Pharmacological comparison of endothelium-dependent relaxation in isolated cerebral and extracerebral arteries


Department of Neurological Surgery, University of Virginia School of Medicine, Charlottesville, Virginia

Endothelium-dependent relaxation was induced by acetylcholine (ACh), adenosine triphosphate (ATP), and thrombin in isolated cerebral and extracerebral arteries obtained from rabbits and dogs. Using an isometric tension-recording method, the authors then examined the difference in the extent of relaxation between the cerebral and extracerebral arteries. In rabbits, the dose-response curve of the basilar artery for ACh was significantly different (p < 0.05) from curves of the femoral and common carotid arteries. The IC50 value (the concentration inducing a one-half inhibition of the initial contractile tone) for the basilar artery in ACh-induced relaxation was significantly higher (p < 0.05) than for the common carotid artery, although the mean maximum relaxation of the basilar artery to ACh was not significantly different from that seen in extracerebral arteries. The relaxing effect of ACh in dogs was much less in the middle cerebral and basilar arteries than in the common carotid, vertebral, and femoral arteries. On the other hand, both ATP (in rabbits and dogs) and thrombin (in dogs) induced significantly more (p < 0.05) relaxation in the cerebral arteries than in the extracerebral arteries.

Endothelium-dependent relaxation induced by ACh or ATP has been demonstrated in a wide range of arteries from a variety of animals. The present results suggest that ATP has a more important role than ACh in the regulation of the vascular tone of the major cerebral arteries in these two species.

Key Words • endothelium dependent relaxation • cerebral artery • extracerebral artery • acetylcholine • adenosine triphosphate • thrombin • dog • rabbit

Intracranial cerebral arteries respond differently to vasoconstrictor and vasodilator agents than do extracerebral arteries. Studies comparing the reactivity of isolated arteries with and without endothelium have demonstrated that the endothelial cells play an obligatory role in the relaxing effect of many vasodilators including acetylcholine (ACh), adenosine triphosphate (ATP), and thrombin. In rabbit aortae precontracted by norepinephrine, it has been reported by Furchgott and Zawadzki that ACh produced a dose-dependent relaxation when administered in concentrations ranging from $10^{-8}$ M to $10^{-6}$ M. Half-maximal relaxation occurred between $3 \times 10^{-8}$ M and $10^{-7}$ M. On the other hand, in a study of rabbit basilar arteries precontracted by serotonin, ACh evoked relaxation over the range of concentration of $10^{-7}$ M to $10^{-4}$ M, with half-maximal relaxation occurring between $10^{-6}$ M and $3 \times 10^{-6}$ M. Thus, there seems to be a marked difference in the reactivity of cerebral and extracerebral arteries to vasoactive agents that can induce endothelium-dependent relaxation.

It has recently been suggested that impairment of endothelium-dependent relaxation of the major cerebral arteries following subarachnoid hemorrhage may be involved in the pathogenesis of cerebral vasospasm. Cerebral vasospasm appears to be different from vasospasm seen in the coronary circulation. For a better understanding of the pathogenesis of cerebral vasospasm, it is important to investigate the difference in endothelium-dependent relaxation between the major cerebral and the extracerebral arteries. The present experiments were designed to examine this difference in vitro in arteries obtained from rabbits and dogs.

Materials and Methods

Artery Preparation and Tension Recording

Adult mongrel dogs of either sex, weighing 13 to 18 kg each, and New Zealand White male rabbits, weighing
Endothelium-dependent relaxation in cerebral and extracerebral arteries

### TABLE 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Rabbit Arteries</th>
<th>Canine Arteries</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basilar</td>
<td>Common</td>
</tr>
<tr>
<td>no. of specimens</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>outer diameter (mm)</td>
<td>0.7-0.8</td>
<td>1.4-1.6</td>
</tr>
<tr>
<td>basal tension (gm)</td>
<td>0.4</td>
<td>3.0</td>
</tr>
<tr>
<td>response to 10^{-3} M serotonin (gm)*</td>
<td>0.78 ± 0.1</td>
<td>4.6 ± 0.3</td>
</tr>
</tbody>
</table>

* Data for response to serotonin are expressed as the mean value ± standard error of the mean. For further explanation see text.

