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Contractile Response of Human Omental Arteries to Endothelin

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Abstract—The effects of endothelin have been studied in isolated arterial segments (0·8–1 mm in external diam.) of human omental arteries obtained during the course of abdominal operations (15 patients, 7 men and 8 women). Paired segments, one normal and the other de-endothelized, were mounted for isometric recording of tension in organ baths. Endothelin produced concentration-dependent contractions with an EC50 value of $5{\cdot}4 \times 10^{-9}$ M. Removal of endothelium did not affect significantly endothelin-induced contractions (EC50, $6{\cdot}7 \times 10^{-9}$ M). Removal of extracellular calcium or addition of the calcium channel blocker nicardipine (10^{-6} M) diminished but did not abolish responses to endothelin. These results indicate that endothelin exerts powerful contractile effects on human isolated omental arteries which are independent of the presence of an intact endothelial cell layer; this contraction cannot be explained solely by voltage-dependent calcium channels.

The release by the endothelium of endothelium-derived relaxing and constrictor factors (Furchgott & Zawadzki 1980; De Mey & Vanhoutte 1982; Hickey et al 1985) suggests a complex role of the endothelium in the regulation of vascular tone as well as in the response of vascular smooth muscle to neurohumoral agents (Furchgott 1984; Vanhoutte et al 1986). Endothelin, a peptide secreted by endothelial cells in response to various stimuli (Yanagisawa et al 1988), contracts vascular strips and produces a long-lasting increase in blood pressure in various animal 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).

Whether the pharmacological intervention of endothelin observed in various species can be extrapolated to human vessels of different regions remains to be determined. Recent observations show that the constriction of human isolated omental (Hughes et al 1989) and rat mesenteric (D'Orleans-Juste et al 1989) arteries induced by endothelin is not abolished by calcium channel blockers or removal of extracellular calcium. On the other hand, it appears that the constriction elicited in human and rat cerebral arteries is particularly sensitive to calcium channel blockers (Hardebo et al 1989). Thus there appear to be substantial differences in the effects and mechanism of action of endothelin among different vascular regions and species which may be relevant in determining the pathophysiological significance of this peptide. The present experiments were designed to elucidate the effects of endothelin on human isolated omental arteries with special emphasis on endothelium-dependent responses as well as extracellular calcium requirements.

Materials and Methods

Arterial segments were taken from portions of human omentum during the course of abdominal operations (15 patients, 7 men and 8 women, aged 30–79 years). The specimens were immediately placed in chilled Krebs–Henseleit solution and transported from the hospital to the laboratory. Only macroscopically normal vascular segments were used. Cylindrical segments (3–4 mm in length and 0·8–1 mm in outside diam.) were cut for isometric recording of tension. 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. 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; gluocse 11·1; and disodium EDTA 0·01. The solution was equilibrated with 95% O_2–5% CO_2 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 rubbed by inserting a roughened stainless-steel wire into the lumen and gently rolling the vessel segment with a wetted filter paper. Functional integrity of the endothelium was confirmed by the presence of relaxation induced by acetylcholine (10^{-7}–10^{-6} M) during steady contractions obtained with noradrenaline (10^{-6}–3×10^{-6} M). Arterial rings with an intact endothelium relaxed 66 ± 4% in response to 10^{-6} M acetylcholine. This relaxation was absent in arteries in which the endothelium had been mechanically removed. After each experiment the arteries were carefully opened flat and stained with $AgNO_3$ to visualize the endothelium (Caplan & Schwartz 1973). 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.

Concentration-response curves for endothelin were determined in a cumulative manner and control and experimental responses were obtained from separate vascular preparations. The calcium antagonist nicardipine was added to the organ bath 10 min before the concentration-response curve to endothelin was determined.

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To determine if endothelin induced significant tachyphylaxis, in a separate group of experiments a single concentration of endothelin was added to each arterial segment (4 segments for each concentration); the segments were washed and vascular tone was allowed to return to basal levels. Repeated addition (up to three times) of endothelin at intervals of 20 to 30 min elicited contractions similar to those obtained in the first trial.

The EC50 values (concentrations of endothelin producing half-maximal contraction) were determined from individual concentration-response curves by non-linear regression analysis, and from these values the geometric means for EC50 with 95% confidence intervals were calculated (Fleming et al 1972).

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

The following drugs were used: acetylcholine chloride, noradrenaline bitartrate (Sigma Chemical Co., USA), endothelin-1-human (Scientific Marketing Associates, London, UK), and nicardipine (Ferrer International, Spain).

