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Endothelium-dependent Vascular Responses in 1,3-Dipropyl-8-sulphophenylxanthine (DPSPX) Hypertensive Rats

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Abstract

The study was undertaken to test the endothelium-mediated vascular responses in rats rendered hypertensive by chronic administration of 1,3-dipropyl-8-sulphophenylxanthine (DPSPX). The relaxant effect of carbachol (an endothelium-dependent relaxing drug) and of sodium nitroprusside (an endothelium-independent relaxing drug) as well as the potentiation of the contractile effect of noradrenaline by N^G-nitro-L-arginine methyl ester (L-NAME) were compared in aortic rings from normotensive and DPSPX-hypertensive rats.

Carbachol and sodium nitroprusside caused concentration-dependent relaxations in aortic rings precontracted by 1 μM noradrenaline. The relaxant effect of carbachol was significantly reduced in tissues of DPSPX-hypertensive rats: the maximal relaxant effect being $86 \pm 3\%$ and $64 \pm 4\%$ (of the pre-existing tone) in normal and hypertensive rats, respectively, while there were no significant differences in the relaxant effect of sodium nitroprusside. L-NAME (100 μM) significantly reduced the EC50 values of noradrenaline (3.71 ± 0.28 times, $n = 8$ and 2.96 ± 0.27 times, $n = 7$, in normal and hypertensive rats, respectively) and significantly enhanced the maximal contractile effect of noradrenaline ($46 \pm 8\%$, $n = 8$ and $35 \pm 6\%$, $n = 7$, in normal and hypertensive rats respectively): the factors of reduction of EC50 values and the percentages of enhancement of the maximal contractile effect in the aorta of normal and hypertensive rats were not significantly different.

The results obtained provide evidence of functional impairment of the endothelium in DPSPX-hypertensive rats.

Chronic treatment of rats with 1,3-dipropyl-8-sulphophenylxanthine (DPSPX), a non selective antagonist of adenosine receptors (Daly et al 1985), causes a hypertensive state which lasts for at least three weeks after the end of the administration of the drug (Albino-Teixeira et al 1991; Matias et al 1991). This hypertensive state is accompanied by altered prejunctional effects of the selective α_2-adrenoceptor agonist, 5-bromo-6(imidazoline-2-ylamino)-quinoxaline (UK 14,304) (Guimarães et al 1994) and enhanced prejunctional β-adrenoceptor-mediated facilitation of noradrenaline release (Guimarães et al 1995). Furthermore, there are marked structural changes with subintimal proliferative response (Albino-Teixeira et al 1991) which are preventable by angiotensin-converting enzyme inhibition (Albino-Teixeira & Osswald 1994). The functional implications of these morphological changes remain to be defined. Due to its anatomical position, the endothelium plays a well known physiological role (Vanhoutte 1989) and also represents a target for vascular injury in states of hypertension. Endothelium-dependent relaxation is impaired in most hypertensive states (for review see Lüscher et al 1991).

The purpose of the present work was to test the endothelial function in isolated aorta taken from rats rendered hypertensive by long-term administration of DPSPX. Two different kinds of experiments were carried out. Firstly, the relaxant effects of carbachol (an endothelium-dependent relaxing substance) and sodium nitroprusside (an endothelium-independent relaxing substance) were examined on contracted thoracic aortic rings from normotensive and DPSPX hypertensive rats. In the other set of experiments the endothelium-dependent enhancement of noradrenaline-induced contractions by N^G-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthase (Rees et al 1990) was compared in the same vascular tissue.

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Materials and Methods

Hypertensive animals

Male Wistar rats, initial weight 200–250 g, were used. Systolic and diastolic blood pressure were measured by the tail-cuff method (LE 500, Letica, Barcelona, Spain) in conscious animals. Blood pressure was measured on seven consecutive days before day 0 and every week thereafter for four weeks. On day 0, an Alzet osmotic minipump (model 2ML1; Alza, Palo Alto, CA, USA) was implanted intraperitoneally under pentobarbitone sodium anaesthesia (30 mg kg^{-1}, i.p.) for continuous infusion for one week of DPSPX (90 μg kg^{-1} h^{-1}) or saline (vehicle of DPSPX). The administration of DPSPX resulted in persistent hypertension that reached a maximum within two weeks (Albino-Teixeira et al 1991). The present experiments were carried out just at this time, i.e. two weeks after starting the infusion.

