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Drugs acting on calcium channels: potential treatment for ischaemic stroke

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Calcium subserves a ubiquitous role in the organisation of cell function. Ca^{2+} channels which control influx may be modified in disease states. Animal models of cerebral ischaemia do present some problems when investigating potential therapies involving Ca^{2+} channels. However, it is important not to be too rigid in searching for models which exactly mimic the human disease state, when even the best experimental approaches fall short of such an ideal. There are differences between different classes of calcium entry blocking drugs with regard to their activity on Ca^{2+} channels and transmembrane Ca^{2+} movement. Some calcium antagonists may also affect ion channels other than Ca^{2+}, and this potential is exemplified by the novel ion channel modulator RS-87476, which affords experimental neurocytoprotection. Limitation of intracellular Na^+ influx during ischaemia-induced depolarization may be useful.

Keywords calcium channel cerebral ischaemia focal and forebrain ischaemia animal models calcium antagonists Na^+/Ca^{2+} ion-channel modulation

Introduction

The ubiquitous role of calcium in the organisation of all types of cell function has long been known and it was already realised by the late 1970s how failure to control calcium-activated events in myocardial cells subjected to ischaemia could lead to irreparable cardiac damage. However by 1980, Hass proposed a, then novel, concept of calcium ion (Ca^{2+}) triggering of ischaemic neuronal cell death (Hass, 1981). This hypothesis was supported simultaneously by Harris *et al.* (1981) and Siesjo (1981). The Hass hypothesis suggested that an unregulated extension of the Ca^{2+} mediated breakdown of the phospholipid neurotransmitter envelope after presynaptic excitation would lead to neuronal death. Raised intracellular calcium activity would stimulate turnover of the phosphatidylinositol cycle, with serious consequences for neurotransmitter metabolism during reperfusion. Increased intracellular calcium activity would also stimulate phospholipase A_2 activity with release of free fatty acids. A major free fatty acid released would be arachidonic acid, the precursor of the prostaglandins and other related substances, which may have further deleterious effects upon ischaemic cerebral tissue. The realisation how failure to control calcium activated events in brain cells subjected to ischaemia would lead to irreparable neuronal damage has prompted much research in recent years into therapies designed to interact with these various pathological features in the way in which they impair normal control mechanisms. Although it is now evident that the whole pathogenic process has been over-simplified, a most exciting development to emerge from drug research in recent years in this respect has been in the area of cytoprotection.

Calcium, the cell and its channels

Because of the complex nature of the environment or matrix in which various cells exist and interact adequate control of ionic gradients across the plasma membrane (and Ca^{2+} is but one such ion) is critical for cell function, irrespective of the nature of the end physiological effect. This is a general cellular prerequisite for the propagation of nerve impulses, release of transmitter substances, secretion from exocrine or endocrine glands, and the contraction of muscle fibres. Several mechanisms serve to control ionic fluxes and these are voltage-operated (sensitive) channels, receptor-operated (agonists) channels, and specific enzyme systems to help eliminate excess calcium from the cell. Only voltage-operated

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channels, activated by cellular depolarization strictly qualify as calcium channels since they have high specificity for Ca^{2+} over other cations.

