The Effects of Calcium Antagonism On The Epicerebral Circulation In Early Vasospasm

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SUMMARY We have studied the effects of the calcium antagonist verapamil on the epicerebral arteriovenous transit time and regional epicerebral circulation of dogs by direct measurement of arterial diameters, fluorescein angiography, and krypton-85 regional epicerebral blood flow analysis. A large craniectomy was performed and vasoconstriction was induced by the subarachnoid injection of human platelet-rich plasma (PRP) pretreated with 25 μ M of ADP to cause maximum aggregation. Once vasoconstriction was established, verapamil (0.1 mg/kg) was topically applied to the perforated arachnoid. The PRP-ADP produced a mean decrease in the arterial diameters of 38.2 ± 1.6% (p < 0.01) at 10 minutes after its injection and verapamil produced a mean dilatation of 19.5 ± 2.5% (p < 0.01), compared to control values. Regional epicerebral blood flow was 54.9 ± 3.4 ml/100 g/min in the control state, 34.8 ± 3.2 ml/100 g/min (p < 0.01) during vasospasm, and 78.2 ± 4.5 ml/100 g/min (p < 0.01) after verapamil. Fluorescein angiography, after verapamil, demonstrated a mean acceleration of the arteriovenous circulation time of 4.5 ± 0.8 seconds (p < 0.01) compared to the spasm value. We concluded that the topical application of verapamil can dilate previously constricted cortical arteries and that this dilatation is associated with acceleration of the epicerebral transit time and increased cerebral blood flow.

CEREBRAL VASOSPASM is the major cause of death and disability in patients with aneurysmal subarachnoid hemorrhage who survive to reach hospital. Although the presence of blood in the subarachnoid space is necessary for its development, the etiology of this phenomenon is unknown. A number of blood products can produce experimental vasospasm, and among them is the platelet fraction which is a source of potent spasmosgens.1-3 Experimentally, vasospasm has been shown to be a biphasic phenomenon with acute and chronic phases.4 The acute phase occurs very rapidly after induced subarachnoid hemorrhage and is short-lasting; the chronic phase begins 4 to 24 hours later and reaches a maximum at 72 hours after hemorrhage.4 Although short-lasting the acute phase can nonetheless be associated with death or neurological deficit in the experimental animal.4 We have previously demonstrated that early experimental vasospasm involving the large arteries at the base of the brain can be reversed by calcium antagonism.5 The present report documents the effect of calcium antagonism on cortical arterial diameters, arteriovenous transit time and regional epicerebral blood flow in the acutely vasoconstricted epicerebral circulation.

Materials and Methods

Twelve mongrel dogs unselected for age or sex were obtained from the McIntyre Animal Center of McGill University. The animals were handled according to established guidelines for humane animal care. They were kept fasting for 24 hours and general anesthesia was induced and maintained by the intravenous injection of 50 mg of phenobarbital. Flaxadil, 10 mg intramuscularly, was given hourly. The animals were intubated and connected to a respirator and the respiratory volume and rate were adjusted to maintain the arterial blood gas values in the normal range. The femoral artery was cannulated for continuous recording of blood pressure, maintained in the normal range throughout the experiments, and for arterial blood gas sampling. The femoral vein was cannulated and served for the infusion of phenobarbital. The sublingual artery was cannulated and the cannula was advanced to the junction of the sublingual and common carotid arteries. This cannula served for the injection of 1.6 cc of 1% fluorescein and of 10 milliCuries of krypton-85 in 3 cc of normal saline. The animals were positioned with the right side up, the temporals muscle was removed and a large hemispheric craniectomy was performed. The dura was opened and reflected so as to tamponade any bleeding from the bone margins. Platelet-rich plasma (PRP) was obtained from healthy human volunteers and prepared as previously described except in one case where PRP was obtained from a rabbit.6 Dog blood was not used because of the difficulty inherent in the preparation of PRP in animals exposed to the distemper virus. Twenty-five μ M of ADP were mixed with 0.4 cc of PRP in a Born aggregometer at a rate of 1000 revolutions/minute for a period of 60 seconds. The PRP was rapidly injected into the subarachnoid space and its effect documented as outlined below. Usually two injections of PRP-ADP were used to assure adequate bathing of the subarachnoid vessels. Pial or subarachnoid bleeding which stopped spontaneously and promptly, was incurred with the injection of PRP-ADP in 6 animals. To 0.1 mg/Kg of verapamil was added enough Elliott's solu-
tion to make a volume of 0.4 cc which covered the whole of the perforated arachnoid at the appropriate time in the experiment.

