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Endothelin action on goat cerebral arteries

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Abstract—Cumulative application of endothelin-1 (human) markedly constricted goat isolated cerebral arteries in a concentration-dependent manner. Contractile responses were not affected by removal of endothelial cells. Removal of extracellular calcium or addition of the calcium channel blocker nicardipine (10^{-7} M) failed to abolish responses to endothelin. The results suggest that the endothelium-independent constriction of cerebral arteries produced by endothelin cannot be explained solely by voltage-dependent calcium channels. The contractile responses are likely to be mediated by stimulation of specific receptors for this peptide.

By releasing endothelium-derived relaxing and constrictor substances (Furchgott & Zawadzki 1980; Yanagisawa et al 1988) the endothelium plays a key role in the local regulation of vascular tone as well as in the response of vascular smooth muscle to neurohumoral agents (Furchgott 1984). Endothelin, a vasoconstrictor peptide discovered and sequenced by Yanagisawa et al (1988), is secreted by endothelial cells in response to various stimuli. Endothelin contracts vascular strips and produces a long-lasting increase in blood pressure in various species (Yanagisawa et al 1988; Miller et al 1989). On the other hand, the effects of endothelin are not limited to vasoconstriction, since experiments in-vivo and in-vitro indicate that endothelin can produce dilation through the release of endothelium-derived relaxing factor (EDRF) (de Nucci et al 1988; Lippton et al 1988).

The possible involvement of endothelin in the local regulation of cerebral blood flow has not been determined. Recent observations have shown that endothelin-1 (ET-1) induces basilar artery contraction in-vivo when applied intracisternally but not when given intraluminally. It was concluded that the endothelium forms a barrier which impedes the access of endothelin to the smooth muscle cells (Shigeno et al 1989). The present experiments were designed to investigate the contractile properties of endothelin on goat isolated cerebral arteries with special emphasis on endothelium-dependent responses as well as extracellular calcium requirements.

Materials and methods

Twelve female goats, 30 to 40 kg, were anaesthetized with 2% sodium thiopental i.v. and killed by injecting a saturated solution of potassium chloride. The brain was removed and both middle cerebral arteries were carefully dissected and cut into cylindrical segments 4 mm in length and approximately 500 μm in outside diameter. Two stainless-steel pins, 150 μm in diameter, were introduced through the arterial lumen. One pin was fixed to the organ bath wall while the other was connected to a strain gauge. The recording system included a Universal transducing cell (UC3), a Statham microscale accessory (U15) and a Beckman type RS recorder. Each arterial segment was set up in a 4 mL bath containing modified Krebs-Henseleit solution with the following composition (mM): NaCl 115; KCl 4·6; KH_2PO_4 1·2; $CaCl_2$ 2·5; $NaHCO_3$ 25; glucose 11·1; and disodium EDTA 0·01. The solution was equilibrated with 95% oxygen and 5% carbon dioxide to give a pH of 7·3 to 7·4. Temperature was held at 37°C. Arterial segments were equilibrated at a passive tension of 1 g for 2 h. Calcium-free medium was prepared by substituting $CaCl_2$ with 1 mM EGTA.

In some experiments the endothelium was removed mechanically by inserting a roughened stainless steel wire into the lumen and gently rolling the vessel segment with a wetted filter paper. After each experiment the arteries were carefully opened flat and stained with $AgNO_3$ following the procedure described by Caplan & Schwartz (1973) to visualize the endothelium. Only results from vessels with more than 70% of the endothelium were considered as control segments. Vessels in which the endothelium had been removed never showed more than 5% of their intima covered with endothelium either before or after the experiment.

Data are expressed as means ± s.e. Statistical evaluation of the results was made by means of Student's *t*-test. $P < 0.05$ was considered significant.

The drugs used were: endothelin-1-human (Scientific Marketing Associates, London), and nicardipine (Ferrer Internacional).

