Role of Prostaglandin I$_2$ and Thromboxane A$_2$ in Recurring Reduction of Carotid and Cerebral Blood Flow in Dogs

YASUMI UCHIDA, M.D., AND SATORU MURAO, M.D.

SUMMARY The roles of PGI$_2$ and TXA$_2$ in recurring reduction of carotid artery and cerebral blood flow induced by partial constriction of the common carotid artery were examined in anesthetized dogs. The recurring reduction was eliminated by OKY 046 and 1580 which inhibit TX synthetase, acetylsalicylic acid which inhibits cyclo-oxygenase and lipooxygenase, PGI$_2$ and by papaverine which enhances PGI$_2$ synthesis. But the recurring reduction was not eliminated by pentolamine. The recurring reduction was induced by epinephrine which activates phospholipase A$_2$ and cyclo-oxygenase and causes platelet aggregation. It was also induced by tranylcypromine which inhibits PGI$_2$ synthetase and, although infrequently, by TXA$_2$. The recurring reduction was also induced by indomethacin that inhibits cyclo-oxygenase. The indomethacin-induced recurring reduction, however, was eliminated not by OKY 046 and 1580 but by PGI$_2$. It is suggested that TXA$_2$ acted as an inducer and PGI$_2$ as an inhibitor in the recurring reduction of carotid artery and cerebral blood flow.

DURING partial constriction of the canine common carotid artery, there are frequent recurring decreases of a transient nature in the blood flow in the constricted artery and in the cerebrum. Participation of vasospasm, platelet aggregation, thrombus formation and embolism in this recurring CBF reduction has been demonstrated angiographically and histologically. This experimental phenomenon resembles transient focal cerebral ischemic attacks in that cerebral artery flow reduction occurs spontaneously and recurrently in both. It is not known whether thromboxane A$_2$, which causes platelet aggregation and contraction of vascular smooth muscles, and prostaglandin I$_2$, which inhibits platelet aggregation and relaxes vascular smooth muscles, participate in this experimental phenomenon. This study was undertaken to clarify the roles of thromboxane A$_2$ and prostaglandin I$_2$ in the recurring reduction of carotid artery and cerebral blood flow induced by partial constriction of the canine common carotid artery.

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This work was supported by a grant-in-aid for scientific research (No. 00548193) from the Ministry of Education, Japanese Government. It was partly reported at the Annual Meeting of the Japanese College of Circulation, Tokyo, March, 1980, and at the International Symposium on Prostaglandins and the Cardiovascular System, Antwerp, Belgium, December, 1980.

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Methods

1. Experimental Preparations

Ninety-five beagle dogs were anesthetized with intravenous pentobarbital sodium (35–40 mg/kg). In each dog the trachea was intubated for artificial positive pressure respiration with air. The left common carotid artery was dissected free and a magnetic flowmeter (Nihonkoden MF-2) was placed around it to measure carotid artery blood flow. The superior thyroidal artery was cannulated to measure carotid blood pressure. A segment of the common carotid artery, 2–3 cm proximal to the thyroidal artery but distal to the flowmeter, was constricted with a cylindrical tube 3 mm in length and of various internal diameters. Usually, the blood flow was reduced by constriction to 30 to 70 percent of the control value. A pair of needle type cross-thermocouples were introduced into the anterior or lateral lobe of the cerebrum for measurement of cerebral blood flow. The cross-thermocouples could measure the flow within 0.1 ml of tissue as reported in a previous study. A catheter was introduced into the right femoral artery to monitor the systemic blood pressure. Heart rate was obtained by a pulse-integrator triggered by the femoral arterial pulse. Another catheter was introduced into the brachiocephalic artery with the tip at the orifice of the left common carotid artery for selective injection of the chemical agents (fig. 1). After the experiments, ventricular fibrillation was induced by electrical stimulation. Following the stimulation, the
cerebral blood flow decreased gradually and attained a stable level. This level was called zero flow and the flow was expressed as μv.

