Effects of a Calcium Antagonist, Lacidipine, on Experimental Focal Cerebral Ischemia in Rats

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ABSTRACT—We investigated the effects of lacidipine on focal cerebral ischemia in rats, and these effects were compared with those of nicardipine. Drugs were administered orally 5 min after middle cerebral artery occlusion (MCAO). Neurological scores as described by Bederson et al. (Stroke 17, 472–476, 1986) and cerebral infarct size (CIS) determined by the 2,3,5-triphenyltetrazolium chloride staining method were measured 24 hr after MCAO. Cerebral blood flow (CBF) and energy metabolites were determined by the hydrogen clearance method and an enzymatic method, respectively. In the drug-untreated group, we observed low-CBF of approximate 13 ml/100 g/min during 0.5–6 hr of occlusion and extensive cerebral infarction associated with severe neurologic deficits (ND). Lacidipine at 1 and 3 mg/kg, although it lowered blood pressure, improved low-CBF to approximate 20 ml/100 g/min during 1.5–6 hr of occlusion and increased tissue levels of ATP 6 hr after MCAO in a dose-dependent manner. Nicardipine at 30 mg/kg also improved low-CBF and increased tissue levels of ATP significantly. However, the improvement of low-CBF by nicardipine was transient. Lacidipine at 3 mg/kg reduced CIS and ameliorated ND significantly. In contrast, nicardipine at 30 mg/kg could not ameliorate ND in spite of a significant reduction of CIS similar to that of lacidipine (3 mg/kg). These results suggest that the improvement of focal cerebral ischemia by lacidipine may be partly due to long-lasting improvement of collateral blood supply to the ischemic area.

Keywords: Lacidipine, Calcium antagonist, Focal cerebral ischemia, Cerebral blood flow, Energy metabolism

Calcium antagonists have been expected to be useful in the treatment of cerebral ischemia because of their potent cerebral vasodilatory activity (1, 2) and because they may protect neurons by preventing the accumulation of intracellular calcium (3, 4), which may serve as a trigger of irreversible cellular injury (5, 6). Some calcium antagonists of the dihydropyridine type such as nilvadipine (7) and nimodipine (8) were found to be effective for the treatment of cerebral ischemia. However, all calcium antagonists of dihydropyridine derivatives do not have always therapeutic effects against ischemic brain damage as reported by Sauter et al. (9).

Lacidipine, a dihydropyridine derivative, is a vascular selective calcium antagonist with potent and long-lasting antihypertensive activity (10). It was also reported to possess a potent cerebral vasodilatory activity (11) and increase cerebral blood flow (CBF) (12). In the previous report (12), we also showed the cerebral protective effects of lacidipine using three different types of cerebral ischemia models. We thought the protective effect in focal cerebral ischemia model was more useful than the other two because an ischemic lesion produced by middle cerebral artery occlusion (MCAO) was thought to be equivalent to a large focal cerebral infarction in humans (13). However, the following questions about lacidipine in the treatment of focal cerebral ischemia in rats have not been clarified yet: 1) Are the neurologic deficits (ND) after MCAO ameliorated or not?; 2) Is the extreme low-CBF in ischemic regions improved or not?; 3) Is the ischemic disruption of cellular energy state in brain ameliorated or not? Therefore, in the present study, we examined the effects of lacidipine on the ND, the regional CBF of the ischemic area and the metabolism of the brain in middle cerebral artery occluded rats, and these effects were compared with those of nicardipine.
MATERIALS AND METHODS

Animals
Male Sprague-Dawley (SD) rats, 10-weeks-old, were purchased from Japan SLC (Hamamatsu). They were kept in an air-conditioned room with a temperature of 23±2°C and humidity of 55±10% on a 12-hr light-dark cycle. They were fed a normal diet (CE-2; Clea Japan, Tokyo) with water provided ad libitum. Animals were housed over one week in cages before use.