Results

Cumulative application of endothelin produced a sustained constrictor response which was concentration-dependent (Fig. 1). The maximal tension developed, as well as the concentration of endothelin producing half-maximal contraction (EC50) were similar ($P > 0.05$) in arteries with and without endothelium (Table 1). The maximal tension induced by endothelin was 114% of the respective maximal contraction induced by KCl 60 mM. There was no significant difference ($P > 0.05$) in the contraction of arteries with and without endothelium to the addition of KCl. The EC50 values were similar to those

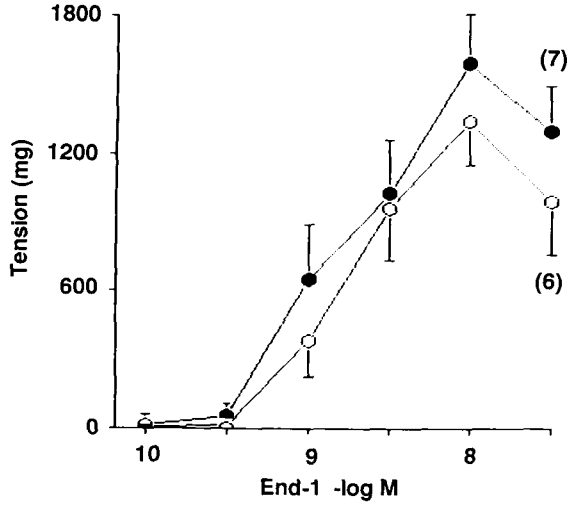

FIG. 1. Concentration-response curves for endothelin determined in goat cerebral arteries with (●) and without (○) endothelium. Values are means ± s.e. Number of experiments are given in parentheses.

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previously reported for vasopressin (Lluch et al 1984) and lower than the values for 5-hydroxytryptamine and noradrenaline (Urquilla et al 1975) in the same vascular preparations.

Endothelin also caused strong contractions in calcium free medium containing 1 mM EGTA and in normal medium containing the calcium antagonist nicardipine (Fig. 2). The differences in the maximal tensions developed, as well as the relative order of potencies, expressed quantitatively in terms of the EC50 values, were not significant ($P > 0.05$, Fig. 3, Table 1).

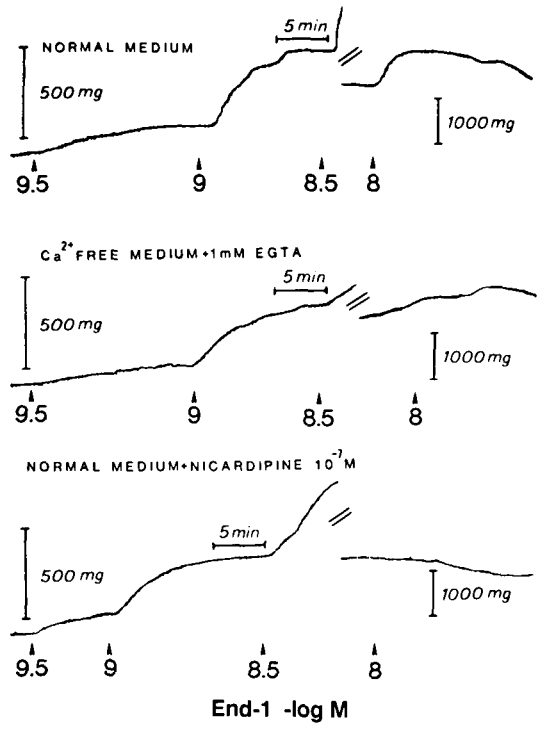

FIG. 2. Representative recordings of contraction produced by endothelin in goat cerebral artery incubated in normal medium, in calcium-free medium, and in normal medium containing nicardipine 10^{-7} M. The break in the tension recordings indicates a change in scale.

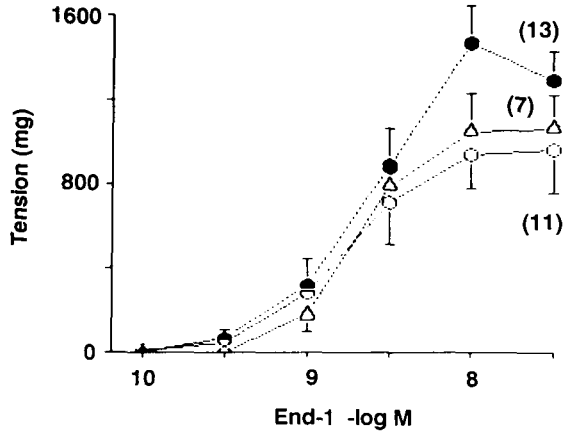

FIG. 3. Concentration-response curves for endothelin determined in goat cerebral arteries incubated in normal medium (●), in calcium-free EGTA medium (○), and in normal medium containing nicardipine 10^{-7} M (△). Values are means ± s.e. Number of experiments are indicated in parentheses.

