The Effects of Nifedipine and Felodipine on Cerebral Blood Flow During Anoxic Episodes

JOHN W. PHILLIS, D.V.SC., ROBERT E. DELONG, PH.D., AND JULIE K. TOWNER, B.SC.

SUMMARY Cerebral blood flow (CBF) in the rat was monitored by a venous outflow technique with an extracorporeal circulation, which allows for the continuous recording of flow over periods of several hours. Brief periods of anoxia increase the rate of flow. The dihydropyridine calcium antagonists did not affect basal flow rate and depressed the increase in CBF elicited by anoxia. These findings may have significant implications for the therapeutic use of dihydropyridine calcium antagonists in brain ischaemia.

In a conceptually important development, it has been hypothesized that calcium antagonists may be of significant benefit in the pharmacotherapy of focal brain ischaemia or vasospasm. The effects of the 1,4-dihydropyridine calcium antagonist, nifedipine, have been amongst the most extensively studied in both the coronary and cerebral circulations. With respect to cerebral vessels nifedipine has been shown to be capable of abolishing the constriction induced in vitro by a number of agents, including prostaglandins, K+, 5-hydroxytryptamine and noradrenaline. In vivo nifedipine is potent in reversing cerebral vasospasm following experimental subarachnoid haemorrhage. The beneficial effects of nifedipine in the treatment of ischaemic heart disease have been attributed to several mechanisms, including dilatation of large coronary arteries and coronary resistance vessels, increased collateral blood flow to ischaemic myocardium and decreased myocardial oxygen requirements. Felodipine is a new dihydropyridine derivative which, like nifedipine, inhibits vascular smooth muscle contraction by interfering with calcium fluxes and/or calcium availability.

Our interest in the effects of these 1,4-dihydropyridines on the cerebral blood flow (CBF) response to anoxia, resulted from the observation that nifedipine is a potent inhibitor of the uptake of adenosine by rat brain cortical synaptosomes and that it potentiates the depressant effects of iontophoretically applied adenosine on the firing of rat cerebral cortical neurons. Nifedipine competes with the adenosine uptake inhibitor, nitrobenzylthioinosine, for binding sites in brain tissue, and it has been suggested that inhibition of adenosine uptake could be a factor in the pharmacological actions of the 1,4-dihydropyridines. Other calcium antagonists (verapamil, D-600, diltiazem) are rather ineffective as inhibitors of calcium uptake by brain synaptosomes.

There is a considerable body of evidence in support of the hypothesis that adenosine, a smooth muscle relaxant, plays a major role in the regulation of cerebral vascular tone, and in particular, in the generation of the reactive hyperaemia which occurs during and after cerebral ischaemia. The possibility that nifedipine and the related calcium antagonist felodipine, as a result of their ability to enhance the action of adenosine, could be of especial therapeutic utility for the treatment of focal brain ischaemias was therefore evaluated.

In this study we have used a venous outflow technique to measure cerebral blood flow and its response to brief periods of anoxia. The method for measuring CBF by cannulation of the retroglenoid vein of the rat was first described by Nilsson and Siesjo and has been refined by the addition of an extracorporeal circulation which allows for continuous recording of CBF over periods of several hours, during which the effects of manipulations of blood gases can be conducted in the presence or absence of pharmacological agents. We report in this paper the unexpected ability of nifedipine and felodipine, to attenuate, in a dose-dependent fashion, the increase in cerebral blood flow produced by a brief period of anoxia. Possible mechanisms for this attenuation are discussed.

Methods

Preparation and Recording

Cerebral blood flow measurements were performed on 22 male Sprague-Dawley rats (Charles River, 350-400 gm wt.). Anaesthesia was induced with 3% halothane to allow tracheotomy and the animals were then maintained during surgery on a mixture of nitrogen (70%), oxygen (30%) and methoxyflurane. Body temperature was maintained at 37°C by a heating pad controlled by a rectal probe. One femoral artery and a femoral vein were cannulated. The artery was used to record arterial blood pressure to obtain periodic small samples (0.4 ml) of arterial blood for pH and blood gas analysis. The femoral vein cannula was used to return cerebral venous blood to the animal. The animal was then heparinized (1 unit/gm wt.).

