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Parallel dose–response studies of the voltage-dependent Na^+ channel antagonist BW619C89, and the voltage-dependent Ca^{2+} channel antagonist nimodipine, in rat transient focal cerebral ischaemia

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Abstract

We have compared two classes of putative neuroprotectants, the voltage-dependent Na^+ channel antagonist BW619C87 [4-amino-2-(4-methyl-1-piperazinyl)-5-(2,3,5-trichlorophenyl) pyrimidine], and the voltage-dependent Ca^{2+} channel antagonist nimodipine, in a rat model of transient focal cerebral ischaemia. BW619C87 (10–50 mg/kg) or nimodipine (10–100 μg/kg) were injected intravenously 5 min before induction of 2 h transient focal cerebral ischaemia via intraluminal thread occlusion of the middle cerebral artery. BW619C87 was a potent neuroprotectant over the range tested, maximally reducing the volume of hemispheric ischaemic damage by 51% at the 50 mg/kg dose. Nimodipine maximally reduced ischaemic damage by 33% at the 50 μg/kg dose, although the maximal level of neuroprotection afforded by BW619C89 and nimodipine was not significantly different. This is the first study to compare these two classes of drug directly in a model of middle cerebral artery occlusion with reperfusion, and it supports the effectiveness of both as neuroprotectants.

Keywords: Middle cerebral artery occlusion; Ischemia; Reperfusion; BW619C89; Nimodipine; (Rat)

1. Introduction

Excitatory amino acids, and glutamate in particular, have been implicated in the mechanism of ischaemia-induced cell death (Simon et al., 1984; Butcher et al., 1990; Choi, 1990). Antagonists of the *N*-methyl-D-aspartate (NMDA) class of glutamate receptor are potent neuroprotectants in in vivo models of stroke (Park et al., 1988; Chen et al., 1995). However, the utility of compounds of this class, for the treatment of stroke, may be limited by neurocytopathological and psychotomimetic side effects (Olney et al., 1991; Lan et al., 1997).

An alternative approach to interrupting the glutamate cascade central to ischaemia-induced cell death, is provided by voltage dependent Na^+ channel antagonists, such as BW619C89 [4-amino-2-(4-methyl-1-piperazinyl)-5-(2,3,5-trichlorophenyl) pyrimidine], and the anticonvulsant lamotrigine (Smith and Meldrum, 1995). BW619C89 reduces glutamate release in in vitro and in vivo models of ischaemia (Leach et al., 1993; Graham et al., 1994; Chen et al., 1995), and is neuroprotective when administered pre- or post middle cerebral artery occlusion (Graham et al., 1994; Swan and Leach, 1995). Inhibition of neurotransmitter release by voltage-dependent Na^+ channel blockade may provide a more efficient means with which to attenuate the deleterious effects of ischaemia-induced excitatory amino acid release, since the action of glutamate at all ionotropic and metabotropic glutamate receptors will be blocked (Graham et al., 1994).

Voltage-dependent Ca^{2+} channel antagonists have also been demonstrated to confer neuroprotection in rat models of focal cerebral ischaemia (Mohamed et al., 1985; Yenari et al., 1996) and subarachnoid haemorrhage (Tsuchida et al., 1996). Among these, dihydropyridine antagonists of the L-type channel such as nimodipine, have undergone clinical trials for stroke (Gelmers et al., 1988; Trust Study Group, 1990; The American Nimodipine Study Group, 1992), and nimodipine is now approved for the treatment of subarachnoid haemorrhage.

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Comparison of antagonists of these voltage-dependent channels has not been performed in a rat model middle cerebral artery (MCA) of occlusion. In addition, previous studies with BW619C89 examined this compound in models of permanent occlusion (Leach et al., 1993; Graham et al., 1994; Chen et al., 1995; Swan and Leach, 1995; Smith et al., 1997). Consequently, we have studied the dose-response relationship for the effects of BW619C89 and nimodipine in a rat model of MCA occlusion with reperfusion.

2. Materials and methods

2.1. Transient focal cerebral ischaemia

All surgical procedures were approved by the University of Pittsburgh Committee on Animal Research. Studies were performed on male Sprague–Dawley rats (Zivic–Miller) weighing 280–300 g, which were allowed free access to food and water before and after surgery. Animals were anaesthetised with 3% isoflurane, intubated endotracheally, and artificially ventilated with a mixture of 67% N_2O, 30% O_2 and 3% isoflurane. Catheters were inserted into the left femoral artery and vein for physiological monitoring and drug administration, respectively. Atropine sulfate (0.15 mg/kg, intraperitoneally) and pancuronium bromide (0.2 mg/kg, intravenously) were then administered. Rectal temperature (Homeothermic blanket; Harvard Apparatus) and brain temperature (29-gauge thermocouple (Omega) implanted into the right striatum) were monitored and kept at 37.0 ± 0.3°C.