Preparation of rat aorta and tension measurements

Normal (vehicle-treated) and hypertensive rats were anaesthetized with pentobarbitone sodium (30 mg kg^{-1}, i.p.). The thoracic aorta was removed, cleaned of fat and connective tissue, and cut into rings of approximately 3 mm length. The endothelium was removed in some rings by gently rubbing the intimal surface with a roll of filter paper. Each ring was suspended between two stainless steel wires under 1 g resting tension and allowed to equilibrate for 2 h in Krebs solution containing (mM): NaCl, 118·6; KCl, 4·70; $CaCl_2$ 2·52;

KH_2PO_4, 1·18: $MgSO_4$, 1·23; $NaHCO_3$, 25·0; glucose 10·0, Na_2EDTA 0·027 and ascorbic acid 0·057 at 37°C and oxygenated with 95% O_2 and 5% CO_2. Tension was recorded with isometric transducers connected to a Harvard Universal Oscillograph (Milles, MA, USA). Twice during the equilibration period, noradrenaline (1 μM) was added to the bath to check tension development and the resting tension of 1 g was adjusted whenever necessary.

Relaxant effect of carbachol and sodium nitroprusside

After the equilibration period aortic rings were submaximally contracted with noradrenaline (1 μM) and the contraction assessed for stability over a period of 12–15 min. The tissues were washed for 60 min. Noradrenaline (1 μM) was added to the bath and when the contraction reached a plateau, cumulative concentration–response curves to carbachol or sodium nitroprusside were obtained.

The EC50 and the maximal relaxant effect of carbachol and of sodium nitroprusside were determined.

Effect of L-NAME on contractile responses to noradrenaline

After the equilibration period, two cumulative concentration–response curves to noradrenaline were obtained per ring. Cocaine (12 μM) and propranolol (0·3 μM) were added to the Krebs solution 30 min before the first concentration–response curve and maintained in the solution until the end of the experiment. L-NAME (100 μM) was added to the bath 30 min before starting to determine the second concentration–response curve. Control experiments carried out in the absence of L-NAME showed no significant changes in the concentration-response curves of both normal and hypertensive rats.

The EC50 and the maximal contractile effect of noradrenaline were determined for the first and the second concentration-response curves. F values (EC50 of noradrenaline (first curve)/EC50 of noradrenaline in the presence of L-NAME (second curve)) were calculated.

Statistics

Results are presented as means ± s.e. Differences between the mean values were determined by Student's *t*-test for paired and unpaired observations, and were regarded as significant when $P < 0.05$.

Drugs

Cocaine hydrocloride (Uquipa, Lisboa, Portugal); carbachol (carbamylcholine chloride), N^G-nitro-L-arginine methyl ester, (−)noradrenaline bitartrate, propranolol hydrochloride (Sigma, St Louis, MD, USA); 1,3-dipropyl-8-sulphophenylxanthine (R.B.I., Natick, USA); sodium nitroprusside (Hoffman-La Roche, Basel, Switzerland). All the stock solutions were made in double-distilled water.

Results

Blood pressure values

In the rats treated with DPSPX the systolic and diastolic blood pressure increased from 106 ± 3/74 ± 2 to 146 ± 3/108 ± 3 mm Hg (n = 10). The increment in blood pressure was significant ($P < 0.05$). In vehicle-treated animals the systolic and diastolic blood pressure values were 107 ± 4/75 ± 5 before and 108 ± 5/76 ± 4 mm Hg after treatment (n = 8).

