Effect of the Thromboxane A$_2$ Mimetic U 46619 on Pial Arterioles of Rabbits and Rats

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Our previous experiments have shown that thromboxane A$_2$ is a strong contractor of cerebral arterial smooth muscle strips. The objective of these experiments was to determine if U 46619, a stable thromboxane A$_2$ mimetic, affects the cerebral microcirculation in vivo. Pial arteriole diameter in rabbits and rats was measured with a microscope using the closed cranial window technique. Topical application of $10^{-11}$ to $10^{-6}$ M U 46619 induced dose-dependent arteriole vasoconstriction in both species. In rabbits and rats the maximum vasoconstriction was $9.7 \pm 1.3\%$ (mean $\pm$ SEM) and $14.0 \pm 0.5\%$, respectively. In rats, $10^{-7}$ and $10^{-6}$ M U 46619 induced intravascular platelet aggregation accompanied by a further decrease in diameter and transient occlusion of the arterioles and venules. U 46619 had no significant effect on rabbit pial arterioles that were predilated by hypercapnia or hypercapnia plus hypoxia. Our data suggest that in animals with a normal vasculature, thromboxane A$_2$ may be a moderate constrictor of cerebral arterioles and that this constrictor effect is prevented by hypercapnia and hypoxia. (Stroke 1987;18:796–800)

Materials and Methods

A total of 21 male New Zealand white rabbits weighing 3.4–4.0 kg and 5 rats weighing 365–427 g were used in these studies. As described previously,$^{14}$ rabbits were anesthetized with 25 mg/kg i.v. sodium pentobarbital, 560 mg/kg subcutaneous urethane, and 38 mg/kg subcutaneous $\alpha$-chloralose. Supplemental doses of pentobarbital were given as needed to maintain anesthesia. Under this regimen, surgical anesthesia was quickly induced with minimum respiratory depression, and the need for additional doses of pentobarbital was reduced. After completion of the tracheotomy, each rabbit was artificially ventilated with room air. End-expiratory CO$_2$ concentration was continuously monitored with a Hewlett-Packard infrared CO$_2$ analyzer and was maintained at approximately 34 mm Hg throughout each experiment by adjusting the respiratory rate and volume, except in instances where CO$_2$ was deliberately changed. Arterial blood pressure was measured with a Statham P23Db pressure transducer connected to a catheter inserted into the left femoral artery. Arterial blood samples were periodically collected for determination of Paco$_2$, Pao$_2$, and pH with an Instrumentation Laboratory blood pH-blood gas analyzer.

Rabbit pial arterioles were visualized using a previously described closed cranial window technique.$^{15}$ After craniectomy and incision of the dura, a stainless steel cranial window was implanted on the midline just caudal to the suture connecting the frontal and parietal bones. The cranial window was equipped with 3 openings; 2 were used as inlet and outlet for filling the space under the cranial window with test solutions, and the third was connected to a Statham pressure transducer for continuous measurement of intracranial pressure (ICP).

Rats were anesthetized with 50 mg/kg i.p. sodium pentobarbital. Supplemental doses were given as needed to prolong anesthesia. Ventilation and recording of blood pressure, blood gases, and pH were as described for rabbits. A closed cranial window was constructed according to the method of Morii et al.$^{16}$ After exposure of the skull, 3 polyethylene tubes were secured to the bone with cyanoacrylate glue such that the ends of the tubes were in close apposition to the medial, fron-
tal, and occipital edges of the right parietal bone. Dental acrylic was placed around the right parietal bone embedding the polyethylene tubes, which were subsequently used as drug inlet, drug outlet, and for measurement of ICP. A craniectomy 5 mm in diameter was performed in the mediocaudal portion of the parietal bone. An additional layer of dental acrylic was placed around the craniectomy and flattened with a glass slide before it dried. The dura-arachnoidea complex was incised. This was the most difficult step in preparation of the rat closed cranial window chamber. We could not avoid cutting large dural vessels in about 50% of the rats, and these animals were subsequently excluded because the bleeding was difficult to stop and blood contacted the pial surface. The cranial window chamber was closed with a glass coverslip and sealed with dental acrylic.

In both rabbits and rats the space under the window and the plastic tubing connected to it were filled with artificial cerebrospinal fluid (CSF) equilibrated with gas containing 6.6% O2, 5.9% CO2, and 87.5% N2, which gives gas tensions and a pH in the normal range for CSF. The outflow tubes of the windows were set at a fixed height to give a 4–5 mm Hg ICP throughout the experiments. Vessel diameters were measured with a Vickers image splitting device (Woburn, Mass.) according to a method described by Baez. Readings were taken at 2 and 5 minutes after topical drug application.