2.8 to 3.3 kg each, were anesthetized with sodium pentobarbital (30 mg/kg) and sacrificed by exsanguination from the femoral arteries. The basilar, common carotid, and femoral arteries were obtained from the rabbits, and the middle cerebral, basilar, common carotid, extracranial vertebral, and femoral arteries were obtained from the dogs. The canine vertebral artery was excised from the subclavian artery after the thorax was opened. Each artery was placed in a dissecting chamber filled with a modified Krebs bicarbonate solution (millimolar composition: NaCl 120, KCl 4.5, MgSO4 1.0, NaHCO3 27.0, KH2PO4 1.0, CaCl2 2.5, and dextrose 10.0). After removal of arachnoid membrane or loose connective tissue, arterial ring segments were cut to 3 mm in length for the rabbit specimens and to 4 mm long for the canine specimens. The outer diameters of these arteries are shown in Table 1.

Ring specimens were suspended between L-shaped stainless steel rods in an organ bath with a 10-ml working volume and gassed with 95% O2/5% CO2. The pH of the bathing solution ranged from 7.4 to 7.5. The preparations were allowed to equilibrate at 37°C for 90 minutes before use. In order to standardize the data for endothelium-dependent relaxation in arteries of different anatomical origin, the optimal basal tension for each artery was determined in preliminary experiments from a tension-strength curve for 10^{-5} M of serotonin. The basal tension that elicited nearly maximal contraction was used as the optimal tension (Table 1). Preliminary experiments also demonstrated that 10^{-5} M of serotonin induced submaximal contraction of each artery both in rabbits and in dogs. Contractile force was recorded isometrically using a force-displacement transducer and was displayed on a polygraph.*

The contractile response to 40 mM of KCl was first obtained on each arterial segment, then each segment was washed repeatedly. After confirming a consistent contractile response to KCl, experiments were begun. For relaxation studies, submaximal tone was induced with 10^{-5} M of serotonin, then ACh, ATP, or thrombin was added in an incremental fashion. The relaxation induced by these agents was expressed as a percentage of the tonic phase of contraction induced by 10^{-5} M of serotonin. Prostaglandin (PG) F2α, was also used in the present experiments in order to obtain a good tonic contraction in canine middle cerebral and basal arteries, and to investigate possible differences in endothelium-dependent relaxation due to agonist difference. In the relaxation studies with ATP, 10^{-3} M of 8-phenylthiophylline, an adenosine antagonist, was used to pretreat the arterial rings 5 minutes before application of 10^{-5} M of serotonin or 10^{-5} M of PGF2α, to exclude the effect of endothelium-dependent relaxation by adenosine. In some preparations, the obligatory role of the endothelium in the relaxation was reconfirmed. The endothelial cells were removed by gentle rubbing with a polyethylene tube after drying the lumen with a 95% O2/5% CO2 gas mixture or by rubbing the intimal surface with a wooden rod.8,10

**Drugs**

Serotonin (5-hydroxytryptamine), ACh chloride, ATP, bovine thrombin, papaverine, PGF2α, and 8-phenylthiophylline were obtained commercially.† To make stock solutions, all drugs (except serotonin) were dissolved in distilled water. They were then diluted in Krebs solution before use, such that volumes less than 0.1 ml were added to the organ bath. Serotonin was dissolved in 0.1 N HCl with 0.1% ascorbic acid.

