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Influence of the Endothelium on Vascular Responses of Aortae from Endotoxic Rats

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Abstract—Intraperitoneal injection of endotoxin diminished the in-vitro contractile response of rat aorta to phenylephrine or clonidine, whether the intimal layer was disrupted or not. The relaxing responses to acetylcholine and sodium nitroprusside in aorta precontracted with 10^{-6} M phenylephrine were similar between control and endotoxic groups. However, when the precontractile force following phenylephrine was adjusted to an equivalent level, the relaxing responses to acetylcholine and sodium nitroprusside were diminished in the endotoxic aorta compared with the controls. There was no significant difference between the two groups in the increase in cyclic GMP levels induced by acetylcholine or by sodium nitroprusside. These results suggest that aortae from endotoxic rats show decreased responsiveness to α-adrenoceptor stimulation not because of enhancement of the endothelium-derived relaxing factor but because of abnormality in the vascular smooth muscle which is not specific for subtypes of the α-adrenoceptor.

Circulatory failure is a major cause of death in patients with sepsis and its prognosis is poor in spite of aggressive medical treatment (Hess et al 1981). Recent in-vivo and in-vitro studies suggest that abnormality in vascular responsiveness is involved in endotoxic shock in addition to myocardial abnormality (Parratt 1973; Pomerantz et al 1982; McKenna et al 1986; Wakabayashi et al 1987a; Litten et al 1988). The common conclusion is that responsiveness to noradrenaline is diminished in endotoxic arteries. However, the precise mechanism of this vascular abnormality remains unknown.

Recently, the vascular endothelium has been shown to play an indispensable role in the regulation of vascular tonus (Furchgott & Zawadzki 1980). Vasocontractile responses to α-adrenoceptor agonists are enhanced by the removal of vascular endothelium and this inhibitory effect of the endothelium on contractile response is due to endothelium-derived relaxing factor (EDRF) from the vascular endothelium (Eglème et al 1984; Lues & Schümann 1984; Martin et al 1986). The vascular endothelium is known to be affected in endotoxaemia. Gaynor et al (1970) reported that treatment of rabbits with endotoxin induces damage to the aortic endothelial cells, which are then detached and circulate in blood. However, it is not known whether modulation of vascular tonus by the endothelium is altered in endotoxaemia.

The purpose of this study was to investigate changes in vasoconstriction mediated by selective α-adrenoceptors and endothelial modulation of vascular tonus using the aorta from endotoxin-injected rats.

Materials and Methods

Tissue preparation and tension recording

Male Wistar rats (14–15 weeks old) were injected intraperitoneally with 10 mg kg^{-1} of endotoxin (lipopolysaccharide from *Escherichia coli*, 026 B6 from Difco) and maintained on water only until killed 4 h later. Rats not treated with endotoxin were used as controls. All animals were killed by a blow to the head. The thoracic aorta was excised and placed in Krebs-Ringer bicarbonate solution (mM: NaCl 118, KCl 4·7, $CaCl_2$ 2·5, KH_2PO_4 1·2, $MgSO_4$ 1·2, glucose 10 and $NaHCO_3$ 25) and excess fat and connective tissue removed. Helical strips (approximately 2mm × 15 mm) were then prepared. In some preparations, the endothelium was removed by gentle abrasion of the intimal surface with ultrafine sandpaper (Nippon Coated Abrasive C-1000). Each strip was then suspended vertically in a 10 mL organ bath filled with the above solution (37°C, pH 7·4), which was bubbled with 95% O_2–5% CO_2. A force-displacement transducer was attached to each strip. After 1 h of equilibrium with a resting tension of 1 g, changes in isometric force were recorded. Contractile agonists were cumulatively added to the bath and the contractile response obtained was expressed in terms of mg force (mg tissue)$^{-1}$. Relaxants were also cumulatively added when the phenylephrine-induced contraction reached a plateau. The relaxant response was expressed in terms of percentage decrease of tension/phenylephrine-induced precontraction force. The vasorelaxation caused by acetylcholine disappeared after removal of the endothelial intimal layer in both the control and endotoxin-injected groups. Denudation of the endothelium was confirmed functionally by the disappearance of the 10^{-5} M acetylcholine-induced relaxant response of the 10^{-7} M noradrenaline-precontracted vessel at the end of each experiment (Wakabayashi et al 1987b). Removal of endothelium by the sandpaper did not affect the relaxant response to sodium nitroprusside.