The right retroglenoid vein (RGV) was exposed through skin incisions placed in front of the external auditory meatus after division of the temporalis muscle. A loose ligature was placed on the right RGV on the cranial side of large facial and retroauricular veins that connect with the RGV. The left RGV was then
exposed and freed from the surrounding tissues at its exit through the retrogleneoid foramen. On this side, a ligature was tied into position to prevent reflux of venous blood into the transverse sinus from the extracranial veins, and the small facial veins emptying into the RGV were coagulated. An angiocatheter (Deseret, 22GA, 1") was inserted into the retrogleneoid vein and advanced in a retrograde direction until its tip was flush with the opening of the retrogleneoid foramen. The angiocatheter was tied into place in the RGV using 6-0 suture thread. The extracorporeal venous flow system was then connected. The femoral catheter was connected to a drop recording chamber via tubing which passed through an adjustable roller pump. The drop chamber and extracorporeal tubing were filled with blood from a donor animal to prevent blood loss from the experimental animal. Blood in the extracorporeal circuit was warmed with a heat lamp. Blood from the retrogleneoid vein flowed through the drop counter into the drop chamber and thus back to the animal. The height of the end of the drainage tube attached to the retrogleneoid angiocatheter was adjusted to be level with the retrogleneoid foramen.

Once flow through the extracorporeal circuit had started, the contralateral (right) RGV was occluded by tying off the previously placed ligature, to ensure that the venous blood from the transverse sinus drained through the angiocatheter and was not contaminated by extracranial blood from the contralateral side of the head. The animal was now administered pancuronium bromide (Pavulon, 1 mg/kg), connected to a ventilator and respired at a frequency of 60-80 strokes/min with a gas mixture of methoxyflurane (0.2%) in 30-40% oxygen and nitrogen. A period of 15-20 min was now allowed for blood pressure and blood gases to stabilize. Mean arterial blood pressures (MABP) were between 105-130 mmHg. The mean values for blood gases in all 22 animals were PaO₂, 139 ± 5.7 mmHg; PaCO₂, 38.3 ± 2.3 mmHg with a pH of 7.38 ± 0.02. Additional doses of heparin and pancuronium bromide were administered as required. Drop rate and arterial blood pressure were recorded on a Grass Polygraph.

Following stabilization, the animals were administered a series of anoxic challenges. Oxygen flow was occluded for periods of 36 sec and, at the same time, the nitrogen concentration was increased to 100%. The total flow rate remained constant. In this way the animals were rendered anoxic, without any accompanying hypercapnia. An interval of 15 min was allowed between anoxic challenges.

**Data Analysis**

The data obtained in these experiments is expressed as a percent of change in cerebral blood flow and the time to recovery following an anoxic challenge. Baseline flow rates were measured as the number of drops occurring in the minute preceding the anoxic challenge. Due to the "dead-space" (anaesthetic jar, respiratory pump, connecting tubing) in the respiratory circuit, there was a delay of about 15 sec before the animals started to respond to the anoxic challenge.

Thus, the start of the anoxic challenge was defined as that point at which there was a change in either arterial blood pressure or in CBF. Peak flow rate represents the rate of flow achieved during the period of anoxia and the percent change in CBF is calculated from the formula: \% change = peak flow rate/baseline flow rate. Recovery time represents the time from the end of the anoxic challenge until the flow rate returned to preanoxia levels.

In evaluating the effects of nifedipine and felodipine on cerebral blood flow during anoxia, the response to anoxia immediately preceding an intraperitoneal drug administration was compared with the response 15-20 min following drug administration. Drug effects were analyzed by comparisons between control data and results at each dosage using a Student’s t test for within-subject data. Results are presented as the means ± SEM.

