

Neuroprotection – rationale for pharmacological modulation of Na⁺-channels

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Summary. The primary factor detrimental to neurons in neurological disorders associated with deficient oxygen supply or mitochondrial dysfunction is insufficient ATP production relative to their requirement. As a large part of the energy consumed by brain cells is used for maintenance of the Na⁺ gradient across the cellular membrane, reduction of energy demand by down-modulation of voltage-gated Na⁺-channels is a rational strategy for neuroprotection. In addition, preservation of the inward Na⁺ gradient may be beneficial because it is an essential driving force for vital ion exchanges and transport mechanisms such as Ca²⁺ homeostasis and neurotransmitter uptake.

Keywords: Neuroprotection – Na⁺-channels – Na⁺-channel blockers – Ischaemia – Energy deprivation – Traumatic brain injury

Introduction

Voltage-gated Na⁺-channels are responsible for initiation and conduction of the neuronal action potential and, therefore, play a fundamental role in the normally functioning 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. This article illustrates that selective down-modulation of voltage-gated Na⁺-channels is, *in itself*, a rational and effective approach to protect brain tissue in conditions associated with defective energy supply (e.g. ischaemia) or metabolism (i.e. mitochondrial abnormalities). For more details on this topic, see Urenjak and Obrenovitch (1996), and Ames III (1997).

Brain cellular ion homeostasis and energy requirement

With anoxia, ischaemia and mitochondrial dysfunction, the primary factor detrimental to neurons is insufficient energy supply relative to their requirement. Accordingly, reduction of energy demand is a rational neuroprotective

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strategy, which probably underlies beneficial effects of hypothermia (Ginsberg et al., 1992) and barbiturates (Spetzler and Hadley, 1989). Down-modulation of voltage-gated Na^+ -channels is another effective way to reduce ATP demand because a large part of the energy consumed by nerve cells is used for the maintenance and replenishment of ionic gradients (especially Na^+ gradient) across the cellular membrane (Erecinska and Silver, 1989; Urenjak et al., 1991; Silver et al., 1997). Even under barbiturate anaesthesia, which markedly reduces the functional state of the brain and hence its metabolic rate, 50% of the residual energy is still required to compensate for fluxes of Na^+ and K^+ across the cellular membrane (Astrup, 1982). *In vitro* studies are consistent with this notion. For example, simultaneous measurement of oxygen consumption and lactate production of a retina preparation in darkness indicated that Na^+ transport by Na^+/K^+ -ATPase accounted for about half of all energy expenditure (Ames et al., 1992). Therefore, a direct positive effect of the modulation of voltage-gated Na^+ -channels in neurological disorders associated with ATP depletion is reduction of brain cells energy demand.

Intracellular Na^+ loading: acute and indirect neurotoxicity

Excessive Na^+ entry into neurons, subsequent to either energy depletion or Na^+ -channel activators (e.g. veratridine), is clearly hazardous to their survival. 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). Microdialysis application of veratridine to the rat striatum produced recurrent spreading depression superimposed on persistent negative shifts of the extracellular direct current potential (Obrenovitch and Urenjak, unpublished observation) and a tetrodotoxin (TTX)-sensitive efflux of amino acid neurotransmitters in the rat striatum (Young et al., 1990) and spinal cord (Skilling et al., 1988). Severe anoxia caused a rapid increase of intracellular concentrations of Ca^{2+} and Na^+ in adult CA1 hippocampal neurons, followed by swelling and bleb formation. Only replacement of extracellular Na^+ with the impermeant *N*-methyl-D-glucamine prevented anoxia-induced neuronal injury (Friedman and Haddad, 1994).

In addition, as the inwardly directed gradient of Na^+ across the cellular membrane is the driving force behind a number of exchange/transport mechanisms, sustaining this gradient implies preservation of several vital processes. These include intracellular Ca^{2+} -homeostasis (Siesjö and Bengtsson, 1989), intracellular acid-base regulation (Hakim and Shoubridge, 1989; Obrenovitch et al., 1990b), control of cellular volume (Hansen, 1985; Kempinski et al., 1988), and neurotransmitter uptake (Nicholls and Attwell, 1990).

