Evidence That \textit{in Vivo} Constriction of Cerebral Arterioles by Local Application of \textit{tert}-Butyl Hydroperoxide Is Mediated by Release of Endogenous Thromboxane

William I. Rosenblum and Doris Bryan

Pial arterioles of mice were suffused \textit{in situ} with \textit{tert}-butyl hydroperoxide (TBHP). This agent has been reported to stimulate synthesis or release of the constrictor, thromboxane. In the present study we observed pial arterioles by \textit{in vivo} microscopy. Locally applied TBHP produced dose-dependent constriction, significantly inhibited by each of three drugs: acetylsalicylic acid, OKY-046, and SQ-29548. These drugs are respectively a cyclooxygenase inhibitor, a thromboxane synthetase inhibitor, and a thromboxane receptor blocker. Since each acts by a different mechanism to interfere with thromboxane-mediated responses and each inhibited the contractile response to TBHP, thromboxane appears to be a mediator of this response. Platelet aggregation was not seen after local application of TBHP, and the dwell time of platelets at the site of TBHP application is less than 1 second. Thus, platelets are an unlikely source of the thromboxane mediating the local constriction. It is much more likely that the source of thromboxane is either the wall of the pial vessels or the underlying brain and/or its vessels. These data do not distinguish between the latter two possibilities, but if this suggestion is correct, then the data show for the first time that thromboxane can be released from normal brain and/or brain vessels in amounts sufficient to cause arteriolar constriction. (\textit{Stroke} 1987; 18:195–199)

Thromboxane has long been known to be a potent constrictor of cerebral arteries and other vessels. However, there has been no published evidence to suggest that cerebral blood vessels can be constricted by thromboxane released from the vessels themselves and/or adjacent brain. Rather, demonstrations of constriction have involved \textit{in vitro} application of thromboxane or its analogs. A similar situation is encountered in the literature for other vascular beds, probably because of the difficulty of selectively releasing endogenous thromboxane from the vessels or organ in question. However, Gurtner et al found that \textit{tert}-butyl hydroperoxide (TBHP) caused marked constriction of the perfused pulmonary vasculature, which was associated with the appearance of increased levels of thromboxane in the effluent. The elevated thromboxane level and the associated vasconstriction were prevented by blocking cyclooxygenase, which was interpreted as indicating that TBHP stimulates cyclooxygenase-dependent arachidonic acid metabolism with a resultant increase in the production of eicosanoids like thromboxane. However, thromboxane production was increased at least eightfold while prostacyclin production was increased only threefold by TBHP. Thus, TBHP might in addition selectively stimulate thromboxane synthetase by acting on cyclooxygenase-dependent precursors of thromboxane. In either case the thromboxane produced by TBHP caused significant constriction.

The source of the thromboxane was thought to be lung "parenchyma"; however, the possibility of thromboxane released from vessel walls cannot be ruled out. Thromboxane can be made by blood vessel walls, but this fact has not been emphasized in the literature because, under the published conditions of measurement, relatively little thromboxane is made compared with other eicosanoids, particularly the potent dilator prostacyclin. We applied TBHP briefly in a superfusate over the brain surface to produce constriction of surface (pial) arterioles and continued to study the phenomenon with pharmacological probes that interfere with thromboxane production or the thromboxane receptor. These studies indicate that TBHP does produce thromboxane-mediated constriction of pial arterioles. The source of the thromboxane must be either the arterioles or the underlying brain. The data do not permit a definitive decision on this point, but the brief period (30 seconds) of exposure to TBHP and the immediate proximity of the pial arterioles to the superfusate suggest that vessel wall thromboxane might indeed be a potentially powerful vasoconstrictor of cerebral arterioles. The alternative explanation, that TBHP stimulates brain parenchyma to produce thromboxane, is equally important. In either case our data demonstrate for the first time that local release of endogenous thromboxane, whether from parenchyma or vessel, is sufficient to cause constriction.

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Materials and Methods

Male mice (Institute for Cancer Research strain, Flow Laboratories) weighing 22–35 g were anesthetized with urethan and subjected to trephectomy and craniotomy as previously described.14–18 The dura was stripped as previously described,14–18 and the cerebral surface (pial) vessels lying in the subarachnoid space between the transparent arachnoid and the brain were observed through a Leitz Ultropak microscope.15 A TV camera and monitor were employed together with an image splitter and strip-chart recorder for measurements of arteriolar diameter and diameter changes as described by Baez.19 This technique can detect diameter changes of 0.5 μm or less,19,20 In each mouse a single arteriole was arbitrarily selected for monitoring. The only criteria for monitoring were size (30–50 μm) and that the vessel could not be an anastomosis between other arterioles. The mice were maintained at 37°C, and the surface of the brain was irrigated with an artificial cerebrospinal fluid (CSF) flowing at 2 ml/min,15,18 37° C, pH 7.35 ± 0.03 (mean ± SD) as measured in the fluid passing across the craniotomy site.