U 46619 (15S-hydroxy-11α,9α-epoxymethano-prosta-5Z,13E-dienoic acid) was donated through the courtesy of the Upjohn Co. (Kalamazoo, Mich.), and indomethacin was purchased from Sigma Chemical Co. (St. Louis, Mo.). U 46619 was dissolved in absolute ethanol to produce stock solutions from 1 to 10^-3 mg/ml; 0.35 μl of these stock solutions was added to 1 ml of artificial CSF to produce final concentrations of 10^-6 to 10^-11 M. The stock solutions were stored at -70°C. The maximum ethanol concentration used in testing the vasoactivity of U 46619 was 0.35 μl/ml CSF, which we found does not affect pial arteriole diameters. Nevertheless, the arteriole responses to U 46619 were compared with the appropriate ethanol-containing drug vehicle. A 10 mg/ml indomethacin stock solution was prepared daily by dissolving 3 parts of indomethacin and 1 part of sodium carbonate (by weight) in distilled water.

To ensure that the U 46619 had its previously described biologic activity, we tested its capacity to induce “sudden death” after i.v. bolus injection in rabbits and irreversible aggregation in human platelet-rich plasma (PRP). In vitro platelet aggregation of PRP was measured with a dual-channel aggregometer (Payton Assos., Buffalo, N.Y.). Blood taken from a drug-free volunteer was collected in citrate and centrifuged at 200g for 10 minutes to prepare PRP, and 10^-6 M U 46619 was added to induce aggregation.

Our experimental design was as follows: Control observations of blood pressure, ICP, and vessel caliber were made until a steady state was reached. In the first series of experiments, we investigated the effect of topically applied 10^-11 to 10^-6 M U 46619 on pial arteriole diameter in rabbits. Each solution was added to 37°C artificial CSF and was slowly flushed under the window. To determine whether there might be species variations in the response to U 46619, the same kind of experiment using U 46619 at concentrations of 10^-9 to 10^-6 M was performed in rats.

In another series of experiments, the responses to U 46619 were measured in rabbit pial arterioles that were diluted by hypercapnia or hypercapnia plus hypoxia to assess whether the effect of U 46619 on pial arterioles is enhanced in conditions that simulate cerebral ischemia. To produce hypercapnia, the rabbits were ventilated with a gas mixture containing 5% CO2 in room air. Another group of rabbits was ventilated with 5% CO2 and 10% O2 in N2 to induce hypercapnia plus hypoxia, referred to as asphyxia. Blood gases and vessel diameters were measured during ventilation with room air and after 10 minutes’ ventilation with the gas mixtures. U 46619 was topically applied when the vessel diameters had stabilized.

The experiments were conducted using groups of 3–9 animals. All results are reported as means ± SEM and were statistically analyzed using Bonferroni’s t-test for paired or unpaired observations.

Results

Intravenous bolus injection of 1.4 μg/kg U 46619 in 1 rabbit induced an almost immediate respiratory arrest, an acute drop in mean arterial blood pressure (MABP), and death within 3 minutes, similar to the events in sudden death described for rabbits and mice after i.v. injection of arachidonic acid and endoperoxide analogs. U 46619 at 10^-6 M induced pronounced and irreversible aggregation of human PRP as reported by Burke. From these data we conclude that the U 46619 used in our study had the previously described biologic activity. MABP, Paco2, PaO2, pH, and control arteriole diameters of rabbits in normocapnia, hypercapnia, and asphyxia are given in Table 1. Topical application of the drugs never affected MABP.

As shown in Figure 1, 10^-11 to 10^-5 M U 46619 induced a concentration-dependent constriction of pial arterioles in normocapnic rabbits. The maximum constriction 2 minutes after topical application was 9.7 ± 1.3% at a dose of 10^-6 M U 46619. In rabbit pial arterioles that were predilated by hypercapnia, 10^-11 to 10^-6 M U 46619 produced slight additional but statistically nonsignificant dilation. Application of U 46619 during asphyxia produced an effect similar to that observed with hypercapnia. In the latter series of experiments, only those U 46619 concentrations that were the most effective during hypercapnia (10^-9 to 10^-7 M) were used since the MABP of the rabbits dropped when the duration of asphyxia was extended. The ethanol-containing vehicle for U 46619 had no effect on arteriole caliber in either normocapnia or hypercapnia.