Potential neuroprotection by inhibition of persistent Na^+ currents

Na^+ -channel blockers may also be protective by reducing Na^+ currents which do not conform to the characteristics of the classic TTX-sensitive, fast Na^+ current responsible for action potential generation. For example, a small fraction of Na^+ currents fails to inactivate even with prolonged depolarization

(Taylor, 1993). These sustained or persistent Na⁺ currents have been characterized in a variety of neurons, and in glial cells where they may provide a substrate for Na⁺-K⁺ exchange (Taylor, 1993). As these persistent currents are TTX-sensitive however, the finding that TTX did not alter the kinetic of Na⁺-influx associated with anoxic depolarization (Xie et al., 1994) conflicts with this hypothesis. Another site of action for Na⁺-channel modulators in ischaemia may be the TTX-insensitive, *slow* Na⁺ current evidenced in striatal and hippocampal neurons (Hoehn et al., 1993).

Down-modulation of Na⁺-channels – an inherent strategy against energy deprivation

Reduced density of Na⁺-channels, and their inherent down-regulation during oxygen deprivation, decrease membrane excitability and cut down energy expenditure. These changes were demonstrated to contribute to the increased tolerance of the immature brain to hypoxia (Xia and Haddad, 1994) and to the remarkable ability of some water turtles to survive anoxia (Xia and Haddad, 1993). Voltage-sensitive Na⁺ currents are much smaller in newborn than in adult cortical neurons (Cummins et al., 1994) and excitatory postsynaptic potentials (EPSPs) were depressed by 90% within two min of anoxia in the adult, but only by 44% in the newborn animals, and post-anoxic recovery was much more rapid in the latter (Cherubini et al., 1989). In turtle synaptosomes, the Na⁺-channel density is about 1/3 of that in rat synaptosomes (Edwards et al., 1989), and a further decline in number and conductance of Na⁺-channels occur in anoxia or whenever Na⁺-influx was increased by Na⁺-channel activators such as veratridine (Pérez-Pinzon et al., 1992; Dargent and Couraud, 1990). Reduction in the number and conductance of Na⁺-channels during anoxia may be mediated by second-messenger systems, via an increase in cAMP-dependent protein kinase or protein kinase C activity or an involvement of adenosine.

Although emphasis is here placed on Na⁺-channels, it is important to note that other changes in neuronal membrane ion conductance contribute to decreasing neuronal excitability during anoxia and metabolic inhibition (e.g. Ca²⁺ and ATP-dependent-K⁺ conductance, Ca²⁺ dependent-Cl⁻ current, L-type Ca²⁺ current) (Obrenovitch et al., 1990a; Obrenovitch, 1997).

Effective neuroprotection by pharmacological down-modulation of Na⁺-channels

Selective blockade of voltage-gated Na⁺-channels by TTX increases the anoxic tolerance of a number of preparations. TTX reduced the fall in ATP concentration in rat hippocampal slices exposed to anoxia, and improved the recovery of evoked population spike from dentate granule neurones and CA1 pyramidal neurones (Boening et al., 1989). It also protected hippocampal cultured neurons against hypoglycaemia- and potassium cyanide-induced injury, even when applied after the insult (Vornov et al., 1994). In the rat optic nerve, TTX substantially improved post-anoxic functional recovery, at concentrations that had little effect on the amplitude of the control compound

action potential (Stys et al., 1992) and protected the axonal cytoskeleton (Waxman et al., 1994). Increased tolerance to ischaemia with TTX was also observed *in vivo*. For example, TTX slowed down extracellular acidosis produced by complete ischaemia in the isolated perfused rat brain and markedly delayed anoxic depolarization (Prenen et al., 1988; Xie et al., 1994). These effects agree with the notion that Na⁺-channel blockade reduces energy demand. Direct application of TTX to the rat hippocampus also reduced, dose-dependently, neuronal death subsequent to transient global ischaemia in rats and gerbils (Yamasaki et al., 1991; Lysko et al., 1993). Finally, TTX (1 μ M) also markedly improved posttraumatic evoked responses in a model where slices from adult rat spinal cord were subjected to fluid percussion injury (Douglas et al., 1996).