Chemicals were applied to the cerebral surface as a bolus of 1.0 ml at 37°C delivered in 30 seconds. The chemicals used in this way were norepinephrine bitartrate (levarterenol), tert-buty1 hydroperoxide (Aldrich), SQ-29548 (15-[1α,2β(S)],3β,4α]-7-[2-[(phenylamino)carbonyl]hydrazino]methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid; a gift from Squibb, Princeton, N.J.), and U-46619 (9,11-didecylxyclo[2.1.1]hept-2-yl]-5-heptenoic acid), a gift from Ono Pharmaceutical Co., Osaka, Japan), OKY-046 ([sodium (£)-3-4-Cl-imidazolylmethyl]-phenyl-2-propanoate; Ono Pharmaceutical Co., Osaka, Japan), and SQ-29548 were used. The first two were soluble in normal saline, the former with pH adjusted to 7.4–7.5 by adding NaHCO3. These diluents served as controls. SQ-29548 was initially dissolved in ethanol and subsequently diluted in saline for i.p. injection. Controls received the equivalent ethanol–saline mixture.

In all the experiments in which i.p. injections were employed, the injection was given prior to craniotomy. Following craniotomy, the pial surface was suffused with artificial CSF for a 20-minute period of equilibration prior to beginning the experiment itself. A similar equilibration period was employed in the experiments in which only locally applied drugs were employed.

At the end of each experiment, 100 μl of blood was obtained from carotid artery for CO2, O2, and pH analysis with a Radiometer Ultramicro Blood Gas Analyzer (Cleveland, Ohio).

Results

Constriction by TBHP and Inhibition by an Inhibitor of Cyclooxygenase

In this experiment 5 mice were treated with vehicle and 5 mice with 100 mg/kg ASA i.p. 1 hour before the test. ASA is a well-established inhibitor of cyclooxygenase21,22 and should therefore reduce the capacity of TBHP to elicit thromboxane production. On the test day TBHP was applied to the brain surface in molar concentrations of 2 × 10⁻³, 2 × 10⁻⁴, and 2 × 10⁻⁵. Three applications of 30 seconds each were separated by a 15-minute washout period during which baseline diameter was reestablished. Prior to these experiments, preliminary studies showed no tachyphylaxis to doses of TBHP given 15 minutes apart. In the experiments comparing ASA-treated with vehicle-treated mice the arteriolar diameter was 40 ± 0.5 μm in each group of mice (mean ± SEM). As shown in Table 1, TBHP constricted pial arterioles in a dose-dependent manner (p < 0.01, analysis of variance), and constriction was significantly reduced by ASA. Arterial CO2, pH, and O2 levels (mean ± SEM) were the same in both groups (31 ± 1 mm Hg, 7.37 ± 0.04, 113 ± 6 mm Hg vs. 33 ± 1, 7.36 ± 0.02, 106 ± 7). In a control study to establish the specificity of the ASA effect, the contractile response to norepinephrine was not affected by the ASA treatment. This negative result with norepinephrine duplicates data we have previously reported.16

Constriction by TBHP and Inhibition by an Inhibitor of Thromboxane Synthesis

In this experiment 5 mice were treated with vehicle and 5 with 100 mg/kg OKY-046 given i.p. 1 hour before the test. OKY-046 is a potent and selective inhibitor of thromboxane synthetase,4 and therefore, it should reduce the capacity of TBHP to elicit thromboxane production. TBHP was suffused over the brain surface in 3 doses. Each application was 30 seconds long and separated by a 15-minute washout period during which baseline diameter was reestablished. The diameter (mean ± SEM) of the arterioles was 42 ± 3 μm in the vehicle-treated mice and 41 ± 0.5 μm in the OKY-treated mice. Arterial CO2, pH, and O2 (mean ± SEM) were also the same in both groups (32 ± 1 mm Hg, 73.7 ± 0.01, 109 ± 4 mm Hg vs. 32 ± 1, 7.36 ± 0.01, 117 ± 3). Significant inhibition of the

Table 1. ASA, a Cyclooxygenase Inhibitor, Inhibits Constriction of Pial Arterioles by TBHP

<table>
<thead>
<tr>
<th>Dose TBHP (molar)</th>
<th>Diameter reduction as percent of baseline</th>
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<tbody>
<tr>
<td></td>
<td>Vehicle-treated</td>
</tr>
<tr>
<td>2 × 10⁻³</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>2 × 10⁻⁴</td>
<td>9 ± 1</td>
</tr>
<tr>
<td>2 × 10⁻⁵</td>
<td>8 ± 2</td>
</tr>
</tbody>
</table>

Values as mean ± SEM.

