Alpha-Receptor Stimulation by Endogenous and Exogenous Norepinephrine and Blockade by Phentolamine in Pial Arteries of Cats

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ABSTRACT
The question regarding the existence of an alpha-adrenergic component of pial arterial tone was investigated using a microapplication technique combined with the measurement of vascular diameter. Concentration-response curves for the alpha-receptor blocker, phentolamine, revealed no vascular reaction for a concentration range from \(2.5 \times 10^{-7}\) to \(2.5 \times 10^{-6}\)M. At higher concentrations (up to \(1.3 \times 10^{-3}\)M) concentration-dependent dilations were observed. Constrictions of pial arteries induced by perivascular injection of \(2.5 \times 10^{-6}\)M norepinephrine could be reduced by 38% and 73% when phentolamine was applied simultaneously in concentrations of \(2.5 \times 10^{-7}\) and \(2.5 \times 10^{-8}\)M, respectively, whereas constrictions due to \(2.5 \times 10^{-4}\)M norepinephrine were not reduced by \(2.5 \times 10^{-6}\)M phentolamine, indicating a competitive antagonism between norepinephrine and phentolamine for pial arteries. Stimulation of the cervical sympathetic chain (90 seconds, 10 v, 1.4 msec, 20 Hz) induced constrictions of pial arteries (mean 12%) which could be reduced by two-thirds during the simultaneous application of \(2.5 \times 10^{-7}\)M phentolamine. Since the constriction induced by norepinephrine applied exogenously or released endogenously could be reduced by a concentration of phentolamine which had no vascular effect per se, we conclude that the resting tone of the pial arteries is not influenced by an alpha-adrenergic component under our experimental conditions. The dilations induced by high concentrations of phentolamine are believed to be nonspecific.

The effects of catecholamines on the regulation of the resistance of cerebral arteries have been a matter of controversy. The discrepant results may be explained by differences in methodological procedures. When they are applied from the intravascular side, catecholamines may induce secondary reactions of cerebral vessels resulting from alterations in blood pressure, acid-base state, or cerebral metabolism. Also the existence of a blood-brain barrier for catecholamines (1-4) may prevent intravascularly infused catecholamines from reaching the smooth muscle receptors, presuming that this barrier is located in the vascular endothelium. These problems can be circumvented by applying the catecholamines directly to the vessel wall with micropuncture of the perivascular space. Using this technique, our group has recently shown that both norepinephrine (5) and epinephrine (6) can induce concentration-dependent constrictions of pial arteries. This constrictor effect is critically dependent on the pH of the mock spinal fluid in which the norepinephrine is dissolved (5). Considering this dependency of the vascular reactions to norepinephrine on the pH, the results of Raper et al. (7) do not contradict our data. These authors were unable to demonstrate any effect of norepinephrine on pial arteries using the window technique. When in our experiments the norepinephrine was dissolved in a mock spinal fluid with a composition comparable to that of Raper et al. (7), we also did not find a constriction in response to norepinephrine.

Since it has been demonstrated that the alpha-receptor stimulating agent, norepinephrine, can induce constrictions of pial arteries, we wanted to test whether the tone of pial arteries is mediated, at least in part, by an alpha-adrenergic component. This question can be answered by application of alpha-receptor blocking substances. The published data about the effects of alpha-receptor blocking agents on cerebral blood flow are conflicting. Gottstein (8), Skinhøj (9), and d’Alecy (10) found no change in cerebral blood flow after the intravenous application of phentolamine (8-10) and phenoxybenzamine (10). Applying phentolamine into the internal carotid artery, Gottstein (8) found an increase in cerebral blood flow after the intravenous application of phentolamine (8-10) and phenoxybenzamine (10). Applying phentolamine into the internal carotid artery, Gottstein (8) found an increase in cerebral blood flow, but Lang and Zimmer (11) found no change. Using the window technique, Fraser et al. (12) found dilations of pial arteries when the cerebral surface was rinsed with...
In a preliminary study, using the microapplication technique, our group (13) demonstrated that high doses of phentolamine induced strong pial arterial dilations. However, as has already been discussed in this paper (13), these dilations observed after phentolamine administration may be due to a nonspecific, nonblocking action of phentolamine; such an effect cannot be excluded from the results of Gottstein (8) and Fraser et al. (12). Therefore, it was necessary to test the effect of an alpha-receptor blocking substance over a wide concentration range and to differentiate between specific alpha-receptor blocking and possible nonspecific effects of this substance.