There are three basic types of calcium channels of the L, N and T types as based on electrophysiological evidence and they have different cellular localisations, metabolic requirements, ion selectivities and sensitivity to toxins and drugs (see reviews by Spedding, 1985; Spedding *et al.*, 1989). T channels appear to be involved in some pacemaker activity and N channels are tightly coupled to neurotransmitter release. L channels are responsible for the many calcium activated events defined by Fleckenstein (1988) which are susceptible to calcium entry blockers. This is a very simplified classification and it is evident from the techniques used to classify and identify these channel types that many different subtypes can potentially exist or be differentiated according to various levels of membrane depolarization. The impact of disease makes it quite difficult to interpret therapeutic effects involving simple and restricted channel types where voltage-operated channels are most likely coupled into a multitude of other ion channels (probably dominated by K^+). For the most part consideration is given to the L channel and its predominant link with the dihydropyridine calcium entry blockers. In this respect nimodipine is the most well known and clinically applied representative, but there are potential problems inherent in the effect that such agents have on peripheral vascular function, and the detracting influence that this property can have in narrowing their therapeutically useful window. It is interesting to note the increased number of [^{3}H]-PN200-110 binding sites labelling L-type Ca^{2+} channels in the ischaemic as opposed to non-ischaemic hippocampus of Sprague-Dawley rats demonstrated by Magnoni *et al.* (1988). Whereas these changes in the L channel are transient in terms of binding data, beginning within an hour of the onset of the ischaemic insult and reversible at 24 h, they may, by facilitating excessive intracellular Ca^{2+} overload, trigger events that could outlast the changes in channel activity. It is also important in conjunction with these changes in Ca^{2+} channel activity to note the additional role that activators of Ca^{2+} channels may play in the pathogenesis of cerebral ischaemia (Patmore *et al.*, 1989b), especially since the lipid metabolite products of ischaemia can behave like the synthetic activators Bay K8644 and CGP 28392. Such agents have been claimed to slow the transition of the Ca^{2+} channel from the active to the closed state (see Kass, 1987; Sanguinetti *et al.*, 1986) and the dihydropyridines do not antagonise this enhanced mode of Ca^{2+} influx (Spedding *et al.*, 1989). Neither do these agents inhibit K^+-evoked release of 5-HT from normal cortical cells and this appears attributable to the function of N channels upon which these agents are inactive (Middlemiss & Spedding, 1985). However, caution must be applied in the interpretation of findings from essentially normal tissue because Bay K8644 can evoke 5-HT release and this is abolished by low concentrations of calcium entry blockers (Middlemiss & Spedding, 1985). The recruitment of L type channels may occur under disease conditions and then be further influenced by calcium channel activators. It is also possible that some calcium entry blockers may be effective under conditions of high frequency neuronal stimulation and therefore demonstrate a disease-switched-on effect on N channels (Spedding & Middlemiss, 1985). Thus, when considering the role of calcium channels *per se*, in the search for new therapies, it is important to reflect on how appropriate it is to expect to have a major impact on this mode of calcium influx. In fact, can the calcium channel *per se* offer a therapeutic pathway? Discussion, therefore, will necessarily revolve around the effect of the three known classes of calcium antagonists (Spedding, 1982, 1985), which have been identified by chemical, biochemical and pharmacological techniques and are further differentiated as to how they interact with voltage-operated channels. In addition, the role that antagonists of receptor-operated channels can play in limiting Ca^{2+} influx must be considered. Receptor-operated channels have a high density and high calcium conductance, but allow passage of other ions, and their regional distribution and neuronal population density may well influence the order of selective vulnerability of different neuronal types to disease states. With regard to voltage-operated channels it is necessary to bear in mind glutamate receptor-gated calcium channels. The therapeutic potential for antagonists of neuroexcitatory amino acid-mediated neuronal damage has attracted a considerable amount of attention (and controversy) in recent years. Therefore, it may be necessary to reflect on the need or advisability to extend therapeutic concepts to include multiple actions in drug profiles. In this regard intriguing results are appearing in the literature with regard to the neuroprotective properties of such agents in combination with more conventional calcium antagonists, such as MK801 and nimodipine (Uematsu *et al.*, 1991).

Animal models of cerebral ischaemia

The question of potential therapies involving Ca^{2+} channels in cerebral ischaemia cannot really be addressed without a brief consideration of the models involved, and in so doing reflect on the wisdom of being too rigid in searching for models which exactly mimic the human disease state.

Transient forebrain ischaemia

One approach has employed rodent models of transient forebrain (global) ischaemia which mimics cerebral catastrophes caused by cardiac arrest. It should be noted that cardiac arrest is not used as the means of causing a failure of blood supply to the brain in the rodent models described here. The underlying complications of involving the myocardium and peripheral circulation would require careful consideration in any new therapy. The gerbil model (Kirino, 1982) has been employed extensively over the years, but caution must be exercised in interpreting the value of positive results obtained for a plethora of compounds worldwide, with probably quite dissimilar mechanisms of action against a highly specialised and vulnerable single neuronal type, the CA_1 cell. In addition, comparatively few compounds have been reported to be active in the rat 4 vessel occlusion model (Alps & Hass, 1987; Poignet *et al.*, 1989; Pulsinelli & Brierley, 1979), which again has predominantly featured the ischaemic changes induced in CA_1 cells. This type of

model has also been used for several years, but in the context of this discussion it has not been operated in the classical Pulsinelli mode. It was evident very early on that several major and independent variables could influence the results of studies carried out with the same drug and using identical dosage regimens.

Focal ischaemia

Another approach has been the use of focal infarct models which appear to be much more acceptable since they are considered to reflect more closely the entity of human stroke. Nevertheless, there are extremes in these various models and species differences in the extent of vascular territory encompassed, and therefore in the neuronal regions involved.