The 45 dogs in which recurring blood flow reduction occurred in either the carotid artery or cerebrum or both were then given the agents listed in table 1.

During the recurring changes in flow, carotid flow decreased gradually and then increased abruptly, while cerebral flow decreased gradually and then increased gradually, and the changes were repeated. The length of 3 cycles of recurring changes was timed and the various agents were injected intravenously, or into the carotid artery at the fourth increase of flow. When the time required for the reappearance of a flow reduction was significantly (Student's t-test, p < 0.05) longer than the average length of the preceding 3 cycles, the agents injected were considered to have eliminated the recurring reduction.

In 3 preparations in which prostaglandin I2 was injected, angiography of the neck and head was performed by injecting the contrast material (Conraxin H) through the catheter which was previously introduced into the brachiocephalic artery, to observe morphological change.

In a previous study, the recurring reduction appeared within one h after the constriction in a majority of the experiments, and rarely appeared after one h. The 50 dogs in which the recurring reduction did not appear within one h of observation were then given the agents listed in table 2 to induce the recurring reduction. Among these agents, thromboxane A2 was synthetized by incubating 10 μg of prostaglandin H2 with 10 mg of platelet microsomes at 0°C for 1 min, and was immediately injected into the common carotid artery. Thromboxane A2, produced from (1-C') prostaglandin H2 by platelet microsomes, was measured as thromboxane B2 by radiochromatography. It was revealed that the dose of thromboxane A2 injected was around 300 ng. Thromboxane B2 was synthetized by incubating 10 μg of prostaglandin H2 with 10 mg platelet microsomes at 37°C for 10 min, and was injected intravenously.

### Table 1: Effect of Various Substances on Recurring Reduction of Carotid and Cerebral Blood Flow

<table>
<thead>
<tr>
<th>Substance</th>
<th>Dose (μg/kg)</th>
<th>n</th>
<th>Control cycle length (min)</th>
<th>Reappearance time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGI2</td>
<td>0.00001</td>
<td>5</td>
<td>8.1 ± 2.3</td>
<td>16.0 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>0.00025</td>
<td>7</td>
<td>9.2 ± 2.5</td>
<td>36.5 ± 5.6†</td>
</tr>
<tr>
<td>OKY 046</td>
<td>10</td>
<td>3</td>
<td>11.0 ± 4.2</td>
<td>25.3 ± 8.0</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>7</td>
<td>9.6 ± 2.8</td>
<td>98.0 ± 19.1†</td>
</tr>
<tr>
<td>OKY 1580</td>
<td>20</td>
<td>7</td>
<td>10.3 ± 4.0</td>
<td>105.5 ± 20.5‡</td>
</tr>
<tr>
<td>Imidazole</td>
<td>10</td>
<td>7</td>
<td>7.3 ± 2.8</td>
<td>20.5 ± 4.8</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>7</td>
<td>8.0 ± 2.5</td>
<td>23.0 ± 12.8</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>10</td>
<td>5</td>
<td>10.3 ± 4.0</td>
<td>21.0 ± 4.8</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>2.5</td>
<td>5</td>
<td>6.8 ± 2.0</td>
<td>17.6 ± 4.1</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>7</td>
<td>10.8 ± 4.0</td>
<td>37.3 ± 5.0‡</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>5</td>
<td>8.0 ± 3.1</td>
<td>16.0 ± 6.4</td>
</tr>
<tr>
<td>Papaverine</td>
<td>0.1</td>
<td>3</td>
<td>9.0 ± 3.3</td>
<td>14.8 ± 3.0</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>7</td>
<td>11.0 ± 3.5</td>
<td>52.7 ± 19.6†</td>
</tr>
<tr>
<td>Phentolamine</td>
<td>0.5</td>
<td>7</td>
<td>10.5 ± 4.0</td>
<td>12.5 ± 5.2</td>
</tr>
<tr>
<td>Methysergide</td>
<td>0.1</td>
<td>4</td>
<td>8.6 ± 2.5</td>
<td>9.0 ± 3.2</td>
</tr>
</tbody>
</table>

*†p < 0.05; *‡p < 0.01; †p < 0.001; n = number of trials.