In a second series of experiments, the effect of electrical stimulation of the cervical sympathetic chain on the diameter of pial arteries was tested. To investigate whether the effect of endogenously released norepinephrine could be blocked by phentolamine, the blocking agent was injected perivascularly during sympathetic stimulation.

Methods

Experiments were performed on 23 cats of both sexes anesthetized with glucochloralose (40-50 mg/kg, iv). The cats were ventilated artificially with a Bird Mark 8 respirator. The carbon dioxide tension (Pco₂), the pH, and the oxygen tension (Po₂) of the arterial blood were measured at 38°C. pH was 7.33 ± 0.03 (sd). Po₂ was 31.3 ± 2.0 mm Hg, and Po₂ was 122 ± 19.5 mm Hg. The value of arterial Pco₂ is close to that obtained in conscious cats (14, 15). End-tidal CO₂ (4.5 ± 0.1 ml/100 ml) and arterial blood pressure were recorded continuously. Only cats with a mean arterial blood pressure of more than 100 mm Hg were used for experiments. Body temperature was maintained between 37° and 38°C. Tyrode's solution (2.5 ml/kg hour⁻¹) was infused intravenously. The brain surface (part of parietal and temporal lobe) was bathed with mineral oil heated to between 37° and 38°C.

Glass micropipettes with sharpened tips (8-10μo.d.) were filled with test solutions and sealed between oil, as has been discussed elsewhere (16). The tip of a micropipette was positioned by a micromanipulator into the immediate vicinity of a superficial artery or arteriole. By applying pressure to a syringe attached to the micropipette, 1-3 μl of fluid was injected into the perivascular space. A Bausch and Lomb stereozoom microscope was used at a magnification of 70x. Vascular diameter was measured by the image-splitting method of Baez using a 625-line Grundig TV camera (equipped with a multidiodal Vidicon) and a Watanabe Multicorder. The reproducibility of the method and the error due to defocusing have been described in a previous paper (16).

In all experiments, with the exception of the stimulation experiments, the vascular diameter was measured before and 20 and 40 seconds after the beginning of the injection of the test solution. The changes in diameter were calculated, and the control value was compared with the mean value of these two measurements.

First, the reactivity of all of the vessels to alterations in pH was tested (17). Then, a test solution with the following millimolar composition was applied: Na⁺ 156, K⁺ 3, Ca²⁺ 1.5, Cl⁻ 151, and HCO₃⁻ 11. The pH of the test solution was 7.15 (38°C), and the osmolarity was 300 mosmoles/liter. This solution served as a solvent solution when norepinephrine, phenolamine, or both were applied. All solutions were bubbled with a gas mixture of 5% CO₂/95% N₂ equilibrated with water during the whole experiment. This procedure prevents auto-oxidation of catecholamines (18). The withdrawal of oxygen did not alter the reactions of pial arteries to the different concentrations of HCO₃⁻. The composition of the mock spinal fluids was determined as described in a previous paper (16).

When stimulation experiments were performed, the cervical sympathetic chain was exposed on both sides of the neck between the superior and the inferior cervical ganglion. Both vagi and sympathetic chains were transected at this site. A platinum electrode was placed under the cut end of that part of the sympathetic chain which leads to the brain and which was situated in a pool of mineral oil contained by the skin of the dorsal side of the neck. Stimulation was performed on that side of the cat from which the skull had been removed using a Grass SD 5 square-wave stimulator. The stimulus parameters, as monitored on an oscilloscope, were rectangular square pulses 1.4 msec in duration, 20 Hz in frequency, and 10 v in magnitude. Only cats that showed maximal pupillary dilations during electrical stimulation were used for these experiments.