Factors exacerbating ischaemic damage

The methodological variables which can have a major impact on the outcome of an experimental study need to be addressed. The problem of controlling brain temperature is a very important feature and must be allowed for during surgery, the ischaemic insult and recovery (Clifton *et al.*, 1989; Kuroiwa *et al.*, 1990; Welsh *et al.*, 1990). Welsh & Harris (1991) have re-investigated the 'protective' effect of hypothermia in gerbils subjected to 5 min bilateral carotid artery occlusion. They observed a lack of protection by acute post-ischaemic hypothermia lasting 1–2 h, and made the comments that the effect on hippocampal injury of pharmacological agents administered during the early phase of recirculation may not be greatly perturbed by transient hypothermia. Assuming this feature is adequately controlled, there are still other variables which can easily override this consideration and abolish previously established neuroprotective profiles for drugs. A major issue is the duration of the insult. This is taken as the period of 'effective' ischaemia resulting from a standard 10 min period of bilateral carotid artery occlusion with a resulting 1000–1200 s (20 min) period of EEG isoelectricity (Alps *et al.*, 1990).

It is important in the rat model of 4 vessel occlusion model (Alps & Hass, 1987) to study a variety of regional neuronal populations and establish reproducible and quantifiable damage, and a discrete order of selective vulnerability in small groups of animals where the injured neurones are therapeutically retrievable. Thus, the model still highlights the exquisite susceptibility of the hippocampal CA_1 cells to ischaemia, but extends this model beyond the gerbil approach (Kirino, 1982) to include neuronal populations also involved in middle cerebral artery occlusion studies. It would appear that the intensity of the ischaemic insult caused by 4 vessel occlusion on the cortex, thalamus and basal ganglia induces a similar degree of injury in cells of these areas to that observed in the penumbra of a focal infarct model (Alps, unpublished observations). Thus, for example, there should be complementary degrees of protection in the cortex of both models in response to identical doses of a test drug, providing appropriate allowance is made for the vascular component in the focal model. In this regard any consideration must take account of the injury to the blood vessels as well and any species variation in anatomical penetration of collateral or adjacent blood vessels to areas at risk.

Another important feature is the level of blood glucose at the time of the ischaemic insult (Pulsinelli *et al.*, 1982). Nominally, in fasted rats (Sprague-Dawley), under fluothane/nitrous oxide/oxygen anaesthesia, it is about 6.5 mM (Alps & Hass, 1987). An increase of 1–2 mM above this level, assuming all other variables are optimally controlled, can reduce the apparent effectiveness of drug treatments (Alps, unpublished observations).

The control of the duration of the isoelectric EEG period is critically dependent upon good and rapid, but flexible, anaesthetic control of peripheral blood pressure once the carotid arteries are occluded. The carotid pressor response must be defeated rapidly and a short-lived combined burst of 5% fluothane from two fluotec vaporizers arranged in parallel can quickly accomplish this aim; usually EEG activity is lost in under 2 min. The provision of respiratory support is vital during the ischaemic episode because once systolic blood pressure falls below 80 mmHg spontaneous respiration ceases in many animals. Peripheral blood pressure is continuously monitored from a tail artery and manipulation of anaesthetic levels is constantly applied and reviewed to maintain blood pressure at about 50 mmHg, or even less,during the 10 min ischaemic period.

Attention must also be given to the effect of environmental stress and handling on animals subjected to forebrain ischaemia, expecially during the first 24 h following the insult. It is during this time that animals recovering from 4 vessel occlusion ischaemia are highly sensitive to touch and very prone to seizures. Our experience shows that too frequent handling for dosing during this time is detrimental to CA_1 cell survival.

Effects of the calcium antagonists

Most calcium entry blockers act on the L type of Ca^{2+} channel with apparently little effect on the N and T types (Tsien *et al.*, 1988). Nimodipine is well known among the calcium entry blockers and has been evaluated in many clinical stroke studies with controversial results (Bougousslavsky *et al.*, 1990; Forsman *et al.*, 1990; Gelmers & Hennerici, 1990; Gelmers *et al.*, 1988; Martinez *et al.*, 1990; Paci *et al.*, 1989; Trust Study Group, 1990). The realisation of the need to institute treatment as early as possible after a stroke has led to the drug being administered in under 12 h from the onset of the injury but its efficacy is still in question. There are obvious difficulties imposed by the hypotensive and cerebrovascular dilatory properties of such drugs in treating this condition. However, there are differences mechanistically between calcium entry blockers which may have some bearing on their apparent effectiveness or otherwise in different models of cerebrovascular disease.

