Unresponsiveness of Pial Precapillary Vessels to Catecholamines and Sympathetic Nerve Stimulation

By A. Jarrell Raper, Hermes A. Kontos, Enoch P. Wei, and John L. Patterson, Jr.

ABSTRACT

A systematic analysis of the possible existence of neurogenic control of precapillary pial vessels was made in three species (cat, dog, and rabbit). In all of these animals, pial vessels failed to respond to externally applied isoproterenol or norepinephrine in high concentrations (up to 100 μg/ml), although the vessels did dilate in response to externally applied histamine. Adrenergic nerve endings on the pial vessels were demonstrated by fluorescent histochemical techniques specific for catecholamines. However, in the absence of changes in arterial blood pressure and arterial blood carbon dioxide tension, pial precapillary vessels showed no change in caliber in response to stimulation of the ipsilateral superior cervical ganglion. These results show that pial precapillary vessels are not subject to vasoconstriction probably because they lack sufficient receptors for the catecholamine neurotransmitter.

KEY WORDS neurogenic control cerebral circulation cerebral sympathetic nerves catecholamine fluorescence isoproterenol norepinephrine rabbit cat dog pial arteries and arterioles

Adrenergic nerves are present on parts of the cerebral arterial tree, but their function is not known (1). According to the classical view (2), neurogenic control of cerebral circulation is absent or weak and physiologically unimportant. This view still predominates (3, 4) even though numerous publications (5-16) have suggested that electrical stimulation of vasomotor nerves causes cerebral vasoconstriction. Rosenblum (17) has reviewed the more recent evidence pertaining to neural regulation of the caliber of cerebral blood vessels. Some investigators (3, 11) who have presented evidence in favor of neurogenic control are reluctant to accept the physiological importance of neurogenic cerebral vasomotor control. We believe that the failure of the concept of neurogenic control of the cerebral vascular bed to gain general acceptance is related to two factors. (1) Neurogenic cerebral vasoconstriction is teleologically unappealing. If a major function of neurogenic vasoconstriction in other vascular beds is the maintenance of arterial blood pressure at normal levels to sustain perfusion of the vital organs, participation of the cerebral vascular bed in such a generalized neurogenic vasoconstriction would compromise the advantage gained from the mechanism. (2) Many of the studies which support the presence of significant neurogenic control of the cerebral vascular bed suffer from one or more deficiencies: the reproducibility and accuracy of the methods of measuring flow were not demonstrated under the conditions of the experiment, extracerebral blood flow was not excluded with certainty, the experiments were
largely uncontrolled and thus it was not clear whether the observed vasoconstriction was directly related to nerve stimulation, indirect effects such as changes in arterial blood CO2 tension or blood pressure were not eliminated or otherwise taken into account. These deficiencies are quite significant because in most studies the decreases in flow and the increases in vascular resistance resulting from nerve stimulation were small in magnitude.

The present study is a systematic examination of the basis for neurogenic control of vasoconstriction of the pial vessels of the cat. If effective neurogenic control of these vessels does exist (1) there must be adrenergic innervation of these vessels, (2) the vessels must be able to respond to the sympathetic neurotransmitter, norepinephrine, in concentrations comparable to those achieved by transmitter release from nerve endings, and (3) there must be changes in vascular caliber as a result of stimulation of the nerves supplying the vessels.

Methods

Experiments were carried out in 40 cats, 3 dogs, and 3 rabbits anesthetized with sodium pentobarbital (30 mg/kg, iv). In 43 animals skeletal muscle paralysis was induced with decamethonium (0.4 mg/kg, iv). Three cats, in which the superior cervical ganglia were stimulated, were allowed to breathe spontaneously. The remaining animals were ventilated with a positive-pressure respirator at rates and tidal volumes adjusted to give an end-tidal Pco2 within the normal range. The CO2 tension in expired air was continuously monitored with a Statham P23Db strain-gauge transducer connected to a catheter introduced into the aorta via the femoral artery. The pial precapillary vessels in the parietal cortex were visualized by the formaldehyde-condensation method of Falck et al. (20). Portions of the superficial part of the parietal cortex were removed and frozen with isopentane cooled by dry ice to −80°C. The specimens were lyophilized in a vacuum at −73°C for 1–2 days and then placed in a 500-ml desiccator containing 5 g of paraformaldehyde (trioxymethylene) kept at a relative humidity of 70%. The desiccator was placed in an oven at 80°C for 18 hours. Paraffin sections 15μ thick were cut and examined with a fluorescence microscope.

