Br. J. Pharmac. (1983), **79**, 141–148

Calcium antagonists: effects on cerebral blood flow and blood-brain barrier permeability in the rat

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1 Because they affect isolated cerebral arteries, some calcium antagonists have been studied on the intact cerebral circulation of the rat.

2 Global cerebral blood flow (^{133}Xe clearance technique) was measured in anaesthetized rats. Neither perhexiline (0.1 μg/kg to 1.0 mg/kg, i.v.) nor diltiazem (0.06–0.6 mg/kg, i.v.) had any significant effect on resting cerebral blood flow when measured 5 min after each dose. A high dose of nifedipine (1.0 mg/kg, i.v.) was administered during induced hypocapnia. Nifedipine failed to modify the hypocapnic vasoconstriction of the cerebral vasculature when compared to vehicle-treated rats.

3 The possibility of discrete changes in *regional* cerebral blood flow was investigated. Local cerebral blood flow was measured in a number of brain regions by the [^{14}C]-ethanol technique 15 min after the administration of nifedipine (20 or 100 μg/kg, i.v.). Nifedipine had no apparent effect on regional blood flow in the rat brain.

4 Acute arterial hypertension increases protein leakage into the brain, a phenomenon susceptible to drugs that act on endothelial pinocytosis which is known to be calcium-dependent. The increase in protein extravasation, induced by the intravenous administration of either angiotensin II or adrenaline, was unchanged in rats previously treated with either nimodipine (20 μg/kg, i.v.) or nifedipine (50 μg/kg, i.v.) when dissolved in ethanol alone. However, nifedipine (20 μg/kg, i.v.) when dissolved in a solution of polyethylene glycol and ethanol further enhanced the hypertension-induced increase in brain albumin permeability.

5 In conclusion, we have been unable to demonstrate any apparent effects of various calcium antagonists on the intact cerebral circulation of the rat, despite the number of different experimental models used.

Introduction

In the accompanying paper (Andersson, Edvinsson, MacKenzie, Skärby & Young, 1983) the calcium antagonists diltiazem, nifedipine, nimodipine and verapamil were shown to be potent vasodilators in feline isolated middle cerebral vessels. These compounds are of considerable interest when employed for the treatment of myocardial disease, partly due to their vasodilator properties and partly to their capacity to reduce heart work; the combination of these two actions might account for the ability of calcium antagonists to reduce a myocardial infarction (Henry, Shuchleib, Borda, Roberts, Williamson & Sobel, 1978; Ferlinz, Easthope & Aronow, 1979; Ichihara, Ichihara & Abiko, 1979).

It has been hypothesized that calcium antagonists may be of benefit in the pharmacotherapy of focal brain ischaemia or vasospasm (du Boulay & Gado, 1974; Craigen, Harper & Kazda, 1981). Prior to undertaking such studies, we considered it important to assess the effects of the calcium antagonists on the intact cerebral circulation and we used both anaesthetized and conscious rats in this study.

Two approaches were used. Firstly, the cerebrovascular effects of known calcium antagonists were studied on global and on regional cerebral blood flow under normocapnic conditions. In some experiments on global flow, increased cerebrovascular tone was induced by hypocapnia.

Secondly, the effects of calcium antagonists were studied on blood-brain barrier function, another im-

portant aspect of the cerebrovascular bed. It is known that acute arterial hypertension can increase the passage of albumin over the blood-brain barrier (Johansson, Li, Olsson, & Klatzo, 1970). The protein leakage into the brain can be modified by drugs changing the cerebrovascular tone (Johansson, 1974) as well as by drugs acting on the endothelial cell membrane, possibly by inhibiting pinocytosis (Hansson & Johansson, 1979; Larsson, Skärby, Edvinsson, Hardebo & Owman, 1980; Hardebo & Johansson, 1980; Johansson, 1981). Since calcium antagonists theoretically could change both the vascular tone and the calcium-dependent pinocytotic process in endothelial cells it was thought worthwhile to examine if nifedipine and nimodipine could modify the extravasation of albumin in acute hypertension in conscious unrestrained rats.