Results

The question of an alpha-adrenergic component of pial arterial tone was tested by microapplication of phenolamine (2.5 x 10⁻¹¹ to 1.3 x 10⁻⁸M) into the perivascular space of single pial arteries. After subtracting the effect of the solvent at each vessel (a mean dilation of 2.5%), the results shown in Figure 1 were obtained. The values of the average curve result in part from the injection of ascending concentrations of phenolamine and in part from injections of random concentrations of phenolamine. The size of the vessels tested ranged from 32μ to 247μ. At concentrations of phenolamine from 2.5 x 10⁻¹¹ to 2.5 x 10⁻⁸M, no vascular reaction occurred. At higher concentrations dilations were observed; they were concentration dependent but independent of the initial vascular diameter.

The evaluation of the specific alpha-receptor blocking effect of phenolamine at pial arteries can be performed by the demonstration of a shift in the concentration-response curve for norepinephrine by phenolamine. However, such an attempt would require the determination of two concentration-response curves at the same vessel, the first for...
norepinephrine alone and the second for norepinephrine together with phentolamine. This approach cannot be used in our preparation, since, in a previous study (5), we have demonstrated that the vascular reactions to norepinephrine are significantly decreased when a second concentration-response curve is taken from the same vessel. Because this study (5) has shown that three applications of norepinephrine can be made at the same vessel without decreasing the vascular effects, another experimental procedure was chosen. After the effect of the solvent solution was tested, the reaction to norepinephrine alone was measured. Then, the reaction to phentolamine was determined, and finally the reaction to both norepinephrine and phentolamine given simultaneously was measured. The effects of $2.5 \times 10^{-6}$M phentolamine on the constrictions induced by $2.5 \times 10^{-4}$M (group A) and $2.5 \times 10^{-6}$M norepinephrine (group B) and the effect of $2.5 \times 10^{-7}$M phentolamine on the constriction induced by $2.5 \times 10^{-6}$M norepinephrine (group C) were tested. The results of these studies are depicted in Figure 2, which shows the values obtained after subtraction of the effects due to the solvent and to phentolamine alone. The effects of the solvent solution for group A were 0.1% dilation, for group B 0.5% constriction, and for group C 1.9% constriction. Phentolamine induced a 2.4% dilation in group A, a 3.5% dilation in group B, and a 0.2% dilation in group C. The size of the vessels tested ranged from 35\(\mu\) to 216\(\mu\). Figure 2 demonstrates that $2.5 \times 10^{-6}$M phentolamine reduced the constriction induced by $2.5 \times 10^{-6}$M norepinephrine by 73% but not the constriction induced by $2.5 \times 10^{-4}$M norepinephrine. The lower concentration of phentolamine ($2.5 \times 10^{-7}$M) reduced the constriction of $2.5 \times 10^{-6}$M norepinephrine by 38%. The statistical analysis of the data was performed using the Wilcoxon matched pairs, signed rank test (19). The reduction of the constriction induced by $2.5 \times 10^{-6}$M norepinephrine was significant ($P < 0.01$) when $2.5 \times 10^{-7}$M or $2.5 \times 10^{-6}$M phentolamine was added.

Figure 3 shows the effects of stimulation of the cervical sympathetic chain on pial arteries. The initial size of the vessels tested ranged from 40\(\mu\) to 221\(\mu\). The arterial blood pressure and the end tidal CO$_2$ were unchanged during the stimulation, which lasted for 90 seconds, and in the poststimulation period compared with the values for the control period. Figure 3 shows a time-dependent decrease in vascular diameter which reached a steady state between 60 and 90 seconds. During the following 90 seconds, after the end of the stimulation, the vascular diameter increased but did not reach its initial value. The fact that the vascular diameter did not completely return to the control value within 90 seconds after stimulation may be explained by the duration of stimulation. In pilot studies (L. G. d’Aley, M. Wahl, and W. Kuschinsky, unpublished observations) in which the stimulation lasted only 65 seconds, the vascular diameter returned to its control value within 2 minutes after the end of the stimulation. The statistical analysis of the values shown in Figure 3 was performed by multiple comparisons of dependent observations (20); the values of vascular dia-
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The stimulation obtained after 40, 60, 75, and 90 seconds of stimulation were significantly \( (P < 0.01) \) different from the control value obtained before stimulation. The poststimulation value 180 seconds after the beginning of the stimulation was significantly different from the values obtained after 60, 75, and 90 seconds \( (P < 0.01) \) but not significantly different from the prestimulation control value.