Relaxant effect of carbachol and sodium nitroprusside

Carbachol caused a concentration–dependent relaxation of rat aorta contracted by noradrenaline (1 μM) in both normal and hypertensive rats (Fig. 1A). The contractions induced by 1 μM noradrenaline (expressed in terms of percentage of maximal response to noradrenaline; Table 1) were not significantly different: 82·0 ± 3·1 (n = 10) and 85·1 ± 4·1 (n = 9) in normal and hypertensive rats, respectively. The EC50 values for carbachol were not significantly different: 0·72 ± 0·08 μM (n = 8) and 0·76 ± 0·12 μM (n = 9) in aortic rings from normal and hypertensive rats, respectively. The relaxant effect of carbachol was significantly reduced in aorta from hypertensive rats: the maximal relaxant effect being 86·0 ± 2.6 (n = 8) in normal rats and 64·4 ± 3·6 (n = 9) in hypertensive rats ($P < 0.01$). Carbachol (up to 24 μM) did not produce any relaxant effect in endothelium-denuded preparations from normal or hypertensive rats.

In aortic rings previously contracted by 1 μM of noradrenaline, sodium nitroprusside caused a concentration-dependent relaxation which completely abolished the previous contraction (100% relaxation) in both normal and hypertensive rats (Fig. 1B). The EC50 values were not significantly different: 62 ± 13 nM (n = 6) and 45 ± 15 nM (n = 6) in tissues from normal and hypertensive rats, respectively. The maximal relaxant effect of sodium nitroprusside in endothelium denuded preparations was 100% in both normal and hypertensive rats.

Effect of L-NAME on the contractile responses to noradrenaline

Noradrenaline caused a concentration–dependent contraction of rat aorta from both normal and hypertensive rats (Fig. 2).

Table 1. EC50 values of noradrenaline and maximal responses to noradrenaline in aorta from normal and DPSPX hypertensive rats in the absence or in the presence of 100 μM L-NAME.

	EC50 values (nM)		Maximal response to noradrenaline (g per mg of tissue)	
	No drug	L-NAME	No drug	L-NAME
Normal rats	26·5 ± 4·2 n = 8	7·1 ± 1·2* n = 8	0·33 ± 0·04 n = 6	0·49 ± 0·05* n = 6
Hypertensive rats	19·3 ± 5·4 n = 7	6·8 ± 2·6* n = 7	0·36 ± 0·05 n = 6	0·50 ± 0·05* n = 6

Shown are means ± s.e. *Significantly different from the corresponding values in the absence of L-NAME; $P < 0.001$ (paired values).

