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The importance of voltage-dependent sodium channels in cerebral ischaemia

A. J. Carter

Department of Central Nervous System Research, Boehringer Ingelheim, Ingelheim am Rhein, Federal Republic of Germany

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Summary. Strategies for the treatment of thromboembolic stroke are based on restoring the blood flow as soon as possible and protecting the neurons from the deleterious consequences of cerebral ischaemia. Interest has focused on blockers of voltage-dependent Na^+ channels as potential neuroprotective agents because they prevent neuronal death in various experimental models of cerebral ischaemia and act cytoprotectively in models of white matter damage. Although several Na^+ blockers are currently being tested in various phases of clinical development, most of these agents are relatively weak and unspecific. I therefore consider it worthwhile to search for molecules which specifically block voltage-dependent Na^+ channels for the treatment of cerebral ischaemia.

Keywords: Neuroprotection – Reperfusion – Na^+ Channels – NMDA Receptors – Ischaemia – Thromboembolic stroke

Introduction

The brain depends on arterial blood for a continuous supply of oxygen and glucose. Even if blood flow is interrupted for only a few minutes, certain highly vulnerable neurons will degenerate. If the interruption is sustained, then all types of brain cells will eventually die. Fundamental to our understanding of the process of cerebral ischaemia has been the assumption that brain cells do not simply die of a simple loss of energy. The link between ischaemia and neuronal cell death is considerably more complicated. Stroke triggers a chain reaction of electrical and chemical activity which is related to ischaemic depolarization, the release of excitatory amino acids and changes in calcium homeostasis (Mattson and Mark, 1996; Tymianski and Tator, 1996). These events act in concert to orchestrate cell death.

The core area of an infarct is probably beyond treatment. This area of the brain is totally deprived of oxygen and glucose and dies within minutes. However, the area at risk surrounding the core, often called the penumbra because it is similar to the partially lit penumbra of a lunar eclipse, does not die immediately. The collateralization or natural redundancy of the brain's blood supply

allows this area to continue functioning with a reduced flow of blood. It is here that the chain reaction of secondary injury, if not stopped, will cause the brain tissue to eventually succumb to the ischaemia. The penumbra therefore represents an opportunity for intervention (Ginsberg, 1997). Strategies for the treatment of acute ischaemic stroke are based on first restoring the blood flow – reperfusion – as soon as possible and, secondly, on protecting the neurons from the deleterious consequences of the blockage (Önal and Fisher, 1996).

Reperfusion

Clinical investigators attempting to treat the consequences of thromboembolic stroke first turned their attention to restoring blood flow to the ischaemic brain by removing the offending blood clot with thrombolytic agents. However, many early studies did not improve survival rate or functioning of patients. Indeed, they actually often worsened outcome. Fortunately, these early trials did not discourage investigators from trying out the newer thrombolytic agents. The feasibilty and safety of intra-arterial or intravenous administration of recombinant tissue plasminogen activator (rt-PA) in patients presenting within 6 to 8 hours of the onset of symptoms of focal cerebral infarction has been demonstrated in several prospective angiographic studies (del Zoppo, 1995). Furthermore, two recent double-blind, placebo-controlled trials of rt-PA in acute thromboembolic stroke have supported a more sanguine view of thrombolytic therapy.

The trial organized by the National Institute of Neurological Disorders and Stroke (NINDS) included 624 patients randomized to receive either placebo or rt-PA within three hours of onset of ischaemic stroke (The Multicenter Acute Stroke Trial, 1996). There was a significant improvement in neurological recovery three months after the event. Patients were a third more likely to have no or minimal disability than control patients. The European cooperative acute stroke study (ECASS) was similar in design and size, except that treatment was initiated within six hours of onset of ischaemia (Hacke et al., 1995). These results were not quite so encouraging. The mortality at three months was higher in the treated group than in the controls without improvement of functional state. However, when the protocol violators, who mainly showed signs of haemorrhagic transformation, were excluded from the analysis the treatment group exhibited a better functional outcome. Although the disparity between the two trials has caused a certain amount of discussion about the clinical implications, most experts agree that further trials of thrombolysis in stroke are needed and that patient groups need to be better defined (Bogousslavsky, 1996). There still may, however, be scope for improving therapy by administering neuroprotective agents.

Neuroprotection

Many pharmaceutical companies and clinicians have concentrated their activities on designing neuroprotective drugs that interfere with the host of biological processes that are set in motion by the original ischaemic event (Barinaga,

1996; Koroshetz and Moskowitz, 1996; Schehr, 1996). The idea behind this is that such agents may slow down the destructive processes to buy more time to administer thrombolytic agents. Furthermore, neuroprotective agents could be used irrespective of the type of stroke because they are theoretically not dangerous for patients with haemorrhagic stroke. Here I shall concentrate on two such strategies, namely NMDA receptor antagonists and blockers of voltage-dependent Na^+ channels, because they are the most clinically advanced.