Transient focal cerebral ischaemia was induced using the intraluminal filament occlusion method (Longa et al., 1989), with some modifications. A 3-0 nylon monofilament was carefully inserted 20–21 mm from the bifurcation of the right common carotid artery, into the internal carotid artery via the external carotid artery, and advanced to block the origin of right MCA. Five minutes before MCA occlusion, BW619C87 (0, 10, 20, 30, 50 mg/kg) or nimodipine (0, 10, 30, 50, 100 μg/kg), in 2 ml vehicle, were injected intravenously at a rate of 2 ml/min. Reperfusion was initiated 2 h later by withdrawal of the suture.

2.2. Quantification of ischaemic damage

Rats were sacrificed 72 h after reperfusion. Animals were anaesthetised with chloral hydrate (400 mg/kg) and decapitated. Brain sections (2 mm thickness) were immersed in 2% 2,3,5-triphenyltetrazolium chloride for 20 min at 37°C, and then examined using an image analysis program to calculate the volume of ischaemic damage. The area of damage was determined by subtracting the non-ischaemic area within the ipsilateral hemisphere, from the

Table 1
Physiological parameters for treatment groups in the BW619C89 study

	Brain temperature (°C)	MABP (mmHg)	P_aO_2 (mmHg)	P_aCO_2 (mmHg)	pH (units)	Plasma glucose (mg/dl)
Control (n = 10)						
Before ischaemia	37.0 ± 0.2	123 ± 12	125 ± 11	33 ± 4	7.32 ± 0.04	
During ischaemia	36.9 ± 0.3	122 ± 9	123 ± 10	34 ± 6	7.34 ± 0.05	132 ± 25
After ischaemia	37.0 ± 0.2	119 ± 11	120 ± 12	35 ± 4	7.35 ± 0.04	
10 mg / kg (n = 10)						
Before ischaemia	37.0 ± 0.3	120 ± 13	123 ± 12	33 ± 5	7.34 ± 0.05	
During ischaemia	37.0 ± 0.2	124 ± 14	119 ± 10	35 ± 6	7.34 ± 0.04	128 ± 31
After ischaemia	36.9 ± 0.3	121 ± 10	121 ± 9	33 ± 4	7.33 ± 0.05	
20 mg / kg (n = 10)						
Before ischaemia	36.9 ± 0.2	124 ± 11	118 ± 12	34 ± 5	7.34 ± 0.05	
During ischaemia	36.9 ± 0.2	120 ± 10	121 ± 10	35 ± 4	7.33 ± 0.04	135 ± 28
After ischaemia	37.0 ± 0.3	121 ± 9	119 ± 12	33 ± 4	7.35 ± 0.04	
30 mg / kg (n = 9)						
Before ischaemia	37.0 ± 0.3	120 ± 12	121 ± 9	34 ± 5	7.33 ± 0.05	
During ischaemia	36.9 ± 0.3	123 ± 8	123 ± 13	34 ± 4	7.34 ± 0.04	126 ± 35
After ischaemia	37.0 ± 0.2	118 ± 9	118 ± 14	33 ± 4	7.34 ± 0.04	
50 mg / kg (n = 8)						
Before ischaemia	37.0 ± 0.3	122 ± 11	124 ± 12	33 ± 5	7.33 ± 0.04	
During ischaemia	36.9 ± 0.2	123 ± 9	121 ± 12	34 ± 6	7.33 ± 0.04	124 ± 24
After ischaemia	36.9 ± 0.3	119 ± 12	120 ± 13	34 ± 5	7.35 ± 0.05	

Data are mean ± S.D.
Parameters were measured 20 min before MCA occlusion, 60 min after, and following 20 min of reperfusion.
No significant differences were found between treatment groups at each time point.

