Pial Arteriolar Responses in the Mouse Brain Revisited

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SUMMARY Arteriolar responses were measured on the cerebral surface of the mouse brain using an image splitter and TV monitor. The response to locally applied norepinephrine (NOR) was significantly more frequent for vessels greater than 30 μm I.D. than for smaller vessels. However, even the smaller vessels were frequently constricted by NOR in doses of 5 μg per milliliter. Reserpine (5 mg per kilogram) failed to alter the response to NOR at either 24 or 72 hours after reserpinization. At 48 hours the threshold dose of NOR was reduced, but the effect was slight (two-tailed, P = 0.08). Both propranolol (10⁻⁶ M) and phentolamine (10⁻⁴ M) blocked responses to 5 μg per milliliter of NOR, but neither agent altered resting arteriolar diameter. Isoproterenol, tyramine, and histamine had no effect. Serotonin (5HT) constricted the arterioles but did not potentiate the response to NOR. Additive or potentiated effects were not observed with NOR, 5HT or histamine in any combination. These data indicate the presence of alpha-adrenergic receptors in murine cerebral surface arterioles, but do not establish a significant tonic effect of norepinephrine. The existence or role of a beta-receptor in these murine cerebral surface arterioles remains an unsettled question.

In 1963, we reported data concerning microcirculation on the surface of the mouse brain. That report represented an initial summary of data concerning flow patterns and pharmacological responses in this species. Since then, only one other laboratory appears to have concerned itself with the pharmacological responses of murine cerebral circulation. Meanwhile, a series of papers have appeared from our laboratory describing flow phenomena within this vascular bed of the mouse and the behavior of the vessels themselves. These papers parallel increasing reports, in other species, of the pharmacological responses of cerebral surface vessels observed in vivo and a renewal of the controversy concerning the adrenergic responses of these vessels.

During our investigations we changed our technique of observation. This change was followed by consistent observations of contractile responses to locally applied norepinephrine (NOR) and to serotonin (5HT), where, previously, we had failed to make such observations. The new data have been presented elsewhere together with an account of the change in methodology. What remained unexplained was the reason for the new results.

During the same time period, Kontos and co-workers, studying the cat, had also reported negative and then positive findings with respect to the response of surface arterioles or arteries to NOR. They suggested that size of the vessel was the factor determining the presence or absence of a response. The series of experiments and observations reported below was undertaken, in part, to determine whether size of the vessel played a role in our own results. In addition, we wished to repeat some other previously negative studies that had employed our older techniques, in order to determine whether these studies would now also provide a positive result. Finally, in view of the continued interest in pharmacological responses of cerebral surface vessels and particularly because of interest in adrenergic influence on these vessels, we included new observations concerning the effects of adrenergic influences on the response to NOR.

Methods

Mice were prepared as previously described after being anesthetized with pentobarbital or urethane. Choice of anesthetic did not influence the results. The internal diameter of the surface vessels (pial vessels) was measured using a TV monitor and image splitter. The drugs used were applied locally for 30 seconds unless otherwise stated. All were dissolved in a physiological salt solution and applied at 37°C with a pH of 7.35 ± 0.05, the same temperature and pH as that of the physiological salt solution continually irrigating the brain when drugs were not being applied. At no time were systematic changes in pH of either drug solutions or irrigant observed, nor could results ever be related to the slight random changes in pH that might be present between irrigant and drug solution. The drugs used were: norepinephrine bitartrate (Levophed), tyramine HCl, serotonin creatinine sulfate, isoproterenol HCl (Isuprel), histamine phosphate, phentolamine mesylate, propranolol HCl, and reserpine (Serpasil). Concentrations and doses are expressed in terms of the free base of the preceding agents, except for serotonin, propranolol and tyramine, where doses are expressed in terms of the salt.

Blood gases were measured at the end of experiments where such measurements were pertinent. An IL micro blood gas analyzer was used. When pertinent, blood pressures were measured using a tail cuff technique.

Results

Effects of Size on the Response to Norepinephrine

We reviewed all data from the negative experiments performed prior to use of the TV camera and image splitter as a system of measurement. These earlier data were obtained by making measurements with an ocular micrometer. Our review indicated that the arterioles examined were consistently smaller in these early negative studies than in the later positive studies with the image splitter. For example, in a study with NOR, the diameter of arterioles was 26 ± 8 μm (N = 9), while in a later series of studies employing TV and image splitter, the diameter was 47 ± 7 μm (N = 89), P < 0.01. A similar difference in vessel size was noted when early studies with 5HT were compared with later studies using TV and image splitter (diameter: 30 ± 9 μm versus 48 ± 8 μm, P < 0.01). These comparisons suggested that the previous failure to observe a response to NOR or 5HT might be due to the lesser responsiveness of the smaller arterioles.
Therefore, we decided to compare in the same mice the response of larger versus smaller vessels.