Methods

Global cerebral blood flow (^{133}Xe)

The experiments were performed on 21 male Wistar rats (Iffa Credo), having free access to food and water, and weighing between 260 and 290 g.
Anaesthesia was induced with 4.0% halothane in O_2. After being tracheotomized under 2.5% halothane in O_2, the rats were placed immediately on a positive pressure ventilation system and maintained on a mixture of $N_2O:O_2 = 3:1$ with the addition of 0.8% halothane. (+)-Tubocurarine (Bruneau Laboratories; 15 mg/kg, i.p.) was administered in order to facilitate the control of ventilation. The rectal temperature was kept constant at 37°C by means of a thermal feedback system coupled to the operating table. A catheter was introduced into the abdominal aorta via the femoral artery and connected to a strain gauge transducer (Bell and Howell) for the continuous recording of the mean and pulsatile arterial pressures (Lectromed MX 412). A second aortic catheter was introduced to allow the removal of arterial samples for the determination of P_{CO_2}, P_{O_2} and pH. In addition, one femoral vein was catheterized to allow the administration of intravenous solutions (to counteract fluid loss) and the drug under study.

In order to reduce artefacts, that arise from extracranial contamination, in the measurement of global cerebral blood flow, it was necessary to ensure that the transport of ^{133}Xe into the brain was carried via the internal carotid artery alone. This was achieved by the ligation of the superior thyroid and the pterygopalatine arteries, and the ligation and section of the occipital artery. Following this, the lingual and facial arteries were ligated just distal to the bifurcation of the external carotid artery after which a catheter was inserted retrogradely into the external carotid artery, its tip being placed at the bifurcation of the common and internal carotid arteries. Care was taken to ensure that the tip of the catheter did not restrict flow in the internal carotid artery.

A scalp incision was made and followed by reflection of the temporal muscle to leave the calvarium exposed. Detection of ^{133}Xe in the expired air, a possible artefact, was avoided by placing a small lead plate over the tracheal cannula. For each measurement of cerebral blood flow, a bolus injection of 25–35 µl (0.1 mCi) of ^{133}Xe, dissolved in saline, was given via the catheter in the external carotid artery. The subsequent γ-emissions were detected by a heavily collimated NaI crystal, placed on the hemisphere ispsilateral to the injection site. The detector was connected to a photomultiplier and a pulse height analyser (Novo Diagnostic Systems). The pulses were registered on a scaler/ratemeter, the latter being connected to a chart recorder to display the clearance trace. Cerebral blood flow was calculated using the initial slope index (Hertz, Hemmingsen & Bolwig, 1977). It was found, in other experiments, that blood flow values, determined by this method and by stochastic analysis, correlated extremely well with each other and thus, in the present study, flow has been uniformly calculated by the initial slope method. The biexponential clearance curve obtained was plotted out on semi-logarithmic paper, and the initial slope calculated using the first 15 s of this curve from the formula:

$$CBF_{init} \text{ (in ml } 100\,g^{-1}\,min^{-1}) = (1n\,2.\lambda.6000)/T_{1/2}$$

where: λ is the blood-brain partition coefficient for xenon, and $T_{1/2}$ is expressed in s.

Regional cerebral blood flow ([^{14}C]-ethanol)

The experiments were performed on 17 adult male Sprague-Dawley rats (250–300 g body weight) with free access to tap water and commercial pellet food until operation. Anaesthesia was induced with halothane (Fluothane, Hoechst; 2–3%) delivered via a Dräger vaporizer and the rats tracheotomized and (+)-tubocurarine (0.5 mg/kg, i.v.) was administered. Thereafter, they were ventilated with a small animal respirator (Braun) on 70% N_2O and 30% O_2. Body temperature was measured rectally and adjusted to 37°C by a heating lamp. The femoral arteries and veins were cannulated bilaterally. One of the arterial catheters was used for continuous recording of arterial pressure via a pressure transducer (Elema, Sweden) and the other for anaerobic sampling of blood for gas measurements (BMS Mk II; Radiometer, Copenhagen) and for sampling of the radioactive tracer. One of the femoral veins was cannulated for infusion of [^{14}C]-ethanol (sp. act.,

60 mCi/mmol; Radiochemical Centre, Amersham), 20 μCi dissolved in physiological saline, and the other femoral vein for administration of the drug to be tested.