In the second series of experiments, the responses of pial precapillary vessels to norepinephrine and isoproterenol were examined. Solutions of norepinephrine bitartrate and isoproterenol hydrochloride were prepared in artificial cerebrospinal fluid (CSF) containing Na+ 150 mEq/liter, K+ 3.0 mEq/liter, Ca2+ 2.5 mEq/liter, Mg2+ 1.2 mEq/liter, Cl− 132 mEq/liter, glucose 3.7 mM, urea 6.0 mM, and HCO3− 25 mEq/liter (21). The solutions were buffered with isosmotic sodium bicarbonate solution so that, when equilibrated with a mixture of 6.5% O2−6% CO2 at 37°C, they had a pH within the physiological limits (7.30–7.40). The solutions were maintained at 37°C and infused through the space under the cranial window with a constant-infusion pump at the rate of 3.8 ml/min. Concentrations of amines were expressed as micrograms per milliliter of base. The bicarbonate content, calculated from pH and Pco2, ranged between 21 and 27 mEq/liter (mean 25 mEq/liter). Volume of the space under the window was very small; it could be completely filled with India ink within 3 seconds at an infusion rate of 3.8 ml/min and also cleared within an equal period of time. In six of the experiments, a modification of the cranial window was used, a third outlet being present for measurement of intracranial pressure. During the infusion of the solution the intracranial pressure did not increase above atmospheric levels by more than 0.5μ for vessels of 20–85μ in diameter.

For two series of experiments (highest local catecholamine concentration and graded sympathetic nerve stimulation), arterial diameter was measured with a Vickers image-splitting device, television camera, and monitor, as described by Baez (19). We have confirmed this system’s linearity and stability. The standard error of repeated measurements of the same vessels under steady-state conditions was consistently less than 0.5μ for vessels of 20–85μ in diameter.

Three types of experiments were carried out. In the first series of experiments, the adrenergic innervation of pial precapillary vessels was examined in five cats by the formaldehyde-condensation method of Falck et al. (20). Portions of the superficial part of the parietal cortex were removed and frozen with isopentane cooled by dry ice to −80°C. The specimens were lyophilized in a vacuum at −73°C for 1–2 days and then placed in a 500-ml desiccator containing 5 g of paraformaldehyde (trioxymethylene) kept at a relative humidity of 70%. The desiccator was placed in an oven at 80°C for 18 hours. Paraffin sections 15μ thick were cut and examined with a fluorescence microscope.

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than 1–2 mm Hg. Each concentration of catecholamines was infused for 4 minutes, and diameter measurements were made during the last 2 minutes of the infusion. The response to norepinephrine (up to 20 μg/ml) was examined in five cats. In each cat, at the end of the experiment India ink was infused under the window to demonstrate that the solutions did indeed reach the vessels which were photographed. As a result of the convexity of the parietal cortex, the brain sometimes touched the glass of the window, thus preventing the solutions from reaching this part of the brain. This was not unusual with the larger window described by Forbes and Wolff (5), but it was a rare occurrence with the smaller modification of the window we used. Such experiments were excluded from further consideration and were not included in the totals. The artificial CSF not containing catecholamines had no effect on pial arterial diameter when it flowed under the window at 3.8 ml/min. In another three cats the effects of the highest concentrations (20 μg/ml) of norepinephrine hydrochloride and norepinephrine bitartrate were studied.

When high concentrations of norepinephrine were applied to the surface of the brain, an increase in arterial blood pressure was observed. We thought that absorption of norepinephrine into the blood stream was a likely explanation for this rise in blood pressure, and we attempted to verify the mechanism and the site of the presumed absorption in preliminary experiments. We found that the increase in blood pressure was accelerated either by an increase in the concentration of norepinephrine applied locally or by dissolving the applied norepinephrine in acidic CSF. In contrast, the increase in blood pressure was delayed or entirely eliminated by decreasing the concentration of norepinephrine or by dissolving the drug in alkaline CSF. The same results were obtained in experiments in which the application of norepinephrine was made without the use of a cranial window and with the space around the opening in the skull sealed off with bone wax so that no loss of fluid from the site of application occurred. We concluded that norepinephrine absorption took place from the surface of the cortex where the norepinephrine was applied. We accordingly modified the method so that we removed only a small portion of the exposed dura thus limiting the area of the brain exposed to the drug. In this manner the application of norepinephrine at a concentration of 100 μg/ml could be carried out long enough to permit observations without the occurrence of a rise in arterial blood pressure. This procedure was carried out in five experiments.