The following drugs were used: nifedipine (Sigma) and felodipine (A.B. Hassle). Both substances were dissolved in a mixture of dimethylsulphoxide (DMSO) and 0.9% NaCl solution (50:50). Both calcium antagonists were kept in a dark environment to minimize light induced decomposition. Two rats were used to study the effects of DMSO on cerebral blood flow and its response to anoxia. In amounts of up to 0.35 ml (twice the highest amount used for the administration of the calcium antagonists), DMSO did not affect basal CBF or its response to anoxia.

The effects of both agents on cerebral blood flow were evaluated in two separate groups of animals. Nifedipine: 0.01 and 0.05 mg/kg was tested in 4 rats; 0.5 and 1.0 mg/kg in 6 rats. Felodipine: 0.01 and 0.05 mg/kg in 4 rats; 0.5 and 1.0 mg/kg in 6 rats. Successive doses of drug were administered at approximately 45 min intervals. The higher doses of both nifedipine and felodipine were associated with small decreases in arterial blood pressure (10-15 mmHg), which then returned to control levels. Nifedipine was administered to an additional group of four animals in order to obtain blood gas measurements during the periods of nitrogen inhalation. Arterial blood samples were withdrawn during the last 5 sec of two control anoxic challenges and then during two anoxic challenges following the administration of nifedipine (0.5 mg/kg) and again after nifedipine (1.0 mg/kg).

**Results**

**Basal CBF**

The relationship between drop rate and blood flow rate has been ascertained in a previous series of experiments. For a wide range of drop rates, there is a linear relationship between rate and flow/min. The mean resting flow rate for all the animals used in this series was 29.6 ± 1.3 drops per min (0.7 ml/min; or 35 ml/100 gm brain/min). This is comparable to the mean rate of 26.2 ± 2.6 drops per min (0.62 ml/min) recorded in our previous series of experiments.

Neither nifedipine nor felodipine (doses of 0.01, 0.05, 0.5 or 1.0 mg/kg) affected basal CBF. With both agents, there was a tendency for flow rates to increase...
in some animals, but overall this trend did not achieve significance (p > 0.05). It is possible that the transient fall in arterial blood pressure following nifedipine or felodipine administration may have negated any increase in CBF resulting from dilation of cerebral resistance vessels.

Responses to Anoxic Challenge

Figure 1 illustrates the sequence of responses to an anoxic challenge. The effects of a brief period of anoxia on arterial blood pressure varied from animal to animal, but were relatively reproducible in a given animal. A frequent pattern, which is illustrated in this figure, was a series of small increases and decreases in arterial blood pressure, often culminating with a brief fall in blood pressure at the end of the period of anoxia. Drop rate increased rapidly, coincident with the onset of alteration in arterial pressure, and then declined slowly following the cessation of anoxia. The increases in CBF elicited by repeated anoxic challenges at 15 min intervals were consistent over periods of 90–120 min.

Nifedipine and Felodipine

When administered intraperitoneally at doses of 0.01 and 0.05 mg/kg, nifedipine did not significantly alter either the % increase in peak flow or the recovery time following an anoxic challenge (table 1). At higher doses (0.5 to 1.0 mg/kg) nifedipine significantly reduced both the increase in flow rate and the duration of the recovery period. This attenuation of the response to anoxia is illustrated in figure 2. The control exposure to nitrogen inhalation (36 sec) elicited a marked increase in venous blood flow which persisted for over 3 min. When repeated 15 min after the administration of nifedipine, anoxic challenge evoked only a minimal increase in flow rate, which rapidly returned to control values. Table 2 presents the blood gas and pH data obtained during anoxic challenges administered before and after nifedipine. Prior to the administration of nifedipine, \( PaO_2 \) fell to 24.0 ± 1.2 mmHg during the anoxic episode. The \( PaO_2 \) values were not altered by the administration of nifedipine. Blood \( PaCO_2 \) and pH values were also unaffected by nifedipine.

