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Pharmacological Modulation of Voltage-Gated Na^+ Channels: A Rational and Effective Strategy Against Ischemic Brain Damage

JUTTA URENJAK AND TIHOMIR P. OBRENOVITCH

Department of Neurological Surgery, Institute of Neurology, London, UK (J.U., T.P.O.); Discovery Biology, Pfizer Central Research, Sandwich, UK (J.U.); and Department of Pharmacology, School of Pharmacy, London, UK (T.P.O.)

I. Introduction

Mortality and neurological disabilities due to stroke, cardiac arrest, or perinatal asphyxia have an enormous impact on public health and resources. Stroke alone is the third most common cause of death in the United States (after heart disease and cancer), the leading cause of serious disability, and it accounts for an estimated one-half of all hospitalizations for acute neurological disease (Drug & Market Development, Inc., 1994). Over the last 10 years, there has been considerable progress in the understanding of the mechanisms underlying ischemic brain damage, and a wide range of drugs has been shown to be neuroprotective in experimental models. Despite these advances, a clinically effective drug capable of protecting neurons against ischemia is still awaited (Zivin and Choi, 1991; Bullock, 1992; White et al., 1993). The fact that brain tissue damage subsequent to ischemia results from multifactorial and interrelated processes justifies the wide range of therapeutic strategies currently under investigation (Kogure et al., 1993; Hallenbeck and Frerichs, 1993). The most common approaches are: free radical scavenging (Watson, 1993), prevention of glutamate-mediated excitotoxicity (Diemer et al., 1993), preservation of intracellular Ca^{2+}-homeostasis (Simon et al., 1984; Choi, 1988; Siesjö and Bengtsson, 1989), and improvement of microvascular perfusion (Dirnagl, 1993). In comparison, pharmacological modulation of voltage-gated Na^+ channels has received very little attention. It seems that this molecular target is restricted to antiarrhythmic drugs, anticonvulsants, and local anesthetics (Catterall, 1987).

Voltage-gated Na^+ channels are responsible for initiation and conduction of the neuronal action potential and therefore play a fundamental role in the normal function of nervous system. More precisely, Na^+ channels in cell bodies and axon initial segments determine the threshold for action potential generation and affect the duration and frequency of repetitive neuronal firing. In addition, in nerve terminals, Na^+ channels can potentially influence neurotransmitter release from presynaptic vesicles. The purpose of this article is to dem-

Address for reprints and correspondence: Dr. Jutta Urenjak, Discovery Biology, Pfizer Central Research, Sandwich, Kent, CT13 9NJ, UK.

onstrate that selective down-modulation of voltage-gated Na^+ channels is a rational and effective approach to protect brain tissue in conditions associated with defective blood supply, and one that may still be beneficial when treatment of transient ischemia or stroke is delayed. The first section (II) is an overview of Na^+ channels in the central nervous system (CNS).[a] By focusing on the molecular determinants of Na^+ channel function and pharmacology, this section makes clear that development of site-directed Na^+ channel modulaters is feasible. Next (section III), we detail the rationale for down-modulation of Na^+ channels in ischemia, concentrating on four major points: (1) Most of the brain's energy consumption is used to maintain Na^+ and K^+ gradients across the cellular membrane, and these gradients are vital to electrical activity and transport mechanisms; (2) sustained Na^+ influx, such as that occurring in ischemia, is neurotoxic both acutely and indirectly; (3) the survival strategy of the turtle and immature mammalian brain to anoxia includes down-regulation of Na^+ currents; and (4) *post*ischemic down-modulation of Na^+ channels is also beneficial. Section IV provides further supporting evidence for the beneficial effect of Na^+ channel down-modulation, based on in vitro experiments with Na^+-free medium, and studies using recognized Na^+ channel blockers such as tetrodotoxin (TTX), local anesthetics and anticonvulsants. Finally, a number of *anti-ischemic* compounds under development are examined in section V, all of which appear to primarily target Na^+ channels at therapeutic concentrations.

II. Overview of Voltage-Gated Na^+ Channels in the Central Nervous System

The ion channels involved in electrical signaling belong to two classes. *Ligand-operated* ion channels (e.g., glutamate-receptor ionophore complexes) mediate local increases in ion conductance at chemical synapses, and thereby depolarize or hyperpolarize the subsynaptic area of the cell. In contrast, *voltage-gated* ion channels mediate rapid, voltage-dependent changes in ion permeability during action potentials in excitable cells, and also modulate membrane potential and ion permeability in many inexcitable cells (e.g., glia) (Hille, 1991). Voltage-gated Na^+ channels are responsible for the initial inward current during the depolarization phase of action potentials, and differences in Na^+ channel kinetics, anatomical distribution, and pharmacology have a major impact on cell signaling and information processing. Ion transport, mediated by the Na^+ channel, is among the most rapid of protein-mediated processes, consistent with the view that ion movement takes place by diffusion through an aqueous pore rather than by a carrier mechanism. Na^+ channels are also characterized by high ion selectivity, and steep voltage sensitivity of their ion conductance activity. Several recent reviews have covered in detail the structure, expression, and molecular determinants of Na^+ channel functions (Patlak, 1991; Catterall, 1992, 1993a, b; Cohen and Barchi, 1993; Kallen et al., 1994; Kirsch, 1994). The purpose of this section is to focus on features of voltage-gated Na^+ channels that may be relevant to the etiology of ischemic brain damage, to neuroprotection by selective Na^+ channel modulators, and to a more selective development of such drugs.

A. Structure and Classification

Brain Na^+ channels are heterotrimeric complexes consisting of a highly glycosylated α-subunit (260 kDa), associated noncovalently with a β_1-subunit (36 kDA) and, via a disulfide-linkage, with a β_2-subunit (33 kDA) (Barchi, 1988; Catterall, 1988a, 1992; Goldin, 1993). Analysis of the hydrophobicity of the amino acid sequence of α-subunits suggests a membrane-folding topology consisting of four homologous membrane-bound repeats (I to IV), each composed of six transmembrane segments (S1 to S6 and additional membrane-associated sequences (fig. 1) (Noda et al., 1984). The N- and C-termini, as well as the linkers between α-subunit repeats, are intracellular, whereas the linkers between segments alternate between intracellular and extracellular surfaces. Potential glycosylation sites are all in putative extracellular loops, and phosphorylation sites in intracellular regions (fig. 1). The primary structure of the β_1-subunit of the rat brain, deduced from the isolation of complementary DNA (cDNA) clones, indicated that it contains a single putative transmembrane repeat and four potential extracellular N-linked glycosylation sites (Isom et al., 1992).

Molecular cloning has revealed four distinct, but highly homologous (> 85% sequence identity), primary structures of adult rat brain α-subunits. They are designated type I, II, IIA and III (Noda et al., 1986; Auld et

[a] Abbreviations: CNS, central nervous system; DNA, deoxyribonucleic acid; cDNA, complementary DNA; RNA, ribonucleic acid; mRNA, messenger RNA; cAMP, cyclic adenosine 3′,5′-monophosphate; cA-PK, cAMP-dependent protein kinase; BTX, batrachotoxin; BTX-B, [^{3}H]batrachotoxinin A 20-α-benzoate; G protein, guanine nucleotide binding protein; LDH, lactate dehydrogenase; IFM, isoleucine-phenylalanine-methionine; CHO, Chinese hamster ovary; PMA, phorbol 12-myristate 13-acetate; ATP, adenosine triphosphate; CMR, cerebral metabolic rate; CMR_{glc}, rate of cerebral glucose utilization; $CMRO_2$, cerebral rate of oxygen utilization; EEG, electroencephalogram; PKC, protein kinase C; STX, saxitoxin; TTX, tetrodotoxin; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; SD, spreading depression; DC, direct current; DC potential, direct current potential; NMDA, *N*-methyl-D-aspartate; MCA, middle cerebral artery; IC_{50}, concentration that produces 50% of its maximum possible inhibition; ED_{50}, median effective dose; LTP, long-term potentiation; CAP, compound action potential; QX314, lidocaine ethochloride; KB-2796, 1-[bis(4-fluorophenyl)methyl]-4-(2,3,4-trimethoxybenzyl)piperazine; R56865, *N*-[1-[(4-fluorophenoxy)-butyl]-4-piperidinyl]-*N*-methyl-2-benzothiazolamine; BW1003C87, 5-(2,3,5-trichlorophenyl)-2,4-diaminopyrimidine ethane sulphonate; BW619C89, 4-amino-2-(4-methyl-1-piperazinyl)-5-(2,3,5-trichlorophenyl pyrimidine); GABA, γ-aminobutyric acid; riluzole, 2-amino-6-trifluoromethoxybenzothiazole, PK 26124, RP 54274; PD85,639, *N*-[3-(2,6-dimethyl-1-piperidinyl)propyl]-α-phenylbenzeneacetamide; vinpocetine, [(3α,16α)-eburnamenine-14-carboxylic acid ethyl ester].

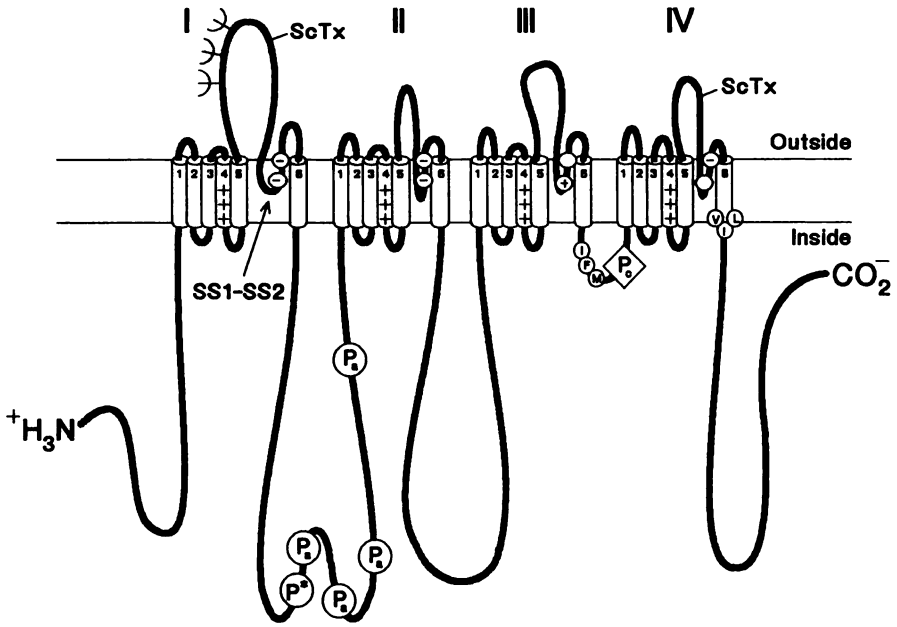


FIG. 1. Primary structure of the α-subunit on a Na^+ channel (rat brain type IIA), illustrated as transmembrane folding diagram, with the major molecular determinants of its function. *Bold lines*, polypeptide chains with length of each segment approximately proportional to its true size in the rat brain Na^+ channel; *Cylinders*, probable transmembrane α-helices—other segments presumably associated with the membrane (i.e., SS1-SS2) are drawn as loops in extended conformation; *Circled P_a in intracellular $L_{III/IV}$*, serine residues that are selectively phosphorylated by cAMP-dependent protein kinase (cA-PK); *P**, this residue (serine^{554}) lies within a cA-PK consensus sequence but is not detectably phosphorylated in vitro, suggesting that it is not accessible to cA-PK in the context of the α-subunit in vitro, or that it is quantitatively phosphorylated under basal conditions in vivo (Murphy et al., 1993); *P_c in the diamond symbol in intracellular $L_{III/IV}$*, site phosphorylated under physiological conditions by PKC—further activation of PKC, however, also phosphorylates serine$_{610}$; *IFM in $L_{III/IV}$*, cluster of three adjacent hydrophic amino acid residues (isoleucine-phenylalanine-methionine) that are critical for fast inactivation; *VIL at the intracellular end of IVS6*, amino acids presumed to be part of the hydrophobic receptor site for the fast inactivation gate; *(+++) in S4*, voltage sensor for activation; *ScTx*, scorpion toxins receptor sites; *Small circles with −, + or open*, amino acid residues required for tetrodotoxin binding (see fig. 2 for details); *(ψ)*, glycosylations sites. Modified from Caterall (1992), with permission of the author and the American Physiological Society.

al., 1988; Kayano et al., 1988). The greatest degree of primary sequence conservation among isoforms occurs in repeats I to IV and in the linker between III and IV ($L_{III/IV}$), regions of the sequence which presumably subserve conserved functions (see section B.6.) (Catterall, 1992). Type II and IIA subunits are alternatively spliced forms, differing in only six amino acids of approximately 2000, and most of these differences are clustered in a *hypervariable* region in the first homologous repeat (Auld et al., 1988).

One α-subtype from rat heart (H1) and two α-subtypes from rat skeletal muscle (SkM1 and SkM2; TTX-sensitive and -insensitive, respectively) have also been documented (Rogart et al., 1989; Trimmer et al., 1989; Kallen et al., 1990). They have a close structural relationship to those in the brain, with the greatest similarity in amino acid sequence in the homologous repeats from transmembrane segments S1 to S6, whereas the intracellular connecting loops are not highly conserved (Trimmer et al., 1989; Rogart et al., 1989). Finally, it is important to mention that two novel Na^+ channel α-subunits have been reported recently: (*a*) Na-g, a distinctive Na^+ channel α-subunit cloned from rat astrocytes cDNA (Gautron et al., 1992); and (*b*) NaCh6, highly expressed in both neurons and glia (Schaller et al., 1995). The amino acid sequence of Na-g retains the four-repeat structure and many conserved features of other brain α-subunits, but it is distinctly more divergent, and has, presumably, a low sensitivity to TTX. In vivo, the mRNA from this glial channel was detected not only in the brain, dorsal root ganglia and sciatic nerve, but also in tissues outside the nervous system including heart, skeletal muscle and lung (Gautron et al., 1992). With regard to NaCh6, its most significant aspects are that it is one of the most abundant Na^+ channel α-subtypes in the nervous system at the RNA level, and that it is expressed in both neurons and glia (Schaller et al., 1995). It is noteworthy that glial cells, although nonexcitable, express a wealth of voltage-gated ion channels, including Na^+ and Ca^{2+} channels that are typically characteristic of excitable cells (Ritchie, 1992; Sontheimer, 1994; Oh and Waxmann, 1994). The primary role of Na^+ channels in these *satellite* cells may be to ensure a controlled leak of Na^+ into cells, thereby fueling the glial Na^+/K^+-ATPase pump, which removes local excess of K^+ in the extracellular space (i.e., spatial buffering) (Sontheimer, 1994; Sontheimer et al., 1994).

An Na^+ channel α-subtype from human brain has been identified from a cDNA library derived from human cerebral cortex, and its expression led to functional

Na^+ channels. The predicted structure exhibits greatest homology (97% sequence identity) and structural similarities to the rat brain type II Na^+ channels (Ahmed et al., 1992). Other studies aiming to identify human brain Na^+ channel α subtypes have suggested a strong homology between these channels and their rat brain counterparts (Lu et al., 1992), and the human β_1-subunit was found 96% identical to the rat brain subunit at the amino acid level (McClatchey et al., 1993; Makita et al., 1994). These structural analogies, supplemented by close functional properties between rat and human Na^+ currents (Cummins et al., 1994), strongly support the view that rat neurons represent a valid model for the study of human Na^+ channel expression and function.

B. Molecular Determinants of Na^+ Channel Function, Toxicology, and Pharmacology

Most Na^+ channels are in a closed, *resting* state at negative membrane potentials. During a step depolarization, the membrane Na^+ conductance rises rapidly due to *voltage-dependent activation* of Na^+ channels, reaches a peak within a few milliseconds, and then declines gradually due to channel *inactivation*. These two fundamental processes ensure a rapid but transient increase of ion conductance in response to membrane potential changes. They can be portrayed by an activation gate that opens rapidly upon depolarization and closes rapidly upon repolarization and an inactivation gate that closes slowly upon depolarization and opens upon repolarization (Hodgkin and Huxley, 1952; Hille, 1991). Voltage-dependent activation controls the timing and voltage-dependence of ion channels opening in response to membrane potential changes; inactivation controls the rate and extent of ion channel closure during maintained depolarization. In addition to these very rapid changes, the ion conductance activity of voltage-gated Na^+ channels can be modulated over longer periods by protein phosphorylation and interaction with guanyl nucleotide binding (G) proteins (see below, section C).

Remarkable progress has been made toward definition of the structural elements that are responsible for the basic functions outlined above, as well as for the binding of specific neurotoxins and local anesthetics (Catterall, 1993a, b). The α-subunit is the principal component of the Na^+ channel, and it forms functional channels when expressed alone in mammalian cells or *Xenopus* oocytes (Goldin et al., 1986; Noda et al., 1986; Scheuer et al., 1990; West et al., 1992b) This feature, and the fact that no drug or toxin has so far been reported to interact with β_1 or β_2 subunits, prompted us to overlook these accessory subunits in this review. However, the β_1 subunit may well be a valuable target for developing therapeutic Na^+ channel modulators, because it appears to modify the inactivation properties of Na^+ channels (Isom et al., 1992; Goldin, 1993), although coexpression of β_1 is required for normal kinetic properties in oocytes (Isom et al., 1992). The α-subunits, therefore, contain all the necessary structural elements to form the transmembrane ion pore, ensure ion selectivity, and carry out the basic functions of the Na^+ channel. They also contain most of the receptor sites for the toxins and many pharmacological agents that modify Na^+ channel functions. In all models for voltage-gated Na^+ channels, the transmembrane pore is at the center of a square array of homologous repeats, with each repeat contributing to a quarter of the channel pore (fig. 2b) (Hille, 1991; Catterall, 1993a, b; Heinemann, 1995).

1. Extracellular opening of the pore: tetrodotoxin and saxitoxin receptor site. TTX and saxitoxin (STX) are water-soluble guanidinium toxins that selectively block Na^+ channels by binding, with high affinity, to the external side of the pore (neurotoxin receptor site 1) (Adams and Olivera, 1994). The fact that protonation or covalent modification of carboxyl residues blocks their binding suggests that these cationic toxins may bind to a ring of carboxyl residues at the extracellular opening of the pore (Hille, 1991). The first of these sites to be identified was glutamate^{387} (rat brain IIA α-subtype),

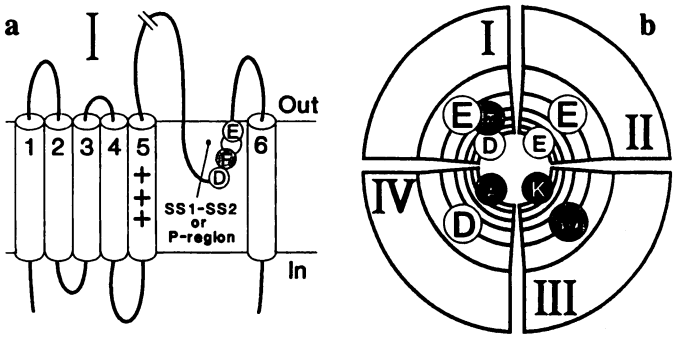


FIG. 2. Current model of the extracellular opening of type II rat brain Na^+ channel. The transmembrane pore is at the center of a square array of homologous repeats, with each repeat contributing to a quarter of the channel pore (*b*). *a* Folding topology of repeat I, illustrating the hairpin-like P-region located in the S5-S6 linker. This segment, previously called short segments SS1-SS2, contributes to the lining of the pore at its extracellular opening. The P-region is critical to the binding of guanidinium toxins (TTX and STX), Na^+ channel conductance, and ion selectivity. Amino acids code and position in the sequence: E, glutamate^{387}; F, tyrosine^{385}; D, aspartate^{384}. *b* Schematic diagram of the extracellular opening of type II rat brain Na^+ channels. The two rings of four amino acid residues that are represented, as well as F^{385} in repeat I, are all essential for TTX and STX binding. They are also determinants of the ion conductance and selectivity, with the inner ring differentiating Na^+-selectivity from Ca^{2+}-selectivity (see text). Amino acids in each ring are located at identical position on each P-region of repeat I-IV. Positive amino acid residues are in black, neutral residues in gray, and negative residues in open circles. Homologous repeats are drawn clockwise, but current experimental data does not exclude a counterclockwise arrangement (Heinemann, 1995; Heinemann et al., 1994). *Repeat I*, outer ring = E, glutamate^{387}, F, tyrosine^{385}; inner ring: D, aspartate^{384}; *Repeat II*, outer ring = E, glutamate^{945}; inner ring = E, glutamate^{942}; *Repeat III*, outer ring: M, methionine^{1425}; inner ring: K, lysine ^{1422}; *Repeat IV*, outer ring: D, aspartate^{1717}; inner ring: A, alanine^{1714}. Diagram *b* is reproduced from Heinemann (1995), with permission of the author and Elsevier Science Publishers (Amsterdam).

located on the S5-S6 linker of repeat I (fig. 2). Mutation of this residue to glutamine reduces 10,000-fold the affinity of TTX for the channel (Noda et al., 1989). The nearby, negatively charged aspartate[384] was also found to be important for guanidinium toxin block. The mutant D384N (i.e., aspartate replaced by asparagine) was no longer blocked by TTX and STX and showed an extremely low single-channel conductance (Pusch et al., 1991). Subsequent studies have identified amino acid residues in the same position as glutamate[387] and aspartate[384] in the S5-S6 linker of the other repeats, which are all required for high-affinity TTX binding and normal single-channel conductance (Terlau et al., 1991). These, and other results obtained from Ca^{2+} and K^+ channel studies, contributed to the current model of voltage-gated ion channels in which a section of the S5-S6 linker forms at least part of the actual pore, dipping into and out of the membrane as a hairpin-like structure (fig. 2a) (Heinemann et al., 1994; Heinemann, 1995). This region corresponds to the short membrane-associated segments SS1 and SS2 (now termed P-region, for pore-region), previously postulated to form the narrow section of the channel ensuring ionic selectivity (Greenblatt et al., 1985; Guy and Seetharamulu, 1986; Guy and Conti, 1990).

Although cardiac Na^+ channels contain the eight residues described above, which are essential for high-affinity TTX binding, they bind to TTX with 200 times lower affinity than brain or skeletal muscle (SkM1) Na^+ channels. This indicates that other residues must contribute in an essential way to the TTX receptor site. Site-directed mutagenesis and toxin assay have located one such residue at position 385 (fig. 2), i.e., two residues from glutamate[387] toward the amino terminus in the repeat I of type IIA brain α-subtype. In the brain and skeletal muscle, this residue is tyrosine and phenylalanine, respectively, but it is cysteine in the heart Na^+ channel. Mutation of this residue from cysteine to phenylalanine or tyrosine causes a 200-fold increase in affinity for TTX in the cardiac Na^+ channel (Heinemann et al., 1992a). The converse mutation in the brain or skeletal muscle channel reduces their affinity for TTX by the same factor (Satin et al., 1992; Backx et al., 1992).

The current structural model depicts the external mouth of the Na^+ channel pore as a funnel-shaped space, in which TTX and STX occlude the pore by binding at multiple attachment points constituting the neurotoxin receptor site 1. This model conforms to the general view of interchangeability between TTX and STX with regard to their binding to the channel. However, the chemical structures of these two toxins differ considerably (Shimizu, 1986) and there is clear indication that the toxin-receptor interaction at microscopic level is different for the two toxins (Kirsch et al., 1994).

2. Divalent cation-blocking sites: selectivity filter. The ionic selectivity of Na^+ channels is well defined (Hille, 1991); protons are the most permeant, as is expected for an aqueous pore, and the alkali cations permeability falls with increasing crystal radius ($Li^+ \sim Na^+ > K^+ > Rb^+ > Cs^+$). Although K^+ and Ca^{2+} ions are selected against in Na^+ channels, these ions are still quite measurably permeant (Meves and Vogel, 1973). A number of observations indicate that the divalent ion-blocking site and the guanidinium toxin receptor site 1 overlap each other: (*a*) divalent cations block within the open channel and compete with toxin binding to the channel, with isoform-specific differences in the blocking affinity and competitive efficacy (Schild and Moczydlowski, 1991; Doyle et al., 1993); (*b*) mutations affecting toxin affinity also change the divalent ion blocking affinity (Pusch et al., 1991; Heinemann et al., 1992a; Satin et al., 1992; Backx et al., 1992); and (*c*) voltage dependence analysis of the divalent ions block (Yamamoto et al., 1984; Nilius, 1988; Sheets and Hanck, 1992; Sheets et al., 1987; Backx et al., 1992) and that of the guanidinium toxins block (Satin et al., 1994) showed that both binding sites are 15 to 30% into the membrane field. In addition, mutation of lysine[1422] and alanine[1714] (K and A on the inner ring in fig. 2b) to negatively charged residues, not only alters TTX binding, but also switches the selectivity of the channel from Na^+- to Ca^{2+}-selectivity (Heinemann et al., 1992b). The corresponding mutant showed no more selectivity among monovalent cations, strong block of monovalent current by divalent cations, and permeation of divalent cations, i.e., features of voltage-gated Ca^{2+} channels. These findings strongly suggest that the inner ring constitutes the selectivity filter of ion channels (Heinemann et al., 1994). To summarize sections 1 and 2, the P-region in each repeat is critical to guanidinium toxin binding, Na^+ channel conductance and ion selectivity.

3. Ion conducting pore: local anesthetics receptor site. Local anesthetics block the propagation of nerve impulse by preventing the normal function of Na^+ channels (Taylor, 1959), and they were also shown to interact with Na^+ channels of central neurons (Butterworth et al., 1993; Capek and Esplin, 1994). The action of local anesthetics on Na^+ currents is complex, with use-dependent and frequency-dependent (i.e., inhibition is potentiated with repetitive stimuli) properties which suggest that binding is modulated by channel state (Catterall, 1987; Butterworth and Strichartz, 1990; Starmer et al., 1990). These drugs were initially thought to act by perturbing the properties of the phospholipid phase of biological membranes, because they are effective only at relatively high concentrations (10^{-6} to 10^{-2} M), and their potency is most strongly correlated with their lipid solubility. However, experiments with impermeant derivatives strongly suggested that local anesthetics bind to specific receptor sites located on the Na^+ channel protein itself. Permanently positively charged derivatives, such as the quaternary analogue of lidocaine QX314 (fig. 3b), are ineffective when applied extracellularly, but potent use-dependent blockers when applied

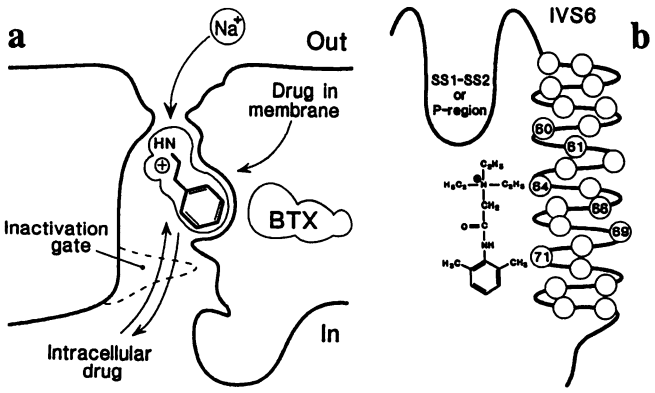


FIG. 3. Molecular mechanism of Na^+ channel blockade by local anesthetics. *a* Two pathways exist for drugs to reach the receptor located in the channel pore: neutral (hydrophobic) compounds can bind and unbind even when the inactivation gate is closed, whereas charged hydrophilic drugs require the gate to be open to access the binding site. BTX, batrachotoxin receptor site: there is an indirect allosteric coupling between this neurotoxin receptor (site 2) and the receptor site for local anesthetics. Adapted from Hille (1991) with permission of the author and Sinauer Associates. *b*, Putative orientation of amino acids in S6 or repeat IV (rat brain type IIA Na^+ channel) with respect to a quaternary analogue of lidocaine (QX314) bound in the ion-conducting pore. In this model, phenylalanine $^{17(64)}$ and tyrosine$^{17(71)}$ are both oriented on the same face of the IVS6 helix, facing the pore lumen, because their mutation to alanine strongly suggests that these hydrophobic aromatic residues contribute to the free energy of drug binding (see text). Binding presumably occurs through hydrophobic of π electron interactions, because effective local anesthetics are characterized by positively charged and hydrophobic moieties at either end of the molecule (Zamponi and French, 1994a). The bulky isoleucine $^{17(60)}$ is oriented on the same face of the helix as 17(64) and 17(71) residues and is therefore well positioned to modulate extracellular access to the local anesthetic binding site. Modified from Ragsdale and coworkers (1994), with permission from the authors and the American Association for the Advancement of Science.

intracellularly (Frazier et al., 1970; Strichartz, 1973; Courtney, 1975; Khodorov et al., 1976). Further experiments confirmed that these drugs act mainly on open Na^+ channels, through a cytoplasmic, hydrophilic pathway which is occluded by the activation and inactivation gates (Catterall, 1987; Butterworth and Strichartz, 1990; Ragsdale et al., 1991; Hille, 1991) (fig. 3a).

The S6 segment in repeat IV of Ca^{2+} channels, and the single S6 segment of K^+ channels as well, have been implicated in the binding of pore blockers (Catterall and Striessnig, 1992). Ragsdale and coworkers (1994) have demonstrated that site-directed mutations in transmembrane segment S6 of repeat IV of the rat brain α-subunit selectively modified the binding of QX314 to resting or to open and inactivated channels when expressed in *Xenopus* oocytes (fig. 3b). Mutation of phenylalanine^{1764} near the middle of this segment to alanine, decreased the affinity of open and inactivated channels to 1% of the wild-type value, resulting in almost complete abolition of both the use-dependence and voltage-dependence of drug block. In contrast, mutation of asparagine^{1769} to alanine increased the affinity of the resting channel 15-fold. Replacement of the bulky isoleucine residue at position 1760 with alanine allowed QX314 to reach the site from the extracellular side, perhaps by passing directly through the pore from the outside. Presumably, this amino acid residue corresponds to a narrow region in the pore, just to the extracellular side of the local anesthetic binding site. Mutations of isoleucine^{1761}, valine^{1766} and asparagine^{1769} to alanine increased QX314 resting block without altering inactivated state affinity, despite being presumably oriented away from the channel pore, suggesting that these mutations may increase channel sensitivity to drugs through indirect effects on the local anesthetic site. Together, these data clearly define the location of the local anesthetic receptor site in the pore of the Na^+ channel and identify molecular determinants of the state-dependent binding of these drugs and related blockers.

4. Veratridine and batrachotoxin receptor site. Grayanotoxin and the alkaloids veratridine, aconitine and batrachotoxin (BTX) compete for binding at neurotoxin receptor site 2 (Catterall, 1980; Catterall et al., 1981). These toxins cause persistent activation of Na^+ channels at the resting membrane potential by blocking Na^+ channel inactivation and shifting the voltage dependence of the channel activation to a more negative membrane potential. These effects presumably result from preferential, high affinity binding of the toxins to the active states of Na^+ channels, and consequent stabilization of those states (Catterall, 1980; Khodorov, 1985; Hille et al., 1987). As the toxins acting at site 2 also alter the ion selectivity of the Na^+ channel, this receptor site is presumably in a region of the α subtype that is involved in voltage-dependent activation and inactivation, and allosterically linked to the transmembrane pore of the channel (Catterall, 1992). As these toxins are large molecules, their multiple effects might also result from an interaction at several microsites of the Na^+ channel (Zamponi and French, 1994).

Due to their lipophilic nature, these toxins can produce their effects when added to either the extracellular or the cytoplasmic side of the membrane. They are thought to bind to the channel at sites buried in the matrix of the lipid bilayer. Local anesthetics do not affect toxin binding to neurotoxin receptor sites 1 (TTX, STX) or 3 (α-scorpion toxins, see fig. 1), but they allosterically inhibit BTX binding to neurotoxin receptor site 2 (Postma and Catterall, 1984; Catterall, 1987; Creveling et al., 1983; McNeal et al., 1985). This inhibition results from indirect allosteric coupling between the receptor site for the drugs and neurotoxin receptor site 2, rather than from direct steric interaction at a common binding site (fig. 3a). This interaction presumably results from the fact that neurotoxins acting at site 2 bind with high affinity to active states of Na^+ channels, whereas local anesthetics bind with highest affinity to either resting or inactivated states of Na^+ channels. By binding preferentially to states with lowest affinity for

the toxins, these drugs therefore stabilize those states of the channel and reduce neurotoxin binding affinity and persistent activation of Na^+ channels. Together, these findings strongly suggest that the BTX site is in close proximity to the local anesthetic site and, thus, of the lumen of the pore.

BTX and veratridine have become very popular tools for the selection of drugs acting on the Na^+ channel, and for the investigation of their mode of action. A BTX analogue ([^{3}H]batrachotoxinin A 20-α-benzoate) (BTX-B) was developed as a radioligand for the neurotoxin site 2 (Brown et al., 1981; Catterall et al., 1981; Creveling and Daly, 1992). Initially, as local anesthetics were known to displace BTX-B from synaptosomal membranes (Creveling et al., 1983; Postma and Catterall, 1984), BTX-B binding was proposed as a rapid assay for local anesthetic activity (McNeal et al., 1985), but it is now clear that binding to site 2 is not a unique feature of local anesthetics (fig. 4) and that high affinity for the BTX site does not imply potent local anesthetic activity (Velly et al., 1987; Pauwels et al., 1986). This is a reminder that *high-affinity binding and potent functional activity are not necessarily coincident* (Adams and Olivera, 1994). Veratridine-induced Na^+ influx is an appropriate functional test to verify whether a given compound inhibits Na^+ currents. Na^+ influx can be assessed by using ^{22}Na (Zimanyi et al., 1989), fluorescent Na^+ indicators (Daniell, 1992; Deri and Adam-Vizi, 1993), [^{14}C]guanidinium ion (Jacques et al., 1980; Reith, 1990), or indirectly, by monitoring oxygen consumption (Urenjak et al., 1991) or toxicity [e.g., lactate dehydrogenase (LDH) release in cultured neurons; Pauwels et al., 1990]. BTX is also used in voltage-clamp studies to eliminate inactivation (Keller et al., 1986).

5. Voltage-dependent activation. Activation of voltage-gated ion channels is thought to result from a voltage-driven conformational change that opens a transmembrane pore through the protein. Channel opening is

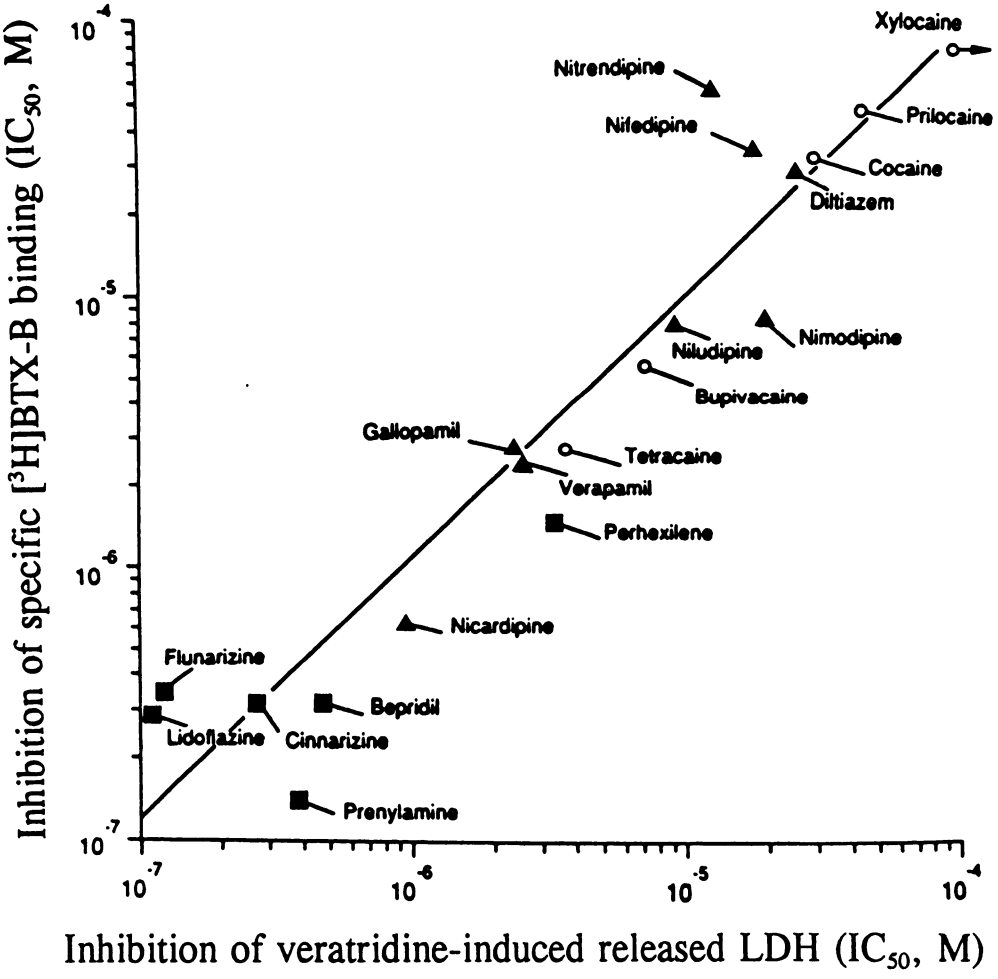


FIG. 4. Correlation between the potencies of Ca^{2+} blockers and local anesthetics to prevent neurotoxicity induced by 30 μM veratridine in brain neuronal cultures [assessed by lactate dehydrogenase (LDH) release], and their binding affinity for the [^{3}H]BTX-B binding site in rat cortex synaptosomal preparation. The plotted values are mean IC_{50}. The correlation between neurotoxicity and binding data was calculated by linear regression analysis (slope = 0.96; correlation coefficient = 0.90, $P < 0.001$. ▲, Ca^{2+} blockers selective for slow Ca^{2+} channels; ■, Ca^{2+} blockers nonselective for slow Ca^{2+} channels; *o*, local anesthetics. Note the tendency of the nonselective Ca^{2+} blockers to strongly interact with Na^+ channels. This figure was taken from Pauwels and coworkers (1990), with permission from the authors and Williams & Wilkens.

triggered by membrane depolarization, exerting an electrical force on gating charges (voltage sensors) located within the transmembrane electrical field (Catterall, 1993a, b). The movement of the gating charges through the membrane has been directly measured as an outward gating current (Armstrong, 1981). Na^+ currents through the channel can be blocked by toxins without affecting these gating currents, suggesting that elements of the channel protein involved in voltage-dependent activation are separate from those that form the pore. Similarly, mutations in the P-region that alter ion conduction have no effect on gating currents (Pusch et al., 1991).

The S4 transmembrane regions of one or more repeats contain voltage sensing structures that are critical to activation gating (fig. 1). Analogous sequences to S4 have been found in Ca^{2+} and K^+ voltage-activated channels, and they all share a unique motif composed of four to eight positively charged amino acids (arginine or lysine), spaced at three-residue intervals along a putative α-helical segment. Activation gating is thought to result from a displacement of these charged residues in response to changes in the transmembrane electrical field (Armstrong and Bezanilla, 1973; Catterall, 1993a, b). Site-directed mutagenesis and in vitro expression of rat brain II Na^+ channels have strongly supported this concept: (*a*) replacement of positively charged amino acid residues in S4 of repeat I, by neutral or negatively charged residues, reduced the steepness of the potential dependence of activation, indicating a decrease in the apparent gating charges (Stühmer et al., 1989); and (*b*) alteration of a single neutral residue in the S4 sequence of repeat II shifted the I-V relationship by 20 to 25 mV toward more positive potentials, reflecting a dramatic change in gating behaviour (Auld et al., 1990).

6. Fast inactivation. A wide range of experimental evidence indicates that activated channels become nonconducting when a blocking site at the inner side of the membrane is physically occupied by an *inactivation particle*, presumably located in the cytoplasmic linker connecting repeats III and IV ($L_{III/IV}$) (fig. 1): (*a*) perfusion of the intracellular surface of Na^+ channels with proteolytic enzymes prevented inactivation (Armstrong et al., 1973; Rojas and Rudy, 1976); (*b*) antibody directed against a peptide sequence of $L_{III/IV}$ markedly slowed inactivation, and the intracellular sequence recognized by the antibody was rendered inaccessible by inactivation, suggesting a conformational change of this sequence during inactivation (Vassilev et al., 1988, 1989); (*c*) mutational cleavage of the linkage between repeats III and IV of the rat brain type II Na^+ channel caused a marked reduction in the rate of inactivation (Stühmer et al., 1989); and (*d*) deletions of some residues (Patton et al., 1992), or mutation of three contiguous hydrophobic residues [isoleucine-phenylalanine-methionine (IFM); fig. 1] to polar glutamine in the amino acid sequence of $L_{III/IV}$, completely removed inactivation (West et al., 1992a). The phenylalanine^{1489} residue at the centre of the IFM cluster seems critical for the presumed hydrophobic interaction, because its conversion to glutamine is sufficient, by itself, to almost completely prevent fast channel inactivation.

The cluster of residues IFM in $L_{III/IV}$ is conserved among α subtype isoforms, and it performs a similar role in heart (Hartmann et al., 1994) and skeletal muscle channels (Orias et al., 1994). In addition, this cluster has affinity for a receptor in the pore, inasmuch as small peptides containing the motif IFM are able to restore fast inactivation to channels mutated in this region (Eaholtz et al., 1994). McPhee and coworkers (1994) have identified three adjacent residues (valine-isoleucine-leucine) at the intracellular end of segment IVS6, which may be part of the hydrophobic receptor site for the fast inactivation gate (fig. 1) because their conversion to alanine markedly disrupted fast inactivation. Interactions between cluster IFM and the receptor for the inactivation particle are likely to be hydrophobic, because there is a close correlation between hydrophobicity of the residue at that position and the extent of fast Na^+ channel inactivation (Scheuer et al., 1993).

Although the charged residues of $L_{III/IV}$ are not critical for fast inactivation (Moorman et al., 1990; Patton et al., 1992), mutations altering them shifted the voltage-dependent properties of the channel, and these shifts appeared to correlate with the charge of the replacement amino acids (Patton et al., 1992). This observation is consistent with the hypothesis that these charge residues interact electrostatically from the cytoplasmic side with a voltage sensor of the channel, possibly one of the S4 segments (Moorman et al., 1990).

C. Physiological Modulation of Na$^+$ Channels

Voltage-gated Na^+ channels are not only responsible for initiation and conduction of neuronal action potentials, but can also influence neurotransmitter release from presynaptic vesicles (Krueger et al., 1980; Abita et al., 1977; see also section III. B.5.). There is strong evidence indicating that these integrative functions of Na^+ channels are subject to neuromodulation by secondary messenger systems, especially by protein phosphorylation, a fundamental regulatory mechanism in the control of membrane properties (Levitan, 1988).

1. Phosphorylation by adenosine 3′,5′-cyclic monophosphate-dependent protein kinase. Rat brain Na^+ channels are readily phosphorylated by 3′,5′-cyclic monophosphate (cAMP)-dependent protein kinase (cA-PK) in purified preparations (Costa and Catterall, 1982), isolated nerve endings (synaptosomes) (Costa and Catterall, 1984a), and following elevation of intracellular cAMP in intact rat brain neurons (Rossie and Catterall, 1987). The fact that the same residues were phosphorylated in vitro, and following activation of cA-PK in vivo, suggested a physiological role for this covalent modification (Rossie et al., 1987; Rossi and Catterall, 1989).

