Nitrendipine Facilitates Recovery of Cerebral Blood Flow, EEG and Metabolites Following Cerebral Ischemia in Anesthetized Rabbits

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Nitrendipine ; nicardipine ; postischemic cerebral blood flow ; EEG ; rabbit

After complete cerebral ischemia, postischemic reduction in cerebral blood flow (CBF) has been well established to occur following reactive hyperemia (Siesjö 1981; Hossmann 1982). The postischemic reduction in CBF has been postulated to be a pathogeny of ischemic neuronal injury, although the precise process between the complete cerebral ischemia and the ischemic neuronal injury remains to be clarified. A provisional hypothesis for the ischemic neuronal injury, proposed in correlation with disturbance of calcium homeostasis in the brain (Meyer 1989; Siesjö 1990), has been supported by pharmacological studies demonstrating that nimodipine, a calcium antagonist, improves the postis-
chemically impaired CBF and neuronal functions (Kazda et al. 1982; Steen et al. 1983). This hypothesis, however, may not be equally supported by the result that nicardipine, a most commonly used calcium antagonist, improved the ischemic CBF but did not enhance the neuronal functions (Sakabe et al. 1986). Since the hypothesis should further be examined by pharmacological studies using other calcium antagonists, the present study was thus undertaken to elucidate the effect of nitrendipine, an antihypertensive calcium antagonist, on the impairment of CBF and EEG occurring after complete cerebral ischemia.

**Materials and Methods**

*Animal preparation*

Studies on complete ischemia and normal circulation in the rabbit brain were performed in the same procedure, except for the induction of ischemia and the monitoring of EEG. Male New Zealand white rabbits weighing between 3.0 and 4.0 kg were used. Each animal was anesthetized by intravenous administration of fentanyl (30 μg/kg) and inhalation of nitrous oxide-oxygen (6:4). Lidocaine (1% solution) was also infiltrated into the skin before the initial incision.

Following a tracheotomy, a hard tube was inserted into the trachea to a level of the first rib. Animals were given alcuronium (0.2 mg/kg) intravenously in order to block their respiratory efforts. Respiration was maintained with a positive pressure respirator (661E; Harvard Apparatus, Millis, MA, USA). End-tidal CO₂ was monitored continuously (1H21A; NEC Sanei, Tokyo) to maintain PaCO₂ at between 30 and 40 mmHg.

The left femoral artery and vein were cannulated to monitor blood pressure, obtain sequential samples for blood gases and pH, and administer the supplementary infusion of fentanyl (1 μg/kg/min) and alcuronium (1 μg/kg/min). The right femoral artery was additionally catheterized to permit the withdrawal and injection of blood while lowering the blood pressure during ischemia. Another catheter was introduced into the duodenum to facilitate administration of calcium antagonists or vehicle. The head of the animal was placed in a stereotaxic instrument using a rabbit adapter (Sawyer et al. 1954). Body temperature was maintained at around 38°C with a heating pad.

*CBF measurement*

Blood flow was measured with the hydrogen-clearance method in the total brain, cortex and thalamus as reported previously (Aihara 1989). A recording electrode for the total brain was placed into the posterior end of the sagittal sinus, two other electrodes were placed on the cortical surface along the temporal branches of the left middle cerebral artery, and the other one was inserted to the right thalamus (6 mm posterior, 3 mm right and 13 mm lower portion of bregma). All craniectomies were closed with dental cement.

To determine CBF, rabbits were inhaled with approximately 10% hydrogen for 3 min. CBF was calculated from the clearance curve of the tissue hydrogen concentration by the height-over-area method (Høsted-Rasmussen et al. 1966).

*EEG analysis*

To monitor EEG activity, two small drill holes were made in the right parietal bone. Two silver-ball electrodes were placed on the dura surface to record the bipolar EEG. The EEG was stored in a data-recorder (SR-51; TEAC, Tokyo) for subsequent off-line frequency analysis. The EEG was digitalized at 64 Hz using Fast Fourier Transform (FFFT523; Ariel Corp., NYC, NY, USA) for 4-sec epochs that were averaged 30 times (9816; Hewlett-Packard Co., Fort Collins, CO, USA). EEG intensity was linearized by calculating the square roots of the spectral power Fourier coefficients. The total intensity was
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expressed as the percentage of preischemic value in the 1.0–30.0-Hz range of EEG frequency. In addition, the frequency index was calculated by dividing the alpha plus beta (7.6–30.0 Hz) by the delta plus theta (1.0–7.5 Hz) power.