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**TABLE 1** The Mean (± SEM) Percent Increase in Cerebral Blood Flow (venous outflow) and Recovery Times Recorded during 36 sec Anoxic Challenges before and after Administration of Nifedipine and Felodipine

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of rats</th>
<th>% Increase in flow*</th>
<th>Time of recovery* (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control anoxia</td>
<td>4</td>
<td>163.8 ± 17.6</td>
<td>291.8 ± 82.4</td>
</tr>
<tr>
<td>nifedipine 0.01 mg/kg</td>
<td>6</td>
<td>171.3 ± 16.9</td>
<td>270.3 ± 51.6</td>
</tr>
<tr>
<td>nifedipine 0.05 mg/kg</td>
<td>4</td>
<td>187.8 ± 34.5</td>
<td>215.5 ± 46.7</td>
</tr>
<tr>
<td>Group II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control anoxia</td>
<td>4</td>
<td>171.5 ± 12.9</td>
<td>233.7 ± 37.7</td>
</tr>
<tr>
<td>nifedipine 0.5 mg/kg</td>
<td>6</td>
<td>124.3 ± 5.3*</td>
<td>122.7 ± 30.0‡</td>
</tr>
<tr>
<td>nifedipine 1.0 mg/kg</td>
<td>4</td>
<td>120.5 ± 7.1*</td>
<td>70.8 ± 21.8‡</td>
</tr>
<tr>
<td>Group III</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control anoxia</td>
<td>4</td>
<td>165.3 ± 16.4</td>
<td>217.5 ± 28.9</td>
</tr>
<tr>
<td>felodipine 0.01 mg/kg</td>
<td>6</td>
<td>197.0 ± 15.5*</td>
<td>239.3 ± 34.4</td>
</tr>
<tr>
<td>felodipine 0.05 mg/kg</td>
<td>4</td>
<td>172.3 ± 15.8</td>
<td>236.0 ± 53.6</td>
</tr>
<tr>
<td>Group IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control anoxia</td>
<td>6</td>
<td>142.8 ± 3.6</td>
<td>136.0 ± 14.9</td>
</tr>
<tr>
<td>felodipine 0.5 mg/kg</td>
<td>6</td>
<td>117.5 ± 7.1*</td>
<td>121.0 ± 17.7</td>
</tr>
<tr>
<td>felodipine 1.0 mg/kg</td>
<td>6</td>
<td>124.5 ± 6.0*</td>
<td>83.7 ± 30.0*</td>
</tr>
</tbody>
</table>

*p < 0.05.
†p < 0.01.
‡p < 0.001.
*See data analysis section of Methods for details.

Similar results were observed with felodipine administration. At a dose of 0.01 mg/kg felodipine administration did result in a small, significant increase in peak CBF, but there was no increase in the duration of the response. The responses following 0.05 mg/kg

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**FIGURE 1.** Responses of cerebral blood flow and arterial blood pressure to an anoxic episode. CBF, as represented by outflow from the retroglenoid vein, is recorded as drops. Anoxia was induced by ventilating the rat with 100% nitrogen (tidal volume remaining constant) for 30 sec.

**FIGURE 2.** Arterial blood pressure (pulse pressures damped electronically) and CBF rates elicited by anoxic challenges (36 sec) before (A) and after (B) nifedipine (0.5 mg/kg) administration intraperitoneally.
felodipine were comparable to the controls. Higher
doses of felodipine (0.5 and 1.0 mg/kg) caused signifi-
cant reductions in both the magnitude of the increase in
cerebral blood flow as measured by a venous outflow tech-
nique. With the higher doses of both agents there was
an initial small fall in systemic blood pressure, and this
may have negated any increase in cerebral blood flow
that might be consistent with this explanation. Alternati-
vely, it is possible that nifedipine may be acting as an adenosine
antagonist at smooth muscle membrane receptors. Ni-
edipine was the most potent of a series of calcium
antagonists (nimodipine) to baboons \(^23\) and humans \(^24\). Con-
versely, it has been claimed that the oral or intraarterial
administration of nimodipine increases cerebral perfu-
sion in cats, dogs, rabbits \(^\text{25-27}\) and primates \(^28, 29\). Species and technical factors may be partially responsible
for these experimental differences.