Studies with the dihydropyridines nicardipine, nimodipine and nifedipine at a concentration of 5 μM (Patmore *et al.*, 1989a) clearly showed that whereas nimodipine and nifedipine were devoid of activity in the embryonic

chick myocyte preparation activated into contracture by veratrine, nicardipine practically blocked this effect. Although this is a myocyte preparation and involves Ca^{2+} overload by Na^{+} activation, it highlights the potential for some conventional calcium entry blockers to affect ion channels other than Ca^{2+}. Because protection of neurones in the rat 4 vessel occlusion model involves principally the hippocampal CA_1 cells which are exquisitely sensitive and nicardipine showed activity, it was of interest to examine the calcium entry blockers in another model where these cells could be provoked into enhanced activity by a non-ischaemic stimulus, and where increased and sustained burst firing would most likely involve Ca^{2+}-mediated mechanisms.

In studies where pentylenetetrazole-induced seizures were treated with the dihydropyridines nicardipine and nimodipine, at i.p. doses of 500 $\mu g\ kg^{-1}$ (a neuroprotective dose of nicardipine in gerbils and rats) nicardipine but not nimodipine offered protection against the incidence of tonic seizures and death. The effects of nicardipine were improved by preloading with the agent (Allely & Alps, 1987). Pentylenetetrazole appears to affect the inhibition of neuronal firing by γ-amino butyric acid in cultured mouse spinal cord and hippocampus (Macdonald, 1984). If, *in vivo*, this involves the hippocampal CA_3 neurones (which possess high Ca^{2+} conductance), it may be that nicardipine, but not nimodipine, can antagonise the effect of this neuronal Ca^{2+} modulator in provoking seizures. It is known that nicardipine and flunarizine can inhibit T-type Ca^{2+} channels of isolated hippocampal cells (Takahashi & Akaike, 1991) whereas the T channel is otherwise largely insensitive to the calcium entry blockers, especially those such as nifedipine and the related dihydropyridines (Bean *et al.*, 1986; Benham *et al.*, 1987; Boll & Lux, 1985; Fedulova *et al.*, 1985; Fox *et al.*, 1987; Friedman *et al.*, 1986; Nilius *et al.*, 1985; Yatani *et al.*, 1987). It is interesting that an increase in stimulation rate facilitates the block of T-type Ca^{2+} currents since it has already been mentioned that some calcium entry blockers may be effective only under conditions of high frequency neuronal stimulation, possibly involving N channels, and some may only be active at low concentrations in inhibiting release of some neurotransmitter substances by calcium channel activators (Spedding & Middlemiss, 1985).

Experiences with nicardipine and nimodipine in the gerbil model of forebrain ischaemia have shown that only nicardipine provided statistically significant protection at 500 $\mu g\ kg^{-1}$ i.p. given 15 min pre-ischaemia and followed twice daily for 3 days (Alps *et al.*, 1988). Here there was 41.8% delayed neuronal death in the hippocampal CA_1 subfield in animals treated with nicardipine compared to 78.3% for controls and 76.5% for nimodipine treatment. This dose level for nimodipine was chosen partly on the basis that Heffez *et al.* (1985) demonstrated it would fully saturate voltage-operated channels in gerbil brain membrane. Thus, it must be suspected that the potent binding *per se* of dihydropyridines to voltage-operated channels in such membranes may not necessarily impart a direct neurocytoprotective action to this class of drug. What was evident in the gerbil model, in addition, was the progressive spread of cytotoxic oedema throughout various brain regions as the level of CA_1 neuronal ischaemic lesions increased (Figure 1). The presence