Four serine residues have been identified as sites of selective cA-PK phosphorylation and dephosphorylation. They are all clustered within the intracellular segment linking homologous repeats 1 and 2 ($L_{I/II}$) of the α-subunit (fig. 1) (Rossie et al., 1987; Rossie and Catterall, 1989; Murphy et al., 1993), and antibodies against this region partially block phosphorylation (Nakayama et al., 1992). Because $L_{I/II}$ is highly conserved among rat brain α subtypes (Noda et al., 1986; Kayano et al., 1988; Auld et al., 1988), modulation by cAMP-dependent phosphorylation is presumably a mechanism common to all brain Na^+ channels.

A number of findings suggest that cA-PK down-regulates Na^+ channel activity in neurons. Increased cytosolic cAMP reduced neurotoxin-activated ^{22}Na influx into rat brain synaptosomes (Costa and Catterall, 1984a). Na^+ currents in acutely dissociated striatonigral neurons were reduced by agonists acting at D_1 dopamine receptors that activate adenylate cyclase, whereas agonists acting at D_2 receptors, which inhibit adenylate cyclase, had no action or increased the evoked currents (Surmeier and Kitai, 1993). The most convincing evidence was obtained by purified cA-PK acting directly on Na^+ channels in excised membrane patches (Gershon et al., 1992; Li et al., 1992). cA-PK phosphorylation of inside-out membrane patches from rat brain neurons, or Chinese hamster ovary (CHO) fibroblasts expressing type IIA Na^+ channels, reduced peak Na^+ current by up to 50%, without change in the kinetics or voltage dependence of activation or inactivation of the channel, thus suggesting a decrease in the open probability of single Na^+ channels during depolarization (Li et al., 1992). A significant reduction in Na^+ channel number and activity was caused by the basal activity of cA-PK in CHO cells, indicating that the activity of Na^+ channels is dynamically modulated by fluctuations in cAMP concentration at or above the resting level in cells (Li et al., 1992). Thus, both basal and stimulated levels of cA-PK, as well as the activity of phosphoprotein phosphatases, are likely to play important roles in controlling the dynamic state of α-subunit phosphorylation and, thereby, the regulation of channel function. This further implies that a wide range of neurotransmitters acting through cAMP as a second messenger can modulate neuronal Na^+ channels, with increased cAMP attenuating Na^+ currents.

2. Phosphorylation by protein kinase C. Protein kinase C (PKC) is a Ca^{2+}/phospholipid-dependent regulatory enzyme activated by diacylglycerol. Its activation is believed to involve a translocation to cell membranes, followed by activation, after which substrate phosphorylation takes place. PKC is highly concentrated in the brain and exists in several different isoforms that are differentially distributed and differentially activated by secondary messengers (Nishizuka, 1988, 1992). The finding that α-subunits of purified Na^+ channels from rat brain are phosphorylated by PKC was first to suggest that Na^+ currents may also be modulated by the Ca^{2+}-diacylglycerol signaling pathway (Costa and Catterall, 1984b). Under physiological conditions, PKC phosphorylates a single serine residue (serine^{1506}) of rat brain α-subunits, located in the inactivation gate formed by the short intracellular loop between repeats III and IV ($L_{III/IV}$) (fig. 1) (West et al., 1991). The sequence surrounding serine^{1506} (lysine-lysine-leucine-glycine-*serine*-lysine-lysine; Auld et al., 1988) favours its phosphorylation by PKC, but not by cA-PK (Kennelly and Krebs, 1991; Kemp and Pearson, 1990; West et al., 1991). Further activation of PKC, however, also phosphorylates serine^{610} in $L_{I/II}$ (Numann et al., 1992), thus implying that this residue may be a phosphorylation site common to cA-PK and PKC (see above, section 1.; Murphy and Catterall, 1992).

PKC activation by arachidonic acid and other *cis*-unsaturated fatty acids, phorbol esters or diacylglycerol-like compounds modulates Na^+ currents in neuroblastoma cells, rat brain neurons and *Xenopus* oocytes and mammalian cells expressing rat brain Na^+ channels or type IIA α-subunits (Linden and Routtenberg, 1989; Godoy and Cukierman, 1994; Numann et al., 1991, 1992; Schreibmayer et al., 1991; Dascal and Lotan, 1991; Sigel and Baur, 1988). Although there are some discrepancies, probably arising from differences in preparations and PKC activators, overall, phosphorylation by PKC slows Na^+ channel inactivation and reduces peak Na^+ currents (West et al., 1991; Schreibmayer et al., 1991; Dascal and Lotan, 1991; Murphy and Catterall, 1992). Phosphorylation by PKC of serine^{1506}, alone, is sufficient to slow inactivation (West et al., 1991), which is consistent with the location of this residue in a protein segment essential to inactivation gating. However, the reduction of peak Na^+ currents required PKC phosphorylation of both serine^{1506} in $L_{III/IV}$ and serine^{610} in $L_{I/II}$ (West et al., 1991). Li and coworkers (1993) showed that the reduction of peak Na^+ currents by cA-PK (see above, section 1.) only occurs when serine^{1506} is phosphorylated by PKC, or substituted with a negatively charged amino acid. This strongly suggests that the effect of cA-PK on peak Na^+ current is conditional, dependent on phosphorylation of serine^{1506} by PKC (Li et al., 1993).

Serine^{1506} and the adjacent amino acids of the PKC phosphorylation site are conserved in Na^+ channel α-subunits from brain, heart and skeletal muscle (Noda et al., 1984; Auld et al., 1988; Kayano et al., 1988; Kallen et al., 1990; Rogart et al., 1989; Trimmer et al., 1989), suggesting that Na^+ channel function may be modulated by activation of PKC in a wide range of excitable tissues.

It is relevant to mention here that, although TTX-sensitive Na^+ channels expressed in neurons and astrocytes exhibit reduced Na^+ currents with PKC activation, the distinct TTX-resistant Na^+ channel expressed in astrocytes showed a strong potentiation of currents following PKC activation (Thio and Sontheimer, 1993).

Morphologically distinguishable subtypes of astrocytes cultured from neonatal rat spinal cord, expressed distinct Na^+ current types that differed by up to 1000-fold in their TTX sensitivity (Thio and Sontheimer, 1993). Activation of PKC by phorbol 12-myristate 13-acetate (PMA) reduced peak TTX-sensitive Na^+ currents by 20 to 60%, whereas PMA potentiated peak TTX-resistant Na^+ currents by 60 to 150%. PMA-induced effects developed within minutes and were typically not reversible (Thio and Sontheimer, 1993). The functional importance of this up-regulation of glial TTX-resistant Na^+ channels by PKC is unknown.

3. Modulation of Na^+ channels by guanine nucleotide binding proteins (G proteins). In addition to protein phosphorylation, there is evidence suggesting that activation of G proteins in response to neurotransmitters can modulate Na^+ channel function. Na^+ currents through brain Na^+ channels expressed in *Xenopus* oocytes were reduced by guanosine 5′-[gamma-thio]-triphosphate, a nonhydrolyzable guanosine triphosphate analogue (Cohen-Armon et al., 1989). Ma and coworkers (1994) have shown that activation of brain Na^+ channels was enhanced by activation of G proteins in both hippocampal neurons and in CHO cells expressing the α-subunit of the type IIA Na^+ channel. Furthermore, basal activation of G proteins (i.e., in absence of agonist) was sufficient to shift significantly the voltage dependence of both activation and inactivation toward more negative potentials. As G proteins were also found to mediate the regulation of cardiac Na^+ channels by β-adrenergic receptors (Schubert et al., 1990; Matsuda et al., 1992), modulation of Na^+ channel activity by G proteins may be a widespread mechanism for regulation of electrical excitability.

III. Rationale for Pharmacological Modulation of Na^+ Channels in Ischemia

A. Brain Cellular Ionic Homeostasis and Energy Requirement

Although the precise meaning of ischemia is interruption or reduction of blood flow, it is more pertinent to consider ischemia as an imbalance between energy supply and demand when considering cerebroprotection. This extended definition encompasses situations without occlusive impairment of blood flow but where energy demand exceeds supply, such as hypoglycemia (Astrup and Norberg, 1976; Pelligrino et al., 1982; Harris et al., 1984), hypotension superimposed upon sustained seizures (Astrup et al., 1979), and deficient mitochondrial ATP synthesis (Beal, 1992; Sparaco et al., 1993). Above all, this concept illustrates that protection may be achieved, not only by improving local perfusion in ischemic territories, but also by decreasing their metabolic demand (Obrenovitch, 1995a). Reduction of cerebral metabolic rate by barbiturates (Bendo et al., 1987; Spetzler and Hadley, 1989; Kass et al., 1992) or hypothermia (Ginsberg et al., 1992; Dietrich, 1992; Dietrich et al., 1993) is probably responsible, at least partly, for their protective effects in cerebral ischemia (see below, sections 2. and 3.). Down-modulation of voltage-gated Na^+ channels is another effective way of reducing energy demand, because a large part of the energy consumed by the brain is used for maintenance of ionic gradients across the cellular membrane (Erecinska and Silver, 1989), even when synaptic activity is abolished (e.g., barbiturate coma) (Astrup, 1982).

The total energy consumption of the brain, expressed as cerebral metabolic rate (CMR) of either oxygen ($CMRO_2$) or glucose (CMR_{glc}), can be considered as the sum of activation metabolism, and residual or basal metabolism (Michenfelder, 1974; Astrup, 1982). *Activation metabolism*, which supports electrical and synaptic activity, varies with the functional state of the brain (see below, sections 1. and 2.). *Residual metabolism* supports the basal cellular processes that persist after functional activity has been abolished (see below, section 3.).

1. Functional activity and energy metabolism. Application of the autoradiographic 2-deoxy-D-glucose method for measurement of local CMR_{glc}, and of its extension to positron emission tomography with 2-[^{18}F]fluoro-2-deoxy-D-glucose in man, have clearly established that local functional activity and activation metabolism are closely linked in nervous tissues. Decreased functional activity reduces local CMR_{glc}, whereas increased activity raises local CMR_{glc} (Sokoloff, 1993). The highest levels of activation metabolism are reached during the synchronous neuronal firing in epileptic seizures; $CMRO_2$ was increased three-fold in the rat brain during bicuculline-induced seizures (Meldrum and Nilsson, 1976).

It is also well documented that most of the energy demand coupled to brain functional activity is used for ion transport. The main function of the CNS is the generation, processing and transmission of action potentials. These do not consume energy by themselves; they are passive electrical consequences of the movement of K^+ from inside the cell to the extracellular space, and of Na^+ in the reverse direction, when the cell membrane is depolarized. Instead, energy metabolism associated with the electrical and functional activities of nervous tissue is used to restore the ionic gradients and resting membrane potentials that were partly degraded during the excitation phase (Erecinska and Silver, 1989; Sokoloff, 1993).

2. Barbiturate inhibition of functional activity and cerebroprotection. The primary protective mechanism of barbiturates is generally attributed to their ability to decrease the cerebral metabolic rate, thus improving the ratio of energy supply to energy demand. Barbiturates depress carbohydrate metabolism (Strang and Bachelard, 1973; Nilsson and Siesjö, 1975; Sokoloff et al., 1977) and increase brain energy reserves (Gatfield et al., 1966), but these actions result from inhibition of synaptic transmission, and not from a direct effect on brain

energy metabolism. Barbiturates dose-dependently reduce glucose consumption, only as long as some EEG activity remains (Michenfelder and Milde, 1975; Astrup et al., 1981a, b). This feature may explain why barbiturates are beneficial against focal ischemia and incomplete global ischemia, especially when administered before the ischemia onset (Spetzler and Hadley, 1989; Hall and Murdoch, 1990). In these conditions, they selectively reduce the energy expenditure required for synaptic transmission while maintaining the component necessary for basic cellular functions (Steen and Michenfelder, 1980), thus favouring a redistribution of cerebral blood flow to ischemic tissue in focal ischemia (Branston et al., 1979; Feustel et al., 1981). In contrast, barbiturate therapy is of no benefit during instances of complete global ischemia, including cardiac arrest (Brain Resuscitation Clinical Trial I Study Group, 1986), during which EEG silence occurs within seconds, and rapid depletion of brain energy stores is little altered by barbiturate (Steen et al., 1978; Mrsulja et al., 1984).

3. Brain utilization of residual energy metabolism. Basal energy metabolism (i.e., residual metabolism after inhibition of synaptic transmission) is especially relevant to protection against cerebral ischemia, because it corresponds to the level of energy necessary for preservation of nonfunctional, but still viable, ischemic brain regions. Residual energy metabolism is a feature of the ischemic penumbra, regions where neuronal function is lost but cellular ionic gradients are maintained (Obrenovitch, 1995a). By superimposing ouabain, a specific inhibitor of Na^+/K^+-ATPase, onto barbiturate inhibition of electrical activity, Astrup and coworkers (1981b) demonstrated that 40 to 50% of residual metabolism in the dog brain was still linked to Na^+/K^+-ATPase activity to compensate for Na^+-K^+ leakage across cellular membranes. The same investigators showed that these residual ion transports were also markedly inhibited by high doses of lidocaine, a local anesthetic blocking Na^+ channels, or hypothermia. This action was referred to as *membrane stabilization or sealing* (Astrup et al., 1981a, b; Astrup, 1982).

In vitro studies are consistent with the notion that approximately half of the residual energy expenditure is used for ion transport. Specific inhibition of Na^+/K^+-ATPase by ouabain, or incubation in Na^+-free medium, lowered the energy consumption of brain slices by 40% (Whittam, 1962). Simultaneous measurement of oxygen consumption and lactate production of a rabbit retina preparation in darkness indicated that Na^+ transport by Na^+/K^+-ATPase accounted for approximately half of all energy expenditure (Ames et al., 1992). Marked reductions in oxidative activity were also observed in nonmyelinated nerves exposed to ouabain or with reduced Na^+ (Ritchie, 1967).

4. Mechanism coupling energy metabolism to transmembrane ion transport. The key component linking energy metabolism to transmembrane ion transport is the Na^+/K^+-ATPase, an enzyme that uses ATP to transport Na^+ back out of the cell and K^+ back into the cell, thus restoring the ionic gradients across the cellular membrane. For example, electrical stimulation of rat posterior pituitary glands in vitro increased 2-deoxy-D-glucose uptake in almost direct proportion to the spike frequency, indicating increased glucose utilization, and the enhancement of uptake was completely blocked by the inhibition of Na^+/K^+-ATPase with ouabain (Mata et al., 1980). The K_m of the Na^+/K^+-ATPase from neurons and synaptosomes is approximately 80 mM for Na^+ and 2 to 3 mM for K^+ (Logan, 1980; reviewed by Erecinska and Silver, 1989), which implies that, with regard to ions, the activity of this enzyme in neurons is predominantly controlled by intracellular Na^+. This was confirmed by experimental procedures that induce Na^+ influx.

The basal consumption of oxygen by rat brain synaptosomes was increased 2.5-fold by veratridine, and this effect was blocked by TTX or ouabain, indicating that the stimulation of respiration was directly linked to Na^+ influx (Erecinska and Dagani, 1990; Urenjak et al., 1991). In the same preparation, veratridine increased the calculated turnover of ATP in oxygenated synaptosomes five-fold (Gleitz et al., 1993). In cultured neurons, veratridine increased CO_2 production three times (Peng and Hertz, 1994). Monensin (an antibiotic that mediates electroneutral exchange of external Na^+ for internal H^+) also increased energy consumption in both neurons and glial cells (Yarowski et al., 1986; Erecinska et al., 1991). Although part of the monensin effect in the glia may be due to direct stimulation of glycolysis by intracellular alkalinization (Erecinska et al., 1991), these cells contain high levels of Na^+/K^+-ATPase activity (Atterwill et al., 1984), and they may therefore contribute significantly to regional energy demand associated with functional activity in the brain.

The high energy requirement of Na^+ homeostasis was also confirmed in freely moving rats, using microdialysis to measure local changes in brain extracellular glucose and lactate (Fellows et al., 1992, 1993). Extracellular glucose levels, which reflect the balance between supply from the blood and local utilization, were significantly increased in the presence of TTX and decreased sharply following veratridine application (Fellows et al., 1992). Five minutes of tail pinch-induced stress nearly doubled extracellular lactate concentration, indicating increased nonoxidative glucose metabolism, and this effect was blocked by TTX (Fellows et al., 1993).

The elements outlined above support the rationale of pharmacological down-regulating voltage-gated Na^+ channels in ischemia: i.e., reduction of Na^+ influx into brain cells, with subsequent energy preservation. The recent study of Xie and coworkers (1995) clearly illustrates this concept: among several compounds selected for their selective actions on ion channels, only those modulating α-amino-3-hydroxy-5-methyl-4-isox-

azolepropionic acid (AMPA)-operated or voltage-gated Na^+ channels delayed anoxic depolarization produced in the rat cortex by circulatory arrest. The data reviewed so far might suggest that cerebroprotection may require drastic down-modulation of Na^+ channels, such as that produced by lidocaine coma, profound hypothermia or TTX application; all associated with severe side-effects (Artru et al., 1991; Ginsberg et al., 1992; Mosher et al., 1964). However, it seems possible to interact selectively with specific Na^+ channels, or Na^+ channel states, to provide neuroprotection without complete blockade of neuronal function. As detailed below (in section C.), Na^+ channels may be inherently down-regulated during O_2 deprivation or metabolic inhibition, and the remarkable tolerance of the immature brain, as well as that of the turtle brain, does not result from complete blockade of Na^+ channels. Some of the data presented in section IV and V also suggests that moderate or selective down-modulation of Na^+ channels is sufficient to confer cerebroprotection, and that this strategy may be effective even when treatment is initiated after the ischemic episode (see below, section D.).

B. Sustained Na^+ Influx Into Neurons: Acute and Indirect Neurotoxicity

1. Intrinsic neurotoxicity of acute Na^+ influx. Veratridine, by blocking Na^+ channel inactivation and shifting activation to more negative membrane potentials (Catterall, 1980), causes Na^+ influx and a persistent tendency for depolarization (Deri and Adam-Vizi, 1993). With energy depletion, extracellular concentrations of Na^+ are maintained within the normal range as long as anoxic depolarization does not occur, but then decrease abruptly, reflecting sudden Na^+ influx into the cells (Harris et al., 1984; Hansen and Nedergaard, 1988; Obrenovitch et al., 1990a). With anoxia or veratridine, excessive Na^+ entry into neurons is clearly hazardous to their survival. For example, in the rat four-vessel occlusion model, EEG recovery from 5 to 20 min transient ischemia was considerably slower when anoxic depolarization occurred during the insult, and even a short period of depolarization was sufficient to produce this effect (Ueda et al., 1992a; Obrenovitch et al., 1993). Anoxic depolarization was also deleterious to neuronal recovery in vitro (Jiang and Haddad, 1992), and previous studies had shown that the severity of ischemic injury to neurons correlated with the length of time during which the preparation was in a depolarized state (Balestrino et al., 1989; Somjen et al., 1990).

Cultured hippocampal neurons from 18-day-old rats were all destroyed within 30 min when treated with 50 μM veratridine, even when the incubating medium was Ca^{2+}-deficient (Rothman, 1985). Only replacement of Na^+ by benzoylcholine, or that of Cl^- by SO_4^{2-}, blocked veratridine toxicity. Elimination of Na^+ presumably prevented the initial depolarization, whereas removing Cl^- decreased Cl^- flux into neurons and allowed them to tolerate depolarization without excessive osmotic load (Rothman, 1985). Similar observations were made by Pauwels and coworkers (1989) in the same preparation, and by Lysko and coworkers (1994) in cultured rat cerebellar granule cells, but toxicity was 25% Ca^{2+}-dependent with 40 μM veratridine (Lysko et al., 1994) and completely Ca^{2+}-dependent with 30 μM of the toxin (Pauwels et al., 1989). From these three studies, it is interesting to note that the Ca^{2+} dependency of veratridine toxicity appears to decrease inversely with the toxin concentration applied to neurons. This suggests that direct, acute neurotoxicity of sustained Na^+ influx may predominate only when it exceeds a given threshold. It is likely that this threshold varies with the efficacy of the Na^+/K^+-ATPase and thus with energy availability.

In hippocampal slices from guinea pigs, veratridine blocked synaptic transmission in CA1 subfield and induced several episodes of spreading depression (SD) followed by persistent loss of ionic homeostasis (Ashton et al., 1990). In vivo, microdialysis application of veratridine to the rat striatum produced recurrent SD superimposed on persistent negative shifts of the extracellular direct current (DC) potential (Obrenovitch T.P. and Urenjak J., unpublished observation), and a TTX-sensitive efflux of amino acid neurotransmitters in the rat striatum (Butcher and Hamberger, 1987; Young et al., 1990) and spinal cord (Skilling et al., 1988).

Intrinsic neurotoxicity of intracellular Na^+ loading was also documented in anoxic neurons (Friedman and Haddad, 1993, 1994a, b). Severe anoxia caused a rapid increase of $[Ca^{2+}]_i$ and $[Na^+]_i$ in adult CA1 hippocampal neurons, followed by swelling and bleb formation. Removal of extracellular Ca^{2+} and addition of Co^{2+} to block Ca^{2+} channels markedly attenuated the increase in $[Ca^{2+}]_i$ in response to anoxia, but did not prevent cell swelling and injury. Antagonists of glutamate-operated ion channels did not affect the increase in $[Ca^{2+}]_i$ induced by anoxia. Only replacement of extracellular Na^+ with the impermeant *N*-methyl-D-glucamine prevented anoxia-induced neuronal injury (Friedman and Haddad, 1993, 1994b). In addition, $[Ca^{2+}]_i$ levels dropped, rather than increased, during anoxia without extracellular Na^+ (Friedman and Haddad, 1993). Influx of Na^+ also contributed to neuronal damage produced by anoxia/aglycemia in cultures of rat basal ganglia cells (Goldberg et al., 1986).

In addition to the potential intrinsic neurotoxicity of acute Na^+ influx, the inward gradient of Na^+ across the cellular membrane is required to drive a number of exchange/transport mechanisms and, therefore, alteration of this gradient threatens several vital processes.

2. Intracellular Na^+ loading and Ca^{2+} homeostasis. The so-called *Ca^{2+}-overload* hypothesis (i.e., increase of intracellular free Ca^{2+}, triggering a cascade of harmful events via activation of proteases, phospholipases and endonucleases) (Siesjö and Bengtsson, 1989) remains

the leading hypothesis of ischemia-induced neuronal death (Choi, 1995). Neurotoxicity of excessive Na^+ influx, such as those provoked by severe energy depletion or veratridine, may include a *Ca^{2+} component*, because these conditions are associated with an influx of extracellular Ca^{2+} (Blaustein and Oborn, 1975; Hansen, 1985; Uematsu et al., 1988; Jacques et al., 1981; Gibson et al., 1991), which may partly result from Na^+ movement.

Voltage-gated Ca^{2+} channels are obvious routes for Ca^{2+} entry under these circumstances, because excessive Na^+ influx depolarizes the cellular membrane (Adam-Vizi and Ligeti, 1986). Unexpectedly, Ca^{2+} influx into hypoxic neurons was only partially dependent on the activity of L-type Ca^{2+} channels (Wauquier et al., 1988; Marcoux et al., 1989) or N-type Ca^{2+} channels (Madden et al., 1990), and that produced by veratridine was even less sensitive to Ca^{2+} channel blockers (Adam-Vizi and Ligeti, 1986). Furthermore, the efficacy of drugs that reduce Ca^{2+} influx through voltage-gated Ca^{2+} channels to protect against cerebral ischemia is, on the whole, negative (Ginsberg, 1988). These findings strongly suggest that Ca^{2+} penetrates the cell by other means during such insults.

Under normal conditions, the Na^+/Ca^{2+} exchanger contributes to the maintenance of low $[Ca^{2+}]_i$ by extruding one Ca^{2+} in exchange for three Na^+, a process driven by the large transmembrane gradient of Na^+ (Carafoli, 1987). Because this mechanism is electrogenic, elevation of $[Na^+]_i$ and depolarization can reverse its operation, i.e., Na^+ is transported out of the cell and Ca^{2+} enters (Blaustein, 1988). Intracellular Ca^{2+} loading subsequent to reversal of the Na^+/Ca^{2+} exchanger is well documented in the anoxic heart (Haigney et al., 1992) and optic nerve (Stys et al., 1992b; see section IV.A.1.). With regard to brain cells, the favoured explanation for Ca^{2+} entry during ischemia remains glutamate/excitotoxin-gated Ca^{2+} channels (Meldrum et al., 1993; Meldrum, 1994). It is interesting to note that *N*-methyl-D-aspartate (NMDA)-receptor channels played a critical part in membrane depolarization-induced death of cultured embryonic spinal neurons and that the contribution of these channels to neurotoxicity was apparently not related to their ability to trigger large Ca^{2+} influx, suggesting the involvement of other properties of these ligand-operated channels (Tymianski et al., 1993). Nevertheless, reversal of Na^+/Ca^{2+} exchange was demonstrated in neurons (Wakade et al., 1993) and synaptosomes (Nachshen and Kongsamut, 1989; Taglialatela et al., 1990; Dagani et al., 1990) in situations that mimic hypoxia or energy depletion.

Another possible link between excessive influx of Na^+ and Ca^{2+} may be the voltage-gated Na^+ channels themselves, because their ion selectivity is not perfect. The Na^+:Ca^{2+} permeability ratio has been estimated to be approximately 10:1 under normal conditions (Hille, 1991; see also section II.B.2.) (note that higher permeability ratios have been suggested; Lederer et al., 1991), and a TTX-sensitive transient Ca^{2+} current has been measured in isolated rat hippocampal CA1 pyramidal cells (Takahashi et al., 1989). Permeation of Ca^{2+} through voltage-gated Na^+ channels may be more important when Na^+ influx is provoked by veratridine or other toxins acting at Na^+ channel site 2, because these toxins alter the ion selectivity of Na^+ channels (Jacques et al., 1981; Adam-Vizi and Ligeti, 1986). It is relevant to mention that alteration of Na^+ channels selectivity is not exclusive to toxins. For example, Sorbera and Morad (1990) found that atrionatriuretic peptide rapidly and reversibly transforms cardiac Na^+ channels into Ca^{2+} conducting channels.

3. Collapse of acid-base regulation with anoxic depolarization. As oxygen is the limiting substrate of brain energy production in normoglycemic conditions, deficient oxygen supply implies anaerobic metabolism and consequent tissue acidosis. Cytosolic pH (pH_i) is a key regulator of numerous cellular processes (Roos and Boron, 1981), and tissue acidosis clearly promotes ischemia-induced tissue damage (Siesjö et al., 1993; see, however, Tombaugh and Sapolsky, 1993), even though extracellular acidosis protects cultured neurons against NMDA-receptor mediated injury (Kaku et al., 1993). Ischemia-induced acidosis is initiated intracellularly, but extends to the extracellular space within seconds of ischemia onset, reflecting increased CO_2 that diffuses freely through all brain tissue compartments and activation of a variety of mechanisms involved in the regulation of pH_i. These include: (*a*) facilitated efflux of intracellular lactic acid (Symon et al., 1994; Taylor et al., 1994); (*b*) extrusion of H^+ by Na^+/H^+ antiporter that is blocked by amiloride and its more potent *N*-5-disubstituted derivatives (Sánchez-Armass et al., 1994); and (*c*) exchanges of HCO_3^- with Cl^- via Na^+-dependent or independent HCO^{3-}/Cl^- antiporters that are sensitive to stilbene derivatives (Schlue and Dorner, 1992; Mellergård et al., 1994). All these mechanisms are activated and retain their efficacy for as long as anoxic depolarization does not occur, to such an extent that the extracellular space becomes more acid than the cytosol (at least for some cells) (Obrenovitch et al., 1990b). However, pH_i regulation collapses abruptly with anoxic depolarization.

Continuous monitoring of extracellular lactate concentration in the striatum of rat showed that K^+-induced depolarization (Taylor et al., 1994) and anoxic depolarization (Taylor et al., 1996) were associated with a decrease in extracellular lactate. These findings indicate that lactate transport out of the cells is impaired, and/or its rate of production reduced (see below, section 6.), when transmembrane ionic gradients are disrupted.

Under physiological conditions, Na^+/H^+ exchangers mediate the uphill extrusion of H^+ coupled to, and driven by, the downhill flow of Na^+ into the cell. It is the predominant mechanism for recovery from intracellular

acidification in neurons and synaptosomes (Tolkovsky and Richards, 1987; Schlue and Dorner, 1992; Sánchez-Armass et al., 1994). This antiporter system is electroneutral (1:1 stoichiometry) and, therefore, insensitive to changes in the membrane potential. Furthermore, a number of studies with vertebrate cells have shown that acid extrusion is not blocked by ouabain, even though $[Na^+]_i$ was markedly raised (reviewed by Roos and Boron, 1981), possibly because of the relatively low K_m of the Na^+/H^+ exchanger for external Na^+ (3 to 50 mM at physiological external pH; Aronson, 1985). Despite this apparent resistance to changes in the transmembrane gradient of Na^+, the efficacy of the Na^+/H^+ exchanger in extruding H^+ may still be reduced with anoxic depolarization, because of the sudden fall in the ratio $[Na^+]_o/[Na^+]_i$ associated with this event (Aronson, 1985), superimposed on extracellular acidosis that also reduces the rate of H^+ extrusion (Jean et al., 1986).

The driving forces for HCO_3^-/Cl^- exchanges are less obvious. Astrocytes apparently express a HCO_3^-/Cl^- exchanger driven by the Na^+ gradient, translocating HCO_3^- inward in response to intracellular acid loading (Mellergård et al., 1994). As with the Na^+/H^+ exchanger, this mechanism is presumably altered by anoxic depolarization. The other HCO_3^-/Cl^- antiporter may not be relevant to hypoxic/ischemic conditions because it is Na^+-independent, and its function may be to translocate HCO_3^- outward and Cl^- inward during alkaline transients (Mellergård et al., 1993).

Whatever the residual efficacy of ion exchangers to control intracellular acidosis after anoxic depolarization, their actions are nullified, because this event is associated with a sudden increase in cellular permeability to H^+ and pH-changing anions, which may be linked to the opening of voltage-gated or ligand-operated ions channels (Obrenovitch et al., 1990b; Symon et al., 1994). Permeation of H^+ through voltage-gated Na^+ channels would be a possibility, particularly as H^+ are the ions most permeant through these channels (Hille, 1991), but this is very unlikely because the amplitude of the extracellular pH (pH_e) alkalotic shift occurring with anoxic depolarization, as with the abrupt drop in $[Na^+]_o$, was not altered by TTX (Xie et al., 1994b) (fig. 5).

4. Intracellular Na^+ loading and cell swelling. Cellular swelling (i.e., cytotoxic edema) is another important component of the pathophysiology of cerebral ischemia and other conditions characterized by an imbalance between energy supply and demand, and/or acidosis (Kimelberg et al., 1989). Acidosis, high extracellular K^+, excitotoxins and free fatty acids initiate cellular swelling through various mechanisms, but all have one important element in common: downhill entry of Na^+.

Acidification of the incubation medium below pH 6.8 immediately induced swelling of cultured glial cells, and this effect was significantly reduced by amiloride or the removal of HCO_3^- and Na^+ from the medium (Kempski et al., 1988). Exposure of glial cells to 30 mM K^+ produced a gradual swelling followed by slow normalization, an action blocked by ouabain or iodoacetate and reduced by amiloride, suggesting that both Na^+/K^+-ATPase and Na^+/H^+ exchange contributed to K^+-induced swelling (Kempski et al., 1991). Glutamate-induced swelling of rat brain cortical slices was dependent upon the presence of Na^+ in the medium (Lund-Andersen and Hertz, 1970), and more recent work suggests that this effect involves Na^+ entry subsequent to stimulation of Na^+-dependent acidic amino acid uptake (see below, section 5.), with subsequent activation of Na^+/K^+-ATPase and ion exchanges ensuring pH_i control (see above, section 3.) (Kimelberg et al., 1989; Hansson et al., 1994). Finally, replacement of Na^+ by choline in the suspension medium completely abolished the swelling of cultured glial cells produced by arachidonic acid, suggesting that activation of lipid peroxidation by fatty

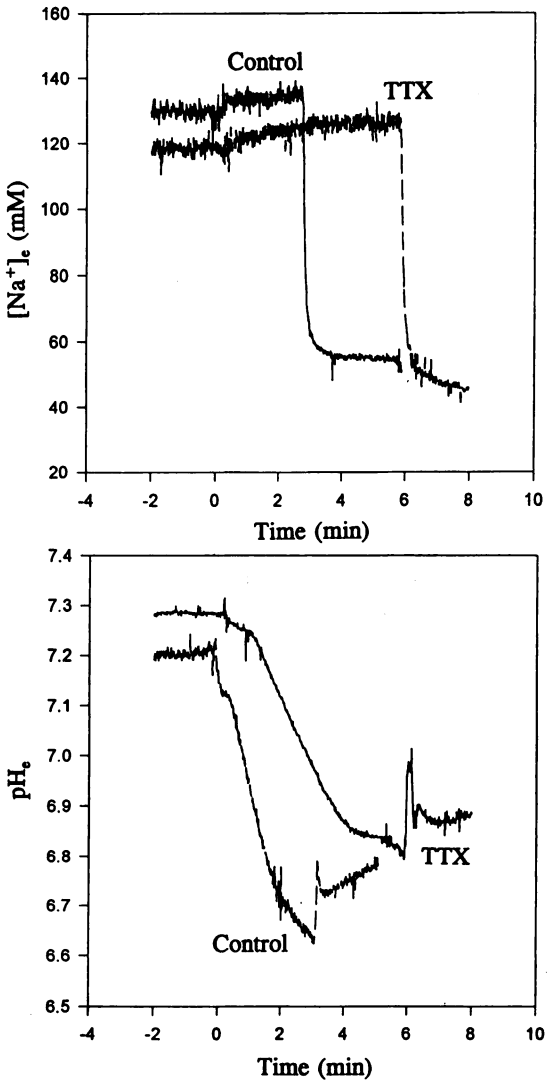


FIG. 5. Effects of TTX on ischemia-induced changes in extracellular Na^+ (*top panel*); $[Na^+_e]$ and acidosis (*bottom panel*; pH_e). Complete ischemia was produced by stopping perfusion with oxygenated fluorocarbon medium of the isolated rat brain. These data were taken from Xie and coworkers (1994b), with permission from the authors and Elsevier Science Publishers BV.

acids leads to increased Na^+ permeability with subsequent influx of water into the cells (Staub et al., 1994).

Although anoxia, iodoacetate glycolysis inhibition and ouabain Na^+/K^+-ATPase inhibition failed to increase the volume of cultured glioma cells (Kempski et al., 1988), this does not apply in vivo (Cornog et al., 1967) or in tissue slices (Okamoto and Quastel, 1970). There is convincing evidence that cellular swelling occurs in the intact brain whenever there is a sudden breakdown of ionic gradients across the cellular membrane (Lux et al., 1986). For example, both anoxic depolarization and SD are associated with a sudden shrinking of the extracellular compartment, reflecting cellular swelling due to influx of Na^+ and Cl^- (Phillips and Nicholson 1979; Hansen and Olsen, 1980).

5. Cellular membrane depolarization, intracellular Na^+ loading and glutamate efflux. It is now accepted that excessive opening of glutamate-operated ion channels plays a major role in ischemia-induced neuronal death, and one possible triggering factor may be increased extracellular concentration of glutamate (Meldrum et al., 1993; Obrenovitch and Richards, 1995). Under resting conditions, the level of glutamate in the cytoplasm of brain cells is approximately 10,000 times higher than that in the extracellular space, a gradient maintained by acidic amino acid carriers present in both presynaptic and glial plasma membranes (Nicholls and Atwell, 1990). These carriers, which are essential for terminating the postsynaptic action of neurotransmitter glutamate, are characterized by a high (20 to 50 μM) affinity for glutamate, and a unique coupling to Na^+ and K^+ (Kanai et al., 1993). The transport of glutamate into presynaptic terminals or neuroglia requires the simultaneous presence of external Na^+ and internal K^+, and its efficacy is dependent on the Na^+/K^+ gradient across the plasma membrane (Kanner and Bendahan, 1982). Any reduction of this gradient, resulting from an alteration of the Na^+/K^+-ATPase (i.e., energy supply) or a sudden change in the permeability of the cellular membrane to Na^+ or K^+, reduces and possibly reverses the action of glutamate transporters (Szatkowski et al., 1990). Thus, moderate ischemic insults are likely to produce an imbalance between glutamate efflux and uptake (Bradford et al., 1987), whereas more severe insults may reverse glutamate uptake processes completely (Szatkowski et al., 1990). This mechanism, which has yet to be demonstrated in vivo (Obrenovitch, 1995b), very likely applies to a wide range of uptake mechanisms driven by the Na^+/K^+ transmembrane gradient.

In addition, repetitive action potentials (i.e., Na^+-influx through voltage-gated Na^+ channels) are the physiological trigger for vesicular release of neurotransmitter glutamate (exocytosis). Glutamate release elicited by short repetitive electrical pulses applied to cerebellar slices was TTX- and Ca^{2+}-dependent (De Barry et al., 1989). Exposure of synaptosomes to 4-aminopyridine (i.e., block of fast K^+ channel), which closely mimics repetitive firing, induced an increase in cytosolic free Ca^{2+} ($[Ca^{2+}]_c$) and glutamate release that was almost entirely blocked by TTX, and 4-aminopyridine failed to increase $[Ca^{2+}]_c$ in low-Na^+ media (Tibbs et al., 1989). Increase in $[Ca^{2+}]_c$ and glutamate release produced in synaptosomes by KCl is insensitive to TTX and low-Na^+ medium, but this stimulus provokes a clamped depolarization of the plasma membrane that is not physiological (Tibbs et al., 1989). Therefore, down-modulation of Na^+ channels reduces vesicular glutamate release, with two potential benefits: (*a*) decreased excitotoxic mechanisms; and (*b*) reduced energy-demand, because all exocytotic processes require ATP hydrolysis (Söllner and Rothman, 1994). One must emphasizes that the latter benefit is not exclusive to glutamate but applies to exocytosis of all neurotransmitters.

6. Inhibition of anaerobic metabolism with anoxic depolarization and Na^+ influx. Na^+ influx stimulates respiration and ATP turnover in normoxia (see section III.A.), and anaerobic metabolism as long as physiological conditions are maintained (Fellows et al., 1993). Paradoxically, during anoxia, excessive intracellular Na^+ loading (i.e., intensive electrical stimulation, anoxic depolarization or treatment with veratridine) can inhibit anaerobic glycolysis. In Ca^{2+}-free medium (i.e., increased membrane permeability to ions), TTX, at concentrations that block action potentials, enhanced the rate of anaerobic glycolysis of brain cortex slices from rats and guinea pigs (by approximately 300%). A similar effect was observed with local anaesthetics at pharmacologically active concentrations, whereas protoveratrine diminished the glycolytic stimulation of TTX (Shankar and Quastel, 1972). These findings indicate that the influx of Na^+ and efflux of K^+ under these conditions blocked anaerobic metabolism, possibly at the level of pyruvate kinase. This enzyme, a regulatory step in glycolysis, is activated by K^+ and inhibited by Na^+ (Takagaki, 1968; Rose and Rose, 1969).

Inhibition of anaerobic metabolism by Na^+ loading was recently confirmed in synaptosomes. Veratridine superimposed onto anoxia dose-dependently reduced anaerobic ATP synthesis. This effect was linked to Na^+ influx as it was blocked by TTX (Gleitz et al., 1993). Microdialysis studies of extracellular lactate in the striatum of rats are also consistent with this hypothesis, although the following observation might also reflect altered efflux of lactate with depolarization (see above, section 3.). Dialysate lactate decreased during transient depolarization, increasing markedly only during the subsequent repolarization phase (Taylor et al., 1994). Similar observations were made with anoxic depolarization provoked by transient ischemia (Taylor et al., 1996). This inhibitory effect of intracellular Na^+ loading on anaerobic metabolism is seldom discussed, despite the potential significance of abolition of this sole remaining source of ATP.

This section clearly shows that anoxic depolarization is detrimental to neuronal survival and that strategies for suppressing or delaying it are bound to be potentially cerebroprotective. The delay from ischemia onset to occurrence of anoxic depolarization, and its cerebral blood flow threshold (Obrenovitch, 1995a), depend on two factors: local rate of energy metabolism (Astrup et al., 1980) and stores of energy-rich substrates, notably glucose (Bures and Buresova, 1957; Siemkowicz and Hansen, 1981; Kristián et al., 1994). When energy demand is high and/or glucose stores low, the threshold for anoxic depolarization is reduced.

Na^+ channel blockade and down-modulation delays anoxic depolarization. This action, clearly demonstrated with TTX both in vitro (Rosen et al., 1994) and in vivo (Prenen et al., 1988; Xie et al., 1994b, 1995) (see section IV. B.), has two important benefits: (*a*) exposure to neurotoxic intracellular Na^+ and Ca^+ loading is avoided or delayed, and (*b*) vital processes such as intracellular acid-base balance, cell volume regulation, and uptake mechanisms are preserved.

C. Increased Tolerance to Ischemia by Down-Regulation of Na^+ Channels

1. Inherent down-regulation of Na^+ currents during anoxia and metabolic inhibition. The fact that functional loss (i.e., EEG silence) occurs within seconds of ischemia onset, whereas energy levels are not depleted for several minutes, strongly suggests that anoxia-induced blockade of neuronal function may be an adaptive process and not a consequence of energy substrate limitation (Neubauer, 1993). As already explained, reducing neuronal metabolism by stabilizing membrane potential and decreasing the production of action potentials potentially restricts the use of metabolic substrates to the maintenance of cellular integrity, thus extending the time to which neurons can be exposed to hypoxia without cellular damage.

Changes in neuronal membrane ion conductance are clearly involved in this survival strategy. In vitro electrophysiological studies of hypoxia and metabolic inhibition have shown that early functional loss is associated with hyperpolarization of neurons, and a rise in extracellular K^+ (Hansen et al., 1982; Fujiwara et al., 1987; Leblond and Krnjevic, 1989). This initial increase in K^+ conductance presumably involves the opening of Ca^{2+}-dependent K^+ channels subsequent to a rise in intracellular free Ca^{2+} (Duchen, 1990), and possibly opening of voltage-independent K^+ channels such as ATP-dependent K^+ channels (Mourre et al., 1989; Xie et al., 1995) (for review, see Obrenovitch et al., 1990a). Other conductances also appear to be altered, including a Ca^{2+}-dependent Cl^- current that may increase (Duchen, 1990) and a blockade of Ca^{2+} current (L-type Ca^{2+} current in particular) (Krnjevic and Leblond, 1989).

Recent findings strongly suggest that reduction in Na^+ conductance may also be an important contributor to decreasing neuronal excitability during anoxia and metabolic inhibition (Cummins et al., 1993). Intracellular recordings of neocortical neurons in human brain slices showed that their excitability was markedly decreased within the first 5 min of anoxia, and this effect could not be explained adequately by increased K^+ conductance, because it was associated with little or no change in membrane input resistance and membrane potential. Whole-cell voltage-clamp studies of acutely isolated human neocortical pyramidal neurons demonstrated that anoxia and cyanide rapidly decreased a voltage-dependent, TTX-sensitive Na^+ current and markedly shifted its steady state inactivation curve toward more negative potentials (Cummins et al., 1993). The effect of anoxia on steady state inactivation was greatly reduced when 2 mM ATP was included, suggesting that anoxic depression of Na^+ currents was linked to reduced oxidative metabolism.