NMDA receptors

Antagonists of excitatory amino acids have attracted a great deal of attention because of their promising therapeutic potential (Danysz et al., 1995). Experiments with hippocampal cell cultures prompted the proposal that enhanced synaptic activity and release of excitatory amino acids causes the death of neurons that have been deprived of oxygen (Rothman, 1983; Rothman, 1984). Microdialysis studies with rats have supported this concept. They demonstrate that high levels of excitatory amino acids are released into the extracellular space during experimental ischaemia and that this release correlates with the neuropathological outcome (Benveniste et al., 1984; Butcher et al., 1990). Finally, investigations with several animal models of cerebral ischaemia have implicated the N-methyl-D-aspartate (NMDA) receptor-channel complex in the neuronal loss which occurs (Simon et al., 1984; Ozyurt et al., 1988; Park et al., 1988).

The NMDA receptor is currently one of the pharmacologically best characterized excitatory amino acid receptor subtypes. Functionally, it consists of a voltage-dependent channel, which is permeable to both Ca^{2+} and Na^+, and at least three different regulatory domains (Reynolds and Miller, 1988). It is therefore referred to as the NMDA receptor-channel complex. The first site, by definition, is the neurotransmitter recognition site to which compounds such as glutamate, aspartate or NMDA bind and can be blocked by various competitive antagonists (Olverman and Watkins, 1989; Watkins et al., 1990). The strychnine-insensitive glycine site constitutes another regulatory domain of the receptor-channel complex (Carter, 1992). There is also a site in the receptor channel itself and can be blocked by Mg^{2+} or various ion-channel blockers (Foster, 1991). There are three structural classes of ion-channel blockers: these are the arylcyclohexylamines, e.g. ketamine, phencyclidine and (+)MK-801 (Anis et al., 1983; Wong et al., 1986), the diarylguanidines, e.g. Cerestat (Reddy et al., 1994), and the benzomorphans, e.g. dextromethorphan, *N*-normetazocine and BIII 277 CL (Foster, 1991; Carter et al., 1995a; Pschorn and Carter 1996).

Although stroke displays considerable diversity in its pathogenesis, manifestations and anatomical sites (Chopp and Zhang, 1995), about 80% of all events are due to ischaemic infarction (Bamford, 1992). Animal models of cerebral ischaemia generally fall into two categories: global and focal. Global models, as their name implies, produce ischaemia in the entire brain and cause selective neuronal necrosis within the most vulnerable brain regions (Ginsberg and Busto, 1989). In contrast, focal models produce lesions in clearly

defined regions of the brain (Ginsberg and Busto, 1989; Macrae, 1992). They are considered to be more relevant to acute ischaemic stroke. At least three different published studies have demonstrated significant neuroprotective effects of Cerestat in various rat models of focal cerebral ischaemia using different intravenous dose regimens with total doses in excess of 1 mg/kg (Minematsu et al., 1993; Aronowski et al., 1994; Hasegawa et al., 1994). This corresponds to drug plasma levels of approximately 1 μmol/L or 370 ng/mL (Minematsu et al., 1993).

Initial tolerability studies have been performed in human beings with several different antagonists of the NMDA receptor-channel complex (Krystal et al., 1994; Muir et al., 1994; Albers et al., 1995; Grotta et al., 1995). Interestingly, the pattern of side effects observed is remarkably similar, irrespective of whether the compound is a competitive antagonist or ion-channel blocker (Muir and Lees, 1995). As predicted from preclinical studies (Carter, 1995), all compounds cause disturbances of motor coordination. Moreover and probably more seriously, these compounds also cause a similar spectrum of neuropsychological symptoms, such as agitation, paranoia and hallucinations, and have important cardiovascular side effects (Muir and Lees, 1995). Cerestat caused significant sedation as well as increases in heart rate and mean arterial blood pressure at plasma levels of 36 μg/mL/h, and these side effects became unacceptable at plasma levels of 39 μg/mL/h and above (Muir et al., 1994).

Results from larger clinical studies with antagonists of the NMDA receptor-channel complex have been disappointing so far. The development of the competitive NMDA antagonist Selfotel was stopped after two parallel phase III studies demonstrated an increased mortality in drug-treated groups (SCRIP, 1995). Moreover, Boehringer Ingelheim and Cambridge Neuroscience have very recently temporarily suspended enrolment of patients into a trial of Cerestat in acute ischaemic stroke because of concerns over the drug's risk-benefit ratio (SCRIP, 1997). Interestingly, the target plasma level of Cerestat of 10 ng/mL in the highest dose group is well below that required for neuroprotection in the model of permanent focal ischaemia mentioned above (Minematsu et al., 1993). Obviously, care should be taken in future trials to define exactly the plasma levels required for neuroprotective effects in several different preclinical studies.