Induction of complete ischemia

Cerebral ischemia was accomplished with the modified tourniquet method originally described by Nemoto et al. (1977) as the simple and noninvasive one. Its duration was selected for a 10 min of the maximal reversible-time in the complete ischemia (Hossmann 1982). After administration of heparin (500 units/kg, i.v.), ischemia was achieved by lowering the mean arterial pressure to between 50 and 60 mmHg by withdrawing of arterial blood via a heparinized syringe, and by tightening a tourniquet (1,140 mmHg) around the neck of the animal. We used the tourniquet 7.5 cm wide (Mizuho Ikakogyo Co., Tokyo), which wrapped the neck from the level of the first rib to that of the foramen magnum, and tightened the common carotid and vertebral arteries near the first rib. EEG activity disappeared within 30 sec after tourniquet inflation. The completeness of the ischemia was confirmed by the inhalation of 10% hydrogen after the onset of ischemia. Ischemia was considered to be complete when the concentration of hydrogen in three brain regions was less than 3% of that of preischemia. After 10 min of ischemia, recirculation was attempted by removing the neck tourniquet and reinfusing the withdrawn arterial blood.

Determination of cerebral metabolites

Following 60-min postischemic recirculation, the brain was frozen in situ with liquid nitrogen through a plastic funnel fixed to the parietal bone (Ponten et al. 1973). According to Lowry et al. (1964), we prepared cortical samples and analyzed their metabolites serving as indices of energy metabolism in the brain. Briefly, the frozen brain was chiseled out and powdered in liquid nitrogen. The powder was transferred into 3 M perchloric acid in an alcohol-dry ice bath. After the addition of 3 mM EDTA, the perchloric acid extract was obtained by centrifugation and neutralized to pH 6.7 with 2 M potassium bicarbonate. Tissue extracts were analyzed by enzymatic fluorometric techniques for ATP, ADP, AMP, phosphocreatine, glucose, glucose-6-phosphate, pyruvate and lactate (Lowry et al. 1964; Lowry and Passonneau 1972).

Experimental protocol

The experiment fundamentally comprised two studies on complete cerebral ischemia and normal cerebral circulation. In the study on complete ischemia, five groups of rabbits were used to evaluate the changes in CBF and EEG recovery. Following preparation, each animal was randomly assigned to receive vehicle (n = 6), 0.3 (n = 4) or 1 mg/kg (n = 4) of nitrendipine, or 3 (n = 4) or 10 mg/kg (n = 4) of nicardipine. Vehicle or each agent was intraduodenally administered 15 min prior to the initiation of ischemia.

In the study on cerebral metabolites conducted 60 min after recirculation, the experimental procedure was the same as that for complete ischemia except for CBF and EEG measurements. Vehicle (n = 4) or nitrendipine 0.3 mg/kg (n = 4) was administered 15 min before ischemia.

In the study on normal cerebral circulation, three groups of rabbits were used to evaluate the change in CBF and arterial pressure after the intraduodenal administration of vehicle (n = 4), nitrendipine (0.03-1 mg/kg, n = 4) or nicardipine (0.3–10 mg/kg, n = 3). Each animal was administered the respective agent or vehicle in consecutive doses 30 min after the recovery of cerebral and systemic responses. Baseline values for CBF, arterial pressure, arterial blood gases and pH were not significantly different between or within each group.

Data analysis

The mean value of the two blood flows of the cortex in each animal was calculated as
a representative. Data are presented as the mean ± s.e. or as the percentage of the baseline. Statistical comparisons within animal groups were accomplished by paired t-test. Between-group comparisons were done by analysis of variance and Dunnett's t-test. A p value of less than 0.05 was considered significant.

Drugs
Nitrendipine (Yoshitomi, Osaka), nicardipine hydrochloride (Yamanouchi, Tokyo), fentanyl citrate (Sankyo, Tokyo), alcuronium chloride (Roche, Basel, Switzerland), lidocaine hydrochloride (Fujisawa, Osaka) and heparin sodium (Nakarai, Kyoto) were used. Nitrendipine and nicardipine were dissolved in the vehicle containing 5% dimethyl sulfoxide and 0.5% solubilizing agent (Cremophor EL®; BASF, Ludwigshafen, Germany) in water, and diluted to the desired concentration with the vehicle.

RESULTS

CBF after ischemia
Physiological parameters measured before and after cerebral ischemia are shown in Table 1, and were not significantly different between or within each group except for the arterial pressure of the vehicle group.

