Determinants of Response of Pial Arteries to Norepinephrine and Sympathetic Nerve Stimulation

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Abstract: Determinants of Response of Pial Arteries to Norepinephrine and Sympathetic Nerve Stimulation

Feline pial arteries larger than 100 μ in diameter constricted in response to cervical sympathetic nerve stimulation or in response to topical application of norepinephrine. Smaller pial arteries were unresponsive to norepinephrine. This unresponsiveness persisted when norepinephrine was dissolved in CSF with high calcium ion concentration, or in CSF with both high calcium ion and zero magnesium ion concentration, or when it was dissolved in the acid fluid used by Wahl et al. and applied by constant infusion or by intermittent application. Comparison of the responses of the larger pial vessels to norepinephrine and to sympathetic nerve stimulation suggests that maximal activation of sympathetic nerves achieves a concentration of released norepinephrine equal to \( 5.9 \times 10^{-8} \) M. The constriction of the larger pial vessels in response to sympathetic nerve stimulation could account for modest reductions in cerebral blood flow.

Additional Key Words: cat vasoconstriction catecholamines neurogenic control of cerebral blood flow

Despite extensive study there is still considerable disagreement concerning the physiological importance of neurogenic control of cerebral blood vessels. Studies utilizing pressure and flow measurements have yielded contradictory results. Much of the disagreement is related to problems with the techniques of measuring blood flow. A different approach, not subject to these objections, is the direct observation of the blood vessels on the surface of the brain. In an earlier study we were unable to establish a physiological basis for neurogenic control of the cerebral circulation via changes in the activity of adrenergic fibers innervating the smaller cerebral arterioles. In that study we found that, although pial arteries smaller than 100 μ in diameter were richly innervated by adrenergic nerve fibers, they were unresponsive to high concentrations of topically applied norepinephrine and isoproterenol and were equally unresponsive to maximal electrical stimulation of the cerebral sympathetic nerves. These results were in apparent conflict with the findings of other investigators who had shown significant vasoconstriction in response to either epinephrine or norepinephrine, applied in much the same fashion as was done in our experiments, and vasoconstriction in response to sympathetic nerve stimulation.

In our earlier study we suggested the possibility that neurogenic control of the cerebral circulation could still be exercised via action of adrenergic nerves on the larger surface arteries of the brain. This possibility was explored in the experiments reported here. In view of the fact that these experiments showed that the larger pial arteries are responsive to both norepinephrine and to sympathetic nerve stimulation, it appeared profitable to explore the influence of certain factors on the responsiveness of smaller pial arteries to norepinephrine. It was hoped that this effort would lead to clarification of the reasons underlying the conflicting results, and therefore might reconcile some of the differences of opinion in this controversial area.

Methods

Experiments were carried out in 47 cats. Forty-one animals were anesthetized with intravenous sodium pentobarbital (30 mg per kilogram) and six animals with intravenous urethane (600 mg per kilogram). After completion of tracheostomy, each animal was ventilated with a positive pressure respirator and received 0.4 mg per kilogram of decamethonium bromide intravenously for skeletal muscle paralysis. The end-expiratory CO₂ of the animal was continuously monitored with a Beckman infrared CO₂ analyzer and maintained at a constant level, between 30 and 40 mm Hg throughout each experiment, by adjusting the respirator. Mean arterial blood gases and pH for all the animals studied were as follows: \( Pao, 116 \pm 6.7 \) mm Hg, \( Paco, 37.4 \pm 0.83 \)
mm Hg, and pH 7.37 ± 0.16. Arterial blood pressure was measured with a Statham P23Db pressure transducer connected to a cannula introduced into the aorta through the femoral artery. Arterial blood samples were periodically collected for the determination of Po2, Pco2, pH, and hematocrit. Blood gases and pH were determined with Radiometer electrodes. Hematocrit was measured with a micromethod.