### Table 2: Induction of the Recurring Reduction of Carotid and Cerebral Blood Flow by Chemical Agents

<table>
<thead>
<tr>
<th>Substance</th>
<th>Dose (μg/kg)</th>
<th>Recurring reduction induced</th>
<th>Sustained reduction induced</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGH2</td>
<td>0.1/0.5/kg</td>
<td>iv</td>
<td>0/2</td>
</tr>
<tr>
<td>TXA2</td>
<td>ic</td>
<td>2/5</td>
<td>3/5*</td>
</tr>
<tr>
<td>PGE2</td>
<td>10/25/kg</td>
<td>iv</td>
<td>0/1</td>
</tr>
<tr>
<td>Leukotriene C-1</td>
<td>0.1/1/kg</td>
<td>iv</td>
<td>0/4</td>
</tr>
<tr>
<td>Tranylcypromine</td>
<td>100/250/kg</td>
<td>iv</td>
<td>2/4</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>0.05/0.1/kg</td>
<td>iv</td>
<td>0/1</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>1/5/kg</td>
<td>iv</td>
<td>0/3</td>
</tr>
<tr>
<td>Ergonovine</td>
<td>20/15/10/kg</td>
<td>iv</td>
<td>5/5</td>
</tr>
<tr>
<td>Serotonin</td>
<td>50/100/kg</td>
<td>iv</td>
<td>4/4</td>
</tr>
</tbody>
</table>

*transient decrease

transient decrease in = intravenous injection, ic = intra-carotid injection.
Results

1. Prostaglandin (PG) I\textsubscript{2}, Thromboxane (TX) Synthetase Inhibitors and Cyclo-oxygenase Inhibitors

Figure 2 shows the effect of an intravenous injection of PG\textsubscript{I\textsubscript{2}} on recurring reduction of carotid and cerebral blood flow. Before the injection, carotid and cerebral flow decreased and then increased spontaneously and recurrently. Following the injection, the recurring reduction disappeared, and reappeared about 100 min after the injection. The recurring reduction was eliminated by PG\textsubscript{I\textsubscript{2}} with a dose of 0.25 \mu g/kg or more (table 1). It was demonstrated angiographically that vasospasm of the internal carotid artery and the cerebral arteries could be eliminated for at least 10 min after the injection of this substance (figs. 3, 4). Similarly, the recurring reduction was eliminated by the TX synthetase inhibitors, (E)-3-[4-(1-imidazolylmethyl)phenyl]-2-propenoic acid (OKY 046), and (E)-3-[4-(3-pyridylmethyl)phenyl]-2-methyl-2-propenoic acid (OKY 1580) (table 1, fig. 5). Imidazole, which also inhibits TX synthetase,\textsuperscript{18} did not eliminate the recurring reduction at the dose levels used in this study. The recurring reduction was also eliminated by acetylsalicylic acid and ketoprofen, both of which inhibit cyclo-oxygenase.\textsuperscript{13} After reappearance of the reduction, however, its magnitude became larger than it was before the injection. In addition, recurring reduction appeared anew in either carotid or cerebral flow in a few preparations (fig. 6). Such an augmentative effect was not observed following the injections of PG\textsubscript{I\textsubscript{2}} or TX synthetase inhibitors.

![Figure 2](image)

FiguRE 2. From top: carotid blood flow (CaBF), carotid blood pressure (CaBP), cerebral blood flow (CBF), systemic blood pressure (SBP) and heart rate (HR). The recurring decreases in CaBF and CBF disappeared after the intravenous injection of PG\textsubscript{I\textsubscript{2}} (0.5 \mu g/kg), at arrow.