**Statistical Analysis**

The data were expressed as mean values ± standard error of the mean. Statistical analysis of the dose-response curves of the relaxation induced by ACh, ATP, or thrombin was performed using the general linear models procedure with the SAS (Statistical Analysis System) computer program; Scheffe’s test was used for subgroup analysis. Multiple comparisons of the initial contractile response to 10^{-5} M of serotonin or 10^{-5} M of PGF2α, in arterial rings of the same anatomical origin, and of the maximal relaxation of the arteries from


† Drugs obtained from Sigma Chemical Co., St. Louis, Missouri.
FIG. 1. Effect of acetylcholine (ACh) and adenosine triphosphate (ATP) on contraction of rabbit basilar (A to C) and common carotid arteries (D to F) induced by 10^{-5} M of serotonin (5-HT). Acetylcholine evoked dose-dependent relaxation in the arterial rings with intact endothelium (A and D) but not in those without endothelium (B and E). Adenosine triphosphate also induced dose-dependent relaxation in the rings with endothelium (C and F). Pap = 10^{-6} M papaverine.

different anatomical origins, were done by Scheffé's test after analysis of variance. Dose-response curves and IC_{50} values were calculated using probit analysis with the SAS computer program. In the present experiments, IC_{50} values were defined as concentrations causing a one-half inhibition of the initial contractile tone. The values were considered to be significantly different if p was less than 0.05.

**Results**

**Initial Contractile Tone Induced by Serotonin**

Serotonin (10^{-5} M) induced submaximal long-lasting tonic contraction in the extracerebral arteries from both species. However, in the cerebral arteries, tonic contraction by serotonin was not as prolonged, demonstrating a gradual spontaneous decrease. The initial contractile force induced by 10^{-5} M of serotonin is shown in Table 1. There were no significant differences in the mean initial contractile tone of each artery between the studies with ACh and ATP in rabbits, and among the studies with ACh, ATP, and thrombin in dogs.

**Endothelium-Dependent Relaxation in Rabbit Arteries**

Acetylcholine (10^{-8} M to 10^{-4} M) evoked dose-dependent relaxation in the basilar, common carotid, and femoral arteries precontracted by 10^{-5} M of serotonin (Figs. 1A and D, and 2). In rings without endothelium, ACh caused no significant changes in tension (Fig. 1B and E). The mean maximum relaxation induced by ACh was not significantly different among these three arteries at approximately 90% (Table 2). However, the dose-response curve of the basilar artery for ACh was significantly different (p < 0.05) from curves of the femoral and common carotid arteries, and the IC_{50} value for the basilar artery was significantly higher (p < 0.05) than that for the common carotid arteries (Table 2).

Adenosine triphosphate (10^{-7} M to 10^{-4} M) also induced dose-dependent relaxation in the basilar, common carotid, and femoral arteries (Figs. 1C and F, and 2). The mean maximum relaxation of the basilar artery induced by ATP was significantly higher (p < 0.05) than for the common carotid or femoral arteries. The IC_{50} value for the basilar artery was the lowest among those three arteries (Table 2).

**Endothelium-Dependent Relaxation in Canine Arteries**

Acetylcholine (10^{-8} M to 10^{-4} M) produced good dose-dependent relaxation in every preparation of the common carotid, vertebral, and femoral arteries precontracted by 10^{-5} M of serotonin (Fig. 3). However,
Endothelium-dependent relaxation in cerebral and extracerebral arteries

**FIG. 2.** Dose-response relationships of rabbit arteries to acetylcholine (ACh, left) and to adenosine triphosphate (ATP, right). Data are expressed as percentages of the contraction induced by $10^{-5}$ M of serotonin (5-HT). Vertical bars indicate 2 standard errors of the mean. Twelve preparations were used for each artery.