The effects of treatment on cortical arterial diameters were documented by colour slide pictures using a photographic system previously described which produces high quality photographic slides of the vessels of the cortex. For each animal a number of cortical arteries in good focus were chosen for measurements of diameters in the normal state, after the subarachnoid injection of PRP-ADP, and after the application of verapamil. The slides were projected at constant magnification and the arteries measured with a millimeter rule. The arteries in the normal state are considered as having a value of 0% and constriction and dilatation are expressed as a negative or positive percentage value of the normal state. Photographs were taken immediately before the injection of PRP-ADP, and at 5 and 10 minutes after. At that point, in six experiments, verapamil was topically applied and photographs were taken at 10 minutes, 30 minutes, and 60 minutes after its application. In six other experiments the verapamil was not applied at 10 minutes but at 30 minutes after the injection of PRP-ADP. Photographs were then taken at similar intervals after its application as outlined above. In six experiments fluorescein angiography was performed in the normal state, 10 minutes after the injection of PRP-ADP, and at 10 minutes and at 60 minutes after verapamil. At that point another application of verapamil was performed and cortical photography and fluorescein angiography were repeated 10 minutes after the second application. The technique of fluorescein angiography has been described in detail elsewhere. In the other six experiments regional epicerebral blood flow studies following the injection of krypton-85 were performed using a system previously described, and a partition coefficient of 0.9 for krypton-85. Blood flow studies were performed in the normal state, 10 minutes after the subarachnoid injection of PRP-ADP, and at 10 and 60 minutes after the application of verapamil. In one blood flow and two transit time experiments Elliott’s solution was applied prior to the application of verapamil to serve as a control for its effects. We have previously demonstrated that platelet-free plasma, ADP alone and Elliott’s solution alone, when injected into the subarachnoid space, have no vasoconstrictive properties, and that Elliott’s solution applied to arteries in spasm, immediately before the application of verapamil, has no dilatory effect. Statistical analysis was performed using the student t test for the comparison of 2 means. Plus and minus values after the mean value represent the standard error of the mean.

Results

A. Arterial Diameter Changes

The effects of PRP-ADP and verapamil on arterial diameters are illustrated in figures 1 and 2 and tables 1 and 2. PRP-ADP produced a mean constriction of 35.1 ± 2.4% at 5 minutes after treatment, 38.2 ± 1.6% at 10 minutes after treatment, and 24.4 ± 3.0% at 30 minutes after treatment.

In the experiments where krypton 85 blood flow studies were performed (fig. 1, table 1), the topical application of verapamil 30 minutes after treatment with PRP-ADP, with the vessel still in spasm, produced a mean dilatation of 13.9 ± 3.1% at 5 minutes after verapamil, 11.9 ± 2.5% 10 minutes after verapamil, 8.5 ± 2.6% 30 minutes after verapamil, and 0.2 ± 3.0% 60 minutes after verapamil.

In the experiments where fluorescein angiography was performed (fig. 2, table 2), the topical application of verapamil 10 minutes after treatment with PRP-ADP, when the blood vessels were maximally constricted, produced a mean dilatation of 19.5 ± 2.5% 10 minutes after treatment. By the end of the 60 minute period of observation the vessels had returned to their resting state. A second application of verapamil at that time again produced prompt vasodilation of a similar magnitude as the first treatment.
TABLE 1  Changes in Cortical Arterial Diameters during Blood Flow Experiments*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time after treatment</th>
<th>Number of observations</th>
<th>% Change in arterial diameter (± SEM)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRP-ADP</td>
<td>5 min.</td>
<td>31</td>
<td>-35.1 ± 2.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>10 min.</td>
<td>77</td>
<td>-38.2 ± 1.6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>30 min.</td>
<td>31</td>
<td>-24.4 ± 3.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Verapamil</td>
<td>5 min.</td>
<td>33</td>
<td>+13.9 ± 3.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>10 min.</td>
<td>39</td>
<td>+11.9 ± 2.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>30 min.</td>
<td>26</td>
<td>+8.5 ± 2.6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>60 min.</td>
<td>26</td>
<td>+0.2 ± 3.0</td>
<td>NS</td>
</tr>
</tbody>
</table>

*PRP = platelet-rich plasma.

B. Epicerebral Blood Flow Analysis (table 3, fig. 3)

The mean value of the epicerebral blood flow in the normal state was 54.9 ± 3.4 ml/100 g/min. This decreased to 34.8 ± 3.2 ml/100 g/min 10 minutes after treatment with PRP-ADP and increased to 78.2 ± 4.5 ml/100 g/min 10 minutes after treatment with verapamil. At 60 minutes after treatment with verapamil the epicerebral blood flow had returned to normal.

C. Arteriovenous Transit Time (table 4, fig. 4)

The mean arteriovenous transit time in the resting state was 5.9 ± 1.4 seconds. This increased to a value of 6.8 ± 1.1 seconds 10 minutes after treatment with PRP-ADP and decreased to a value of 2.3 ± 0.3 seconds at 10 minutes after treatment with verapamil. Sixty minutes after treatment with verapamil, when the blood vessel diameters had returned to their resting state, the arteriovenous transit time had also returned to normal. A second application of verapamil at that time again produced a decrease in the arteriovenous transit time to a value of 1.9 ± 0.4 seconds.