NOR (5 µg per milliliter) was applied to the cerebral surface after first selecting an arteriole for measurement with the image splitter technique. The "large" vessels were 32 to 64 µ I.D. The "small" arterioles were 8 to 24 µ. Sometimes the response of a small vessel was observed first and sometimes that of a large vessel. Twenty-four animals were studied, one pair of vessels per animal. Twenty-two of 24 large vessels responded compared to 14 of 24 small vessels. This difference is significant (P = 0.02, chi square = 5.4). All vessels gave large contractile responses to locally applied Ba++, thus indicating their capacity to constrict. Following the application of NOR, the mean percentage change in diameter for the group of small vessels was the same as that of the group of larger vessels (20 ± 22% versus 23 ± 9% of resting diameter).

Response to NOR After Reserpinization

Since we observed a response to NOR in "larger" and even in a substantial number of "small" vessels, it was of interest to see whether depletion of NOR from perivascular nerves would result in hypersensitivity to NOR. Reserpine was given intraperitoneally in a dose (5 mg per kilogram) which depleted all NOR from nerves to cerebral vessels within 24 hours. This was checked by examination of whole mounts using the Falck-Hillarp technique for NOR fluorescence. Mice were studied 24 hours (13 animals), 48 hours (14 animals), and 72 hours (five animals) after injection. Similar numbers of control animals also were observed. The response of pial arterioles above 32 µ I.D. was observed at increasing doses of NOR (0.1, 0.5, 1.0 and 5 µg per milliliter) until a constriction was observed. Constriction was first seen at doses varying from 0.1 µg to 5 µg per milliliter for reserpinized mice and from 0.1 µg to 5.0 µg per milliliter for control mice. In spite of the fact that some controls displayed higher effective doses than reserpinized mice, the overall distribution of effective doses did not differ significantly for the two groups. This was true at 24, 48 and 72 hours, and it was also true when the 24-hour, 48-hour and 72-hour animals were combined. It may be of interest, however, that the 48-hour data (table 1) provided a distribution of effective doses that came quite close to the 0.05 significance level (P = 0.08 Fisher test, two-tailed). This tendency toward a reduction in effective dose following reserpine could not be ascribed to altered blood pressures or to altered CO2 levels, since these parameters were virtually identical in the two groups (CO2 = 57 ± 4 and 52 ± 4, BP = 86 ± 7 and 76 ± 9, mean ± SEM in the controls and reserpinized groups, respectively).

Although the lowest measured effective dose of NOR appears to be somewhat reduced in reserpinized mice at 48 hours, the magnitude of constriction produced by these doses did not differ between control and reserpinized mice (22% of initial diameter in the controls and 20% of initial diameter in the reserpinized mice).

Effects of Adrenergic Blockers on Response to NOR

We have previously reported\(^4\) that locally applied phentolamine, an alpha blocker, inhibits the response to locally applied NOR. A dose of 10^-4 M of phentolamine was required to totally block 10 µg per milliliter of NOR. (An error in the published report gives 10^-4 M as the blocking dose.) However, data were not presented describing the effect of phentolamine itself on arteriolar diameter. In 20 experiments, a dilation of arterioles was observed in only three cases, although phentolamine (10^-4 M) remained in contact with the vessels for at least five minutes and blocked NOR. Consequently, the data provided no support for the concept of adrenergic tone in these vessels, which ranged in size from 32 to 64 µ I.D.

Propranolol, a beta-blocker, failed to alter the diameter of the pial arterioles at a dose of 3 × 10^-4 M, which, surprisingly, also blocked the response to NOR. These findings were noted in a study of ten mice which received only propranolol and NOR locally applied to the cerebral surface. The response to NOR (1 µg per milliliter or 5 µg per milliliter) was determined first in each animal, followed by three to five minutes of propranolol, and then another test with NOR at the same dose as that applied prior to propranolol. Following propranolol, the response to NOR could no longer be recognized in five of the ten animals, and the before-and-after responses differed significantly when compared with a paired "t"-test (P < 0.05). After propranolol was washed out, the vessels were tested with BaCl and all contracted vigorously, demonstrating that the effect of propranolol was not due merely to some factitious nonspecific decline in the viability of the vascular smooth muscle.