Whether the pial arterial constriction induced by the stimulation of the cervical sympathetic chain can be reduced by an alpha-receptor blocking agent was investigated by perivascular application of phentolamine during stimulation. After testing the effect of the solvent solution without phentolamine (mean constriction of 0.4%), the cervical sympathetic chain was stimulated for 90 seconds. During stimulation (62 seconds after the beginning of the stimulation), phentolamine was applied extravascularly on the constriction of pial arteries induced by stimulation of the cervical sympathetic chain. The stimulation lasted 90 seconds, as described for Figure 3. From the sixty-second to the ninety-second phentolamine was applied by microapplication to the vessel studied during stimulation. The curve shows means ± SE; \( n \) = number of vessels tested.

**FIGURE 3**
Time course of change in pial arterial diameter during and after 90 seconds of stimulation of the ipsilateral cervical sympathetic chain. The curve shows means ± SE; \( n \) = number of vessels tested.

**FIGURE 4**
Effect of \( 2.5 \times 10^{-7} \text{M} \) phentolamine applied extravascularly on the constriction of pial arteries induced by stimulation of the cervical sympathetic chain. The stimulation lasted 90 seconds, as described for Figure 3. From the sixty-second to the ninety-second phentolamine was applied by microapplication to the vessel studied during stimulation. The curve shows means ± SE; \( n \) = number of vessels tested.
perivascularly in a concentration \((2.5 \times 10^{-7} \text{M})\) which has no vascular effect when it is applied without sympathetic stimulation, as can be seen from Figure 1. The results are shown in Figure 4. During the application of phentolamine, the constrictor effect due to the stimulation of the sympathetic nerves was reduced by 67%. This reduction can be explained by the alpha-receptor blocking action of phentolamine, since stimulation without phentolamine leads to a steady state of constriction between 60 and 90 seconds, as shown in Figure 3. The reduction of the stimulation-induced constriction by phentolamine was statistically analyzed by comparison of the data obtained after 60 and 75 seconds. The Wilcoxon matched pairs, signed rank test (19) revealed that the decrease in constriction obtained was statistically significant \((P < 0.01)\).

Figure 5 demonstrates the relationship between the vessel size during the control period and the change in vascular diameter after application of \(2.5 \times 10^{-6} \text{M}\) norepinephrine (top) and during stimulation (60 seconds after the beginning of the stimulation) of the cervical sympathetic chain (bottom). The calculation of the regression lines revealed the existence of a significant correlation \((P < 0.01)\) between the vessel size and the percent decrease in vascular diameter during sympathetic stimulation \((r = -0.49)\) but not during application of \(2.5 \times 10^{-6} \text{M}\) norepinephrine \((r = 0.20)\). These results show a different pattern of vascular reactions when norepinephrine is applied exogenously or released endogenously.