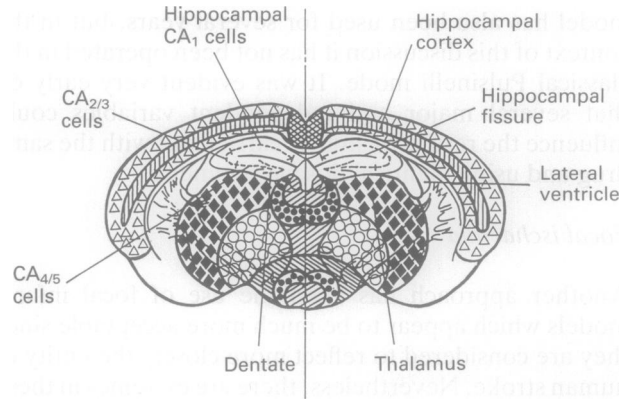

Figure 1 Diagrammatic (coronal section) representation of the ordered progressive regional appearance of perinuclear 'halos' (taken as evidence of cytotoxic oedema) outside the hippocampus in the brain of gerbils subjected to 5 min bilateral carotid artery occlusion, followed by 72 h survival. The order of spread, and therefore degree of involvement, of neurones showing shrunken nuclei was graded (additive) as follows: ▨0.5, ⊡1.0, ▧1.5, ⊡2.0, ◆2.5, ▩3.0, ▥3.5, △4.0. Other landmarks, graded for signs of oedematous change, included splitting of the hippocampal fissure and dentate, and dilatation of the lateral ventricle.

and progressive distribution of perinuclear 'halos' scored on a 0–4 basis, was taken as evidence of previous intracellular swelling. Combined with three other 'oedema' scores for recording splitting of the hippocampal fissure, dilatation of the lateral ventricles, and breaking up of the dentate neurophil in the hippocampus, it can be seen in Figure 2 for a variety of agents tested (nicardipine lidoflazine, flunarizine and nimodipine at 500 $\mu g\ kg^{-1}$ i.p.) that there was an interesting linear relationship ($r = 0.96$) between the mean of the total 'oedema' scores and extent of percentage delayed neuronal death for the CA_1 subfield. It is likely that nicardipine's protective effect combines a direct neuronal action and an anti-oedema effect.

Nicardipine has been shown to protect both the CA_1 neurones and other regional brain areas, including the cortex, thalamus, striatum and cerebellar Purkinje cells

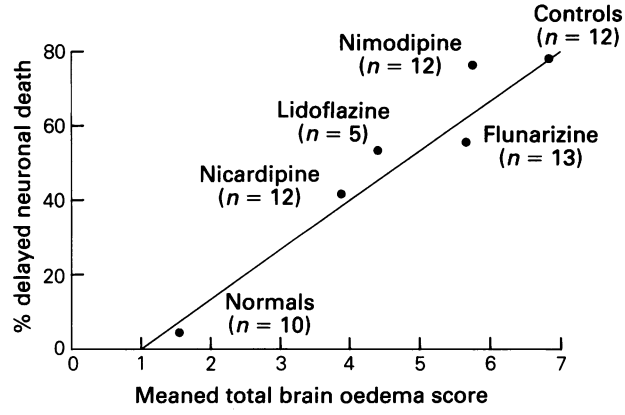

Figure 2 The effect of drug treatment on the relationship between percentage delayed neuronal death of hippocampal CA_1 neurones and meaned total brain 'oedema' score in gerbils subjected to 5 min bilateral carotid artery occlusion, with 72 h survival. All drugs (base substance) were administered i.p. using a loading dose of 500 $\mu g\ kg^{-1}$ 15 min pre-ischaemia followed by 500 $\mu g\ kg^{-1}$ i.p. twice daily for 72 h. Calculated linear regression: $y = 14.1x - 14.2$, $r = 0.96$.