Table 2
Physiological parameters for treatment groups in the nimodipine study

	Brain temperature (°C)	MABP (mmHg)	P_aO_2 (mmHg)	P_aCO_2 (mmHg)	pH (units)	Plasma glucose (mg/dl)
Control (n = 8)						
Before ischaemia	36.9 ± 0.3	125 ± 11	121 ± 12	33 ± 4	7.34 ± 0.03	
During ischaemia	37.0 ± 0.3	122 ± 11	117 ± 14	35 ± 4	7.33 ± 0.05	125 ± 31
After ischaemia	37.0 ± 0.2	123 ± 13	119 ± 9	34 ± 6	7.35 ± 0.05	
10 μg / kg (n = 8)						
Before ischaemia	36.9 ± 0.3	119 ± 10	119 ± 13	34 ± 6	7.33 ± 0.04	
During ischaemia	37.0 ± 0.3	123 ± 8	124 ± 12	33 ± 4	7.35 ± 0.05	134 ± 33
After ischaemia	37.0 ± 0.2	123 ± 13	120 ± 10	34 ± 5	7.34 ± 0.03	
30 μg / kg (n = 8)						
Before ischaemia	37.0 ± 0.3	121 ± 12	124 ± 11	33 ± 5	7.34 ± 0.05	
During ischaemia	36.9 ± 0.2	121 ± 10	123 ± 13	34 ± 4	7.32 ± 0.05	128 ± 25
After ischaemia	37.0 ± 0.3	125 ± 8	118 ± 13	33 ± 4	7.35 ± 0.04	
50 μg / kg (n = 8)						
Before ischaemia	37.1 ± 0.3	122 ± 10	125 ± 14	34 ± 4	7.34 ± 0.03	
During ischaemia	37.0 ± 0.3	113 ± 13	120 ± 12	33 ± 5	7.35 ± 0.05	131 ± 32
After ischaemia	37.0 ± 0.2	116 ± 10	121 ± 8	36 ± 5	7.32 ± 0.06	
100 μg / kg (n = 8)						
Before ischaemia	37.0 ± 0.3	121 ± 10	121 ± 11	35 ± 6	7.33 ± 0.04	
During ischaemia	37.0 ± 0.3	93 ± 10**	121 ± 13	34 ± 4	7.35 ± 0.05	124 ± 26
After ischaemia	37.1 ± 0.3	98 ± 11*	123 ± 10	35 ± 4	7.34 ± 0.03	

Data are mean ± S.D.
Parameters were measured 20 min before MCA occlusion, 60 min after, and following 20 min of reperfusion.
* $P < 0.005$, ** $P < 0.0001$ vs. control (One-way ANOVA followed by Fisher's PLSD test).

undamaged contralateral side. Areas of ischaemic damage at 7 stereotaxic levels were summed, and the volume of ischaemic damage calculated by multiplying the area of damage at each level, by the section thickness, as previously described (Swanson et al., 1990). Analysis of brain sections was performed by an observer blinded to treatment group.

2.3. Reagents

All drug solutions were made fresh on the day of study. BW619C89 [4-amino-2-(4-methyl-1-piperazinyl)-5-(2,3,5-trichlorophenyl)pyrimidine] was obtained from Wellcome Research Laboratories (Beckenham, Kent, UK), and dissolved in 0.9% saline. Nimodipine (isopropyl(2-methoxyethyl)1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine dicarboxylate) was from Bayer (West Haven, CT, USA), and was dissolved in PEG400 (with sonication), and then diluted with 0.9% saline. Due to the photosensitivity of nimodipine, drug preparation was performed under a sodium lamp, and syringes were covered with aluminium foil at all times.

2.4. Statistical analysis

Data from physiological parameters and infarct volume quantification were analysed using One-way analysis of variance (ANOVA), followed by Fisher's PLSD post-hoc test for each drug studied. Values are expressed as mean ± S.D. $P < 0.05$ was considered significantly different.