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Measurement of cyclic (c)GMP

Endothelium-intact aortic strips were set up and equilibrated in organ baths as described above. At various times after exposure to the drugs, the tissues were frozen in liquid nitrogen and stored at −80°C until extraction of cGMP. The frozen tissues were homogenized in 1 mL of 6% trichloroacetic acid and centrifuged at 3000 *g* for 15 min. The supernatants were extracted four times in 5 volumes of water-saturated ether, and samples were then evaporated to dryness. These were stored at −80° until assayed for cGMP.

After reconstitution of the samples in a sodium acetate buffer (50 mM, pH 6·2) containing theophylline (1 mM) and acetylation, cGMP levels were determined using radioimmunoassay kits obtained from New England Nuclear. The tissue residue was dissolved in 2 M NaOH and protein content was determined by the method of Lowry et al (1951), using bovine serum albumin as standard.

Drugs

Phenylephrine hydrochloride, clonidine hydrochloride and noradrenaline hydrochloride were purchased from Sigma Chemical Co. Acetylcholine chloride, sodium nitroprusside and theophylline were purchased from Wako Pure Chemical Co.

Statistical analysis

All the values represent mean ± s.e.m. The potency of the agonists was expressed as EC50 (concentration producing a half-maximal response). The data were analysed by Student's *t*-test, and $P < 0.05$ was defined as significant.

Results

Contractile responses to phenylephrine and clonidine

Fig. 1A shows the contractile force induced by phenylephrine in aortae with and without endothelium from the endotoxic and control rats. By removal of endothelium, the EC50 value for the phenylephrine-induced contracture was reduced in the control aortae ($4.95 \pm 1.03 \times 10^{-8}$ M with endothelium, $1.20 \pm 0.26 \times 10^{-8}$ M without endothelium) but not changed in the endotoxic aortae ($3.04 \pm 0.60 \times 10^{-7}$ M with endothelium, $2.80 \pm 1.00 \times 10^{-7}$ M without endothelium). Removal of endothelium produced no significant increase in maximal contractile force by phenylephrine in aortae from both groups. Whether the endothelium was present or not, the aortic contractile response to phenylephrine was markedly reduced in the endotoxin-injected group. The clonidine-induced contraction was significantly enhanced by removal of the intimal layer in both control and endotoxin-injected rat aortae. Whether the intimal layer was disrupted or not, the aortic contractile response to clonidine was significantly reduced in the endotoxin-injected group. The potency of clonidine was significantly different between the two groups when the endothelium was removed [endotoxic group, $7.79 \pm 0.89 \times 10^{-7}$ M; control, $5.58 \pm 0.86 \times 10^{-8}$ M], but not when it was present [endotoxic group, $2.06 \pm 0.29 \times 10^{-7}$ M; control, $2.48 \pm 0.26 \times 10^{-7}$ M] (Fig. 1B).