The flow determinations were not performed until the P_{CO_2} had been stable for at least 15 min. The regional blood flow was determined according to Reivich, Jehle, Sokoloff & Kety, (1969) as modified by Eklöf, Lassen, Nilsson, Norberg & Siesjö, (1973). The [^{14}C]-ethanol solution was injected into the circulatory bed via a femoral vein during 30 s using a constant rate infusion pump. Arterial samples were collected into 10 μl glass capillaries from a femoral artery every 3 s during the isotope infusion.

Immediately after the last sample had been taken, 1–2 ml of saturated potassium chloride was rapidly injected to stop the heart. The animal was decapitated instantly, the head frozen *in toto* in liquid nitrogen, and stored at −25°C in a cryostat for later preparation (1–4 days). The brain was removed and various regions dissected out in a cryostat at a temperature of −15°C in which the tissue pieces were weighed on a microbalance (Kahn). The telencephalic samples taken for analysis consisted mainly of grey matter. These pieces of brain tissue as well as the previously withdrawn blood samples were added to scintillation vials and counted in a Nuclear Chicago liquid scintillation counter. The blood samples were, after blenching with H_2O_2, mixed directly with scintillation fluid (Instagel; Radiochemical Centre, Amersham), whereas the tissue pieces were dissolved in Soluene (Packard) before the scintillation fluid was added. Quenching was corrected according to standard procedures (Eklöf *et al.*, 1973).

The partition coefficient for [^{14}C]-ethanol has been determined previously and found to be 1.14 for brain tissue (Eklöf, Lassen, Nilsson, Norberg, Siesjö & Torlöf, 1974). Calculations of the flow values were performed as described by Reivich *et al.*, (1969). The flow values were calculated on a Wang desk computer according to the protocol used by Eklöf *et al.*, (1974), and the results are expressed on the basis of tissue net weight as ml $100\,g^{-1}\,min^{-1}$.

Blood-brain barrier permeability

Under methohexitone anaesthesia (Brietal Sodium, 50 mg/kg, i.p.), indwelling catheters were inserted into the abdominal aorta via the left femoral artery and in the jugular vein of male Sprague-Dawley rats (weight 200–250 g, $n = 86$). The free end of the catheter was exteriorized on the back of the neck. Two days later the aortic catheter was connected to a transducer and mean arterial pressure (MAP) recorded in awake, unrestrained rats. [^{125}I]-human serum albumin (IHSA, 100 μCi/kg) and Evans blue (2 ml/kg of a 2% solution in saline) were given intravenously as indicators of the blood-brain barrier (BBB) function. Evans blue binds to serum albumin *in vivo* and is easily detected macroscopically providing a convenient gross evaluation of leakage.

Rats were pretreated with nifedipine, (20 μg/kg when dissolved in the commercial solvent or 50 μg/kg when dissolved in ethanol 100 μg/ml) or with nimodipine (20 μg/kg, in ethanol 100 μg/ml). The substances were handled in sodium light to prevent degradation of the drug. Fifteen min later, acute hypertension was induced by an intravenous injection of either angiotensin (20 μg/kg) or adrenaline (20 μg/kg) or by a continuous infusion of adrenaline ($10\,\mu g\,kg^{-1}\,min^{-1}$). A continuous infusion of the lower dose of adrenaline gives a less rapid increase in blood pressure and under control conditions a lower degree of protein leakage in the brain (Johansson & Martinsson, 1979).

Three min after the elevation of arterial pressure, the rats were given methohexitone (30 mg/kg, i.v.) and the brains perfused *in situ* with saline given through the heart for one min to remove the tracers from cerebral blood vessels. The control rats not subjected to hypertension were killed 30 min after the injection of the tracers. The brains were divided as shown in Table 3, the samples weighed, and the radioactivity determined in a scintillation counter. The tracer content in the brain was calculated as a ratio of activity in brain and blood, *i.e.* $100 \times ct\,min^{-1}\,mg^{-1}$ brain tissue over $ct\,min^{-1}\,mg^{-1}$ blood. Statistical differences were evaluated with Wilcoxon's rank sum test.

Results

Global cerebral blood flow

Each successive dose of perhexiline, (0.1 μg/kg–1.0 mg/kg) was given as an intravenous infusion over 1 min, to a group of six anaesthetized rats. There was no apparent effect on either cerebral blood flow or mean arterial pressure at any of the doses studied (Table 1). Normocapnia was maintained throughout the investigation.

