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Research Articles

Length-tension dependence in vascular smooth muscle: possible participation of endothelin

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Abstract. The results of experiments with isolated strips of rat portal vein, and of 'sandwich' experiments involving strips of the portal vein or aorta, revealed the involvement of endothelin in the development of myogenic reactions of the vascular smooth muscle, observed on distension of the vascular wall. Released by the endothelial cells, endothelin is capable of stimulating the contraction of these muscles.
Key words. Vascular smooth muscles; length-tension dependence; endothelin.

As was shown recently, the endothelium is a modulator of the vascular tone[1-3] and can participate in such basic vascular reactions as reactive and functional hyperemia[4-8]. It is obvious that the endothelium can take part in the realization of length-tension dependence in vascular smooth muscles by modulating the distension-related contractile reactions[9]. The endothelium participates in the vascular reactions via the biosynthesis and release of both vasodilator (prostacycline, nitric oxide), and vasoconstrictor substances (endothelin, thromboxan A2, platelet-activating factor, angiotensin II)[3,10,11]. These substances are released in response to various agonists and mechanical factors[1,12,13].

Apart from changes in pressure, which lead to the synthesis and release of nitric oxide, the mechanical factors which influence the endothelium include the distension of the vascular wall, resulting from a sharp increase in the intravascular pressure. Such cases are known to be accompanied by the inhibition of nitric oxide synthesis[14] and an increase of endothelin[15]. The endothelin is also capable of increasing the vascular tone via the activation of Ca^{2+} channels in the plasma membrane, mobilization of Ca^{2+} from the intracellular depot as a result of activation of phospholipase C, and the formation of inositol-1,4,5-triphosphate and an increase of the sensitivity of the contractile proteins to Ca^{2+} [11,16]. The purpose of the present study was to elucidate the possible involvement of endothelin in the modulation of length-tension dependence.

Methods

Albino Wistar rats aged 6–8 months weighing 150–200 g were used. They were killed by a blow to the head or by cervical dislocation.

Experiments were performed on intact and mechanically denuded preparations of the rat portal vein and the thoracic part of the aorta, 4–6 mm long and 1.0–1.5 mm wide, each weighing 2–3 mg. The vascular preparations were placed in the thermostatic perfusion cell (1 ml) and subjected to passive distension (2–3 mN). The preparations were perfused with Krebs' solution at 36.4-37 °C.

5 series of experiments were performed.

1) To study the length-tension dependence of the intact and denuded strips of the portal vein, 'sandwich' experiments were made to elucidate the involvement of biologically active substances of endothelial origin. The contractile activity of a 'recipient' strip was registered when an adjacent 'donor' was distended. Close contact was made between the vascular strips and intimal surfaces. The changes were compared with the shifts observed following the deendothelization of the donor strip.

2) and 3) Preparations of the portal vein and aorta were used as donors, whereas the recipients were strips of the portal vein.

4) and 5) A study was made of the effect of human endothelin (10^{-9} M), and of phosphoramidon ($3 \cdot 10^{-6}$ M), which is an inhibitor of the endothelin-converting enzyme[17-19], on the shape of the length-tension curve of intact strips of the portal vein.

The contractile activity of the smooth muscles was registered using a 6MX1C mechanoelectric tranducer in the mode close to isometric. The preparations were subjected to graded distension to 8–13 mN. After the registration of the values of the phasic contraction amplitude of the strip it was denuded, and the experimental procedure repeated again. A special series of experiments was performed to register the curve indices on freshly denuded vein strips. The endothelium was removed mechanically by rolling the preparation along