Anticonvulsants (e.g. phenytoin and carbamazepine) and local anaesthetics (e.g. lidocaine and procaine) (Catterall, 1987) also block neuronal Na⁺-channels and have been shown to be cerebroprotective in various models of cerebral ischaemia. They provide significant protection from oxygen deprivation in the rat optic nerve and hippocampal slices at concentrations that caused little suppression of the normal compound action potential (Lucas et al., 1989; Fern et al., 1993). Administration of phenytoin (at effective anticonvulsive doses) pre- and postischaemia markedly reduced brain damage induced by occlusion of the middle cerebral artery in rodents (Rataud et al., 1994). In a cat model of acute cerebral ischaemia produced by air embolism, pretreatment with lidocaine attenuated the decrement in cortical somatosensory evoked responses during ischaemia, and improved their recovery after insult (Evans et al., 1984). Lidocaine also accelerated neuroelectrical recovery after incomplete global ischaemia in rabbits (Rasool et al., 1990) and continuous perfusion of high doses of lidocaine was proposed for protection against high intracranial pressure and against cerebral ischaemia in man (Artru et al., 1991).

“Novel” neuroprotective drugs, acting on voltage-gated Na⁺-channels

A variety of compounds such as lamotrigine derivatives and riluzole demonstrate that it is possible to interact with specific Na⁺-channel states, or possibly with specific Na⁺-channel types, to provide neuroprotection without unacceptable deficits of neuronal function, or cardiotoxic effects (Urenjak and Obrenovitch, 1996). Here we focus on lamotrigine and its derivatives. For a detailed review of the actions of riluzole in animal models of CNS ischaemia and trauma, see Obrenovitch and Urenjak (1997).

Lamotrigine has modest neuroprotective actions against ischaemia (Rataud et al., 1994; Smith and Meldrum, 1995; Wiard et al., 1995), trauma (Douglas et al., 1996) and mitochondrial poisoning (Schulz et al., 1996), relative to its effectiveness against seizures (Messenheimer, 1994). 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)], structurally related to lamotrigine, potently reduced tissue injury subsequent to global and focal ischaemia (Meldrum et al., 1992;

Leach et al., 1993; Lekieffre and Meldrum, 1993; Smith et al., 1993; Graham et al., 1993, 1994; Gilland et al., 1994) and both analogues appear more potent neuroprotectors than lamotrigine. Besides their “anti-ischaemic” effects, BW1003C87 and BW619C89 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).

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; Schulz et al., 1996), ligand-binding experiments with rat brain synaptosomes (Cheung et al., 1992) and voltage-clamp recordings with cultured neurons and recombinant rat brain type IIa Na⁺-channels expressed in CHO cells (Cheung et al., 1992; Lang et al., 1993; Lees and Leach, 1993; Xie et al., 1995; Xie and Garthwaite, 1996) show that their actions actually originates from use-dependent inhibition of Na⁺ conductance, presumably by stabilization of the Na⁺-channel in its inactivation state.

It is important to stress that Na⁺-channel “blockers” are also cerebroprotective when their administration is delayed (e.g. after transient ischaemia). This suggests that they help damaged or vulnerable brain regions to cope with secondary pathological processes (e.g. recurrent spreading depression, inflammation, delayed impairment of microvascular perfusion) or persistent abnormalities (e.g. upregulation of voltage-gated Na⁺-channels, enhanced synaptic efficacy). In these situations, the basis for protection may still be linked, at least partly, to reduced energy demand and preservation of ionic gradients (Kozłowski et al., 1996).

Conclusion

Down-regulation of voltage-gated Na⁺-channels is an inherent mechanism to reduce the energy expenditure of neurons and favour their survival during periods of anoxia or energy metabolism deficiency. The fact that a number of neuroprotective drugs, which are structurally unrelated, share the property of down-modulating Na⁺-channels, indicates that selective modulation of these channels is a valid strategy for the protection of the CNS against ischaemic damage. In addition, a number of findings suggest that neuroprotection can be achieved without conspicuous adverse effects on the normal function of the brain and heart.