Drugs
Lacidipine was supplied by Nippon Glaxo Co., Ltd. (Tsukuba), and nicardipine hydrochloride was purchased from Sigma Chemical Co. (St. Louis, Mo, USA). These two agents were suspended in 5% arabic gum distilled water and administered orally in a volume of 5 ml/kg at 5 min after MCAO. Sodium pentobarbital (Nembutal®) was obtained from Dinabot, Inc. (North Chicago, IL, USA). All other agents used in this study were of reagent grade.

Induction of focal cerebral ischemia
Anesthesia was induced with inhalation of 3.5% halothane in air and maintained with 1% halothane in a nitrous oxide/oxygen mixture (2:1). During the procedures, body temperature was maintained within normal limits with a heating pad. Using an operating microscope under high magnification, the left middle cerebral artery (MCA) was exposed transcranially (14) without damage to the zygomatic bone. The segment of MCA between the medial and lateral borders of the crossing olfactory tract was occluded with a bipolar coagulator (MICRO-1D; Mizuho, Tokyo), and cortical branches arising from this segment, if any, were inevitably obliterated by this procedure. MCA was also transected at the lateral margin of the olfactory tract to avoid recanalization. The craniectomy site was covered with a small piece of gelatin sponge, the soft tissues were allowed to fall back into place, and the skin was sutured. The rats were allowed to recover from anesthesia on a heating pad. In the sham operation, rats were treated exactly the same way except for closing the middle cerebral artery. After recovery from anesthesia, each rat was placed in an individual cage.

Effects of lacidipine on the ND
SD rats weighing 299–450 g were used. Rats were fasted overnight with free access to tap water before use and divided into 7 groups as follows: drug-untreated (n=18); lacidipine, 0.3 mg/kg (n=18); lacidipine, 1.0 mg/kg (n=18); lacidipine, 3.0 mg/kg (n=18); nicardipine, 3 mg/kg (n=18); nicardipine, 10 mg/kg (n=18); and nicardipine, 30 mg/kg (n=18). Drugs were administered in a double-blind manner 5 min after MCAO. In addition, another 18 rats received a sham operation.

Twenty four hours after MCAO, the neurological status of each rat was carefully evaluated by a person who did not know to which group the rat had been assigned. Neurological scores were determined according to the method described by Bederson et al. (15) and graded into the following four groups: grade 0: no neurologic deficit observed; grade 1: flexion of forelimb; grade 2: flexion of forelimb, decreased resistance to lateral push, but no circling; grade 3: flexion of forelimb, decreased resistance to lateral push, and circling. After neurological evaluation, the rats were sacrificed by decapitation, and the brains were quickly removed. According to the method described by Lundy et al. (16), each cerebrum was cut into five coronal slices and incubated in 2% 2,3,5-triphenyl tetrazolium chloride (TTC) solution at 37°C for 30 min. Both the infarction area (mm²) and coronal section area (mm²) on the caudal surface of each TTC-stained slice were measured using an image analyzer (Luzex-5000; Nireco, Tokyo). The ratio (%) of total infarction area to total coronal section area in each rat was calculated and was represented in the text by "cerebral infarct size (CIS)".

Effect of lacidipine on regional CBF after MCAO
Male SD rats weighing 313–498 g were used. Regional CBF in the frontal cortex was measured according to the method described previously (12). Two weeks prior to the experiment, rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). The animal's head was fixed in a head-holder by a stereotaxic apparatus (SR-6; Narishige, Tokyo). A small burr hole was made in the position showed in Fig. 1. A teflon-coated platinum electrode,
0.2 mm in diameter, with a 1-mm portion at its tip uncoated and plated with platinum black, was chronically placed in the cortex (2 mm in depth from the brain surface). Each rat was placed in an individual cage after the surgery and allowed free access to food and water. One day prior to the experiment, the rats were reanesthetized with sodium pentobarbital (50 mg/kg, i.p.). According to the method described by Kawasaki et al. (17), a catheter, filled with saline containing sodium heparin (500 U/ml), was chronically placed in the abdominal aorta through the left femoral artery for the measurement of blood pressure (BP) and heart rate (HR). The other end of catheter was led under the skin and exteriorized at the back of the neck. Each animal was returned to an individual cage after the surgery.