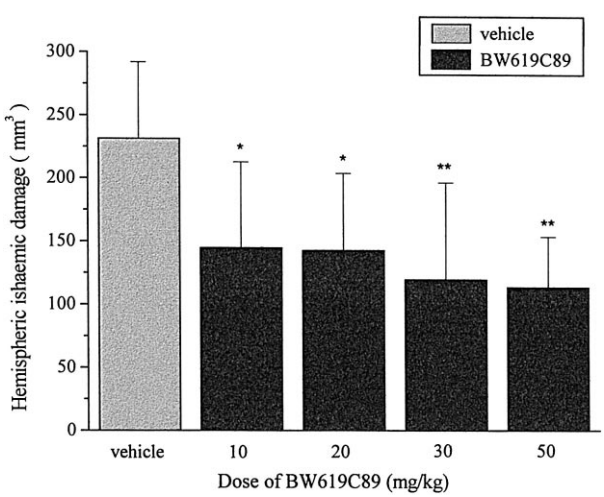

Fig. 1. Volume of hemispheric ischaemic damage following 2 h of temporary focal cerebral ischaemia. Rats were treated with vehicle or ascending doses of BW619C89, 5 min before MCA occlusion. Seventy-two hours following reperfusion, animals were sacrificed and brains sectioned for 2,3,5-triphenyltetrazolium chloride staining. Data are mean ± S.D. for $n = 8–10$ animals per group. * $P < 0.005$, ** $P < 0.001$, compared to vehicle treatment (ANOVA followed by post-hoc Fisher's PLSD test).

3. Results

3.1. Physiological parameters

Physiological parameters from the BW619C89 treatment groups are shown in Table 1. There were no significant differences in brain temperature, mean arterial blood pressure (MABP), P_aO_2, P_aCO_2, pH or plasma glucose, between groups. However, BW619C89 did cause transient hypotension (to ~70 mmHg) in a dose-dependent manner which lasted for 1–3 min immediately after infusion (data not shown).

Table 2 shows physiological parameters for the nimodipine study. No significant differences in P_aO_2, P_aCO_2, pH or plasma glucose were found between drug and vehicle treatments. Nimodipine similarly produced transient hypotension (to ~70 mmHg) which lasted for 2–4 min immediately after administration. In animals receiving 100 μg/kg nimodipine, MABP was significantly reduced during and after ischaemia compared to vehicle-treated animals.

3.2. Histopathology

Pretreatment with BW619C89 at all doses tested, significantly reduced the volume of ischaemic damage following 2 h transient occlusion, compared to vehicle injection (Fig. 1). The volume of hemispheric ischaemic damage (mm^3) in vehicle-treated animals was 232 ± 60 ($n = 8$). This was reduced to 145 ± 68 ($n = 10$), 143 ± 61 ($n = 10$), 120 ± 77 ($n = 9$) and 114 ± 40 ($n = 8$), for animals injected with 10, 20, 30, 50 mg/kg, respectively.

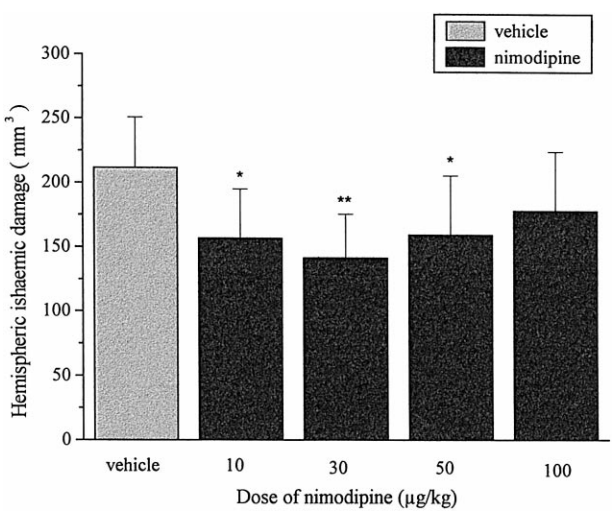

Fig. 2. Volume of hemispheric ischaemic damage following 2 h of temporary focal cerebral ischaemia. Rats were treated with vehicle or ascending doses of nimodipine, 5 min before MCA occlusion. Seventy-two hours following reperfusion, animals were sacrificed and brains sectioned for 2,3,5-triphenyltetrazolium chloride staining. Data are mean ± S.D. for $n = 8$ animals per group. $^{*}P < 0.05$, $^{**}P < 0.005$, compared to vehicle treatment (ANOVA followed by post-hoc Fisher's PLSD test).

Intravenous injection of nimodipine, immediately before occlusion, affected the volume of hemispheric ischaemic damage in a bi-phasic manner (Fig. 2). The volume of hemispheric ischaemic damage (mm^3) in vehicle-treated animals was 212 ± 39 ($n = 8$). Administration of nimodipine at a dose of 10, 30, 50 and 100 μg/kg significantly reduced hemispheric ischaemic damage to 157 ± 38 ($n = 8$), 142 ± 34 ($n = 8$) and 160 ± 46 ($n = 8$), respectively (Fig. 2). The reduction in damage at the 100 μg/kg dose (179 ± 46; $n = 8$) did not reach statistical significance.