References

- Ames III A (1997) Energy requirements of brain function: when is energy limiting? In: Beal MF, Howell N, Bodis-Wollner I (eds) *Mitochondria and free radicals in neurodegenerative diseases*. Wiley-Liss, Baltimore, pp 17–27
- Ames III A, Li Y-Y, Heher EC, Kimble CR (1992) Energy metabolism of rabbit retina as related to function: high cost of Na⁺ transport. *J Neurosci* 12: 840–853
- Artru F, Terrier A, Tixier S, Jourdan Ch, Deleuze R (1991) The use of intravenous lidocaine in neuro-anesthesia and neuro-intensive care. *Agressol* 32: 439–443
- Astrup J (1982) Energy-requiring cell functions in the ischemic brain. Their critical supply and possible inhibition in protective therapy. *J Neurosurg* 56: 482–487

- Boening JA, Kass IS, Cottrell JE, Chambers G (1989) The effect of blocking sodium influx on anoxic damage in the rat hippocampal slice. *Neuroscience* 33: 263–268
- Catterall WA (1987) Common modes of drug action on Na⁺ channels: local anesthetics, antiarrhythmics and anticonvulsants. *Trends Pharmacol Sci* 8: 57–65
- Cherubini E, Ben-Ari Y, Krnjevic K (1989) Anoxia produces smaller changes in synaptic transmission, membrane potential, and input resistance in immature rat hippocampus. *J Neurophysiol* 62: 882–895
- Cheung H, Kamp D, Harris E (1992) An *in vitro* investigation of the action of lamotrigine on neuronal voltage-activated sodium channels. *Epilepsy Res* 13: 107–112
- Cummins TR, Xia Y, Haddad GG (1994) Functional properties of rat and human neocortical voltage-sensitive sodium currents. *J Neurophysiol* 71: 1052–1064
- Dargent B, Couraud F (1990) Down-regulation of voltage-dependent sodium channels initiated by sodium influx in developing neurons. *Proc Natl Acad Sci USA* 87: 5907–5911
- Douglas SM, Panizzon KL, Wallis RA (1996) Sodium channel blockers protect against traumatic neuronal injury in the adult rat spinal cord. *Soc Neurosci Abstr* 22: 230
- Edwards RA, Lutz PL, Baden DG (1989) Relationship between energy expenditure and ion channel density in the turtle and rat brain. *Am J Physiol* 257 (6 Pt 2): R1354–R1358
- Erecinska M, Silver IA (1989) ATP and brain function. *J Cereb Blood Flow Metab* 9: 2–19
- Evans DE, Kobrine AI, Legrys DC, Bradley ME (1984) Protective effect of lidocaine in acute cerebral ischemia induced by air embolism. *J Neurosurg* 60: 257–263
- Fern R, Ransom BR, Stys PK, Waxman SG (1993) Pharmacological protection of CNS white matter during anoxia: actions of phenytoin, carbamazepine and diazepam. *J Pharmacol Exp Ther* 266: 1549–1555
- Friedman JE, Haddad GG (1994) Removal of extracellular sodium prevents anoxia-induced injury in freshly dissociated rat CA1 hippocampal neurons. *Brain Res* 641: 57–64
- Gilland E, Malgorzata P-S, Andiné P, Bona E, Hagberg H (1994) 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
- Ginsberg MD, Sternau LL, Globus MY-T, Dietrich WD, Busto R (1992) Therapeutic modulation of brain temperature: relevance to ischemic brain injury. *Cerebrovasc Brain Metab Rev* 4: 189–225
- Graham SH, Chen J, Sharp FR, Simon RP (1993) Limiting injury by inhibition of excitatory amino acid release. *J Cereb Blood Flow Metab* 13: 88–97
- Graham SH, Chen J, Lan J, Leach MJ, Simon RP (1994) 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
- Hakim AM, Shoubridge EA (1989) Cerebral acidosis in focal ischemia. *J Cereb Blood Flow Metab* 1: 115–119
- Hansen AJ (1985) Effect of anoxia on ion distribution in the brain. *Physiol Rev* 65: 101–148
- Hoehn K, Watson TWJ, MacVitar BA (1993) A novel tetrodotoxin-insensitive, slow sodium current in striatal and hippocampal neurons. *Neuron* 10: 543–552
- Kempinski O, Staub F, v Rosen F, Zimmer M, Neu A, Bachtmann A (1988) Molecular mechanisms of glial cell swelling *in vitro*. *Neurochem Path* 9: 109–125
- Kozłowski DA, James DC, Schallert T (1996) Use-dependent exaggeration of neuronal injury after unilateral sensorimotor cortex lesions. *J Neurosci* 16: 4776–4786
- Lang DG, Wang CM, Cooper BR (1993) Lamotrigine, phenytoin and carbamazepine interactions on the sodium current present in N4TG1 mouse neuroblastoma cells. *J Pharmacol Exp Ther* 266: 829–835
- Leach MJ, Marden CM, Miller AA (1986) Pharmacological studies on Lamotrigine, a novel antiepileptic drug: II. Neurochemical studies on the mechanism of action. *Epilepsia* 27: 490–497