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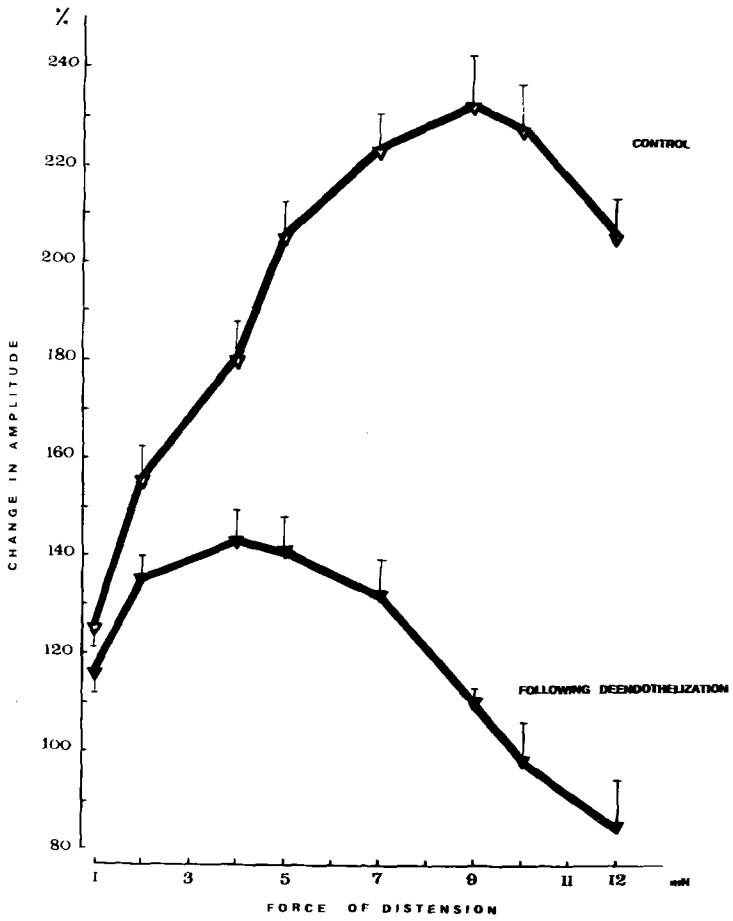

Figure 1. Changes in the amplitude of the phasic contractions of the portal vein strips after distension, with and without deendothelization. Abscissa, force of graded distension; ordinate, increment of the phasic contraction amplitude (% of initial level).

a piece of paper[1]. This method of deendothelization was found to be the most appropriate because the muscle layer and the inner elastic membrane remained intact. The level of deendothelization was controlled morphologically. Human endothelin-1 was from Novabiochem, Switzerland, and phosphoramidon from Sigma, USA. Data are presented as the mean +SEM. Differences with $p < 0.05$ were considered as significant.

Results and discussion

Additional distension of the intact strips of the portal vein (with an initial tension of 2–3 mN) resulted in an increase of the amplitude of phasic contractions of the vascular smooth muscles by $28 \pm 3\%$–$128 \pm 8\%$ above the initial level. When the distension force was increased to 9–10 mN the maximum increment of the amplitude was reached (fig. 1), with a subsequent fall at 12 mN. In deendothelized strips, the increase of the contractile activity with increasing distension force was less marked than in intact strips. The amplitude of the contractions reached a maximum more readily (at 4–5 mN), but the absolute values were much lower than those of the control. At 5–10 mN the denuded strips showed a decrease of the phasic contraction amplitude from the initial level.

The significant difference between the level of increase of the amplitude of phasic contractions of the intact and deendetholized strips of the portal vein ($p < 0.05$–$p < 0.01$) suggested the involvement of the endothelium, and of endothelium-synthesized biologically active substances, in the active myogenic reactions studied. To verify this possibility experiments were carried out with pairs of vessels or their fragments, with an intact or removed endothelium (sandwich experiments)[2,20]. In these experiments the distension of the donor vascular strip was accompanied by an increase of the phasic contraction amplitude of the recipient strip (fig. 2). Mechanical removal of the endothelium from the donor strip resulted in a decrease in the increment of the contraction amplitude of the recipient strip. This occurred when the preparations of either the portal vein (fig. 2A), or the aorta (fig. 2B) were used as the donor ($p < 0.01$).