Pial precapillary vessels were visualized through a cranial window technique described in detail previously. The window was equipped with three outlets. One of these was connected to a Statham-gauge for continuous measurement of intracranial pressure. The other two openings served as the inlet and outlet for perfusion of the space under the window with artificial cerebrospinal fluid (CSF). The outflow from the window was connected to plastic tubing whose distal end was set to a fixed height to maintain the intracranial pressure at 7 mm Hg throughout the experiment. In addition, this positive pressure prevented the brain surface from making contact with the glass plate of the cranial window and assured that the perfusing solution covered the area of the vessels under observation. The cranial window was implanted on the vertex of the skull just caudal to the suture connecting the frontal and parietal bones in experiments in which each solution was applied for four minutes and the order in which each solution was applied was chosen at random. It is important to note that calcium ion concentration in the fluid in which norepinephrine was dissolved had a determinative influence on the responsiveness of pial vessels to this agent. It is known that the responsiveness of vascular smooth muscle to norepinephrine is influenced markedly by the prevailing calcium ion concentration. Since the concentration of calcium in the solution used by Wahl et al.7 was higher than what we used, this might account for the differences in results. Solutions containing 0, 10 and 100 μg per milliliter of norepinephrine were prepared in normal CSF and in fluid with the same composition as normal CSF but with the concentration of calcium increased to 10 mEq per liter. Wahl et al. used a calcium ion concentration equal to 5 mEq per liter. In preliminary experiments increase in calcium ion concentration from 2.5 to 5 mEq per liter did not alter responsiveness to norepinephrine. For this reason the final experiments were carried out using a calcium ion concentration of 10 mEq per liter. Each solution was applied for a period of four minutes. The application of the solutions was made in random order.

In a second series of six animals we examined the effect of norepinephrine dissolved in normal artificial CSF on pial arteries ranging in diameter from 167 to 252 μ. Norepinephrine dissolved in normal artificial CSF in concentrations of 0, 1, 10 and 20 μg per milliliter was used.

The next four groups of experiments were used to explore whether or not certain differences between our methods and those of Wahl and his colleagues had a determining influence on the responsiveness of smaller pial arteries to topically applied norepinephrine.

(1) In 12 animals we explored the effect of the calcium ion concentration in the fluid in which norepinephrine was dissolved on the responsiveness of pial vessels to this agent. The first series of experiments was carried out in nine animals in which, prior to the implantation of the cranial window, the cervical sympathetic nerve chain was dissected free and a bipolar electrode was attached to the superior cervical ganglion. Electrical stimulation was carried out with a Phipps & Bird stimulator by delivering square-wave stimuli 1 msec in duration at 5 to 10 v at frequencies ranging from 1 to 15 cps. In each cat the effectiveness of stimulation was judged by sustained dilation of the ipsilateral pupil. Stimulation was continued for a period of three minutes. Only experiments in which no major changes in blood pressure took place were used. The effect of sympathetic nerve stimulation on pial arteries 128 to 240 μ in diameter was determined.

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The next four groups of experiments were used to explore whether or not certain differences between our methods and those of Wahl and his colleagues had a determining influence on the responsiveness of smaller pial arteries to topically applied norepinephrine.

(2) In eight animals norepinephrine in concentrations of 0, 10 and 100 μg per milliliter was prepared in CSF identical with normal CSF in composition except that the calcium ion concentration was increased to 10 mEq per liter and the concentration of magnesium ion was reduced to zero. Again, each solution was applied for four minutes and the order in which each solution was applied was chosen at random. It is

<table>
<thead>
<tr>
<th>TABLE 1 Composition of Solutions Used to Dissolve Norepinephrine</th>
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<tbody>
<tr>
<td>Normal artificial CSF</td>
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<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>Na+ (mEq/liter)</td>
</tr>
<tr>
<td>K+ (mEq/liter)</td>
</tr>
<tr>
<td>Ca2+ (mEq/liter)</td>
</tr>
<tr>
<td>Mg2+ (mEq/liter)</td>
</tr>
<tr>
<td>HCO3- (mEq/liter)</td>
</tr>
<tr>
<td>Cl- (mEq/liter)</td>
</tr>
<tr>
<td>Glucose (mM)</td>
</tr>
<tr>
<td>Urea (mM)</td>
</tr>
<tr>
<td>pH</td>
</tr>
<tr>
<td>Osmolality (mOsm/kg)</td>
</tr>
<tr>
<td>P50 (mm Hg)</td>
</tr>
</tbody>
</table>
known that decrease in magnesium ion concentration enhances the effect of calcium ions on responsiveness to norepinephrine. Wahl et al. used fluid not containing magnesium.