Somewhat surprisingly, nifedipine and felodipine
agonized the increase in CBF induced by an anoxic
challenge to the rats in this series. This observation
was unexpected, in that nifedipine is a potent inhibitor
of adenosine uptake by rat brain synaptosomes, and
can potentiate the depressant actions of exogenous
adenosine on the firing of cerebral cortical neurons.\(^14\)
Adenosine release from hypoxic brain tissues has been
established as a major factor in the production of the
reactive hyperaemia in the brain which follows hy-
poxia or systemic hypotension.\(^20\) Furthermore, the in-
crease in CBF in rats elicited by anoxia is potentiated
by the adenosine uptake inhibitors dipyridamole and
papaverine and antagonized by the adenosine antago-
nist, caffeine.\(^21\) The literature did, however, contain
some prior indications that dihydropyridine calcium
antagonists might attenuate vascular responsiveness.
Harris et al.\(^22\) found that the ability of the cerebral
vasculature to autoregulate during decreased blood
pressure was greatly reduced during the administration of
nimodipine. Nifedipine has also been observed to
attenuate the reactive hyperaemia following coronary
artery occlusion in vivo in the dog heart.\(^30,31\)

The loss of a hyperaemic response to anoxia follow-
ing nifedipine administration may be a result of this
agent's interaction with the "transporter" system which
mediates the facilitated diffusion of adenosine
across cell membranes.\(^32\) The "transporter" mediates
adenosine efflux as well as its uptake, and inhibition of
the transporter could result in a failure of the hypoxia-
induced release of adenosine. A reduction in the re-
lease of adenosine from ischaemic rat hearts in the
presence of nifedipine has been observed\(^33\) and would
be consistent with this explanation. Alternatively, it is
possible that nifedipine may be acting as an adenosine
antagonist at smooth muscle membrane receptors. Ni-
edipine was the most potent of a series of calcium
antagonists at displacing \(^3\text{H}-\text{cyclohexyladenosine}\)
and \(^3\text{H}-\text{diethylphenylxanthine}\) binding to bovine brain tis-
ues, and it was suggested that the nifedipine binding
site may be linked to adenosine receptors.\(^34\)

### Table 3

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Control</th>
<th>Nifedipine (0.01 mg/kg)</th>
<th>Nifedipine (0.05 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>102.7 ± 4.5</td>
<td>113.3 ± 4.1*</td>
<td>116.9 ± 5.3*</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>98.4 ± 4.1</td>
<td>105.8 ± 4.1</td>
<td>103.8 ± 2.9</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>101.8 ± 3.5</td>
<td>112.2 ± 2.1*</td>
<td>112.0 ± 8.5</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>102.2 ± 7.7</td>
<td>99.2 ± 4.0</td>
<td>96.2 ± 3.9</td>
</tr>
</tbody>
</table>

*The post-drug values are significantly different from the controls using a paired t test \((p < 0.05)\).
†MABP was calculated by averaging values observed at 6 sec intervals during the 36 sec anoxic challenge.
reduces the increase in dog coronary arterial flow elicited by adenosine, but it failed to antagonize the relaxing effect of adenosine on norepinephrine- and K+-contracted rabbit femoral arterial rings. An antagonism between nifedipine and adenosine is also difficult to reconcile with its potentiation of adenosine's actions on rat cortical neurons, and it is possible that the apparent antagonism of the adenosine elicited increase in dog coronary arterial flow in vivo was a result of a reduction in the release of endogenous adenosine and the withdrawal of its contribution to coronary relaxation.