The time courses of the changes in blood flow of the total brain are shown in Fig. 1. After a 10-min ischemia of the vehicle group, we observed transient reactive hyperemia followed by a sustained decrease in total CBF starting from 30 min after recirculation. The reduction in blood flow reached the maximum of 58 ± 4, 65 ± 4 and 73 ± 7% in the total brain, cortex and thalamus, respectively, 2 hr after recirculation (Fig. 2). Mean arterial pressure decreased maximally by 17 ± 5 mmHg (Table 1). Estimated vascular resistance in the total brain, cortex and thalamus increased significantly from 2.1 ± 0.2, 1.8 ± 0.4 and 3.4 ± 0.6 to 3.2 ± 0.5, 2.4 ± 0.4 and 4.4 ± 0.6 mmHg/ml/100 g/min, respectively.

Pretreatment with nitrendipine or nicardipine had no significant effect on the transient reactive hyperemia (Fig. 1A, 1B). However, pretreatment with nitrendipine (0.3-1 mg/kg) or nicardipine (3-10 mg/kg) reversed dose-dependently the postischemic decrease followed by transient reactive hyperemia in the total brain, cortex and thalamus (Fig. 2). Pretreatment with nitrendipine or nicardipine caused little, if any, change in the arterial pressure (Table 1).

EEG after ischemia
The results obtained by EEG frequency analysis are summarized in Fig. 3. In the vehicle group, the total intensity recovered promptly to 61 ± 10% 30 min after recirculation (Fig. 3A). However, the frequency index was 35 ± 9% of the control value (Fig. 3B), which indicates a low ratio of alpha and/or beta to delta and theta power i.e., a slow-wave activity. The frequency index gradually returned to the preischemic value, but the total intensity was still reduced to about 64 ± 9% 4 hr after recirculation. Pretreatment with nitrendipine (0.3 mg/kg) accelerated the recovery of both parameters until they reached more than 80% of the control value within 60 min after the start of recirculation (Fig. 3A, 3B).
**Table 1. Physiological values before and after complete ischemia in rabbits**

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Vehicle</th>
<th>Nitrendipine (0.3)</th>
<th>Nitrendipine (1)</th>
<th>Nicardipine (3)</th>
<th>Nicardipine (10)</th>
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<tbody>
<tr>
<td>Number</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
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<tr>
<td>Preischemic CBF (ml/100 g/min)</td>
<td></td>
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<tr>
<td>Total</td>
<td>54 ± 4</td>
<td>47 ± 3</td>
<td>52 ± 4</td>
<td>49 ± 2</td>
<td>46 ± 3</td>
</tr>
<tr>
<td>Cortex</td>
<td>75 ± 13</td>
<td>60 ± 8</td>
<td>72 ± 14</td>
<td>61 ± 6</td>
<td>77 ± 12</td>
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<tr>
<td>Thalamus</td>
<td>35 ± 4</td>
<td>43 ± 9</td>
<td>36 ± 3</td>
<td>33 ± 1</td>
<td>27 ± 2</td>
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<tr>
<td>Mean arterial pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Preischemia</td>
<td>110 ± 5</td>
<td>94 ± 3</td>
<td>97 ± 5</td>
<td>100 ± 2</td>
<td>94 ± 3</td>
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<tr>
<td>Postischemia 2 hr</td>
<td>93 ± 6*</td>
<td>90 ± 7</td>
<td>88 ± 6</td>
<td>102 ± 8</td>
<td>88 ± 2</td>
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<td>PaCO₂ (mmHg)</td>
<td></td>
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<tr>
<td>Preischemia</td>
<td>37 ± 1</td>
<td>37 ± 2</td>
<td>38 ± 1</td>
<td>40 ± 1</td>
<td>41 ± 2</td>
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<tr>
<td>Postischemia 2 hr</td>
<td>40 ± 3</td>
<td>39 ± 2</td>
<td>40 ± 2</td>
<td>41 ± 1</td>
<td>43 ± 1</td>
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<td>pH</td>
<td></td>
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<tr>
<td>Preischemia</td>
<td>7.40 ± 0.01</td>
<td>7.41 ± 0.02</td>
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<td>Postischemia 2 hr</td>
<td>7.34 ± 0.02</td>
<td>7.34 ± 0.04</td>
<td>7.32 ± 0.03</td>
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<td>PaO₂ (mmHg)</td>
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<tr>
<td>Preischemia</td>
<td>156 ± 10</td>
<td>150 ± 16</td>
<td>148 ± 15</td>
<td>160 ± 6</td>
<td>147 ± 15</td>
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<td>Postischemia 2 hr</td>
<td>147 ± 13</td>
<td>134 ± 19</td>
<td>152 ± 4</td>
<td>141 ± 2</td>
<td>133 ± 6</td>
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<tr>
<td>Frequency index of EEG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preischemia</td>
<td>2.8 ± 0.4</td>
<td>2.3 ± 0.1</td>
<td>2.7 ± 0.2</td>
<td>3.6 ± 0.5</td>
<td>2.8 ± 0.5</td>
</tr>
</tbody>
</table>