Effects of Isoproterenol

Since propranolol interfered with the response to NOR, one wondered about the possibility of some peculiar beta-receptor modulating constriction or the possibility that the contractile machinery could be activated, whatever the mechanism, by beta agonists as well as alpha agonists. Therefore, we tested the effect of isoproterenol, the prototype of beta agonists. Five mice were tested, each at four doses of isoproterenol (1, 5, 10 and 100 µg per milliliter). Dilation was noted on only four of the 20 occasions, unrelated to dose, and constriction only once. On the 15 remaining trials no change in diameter was recognized. The five changes in diameter were thought to be random changes in diameter occurring coincidentally during the trials, and isoproterenol, therefore, was found to lack an ability to constrict or dilate pial arterioles from their "resting" state. No attempt was made to test the effect of isoproterenol on vessels with markedly increased tone. Phentolamine (10^-4 M) failed to alter the response to isoproterenol (1 to 100 µg per milliliter).

Effects of Tyramine

Tyramine is known to release NOR from sympathetic nerve terminals and intravenous injection of tyramine has

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**Table 1** Lowest Measured Effective Dose of Norepinephrine 48 Hours After Reserpine

<table>
<thead>
<tr>
<th>Effective dose</th>
<th>Control*</th>
<th>Reserpinized*</th>
</tr>
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<tbody>
<tr>
<td>0.1 µg/ml</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>0.5 µg/ml or greater</td>
<td>11</td>
<td>5</td>
</tr>
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*Number of mice falling into this group.
been reported to reduce cerebral blood volume in mice on the basis of a cerebral vasoconstriction produced by NOR released by the tyramine. Since no direct in vivo observations of cerebral vessels were reported following local applications of tyramine, and since mice are the subject of our own intensive studies, we tested the local application of tyramine. Twenty mice were tested at a single dose of tyramine (either 10, 100, or 1,000 µg per milliliter). Two years later another five mice were tested, each mouse having the following doses of tyramine locally applied to the pial surface (1, 5, 10, 50, 100 µg per milliliter). The arterioles tested measured 49 ± 14 µm I.D. Constriction was not observed at any dose in the last group of studies and was observed in only five of the 20 earlier studies. These five constrictions were not dose-related and were thought to represent random fluctuations in diameter. In short, we could not demonstrate a contractile effect of tyramine in vivo on pial arterioles of the size tested, though vessels of this size and in this species do respond to NOR.

Effects of Histamine

Local histamine has been reported to dilate surface arterioles in vivo in other species, but to constrict cerebral arteries in vitro. Prior to our use of the image splitting-TV technique, we failed to find either a constricting or dilating effect of histamine. We repeated our studies using the newer technique and the larger-sized vessels that have been the major focus since we began using the technique. These arterioles were 30 to 56 µm I.D. Five mice were tested, each mouse at four dose levels (1, 5, 10, and 100 µg per milliliter). Out of a total of 20 trials, only one response was recognized — a dilation at the highest dose in a single mouse. In short, we confirmed our earlier studies and were unable to demonstrate an effect of histamine in vivo on the resting tone of mouse cerebral arterioles.

Effects of Combined Amines

In vitro, histamine has been reported to potentiate the contractile response of rabbit cerebral arteries to NOR or 5HT. In earlier studies we failed to find such an effect on surface arterioles of the mouse brain. We wished to repeat these studies on somewhat larger arterioles, using the image splitting technique. Moreover, since we now consistently find that NOR and 5HT induce constriction in the larger arterioles of the mouse, and often in smaller arterioles as well, we wished to repeat our previous negative studies using these two amines in combination. These combination studies were of particular interest to us since we have been able to demonstrate that the contractile response of surface arterioles to some prostaglandins, added either to NOR or 5HT, is greater than the response to either the amine or the prostaglandin administered alone. We tested each of three mice with 5 µg per milliliter of 5HT, 10 µg per milliliter of 5HT, 10 µg per milliliter of NOR, and then the two drugs combined at these dosages. In no case was the response to the combination greater than the response to each of the amines applied alone. Though no evidence of summation or potentiation was obtained, responses to BaCl2 at the end of the experiments always showed that the vessels had the capacity to constrict to a greater degree than that elicited by the amines in combination.

