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THE ROLE OF THE ENDOTHELIUM IN THE EFFECTS OF BIOGENIC AMINES AND THEIR ANTAGONISTS ON THE ISOLATED BOVINE ABDOMINAL AORTA

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Noradrenaline, 5-hydroxytryptamine and histamine produce concentration-dependent contraction of the isolated bovine abdominal aorta, both in intact preparations and after deendothelialization. In endothelium-free preparations the contractile effects of noradrenaline and of 5-hydroxytryptamine were significantly depressed, whereas the response to histamine was unaffected by removing the endothelium. The actions of antagonists of biogenic amines (phentolamine, cyproheptadine, chlorpyramine) were significantly less pronounced in endothelium-free preparations. Evidently, endothelium plays a significant role in modulating the effects of biogenic amines on the isolated bovine abdominal aorta. This role can be explained, either by differences between the endothelium and vascular smooth muscle in the density of specific receptors responsible for contractile effects of biogenic amines, or by the release of the endothelium-derived relaxing factor (EDRF).

Key words: EDRF, abdominal aorta, phentolamine, cyproheptadine, chlorpyramine.

INTRODUCTION

It has been already found that in various isolated blood vessel preparations the responses to noradrenaline and adrenaline are increased after removing the vascular endothelium (Godfraind et al., 1985; Vanhoutte et al., 1986; Bullock et al., 1986). It has been postulated that these findings might indicate a different distribution of adrenoceptors in the endothelium and smooth muscle of the blood vessels (Cohen et al., 1988). It was also suggested that all spasmogenic agonists acted by releasing the endothelium derived relaxing factor (EDRF ; Bullock et al., 1986).

The vascular endothelium has been found to play an important modulatory role in the response of vascular smooth muscle to a variety of stimuli (Furchgott, 1984; Bullock et al., 1986). Thus, it has been shown that the endothelium has an inhibitory effect on contractile responses to alpha-adrenoceptor stimulation, either by exogenous administration of noradrenaline and other adrenoceptor

agonists (Martin et al., 1986), or by adrenergic nerve stimulation (Cohen and Weisbrod, 1988).

It was, therefore, of interest to investigate the role of the endothelium in the effects of several biogenic amines on the isolated bovine abdominal aorta. This was even more interesting because the function of the endothelium differs between various blood vessels (Gardiner et al., 1990a, 1990b).

MATERIAL AND METHODS

The isolated rings of bovine abdominal aorta were incubated in an isolated organ bath (20 ml). The aorta was always dissected from the same anatomical region between the last thoracic and the second lumbar vertebra. The material was obtained from the local abattoir. The rings of aorta were 0.5 cm wide. The bathing medium was Tyrode solution of the following composition (in mmol. l^{-1}); NaCl 136.7, KCl 2.81, $CaC1_2$ 1.8, $MgC1_2$ 0.105, NaH_2PO_4 0.417, $NaHCO_3$ 11.9 and glucose 11.1. This medium was aerated with a mixture of oxygen (95%) and carbon dioxide (5%). The temperature of the medium was 37^0C.

In dissecting the aorta particular care was taken not to produce any lesion to the endothelium. In a separate series of experiments the endothelium was removed by rubbing it off with a wooden rod. The isometric contractions of the isolated aortal rings were recorded by a microdisplacement myograph transducer (F-50, Narco-Bio-Systems). The initial preloading of the preparation was about 5 g.

The following substances were used: noradrenaline hydrochloride (Serva), 5-hydroxytryptamine-creatine-sulphate (BDH Biochemicals), histamine hydrochloride (Serva), chlorpyramine (Synopen, Pliva), phentolamine (Regitin, Ciba) and cyproheptadine (Merck).

Statistical evaluation was made using Student's *t*-test.