Relaxant responses to acetylcholine and sodium nitroprusside

The vasorelaxant responses to acetylcholine and sodium nitroprusside in phenylephrine (10^{-6} M)-contracted tissue did not differ between the two groups (Fig. 2A, B). As indicated in Fig. 1, endotoxic aortae showed decreased contractility to 10^{-6} M phenylephrine. Since it has been reported that relaxant responses are influenced by the precontractile force (Cohen & Berkowitz 1974; Herlihy et al 1976), we also studied the relaxing responses to acetylcholine and sodium nitroprusside in control aortae precontracted with 5×10^{-8} M phenylephrine, which shows a contractile force similar to that of 10^{-6} M phenylephrine in endotoxic aortae (82·8 ± 5·6, 78·2 ± 7·6 mg tension (mg tissue)$^{-1}$, respectively). The relaxing responses to acetylcholine and sodium nitroprusside were diminished in the endotoxic aorta precontracted with 10^{-6} M phenylephrine compared with those in the control aorta precontracted with 5×10^{-8} M phenylephrine (Fig. 2A, B).

cGMP accumulation

The cGMP levels in aortic strips without any precontraction were compared between the endotoxic and control rats (Fig. 3). The basal levels of cGMP in the aortae did not differ

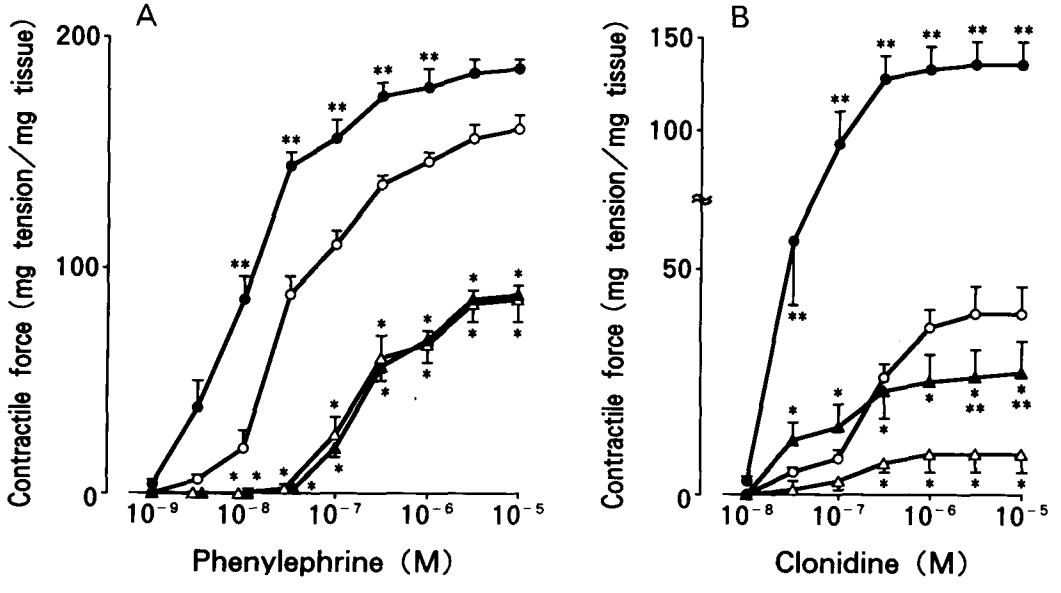

FIG. 1. Effect of endotoxin administration on contractile responses to α-adrenoceptor agonists, phenylephrine (A) and clonidine (B) (○——○: control aortae with endothelium, ●——●: control aortae without endothelium, △——△: endotoxic aortae with endothelium, ▲——▲: endotoxic aortae without endothelium). Asterisks denote significant differences between aortae from endotoxin-injected and control rats (*), and endothelium-intact and denuded aortae (**). n = 8–12.