The results of sandwich experiments revealed that the increase in the amplitude of phasic contractions of the

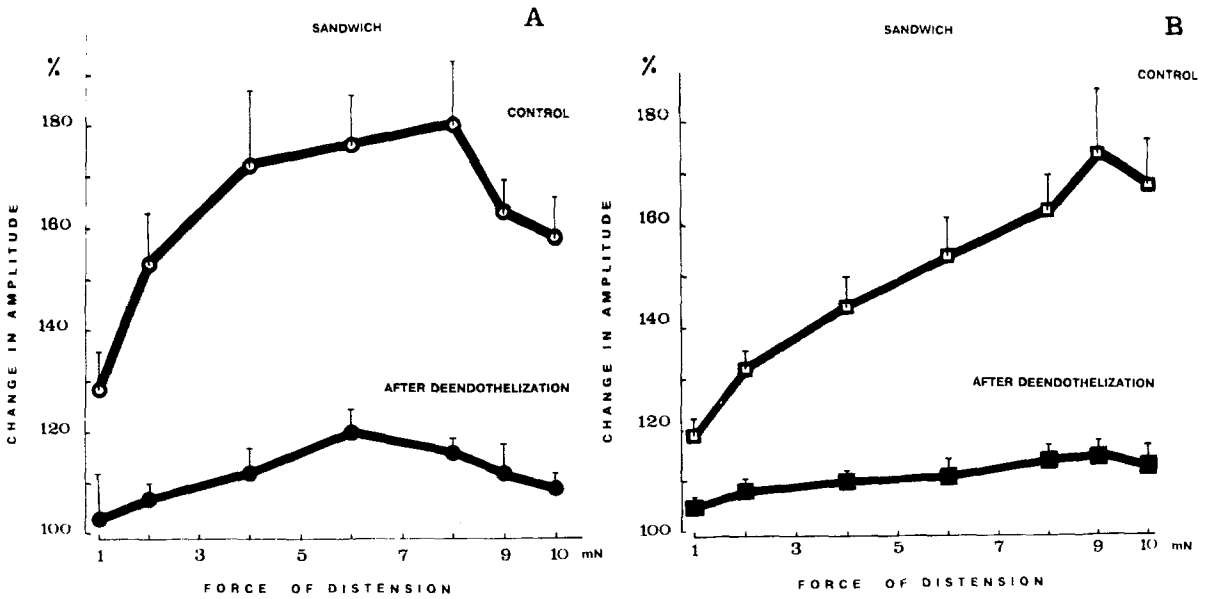

Figure 2. Effect of 'donor'-strip distension upon the phasic contraction amplitue of a 'recipient'-strip. *A* Donor and recipient strips both from the portal vein. *B* Donor strips of the aorta; recipient strips of the portal vein. Upper curve, donor control; lower curve, donor after deendothelization.

recipient strip was partly conditioned by biologically active substances released by the endothelium of the donor strip, which stimulated the contractile reactions of the recipient strips. As is evident from the pattern of changes that occur in the amplitude of phasic contraction of the recipient strip, the degree of distension of the donor strip affects the release of biologically active substances from its endothelium. These substances are capable of producing an inotropic effect on the contractile elements of the smooth muscle cells.

A number of factors are known to be synthesized by endothelial cells which affect the contraction of vascular smooth muscles (endothelins, endothelium-derived relaxing factor (EDRF) or nitric oxide, prostacyclin, platelet-activating factor (PAF), etc.). In view of their known activities, it may be assumed that endothelin or PAF are most likely to be responsible for the increase of the recipient strip phasic contractions, since they are potent vasoconstrictors acting via the stimulation of the contractile activity of the smooth muscle cells. Their effect is mainly conditioned by an increase influx of the external Ca^{2+} and by the level of release of Ca^{2+} from the intracellular depot[21]. However, an increase in the sensitivity of the contractile proteins of the smooth muscle cells to Ca^{2+} in the distension-related increase of the amplitude of phasic contractions of the portal vein should not be ruled out[22]. The contribution of this mechanism can be assessed from the reaction of the denuded strips. However, the rise of sensitivity of the contractile proteins of the smooth muscle cells to Ca^{2+} may result from the vasoconstrictor effect of the endothelial substances.