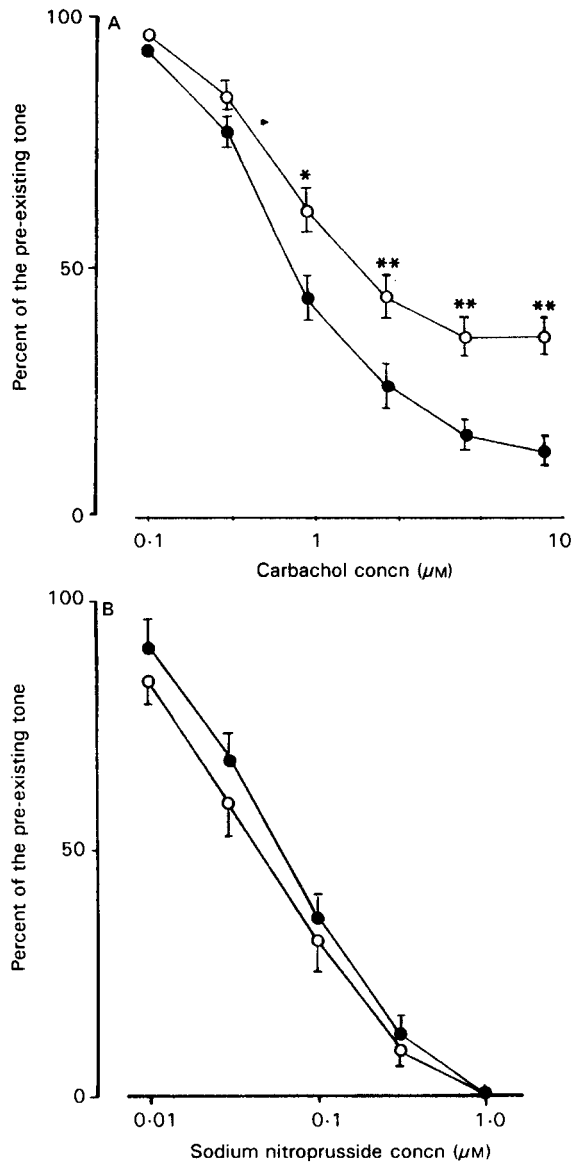

FIG. 1. Concentration–response curves for the relaxant effect of carbachol (A) and of sodium nitroprusside (B) obtained in aortic rings previously contracted by noradrenaline (1 μM), from normal (●) and hypertensive (○) rats. Means ± s.e.; n = 6–8. Significant differences from corresponding values in normal rats: $*P < 0.05$; $**P < 0.01$.

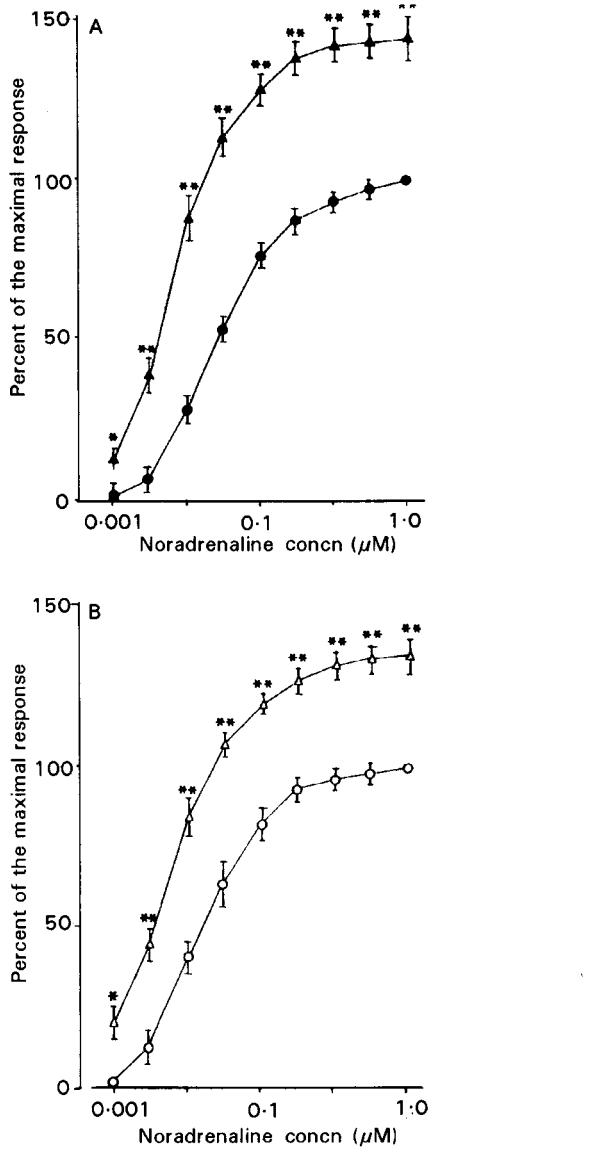

FIG. 2. Concentration–response curves to noradrenaline in aortic rings from normal (A) and hypertensive (B) rats. Responses in the absence (●, ○) or in the presence (▲, △) of 100 μM L-NAME. Means ± s.e.; n = 7–8. Significant differences from corresponding values in the absence of L-NAME: $*P < 0.05$; $**P < 0.001$ (paired values).

The EC50 values and the maximal responses to noradrenaline (in g (mg tissue)$^{-1}$) were not significantly different in tissues from normal and hypertensive rats (Table 1). L-NAME (100 μM) did not change the basal tonus of aortic rings either in normal or hypertensive rats, but potentiated the responses to noradrenaline (Fig. 2; Table 1). The F values were not significantly different: 3·71 ± 0·29 (n = 8) and 2·96 ± 0·27 (n = 7) in normal and hypertensive rats, respectively. L-NAME enhanced significantly the maximal response to noradrenaline in both normal and hypertensive rats (Fig. 2); the increases of the maximal response expressed as percent of the initial value were not significantly different: 46·8 ± 8 (n = 8) and 35 ± 6 (n = 7), in normal and hypertensive rats, respectively.