*Values are mean ± s.e.  *Significantly different from the preischemia value (p < 0.05).
Fig. 1. The time courses of cerebral blood flow (CBF) in the total brain following complete ischemia in rabbits. Panel A shows vehicle (●) and nitrendipine (○, 0.3 mg/kg; ▲, 1 mg/kg). Panel B shows vehicle (●) and nicardipine (△, 0.3 mg/kg; ◇, 1 mg/kg). These agents were administered intraduodenally 15 min before ischemia. Each value represents the percentage of the preischemic baseline value and is the mean ± s.e. of four to six experiments. *Significantly different from the value of the vehicle group at the corresponding time (p < 0.05). †Significantly different from the baseline value (p < 0.05).

Fig. 2. Maximum values of the postischemic decrease in cerebral blood flow (CBF) in the total brain, cortex and thalamus are plotted against doses of pretreatment with nitrendipine (○) or nicardipine (△). Each value represents the percentage of the preischemic baseline value and is the mean ± s.e. of four to six experiments. *Significantly different from the value of the vehicle group at the corresponding maximum value (p < 0.05).
Pretreatment with nitrendipine (1 mg/kg) recovered total intensity in excess of 120%, which was not significantly different from the preischemic value (Fig. 3A). Notably, nicardipine did not accelerate the recovery of EEG (Fig. 3A, 3B).

**Cerebral metabolism after ischemia**

The cortical level of cerebral metabolites 60 min after recirculation is summarized in Table 2. Nitrendipine (0.3 mg/kg) significantly reduced the content of lactate, elevated that of phosphocreatine, and tended to increase the contents

| Table 2. Content (μmol/g) of cerebral metabolites 60 min after complete ischemia in rabbits |
|---------------------------------|-----------------|----------------|-----------------|----------------|-----------------|----------------|
| ATP | ADP | AMP | PCr | Glc | G6P | Pyr | Lac |
|-----------------|-----------------|----------------|----------------|----------------|----------------|----------------|
| Vehicle | 2.25 | 0.303 | 0.002 | 5.10 | 4.60 | 0.146 | 0.175 | 2.48 |
| ±0.08 | ±0.033 | ±0.002 | ±0.25 | ±0.34 | ±0.018 | ±0.022 | ±0.31 |
| Nitrendipine | 2.38 | 0.325 | 0.003 | 5.90* | 6.34 | 0.140 | 0.156 | 1.29* |
| (0.3 mg/kg) | ±0.14 | ±0.043 | ±0.003 | ±0.09 | ±0.49 | ±0.005 | ±0.028 | ±0.21 |

*Values are means±s.e. of four experiments. *PCr, phosphocreatine; Glc, glucose; G6P, glucose-6-phosphate; Pyr, pyruvate; Lac, lactate. *Significantly different between vehicle and nitrendipine treated-rabbits (p <0.05).
of ATP, ADP and glucose.

**CBF in normal rabbits**

Intraduodenal administration of nitrendipine (0.03–0.3 mg/kg) increased dose-dependently the blood flow in the total brain, cortex and thalamus (Fig. 4A, 4B). The responses in CBF and arterial pressure reached the maximum in 15 to 30 min, and then gradually returned to the baseline. At a higher dose (1 mg/kg), the increase in blood flow of the total brain was slightly reduced but prolonged. Nicardipine also increased the CBF (Fig. 4B), and decreased the arterial pressure at a dose in the 0.3 to 10 mg/kg range. Thus, nitrendipine or nicardipine induced the maximum increase in normal CBF at a dose that restored the postischemic CBF (Fig. 2).