4. Discussion

In the present study, BW619C89 and nimodipine were effective in attenuating the volume of hemispheric ischaemic damage when administered immediately before transient focal cerebral ischaemia. BW619C89 exhibited dose-dependent increases in neuroprotection up to 50 mg/kg, while the dose–response relationship for nimodipine was biphasic in nature, loosing efficacy at the highest treatment dose (100 μg/kg). These data provide the first head-to-head comparison of these voltage-dependent channel antagonists in a model of focal cerebral ischaemia with reperfusion.

The efficacy of both drugs was studied using a reperfusion model of stroke. Examining putative neuroprotectants in a reperfusion model is warranted since permanent vessel occlusion has been shown to be the exception rather than the rule, following human stroke (Overgaard, 1994). Furthermore, the pathophysiological profile of transient occlusion differs to that found when vessel occlusion is permanent (Clark et al., 1994; Zhang et al., 1995).

Nimodipine was selected as a reference compound due to its clinical use for subarachnoid haemorrhage, and its efficacy as a neuroprotectant in animal models of stroke (Mohamed et al., 1985; Jacewicz et al., 1990; Herz et al., 1996; Feigin et al., 1998). While the mechanism of action is still unknown, antagonists of voltage-dependent Ca^{2+} channels have potent vasodilator effects (Brandt et al., 1983), and nimodipine has been shown to increase cerebral blood flow (CBF) in rats when administered before MCA occlusion (Mohamed et al., 1985; Herz et al., 1996). However, studies have shown that nimodipine does not affect CBF when administered after vessel occlusion (Gotoh et al., 1986; Hakim, 1986; Berger and Hakim, 1988, 1989; Dirnagl et al., 1990; Herz et al., 1996), while still conferring neuroprotection in post-treatment paradigms (Germano et al., 1987). This suggests the neuroprotective mechanism of nimodipine is unrelated to its haemodynamic effects. Disruption of intracellular Ca^{2+} homeostasis has been suggested to account for some cell death following stroke (Siesjö and Bengtsson, 1989), and the neuroprotective effects of nimodipine may be partly due to

a reduction in ischaemia-induced Ca^{2+} entry into neurons (Koroshetz and Moskowitz, 1996; Feigin et al., 1998).

In the present study, nimodipine (10–50 μg/kg) reduced the volume of ischaemic damage when given prior to transient occlusion. The dose-response study for nimodipine yielded a 'U'-shaped curve of effectiveness, with a reduction in efficacy at the highest dose tested (100 μg/kg). This observation might be explained by the significant hypotensive effect of nimodipine found at the highest dose. Hypotension is an aggravating factor during ischaemia, and has been proposed to impair the neuroprotective effects of nimodipine (Mohamed et al., 1985). However, Jacewicz et al. (1990) reported that the hypotensive effects of nimodipine did not correlate with histopathological outcome, and the reduction in efficacy of nimodipine at the highest dose may be unrelated to the hypotensive effects.

Clinical trials with nimodipine for stroke have yielded disappointing results (Bogousslavsky et al., 1990; Martinez-Vila et al., 1990; Trust Study Group, 1990). In the present study, the maximal level of neuroprotection afforded by nimodipine was only 33%, and other studies have reported relatively mild levels of protection of below 40% (Jacewicz et al., 1990). Indeed, in the study by Jacewicz et al. (1990), the use of 3 different rat strains, masked the neuroprotective effects of nimodipine when results were pooled. However, nimodipine is clinically effective for subarachnoid haemorrhage, and so the mechanism of neuroprotection, and not just the level of efficacy, may be responsible for the disappointing stroke trials, with nimodipine better suited to the treatment of the ischaemia associated with subarachnoid haemorrhage (Trust Study Group, 1990; Feigin et al., 1998).

In the present study, BW619C89 was an effective neuroprotectant within the 10–50 mg/kg dose range tested. Previous studies have demonstrated neuroprotective effects within this dose range when administered before or after permanent MCA occlusion (Graham et al., 1994; Chen et al., 1995; Swan and Leach, 1995; Smith et al., 1997), before traumatic brain injury (Sun and Faden, 1995), or following subdural haematoma (Tsuchida et al., 1996). The level of maximal neuroprotection for BW619C89 (51%) in the present study also compares well to results from previous studies in models of permanent MCA occlusion, when administered before or after vessel occlusion (Graham et al., 1994; Smith et al., 1997). Higher doses of BW619C89 were not studied since the difference in protection between the 30 and 50 mg/kg doses was minimal (3%), and previous studies support the optimally effective dose as 30 mg/kg (Graham et al., 1994).