Reduced membrane excitability with preservation of membrane potential and input resistance has been previously observed in neurons from rat cortex and hippocampus CA1 exposed to 4 to 5 min of O_2 deprivation, but not in adult rat brainstem neurons, which are more sensitive to anoxia (Cummins et al., 1991; Jiang and Haddad, 1992), suggesting that these features are important for neuronal tolerance to energy depletion.

The apparent sensitivity of Na^+ channel conductance to ATP depletion in neocortical neurons contrasts with previous findings: (*a*) when the peripheral nerve was rendered anoxic, the rate of impulse conduction fell markedly before there had been more than a small decrease in ATP levels (Stewart et al., 1965); (*b*) conduction through the superior cervical ganglion was blocked by hypoxia long before high energy phosphates had been exhausted (Härkönen et al., 1969); and (*c*) brain function was severely depressed in severe hypoglycemia with little change in ATP levels (Tarr et al., 1962; King et al., 1967). All these findings indicate that changes in ion conductance with reduced energy availability, and the mechanisms leading to these changes, may vary markedly with the type of neuron considered.

Early down-regulation of Na^+ channels during periods of limited O_2 supply, in order to decrease membrane excitability and reduce energy expenditure, is a cellular mechanism that appears particularly efficient in species adapted to long periods of anoxia and in the immature brain.

2. Survival strategy of the turtle brain to anoxia: adaptive down-regulation of Na^+ channels. Reptiles and especially some fresh-water turtles show an extraordinary capacity to survive prolonged anoxia (Belkin, 1963). The near-arctic turtle *Chrysemys picta* can survive anoxic dives for over 6 months at low-temperature (3°C) (Ultsch, 1985) and up to 48 h at 25°C (Musacchia, 1959). Under these conditions, the turtle brain EEG and

evoked field potentials are reduced, but anoxic depolarization does not occur, and ATP levels are preserved (Sick et al., 1982; Lutz et al., 1984; Chih et al., 1989a). The large glycolytic capacity of the turtle brain (Kelly and Storey, 1988; Suarez et al., 1989) certainly aids in maintaining ATP concentration in anoxic situations, because anoxic depolarization occurs when glycolysis is blocked with iodoacetate (Sick et al., 1982; Doll et al., 1991). However, another important mechanism contributing to the adaptation of the turtle brain to anoxia is ion channel down-regulation. *Ion channel arrest* (Hochachka, 1986) reduces energy demand for ion pumping, promotes stabilization of transcellular ion and electrical gradients, and prevents cytotoxic elevations of intracellular Ca^{2+}. For example, anoxia in isolated turtle cerebellum provoked partial depolarization of Purkinje cells transmembrane potentials, a shift of Na^+ (and probably Ca^{2+}) spike thresholds to a more depolarized membrane potential, and depression of postsynaptic responses (Pérez-Pinzon et al., 1992a). In addition, efflux of K^+ produced by superfusion of intact turtle brain with ouabain was slower during anoxia than in normoxia (Chih et al., 1989b).

Although a number of mechanisms involving Ca^{2+} channels, K^+ channels and inhibitory neurotransmitters also participate in ion channel arrest (Bickler and Gallego, 1993; Nilsson and Lutz, 1992; Sakurai et al., 1993), down-regulation of voltage-gated Na^+ channels appears to be a key element. The turtle brain has a much lower density of STX-sensitive Na^+ channels than the rat brain, especially in the rostral areas such as the cortex (fig. 6a) (Xia and Haddad, 1993). In turtle synaptosomes, the maximum binding capacity for brevetoxin is only approximately ⅓ that in rat synaptosomes (Edwards et al., 1989). However, low Na^+ channel density cannot account for the 80 to 90% reduction in brain metabolism of turtle brain in anoxia (Edwards et al., 1989; Xia and Haddad, 1993). Additional reduction of Na^+ currents is necessary, achieved by a further decline in Na^+ channel density in anoxia (fig. 6b) (Pérez-Pinzon et al., 1992b), to which *functional* down-regulation may be superimposed (Edwards et al., 1989). How the number and conductance of Na^+ channels are reduced during anoxia in turtle brains remains undefined. This process may be mediated by second-messenger systems, via an increase in cA-PK or PKC activity (See section II.C.). Adenosine could also be involved in this mechanism, because it was transiently released into the extracellular space in the anoxic turtle brain (Nilsson and Lutz, 1992) and adenosine-modulated cAMP levels in CNS neurons (Stone, 1991), and specific adenosine A_1 receptor antagonists caused the isolated turtle cerebellum to depolarize during anoxia (Pérez-Pinzón et al., 1993a).

3. Tolerance of the immature brain to anoxia and ischemia. The fact that newborn central mammalian neurons are more tolerant to anoxia/ischemia than their adult counterpart is well documented (Haddad and Jiang, 1993). Infant primates, especially those that are preterm, can maintain the viability of cerebral neurons with blood flow levels that would rapidly produce infarction in the adult brain (Altman, et al., 1993; Powers, 1993). In rat hippocampal slices, anoxia depresses excitatory postsynaptic potentials, causes an initial hyperpolarization and decreases membrane resistance, but the magnitude of these changes are much smaller in tissue slices from immature versus mature animals (Cherubini et al., 1989; Krnjevic et al., 1989; Cummins et al., 1991). For example, excitatory postsynaptic potentials were depressed by 90% within 2 min of anoxia in the adult, but only by 44% in the newborn animals, and

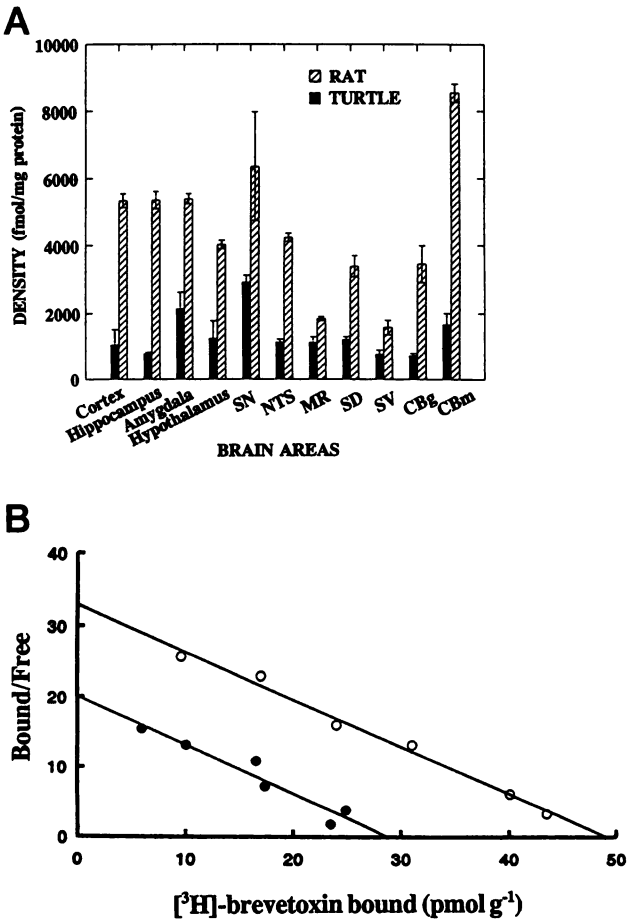


FIG. 6. Quantitative differences in STX binding density of major CNS regions between turtle and rat (*a*) and reduction of [^{3}H]brevetoxin binding in turtle cerebellum by anoxia (*b*). (*a*) Note STX binding density is much higher in all rat CNS areas when compared with that of the turtle. SN, substantia nigra; NTS, nucleus of the solitary tract; MR, medullary reticular nucleus; SD, spinal cord—dorsal horn; SV, spinal cord—ventral horn; CVg, cerebellum—granular layer; CBm, cerebellum—molecular layer. (*b*) Open circles, normoxia; closed cirles, anoxia. Brevetoxin binds to site 5 of voltage-gated Na^+ channels, independently of membrane potential (i.e., it binds to active and inactive Na^+ channels) (Baden, 1989). Values of B_{max} for [^{3}H]brevetoxin, determined from the Scatchard plot indicated a 42% decline in Na^+ channel density by anoxia. From Xia and Haddad (1993) (*a*) and Pérez-Pinson and coworkers (1992b) (*b*), with permission from the authors, Wiley-Liss, Inc. (a subsidiary of John Wiley & Sons, Inc.) and the American Physiological Society.

postanoxic recovery was much more rapid in the latter (Cherubini et al., 1989). As with the turtle brain, several mechanisms contribute to the remarkable anoxic tolerance of the immature brain (Haddad and Jiang, 1993); its ability to use alternative substrates (e.g., lactate and ketone bodies) as a source of energy is certainly one of them (Nehlig and Pereira de Vasconcelos, 1993). However, the immature brain also has much lower energy requirements than that of the adult. This was recently confirmed in the human by positron emission tomography measurements of oxygen consumption (Altman et al., 1993; Powers, 1993).

Low energy expenditure of immature brain is probably linked to reduced electrical and synaptic activity, partly because there are fewer neurons, dendritic processes, and synapses in the newborn. Resting cellular membrane potentials, DC potential and EEG activity are all lower in the brain of neonatal rats as compared with adults, and action potentials from cortical neurons do not appear until the postnatal day 4 (Deza and Eidelberg, 1967). There is also good correlation between development of Na^+/K^+-ATPase activity and maturation of electrical activity in the developing brain (Abdel-Latif et al., 1967). In common with the turtle brain, the tolerance to anoxia of the immature brain is associated with a remarkable delay in the development of membrane failure (i.e., anoxic depolarization) (Hansen, 1977; Haddad and Donnelly, 1990). This is supported by a low rate of K^+ efflux (Hansen, 1977; Haddad and Donnelly, 1990; Trippenbach et al., 1990; Pérez-Pinzón et al., 1993b), markedly delayed influx of Na^+ and Cl^- (Jiang et al., 1992), and a delayed increase of intracellular Ca^{2+} (Friedman and Haddad, 1993) in the neonatal brain or neurons subjected to anoxia. There is also a slower rate of ATP depletion during complete ischemia in the newborn brain as compared with adult (Thurston and McDougal, 1969).

Down-regulation of Na^+ channels may play an important role in the tolerance of the newborn CNS. Voltage-sensitive Na^+ currents are much smaller in newborn than in adult cortical neurons (Cummins et al., 1994), and Na^+ channel density is markedly lower at birth than in the mature brain (Xia and Haddad, 1994). Furthermore, Dargent and Couraud (1990) have demonstrated, in fetal brain neurons developing in vitro, a rapid down-regulation of Na^+ channels whenever Na^+-influx was increased. Application of Na^+ channel activators (scorpion α toxin, BTX, and veratridine) produced a partial but rapid ($t_{1/2}$, 15 min) disappearance of surface Na^+ channels as measured by a decrease in the specific binding of [^{3}H]STX or ^{125}I-labeled scorpion β toxin, and a decrease in specific ^{22}Na uptake. The induced disappearance of Na^+ channels was abolished by TTX and dependent on external Na^+ concentration. Amphotericin B, a Na^+ ionophore, and monensin were able to mimic the effect of the Na^+ channel activators, whereas KCl depolarization failed to do so. Another interesting aspect of this phenomenon is its apparent specificity to neurons, inasmuch as it has not been observed in astrocytes or skeletal muscle cells. Here also, as proposed by Dargent and Couraud (1990), second-messenger systems may be involved in the mechanism of Na^+ channel down-regulation, especially because Na^+ channel activators stimulated phosphatidylinositol diphosphate breakdown in a brain synaptosomal preparation (Gusovsky et al., 1986).

D. Potential Benefit from Postischemic Down-Modulation of Na^+ Channels

So far, our analysis has focused on down-modulation of Na^+ channels as a neuroprotective intervention *during* or *preceding* ischemia. As such, it is relevant to patients at high risk of cerebral ischemia (e.g., those undergoing cardiopulmonary bypass, carotid endarterectomy or aneurysm surgery), and represents both a fertile area for trials and a potentially major clinical application (Fisher et al., 1994). In contrast, it appears less relevant to stroke therapy because most patients suffering from this condition are admitted to a hospital several hours after the onset of symptoms (Hantson et al., 1994; Panayiotou et al., 1994). However, theoretical considerations, as well as experimental data (see also sections IV. and V.), strongly suggest that down-modulation of Na^+ channels remains beneficial even when it is delayed, i.e., after occlusion of a major cerebral artery (stroke) or following transient global ischemia. In these situations, the basis for protection may still be linked, at least partly, to reduced energy demand and preservation of ionic gradients. However, the rationale for Na^+ channel down-modulation differs between delayed intervention after stroke, and postischemic treatment after transient ischemia. We focus here on three possible mechanisms.

1. Focal ischemia: Na^+ channel modulation and recurrent spreading depression. SD is a transient suppression of electrical activity with membrane depolarization, propagating across the cerebral cortex or other grey matter regions (Lauritzen, 1994). Experimental studies of focal ischemia have demonstrated that recurrent SD propagates from the ischemic core to adjacent regions, contributing to the development of tissue damage, and that selective protection of the penumbra by NMDA-receptor antagonists may well be linked to inhibition of SD (for review, see Hossmann, 1994a, b; Obrenovitch, 1995a). Although repeated SD may be a sublethal stress to neurons in otherwise normal tissue (Nedergaard and Hansen, 1988), sensitive markers show that it remains a formidable challenge, and in stroke models, the frequency of SD occurrence correlated well with infarct size, whether the latter was modulated by dizocilpine (MK-801) (Iijima et al., 1992), hypothermia (Chen et al., 1993b), AMPA-receptor antagonist (Mies et al., 1994) or hyperglycaemia (Nedergaard and Astrup, 1986). It is probable that SD is deleterious in focal ischemia, be-

cause it produces marked disruption in ionic homeostasis, increased energy demand, acidosis and neurotransmitter efflux in regions where residual blood flow can only sustain basal ionic homeostasis (Obrenovitch, 1995a).

Within this context, down-modulation of Na^+ channels may reduce energy demand, improve ion homeostasis, and thus enhance the resistance of brain tissue to SD. In addition, inhibition of Na^+ currents inhibit SD to some extent (Marrannes et al., 1993) (see also the sections on flunarizine and KB-2796 in section V.A.). Tetrodotoxin (> 0.1 μM) had no effect on SD elicited in the chicken retina (Sheardown, 1993). In contrast, local application of this toxin to the rat cerebellum inhibited SD elicitation by electrical stimulation, but not that elicited by KCl application or SD propagation (Bures et al., 1974; Tobiasz and Nicholson, 1982). Two local anaesthetics (benzocaine and lidocaine) dose-dependently reduced the rate of propagation, amplitude, and duration of retinal SD elicited by mechanical stimulation (Chebabo et al., 1993). Phenytoin (an anticonvulsant with Na^+ channel blocking properties; see section IV.D.), applied to the retina at a concentration range comparable to therapeutic plasma levels of epileptic patients, increased the threshold concentration of KCl to initiate SD, decreased the velocity of its propagation, and shortened the duration of the depolarization wave (Chebabo and Do Carmo, 1991).

Ischemia-induced SD occurs in cats (Strong et al., 1983) and primates (Branston et al., 1977) and is not exclusive to the cortex, because it was recorded in the striatum of rats subjected to middle cerebral artery (MCA) occlusion (Wahl et al., 1994) and in hippocampal slices (Jing et al., 1993). However, whether or not SD occurs in man, especially in the neocortex, remains an unanswered question. All attempts to elicit SD in the human cortex so far have failed, even in the absence of general anaesthesia (McLachlan and Girvin, 1994). However, this paradox may soon be resolved by recent advances in noninvasive techniques such as magnetoencephalography and magnetic resonance imaging.

2. Na^+ channel modulation and postischemic enhancement of synaptic efficiency. Transient cerebral ischemia induces delayed neuronal loss in the brain, particularly in selectively vulnerable neurons of the hippocampus (Kirino, 1982; Pulsinelli et al., 1982; Petito et al., 1987; Hsu and Buzsáki, 1993). A number of mechanisms based upon the accumulation of intracellular Ca^{2+} have been proposed to explain postischemic neuronal death (Siesjö and Bengtsson, 1989; Lee et al., 1991), and delayed excessive release of excitatory amino acids is a widely quoted triggering event (Szatkowski and Attwell, 1994; see, however, Obrenovitch and Richards, 1995).

More recently, it has been suggested that synchronous and long-lasting enhancement of the efficiency of excitatory synapses may contribute to delayed ischemia-induced damage to neurons (Crépel et al., 1993; Obrenovitch and Richards, 1995). This phenomenon may be a pathological extension of long-term potentiation (LTP) or other forms of synaptic potentiation, which underlies information storage in the brain, i.e., learning and memory (Bliss and Collingridge, 1993). Ischemia-induced enhancement of synaptic efficiency is compatible with the protective action demonstrated by glutamate-receptor antagonists administered after the insult (Sheardown et al., 1990; Swan and Meldrum, 1990; Nellgård and Wieloch, 1992) and is supported by several key findings: (*a*) exposure of rat hippocampal slices to a short anoxic-aglycemic episode potentiated NMDA-receptor-mediated excitatory responses (Crépel et al., 1993); (*b*) Ca^{2+} uptake evoked by electrical stimulation was also enhanced postischemia (Andiné et al., 1988); (*c*) the degree of mossy fibre innervation (major excitatory input) correlated with ischemic vulnerability in the hippocampus hilus neurons and CA3 interneurons (Hsu and Buzsáki, 1993); (*d*) glucose utilization is increased for longer in the hippocampus early postischemia (Levy and Duffy, 1977; Diemer and Siemkowicz, 1980; Choki et al., 1983) and postischemic hypermetabolism in the CA1-subfield is dependent on its excitatory input (Jørgensen, et al., 1990); and (*e*) LTP can be induced by transient exposure to a variety of agents, including Ca^{2+} (Turner et al., 1982), arachidonic acid (Williams et al., 1989), the metabotropic glutamate receptor agonist aminocyclopentane-1S,3R-dicarboxylate (Bortolotto and Collingridge, 1993), and the K^+ channel blocker, tetraethylammonium (Aniksztejn and Ben-Ari, 1991), all of which are involved or mimic ischemic-induced events. The fact that the cytopathology of excitotoxicity is entirely postsynaptic, sparing axons and presynaptic terminals (Simon et al., 1984; Kirino et al., 1990), is also in line with this hypothesis, because the induction mechanisms for long-term and short-term potentiation reside, at least in part, in the postsynaptic membrane (Zilberter, et al., 1990; Cormier et al., 1993). These arguments, however, conflict with a number of electrophysiological studies that failed to demonstrate neuronal hyperexcitability in the hippocampus before CA1 cell loss (Chang et al., 1989a, b; Buzsáki et al., 1989; Urban et al., 1989; Mitani et al., 1989, 1990a; Imon et al., 1991; Jensen et al., 1991).

Both of these hypotheses, delayed release of excitatory neurotransmitters and long-lasting enhancement of synaptic efficiency, imply detrimental intracellular Ca^{2+} and Na^+ overload (Simon et al., 1984; Crowder et al., 1987; Siesjö and Bengtsson, 1989) and increased energy demand, which may be alleviated by down-modulation of Na^+-channels. Within this context, it is relevant to mention that induction of LTP in the dentate gyrus is accompanied by a persistent enhancement in the ability of synaptosomes to release glutamate in response to a depolarizing stimulus and that the most significant enhancement of release from potentiated synaptosomes was seen with veratridine (Canevari et al., 1994). Fi-

nally, although TTX did not antagonize the initiation or the maintenance of hippocampal LTP induced by tetanizing stimulation, several local anesthetics blocked the induction of LTP without any effect on the established potentiated responses, possibly via calmodulin inhibition (Smith et al., 1993).

3. Persistent up-regulation of voltage-gated Na^+ currents following ischemia: a speculative hypothesis. Several studies with a variety of animal models concur: brain ischemia causes an initial translocation of PKC protein from the cytosol to the plasma membrane (i.e., a prerequisite for PKC activation), followed by rapid inhibition of its catalytic activity measured in vitro (Louis et al., 1991; Cumrine et al., 1990; Cardell et al., 1990; Wieloch et al., 1991; Domanska-Janik and Zalewska, 1992). The molecular mechanisms underlying these changes remain unclear, although the large influx of Ca^{2+} and increased levels of free arachidonic acid and diacylglycerols produced by ischemia are probably involved (Siesjö and Bengtsson, 1989; Aveldaño and Bazán, 1975; Abe et al., 1987). The significance of the observed translocation and down-regulation of PKC with ischemia for the development of ischemic neuronal damage is also uncertain, because the effects of PKC inhibitors on postischemic neuronal damage are conflicting: some studies claim that PKC inhibitors aggravate neuronal damage (Madden et al., 1991). Others claim that staurosporine prevents neuronal cell death (Hara et al., 1990) and reduces the postischemic impairment of working memory in rats exposed to cerebral ischemia (Ohno et al., 1991). Nevertheless, as phosphorylation was shown to modulate voltage-gated Na^+ channels (see section II.C.), the possibility that persistent alterations of Na^+ currents may contribute to postischemic neuronal deficit remains a possibility that deserves investigation.

IV. Effective Cerebroprotection by Down-Modulation of Excessive Na^+ Currents in Ischemia

A. Anoxic Injury to CNS White Matter

Within the mammalian CNS, both gray *and* white (i.e., myelinated fiber tracts) matter suffer irreversible injury after anoxia/ischemia (Waxman et al., 1991), even though axons are less sensitive than neurons to reduced blood flow (Marcoux et al., 1982; Branston et al., 1984). In comparison to neuronal loss, ischemic white matter injury has received little attention. This should be rectified, firstly because action potential in white matter is essential for normal neural signaling and is altered in stroke, traumatic brain damage and spinal cord injury (Fisher, 1982; Bamford et al., 1987; Povlishock, 1992; Maxwell et al., 1993); and secondly, because the simple and well defined structure of myelinated axons (no neuronal cell bodies or synapses) may help in distinguishing some of the complex processes contributing to neuronal damage (see section III.B.).

1. Na^+- and Ca^{2+}-dependency. The ionic mechanisms underlying anoxic injury to white matter were recently characterized by Waxman and coworkers. They measured changes in the compound action potentials (CAPs) of the rat optic nerve in vitro, a parameter that provides a reliable estimate of the number of functional fibres (Ransom et al., 1990). Application of Na^+-free medium before 60 min of anoxia markedly improved the recovery of CAP, whereas increasing the transmembrane Na^+ gradient at various times before or during anoxia worsened the injury (Stys et al., 1991, 1992b). As anoxia-induced alteration of CAP had previously been found to be critically dependent on Ca^{2+} influx (Stys et al., 1990b), these results indicate that both extracellular Ca^{2+} and a finite Na^+ permeability are required before irreversible axonal injury.

Several lines of evidence suggest that Ca^{2+} does not penetrate via conventional voltage-gated or ligand-operated Ca^{2+}-channels in white matter cells: (*a*) Ca^{2+} seemed to enter the intracellular compartment gradually during anoxic conditions, despite its large electrochemical gradient (Stys et al., 1992b); (*b*) Ca^{2+} channel blockers such as polyvalent cations (Mn^{2+}, Co^{2+}, or La^{3+}) or dihydropyridines did not protect the optic nerve from anoxia (Stys et al., 1990a); and (*c*) sustained exposure to high concentrations of glutamate or aspartate did not affect the optic nerve CAP, supporting the notion that NMDA receptors do not contribute to anoxic injury in this preparation (Ransom et al., 1990) (see, however: Kriegler and Chiu, 1993; Jensen and Chiu, 1993). Stys and coworkers (1991) thus proposed that anoxia-induced Ca^{2+}-dependent neurotoxicitity may result from membrane depolarization subsequent to Na^+ influx through Na^+ channels, leading to Ca^{2+} influx through reversal of the Na^+/Ca^{2+}-exchanger (Stys et al., 1991) (see section III.B.2.). Two other findings have further strengthened this hypothesis: (*a*) introduction of Na^+-free medium 20 to 40 min *after* the start of anoxia was deleterious, presumably because this enhanced the gradient $[Na^+]_i > [Na^+]_o$ and forced more Ca^{2+} into cells via the exchanger (Stys et al., 1992b); and (*b*) pharmacological inhibition of the Na^+/Ca^{2+} exchanger with bepridil or benzamil significantly protected the optic nerve from anoxic damage (Stys et al., 1991).

2. Route for Na^+ entry during ischemia. The mechanism of sustained penetration of Na^+ into white matter cells during anoxia is an intriguing question, because the *classic* voltage-gated Na^+ channels that initiate the rapid upstroke of axonal action potential (i.e., membrane depolarization) also contribute to its termination by fast and complete inactivation (Chiu et al., 1979), implying that these channels should also rapidly inactivate during anoxic depolarization. Stys and coworkers (1991) speculated that a noninactivating Na^+ conductance, persisting at depolarized membrane potentials,

was involved. Measurements of the compound membrane potential of rat optic nerves at rest, or depolarized by 15 to 40 mM K^+, confirmed this hypothesis. A TTX-sensitive Na^+ conductance that was present at rest persisted in nerves depolarized sufficiently to abolish classical transient Na^+ currents (Stys et al., 1993). It is important to mention that noninactivating Na^+ conductance, which rapidly activates like the *classical* Na^+ channels, but inactivates either very slowly or incompletely, even with prolonged depolarization, was previously identified in cerebellar (Llinás and Sugimori, 1980; Sugimory and Llinás, 1980), cortical (Connors et al., 1982; Stafstrom et al., 1985; Lynch et al., 1995) and hippocampal neurons (French et al., 1990). Although such noninactivating Na^+ current (also called sustained or persistent Na^+ current) may only represent 1 to 3% of the peak amplitude of Na^+ current, they may play a critical role in situations where membrane depolarization is sustained (Taylor, 1993). Also relevant are reports suggesting that oxidative stress and ischemia modify inactivation gating properties of the Na^+ channel in cardiac cells (Burnashev et al., 1989; Bhatnagar et al., 1990).

B. Protection Against Ischemic Damage by Tetrodotoxin

1. In vitro preparations. Selective blockade of voltage-gated Na^+ channels by TTX clearly increases the anoxic tolerance of a number of preparations. In rat hippocampal slices, TTX reduced the fall in ATP concentration during anoxia (5 to 10 min) and improved the recovery of evoked population spikes from dentate granule neurons and CA1 pyramidal neurons (Boening et al., 1989). Some of these observations were confirmed by Weber and Taylor (1994), and their histological examinations showed that TTX also prevented CA1 pyramidal cell damage produced by anoxia/aglycemia. In the same preparation exposed to 10 min of anoxia, TTX provided better protection than thiopental against long-term loss of the population spike of the CA1 region, even though thiopental was more potent at blocking anoxic Ca^{2+} influx (Kass et al., 1992). In rat neocortical slices, TTX markedly attenuated the depolarization of layer II to III pyramidal neurons evoked by brief anoxia (Rosen et al., 1994). This specific Na^+ channel blocker also protected cultured hippocampal neurons against hypoglycaemia- and cyanide-induced injury, even when applied after the insult (Tasker et al., 1992; Vornov et al., 1994). In contrast, TTX alone produced a small and variable reduction in neuronal death subsequent to 40 to 50 min oxygen-glucose deprivation in murine mixed neocortical cell cultures (Goldberg and Choi, 1993; Lynch et al., 1995), despite attenuating the increase in $[Na^+]_i$ associated with this insult (Lynch et al., 1995). Finally, in the anoxic rat optic nerve, a representative white matter tract (see above, section A.), TTX substantially improved postanoxic functional recovery, even at concentrations that had little effect on the amplitude of the normal CAP (Stys et al., 1992a) and protected the axonal cytoskeleton (Waxman et al., 1994).

2. In vivo experimental models. Increased tolerance to ischemia with TTX was also observed in vivo. TTX slowed down extracellular acidosis produced by complete ischemia in the isolated perfused rat brain, indicating a reduction of the anaerobic metabolic rate (fig. 5) (Xie et al., 1994b). It also markedly delayed anoxic depolarization (Prenen et al., 1988; Xie et al., 1994b) and, as a direct consequence, the critical ionic changes associated with anoxic depolarization, i.e., Na^+-entry (fig. 5), Ca^{2+}-entry and K^+-efflux (Xie et al., 1994b). These effects of TTX confirm that Na^+ channel blockade reduces energy demand, but it is important to note that TTX does not *prevent* anoxic depolarization, nor does it reduce its magnitude when such depolarization does occur (Haddad and Donnelly, 1990; Xie et al., 1994b) (fig. 5). This implies that TTX-sensitive voltage-gated Na^+ channels do not play an essential role in the sudden increase in the ionic permeability of the cellular membrane that provokes anoxic depolarization. Finally, direct application of TTX to the rat hippocampus dose-dependently reduced neuronal death following transient global ischemia in rats and gerbils (Yamasaki et al., 1991; Lysko et al., 1993, 1994) and improved functional recovery (Prenen et al., 1988), again supporting the neuroprotective potential of Na^+ channel blockade.

The experimental findings outlined above clearly demonstrate that selective blockade of voltage-gated Na^+ channels reduces the rate of ATP depletion during ischemia and is potentially neuroprotective. Conversely, persistent drug-induced Na^+ influx is neurotoxic, even when energy supply to the brain is not compromised (see section III.B.1.).

C. Local Anesthetics

Local anesthetics [i.e., lidocaine, procaine, lidocaine ethochloride (QX314), etc.] *reversibly* block Na^+ channels with complex voltage-and frequency-dependent properties. This is an important feature for their clinical efficacy, which also indicates that drug binding is modulated by channel state (Catterall, 1987; Butterworth and Strichartz, 1990; Starmer et al., 1990). Indeed, it is now established that local anesthetics bind to a specific site inside the pore of the channel (see section II.B.3 and fig. 3), and with a higher affinity when channels are open or inactivated, thereby promoting inactivation. These agents also alter the conductance of K^+ channels, although to a lesser extent (Swenson, 1981; Stolc, 1988). Despite some conflicting in vivo results (see below, section 2.), studies with local anesthetics generally support the concept that down-modulation of Na^+ channels is potentially neuroprotective.

1. In vitro preparations: action mechanism. Local anesthetics have been shown to be neuroprotective against ischemia in several in vitro models. In rat hippocampal

slices, the recovery rate of synaptic function following 15 min of hypoxia was significantly improved by previous incubation for 60 min with 0.1 mM of lidocaine, 2-chloroprocaine or cocaine. It is important to record that 0.1 mM of these agents preserved normal synaptic function (Schurr et al., 1986; Lucas et al., 1989). The protective effect of lidocaine in this preparation was recently confirmed by Weber and Taylor (1994), who also showed that this drug prevented CA1 pyramidal cell damage produced by 12 min of anoxia/aglycaemia.

In primary cultures of rat brain neurons, the neurotoxic effect of 16 h exposure to 30 μM veratridine was Ca^{2+}-dependent and only partially inhibited by local anesthetics. Nevertheless, there was good correlation between the potency of these drugs and several Ca^{2+} antagonists, and their binding affinity for the BTX binding site of Na^+ channels in rat cortex synaptosomal preparations (fig. 6) (Pauwels et al., 1990). In cultured mixed neocortical cells exposed to transient oxygen-glucose deprivation, lidocaine (100 μM), quinidine (100 μM), lorcainide (10 or 100 μM) and, unexpectedly, 1 mM of the impermeant QX314 (see section II.B.3.) further reduced neuronal death when superimposed upon glutamate receptor blockade (Lynch et al., 1995).

The ability of local anesthetics to protect CNS white matter (i.e., myelinated fibre tract) was also studied, using rat optic nerves subjected to 60 min of anoxia (Stys et al. 1992b; Stys, 1995). Lidocaine and procaine (0.1 and 1 mM) applied for 1 h before anoxia significantly improved postanoxic recovery of the CAP, but preanoxic CAP was also depressed. The quaternary derivatives QX314 (fig. 3) (0.1 to 1.0 mM) and QX222 (0.3 to 3 mM), as well as the tertiary amine bupivacaine (10 and 30 μM), were more effective; they further improved the recovery from anoxia at concentrations that did not block conduction. Lidocaine (0.1 mM) had previously been shown to delay the onset of CAP depression and reduce K^+ and Na^+ changes caused by glucose-free medium in rabbit vagus nerve (Fink, 1982).

The fact that, at least in some cases, local anesthetics were protective at concentrations that did not block normal conduction is promising and particularly relevant to pharmacological neuroprotection. It clearly suggests that it may be possible to block Na^+ channels selectively in anoxic regions of the CNS (i.e., with compromised membrane potential) while sparing normal tissue and function. This can be explained in two different ways: (*a*) the blockade of Na^+ channels by local anesthetic is enhanced with membrane depolarization because their actions are use- and frequency-dependent; and/or (*b*) some of these agents may be more selective to noninactivating (persistent) Na^+ channels. The first hypothesis may apply more to lidocaine and procaine, as they were reported to preferentially block fast Na^+ channels while preserving the noninactivating Na^+ conductance (Llinás and Sugimori, 1980; Sugimori and Llinás, 1980). The second hypothesis deserves more attention because, as already stated (see section A.2.), fast-inactivating Na^+ channels should not remain open in membranes depolarized by anoxia. This is supported by the finding that TTX delayed but did not modify anoxic depolarization (see above, section B.). There is also evidence that Na^+ conductance is not completely inactivated by prolonged depolarization, presumably because of a subpopulation of persistent Na^+ channels (Stafstrom et al., 1985; French et al., 1990). As the persistent Na^+ conductance is activated at more negative membrane potentials than transient Na^+ currents (French et al., 1990), the former may play a dominant role in mediating Na^+ influx during the initial stages of anoxia and energy failure (Stys et al., 1992b). Indeed, in contrast to lidocaine, QX314, which was more effective in protecting the optic nerve against anoxia, was found to be relatively specific for noninactivating Na^+ channels (Stafstrom et al., 1985). Finally, and independent of these considerations, concomitant, partial block of K^+ conductance by local anesthetics may also contribute to preservation of electrogenesis (Swenson, 1981; Stolc, 1988; Stys et al., 1992b).

2. In vivo experimental models. High doses (i.e., 160 mg kg^{-1}) of lidocaine rapidly abolished electrical activity in dogs and, unlike barbiturates, reduced the brain metabolic rate 15 to 20% beyond that achieved by eliminating synaptic transmission (Astrup et al. 1981a; Astrup and Sørensen, 1981). Lidocaine also delayed and slowed K^+ efflux produced by circulatory arrest in the dog brain (Astrup et al., 1981c; Lantos et al., 1990b). These pioneering findings, attributed to reduction of ion leakage (*membrane stabilizing or sealing* effect; Astrup et al., 1981a, c), triggered much interest, and lidocaine was subsequently tested in various models of cerebral ischemia.

In contrast to the beneficial effects observed following circulatory arrest in dogs, high prophylactic doses of lidocaine (50 mg bolus followed by 50 mg kg^{-1} h^{-1}) failed to reduce the size of infarct produced by MCA occlusion in cats (Shokunbi et al., 1986). A much lower dose, administered as a single bolus (10 mg kg^{-1}), did not alter the infarct volume, but temporarily preserved somatosensory evoked potentials (Gelb et al., 1988). In this model, only intermediary, prolonged dosage (5 mg kg^{-1} in 3 to 5 min, immediately followed by 3 mg kg^{-1} h^{-1} for 25 min before MCA occlusion; and 2 mg kg^{-1} h^{-1} for the rest of the experiment) reduced the infarct volume produced by 3 h of MCA occlusion and preserved local cerebral blood flow in the infarct border (Shokunbi et al., 1990). The lack of protection with high doses of lidocaine in focal ischemia may be due to hypotension, which may further impair cerebral perfusion (Shokunbi et al., 1986). With the single bolus of 10 mg kg^{-1} lidocaine, plasma concentrations of lidocaine may have decayed rapidly because of extensive hepatic metabolism and rapid distribution to all tissues (Gelb et al., 1988).

The protective effect of prolonged lidocaine treatment reported by Shokunbi and coworkers (1990) may be due

to prevention of Na^+ influx with a concomitant reduction in energy demand, supplemented by *membrane stabilization*. However, prevention of high intracranial pressure and improved perfusion of the infarct border could also have played a part (Shokunbi et al., 1990). The latter must be emphasized: lidocaine pretreatment (5 to 15 mg kg^{-1}) was not beneficial in rats subjected to transient global cerebral ischemia, because there was no potential for collateral circulation (Warner et al., 1988; Sutherland et al., 1989).

In a rabbit model of 20-min incomplete global ischemia, sustained treatment with lidocaine (12 mg kg^{-1} h^{-1} starting 15 min before ischemia and continuing for 60 min after the ischemia onset) significantly accelerated the recovery of EEG and evoked-potential amplitudes (Rasool et al., 1990). However, this effect may not represent strict cerebral protection, because 20 min of incomplete global ischemia may not lead to histopathological changes in this model (Rasool et al., 1990). Similarly, in dogs subjected to repeated short episodes of circulatory arrest, 100 mg kg^{-1} lidocaine favourably influenced both the cessation of EEG activity and its restitution during reperfusion (Lantos et al., 1990a).

Intravenous administration of lidocaine (5 mg kg^{-1}) before acute cerebral ischemia induced by air embolism in cats reduced the decrement in cortical somatosensory evoked responses and improved their recovery (Evans et al., 1984). A similar effect was observed with this treatment after experimental spinal cord injury in cats (Kobrine et al., 1984). Repeated administration of lidocaine, superimposed upon hyperbaric treatment, ameliorated the delayed deterioration of evoked potential associated with internal carotid air embolism in dogs (Dutka et al., 1992). However, as the vascular endothelium is damaged in all of these models, lidocaine protection could be linked to its effect on granulocyte adherence and migration (MacGregor et al., 1980).

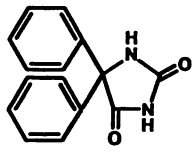
The variety of animal models and dose regimens used in these studies preclude a reliable synthesis of the data. Nevertheless, several important features emerge: (*a*) as with other drugs, lidocaine treatment may be effective only with focal or incomplete global ischemia; (*b*) systemic administration of lidocaine must be sustained; and (*c*) high doses may not be effective because of cardiovascular toxicity.

3. Clinical observations. Artru and coworkers (1991) have advocated i.v. perfusion of lidocaine at high dose, to replace or supplement barbiturate (thiopental) treatment of refractory intracranial hypertension and acute cerebral ischemia. One major advantage of lidocaine over barbiturate is its rapid elimination upon cessation of treatment. Naturally, the authors have stressed the necessity for simultaneous anticonvulsant therapy because of lidocaine epileptogenicity and for close cardiovascular monitoring to detect early signs of cardiotoxicity.

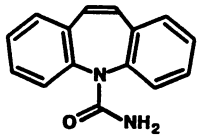
D. Anticonvulsants Acting on Na^+ Channels

This section focuses on phenytoin (and carbamazepine to a lesser extent) because large amounts of data are available for this drug (fig. 7). However, the rationale developed here applies to other anticonvulsants that primarily target Na^+ channels at therapeutic concentrations (Catterall, 1987). These include valproate (Van den Berg et al., 1993), flunarizine (fig. 8) (see section V.A.1.) that has also anticonvulsant activity (Wauquier et al., 1986; Rogawski and Porter, 1990), lamotrigine (fig. 9; see section V.B.), and compounds under development, such as remacemide (FPL 12924; Palmer et al., 1993; Bannan et al., 1994). Our analysis is also intentionally focused on anticonvulsant interactions with Na^+ channels, but, as clinically effective anticonvulsants are all lipophilic compounds, they are expected to have multiple actions on excitable membranes, particularly at supratherapeutic concentrations. For example, phenytoin was reported to displace [^{3}H]benzodiazepine from synaptosomal receptors (Bowling and DeLorenzo, 1982), and to block voltage-sensitive Ca^{2+} channels (Messing et al., 1985; Yaari et al., 1986), even at the relatively low (3 to 30 μM) concentrations in some preparations (Twombly et al., 1988). Wamil and McLean (1993) reported that phenytoin inhibited NMDA receptor-mediated depolarizing responses of mouse spinal neurons in culture, but this observation was not confirmed (Sheridan et al., 1994; Laffling et al., 1995). These *secondary* actions may contribute significantly to the therapeutic effects of this type of drug in epilepsy and ischemia.

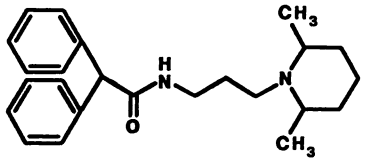
1. Phenytoin and carbamazepine: interactions with Na^+ channels. Phenytoin and carbamazepine (fig. 7) are particularly effective anticonvulsants in the management of partial seizures and generalized tonic-clonic seizures. Their effectiveness is probably related to their ability to inhibit high-frequency action potential firing, while having little effect on lower-frequency firing



Phenytoin



Carbamazepine



PD85,639

FIG. 7. Structure of the anticonvulsants phenytoin and carbamazepine, acting primarily via Na^+ channel block at therapeutic concentrations, and of PD85,639, a novel phenylacetamide structurally related to tertiary amine local anesthetics.

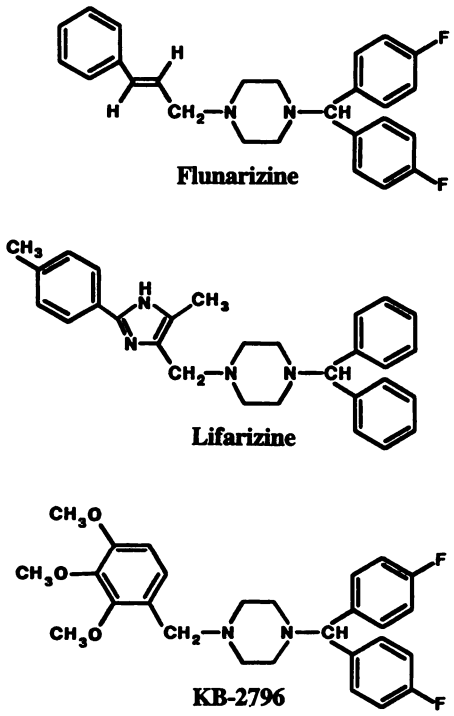


FIG. 8. Structures of the diphenylpeperazine analogues flunarizine, lifarizine and KB-2796.

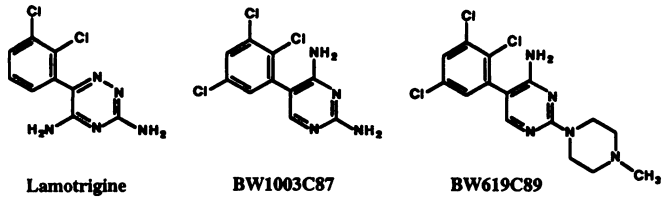


FIG. 9. Structures of the anticonvulsant lamotrigine and its derivatives BW100CC87 and BW619C89 (often named *glutamate-release inhibitors*).

(Yaari et al., 1986; Rogawski and Porter, 1990). At therapeutic concentrations, phenytoin and carbamazepine interact preferentially with neuronal Na^+ channels (Catterall, 1987; Rogawski and Porter, 1990). They displace BTX, partly by increasing the rate of dissociation of the toxin-receptor complex (Willow and Catterall, 1982; Francis and McIntyre-Burnham, 1992) and inhibit veratridine- and BTX-induced Na^+ and Ca^{2+} uptake in mouse neuroblastoma and rat brain synaptosomes (Catterall, 1981; Ferrendelli and Daniels-McQueen, 1982; Willow et al., 1984). Taken together, these studies demonstrate that phenytoin and carbamazepine, like local anesthetics, are allosteric inhibitors of neurotoxin binding and action at receptor 2 on Na^+ channels (fig. 3). Specific [^{3}H]phenytoin binding to rat brain membranes was also inhibited by drugs known to interact with Na^+ channels, including site-specific neurotoxins, local anesthetics and antiarrhythmics (Francis and McIntyre-Burnham, 1992).