Likewise, our preliminary results with diltiazem (0.06–0.6 mg/kg, i.v.) failed to reveal any change in resting cerebral blood flow. However, there was a tendency for the mean arterial pressure to fall with increasing doses of diltiazem.

The studies with perhexiline and diltiazem, described above, were both carried out during normocapnia and hence with normal cerebral tone. It was therefore considered worthwhile to study the effects of a calcium antagonist under conditions of increased cerebrovascular resistance, which was achieved by induced hypocapnia.

Table 1 The effects of perhexiline on the cerebral circulation of the anaesthetized rat

Parameter	*Control*	*Perhexiline* (mg/kg, i.v.)				
		0.0001	0.001	0.01	0.1	1.0
CBF (ml $100\,g^{-1}\,min^{-1}$)	63±3	58±4	64±6	64±3	66±4	72±4
CBF (%)	100	90±5	105±12	105±6	108±6	112±12
P_{CO_2} (mmHg)	40.0±0.3	37.9±0.6	39.7±1.0	39.6±0.8	38.1±0.5	38.9±0.9
MAP (mmHg)	120±4	117±6	111±5	123±5	123±2	135±5

Values are expressed as the mean ±s.e.mean; $n = 6$.
Global cerebral blood flow (CBF) was measured by a ^{133}Xe-clearance technique.

In 6 control rats, hypocapnia was induced by increasing the stroke volume of the respirator ($P_{CO_2} = 21.4-22.6$ mmHg) which resulted in a fall in global cerebral blood flow to 70% of base line values. It was possible, under hypocapnia, to maintain these levels of cerebral blood flow for at least 1 h. During this period MAP and cerebral blood flow remained constant at approximately 115 mmHg and 56 ml $100\,g^{-1}\,min^{-1}$, respectively (Table 2).

In 6 other rats, nifedipine (1.0 mg/kg, i.v.) was administered during induced hypocapnia and cerebral blood flow was thereafter measured at 10 min intervals for up to 1 h. Even under conditions of decreased global cerebral blood flow produced by hypocapnia, nifedipine had no significant effect on the cerebral circulation and blood flow remained constant at approximately 60% of normocapnic values. Mean arterial pressure showed a slight reduction (−17%) following the administration of nifedipine. However, after 10 min arterial pressure had returned to within normal limits (Table 2). This negative result could not be explained by an inadequate dose as nifedipine was administered in a dose 20 times greater than that which was used in the subsequent investigations when dissolved in the commercial solvent.

Regional cerebral blood flow

The values of the physiological parameters remained stable throughout the experimental period in control animals (MAP 135±4 mmHg; pH 7.407±0.048; P_{CO_2} 37.1±2.4 mmHg; P_{O_2} 122±8 mmHg). Administration of the vehicle or 20 μg/kg nifedipine did not significantly change these parameters. A slight reduction in MAP by 9±5%, was seen after 100 μg/kg nifedipine ($P > 0,05$) while the other parameters remained unchanged.

The resting regional cerebral blood flows were all within the limits published previously (Eklöf *et al.*, 1973; 1974; Edvinsson, Lacombe, Owman, Reynier-Rebuffel & Seylaz, 1979).

Neither of the two doses of nifedipine caused a significant change in cerebral blood flow in any of the five regions examined, as compared with vehicle-injected animals (Figure 1).

Blood-brain barrier permeability

The mean decrease in MAP induced by intravenous nifedipine or nimodipine was 20 mmHg (range 15–32 mmHg) in conscious rats. Prior to the ad-

Table 2 The effects of induced hypocapnia ($n = 6$) and hypocapnia plus nifedipine (1.0 mg/kg, i.v., $n = 6$) on the cerebral circulation of the anaesthetized rat