![Figure 3](image)

FIGURE 3. A: during an increase of CBF, B: during a decrease of CBF. The diameter of the internal carotid artery (white arrow) became significantly smaller than that in A. C: 10 min after intravenous injection of 0.5 \mu g/kg of PG\textsubscript{I\textsubscript{2}}. Dilatation of the internal carotid artery is obvious. Black arrow indicates the constricted segment.
Indomethacin inhibits cyclo-oxygenase as does acetylsalicylic acid, but this agent did not eliminate recurring reduction. Papaverine is a vasodilating agent and enhances PGI synthesis in vitro. This agent also eliminated recurring reduction (table 1). Phentolamine, an α-receptor blocking agent, and methysergide, an antagonist of serotonin, did not eliminate the recurring reduction.

2. Induction of Recurring Reduction of Carotid and Cerebral Blood Flow

Intra-carotid injection of PGH₂ in doses of up to 0.5 μg did not induce the recurring reduction. TXA₄ induced recurring reduction in 2 of 5 preparations (fig. 7, table 2). A transient reduction in flow was induced by this substance in the remaining preparations. TXB₂ did not cause any change in flow. Intravenous injec-
tion of PGF\textsubscript{2\alpha} and intra-carotid injection of PGE\textsubscript{2} that caused contraction of cerebral arteries\textsuperscript{18} also induced recurring reduction, although not always (table 2). Tranlynpyromine, which inhibits PGI\textsubscript{2} synthetase \textit{in vitro},\textsuperscript{9} also induced recurring reduction when a small dose was used. With large doses there was a sustained decrease in flow followed by recurring reduction during recovery from the decrease. During the tranlynpyromine-induced flow decrease, diffuse narrowing of the carotid and cerebral arteries was observed angiographically. Epinephrine, which activates phospholipase A\textsubscript{2} and cyclo-oxygenase and causes platelet aggregation, frequently induced recurring reduction. The number of reductions produced was only up to 4 cycles. The time required for epinephrine-induced reduction ranged from 2 to 18 (6.1 ± 2.2, mean ± SD) min. In contrast with epinephrine, norepinephrine did not induce recurring reduction. Unexpectedly recurring reduction was induced by indomethacin which inhibits cyclo-oxygenase and enhances production of slow reacting substance of anaphylaxis (SRS-A) such as leukotriene C and D\textsubscript{4-18} (fig. 8). The time required for the appearance of the reduction induced by this agent ranged from 3 to 45 (20.3 ± 4.3) min, and did not disappear for more than 2 h (table 2). Recurring reduction was induced by indomethacin even after administration of 30 mg/kg of OKY 046 in 3 preparations. The indomethacin-induced recurring reduction was not eliminated by OKY 046 and 1580 at dose levels otherwise high enough to eliminate recurrent reduction in all 7 of the preparations tested. The indomethacin-induced recurring reduction was eliminated by 0.5 \mu g/kg of PGI\textsubscript{2} in all these preparations (fig. 9). Leukotriene C-1 induced the recurring reduction, although not always (table 2). However, 10 mg/kg of cromoglycate which inhibits release of SRS-A\textsuperscript{17} could not eliminate indomethacin-induced recurring reduction in all 3 preparations tested.

**Figure 7.** Recurring reduction of CaBF and CBF induced by selective injection of TXA\textsubscript{2} into the left common carotid artery.

**Figure 8.** Indomethacin-induced recurring reduction of CaBF and CBF.
Figure 9. Indomethacin-induced recurring reduction of CaBF and CBF, and its elimination by intravenous injections of PGI,