RESULTS

The effects of noradrenaline and phentolamine on intact and deendothelialized bovine abdominal aorta. — Noradrenaline (10^{-9} to $1.8x10^{-7}$ mol.l^{-1}) produced a concentration-dependent contraction of the isolated bovine abdominal aorta, whether it was intact or deendothelialized. However, removing the endothelium significantly decreased the contractile responses to noradrenaline, particularly in the range of concentrations between $2x10^{-8}$ and $1.2x10^{-6}$ mol.l^{-1} (Figure 1)

Phentolamine ($7x10^{-8}$ mol.l^{-1}) significantly shifted the concentration-response curves of noradrenaline to the right both in intact and deendothelialized preparations. In intact preparations the effect of phentolamine was particularly pronounced in the range of concentrations between $1.2x10^{-9}$ and $1.8x10^{-7}$ mol.l^{-1} of noradrenaline. In deendothelialized preparations the effect of phentolamine was particularly marked in the range of noradrenaline concentrations between $4.6x10^{-7}$ and $3.0x10^{-6}$ mol.l^{-1} (Figure 1).

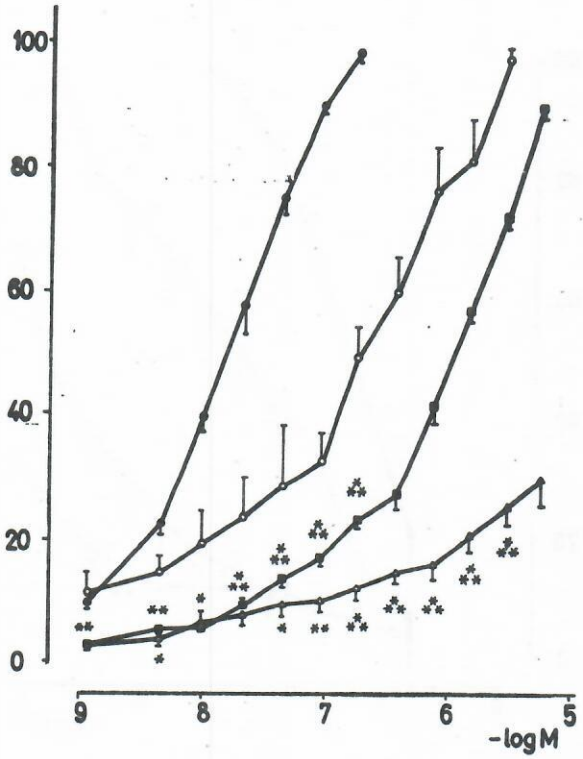

Figure 1. Concentration-response curves for increasing concentrations of noradrenaline alone (●) and noradrenaline in the presence of phentolamine (■), in intact (● ■) and endothelium-free preparations (o Δ). Abscissa: concentrations of noradrenaline in the bath. Ordinate: percentage increase of the isometric contraction of the isolated bovine abdominal aorta *(P<0.05; **P<0.01; ***P<0.001). Each point is the mean value of 6 experiments ± s.e.m.

The effects of 5-hydroxytryptamine and cyproheptadine on intact and deendothelialized bovine abdominal aorta. — In the range of concentrations between $6.0x10^{-8}$ and $1.7x10^{-7}$ $mol.l^{-1}$ 5-hydroxytryptamine produced a concentration-dependent contraction of the isolated bovine abdominal aorta, both in preparations with and without endothelium (Figure 2).

Cyproheptadine ($3.5x10^{-7}$ $mol.l^{-1}$) produced a statistically significant depression in the response to 5-hydroxytryptamine. Thus, the concentration-response curves for 5-hydroxytryptamine, in the presence of cyproheptadine, were significantly shifted to the right. The effects of 5-hydroxytryptamine and of cyproheptadine were significantly depressed in endothelium-free preparations (Figure 2).*The effects of histamine and chlorpyramine on intact and deendothelialized bovine abdominal aorta.* — Histamine ($5.0x10^{-9}$ to $6.9x10^{-7}$ $mol.l^{-1}$)