Voltage-clamp studies have shown that phenytoin and carbamazepine block, in a voltage- and frequency-dependent manner, Na^+ channels in central neurons, with a stronger block at more depolarized voltage and higher frequencies (Connors, 1981; McLean and MacDonald, 1983; Matsuki et al., 1984; Willow et al., 1985). Several features of phenytoin's action on Na^+ currents have suggested preferential binding of the drug to the inactivated state of the channel. For example, phenytoin shifted the steady state inactivation curve for Na^+ current to more hyperpolarized potentials and also markedly slowed recovery from inactivation (Matsuki et al., 1984; Willow et al., 1985; Shwartz and Grigat, 1989; Ragsdale et al., 1991; Lang et al., 1993). A feature that may be peculiar to phenytoin is that effective blockade requires relatively long depolarizations (at least 1 s) (Matsuki et al., 1984; Willow et al., 1985; Kuo and Bean, 1994). Kuo and Bean (1994) showed in voltage-clamped rat hippocampal neurons that slow development of blockade does not reflect selective binding of phenytoin to slow inactivated states of the channels, because blockade developed faster, and required less depolarized voltages, than did slow inactivation. Instead, it appears that phenytoin binds tightly but slowly to fast inactivated states of the Na^+ channels. Regardless of how phenytoin blocks inactivated Na^+ channels, the end result is a delayed transition from inactivated to closed and available channels and therefore to an increased fraction of channels in the inactivated state (Matsuki et al., 1984; Catterall, 1987; Rogawski and Porter, 1990).

2. Neuroprotection in vitro. In the rat optic nerve, a representative white matter tract (see section IV.A.), both phenytoin and carbamazepine protected against anoxic injury at concentrations below those inhibiting CAP and below (⅙) the therapeutic range used to control epilepsy (Fern et al., 1993). Pretreatment with phenytoin (20 μM) also protected rat hippocampal slices against 10 min of hypoxia, as assessed by improved recovery of synaptically evoked population spikes (Kenny and Sheridan, 1992). In the same preparation exposed to hypoxia/glucose-free medium, phenytoin (5 to 100 μM) concentration-dependently delayed negative DC shifts (i.e., anoxic depolarization), improved recovery of synaptic potentials and protected against histological damage (Weber and Taylor, 1994). It is important to emphasize that, as with TTX (in some models) and lidocaine (see above, sections B. and C.), effective neuroprotection by phenytoin was achievable without blocking synaptic potentials or presynaptic fiber volleys. In contrast, 10 and 100 μM of phenytoin alone failed to protect murine cultured cortical neurons from injury induced by oxygen-glucose deprivation; phenytoin only became effective when combined with glutamate receptor blockade (Lynch et al., 1995).

Phenytoin was reported to have limited antagonist efficacy against kainate neurotoxicity in vivo (Zaczek et al., 1978), and studies of its action on excitotoxicity in vitro have produced conflicting results. Cell death, provoked by exposing primary cultures of rat cortex cells to

1 mM of kainic acid or NMDA for 24 h, was reduced in an incomplete but concentration-dependent manner by pretreatment with phenytoin (10 to 100 μM) and carbamazepine (10 to 1000 μM) (Lakics and Erdö, 1994). In contrast, 100 μM phenytoin was ineffective against acute glutamate neurotoxicity (500 μM for 5 min) in cortical cell culture (Koh and Choi, 1987).

3. Beneficial effects in experimental models of cerebral ischemia. Phenytoin pretreatment (200 mg kg^{-1}) significantly protected hippocampal CA1 neurons in gerbils subjected to 5 min of forebrain ischemia produced by bilateral carotid artery occlusion under various experimental conditions (Clifton et al., 1989; Taft et al., 1989), but Deshpande and Wieloch (1986) failed to demonstrate protection by phenytoin in a rat model of global ischemia. A much lower dose (15 mg kg^{-1}) still attenuated both necrosis and neurological deficits in the rabbit brain subjected to transient global ischemia (Aldrete et al., 1979; Cullen et al., 1979). At 50 to 300 mg kg^{-1}, phenytoin attenuated the accumulation of brain free fatty acids during 10 min of complete ischemia produced by decapitation in rats (Shiu et al., 1983). Finally, phenytoin was also neuroprotective in a model of in utero hypoxic brain injury as assessed by maintenance of Na^+/K^+-ATPase activity and reduced lipid peroxidation (Lampley et al., 1995).

Phenytoin (28 mg kg^{-1}, i.v.; i.e., an effective anticonvulsant dose) administered 30 min and 24 h *post*ischemia markedly reduced ischemic damage subsequent to permanent occlusion of the MCA in mice (Kenny et al., 1992). This action was confirmed in rats. Phenytoin and carbamazepine (but not valproic acid) preserved the activity of Na^+/K^+-ATPase measured in several brain regions of rats killed after 30 min of MCA occlusion (Murakami and Furui, 1994). When administered 30 min and 24.5 h after insult, phenytoin (2 × 100 mg kg^{-1} i. p.) and carbamazepine (2 × 50 mg kg^{-1}) reduced the infarct size produced by MCA occlusion in rats by 40 and 24%, respectively (Rataud et al., 1994). With dual ipsilateral occlusion of MCA and common carotid artery in the same species, phenytoin (28 mg kg^{-1}, i.v., 30 min and 24 h after arterial occlusion) reduced the infarct volume by 45%, i.e., was more effective in this model than the noncompetitive NMDA-receptor antagonists dizocylpine maleate (MK-801, 0.1 mg kg^{-1}) and phencyclidine (3 mg kg^{-1}) (Boxer et al., 1990). A single dose of this anticonvulsant (28 mg kg^{-1}) administered 30 min after occlusion was neuroprotective, but delaying its administration by more than 2 h rendered it ineffective (Boxer et al., 1990). Finally, 30 mg kg^{-1} of phenytoin was as efficient as steroid therapy in preventing both edema and infarction in the primate cortex after embolization of the internal carotid artery (Bremer et al., 1980).

4. Increased tolerance to anoxia/ischemia and delayed anoxic depolarization. Several studies indicate that phenytoin minimizes residual energy demand and thereby increases the tolerance to ischemia (presumably by reducing Na^+ influx) (see section III.A.). As early as 1944, Hoff and Yahn reported that phenytoin pretreatment of mice and rats increased their resistance to hypoxia produced by hypobaric exposure. This was confirmed by measuring survival time of mice breathing 5% oxygen; the optimal dose of 200 mg kg^{-1} phenytoin increased survival time by 123% (Artru and Michenfelder, 1980). It is interesting to note that this effect was significantly greater than that of diazepam (benzodiazepine, with little action on Na^+ channels at therapeutic concentrations), even though the latter was more effective in suppressing hypoxemic convulsions in this model, which suggested that phenytoin protection was not dependent on its anticonvulsant effect *per se* (Artu and Michenfelder, 1980). Increased survival time following anoxia and global ischemia by phenytoin was also reported in cats and guinea pigs (Naiman and Williams, 1964; Baldy-Moulinier, 1971–1972). In dog studies, pretreatment with phenytoin decreased the rate of K^+ accumulation in cisternal cerebrospinal fluid following 20 min of anoxia, suggesting that this drug prevented or delayed K^+ efflux (Artru and Michenfelder, 1980). This latter effect was confirmed in rabbits subjected to circulatory arrest provoked by complete anoxia. Phenytoin (50 and 150 mg kg^{-1}) dose-dependently reduced the increase in $[K^+]$ (and the slight drop in $[Na^+]$) in the cisterna magna 10 and 20 min after cardiac arrest. Interestingly, phenytoin was more potent than either phenobarbital (33 mg kg^{-1}) or mild hypothermia (35°C) and did not markedly alter the time to loss of EEG activity (Artru and Michenfelder, 1981), thus suggesting a selective effect on residual energy demand (see section III.A.).

Carbamazepine (13 to 50 mg kg^{-1}) was also found to markedly prolong the survival time of mice subjected to severe hypoxia or bilateral carotid artery occlusion (Dong et al., 1994). At 25 to 70 mg kg^{-1}, carbamazepine also reduced both ATP and phosphocreatine depletion, and lactic acid accumulation, in mouse brain 30 s after decapitation (Dong et al., 1994).

V. Neuroprotective Drugs, Presumably Acting on Na^+ Channels

A. Ca^{2+} channel modulators with actions on Na^+ channels

Voltage-gated Na^+, Ca^{2+} and K^+ channels are members of a related gene family (e.g., 55% sequence homology remains between the principal subunits of Na^+ and Ca^{2+} channels; Tanabe et al., 1987), and they share strong functional analogies such as voltage-dependent activation and inactivation (Catterall, 1988a). With regard to ion conductance, Na^+ channel selectivity is altered by neurotoxins (see section II.B.4.), and mutation of only two amino acid residues in the Na^+ channel is sufficient to confer Ca^{2+} channel-like permeability properties (see section II.B.2). Therefore, it is not surprising that a number of drugs classified as Ca^{2+} channel block-

ers also interact strongly with Na^+ channels (McNeal et al., 1985; Velly et al., 1987, Pauwels et al., 1991; Ragsdale et al., 1991) (fig. 6), and that the anti-ischemic properties of such Ca^{2+} antagonists may be linked, at least in part, to down-modulation of Na^+ channels (Velly et al., 1987).

1. Flunarizine. The diphenylalkylamine flunarizine (fig. 8) has beneficial effects against global (Desphande and Wieloch, 1985; Kumar et al., 1987; Alps et al., 1988; Tegtmeier et al., 1989) and focal cerebral ischemia (Van Reempts et al., 1987; De Ryck et al., 1989, 1991). Flunarizine (40 mg kg^{-1}, p.o., 3 h before insult) decreased by half the size of infarcts produced photochemically in the cortex of rats (Van Reempts et al., 1987) and reduced neurological deficit in this model, even when administered 5 to 30 min after the insult [0.16 to 5 mg kg^{-1}, i.v.; median effective dose (ED_{50}) approximately 0.85 mg kg^{-1}] (De Ryck et al., 1989, 1991). In contrast, flunarizine showed no clear neuroprotective potential when administered repeatedly after coagulation of the MCA in mice (2.5 to 20 mg kg^{-1} i.p., starting 5 min after coagulation) (Gotti et al., 1990). In global ischemia, flunarizine decreased the incidence of delayed neuronal death in the hippocampus of rats (Desphande and Wieloch, 1985), dogs (Kumar et al., 1987) and gerbils (Alps et al., 1988) (see however Hossmann et al., 1983; Newberg et al., 1984; Araki et al., 1990; Xie et al., 1992). In gerbil models of cerebral ischemia, pretreatment with flunarizine also improved neurological symptoms (Cohan et al., 1992; Ito et al., 1994). The neuroprotective potential of flunarizine was confirmed in vitro. Pretreatment with this drug prevented glutamate-induced toxicity in rat hippocampal primary cell cultures (Hara et al., 1993b), inhibited ouabain-induced Na^+ influx into synaptosomes (Cousin et al., 1995) and protected neuroblastoma cells against cytotoxic hypoxia (Peruche and Krieglstein, 1991), providing the insults remained moderate (Pauwels et al., 1989). Flunarizine, however, failed to prevent neurotoxicity induced by depolarization with 90 mM K^+ for 30 min in rat hippocampal organotypic cultures (Takahashi et al., 1995).

Despite being classified as a nonselective Ca^{2+} channel modulator (Pauwels et al., 1991; Spedding and Paoletti, 1992; Akaike et al., 1993; Panchenko et al., 1993), flunarizine clearly interacts with voltage-gated Na^+ channels. Among 180 compounds, including classical local anesthetics, anticonvulsants and Ca^{2+} channel blockers, this drug was among the most potent inhibitors of BTX-B (see section II.B.4.) binding to guinea pig cerebral cortex vesicles [flunarizine IC_{50}(concentration that produces 50% of its maximum possible inhibition) = 0.6 μM versus lidocaine IC_{50} = 240 μM] (McNeal et al., 1985), and this property was confirmed with rat brain synaptosomes (fig. 6) (Velly et al., 1987; Pauwels et al., 1986, 1990; Roufos et al., 1994). In contrast, flunarizine was a poor inhibitor of [^{3}H]-tetracaine binding in rat brain synaptosomes (IC_{50} > 0.1 mM) (Velly et al., 1987) and demonstrated relatively weak local anesthetic potency (Hay and Wadworth, 1982; Pauwels et al., 1986), thus illustrating that high affinity to the BTX binding site is *not* a property exclusive to local anesthetics (McNeal et al., 1985).

Flunarizine inhibited also the effects of veratridine in several in vitro preparations, for example: (*a*) Na^+ influx in synaptosomes and cultured cerebellar granule cells (Pauwels et al., 1986; Velly et al., 1987; Cousin et al., 1995); (*b*) increased synaptosomal respiration (Urenjak et al., 1991; Wermelskirchen et al., 1992); (*c*) stimulation of [^{14}C]guanidine uptake (i.e., a representative measure of Na^+ fluxes; Reith, 1990) in synaptosomes (Pauwels et al., 1986) and CHO cells expressing type IIA Na^+ channels (Roufos et al., 1994); (*d*) neurotransmitter release from synaptosomes and cultured neurons (Cousin et al., 1995); (*e*) spreading depression and ion fluxes across the cellular membrane in hippocampal slices (Ashton et al., 1990); and (*f*) neuronal cell degeneration (fig. 6) (Pauwels et al., 1989, 1990, 1991; Roufos et al., 1994).

Patch-clamp studies with isolated rat cerebellar neurons clearly confirmed that flunarizine blocks Na^+ currents and revealed that this drug interacts predominantly with inactivated Na^+ channels (Kiskin et al., 1993). Supporting findings include: (*a*) increased Na^+ channel block with the frequency of stimulation and membrane depolarization; (*b*) shift of the steady state voltage-dependence of Na^+ channel inactivation toward more negative potentials; and (*c*) marked slowdown of Na^+ channel recovery from inactivation. As with local anesthetics (Catterall, 1987), this high affinity for inactivated states probably increases the probability of channel inactivation at negative membrane potentials and thereby stabilizes the Na^+ channels in this state relative to resting or active states. This property may also explain why flunarizine did not interfere with Na^+ currents under normal conditions in both isolated cardiac cells and synaptosomes (Tytgat et al., 1990; Urenjak et al., 1991; Wermelskirchen et al., 1992).

Finally, investigations into the mechanism of neuroprotection by flunarizine have shown that it markedly delays anoxic depolarization (Marrannes et al., 1989; Scheller et al., 1989; Xie et al., 1995), a characteristic feature of Na^+ channel down-modulation in ischemia (see sections III and IV.B.), independently of any direct actions on key enzymes of energy metabolism (Bielenberg et al., 1986) or Na^+/K^+-ATPase activity (Urenjak et al., 1991; Wermelskirchen et al., 1992). It is interesting to note that flunarizine protected the gerbil hippocampus against delayed neuronal loss following carotid artery occlusion, whereas the dihydropyridine Ca^{2+} antagonist nimodipine, which interacts only slightly with Na^+ channels (fig. 6) (Pauwels et al., 1990), was ineffective in this model (Alps et al., 1988). Similarly, nimodipine did not delay anoxic depolarization, nor did it alter any of the ionic changes associated with this event (Xie et al., 1995). However, it is important to mention that, in ad-

dition to interacting with Ca^{2+} and Na^+ channels, flunarizine has other pharmacological properties that may also contribute to neuroprotection, such as inhibition of adenosine exchange systems and/or catabolism (Di-perri et al., 1989; Ferrandon et al., 1994; Rudolphi et al., 1992) and, possibly, free-radical scavenging (Aruoma et al., 1991; Goncalves et al., 1991; Nemoto and Chavko, 1993).

2. Lifarizine. Lifarizine (RS-87476) is another diphenylpiperazine (fig. 8) that has a broad neuroprotective profile in both global and focal models of cerebral ischemia (Alps, 1992): a phase 2 (efficacy) trial in stroke patients is nearing completion (Lipton and Rosenberg, 1994). Lifarizine has been shown to protect dose-dependently the gerbil striatum against ischemia-induced dopamine depletion (Brown et al., 1993) and several brain regions against damage produced by 10 min of four-vessel occlusion in rats (Alps et al., 1990, 1995). In severe global ischemia, provoked by two-vessel occlusion superimposed on halothane-induced reduction of arterial blood pressure (50 mmHg), repeated administration of lifarizine (0.1 mg kg^{-1}, 5 min postocclusion; 0.5 mg kg^{-1}, 15 min postocclusion and twice daily for 72 h) was able to achieve significant protection of hippocampal CA1, cortex, thalamus, and cerebellar brainstem (McBean et al., 1995a). With permanent MCA occlusion in cats, 2 $\mu g\ kg^{-1}$ of lifarizine administered i.v. after ischemia onset, followed by infusion at 0.7 $\mu g\ kg^{-1}\ h^{-1}$ over 12 h, reduced by 70% the size of the infarct. The highest dose tested in this study (50 $\mu g\ kg^{-1}$ i.v., with 17.5 $\mu g\ kg^{-1}\ h^{-1}$ maintenance dose) was able to reduce the infarct size by as much as 88% (Kucharczyk et al., 1990, 1991). Monitoring of the developing lesions using magnetic resonance imaging and spectroscopy also suggested that energy levels were preserved and tissue acidosis and edema reduced in animals treated with lifarizine (Kucharczyk et al., 1990, 1991). Repeated administration of lifarizine also reduced the size of the infarct produced by MCA occlusion in mice (Brown et al., 1994a, 1995), but high doses of lifarizine (200 times the dose effective in the mouse) failed to protect the corresponding rat model (Rataud et al., 1994). Finally, lifarizine was also beneficial when administered after induction of photothrombotic infarcts in the rat cortex (McBean et al., 1995b).

Like flunarizine, lifarizine was initially introduced as Ca^{2+}-modulator (Alps et al., 1990). This drug however did not act as a conventional dihydropyridine Ca^{2+} antagonist in the taenia smooth muscle preparation, because it potently inhibited the responses to Ca^{2+} channel activators such as Bay K 8644 but had little effect on responses to Ca^{2+} (Fraser and Spedding, 1991). In contrast to these indistinct and relatively weak effects on Ca^{2+} channels, ligand binding and functional assays have clearly suggested that lifarizine interacts potently with voltage-gated Na^+ channels: (*a*) lifarizine displaced BTX-B from rat cortical membranes with an IC_{50} of 55 nM (McGivern et al., 1994; MacKinnon et al., 1995); (*b*) it protected cultured cortical neurons against veratridine neurotoxicity with an IC_{50} of 0.4 μM (versus 30 nM for TTX, and 30 μM for the dihydropyridine Ca^{2+} channel antagonist nitrendipine) (May et al., 1995); and (*c*) similar to TTX and flunarizine, lifarizine inhibited veratridine-induced contractions of embryonic chick cardiac myocytes (IC_{50} = 1.6 μM) (Patmore et al., 1991).

Patch-clamp studies in mouse neuroblastoma cells have confirmed and clarified the potent actions of lifarizine on Na^+ currents (McGivern et al., 1995b): (*a*) at holding potentials, the potency of lifarizine to reduce the peak Na^+ current evoked by a 10 ms depolarizing step was markedly increased when the holding potential became increasingly less negative (IC_{50} of 7.3, 1.3 and 0.3 μM for a corresponding holding potential of −100, −80 and −60 mV); (*b*) recovery from inactivation following a 20 s depolarization from −100 to 0 mV was markedly slowed by 3 μM lifarizine; (*c*) the apparent affinity of lifarizine for the inactivated state of the channel (K_I) was 0.19 μM, i.e., much higher than that estimated for resting channels (K_R = 7.3 μM); and (*d*) 1 μM lifarizine had no effect on Na^+ currents modified by chloramine-T (i.e., oxidizing agent producing currents that decay very slowly), suggesting no significant interaction with open Na^+ channels. Taken together, these data demonstrate that lifarizine is a potent voltage-dependent inhibitor of Na^+ currents and that the voltage dependence arises from an interaction with the inactivated state of the channel (McGivern et al., 1995b). Voltage-dependent inhibition of Na^+ currents was also demonstrated in human neuroblastoma cells (Brown et al., 1994b), and this study clarified further the action mechanism of lifarizine. The fact that TTX did not prevent the inhibition caused by a subsequent application of lifarizine, and that the block of Na^+ currents by lifarizine did not require Na^+ channel activation, confirmed that this drug does not interact with the open state of the channel. In contrast, the depression of Na^+ currents caused by the local anesthetic lignocaine was readily reversible after pretreatment of cells with lifarizine, suggesting an allosteric interaction of lifarizine with the local anesthetic binding site (fig. 3), or selective binding to a state of the channel that is modulated by local anesthetics. It is interesting to note that, in comparison to the anticonvulsants phenytoin and lamotrigine (see sections IV.D.1. and V.B.2.), the lifarizine block of Na^+ channels showed limited use dependence and frequency dependence that suggests that this property may not be essential for neuroprotection.

3. Lomerizine hydrochloride. Lomerizine hydrochloride (KB-2796) {1-[bis(4-fluorophenyl)methyl]-4-(2,3,4-trimethoxybenzyl)piperazine dihydrochloride} (fig. 8) is another diphenylpiperazine Ca^{2+} antagonist (Akaike et al., 1993; Handa et al., 1990) with proven protective effects against cerebral ischemia, both in vitro and in vivo (Handa et al., 1990; Hara et al., 1993a, b; Yamashita et al., 1993). Pretreatment of hippocampal

guinea pig slices with KB-2796 (0.1 and 1 μM) significantly accelerated the recovery of population spikes of dentate granule cell layers following mild hypoxia (Hara et al., 1988). The same treatment prevented glutamate-induced neurotoxicity in rat hippocampal primary cell cultures, even though KB-2796 has no affinity for ionotropic glutamate receptors (Hara et al., 1993b). In vivo, KB-2796 (10 mg kg^{-1} i.p.) reduced infarct size and significantly improved neurological recovery in the rat MCA occlusion model (Harada et al., 1989; Hara et al., 1993a); it also prevented delayed neuronal death produced by 5-min bilateral carotid occlusion in the gerbil hippocampal CA1 subfield, even when the drug was administered postischemia (Yoshidomi et al., 1989).

With some experimental models, the anti-ischemic properties of KB-2796 may be partly linked to Ca^{2+} antagonistic and cerebrovascular effects (Handa, 1990). Nevertheless, strong evidence points to Na^+ channel down-modulation as the primary neuroprotective mechanism: (*a*) KB-2796 minimized the deficits in brain energy metabolism during ischemia produced by decapitation or four-vessel occlusion (Yamashita et al., 1993), suggesting a reduction of residual energy demand by the drug (see sections III.A. and B.); (*b*) similarly, KB-2796 concentration-dependently prolonged both latency and interval of spreading depression produced by a brief period of hypoxia: it was 10 and 1000 times more potent than flunarizine in prolonging the latency and interval of SD, respectively (Takagi et al., 1994); (*c*) this compound was proposed to potently displace the Na^+ channel ligand BTX-B from rat brain synaptosomes (Brown C. M. et al., unpublished data; cited by McGivern et al., 1995a); and (*d*) voltage-clamp studies in N1E-115 cells showed that KB-2796 potently inhibits Na^+ currents by interacting predominantly with the inactivated state of Na^+ channels, but also with their open and resting states (McGivern et al., 1995a). Finally, KB-2796 (1 mg kg^{-1} i.p.), administered before elicitation of cortical SD by topical application of KCl, inhibited the subsequent hypoperfusion and reduced the expression of c-Fos-like immunoreactivity (Shimazawa et al., 1994), suggesting a depressing effect of KB-2796 on SD elicitation (see section III.D.1.).

4. R56865 and related benzothiazoles. The benzothiazolamine R56865 [*N*-(1-(4-(4-fluorophenoxy)butyl)-4-piperidinyl)-*N*-methyl-2-benzothiazolamine] (fig. 10) is another Ca^{2+} channel modulator (Panchenko et al., 1993) that interacts strongly with Na^+ channels (Verdonck et al., 1991; Kiskin et al., 1993). Primarily studied as an antianginal drug (Vollmer et al., 1987; Verdonck et al., 1991; Chen et al., 1993a), R56865 also showed beneficial effects in cerebral ischemia, and a number of studies clearly suggested that its anti-ischemic properties in heart and brain may be linked to actions on Na^+ channels. As with flunarizine, R56865 dose-dependently (10^{-6}-10^{-5} M) inhibited the marked increase in synaptosomal respiration associated with veratridine-induced

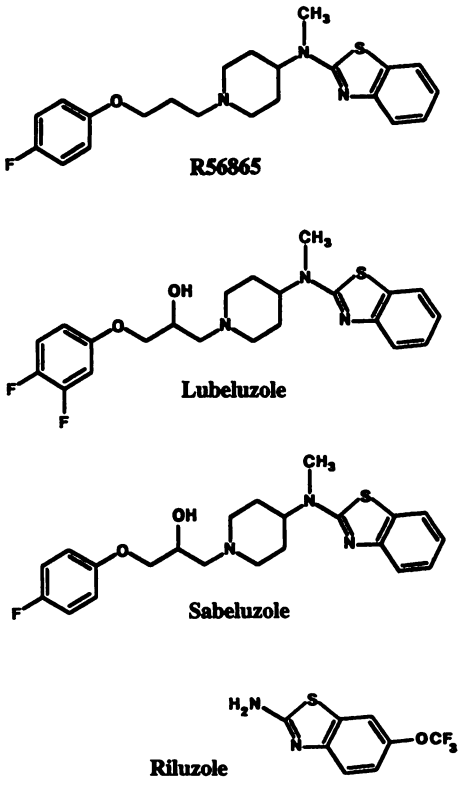


FIG. 10. Structures of benzothiazole derivatives with *anti-ischemic* properties.

Na^+ influx (Urenjak et al., 1991; Wermelskirchen et al., 1992), and a similar action was reported with adult cardiac myocytes (Haigney et al., 1994). R56865 consistently delayed anoxic depolarization in several models of global cerebral ischemia (Scheller et al., 1989; Tegtmeier et al., 1992; Xie et al., 1995). Furthermore, R56865 was the only drug among various selective inhibitors of voltage- and ligand-operated ion channels to reduce slightly, but significantly, the amplitude of the drop in $[Na^+]_o$ measured 5 min after anoxic depolarization (Xie et al., 1995). As this reduction was not obtained with TTX (fig. 5) (Xie et al., 1994b), these data suggested that R56865 may be capable of reducing Na^+ currents through non-inactivating, TTX-resistant Na^+ channels, which may become predominant with depolarized CNS membranes. It is relevant to mention here that sustained depolarization (3 min) of *Xenopus* oocytes resulted in the development of a slow inward current, TTX-resistant (5 to 30 μM), caused by the induction of slowly activated TTX-insensitive Na^+ channels (native), possibly mediated by PKC phosphorylation (Charpentier et al., 1993). Voltage-clamp studies are consistent with an action of R56865 on slow Na^+ currents. In isolated cardiac Purkinje cells, voltage-clamped in the presence of veratridine, R56865 was more effective in inhibiting the non-inactivating Na^+ current than in inhibiting the time-dependent Na^+ currents elicited by short depolarizing pulses (Verdonck et al., 1991; see also Rambold et al., 1993). In acutely isolated Purkinje neurons of rat cere-

bellum, R56865 showed similar actions on Na^+ currents to flunarizine (Kiskin et al., 1993; see above section 1.) but with a greater ability to block open channels (Na^+ current inactivation suppressed by treatment with pronase) and a greater frequency dependence.

Lubeluzole (the S-isomer of a 3,4-difluoro-benzothiazole) is a close structural analogue of R56865 (fig. 10). When administered as a single i.v. bolus, 5 min after induction of photochemical infarcts in the rat sensorimotor cortex, lubeluzole protected neurological function with an ED_{50} of 0.16 mg kg^{-1} (De Ryck et al., 1994). Protection remained near-maximal when treatment was delayed for up to 1 h after infarct induction, and then progressively diminished as measured as 3 and 6 h postinfarct. With a different regimen (i.v. bolus of 0.31 mg kg^{-1} starting 5 min after infarct, followed by a 1-h infusion of 0.31 or 0.63 mg kg^{-1}) the infarct volume was reduced by approximately 22 to 24% (De Ryck et al., 1994). An interesting feature of lubeluzole is its strong stereospecific effect, because the R-isomer was virtually inactive in this model. A phase II trial has investigated the effect of 5-day treatment with lubeluzole (10 or 20 mg per day, versus placebo) on neurological and functional recovery, and mortality in 232 ischemic stroke patients. The low dose of lubeluzole showed a trend for more favourable outcome than placebo for all the efficacy parameters considered (Diener et al., 1995; Muir and Lees, 1995). The molecular target(s) of this compound remain speculative (Lesage et al., 1994). However, the structural analogy with R56865 and the fact that lubeluzole displaced BTX (0.1 to 1 μM) (Lesage et al., 1994) warrant further studies on its action on voltage-gated Na^+ channels.

Sabeluzole is another close structural analogue of R56865 (fig. 10) with cerebroprotective effects in several models of hypoxia and ischemia (Wauquier et al., 1986; Van Reempts et al., 1986; Nikolov et al., 1991; Werbrouck et al., 1991). For example, pretreatment of mice with sabeluzole (20 to 40 mg kg^{-1} i.p.) significantly prolonged their survival to hypoxia and increased the gasping time after decapitation (Nikolov et al., 1991; Werbrouck et al., 1991). Positive effects in the gasping test suggest that this compound increases the tolerance of brain tissue to oxygen deprivation via local mechanisms within the CNS, without an involvement of the cardiovascular system. Sabeluzole (19 mg kg^{-1}, i.p., 1 h before ischemia) also reduced significantly the neuronal damage in the ipsilateral cortex of rats, as assessed 24 h after unilateral carotid artery occlusion and hypoxia (Van Reempts et al., 1986). This compound was originally proposed as a Ca^{2+} entry blocker on the basis of functional and electrophysiological data (Wilhelm et al., 1986; Boddeke et al., 1986). However, its structural similarity with R56865 (fig. 10) and the fact that sabeluzole delayed anoxic depolarization (Nikolov et al., 1991) and protected cultured neurons against veratridine (Pauwels et al., 1992) strongly suggest an action on voltage-gated Na^+ channels.

B. Lamotrigine and Structural Analogues BW1003C87 and BW619C89

Lamotrigine and its derivatives BW1003C87 [5-(2,3,5-trichlorophenyl)-2,4-diaminopyrimidine ethane sulphonate] and BW619C89 [4-amino-2-(4-methyl-1-piperazinyl)-5-(2,3,5-trichlorophenyl pyrimidine)] (fig. 9) were initially selected for their anticonvulsant efficacy (Messenheimer, 1994; Meldrum et al., 1994), but they also protect the CNS against ischemic and traumatic injury. Although these compounds are often referred to as *(presynaptic) glutamate release inhibitors* (Leach et al., 1986; 1993; Meldrum et al., 1992; Graham et al., 1993), strong evidence suggests that their actions actually originate from use-dependent inhibition of Na^+ channels.

1. Action on glutamate release. Lamotrigine dose-dependently inhibited the release of endogenous glutamate evoked by veratrine in rat cortical slices (IC_{50} = 21 μM), but this action was not exclusive to glutamate (lamotrigine was only 2 times less potent in its inhibition of γ-aminobutyric acid (GABA) release; IC_{50} = 44 μM) and, at concentrations of up to 300 μM, lamotrigine had no effect on K^+-induced glutamate release (Leach et al., 1986, 1991). These effects of lamotrigine on neurotransmitter release were recently confirmed in vivo by microdialysis (Ahmad et al., 1995). BW1003C87 and BW619C89 also inhibited veratrine- (but not K^+-) evoked glutamate release from rat cortical slices (Meldrum et al., 1992; Leach et al., 1993). In comparison to lamotrigine, however, BW619C89 was more potent in reducing the release of glutamate and aspartate (IC_{50} = 5 μM) than that of acetylcholine (IC_{50} = 21 μM) and GABA (IC_{50} = 51 μM) (Leach et al., 1993).

Although lamotrigine and BW619C89 were slightly more potent in reducing veratrine-induced glutamate release, this property by itself is not sufficient to classify these compounds as *glutamate release inhibitors*. The fact that TTX (1 μM) completely blocks veratrine-induced neurotransmitter release (Leach et al., 1986), and that lamotrigine analogues were ineffective against K^+-induced glutamate release, strongly suggested that their actions on neurotransmitter release were *secondary* to down-modulation of voltage-gated Na^+ conductance. This action mechanism is now largely substantiated.

2. Down-modulation of Na^+ channels. Binding experiments carried out with rat brain synaptosomes showed a concentration-dependent inhibition of BTX-B binding by lamotrigine (K_D = 114 μM) (Cheung et al., 1992), confirming a preliminary report by Leach and coworkers (1991). BW1003C87 was also mentioned to share this property (M. Leach, personal communication to Graham et al., 1994a). Voltage-clamp experiments in cultured rat cortical neurons showed that 100 μM lamotrigine, but not 10 μM, inhibited indiscriminately both excitatory and inhibitory spontaneous synaptic events (Lees and

Leach, 1993). This suggested that, even at high doses, lamotrigine did not preferentially depress excitatory, probably glutamate-mediated, synaptic events. In the same preparation, presumptive Na^+ spikes evoked at low frequencies were not blocked significantly by lamotrigine. In contrast, 10 μM of lamotrigine already depressed burst firing produced by either glutamate or K^+ (Lees and Leach, 1993). Sustained, repetitive firing produced by depolarizing pulses in mouse spinal cord cultured neurons were also blocked by lamotrigine in a concentration- (IC_{50} = 20 μM), voltage-, and use-dependent manner (i.e., lamotrigine had no effect on the first action potential elicited by a depolarizing step but reduced firing of subsequent action potentials) (Cheung et al., 1992). In mouse neuroblastoma cells, Lang and coworkers (1991, 1993) found that at 100 μM, lamotrigine as well as phenytoin and carbamazepine produced a use-dependent inhibition of Na^+ channels, even at low-frequency stimulation, shifting the voltage dependency of steady state inactivation toward more negative potentials by 7 to 15 mV, and slowing the rate of recovery from inactivation. Weak actions on Ca^{2+} and K^+ conductance by high concentrations of lamotrigine were also reported but remained inadequately characterized (Lang and Wang, 1991; Lees and Leach, 1993).

In spite of the conflicting finding of Lang and coworkers (i.e., inhibition of Na^+ conductance at *low* frequency stimulation), possibly resulting from different experimental procedures and cell types, all these studies indicated that the primary action of lamotrigine is use-dependent inhibition of Na^+ conductance, presumably by stabilization of the channel inactivation state. Whole-cell voltage clamp recordings of recombinant rat brain type IIA Na^+ channels expressed in CHO cells have recently suggested that BW619C89 has similar actions and may be more potent than lamotrigine (Xie et al., 1994a; Xie and Garthwaite, 1995). Note that the displacement of BTX-B by lamotrigine required concentrations approximately 5 times higher than those for functional inhibition of neurotransmitter release (see above, section 1.) and burst firing. This apparent discrepancy is consistent with use-dependent action because the neurotoxin site 2 is highly sensitive to the conformational state of the Na^+ channel protein.

3. Neuroprotective actions. In comparison to its effectiveness against seizures, lamotrigine has only modest neuroprotective potency against ischemia. In the gerbil model of global ischemia, high doses of lamotrigine (30 to 50 mg kg^{-1} p.o.; i.e., 6 times the anticonvulsant ED_{50}) given 2 h before and immediately after 5 min of carotid occlusion, provided some protection against hippocampal CA1 damage (Beek, personal communication to Leach et al., 1991). This observation was substantiated by Wiard and coworkers (1995): 100 mg kg^{-1}, p.o., administered immediately after 5 to 15 min bilateral carotid occlusion, or in two equal doses of 30 to 50 mg kg^{-1} 2 h before and immediately after reperfusion, protected gerbils against behavioural deficits and greatly reduced hippocampal damage. With permanent MCA occlusion in rats, when lamotrigine was administered i.v. over 10 min immediately after ischemia onset, the optimum dose (20 mg kg^{-1}) reduced the volume of total infarct by 31% and cortical infarct volume by 52% (Smith and Meldrum, 1995). At 50 mg kg^{-1}, lamotrigine was no longer cerebroprotective, presumably because arterial blood pressure was significantly reduced. The optimal dose of 20 mg kg^{-1} remained protective when administered with 1-h delay after MCA occlusion but was ineffective when this delay was extended to 2 h (Smith and Meldrum, 1995; see also Rataud et al., 1994).

The protective effects of BW1003C87 and BW619C89 have been studied extensively in models of global (Meldrum et al., 1992; Lekieffre and Meldrum, 1993; Gilland et al., 1994) and focal ischemia (Leach et al., 1993; Graham et al., 1993, 1994b), and both analogues appear to be more potent neuroprotectors than lamotrigine. For example, at 20 mg kg^{-1} (i.v., 5 min after MCA occlusion), BW619C89 reduced total infarct volume by 57% and protected even the basal ganglia, a region reputedly refractory to protection in this model (Leach et al., 1993). In the optic nerve preparation, BW619C89 (1 to 100 μM) dose-dependently prevented the axonopathy induced by oxygen and glucose deprivation, without impairing axonal conduction (Garthwaite et al., 1995). In addition to their *anti-ischemic* effects, BW1003C87 (10 mg kg^{-1}, i.v.) and BW619C89 (30 mg kg^{-1}, i.v.) protected against brain injury induced in rats by lateral fluid percussion, reducing regional edema, astrocytic activation, neuronal loss and neurological deficit (Sun and Faden, 1995; Okiyama et al., 1995). Lamotrigine and BW1003C87 were also found to protect against excitotoxic lesions induced in the rat striatum by kainate (McGeer and Zhu, 1990; Moncada et al., 1994). Here, it is relevant to mention that the mechanisms of excitotoxin-induced neuronal damage also includes a Na^+-Cl^- component (Rothman, 1985; Dessi et al., 1994), whose deleterious action may be reduced and/or better tolerated when further Na^+ influx through voltage-gated Na^+ channels is inhibited. Finally, as one could expect, BW1003C87 reduced veratridine toxicity but failed to protect against depolarization with KCl (Lustig et al., 1992).

4. Reduction of ischemia-induced glutamate efflux and neuroprotection: a critical appraisal. The reduction of ischemia-induced glutamate efflux in the extracellular fluid by lamotrigine and its analogues is often considered as causative of neuroprotection (Lekieffre and Meldrum, 1993; Graham et al., 1993, 1994a, b), but this prevalent interpretation conflicts with key findings (Obrenovitch and Urenjak, 1994; Obrenovitch and Richards, 1995):

- The action of these drugs on high extracellular levels of glutamate during ischemia is not specific;

high extracellular aspartate, GABA, glycine and taurine are reduced to a similar extent (Lekieffre and Meldrum, 1993; Graham et al., 1993, 1994a). Presumably, the unspecific reduction in neurotransmitter efflux is due to these compounds reducing the severity of ischemia through their interaction with Na^+ channels (see above, section III.).

- BW1003C87 reduced hippocampal CA1 lesions even when administered up to 2 h after forebrain ischemia, i.e., long after complete return of extracellular glutamate to normal levels (Lekieffre and Meldrum, 1993; Ueda et al., 1992a, b; Mitani et al., 1990b).
- BW1003C87 reduced extracellular glutamate levels in both cortex and caudate during MCA occlusion in rats, but only the cortex was protected (Graham et al., 1993). Similarly, the reduction in microdialysate glutamate concentration by lamotrigine did not relate to cerebroprotection in the striatum (Graham et al., 1994b).

In addition, it is becoming increasingly clear that increased extracellular glutamate levels may not be the key to excitotoxicity in ischemia (Hossmann, 1994a, b; Obrenovitch and Richards, 1995). Apart from increased extracellular glutamate, the exceptional complexity of glutamate-operated ion channels can give rise to many potentially damaging mechanisms (Obrenovitch and Richards, 1995). It is also established that most of glutamate released in ischemia is of metabolic origin, which questions the validity of therapeutic strategies aimed at preventing or reducing excessive release of *neurotransmitter* glutamate in ischemia (Wahl et al., 1994; Obrenovitch and Richards, 1995; Obrenovitch, 1995b).

This analysis strongly suggests that the cerebroprotective actions of lamotrigine and structural analogues are a *direct* consequence of use-dependent down-modulation of voltage-gated Na^+ channels, and this mechanism of action should be recognized as such. Reduction of ischemia-induced neurotransmitter efflux by putative neuroprotective agents does not imply that the former is causative. Testing whether glutamate neuroprotection correlates with reduced glutamate efflux during ischemia is clearly beset by the difficulty of separating cause from effect (Obrenovitch and Richards, 1995).

C. Riluzole

The benzothiazole derivative riluzole (2-amino-6-trifluoromethoxybenzothiazole, PK 26124, RP 54274) was first described as an anticonvulsant because it protected animals against convulsions induced by maximal electroshock, inhibitors of GABA synthesis, glutamate and kainate (Mizoule et al., 1985). Subsequently, it was reported to also exhibit hypnotic (Stutzmann et al., 1988), anxiolytic (Stutzmann et al., 1989), anesthetic (Mantz et al., 1992), and anti-ischemic properties.

1. Neuroprotective actions of riluzole in ischemia. Repeated administration of riluzole, starting before or shortly after ischemia onset and pursued for up to several days, was effective in a number of experimental models. For example, repeated doses of riluzole (4 to 8 mg kg^{-1}, i.p.) significantly reduced degeneration of hippocampal CA1 pyramidal cells, prevented memory loss (Malgouris et al., 1989) and improved the EEG (Pratt et al., 1992) in gerbils subjected to transient bilateral carotid artery occlusion. In the rat MCA occlusion model, riluzole reduced the volume of infarcted cortex (Pratt et al., 1992; Wahl et al., 1993; Rataud et al., 1994) with an efficacy similar to that of lamotrigine and carbamazepine (Rataud et al., 1994). However, it failed to improve neurological and memory deficits in this model (Wahl et al., 1993) and, as other drugs, did not reduce the striatal lesion (Pratt et al., 1992). Despite the inhibitory action of riluzole on various responses evoked in vitro by excitatory amino acids (see below), riluzole was found ineffective against lesions produced by direct intracerebral injection of quinolinic or kainic acid (Doble et al., 1993).

2. Actions on amino acid neurotransmitters. The actions of riluzole against seizures and ischemia-induced brain damage are often attributed to an *antiglutamate action* because of unusual effects on glutamatergic transmission (Malgouris et al., 1989; Pratt et al., 1992; Wahl et al., 1993; Stutzmann et al., 1993; Rataud et al., 1994). Riluzole inhibited various responses evoked by excitatory amino acids (Benavides et al., 1985; Drejer et al., 1986; Hubert and Doble, 1989; Debono et al., 1993; Malgouris et al., 1994; Hubert et al., 1994), but without binding to AMPA, kainate or NMDA receptors (Benavides et al., 1985; Hays et al., 1991b; Doble et al., 1993), nor to any of the known NMDA-receptor modulatory sites (Hays et al., 1991b). It is interesting to note that riluzole could no longer block responses to excitatory amino acids when cultured neurons were pretreated with pertussis toxin (pertussis toxin reduces the level of the activated form of pertussis toxin-sensitive G proteins; Gilman, 1987), which suggested an action of riluzole on G proteins-dependent processes (Hubert et al., 1994). In addition, riluzole was shown to reduce spontaneous glutamate release and to increase the size of its K^+-releasable pool (Chéramy et al., 1992), although this study did not rule out the possibility that riluzole may have similar effects on other neurotransmitters. Finally, Mantz and coworkers (1994) have suggested that riluzole may promote GABA accumulation in the synaptic cleft because the compound dose-dependently inhibited GABA uptake by striatal synaptosomes (IC_{50} of 43 μM; versus 3.6 μM for nipecotic acid, a classical blocker of GABA uptake).