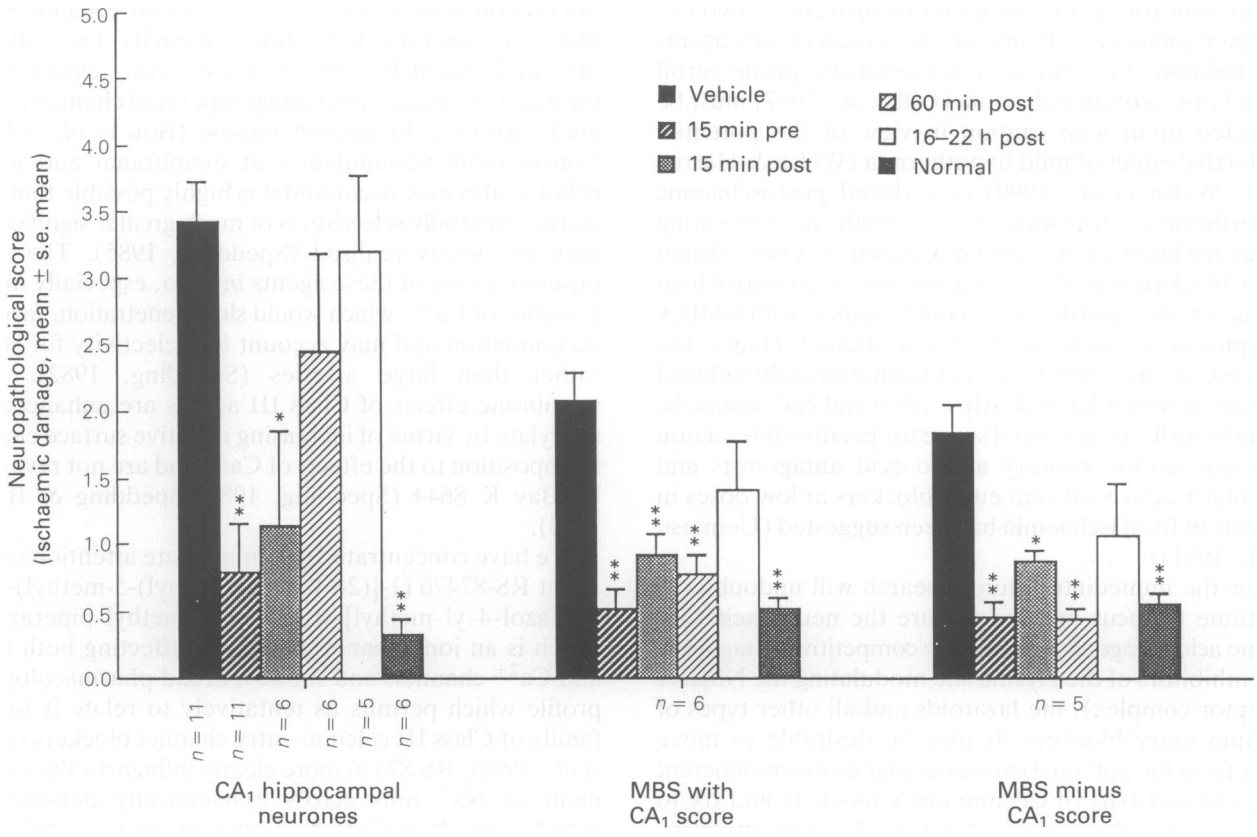

Figure 3 The protective effect of nicardipine on ischaemic damage induced in brain cells by forebrain ischaemia in rats subjected to 10 min four vessel occlusion. Nicardipine was administered as a loading dose of 500 μg kg^{-1} i.p. according to the treatment schedules shown in the figure, and followed in each case by twice daily repeat dosing at 500 μg kg^{-1} i.p. for 72 h. Note the mean score for the CA_1 neurones is based on individual group numbers, whereas the group mean brain scores (MBS) are based on the number of regions observed (anterior and posterior neocortex, thalamus, striatum, cerebellar Purkinje cells), with and without CA_1 scores added. $*P < 0.05$, $**P < 0.0025$.

(Figure 3) in rats subjected to 4 vessel occlusion (Alps & Hass, 1987). Pre-ischaemia treatment was quite effective in limiting ischaemic damage to the CA_1 cells but successive periods of deferred treatment beyond 15 min post-ischaemia were less protective (Alps *et al.*, 1987). In normal brains there was about a 5% level of artefact staining. The mean brain score values were averaged from six different neuronal populations including the anterior and posterior neocortex, thalamus, striatum, hippocampal CA_1 subfield and cerebellar Purkinje cells. The mean brain scores for each treatment or comparator group were calculated with, and without, the CA_1 component value to demonstrate that the other brain areas were protected independently of the CA_1 cells. There was still overall protection even when treatment was deferred for 16–22 h.

It is interesting to note recent attention being drawn to the P type Ca^{2+} channel in the Purkinje cells (Llinas *et al.*, 1989). Incurring damage to the Purkinje cells is really an unnecessary complication in what should be a model of forebrain ischaemia, but it is inevitable that lowering blood pressure sufficiently to induce an isoelectric EEG will reduce cerebellar hindbrain blood flow to a point where respiratory function is depressed and spontaneous breathing ceases. Instituting and maintaining positive pressure ventilation prior to and following the ischaemic insult is very important. With regard to the deferred treatment with nicardipine, it is likely that the mean brain score for animals treated 16–22 h post-ischaemia was higher than necessary because the Purkinje cells were not well protected procedurally. Certainly, under these conditions the Purkinje cells demonstrate a similar order of selective vulnerability to CA_1 neurones. The damage to this area *per se* is now minimised by instituting good pulmonary ventilation in advance of inducing the ischaemic insult and maintaining support until spontaneous breathing returns and is well established. It is still possible to demonstrate an additional element of drug-related protection but the difference is not necessarily expected to show statistical significance.