Discussion

Effects of Size

The preceding data indicate that size is a determinant of the response to cerebral surface arterioles in the mouse. The smaller arterioles responded less frequently to NOR than did the larger vessels. Others also have reported a difference in the response of surface arterioles to catechol. Prior to the availability of NOR, Fogg and Forbes et al. demonstrated, in cats and monkeys, a more consistent contractile effect of adrenaline on larger arterioles or arteries than on smaller pial arterioles. Recently, after initially reporting failure of pial arterioles to constrict, Kontos and co-workers have reported that these arterioles do constrict, provided they are more than 100 µm in diameter. Our own previous failure to demonstrate an effect of NOR cannot be wholly ascribed to the smaller size of the vessels originally examined by us, since, as the present data show in the mouse, many of the smaller arterioles also constrict with NOR. Our earlier, total failure to recognize such constrictions thus remains unexplained.

The difference between our results and the recent report of Kontos and co-workers might be explained on the basis of a species difference. In the mouse the entire range of pial arterial diameters is below 80 microns. Thus, in this 80-micron range, one includes vessels that are analogous to both the smallest arterioles of the cat and the much larger surface vessels (200 or more). It may be that in the compressed size range of the murine vessels, functional differences between classes of vessels are not so well demarcated on the basis of size. On the other hand, while our results are similar to those of Kontos and co-workers insofar as we do show a difference between the frequency of response of "large" and "small" arterioles, we must also point out that other workers have not reported such a difference, and, though using the cat like Kontos and co-workers, they have successfully demonstrated constriction of arterioles after application of NOR to vessels less than 100 µm and even less than 50 µm in diameter.

Effects of Reserpine

One might have expected a dramatic increase in responsivity to NOR, because of hypersensitivity of vascular smooth muscle induced by reserpine. Smooth muscle has been shown to have such hypersensitivity. This is thought due, at least in part, to a reduction of NOR by the reserpine, and a consequent reduction in tonic stimulation of the receptors. Physical sympathectomy has been shown to increase the effect of NOR on cerebral blood volume in the mouse. Nevertheless, our data revealed only a slight effect of reserpine (P = 0.08) at one time interval (48 hours after reserpine). Others have failed to show an effect of cocaine on the cerebrovascular response to catecholamines or to sympathetic stimulation, yet cocaine is an agent producing an even greater "denervation" hypersensitivity than reserpine on other test objects. Some suggest that reuptake of NOR...
by nerves in the vicinity of cerebral vessels is not as important for inactivation of NOR as is reuptake at other sites. It is evident that questions remain concerning the effect of reserpine, cocaine and denervation on the cerebrovascular response to NOR, not only in the mouse but also in other species.

Effects of Adrenergic Blockers

The effect of phentolamine on the response to NOR was similar to that shown by Wahl et al. in the cat. That is, the alpha blocker inhibited the response to NOR. These data also correlate with in vitro data obtained from much larger cerebral vessels in cats and goats. However, unlike Wahl et al., we were unable to demonstrate any dilatation produced by phentolamine, hence we could not provide evidence for adrenergic tone in "resting" vessels. This may be because Wahl et al. used doses 10 to 50 times greater than ours. Our dose was sufficient to inhibit the response to NOR and should have produced dilatation if that dose of NOR was present in the vascular environment and maintained a tonic effect on the vessel.

It is of interest that we also observed an inhibiting effect of propranolol, a beta blocker, on the response to NOR. Though the dose of propranolol (10^-6M) was within the pharmacological range, this effect may have been "nonspecific." We had previously observed blockade by beta blockers (at higher doses than those used here) of the contractile response to BaCl2. Edvinsson and Owman have recorded blockade of isoproterenol (1) induced contractions of larger cerebral arteries in vitro, by beta blockers, including propranolol. The low effective dose of propranolol in their study (10^-6M) suggests the futility of using dosage itself to distinguish "specific" from "nonspecific" effects. Like Wahl et al., we found no effect in vivo with propranolol alone.

Effects of Isoproterenol, Tyramine, and Histamine

Like Wahl et al. or Raper et al., we were unable to demonstrate significant effects of isoproterenol in vivo. Wahl et al. using the cat, found contractions of up to 5% at some doses and dilatations of up to 5% at others. Also, like these workers, we failed to find any effect of phentolamine on the response to isoproterenol, and concluded, as they did, that the absence of significant dilatation to isoproterenol was not due to simultaneous stimulation of contractile alpha receptors.