**FIG. 3.** Dose-response relationships of canine arteries to acetylcholine (ACh, left) and to adenosine triphosphate (ATP, right). Data are expressed as percentages of the contraction induced by $10^{-5}$ M of serotonin (5-HT). Vertical bars indicate 2 standard errors of the mean. Twelve preparations were used for each artery. Specimens: MCA = middle cerebral artery; BA = basilar artery; VA = vertebral artery; CCA = common carotid artery; FA = femoral artery.
FIG. 4. Effect of acetylcholine (ACh), adenosine triphosphate (ATP), and thrombin on contraction of canine middle cerebral arteries induced by $10^{-5}$ M of serotonin (5-HT) (A to C) and on contraction of canine basilar arteries induced by $10^{-5}$ M of prostaglandin (PG) $F_{2\alpha}$ (D and E). Acetylcholine induced little relaxation (A, D, and E), whereas ATP and thrombin evoked relaxation in a dose-dependent manner both in rabbits and dogs (B to E). Pap = $10^{-4}$ M papaverine.

### TABLE 2

*Mean maximum relaxation and ICso value in rabbits*

<table>
<thead>
<tr>
<th>Artery</th>
<th>ACh-Induced Relaxation</th>
<th>ATP-Induced Relaxation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MMR (%)</td>
<td>ICso (M)</td>
</tr>
<tr>
<td>basilar</td>
<td>87.4 ± 1.4</td>
<td>4.6 × $10^{-6}$ (1.6–13.8)</td>
</tr>
<tr>
<td>common carotid</td>
<td>87.5 ± 2.0</td>
<td>3.8 × $10^{-7}$ (0.8–13.3)</td>
</tr>
<tr>
<td>femoral</td>
<td>89.6 ± 2.0</td>
<td>1.0 × $10^{-4}$ (0.4–35.3)</td>
</tr>
</tbody>
</table>

* Data for the mean maximum relaxation (MMR) are expressed as the mean value ± standard error of the mean: 12 preparations from different animals were used for each artery with each vasodilator. The ICso value is defined as the concentration that gives a one-half relaxation of the initial contractile tone induced by $10^{-5}$ M of serotonin; 95% confidence limits are shown in parentheses. Significance: † = p < 0.05 vs. basilar; ‡ = p < 0.01 vs. basilar. ACh = acetylcholine; ATP = adenosine triphosphate.

### TABLE 3

*Mean maximum relaxation in canine arteries*

<table>
<thead>
<tr>
<th>Artery</th>
<th>Mean Maximum Relaxation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ACh-Induced Relaxation</td>
</tr>
<tr>
<td>middle cerebral</td>
<td>19.7 ± 3.3</td>
</tr>
<tr>
<td>basilar</td>
<td>18.5 ± 4.3</td>
</tr>
<tr>
<td>common carotid</td>
<td>89.8 ± 2.0</td>
</tr>
<tr>
<td>vertebral</td>
<td>80.3 ± 2.0</td>
</tr>
<tr>
<td>femoral</td>
<td>85.0 ± 2.0</td>
</tr>
</tbody>
</table>

* Data are expressed as mean values ± standard error of the mean: 12 preparations from different animals were used for each artery with each vasodilator. ACh = acetylcholine; ATP = adenosine triphosphate.
Endothelium-dependent relaxation in cerebral and extracerebral arteries

in the middle cerebral and basilar arteries, ACh induced only minor relaxation or, sometimes, no relaxation at all (Fig. 4A). The dose-response curves of these cerebral arteries were significantly different (p < 0.01) from those of the extracerebral arteries.

Low concentrations of ATP induced relaxation in vessels precontracted by 10^{-5} M of serotonin for all types of canine arteries studied. At higher concentrations, however, serotonin evoked contraction in the middle cerebral and basilar arteries (Figs. 3 and 4B). The mean maximum relaxations of the cerebral arteries were significantly higher (p < 0.05) than those of the extracerebral arteries (Table 3).

FIG. 5. Dose-response relationships of canine arteries to thrombin. Data are expressed as percentages of the contraction induced by 10^{-5} M of serotonin (5-HT). Vertical bars indicate 2 standard errors of the mean. Twelve preparations were used for each artery. CCA = common carotid artery; VA = vertebral artery; BA = basilar artery; MCA = middle cerebral artery.

