Effects of nicardipine on the ex vivo release of eicosanoids after experimental subarachnoid hemorrhage

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The activation of lipid peroxidation and the enhancement of arachidonic acid metabolism have been demonstrated as indicators of brain damage after subarachnoid hemorrhage (SAH). Meanwhile, the final common pathway of neuronal damage seems to be related to the impaired homeostasis of Ca++. The present study evaluated the effect of the calcium-antagonist nicardipine on arachidonate metabolism after experimental induction of SAH. The ex vivo release of four eicosanoids (prostaglandin (PG)D2, PGE2, 6-keto-PGF1α, and leukotriene (LT)C4) was measured at different intervals after SAH induction. Rats were separated into the following three groups: a sham-operated group, an SAH group (rats were injected with 0.3 ml autologous arterial blood), and an SAH-treated group (after SAH induction, rats were treated with nicardipine 1.2 mg/kg intraperitoneally). Nicardipine significantly decreased the ex vivo release of PGD2 at 48 hours after SAH (p < 0.01). The release of PGE2 was significantly enhanced at 6 hours after SAH, while in the nicardipine-treated group PGE2 release is significantly reduced. Nicardipine also affects the lipoxygenase pathway, reducing the release of LTC4 at 1, 6, and 48 hours after SAH induction. The results of the present study show that nicardipine treatment exerts an inhibitory effect on both biochemical pathways of arachidonic acid metabolism; aside from vascular effects, nicardipine could exert a protective role against the release of arachidonate metabolites, which could play a significant role in the pathogenesis of brain damage after SAH.

KEY WORDS • subarachnoid hemorrhage • arachidonic acid • nicardipine • leukotriene • eicosanoid • rat
FIG. 1. Ex vivo release of prostaglandin D₂ (pg/mg of protein: mean ± standard error of the mean) in rats subjected to an experimental subarachnoid hemorrhage (SAH) procedure and treated with the calcium-antagonist nicardipine. Asterisks: p < 0.01, SAH rats vs. sham-operated rats; triangles: p < 0.001, SAH rats vs. rats with SAH and nicardipine treatment.

FIG. 2. Ex vivo release of prostaglandin E₂ (pg/mg of protein: mean ± standard error of the mean) in rats subjected to an experimental subarachnoid hemorrhage (SAH) procedure and treated with the calcium-antagonist nicardipine. Asterisks: p < 0.01, SAH rats vs. sham-operated rats; triangles: p < 0.001, SAH rats vs. rats with SAH and nicardipine treatment.

FIG. 3. Ex vivo release of 6-keto-prostaglandin F₁α (pg/mg of protein: mean ± standard error of the mean) in rats subjected to an experimental subarachnoid hemorrhage (SAH) procedure and treated with calcium-antagonist nicardipine. Single asterisk: p < 0.05, and double asterisk: p < 0.02, sham-operated rats vs. rats with SAH or SAH and nicardipine treatment; single triangle: p < 0.05, and treble triangle: p < 0.01, SAH rats vs. rats with SAH and nicardipine treatment.

TABLE 1

<table>
<thead>
<tr>
<th>Group &amp; Time of Study</th>
<th>MABP (mm Hg)</th>
<th>pH</th>
<th>PaO₂ (mm Hg)</th>
<th>PaCO₂ (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>130 ± 4</td>
<td>7.40 ± 0.02</td>
<td>114 ± 7</td>
<td>36.6 ± 2</td>
</tr>
<tr>
<td>SAH, 15 min</td>
<td>138 ± 5</td>
<td>7.41 ± 0.01</td>
<td>110 ± 8</td>
<td>37.7 ± 1</td>
</tr>
<tr>
<td>control</td>
<td>125 ± 4</td>
<td>7.39 ± 0.02</td>
<td>120 ± 10</td>
<td>36.4 ± 1</td>
</tr>
<tr>
<td>SAH, 30 min</td>
<td>120 ± 6</td>
<td>7.38 ± 0.02</td>
<td>108 ± 7</td>
<td>37.7 ± 1</td>
</tr>
<tr>
<td>control</td>
<td>130 ± 6</td>
<td>7.39 ± 0.01</td>
<td>113 ± 6</td>
<td>36.7 ± 2</td>
</tr>
</tbody>
</table>

* Values are the mean ± standard error of the mean (in a group of six rats). MABP = mean arterial blood pressure. Subarachnoid hemorrhage (SAH) produced by cisternal injection of 0.30 ml of autologous arterial blood.

improved neuronal function (evaluated by means of cortical evoked potentials). Moreover, nicardipine showed a significant vasodilating effect in experimental models of SAH, reducing angiographic vasospasm. The aim of the present study was to verify whether nicardipine treatment could reduce the activation of arachidonic acid metabolism associated with experimental SAH, providing metabolic brain protection after the hemorrhagic insult against one of the most important Ca++-dependent patterns of SAH.