Parameter	*Control*	*Hypocapnia*						
Time (min)	0	+5	+15	+25	+35	+45	+55	+65
CBF (ml $100\,g^{-1}\,min^{-1}$)	81±7	60±4	54±8	56±5	54±6	52±7	56±4	58±7
CBF (%)	100	74±8	67±11	72±10	68±11	67±11	71±8	74±14
P_{CO_2} (mmHg)	40.4±0.6	22.4±2.3	21.4±2.1	21.8±5	22.6±2.0	22.3±2.3	21.6±1.8	21.8±1.7
MAP (mmHg)	110±3	122±11	120±10	109±5	116±5	110±6	114±6	117±7
Parameter	*Control*	*Hypocapnia + nifedipine*						
Time (min)	0	+5	+15	+25	+35	+45	+55	+65
CBF (ml $100^{-1}\,min^{-1}$)	89±6	53±5	51±2	56±3	57±4	52±3	52±3	55±6
CBF (%)	100	61±7	59±5	65±6	65±5	60±6	60±5	66±10
P_{CO_2} (mmHg)	39.7±0.5	21.3±0.9	21.4±1.5	20.3±0.7	19.6±0.6	19.5±0.3	19.2±0.4	19.2±0.7
MAP (mmHg)	104±5	86±2	90±1	89±3	89±3	92±5	92±4	89±4

Values are expressed as the mean ± s.e.mean. Global cerebral blood flow (CBF) was measured by a ^{133}Xe-clearance technique.

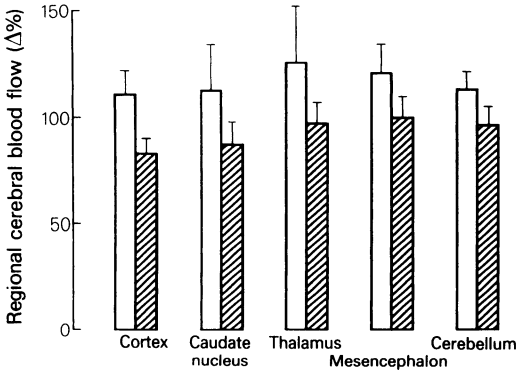

Figure 1 Regional cerebral blood flow of rats injected intravenously with 20 μg/kg, (open columns, $n = 5$) or 100 μg/kg (hatched columns, $n = 6$) nifedipine 15 min prior to measurement. The data are expressed as a percentage of values in vehicle injected animals (0.1 ml solvent, $n = 6$). Mean values shown; s.e.mean indicated by vertical lines.

ministration of angiotensin II or adrenaline, the pressure had normalized or was, at maximum, 10 mmHg lower than the initial MAP (Table 3). Nifedipine, in the solution provided by the drug company, significantly increased albumin leakage in angiotensin-induced hypertension as well as in normotensive control rats. Thus, with this solution, there was no difference between rats given angiotensin and those given adrenaline, in contrast to the other treatment groups in the present study, and to earlier findings (Johansson & Martinsson, 1979). It was obvious that the solvent, and not the drug, was responsible for the enhancement of protein extravasation as nifedipine, in a higher dose but dissolved in ethanol, had no effect on normotensive rats and the angiotensin-induced increase in extravasation was the same as in control rats. (The small amount of ethanol used does not change blood-brain barrier function according to earlier, unpublished observations). A slightly higher tracer content was found in all the brain regions of rats given nimodipine than in the corresponding control rats, but the values were not significantly different (Table 3).

Discussion

Despite the large number of studies carried out on isolated peripheral vessels and myocardial perfusion, the effects of calcium antagonists on the cerebral circulation have not been extensively investigated.

Table 3 Mean arterial pressure (MAP) and brain radioactivity in conscious rats given ^{125}I-labelled serum albumin and subjected to acute hypertension