Thrombin (0.1 to 1.0 U/ml) also produced a dose-dependent relaxation followed by slight contraction in middle cerebral, basilar, common carotid, and vertebral arteries precontracted by 10^{-3} M of serotonin (Figs. 4C and 5). There were significant differences (p < 0.05) in the dose-response curves between cerebral and extracerebral arteries. The mean maximum relaxations of the cerebral arteries were significantly higher (p < 0.01) than those of the extracerebral arteries (Table 3).

Prostaglandin F_2, produced submaximal tonic contractions of the middle cerebral and basilar arteries. There was no significant difference in the initial contractile tone between the studies with ACh and ATP. Acetylcholine failed to evoke good relaxation in these two cerebral arteries precontracted by 10^{-5} M of PGF_2 (Figs. 4D and E, and 6). On the other hand, both ATP and thrombin induced good relaxation in those arteries, with contractions at higher concentrations.

Discussion

The present study demonstrates a difference in endothelium-dependent relaxation induced by ACh, ATP, and thrombin between cerebral and extracerebral arteries, both in rabbits and in dogs. In rabbits, the dose-response curve of the basilar artery for ACh was significantly different (p < 0.05) from curves of the femoral and common carotid arteries, and the IC_{50} value for basilar artery in ACh-induced relaxation was significantly higher (p < 0.05) than that for the common carotid artery, although the mean maximum relaxation produced by ACh was not significantly different for the basilar artery or these extracerebral arteries. The relaxing effect of ACh on canine vessels was much less in both the middle cerebral and basilar arteries than in the

FIG. 6. Dose-response relationships of canine cerebral arteries to acetylcholine (ACh, left) and to adenosine triphosphate (ATP, right). Data are expressed as percentages of the contraction induced by 10^{-5} M of prostaglandin (PG) F_2. Vertical bars indicate 2 standard errors of the mean. Twelve preparations were used for each artery. BA = basilar artery; MCA = middle cerebral artery.

J. Neurosurg. / Volume 69 / October, 1988
common carotid, vertebral, or femoral arteries. On the other hand, both ATP (in rabbits and dogs) and thrombin (in dogs) induced significantly more potent relaxation in the cerebral arteries than in the extracerebral arteries. The difference between ACh-induced relaxation and relaxations induced by ATP or thrombin in canine cerebral arteries seems to be independent of the vasoconstrictor agents used to produce the initial contractile tone, since the same results were obtained from the PGF_{2α}-induced contraction.

De Mey and Vanhoutte have reported that extracerebral arteries of different anatomical origin respond dissimilarly to the same pharmacological agent that can induce endothelium-dependent relaxation. They revealed that the maximum relaxation induced by ACh, ATP, or thrombin is different in isolated canine femoral, pulmonary, saphenous, and splenic arteries.

It is known that cerebral arteries respond differently to vasoconstrictor and vasodilator agents than do extracerebral arteries. Several years before the essential role of the endothelium in vasorelaxation was suggested by Furchgott and Zawadzki, Toda reported that relaxation by ACh in spiral strips of canine basilar artery is much less than that seen in canine coronary and mesenteric arteries. The present study has confirmed these results.

A difference in thrombin-induced relaxation between cerebral and extracerebral arteries has been previously documented by De Mey, et al. They reported that 1.0 U/ml of thrombin caused approximately a 60% relaxation of canine femoral arteries, whereas approximately a 75% relaxation was observed in canine basilar arteries at the same thrombin dose. In the present experiments, 1.0 U/ml of thrombin elicited relaxations of approximately 40% to 50% and 90%, respectively, in canine extracerebral and cerebral arteries. Thus, the cerebral vessels studied consistently showed more sensitivity to the relaxing effects of thrombin than did extracerebral arteries. The difference between the degree of thrombin-induced relaxation seen in the present study and that reported by De Mey, et al., may be attributed to different experimental conditions.