Materials and Methods

The experiments were conducted on male Sprague-Dawley rats (Charles River strain), each weighing 375 to 425 gm.

Experimental SAH Induction

The experimental model of SAH was prepared according to Solomon, et al., with few modifications. General anesthesia was induced with 3% halothane (70% N₂O:30% O₂) and maintained with 0.75% halothane in the gas mixture. A burr hole was performed by refrigerated twist-drill at the interparietal/occipital suture connection, and a small catheter was inserted into the cisterna magna. Suitable placement of the catheter was assessed by testing the flow of CSF through the catheter and by confirming under magnification that the lower distal part of the catheter was through the atlanto-occipital membrane. A femoral artery was cannulated for anaerobic sampling of blood to measure the pH, PaCO₂, and PaO₂, while the mean arterial blood pressure (MABP) was monitored with an indirect blood pressure sensor (Table 1). Body temperature, monitored by a rectal thermometer, was adjusted and maintained near 37°C by external heating. When the rats were in a steady respiratory state (PaO₂ 90 mm Hg and PaCO₂ 35 to 40 mm Hg), autologous arterial blood was collected (0.35 ml) from the femoral artery and an aliquot of 0.30 ml was injected into the cisterna magna via the catheter within about 2 minutes. Before SAH induction, a CSF sample of 0.01 to 0.03 ml was gently drawn in order to limit modifications in intracranial pressure. The animals were kept in a 20° head-down position.
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**Analytical Methods**

**Experimental Groups**

Samples were kept at -80°C until analysis. The brains were immediately frozen in dry ice and maintained in a pre-refrigerated glove box at -22°C. The medium was decanted and centrifuged at 3000 rpm in a centrifuge refrigerated at 0°C. Three aliquots of the medium were subjected to SAH induction with injection of 0.3 ml autologous arterial blood; and 3) an SAH-treatment group, subjected to SAH induction and treated with nicardipine (1.2 mg/kg intraperitoneally) immediately after the surgical procedure and every 8 hours for the 48 hours sacrificed at 48 hours. Biochemical evaluations were performed at 1, 6, and 48 hours after the experimental SAH procedure.

**Methods**

The rats were sacrificed by decapitation, and the brains were immediately frozen in dry ice and maintained in a pre-refrigerated glove box at -22°C. The slices of cortex, weighing approximately 10 to 15 mg, were bound-cut, weighed, and placed immediately in 1 ml of oxygenated (95% O₂:5% CO₂) Krebs solution (pH 7.4) containing: NaCl 118 mM; KCl 4.7 mM; MgSO₄ 7 mM; H₂O 1.2 mM; KH₂PO₄ 1.2 mM; NaHCO₃ 25 mM; and glucose 1 gm/liter. The tubes containing medium and slices were tightly closed. The time interval between decapitation and incubation was about 3 minutes. Slices were incubated at 37°C in a shaking water bath up to 1 hour. At the end of the incubation period, the medium was decanted and centrifuged at 3000 rpm in a centrifuge refrigerated at 0°C. Three aliquots of the supernatant were kept at -80°C until analysis.

**Determination of Arachidonate Metabolites**

Levels of arachidonic acid metabolites were determined by radioimmunoassay (RIA). RIA for prostaglandin (PG)D₂ was performed as previously described in detail.**35,36** RIA kits for PGE₂ and 6-keto-PGF₁α were obtained commercially.** The radioactive labels used were ¹²⁵I for PGE₂ and ³H for 6-keto-PGF₁α. Antiserum for these metabolites had less than 2.5% of cross-reactivity with other prostaglandins. Immunoreactive leukotriene (LT)C₄-like activity was detected by RIA, as described by Levine, et al., using an antiserum to LTC₄ which has a cross reactivity of 10.1% with LTD₄, 2.3% with LTE₄, 0.07% with hydroxyeicosatetraenoic acid (HETE), and 0.006% with LTB₄. Ten ml of high sample-capacity scintillation solution† was added to each sample. Radioactivity was measured using a liquid scintillation spectrometer,‡ as described previously. The results are expressed in pg/mg of protein. The protein content of homogenate was assayed according to Lowry, et al.,† with serum albumin as a standard. The assay sensitivity was 6 pg/mg of protein. Statistical analysis was performed using the analysis of variance (ANOVA) and a Tukey test for multiple comparisons. Statistical significance was accepted for p < 0.05.