In the third series of experiments (13 cats), prior to the installation of the cranial window the ipsilateral 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 and Bird stimulator by delivering square-wave stimuli 1 msec in duration at 50 cps and 5–10 v in 8 cats and at 5, 10, 20, and 50 cps in 5 cats. In each cat the effectiveness of stimulation was judged by sustained dilation of the ipsilateral pupil. For the single-frequency experiments, diameter measurements were made during the control period prior to stimulation, during 3 minutes of stimulation, and during a second control period after the stimulation was discontinued. In the experiments with graded stimulation, measurements were made prior to stimulation and continuously during each stimulation period. In addition, 3 more cats prepared for sympathetic nerve stimulation were allowed to breathe spontaneously to ascertain the respiratory effects of such stimulation.

Results

Catecholamine-specific fluorescence was present in the walls of virtually all pial arteries examined. We estimated that these adrenergic nerve fibers were more numerous than in skeletal muscle and equally as numerous as in mesenteric vessels. These results are in general agreement with studies previously reported for pial arteries by others (1, 22, 23).

Figure 1 shows the results of a typical experiment in which artificial CSF containing norepinephrine was injected beneath the window; diameters of a larger and a smaller arterial vessel are shown. There was no response to local exposure of the vessels to norepinephrine even in very large concentrations (20 μg/ml).

Figure 2 summarizes the results of application of norepinephrine and isoproterenol to the brain surface. Control (normal CSF) diameters are compared with those seen during exposure to 1, 10, and 20 μg/ml of the test drugs. With the highest dose of norepinephrine significant hypertension occurred in three animals, presumably because of the absorption of the amine into the blood stream. Measurements were made before the rise in blood pressure occurred. There was no change in diameter after administration of either drug.
even at these supraphysiological concentrations. The potency of the solutions was checked by intravenous administration after the end of the experiment; a marked rise in blood pressure (norepinephrine) or tachycardia (isoproterenol) occurred in all animals.

In five additional animals, 100 \( \mu \text{g/mL} \) of norepinephrine in artificial CSF was administered. As noted in the Methods, in these experiments we removed only a small area of the exposed dura to limit absorption of norepinephrine. Repeated measurements of diameter were made from the onset of application of norepinephrine and for several minutes thereafter. No significant change in diameter was observed at any time. Average diameter was \( 67.2 \pm 7.0 \mu \text{m} \) during the control period and \( 67.4 \pm 6.8 \mu \text{m} \) during the second minute of norepinephrine application. Arterial blood \( \text{PCO}_2 \) during the control period was \( 38.9 \pm 1.6 \text{ mm Hg} \) and did not change during norepinephrine application.

To check for species differences, we tested the pial vessels of three rabbits and three dogs with catecholamines at a concentration of 20 \( \mu \text{g/mL} \). There was no significant change in diameter in any of these experiments. Application of a commercial preparation of norepinephrine bitartrate (U.S.P.) in undiluted form (2 mg/ml) caused vasodilation, presumably because of its acidic pH (pH = 3.5); the animals died a few minutes later of the effects of absorbing the drug.

Wahl et al. (24) reported that local application of norepinephrine hydrochloride in high concentrations (10-1000 \( \mu \text{g/mL} \)) produced constriction of pial arteries. To exclude the possibility that the use of the bitartrate salt of norepinephrine accounted for our negative results, in three cats we studied the effects of norepinephrine hydrochloride (20...
Effects of norepinephrine (A) and isoproterenol (B) on the internal diameter of pial precapillary vessels. Means ± se, obtained from five animals in A and from six animals in B are shown. Arterial blood Pco2 during the control period was 38.6 ± 1.3 mm Hg in A and 35.6 ± 0.5 in B and did not change during application of norepinephrine or isoproterenol.

\[ \mu g/ml \] on pial arteries. No change in arterial diameter was observed during application of norepinephrine hydrochloride to the brain surface.

Figure 3 shows the results of exposure to histamine. A significant increase in diameter occurred at 1 \[ \mu g/ml \], and a large increase occurred at the highest concentration (3 \[ \mu g/ml \]).

Figure 4 summarizes the results of cerebral sympathetic nerve stimulation in eight animals. There was no response to the stimulation. In one animal, hypocapnia (Pco2 of 34 mm Hg) was inadvertently allowed to develop during the second control period, and significant constriction (10%) occurred.

Five additional animals were subjected to graded cerebral sympathetic nerve stimulation at 5, 10, 20, and 50 cps (Fig. 5). There was no significant vasoconstriction at any frequency regardless of whether the animal was ventilated with room air or 7.5% CO2. The small increase in diameter in Figure 5B is attributed to a slow increase in arterial CO2 tension observed during sympathetic nerve stimulation when the animals breathed 7.5% CO2.