Other workers (Vibulsresth *et al.*, 1986) have shown nimodipine not to protect the CA_1 neurones in the Pulsinelli version (Pulsinelli & Brierley, 1979) of the 4 vessel occlusion model, using a 20 min period of vessel occlusion. According to the criterion for an optimal insult of 1000–1200 s resulting from a 10 min vessel occlusion (Alps *et al.*, 1990), the reported insult duration of 20 min for the nimodipine study may well have been excessive for hoping to demonstrate a therapeutic benefit for this agent.

In addition, animals with forebrain ischaemia induced straight from the conscious state will satisfy criteria for losing their righting reflex and appearing to lose consciousness, but often demonstrate practically continuous clonic, bordering on tonic general muscular contractures during the ischaemic episode. Perhaps there is an element of damage induced in this version of the model by excessive neuronal excitation and firing which may be

better controlled by *N*-methyl-D-aspartate (NMDA) receptor antagonists. However, the success of such agents and calcium entry blockers in the seizure-prone gerbil model of forebrain ischaemia (Gill *et al.*, 1987) must be reflected upon with caution in view of the potential protective effect of mild hypothermia (Welsh & Harris, 1991; Welsh *et al.*, 1990) or reduced post-ischaemic hyperthermia (Kuroiwa *et al.*, 1990) in preventing ischaemic injury in the gerbil hippocampus. Only calcium entry blockers with the additional ability to control high frequency neuronal discharge could compete with NMDA receptor antagonists and positively interact. Hence, the interest in the effect on pentylenetetrazole-induced seizures in mice (Allely & Alps, 1987) and Na^+ channels. Equally well, the potential scope for positive interaction between neuroexcitatory amino acid antagonists and vascularly active calcium entry blockers at low doses in models of focal ischaemia has been suggested (Uematsu *et al.*, 1991).

For the immediate future research will undoubtedly continue to focus on, and feature the neuroexcitatory amino acid antagonists (especially competitive antagonists and inhibitors of the glycine site modulating the NMDA receptor complex), the lazaroids and all other types of calcium entry blockers. It may be desirable to move away from the potential cardiovascular problems inherent in L channel type of calcium entry blockers and try to broaden the therapeutic window. In contemplating potential therapeutic success with agents having a more broad spectrum site or sites of action, there should be effects on multiple ionic fluxes and a more general (but nevertheless with central selectivity) effect on the stability of the cell membrane. Desirably, agents should be more selective in affecting injured rather than normal membranes and be capable of stabilising neuronal membranes over a period of several hours to limit toxic intracellular accumulation of Na^+ and Ca^{2+}. In fact, the properties of Class III calcium entry blockers as defined by Spedding (1985) are very attractive in potentially offering a reasonably long-lasting influence on membrane stability.

In a study by Poignet *et al.* (1989) where the effects of repeated oral dosing with cinnarizine and flunarizine were examined in the rat 4 vessel occlusion model, according to the procedure advocated by Pulsinelli & Brierley (1979), flunarizine (50 mg kg^{-1}) demonstrated marked protective effects in parietal and frontal cortex, striatum and the CA_1 subfield. Cinnarizine (100 mg kg^{-1}) had little or no effect in the hippocampus, striatum and parietal cortex, but did tend to reduce minor damage in the frontal cortex. Whilst the site and mechanism of action of these drugs was not explained, it was suggested that it might involve effects at vascular and neuronal cell levels. It was concluded that Class III calcium entry blockers can improve post-ischaemic neuronal survival after a transient cerebral ischaemia. It was further suggested that more selective Class III compounds may provide a better way forward.

As a class, the diphenylalkylamines bind to voltage-operated channels at relatively low concentrations and to calmodulin (Bolger *et al.*, 1983; Sarmiento *et al.*, 1983). The link here may reflect a non-specific membrane-stabilising property by virtue of the non-specific lipophilic binding nature of these agents. This property can permit interaction with multiple sites. Class III calcium entry blockers generally have low selectivity for voltage-operated channels compared with Na^+ channels in the heart or against non voltage-operated channel mediated activation in smooth muscle (Bou *et al.*, 1983). Considerable accumulation at membranal and intracellular sites may occur and it is highly possible that this feature, centrally selective, is of much greater significance than previously realised (Spedding, 1985). The slow onset of action of these agents *in vitro*, especially in the presence of Ca^{2+}, which would slow penetration, favours accumulation and may account for selectivity for small rather than large arteries (Spedding, 1982). The membrane effects of Class III agents are enhanced by salicylate by virtue of increasing negative surface charge in opposition to the effects of Ca^{2+} and are not reversed by Bay K 8644 (Spedding, 1984; Spedding & Berg, 1985).

