Role of endothelium and calcium channels in endothelin-induced contraction of human cerebral arteries

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Endothelin constricted human isolated cerebral arteries in a concentration-dependent manner. The maximal tension developed, as well as EC_{50} values were similar in arteries with and without endothelium. Removal of extracellular calcium or addition of the calcium antagonist nicardipine (10^{-6} M), attenuated but did not abolish responses to endothelin. These experiments show that the endothelin-induced contraction in human cerebral arteries is not linked to the presence of intact endothelial cells. The data also show that the contractile effects of endothelin cannot be explained solely by an action on voltage-dependent calcium channels.

Introduction Endothelin, a peptide secreted by endothelial cells in response to various stimuli, contracts vascular strips and produces a long-lasting increase in blood pressure (Yanagisawa *et al.*, 1988). With regard to the cerebral vessels, recent observations have shown that endothelin-1 contracts canine and feline basilar arteries when applied intracisternally but not when is given intraluminally (Shigeno *et al.*, 1989). It was concluded from these experiments that the endothelium forms a barrier which impedes the access of endothelin to the smooth muscle cells. The present experiments were designed to elucidate the effects of endothelin on human isolated cerebral arteries with special emphasis on endothelium-dependent responses as well as extracellular calcium requirements.

Methods Human cerebral arteries were obtained during autopsy of 12 patients (8 men and 4 women, aged 23–79 years) who had died 4 to 12 h before. The cause of death was variable: 4 were victims of car accidents, 5 died from myocardial infarction and 3 from cancer of the stomach. Cylindrical segments (4 mm in length and 0.7–1 mm in outside diameter) were cut from branches of the middle cerebral artery for isometric recording of tension. Each arterial segment was set up in a 4 ml bath containing Krebs-Henseleit solution of the following composition (mM): NaCl 115, KCl 4.6, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, glucose 11.1 and disodium EDTA 0.01, equilibrated with 95% O_2 and 5% CO_2 to give a pH of 7.3 to 7.4. Temperature was held at 37°C. To select resting tension for maximal force development, the arterial segments were exposed repeatedly to 60 mM KCl. Basal tension was increased gradually until contractions were maximal. The optimal resting tension was 1 g. The vessels were allowed to attain the steady level of tension during a 2 h accommodation period before testing. 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 on a damp filter paper. This procedure removed more than 90% of the endothelial cells as observed from silver-stained whole-mount sections for light microscopy (Caplan & Schwartz, 1973). Functional integrity of the endothelium was confirmed by the presence of relaxation induced by acetylcholine (10^{-6} M) during contraction obtained with prostaglandin $F_{2\alpha}$ (3×10^{-6} M). The following drugs were used: endothelin-1-human (Scientific Marketing Associates); nicardipine (Ferrer International); acetylcholine chloride (Sigma) and prostaglandin $F_{2\alpha}$ (Sigma).

Data are expressed as means $\pm$ s.e.mean and were analyzed by Student's *t* test. A probability value less than 0.05 was considered as significant.

Results Cumulative application of endothelin produced a sustained constrictor response which was concentration-dependent (Figure 1a). Maximal tensions developed were of similar size in arteries with and without endothelium: 2589 ± 434 and 2104 ± 323 mg ($P > 0.05$). Geometric mean EC_{50} values (95% confidence interval) were of comparable magnitude ($P > 0.05$) in arteries with and without endothelium: 1.1×10^{-9} M (4.5×10^{-10} – 2.4×10^{-9} M) and 6.9×10^{-10} M (3.5×10^{-10} – 1.3×10^{-9} M). There was no significant difference ($P > 0.05$) in the contraction of arteries with and without endothelium to the addition of KCl 60 mM (2312 ± 151 versus 1972 ± 216 mg). Removal of extracellular calcium or addition of nicardipine (10^{-6} M) to the baths 10 min before the endothelin concentration-response curve was determined, reduced significantly ($P < 0.05$) the maximal response to endothelin to about 20 and 35%, respectively (Figure 1b).

Figure 1 (a) Dose-response curves for endothelin-1 in human cerebral arteries with (○) and without (●) endothelium. (b) Dose-response curves for endothelin-1 in arteries incubated in normal medium (○), in calcium-free EGTA medium (●), and in normal medium containing nicardipine 10^{-6} M (□). Values are means with s.e.mean shown by vertical bars. Number of experiments are indicated within parentheses.

Discussion The present experiments demonstrate that endothelin, a vasoconstrictor peptide derived from endothelial

cells, is a potent agonist for cerebral smooth muscle contraction. The EC_{50} values are similar to those previously obtained for vasopressin (Lluch *et al.*, 1984) and lower than the values for 5-hydroxytryptamine, angiotensin II and noradrenaline (Conde *et al.*, 1981) in the same vascular preparations. The endothelin-induced contraction is not affected by the presence of endothelium 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 interpretation is consistent with the endothelium-independent contraction observed in human and rat cerebral arteries (Hardebo *et al.*, 1989). In contrast, in canine and feline cerebral arteries, the endothelium attenuates this contraction (Shigeno *et al.*, 1989; Edvinsson *et al.*, 1989).

The contraction of some vascular preparations induced by endothelin is completely inhibited in a calcium-free medium (Yanagisawa *et al.*, 1988). Furthermore, a previous report indi-

cates that maximal endothelin-induced contractions in human isolated cerebral arteries are reduced to 15% in the presence of nimodipine 10^{-8} M (Hardebo *et al.*, 1989). The present experiments in human cerebral arteries indicate that the contraction produced by endothelin is, at least in part, independent of calcium influx since this peptide induced a slow tonic contraction in a calcium-free medium or after the addition of the calcium antagonist nifedipine 10^{-6} M. These results support previous experiments 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.

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