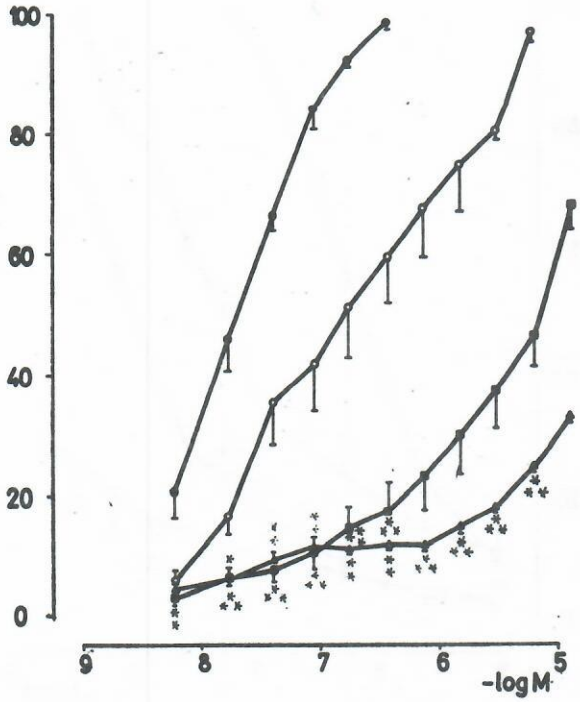

Figure 2. Concentration-response curves for increasing concentrations of 5-hydroxytryptamine alone (●) and 5-hydroxytryptamine in the presence of cyproheptadine (■), in intact (● ■) and endothelium-free preparations (O Δ.) Abscissa: Concentrations of 5-hydroxytryptamine in the bath. Ordinate: percentage increase of the isometric contraction of the isolated bovine abdominal aorta. Each point is the mean value of 6 experiments +s.e.m. Statistical significance as in Figure 1.

was found to produce a concentration-dependent contraction of the isolated bovine abdominal aorta. The curves obtained in intact and deendothelialized preparations were practically identical (Figure 3).

Chlorpyramine ($1.7x10^{-8}$ mol.l^{-1}) produced a significant depression of the response to histamine in the isolated bovine abdominal aorta, both in intact and deendothelialized preparations (Figure 3). In the preparations without endothelium the graded response to histamine was almost completely blocked. In intact preparations the response to histamine was highly significantly depressed only in the range of concentrations between $4.0x10^{-8}$ and $7.0x10^{-7}$ mol.l^{-1}. In the higher range of histamine concentrations the blocking effect of chlorpyramine was found to be surmountable, as shown in Figure 3.

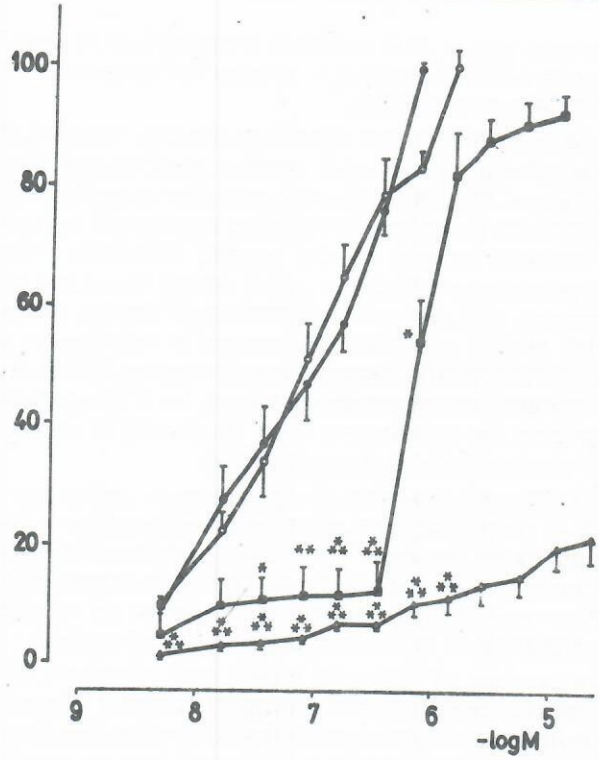

Figure 3. Concentration-response curves for increasing concentrations of histamine alone (●) and histamine in the presence of chlorpyramine (■), in intact (● ■) and endothelium-free preparations (o Δ). Abscissa: concentrations of histamine in the bath. Ordinate: percentage increase of the isometric contraction of the isolated bovine abdominal aorta. Each point is the mean value of 5 preparations ± s.e.m. Statistical significance as in Figure 1.