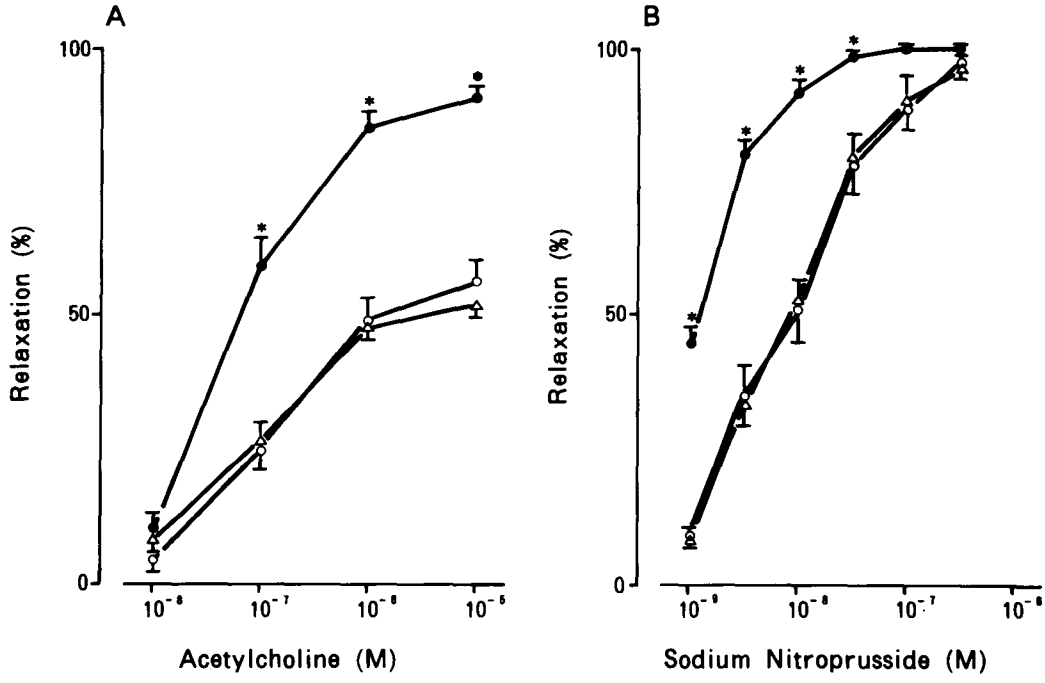

FIG. 2. Effect of endotoxin administration on relaxing response to acetylcholine (A) and sodium nitroprusside (B) (○——○: control aortae precontracted with 10^{-6} M phenylephrine, ●——●: control aortae precontracted with 5×10^{-8} M phenylephrine, △——△: endotoxic aortae precontracted with 10^{-6} M phenylephrine). Asterisks denote significant differences between endotoxin-injected and control rats. n = 10–13.

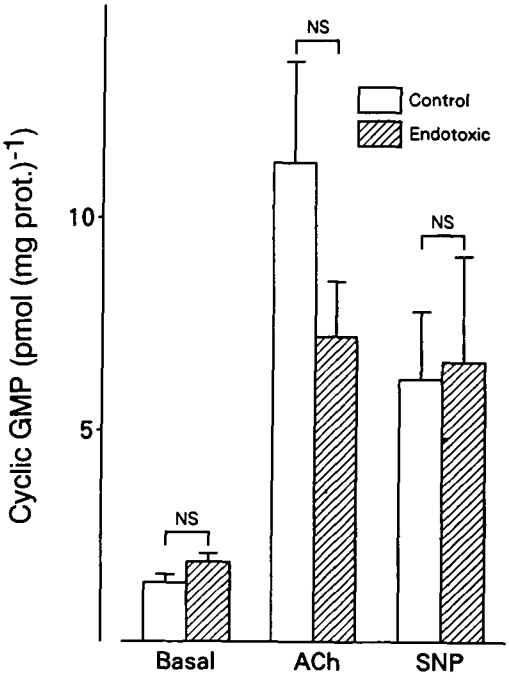

FIG. 3. Effect of endotoxin administration on levels of cGMP in rat aorta. Basal, tissues frozen without pretreatment. ACh, tissues frozen after treatment with 10^{-6} M acetylcholine for 1 min. SNP, tissues frozen after treatment with 5×10^{-8} M sodium nitroprusside for 1 min. NS denotes no significant difference between aortas from endotoxin-injected and control rats. n = 6–9.

between the control and endotoxic groups. Also, no significant difference was shown in the increase in cGMP levels induced by acetylcholine or sodium nitroprusside between the two groups.

