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Enhanced responsiveness of rat isolated aorta to clonidine after removal of the endothelial cells

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With the endothelium present, the maximum response of rat isolated aorta to clonidine was much lower than that to noradrenaline. Removal of endothelium enhanced the response to both adrenoceptor agonists and the clonidine-induced maximum contraction became almost equal to that produced by noradrenaline, although it was much more sensitive to inhibition by flunarizine and nifedipine. These results indicate that clonidine and noradrenaline activate receptors present in the endothelial cells and that these receptors are highly sensitive to clonidine.

Introduction The essential role of endothelium as a mediator of relaxant responses induced in isolated vascular tissues by agonists such as acetylcholine, substance P and the calcium ionophore A23187 is well accepted (Furchgott & Zawadzki, 1980). Bradykinin produces a relaxation dependent on the endothelium in arteries of some species but not others (Cherry *et al.*, 1982). Other mediators of vascular relaxation such as ATP and ADP are partially dependent on the endothelium in some species whilst AMP, adenosine, vasoactive intestinal peptide and isoprenaline exert a direct relaxant effect on the smooth muscle (see Vanhoutte & Rimele, 1983). The role of endothelial tissue as a modulator of contractile agonist effects on vascular smooth muscle has not so far been widely investigated. In one study on rat aorta, removal of endothelium potentiated noradrenaline EC_{50} values by about 17 fold and significantly increased the maximal tension developed (Zuleica *et al.*, 1983). In another study a 49 fold reduction of the noradrenaline EC_{50} value was observed with no change in the maximal response (Allan *et al.*, 1983). In view of the differences in the dependency of contractions evoked by noradrenaline and the α_2- agonist clonidine on extracellular calcium in the rat aorta (Godfraind *et al.*, 1982), we have examined the influence of the endothelium on the contractile responses of these two agonists. The results show that the contractile response to clonidine is dramatically enhanced by removal of the endothelium.

Methods Pairs of rings (2 mm wide) were cut from the thoracic aorta of male Wistar rats (330–350 g). One ring of this pair was left intact, but the other was stripped of its endothelial cells by mechanical rubbing. Each ring was carefully suspended under a tension of 2 g, in a 50 ml organ bath containing physiological solution (mM: NaCl 112, KCl 5, $NaHCO_3$ 25, KH_2PO_4 1.0, $MgSO_4$ 1.2, $CaCl_2$ 1.25, glucose 11.5) at 37 °C, gassed with 95% O_2 and 5% CO_2. Contractile responses were measured with an isometric strain gauge coupled to a potentiometric pen recorder. After an equilibration period of 60 min the artery preparations were contracted maximally in a depolarizing medium (mM:NaCl 17, KCl 100, $NaHCO_3$ 25, KH_2PO_4 1.0, $MgSO_4$ 1.2, $CaCl_2$ 1.25, glucose 11.5) and selective removal of the endothelial cells was confirmed by demonstrating the presence ($50.6 \pm 2.8\%$, $n = 18$) or the lack of a relaxation response to acetylcholine (10^{-6}M). Preparations were then washed and allowed a further 60 min period of equilibration. A single cumulative concentration-response curve to the chosen vasoconstrictor substance was then established in the pairs of rings.

The action of calcium entry blocking agents was studied on the response evoked by a maximum concentration (10^{-6} M) of each agonist; the maximum effective concentration (3×10^{-6} M) of flunarizine (Godfraind & Dieu, 1981) or nifedipine (Godfraind, 1983) was used after a preincubation period of 90 min.

Noradrenaline bitartrate (Flucker) was dissolved in distilled water containing 7.9 mM Na_2SO_3 and 34 mM HCl as stock solution of 10 mM. Clonidine HCl (Boehringer) and acetylcholine HCl (Roche) were prepared each day as a 10 mM stock solution dissolved in distilled water. Flunarizine (Janssen Pharmaceutica) was dissolved in an aqueous solution of 100 mM tartaric acid (pH 3.1) to a concentration of