In the three additional animals allowed to breathe spontaneously, various combinations of apnea, hyperpnea, and bradypnea along with variable and unpredictable changes in arterial blood Pco2 occurred during stimulation. These data were excluded from further consideration.
Effect of cerebral sympathetic nerve stimulation at 50 cps on the internal diameter of pial arteries. There was no significant change during stimulation. The solid circles represent diameter values for individual experiments, the triangles are the mean diameters, the bars represent the SE, and the open circles represent the means for arterial CO₂ tension. Note that in the experiment shown at the top of the figure, there was a decrease in diameter which was accompanied by decrease in arterial P₇. The numbers in parentheses indicate the P₇₀ at each condition for this experiment.

From these results it appears to us that rigid control must be exercised over non-neurogenic variables to avoid difficulties in interpretation of diameter changes.

Discussion

Of the three requisites which we considered essential to establish the presence of significant neurogenic control of pial blood vessels, only the presence of adrenergic innervation could be demonstrated. The pial vessels we studied did not respond to norepinephrine or isoproterenol nor did they respond to electrical stimulation of the superior cervical ganglion. We therefore conclude that the small pial arterial vessels of the cat, and probably those of the rabbit and the dog, are not subject to neurogenic vasoconstriction, most likely because their smooth muscle is unresponsive to the sympathetic neurotransmitter, norepinephrine. Other explanations for our negative results are much less appealing. For example, it is clear that our results were not due to general unresponsiveness of the vessels in our preparation. The vessels which did not respond to norepinephrine and isoproterenol showed substantial vasodilation when lower molar concentrations of histamine were applied. In an earlier investigation (18) on an
identical preparation, we demonstrated that vessels of similar size readily responded to alterations in arterial blood \( \text{CO}_2 \) tension. In addition, in unpublished observations we showed that pial vessels of similar size and in an identical preparation readily displayed the expected responses to alterations in CSF pH. We have also shown (unpublished data) that these vessels, in an identical preparation, responded to systemic and local hypoxia. The evidence clearly indicated that the catecholamines had easy access to the walls of the vessels we studied. In each experiment a marking solution (ink) readily covered the area of the blood vessels studied. The responsiveness to histamine, applied in a similar manner as the catecholamines, further corroborated this view. Furthermore, in several of the experiments with the high concentrations of norepinephrine, there was significant absorption into the blood stream, evidently from vessels within the area of observation (see Methods).

The accuracy, reproducibility, and discriminatory capacity of the methods employed for measuring pial blood vessel diameter, which were evaluated previously (18, 19), allow the reasonable assertion that changes on the order of 5–10% could have been readily demonstrated. In this respect, we emphasize the use of a pial window and the compound microscope equipped with a short focal-length objective having a shallow plane of focus. These attributes are necessary to achieve the accuracy and reproducibility we described previously. If one employed an open method without a window, in which the vessels were exposed to atmospheric pressure, the movement of the brain as a result of respiration would necessitate use of a dissecting microscope equipped with a long focal-length lens, resulting in a deep plane of focus. Unpredictable changes in object location and magnification could occur, which might result in errors of such magnitude that only gross changes from resting diameter could be reliably detected.

Although the concentration of neurogenically released norepinephrine at its site of action is not known, the fact that the net release of norepinephrine from granules is inhibited by concentrations on the order of 20 \( \mu \text{g/ml} \) suggests that it is unlikely that higher concentrations are achieved in vivo (25). Hence the concentrations of catecholamines we used should have been sufficient to produce maximal effects.

The absence of a response to electrical stimulation of the superior cervical ganglion occurred even though (1) the innervation of the blood vessels has been shown (22), by denervation experiments, to originate in this ganglion, (2) the stimulation employed was effective in producing pupillary dilation, and (3) the frequencies of stimulation used included those that produce maximum vasoconstriction in other vascular beds.

The response of cerebral blood vessels to catecholamines has been studied previously by numerous investigators either by direct observation or by indirect methods based on measurement of flow and pressure. Although catecholamines injected into the blood stream do not reach the brain tissue or the walls of intracerebral vessels, they do have access to the walls of pial vessels (26–29). Our results concerning sites of absorption of norepinephrine, which occurred in some of our experiments, were consistent with the view that catecholamines have access to pial vascular walls. However, intravascular administration of catecholamines frequently produces changes in blood pressure or in arterial blood \( \text{CO}_2 \) tension, which may have their own cerebrovascular effects and hence may render interpretation of the results difficult (30). For example, in the two human studies on the effect of intravenous administration of catecholamines on cerebral flow, decreases in arterial \( \text{CO}_2 \) tension occurred (31, 32).

