Endothelium-Dependent and -Independent Responses to Vasodilators of Isolated Dog Cerebral Arteries

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We compared responses to calcium ionophore A23187, vasopressin, and substance P in helical strips of dog middle cerebral, basilar, and posterior communicating arteries to obtain a better understanding of humoral control of cerebrovascular tone in different brain regions and its potential impact on mechanisms of cerebral vasospasm. A23187 relaxed these different arterial strips partially precontracted with prostaglandin F$_2$α to a similar extent. Vasopressin produced concentration-dependent relaxation in basilar and posterior communicating arterial strips, whereas middle cerebral arterial strips either contracted or relaxed slightly. Relaxations induced by A23187 and vasopressin were either abolished or converted to contractions by removal of the endothelium. In contrast, the relaxation of cerebral arterial strips to substance P was markedly attenuated but not abolished by endothelium denudation; the remaining relaxation was suppressed by indomethacin. In some cerebral arterial strips with intact endothelium, substance P caused a transient contraction that was reversed to a relaxation by indomethacin or ONO-3708, a prostaglandin antagonist. In arterial strips denuded of endothelium from the same dogs, substance P always produced relaxations. Relaxations of cerebral arterial strips to A23187 and vasopressin appear to be mediated by endothelium-derived relaxing factor. The function of vasopressin receptors in endothelial cells differs markedly in basilar and posterior communicating arteries versus middle cerebral arteries. Substance P-induced relaxations appear to be primarily associated with endothelium-derived relaxing factor and with prostaglandin I$_2$, whereas contractions appear to be mediated by endothelium-derived prostaglandins. (Stroke 1988;19:1388-1394)
Materials and Methods

Forty-five mongrel dogs of either sex weighing 8–15 kg were anesthetized with 50 mg/kg i.p. thiopental sodium and were killed by bleeding from the common carotid arteries; the brains were rapidly removed. Middle cerebral and basilar arteries and posterior portions of the circle of Willis (posterior communicating arteries) were isolated from the brains and were helically cut into strips approximately 20 mm long. The arterial strips were fixed vertically between hooks in a muscle bath containing a modified Locke-Ringer solution of the following millimolar composition: 120 NaCl, 5.5 KCl, 2.2 CaCl₂, 1.0 MgCl₂, 25.0 NaHCO₃, and 5.6 dextrose, maintained at 37 ± 0.3°C and aerated with 95% O₂ and 5% CO₂. The hook anchoring the upper end of the arterial strips was connected to the lever of a force-displacement transducer (Nihon-kohden Kogyo Co., Tokyo, Japan). Resting tension was adjusted to 1.5 g, which is optimal for inducing maximal contraction.¹⁷ Before the start of the experiments, all arterial strips were allowed to equilibrate for 90–120 minutes in the solution, which was replaced every 10–15 minutes.

Isometric contractions and relaxations were displayed on an ink-writing oscillograph (Sanei Sokki Co., Tokyo). Contraction to 30 mM K⁺ was measured, then the arterial strips were repeatedly washed and equilibrated for 30 minutes. Arterial strips were partially contracted with 3 × 10⁻⁷ to 3 × 10⁻⁴ M PGF₂α before the addition of vasodilator agents; contractions ranged between 25% and 35% of that induced by 30 mM K⁺. The concentration–response relations for vasopressin and A23187 were obtained by adding the agents directly to the solution in cumulative concentrations. However, substance P in single experiments because of tachyphylaxis. At the end of each experiment, 10⁻⁴ M papaverine was added to obtain maximal relaxation.¹⁸ The agonist-induced relaxation relative to that induced by papaverine and the contraction relative to that induced by 30 mM K⁺ are presented in the text and figures.

The endothelium was removed by gently rubbing the intimal surface of the arterial strips with a cotton pellet. Unrubbed arterial strips from the same dogs were compared. Preservation or removal of the endothelium was verified by the AgNO₃ staining method.¹⁹ Results are expressed as mean ± SEM and were compared using Student’s paired and unpaired t tests and Tukey’s method after one-way analysis of variance. The agents used were substance P, [Arg⁸]-vasopressin (Protein Research Foundation, Osaka, Japan), calcium ionophore A23187 (C.H. Boehringer Ingelheim Ltd., Elmsford, New York), indo methacin, methylene blue trihydrate (Nakarai Chemicals, Ltd., Kyoto, Japan), ONO-3708 [9,11],(11,12)-dideoxa-9a,11a-dimethylmethano-11,12-methano-13,14-dihydro-13-azo-14-oxo-15-cyclopentyl-16,17,18,19,20-pentanor-15-epithromboxane A₂ (Ono Pharmaceutical Co., Osaka), PGF₂α, tris(hydroxy-

Results

The addition of 10⁻¹⁰ to 10⁻⁸ M substance P produced a concentration-dependent relaxation in two middle cerebral, two basilar, and one posterior communicating arterial strips partially contracted with 3 × 10⁻⁷ to 3 × 10⁻⁶ M prostaglandin F₂α. The concentration–response relations obtained from the different arteries were similar and therefore were combined to generate one curve (Figure 1). Maximal relaxation was obtained at 10⁻⁸ or 10⁻⁷ M substance P; increasing the concentration to 10⁻⁶ M resulted in contraction. Removal of the endothelium markedly suppressed the relaxation. Middle cerebral, basilar, and posterior communicating arterial strips with intact endothelium responded to 10⁻⁷ M substance P with similar magnitudes of relaxation, 59.8 ± 3.6% (n = 12), 69.7 ± 2.9% (n = 12), and 64.8 ± 3.0% (n = 10), respectively.