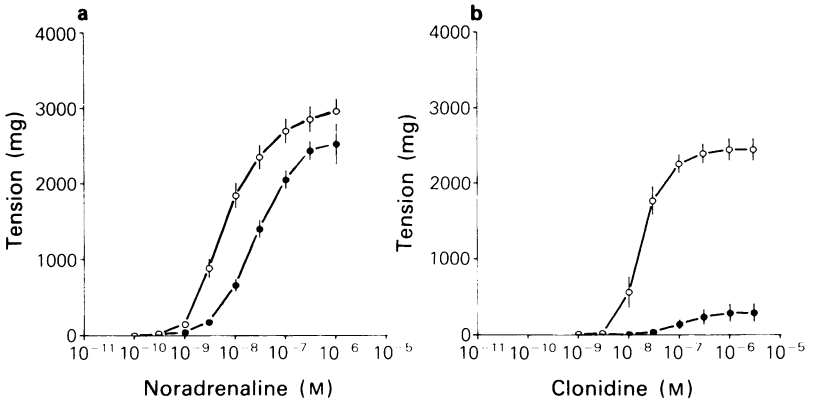

Figure 1 Comparison of cumulative concentration-effect curves with (●) and without (○) endothelium elicited by noradrenaline (a) and clonidine (b) in the rat isolated aorta. Responses are expressed in mg of isometric tension. Each curve is the mean of 12 observations for noradrenaline and 6 observations for clonidine. Vertical lines indicates standard errors where they exceed the size of the symbol.

1 mM. Nifedipine (Bayer) was dissolved in acetone (0.01 M) and diluted in physiological solution.

Results Concentration-effect curves obtained with noradrenaline and clonidine in rat isolated aorta with and without endothelium are illustrated in Figure 1. Noradrenaline concentration-effect curves obtained in the absence of endothelium were shifted to the left as compared to the concentration-effect curves obtained in the presence of endothelium, EC_{50} values being respectively equal to $3.2 \pm 0.3 \times 10^{-8}$ M and $6.7 \pm 0.9 \times 10^{-9}$ M ($n = 12$, $P < 0.01$). The maximum tension developed by the preparations without endothelium tended to be higher than that developed in preparations with endothelium but this difference was not significant.

On the other hand, removal of endothelium evoked a very dramatic effect on the contractile responsiveness of rat isolated aorta to clonidine (Figure 1b). As is obvious, the maximum response in the absence of endothelium increased by a factor of 9 ($P < 0.01$). EC_{50} values were significantly different ($n = 6$, $P < 0.01$) and equal to $1.1 \pm 0.1 \times 10^{-7}$ M in the presence and to $1.9 \pm 0.3 \times 10^{-8}$ M in the absence of endothelium.

It has been shown by Godfraind *et al.*, (1982) that the maximal inhibition of responses to α_2-agonists by calcium entry blocking drugs was much greater than was the maximal inhibition of noradrenaline responses. Nifedipine 3×10^{-6} M and flunarizine 3×10^{-6} M were equiactive in inhibiting the contractile responses to noradrenaline and clonidine elicited in the absence of endothelium. However, the residual responses were then respectively $69.5 \pm 5.5\%$ ($n = 7$) and $17.7 \pm 1.4\%$ ($n = 6$) of control.

Discussion The present results indicate that the endothelium plays a major role in the contractile response of rat isolated aorta to clonidine and a much smaller one when noradrenaline is the agonist. For the latter agonist our observations are in general agreement with those of other authors (Allan *et al.*, 1983; Zuleica *et al.*, 1983). These observations suggest that clonidine activates receptors present on the endothelial cell and that their activation leads to inhibition of clonidine responses elicited by its interaction with receptors located on the smooth muscle membrane. This is consistent with the reports by Furchgott & Zawadzki (1980) and De Mey & Vanhoutte (1981) of the possible diffusion of an inhibitory substance formed in the endothelium as a result of the stimulation of muscarinic receptors which acts on the smooth muscle cell to inhibit contraction. Such an inhibitory process could be activated not only by muscarinic receptors but, as shown in the present paper, by α-adrenoceptors likely to be of the α_2-type since they appear to be less sensitive to noradrenaline than to clonidine. The present results also confirm our previous report (Godfraind *et al.*, 1982) that receptor response coupling stimulated by noradrenaline is dependent on extracellular calcium to a different extent from that stimulated by clonidine.

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