It is less commonly realized that the topical application of catecholamines might have indirect effects which could affect the caliber of cerebral blood vessels and falsely create the impression of significant direct cerebral vascular action. If higher concentrations of catecholamines are employed, as was previously the case in many experiments, significant absorption into the blood stream may take place.
This may change the arterial blood pressure, which in turn may result in autoregulatory alterations in cerebral vessel caliber. In addition, if the animals breathe spontaneously, catecholamines may stimulate respiration and result in hyperventilation and hypocapnia. If significant absorption occurs in animals whose ventilation is kept constant, the well-known calorigenic effect of catecholamines may cause increased CO₂ production and arterial hypercapnia with resultant dilation of the cerebral blood vessels. These effects were meticulously watched for in our study, and they were either prevented or the experiments in which they occurred were excluded from consideration because of the obvious difficulties in interpretation. Furthermore, catecholamines are usually commercially available in the form of salts with strong acids, which, on dissociation in the unbuffered solution have an acidic pH and hence may produce effects as a result of changes in local pH, unless they are used in buffered solution, as was the case in our experiment. Direct application of these solutions in undiluted form causes marked vasodilation of the pial vessels, as we have demonstrated. Although in the case of norepinephrine this may be easily recognized as an artifact, in the case of isoproterenol it may create the false impression of a significant effect related to stimulation of beta receptors.

There are no previous studies of the effects of topical application of norepinephrine or isoproterenol on pial vessels. All previous studies of the effects of topical application of catecholamines on these vessels have used epinephrine (33-36). Results have been conflicting; vasoconstrictor, vasodilator, or biphasic responses have been described and in some studies no significant effect on vessel diameter was found. In none of these studies were the indirect effects mentioned above satisfactorily controlled. Most frequently there was absorption of epinephrine into the blood stream, and, consequently, an increase in blood pressure and alterations in respiration occurred. In other studies in which predominant vasodilation was obtained, it is evident that the catecholamine was applied in unbuffered solution probably having an acidic pH. It is not possible to state confidently whether these factors account fully for the positive findings. Of particular interest is that in two studies epinephrine application was associated with vasoconstriction of the larger pial vessels but not of the smaller ones (33, 35). Since the percent decrease in diameter reported was small, it is possible that the absence of a response of the small pial vessels was related to inability of the method to detect relatively small changes. Two additional in vitro studies are relevant. Uchida and his colleagues (37) studied the responses of isolated pial arteries to norepinephrine. Half of the vessels studied did not respond even to very high concentrations (over 20 μg/ml) of the catecholamine. The other half showed vasocostriction, but the threshold was sometimes very high (5-20 μg/ml). Also the response was much smaller than that of mesenteric vessels of comparable size. The size of the vessels studied was between 50 and 200μ, but it is not known how this corresponds to the in vivo size. Nielsen and Owman (38) found that the proximal portion of the middle cerebral artery in cats constricted in response to norepinephrine and tyramine in vitro. These positive results raise the question of effective neurogenic control of the larger cerebral vessels. The importance of the in vitro studies and the possibility that the larger cerebral vessels may account for the increase in cerebrovascular resistance found by some investigators in response to nerve stimulation remains to be investigated.

There are several earlier studies of the effect of sympathetic nerve stimulation on pial vessel caliber. Forbes and Wolff reviewed the older studies (5). The results of these studies have been conflicting. Since they were largely uncontrolled and the effects of indirect factors were not considered, no reliable conclusions can be reached. Forbes and Wolff (5) found that sympathetic nerve stimulation constricted the larger pial arteries but not the smaller ones. It is clear from their findings, however,
that sympathetic nerve stimulation was accompanied by large changes in blood pressure and alterations in respiration. Although the authors claim that these did not account for the observed vasoconstriction, we remain unconvinced. A more recent study by Kobayashi and his associates (11) of the effects of sympathetic nerve stimulation on pial vessel caliber found constriction in pial vessels in 9 of 15 stimulations. The investigators were aware that indirect effects could produce vasoconstriction in the absence of a direct action on the pial vessels, and they took measures to eliminate these effects. They reported their results in qualitative fashion since they used a dissecting microscope which had a resolution capable of detecting changes in the diameter in excess of 20 μ for vessels between 50 and 250 μ. We cannot account for the differences between their results and ours. The resolving power of our method was superior, and, had changes of the magnitude reported by them occurred in our study, we surely would have detected them.

Our study does not exclude the possibility of effective neurogenic control which might be physiologically important. However, we believe that such control, if present, cannot be exercised at the small arterial and arteriolar level. Whether neurogenic control could be exercised through action on the larger cerebral vessels or through the action of cholinergic nerves remains to be demonstrated.

References


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