3 In six animals the effect of norepinephrine dissolved in solution identical in composition to that used by Wahl and his associates was tested. The concentrations of 0, 10 and 100 μg per milliliter of norepinephrine were prepared as described by Wahl et al. Each solution was applied for four minutes at the rate of 3.8 ml per minute. The effects of these solutions were compared to the effect of normal artificial CSF passed under the window at the same rate.

4 In six animals the effect of the method of application of norepinephrine solution was explored. Wahl and his colleagues applied norepinephrine transiently while we used continuous application for longer periods of time. Since they found that repeated application of norepinephrine resulted in vessel unresponsiveness, it was important to determine whether or not in our experiments the continued application led to such unresponsiveness. For this purpose solutions of 10 μg per milliliter of norepinephrine were prepared in fluid with a composition identical to that used by Wahl and his colleagues. The space under the cranial window was perfused with a solution identical to that used by Wahl at a rate of 3.8 ml per minute and intermittently a small volume of solution of the same composition with or without norepinephrine was applied through a side arm as bolus. This method was designed to result in application of the norepinephrine solution for only 15 seconds. During this period and for two minutes thereafter vessel diameter was measured continuously. The administration of solution with and without norepinephrine was carried out in random order. To simulate more closely the experimental conditions used by Wahl et al., the animals were anesthetized with urethane.

**Results**

Table 2 shows that sympathetic nerve stimulation produced significant vasoconstriction of pial arteries larger than 100 μm in diameter. This vasoconstriction was sustained as long as the stimulation was continued and was reproducible on repeated stimulation. Its magnitude increased with the frequency of stimulation, but even at 15 cps it was modest, amounting to only 7% of the control diameter. In six cats we were able to measure the diameter of a smaller artery branching off from the larger vessel. As seen in table 2, there was no significant change in the caliber of these smaller vessels at stimulation rates of 15 cps. The effect of topical norepinephrine on large pial arteries when the norepinephrine concentration (μg/ml) was 0, 1, 10, and 20 was to produce a vessel diameter, respectively, of 217.7 ± 11.9, 201.7 ± 15.4, 182.5 ± 16.3, and 179.3 ± 13.4 μm. This vasoconstriction was dose dependent. At the highest concentration of norepinephrine used it amounted to 18% of the control diameter. As was the case with nerve stimulation, the norepinephrine-induced vasoconstriction was reproducible on repeated application and was sustained for as long as norepinephrine was applied.

Norepinephrine up to a concentration of 100 μg per milliliter had no significant effect on the caliber of pial arteries smaller than 100 μm in diameter when dissolved in normal artificial CSF, when the calcium ion concentration was raised to 10 mEq per liter (table 3), when the calcium ion concentration was raised to 10 mEq per liter and the magnesium ion concentration was reduced to zero (table 4), or when it was dissolved in the solution used by Wahl et al. at pH 7.15 and applied either by sustained perfusion of the space under the cranial window (table 5) or transiently (the effect of transient application of norepinephrine dissolved in the solution of Wahl et al. on small arteries was to produce a vessel diameter in the control of 50.5 ± 3.9, with norepinephrine 50.5 ± 3.3, and a dummy 49.0 ± 4.9 μm).

**Discussion**

The main finding of the experiments reported above is that pial arteries larger than 100 μm in diameter contract in response to norepinephrine or in response to cerebral sympathetic nerve stimulation. This behavior contrasts with the unresponsiveness of pial arteries smaller than 100 μm to either of these stimuli. This unresponsiveness of the smaller pial arteries could not be altered by means which enhance the responsiveness of vascular smooth muscle to norepinephrine, such as increase in calcium ion concentration, or simultaneous increase in calcium ion concentration and decrease in magnesium ion concentration. These smaller vessels remained unresponsive when norepinephrine was dissolved in acid solution and when it was applied transiently. The results with respect to the smaller pial arteries confirm our earlier findings. Our inability to alter the unresponsiveness of the smaller pial arteries to norepinephrine and to sympathetic nerve stimulation reinforces our earlier belief that these smaller vessels lack a sufficient number of alpha-adrenergic receptors and that this is the reason for their unresponsiveness.

