It is now clear that the wide variety of neuronal voltage-dependent calcium channels (Table 1) are determinants of transmitter release (N-type, P-type, Q-type channels), repetitive or burst neuronal firing (T-type channels) and perhaps of some aspects of gene expression (L-type channels). However, although the calcium overload hypothesis of neuronal cell death has been around for 14 years, there is little clinical acceptance of a role for calcium channel modulators in the treatment of stroke or other forms of neurodegeneration. Indeed, in a recent article Grosset [11] concluded that there was no current useful therapy for acute stroke. In this article, we review why initial approaches have failed to provide robust clinical benefit, but why newer approaches, which are either in early clinical trials or in preclinical evaluation, may be of benefit. These approaches are either ion channel selective or, alternatively, gain selectivity by appropriate kinetics at different ion channel states.

**L-type calcium channel blockers**

L-type calcium channels are predominantly located on neuronal cell bodies [2] and under certain circumstances can modify transmitter release [3]. Consequently, highly selective L-type channel blockers such as the dihydropyridine nimodipine have been investigated extensively in animal models of cerebral ischaemia and in several clinical stroke trials. All the dihydropyridines are potent vasodilators and are, thus, highly effective against cerebral vasospasm [4]. Consequently, such drugs are effective in subarachnoid haemorrhage, in which delayed vasospasm due to iron-induced free radical formation and endogenous vasoactive compound release can exacerbate damage and cause dissemination several days after the initial event. However, in ischaemic stroke the vasodilator effects are modified by local oedema, and dihydropyridines may cause heterogeneous vasodilator effects in the infarcted area [5]. Furthermore, patients with marked carotid atherosclerosis may be close to the threshold for global ischaemic damage under resting conditions, and additional peripheral vasodilatation may precipitate ischaemia and/or create a 'steal syndrome' in the infarct area in a subset of patients. Thus, marked vasodilator effects appear to be contraindicated in the therapy of ischaemic stroke, which explains why, in man, despite initially encouraging results, the use of nimodipine has not revealed major benefit [4,6,7], although, of course, admission of patients into stroke clinics with the time window necessary (< 6 h) is only possible in a few centres and was not a criterion for many of the nimodipine trials. Other selective L-type channel inhibitors such as diltiazem and fantofarone are poorly active in models of cerebral ischaemia (mouse middle cerebral artery model, gerbil two-vessel occlusion model; M. Spedding and P. Chatelain, unpublished work).

**N-, P-, Q-type calcium channel blockers in ischaemia**

N-type channels are critically involved in transmitter release [8-12] and α-conotoxin GVIA inhibits the N-type channel component of transmitter release, but its very slow kinetics at the channels means that the compound cannot be used in the clinic. However, α-conotoxin GVIA (10 and 20 pmol injected intracisternally) caused a dose-dependent reduction of CA1 neuronal loss after 5 min bilateral carotid ligation in the gerbil [13]; this procedure was ineffective against neurodegeneration induced by intrahippocampal quinolinic acid. A synthetic α-conotoxin, SNX-111, is being developed for therapy in ischaemic stroke and has shown protective effects in animal models [14], but the effects of N-type channel blockade on transmitter release in the peripheral nervous system and consequent cardiovascular control must be carefully monitored; the influence of hypotension and hypothermia in central models must also be tightly controlled. SNX-230 has been claimed to be a selective blocker of Q-type calcium channels controlling transmitter release [11], but the evidence for Q-type channels as distinct entities is still not entirely convincing [9,10]. N-, P- and Q-type channels may all contribute to different extents to calcium current in different neurons, with different contributions in various brain areas. For example, P-type channels may be involved in the activation of nitric oxide synthesis in the frontal cortex [15]. Nevertheless, Ca2+ entry in synaptosomes following a depolarization is inhibited by a combination of
Table I
Classification of calcium channels [8–11]

<table>
<thead>
<tr>
<th></th>
<th>L-type</th>
<th>T-type</th>
<th>N-type</th>
<th>P-type</th>
<th>Q-type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conductance (pS)</td>
<td>25</td>
<td>8</td>
<td>13</td>
<td>9, 14, 19</td>
<td>-</td>
</tr>
<tr>
<td>Dihydropyridines</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GVIA conotoxin</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Aga IVA toxin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>SNX 230</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Location/role</td>
<td>Cell body</td>
<td>Pacemaker</td>
<td>Terminals</td>
<td>Cerebellar</td>
<td>Terminals</td>
</tr>
<tr>
<td>Gene activation?</td>
<td>burst firing</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

There are two main subtypes of L-type channels in the brain. N-, P-, and Q-type channels are involved in transmitter release.

T-type channel blockers
There are no selective inhibitors of T-type channels, which are involved in burst firing and repetitive discharges. NC-1100 [18] and U-92032 [19] have been shown to inhibit T-type channel activity and to have some anti-ischaemic activity in stroke models. However, T-type channels do have some electrophysiological similarities to sodium channels and there is some overlap between the inhibitory effects on the two classes of channel.