Table 1. Maximal responses and EC50 values of endothelin in goat cerebral arteries.

	EC50 Values (M)	Maximal responses (mg ± s.e.)
With endothelium (n = 7)	2.0×10^{-9} (6.1×10^{-10}–6.4×10^{-9})	1600 ± 172
Without endothelium (n = 6)	1.7×10^{-9} (7.6×10^{-10}–3.4×10^{-9})	1340 ± 154
With Ca^{2+} (n = 13)	1.8×10^{-9} (9.8×10^{-10}–3.4×10^{-9})	1480 ± 145
Without Ca^{2+} (n = 11)	1.7×10^{-9} (9.4×10^{-10}–3.2×10^{-9})	976 ± 230
With Nicardipine 10^{-7} M (n = 7)	2.3×10^{-9} (1.9×10^{-9}–2.7×10^{-9})	1085 ± 173

EC50 values are expressed as geometric means according to Fleming et al (1972). n, number of experiments. Numbers in parentheses, 95% confidence interval.

Discussion

Studies designed to examine the influence that endothelin and endothelin receptors might have in the regulation of cerebral blood flow are still scanty. Recent observations indicate that removal of the endothelium attenuates the constrictor effects of endothelin in cat cerebral arteries (Edvinsson et al 1989) whereas in human and rat cerebral arteries (Hardebo et al 1989) the response was not altered by endothelium removal. The present experiments demonstrate that endothelin, a vasoconstrictor peptide derived from endothelial cells, is a potent agonist for cerebral smooth muscle contraction. The maximal tensions attained are higher than those of KCl-induced contraction. It appears that endothelin is one of the most potent cerebral vasoconstrictors known. The endothelin-induced contraction is not affected by the presence of endothelial cells indicating that vasodilator agents secreted by endothelial cells under basal, spontaneous conditions (Martin et al 1986) do not appear to counteract the local effects of endothelin. Therefore, it is likely that the contractile effects of endothelin are due to direct stimulation of vascular smooth muscle. This endothelium-independent contraction has also been observed in rabbit isolated aortic rings (Marsden et al 1989). In contrast, the vasoconstriction induced by endothelin in the rat isolated perfused mesentery is significantly potentiated by removal of the endothelial cells (Warner et al 1989) or by the presence of haemoglobin or methylene blue, two reported inhibitors of EDRF activity on vascular smooth muscle (Martin et al 1985). These findings would indicate that the release of EDRF in the perfused mesentery can limit the vasopressor response to endothelin.

The contraction of some vascular preparations induced by endothelin is inhibited in a calcium-free medium (Yanagisawa et al 1988; Borges et al 1989). Furthermore, previous reports indicate that calcium antagonists produce different degrees of the endothelin-induced contraction (Hardebo et al 1989; Saito et al 1989). Although these observations are in agreement with the idea that endothelin is an agonist for dihydropyridine-sensitive calcium channels in the smooth muscle cells, it is possible that endothelin could initially stimulate a specific receptor which would lead to the activation of calcium channels and smooth muscle contraction (Marsden et al 1989). The present experiments in goat cerebral arteries indicate that the contraction produced by endothelin is independent of calcium influx. Endothelin induces a slow tonic contraction in a calcium-free

medium or after addition of the calcium antagonist nicardipine. These results support similar findings in rat aortic rings indicating that the contractile response to endothelin has two components, one independent of calcium influx and the other dependent on calcium influx (Auguet et al 1988). This latter component is only partially inhibited by calcium channel blockers, indicating that the action of endothelin may not be limited to opening of a calcium channel. In rabbit aortic strips endothelin also causes vasoconstriction in the absence of extracellular calcium (Sugiura et al 1989). There appears to be substantial differences in the effects and mechanism of action of endothelin among different species which may be relevant in determining the pathophysiological significance of this peptide.

Autoradiographic studies with [^{125}I]endothelin-1 indicate that putative endothelin-1 receptors are located in brain blood vessels of various species including man (Hoyer et al 1989). The localization of binding sites in brain vessels is consistent with the contractile effects observed in the present study and suggests a direct action of endothelin on specific receptors.

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