Our results with norepinephrine are consistent

**Table 2**

<table>
<thead>
<tr>
<th>Diameter</th>
<th>n</th>
<th>Frequency of stimulation (cps/sec)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Large arteries</td>
<td>9</td>
<td>197.2 ± 10.7</td>
<td>195.1 ± 10.6</td>
</tr>
<tr>
<td>Small arteries</td>
<td>6</td>
<td>45.7 ± 2.6</td>
<td>54.3 ± 2.7</td>
</tr>
</tbody>
</table>

All values are mean ± SE.

n = number of animals.
DETERMINANTS OF RESPONSE OF PIAL ARTERIES

TABLE 3
Effect of Topical Norepinephrine Dissolved in Normal CSF and in CSF Containing 10 mEq/Liter of Ca\(^{2+}\) on Small Pial Arteries

<table>
<thead>
<tr>
<th>Norepinephrine concentration ((\mu)g/ml)</th>
<th>0</th>
<th>10</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vessel diameter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A: normal CSF</td>
<td>44.4</td>
<td>1.8</td>
<td>43.3</td>
</tr>
<tr>
<td>B: CSF with Ca(^{2+}) at 10 mEq/liter</td>
<td>43.3</td>
<td>2.1</td>
<td>43.2</td>
</tr>
</tbody>
</table>

A: normal CSF.
B: CSF with Ca\(^{2+}\) at 10 mEq/liter.
Values are mean ± SE from 12 animals.

with the findings of Fog\(^6\) and of Forbes et al.\(^8\) who used topical epinephrine in the cat. Fog\(^6\) found that topical epinephrine produced vasoconstriction of pial arteries larger than 100 \(\mu\) in diameter, but had no effect on arteries smaller than 50 \(\mu\) in diameter. Similar, but not as uniform, results were obtained by Forbes and his colleagues.\(^5\) Kapp and his colleagues\(^15\) found small vasoconstrictor responses from direct application of norepinephrine to the basilar artery. Our findings also are consistent with the results of three in vitro studies. Uchida and his colleagues\(^16\) found that cerebral arteries 50 to 250 \(\mu\) in diameter displayed weak vasoconstrictor responses to large concentrations of norepinephrine. Similarly, Nielsen and Owman\(^17\) and Toda and Fujita\(^18\) found that strips from large cerebral arteries showed relatively weak contractile responses to norepinephrine.

Our findings differ from the findings of two other investigations. Rosenblum\(^19\) found vasoconstriction of small pial arteries in response to topical application of norepinephrine in mice. Because of the markedly different size of mice and cats, it is likely that the results are due to the species differences and are not inherently contradictory. Despite considerable effort, we were unable to reconcile our findings with those of Wahl et al.\(^7\) These investigators found dose-related vasoconstriction of small and large pial arteries in the cat in response to topical application of norepinephrine. This vasoconstriction had certain characteristics not found in other vascular beds. It was present only at pH 7.15, being eliminated at either considerably lower pH or slightly higher pH. Furthermore, it displayed tachyphylaxis, disappearing on repeated application. These authors ascribed the differences between their results and ours to the lower pH of their solution. They also assumed that the prevailing pH in the vicinity of the small pial vessels was 7.15, because application of solution with this pH not containing norepinephrine produced no significant change in vessel caliber. They concluded that their results were more appropriate for physiological conditions. We do not agree with these conclusions. First, we showed that the lack of responsiveness to norepinephrine on the part of the smaller arteries in our experiments was due neither to the higher pH nor to other obvious differences in the experimental conditions between their experiments and ours. Also, vasoconstriction in response to norepinephrine was observed by us in larger pial arteries at pH 7.35 where they did not obtain vasoconstriction. Similarly, Rosenblum\(^19\) found vasoconstriction in mice at pH 7.35. It also must be noted that in addition to the differences in pH, calcium and magnesium ion concentrations, the solution used by Wahl et al.\(^7\) differed from normal CSF in other significant respects. It had a lower osmolality, a lower Po\(_2\), and a higher potassium concentration. In other experiments Wahl and his colleagues\(^20\) found that a decrease in osmolality from 315 to 280 mOsm per kilogram would produce a 20% reduction in pial arterial diameter. Similarly, the same investigators found that increase in potassium ion concentration from 3 to 5 mEq per liter would produce a 20% increase in pial arterial diameter.\(^21\) We found that reduction in CSF Po\(_2\) from 45 to 0 mm Hg produced a 15% increase in pial arterial diameter. One might reasonably expect, therefore, that the application of such a solution would subject the vessels to multiple, strong, opposing and abnormal vasoactive stimuli. Surprisingly, none of these differences accounted for the differences between our results and theirs. It should be noted that the application of the solution of Wahl et al. in our preparation does not produce any change in arterial diameter when compared to normal artificial CSF with a pH of 7.35. Therefore, the fact that their solution does not change vessel caliber in their experiments need not imply necessarily that the prevailing pH at the vessel wall is 7.15.