After an overnight fast with free access to tap water, each rat was placed in an individual acrylic case (20 x 20 x 20 cm). Then, the aortic catheter was connected to a pressure transducer (DTXPLUSMB; Viggo-Spectramed Co., Ltd., Singapore), and the electrodes for CBF were connected to a flow meter (MHG-D1; Unique Medical, Tokyo). CBF in conscious rats was measured using a hydrogen clearance method by giving 20% hydrogen gas mixture for 2 min under spontaneous breathing. CBF was calculated using the initial slope method described by Auckland et al. (18). The first 1-min portion of the desaturation curve after termination of hydrogen administration was excluded in order to avoid contamination due to hydrogen recirculation.

After allowing more than 30 min for the steady state, arterial PaCO2, PaO2 and pH (Model 170; Ciba Corning Diagnostics Corp., Medfield, MA, USA), 3 baseline CBF at intervals of about 15 min, BP and HR were measured. Thereafter, each rat was taken out from its acrylic case and placed in the cortex (2 mm in depth from the brain surface). Each rat was sacrificed by decapitation after completion of the measurement, and CIS was determined.

Effect of lacidipine on energy metabolism in rat brain after MCAO
Male SD rats weighing 330–408 g were fasted overnight with free access to tap water before use. Left MCA was occluded in the same way as described above. Rats were divided into 5 groups as follows: drug-untreated (n=10); lacidipine, 1.0 mg/kg (n=10); lacidipine, 3.0 mg/kg (n=10); nicardipine, 10 mg/kg (n=10); and nicardipine, 30 mg/kg (n=10); and each drug was administered orally 5 min after MCAO. In addition, another 10 rats received a sham operation. Six hours after MCAO, the rat was instantly sacrificed with a focal microwave irradiation to the head at 5 kW for 1.6 sec by a microwave applicator (TMW-6402C; Toshiba Electric Co., Ltd., Tokyo). The brain was removed immediately after the irradiation, and the left cerebral hemisphere was cut into five coronal slices according to the method described by Lundy et al. (16). Then, each slice was weighed and frozen in liquid N2. The frozen slices were stored at −70°C until time of analysis. The second and third slices from the rostral side were put together as the sample of each rat and extracted as described by Miyake et al. (19) with some modifications. Briefly, each sample was separately homogenated with 1 ml 0.3 N perchloric acid containing 1 mM EDTA, and the homogenate was centrifuged (15,000 x g, 5 min). The supernatant fluid was neutralized with 1.5 M K2CO3, and the resultant solution was centrifuged (10,000 x g, 5 min). ATP, ADP, AMP, glucose, pyruvate and lactate in the supernatant solution were determined by the enzymatic, fluorometric method described by MacMillan and Siesjö (20). The adenylate energy charge (EC) was calculated according to the method described by Atkinson (21).

In another experiment (3 rats per group), we further determined energy metabolites in the sham-operated rats 6 hr after oral administration of lacidipine (3 mg/kg) or nicardipine (30 mg/kg).

Physiological parameters
In separate preliminary experiments where we used SD rats weighing 352–484 g, systemic arterial pressure and blood gasses were monitored using a femoral arterial catheter before anesthesia, just before and 30 min after MCAO. Middle cerebral artery in each rat was occluded under identical conditions during this study. No significant differences between physiological parameters before anesthesia and 30 min after MCAO were noted (Table 1), and each parameter 30 min after MCAO was within the normal range.

Statistical analyses
All data are expressed as the mean ± S.E.M. Statistical comparisons between two groups were carried out by the
paired t-test or Aspin-Welch's t-test. In order to compare drug-untreated group with more than two other treated groups, statistical analysis was performed with Bonferroni's multiple comparison test following the one-way analysis of variance (ANOVA) for parametric or Kruskal-Wallis' test for non-parametric comparisons.