3. Modulation of Na^+ currents. Besides this unclear action of riluzole on glutamatergic transmission, a strong body of evidence has indicated that this drug acts on Na^+ channels at *therapeutic* concentrations. Riluzole was reported to displace BTX-B in binding studies and

to inhibit veratridine-induced acetylcholine release from rat striatal slices (Hays et al., 1991b). In a Na^+ flux assay using rat cortical slices, riluzole was the most potent in a series of 32 substituted 2-benzothiazolamines inhibiting [^{14}C]guanidine uptake with an IC_{50} of 4.1 μM (versus 23 μM for phenytoin) (Hays et al., 1994). It also inhibited veratridine-induced (but not K^+-induced) increase of intracellular Ca^{2+} in cultured rat cerebellar granule neurons with an IC_{50} of 0.31 μM (Hubert et al., 1994), and veratridine neurotoxicity as measured by LDH release from immature rat hippocampal slices (Malgouris et al., 1994), although a single high (100 μM) concentration of riluzole was used in the latter study. Voltage-clamp studies clearly confirmed the interaction of riluzole with voltage-gated Na^+ channels: (*a*) in isolated myelinated nerve fibres of the frog, riluzole was a highly specific blocker of inactivated Na^+ channels, 300 times more effective on these channels than on K^+ or resting Na^+ channels (Benoit and Escande, 1991); (*b*) in cultured neurons, riluzole (1 to 30 μM) produced a 5 to 30 mV negative shift of the Na^+ current steady state inactivation curve, with modest effects on Na^+ channel activation and recovery from inactivation—inhibition of Na^+ currents was frequency-dependent only at activation frequencies exceeding 30 Hz (Randle et al., 1994); and (*c*) with these effects were confirmed with rat brain IIA Na^+ channel α-subunit expressed in *Xenopus* oocytes (Hebert et al., 1994).

RP 66055 (3-{2-[4-(4-fluorophenyl)-1-piperazinyl]-ethyl}-2-imino-6-trifluoromethoxy-benzothiazoline), a riluzole derivative, is also a potent neuroprotective agent in rodent models of hypoxia and ischemia with anticonvulsant properties (Stutzmann et al., 1993; Rataud et al., 1994). So far, this compound is described as a Na^+ channel blocker, because binding assays only revealed an affinity for voltage-gated Na^+ channels (Stutzmann et al., 1993; Rataud et al., 1994).

D. Miscellaneous Neuroprotective Agents Acting on Na^+ Channels

1. PD85,639. PD85,639 (*N*-[3-(2,6-dimethyl-1-piperidinyl)propyl]-α-phenylbenzeneacetamide) belongs to a novel series of phenylacetamides structurally related to both local anesthetics and phenytoin (fig. 10) (Thomsen et al., 1993; Roufos et al., 1994). Preliminary in vitro biological assays have suggested that this compound may have some neuroprotective potential, as it inhibited hypoxia-induced LDH release from cultured rat brain neurons with an IC_{50} of 89 μM (versus 7.3 μM for flunarizine) (Roufos et al., 1994). PD85,639 interacts strongly with voltage-gated Na^+ channels, especially with the neurotoxin binding site 2: (*a*) it displaced BTX-B binding to rat neocortical membranes with a K_i of 0.26 μM (versus 0.053 μM for flunarizine, and 0.077 μM for lidoflazine in the same test) (Roufos et al., 1994); (*b*) PD85,639 inhibited veratridine-stimulated influx of [^{14}C]guanidine into rat neocortical slices with an IC_{50} of 2 nM, with an inhibition curve featuring both high (nM) and low (μM) affinity (Hays et al., 1991a); (*c*) it blocked veratridine-stimulated Na^+ influx into CHO cells expressing rat brain type IIA Na^+ channel with an IC_{50} of 2.7 μM (TTX, 0.014 μM; flunarizine, 0.42 μM; lidoflazine, 2.4 μM) (Roufos et al., 1994); and (*d*) PD85,639 inhibited veratridine induced LDH release in rat brain neuronal cell cultures with an IC_{50} of 5 μM (flunarizine 2 μM) (Roufos et al., 1994).

Thompsen and coworkers (1993) have demonstrated that PD85,639 binds directly and specifically to reconstituted Na^+ channels and type IIA Na^+ channels α-subunits expressed in transfected CHO cells. Binding of PD85,639 to these preparations was inhibited by several tertiary amine local anesthetics at concentrations in the same range as those that inhibits Na^+ channels. Furthermore, veratridine and BTX inhibited completely specific PD85,639 binding. Thus, PD85,639 appears to bind specifically, and with high affinity, to the local anesthetic receptor that is allosterically linked to neurotoxin receptor site 2 (see section II.B.3.; fig. 3).

As it could be expected from the structural analogies between PD85,639, lidocaine and tetracaine (Ragsdale et al., 1993), as well as from the data outlined above, voltage-clamp recordings from CHO cells expressing brain type IIA Na^+ channel and dissociated rat brain neurons have confirmed that PD85,639 strongly attenuated Na^+ currents when applied either in the external bath or in the internal pipette solution, with properties close to those of local anesthetics (Ragsdale et al., 1993). The most striking effect of PD85,639 was its pronounced use-dependent block of Na^+ currents, which was detectable at much lower frequencies than those required for use-dependent block with tertiary amine local anesthetics, and far below those that are physiologically relevant in cardiac cells or brain neurons. In addition, tonic block with PD85,639 was not strongly dependent on holding potential. In theory, these properties are not favourable to antiarrhythmic and anticonvulsant therapy for which the strategy is to induce a more potent block at the more depolarized membrane potentials, and to filter out abnormal high frequency depolarizations with nominal effect on normal activity. Nevertheless, it would be interesting to explore further the neuroprotective action of PD85,639 in stroke models.

2. Vinpocetine. Vinpocetine [(3α,16α)-eburnamenine-14-carboxylic acid ethyl ester], a synthetic ethyl ester of apovincamine, is beneficial in a series of cerebrovascular diseases that are accompanied by hypoxia and ischemia (Werner et al., 1986; Otomo et al., 1985; Hadjiev and Yancheva, 1976). It is already marketed as a nootropic peripheral vasodilator for treatment of senile dementia in a number of countries, including Japan and Portugal. It is awaiting Food and Drug Administration approval in the United States. In experimental animal models, vinpocetine increased the tolerance to anoxia (King, 1987; Biró et al., 1976), reduced delayed neuronal death subsequent to global cerebral ischemia (Sauer et al., 1988;

Rischke and Krieglstein, 1990; Araki et al., 1990) and reduced the infarct size in stroke models (Rischke and Krieglstein, 1990; Karkoutly et al., 1990). For example, vinpocetine increased dose-dependently the number of mice surviving 80 s of exposure to pure nitrogen gas (i.e., a lethal insult in control animals) with 100% survival at 50 mg kg^{-1} (i.p.) (King, 1987). In global ischemia, administration of vinpocetine (10 mg kg^{-1}, i.p.) 15 min before or immediately after the insult reduced delayed neuronal death in the rat CA1 subfield (Sauer et al., 1988; Rischke and Krieglstein, 1990). Doubling this dose became ineffective however, presumably because arterial blood pressure was lowered (Rischke and Krieglstein, 1990). With MCA occlusion in mice and rats, 10 mg kg^{-1} (i.p.) reduced significantly the volume of cortical infarct (Rischke and Krieglstein, 1990; Karkoutly et al., 1990). Finally, vinpocetine showed anticonvulsant activity in mice, preventing maximal electroshock (IC_{50} = 18.3 mg kg^{-1}, i.p.); metrazol induced convulsions (IC_{50} = 62.1 mg kg^{-1}, i.p.) (Pálosi and Szporny, 1976).

In vitro, vinpocetine potentiated the neuroprotective effect of adenosine against chemical hypoxia (1 mM NaCN) in primary neuronal cultures from chick embryo cerebral hemispheres, suggesting that the neuroprotective effect of vinpocetine may be mediated by adenosine (Krieglstein and Rischke, 1991). In contrast, up to 100 μmol l^{-1} of this drug did not prevent protein and ATP loss in neuroblastoma cells during cytotoxic hypoxia (1 mM NaCN for 30 min) superimposed on glucose deprivation (Peruche and Krieglstein, 1991)

The cerebroprotective mechanism of vinpocintine remains unresolved. It may result partly from cerebrovascular effects in some models (e.g., improvement of postischemic local cerebral blood flow) (Béncsáth et al., 1976), but a direct action on brain parenchyma seems also plausible (Rischke and Krieglstein, 1990). Attempts to identify the molecular target(s) of vinpocetine have shown a wide range of effects, including: (*a*) Ca^{2+} antagonistic activity at 100 μM but not 10 μM (Kaneko et al., 1990); (*b*) interactions with glutamate receptors (e.g., at approximately 100 μM, vinpocetine displaced [^{3}H]MK-801 from guinea pig forebrain membranes) (Kaneko et al., 1991; Kiss et al., 1991); and (*c*) protection against excitotoxins (Miyamoto et al., 1989; Erdö et al., 1990; Kiss et al., 1991).

However, all these actions only occurred at high concentrations and remained weak or incomplete to such an extent that none of them has been accepted as the primary origin of the neuroprotective effects of the drug. A recent study by Lakics and coworkers (1995) strongly suggested that voltage-gated Na^+ channels may be the predominant target of vinpocetine at therapeutic concentrations. Vinpocetine inhibited veratridine-mediated cell death in primary cultures of rat cerebral cortex in a concentration-dependent and complete manner; in contrast to the high doses required for the actions outlined above, IC_{50} against 100 μM and 50 μM veratridine (i.e., maximal and half-maximal toxicity) toxicity were 490 nM and 63 nM, respectively (Lakics et al., 1995). This strong protective effect of vinpocetine against veratridine-induced cell death exceeded by two orders of magnitude the potency of phenytoin (Lakics et al., 1995), despite the equipotency of these two drugs in blocking voltage-gated Na^+ channels in rat cortical neurons (Molnár and Erdö, 1995). In an earlier study, 10 μM vinpocetine inhibited veratridine-induced purine release from rat hypothalamic synaptosomes (Fredholm et al., 1983).

VI. Concluding Remarks

As it is apparent from this analysis, a wide array of evidence suggests that selective down-modulation of voltage-gated Na^+ channels is a rational and effective strategy against ischemic brain damage. It is therefore surprising that among all the neuroprotectors interacting with Na^+ channels at therapeutic concentrations, only lifarizine is promoted unambiguously as acting in this way (see section V.A.2.). Despite their strong interactions with Na^+ channels, the favored action mechanism advanced for the prominent compounds BW1003C87, BW619C89 and riluzole remains inhibition of glutamate release (see section V.B. and C.) and, more recently, secondary inhibition of the synthesis of nitric oxide (Lizasoain et al., 1995). Why does Na^+ channel modulation fail to be recognized as a valid action mechanism against ischemic damage? One possibility may be the concern of potential side effects of Na^+ channel blockers, examplified by the cardiotoxicity of lidocaine. However, it is now clear that neuroprotection can be achieved by *selective* down-modulation of Na^+ channels, without conspicuous undesirable effects on the normal function of brain and heart. Another possibility may be the investigators' tendency to favour action mechanisms related to novel and exciting hypotheses. Over the last 20 years, several classes of drugs have successively been the focus of attention, including cerebral vasodilators, voltage-sensitive Ca^{2+}-blockers, and free-radical scavengers. At present, a large number of compounds under development are either glutamate receptor antagonists or so-called glutamate release inhibitors (Muir and Lees, 1995), and there is little doubt that the next trend will be selective nitric oxide-synthase inhibitors and *anti-apoptosis* drugs.

Selective down-modulation of voltage-gated Na^+ channels should be recognized, not only as a valid approach to cerebroprotection, but also as a timely one. Firstly, because our understanding of the structure and function of voltage-gated Na^+ channels has improved to a point where it should allow development of site-directed Na^+ channel blockers. Secondly, because selective modulation of voltage-gated Na^+ channels, in addition to ischemia, may be relevant to a wide range of neurological disorders. For example, riluzole was recently reported to slow the progression of amyotrophic lateral sclerosis, a progressive motor neuron disease for

which there is no adequate treatment (Bensimon et al., 1994) (See however Rowland, 1994; and the related correspondence in N. Engl. J. Med. **331:** 272–274, 1994) (Note also that in the study of Eisen and coworkers, 1993, 100 mg oral daily lamotrigine did not alter the course of amyotrophic lateral sclerosis). Here, again, riluzole was presented as an *antiglutamate agent* (Bensimon et al., 1994; Couratier et al., 1994), but the recent discovery of axonal ion channel dysfunction in amyotrophic lateral sclerosis patients (Bostock et al., 1995) strongly suggests that the beneficial effects of riluzole against this disorder may rather result from its direct action on voltage-gated Na^+ channels. Another relevant finding is that of Takigawa and coworkers (1995), who showed that antibodies against GM_1 gangliosides, which are found in patients with lower motor neuron disease, motor neuropathy and Guillain-Barré syndrome, can alter voltage-gated Na^+ currents, suggesting that Na^+ channels may be targets of immune attack in some neurological disorders (Waxman, 1995). In the study of Takigawa and coworkers, Na^+ currents were suppressed by antibodies against GM_1 gangliosides, but immune effects on Na^+ channels could also render neurons hyperexcitable by interfering with deactivation or inactivation (Waxman, 1995).

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REFERENCES

ABDEL-LATIF, A. A., BRODY, J., AND RAMAHI, H.: Studies on sodium-potassium adenosine triphosphate of the nerve endings and appearance of electrical activity in developing rat brain. J. Neurochem. **14:** 1133–1141, 1967.

ABE, K., KOGURE, K., YAMAMOTO, H., IMAZAWA, M., AND MIYAMOTO, K.: Mechanism of arachidonic acid liberation during ischemia in gerbil cerebral cortex. J. Neurochem. **48:** 503–509, 1987.

ABITA, J. P., CHICHEPORTICHE, R., SCHWEITZ, H., AND LAZDUNSKI, M.: Effects of neurotoxins (veratridine, sea anemone toxin, tetrodotoxin) on transmitter accumulation and release by nerve terminals in vitro. Biochemistry **16:** 1838–1844, 1977.

ADAMS, M. E., AND OLIVERA, M. B.: Neurotoxins: overview of an emerging research technology. Trends Neurosci. **17:** 151–155, 1994.

ADAM-VIZI, V., AND LIGETI, E.: Calcium uptake of rat brain synaptosomes as a function of membrane potential under different depolarizing conditions. J. Physiol. (Lond.) **372:** 363–377, 1986.

AHMAD, S., FOWLER, L. J., LEACH, M. J., AND WHITTON, P. S.: Lamotrigine alters veratridine- but not K^+-evoked amino acid release in the ventral hippocampus of the rat in vivo. Br. J. Pharmacol. (suppl.) **115:** 116P, 1995.

AHMED, C. M. I., WARE, D. H., LEE, S. C., PATTEN, C. D., FERRER-MONTIEL, A. V., SCHINDER, A. F., MCPHERSON, J. D., WAGNER-MCPHERSON, C. B., WASMUTH, J. J., EVANS, G. A., AND MONTAL, M.: Primary structure, chromosomal localization, and functional expression of a voltage-gated sodium channel from human brain. Proc. Natl. Acad. Sci. USA **89:** 8220–8224, 1992.

AKAIKE, N., ISHIBASHI, H., HARA, H., OYAMA, Y., AND UEHA, T.: Effect of KB-2796, a new diphenylpiperazine Ca^{2+} antagonist, on voltage-dependent Ca^{2+} currents and oxidative metabolism in dissociated mammalian CNS neurons. Brain Res. **619:** 263–270, 1993.

ALDRETE, J. A., ROMO-SALAS, F., JANKOVSKY, L., AND FRANATOVIC, Y.: Effect of pretreatment with thiopental and phenytoin on postischemic brain damage in rabbits. Crit. Care Med. **7:** 466–470, 1979.

ALPS, B. J.: Drugs acting on calcium channels: potential treatment for ischaemic stroke. Br. J. Clin. Pharmacol. **34:** 199–206, 1992.

ALPS, B. J., CALDER, C., HASS, W. K., AND WILSON, A. D.: Comparative protective effects of nicardipine, flunarizine, lidoflazine and nimodipine against ischaemic injury in the hippocampus of the Mongolian gerbil. Br. J. Pharmacol. **93:** 877–883, 1988.

ALPS, B. J., CALDER, C., WILSON, A. D., MCBEAN, D. E., AND ARMSTRONG, J. M.: Reduction by lifarizine of the neuronal damage induced by cerebral ischaemia in rodents. Br. J. Pharmacol. **115:** 1439–1446, 1995.

ALPS, B., CALDER, C., WILSON, A., AND PASCAL, J.-C.: Cerebral protection with a novel calcium blocker in rats. *In* Proceedings of the XXth International Congress of Medicine, p. C17, ISIM Satellite, Stockholm, Sweden, 1990.

ALTMAN, D. I., PERLMAN, J. M., VOLPE, J. J., AND POWERS, W. J.: Cerebral oxygen metabolism in newborns. J. Pediatr. **92:** 99–104, 1993.

AMES, A., III, LI, Y.-Y., HEHER, E. C., AND KIMBLE, C. R.: Energy metabolism of rabbit retina as related to function: high cost of Na^+ transport. J. Neurosci. **12:** 840–853, 1992.

ANDINÉ, P., JACOBSON, I., AND HAGBERG, H.: Calcium uptake evoked by electrical stimulation is enhanced postischemically and precedes delayed neuronal death in CA1 of rat hippocampus: involvement of N-methyl-D-aspartate receptors. J. Cereb. Blood Flow Metab. **8:** 799–807, 1988.

ANIKSZTEJN, L., AND BEN-ARI, Y.: Novel form of long-term potentiation produced by a K^+ channel blocker in the hippocampus. Nature (Lond.) **349:** 67–69, 1991.

ARAKI, T., KOGURE, K., AND NISHIOKA, K.: Comparative neuroprotective effects of pentobarbital, vinpocetine, flunarizine and ifenprodil on ischemic neuronal damage in the gerbil hippocampus. Res. Exp. Med. **190:** 19–23, 1990.

ARMSTRONG, C. M.: Sodium channels and gating currents. Physiol. Rev. **61:** 644–682, 1981.

ARMSTRONG, C. M., AND BEZANILLA, F.: Currents related to movement of the gating particles of the sodium channels. Nature (Lond.) **242:** 459–461, 1973.

ARMSTRONG, C. M., BEZANILLA, F., AND ROJAS, E.: Destruction of sodium conductance inactivation in squid axons perfused with pronase. J. Gen. Physiol. **62:** 375–391, 1973.

ARONSON, P. S.: Kinetic properties of the plasma membrane Na^+-H^+ exchanger. Annu. Rev. Physiol. **47:** 545–560, 1985.

ARTRU, A. A., AND MICHENFELDER, J. D.: Cerebral protective, metabolic, and vascular effects of phenytoin. Stroke **11:** 377–382, 1980.

ARTRU, A. A., AND MICHENFELDER, J. D.: Anoxic cerebral potassium accumulation reduced by phenytoin: mechanism of cerebral protection? Anesth. Analg. **60:** 41–45, 1981.

ARTRU, F., TERRIER, A., TIXIER, S., JOURDAN, C., AND DELEUZE, R.: Use of intravenous lidocaine in neuro-anesthesia and neuro-resuscitation. Agressologie **32:** 439–443, 1991.

ARUOMA, O. I., SMITH, C., CECCHINI, R., EVANS, P. J., AND HALLIWELL, B.: Free radical scavenging and inhibition of lipid peroxidation by beta-blockers that interfere with calcium metabolism. A physiologically significant process? Biochem. Pharmacol. **42:** 735–743, 1991.

ASHTON, D., WILLEMS, R., MARRANNES, R., AND JANSSEN, P. A. J.: Extracellular ions during veratridine-induced neurotoxicity in hippocampal slices: neuroprotective effects of flunarizine and tetrodotoxin. Brain Res. **528:** 212–222, 1990.

ASTRUP, J.: Energy-requiring cell functions in the ischemic brain. Their critical supply and possible inhibition in protective therapy. J. Neurosurg. **56:** 482–497, 1982.

ASTRUP, J., BLENNOW, G., AND NILSSON, B.: Effects of reduced cerebral blood flow upon EEG pattern, cerebral extracellular potassium, and energy metabolism in the rat cortex during bicuculline-induced seizures. Brain Res. **177:** 115–126, 1979.

ASTRUP, J., MØLLER SØRENSEN, P., AND RHABEK SØRENSEN, H.: Inhibition of cerebral oxygen and glucose consumption in the dog by hypothermia, pentobarbital, and lidocaine. Anesthesiology **55:** 263–268, 1981a.

ASTRUP, J., MØLLER SØRENSEN, P., AND RHABEK SØRENSEN, H.: Oxygen and glucose consumption related to Na^+-K^+ transport in canine brain. Stroke **12:** 726–730, 1981b.

ASTRUP, J., AND NORBERG, K.: Potassium activity in cerebral cortex in rats during progressive severe hypoglycemia. Brain Res. **103:** 418–423, 1976.

ASTRUP, J., REHNCRONA, S., AND SIESJÖ, B. K.: The increase in extracellular potassium concentration in the ischemic brain in relation to the preischemic functional activity and cerebral metabolic rate. Brain Res. **199:** 161–174, 1980.

ASTRUP, J., SKØVSTED, P., GJERRIS, F. AND SØRENSEN, H. R.: Increase in extracellular potassium in the brain during circulatory arrest: effects of hypothermia, lidocaine and thiopental. Anesthesiology **55:** 256–262, 1981c.

ASTRUP, J., AND SØRENSEN, H. R.: Inhibition of cerebral metabolism by lidocaine. Eur. Neurol. **20:** 221–224, 1981.

ATTERWILL, C. K., CUNNINGHAM, V. J., AND BALAZS, R.: Characterization of Na^+, K^+-ATPase in cultured and separated neuronal and glial cells from rat cerebellum. J. Neurochem. **43:** 8–18, 1984.

AULD, V. J., GOLDIN, A. L., KRAFTE, D. S., CATTERALL, W. A., LESTER, H. A., DAVISON, N., AND DUNN, R. J.: A neutral amino acid change in segment IIS4 dramatically alters the gating properties of the voltage-dependent sodium channel. Proc. Natl. Acad. Sci. USA **87:** 323–327, 1990.

AULD, V. J., GOLDIN, A. L., KRAFTE, D. S., MARSHALL, J, DUNN, J. M., CATTERALL, W. A., LESTER, H. A., DAVIDSON, N., DUNN, R. J.: A rat brain Na^+ channel α subunit with novel gating properties. Neuron **1:** 449–461, 1988.

AVELDANO, M. I., AND BAZAN, N. G.: Differential lipid deacylation during brain ischemia in a homeotherm and a poikilotherm. Content and composition of free fatty acids and triacylglycerols. Brain Res. **100:** 99–110, 1975.

BACKX, P. H., YUE, D. T., LAWRENCE, J. H., MARBAN, E., AND TOMASELLI, G. F.: Molecular localization of an ion-binding site within the pore of mammalian sodium channels. Science (Wash. DC) **257:** 248–251, 1992.
BADEN, D.: Breve toxins: unique polyether dinoflagellate toxins. FASEB J. **3:** 1807–1817, 1989.
BALDY-MOULINIER, M.: Cerebral blood flow and membrane ionic pump. Eur. Neurol. **6:** 107–113, 1971.
BALESTRINO, M., AITKEN, P. G., AND SOMJEN, G. G.: Spreading depression-like hypoxic depolarization in CA1 and fascia dentata of hippocampal slices: relationship to selective vulnerability. Brain Res. **497:** 102–107, 1989.
BAMFORD, J., SANDERCOCK, P., JONES, L., AND WARLOW, C.: The natural history of lacunar infarction: the Oxfordshire community stroke project. Stroke **18:** 545–551, 1987.
BANNAN, P. E., GRAHAM, D. I., LEES, K. R., AND MCCULLOCH, J.: Neuroprotective effect of remacemide hydrochloride in focal cerebral ischemia in the cat. Brain Res. **664:** 271–275, 1994.
BARCHI, R. L.: Probing the molecular structure of the voltage-dependent sodium channel. Annu. Rev. Neurosci. **11:** 455–495, 1988.
BEAL, M. F.: Does impairment of energy metabolism result in excitotoxic neuronal death in neurodegenerative illnesses? Ann. Neurol. **31:** 119–130, 1992.
BELKIN, D. A.: Anoxia: tolerance in reptiles. Science (Wash. DC) **133:** 492–493, 1963.
BENAVIDES, J., CAMELIN, J. C., MITRANI, N., FLAMAND, F., UZAN, A., LEGRAND, J.-J., GUEREMY, C., AND LE FUR, G.: 2-amino-6-trifluoromethoxy benzothiazole, a possible antagonist of excitatory amino acid neurotransmission. II. Biochemical properties. Neuropharmacology **24:** 1085–1092, 1985.
BENCSATH, L., DEBRECZENI, L., AND TAKACS, L.: Effect of ethyl apovincaminate on cerebral circulation of dogs under normal conditions and in arterial hypoxia. Arzneimittelforschung **26:** 1920–1926, 1976.
BALESTRINO, M., AITKEN, P. G., AND SOMJEN, G. G.: Spreading depression-like hypoxic depolarization in CA1 and fascia dentata of hippocampal slices: relationship to selective vulnerability. Brain Res. **497:** 102–107, 1989.
BENDO, A. A., KASS, I. S., AND COTTRELL, J. E.: Anesthetic protection against anoxic damage in the rat hippocampal slice. Brain Res. **403:** 136–141, 1987.
BENOIT, E., AND ESCANDE, D.: Riluzole specifically blocks inactivated Na channels in myelinated nerve fibre. Pflueg. Arch. Eur. J. Physiol. **419:** 603–609, 1991.
BENSIMON, G., LACOMBLEZ, L., MEININGER, V., AND THE ALS/RILUZOLE STUDY GROUP: A controlled trial of riluzole in amyotrophic lateral sclerosis. N. Engl. J. Med. **330:** 585–591, 1994.
BHATNAGAR, A., SRIVASTAVA, S. K., AND SZABO, G.: Oxidative stress alters specific membrane currents in isolated cardiac myocytes. Circ. Res. **67:** 535–549, 1990.
BICKLER, P. E., AND GALLEGO, S. M.: Inhibition of brain calcium channels by plasma proteins from anoxic turtles. Am. J. Physiol. **265**(2 Pt 2): R277–R281, 1993.
BIELENBERG, G. W., HAYN, C., AND KRIEGLSTEIN, J.: Effects of cerebro-protecive agents on enzyme activities of rat primary glial cultures and rat cerebral cortex. Biochem. Pharmacol. **35:** 2693–2702, 1986.
BIRO, K., KARPATI, E., AND SZPORNY, L.: Protective activity of ethyl apovincaminate on ischaemic anoxia of the brain. Arzneimittelforschung **26:** 1918–1929, 1976.
BLAUSTEIN, M. P.: Calcium transport and buffering in neurons. Trends Neurosci. **11:** 438–443, 1988.
BLAUSTEIN, M. P., AND OBORN, C. J.: The influence of sodium on calcium fluxes in pinched-off nerve terminals in vitro. J. Physiol. (Lond.) **247:** 657–686, 1975.
BLISS, T. V. P., AND COLLINGRIDGE, G. L.: A synaptic model of memory: long-term potentiation in the hippocampus. Nature (Lond.) **361:** 31–39, 1993.
BODDEKE, H. W. G. M., HEYNIS, J. B., JONKMAN, F. A. M., VAN ZWIETEN, P. A.: Protective activity of R58735 upon ouabain intoxication in anaesthetized guinea-pigs. Br. J. Pharmacol. **89**(suppl.): 597P, 1986.
BOENING, J. A., KASS, I. S., COTTRELL, J. E., AND CHAMBERS, G.: The effect of blocking sodium influx on anoxic damage in the rat hippocampal slice. Neuroscience **33:** 263–268, 1989.
BORTOLOTTO, Z. A., AND COLLINGRIDGE, G. L.: Characterisation of LTP induced by the activation of glutamate metabotropic receptors in area CA_1 of the hippocampus. Neuropharmacology **32:** 1–9, 1993.
BOSTOCK, H., SHARIEF, M. K., RIED, G., AND MURRAY, N. M. F.: Axonal ion channel dysfunction in amyotrophic lateral sclerosis. Brain **118:** 217–225, 1995.
BOWLING, A. C., AND DELORENZO, R. J.: Micromolar affinity benzodiazepine receptors: identification and characterization in central nervous system. Science (Wash. DC) **216:** 1247–1250, 1982.
BOXER, P. A., CORDON, J. J., MANN, M. E., RODOLOSI, L. C., VARTANIAN, M. G., ROCK, D. M., TAYLOR, C. P., AND MARCOUX, F. W.: Comparison of phenytoin with noncompetitive N-methyl-D-aspartate antagonists in a model of focal brain ischemia in rat. Stroke **21**(suppl. III): 47–51, 1990.
BRADFORD, H. F., YOUNG, A. M., AND CROWDER, J. M.: Continuous leakage from brain cells is balanced by compensatory high-affinity reuptake transport. Neurosci. Lett. **81:** 296–302, 1987.
BRAIN RESUSCITATION CLINICAL TRIAL I STUDY GROUP: Randomized clinical study of thiopental loading in comatose survivors of cardiac arrest. N. Engl. J. Med. **314:** 397–403, 1986.
BRANSTON, N. M., HOPE, D. T., AND SYMON, L.: Barbiturates in focal ischemia of primate cortex: effects on blood flow distribution, evoked potential, and extracellular potassium. Stroke **10:** 647–653, 1979.
BRANSTON, N. M., LADDS, A., SYMON, L., AND WANG, A. D.: Comparison of the effects of ischaemia on early components of the somatosensory evoked potential in brainstem, thalamus and cerebral cortex. J. Cereb. Blood Flow Metab. **4:** 68–81, 1984.
BRANSTON, N. M., STRONG, A. J., AND SYMON, L.: Extracellular potassium activity, evoked potential and tissue blood flow: relationship during progressive ischaemia in baboon cerebral cortex. J. Neurol. Sci. **32:** 305–321, 1977.
BREMER, A. M., YAMADA, K., AND WEST, C. R.: Ischemic cerebral edema in primates: effects of acetazolamide, phenytoin, sorbital, dexamethasone, and methylprednisolone on brain water and electrolytes. Neurosurgery **6:** 149–154, 1980.
BROWN, C. M., CALDER, C., ALPS, B. J., AND SPEDDING, M.: The effect of lifarizine (RS-87476), a novel sodium and calcium channel modulator, on ischaemic dopamine depletion in the corpus striatum of the gerbil. Br. J. Pharmacol. **109:** 175–177, 1993.
BROWN, C. M., CALDER, C., LINTON, C., SMALL, C., KENNY, B. A., SPEDDING, M., AND PATMORE, L.: Neuroprotective properties of lifarizine compared with those of other agents in a mouse model of focal cerebral ischaemia. Br. J. Pharmacol. **115:** 1425–1432, 1995.
BROWN, C. M., SMALL, C., CALDER, C., AND PATMORE, L.: Neuroprotective properties of lifarizine (RS-87476) in a mouse model of focal ischaemia. Can. J. Physiol. Pharmacol. **72**(suppl. 1): 435, 1994a.
BROWN, G. B., TIESZEN, S. C., DALY, J. W., WARNICK, J. E., AND ALBUQUERQUE, E. X.: Batrachotoxinin-A 20-α-benzoate: a new radioactive ligand for voltage sensitive sodium channels. Cell. Mol. Neurobiol. **1:** 19–40, 1981.
BROWN, N. A., KEMP, J. A., AND SEABROOK, G. R.: Block of human voltage-sensitive Na+ currents in differentiated SH-SY5Y cells by lifarizine. Br. J. Pharmacol. **113:** 600–606, 1994b.
BULLOCK, R: Introducing NMDA antagonists into clinical practice: why head injury trials? Br. J. Clin. Pharmacol. **34:** 396–401, 1992.
BURES, J., AND BURESOVA, O.: Die anoxische Terminaldepolarisation als Indikator der Vulnerabilität der Großhirnrinde bei Anoxie und Ischämie. Pflueg. Arch. Eur. J. Physiol. **264:** 325–344, 1957.
BURES, J., BURESOVA, O., AND KRIVANEK, J.: The Mechanisms and Applications of Leao's Spreading Depression of Electroencephalographic Activity. Academic Press, New York, 1974.
BURNASHEV, N. A., UNDROVINAS, A. I., FLEIDERVISH, I. A., AND ROSENSHTRAUKH, L. V.: Ischemic poison lysophosphatidylcholine modifies heart sodium channels gating inducing long-lasting bursts of openings. Pflueg. Arch. Eur. J. Physiol. **415:** 124–126, 1989.
BUTCHER, S. P., AND HAMBERGER, A.: In vivo studies on the extracellular, and veratrine-releasable, pools of endogenous amino acids in the rat striatum: effects of corticostriatal deafferentation and kainic acid lesion. J. Neurochem. **48:** 713–721, 1987.
BUTTERWORTH, J., COLE, L., AND MARLOW, G.: Inhibition of brain cell excitability by lidocaine, QX314, and tetrodotoxin: a mechanism for analgesia from infused local anesthetics? Acta Anaesthesiol. Scand. **37:** 516–523, 1993.
BUTTERWORTH, J. F., AND STRICHARTZ, G. R.: Molecular mechanisms of local anesthesia: a review. Anesthesiology **72:** 711–734, 1990.
BUZSAKI, G., FREUND, T. F., BAYARDO, F., AND SOMOGYI, P.: Ischemia-induced changes in the electrical activity of the hippocampus. Exp. Brain Res. **78:** 268–278, 1989.
CANEVARI, L., RICHTER-LEVIN, G., AND BLISS, T. V. P.: LTP in the dentate gyrus is associated with a persistent NMDA receptor-dependent enhancement of synaptosomal glutamate release. Brain Res. **667:** 115–117, 1994.
CAPEK, R., AND ESPLIN, B.: Effects of lidocaine on hippocampal pyramidal cells: depression of repetitive firing. Neuroreport **5:** 681–684, 1994.
CARAFOLI, E.: Intracellular calcium homeostasis. Annu. Rev. Biochem. **56:** 395–433, 1987.
CARDELL, M., BINGREN, H., WIELOCH, T., ZIVIN, J., AND SAITOH, T.: Protein kinase C is translocated to cell membranes during cerebral ischemia. Neurosci. Lett. **119:** 228–232, 1990.
CATTERALL, W. A.: Neurotoxins that act on voltage-sensitive sodium channels in excitable membranes. Annu. Rev. Pharmacol. Toxicol. **20:** 15–43, 1980.
CATTERALL, W. A.: Inhibition of voltage-sensitive sodium channels in neuroblastoma cells by antiarrhythmic drugs. Mol. Pharmacol. **12:** 356–362, 1981.
CATTERALL, W. A.: Common modes of drug action on Na^+ channels: local anaesthetics, antiarrhythmics and anticonvulsants. Trends Pharmacol. Sci. **8:** 57–65, 1987.
CATTERALL, W. A.: Structure and function of voltage-sensitive ion channels. Science (Wash. DC) **242:** 50–61, 1988a.
CATTERALL, W. A.: Molecular pharmacology of voltage-sensitive sodium channels. ISI Atlas Sci. Pharmacol. **2:** 190–195, 1988b.
CATTERALL, W. A.: Cellular and molecular biology of voltage-gated sodium channels. Physiol. Rev. **72**(suppl. 4): 15S–48S, 1992.
CATTERALL, W. A.: Structure and function of voltage-gated ion channels. Trends Neurosci. **16:** 500–506, 1993a.
CATTERALL, W. A.: Structure and modulation of Na^+ and Ca^{2+} channels. Ann. N. Y. Acad. Sci. **707:** 1–19, 1993b.
CATTERALL, W. A., MORROW, C. S., DALY, J W., AND BROWN, G. B.: Binding of

batrachotoxinin A 20-α-benzoate to a receptor site associated with sodium channels in synaptic nerve ending particles. J. Biol. Chem. **256:** 8922–8927, 1981.

CATTERALL, W. A., AND STRIESSNIG, J.: Receptor sites for Ca^{2+} channel antagonists. Trends Pharmacol. Sci. **13:** 256–262, 1992.

CHANG, H. S., SASAKI, T., KASSELL, N. F.: Hippocampal unit activity after transient cerebral ischemia in rats. Stroke **20:** 1051–1058, 1989a.

CHANG, H. S., STEWART, O., AND KASSEL, N. F.: Decreases in excitatory synaptic transmission and increases in recurrent inhibition in the rat dentate gyrus after transient cerebral ischemia. Brain Res. **505:** 220–224, 1989b.

CHARPENTIER, G., FOURNIER, F., BEHUE, N., MARLOT, D., AND BRULE, G.: Positive regulation by protein kinase C of slow Na current in Xenopus oocytes. Proc. R. Soc. Lond. B Biol. Sci. **254:** 15–20, 1993.

CHEBABO, S. R., AND DO CARMO, R. J.: Phenytoin and retinal spreading depression. Brain Res. **551:** 16–19, 1991.

CHEBABO, S. R., DO CARMO, R. J., AND MARTINS-FERREIRA, H.: Effects of local anaesthetics on retinal spreading depression. Exp. Brain Res. **96:** 363–364, 1993.

CHEN, C. C., MORISHIGE, N., MASUDA, M., LIN, W., WIELAND, W., THONE, F., MUBAGWA, K., BORGERS, M., AND FLAMENG, W.: R56865, a Na^+- and Ca^{2+}-overload inhibitor, reduces myocardial ischemia-reperfusion injury in blood-perfused rabbit hearts. J. Mol. Cell. Cardiol. **25:** 1445–1459, 1993a.

CHEN, Q., CHOPP, M., BODZIN, G., AND CHEN, H.: Temperature modulation of cerebral depolarization during focal cerebral ischemia in rats: correlation with ischemic injury. J. Cereb. Blood Flow Metab. **13:** 389–394, 1993b.

CHÉRAMY, A., BARBEITO, L., GODEHEU, P., AND GLOWINSKI, J.: Riluzole inhibits the release of glutamate in the caudate nucleus of the cat in vivo. Neurosci. Lett. **147:** 209–212, 1992.

CHERUBINI, E., BEN-ARI, Y., AND KRNJEVIC, K.: Anoxia produces smaller changes in synaptic transmission, membrane potential, and input resistance in immature rat hippocampus. J. Neurophysiol. **62:** 882–895, 1989.

CHEUNG, H., KAMP, D., AND HARRIS, E.: An in vitro investigation of the action of lamotrigine on neuronal voltage-activated sodium channels. Epilepsy Res. **13:** 107–112, 1992.

CHIH, C.-P., FENG, Z.-C., ROSENTHAL, M., LUTZ, P. L. AND SICK, T. J.: Energy metabolism, ion homeostasis, and evoked potentials in anoxic turtle brain. Am. J. Physiol. **257:** R854–R860, 1989a.

CHIH, C.-P., ROSENTHAL, M., AND SICK, T. J.: Ion leakage is reduced during anoxia in turtle brain: a potential survival strategy. Am. J. Physiol. **257**(6 Pt 2): R1562–R1564, 1989b.

CHIU, S. Y., RITCHIE, J. M., ROGART, R. B., AND STAGG, D.: A quantitative description of membrane currents in rabbit myelinated nerve. J. Physiol. (Lond.) **292:** 149–166, 1979.

CHOI, D. W.: Calcium-mediated neurotoxicity: relationship to specific channel types and role in ischemic damage. Trends Neurosci. **11:** 465–469, 1988.

CHOI, D. W.: Calcium: still center-stage in hypoxic-ischemic neuronal death. Trends Neurosci. **18:** 58–60, 1995.

CHOKI, J., GREENBERG, J., AND REIVICH, M.: Regional cerebral glucose metabolism during and after bilateral cerebral ischemia in the gerbil. Stroke **14:** 568–574, 1983.

CLIFTON, G. L., TAFT, W. C., BLAIR, R. E., CHOI, S. C., AND DELORENZO, R. J.: Conditions for pharmacologic evaluation in the gerbil model of forebrain ischemia. Stroke **20:** 1545–1552, 1989.

COHAN, S. L., REDMOND, D., AND CHEN, M.: Effect of flunarizine on electroencephalogram recovery and brain temperature in gerbils after brain ischemia. Stroke **23:** 229–233, 1992.

COHEN, S. A., AND BARCHI, R. L.: Voltage-dependent sodium channels. Int. Rev. Cytol. **137C:** 55–103, 1993.

COHEN-ARMON, M., SOKOLOVSKY, M., AND DASCAL, N.: Modulation of the voltage-dependent sodium channel by agents affecting G-proteins: a study in Xenopus oocytes injected with brain RNA. Brain Res. **496:** 197–203, 1989.

CONNORS, B. W.: A comparison of the effects of pentobarbital and diphenylhydantoin on the GABA sensitivity and excitability of adult sensory ganglion cells. Brain Res. **207:** 357–369, 1981.

CONNORS, B. W., GUTNICK, M. J., AND PRINCE, D. A.: Electrophysiological properties of neocortical neurons in vitro. J. Neurophysiol. **48:** 1302–1320, 1982.

CORMIER, R. J., MAUK, M. D., KELLY, P. T.: Glutamate iontophoresis induces long-term potentiation in the absence of evoked presynaptic activity. Neuron **10:** 907–919, 1993.

CORNOG, J. L., GONATAS, N. K., AND FEIERMAN, J. R.: Effects of intracerebral injection of ouabain on the fine structure of rat cerebral cortex. Am. J. Pathol. **51:** 573–590, 1967.

COSTA, M. R., AND CATTERALL, W. A.: Characterization of variant neuroblastoma clones with missing or altered sodium channels. Mol. Pharmacol. **22:** 196–203, 1982.

COSTA, M. R., AND CATTERALL, W. A.: Cyclic AMP-dependent phosphorylation of the alpha subunit of the sodium channel in synaptic nerve ending particles. J. Biol. Chem. **259:** 8210–8218, 1984a.

COSTA, M. R., AND CATTERALL, W. A.: Phosphorylation of the alpha subunit of the sodium channel by protein kinase C. Cell. Mol. Neurobiol. **4:** 291–297, 1984b.

COURATIER, P. H., SINDOU, P. H., ESCLAIRE, F., LOUVEL, E., AND HUGON, J.: Neuroprotective effects of riluzole in ALS CSF toxicity. Neuroreport **5:** 1012–1014, 1994.

COURTNEY, K. R.: Mechanism of frequency-dependent inhibition of sodium currents in frog myelinated nerve by the lidocaine derivative GEA 968. J. Pharmacol. Exp. Ther. **195:** 225–236, 1975.

COUSIN, M. A., NICHOLLS, D. G., AND POCOCK, J. M.: Flunarizine inhibits both calcium-dependent and -independent release of glutamate from synaptosomes and cultured neurones. Brain Res. **606:** 227–236, 1995.

CRÉPEL, V., HAMMOND, C., KRNJEVIC, K., CHINESTRA, P., AND BEN-ARI, Y.: Anoxia-induced LTP of isolated NMDA receptor-mediated synaptic responses. J. Neurophysiol. **69:** 1774–1778, 1993.