DISCUSSION

It was found in the present experiments that all the biogenic amines used (noradrenaline, 5-hydroxytryptamine, histamine) produced concentration-dependent contractions of the isolated bovine abdominal aorta. The effects of noradrenaline and of 5-hydroxytryptamine were more pronounced on the preparations with endothelium than on deendothelialized preparations. Contrary to this, the effects of histamine were the same both in intact and deendothelialized preparations.

Other authors, mainly using isolated aorta of the rat, found that contractions produced by noradrenaline and adrenaline were increased after removing the endothelium from the aorta (Fortes et al., 1983; Godfraind et al., 1985; Carrier et al., 1985; Vanhoutte et al., 1986; Bullock et al., 1986). It was therefore postulated that spasmogens probably produced a release of EDRF from the endothelium, thus decreasing the effects of noradrenaline and adrenaline while

the endothelium was intact. The vascular endothelium is also supposed to modulate adrenergic neurotransmission, at least in the vascular smooth muscle of the dog (Greenberg et al., 1990).

In the isolated coronary blood vessels of the pig, noradrenaline produced a relaxing effect which was more pronounced in intact than in deendothelialized preparations (Cohen et al., 1988). These results were explained as being due to different distributions of alpha$_1$ and alpha$_2$ adrenergic receptors in the endothelium and smooth muscle of the vessel. Therefore, our results with noradrenaline (the stronger effect on intact aorta) might be due to species differences (bovine vs. rat), to type and anatomical region of the blood vessel (abdominal aorta) and to probable differences in distribution of alpha$_1$ and alpha$_2$ receptors in the endothelium and smooth muscle of the abdominal aorta. Removing the endothelium would also remove to a great extent the alpha$_1$ receptors responsible for contraction, thus producing a weaker response to noradrenaline in deendothelialized preparations.

Phentolamine has been known to depress both alpha$_1$ and alpha$_2$ receptors equally well. It depressed the effect of noradrenaline in the intact aorta, but its effect was much stronger on deendothelialized preparations. These results support our contention about the different distribution of alpha receptors in the endothelium and smooth muscle of the aorta as significant factors in the role of the endothelium in the response to noradrenaline.

Due to the variety of different types and subtypes of receptors, 5-hydroxytryptamine has been known to produce various effects depending on the species, type of blood vessel and method of application. In our experiments 5-hydroxytryptamine produced a concentration-dependent contraction of bovine abdominal aorta. This effect was significantly weaker in preparations without endothelium. Here again, removing of endothelium probably removes the 5-HT receptors which are mainly responsible for the contractile effect of 5-hydroxytryptamine. Cyproheptadine, a nonspecific 5-hydroxytryptamine antagonist, significantly counteracted the contractile effect of 5-hydroxytryptamine. The effect was significantly stronger on deendothelialized than on intact preparations. This difference can be attributed to different distribution of 5-HT receptors responsible for contraction of the aorta in the endothelium and smooth muscle.

Histamine also produced a concentration-dependent contraction of the isolated bovine abdominal aorta. It should be pointed out that, contrary to noradrenaline and 5-hydroxytryptamine, the responses to histamine were nearly identical in intact and deendothelialized preparations. Evidently, the contractile responses to histamine, at least in the isolated bovine abdominal aorta, are endothelium-independent. This also means that the histamine receptors are equally distributed in the endothelium and the smooth muscle of the aorta, or even dominantly present in the smooth muscle. Meanwhile, the relaxing effect of histamine is predominantly endothelium-dependent (Krstić et al., 1989).

The contractile effects of histamine on the bovine aorta were significantly antagonized by chlorpyramine, an H_1 antagonist. This effect was particularly strong in preparations without endothelium, but easily surmountable by higher concentrations of histamine on the intact preparations of aorta. These findings

indicate that the histamine H_1 receptors responsible for the contractile effect of histamine on bovine abdominal aorta are predominantly located in the vascular smooth muscle.