Discussion

In aortae from rats 4 h after injection of endotoxin, both the contractile responses to phenylephrine, an α_1-adrenoceptor agonist, and clonidine, an α_2-adrenoceptor agonist, were decreased compared with the control. Auclair et al (1986) showed that endotoxin reduces both the pressor effects of α_1- and α_2-adrenoceptor agonists in pithed rats and isolated perfused rat kidney. Those results suggest that the decrease in the α-adrenoceptor contractile responses in endotoxic aortae is not selective between α_1- and α_2-subtypes.

Vascular contraction mediated by the α-adrenoceptor has been shown to be potentiated by removal of the endothelium and the inhibitory effect of the endothelium is attributed to EDRF (Eglème et al 1984; Lues & Schümann 1984; Martin et al 1986). By removal of endothelium, the sensitivity of phenylephrine-contracture was potentiated in the control rat aortae, but not altered in the endotoxic aortae. Whether the endothelium was present or not, the aortic contractile response to phenylephrine was markedly reduced in the endotoxin-injected group. Both in endotoxic and control aortae, removal of the endothelium resulted in enhancement of responses to clonidine. Endotoxic aortae showed a much lower contractile response to clonidine compared with the control, regardless of whether or not the endothelium was removed. Thus, the decreased responsiveness of aorta from endotoxic rat to α-adrenoceptor agonists is attributed to the abnormal contractility of vascular smooth muscle cells. McKenna et al (1986) reported that in aorta from rats with sepsis induced by caecal ligation with puncture, removal of the endothelium improved the decreased vasocontractility in response to noradrenaline, with a high concentration giving a response equivalent to that on control rat aortae. One

possible reason for this disparity is the differences in the experimental conditions (the procedures for inducing endotoxaemia and its duration).

Furchgott & Zawadzki (1980) found that acetylcholine could relax vascular smooth muscle indirectly via the release of EDRF, which stimulates guanylate cyclase and elevates cGMP (Rapoport & Murad 1983). EDRF is detectable only by bioassay because of its short half-life (Griffith et al 1984). It is known that the force of precontraction affects the percentage of the subsequent relaxation (Cohen & Berkowitz 1974; Herlihy et al 1976). Since the contractile force is decreased in endotoxic aorta compared with the control, it is difficult to determine whether EDRF is altered in endotoxaemia. In our study, we observed the relaxing responses after the precontraction with phenylephrine of the same concentration or equivalent tension. When vessels were precontracted with the same concentration (10^{-6} M) of phenylephrine, the relaxing response to acetylcholine did not differ between endotoxic and control groups. In aortae with an equivalent phenylephrine-precontracted tension, the endotoxic group displayed a decreased relaxing response to acetylcholine compared with the control. These results suggest that the relaxing response to acetylcholine is not increased in the endotoxic group. The vasorelaxant response to sodium nitroprusside is thought to be independent of the endothelium but mediated by direct stimulation of guanylate cyclase and elevation of cGMP in vascular smooth muscle (Rapoport & Murad 1983). As with acetylcholine, the relaxing response with sodium nitroprusside did not differ in the aortae precontracted with the same concentration of phenylephrine (10^{-6} M) between the two groups and decreased in endotoxic aortae when the precontracted tension was equivalent. Furthermore, cGMP levels in aortic strips without any precontraction were compared between the endotoxic and control rats. There was no significant difference in the increase in cGMP levels induced by acetylcholine or sodium nitroprusside between the two groups, suggesting that EDRF from endothelium is not altered in the endotoxic aortae compared with the controls. Thus, endothelial modulation may not be involved in the altered vascular tonus of endotoxic aortae.

In conclusion, the results from this study suggest that the decreased contractility of aortae from endotoxin-injected rats in response to α-adrenergic agonists can be attributed not to endothelial modulation but to an abnormality in vascular smooth muscle which is not selective for subtypes of the α-adrenoceptor.

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