Results

Cumulative applications of endothelin produced a constrictor response which was concentration-dependent. Fig. 1A shows the effects of increasing concentrations of endothelin on one arterial segment with intact endothelium and one arterial segment with endothelium removed and Fig. 1B summarizes the results obtained from all the experiments. The maximal tension developed, as well as the concentration producing half-maximal contraction (EC50) were similar in arteries with and without endothelium (Table 1). The maximal tension induced by endothelin was about 245% of the respective maximal contraction induced by 60 mM KCl. There was no significant difference in the contraction of arteries with and without endothelium to the addition of KCl (1135 ± 240 mg in arteries with endothelium vs 1149 ± 323 mg in arteries without endothelium).

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 55 and 59%, respectively (Fig. 2, Table 1).

Discussion

The present experiments demonstrate that endothelin, a vasoconstrictor peptide derived from endothelial cells, is a potent agonist for human omental arteries. The maximal tensions attained were higher than those of KCl-induced contraction and EC50 values observed were lower than those previously reported for noradrenaline (Mikkelsen et al 1979) in the same vascular preparations. The potent constrictor action of endothelin has also been reported in strips of human coronary arteries (Davenport et al 1989) and in human isolated subcutaneous and omental resistance vessels (Hughes et al 1989).

One main concern of our study was to examine the possible modulation by the endothelium of the endothelin-induced responses. Our experiments show that this contraction is not affected by the presence of endothelial cells, indicating that vasodilator substances secreted by endothelial cells under basal, spontaneous conditions (Martin et al 1986) do not appear to counteract the local effects of endothelin. This endothelium-independent contraction has also been observed in rabbit isolated aortic rings (Marsden et al 1989) and in cerebral arteries of man (Martin de Aguilera et al 1990) and goat (Vila et al 1990). 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). The reduced constriction observed in the latter experiments in vessels with endothelium may result from stimulation of putative endothelin receptors on the endothelial cells which would lead to release of EDRF and consequently limit the vasopressor response to endothelin

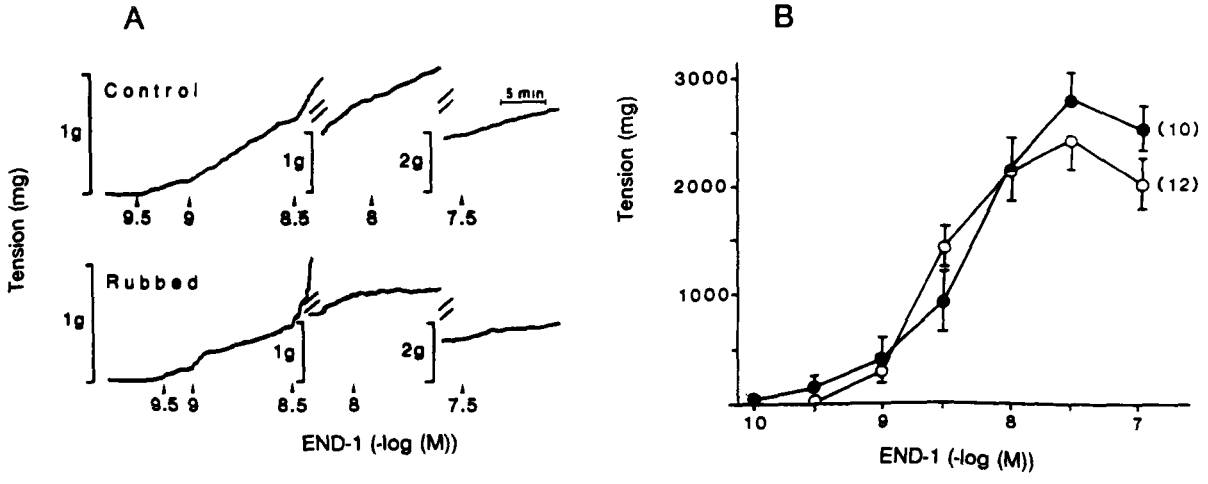

FIG 1. A. Actual recordings showing the effect of endothelin-1 (END-1) on one arterial segment with intact endothelium (control) and one arterial segment with endothelium removed. The break in the tension recordings indicates a change in the scale. B. Concentration-response curves for endothelin in control and rubbed arteries. Number of arterial segments are given within parentheses. Control, ●; rubbed, ○.