We also failed to find any response to tyramine. Tyramine constricts larger cerebral arteries in vitro. Intravenous tyramine also has been reported to reduce cerebral blood volume (CBV) in the mouse in vivo and the latter has been interpreted as an effect of NOR, released from cerebrovascular nerves by the tyramine. However, intravenous tyramine also might raise blood pressure, and this would, in itself, reduce CBV because of autoregulation. The authors ignore this possibility, perhaps because they could not integrate such an hypothesis into their additional data, which showed that the response to tyramine was abolished by cervical sympathectomy performed two days earlier. Nevertheless, one wonders whether cervical sympathectomy might have modified in some way either autoregulation and/or the pressor response to tyramine. In short, data from experiments using intravenous tyramine cannot, as yet, be interpreted as contradicting the data presented here. It should be noted that both our earlier studies and our recent studies with tyramine employed arteries in the size range where NOR gave the greatest frequency of response (44 ± 13 μ I.D.).

Histamine, in our earlier studies and in the present studies, failed to alter the diameter of the pial arterioles. These in vivo data thus differ from in vitro data on larger cerebral arteries, where constriction was observed. However, from in vivo studies in the cat, where dilation was observed. Our studies cannot account for the differences between in vitro and in vivo results in other species; however, such differences are well known, as are differences between species where histamine is the agent of concern.

Combination of Amines

As in our previous studies we failed to observe any additive or potentiating effects of NOR, SHT, or histamines in any combination. The subject of potentiation is complex. For example, as pointed out by Bevan et al. in the same animal, drug combinations may demonstrate potentiation in one set of arteries but not in another. Our data shed no further light on this problem.

References

Studies on the Cerebral Circulation of the Baboon in Acutely Induced Hypertension

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SUMMARY The upper limit of autoregulation of cerebral blood flow was investigated in eight young baboons with the intracarotid \(^{133}\)xenon clearance method. Blood pressure was increased by intravenous angiotensin infusion. Autoregulation was effective during blood pressure increase from normotensive levels to a mean pressure of 130 to 139 mm Hg. At this pressure, cerebrovascular resistance reached a maximum. With further blood pressure increase, autoregulation was broken, and the vascular resistance dropped significantly. This flow increase was restricted to the fast component of the \(^{133}\)xenon clearance curve, leaving the slow component unchanged.

Introduction

CEREBRAL BLOOD FLOW (CBF) is normally autoregulated: that is, it remains relatively constant over a wide range of perfusion pressure. It has been demonstrated that there is an upper blood pressure limit of autoregulation beyond which CBF increases with increasing perfusion pressure.\(^1\)\(^4\) This has been called the hypertensive "breakthrough" of autoregulation and has been postulated to be of relevance in the pathogenesis of acute hypertensive encephalopathy.\(^2\)\(^4\)

The present investigation describes in greater detail the events occurring in the cerebral circulation at, and beyond, the upper limit of autoregulation. In particular, the fast and slow components of the \(^{133}\)xenon clearance curves and their respective contributions to the observed increase in total CBF were examined. Further, attempts were made to maintain blood pressure above the point of "breakthrough" for prolonged periods of time. Following sustained hypertension, blood pressure was returned to normotensive levels and the pattern of CBF was examined throughout.

Methods

The study was carried out in eight young baboons (Papio cynocephalus or Papio anubis) weighing 7 to 14 kg. The animals were sedated with phencyclidine (12 mg i.m.) and then anesthetized with sodium thiopental before being paralyzed with suxamethonium (100 mg i.v. or i.m.). They were then intubated and connected to an intermittent positive-pressure respirator pump (Starling) which delivered a mixture of 75% \(N_2O\) and 25% \(O_2\) in open circuit. Phencyclidine (2 to 4 mg i.m.) and suxamethonium (50 to 100 mg i.m.) were administered at 30-minute intervals. Body temperature was controlled (36° to 38°C) by heating lamps.

A catheter was placed in one common carotid artery via the lingual artery, all other branches of the external carotid artery being ligated. The scalp and temporal muscle were removed on the same side. In some animals a catheter was placed in the superior sagittal sinus through a burr hole. The abdominal aorta was cannulated, via one femoral artery, for pressure measurement and blood sampling. A femoral vein was cannulated for saline and drug administration. The animal was then allowed to stabilize for one hour before being studied.

Aortic and sinus pressures were measured with Statham strain gauge transducers. \(^{133}\)Xenon, dissolved in saline, was injected via the lingual artery catheter and the clearance of the isotope was measured by a lead-collimated detector placed over the denuded skull in the parietal region. CBF was calculated by the height/area (H/A) equation.\(^7\) The linearly displayed clearance curve was manually transformed into a semilogarithmic coordinate system for compartmental analysis and calculation of the relative weight of gray matter.\(^7\) These calculations were carried out by the technical staff at a later date.

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