D. Summary and Correlation of Results

Treatment with PRP-ADP produced vasoconstriction that attained a maximum 10 minutes after its injection and persisted for the 30 minute period of observation. This was associated with decreased epicerebral blood flow. Reversal of this vasoconstriction with verapamil, either when it was at its maximum, or when it had been established for a longer period of time, produced prompt and dramatic vasodilatation associated with diminished arteriovenous transit time and increased epicerebral blood flow. This persisted in a decremental fashion and by 60 minutes after treatment the blood vessel diameters, arteriovenous circulation time and epicerebral blood flow returned to normal values. Repeated treatment with verapamil again produced vasodilatation, decreased arteriovenous transit time and increased epicerebral blood flow of the same magnitude as the first treatment with verapamil. Elliot's 'A' solution produced no significant change in the blood vessel diameters, arteriovenous circulation time or epicerebral blood flow values when it was applied immediately before treatment with verapamil.

The degree of vasodilatation, the speed of the arteriovenous transit time and the initial slope of the

TABLE 2  Changes in Cortical Arterial Diameters during Transit Time Experiments*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time after treatment</th>
<th>Number of observations</th>
<th>% Change in diameters (± SEM)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRP-ADP</td>
<td>5 min.</td>
<td>31</td>
<td>-35.1 ± 2.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>10 min.</td>
<td>77</td>
<td>-38.2 ± 1.6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Verapamil</td>
<td>10 min.</td>
<td>44</td>
<td>+19.5 ± 2.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>(1st application)</td>
<td>60 min.</td>
<td>41</td>
<td>+1.7 ± 2.9</td>
<td>NS</td>
</tr>
<tr>
<td>Verapamil</td>
<td>10 min.</td>
<td>45</td>
<td>+18.7 ± 3.2</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*PRP = platelet-rich plasma.

FIGURE 3. Effects of PRP-ADP and of verapamil on cerebral blood flow.

FIGURE 4. Cortical photographs (left column) and corresponding fluorescein angiograms (right column) demonstrating the normal state (top), vasoconstriction and delayed arterial filling after PRP-ADP (middle) and vasodilatation and acceleration of transit time after verapamil (bottom). The numbers at the bottom right of each fluorescein angiogram indicate the time, in seconds, after the injection of fluorescein.
blood flow curves early after treatment with verapamil are suggestive of hyperemia and of a shunting phenomenon.

Discussion

The degree of neurological deficit produced by cerebral vasospasm depends on the extent and severity of the vasospasm which is in turn dependent on the amount of blood in the subarachnoid space.10,11 It is appropriate, therefore, to consider that the extravasated blood in the subarachnoid space contributes to the development of cerebral vasospasm. Platelets contain or can synthesize upon activation a number of vasoactive compounds such as thromboxanes, leukotrienes, prostaglandins, and serotonin. We have, therefore, used platelet-rich plasma stimulated to aggregate, release, or secrete to produce acute vasostriction of the epicerebral circulation.

Cerebral arteries require the entry of extracellular calcium into the smooth muscle cell to initiate and maintain vasoconstriction.12,13 Blood in the subarachnoid space may result in the release of a number of compounds capable of initiating this process.14 We have previously demonstrated that stimulation of the adenyl cyclase-cyclic-AMP system, which promotes the sequestration of calcium by the sarcoplasmic reticulum, reverses the acute cerebral vasoconstriction induced by blood or by prostaglandin F2α.15 The present experiments demonstrate that interference with the entry of calcium into the cell by calcium channel blockade results in vasodilatation of previously constricted arteries. This dilatation is prompt, dramatic, decremental with time and reproducible with repeated treatment. It is associated with acceleration of the arteriovenous transit time and with increased regional epicerebral blood flow.

These experiments were designed to investigate the hypothesis that acute vasoconstriction of the epicerebral arteries is a calcium-mediated phenomenon that can be reversed by calcium channel blockade. They do not address the chronic vasospasm that follows subarachnoid hemorrhage although this has also been shown to respond favorably to calcium antagonism.17,18

Acknowledgments

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References


Table 3 Changes in Cerebral Blood Flow*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of observations</th>
<th>CBF (± SEM) (ml/100g/min)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13</td>
<td>54.9 ± 3.4</td>
<td></td>
</tr>
<tr>
<td>PRP-ADP</td>
<td>13</td>
<td>34.8 ± 3.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Verapamil</td>
<td>10 minutes</td>
<td>78.2 ± 4.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>60 minutes</td>
<td>41.4 ± 3.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

*PRP = platelet-rich plasma.

Table 4 Changes in Arteriovenous Transit Time*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of observations</th>
<th>A-V. time (± SEM) (seconds)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>5.9 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>PRP-ADP</td>
<td>6</td>
<td>6.8 ± 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>Verapamil: 1st application</td>
<td>6</td>
<td>2.3 ± 0.3</td>
<td>&lt;0.01†</td>
</tr>
<tr>
<td>at 10 minutes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at 60 minutes</td>
<td></td>
<td>6.1 ± 0.1</td>
<td>NS</td>
</tr>
<tr>
<td>Verapamil: 2nd application</td>
<td>5</td>
<td>1.9 ± 0.4</td>
<td>&lt;0.01†</td>
</tr>
<tr>
<td>at 10 minutes</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*A-V. = arteriovenous; PRP = platelet rich plasma.
†Compared to PRP-ADP.
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