CREVELING, C. R., AND DALY, J. W.: Batrachotoxinin A [^{3}H]benzoate binding to sodium channels. *In* Methods in Neuroscience: Neurotoxins, ed. by M. P. Conn, vol. 8, pp. 25–37, Academic Press, San Diego, 1992.

CREVELING, C. R., MCNEAL, E. T., DALY, J. W., AND BROWN, G. B.: Batrachotoxin-induced depolarization and [^{3}H]batrachotoxin-A 20α-benzoate binding in a vesicular preparation from guinea pig cerebral cortex. Inhibition by local anesthetics. Mol. Pharmacol. **23:** 350–358, 1983.

CROWDER, J. M., CROUCHER, M. J., BRADFORD, H. F., AND COLLINS, J. F.: Excitatory amino acid receptors and depolarization-induced Ca^{2+} influx into hippocampal slices. J. Neurochem. **48:** 1917–1924, 1987.

CULLEN, J. P., ALDRETE, J. A., JANKOVSKY, L., AND ROMOS-SALAS, F.: Protective action of phenytoin in cerebral ischemia. Anesth. Analg. **58:** 165–169, 1979.

CUMMINS, T. R., DONNELLY, D. F., AND HADDAD, G. G.: Effect of metabolic inhibition on the excitability of isolated hippocampal CA1 neurons: developmental aspects. J. Neurophysiol. **66:** 1471–1482, 1991.

CUMMINS, T. R., JIANG, C., AND HADDAD, G. G.: Human neocortical excitability is decreased during anoxia via sodium channel modulation. J. Clin. Invest. **91:** 608–615, 1993.

CUMMINS, T. R., XIA, Y., AND HADDAD, G. G.: Functional properties of rat and human neocortical voltage-sensitive sodium currents. J. Neurophysiol. **71:** 1052–1064, 1994.

CUMRINE, R. C., DUBYACK, G., LAMANNA, J. C.: Decreased protein kinase C activity during cerebral ischemia and after reperfusion in the adult rat. J. Neurochem. **55:** 2001–2007, 1990.

DAGANI, F., FERRARI, R., AND CANEVARI, L.: A pharmacological model for studying the role of Na^+ gradients in the modulation of synaptosomal free $[Ca^{2+}]_i$ levels and energy metabolism. Brain Res. **530:** 261–266, 1990.

DANIELL, L. C.: Determination of the intravascular ionized sodium concentration in a cell-free brain membrane vesicle preparation using the fluorescent indicator, SBFI. Anal. Biochem. **202:** 239–244, 1992.

DARGENT, B., AND COURAUD, F.: Down-regulation of voltage-dependent sodium channels initiated by sodium influx in developing neurons. Proc. Natl. Acad. Sci. USA **87:** 5907–5911, 1990.

DASCAL, N., AND LOTAN, I.: Activation of protein kinase C alters voltage dependence of a Na^+ channel. Neuron **6:** 165–175, 1991.

DE BARRY, J., GOMBOS, G., AND VIZI, E. S.: Release of [^{3}H]L-glutamate and [^{3}H]L-glutamine in rat cerebellum slices: a comparison of the effect of veratridine and electrical stimulation. Neurochem. Res. **14:** 1053–1060, 1989.

DEBONO, M. W., LE GUERN, J., CANTON, T., DOBLE, A., AND PRADIER, L.: Inhibition by riluzole of electrophysiological responses mediated by rat kainate and NMDA receptors expressed in Xenopus oocytes. Eur. J. Pharmacol. **235:** 283–289, 1993.

DERI, Z., AND ADAM-VIZI, V.: Detection of intracellular free Na^+ concentration of synaptosomes by a fluorescent indicator, Na^+-binding benzofuran isophthalate: the effect of veratridine, ouabain, and α-latrotoxin. J. Neurochem. **61:** 818–825, 1993.

DE RYCK, M., DUYTSCHAEVER, H., PAUWELS, P. J., AND JANSSEN, P. A. J.: Ionic channels, cholinergic mechanisms, and recovery of sensorimotor function after neocortical infarcts in rats. Stroke **21**(suppl. III): 58–63, 1991.

DE RYCK, M., KEERSMAEKERS, R., CLINCKE, G., JANSSEN, M., AND VAN REET S.: Lubeluzole, a novel benzothiazole, protects neurologic function after cerebral thrombotic stroke in rats: an apparent stereospecific effect. Soc. Neurosci. Abstr. **20:** 185, 1994.

DE RYCK, M., VAN REEMPTS, J., BORGERS, M., WAUQUIER, A., AND JANSSEN, P. A. J.: Photochemical stroke model: flunarizine prevents sensorimotor deficits after neocortical infarcts in rats. Stroke **20:** 1383–1390, 1989.

DESHPANDE, J. K., AND WIELOCH, T.: Amelioration of ischaemic brain damage by postischaemic treatment with flunarizine. Neurol. Res. **7:** 27–29, 1985.

DESHPANDE, J. K., AND WIELOCH, T.: Flunarizine, a calcium entry blocker, ameliorates ischemic brain damage in the rat. Anesthesiology **64:** 215–224, 1986.

DESSI, F., CHARRIAUT-MARLANGUE, C., AND BEN-ARI, Y.: Glutamate-induced neuronal death in cerebellar culture is mediated by two distinct components: a sodium-chloride component and a calcium component. Brain Res. **650:** 49–55, 1994.

DEZA, L., AND EIDELBERG, E.: Development of cortical electrical activity in the rat. Exp. Neurol. **17:** 425–438, 1967.

DIEMER, N. H., AND SIEMKOWICZ, E.: Regional glucose metabolism and nerve cell damage after cerebral ischemia in normo- and hypoglycemic rats. *In* Circulatory and Developmental Aspects of Brain Metabolism, ed. by M. Spatz, B. B. Mrsulja, L. M. Rakic, and W. D. Lust, pp. 23–32, Plenum Press, New York, 1980.

DIEMER, N. H., VALENTE, E., BRUHN, T., BERG, M., JORGENSEN, M. B., AND JOHANSEN, F. F.: Glutamate receptor transmission and ischemic nerve cell

damage: evidence for involvement of excitotoxic mechanisms. Prog. Brain Res. **96:** 105–123, 1993.

DIENER, C.-H., HACKE, W., HENNERICI, M., DE KEYSER, J., RADBERG, J., AND HANTSON, L. FOR THE LUBINT-4 STUDY GROUP: The effects of lubeluzole in the acute treatment of ischemic stroke: results of a phase-2 trial. Stroke **26:** 185, 1995.

DIETRICH, W. D.: The importance of brain temperature in cerebral injury. J. Neurotrauma **9**(suppl. 2)**:** 475S–485S, 1992.

DIETRICH, W. D., BUSTO, R., ALONSO, O., GLOBUS, M. Y.-T., AND GINSBERG, M. D.: Intraischemic but not postischemic brain hypothermia protects chronically following global forebrain ischemia in rats. J. Cereb. Blood Flow Metab. **13:** 541–549, 1993.

DI PERRI, T., PASINI, F. L., PECCHI, S., DE FRANCO, V., DAMIANI, P., PASQUI, A. L., CAPECCHI, P. L., ORRICO, A., MATERAZZI, M., AND DOMINI, L.: In vivo and in vitro evidence of an adenosine-mediated mechanism of calcium entry blocker activities. Angiology **40:** 190–198, 1989.

DIRNAGL, U.: Cerebral ischemia: the microcirculation as trigger and target. Prog. Brain Res. **96:** 49–65, 1993.

DOBLE, A., BLANCHARD, J. C., BOIREAU, A., DEBONO, M., ESCANDE, D., HUBERT, J. P., MALGOURIS, C., PRATT, J., RATAUD, J., AND STUTZMANN, J. M.: Neuroprotective effects of riluzole, a blocker of glutamatergic transmission. J. Neurochem. **61**(suppl.)**:** 283S, 1993.

DOLL, C. J., HOCHACHKA P. W., AND REINER, P. B.: Effects of anoxia and metabolic arrest on turtle and rat cortical neurons. Am. J. Physiol. **260**(4 Pt 2)**:** R747–R755, 1991.

DOMANSKA-JANIK, K., AND ZALEWSKA, T.: Effect of brain ischemia on protein kinase C. J. Neurochem. **58:** 1432–1439, 1992.

DONG, L. P., WANG, T. Y., AND ZHU, J.: Effects of carbamazepine on hypoxic and ischemic brain damage in mice. Chung Kuo Yao Li Hsueh Pao **15:** 257–259, 1994.

DOYLE, D. D., GUO, Y., LUSTIG, S. L., SATIN, J., ROGART, R. B., AND FOZZARD, H. A.: Divalent cation competition with [^{3}H]-saxitoxin binding to tetrodotoxin-resistant and -sensitive sodium channels. A two-site structural model of ion/toxin interaction. J. Gen. Physiol. **101:** 153–182, 1993.

DREJER, J., HONORÉ, T., MEIER, E., AND SCHOUSBOE, A.: Pharmacologically distinct glutamate receptors on cerebellar granule cells. Life Sci. **38:** 2077–2085, 1986.

DRUG & MARKET DEVELOPMENT, INC.: Stroke and cerebral circulation: Epidemiology and AHA Joint Conference highlights. Drug Market Dev. **4:** 257–262, 1994.

DUCHEN, M. R.: Effects of metabolic inhibition on the membrane properties of isolated mouse primary sensory neurones. J. Physiol. (lond.) **424:** 387–409, 1990.

DUTKA, A. J., MINK, R., MCDERMOTT, J., CLARK, J. B., AND HALLENBECK, J. M.: Effect of lidocaine on somatosensory evoked response and cerebral blood flow after canine cerebral air embolism. Stroke **23:** 1515–1520, 1992.

EAHOLTZ, G., SCHEUER, T., AND CATTERALL, W. A.: Restoration of inactivation and block of open sodium channels by an inactivation gate peptide. Neuron **12:** 1041–1048, 1994.

EDWARDS, R. A., LUTZ, P. L., AND BADEN, D. G.: Relationship between energy expenditure and ion channel density in the turtle and rat brain. Am. J. Physiol. **257**(6 Pt 2)**:** R1354–R1358, 1989.

EISEN, A., STEWART, H., SCHULZER, M., AND CAMERON, D.: Anti-glutamate therapy in amyotrophic lateral sclerosis: a trial using lamotrigine. Can. J. Neurol. Sci. **20:** 297–301, 1993.

ERDŐ, S. L., NING-SHENG, C., WOLFF, J. R., AND KISS, B.: Vinpocetine protects against excitotoxic cell death in primary cultures of rat cerebral cortex. Eur. J. Pharmacol. **187:** 551–553, 1990.

ERECINSKA, M., AND DAGANI, F.: Relationships between the neuronal sodium/potassium pump and energy metabolism. Effects of K+, Na+, and adenosine triphosphate in isolated brain synaptosomes. J. Gen. Physiol. **95:** 591–616, 1990.

ERECINSKA, M., DAGANI, F., NELSON, D., DEAS, J., AND SILVER, I. A.: Relations between intracellular ions and energy metabolism: a study with monensin in synaptosomes, neurons, and C6 glioma cells. J. Neurosci. **11:** 2410–2421, 1991.

ERECINSKA, M., AND SILVER, I. A.: ATP and brain function. J. Cereb. Blood Flow Metab. **9:** 2–19, 1989.

EVANS, D. E., KOBRINE, A. I., LEGRYS, D. C., AND BRADLEY, M. E.: Protective effect of lidocaine in acute cerebral ischemia induced by air embolism. J. Neurosurg. **60:** 257–263, 1984.

FELLOWS, L. K., BOUTELLE, M. G., AND FILLENZ, M.: Extracellular brain glucose levels reflect local neuronal activity: a microdialysis study in awake, freely moving rats. J. Neurochem. **59:** 2141–2147, 1992.

FELLOWS, L. K., BOUTELLE, M. G., AND FILLENZ, M.: Physiological stimulation increases nonoxidative glucose metabolism in the brain of the freely moving rat. J. Neurochem. **60:** 1258–1263, 1993.

FERN, R., RANSOM, B. R., STYS, P. K., AND WAXMAN, S. G.: Pharmacological protection of CNS white matter during anoxia: actions of phenytoin, carbamazepine and diazepam. J. Pharmacol. Exp. Ther. **266:** 1549–1555, 1993.

FERRANDON, P., BARCELO, B., PERCHE, J. C., AND SCHOFFS, A. R.: Effects of dipyramidole, soluflazine and related molecules on adenosine uptake and metabolism by isolated human red blood cells. Fundam. Clin. Pharmacol. **8:** 446–452, 1994.

FERRENDELLI, J. A., AND DANIELS-MCQUEEN, S.: Comparative action of phenytoin and other anticonvulsant drugs on potassium- and veratridine-stimulated calcium uptake in synaptosomes. J. Pharmacol. Exp. Ther. **220:** 29–34, 1982.

FEUSTEL, P. J., INGVAR, M. C., AND SEVERINGHAUS, J. W.: Cerebral oxygen availability and blood flow during middle cerebral artery occlusion: effects of pentobarbital. Stroke **12:** 858–863, 1981.

FINK, B. R.: Paradoxical preservation of neural conduction by lidocaine. Anesthesiology **57:** 167–171, 1982.

FISHER, C. M.: Lacunar strokes and infarcts: a review. Neurology **32:** 871–876, 1982.

FISHER, M., JONES, S., AND SACCO, R. L.: Prophylactic neuroprotection for cerebral ischemia. Stroke **25:** 1075–1080, 1994.

FRANCIS, J., AND MCINTYRE-BURNHAM W.: [^{3}H]Phenytoin identifies a novel anticonvulsant-binding domain on voltage-dependent sodium channels. Mol. Pharmacol. **42:** 1097–1103, 1992.

FRASER, S., AND SPEDDING, M.: RS-87476, a novel neuroprotective agent, antagonises the effects of calcium channel activators in guinea-pig taenia. Br. J. Pharmacol. **104**(suppl.)**:** 156P, 1991.

FRAZIER, D. T., NARAHASHI, T., AND YAMADA, M.: The site of action and active form of local anesthetics. II. Experiments with quaternary compounds. J. Pharmacol. Exp. Ther. **171:** 45–51, 1970.

FREDHOLM, B. B., LINDGREN, E., LINDSTRÖM, K., AND VERNET, L.: The effect of some drugs with purported antianoxic effect on veratridine-induced purine release from isolated rat hypothalamic synaptosomes. Acta Pharmacol. Toxicol. **53:** 236–244, 1983.

FRENCH, C. R., SAH, P., BUCKETT, K. J., AND GAGE, P. W.: A voltage-dependent persistent sodium current in mammalian hippocampal neurons. J. Gen. Physiol. **95:** 1139–1157, 1990.

FRIEDMAN, J. E., AND HADDAD, G. G.: Major differences in Ca^{2+}_i response to anoxia between neonatal and adult rat CA1 neurons: role of Ca^{2+}_o and Na^+_o. J. Neurosci. **13:** 63–72, 1993.

FRIEDMAN, J. E., AND HADDAD, G. G.: Removal of extracellular sodium prevents anoxia-induced injury in freshly dissociated rat CA1 hippocampal neurons. Brain Res. **641:** 57–64, 1994a.

FRIEDMAN, J. E., AND HADDAD, G. G.: Anoxia induces an increase in intracellular sodium in rat central neurons in vitro. Brain Res. **663:** 329–334, 1994b.

FUJIWARA N., HIGASHI, H., SHIMOJI, K., AND YOSHIMURA, M.: Effects of hypoxia on rat hippocampal neurones in vitro. J. Physiol. (Lond.) **384:** 131–151, 1987.

GARTHWAITE, G., WIGHTMAN, G., AND GARTHWAITE, J.: Protection of CNS white matter from ischaemia-induced damage in vitro by 619C89, a Na^+ channel blocker. Brain Res. Assoc. Abstr. **12:** 73, 1995.

GATFIELD, P. D., LOWRY, O. H., SCHULZ, D. W., AND PASSONNEAU, J. V.: Regional energy reserves in mouse brain and changes with ischaemia and anaesthesia. J. Neurochem. **13:** 185–195, 1966.

GAUTRON, S., DOS SANTOS, G., PINTO-HENRIQUE, D., KOULAKOFF, A., GROS, F., BERWALD-NETTER, Y.: The glial voltage-gated sodium channel: Cell- and tissue-specific mRNA expression. Proc. Natl. Acad. Sci. USA **89:** 7272–7276, 1992.

GELB, A. W., STEINBERG, G. K., LAM, A. M, MANNINEN, P. H., PEERLESS, S. J., AND RASSI-NETO, A.: The effects of a prophylactic bolus of lidocaine in focal cerebral ischemia. Can. J. Anaesth. **35:** 489–493, 1988.

GERSHON, E., WEIGL, L., LOTAN, I., SCHREIBMAYER, W., DASCAL, N.: Protein kinase A reduces voltage-dependent Na^+ current in xenopus oocytes. J. Neurosci. **12:** 3743–3752, 1992.

GIBSON, G., TORAL-BARZA, L., AND HUANG, H.-M.: Cytosolic free calcium concentrations in synaptosomes during histotoxic hypoxia. Neurochem. Res. **16:** 461–467, 1991.

GILLAND, E., MALGORZATA, P.-S., ANDINÉ, P., BONA, E., AND HAGBERG, H.: Hypoxic-ischemic injury in the neonatal rat brain: effects of pre- and post-treatment with the glutamate release inhibitor BW1003C87. Brain Res. Dev. Brain Res. **83:** 79–84, 1994.

GILMAN, A. G.: G proteins: transducers of receptor-generated signals. Annu. Rev. Biochem. **56:** 615–649, 1987.

GINSBERG, M. D.: Efficacy of calcium channel blockers in brain ischemia: a critical assessment. *In* Pharmacology of Cerebral Ischemia 1988, ed. by J. Krieglstein, pp. 65–73, Wissenschaftliche Verlagsgesellschaft, Stuttgart, Germany, 1988.

GINSBERG, M. D., STERNAU, L. L., GLOBUS, M. Y., DIETRICH, W. D., AND BUSTO, R.: Therapeutic modulation of brain temperature: relevance to ischemic brain injury. Cerebrovasc. Brain Metab. Rev. **4:** 189–225, 1992.

GLEITZ J., BEILE, A., KHAN, S., WILFFERT, B., AND TEGTMEIER, F.: Anaerobic glycolysis and postanoxic recovery of respiration of rat cortical synaptosomes are reduced by synaptosomal sodium load. Brain Res. **611:** 286–294, 1993.

GODOY, C. M., AND CUKIERMAN, S.: Multiple effects of protein kinase C activators on Na^+ currents in mouse neuroblastoma cells. J. Membr. Biol. **140:** 101–110, 1994.

GOLDBERG, M. P., AND CHOI, D. W.: Combined oxygen and glucose deprivation in cortical cell culture: calcium-dependent and calcium-independent mechanisms of neuronal injury. J. Neurosci. **13:** 3510–3524, 1993.

GOLDBERG, W. J., KADINGO, R. M., AND BARRETT, J. N.: Effects of ischemia-like conditions on cultured neurons: protection by low Na^+, low Ca^{2+} solutions. J. Neurosci. **6:** 3144–3151, 1986.

GOLDIN, A. L.: Accessory subunits and sodium channel inactivation. Curr. Opin. Neurobiol. **3:** 272–277, 1993.

GOLDIN, A. L., SNUTCH, T, LÜBBERT, H., DOWSETT, A., MARSHALL, J., AULD, V., DOWNEY, W., FRITZ, L. C., LESTER, H. A., DUNN, R., CATTERALL, W. A., DAVIDSON, N.: Messenger RNA coding for only the α subunit of the rat brain Na channel is sufficient for expression of functional channels in Xenopus oocytes. Proc. Natl. Acad. Sci. USA **83:** 7503–7507, 1986.

GONCALVES, T., CARVALHO, A. P., OLIVEIRA, C. R.: Antioxidant effect of calcium antagonists on microsomal membranes isolated from different brain areas. Eur. J. Pharmacol. **204:** 315–322, 1991.

GOTTI, B., BENAVIDES, J., MACKENZIE, E. T., AND SCATTON, B.: The pharmacotherapy of focal cortical ischaemia in the mouse. Brain Res. **522:** 290–307, 1990.

GRAHAM, J. L., SMITH, S. E., CHAPMAN, A. G., AND MELDRUM, B. S.: Effect of lamotrigine on microdialysate amino acid concentrations in the striatum after focal cerebral ischaemia in the rat. Br. J. Pharmacol. **112**(suppl.): 278P, 1994a.

GRAHAM, S. H., CHEN, J., LAN, J., LEACH, M. J., AND SIMON, R. P.: Neuroprotective effects of a use-dependent blocker of voltage dependent sodium channels, BW619C89, in rat middle cerebral artery occlusion. J. Pharmacol. Exp. Ther. **269:** 854–859, 1994b.

GRAHAM, S. H., CHEN, J., SHARP, F. R., AND SIMON, R. P.: Limiting ischemic injury by inhibition of excitatory amino acid release. J. Cereb. Blood Flow. Metab. **13:** 88–97, 1993.

GREENBLATT, R. E., BLATT, Y., AND MONTAL, M.: The structure of the voltage-sensitive sodium channel. Inferences derived from computer-aided analysis of the Electrophorus electricus channel primary structure. FEBS Lett. **193:** 125–134, 1985.

GUSOVSKY, F., HOLLINGSWORTH, E. B., AND DALY, J. W.: Regulation of phosphatidylinositol turnover in brain synaptoneurosomes: stimulatory effects of agents that enhance influx of sodium ions. Proc. Natl. Acad. Sci. USA **83:** 3003–3007, 1986.

GUY, H. R., AND CONTI, F.: Pursuing the structure and function of voltage-gated channels. Trends Neurosci. **13:** 201–206, 1990.

GUY, H. R., AND SEETHARAMULU, P.: Molecular model of the action potential sodium channel. Proc. Nat. Acad. Sci. USA **83:** 508–512, 1986.

HADDAD, G. G., AND DONNELLY, D. F.: O_2 deprivation induces a major depolarization in brainstem neurons in the adult but not in the neonatal rat. J. Physiol. (Lond.) **429:** 411–428, 1990.

HADDAD, G. G., AND JIANG, C.: O_2 deprivation in the central nervous system: on mechanisms of neuronal response, differential sensitivity and injury. Prog. Neurobiol. **40:** 277–318, 1993.

HADJIEV, D., AND YANCHEVA, S.: Rheoencephalographic and psychological studies with ethyl apovincaminate in cerebral vascular insufficiency. Arzneimittelforschung **26:** 1947–1950, 1976.

HAIGNEY, M. C. P., LAKATTA, E. G., STERN, M. D., AND SILVERMAN, H. S.: Sodium channel blockade reduces hypoxic sodium loading and sodium-dependent calcium loading. Circulation **90:** 391–399, 1994.

HAIGNEY, M. C., MIYATA, H., LAKATTA, E. G., STERN, M. D., AND SILVERMAN, H. S.: Dependence of hypoxic cellular calcium loading on Na^+-Ca^{2+} exchange. Circ. Res. **71:** 547–557, 1992.

HALL, R., AND MURDOCH, J.: Brain protection: physiological and pharmacological considerations. Part II. The pharmacology of brain protection. Can. J. Anaesth. **37:** 762–777, 1990.

HALLENBECK, J. M., FRERICHS, K. U: Stroke therapy: it may be time for an integrated approach. Arch. Neurol. **50:** 768–770, 1993.

HANDA, J., MATSUDA, M., NAKASU, Y., SHIINO, A., KANAZAWA, T., AND HARADA, K.: KB-2796, a new Ca^{2+} antagonist. Cardiovasc. Drug Rev. **8:** 105–116, 1990.

HANSEN, A. J.: Extracellular potassium concentration in juvenile and adult rat brain cortex during anoxia. Acta Physiol. Scand. **99:** 412–420, 1977.

HANSEN, A. J.: Effect of anoxia on ion distribution in the brain. Physiol. Rev. **65:** 101–148, 1985.

HANSEN, A. J., HOUNSGAARD, J., AND JAHNSEN, H.: Anoxia increases potassium conductance in hippocampal nerve cells. Acta Physiol. Scand. **115:** 301–310, 1982.

HANSEN, A. J., AND NEDERGAARD, M.: Brain ion homeostasis in cerebral ischemia. Neurochem. Pathol. **9:** 195–209, 1988.

HANSEN, A. J., AND OLSEN, C. E.: Brain extracellular space during spreading depression and ischemia. Acta Physiol. Scand. **108:** 355–365, 1980.

HANSSON, E., JOHANSSON, B. B., WESTERGREN, I., AND RÖNNBÄCK, L.: Mechanisms of glutamate induced swelling in astroglial cells. Acta Neurochir. Suppl. (Wien) **60:** 12–14, 1994.

HANTSON, L., GHEUENS, J., TRITSMANS, L., AND D. E. KEYSER, J.: Hospital referral of stroke patients: a survey of attitudes in general practice, and consideration of entry times for clinical trials. Clin. Neurol. Neurosurg. **96:** 32–37, 1994.

HARA, H., HARADA, K., AND SUKAMATO, T.: Chronological atrophy after transient middle cerebral artery occlusion in rats. Brain Res. **618:** 251–260, 1993a.

HARA, H., ONODERA, H., YOSHIDOMI, M., MATSUDA, Y., AND KOGURE, K.: Staurosporine, a novel protein kinase C inhibitor, prevents post-ischemic neuronal damage in the gerbil and rat. J. Cereb. Blood Flow Metab. **10:** 646–653, 1990.

HARA, H., OZAKI, A., YOSHIDOMI, M., ITO, K., AND SUKAMOTO, T.: Protective effect of KB-2796, a new dilator, on cerebral hypoxia and ischemia. Jpn. J. Pharmacol. **46:** 134P, 1988.

HARA, H., YOKOTA, K., SHIMAZAWA, M., AND SUKAMOTO, T.: Effect of KB-2796, a new diphenylpiperazine Ca^{2+} antagonist, on glutamate-induced neurotoxicity in rat hippocampal primary cell cultures. Jpn. J. Pharmacol. **61:** 361–365, 1993b.

HARADA, K., SHIINO, A., MATSUDA, M., AND HANDA, J.: Effects of a novel calcium antagonist, KB-2796, on neurological outcome and size of experimental cerebral infarction in rats. Surg. Neurol. **32:** 16–20, 1989.

HÄRKÖNEN, M. H. A., PASSONNEAU, J. V., AND LOWRY, O. H.: Relationships between energy reserves and function in rat superior cervical ganglion. J. Neurochem. **16:** 1439–1450, 1969.

HARRIS, R. J., WIELOCH, T., SYMON, L., AND SIESJO, B. K.: Cerebral extracellular calcium activity in severe hypoglycemia: relation to extracellular potassium and energy state. J. Cereb. Blood Flow Metab. **4:** 187–193, 1984.

HARTMANN, H. A., TIEDEMAN, A. A., CHEN, S. F., BROWN, A. M., AND KIRSCH, G. E.: The effects of III-IV linker mutations on human heart Na^+ channel inactivation gating. Circ. Res. **75:** 114–122, 1994.

HAY, D. W., AND WADSWORTH, R. M.: Local anaesthetic activity of organic calcium antagonists: relevance to their actions on smooth muscle. Eur. J. Pharmacol. **77:** 221–228, 1982.

HAYS, S. J., RICE, M. J., ORTWINE, D. F., JOHNSON, G., SCHWARZ, R. D., BOYD, D. K., COPELAND, L. F., VARTANIAN, M. G., AND BOXER, P. A.: Substituted 2-benzothiazolamines as sodium flux inhibitors: quantitative structure-activity relationships and anticonvulsant activity. J. Pharm. Sci. **83:** 1425–1432, 1994.

HAYS, S. J., SCHWARZ, R. D., BOYD, D. K., COUGHENOUR, D., DOOLEY, D. ROCK, TAYLOR C., VARTANIAN, M., AND MOOS, W.: PD 85639: a potent inhibitor of Na^+ influx into rat neocortical slices. Soc. Neurosci. Abstr. **17:** 956, 1991a.

HAYS, S. J., SCHWARZ, R. D., COUGHENOUR, L. L., BOYD, D. K., ANDERSON, R. J., AND BOXER, P. A.: Is riluzole a glutamate antagonist? (Abstract) Abstr. Pap. Am. Chem. Soc. **201:** 14, 1991b.

HEBERT, T., DRAPEAU, P., PRADIER, L., AND DUNN, R. J.: Block of the rat brain IIA sodium channel alpha subunit by the neuroprotective drug riluzole. Mol. Pharmacol. **45:** 1055–1060, 1994.

HEINEMANN, S. H.: Pore structure of voltage-gated ion channels. *In* Progress in Cell Research: Molecular Biophysics of Ion Channel, ed. by M. Sokabe and A. Auerbach, Elsevier Science, Amsterdam, in press, 1995.

HEINEMANN, S. H., SCHLIEF, T., MORI, Y., AND IMOTO, K.: Molecular pore structure of voltage-gated sodium and calcium channels. Braz. J. Med. Biol. Res. **27:** 2781–2802, 1994.

HEINEMANN, S. H., TERLAU, H., AND IMOTO, K.: Molecular basis for pharmacological differences between brain and cardiac sodium channels. Pflueg. Arch. Eur. J. Physiol. **422:** 90–92, 1992a.

HEINEMANN, S. H., TERLAU, H., STÜHMER, W., IMOTO, K., AND NUMA, S.: Calcium channel characteristics conferred on the sodium channel by single mutations. Nature (Lond.) **356:** 441–443, 1992b.

HILLE, B.: Ionic Channels of Excitable Membranes. Sinauer Associates, Sunderland, MA, 1991.

HILLE, B., LEIBOWITZ, M. D., SUTRO, J. B., SCHWARZ, J. R., HOLAN, G.: State-dependent modifications of sodium channels by lipid-soluble agonists. *In* Proteins of Excitable Membranes, ed. by B. Hille and D. M. Fambrough, vol. XLI, pp. 109–124, John Wiley & Sons, New York, 1987.

HOCHACHKA, P. W.: Defense strategies against hypoxia and hypothermia. Science (Wash. DC) **231:** 234–241, 1986.

HODGKIN, A. L., AND HUXLEY, A. F.: Quantitative description of membrane current and its application to conduction and excitation in nerve. J. Physiol. (Lond.) **117:** 500–544, 1952.

HOFF, E. C., AND YAHN, C.: The effect of sodium, 5,5-diphenyl hydantoinate (dilantin sodium) upon the tolerance of rats and mice to decompression. Am. J. Physiol. **141:** 7–16, 1944.

HOSSMANN, K.-A.: Glutamate-mediated injury in focal cerebral ischemia: the excitotoxin hypothesis revised. Brain Pathol. **4:** 23–36, 1994a.

HOSSMANN, K.-A.: Mechanisms of ischemic injury: is glutamate involved? *In* Pharmacology of Cerebral Ischemia 1994, ed. by J. Krieglstein and H. Oberpichler-Schwenk, pp. 239–251, Wissenschaftliche Verlagsgesellschaft, Stuttgart, Germany, 1994b.

HOSSMANN, K.-A. PASCHEN, W., AND CSIBA, L.: Relationship between calcium accumulation and recovery of cat brain after prolonged cerebral ischemia. J. Cereb. Blood Flow Metab. **3:** 346–353, 1983.

HSU, M., AND BUSZAKI, G.: Vulnerability of mossy fiber targets in the rat hippocampus to forebrain ischemia. J. Neurosci. **13:** 3964–3979, 1993.

HUBERT, J. P., DELUMEAU, J. C., GLOWINSKI, J., PRÉMONT, J., AND DOBLE, A.: Antagonism by riluzole of entry of calcium evoked by NMDA and veratridine in rat cultured granule cells: evidence for a dual mechanism of action. Br. J. Pharmacol. **113:** 261–267, 1994.

HUBERT, J. P., AND DOBLE, A.: Ibotenic acid stimulates D-[^{3}H]aspartate release from cultured cerebellar granule cells. Neurosci. Lett. **30:** 345–350, 1989.

ILJIMA, T., MIES, G., AND HOSSMANN, K.-A.: Repeated negative DC deflections in rat cortex following middle cerebral artery occlusion are abolished by MK-801: effect on volume of ischemic injury. J. Cereb. Blood Flow Metab. **12:** 727–733, 1992.

IMON, H., MITANI, A., ANDOU, Y., ARAI, T., AND KATAOKA, K.: Delayed neuronal death is induced without postischemic hyperexcitability: continuous multi-

ple-unit recording from ischemic CA_1 neurons. J. Cereb. Blood Flow Metab. **11:** 819–823, 1991.

ISOM, L. L., DE JONGH, K. S., PATON, D. E., REBER, B. F. X., OFFORD, J., CHARBONNEAU, H., WALSH, K., GOLDIN, A. L., AND CATTERALL, W. A.: Primary structure and functional expression of the β_1 subunit of the rat brain sodium channel. Science (Wash. DC) **256:** 839–842, 1992.

ITO, C., IM, W. B., TAKAGI, H., TAKAHASHI M., TSUZUKI, K., LIOU, S.-Y., AND KUNIHARA, M.: U-92032, a T-type Ca^{2+} channel blocker and antioxidant, reduces neuronal ischemic injuries. Eur. J. Pharmacol. **257:** 203–210, 1994.

JACQUES, Y., FRELIN, C., VIGNE, P., ROMEY, G., PARJARI, M., AND LAZDUNSKI, M.: Neurotoxins specific for the sodium channel stimulate calcium entry into neuroblastoma cells. Biochemistry **20:** 6219–6225, 1981.

JACQUES, Y., ROMEY, G., FOSSET, M., AND LAZDUNSKI, M.: Properties of the interaction of the sodium channel with permeant monovalent cations. Eur. J. Biochem. **106:** 71–83, 1980.

JEAN, T., FRELIN, C., VIGNE, P., AND LAZDUNSKI, M.: The Na^+/H^+ exchange system in glial cell lines. Properties and activation by an hyperosmotic shock. Eur. J. Biochem. **160:** 211–219, 1986.

JENSEN, A. M., AND CHIU, S. Y.: Expression of glutamate receptor genes in white matter: developing and adult rat optic nerve. J. Neurosci. **13:** 1664–1675, 1993.

JENSEN, M. S., LAMBERT, J. D. C., AND JOHANSEN, F. F.: Electrophysiological recordings from rat hippocampus slices following in vivo brain ischemia. Brain Res. **554:** 166–175, 1991.

JIANG, C., AGULIAN, S., AND HADDAD, G. G.: Cl^- and Na^+ homeostasis during anoxia in hypoglossal neurons: intracellular and extracellular in vitro studies. J. Physiol. (Lond.) **448:** 697–708, 1992.

JIANG, C., AND HADDAD, G. G.: Differential responses of neocortical neurons to glucose and/or O_2 deprivation in the human and rat. J. Neurophysiol. **68:** 2165–2173, 1992.

JING, J., AITKEN, P. G., AND SOMJEN, G. G.: Role of calcium channels in spreading depression in rat hippocampal slices. Brain Res. **604:** 251–259, 1993.

JØRGENSEN M. B., WRIGHT, D. C., AND DIEMER, N. H.: Postischemic glucose metabolism is modified in the hippocampal CA_1 region depleted of excitatory input or pyramidal cells. J. Cereb. Blood Flow Metab. **10:** 243–251, 1990.

KAKU, D. A., GIFFARD, R. G., AND CHOI, D. W.: Neuroprotective effects of glutamate antagonists and extracellular acidity. Science (Wash. DC) **260:** 1516–1518, 1993.

KALLEN, R. G., COHEN, S. A., AND BARCHI, R. L.: Structure, function and expression of voltage-dependent sodium channels. Mol. Neurobiol. **7:** 383–428, 1994.

KALLEN, R. G., SHENG, Z. H., YANG, J., CHEN, L. Q., ROGART, R. B., AND BARCHI, R. L.: Primary structure and expression of a sodium channel characteristic of denervated and immature rat skeletal muscle. Neuron **4:** 233–242, 1990.

KANAI, Y., SMITH, C. P., AND HEDIGER, M. A.: The elusive transporters with a high-affinity for glutamate. Trends Neurosci. **16:** 365–370, 1993.

KANEKO, S., SUGIMURA, M., INOUE, T., AND SATOH, M.: Effects of several cerebroprotective drugs on NMDA channel function: evaluation using Xenopus oocytes and [^{3}H]MK-801 binding. Eur. J. Pharmacol. **207:** 119–128, 1991.

KANEKO, S., TAKAHASHI, H., AND SATOH, M.: The use of Xenopus oocytes to evaluate drugs affecting brain Ca^{2+} channels: effects of bifemelane and several nootropic agents. Eur. J. Pharmacol. **189:** 51–58, 1990.

KANNER, B. I., AND BENDAHAN, A.: Binding order of substrates to the sodium and potassium ion coupled L-glutamic acid transporter from rat brain. Biochemistry **21:** 6327–6330, 1982.

KARKOUTLY, C., BACKHAUB, C., NUGLISCH, J., AND KRIEGLSTEIN, J.: The measurement of the infarcted area after middle cerebral artery occlusion in the mouse: a screening model. *In* Pharmacology of Cerebral Ischemia 1990, ed. by J. Krieglstein and H. Oberpichler, pp. 63–69, Wissenschaftliche Verlagsgesellschaft, Stuttgart, Germany, 1990.

KASS, I. S., ABRAMOWICZ, A. E., COTTRELL, J. E., AND CHAMBERS, G.: The barbiturate thiopental reduces ATP levels during anoxia but improves electrophysiological recovery and ionic homeostasis in the rat hippocampal slice. Neuroscience **49:** 537–543, 1992.

KAYANO, T., NODA, M., FLOCKERZI, V., TAKAHASHI, H., AND NUMA, S.: Primary structure of rat brain sodium channel III deduced from the cDNA sequence. FEBS Lett. **228:** 187–194, 1988.

KELLER, B. U., HARTSHORNE, R. P., TALVENHEIMO, J. A., CATTERALL, W. A., AND MONTAL, M.: Sodium channels in planar lipid bilayers. Channel gating kinetics of purified sodium channels modified by batrachotoxin. J. Gen. Physiol. **88:** 1–23, 1986.

KELLY, D. A., AND STOREY, K. B.: Organ-specific control of glycolysis in anoxic turtles. Am. J. Physiol. **255**(5 Pt 2): R774–R779, 1988.

KEMP, B. E., AND PEARSON, R. E.: Protein kinase recognition sequence motifs. Trends Biochem. Sci. **15:** 342–346, 1990.

KEMPSKI, O., STAUB, F., V. ROSEN F., ZIMMER, M., NEU, A., AND BAETHMANN, A.: Molecular mechanisms of glial swelling in vitro. Neurochem. Pathol. **9:** 109–125, 1988.

KEMPSKI, O., VON ROSEN, F., WEIGT, H., STAUB, F., PETERS, J., AND BAETHMANN, A.: Glial ion transport and volume control. Ann. N. Y. Acad. Sci. **633:** 306–317, 1991.

KENNELLY, P. J., AND KREBS, E. G.: Consensus sequences as substrate specificity determinants for protein kinases and protein phosphatases. J. Biol. Chem. **266:** 15555–15558, 1991.

KENNY, B. A., CALDER, C., AND BROWN, C. M.: Neuroprotective properties of phenytoin in a mouse model of focal cerebral ischaemia. Br. J. Pharmacol. **107**(suppl.): 60P, 1992.

KENNY, B. A., AND SHERIDAN, R. D.: D-CPP and phenytoin protect against hypoxia-induced failure of synaptic transmission in the rat hippocampus in vitro. Br. J. Pharmacol. **107**(suppl.): 203P, 1992.

KHODOROV, B., SHISHKOVA, L., PEGANOV, E., AND REVENKO, S.: Inhibition of sodium currents in frog Ranvier node treated with local anesthetics. Role of slow sodium inactivation. Biochim. Biophys. Acta **433:** 409–435, 1976.

KHODOROV, B. I.: Batrachotoxin as a tool to study voltage-sensitive sodium channels of excitable membranes. Proc. Biophys. Mol. Biol. **45:** 57–148, 1985.

KIMELBERG, H. K., ROSE, J. W., BARRON, K. D., WANIEWSKI, R. A., AND CRAGOE, E. J.: Astrocytic swelling in traumatic-hypoxic brain injury. Beneficial effects of an inhibitor of anion exchange transport and glutamate uptake in glial cells. Mol. Chem. Neuropathol. **11:** 1–31, 1989.

KING, G. A.: Protective effects of vinpocetine and structurally related drugs on the lethal consequences of hypoxia in mice. Arch. Int. Pharmacodyn. Ther. **286:** 299–307, 1987.

KING, L. J., LOWRY, O. H., PASSONNEAU, J. V., AND VENSON, V.: Effects of convulsants on energy reserves in the cerebral cortex. J. Neurochem. **14:** 599–611, 1967.

KIRINO, T.: Delayed neuronal death in the gerbil hippocampus following ischemia. Brain Res. **239:** 57–69, 1982.

KIRINO, T., TAMURA, A., AND SANO, K.: Chronic maintenance of presynaptic terminals in gliotic hippocampus following ischemia. Brain Res. **510:** 17–25, 1990.

KIRSCH, G. E.: Na^+ channels: structure, function, and classification. Drug Dev. Res. **33:** 263–276, 1994.

KIRSCH, G. E., ALAM, M., AND HARTMANN, H. A.: Differential effects of sulfhydryl reagents on saxitoxin and tetrodotoxin block of voltage-dependent Na channels. Biophys. J. **67:** 2305–2315, 1994.

KISKIN, N. I., CHIZHMAKOV, I. V., TSYNDRENKO, A. Y., KRISHTAL, O. A., AND TEGTMEIER, F.: R56865 and flunarizine as Na^+-channel blockers in isolated purkinje neurons of rat cerebellum. Neuroscience **54:** 575–585, 1993.

KISS, B., CAI, N.-S., AND ERDÖ, S. L.: Vinpocetine preferentially antagonizes quisqualate/AMPA receptor responses evidence from release and ligand binding studies. Eur. J. Pharmacol. **209:** 109–112, 1991.

KOBRINE, A. I., EVANS, D. E., LEGRYS, D. C., YAFFE, L. J., AND BRADLEY, M. E.: Effect of intravenous lidocaine on experimental spinal cord injury. J. Neurosurg. **60:** 595–601, 1984.

KOGURE, K., HOSSMANN, K.-A., AND SIESJÖ, B. K.: Neurobiology of ischemic brain damage. *In* Progress in Brain Research, vol. 96, Elsevier, London, 1993.

KOH, J.-Y., AND CHOI, D. W.: Effect of anticonvulsant drugs on glutamate neurotoxicity in cortical cell culture. Neurology **36:** 319–322, 1987.

KRIEGLER, S., AND CHIU, S. Y.: Calcium signalling of glial cells along mammalian axons. J. Neurosci. **13:** 4229–4245, 1993.

KRIEGLSTEIN, J., AND RISCHKE, R.: Vinpocetine increases the neuroprotective effect of adenosine in vitro. Eur. J. Pharmacol. **205:** 7–10, 1991.

KRISTIÁN, T., KATSURA, K., GIDÖ, G., AND SIESJÖ, B. K.: The influence of pH on cellular calcium influx during ischemia. Brain Res. **641:** 295–302, 1994.

KRNJEVIC, K., CHERUBINI, E., AND BEN-ARI, Y.: Anoxia on slow inward currents of immature hippocampal neurons. J. Neurophysiol. **62:** 896–906, 1989.

KRNJEVIC, K., AND LEBLOND, J.: Changes in membrane currents of hippocampal neurons evoked by brief anoxia. J. Neurophysiol. **62:** 15–30, 1989.