Experimental group	*Dose* (ug/kg)	n	*Init. MAP* (mmHg)	*Max. MAP* (mmHg)	*Telencephalon*	*Diencephalon, Mesencephalon*	*Pons, Medulla oblongata*	*Cerebellum*
Controls								
Initial	—	6	108± 3	—	0.04±0.01	0.05±0.01	0.04±0.01	0.05±0.01
Angiotensin	20	10	109± 5	180±5	0.08±0.01	0.07±0.01	0.06±0.01	0.11±0.02
Adrenaline	20	6	109± 3	183±6	0.20±0.06	0.27±0.06	0.22±0.07	0.25±0.07
Adrenaline†	10	8	107± 3	170±2	0.09±0.02	0.14±0.02	0.07±0.01	0.10±0.02
Nifedipine (Soln. A)	(20)							
Initial	—	6	105± 4	—	0.11±0.01**	0.10±0.01**	0.10±0.01**	0.10±0.01**
Angiotensin	20	6	108± 4	183±5	0.21±0.03**	0.25±0.04**	0.22±0.03**	0.25±0.02**
Adrenaline	20	6	100± 7	170±7	0.25±0.03	0.30±0.03	0.27±0.03	0.27±0.04
Nifedipine (Soln. B)	(50)							
Initial	—	6	93±12	—	0.03±0.01	0.03±0.01	0.04±0.01	0.04±0.01
Angiotensin	20	6	108± 3	182±4	0.07±0.01	0.06±0.04	0.06±0.01	0.08±0.01
Nimodipine	(20)							
Initial	—	6	96± 2	—	0.06±0.02	0.06±0.01	0.07±0.01	0.07±0.02
Angiotensin	20	8	98± 2	179±4	0.12±0.02	0.16±0.02	0.11±0.01	0.17±0.04
Adrenaline	20	6	108± 4	182±3	0.28±0.03	0.37±0.03	0.32±0.04	0.31±0.05
Adrenaline†	10	6	98± 4	172±4	0.12±0.02	0.15±0.02	0.12±0.01	0.16±0.03

Nifedipine A is the solution provided by the drug company. Nifedipine B and nimodipine were dissolved in ethanol (100 μg/ml); the doses of the calcium antagonists used are given in parentheses. The activity is presented as the ratio between brain and blood × 100. Normal physiological levels of P_{CO_2}, P_{O_2} and pH were observed before the induced rise in pressure. Values are expressed as mean ± s.e. and n = number of rats.
*$P < 0.05$; **$P < 0.01$ (Wilcoxon's rank sum test). † = Dose expressed as an i.v. infusion per minute.

Nonetheless, interactions between calcium antagonists and the cerebral circulation have been studied at three levels: firstly, basic mechanisms *in vitro*; secondly, the effects of calcium antagonists on experimental cerebral vasospasm; and thirdly, their effects on global cerebral blood flow in either the normal or the ischaemic brain.

In the present paper, we have tried to evaluate the actions of four known calcium antagonists (perhexiline, diltiazem, nifedipine and nimodipine) on the intact cerebral circulation of the rat, using a variety of approaches and techniques.

To the best of our knowledge, the effects of perhexiline on the cerebral circulation have not hitherto been studied. In the present investigation we found no change in global cerebral blood flow, following the intravenous administration of the compound at doses ranging from 0.1 μg/kg to 1 mg/kg. These results would tend to support *in vitro* studies which demonstrated the comparatively weak potency of perhexiline in relaxing isolated cerebrovascular smooth muscle (Nowicki, MacKenzie & Young, 1982). However, we cannot exclude the possibility that the weak effect of perhexiline on the cerebral circulation, may be due to a limited passage across the blood-brain barrier. Furthermore, with our present studies, it is impossible to exclude a very transient and limited effect of perhexiline on the cerebrovascular bed; this criticism holds true for the other calcium antagonists that were investigated in the current study.

Diltiazem has a pharmacological profile similar to that of perhexiline (Fleckenstein, 1977) although it was about 20 times more potent than perhexiline when studied on isolated segments of cat pial arteries (Nowicki *et al.*, 1982). Our preliminary studies with diltiazem (0.06–0.6 mg/kg, i.v.) showed no effect on the cerebral circulation of the anaesthetized rat. There was, however, a tendency for the blood pressure to be reduced at the highest dose studied.

Of all the calcium antagonistic compounds, nifedipine has been studied most extensively in both the coronary and the cerebral circulation. With respect to the cerebral vessels, nifedipine has been shown capable of abolishing the constriction induced *in vitro* by a number of agents (prostaglandins, K^+, 5-hydroxytryptamine, phenylephrine, noradrenaline, plasma and blood). This reversal of intense vasoconstriction has been observed consistently in arteries from various species, including cat, dog and man (Allen & Banghart, 1979; Edvinsson, Brandt, Andersson & Bengtsson, 1979; Brandt, Andersson, Bengtsson, Edvinsson, Ljunggren & MacKenzie, 1980; Brandt, Andersson, Edvinsson & Ljunggren, 1981; Andersson *et al.*, 1982). No information is available on the effects of nifedipine on rat cerebral arteries and, as such, the possibility that this drug has a unique effect on the cerebral resistance vessels of the rat remains to be clarified.