**DISCUSSION**

Three principal results were obtained in the present study. First, both nitrendipine at the dose of 0.3 to 1 mg/kg and the nicardipine in the dose range 10 times higher than that of nitrendipine reversed the postischemic reduction in CBF. Second, nitrendipine accelerated the recoveries of EEG after ischemia whereas nicardipine did not. Third, nitrendipine increased the cerebral content of phosphocreatine and decreased the content of lactate after restoration of blood
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These results indicate that pretreatment with nitrendipine prevents postischemic impairment in CBF and EEG, and improves the postischemic cerebral energy metabolism.

These results correspond to the previous reports that a dose of nimodipine or nicardipine suppressed postischemic impairment of blood flow of the total brain in cats or dogs (Kazda et al. 1982; Steen et al. 1983; Sakabe et al. 1986). The present study provides novel information that these effects of the agents on the cortex and thalamus in rabbit are essentially similar to the effect confirmed by previous investigators who estimated the blood flow in the total brain.

The estimated vascular resistance in the total brain, cortex and thalamus significantly increased 2 hr after recirculation despite the decrease in arterial pressure (17 mmHg). Nitrendipine and nicardipine should suppress this increase in vascular resistance, however, because both agents reversed the postischemic CBF without a significant change in arterial pressure. Previous studies have shown that these agents potently relax the depolarized contraction of cerebral vessels (Fleckenstein-Grün et al. 1985) which is due to the facilitation of calcium influx (Van Breemen et al. 1985), and that nitrendipine inhibits the opening of voltage-dependent calcium channels in vascular smooth muscle cells (Bean et al. 1986). These findings are thus consistent with the view that the effect of nitrendipine and nicardipine on the postischemic decrease in blood flow of the cortex, thalamus and total brain may be due to the inhibition of the calcium-induced contraction in cerebral vessels.

Nitrendipine remarkably accelerated the recovery of the total intensity and frequency index in EEG. The rapid recovery of EEG parameters necessitates careful consideration because the EEG parameters may be accelerated by seizure activity (Leniger-Follert 1984). The present study showed that nitrendipine (0.3 mg/kg) significantly decreased production of lactate, and significantly increased the content of phosphocreatine (Table 2). These results indicate that the nitrendipine dose significantly improves the oxidative phosphorylation process. This metabolic improvement is clearly distinguished from the degradative effect of seizures that induces the prominent increase in lactate and the decrease in phosphocreatine (Howse et al. 1974). The acceleration of EEG recovery by nitrendipine should therefore reflect the improvement in electrical activity of the brain after recirculation.

With respect to nicardipine, the present results accord with those cited in the report by Sakabe et al. (1986), specifically that nicardipine increases ischemic CBF but does not improve neurological recovery from complete ischemia in dogs.

Though the difference between the effect of nicardipine and that of nitrendipine on EEG recoveries remains to be clarified, the following explanations are possible. First, nicardipine may not uniformly restore nutrient blood flow to brain tissues after recirculation. A previous study has shown that the total intensity and the frequency index of EEG positively correlate with the blood flow
in the cortex and the subcortical structures during focal ischemia (Mies et al. 1984). However, this is unlikely inasmuch as the improvement in regional blood flow in the cortex was not apparently different from that in the total brain after pretreatment with the agents (Fig. 2).

A second explanation is linked to the report of EEG activity being inversely correlated with the lactate content after cerebral ischemia (Schmidt-Kastner et al. 1986). The significant reduction in cortical lactate 60 min after recirculation by nitrendipine may explain the rapid recovery of EEG brought on this agent. However, precise elucidation of the difference between the effect of the two agents on EEG recovery awaits clarification of the effect of nicardipine on cerebral metabolism.

A third explanation for the difference in the effects of the two agents is that nitrendipine, unlike nicardipine, may protect neuronal activity from ischemic damage. During energy failure occurring through cerebral ischemia, the elevation of calcium influx through neuronal calcium channels is suggested to lead to a rise in the free cytosolic calcium concentration, so that the latter may cause neuronal hyperexcitability or ischemic seizure which promotes neuronal damage (Meyer 1989). Pharmacological studies have shown that nitrendipine, unlike nicardipine, suppresses seizure activity via oral as well as central route (Wauquier et al. 1985; Morón et al. 1990). Therefore, the discrepancy between the effect of nitrendipine and nicardipine on postischemic EEG recovery might be explained by the difference between the direct effect of these agents on neuronal excitability.

In conclusion, pretreatment with nitrendipine or nicardipine reverses the postischemic decrease in CBF after 10-min cerebral ischemia. However, nitrendipine is more beneficial due to its acceleration of EEG recovery, which would seem to be the principal therapeutic merit of this drug.

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