Repeated applications of 10⁻⁷ M substance P altered the pattern of response in one of seven middle cerebral, three of nine basilar, and two of seven posterior communicating arterial strips; that is, relaxation was elicited by the first trial, and after
the second or third trial, $10^{-7} \text{M}$ substance P produced a phasic contraction followed by relaxation. The remaining 17 (six middle cerebral, six basilar, and five posterior communicating) arterial strips responded only with relaxation of similar magnitudes during repeated trials. Maximal relaxation was obtained $0.65 \pm 0.08$ minutes ($n=14$) after the addition of $10^{-7} \text{M}$ substance P, and the initial level of tension was slowly restored in 5–12 minutes. The relaxation in endothelium-denuded arterial strips developed more slowly than in intact arterial strips; the peak response was obtained $1.71 \pm 0.18$ minutes ($n=14$, $p<0.001$) after the addition of $10^{-7} \text{M}$ substance P.

In the 17 arterial strips responding only with relaxation, effects of indomethacin and methylene blue on substance P–induced relaxation were investigated. When endothelium was present, treatment with $10^{-6} \text{M}$ indomethacin failed to significantly reduce the relaxation (Figure 2) but tended to slow the recovery of tension from the maximally relaxed level; recovery half times before and after indomethacin treatment were $2.76 \pm 0.40$ and $3.83 \pm 0.50$ minutes, respectively ($57.0 \pm 19.5\%$ prolongation, $n=14$, $p<0.02$). Relaxation was markedly attenuated by additional treatment with $10^{-5} \text{M}$ methylene blue (Figure 2, left). In contrast, relaxation obtained in endothelium-denuded arterial strips was significantly suppressed by treatment with $10^{-6} \text{M}$ indomethacin alone (Figure 2, right).

In six (one middle cerebral, three basilar, and two posterior communicating) arterial strips with intact endothelium from six separate dogs in which phasic contractions became dominant during repeated applications of $10^{-7} \text{M}$ substance P, the response was reversed to relaxation by treatment with $10^{-6} \text{M}$ indomethacin and $10^{-7} \text{M}$ ONO-3708, an antagonist to vasoconstrictor PGs (Figure 3). In the endothelium-denuded arterial strips obtained from these same dogs, $10^{-7} \text{M}$ substance P always produced relaxations during repeated applications. The addition of $10^{-8}$ and $10^{-7} \text{M}$ A23187 caused a concentration-dependent relaxation in arterial strips partially contracted with PGF$_{2\alpha}$. There was no significant difference in the magnitudes of relaxation in the middle cerebral, basilar, and posterior communicating arteries (Figure 4). Relaxation was abolished almost completely by removal of the endothelium.

**FIGURE 2.** Bar graph of effects of $10^{-6} \text{M}$ indomethacin with (filled bar) and without $10^{-5} \text{M}$ methylene blue (shaded bars) on relaxation of dog cerebral (six middle cerebral, six basilar, and five posterior communicating) arterial strips with (left) and without (right) endothelium in response to $10^{-7} \text{M}$ substance P after partial contraction with $3 \times 10^{-7}$ to $3 \times 10^{-6} \text{M}$ prostaglandin F$_{2\alpha}$. Relaxation induced by $10^{-4} \text{M}$ papaverine was taken as 100%; mean±SEM absolute values in left are $311\pm 27$ (n=17), $317\pm 23$ (n=17), and $344\pm 51$ (n=10) mg, respectively; values in right are $273\pm 27$ (n=17) and $287\pm 24$ (n=17) mg. *Significantly different from control (open bars) and indomethacin-treated strips (left) and from control (right), $p<0.001$.

**FIGURE 3.** Modification of contractile response to $10^{-7} \text{M}$ substance P (SP) in dog basilar arterial strips partially contracted with $1$ to $2 \times 10^{-7} \text{M}$ prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) or $0.1$ to $1.2 \times 10^{-8} \text{M}$ serotonin by application of $10^{-6} \text{M}$ indomethacin and $10^{-7} \text{M}$ ONO-3708. Right: arterial strip was partially contracted with serotonin (5HT) because of inhibition of contractile response to PGF$_{2\alpha}$ by ONO-3708. Horizontal bars, level before addition of PGF$_{2\alpha}$ or 5HT; PA, $10^{-4} \text{M}$ papaverine.
In basilar and posterior communicating arterial strips partially contracted with PGF$_{2\alpha}$, the addition of $10^{-10}$ to $10^{-8}$ M vasopressin caused concentration-dependent relaxations (Figure 5, middle and right). On the other hand, in seven of 12 middle cerebral arterial strips, the same doses of vasopressin produced concentration-dependent contractions despite the presence of endothelium. In the remaining five middle cerebral arterial strips, up to $10^{-8}$ M vasopressin produced only relaxation, which, however, was significantly less than that in the basilar and posterior communicating arterial strips (Figure 5, left). Increasing the concentration of vasopressin to $10^{-7}$ M produced a contraction from the maximally relaxed level. Relaxation induced in the posterior communicating arterial strips by vasopressin was abolished by removal of the endothelium, and relaxations in the middle cerebral and basilar arterial strips were reversed to slight contractions (Figure 5). The concentration-dependent contraction seen in seven middle cerebral arterial strips with intact endothelium was not influenced by endothelium denudation (Figure 5, left).