In vivo, nifedipine is potent in reversing cerebral vasospasm, as defined angiographically, following experimental subarachnoid haemorrhage (Allen & Bahr, 1979); Takagi, Kamiya, Fukuoka, Mabe, Nagai & Hotta, 1979; Brandt *et al.*, 1980). As in some of these studies nifedipine was administered systemically (by sublingual and intravertebral routes), it would appear that the drug has access at least to the large arteries in which vasospasm occurs. In the present investigation, nifedipine had no effect whatsoever despite the induction of vasoconstriction by hypocapnia, a stimulus chosen to approximate the severe vasoconstriction induced by subarachnoid blood. It is surprising that nifedipine had no effect on this type of vasoconstriction, since *in vitro* the drug abolishes the vascular contractions induced by a number of agents.

Two possibilities may account for these findings. Firstly, hypocapnia might contract cerebrovascular smooth muscle by a mechanism entirely different from any of the agents used *in vitro* and this type of vasoconstriction may not be susceptible to calcium blockade. This, however, seems unlikely. Secondly, the systemic administration of nifedipine might permit access to the large cerebral vessels (those contracted by subarachnoid blood) while the smaller resistance vessels (contracted by hypocapnia) are protected by the blood-brain barrier (Edvinsson & MacKenzie, 1976), namely, the endothelial cells characteristic of brain. This second hypothesis would be supported by the fact that regional cerebral blood flow remained unaltered following the intravenous administration of nifedipine (see Figure 1). This lack of effect is surprising as, even normocapnic cerebral vessels have a considerable tone.

The hypothesis that systemically administered calcium antagonists fail to gain access to the smooth muscle of the cerebral arterioles would be supported by their lack of effect on the hypertension-induced increase in blood-brain barrier permeability. Severe hypertension induces a measurable extravasation of protein into the brain and this extravasation is exacerbated if the cerebral arterioles are dilated. However, in the present study the only significant modification of barrier penetration could be attributed to the commercial excipient of nifedipine.

Nimodipine is a substituted pyridine compound which is structurally similar to nifedipine and has been shown to be approximately 10 times more potent than nifedipine (Andersson *et al.*, 1982). Although it has been claimed that the oral and intra-arterial administration of nimodipine markedly increased cerebral perfusion in cats and dogs (Hoffmeister, Kazda & Krausse, 1979; Kazda, Hoffmeister, Garthoff & Towart, 1979), these results must be

interpreted with extreme caution as blood flow measured by ^{133}Xe is invalid in these species (Edvinsson & MacKenzie, 1976). A more rigorous study in the primate showed only a modest increase in cerebral perfusion after the prolonged intravenous administration of nimodipine (Harper, Craigen & Kazda, 1981). More interestingly, they showed a marked increase in cerebral blood flow with the intracarotid administration of nimodipine, following the osmotic opening of the blood-brain barrier. These changes in cerebral perfusion were not accompanied by any modification of cerebral metabolism. The observations of Harper and his colleagues (1981) would tend to support the hypothesis advanced above, namely, that the effects of calcium antagonists are predominantly on large cerebral vessels and that there is a limited penetration of these agents to the smooth muscle of cerebrovascular resistance vessels. Similarly, these findings would strengthen the view that calcium antagonists do not reduce cerebral metabolism unlike their action on myocardial oxygen consumption.

In conclusion, our results indicate that calcium antagonists failed to modify cerebral tissue perfusion and the integrity of the blood-brain barrier, both of which are sensitive indices of the efficacy of drugs in the normal brain. Although there can be no doubt about the efficacy of calcium antagonists *in vitro*, we hypothesize that, in intracerebral arterioles, these agents fail to reach the vascular smooth muscle which is protected by the blood-brain barrier.

Future research might be more profitably directed towards the actions of calcium antagonists on major cerebral vessels, not protected by the blood-brain barrier in diseased conditions such as vasospasm.

We are most grateful to Mr J.-P. Robert who assisted with some of the experiments and to Mme M.-L. Pernelet who was kind enough to type the manuscript.

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(Received August 23, 1982.
Revised October 18, 1982.)