Voltage-dependent Na^+ channel block has been predicted to be the principal mechanism of action of BW619C89 (Graham et al., 1994), reducing vesicular excitatory amino acid release, as well as functioning to reduce Ca^{2+} entry through voltage-dependent Ca^{2+} channels by blocking membrane depolarisation (Tsuchida et al., 1996). Models of focal cerebral ischaemia have demonstrated that BW619C89 reduces the release of glutamate and other excitatory amino acids in vivo (Graham et al., 1994; Chen et al., 1995). Electrophysiological studies with BW619C89 demonstrated that the Na^+ channel block increases at more depolarised membrane potentials (Xie and Garthwaite, 1996), and this use-dependency has been predicted to maximise drug effectiveness within ischaemic tissue, while limiting effects within non-ischaemic tissue (Graham et al., 1994). BW619C89 has been shown not to affect regional CBF as measured by laser Doppler flowmetry (Graham et al., 1994), supporting a neurone-specific mechanism of action. BW619C89 may also lack the psychotomimetic and cytopathologic side effects associated with some glutamate receptor antagonists (Olney et al., 1991; Muir et al., 1995; Graham et al., 1994; Lan et al., 1997).

Antagonists of voltage-dependent Na^+ channels may have significant advantages over glutamate receptor antagonists since they limit the effects of excitatory amino acids at all glutamate receptors (Graham et al., 1994). The degree of neuroprotection exhibited by BW619C89 compares well to the maximal protection afforded by NMDA and non-NMDA receptor antagonists (Park et al., 1988; Gill et al., 1992), but it is not superior. Chen et al. (1995) examined BW619C89 with reference to the NMDA antagonist dextrorphan, finding there was no significant difference in the degree of neuroprotection exhibited by these compounds. That the level of neuroprotection is not greater might be explained by the contribution of glutamate released by non-exocytotic mechanisms, such as following ischaemia-induced cell lysis (Wahl et al., 1994), or reversal of the glutamate transporter (Torgner and Kvamme, 1990). Reperfusion models are also associated with a different pathophysiology and neuroprotection profile, with a significant proportion of cell death attributable to leukocyte-mediated damage (Clark et al., 1994; Zhang et al., 1995).

Direct comparison of BW619C89 with nimodipine is of interest since no previous experimental studies have compared Na^+ and Ca^{2+} channel antagonists in a model of transient focal cerebral ischaemia. In the present studies, vehicle injected animals exhibited similar infarct volumes (232 ± 60 mm^3 and 216 ± 39 mm^3), validating relative comparisons between drug effectiveness. Nimodipine, at its maximally effective dose, reduced ischaemic damage by 33%, while the maximally effective dose of BW619C89 reduced injury by 51%. While BW619C89 appeared more potent, additional studies directly comparing maximally effective doses of each were not performed. Power analysis demonstrated a need for 46 animals per group to demonstrate the superiority of one compound over another at the $P < 0.05$ level, and therefore differences between the two drugs might be considered modest. This may lend support to either (1) the dual mechanism of action of nimodipine, increasing and decreasing intracellular calcium accumulation, or (2) the importance of non exocyto-

sis-originated glutamate as a significant source of neurotoxicity.

The neuroprotective efficacy of these drugs may also be dependent on the degree of ischaemia associated with a particular model. Osuga and Hakim (1996), demonstrated that depolarisation-induced opening of voltage-dependent Ca^{2+} channels (as assessed by increased in vivo [^{3}H] nimodipine binding), occurred when CBF was reduced by 49% of control. In contrast, extracellular glutamate (measured by in vivo microdialysis), did not increase until CBF levels fell to less than 33% of control (Osuga and Hakim, 1996). Thus, voltage-dependent Ca^{2+} channel block by nimodipine may be proportionally more effective in models in which the ischaemic insult is mild, such as following occlusion with reperfusion, while BW619C89 may show greater efficacy in models of permanent occlusion, in which the ischaemic severity is greater. However, previous studies using a model of permanent MCA occlusion reported the degree of neuroprotection with BW619C89 to be very similar to the present study (Graham et al., 1994), and the significance of these blood flow-dependent effects is unclear.

In summary, we have compared voltage-dependent Na^+ (BW619C89) and Ca^{2+} (nimodipine) channel antagonists in a model of transient focal cerebral ischemia. Both BW619C89 and nimodipine were neuroprotective over the dose ranges tested, although there was no significant difference in the level of protection afforded by one compound over the other.

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