Table 1. Cardiovascular and respiratory status of rats before, during and after operation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before operation</th>
<th>During operation</th>
<th>After operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBP (mmHg)</td>
<td>127±4</td>
<td>104±2**</td>
<td>119±2</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>225±4</td>
<td>166±5**</td>
<td>233±5</td>
</tr>
<tr>
<td>PaO2 (mmHg)</td>
<td>80.8±3.4</td>
<td>138.2±6.8**</td>
<td>79.7±3.6</td>
</tr>
<tr>
<td>PaCO2 (mmHg)</td>
<td>37.2±1.5</td>
<td>43.9±1.7*</td>
<td>38.9±1.7</td>
</tr>
<tr>
<td>BT (°C)</td>
<td>37.1±0.1</td>
<td>36.8±0.1</td>
<td>37.1±0.1</td>
</tr>
</tbody>
</table>

MBP: mean blood pressure, HR: heart rate, BT: body temperature. Each value represents the mean±S.E.M. of 8 rats. *P<0.05, **P<0.01 vs before operation by the paired t-test.

RESULTS

Effects of lacidipine on CIS and ND

CIS of each group was shown in Fig. 2. In the sham-operated rats, few infarctions were observed, and CIS was only 0.4±0.1%. On the contrary, in the drug-untreated group, extensive infarction was observed, and CIS was 14.7±0.7%. In the lacidipine-treated groups, CIS was reduced in a dose-dependent manner, and a significant reduction was observed at 1 and 3 mg/kg. Nicardipine at 30 mg/kg also reduced CIS significantly, while no reduction was observed in the groups treated with nicardipine at 3 and 10 mg/kg.

The neurological score in each group is also shown in Fig. 2. In the sham-operated group, no ND was observed, and all rats were evaluated as score 0. On the contrary, in the drug-untreated group, severe ND was observed, and the average score was 2.3±0.1. Lacidipine ameliorated ND in a dose-dependent manner, and a significant reduction of neurological score was observed at 3 mg/kg. However, nicardipine, even at 30 mg/kg, could not ameliorate ND significantly. Then, we measured the infarct size of the frontal cortex and striatum in each group (Table 2). The total infarct size on the caudal surface of

![Fig. 2. Effects of post-ischemic administration of drug on cerebral infarct size (CIS) (A) and neurologic deficits (B) in rats 24 hr after middle cerebral artery occlusion (MCAO). Each drug was administered orally 5 min after MCAO. Neurological score was determined according to the method described by Bederson et al. (15), and CIS was measured by the TTC-staining method. Each column represents the mean±S.E.M. of 18 animals. *P<0.05, **P<0.01 vs drug-untreated group (Bonferroni's multiple comparison test).](image-url)
the second slice from the rostral side (slice 2) was reduced significantly in the lacidipine (3 mg/kg) and nicardipine (30 mg/kg) groups. Infarct size of cortex was also reduced significantly in these groups.

Table 2. Infarction distribution in cortex and basal ganglia on the surface of the second slice from the rostral side (slice 2)

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Infarct size (% of coronal section)</th>
<th>Cortex Infarct size (% of coronal section)</th>
<th>Basal ganglia Infarct size (% of coronal section)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug-untreated</td>
<td>31.6±0.9</td>
<td>18.7±0.9</td>
<td>12.8±0.8</td>
</tr>
<tr>
<td>Lacidipine, 0.3 mg/kg</td>
<td>28.5±1.8</td>
<td>15.5±1.2</td>
<td>13.0±1.0</td>
</tr>
<tr>
<td>Lacidipine, 1 mg/kg</td>
<td>26.3±1.5</td>
<td>12.7±1.2</td>
<td>13.7±1.0</td>
</tr>
<tr>
<td>Lacidipine, 3 mg/kg</td>
<td>24.0±2.0**</td>
<td>11.2±1.3**</td>
<td>12.8±1.2</td>
</tr>
<tr>
<td>Nicardipine, 3 mg/kg</td>
<td>27.6±1.2</td>
<td>13.9±1.0*</td>
<td>13.7±0.9</td>
</tr>
<tr>
<td>Nicardipine, 10 mg/kg</td>
<td>28.8±1.0</td>
<td>15.3±0.9</td>
<td>13.4±0.6</td>
</tr>
<tr>
<td>Nicardipine, 30 mg/kg</td>
<td>23.6±1.4**</td>
<td>11.5±1.0**</td>
<td>12.1±1.0</td>
</tr>
</tbody>
</table>

Infarcted area and coronal section area were measured on the surface of slice 2. Each value represents the mean±S.E.M. of 18 animals. *P<0.05, **P<0.01 vs drug-untreated group (Bonferroni’s multiple comparison test).