KRUEGER, B. K., BLAUSTEIN, M. P., AND RATZLAFF, R. W.: Sodium channels in presynaptic nerve terminals. Regulation by neurotoxins. J. Gen. Physiol. **76:** 287–313, 1980.

KUCHARCZYK, J., MINTOROVITCH, J., MOSELEY, M. E., ASGARI, H. S., SEVICK, R. J., DERUGIN, N., AND NORMAN, D.: Ischemic brain damage: reduction by sodium-calcium channel modulator RS-87476. Radiology **179:** 221–227, 1991.

KUCHARCZYK, J., MINTOROVITCH, J., SEVICK, R., ASGARI, H. AND MOSELEY, M.: MR evaluation of calcium entry blockers with putative cerebroprotective effects in acute cerebral ischaemia. Acta Neurochir. Suppl. (Wien) **51:** 254–255, 1990.

KUMAR, K., KRAUSE, G., KOESTNER, A., HOEHNER, T., AND WHITE, B.: Effect of flunarizine on global brain ischemia in the dog: a quantitative morphologic assessment. Exp. Neurol. **97:** 115–127, 1987.

KUO, C.-C., AND BEAN, B. P.: Slow binding of phenytoin to inactivated sodium channels in rat hippocampal neurons. Mol. Pharmacol. **46:** 716–725, 1994.

LAFFLING, A. J., SCHERR, P., MCGIVERN, J. G., PATMORE, L., AND SHERIDAN, R. D.: Electrophysiological actions of phenytoin on N-methyl-D-aspartate receptor-mediated responses in rat hippocampus in vitro. Br. J. Pharmacol. **115:** 67–72, 1995.

LAKICS, V., AND ERDÖ, S. L.: Attenuation by phenytoin and carbamazepine of kainate- and NMDA-induced cell death in primary cultures of rat cerebral cortex. Eur. J. Neurosci. **7**(suppl.): 115, 1994.

LAKICS, V., SEBESTYÉN, M. G., AND ERDÖ, S. L.: Vinpocetine is a highly potent neuroprotectant against veratridine-induced cell death in primary cultures of rat cerebral cortex. Neurosci. Lett. **185:** 127–130, 1995.

LAMPLEY, E. C., MISHRA, O. M., GRAHAM, E., AND DELIVORIA-PAPADOPOULOS,

M.: Neuroprotective effect of phenytoin against in utero hypoxic brain injury in fetal guinea pigs. Neurosci. Lett. **186:** 192–196, 1995.

LANG, D. G., AND WANG, C. M.: Lamotrigine and phenytoin interactions on ionic currents present in N4TG1 and GH3 clonal cells. Soc. Neurosci. Abstr. **17:** 1256, 1991.

LANG, D. G., WANG, C. M., AND COOPER, D. R.: Lamotrigine, phenytoin and carbamazepine interactions on the sodium current present in N4TG1 mouse neuroblastoma cells. J. Pharmacol. Exp. Ther. **266:** 829–835, 1993.

LANTOS, J., SAROSI, I., TEMES, G., AND TOROK, B., JR.: Influence of protective drugs on the EEG during short-term transient ischaemia. Acta Physiol. Hung. **75:** 217–227, 1990a.

LANTOS, J., TEMES, G., KELEMEN, D., SAROSI, I., AND TOROK, B., JR.: Influence of protective drugs on the elevation of extracellular potassium ion concentration in the brain during ischaemia. Acta Physiol. Hung. **76:** 49–59, 1990b.

LAURITZEN, M.: Pathophysiology of the migraine aura: the spreading depression theory. Brain **117:** 199–210, 1994.

LEACH, M. J., BAXTER, M. G., AND CRITCHLEY, M. A. E.: Neurochemical and behavioral aspects of lamotrigine. Epilepsia **32**(suppl. 2)**:** 4S–8S, 1991.

LEACH, M. J., MARDEN, C. M., AND MILLER, A. A.: Pharmacological studies on lamotrigine, a novel potential antiepileptic drug. II. Neurochemical studies on the mechanism of action. Epilepsia **27:** 490–497, 1986.

LEACH, M. J., SWAN, J. H., EISENTHAL, D., DOPSON, M., AND NOBBS, M.: BW619C89, a glutamate release inhibitor, protects against focal cerebral ischemic damage. Stroke **24:** 1063–1067, 1993.

LEBLOND, J. AND KRNJEVIC, K.: Hypoxic changes in hippocampal neurons. J. Neurophysiol. **62:** 1–14, 1989.

LEDERER, W. J., NIGGLI, E., AND HADLEY, R. W.: Sodium-calcium exchange: response. Science (Wash. DC) **251:** 1371, 1991.

LEE, K. S., FRANK, S., VANDERKLISH, P., ARAI, A., AND LYNCH, G.: Inhibition of proteolysis protects hippocampal neurons from ischemia. Proc. Natl. Acad. Sci. USA **88:** 7233–7237, 1991.

LEES, G., AND LEACH, M. J.: Studies on the mechanism of action of the novel anticonvulsant lamotrigine (Lamictal) using primary neuroglial cultures from rat cortex. Brain Res. **612:** 190–199, 1993.

LEKIEFFRE, D., AND MELDRUM, B. S.: The pyrimidine-derivative, BW1003C87, protects CA1 and striatal neurons following transient severe forebrain ischaemia in rats. A microdialysis and histological study. Neuroscience **56:** 93–99, 1993.

LESAGE, A. S., DE LOORE, K. L., OSIKOWSKA-EVERS, B., PEETERS, L., AND LEYSEN, J. E.: Lubeluzole, a novel neuroprotectant, inhibits the glutamate-activated NOS pathway. Soc. Neurosci. Abstr. **20:** 185, 1994.

LEVITAN, I. B.: Modulation of ion channels in neurons and other cells. Annu. Rev. Neurosci. **11:** 119–136, 1988.

LEVY, D. E., AND DUFFY, T. E.: Cerebral energy metabolism during transient ischemia and recovery in the gerbil. J. Neurochem. **28:** 63–70, 1977.

LI, M., WEST, J. W., LAI, Y., SCHEUER, T., AND CATTERALL, W. A.: Functional modulation of brain sodium channels by cAMP-dependent phosphorylation. Neuron **8:** 1151–1159, 1992.

LI, M., WEST, J. W., NUMANN, R., MURPHY, B. J., SCHEUER, T., AND CATTERALL, W. A.: Convergent regulation of sodium channels by protein kinase C and cAMP-dependent protein kinase. Science (Wash. DC) **261:** 1439–1442, 1993.

LINDEN, D. J., AND ROUTTENBERG, A.: *Cis*-fatty acids, which activate protein kinase C, attenuate Na^+ and Ca^{2+} currents in mouse neuroblastoma cells. J. Physiol. (Lond.) **419:** 95–119, 1989.

LIPTON, S. A., AND ROSENBERG, P. A.: Excitatory amino acids as a final common pathway for neurologic disorders. N. Engl. J. Med. **330:** 613–622, 1994.

LIZASOAIN, I., KNOWLES, R. G., AND MONCADA, S.: Inhibition by lamotrigine of the generation of nitric oxide in rat forebrain slices. J. Neurochem. **64:** 636–642, 1995.

LLINAS, R., AND SUGIMORI, M.: Electrophysiological properties of in vitro purkinje cell somata in mammalian cerebellar slices. J. Physiol. (Lond.) **305:** 171–195, 1980.

LOGAN, J. G.: The extrusion of sodium ions from presynaptic nerve endings of rat cerebral cortex. J. Neurochem. **35:** 349–353, 1980.

LOUIS, J.-C., MAGAL, E., BRIXI, A., STEINBERG, R., YAVIN, E., VINCENDON, G.: Reduction of protein kinase C activity in the adult rat brain following transient forebrain ischemia. Brain Res. **541:** 171–174, 1991.

LU, C.-M., HAN, J., RADO, T. A., AND BROWN, G. B.: Differential expression of two sodium channel subtypes in human brain. FEBS Lett. **303:** 53–58, 1992.

LUCAS, L. F., WEST, C. A., RIGOR, B. M., AND SCHURR, A.: Protection against cerebral hypoxia by local anesthetics: a study using brain slices. J. Neurosci. Methods **28:** 47–50, 1989.

LUND-ANDERSEN, H., AND HERTZ, L.: Effects of potassium and of glutamate on swelling and on sodium and potassium content in brain-cortex slices from adult rats. Exp. Brain Res. **11:** 199–212, 1970.

LUSTIG, H. S., VON BRAUCHITSCH, K. L., CHAN, J., AND GREENBERG, D. A.: A novel inhibitor of glutamate release reduces excitotoxic injury in vitro. Neurosci. Lett. **143:** 229–232, 1992.

LUTZ, P. L., MCMAHON, P., ROSENTHAL, M., SICK, T. J.: Relationship between aerobic and anaerobic energy production in turtle brain in situ. Am. J. Physiol. **247**(4 Pt 2)**:** R740–R744, 1984.

LUX, H. D., HEINEMANN, U., AND DIETZEL, I.: Ionic changes and alterations in the size of the extracellular space during epileptic activity. Adv. Neurol. **44:** 619–639, 1986.

LYNCH, J. J., III, YU, S. P., CANZONIERO, L. M. T., SENSI, S. L., AND CHOI, D. W.: Sodium channel blockers reduce oxygen-glucose deprivation-induced cortical neuronal injury when combined with glutamate receptor antagonists. J. Pharmacol. Exp. Ther. **273:** 554–560, 1995.

LYSKO, P. G., WEBB, C. L., YUE, T.-L., GU, J.-L., AND FEUERSTEIN, G.: Neuroprotective effects of tetrodotoxin as a Na^+ channel modulator and glutamate release inhibitor in cultured rat cerebellar neurons and in gerbil global brain ischemia. Stroke **25:** 2476–2482, 1994.

LYSKO, P. G., YUE, T. L., GU, J. L., WEBB, C. L., AND FEUERSTEIN, G.: Neuroprotective effects of tetrodotoxin in cultured cerebellar neurons and in gerbil global brain ischemia. Soc. Neurosci. Abstr. **19:** 286, 1993.

MA, J. Y., LI, M., CATTERAL, W. A., AND SCHEUER, T.: Modulation of brain Na^+ channels by a G-protein-coupled pathway. Proc. Natl. Acad. Sci. USA **91:** 12351–12355, 1994.

MACGREGOR, R. R., THORNER, R. E., AND WRIGHT, D. M.: Lidocaine inhibits granulocyte adherence and prevents granulocyte delivery to inflammatory sites. Blood **56:** 203–209, 1980.

MACKINNON, A. C., WYATT, K. M., MCGIVERN, J. G., SHERIDAN, R. D., AND BROWN, C. M.: [^{3}H]-lifarizine, a high affinity probe for inactivated sodium channels. Br. J. Pharmacol. **115:** 1103–1109, 1995.

MADDEN, K. P., CLARK, W. M., KOCHLAR, A., AND ZIVIN, J. A.: Effect of protein kinase C modulation on outcome of experimental CNS ischemia. Brain Res. **547:** 193–198, 1991.

MADDEN, K. P., CLARK, W. M., MARCOUX, F. W., PROBERT, A. W., JR., WEBER, M. L., RIVIER, J., AND ZIVIN, J. A.: Treatment with conotoxin, and 'N-type' calcium channel blocker, in neuronal hypoxic-ischemic injury. Brain Res. **537:** 256–262, 1990.

MAKITA, N., BENNETT, P. B., JR., AND GEORGE, A. L., JR.: Voltage-gated Na^+ channel β_1 subunit mRNA expressed in adult human skeletal muscle, heart, and brain is encoded by a single gene. J. Biol. Chem. **269:** 7571–7578, 1994.

MALGOURIS, C., BARDOT, F., DANIEL, M., PELLIS, F., RATAUD, J., UZAN, A., BLANCHARD, J.-C., AND LADURON, P. M.: Riluzole, a novel anti-glutamate, prevents memory loss and hippocampal neuronal damage in ischemic gerbils. J. Neurosci. **9:** 3720–3727, 1989.

MALGOURIS, C., DANIEL, M., AND DOBLE, A.: Neuroprotective effects of riluzole on N-methyl-D-aspartate- or veratridine-induced neurotoxicity in rat hippocampal slices. Neurosci. Lett. **177:** 95–99, 1994.

MANTZ, J., CHERAMY, A., THIERRY, A. M., GLOWINSKI, J., DESMONTS, J. M.: Anesthetic properties of riluzole (54274 RP); a new inhibitor of glutamate neurotransmission. Anesthesiology **76:** 844–848, 1992.

MANTZ, J., LAUDENBACH, V., LECHARNY J.-B., HENZEL, D., AND DESMONTS, J. M.: Riluzole, a novel antiglutamate, blocks GABA uptake by striatal synaptosomes. Eur. J. Pharmacol. **257:** R7–R8, 1994.

MARCOUX, F. W., MORAWETZ, R. B., CROWELL, R. M., DEGIROLAMI, U., AND HALSEY, J. H., JR.: Differential regional vulnerability in transient focal cerebral ischemia. Stroke **13:** 339–346, 1982.

MARCOUX, F. W., PROBERT, A. W., AND WEBER, M. L.: Hypoxic neuronal injury in cell culture: calcium accumulation blockade and neuroprotection by NMDA antagonists but not calcium-channel antagonists. *In* Cerebravascular Diseases: Proceedings of the Sixteenth Research Conference, Princeton, NJ, ed. by M. D. Ginsberg and W. D. Dietrich, pp. 135–141, Raven Press, New York, 1989.

MARRANNES, R., DE PRINS, E., FRANSEN, J., AND CLINCKE, G.: Neuropharmacology of spreading depression. *In* Migraine: Basic Mechanisms and Treatment, ed. by A. Lehmenkühler, K.-H. Grotemeyer and F. Tegtmeier, pp. 431–443, Urban & Schwarzenberg, Munich, Germany, 1993.

MARRANNES, R., DE PRINS, E., FRANSEN, J., AND WAUQUIER, A.: Comparison of the influence of calcium antagonists and NMDA antagonists on the cortical extracellular changes in ion concentration and DC-potential during ischemia. Acta Physiol. Scand. **136**(suppl. 582)**:** 75, 1989.

MATA, M., FINK, D. J., GAINER, H., SMITH, C. B., DAVIDSEN, L., SAVAKI, H., SCHWARTZ, W. J., AND SOKOLOFF, L.: Activity-dependent energy metabolism in rat posterior pituitary primarily reflects sodium pump activity. J. Neurochem. **34:** 213–215, 1980.

MATSUDA, J. J., LE, E. H., AND SHIBATA, E. F.: Enhancement of rabbit cardiac sodium channels by β-adrenergic stimulation. Circ. Res. **70:** 199–207, 1992.

MATSUKI, N., QUANDT, F. N., TEN EICK, R. E., AND YEH, J. Z.: Characterization of the block of sodium channels by phenytoin in mouse neuroblastoma cells. J. Pharmacol. Exp. Ther. **228:** 523–530, 1984.

MAXWELL, W. L., WATT, C., GRAHAM, D. I., AND GENNARELLI, T. A.: Ultrastructural evidence of axonal shearing as a result of lateral acceleration of the head in non-human primates. Acta Neuropathol. (Berl.) **86:** 136–144, 1993.

MAY, G. R., ROWAND, W. S., MCCORMACK, J. G., AND SHERIDAN, R. D.: Neuroprotective profile of lifarizine (RS-87476) in rat cerebrocortical neurones in culture. Br. J. Pharmacol. **114:** 1365–1370, 1995.

MCBEAN, D. E., WINTERS, V., WILSON, A. D., OSWALD, C. B. AND ARMSTRONG, J. M.: Neuroprotective efficacy of the ion channel modulator lifarizine (RS-87476) in a rodent model of global cerebral ischaemia. Br. J. Pharmacol. **114**(suppl.)**:** 331P, 1995a.

MCBEAN, D. E., WINTERS, V., WILSON, A. D., OSWALD, C. B., AND ARMSTRONG, J. M.: The ion channel modulator lifarizine (RS-87476) reduces the size of the infarct produced in the rat by the Rose Bengal photochemical focal of cerebral ischaemia. Br. J. Pharmacol. **114**(suppl.)**:** 332P, 1995b.

MCCLATCHEY, A. I., CANNON, S. C., SLAUGENHAUPT, S. A., AND GUSELLA, J. F.:

The cloning and expression of a sodium channel β_1-subunit cDNA from human brain. Hum. Mol. Genet. **2:** 745–749, 1993.

McGeer, E. G., and Zhu, S. G.: Lamotrigine protects against kainate but not ibotenate lesions in the rat striatum. Neurosci. Lett. **112:** 348–351, 1990.

McGivern, J. G., Patmore, L., and Sheridan, R. D.: Effects of the neuroprotective agent, KB-2796, on the voltage-dependent sodium current in mouse neuroblastoma, N1E-115. Br. J. Pharmacol. **114**(suppl.): 137P, 1995a.

McGivern, J. G., Patmore, L., and Sheridan, R. D.: Actions of the novel neuroprotective agent, lifarizine (RS-87476), on voltage-dependent sodium currents in the neuroblastoma line, N1E-115. Br. J. Pharmacol. **114:** 1738–1744, 1995b.

McGivern, J. G., Sheridan, R. D., and Patmore, L.: The neuroprotective agent, Lifarizine (RS-87476), inhibits sodium currents via an interaction with the slowly inactivating state of the channel. Br. J. Pharmacol. **112**(suppl.): 485P, 1994.

McLachlan, R. S., and Girvin, J. P.: Spreading depression of Leao in rodent and human cortex. Brain Res. **666:** 133–136, 1994.

McLean, M. J., and Macdonald, R. L.: Multiple actions of phenytoin on mouse spinal cord neurons in cell culture. J. Pharmacol. Exp. Ther. **227:** 779–789, 1983.

McNeal, E. T., Lewandowski, G. A., Daly, J. W., and Creveling, C. R.: [^{3}H]Batrachotoxinin A20 α-benzoate binding to voltage-sensitive sodium channels: a rapid and quantitative assay for local anesthetic activity in a variety of drugs. J. Med. Chem. **28:** 381–388, 1985.

McPhee, J. C., Ragsdale, D. S., Scheuer, T., and Catterall, W. A.: A mutation in segment IVS6 disrupts fast inactivation of sodium channels. Proc. Natl. Acad. Sci. USA **91:** 12346–12350, 1994.

Meldrum, B. S.: The role of glutamate in epilepsy and other CNS disorders. Neurology **44**(suppl. 8): 14S–23S, 1994.

Meldrum, B. S., Millan, M. H., and Obrenovitch, T. P.: Injury-induced excitatory amino acid release. *In* The Role of Neurotransmitters in Brain Injury, ed. by M. Y. T. Globus and W. D. Dietrich, pp. 1–7, Plenum Press, New York, 1993.

Meldrum, B. S., and Nilsson, B.: Cerebral blood flow and metabolic rate early and late in prolonged epileptic seizures induced in rats by bicuculline. Brain **99:** 523–542, 1976.

Meldrum, B. S., Smith, S. E., Lekieffre, D., Chapman, A. G., Graham, J. L., Pearce, P. C.: Sodium-channel blockade and glutamate release: the mechanism of cerebroprotection by lamotrigine, BW 1003C87 and BW 619C89. *In* Pharmacology of Cerebral Ischemia 1994, ed. by J. Krieglstein and H. Oberpichler-Schwenk, pp. 203–209, Wissenschaftliche-Verlagsgesellshaft, Stuttgart, Germany, 1994.

Meldrum, B. S., Swan, J. H., Leach, M. J., Millan, M. H., Gwinn, R., Kadota, K., Graham, S. H., Chen, J., and Simon, R. P.: Reduction of glutamate release and protection against ischemic brain damage by BW 1003C87. Brain Res. **593:** 1–6, 1992.

Mellergård, P., Ouyang, Y.-B., and Siesjö, B. K.: Intracellular pH regulation in cultured rat astrocytes in CO_2/HCO_3^- containing media. Exp. Brain Res. **95:** 371–380, 1993.

Mellergård, P., Ouyang, Y.-B., and Siesjö, B. K.: The regulation of intracellular pH is strongly dependent on extracellular pH in cultured rat astrocytes and neurons. Acta Neurochir. Suppl. (Wien) **60:** 34–37, 1994.

Messenheimer, J. A.: Lamotrigine. Clin. Neuropharmacol. **17:** 548–559, 1994.

Messing, R. O., Carpenter, C. L., and Greenberg, D. A.: Mechanism of calcium channel inhibition by phenytoin: comparison with classical calcium channel antagonists. J. Pharmacol. Exp. Ther. **235:** 407–411, 1985.

Meves, H., and Vogel, W.: Calcium inward currents in internally perfused giant axons. J. Physiol. (Lond.) **235:** 225–265, 1973.

Michenfelder, J. D.: The interdependency of cerebral function and metabolic effects following massive doses of thiopental in the dog. Anesthesiology **41:** 231–236, 1974.

Michenfelder, J. D., and Milde, J. H.: Cerebral protection by anaesthetics during ischaemia: a review. Resuscitation **4:** 219–233, 1975.

Mies, G., Kohno, K., and Hossmann, K.-A.: Prevention of periinfarct direct current shifts with glutamate antagonist NBQX following occlusion of the middle cerebral artery in the rat. J. Cereb. Blood Flow Metab. **14:** 804–807, 1994.

Mitani, A., Imon, H., Iga, K., Kubo, H., and Kataoka, K.: Gerbil hippocampal extracellular glutamate and neuronal activity after transient ischemia. Brain Res. Bull. **25:** 319–324, 1990a.

Mitani, A., Imon, H., and Kataoka, K.: High frequency discharges of gerbil hippocampal CA1 neurons shortly after ischemia. Brain Res. Bull. **23:** 569–572, 1989.

Mitani, A., Kubo, H., Iga, K., Imon, H., Kadoya, F., and Kataoka, K.: A new enzymatic cycling technique for glutamate determination in brain microdialysates. J. Neurochem. **54:**.709–711, 1990b.

Miyamoto, M., Murphy, T. H., Schnaar, R. L., and Coyle, J. T.: Antioxidants protect against glutamate-induced cytotoxicity in a neuronal cell line. J. Pharmacol. Exp. Ther. **250:** 1132–1140, 1989.

Mizoule, J., Meldrum, B., Mazadier, M., Croucher, M., Ollat, C., Uzan, A., Legrand, J.-J., Gueremy, C., and Le Fur, G.: 2-Amino-6-trifluoromethoxy benzothiazole, a possible antagonist of excitatory amino acid neurotransmission-I. Neuropharmacology **24:** 767–773, 1985.

Molnar, P., and Erdö, S. L.: Vinpocetine is as potent as phenytoin to block voltage-gated Na^+ channels in rat cortical neurons. Eur. J. Pharmacol. **273:** 303–306, 1995.

Moncada, C. R., Arvin, B., and Meldrum, B. S.: The pyrimidine derivative BW 1003C87 protects against excitotoxic lesions induced by kainate in the rat striatum. Neurosci. Lett. **179:** 17–20, 1994.

Moorman, J. R., Kirsch, G. E., Brown, A. M., and Joho, R. H.: Changes in sodium channel gating produced by point mutations in a cytoplasmic linker. Science (Wash. DC) **250:** 688–691, 1990.

Mosher, H. S., Furhman, F. A., Buchwald, H. D., and Fischer, H. G.: Tarichatoxin-tetrodotoxin: a potent neurotoxin. Science (Wash. DC) **144:** 1100–1110, 1964.

Mourre, C., Ben Ari, Y., Bernardi, H., Fosset, M., and Lazdunski, M.: Antidiabetic sulfonylureas: localization of binding sites in the brain and effects on the hyperpolarization induced by anoxia in hippocampal slices. Brain Res. **486:** 159–164, 1989.

Mrsulja, B. B., Ueki, Y., Wheaton, A., Passonneau, J. V., and Lust, W. D.: Release of pentobarbital-induced depression of metabolic rate during bilateral ischemia in the gerbil brain. Brain Res. **309:** 152–155, 1984.

Muir, K. W., and Lees, K. R.: Clinical experience with excitatory amino acid antagonist drugs. Stroke **26:** 503–513, 1995.

Murakami, A., and Furui, T.: Effects of the conventional anticonvulsants, phenytoin, carbamazepine, and valproic acid, on sodium-potassium-adenosine triphosphatase in acute ischemic brain. Neurosurgery **34:** 1047–1051, 1994.

Murphy, B. J., and Catterall, W. A.: Phosphorylation of purified rat brain Na^+ channel reconstituted into phospholipid vesicles by protein kinase Can. J. Biol. Chem. **267:** 16129–16134, 1992.

Murphy, B. J., Rossie, S., De Jongh, K. S., and Catterall, W. A.: Identification of the sites of selective phosphorylation and dephosphorylation of the rat brain Na^+ channel α-subunit by cAMP-dependent protein kinase and phosphoprotein phosphatases. J. Biol. Chem. **268:** 27355–27362, 1993.

Musacchia, X. J.: The viability of *Chrysemys picta* submerged at various temperature. Physiol. Zool. **32:** 47–50, 1959.

Nachshen, D. A., and Kongsamut, S.: 'Slow' K^+-stimulated Ca^{2+} influx is mediated by Na^+-Ca^{2+} exchange: a pharmacological study. Biochim. Biophys. Acta **979:** 305–310, 1989.

Naiman, J. G., and Williams, H. L.: Effects of diphenylhydantoin on the duration of respiratory activity during anoxia. J. Pharmacol. Exp. Ther. **145:** 34–41, 1964.

Nakayama, H., Shikano, H., and Kanoaka, Y.: A site-directed antibody that inhibits phosphorylation of the rat-brain sodium channel by cyclic-AMP-dependent protein kinase. Biochim. Biophys. Acta **1175:** 67–72, 1992.

Nedergaard, M., and Astrup, J.: Infarct rim: effect of hyperglycemia on direct current potential and [^{14}C]2-deoxyglucose phosphorylation. J. Cereb. Blood Flow Metab. **6:** 607–615, 1986.

Nedergaard, M., and Hansen, A. J.: Spreading depression is not associated with neuronal injury in the normal brain. Brain Res. **449:** 395–398, 1988.

Nehlig, A., and Pereira de Vasconcelos, A.: Glucose and ketone body utilization by the brain neonatal rats. Prog. Neurobiol. **40:** 163–221, 1993.

Nellgård B., and Wieloch, T.: Postischemic blockade of AMPA but not NMDA receptors mitigates neuronal damage in the rat brain following transient severe cerebral ischemia. J. Cereb. Blood Flow Metab. **12:** 2–11, 1992.

Nemoto, E. M., and Chavko, M.: Membrane lipid degradation during ischemia and impact on the monolayer surface area diagram (SPAD) Mol. Chem. Neuropathol. **19:** 25–35, 1993.

Neubauer, J. A.: Anoxia? Don't get excited! J. Clin. Invest. **91:** 377, 1993.

Newberg, L. A., Steen, P. A., Milde, J. H., Michenfelder, J. D.: Failure of flunarizine to improve cerebral blood flow or neurologic recovery in a canine model of complete cerebral ischemia. Stroke **15:** 666–671, 1984.

Nicholls, D., and Attwell, D.: The release and uptake of excitatory amino acids. Trends Pharmacol. Sci. **11:** 462–468, 1990.

Nikolov, R., Scheller, D., and Nikolova, M.: Study on the cerebral effects of sabeluzole. Methods Find. Exp. Clin. Pharmacol. **13:** 385–390, 1991.

Nilius, B.: Calcium block of guinea-pig heart sodium channels with and without modification by the piperazinylindole DPI 201–106. J. Physiol. (Lond.) **399:** 537–558, 1988.

Nilsson, G. E., and Lutz, P. L.: Adenosine release in the anoxic turtle brain: a possible mechanism for anoxic survival. J. Exp. Biol. **162:** 345–351, 1992.

Nilsson, L., and Siesjö, B. K.: The effect of phenobarbitone anaesthesia on blood flow and oxygen consumption in the rat brain. Acta Anaesthesiol. Scand. Suppl. **57:** 18–24, 1975.

Nishizuka, Y.: The molecular heterogeneity of protein kinase C and its implications for cellular regulation. Nature (Lond.) **334:** 661–665, 1988.

Nishizuka, Y.: Intracellular signaling by hydrolysis of phospholipids and activation of phospholipase C. Science (Wash. DC) **258:** 607–614, 1992.

Noda, M., Ikeda, T., Kayano, T., Suzuki, H., Takeshima, H., Kurasaki, M., Takahashi, H., and Numa, S.: Existence of distinct sodium channel messenger RNAs in rat brain. Nature (Lond.) **320:** 188–192, 1984.

Noda, M., Ikeda, T., Suzuki, H., Takeshima, H,, Takahashi, T., Kuno, M., and Numa, S.: Expression of functional sodium channels from cloned cDNA. Nature (Lond.) **322:** 826–828, 1986.

Noda, M., Suzuki, H., Numa, S., and Stühmer, W.: A single point mutation confers tetrodotoxin and saxitoxin insensitivity on the sodium channel II. FEBS Lett. **259:** 213–216, 1989.

NUMANN, R., CATTERALL, W. A., AND SCHEUER, T.: Functional modulation of brain sodium channels by protein kinase C phosphorylation. Science (Wash. DC) **254:** 115–118, 1991.

NUMANN, R., WEST, J. W., LI, M., SMITH, R. D., GOLDIN, A. L., SCHEUER, T., AND CATTERALL, W. A.: Biphasic modulation of sodium channels by phosphorylation at two different sites. Soc. Neurosci. Abstr. **18:** 1133, 1992.

OBRENOVITCH, T. P.: The ischaemic penumbra: twenty years on. Cerebrovasc. Brain Metab. Rev. **7:** 297–322, 1995a.

OBRENOVITCH, T. P.: Origins of glutamate release in ischaemia. Acta Neurochir. Suppl. (Wien), in press, 1995b.

OBRENOVITCH, T. P., AND RICHARDS, D. A.: Extracellular neurotransmitter changes in cerebral ischaemia. Cerebrovasc. Brain Metab. Rev. **7:** 1–54, 1995.

OBRENOVITCH, T. P., SARNA, G. S., AND SYMON, L.: Ionic homeostasis and neurotranmitter changes in ischaemia. *In* Pharmacology of Cerebral Ischemia 1990, ed. by J. Krieglstein and H. Oberpichler, pp. 97–112, Wissenschaftliche Verlagsgesellschaft, Stuttgart, Germany, 1990a.

OBRENOVITCH, T. P., SCHELLER, D., MATSUMOTO, T., TEGTMEIER, F., HÖLLER, M., AND SYMON, L.: Rapid redistribution of hydrogen ions is associated with depolarization and repolarization subsequent to cerebral ischaemia-reperfusion. J. Neurophysiol. **64:** 1125–1133, 1990b.

OBRENOVITCH, T. P., AND URENJAK, J.: Na^+-channel blockade: an underrated approach to cerebroprotection. *In* Pharmacology of Cerebral Ischemia 1994, ed. by J. Krieglstein and H. Oberpichler-Schwenk, pp. 551–563, Wissenschaftliche Verlagsgesellschaft, Stuttgart, Germany, 1994.

OBRENOVITCH, T. P., URENJAK, J., RICHARDS, D. A., UEDA, Y., CURZON, G., AND SYMON, L.: Extracellular neuroactive amino acids in the rat striatum during ischaemia: comparison between penumbral conditions and ischaemia with sustained anoxic depolarization. J. Neurochem. **61:** 178–186, 1993.

OH, Y., AND WAXMAN, S. G.: The β_1 subunit mRNA of the rat brain Na^+ channel is expressed in glial cells. Proc. Natl. Acad. Sci. USA **91:** 9985–9989, 1994.

OHNO, M., YAMAMOTO, T., WATANABE, S.: Effect of staurosporine, a protein kinase C inhibitor, on impairment of working memory in rats exposed to cerebral ischemia. Eur. J. Pharmacol. **204:** 113–116, 1991.

OKAMOTO, K., AND QUASTEL, J. H.: Tetrodotoxin-sensitive uptake of ions and water by slices of rat brain in vitro. Biochem. J. **120:** 37–47, 1970.

OKIYAMA, K., SMITH, D. H., GENNARELLI, T. A., SIMON, R. P., LEACH, M., AND MCINTOSH, T. K.: The sodium channel blocker and glutamate release inhibitor BW1003C87 and magnesium attenuate regional cerebral edema following experimental brain injury in the rat. J. Neurochem. **64:** 802–809, 1995.

ORIAS, D. W., NUSS, H. B., XU, R. H., TOMASELLI, G. F., BACKX, P. H., MARBAN, E., AND LAWRENCE, J. H.: Characterization of an "inactivation defective" voltage-gated sodium channel. Biophys. J. **66:** A357, 1994.

OTOMO, E., ATARASHI, J., ARAKI, G., ITO, E., OMAE, T., KUZUYA, F., NUKADA, T., AND EBI, O.: Comparison of vinpocetine with ifenprodil tartrate and dihydroergotoxine mesylate treatment and results of long-term treatment with vinpocetine. Curr. Ther. Res. **37:** 811–821, 1985.

PALMER, G. C., CLARK, B., AND HUTCHINSON, J. B.: Antiepileptic and neuroprotective potential of remacemide hydrochloride. Drugs Future **18:** 1021–1042, 1993.

PALOSI, E., AND SZPORNY, L.: Effects of ethyl apovincaminate on the central nervous system. Arzneimittelforschung **26:** 1926–1929, 1976.

PANAYIOTOU, B. N., FOTHERBY, M. D., POTTER, J. F., AND CASTELDEN, C. M.: Eligibility of acute stroke patients for pharmacological therapy. Age Ageing **23:** 384–387, 1994.

PANCHENKO, V. A., KRISHTAL, O. A., TEGTMEIER, F., AND TSYNDRENKO, A. Y.: R56865 as Ca^{2+}-channel blocker in purkinje neurons of rat: comparison with flunarizine and nimodipine. Neuroscience **54:** 587–594, 1993.

PATLAK, J.: Molecular kinetics of voltage-dependent Na^+ channels. Physiol. Rev. **71:** 1047–1080, 1991.

PATMORE, L., DUNCAN, G. P., KENNY, B. A., AND SPEDDING, M.: RS-87476, a novel neuroprotective agent, inhibits veratrine-induced calcium overload in embryonic chick cardiac myocytes. Br. J. Pharmacol. **104**(suppl.): 175P, 1991.

PATTON, D. E., WEST, J. W., CATTERALL, W. A., AND GOLDIN, A. L.: Amino acid residues required for fast Na^+-channel inactivation: charge neutralizations and deletions in the III-IV linker. Proc. Natl. Acad. Sci. USA **89:** 10905–10909, 1992.

PAUWELS, P. J., LEYSEN, J. E., AND JANSSEN, P. A. J.: Ca^{++} and Na^+ channels involved in neuronal cell death. Protection by flunarizine. Life Sci. **48:** 1881–1893, 1991.

PAUWELS, P. J., LEYSEN, J. E., AND LADURON, P. M.: [^{3}H]Batrachotoxinin A20-α-benzoate binding to sodium-channels in rat brain: characterization and pharmacological significance. Eur. J. Pharmacol. **124:** 291–298, 1986.

PAUWELS, P. J., VAN ASSOUW, H., LEYSEN, J. E., AND JANSSEN, P. A. J.: Ca^{2+}-mediated neuronal death in rat brain neuronal cultures by veratridine: protection by flunarizine. Mol. Pharmacol. **36:** 525–531, 1989.

PAUWELS, P. J., VAN ASSOUW, H. P., PEETERS, L., AND LEYSEN, J. E.: Neurotoxic action of veratridine in rat brain neuronal cultures: mechanism of neuroprotecion by Ca^{++} antagonists nonselective for slow Ca^{++} channels. J. Pharmacol. Exp. Ther. **255:** 1117–1122, 1990.

PAUWELS, P. J., VAN ASSOUW, H. P., PEETERS, L., MOEREMANS, M., AND LEYSEN, J. E.: Chronic treatment with sabeluzole protects cultured rat brain neurons from the neurotoxic effects of excitatory amino acids. Synapse **12:** 271–280, 1992.

PELLIGRINO, D., YOKOHAMA, H., INGVAR, M., AND SIESJÖ, B. K.: Moderate arterial hypotension reduces cerebral cortical blood flow and enhances cellular release of potassium in severe hypoglycaemia. Acta Physiol. Scand. **115:** 511–513, 1982.

PENG, L., AND HERTZ, L.: Stimulation of energy metabolism in primary cultures of mouse cerebellar granule cell neurons. J. Neurochem. **62**(suppl.): S98D, 1994.

PÉREZ-PINZÓN, M. A., CHAN, C. Y., ROSENTHAL, M., AND SICK, T. J.: Membrane and synaptic activity during anoxia in the isolated turtle cerebellum. Am. J. Physiol. **263**(5 Pt 2): R1057–R1063, 1992a.

PÉREZ-PINZÓN, M. A., LUTZ, P. L., SICK, T. J., AND ROSENTHAL, M.: Adenosine, a "retaliatory" metabolite, promotes anoxia tolerance in turtle brain. J. Cereb. Blood Flow Metab. **13:** 728–732, 1993a.

PÉREZ-PINZÓN, M. A., NILSSON, G. E., AND LUTZ, P. L.: Relationship between ion gradients and neurotransmitter release in the newborn rat striatum during anoxia. Brain Res. **602:** 228–233, 1993b.

PÉREZ-PINZÓN, M. A., ROSENTHAL, M., SICK, T. J., LUTZ, P. L., PABLO, J., AND MASH, D.: Downregulation of sodium channels during anoxia: a putative survival strategy of turtle brain. Am. J. Physiol. **262**(4 Pt 2): R712–R715, 1992b.

PERUCHE, B., AND KRIEGLSTEIN, J.: Neuroblastoma cells for testing neuroprotective drug effects. J. Pharmacol. Methods **26:** 139–148, 1991.

PETITO, C. K., FELDMANN, E., PULSINELLI, W. A., AND PLUM, F.: Delayed hippocampal damage in humans following cardiorespiratory arrest. Neurology **37:** 1281–1286, 1987.

PHILLIPS, J. M., AND NICHOLSON, C.: Anion permeability in spreading depression investigated with ion-sensitive microelectrodes. Brain Res. **173:** 567–571, 1979.

POSTMA, S. W., AND CATTERALL, W. A.: Inhibition of binding of [^{3}H]-batrachotoxinin A 20-α-benzoate to sodium channels by local anesthetics. Mol. Pharmacol. **25:** 219–227, 1984.

POVLISHOCK, J. T.: Traumatically induced axonal injury: pathogenesis and pathobiological implications. Brain Pathol. **2:** 1–12, 1992.

POWERS, W. J.: Cerebral blood flow and metabolism in newborn infants. What can they teach us about stroke? Stroke **24**(suppl. 12): I33–I37, 1993.

PRATT, J., RATAUD, J., BARDOT, F., ROUX, M., BLANCHARD, J.-C., LADURON, P. M., AND STUTZMANN, J.-M.: Neuroprotective actions of riluzole in rodent models of global and focal ischaemia. Neurosci. Lett. **140:** 225–230, 1992.

PRENEN, G. H. M., GO, G. K., POSTEMA, F., ZUIDERVEEN, F., AND KORF, J:. Cerebral cation shifts in hypoxic-ischemic brain damage are prevented by the sodium channel blocker tetrodotoxin. Exp. Neurol. **99:** 118–132, 1988.

PULSINELLI, W. A., BRIERLEY, J. B., AND PLUM, F.: Temporal profile of neuronal damage in a model of transient forebrain ischemia. Ann. Neurol. **11:** 491–498, 1982.

PUSCH, M., NODA, M., STÜHMER, W., NUMA, S., AND CONTI, F.: Single point mutations of the sodium channel drastically reduce the pore permeability without preventing its gating. Eur. Biophys. J. **20:** 127–133, 1991.

RAGSDALE, D. S., MCPHEE, J. C., SCHEUER, T., AND CATTERALL, W. A.: Molecular determinants of state-dependent block of Na^+ channels by local anesthetics. Science (Wash. DC) **265:** 1724–1728, 1994.

RAGSDALE, D. S., NUMANN, R., CATTERAL, W. A., AND SCHEUER, T: Inhibition of Na^+ channels by the novel blocker PD85,639. Mol. Pharmacol. **43:** 949–954, 1993.

RAGSDALE, D. S., SCHEUER, T., AND CATTERALL, W. A.: Frequency and voltage-dependent inhibition of type IIA Na^+-channels, expressed in a mammalian cell line, by local anesthetic, antiarrhythmic, and anticonvulsant drugs. Mol. Pharmacol. **40:** 756–765, 1991.

RAMBOLD, H., RIEDEL, K., WILHELM, D., WILFFERT, B., AND HONERJÄGER, P.: R56865 blocks open and inactivated states of the cardiac sodium channel. Arch. Pharmacol. **347**(suppl.): 352, 1993.

RANDLE, J. C. R., LEGUERN, S., BOUDEAU, P. H., AND BÖHME, G. A.: Riluzole inhibition of sodium channel currents and synaptic transmission is increased at high frequencies of activation. Can. J. Physiol. Pharmacol. **72**(suppl. 1): 425, 1994.

RANSOM, B. R., WAXMAN, S. G., AND DAVIS, P. K.: Anoxic injury of CNS white matter: protective effect of ketamine. Neurology **40:** 1399–1403, 1990.

RASOOL, N., FAROQUI, M., AND RUBINSTEIN, E. H.: Lidocaine accelerates neuroelectrical recovery after incomplete global ischemia in rabbits. Stroke **21:** 929–935, 1990.

RATAUD, J., DEBARNOT, F., MARY, V., PRATT, J., AND STUTZMANN, J.-M.: Comparative study of voltage-sensitive sodium channel blockers in focal ischaemia and electric convulsions in rodents. Neurosci. Lett. **172:** 19–23, 1994.

REITH, M. E.: [^{14}C]guanidinium ion influx into Na^+ channel preparations from mouse cerebral cortex. Eur. J. Pharmacol. **188:** 33–41, 1990.

RISCHKE, R., AND KRIEGLSTEIN, J.: Protective effect of vinpocetine against brain damage caused by focal or global ischemia. *In* Pharmacology of Cerebral Ischemia 1990, ed. by J. Krieglstein and H. Oberpichler-Schwenk, pp. 527–532, Wissenschaftliche Verlagsgesellschaft, Stuttgart, Germany, 1990.

RITCHIE, J. M.: The oxygen consumption of mammalian non-myelinated nerve fibres at rest and during activity. J. Physiol. (Lond.) **188:** 309–329, 1967.

RITCHIE, J. M.: Voltage-gated ion channels in Schwann cells and glia. Trends Neurosci. **15:** 345–351, 1992.

ROGART, R. B., CRIBBS, L. L., MUGLIA, L. K., KEPHART, D. D., AND KAISER, M.

W.: Molecular cloning of a putative tetrodotoxin-resistant rat heart Na^+ channel isoform. Proc. Natl. Acad. Sci. USA **86:** 8170–8174, 1989.

ROGAWSKI, M. A., AND PORTER, R. J.: Antiepileptic drugs: pharmacological mechanisms and clinical efficacy with consideration of promising developmental stage compounds. Pharmacol. Rev. **42:** 223–287, 1990.

ROJAS, E., AND RUDY, B.: Destruction of sodium conductance inactivation by a specific protease in perfused nerve fibres from Loligo. J. Physiol. (Lond.) **262:** 501–531, 1976.

ROOS, A., AND BORON, W. F.: Intracellular pH. Physiol. Rev. **61:** 296–434, 1981.