Our finding of significant but modest vasoconstriction of large pial arteries in response to sympathetic nerve stimulation confirms the findings of Forbes and Wolff\(^8\) who showed that sympathetic nerve

TABLE 4
Effect of Topical Norepinephrine Dissolved in CSF With Ca\(^{2+}\) at 10 mEq/Liter and Mg\(^{2+}\) at 0 mEq/Liter

<table>
<thead>
<tr>
<th>Norepinephrine concentration ((\mu)g/ml)</th>
<th>0</th>
<th>10</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vessel diameter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>49.8 ± 2.3 47.5 ± 3.1 47.0 ± 4.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SE from eight animals.
Vessel diameter when normal CSF was applied was 51.9 ± 2.1 \(\mu\).
stimulation in the cat produced 3% to 18% reduction in diameter of pial arteries 110 to 340 \( \mu \text{m} \) in diameter. Also, Kobayashi and his colleagues\(^2\) found significant vasoconstriction of pial arteries 50 to 250 \( \mu \text{m} \) in diameter in response to sympathetic nerve stimulation. They reported their results only qualitatively.

Comparison of the results of electrical stimulation of ipsilateral superior cervical ganglion on large and small pial arteries and of the effect of topical norepinephrine on large pial arteries shows that the magnitude of the vasoconstriction induced by sympathetic nerve stimulation at the highest stimulation rate was comparable to that produced by the application of 1 \( \mu \text{g} \) per milliliter of norepinephrine. This suggests that the intrasynaptic concentration of norepinephrine achieved by maximal nerve stimulation was 1 \( \mu \text{g} \) per milliliter (5.9 \( \pm \) 10\(^{-6} \text{M} \)) or less, depending on the magnitude of possible concentration gradients between the surface of the brain and the vessel media. Bevan and Su\(^2\) found that the intrasynaptic concentration of norepinephrine achieved by nerve stimulation in various vessels is dependent on the width of the synaptic cleft. According to these investigators the intrasynaptic concentration of norepinephrine for the portal vein having a synaptic cleft width of 1,000 \( \text{Å} \) is 1.4 \( \times \) 10\(^{-6} \text{M} \) and for muscular uterine arteries with a synaptic cleft width of 2,000 \( \text{Å} \) equal to 8.4 \( \times \) 10\(^{-6} \text{M} \). If the relationship between synaptic cleft width and intrasynaptic norepinephrine concentration found by Bevan and Su\(^2\) applies to the cerebral arteries, one would expect the concentration of norepinephrine to be in the range of 10\(^{-8} \) to 10\(^{-6} \text{M} \) since the width of the synaptic clefts in feline cerebral arteries ranges from 780 to 3,000 \( \text{Å} \).\(^2\) The concentration of 5.9 \( \times \) 10\(^{-6} \text{M} \) we estimated compares favorably with this expectation.

Under physiological conditions it is not likely that the constriction of cerebral arteries in response to sympathetic nerve stimulation would on the average exceed the 7% reduction in caliber we found in our experiments. However, the fact that norepinephrine in high concentration is capable of producing substantially greater constriction of these vessels than the 7% reduction in diameter produced by sympathetic nerve stimulation suggests that the potential exists for norepinephrine-induced reductions in CBF of considerable magnitude under abnormal conditions, such as in response to brain injury. This must at present remain only a theoretical possibility, since the concentrations of norepinephrine in CSF found in patients with head trauma are far below those required to produce significant vasoconstriction.\(^2\)

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658

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