REFERENCES

1. *Bullock, G. R., Taylor, S. G., Weston, A. H.* 1986. Influence of the vascular endothelium on agonist-induced contractions and relaxations in rat aorta. *Br. J. Pharmacol. 89,* 819-830.
2. *Carrier, G. O., White, R. E.* 1985. Enhancement of alpha$_1$ and alpha$_2$ adrenergic agonist induced vasoconstriction by removal of endothelium in rat aorta. *J. Pharmacol. Exp. Ther. 232,* 682-687.
3. *Cohen, R. A. and Weisbrod, R. M.* 1988. Endothelium inhibits norepinephrine release from adrenergic nerves of rabbit carotid artery. *Am. J. Physiol. 254,* H871-H878.
4. *Cohen, R. A.,Zitnay, K. M., Weisbrod, R. M. and Testamariam, B.* 1988. Influence of the endothelium on tone and response of isolated pig coronary artery to norepinephrine. *J. Pharmacol. Exp. Therap. 244,* 550-555.
5. *Fortes, Z. B., Leme, J. G. and Scivoletto, R.* 1983. Vascular reactivity in diabetes mellitus - role of the endothelial cell. *Br. J. Pharmacol. 79,* 771-781.
6. *Furchgott, R. F.* 1984. The role of endothelium in the responses of vascular smooth muscles to drugs. *Ann. Rev. Pharmacol. Toxicol. 24,* 175-197.
7. *Gardiner, S. M., Compton, A. M. Bennett, T., Palmer, R. M. J. and Moncada, S.* 1990 b. Regional haemodynamic changes during oral ingestion of N^G-mono-methyl-L-arginine or N^G -nitro-L-arginine methyl ester in conscious Brattleboro rats. *Br. J. Pharmacol. 101,* 10-12.
8. *Gardiner, S. M., Compton, A. M., Kemp, P.A. and Bennett, T.* 1990a. Regional and cardiac haemodynamic effects of N^G -nitro-L-arginine methyl ester in conscious Long Evans rats. *Br. J. Pharmacol. 101,* 625-631.
9. *Godfraind, T., Egleme, C. and Alosachie, I. H.* 1985. Role of endothelium in contractile response of the rat aorta to alpha-adrenoceptor agonists. *Clin. Sci. 69,* Suppl. 10, 65S-71S.
10. *Greenberg, S. S., Diecke, F. P. J., Peevy, K. and Tanaka, T.P.* 1990. Release of norepinephrine from adrenergic nerve endings of blood vessels is modulated by endothelium-derived relaxing factor. *Am. J. Hyperten. 3,* 211-218.
11. *Krstić, M., Stepanović, R. M., Krstić, S. K. and Katušić, Z. S.* 1989. Endothelium-dependent relaxation of the rat artery caused by activation of histamine H_1 -receptors. *Pharmacology 38,* 113-120.
12. *Martin, W., Furchgott, R.F., Vilani, G. M. and Jothianadan, D.* 1986. Depression of contractile responses in rat aorta by spontaneously relased endothelium-derived-relaxing factor. *J. Pharmacol. Exp. Ther. 237,* 529-538.
13. *Vanhoutte, P. M., Rubanyi, G. M., Miller, V. M. and Houston, D. S.* 1986. Modulation of vascular smooth muscle contraction by the endothelium. *Ann. Rev. Physiol. 48,* 307-320.

ULOGA ENDOTELA U EFEKTIMA BIOGENIH AMINA I NJIHOVIH ANTAGONISTA NA IZOLOVANU GOVEĐU ABDOMINALNU AORTU

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SADRŽAJ

Noradrenalin, 5-hidroksitriptamin i histamin prouzrokuju koncentracijski-zavisnu kontrakciju izolovane goveđe abdominalne aorte, i to kako u preparatima sa endotelom, tako i u preparatima kod kojih je endotel bio odstranjen. Uklanjanje endotela je značajno slabilo kontraktilne efekte noradrenalina i 5-hidroksitriptamina, ali nije menjalo dejstva histamina. Dejstvo antagonista biogenih

amina (fentolamin, ciproheptadin, hlorpiramin) bilo je značajno jače izraženo na preparatima bez endotela. Prema tome, endotel ima značajnu ulogu u modulaciji dejstva biogenih amina na izolovanu goveđu aortu. Ovaj efekt se može objasniti, ili razlikama između endotela i glatkog mišića aorte u gustini specifičnih receptora preko kojih se odvijaju kontraktilni efekti biogenih amina, ili posredstvom oslobađanja endotelnog relaksantnog faktora.