Endothelium-Dependent Contractions to Calcium Ionophore A23187, Arachidonic Acid, and Acetylcholine in Canine Basilar Arteries

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The effects of the calcium ionophore A23187, arachidonic acid, and acetylcholine were studied in isolated canine basilar arteries. Rings with and without endothelium were suspended for isometric tension recording in physiological saline. In unstimulated rings, A23187, arachidonic acid, and acetylcholine caused endothelium-dependent contractions. The contractions of rings caused by uridine 5'-triphosphate were not affected by removal of the endothelium. An inhibitor of cyclooxygenase, indomethacin (10⁻⁴ M), prevented excitatory responses to A23187, arachidonic acid, and acetylcholine but did not alter contractions caused by KCl. An inhibitor of thromboxane synthetase, dazoxiben (10⁻⁴ M), significantly reduced endothelium-dependent contractions to A23187 and arachidonic acid but did not significantly affect contractions caused by acetylcholine. These results demonstrate that A23187, arachidonic acid, and acetylcholine cause excitatory endothelium-dependent responses in canine cerebral blood vessels by increasing the release of product(s) of cyclooxygenase from endothelial cells; in the case of A23187 and arachidonic acid, thromboxane A₂ contributes to the endothelium-dependent contractions. (Stroke 1988;19:476–479)

In most peripheral arteries the Ca²⁺ ionophore A23187, arachidonic acid, and acetylcholine cause endothelium-dependent relaxations. However, acetylcholine causes endothelium-dependent contractions in the aorta of spontaneously hypertensive rats and in the pulmonary artery of rabbits. Likewise, in isolated veins of dogs, arachidonic acid causes increases in tension that are prevented by the removal of the endothelium. Endothelium-dependent contractions to A23187 have been observed in the aorta of turtles. In the canine basilar artery both endothelium-dependent relaxations and contractions can be evoked. In this artery, acetylcholine does not evoke endothelium-dependent relaxations, and arachidonic acid causes endothelium-dependent contractions. These findings prompted our present study, in which we compared the endothelium-dependent responses of the canine basilar artery to the Ca²⁺ ionophore A23187, to arachidonic acid, and to acetylcholine.

Materials and Methods

The experiments were performed on 4-mm-long rings of basilar arteries taken from dogs (20–30 kg) anesthetized with 30 mg/kg i.v. sodium pentobarbital. The arteries were placed in physiological saline (control solution; millimolar composition: 118.3 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄, 25.0 NaHCO₃, 0.26 CaEDTA, and 11.1 glucose). In certain rings, the endothelium was removed mechanically by gentle rubbing of the intimal surface with a 31-gauge stainless steel wire. Each ring was connected to a Gould UTC-2 force transducer (Cleveland, Ohio) and suspended in an organ chamber filled with 25 ml control solution (37° C, pH 7.4) gassed with 95% O₂-5% CO₂. Isometric force was continuously recorded.

The rings cut from the basilar arteries were allowed to stabilize at a resting tension of 200–400 mg for 1 hour. This was followed by another 1-hour period of equilibration. In each ring the responsiveness to 10⁻⁵ M uridine 5'-triphosphate (UTP) was tested several times until stabilization. The functional integrity of the endothelium was checked by the presence of relaxation induced by vasopressin.

Concentration–response curves for A23187, arachidonic acid, and acetylcholine were obtained in a cumulative fashion. The preparations were washed at least three times with 25 ml control solution and allowed to equilibrate for 30 minutes after each exposure to vasoactive substances.

Drugs

The following pharmacological agents were used: A23187 (Sigma Chemical Co., St. Louis, Missouri), arachidonic acid (Sigma), acetylcholine chloride (Sigma), indomethacin (Sigma), dazoxiben (Pfizer Inc., New York, New York), sodium pentobarbital (Fort Dodge Laboratories, Fort Dodge, Iowa), and UTP (Sigma). Drugs were dissolved in distilled water such that volumes of <0.2 ml/dose were added to the organ chambers. Stock solutions of 10⁻³ M indomethacin were prepared in equal molar concentrations of Na₂CO₃. The stock solution of 10⁻⁴ M A23187 was prepared in 1.5 × 10⁻⁴ M diethylsulfate. The solvents (Na₂CO₃ and diethylsulfate) did not affect unstimulated rings of the basilar arteries (data not shown). The concentrations of drugs are expressed as final molar bath concentrations.
**Statistical Analysis**

The data are expressed as mean ± SEM; n refers to the number of dogs. Statistical comparisons between responses of rings from the same artery with or without endothelium or in the presence and absence of indomethacin or dazoxiben were made using Student's t test for paired comparisons. Probability values of <0.05 were considered to be significant.