To determine whether dilator prostaglandins formed during hypercapnia might override the constrictive response to U 46619, the cyclooxygenase inhibitor indomethacin was used in the experiments in rabbits to limit the formation of prostaglandins. The results of experiments performed in 3 rabbits are given in Table 1. In rabbits ventilated with a gas mixture containing 5% CO2 and 10% O2 in N2, U 46619 had no effect on cerebral arteriole diameter.
Table 1. Physiologic Parameters of the Rabbits

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Ventilation</th>
<th>Mean arterial blood pressure (mm Hg)</th>
<th>PCO₂ (mm Hg)</th>
<th>PO₂ (mm Hg)</th>
<th>pH</th>
<th>Control diameter (μm)</th>
<th>% Change in diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normocapnia</td>
<td>8</td>
<td>air</td>
<td>90 ± 4</td>
<td>29.9 ± 0.5</td>
<td>90 ± 4</td>
<td>7.490 ± 0.01</td>
<td>71 ± 6</td>
<td></td>
</tr>
<tr>
<td>Hypercapnia</td>
<td>9</td>
<td>air</td>
<td>84 ± 3</td>
<td>29.6 ± 0.7</td>
<td>96 ± 3</td>
<td>7.484 ± 0.01</td>
<td>70 ± 5</td>
<td></td>
</tr>
<tr>
<td>Hypercapnia</td>
<td>5% CO₂ in air</td>
<td>94 ± 5</td>
<td>54.1 ± 0.9</td>
<td>102 ± 5</td>
<td>7.279 ± 0.02</td>
<td>82 ± 5</td>
<td>17.3 ± 2.3</td>
<td></td>
</tr>
<tr>
<td>Asphyxia</td>
<td>4</td>
<td>air</td>
<td>96 ± 7</td>
<td>31.6 ± 1.2</td>
<td>92 ± 8</td>
<td>7.450 ± 0.02</td>
<td>70 ± 9</td>
<td></td>
</tr>
<tr>
<td>Asphyxia</td>
<td>5% CO₂, 10% O₂ in N₂</td>
<td>101 ± 8</td>
<td>50.3 ± 1.0</td>
<td>40 ± 5</td>
<td>7.298 ± 0.01</td>
<td>80 ± 9</td>
<td>16.9 ± 3.0</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

methacin (3 μg/ml, 8.4 μM) and 10⁻⁹ and 10⁻⁸ M U 46619 were simultaneously applied during hypercapnia. Topical indomethacin itself induced a slight constriction of 7 arterioles in 3 rabbits (6.2 ± 1.6%). Subsequent application of indomethacin plus U 46619 had no effect on rabbit pial arterioles (data not shown).

We next measured the effect of 10⁻⁹ to 10⁻⁶ M U 46619 on arteriole diameter in 5 rats. The control MABP, Paco₂, Po₂, and pH in these rats was 102 ± 6, 29.2 ± 0.3, 106 ± 3 mm Hg, and 7.414 ± 0.018, respectively. The pial arterioles that we measured ranged in diameter from 30 to 84 μm; the mean diameter was 54 ± 9 μm. U 46619 at 10⁻⁶ to 10⁻⁷ M induced a dose-dependent vasoconstriction similar to that induced in rabbit pial arterioles (Figure 2). Two minutes after topical application, 10⁻⁷ M U 46619 resulted in a constriction of 14.0 ± 0.5%. While observing the pial surface between 2 and 5 minutes after application of 10⁻⁷ M U 46619, intravascular aggregates appeared in the pial venules and arterioles. The pial arteriole caliber fluctuated, and the blood flow in the venules decreased. Five minutes after application of 10⁻⁷ M U 46619, the pial arterioles had decreased in diameter by 24 ± 2%. These responses occurred even more quickly, within 2 minutes, after topical application of 10⁻⁶ M U 46619. The intravascular aggregates were periodically flushed away by the blood stream and did not occlude the vessels permanently.

**Discussion**

Whereas previous experiments in our own and other laboratories have shown that TxA₂ is a potent constrictor of cerebral artery strips,¹² our present data provide evidence that topical application of a specific TxA₂ mimetic induces moderate constriction of pial arterioles in rabbits and rats. These results are in agreement with the recent study by Rosenblum and Bryan,¹² who reported vasocstriction by U 46619 in the mouse open pial arteriole preparation. Moreover, our present results show that the constrictor response to
U 46619 was prevented, rather than enhanced, in rabbit pial arterioles that were dilated by hypercapnia or asphyxia.

There are several lines of evidence that the synthetic TxA₂ mimetic U 46619, which was used in our study, acts on the same receptor(s) as natural TxA₂. First, U 46619 and authentic TxA₂ display the same pattern of selectivity in their contractile effect on a wide range of isolated smooth muscle preparations. Second, both U 46619 and TxA₂ induce pronounced, irreversible aggregation of platelets from various species. Third, the contractile and proaggregatory effect of U 46619 can be blocked in a competitive manner by the specific TxA₂ receptor blocker SQ 29,545. Fourth and finally, i.v. bolus injection of U 46619 results in sudden death. Sudden death was initially described after i.v. injection of arachidonic acid into rabbits, and evidence has been provided that it is related to pulmonary thrombosis produced by TxA₂. In our study the capacity of U 46619 to induce platelet aggregation and sudden death was used to ascertain its biologic efficacy.