To confirm this notion a series of experiments was carried out, aimed at studying the effect of endothelin-1 and of phosphoramidon, which inhibits endothelin-converting enzyme, on the length-force relationship[19]. After 10 to 15 min of perfusion of the isolated portal vein preparations with a solution containing human endothelin-1 (10^{-9} M), the amplitude of phasic contractions of smooth muscles increased by $60 \pm 4.5\%$. The application of distending forces resulted in a more marked increase in the contractions. The maximum increment of the contraction amplitude was attained at 5 mN ($+153 \pm 14\%$ vs initial level) (fig. 3), and it should be noted that this increment remained high with forces up to 10–12 mN. The change of the length of the vascular preparation led to a steeper rise of the curve of phasic contraction amplitude (in % of initial level) versus the force of graded distension ($p < 0.02$). Thus, the perfusion with endothelin resulted in less distension being needed to be applied to the smooth muscle to produce a given level of contraction.

Phosphoramidon is known to inhibit the vasoconstriction induced by 'big' endothelin-1, and to decrease the arterial blood pressure in conscious rats with spontaneous hypertension[17–19]. In our experiments, by the 15th minute of perfusion of the portal vein strips with phosphoramidon-containing solution, the spontaneous contractile activity of the latter decreased by $23 \pm 5\%$. The increment of the amplitude of contractions of the vascular smooth muscles reached its maximum with an additional distension force of 7 mN ($+70 \pm 6\%$ vs initial level, $p < 0.001$).

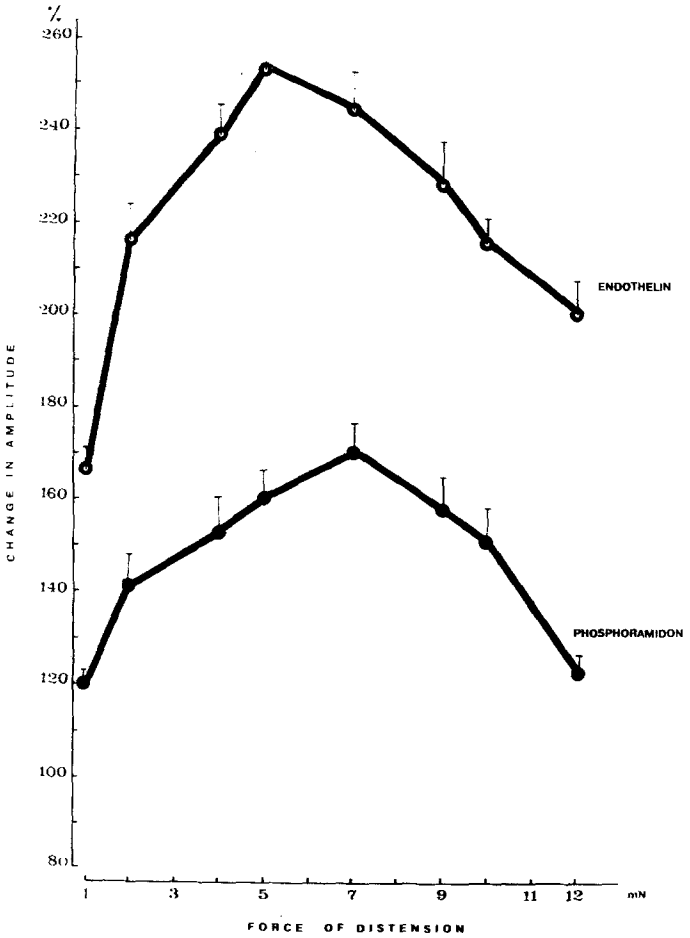

Figure 3. Changes in the amplitude of the phasic contractions of portal vein strips after distension; effect of endothelin (10^{-9} M) and effect of phosphoramidon (3×10^{-6} M).

A further increase of the distending force was accompanied by a gradual decrease of the increment of the amplitude of contractions of the vascular strips (fig. 3). It is possible that other endothelin-released biologically active substances, such as prostacyclin and nitric oxide, are less likely to be involved in the above reactions in view of their relaxing effect upon the vascular smooth muscles. In our experiments, the deendothelization of the vascular strips decreased the force of their contraction. At the same time, distension of the vascular wall, which occurs in hypertension, was accompanied by an inhibition of nitric oxide synthesis[14].

In conclusion, the data presented suggest the involvement of endothelin, released by the endothelial cells and stimulating the contraction of the vascular smooth muscular cells, in the development of the myogenic reactions of the vascular smooth muscles which are observed when the vascular wall is distended.

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