**Results**

**Prostaglandins D₂ and E₂**

Table 2 presents data on the PGD₂ ex vivo release after the experimental procedure. The release was enhanced after SAH, and at 48 hours after SAH was significantly higher than levels in sham-operated animals (p < 0.01). Nicardipine significantly inhibited PGD₂ release at 48 hours (Fig. 1) and values in the nicardipine group did not differ from those of the sham-operated animals.

Table 2 shows that PGE₂ release was significantly enhanced at 6 hours after the SAH procedure (p < 0.01) and that nicardipine significantly reduced the synthesis capacity of this metabolite at that time (Fig. 2).

**6-Keto-Prostaglandin F₁α**

The release of prostacyclin was significantly enhanced at 48 hours after SAH induction, while nicardipine significantly inhibited the release at 48 and also at 6 hours (Table 2, Fig. 3).

* RIA kits No. NEK-020 (for PGE₂) and NEK-008 (for 6-keto-PGF₁α) obtained from New England Nuclear Chemicals GmbH, Dreieich, West Germany.

† Atomlight scintillation solution NEF-968 supplied by United Technologies Packard, Packard Instruments, Downers Grove, Illinois.

‡ Liquid scintillation spectrometer, Model 3320, manufactured by Packard Instruments, Downers Grove, Illinois.

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**TABLE 2**

<table>
<thead>
<tr>
<th>Arachidonic Acid Metabolite</th>
<th>1 Hr Postop</th>
<th>6 Hrs Postop</th>
<th>2 Days Postop</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PGD₂</strong></td>
<td>Sham-Op</td>
<td>SAH</td>
<td>SAH + NIC</td>
</tr>
<tr>
<td>prostaglandin D₂</td>
<td>182.4 ± 15.7</td>
<td>256.5 ± 33.1</td>
<td>219.8 ± 35.2</td>
</tr>
<tr>
<td>prostaglandin E₂</td>
<td>130.0 ± 11.3</td>
<td>132.0 ± 15.0</td>
<td>147.1 ± 16.1</td>
</tr>
<tr>
<td>6-keto-PGF₁α</td>
<td>100.9 ± 17.7</td>
<td>113.9 ± 11.5</td>
<td>84.5 ± 12.4</td>
</tr>
<tr>
<td>leukotriene C₄</td>
<td>21.8 ± 4.0</td>
<td>39.0 ± 2.3</td>
<td>30.2 ± 2.1</td>
</tr>
</tbody>
</table>

* Results are expressed in pg/mg of protein and are the mean ± standard error of the mean of groups of six to seven animals. Sham-operated animals: sham-op; rats with subarachnoid hemorrhage: SAH; SAH rats treated with nicardipine (SAH + NIC): Sham-Op vs. SAH or SAH + NIC: * p < 0.01; † p < 0.02; ‡ p < 0.05. SAH vs. SAH + NIC: # p < 0.01; $ p < 0.02; ~ p < 0.05.
Leukotriene C4

The release of the lipoxygenase metabolite is significantly enhanced at 1, 6, and 48 hours after SAH induction (Table 2). Nicardipine significantly reduces the release at each of the times studied. The inhibitory effect on LTC4 synthesis capacity is characteristic, as seen by comparing data of SAH-treated and sham-operated rats (Fig. 4).

Discussion

After hemorrhagic and ischemic insult to the brain, several physiopathological and biochemical parameters change simultaneously.47,49 The search for factors leading to neuronal damage is important in attempts to define new therapeutic strategies for brain protection. Arachidonic acid metabolites may play a primary role in microcirculatory regulation, in modulating neurotransmitter release, and in promoting blood-brain barrier alterations leading to brain edema.21,22,34,50,51 Many experimental studies on models of cerebral ischemia have shown that the derangement of neuronal cell membrane enhances the liberation of large amounts of arachidonic acid from membrane phospholipids.8,19,44 The question is whether and which arachidonic acid metabolic pathway is preferentially activated after SAH and which is the relationship between different metabolites and the physiological aspects of the disease.