*Significantly (p < 0.05) smaller responses than those in vehicle-treated mice (analysis of variance).
Table 2. OKY-046, a Thromboxane Synthesis Inhibitor, Inhibits Constriction of Pial Arterioles by TBHP

<table>
<thead>
<tr>
<th>Dose TBHP (molar)</th>
<th>Diameter reduction as percent of baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 × 10⁻³</td>
<td>14 ± 2</td>
</tr>
<tr>
<td>2 × 10⁻⁴</td>
<td>9 ± 1</td>
</tr>
<tr>
<td>2 × 10⁻⁵</td>
<td>7 ± 1</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.
*Significantly (p < 0.01) smaller responses than in vehicle-treated mice (analysis of variance).

Table 3. SQ-29548, a Blocker of Thromboxane Receptors, Inhibits Constriction of Pial Arterioles by U-46619, a Thromboxane Receptor Agonist

<table>
<thead>
<tr>
<th>Dose U-46619 (molar)</th>
<th>Diameter reduction as percent of baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.4 × 10⁻⁸</td>
<td>18 ± 2</td>
</tr>
<tr>
<td>1.4 × 10⁻⁹</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>1.4 × 10⁻¹⁰</td>
<td>8 ± 1</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.
*Significantly (p < 0.01) smaller responses than in the mice given U-46619 plus vehicle (analysis of variance).
release by TBHP of thromboxane from vessel wall or brain parenchyma would not be expected to aggregate passing platelets or to stimulate them to release additional thromboxane, again because contact of the moving platelets with locally produced thromboxane would last less than a second. For these reasons it seems most probable that the local constriction produced by TBHP is a result of thromboxane produced from the stationary vessel wall\(^9,10\) and/or brain,\(^13\) but not from rapidly passing platelets.

Thromboxane has been produced in both brain slices and homogenates,\(^13-25\) and from large cerebral vessels\(^9\) as well as from larger vessels of other organs.\(^7,8,11,12\) While brain parenchyma may produce thromboxane, it seems more likely that the effects of a 30-second bolus of TBHP in the suffusate of the pial vessels can best be explained by release of thromboxane from the pial vessels immediately exposed to the suffusate. However, the suffusate does reach the brain parenchymal surface, so release of thromboxane from brain cells or from vessels within the underlying brain cannot be ruled out. Whatever the site of thromboxane production and release, the present data appear to be the first evidence that thromboxane may be mobilized from brain vessels and/or normal brain in amounts sufficient to cause arteriolar vasoconstriction.

Additional findings of interest in our study include the demonstration of dose-dependent constriction of pial arterioles by U-46619, a thromboxane receptor agonist.\(^21,22\) Others have shown constriction of cerebral arteries in vitro by thromboxane or its stable analogs,\(^1,23\) but we are unaware of studies showing constriction of pial arterioles by thromboxane receptor agonists in situ. We used U-46619 to establish that SQ-29548, a selective blocker of the thromboxane receptor at other sites,\(^21,22\) did block the thromboxane receptor in pial arterioles. In fact, it has not been possible to separate the endoperoxide receptor from the thromboxane receptor,\(^23\) and therefore SQ-29548 might interfere with endoperoxide action as well as with thromboxane’s action. However, in our study the constriction elicited by TBHP must be related to production of thromboxane because the constriction was inhibited by OKY-046, a thromboxane synthetase inhibitor.

SQ-29548, the endoperoxide–thromboxane antagonist, worked not only when applied locally with TBHP, but also when given intraperitoneally. Therefore, it must cross either the blood–brain or the blood–CSF barriers in amounts sufficient to reach effective concentrations.

The present data, suggesting the constricting potential of thromboxane mobilized from brain or brain vessels, provides evidence that endogenous thromboxane could play a role in controlling cerebrovascular tone. Whether such control is actually manifest in either health or disease remains an open question. Others have suggested a role for thromboxane in producing cerebral vasocostriction seen in pathologic states.\(^6,27\) However, the brain is frequently injured in these experimental models of pathologic states, and many different putative vasoconstrictors are simultaneously mobilized. It becomes difficult to then ascribe constriction to the thromboxane. The present study is unique in that it provides a demonstration of cerebral vasocostriction elicited by an agent thought to selectively release thromboxane, and this agent, TBHP, has been applied in the absence of brain injury.

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Endogenous Thromboxane Constricts Pial Arteries


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