The mechanisms underlying the differences in the responsiveness of cerebral and extracerebral arteries to ACh, ATP, or thrombin are unclear at present. The differences may exist at the level of the endothelium, the vascular smooth muscle, or both. It is now well established that the endothelial cells produce and release a relaxing factor (endothelium-dependent relaxing factor, EDRF) or factors in response to activation of specific receptors located on the endothelium itself. Acetylcholine induces relaxation via muscarinic receptors, and ATP does so via P_{2}-purinoceptors. A difference in the responsiveness of the endothelium of cerebral and extracerebral arteries could be due to variations in the number or affinity of receptors or to variations in the quantity or type of EDRF produced following a given stimulus. Alternatively, the sensitivity of smooth-muscle cells to an EDRF may be different between cerebral and extracerebral arteries. In the latter case, as suggested by the present data, the EDRF induced by ACh must be different from that elicited by ATP or thrombin. If the EDRF induced by ACh is the same as those induced by ATP or thrombin, the degree of relaxation by ACh should eventually be the same as that produced by ATP or thrombin in a given artery. Although Palmer, et al., have recently reported that an EDRF may be nitric oxide, it is still unclear that the EDRF induced by ACh is the same as that induced by ATP or thrombin. Further studies are needed to determine which mechanism is responsible for the present results.

The role of endothelium-dependent relaxation in the physiological or pathophysiological modulation of cerebrovascular tone is poorly understood. Both ACh and ATP evoke endothelium-dependent relaxation in a wide range of arteries from a variety of animal species. Cholinergic activity of the cerebral arteries has been reported. However, it is unlikely that ACh released from periartrial nerves diffuses all the way through the media and acts on endothelial cells to release EDRF. Acetylcholine and ATP are likely to be released close to their site of action, since they are rapidly degraded in the circulation. Parnavas, et al., have demonstrated the immunocytochemical localization of choline acetyltransferase in about 10% of the endothelial cells of small blood vessels and capillaries of rat visual cortex. Moreover, they suggested that the release of ACh following damage to endothelial cells during ischemia contributes to a pathophysiological mechanism of vasodilation. They postulated that this response protects that segment of vessel as well as distally located cerebral tissue from further hypoxic injury. It is uncertain at present, however, that the concentration of ACh in the vicinity of endothelial receptors could become sufficiently high.

Alternatively, studies demonstrate that it is likely that the main source of intraluminal ATP is endothelial cells and that, during pathological conditions such as ischemia and hypoxia, its release can be measured in amounts likely to activate endothelial P_{2}-purinoceptors. Adenosine triphosphate can also be released during intravascular platelet aggregation.

The present study demonstrates that ATP evoked significantly more potent relaxation in major cerebral arteries than did ACh. Together with the findings mentioned above, the present results suggest that, as far as major cerebral arteries of rabbit and dog are concerned, ATP has a more important role in the regulation of vascular tone than does ACh. The role of impaired endothelium-dependent relaxation of the major cerebral arteries in the pathogenesis of cerebral vasospasm has been suggested. However, it is still unclear which vasoactive agent that can induce endothelium-dependent relaxation is important in the regulation of the cerebrovascular tone.

586 J. Neurosurg. / Volume 69 / October, 1988
Endothelium-dependent relaxation in cerebral and extracerebral arteries

Based upon the present results it is likely that, in the pathogenesis of cerebral vasospasm, impairment of ATP-induced relaxation following subarachnoidal hemorrhage probably plays a more important role than that of ACh-induced relaxation. In light of significant interspecies differences, as again demonstrated in the present study, extrapolation of these results to human cerebrovascular physiology must be performed with caution.

Acknowledgments

The authors thank Mrs. Sarah Hudson for technical assistance and Mrs. Lucille Staiger for manuscript preparation.

References

20. Toda N: The action of vasodilating drugs on isolated basilar, coronary and mesenteric arteries of the dog. J Pharmacol Exp Ther 191:139-146, 1974