The effects of felodipine on adenosine transport have not been reported. However, as a number of related dihydropyridines can compete with a putative ligand for the transport site (H-nitrobenzylthioinosine), it is reasonable to propose that felodipine would share this action. A reduction in the hypoxia-induced release of adenosine would account for felodipine's antagonism of the cerebral reactive hyperaemia elicited by an anoxic challenge. Again, it is also possible that felodipine may act as an adenosine antagonist at smooth muscle receptors.

In regard to the possible therapeutic use of nifedipine and felodipine to alleviate the effects of cerebral vascular insufficiency, the present results indicate a need for caution. Specifically, the administration of these agents in therapeutically relevant amounts not only failed to increase cerebral blood flow, but severely reduced the cerebrovascular responses to anoxia. Our findings serve to emphasize the concerns noted by previous investigators. A severe impairment of CBF responses to arterial pCO2 changes and autoregulation to reduced blood pressure following nimodipine administration has been observed in baboons and it was suggested that this agent might increase the susceptibility of tissue to ischaemic damage. The effects with nifedipine are also in keeping with those observed on leg blood flow in normal subjects and in patients with peripheral occlusive arterial disease. In both groups nifedipine administration (10 and 1 mg) was followed by decrease in post-ischemic flow.

Acknowledgment
We are grateful to AB Hassle for the gift of felodipine.

References
Trends in Mortality from Cerebrovascular Disease in Italy, 1955-78

CARLO LA VECCHIA, M.D.,* AND ADRIANO DECARLI, Sc.D.†

SUMMARY Trends in age-specific and age-standardized death certification rates from all cerebrovascular diseases and various diagnostic subcategories in Italy during the period 1955-78 have been analysed. In both sexes, a decrease in excess of 25% was evident in the overall age-standardized cerebrovascular disease mortality. However, rates were roughly stable in males up to age 50 and in females up to age 45, and slightly but consistently increasing in the younger age groups (under 40), mostly in females. The largest downward trends were for both sexes in the 55 to 74 age groups, and the declines were more marked in females, averaging 3% per year.

Since death certification is most reliable in the younger age groups and it is difficult to imagine any modification of risk factors which should affect mortality in later middle age but not in younger age groups, there is no obvious and simple interpretation of this pattern of trends. A comparison with similar trends in ischemic heart disease and other causes of death suggests that the decline in overall cerebrovascular disease mortality might be partially or largely artefactual, though a between-sexes comparison indicates that at least part of the decrease registered in females may well be real. The extent of the decline, however, has been almost certainly more limited in Italy than in most other Western countries. Only in the younger age group (30-34) did rates show a larger increase in females, which might be related to increased prevalence of cigarette smoking, or the use of oral contraceptives.

DATA FROM THE UNITED STATES.1-3 Australia4 and several other Western countries5-9 indicate that registered incidence and certified mortality from cerebrovascular disease have been largely decreasing over recent decades. The decreasing mortality from cerebrovascular disease was observed earlier than the fall in coronary heart disease mortality, possibly since the beginning of the century in the United States.5 Several potential determinants of this fall have been considered, improved control of hypertension being the most consistent and important.5-9 Nevertheless, there appears to be no unequivocal explanation for the large changes observed in registered incidence and mortality.

Further trends in death certification rates from cerebrovascular disease have been largely heterogeneous in various other countries, with a general tendency towards increases in nations with originally low rates (such as Belgium, Portugal or Greece), at least as far as all-age rates are concerned.8 It is therefore of interest to analyse recent trends in mortality from cerebrovascular disease in Italy, since relatively little data have been, to our knowledge, published, and also in consideration of the differences in rates and recent trends in mortality from ischemic heart disease in Italy compared with other western countries.10

In the present study, we analyzed age-specific and
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