Discussion

It is generally accepted that TXA₂ is mainly formed in platelets and causes platelet aggregation and contraction of vascular smooth muscles while PGI₁ is mainly formed in vascular endothelium and antagonizes the action of TXA₂.\(^\text{[6-7]}\) In this study, the recurring reduction of carotid and cerebral blood flow, which was considered to be due to platelet aggregation, thrombus formation, embolism and/or vasospasm, was induced by synthetized TXA₂, although not always. Since TXA₂ is very unstable, it may have been converted into TXB₂ during preparation, and did not induce recurring reduction in some preparations. This possibility is also supported by the fact that TXB₂ itself did not induce the recurring reduction. It is likely that TXA₂ acted as an inducer of recurring reduction since TX synthetase inhibitors such as OKY 046 and 1580 eliminated the reduction. There is another possibility, namely that enhanced PGI₁ synthesis caused by the inhibition of TXA₂ synthesis by these inhibitors,\(^\text{[6-13]}\) results in the elimination of the recurring reduction. Recurring reduction was also eliminated by PGI₁ and was induced by tranylcypromine, which inhibits PGI₁ synthetase \textit{in vitro}.\(^\text{[6]}\) Although direct evidence which supports inhibition of PGI₁ synthetase by tranylcypromine \textit{in vivo} is lacking, it is likely that PGI₁ acted as an inhibitor of the recurring reduction. The recurring decreases in the ratio, PGI₁/TXA₂, may have resulted in the recurring formation of platelet aggregates and thrombi and in recurring vasospasm which led to the recurring reduction of carotid and cerebral blood flow.

Acetylsalicylic acid and ketoprofen, which inhibit cyclo-oxygenase and, accordingly, the synthesis of PGI₁ and TXA₂,\(^\text{[11]}\) also eliminated the recurring reduction. Because of relative difficulty in affecting the PGI, generating system in the vascular wall, as was suggested by Livio et al.,\(^\text{[21]}\) these agents may have preferentially inhibited the TXA₂ synthesis in platelets, thus increasing the ratio of PGI₁ to TXA₂ and eliminating the flow reduction. The augmentatory action of these agents on the reappearing recurring reduction might be due to inhibition of PGI₁ which occurred later than their action on the TXA₂ synthesis. Although indomethacin is a cyclo-oxygenase inhibitor as is acetylsalicylic acid, it frequently induced recurring reduction. One possibility is that this agent acted not only on cyclo-oxygenase in platelets but also on cyclo-oxygenase in vascular endothelium, thus reducing PGI₁ much more than TXA₂ and decreasing the ratio of PGI₁ to TXA₂. This possibility is supported by the fact that the indomethacin-induced recurring reduction was little influenced by a large dose of TX synthetase inhibitors and was eliminated by PGI₁. Another possibility is that one or more of SRS-A were increased secondary to inhibition of cyclo-oxygenase by indomethacin,\(^\text{[18]}\) resulting in recurring flow reduction. Although leukotriene C-1, a SRS-A, induced recurring reduction in a few preparations, cromoglycate that inhibits release of SRS-A\(^\text{[17]}\) failed to eliminate the indomethacin-induced recurring reduction. It is too early to consider that indomethacin induced the recurring reduction through augmentation of SRS-A synthesis. PGI₁ has an action to suppress SRS-A synthesis,\(^\text{[18]}\) but whether PGI₁ eliminated the indomethacin-induced reduction through inhibition of SRS-A synthesis was not studied.

It has been suggested that vasospasm and embolism of the cerebral arteries with platelet aggregates or thrombi which flowed distally from the atherosclerotic carotid, vertebral or cerebral arteries act as causative factors in transient focal cerebral ischemic attacks.\(^\text{[25-28]}\) In this experimental model, vasospasm, platelet aggregates, thrombi and emboli were observed during the recurring reduction of carotid and cerebral blood flow.\(^\text{[3]}\) The duration of flow reduction in this model was of minutes as in the case of transient focal cerebral ischemic attacks.\(^\text{[3, 25]}\) The experimental and clinical phenomena closely resemble each other. It is likely that recurring changes in production of PGI₁ and TXA₂ cause recurrent vasospasm, platelet aggregation, thrombus formation and resultant embolism, leading to the recurring reduction of carotid
artery and cerebral blood flow which occurs during partial constriction of the canine common carotid artery.

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Role of prostaglandin I2 and thromboxane A2 in recurring reduction of carotid and cerebral blood flow in dogs.
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Stroke. 1981;12:786-792
doi: 10.1161/01.STR.12.6.786

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