We have concentrated our immediate attention on an agent RS-87476 (1-[(2-(4-methylphenyl)-5-methyl)-1H-imidazol-4-yl-methyl]-4-diphenyl-methyl-piperazine) which is an ion channel modulator affecting both Na^+ and Ca^{2+} channels and shows a broad pharmacological profile which permits us tentatively to relate it to the family of Class III calcium entry channel blockers (Alps *et al.*, 1990). RS-87476 more clearly influences the movement of Na^+ ions across ischaemically depolarized membranes. It inhibits Na^+ currents in neuronal cells (Sheridan *et al.*, 1991), reduces veratrine-induced contractures in embryonic chick myocytes and is a relatively potent inhibitor of [^{3}H]-Batrachotoxin-A-20-benzoate binding (IC_{50}119 nM) (Patmore *et al.*, 1991). Whilst it has only relatively weak effects on Ca^{2+} channels it does markedly inhibit Ca^{2+} channel activators (Fraser & Spedding, 1991). Currently it is thought to exert its neuroprotective effects by interactions with both Na^+ and Ca^{2+} channels. Its broad neuroprotective profile in the rat model (Alps *et al.*, 1990) prompted a study in a cat model of permanent middle cerebral artery occlusion (Kucharczyk *et al.*, 1991) where a remarkable 70% protection against infarct size development was demonstrated at a post-ischaemia i.v. dose as low as 2 μg kg^{-1}, with i.v. maintenance infusion at 0.7 μg kg^{-1} h^{-1} over 12 h (Figure 4). A top dose of 50 μg kg^{-1} i.v., with

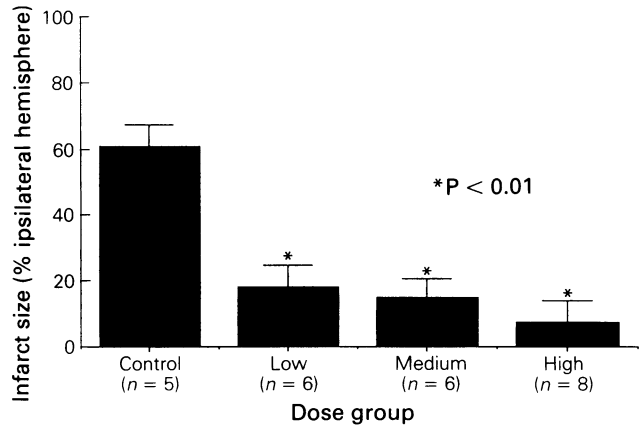

Figure 4 The protective effect of post-ischaemia i.v. treatment with RS-87476 on cerebral infarct size over 12 h in cats subjected to permanent middle cerebral artery occlusion. (Low dose: 2 μg kg^{-1} + 0.7 μg kg^{-1} h^{-1}; medium dose: 10 μg kg^{-1} + 3.5 μg kg^{-1} h^{-1}; high dose: 50 μg kg^{-1} + 17.5 μg kg^{-1} h^{-1}). *$P < 0.01$.

maintenance at 17.5 $\mu g\,kg^{-1}\,h^{-1}$, reduced infarct size by as much as 88%. Progressive monitoring of the developing lesions over 12 h using magnetic resonance imaging/spectroscopy quite clearly demonstrated preservation of ATP levels, reduced tissue acidity and removal of oedematous fluid associated with vasogenic oedema. RS-87476 is not a cerebrovascular dilator but does exhibit some anti-vasopastic properties.

Conclusion

Some broad differences between calcium entry blockers have been observed which may well relate to their potential beneficial effects involving Ca^{2+} channels, but the need for effects on multiple sites involving other ion channels or portals for Ca^{2+} influx must be considered. It is realised that some drugs have probably shown false positive activities in the past by virtue of poorly designed or controlled procedures in animal studies. The possibility for combining therapies exists, and the potential effect that drugs can have on ischaemic processes are evident if stability of membrane function is preserved by reducing its response to ischaemic depolarisation, particularly with regard to the differentiation of Ca^{2+} channels. However, the Ca^{2+} channel *per se* does not appear to offer a simple solution for potential therapies. The role of the Na^{+} channel and its interaction with Ca^{2+} channels opens up interesting prospects for new therapies and the testing of this hypothesis in human patients is awaited.

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