**Discussion**

The present data demonstrate the absence of an alpha-receptor-mediated component of pial arterial resting tone under our experimental conditions. Resting tone is defined as tone without application of norepinephrine or electrical stimulation of the cervical sympathetic chain. The absence of a component mediated by alpha-receptors is evident, since, in this study, the specific and nonspecific effects of the alpha-receptor blocking agent, phentolamine, can be differentiated. The fact that a dose of phentolamine of \(2.5 \times 10^{-7} \text{M}\) exerted no vascular effect per se (Fig. 1) but was able to reduce constrictions induced by \(2.5 \times 10^{-6} \text{M}\) norepinephrine (Fig. 2) can be explained in two different ways. First, it is possible that no endogenous norepinephrine was released from the perivascular nerves during our experimental conditions. Second, norepinephrine could be secreted continuously in such high concentrations (for instance \(10^{-4} \text{M}\)) that this low dose of phentolamine is ineffective in blocking this effect; a similar situation occurred during the application of \(2.5 \times 10^{-6} \text{M}\) phentolamine simultaneously with \(2.5 \times 10^{-6} \text{M}\) norepinephrine. The second possibility can be excluded, because lower doses of norepinephrine (for instance \(2.5 \times 10^{-6} \text{M}\)) were sufficient to induce constrictions of pial arteries. This fact indicates that norepinephrine concentrations resulting from any continuous secretion cannot exceed \(2.5 \times 10^{-6} \text{M}\), a concentration of norepinephrine which can be blocked by phentolamine. From these considerations, it is evident that an alpha-receptor-mediated component of pial arterial tone does not exist under our experimental conditions. Therefore, the minimal dilatatory effect of \(2.5 \times 10^{-6} \text{M}\) phentolamine appears to be due not to a
blockade of an alpha-adrenergically mediated constrictory component but rather to nonspecific dilatory effects; this nonspecific action of phentolamine also holds for all higher concentrations of the drug. Nonspecific effects of high doses of phentolamine are well known and may be due to the drug's histaminelike or serotonin-antagonistic effects. We have tried to test other alpha-receptor blocking agents which may have fewer nonspecific effects, such as phenoxybenzamine and hydergine. Unfortunately, these substances are only soluble in acidic mock spinal fluids (pH 3-4) and therefore cannot be tested because of the strong dilatatory effect of such an acidic solution (21).

The catecholamine-induced constrictions of pial arteries are mediated by stimulation of alpha receptors. This mechanism can be deduced not only from the fact that norepinephrine constricts pial arteries but also from the fact that the norepinephrine-induced constriction is blocked by competitive antagonism by the alpha-receptor blocking agent, phentolamine. The competitive action of phentolamine, which is well known for other organs, is indicated by the results shown in Figure 2.

The beta receptors do not seem to play an important role in the catecholamine-induced reactions of pial arteries, as has been demonstrated in a former study (18). In this paper (18), it was shown that microapplication of isoproterenol induced only minimal vascular reactions and that no beta-receptor-mediated component of pial arterial resting tone could be detected when propranolol was applied perivascularly.

The constrictor effect caused by stimulation of the cervical sympathetic chain is consistent with the data obtained by Forbes and Wolff (22) and Forbes and Cobb (23). These authors measured constrictions of pial arteries 108-342 μ in diameter (22). A comparison of our results with those of Kobayashi et al. (24), who found constriction of pial arteries during stimulation in 60% of the vessels tested, is difficult for several reasons. (1) The experiments of Kobayashi et al. (24) were performed at different stimulation voltages (1-10 V). (2) Their arterial Pco2 varied from 26 to 77 mm Hg. (3) Only a decrease in vascular diameter of more than 20 μ could be detected with accuracy and was defined as a constriction in their experiments. Our results contradict those of Raper et al. (7), who found no alteration of vascular diameter in cats during stimulation of the cervical sympathetic chain. In our opinion this discrepancy is not surprising since in their experiments the pial arter-
cal data (5) indicate the existence of sympathetic perivascular nerves at pial arteries, but under what conditions are these nerves activated? From studies using stimulation, denervation, and alpha-receptor blockers, it has been suggested that the tone of cerebral arteries may be influenced by sympathetic constrictor nerves under conditions such as changes in arterial PCO₂ (26-28) or blood pressure (26) or during subarachnoidal hemorrhage or experimental spasm (12, 29, 30).

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References

10. d’ALECY LG: Sympathetic cerebral vasocostruction blocked by adrenergic alpha receptor antagonists. Stroke 4:30-37, 1973
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