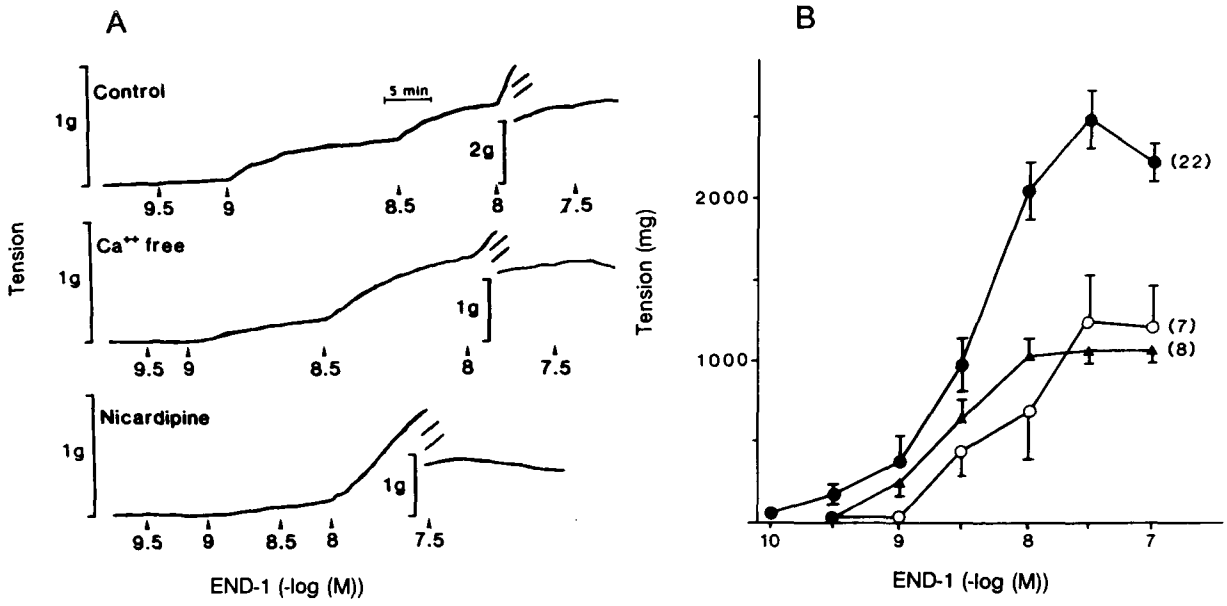

FIG. 2. A. Representative recordings of contraction produced by endothelin (END-1) in human omental arteries incubated in normal medium (control), in calcium-free EGTA medium, and in normal medium containing nicardipine 10^{-6} M. The break in the tension recordings indicates a change in scale. B. Concentration-response curves for endothelin determined in human omental arteries incubated in normal medium (control), in calcium-free EGTA medium, and in normal medium containing nicardipine 10^{-6} M. Number of arterial segments is indicated within parentheses. Control, ●; Ca^{2+} free, ○; nicardipine, ▲.

Table 1. Maximal responses and EC50 values of endothelin in human omental arteries.

	EC50 (M) values	Maximal responses (mg ± s.e.)
With endothelium (n = 10)	$5{\cdot}4 \times 10^{-9}$ ($2{\cdot}1 \times 10^{-9}$–$1{\cdot}4 \times 10^{-8}$)	2792 ± 222
Without endothelium (n = 12)	$6{\cdot}7 \times 10^{-9}$ ($3{\cdot}5 \times 10^{-9}$–$1{\cdot}3 \times 10^{-8}$)	2418 ± 274
Without Ca^{2+} (n = 7)	$1{\cdot}0 \times 10^{-8}$ ($3{\cdot}9 \times 10^{-9}$–$2{\cdot}7 \times 10^{-8}$)	1233 ± 300
With nicardipine 10^{-6} M (n = 6)	$3{\cdot}3 \times 10^{-9}$ ($1{\cdot}4 \times 10^{-9}$–$7{\cdot}5 \times 10^{-9}$)	1060 ± 85

n = Number of arterial segments. Numbers in parentheses, 95% confidence interval.

(Warner et al 1989). In the present experiments the possible release of EDRF was not investigated. Nevertheless, it appears that endothelin, in the doses used, has no significant effect on the endothelial release of EDRF in human vessels. The condition of the experiments and the type of artery used may be particularly relevant in the assessment of the vascular effects of endothelin, and emphasize the need for caution in extrapolating the effects from experimental animals to man.

The contraction of some vascular preparations induced by endothelin is inhibited by calcium-channel blockers such as nicardipine (Yanagisawa et al 1988) or nitrendipine (Borges 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 human mesenteric 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 after the addition of the calcium antagonist nicardipine (10^{-6} M). These results support recent experiments in human cerebral arteries (Martin de Aguilera et al 1990) indicating that the action of endothelium in these particular human vessels may not be limited to opening of a calcium channel. Autoradiographic studies with [^{125}I]endothelin-1 indicate that putative endothelin-1 receptors are located in vascular smooth muscle of various species including man (Hoyer et al 1989; Power et al 1989). The localization of binding sites in human vessels is consistent with the contractile effects observed in the present study being due to direct action of endothelin on specific receptors.

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