ROSE, I. A., AND ROSE, Z. B.: Glycolysis: regulation and mechanisms of the enzymes. *In* Comprehensive Biochemistry: Carbohydrate Metabolism, ed. by M. Florkin and E. H. Stotz, vol. 17, pp. 93–161, Elsevier Publishing, Amsterdam, 1969.

ROSEN, A. S., MORRIS, M. E., AND WEAVER, D. F.: Effects of Na^+ channel blockers on the anoxic depolarization in neocortical pyramidal neurons. Soc. Neurosci. Abstr. **20:** 184, 1994.

ROSSIE, S., AND CATTERALL, W. A.: Cyclic-AMP-dependent phosphorylation of voltage-sensitive sodium channels in primary cultures of rat brain neurons. J. Biol. Chem. **262:** 12735–12744, 1987.

ROSSIE, S., AND CATTERALL, W. A.: Phosphorylation of the alpha subunit of rat brain sodium channels by cAMP-dependent protein kinase at a new site containing Ser686 and Ser 687. J. Biol. Chem. **264:** 14220–14224, 1989.

ROSSIE, S., GORDON, D., AND CATTERALL, W. A.: Identification of an intracellular domain of the sodium channel having multiple cyclic AMP-dependent phosphorylation sites. J. Biol. Chem. **262:** 17530–17535, 1987.

ROTHMAN, S. M.: The neurotoxicity of excitatory amino acids is produced by passive chloride influx. J. Neurosci. **5:** 1483–1489, 1985.

ROUFOS, I., HAYS, S. J., DOOLEY, D. J., SCHWARZ, R. D., CAMPBELL, G. W., AND PROBERT, A. W., JR.: Synthesis and pharmacological evaluation of phenylacetamides as sodium-channel blockers. J. Med. Chem. **37:** 268–274, 1994.

ROWLAND, L. P.: Riluzole for the treatment of amyotrophic lateral sclerosis: too soon to tell? N. Engl. J. Med. **330:** 636–637, 1994.

RUDOLPHI, K. A., SCHUBERT, P., PARKINSON, F. E., AND FREDHOLM, B. B.: Adenosine in brain ischemia. Cerebrovasc. Brain Metab. Rev. **4:** 346–369, 1992.

SAKURAI, S. Y., LUTZ, P. L., SCHULMAN, A., AND ALBIN, R. L.: Unchanged (^{3}H)MK-801 binding and increased (^{3}H)flunitrazepam binding in turtle forebrain during anoxia. Brain Res. **625:** 181–185, 1993.

SANCHEZ-ARMASS, S., MARTINEZ-ZAGUILAN, R., AND MARTINEZ, G. M.: Regulation of pH in rat brain synaptosomes. I. Role of sodium, bicarbonate, and potassium. J. Neurophysiol. **71:** 2236–2248, 1994.

SATIN, J., KYLE, J. W., CHEN, M., BELL, P., CRIBBS, L. L., FOZZARD, H. A., AND ROGART, R. B.: A mutant of TTX-resistant cardiac sodium channels with TTX-sensitive properties. Science (Wash. DC) **256:** 1202–1205, 1992.

SATIN, J., LIMBERIS, J. T., KYLE, J. W., ROGART, R. B., AND FOZZARD, H. A.: The saxitoxin/tetrodotoxin binding site on cloned rat brain IIa Na channels is in the transmembrane electric field. Biophys. J. **67:** 1007–1014, 1994.

SAUER, D., RISCHKE, R., BECK, T, ROBBERG, C., MENNEL, H.-D., BIELENBERG, G. W., AND KRIEGLSTEIN, J.: Vinpocetine prevents ischemic cell damage in rat hippocampus. Life Sci. **43:** 1733–1739, 1988.

SCHELLER, D., XIE, Y., BOCK, A., TEGTMEIER, F., AND HÖLLER, M.: Flunarizine and R 56865 improve antiischemic/antianoxic resistance of brain cells. Naunyn Schmiedeberg's Arch. Pharmacol. **339**(suppl.): R107, 1989.

SCHEUER, T., AULD, V. J., BOYD, S., OFFORD, J., DUNN, R., AND CATTERALL, W. A.: Functional properties of rat brain sodium channels expressed in a somatic cell line. Science (Wash. DC) **247:** 854–858, 1990.

SCHEUER, T., WEST, J. W., WANG, Y. L., AND CATTERALL, W. A.: Biophys. J. **64:** A88, 1993.

SCHILD, L., AND MOCZYDLOWSKI, E.: Competitive binding interaction between Zn^{2+} and saxitoxin in cardiac Na^+ channels. Evidence for a sulfhydryl group in the Zn2+/saxitoxin binding site. Biophys. J. **59:** 523–537, 1991.

SCHLUE, W. R., AND DORNER, R.: The regulation of pH in the central nervous system. Can. J. Physiol. Pharmacol. **70**(suppl.): 278S–285S, 1992.

SCHREIBMAYER, W., DASCAL, N., LOTAN, I., WALLNER, M., AND WEIGL, L.: Molecular mechanisms of protein kinase C modulation of sodium channel alpha-subunits expressed in Xenopus oocytes. FEBS Lett. **291:** 341–344, 1991.

SCHUBERT, B., VANDONGEN, A. M., KIRSCH, G. E., AND BROWN, A. M.: Inhibition of Na^+ currents by isoproterenol. Am. J. Physiol. **258**(4 Pt 2): H977–H982, 1990.

SCHURR, A., SPEARS, B., REID, K. H., WEST, C. A., EDMONDS, H. L. JR., AND RIGOR, B. M.: Lidocaine depresses synaptic activity in the rat hippocampal slice. Anesthesiology **64:** 501–503, 1986.

SCHWARZ, J. R., AND GRIGAT, G.: Phenytoin and carbamazepine: potential- and frequency-dependent block of Na currents in mammalian myelinated nerve fibers. Epilepsia **30:** 286–294, 1989.

SCHALLER, K. L., KRZEMIEN, D. M., YAROWSKI, P. J., KRUEGER, B. K., AND CALDWELL, J. H.: A novel, abundant sodium channel expressed in neurons and glia. J. Neurosci. **15:** 3231–3242, 1995.

SHANKAR, R., AND QUASTEL, J. H.: Effects of tetrodotoxin and anaesthetics on brain metabolism and transport during anoxia. Biochem. J. **126:** 851–867, 1972.

SHEARDOWN, M. J.: The triggering of spreading depression in the chicken retina: a pharmacological study. Brain Res. **607:** 189–194, 1993.

SHEARDOWN, M. J., NIELSEN, E. Ø., HANSEN, A. J., JACOBSEN, P., AND HONORÉ, T.: 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline: a neuroprotectant for cerebral ischemia. Science (Wash. DC) **247:** 571–574, 1990.

SHEETS, M. F., AND HANCK, D. A.: Mechanisms of extracellular divalent and trivalent cation block of the sodium current in canine cardiac Purkinje cells. J. Physiol. (Lond.) **454:** 299–320, 1992.

SHEETS, M. F., SCANLEY, B. E., HANCK, D. A., MAKIELSKI, J. C., AND FOZZARD, H. A.: Open sodium channel properties of single canine cardiac purkinje cells. Biophys. J. **52:** 13–22, 1987.

SHERIDAN, R. D., LAFFLING, A. J., MCGIVERN, J. G., AND PATMORE, L.: Does inhibition of N-methyl-D-aspartate (NMDA) receptor-mediated responses contribute to the anticonvulsant action of phenytoin? Br. J. Pharmacol. **113**(suppl.): 32P, 1994.

SHIMAZAWA, M., HARA, H., AND SUKAMOTO, T.: Effect of KB-2796, a novel calcium channel blocker, on cortical hypoperfusion and expression of c-Fos-like immunoreactivity following KCl-induced cortical spreading depression in rats. Jpn. J. Pharmacol. **64**(suppl. 1): O-153, 1994.

SHIMIZU, Y.: Chemistry and biochemistry of saxitoxin analogues and tetrodotoxin. Ann. N. Y. Acad. Sci. **479:** 24–31, 1986.

SHIU, G. K., NEMOTO, E. M., AND NEMMER, J.: Dose of thiopental, pentobarbital, and phenytoin for maximal therapeutic effects in cerebral ischemic anoxia. Crit. Care Med. **11:** 452–459, 1983.

SHOKUNBI, M. T., GELB, A. W., PEERLESS, S. J., MERVART, M., AND FLOYD, P.: An evaluation of effect of lidocaine in experimental focal cerebral ischemia. Stroke **17:** 962–966, 1986.

SHOKUNBI, M. T., GELB, A. W., WU, X. M., AND MILLER, D. J.: Continuous lidocaine infusion and focal feline cerebral ischemia. Stroke **21:** 107–111, 1990.

SICK, T. J., ROSENTHAL, M., LAMANNA, J. C., AND LUTZ, P. L.: Brain potassium ion homeostasis during anoxia and metabolic inhibition in the turtles and rats. Am. J. Physiol. **243:** R281–R288, 1982.

SIEMKOWICZ, E., AND HANSEN, A. J.: Brain extracellular ion composition and EEG activity following 10 minutes ischemia in normo- and hyperglycemic rats. Stroke **12:** 236–240, 1981.

SIESJÖ, B. K., AND BENGTSSON, F.: Calcium fluxes, calcium antagonists and calcium-related pathology in brain ischemia, hypoglycemia and spreading depression: a unifying hypothesis. J. Cereb. Blood Flow Metab. **9:** 127–140, 1989.

SIESJÖ, B. K., KATSURA, K., MELLERGÅRD, P, EKHOLM, A., LUNDGREN, J., AND SMITH, M.-L.: Acidosis-related brain damage. Prog. Brain Res. **96:** 23–48, 1993.

SIGEL, E., AND BAUR, R.: Activation of protein kinase C differentially modulates neuronal Na^+, Ca^{2+}, and gamma-aminobutyrate type A channels. Proc. Natl. Acad. Sci. USA **85:** 6192–6196, 1988.

SIMON, R. P., GRIFFITHS, T., EVANS, M. C., SWAN, J. H., AND MELDRUM, B. S.: Calcium overload in selectively vulnerable neurons of the hippocampus during and after ischemia: an electron microscopic study in the rat. J. Cereb. Blood Flow Metab. **4:** 350–361, 1984.

SKILLING, S. R., SMULLIN, D. H., BEITZ, A. J., AND LARSON, A. A.: Extracellular amino acid concentrations in the dorsal spinal cord of freely moving rats following veratridine and nociceptive stimulation. J. Neurochem. **51:** 127–132, 1988.

SMITH, D. A., BROWNING, M., AND DUNWIDDIE, T. V.: Cocaine inhibits hippocampal long-term potentiation. Brain Res. **608:** 259–265, 1993.

SMITH, R. D., AND GOLDIN, A. L.: Protein kinase-A phosphorylation enhances sodium channel currents in Xenopus oocytes. Am. J. Physiol. **263:** C660–C666, 1992.

SMITH, S. E., AND MELDRUM, B. S.: Cerebroprotective effect of lamotrigine after focal ischemia in rats. Stroke **26:** 117–122, 1995.

SOKOLOFF, L.: Sites and mechanisms of function-related changes in energy metabolism in the nervous system. Dev. Neurosci. **15:** 194–206, 1993.

SOKOLOFF, L., REIVICH, M., KENNEDY, C., DES ROSIERS, M. H., PATLAK, C. S., PETTIGREW, K. D., SAKURADA, O., AND SHINOHARA, M.: The [^{14}C]deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat. J. Neurochem. **28:** 897–916, 1977.

SÖLLNER, T., AND ROTHMAN, J. E.: Neurotransmission: harnessing fusion machinery at the synapse. Trends Neurosci. **17:** 344–349, 1994.

SOMJEN, G. G., AITKEN, P. G., BALESTRINO, M., HERRERAS, O., AND KAWASAKI, K.: Spreading depression-like depolarization and selective vulnerability of neurons: a brief review. Stroke **21**(suppl. III): 179–183, 1990.

SONTHEIMER, H.: Voltage-dependent ion channels in glial cells. Glia **11:** 156–172, 1994.

SONTHEIMER, H., FERNANDEZ-MARQUES, E., ULLRICH, N., PAPPA, C. A., AND WAXMAN, S. G.: Astrocyte Na^+ channels are required for maintenance of Na^+/K^+-ATPase activity. J. Neurosci. **14:** 2464–2475, 1994.

SORBERA, L. A., AND MORAD, M.: Atrionatriuretic peptide transforms cardiac sodium channels into calcium-conducting channels. Science (Wash. DC) **247:** 969–973, 1990.

SPARACO, M., BONILLA, E., DIMAURO, S., AND POWERS, J. M.: Neuropathology of mitochondrial encephalomyopathies due to mitochondrial DNA effects. J. Neuropathol. Exp. Neurol. **52:** 1–10, 1993.

SPEDDING, M., AND PAOLETTI, R.: III. Classification of calcium channels and the sites of action of drugs modifying channel function. Pharmacol. Rev. **44:** 363–375, 1992.

SPETZLER, R. F., AND HADLEY, M. N.: Protection against cerebral ischemia: the role of barbiturates. Cerebrovasc. Brain Metab. Rev. **1:** 212–229, 1989.
STAFSTROM, C. E., SCHWINDT, P. C., CHUBB, M. C., AND CRILL, W. E.: Properties of persistent sodium conductance and calcium conductance of layer V neurons from cat sensorimotor cortex in vitro. J. Neurophysiol. **53:** 153–170, 1985.
STARMER, C. F., NESTERENKO, V. V., GILIAN, F. R., AND GRANT, A. O.: Use of ionic currents to identify and estimate parameters in models of channel blockade Am. J. Physiol. **259:** H626–H634, 1990.
STAUB, F., WINKLER, A., PETERS, J., KEMPSKI, O., AND BAETHMANN, A.: Mechanisms of glial swelling by arachidonic acid. Acta Neurochir. Suppl. (Wien) **60:** 20–23, 1994.
STEEN, P. A., AND MICHENFELDER, J. D.: Mechanisms of barbiturate protection. Anesthesiology **53:** 183–185, 1980.
STEEN, P. A., MILDE, J. H., AND MICHENFELDER, J. D.: Cerebral metabolic and vascular effects of barbiturate therapy following complete global ischemia. J. Neurochem. **31:** 1317–1324, 1978.
STEWART, M. A., PASSONNEAU, J. V., AND LOWRY, O. H.: Substrate changes in peripheral nerve during ischaemia and Wallerian degeneration. J. Neurochem. **12:** 719–727, 1965.
STOLC, S.: Comparison of effects of selected local anesthetics on sodium and potassium channels in mammalian neurons. Gen. Physiol. Biophys. **7:** 177–189, 1988.
STONE, T. W.: Receptors for adenosine and adenine nucleotides. Gen. Pharmacol. **22:** 25–31, 1991.
STRANG, R. H. C., AND BACHELARD, H. S.: Rates of cerebral glucose utilization in rats anaesthetized with phenobarbitone. J. Neurochem. **20:** 987–996, 1973.
STRICHARTZ, G. R.: The inhibition of sodium currents in myelinated nerve by quaternary derivatives of lidocaine. J. Gen. Physiol. **62:** 37–57, 1973.
STRONG, A. J., VENABLES, G. S., AND GIBSON, G.: The cortical ischaemic penumbra associated with occlusion of the middle cerebral artery in the cat. 1. Topography of changes in blood flow, potassium ion activity, and EEG. J. Cereb. Blood Flow Metab. **3:** 86–96, 1983.
STÜHMER, W., CONTI, F., SUZUKI, H., WANG, X., NODA, M., YAHAGI, N., KUBO, H., AND NUMA, S.: Structural parts involved in activation and inactivation of the sodium channel. Nature (Lond.) **339:** 597–603, 1989.
STUTZMANN, J. M., CINTRAT, P., LAUDURON, P. M., AND BLANCHARD, J. C.: Riluzole antagonizes the anxiogenic properties of the beta-carboline FG 7142 in rats. Psychopharmacology **99:** 515–519, 1989.
STUTZMANN, J. M., LUCAS, M., BLANCHARD, J. C., AND LADURON, P. M.: Riluzole, a glutamate antagonist, enhances slow wave and REM sleep in rats. Neurosci. Lett. **88:** 195–200, 1988.
STUTZMANN, J. M., MIGNANI, S., DEBARNOT, F., RATAUD, J., PIOT, O., PAUCHET, C., JIMONET, P., REIBAUD, M., MALGOURIS, C., UZAN, A., PRATT, J., BLANCHARD, J. C., AND BARREAU, M.: Pharmacological profile and neuroprotective activities of RP 66055, a riluzole derivative, in rodents. Soc. Neurosci. Abstr. **19:** 1647, 1993.
STYS, P. K.: Protective effects of antiarrhythmic agents against anoxic injury in CNS white matter. J. Cereb. Blood Flow Metab. **15:** 425–432, 1995.
STYS, P. K., RANSOM, B. R., AND WAXMAN, S. G.: Effects of polyvalent cations and dihydropyridine calcium channel blockers on recovery of CNS white matter from anoxia. Neurosci. Lett. **115:** 293–299, 1990a.
STYS, P. K., RANSOM, B. R., AND WAXMAN, S. G.: Tertiary and quaternary local anesthetics protect CNS white matter from anoxic injury at concentrations that do not block excitability. J. Neurophysiol. **67:** 236–240, 1992a.
STYS, P. K., RANSOM, B. R., WAXMAN, S. G., AND DAVIES, P. K.: Role of extracellular calcium in anoxic injury of mammalian central white matter. Proc. Natl. Acad. Sci. USA **87:** 4212–4216, 1990b.
STYS, P. K., SONTHEIMER, H., RANSOM, B. R., AND WAXMAN, S. G.: Noninactivating, tetrodotoxin-sensitive Na^+ conductance in rat optic nerve axons. Proc. Natl. Acad. Sci. USA **90:** 6976–6980, 1993.
STYS, P. K., WAXMAN, S. G., AND RANSOM, B. R.: Na^+-Ca^{2+} exchanger mediates Ca^{2+} influx during anoxia in mammalian central nervous system white matter. Ann. Neurol. **30:** 375–380, 1991.
STYS, P. K., WAXMAN, S. G., AND RANSOM, B. R.: Ionic mechanism of anoxic injury in mammalian CNS white matter: role of Na^+ channels and Na^+-Ca^{2+} exchanger. J. Neurosci. **12:** 430–439, 1992b.
SUAREZ, R. K., DOLL, C. J., BUIE, A. E., WEST, T. G., FUNK, G. D., AND HOCHACHKA, P. W.: Turtles and rats: a biochemical comparison of anoxia-tolerant and anoxia-sensitive brains. Am. J. Physiol. **257:** R1083–R1088, 1989.
SUGIMORI, M., AND LLINAS, R.: Lidocaine differentially blocks fast and slowly inactivating sodium conductance in Purkinje cells: an in vitro study in guinea pig cerebellum using iontophoretic glutamic acid. Soc. Neurosci. Abstr. **6:** 468, 1980.
SUN, F.-Y., AND FADEN, A. I.: Neuroprotective effect of 619C89, a use-dependent sodium channel blocker, in rat traumatic brain injury. Brain Res. **673:** 133–140, 1995.
SURMEIER, D. J., AND KITAI, S. T.: D_1 and D_2 dopamine receptor modulation of sodium and potassium currents in rat neostriatal neurons. Prog. Brain Res. **99:** 309–324, 1993.
SUTHERLAND, G., ONG, B. Y., LOUW, D., AND SIMA, A. A. F.: Effect of lidocaine on forebrain ischemia in rats. Stroke **20:** 119–122, 1989.
SWAN, J. H., AND MELDRUM, B. S.: Protection by NMDA antagonists against selective cell loss following transient ischaemia. J. Cereb. Blood Flow Metab. **10:** 343–351, 1990.
SWENSON, R. P., JR.: Inactivation of potassium current in squid axon by a variety of quaternary ammonium ions. J. Gen. Physiol. **77:** 255–271, 1981.
SYMON, L., TAYLOR, D. L., AND OBRENOVITCH, T. P.: Aspect of acid-base homeostasis in ischemia. *In* Cerebral Ischemia and Basic Mechanisms, ed. by A. Hartmann, F. Yatsu and W. Kuschinski, pp. 51–57, Springer-Verlag, Berlin, 1994.
SZATKOWSKI, M., AND ATTWELL, D.: Triggering and execution of neuronal death in brain ischaemia: two phase of glutamate release by different mechanisms. Trends Neurosci. **17:** 359–365, 1994.
SZATKOWSKI, M., BARBOUR, B., AND ATTWELL, D.: Non-vesicular release of glutamate from glial cells by reversed electrogenic glutamate uptake. Nature (Lond.) **348:** 443–446, 1990.
TAFT, W. C., CLIFTON, G. L., BLAIR, R. E., AND DELORENZO, R. J.: Phenytoin protects against ischemia-produced neuronal cell death. Brain Res. **483:** 143–148, 1989.
TAGLIALATELA, M., DI RENZO, G., AND ANNUNZIATO, L.: Na^+-Ca^{2+} exchange activity in central nerve endings. I. Ionic conditions that discriminate 45Ca2+ uptake through the exchanger from that occurring through voltage-operated Ca2+ channels. Mol. Pharmacol. **38:** 385–392, 1990.
TAKAGAKI, G.: Control of aerobic glycolysis and pyruvate kinase activity in cerebral cortex slices. J. Neurochem. **15:** 903–916, 1968.
TAKAGI, H., TAHASHIMA, M., LIOU, S.-Y., AND KUNIHARA, M.: Effects of KB-2796, a novel Ca^{2+} channel blocker, on spreading depression and Ca^{2+} uptake in rat hippocampal slices. Jpn. J. Pharmacol. **64**(suppl. 1)**:** O-302, 1994.
TAKAHASHI, K., WAKAMORI, M., AND AKAIKE, N.: Hippocampal CA1 pyramidal cells of rats have four voltage-dependent calcium conductances. Neurosci. Lett. **104:** 229–234, 1989.
TAKAHASHI, M., LIOU, S.-Y., AND KUNIHARA, M.: Ca^{2+}- and Cl^--dependent, NMDA receptor-mediated neuronal death induced by depolarization in rat hippocampal organotypic cultures. Brain Res. **675:** 249–256, 1995.
TAKIGAWA, T., YASUDA, H., KIKKAWA, R., SHIGETA, Y., SAIDA, T., AND KITASATO, H.: Antibodies against GM_1 ganglioside affect K^+ and Na^+ currents in isolated rat myelinated nerve fibers. Ann. Neurol. **37:** 436–442, 1995.
TANABE, T., TAKESHIMA, H., MIKAMI, A., FLOCKERZI, V., TAKAHASHI, H., KANGAWA, K., KOJIMA, M., MATSUO, H., HIROSE, T., AND NUMA, S.: Primary structure of the receptor for calcium channel blockers from skeletal muscle. Nature (Lond.) **328:** 313–318, 1987.
TARR, M., BRADA, D., SAMSON, F. E., JR.: Cerebral-high energy phosphate. Am. J. Physiol. **203:** 690–692, 1962.
TASKER, R. C., COYLE, J. T., AND VORNOV, J. J.: The regional vulnerability to hypoglycemia-induced neurotoxicity in organotypic hippocampal culture: protection by early tetrodotoxin or delayed MK-801. J. Neurosci. **12:** 4298–4308, 1992.
TAYLOR, C. P.: Na^+ currents that fail to inactivate. Trends Neurosci. **16:** 455–460, 1993.
TAYLOR, D. L., RICHARDS, D. A., OBRENOVITCH, T. P., AND SYMON, L.: Time course of changes in extracellular lactate evoked by transient K^+-induced depolarisation in the rat striatum. J. Neurochem. **62:** 2368–2374, 1994.
TAYLOR, R. E.: Effect of procaine on electrical properties of squid axon membrane. Am. J. Physiol. **196:** 1071–1078, 1959.
TEGTMEIER, F., SCHELLER, D., URENJAK, J., KOLB, J., PETERS, U., BOCK, A., AND HÖLLER, M.: Flunarizine improves postischemic energy metabolism and flow after global ischemia of the rat brain. *In* Pharmacology of Cerebral Ischemia 1988, ed. by J. Krieglstein, pp. 91–96, Elsevier, Amsterdam, 1989.
TEGTMEIER, F., XIE, Y., SCHELLER, D., GLEITZ, J., OSIKOWSKA-EVERS, B., AND PETERS, T.: The role of sodium channels in cerebral ischemia. Wiss. Zeitschrift Humboldt-Uni, Berlin **41:** 45–53, 1992.
TERLAU, H., HEINEMANN, S. H., STÜHMER, W., PUSCH, M., CONTI, F., IMOTO, K., AND NUMA, S.: Mapping the site of block by tetrodotoxin and saxitoxin of sodium channel-II. FEBS Lett. **293:** 213–216, 1991.
THIO, C. L., AND SONTHEIMER, H.: Differential modulation of TTX-sensitive and TTX-resistant Na^+ channels in spinal cord astrocytes following activation of protein kinase C. J. Neurosci. **13:** 4889–4897, 1993.
THOMSEN, W., HAYS, S. J., HICKS, J. L., SCHWARTZ, R. D., AND CATTERALL, W. A.: Specific binding of the novel Na^+ channel blocker PD85,639 to the α subunit of the rat brain Na^+ channel. Mol. Pharmacol. **43:** 955–964, 1993.
THURSTON, J. H., AND MCDOUGAL, D. B., JR.: Effect of ischemia on metabolism of the brain of the newborn mouse. Am. J. Physiol. **216:** 348–352, 1969.
TIBBS, G. R., BARRIE, A. P., VAN MIEGHEM, F. J. E., MCMAHON, H. T., AND NICHOLLS, D. G.: Repetitive action potentials in isolated nerve terminals in the presence of 4-aminopyridine: effects on cytosolic free Ca^{2+} and glutamate release. J. Neurochem. **53:** 1693–1699, 1989.
TOBIASZ, C., AND NICHOLSON, C.: Tetrodotoxin resistant propagation and extracellular sodium changes during spreading depression in rat cerebellum. Brain Res. **241:** 329–333, 1982.
TOLKOVSKY, A. M., AND RICHARDS, C. D.: Na^+/H^+ exchange is the major mechanism of pH regulation in cultured sympathetic neurons: measurements in single cell bodies and neurites using a fluorescent pH indicator. Neuroscience **22:** 1093–1102, 1987.
TOMBAUGH, G. C., AND SAPOLSKY, R. M.: Evolving concepts about the role of acidosis in ischemic neuropathology. J. Neurochem. **61:** 793–803, 1993.
TOWMBLY, D. A., YOSHII, M., AND NARAHASHI, T.: Mechanisms of calcium

channel block by phenytoin. J. Pharmacol. Exp. Ther. **246:** 189–195, 1988.
TRIMMER, J. S., AND AGNEW, W. S.: Molecular diversity of voltage-sensitive Na channels. Annu. Rev. Physiol. **51:** 401–418, 1989.
TRIMMER, J. S., COOPERMAN, S. S., TOMIKO, S. A., ZHOU, J. Y., CREAN, S. M., BOYLE, M. B., KALLEN, R. G., SHENG, Z. H., BARCHI, R. L., SIGWORTH, F. J., GOODMAN, R. H., AGNEW, W. S., AND MANDEL, G.: Primary structure and functional expression of a mammalian skeletal muscle sodium channel. Neuron **3:** 33–49, 1989.
TRIPPENBACH, T., RICHTER, D. W., AND ACKER, H.: Hypoxia and ion activities within the brain stem of newborn rabbits. J. Appl. Physiol. **68:** 2494–2503, 1990.
TURNER, R. W., BAIMBRIDGE, K. G., AND MILLER, J. J.: Calcium-induced long-term potentiation in the hippocampus. Neuroscience **7:** 1411–1416, 1982.
TYMIANSKI, M., CHARLTON, M. P., CARLEN, P. L., AND TATOR, C. H.: Source specificity of early calcium neurotoxicity in cultured embryonic spinal neurons. J. Neurosci. **13:** 2085–2104, 1993.
TYTGAT, J., VEREECKE, J., AND CARMELIET, E.: A combined study of sodium current and T-type calcium current in isolated cardiac cells. Pflueg. Arch. Eur. J. Physiol. **417:** 142–148, 1990.
UEDA, Y., OBRENOVITCH, T. P., LOK, S.-Y., SARNA, G. S., AND SYMON, L.: Efflux of glutamate produced by short ischemia of varied severity in the rat striatum. Stroke **23:** 253–259, 1992a.
UEDA, Y., OBRENOVITCH, T. P., LOK, S.-Y., SARNA, G. S., AND SYMON, L.: Changes in extracellular glutamate concentration produced in the rat striatum by repeated ischemia. Stroke **23:** 1125–1131, 1992b.
UEMATSU, D., GREENBERG, J. H., REIVICH, M., AND KARP, A.: In vivo measurement of cytosolic free calcium during ischemia and reperfusion. Ann. Neurol. **24:** 420–428, 1988.
ULTSCH, G. R.: The viability of neartic freshwater turtles submerged in anoxia and normoxia at 3 and 10°C. Comp. Biochem. Physiol. A. Comp. Physiol. **81:** 607–611, 1985.
URBAN, L., NEILL, K. H., CRAIN, B. J., NADLER, J. V., AND SOMJEN, G. G.: Postischemic synaptic physiology in area CA1 of the gerbil hippocampus studied in vitro. J. Neurosci. **9:** 3966–3975, 1989.
URENJAK, J., TEGTMEIER, F., BEILE, A., KHAN, S., AND PETERS, T. H.: Synaptosomal respiration: a potent indicator of veratridine-induced Na influx. Pharmacology **43:** 26–35, 1991.
VAN DEN BERG, R. J., KOK, P., AND VOSKUYL, R. A.: Valproate and sodium currents in cultured hippocampal neurons. Exp. Brain Res. **93:** 279–287, 1993.
VAN REEMPTS, J., VAN DEUREN, B., HASELDONCKX, M., VAN DE VEN, M., AND BORGERS, M.: Structural cell damage and regional blood flow in the hypoxic brain: effects of treatment with R 58 735, a novel antihypoxic drug. *In* Pharmacology of Cerebral Ischemia, ed. by J. Krieglstein, pp. 385–390, Elsevier, Amsterdam, 1986.
VAN REEMPTS, J., VAN DEUREN, B., VAN DE VEN, M., CORNELISSEN, F., AND BORGERS, M.: Flunarizine reduces cerebral infarct size after photochemically induced thrombosis in spontaneously hypertensive rats. Stroke **18:** 1113–1119, 1987.
VASSILEV, P. M., SCHEUER, T., AND CATTERALL, W. A.: Identification of an intracellular peptide segment involved in sodium channel inactivation. Science (Wash. DC) **241:** 1658–1661, 1988.
VASSILEV, P., SCHEUER, T., AND CATTERALL, W. A.: Inhibition of inactivation of single sodium channels by a site-directed antibody. Proc. Natl. Acad. Sci. USA **86:** 8147–8151, 1989.
VELLY, J., GRIMA, M., MARCINIAK, G., SPACH, M. O., AND SCHWARTZ, J.: Effects of some antianginal and vasodilating drugs on sodium influx and on the binding of ^{3}H-batrachotoxinin-A 20-α-benzoate and ^{3}H-tetracaine. Naunyn Schmiedeberg's Arch. Pharmacol. **335:** 176–182, 1987.
VERDONCK, F., BIELEN, F. V., AND VER DONCK, L.: Preferential block of the veratridine-induced, non-inactivating Na^+ current by R56865 in single cardiac Purkinje cells. Eur. J. Pharmacol. **203:** 371–378, 1991.
VOLLMER, B., MEUTER, C., AND JANSSEN, P. A.: R 56865 prevents electrical and mechanical signs of ouabain intoxication in guinea-pig papillary muscle. Eur. J. Pharmacol. **142:** 137–140, 1987.
VORNOV, J. J., TASKER, R. C., AND COYLE, J. T.: Delayed protection by MK-801 and tetrodotoxin in a rat organotypic hippocampal culture model of ischemia. Stroke **25:** 457–465, 1994.
WAHL, F., ALLIX, M., PLOTKINE, M., AND BOULU, R. G.: Effect of riluzole on focal cerebral ischemia in rats. Eur. J. Pharmacol. **230:** 209–214, 1993.
WAHL, F., OBRENOVITCH, T. P., HARDY, A. M., PLOTKINE, M., BOULU, R., AND SYMON, L.: Extracellular glutamate during focal cerebral ischaemia in rats: time course and calcium-dependency. J. Neurochem. **63:** 1003–1011, 1994.
WAKADE, A. R., PRZYWARA, D. A., BHAVE, S. V., CHOWDHURY, P. S., BHAVE, A., AND WAKADE, T. D.: Massive exocytosis triggered by sodium-calcium exchange in sympathetic neurons is attenuated by co-culture with cardiac cells. Neuroscience **55:** 813–821, 1993.
WAMIL, A. W., AND MCLEAN, M. J.: Phenytoin blocks N-methyl-D-aspartate responses of mouse central neurons. J. Pharmacol. Exp. Ther. **267:** 218–227, 1993.
WARNER, D. S., GODERSKY, J. C., AND SMITH, M. L.: Failure of preischemic lidocaine administration to ameliorate global ischemic brain damage in the rat. Anesthesiology **68:** 73–78, 1988.
WATSON, B. D.: Evaluation of the concomitance of lipid peroxidation in experimental models of cerebral ischemia and stroke. Prog. Brain Res. **96:** 69–95, 1993.
WAUQUIER, A., ASHTON, D., AND CLINCKE, G. H.: Brain ischemia as a target for Ca^{2+} entry blockers. Ann. N. Y. Acad. Sci. **522:** 478–490, 1988.
WAUQUIER, A., ASHTON, D., CLINCKE, G., FRANSEN, J., GILLARDIN, J. M., AND JANSSEN, P. A. J.: Anticonvulsant profile of flunarizine. Drug Dev. Res. **7:** 49–60, 1986.
WAUQUIER, A., CLINCKE, G., ASHTON, D., RYCK, M., DE FRANSEN, J., VAN CLEMEN, G., AND JANSSEN, C. O.: A new antihypoxic drug with anticonvulsant properties and possible effects on cognitive functions. Drug Dev. Res. **8:** 373–380, 1986.
WAXMAN, S. G.: Sodium channel blockade by antibodies: A new mechanism of neurological disease? Ann. Neurol. **37:** 421–423, 1995.
WAXMAN, S. G., BLACK, J. A., RANSOM, B. R., AND STYS, P. K.: Anoxic injury of rat optic nerve: ultratructural evidence for coupling between Na^+ influx and Ca^{2+}-mediated injury in myelinated CNS axons. Brain Res. **644:** 197–204, 1994.
WAXMAN, S. G., RANSOM, B. R., STYS, P. K.: Non-synaptic mechanisms of Ca^{2+}-mediated injury in CNS white matter. Trends Neurosci. **14:** 461–468, 1991.
WEBER, M. L., AND TAYLOR C. P.: Damage from oxygen and glucose deprivation in hippocampal slices is prevented by tetrodotoxin, lidocaine and phenytoin without blockade of action potentials. Brain Res. **664:** 167–177, 1994.
WENER, J., APECECHEA, M., SCHALTENBRAND, R., AND FENZL, E.: Efficacy and tolerance of vinpocetine administered intravenously, in addition to standard therapy, to patients suffering from an apoplectic insult. *In* Pharmacology of Cerebral Ischemia, ed. by J. Krieglstein, pp. 430–434, Elsevier, Amsterdam, 1986.
WERBROUCK, L., MEGENS, A. A. H. P., STOKBROEKX, R. A., AND NIEMEGEERS, C. J. E.: Comparison of the in vivo pharmacological profiles of sabeluzole and its enantiomers. Drug Dev. Res. **24:** 41–51, 1991.
WERMELSKIRCHEN, D., GLEITZ, J., URENJAK, J., WILFFERT, B., TEGTMEIER, F., AND PETERS, T. H.: Flunarizine and R 56865 suppress veratridine-induced increase in oxygen consumption and $^{45}Ca^{2+}$ uptake in rat cortical synaptosomes. Neuropharmacology **31:** 235–241, 1992.
WEST, J. W., NUMANN, R., MURPHY, B. J., SCHEUER, T., AND CATTERALL, W. A.: A phosphorylation site in the Na^+ channel required for modulation by protein kinase C. Science (Wash. DC) **254:** 866–868, 1991.
WEST, J. W., PATTON, D. E., SCHEUER, T., WANG, Y., GOLDIN, A. L., AND CATTERALL, W. A.: A cluster of hydrophobic amino acid residues required for fast Na^+-channel inactivation. Proc. Natl. Acad. Sci. USA **89:** 10910–10914, 1992a.
WEST, J. W., SCHEUER, T., MAECHLER, L., CATTERALL, W. A.: Efficient expression of rat brain type IIA Na^+ channel α subunits in a somatic cell line. Neuron **8:** 59–70, 1992b.
WHITE, B. C., GROSSMAN, L. I., AND KRAUSE, G. S.: Brain injury by global ischemia and reperfusion: a theoretical perspective on membrane damage and repair. Neurology **43:** 1656–1665, 1993.
WHITTAM, R.: The dependence of the respiration of brain cortex on active cation transport. Biochem. J. **82:** 205–212, 1962.
WIARD, R. P., DICKERSON, M. C., BEEK, O., NORTON, R., AND COOPER, B. R.: Neuroprotective properties of the novel antiepileptic lamotrigine in a gerbil model of global cerebral ischemia. Stroke **26:** 466–472, 1995.
WIELOCH, T., CARDELL, M., BINGREN, H., ZIVIN, J., SAITOH, T.: Changes in the activity of protein kinase C and the differential subcellular redistribution of its isozymes in the rat striatum during and following transient forebrain ischemia. J. Neurochem. **56:** 1227–1235, 1991.
WILHELM, D. I., LOER, G., AND PETERS, D.: Differential influence of various calcium entry blockers on ouabain toxicity in rat left atria. Naunyn Schmiedeberg's Arch. Pharm. **332:** 44R, 1986.
WILLIAMS, J. H., ERRINGTON, M. L., LYNCH, M. A., AND BLISS, T. V. P.: Arachidonic acid induces a long-term activity-dependent enhancement of synaptic transmission in the hippocampus. Nature (Lond.) **341:** 739–742, 1989.
WILLOW, M., AND CATTERALL, W. A.: Inhibition of binding of [^{3}H]batrachotoxinin A 20-α-benzoate to sodium channels by the anticonvulsant drugs diphenylhydantoin and carbamazepine. Mol. Pharmacol. **22:** 627–635, 1982.
WILLOW, M., GONOI, T., AND CATTERALL, W. A.: Voltage clamp analysis of the inhibitory actions of diphenylhydantoin and carbamazepine on voltage-sensitive sodium channels in neuroblastoma cells. Mol. Pharmacol. **27:** 549–558, 1985.
WILLOW, M., KUENZEL, E. A., AND CATTERALL, W. A.: Inhibition of voltage-sensitive sodium channels in neuroblastoma cells and synaptosomes by the anticonvulsant drugs diphenylhydantoin and carbamazepine. Mol. Pharmacol. **25:** 228–234, 1984.
XIA, Y, AND HADDAD, G. G.: Neuroanatomical distribution and binding properties of saxitoxin sites in the rat and turtle CNS. J. Comp. Neurol. **330:** 363–380, 1993.
XIA, Y., AND HADDAD, G. G.: Postnatal development of voltage-sensitive Na^+ channels in rat brain. J. Comp. Neurol. **345:** 279–287, 1994.
XIE, X., PEAKMAN, T., GARTHWAITE, J., AND LANCASTER, B.: Inactivated sodium channels and use-dependent action of the anticonvulsant lamotrigine. Soc. Neurosci. Abstr. **20:** 718, 1994a.
XIE, X. M., AND GARTWAITE, J.: State-dependent block of recombinant rat brain type IIA Na channels by the neuroprotectant 619C89. Brain Res. Assoc. Abstr. **12:** 74, 1995.

XIE, Y., DENGLER, K., ZACHARIAS, E., WILFFERT, B., AND TEGTMEIER, F.: Effects of the sodium channel blocker tetrodotoxin (TTX) on cellular ion homeostasis in rat brain subjected to complete ischemia. Brain Res. **652:** 216–224, 1994b.

XIE, Y., SEO, K., ISHIMARU, K., AND HOSSMANN, K. -A.: Effect of calcium antagonists on postischemic protein biosynthesis in gerbil brain. Stroke **23:** 87–92, 1992.

XIE, Y., ZACHARIAS, E., HOFF, P., AND TEGTMEIER, F.: Ion channel involvement in anoxic depolarization induced by cardiac arrest in rat brain. J. Cereb. Blood Flow Metab. **15:** 587–594, 1995.

YAARI, Y., SELZER, M. E., AND PINCUS, J. H.: Phenytoin: mechanisms of its anticonvulsant action. Ann. Neurol. **20:** 171–184, 1986.

YAMAMOTO, D., YEH, J. Z., AND NARAHASHI, T.: Voltage-dependent calcium block of normal and tetramethrin-modified single sodum channels. Biophys. J. **45:** 337–344, 1984.

YAMASAKI, Y., KOGURE, K., HARA, H., BAN, H., AND AKAIKE, N.: The possible involvement of tetrodotoxin-sensitive ion channels in ischemic neuronal damage in the rat hippocampus. Neurosci. Lett. **121:** 251–254, 1991.

YAMASHITA, A., OZAKI, A., IKEGAMI, A., HAYASHI, A., HARA, H., SUKAMOTO, T., AND ITO, K.: Effects of a new diphenylpiperazine calcium antagonist, KB-2796, on cerebral ischemic neuronal damage in rats. Gen. Pharmacol. **24:** 1473–1480, 1993.

YAROWSKI, P., BOYNE, A. F., WIERWILLE, R., AND BROOKES, N.: Effect of monensin on deoxyglucose uptake in cultured astrocytes: energy metabolism is coupled to sodium entry. J. Neurosci. **6:** 859–866, 1986.

YOSHIDOMI, M., HAYASHI, T., ABE, K., AND KOGURE, K.: Effect of a new calcium channel blocker, KB-2796, on protein synthesis of the CA1 pyramidal cell and delayed neuronal death following transient forebrain ischemia. J. Neurochem. **53:** 1589–1594, 1989.

YOUNG, A. M., FOLEY, P. M., AND BRADFORD, H. F.: Preloading in vivo: a rapid and reliable method for measuring gamma-aminobutyric acid and glutamate fluxes by microdialysis. J. Neurochem. **55:** 1060–1063, 1990.

ZACZEK, R., NELSON, M. F., AND COYLE, J. T.: Effects of anaesthetics and anticonvulsants on the action of kainic acid in the rat hippocampus. Eur. J. Pharmacol. **52:** 323–327, 1978.

ZAMPONI, G. W., AND FRENCH, R. J.: Transcainide causes two modes of open-channel block with different voltage sensitivities in batrachotoxin-activated sodium channels. Biophys. J. **67:** 1028–1039, 1994.

ZILBERTER, Y. I., UTESHEV, V. V., SOKOLOVA, S. N., MOTIN, L. G., EREMHJAN, H. H.: Potentiation of glutamate-activated currents in isolated hippocampal neurons. Neuron **5:** 597–602, 1990.

ZIMANYI, I., WANG, E., LAJTHA, A., AND REITH, M. E.: Effect of cocaine and cocaine congeners on veratridine-induced depolarization in mouse cerebrocortical synaptoneurosomes. J. Neurosci. Res. **22:** 201–208, 1989.

ZIVIN, J. A., AND CHOI, D. W.: Stroke therapy. Sci. Am. **265:** 36–43, 1991.