NMDA receptor antagonists possess several drawbacks. They have a narrow therapeutic index, do not prevent the initial depolarization or massive release of excitatory amino acids and do not act neuroprotectively in the white matter of the brain. We know that human stroke encompasses both white and grey matter. And yet strategies based on antagonizing the postsynaptic effects of excitatory amino acids concentrate on protecting grey matter only. Therefore, interest has been focused on blockers of voltage-dependent Na^+ channels as an alternative.

Voltage-dependent Na^+ channels

Voltage-dependent Na^+ channels are thought to play a key role in excitotoxic damage because blockers of such channels inhibit depolarization, thereby

reducing Ca^{2+} influx through voltage-dependent Ca^{2+} and NMDA receptor channels, and prevent the reversal of the Ca^{2+}/Na^+ exchanger. Furthermore, they inhibit the release of excitatory amino acids and reduce neuronal damage. Hence, they should be useful for the treatment of thromboembolic stroke (Taylor and Meldrum, 1995; Urenjak and Obrenovitch, 1996).

Recent cellular and molecular biological advances, as well as the availability of specific toxins, have contributed to a better understanding of voltage-dependent Na^+ channels (Catterall, 1980, 1988, 1992). Na^+ channels are large glycoproteins which exist as heterotrimers consisting of one 250 kDA α subunit, one 39 kDA β_1 subunit and one 37 kDA β_2 subunit (Hartshorne and Catterall, 1984). Several different subtypes of Na^+ channel have now been cloned. Indeed, five different types have been found in rat brain alone. They are designated type NaCh I, NaCh II, NaCh III, NaCh 6 and Na-G (Noda et al., 1986; Kayano et al., 1988; Yarowski et al., 1991; Gautron et al., 1992; Schaller et al., 1995), and appear to have a unique distribution: type I and III Na^+ channels are found primarily in neuronal cell bodies, whereas type II channels are present in fibre tracts or axons (Catterall, 1980; Westenbroek et al., 1992); type Na-G are specific for glial cells (Gautron et al., 1992). The structure of NaCh 6 has recently been published and is broadly distributed throughout the nervous system (Schaller et al., 1995).

Several neurotoxins bind to Na^+ channels and either cause blocking or opening of the channel. The sites of their action are defined as follows: neurotoxin site 1, where water soluble tetrodotoxin and saxitoxin act by blocking Na^+ permeation; neurotoxin site 2 where lipid-soluble toxins bind such as veratridine, aconitine and batrachotoxin causing prolonged activation; neurotoxin site 3 where α-scorpion and sea anemone neumatocyte toxins bind inhibiting inactivation; neurotoxin site 4 where β-scorpion toxins act by shifting activation; and neurotoxin site 5 where ciguatoxins act to prolong activation (Catterall, 1980, 1992). The Na^+ channel can shift between three distinct conformational states – namely active, resting and inactivated – which determine the Na^+ channel permeability (Catterall, 1987). The site of activation of antiarrhythmic, anticonvulsant and local anaesthetic drugs is thought to be the intracellular side of the sodium channel; they allosterically inhibit interaction with neurotoxin site 2 in a frequency and voltage-dependent manner (Catterall, 1980; Ragsdale et al., 1994). Indeed, frequency and use dependence may be very useful attributes for a voltage-dependent Na^+ channel blocker because they would preferentially block Na^+ channels in the open or inactivated state.

Lamotrigine is a drug which has recently been introduced onto the market as an anticonvulsant agent. The compound is reported to block Na^+ channels in a manner similar to phenytoin and carbamazepine (Lang et al., 1993). Recently, the anticonvulsant effects of two more potent derivatives of lamotrigine have been published: namely BW1003C87 and BW619C89 (Smith et al., 1993a). Interestingly, these compounds have also been shown to inhibit glutamate release and prevent neuronal damage after ischaemia (Graham et al., 1993; Leach et al., 1993; Lekieffre and Meldrum, 1993; Smith et al., 1993b; Graham et al., 1994). Two other Na^+ channel blockers are known to possess neuroprotective activity, namely lifarizine (Kucharczyk

et al., 1991; Alps et al., 1995; May et al., 1995; McBean et al., 1995) and lubeluzole (Aronowski et al., 1996; De Ryck et al., 1996; Haseldonckx et al., 1997) and have been tested in phase II clinical trials. Unfortunately, lifarizine caused hypotension in elderly female patients (Squire et al., 1996) and clinical trials have since been discontinued. The cardiovascular side effects are perhaps indicative of the specificity problems associated with these agents. Our own unpublished experiments indicate that BW619C89, lubeluzole and lifarizine all bind to classical voltage-dependent Ca^{2+} channels in addition to voltage-dependent Na^+ channels. There is therefore considerable scope for improvement in this area.