**Results**

In unstimulated rings of the canine basilar artery, A23187, arachidonic acid, and acetylcholine cause concentration-dependent contractions. Acetylcholine-induced contractions were preceded by transient relaxations. Removal of endothelium abolished the contractions induced by A23187 (Figure 1) and reversed the contractions induced by arachidonic acid (not shown) and acetylcholine (Figure 2). Removal of endothelium did not affect the contractions evoked by UTP (EC50 = 3.2 ± 0.8 × 10^{-6} and 5.1 ± 0.6 × 10^{-6} M for rings with and without endothelium, respectively; n = 6). Contractions induced by A23187, arachidonic acid, and acetylcholine were reproducible after 30 minutes (data not shown).

In rings with endothelium, the inhibitor of cyclooxygenase indomethacin did not affect resting tension (1.5 ± 0.1 and 1.7 ± 0.2 g before and after exposure for 40 minutes, respectively; n = 6). It abolished endothelium-dependent contractions induced by A23187 (Figure 3) and arachidonic acid (Figure 4) and reversed acetylcholine-induced contractions to relaxations (Figure 5).

Dazoxiben (10^{-4} M), a thromboxane synthetase inhibitor, did not affect resting tension (2.2 ± 0.5 and 2.7 ± 0.4 g before and after exposure for 30 minutes, respectively; n = 6). It significantly reduced the endothelium-dependent contractions to A23187 (Figure 3) and arachidonic acid (Figure 4) but did not affect those evoked by acetylcholine (Figure 5).

**Discussion**

Our present experiments demonstrate that A23187 and acetylcholine contract canine basilar artery only if endothelium is present and confirm that this is the case also for arachidonic acid. Removal of endothelium did not affect contractions caused by UTP, indicating that the damage to vascular smooth muscle during mechanical removal of endothelium is not responsible for the abolition of contraction caused by A23187, arachidonic acid, and acetylcholine. Maxi-
mal response obtained with A23187 represents 50% of the maximal response to 60 mM KCl. The amplitude of the contractions evoked by arachidonic acid and acetylcholine was smaller than that evoked by the Ca²⁺-ionophore. However, arachidonic acid and acetylcholine caused relaxations of the preparations without endothelium. Hence, it is logical to assume that the direct inhibitory effect on vascular smooth muscle explains the smaller endothelium-dependent contractions that these two agents cause.

The inhibitory effect of indomethacin on endothelium-dependent contractions could be due to calcium entry blockade. However, the concentration of indomethacin used did not affect the contractile responses to KCl and 5-hydroxytryptamine, which are dependent on the influx of extracellular calcium. The abolition of the endothelium-dependent contractions induced by A23187, arachidonic acid, and acetylcholine by the inhibition of cyclooxygenase suggests that these agents activate the metabolism of arachidonic acid through the cyclooxygenase pathway in endothelial cells. These cells in turn release a prostanoid, which then causes contraction of vascular smooth muscle. The presence of products of cyclooxygenase (prostacyclin, prostaglandins, and thromboxane A₂) has been demonstrated in the vascular wall of canine peripheral and cerebral arteries. Furthermore, it has been shown that prostaglandins P₂, E₂, and D₂ and thromboxane A₂ cause contractions of the canine basilar artery.

Endothelium-dependent contractions caused by cyclooxygenase products other than prostacyclin have been observed in response to acetylcholine in veins of dogs, aortas of spontaneously hypertensive rats, and pulmonary arteries of rabbits. In the canine basilar artery, endothelium-dependent contractions to stretch also are prevented by indomethacin. Unlike in the pulmonary artery of rabbits, the endothelium-dependent contractions induced by acetylcholine were not altered by dazoxiben, an inhibitor of thromboxane synthetase, indicating that the release of thromboxane A₂ was not responsible for the response to acetylcholine. By contrast, the reduction of the endothelium-dependent effect of A23187 and arachidonic acid by dazoxiben suggests that these contractions are partly due to the release of thromboxane A₂ from the endothelial cells.

Our present study demonstrates that substances that inhibit endothelium-dependent responses in peripheral arteries induce excitatory endothelium-dependent responses in canine cerebral arteries. The physiologic and pathologic importance of the heterogeneous endothelium-dependent behavior of peripheral and cerebral arteries remains to be established.

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References


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