Sodium channel blockers
Sodium channel down-regulation is a mechanism which is used physiologically under certain circumstances to resist some of the deleterious effects of ischaemia by reducing neuronal activity. Thus, some strains of turtle down-regulate sodium channels in order to survive a 3-h period of anoxia [20], and this mechanism has also been shown to occur in the human cortex, albeit to a limited extent [21]. The possibility, therefore, exists that inhibition of sodium channels may be a powerful neuroprotective mechanism, if selectivity for ischaemic tissue can be obtained and ongoing neuronal activity in non-ischaemic tissue left unmodified. The classic sodium channel inhibitor, tetrodotoxin, has negligible voltage dependency, and as such will inhibit all susceptible channels and have marked effects on ongoing neuronal activity. Nevertheless, this toxin delayed calcium overload and the massive membrane depolarization after ischaemia in an isolated perfused rat brain preparation [22], suggesting that the early phases of ischaemia are exacerbated by repetitive sodium channel activity. The depolarization due to complete energy failure would evidently be resistant to this approach, but the point at which energy failure occurs may be delayed and, thus, the size of the infarcted area may be greatly reduced. Tetrodotoxin has been shown to be effective in several in vivo models after local administration [23–25]. Protective effects after oxygen and glucose deprivation in hippocampal slices have also been demonstrated for the local anaesthetic lidocaine [25], and protective effects were also obtained following intracerebroventricular administration in the gerbil model [26]. Several anti-epileptic agents inhibit sodium channels (phenytoin [26], riluzole [27–29], and to a lesser extent lamotrigine [27], BW619C89 [30]), and these agents have been re-evaluated in models of cerebral ischaemia with positive results [30–32]. However, the utility of each agent will be dependent on the kinetics at the sodium channel, with state and use dependence playing critical roles in the therapeutic profile. Nevertheless, these drugs are not highly selective for sodium channels, which renders analysis of the utility of selective sodium channel blockade difficult. Thus eliprodil blocks sodium and calcium channels, as well as modulating N-methyl-D-aspartate (NMDA) and sigma receptors, and it is not clear which property is responsible for the anti-ischaemic effectiveness of the molecule [32]. Similarly, riluzole has been claimed to be active in amyotrophic lateral sclerosis [33], but the compound is also a potent NMDA antagonist, which could account for some of the efficacy [28]. These compounds also block at least part of the glutamate release caused by forebrain ischaemia, which may be due to the sodium channel blockade [34,35]. Nevertheless, the pro-
gress of these agents in clinical trials will be very interesting, and it may well be that a range of properties are needed to counteract the catastrophic events associated with an ischaemic stroke.

**Sodium/calcium channel blockers**

Several compounds may belong to this class, showing various degrees of selectivity between the different types of calcium channel and sodium channels. Lifarizine is a sodium/calcium channel inhibitor with potent use- and voltage-dependent effects on sodium and calcium channels which has shown efficacy in a wide range of animal models [36-40]. The compound has use-dependent effects on sodium channels with relatively slow kinetics, which will, therefore, not affect ongoing neuronal activity [37,38] and the compound is devoid of overt behavioural effects. As the affinity for sodium channels is markedly increased at depolarizing holding potentials, the compound is selective for ischaemic tissue [37,38]. Interaction of lifarizine with ionic channels was confirmed by binding studies using batrachotoxin as a marker of Na⁺ channels and PN 200-110 as a marker of skeletal muscle Ca²⁺ channels [41].

Lifarizine inhibits T-type calcium channels in N1E-115 cells [41]. T-type channel inhibition may be responsible for inhibition of repetitive neuronal firing in the penumbra zone. Thus, lifarizine may block several types of Ca²⁺ current and the effects of the drug will depend on its kinetics at the channels. Although lifarizine is in clinical trials the efficacy of the compound has not been published. It will remain a benchmark for this class of compound. Nevertheless, because of the multiplicity of channels inhibited by this class of drug, there are many variables which can be modified to vary the pharmacological profile and consequently the clinical utility.

**Role of calcium channels in neurodegeneration**

L-type calcium channels are located on the cell bodies and dendritic processes of CA1 neurons in the hippocampus [2], which are selectively vulnerable after global cerebral ischaemia and also in the early stages of Alzheimer's disease. Studies on the neuroprotective effects of classical L-type calcium channel modulators in ageing have produced a very large body of literature, but without clear protective effects being demonstrated in man. There have been sporadic and controversial reports of activation of L-type calcium channels by βA4 amyloid using *in vitro* models [42,43], but it is unclear whether this effect is secondary to a cellular depolarization or a more specific intervention. However, high doses of glucocorticoid can activate calcium channels [44] and are associated with CA1 hippocampal damage and memory impairment in 1-year-old Wistar rats [45]. In this respect, L- and N-type calcium channels are activated by neurosteroids via a G-protein link in dissociated hippocampal CA1 neurons [46]. Furthermore, corticotrophin-releasing factor antagonists inhibit neuronal damage after focal cerebral ischaemia in rats [47]. α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)-induced neurotoxicity in rat primary cortical cell cultures has been reported to be due to involvement of L-type channels [48]. It is therefore possible that calcium channel modulators may be protective against certain insults to the hippocampus. McEwen's group [49,50] have shown in the rat that phenytoin, and the atypical anti-depressant tianeptine, protect against the loss of CA3 dendritic arborization caused by repeated stress or administration of high doses of steroids. However, it remains to be seen whether the protective effects are due to primary or secondary effects on channel function. The involvement of voltage-dependent calcium channels in a wide range of neuronal processes remains a key area for future research, but the use of drugs which are selective for neuronal channels remains a prerequisite.

43 Davidson, R. M., Shajenko, J. C., and Donta, T. S. (1994) Brain Res. 643, 324–327

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