- Leach MJ, Swann JH, Eisenthal D, Dopson M, Nobbs M (1993) BW619C89, a glutamate release inhibitor, protects against focal cerebral ischaemic damage. *Stroke* 24: 1063–1067
- Lees G, Leach MJ (1993) Studies on the mechanism of action of the novel anticonvulsant lamotrigine (Lamictal) using primary neuroglial cultures from rat cortex. *Brain Res* 612: 190–199
- Lekieffre D, Meldrum BS (1993) 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
- Lucas LF, West CA, Rigor BM, Schurr A (1989) Protection against cerebral hypoxia by local anesthetics: a study using brain slices. *J Neurosci Meth* 28: 47–50
- Lysko PG, Yue TL, Gu JL, Webb CL, Feuerstein G (1993) Neuroprotective effects of tetrodotoxin in cultured cerebellar neurons and in gerbil global brain ischemia. *Soc Neurosci Abstr* 19: 286
- Meldrum BS, Swann JH, Leach MJ, Millan MH, Gwinn R, Kadota K, Graham SH, Chen J, Simon RP (1992) Reduction of glutamate release and protection against ischaemic brain damage by BW1003C87. *Brain Res* 593: 1–6
- Messenheimer JA (1995) Lamotrigine. *Epilepsia* 36 [Suppl 2]: S87–S94
- Nicholls DG, Attwell D (1990) The release and uptake of excitatory amino acids. *Trends Neurosci* 11: 462–468
- Obrenovitch TP (1997) Sodium and potassium channel modulators: their role in neuroprotection. In: Cross AJ, Green AR (eds) *Neuroprotective agents and cerebral ischaemia*. Academic Press, London, pp 109–135
- Obrenovitch TP, Urenjak J (1997) Actions of riluzole in animal models of CNS ischaemia and trauma. *Rev Contemp Pharmacother* 8: 227–235
- Obrenovitch TP, Sarna GS, Symon L (1990a) Ionic homeostasis and neurotransmitter changes in ischaemia. In: Kriegstein J, Oberpichler H (eds) *Pharmacology of cerebral ischemia 1990*. Wissenschaftliche Verlagsgesellschaft, Stuttgart, pp 97–112
- Obrenovitch TP, Scheller D, Matsumoto T, Tegtmeier F, Höller M, Symon L (1990b) Rapid redistribution of hydrogen ions is associated with depolarization and repolarization subsequent to cerebral ischaemia-reperfusion. *J Neurophysiol* 64: 1125–1133
- Okiyama K, Smith DH, Gennarelli TA, Simon RP, Leach M, McIntosh TK (1995) 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
- Pérez-Pinzón MA, Rosenthal M, Sick TJ, Lutz PL, Pablo J, Mash D (1992) Downregulation of sodium channels during anoxia: a putative survival strategy of turtle brain. *Am J Physiol* 262: R712–R715
- Prenen GHM, Go GK, Postema F, Zuiderveen F, Korf J (1988) Cerebral cation shifts in hypoxic-ischemic brain damage are prevented by the sodium channel blocker tetrodotoxin. *Exp Neurol* 99: 118–132
- Rasool N, Farouqi M, Rubinstein, EH (1990) Lidocaine accelerates neuroelectrical recovery after incomplete global ischemia in rabbits. *Stroke* 21: 929–935
- Rataud J, Debarnot F, Mary V, Pratt J, Stutzmann J-M (1994) Comparative study of voltage-sensitive sodium channel blockers in focal ischaemia and electric convulsions in rodents. *Neurosci Lett* 172: 19–23
- Rothman SM (1985) The neurotoxicity of excitatory amino acids is produced by passive chloride influx. *J Neurosci* 5: 1483–1489
- Schulz JB, Matthews RT, Henshaw DR, Beal MF (1996) Neuroprotective strategies for treatment of lesions produced by mitochondrial toxins: implications for neurodegenerative diseases. *Neuroscience* 71: 1043–1048
- Siesjö BK, Bengtsson F (1989) 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