To confirm the different responses of middle cerebral arterial strips, the effects of substance P, A23187, and vasopressin were compared in the
same preparations from 16 middle cerebral, 16 basilar, and 14 posterior communicating arterial strips. Relaxations caused by 10⁻⁷ M substance P and 10⁻⁸ and 10⁻⁷ M A23187 in these arterial strips did not differ significantly. However, 10⁻¹⁰ to 10⁻⁸ M vasopressin relaxed only six of 16 middle cerebral arterial strips but relaxed all 16 basilar and all 14 posterior communicating arterial strips in a dose-dependent manner. Maximal relaxation in these middle cerebral arterial strips (21.5 ± 6.5% at 10⁻⁸ M vasopressin) was <50% of that in the basilar and posterior communicating arterial strips (60.3 ± 4.2% and 55.5 ± 4.4% at 10⁻⁸ M vasopressin, respectively). In the other 10 middle cerebral arterial strips, 10⁻¹⁰ to 10⁻⁸ M vasopressin caused a concentration-dependent contraction (42.3 ± 7.4% contraction at 10⁻⁸ M vasopressin) that was not influenced by treatment with 10⁻⁶ M indomethacin (n = 5) or 10⁻⁷ M ONO-3708 (n = 4).

Discussion

Our study demonstrates that substance P produced a concentration-dependent relaxation in helical strips of dog cerebral arteries partially contracted with PGF₁α that was markedly inhibited by endothelium denudation and treatment with methylene blue, a guanylate cyclase inhibitor. Removal of the endothelium markedly attenuated but did not abolish substance P–induced relaxation. The remaining relaxation after endothelium denudation was abolished by application of indomethacin, a cyclooxygenase inhibitor. Our findings suggest that the relaxation of dog cerebral arteries in response to substance P is mainly mediated by EDRF and vasodilator PGs, such as PG1₂, produced in smooth muscle cells.

During repeated applications, 10⁻⁷ M substance P caused contraction in some basilar arterial strips. The contractile response depended on the endothelium and was reversed to relaxation by treatment with indomethacin or ONO-3708. Contractions of dog cerebral arteries caused by arachidonic acid and PGH₂ depend on the endothelium, suggesting that vasoconstrictor PGs are synthesized primarily in the endothelium in cerebral arteries. Involvement of thromboxane A₂ in the contractile response is excluded because of the inability of OKY-046, a thromboxane synthase inhibitor, to attenuate the response. Therefore, substance P produces basilar artery contractions, possibly by releasing vasoconstrictor PGs from endothelial cells. The findings obtained so far suggest the involvement of three mechanisms underlying the response of cerebral arteries to substance P (Figure 6): substance P releases EDRF to produce a rapid relaxation (A), releases vasoconstrictor PGs such as PGF₂α, PGE₂, PGD₂, and PGA₂ to produce a transient contraction (B), and releases vasodilator PGs, possibly PG1₂, to produce a slow and small relaxation (C). Substance P does not always act as a cerebral vasodilator, and its effect appears to be modulated by functions of the endothelium, which are impaired by oxyhemoglobin.

A23187 relaxed dog middle cerebral, basilar, and posterior communicating arterial strips in a similar pattern and to similar magnitudes. Since the relaxation was abolished almost completely by removal of the endothelium, A23187 does not seem to act directly on smooth muscle cells. A23187 also produces relaxations that depend on the endothelium in rabbit aortas and in guinea pig pulmonary arteries. The relaxation induced by A23187 in isolated dog basilar arteries is not affected by indomethacin. Therefore, the A23187-induced relaxation appears to be mediated exclusively by EDRF.

Concentrations of vasopressin in plasma and cerebrospinal fluid obtained from patients with subarachnoid hemorrhage or from cats with experimental subarachnoid hemorrhage induced by injection of blood into the subarachnoid space are increased. In our study, vasopressin relaxed basilar and posterior communicating arterial strips with intact endothelium, whereas vasopressin produced a contraction in 10 of 16 middle cerebral arterial strips and a slight relaxation in the remaining six. The concentration-dependent contraction caused by vasopressin in middle cerebral arterial strips was endothelium-independent and was not influenced by indomethacin or ONO-3708, indicating that vasopres-
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