Discussion

The results show that in DPSPX hypertensive rats the endothelium-mediated relaxant effect of carbachol in aorta preparations previously contracted by noradrenaline is depressed. This result is not a consequence of differences of the tone induced by noradrenaline in normal and hypertensive rats. In fact the magnitude of contraction caused by noradrenaline (1 μM) in tissues of normal or hypertensive rats was not different.

Carbachol, apart from its endothelium-mediated relaxant effect, can contract vascular smooth muscle cells of isolated preparations. However it is not likely that the decreased

relaxant effect obtained in aortic rings of hypertensive rats was due to an increased sensivity to the direct contractile effect since in relaxed endothelium-free preparations (from normal and hypertensive rats) carbachol was unable to cause any contraction in concentrations up to 24 μM.

The present results confirm that the relaxant effect of carbachol is dependent on the endothelium since in endothelium-free rings from normal or hypertensive rats it did not cause any relaxant effect even at the highest concentration used.

As far as the relaxant effect of sodium nitroprusside is concerned, the results show that the concentration-response curves to nitroprusside obtained in the aorta from both normal and hypertensive rats were not different (the EC50 and the maximal effect of sodium nitroprusside were similar). The maximal relaxant effect of sodium nitroprusside was similar in intact and endothelium-free preparations both in normal and hypertensive rats. Thus, it appears that the reduction of the relaxant effect of carbachol obtained in the aorta from hypertensive rats is not due to the decreased relaxing capacity of this vessel since there was no reduction of relaxant effect of sodium nitroprusside.

A possible explanation for the impairment of endothelial-dependent relaxation to carbachol in hypertensive rats may be a reduced production, reduced release, or both, of endothelium-derived relaxing factor (EDRF). Other possibilities might include alterations in the transport of EDRF to smooth muscle cells, in the acetylcholine receptor density or in the transduction mechanisms.

The second part of our study deals with the effect of L-NAME on the contractile response of aortic rings to noradrenaline.

In rat isolated aorta the presence of functional endothelium has been shown to depress the contractile action of α-adrenoceptor agonists (Allan et al 1983; Carrier & White 1985; Martin et al 1986), an effect attributed to the spontaneous release of EDRF and subsequent physiological antagonism of the contractions.

Our data show that L-NAME (an inhibitor of EDRF formation) significantly reduced the EC50 for noradrenaline and significantly enhanced the maximal contractile response to noradrenaline in endothelium intact rings from both normal and hypertensive rats. Furthermore no significant differences were found between aortic rings of normal and hypertensive rats either in the factor of reduction of the EC50 (F values were not significantly different) or in the percentage of the enhancement of the maximal response to noradrenaline. These results, although pointing to an intact function of the endothelium in hypertensive rats, are not in conflict with the results obtained with carbachol. In fact, it is known that the relaxant effect of carbachol is due to the evoked release of EDRF by this substance (Furchgott 1984) whereas the depression on the contractile effect of noradrenaline was attributed to the spontaneous release of EDRF (Martin et al 1986).

To explain the impairment of endothelial-dependent relaxation by carbachol in hypertensive rats some possible mechanisms were invoked. Although our data cannot exclude the possibility of a reduced release of EDRF by carbachol in hypertensive rats (the amount of EDRF released was not estimated) one can speculate that the disturbed mechanism is beyond the production (or release) of the EDRF since the spontaneous release is not affected in hypertensive rats.

In summary this study shows that the relaxant effect of carbachol (an endothelium-dependent relaxing substance) is impaired in aorta from DPSPX hypertensive rats, while the relaxant effect of nitroprusside (an endothelium-independent relaxing substance) is not, and also that treatment with L-NAME significantly enhanced noradrenaline-induced contractions in aorta from both normal and DPSPX hypertensive rats. The extent of the enhancement was not significantly different in both normal and hypertensive animals.

The results obtained provide evidence supporting the existence of some functional impairment of the endothelium in DPSPX hypertensive rats.

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