- Silver IA, Deas J, Erecinska M (1997) Ion homeostasis in brain cells: differences in intracellular ion responses to energy limitation between cultured neurons and glial cells. *Neuroscience* 78: 589–601
- Skilling SR, Smullin DH, Beitz AJ, Larson AA (1988) Extracellular amino acid concentrations in the dorsal spinal cord of freely moving rats following veratridine and nociceptive stimulation. *J Neurochem* 51: 127–132
- Smith SE, Meldrum BS (1995) Cerebroprotective effect of lamotrigine after focal ischemia in rats. *Stroke* 26: 117–122
- Smith SE, Lekieffre D, Sowinski P, Meldrum BS (1993) Cerebroprotective effect of BW619C89 after focal or global cerebral ischaemia in the rat. *Neuroreport* 4: 1339–1342
- Spetzler RF, Hadley MN (1989) Protection against cerebral ischemia: the role of barbiturates. *Cerebrovasc Brain Metabol Rev* 1: 212–229
- Stys PK, Waxman SG, Ransom BR (1992) Ionic mechanism of anoxic injury in mammalian CNS white matter: the role of Na⁺ channels and Na⁺-Ca²⁺ exchanger. *J Neurosci* 12:430–439
- Sun F-Y, Faden AI (1995) Neuroprotective effect of 619C89, a use-dependent sodium channel blocker, in rat traumatic brain injury. *Brain Res* 673: 133–140
- Taylor CP (1993) Na⁺ currents that fail to inactivate. *Trends Neurosci* 16: 455–460
- Urenjak J, Obrenovitch TP (1996) Pharmacological modulation of voltage-gated Na⁺-channels: a rational and effective strategy against ischemic brain damage. *Pharmacol Rev* 48: 21–67
- Urenjak J, Tegtmeier F, Beile A, Khan S, Peters T (1991) Synaptosomal respiration: a potent indicator of veratridine-induced Na⁺ influx. *Pharmacology* 43: 26–35
- Vornov JJ, Tasker RC, Coyle JT (1994) Delayed protection by MK-801 and tetrodotoxin in a rat organotypic hippocampal culture model of ischemia. *Stroke* 25: 457–465
- Waxman SG, Black JA, Ransom BR, Stys PK (1994) Anoxic injury of rat optic nerve: ultrastructural evidence for coupling between Na⁺ influx and Ca²⁺-mediated injury in myelinated CNS axons. *Brain Res* 644: 197–204
- Wiard RP, Dickerson MC, Beek O, Norton R, Cooper BR (1995) Neuroprotective properties of the novel antiepileptic lamotrigine in a gerbil model of global cerebral ischemia. *Stroke* 26: 466–472
- Xia Y, Haddad GG (1993) Neuroanatomical distribution and binding properties of saxitoxin sites in the rat and turtle CNS. *J Comp Neurol* 330: 363–380
- Xia Y, Haddad GG (1994) Postnatal development of voltage-sensitive Na⁺ channels in rat brain. *J Comp Neurol* 345: 279–287
- Xie XM, Garthwaite J (1996) State-dependent inhibition of Na⁺ currents by the neuroprotective agent 619C89 in rat hippocampal neurons and in a mammalian cell line expressing rat brain type IIa Na⁺ channels. *Neuroscience* 73: 951–962
- Xie XM, Lancaster B, Peakman T, Garthwaite J (1995) Interaction of the antiepileptic drug lamotrigine with recombinant rat brain type IIa Na⁺ channels and with native Na⁺ channels in rat hippocampal neurons. *Eur J Physiol* 430: 437–446
- Xie Y, Dengler K, Zacharias E, Wilffert B, Tegtmeier F (1994) Effects of the sodium channel blocker tetrodotoxin (TTX) on cellular ion homeostasis in rat brain subjected to complete ischemia. *Brain Res* 652: 216–224
- Yamasaki Y, Kogure K, Hara H, Ban H, Akaike N (1991) The possible involvement of tetrodotoxin-sensitive ion channels in ischemic neuronal damage in the rat hippocampus. *Neurosci Lett* 121: 251–254
- Young AM, Foley PM, Bradford HF (1990) Preloading *in vivo*: a rapid and reliable method for measuring gamma-aminobutyric acid and glutamate fluxes by microdialysis. *J Neurochem* 55: 1060–1063

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