Effects of lacidipine on BP and regional CBF

Table 3 shows arterial PaCO₂, PaO₂ and pH before the operation. No significant differences among groups were noted in these parameters.

Figure 3 shows the changes of CBF after MCAO and sham operation. A significant reduction of CBF was not observed after sham operation. In contrast, CBF decreased significantly after MCAO and remained extremely low at about 13 ml/100 g/min during 0.5–6 hr of occlusion. It became 16.2±1.6 ml/100 g/min 24 hr after MCAO.

MCAO.

Few changes of BP were observed not only after sham operation but also after MCAO. Changes of BP in drug-treated groups were analyzed in Table 4. Both lacidipine and nicardipine lowered BP in a dose-dependent manner. A significant reduction of BP was also observed even at 24 hr after administration of 3 mg/kg lacidipine or 30 mg/kg nicardipine.

CBF in each drug-treated group is shown in Fig. 4. Lacidipine at 1 and 3 mg/kg improved a low-CBF after MCAO significantly and preserved CBF at about 20 ml/100 g/min during 1.5–6 hr of occlusion. Nicardipine also improved a low-CBF in a dose-dependent manner, and a significant increment of CBF was observed at 30 mg/kg. However, the effect of nicardipine was transient,
and CBF at a level more than 20 ml/100 g/min in the 10 mg/kg and 30 mg/kg group was seen only at 1.5 hr and at 0.5, 1.5 and 3.0 hr after MCAO, respectively.

Table 4. Analysis of the reduction in blood pressure caused by lacidipine or nicardipine in conscious rats with focal cerebral ischemia

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose (mg/kg)</th>
<th>Maximum response (mmHg)</th>
<th>Peak time (min)</th>
<th>T_{1/2} (hr)</th>
<th>ED_{20} (mg/kg, p.o.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lacidipine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.3</td>
<td>-15 ±3</td>
<td>96 ±24</td>
<td></td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-24 ±3</td>
<td>133 ±31</td>
<td>1.5</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>-33 ±2</td>
<td>134 ±33</td>
<td>0.5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>-33 ±3</td>
<td>30</td>
<td>3.4</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>-37 ±2</td>
<td>51 ±21</td>
<td>6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Peak time: Time to maximal response. T_{1/2}: Recovery time by 50% of maximal response. ED_{20}: The dose that reduces the blood pressure of the animal by 20% of the initial value.

Effect of lacidipine on cerebral energy metabolism

Table 5 shows the effect of drugs on cerebral energy metabolism 6 hr after MCAO. Both lacidipine and nicardipine ameliorated the low-level of ATP after MCAO in a dose-dependent manner. In comparison with the drug-untreated group, lacidipine at 3 mg/kg and nicardipine at 30 mg/kg increased the tissue glucose level of ischemic brain significantly, and the level in each group was far higher than that in the sham operated group. In spite of a significant increase of tissue glucose level in these two groups compared with the sham operated group, each tissue lactate level was similar to that of the

![Fig. 4. Effects of post-ischemic administration of lacidipine (A) or nicardipine (B) on cerebral blood flow (CBF) in conscious rats after middle cerebral artery occlusion (MCAO). Each drug was administered orally 5 min after MCAO, and CBF in the frontal cortex was measured using a hydrogen clearance method at the scheduled time. Each point represents the mean ±S.E.M. of 7 animals. In panel A: ○: drug-untreated, ●: lacidipine (0.3 mg/kg), □: lacidipine (1 mg/kg), ■: lacidipine (3 mg/kg). In panel B: ○: drug-untreated, ●: nicardipine (3 mg/kg), □: nicardipine (10 mg/kg), ■: nicardipine (30 mg/kg). *P <0.05, **P <0.01 vs drug-untreated group (Bonferroni's multiple comparison test).](image-url)
Table 6 shows the effect of drugs on cerebral energy metabolism after sham operation. Both lacidipine and nicardipine did not influence the level of ATP and did not increase tissue glucose level significantly.