Investigations of the effects of TxA₂ or U 46619 on arterial tone in vivo are scarce. Kaley et al and Wang et al report that the coronary artery resistance in adult dogs was not changed by TxA₂ generated from the cyclic endoperoxide prostaglandin (PG) H₂ and then immediately injected into the coronary artery. Their failure to demonstrate significant effects may be due to the rapid degradation of natural TxA₂. Also, PGH₂ spontaneously isomerizes to PGE₂ in an aqueous medium, and PGE₂ may counteract TxA₂ effects. Therefore, we used the stable TxA₂ mimetic U 46619, which has been reported to induce strong in vivo constriction of all peripheral vessels in all species studied, including canine coronary and pulmonary arteries and the rabbit skin microvasculature. Our failure to show a potent effect of U 46619 in the cerebral microcirculation in two different species indicates that pial arterioles may have fewer TxA₂ receptors than peripheral vessels or that there might be a specific mechanism in cerebral vessels that opposes the U 46619-induced constriction.

One putative candidate for such a mechanism would be an endothelium-derived relaxing factor (EDRF). EDRF is released from mouse cerebral arterioles after topical application of acetycholine and bradykinin. Cocks and Angus reported that U 46619 did not release a dilator substance from isolated coronary arteries with intact endothelium. However, U 46619 might act as a stimulator of EDRF in other vascular beds such as the cerebral vasculature. A precedent for this mechanism is vasopressin, which has been shown to induce an endothelium-dependent vasodilation in canine basilar arteries and a constriction, unaffected by endothelium removal, in dog femoral arteries. The hypothesis that an EDRF is released from the endothelium of cerebral arteries in response to U 46619 is suggested by the discrepancy between the present results and our previous study showing strong TxA₂–induced contraction of isolated cerebral arteries. In our previous studies, the endothelium was destroyed by cutting helical arterial strips. Thus, the direct effect of TxA₂ on arterial smooth muscles may be strong contraction, whereas in intact cerebral arteries this effect may be antagonized by a relaxing substance released from the endothelium.

Another explanation for the weak effect of the analog in our study might be that pial arterioles respond relatively mildly to constrictive stimuli in general. However, experiments in our laboratory using the same rabbit arteriole preparation showed that topical application of high doses of histamine consistently induced a 31% constriction. Similarly, rat pial arterioles constricted by 24% in response to topically applied PGF₂α. These data argue against the possibility that pial arterioles of the species currently studied lack the capacity to constrict.

Our approach to determine the effect of U 46619 during hypercapnia and asphyxia was based on the assumption that autoregulatory vasodilation might render the cerebral arterioles more reactive to vasoconstrictors. In contrast, hypercapnia and hypoxia prevented the contractile response to U 46619. We hypothesize that, in conditions simulating cerebral ischemia, a mechanism might exist that protects the brain from additional harm by TxA₂ actions. Since U 46619 stimulated vascular prostacyclin synthesis in vitro, we tested the possibility that this vasodilator prostaglandin might counteract the vasoconstrictor response to U 46619 in hypercapnia. However, inhibition of prostacyclin synthesis by topical indomethacin failed to reveal constriction to U 46619 during hypercapnia. Therefore, the reason for our failure to observe vasoconstriction to U 46619 in hypercapnia and asphyxia remains uncertain.

In the rat cerebral microcirculation, high doses of U 46619 induced fluctuations of the arteriole diameter, transient occlusions of venules and arterioles by aggregates, and an additional decrease in arteriole diameter. We believe that the changes in vascular diameter were likely the result of intravascular platelet aggregation. Whether the fluctuations in diameter were due to alterations in pressure within the arterioles or were due to active vasoconstriction by substances released from the platelets is uncertain. Our data do not permit us to distinguish between these possibilities. Interestingly, we never observed platelet aggregation in rabbit pial arterioles in response to U 46619. This may indicate that topical U 46619 penetrates the rabbit pia mater and pial vascular wall less than it does in rats. The finding that platelet aggregation was induced at 5 but not at 2 minutes after application of 10⁻⁷ M U 46619 suggests that a diffusion barrier also exists in rat pial arterioles and that U 46619 needs time to pass this barrier.

Our data do not strongly support the concept that TxA₂ is a critical mediator of cerebrovascular events such as vasospasm or postischemic hyperperfusion. We caution, however, that in injured vessels the effect of TxA₂ may be different than that currently observed in normal blood vessels. Since ultrastructural damage of the endothelium and vascular smooth muscle following subarachnoid hemorrhage is known to occur, our
ongoing studies are addressing the possible role of the endothelium in the cerebral arteriole response to TxA2.

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References


KEY WORDS • brain microcirculation • thromboxane A2 • hypoxia • hypercapnia • platelet aggregation
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