The white matter of the mammalian brain is also susceptible to ischaemic injury. However, little is known about the pathophysiology of this process, despite the fact that the functional integrity of the central nervous system as a whole depends on the ability of neurons to communicate with each other via their axons located in the white matter (Stys, 1996). Initially, evidence suggests that the Ca^{2+} influx that occurs in white matter during anoxia is not caused by conventional Ca^{2+} channels, but rather by other channels which are imperfectly impermeable to Ca^{2+} or via the Na^+/Ca^{2+} exchanger (Ransom et al., 1990). Subsequent work has led to the conclusion that depriving white matter of oxygen causes rapid depletion of energy-rich phosphates and membrane depolarization leading to Na^+ influx through voltage-dependent Na^+ channels (Stys et al., 1992). The resulting rise in the intracellular [Na^+] and membrane depolarization encourages damaging levels of Ca^{2+} to enter the axons by reversal of the Na^+/Ca^{2+} exchanger. Indeed, blockers of voltage-dependent Na^+ channels such as tetrodotoxin and saxitoxin prevent anoxic damage to mammalian white matter (Stys et al., 1992).

Microdialysis studies have demonstrated that large amounts of glutamate and aspartate are released into the extracellular space during ischaemia (Benveniste et al., 1984; Globus et al., 1988), and that this release correlates with the neuropathological outcome (Butcher et al., 1990; Takagi et al., 1993). Glutamate can be released from cells by at least two distinct mechanisms: one mechanism involves Ca^{2+}-dependent vesicular release from synapses in neurons (Nicholls and Attwell, 1990); the other mechanism is Ca^{2+} independent and works by reversal of the plasma membrane transporter in glial cells and neurons (Nicholls and Attwell, 1990; Attwell et al., 1993). The Ca^{2+}-independent release of glutamate probably makes the major contribution to the excitotoxic release of glutamate that occurs during ischaemic conditions (Kauppinen et al., 1988; Szatkowski et al., 1990; Taylor et al., 1992). Glutamate transport is an electrogenic process. The proposed stoichiometry is that one glutamate anion and two Na^+ are coupled to the transport of one K^+ and one OH^- (Bouvier et al., 1992). Because of this unique ionic dependence, significant changes in the distribution of Na^+ or K^+ can have dramatic effects on the transporter. And this is just what can happen during ischaemia. An increase in the extracellular [K^+] and intracellular [Na^+], together with membrane depolarization, favours reversal of the transport system. Glutamate starts to leak out of the glial cells and neurons, and things get out of hand.

More recent evidence indicates that excitotoxic and hypoxic injury can be reduced by blocking voltage-dependent Na^+ channels directly or by removing extracellular Na^+ (Lustig et al., 1992; Dessi et al., 1994; Friedman and Haddad, 1994). The protective effects of Na^+ channel blockers suggest that an early period of Na^+ entry into cells after ATP depletion may be responsible for a more prolonged toxic NMDA receptor activation (Vornov et al., 1994). Indeed, our work has shown that artificially maintaining high levels of energy-rich phosphates can potentiate the protective effects of NMDA receptor antagonists (Carter et al., 1995b). Moreover, at least two separate groups have proposed that blockers of Na^+ channels improve the efficacy of excitatory amino acid antagonists in cell culture models of oxygen/glucose deprivation (Zeevalk and Nicklas, 1991; Lynch et al., 1995). In summary, these findings, together with establishment of the ionic dependence of the glutamate transporter, implicate voltage-dependent Na^+ channels in excitotoxic neuronal death after ischaemia.

Conclusions

Blockers of voltage-dependent Na^+ channels can be used for the treatment of thromboembolic stroke because they prevent neuronal death in experimental models of cerebral ischaemia and act cytoprotectively in models of white matter damage. However, most of the Na^+ blockers currently being tested in various clinical phases of development are relatively weak and unspecific. I therefore believe that there is a need to identify new molecules which specifically block voltage-dependent Na^+ channels in a use- and frequency-dependent manner.

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Author's address: Dr. A. J. Carter, Department of Central Nervous System Research, Boehringer Ingelheim, D-55216 Ingelheim am Rhein, Federal Republic of Germany.

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