Table 6. Cerebral concentrations of energy metabolites, energy charge (EC) and lactate/pyruvate (L/P) ratio 6 hr after middle cerebral artery occlusion (MCAO)

<table>
<thead>
<tr>
<th></th>
<th>Sham-operated</th>
<th>Drug-untreated</th>
<th>Lacidipine (1 mg/kg)</th>
<th>Lacidipine (3 mg/kg)</th>
<th>Nicardipine (10 mg/kg)</th>
<th>Nicardipine (30 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>2.14±0.08**</td>
<td>1.22±0.08</td>
<td>1.42±0.09</td>
<td>1.53±0.06*</td>
<td>1.39±0.07</td>
<td>1.57±0.08**</td>
</tr>
<tr>
<td>ADP</td>
<td>1.06±0.05</td>
<td>0.68±0.06</td>
<td>0.74±0.04</td>
<td>0.77±0.03</td>
<td>0.77±0.03</td>
<td>0.82±0.03</td>
</tr>
<tr>
<td>AMP</td>
<td>0.29±0.02**</td>
<td>0.23±0.02</td>
<td>0.23±0.01</td>
<td>0.23±0.01</td>
<td>0.24±0.01</td>
<td>0.24±0.01</td>
</tr>
<tr>
<td>EC</td>
<td>0.77±0.01**</td>
<td>0.73±0.01</td>
<td>0.75±0.01</td>
<td>0.76±0.00*</td>
<td>0.74±0.01</td>
<td>0.75±0.01</td>
</tr>
<tr>
<td>Glucose</td>
<td>2.04±0.08</td>
<td>1.68±0.13</td>
<td>1.85±0.12</td>
<td>2.56±0.09**</td>
<td>1.86±0.08</td>
<td>2.84±0.21**</td>
</tr>
<tr>
<td>Lactate</td>
<td>2.09±0.20**</td>
<td>7.40±0.45</td>
<td>7.01±0.82</td>
<td>8.70±0.63</td>
<td>6.80±0.57</td>
<td>7.65±0.96</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0.22±0.20</td>
<td>0.18±0.02</td>
<td>0.19±0.01</td>
<td>0.21±0.01</td>
<td>0.19±0.01</td>
<td>0.19±0.02</td>
</tr>
<tr>
<td>L/P ratio</td>
<td>9.95±1.00**</td>
<td>46.46±7.39</td>
<td>38.96±6.33</td>
<td>42.96±4.20</td>
<td>37.07±4.27</td>
<td>42.86±6.37</td>
</tr>
</tbody>
</table>

Each drug was administered orally 5 min after MCAO. Rats were sacrificed with microwave irradiation 6 hr after administration, and energy metabolites were measured. Each value represents the mean±S.E.M. of 10 animals, and concentrations of energy metabolites were expressed as µmol/g wet weight. *P<0.05, **P<0.01 vs drug-untreated group (Bonferroni’s multiple comparison test).

DISCUSSION

Questions about lacidipine in experimental focal cerebral ischemia in rats were clarified as follows: 1) Lacidipine reduced CIS and ameliorated ND significantly. 2) Lacidipine improved low-CBF after MCAO significantly. 3) Lacidipine ameliorated the low-levels of ATP after MCAO significantly.