The accumulation of arachidonic acid causes a characteristic elevation of the eicosanoid level in brain tissue.19,44 Kempski et al.19 have recently demonstrated that brain tissue maintains a residual capacity for synthesizing arachidonic acid metabolites after ischemia and reperfusion, and that synthesis and release of each arachidonic acid metabolite are strictly time-dependent. Many reports on the vascular effects of eicosanoids after aneurysmal SAH and their relevance in the pathogenesis of vasospasm have appeared in the literature.10,29,30,48,50,53 Arachidonic acid metabolites are synthesized and released by the arterial wall, and also, importantly, by brain tissue. Kiwak et al.21 have shown that the lipoxygenase pathway is significantly enhanced after experimental SAH and that leukotrienes are preferentially synthesized in the gray matter.

The present study demonstrates that, after experimental SAH induction, brain tissue preserves a residual capacity of arachidonic acid metabolism and that the activation of biochemical pathways is quite different. The cyclooxygenase pathway seems to be activated in the late phase, while the lipoxygenase pathway shows an increasing activity in the early phase which persists at 2 days. In experimental conditions, nicardipine significantly reduces Ca++-dependent enzymatic reactions generating free fatty acids from membrane phospholipids, leading to peroxidative damage.20 Prostacyclin has a protective effect on brain physiological homeostasis: the former arachidonic acid metabolite is a potent vasodilating agent,10,32 reduces platelet adhesion via a cyclic adenosine monophosphate-mediated mechanism and, in association with indomethacin and heparin, significantly reduces the extension of ischemic damage after reperfusion following four-vessel occlusion in the dog.13,16 Moreover, prostacyclin reduced blood-brain barrier derangement after carotid occlusion in cat.5

In our experience, the increase of PGH2 release is evident only at 48 hours after SAH induction, and this suggests a protective effect on the arterial compartment against the late phase of vasospasm developing in the rat SAH model.11,41 Nicardipine significantly reduces 6-keto-PGF1α release at 6 and 48 hours; this could be an unwanted pharmacological effect but, in view of the global effect of nicardipine on the release of vasoactive arachidonic acid metabolites, we have also to consider the reduction of the synthesis and release of vasoconstrictor agents such as PGD2 and LTC4. The protective role of PGD2 after an ischemic or hemorrhagic insult may be related to a decreasing effect on glutamate-dependent excitatory effects on neuronal cells: the release of this metabolite was not significantly affected at 1 and 6 hours after SAH, while at 48 hours the release was significantly reduced in nicardipine-treated rats. The calcium channel blocker significantly reduces the protective effect of PGD2 on brain, but is useful in reducing the release of this well-demonstrated spasmoden.21

The enhanced release of PGE2 may raise some questions. Prostaglandin E2 is a vasoactive compound which may exert vasoconstrictor or vasodilating effects, depending on different species and experimental conditions.51 The E class of prostaglandins may be involved in edema pathogenesis and in inflammatory responses,19 and could potentiate the action of other mediators such as leukotrienes.9,12,27

In our experience, LTC4 release is significantly enhanced at 1, 6, and 48 hours after SAH induction. The protective effect of nicardipine could be exerted against arachidonic acid-dependent edema reactions. Recently,
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in a clinical experience, we demonstrated that cisternal CSF levels of LTC₄ are significantly higher in patients with symptomatic LTC₄ and that LTC₄ levels in the CSF are related to an enhanced release of the metabolite from brain cortex (unpublished data). Moreover, there is experimental and clinical evidence that leukotrienes are important factors for the development of anoxic-ischemic and hemorrhagic damage and in brain edema formation, after aneurysm rupture.

Nicardipine exerts a significant inhibitory effect on both biochemical pathways. Thereafter, the effect on the release of LTC₄ is evident in the early phase and persists with progressive significance. Thus, we can hypothesize that nicardipine, aside from its vascular effect, may exert a protective role against the release of compounds involved in the pathogenesis of neuronal damage and in brain edema formation, after aneurysm rupture.

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