Bederson et al. (15) and Germano et al. (8) reported that there was a linear relationship between CIS and ND in MCA-occluded rats. We also confirmed a significant correlation between CIS and ND (P<0.01, r=0.725) according to the analysis of values obtained from all 144 rats. However, nicardipine at 30 mg/kg could not ameliorate ND significantly in spite of a significant reduction of CIS similar to that of lacidipine (3 mg/kg). Because motor disturbances including hemiparesis are well-known sequelae of lesions of the frontal cortex and striatum in the rat, we also measured the cortical and striatal infarction on slice 2. However, the reduction of cortex infarction in the nicardipine (30 mg/kg), group was almost the same as that in the lacidipine (3 mg/kg) group, and no reduction of striatal infarction was observed in either group. Jones et al. (22), studying awake unanesthetized monkey, found that mild hemiparesis appeared in the flow range of 20 to 25 ml/100 g/min, with progression to complete hemiplegia occurring first at flows as low as 8 ml/100 g/min. The threshold in rats may lie higher, because the flow threshold of membrane and metabolic failure was reported to be approximately 15 ml/100 g/min in rats (23) in contrast to that of 8 to 10 ml/100 g/min in lightly anesthetized baboon (24). Both lacidipine and nicardipine improved low-CBF after MCAO significantly. Lacidipine (3 mg/kg) preserved CBF at about 20 ml/100 g/min during 1.5–6 hr of occlusion. In contrast, nicardipine (30 mg/kg) preserved CBF at a level more than 20 ml/100 g/min during 0.5–3 hr of occlusion. We already showed that the increment of CBF induced by lacidipine at a given level of reduced-blood pressure was greater than that induced by nicardipine (12). A significant reduction of BP was observed even at 24 hr after administration of lacidipine (3 mg/kg) or nicardipine (30 mg/kg), and the degree of reduction in...
the lacidipine was similar to that in nicardipine. CBF at 24 hr after administration in the lacidipine (3 mg/kg) group and nicardipine (30 mg/kg) group was 18.1 ± 1.4 ml/100 g/min and 16.8 ± 2.6 ml/100 g/min, respectively. Owing to the long-lasting improvement of collateral blood supply to the ischemic area, it seemed to be natural that lacidipine at 3 mg/kg could ameliorate ND 24 hr after MCAO significantly.

It was reported that damage after MCAO in rats was exaggerated by hyperglycemia because it allowed additional lactate to accumulate in the partially substrate-depleted tissue (25). At 2 hr after oral administration, nicardipine at 30 mg/kg increased the tissue glucose level in MCA-occluded rats to a level far over that in sham-operated rats and also increased tissue lactate level in ischemic brain significantly (H. Kawano et al., unpublished data). A rapid improvement of low-CBF in rats with focal cerebral ischemia was also likely to cause an additional increment of tissue lactate level in the ischemic region, because nicardipine (30 mg/kg) improved low-CBF after MCAO rapidly. However, in spite of a significant increment of tissue glucose level, lacidipine at 3 mg/kg did not increase tissue lactate level significantly in MCA-occluded rats 6 hr after administration. A mild improvement of low-CBF after MCAO did not cause an additional increment of tissue lactate level in the ischemic region. So, we thought it was beneficial to treat the focal cerebral ischemia in rats with lacidipine.

Mies et al. (26) reported that the threshold of energy failure gradually increased from 18.5 ml/100 g/min after 1 hr to 23.2 ml/100 g/min after 6 hr following MCAO in rats. Although CBF remained at a level less than 23.2 ml/100 g/min during 6 hr following MCAO, energy status in the lacidipine 3 mg/kg group was ameliorated at 6 hr after MCAO. In addition, a significant increase of tissue glucose level in ischemic brain did not induce a significant additional lactate accumulation in the lacidipine (3 mg/kg) group. These results suggested to us that the improvement of lacidipine may be due to not only the long-lasting improvement of collateral blood supply to the ischemic area but also amelioration of energy metabolism by direct action. Because other calcium antagonists such as isradipine (27), nimodipine (28) and nilvadipine (29) were reported to prevent metabolic alterations in the ischemic brain without affecting CBF, some calcium antagonists, especially the long-lasting type, seemed to posses a direct amelioration effect on energy metabolism.

REFERENCES

2. Takenau T, Usuda S, Nomura T, Maeno H and Sado T: Vasodilator profile of a new 1,4-dihydropyridine derivative, 2,6-dimethyl-4-(3-nitrophenoxy)-1,4-dihydropyridine-3,5-dicarboxylic acid 3-[2-(N-benzyl-N-methylamino)-ethyl ester 5-methyl ester hydrochloride (YC-93). Arzneimittelorschung 26, 2172–2178 (1976)
18. Auckland K, Bower BF and Berliner RW: Measurement of local