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Enhanced sympathetic neurotransmission in the tail artery of 1,3-dipropyl-8-sulphophenylxanthine (DPSPX)-treated rats

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1 Sympathetic neurotransmission and noradrenaline content of the tail artery of Wistar rats treated for 7 days with the adenosine antagonist, 1,3-dipropyl-8-sulphophenylxanthine (DPSPX), were examined.

2 Systolic blood pressure of the DPSPX-treated rats (164.0 ± 2.9 mmHg; $n=6$) was significantly greater than saline-treated controls (140.0 ± 2.8 mmHg; $n=5$) after 7 days treatment.

3 The pressor responses of the arterial rings to transmural nerve stimulation (65 V, 0.1 ms, 4-64 Hz, for 1 s) were markedly enhanced in the DPSPX-treated compared with the saline-treated animals. Both noradrenergic and purinergic components of perivascular sympathetic neurotransmission were enhanced during DPSPX-induced hypertension.

4 Vasoconstrictor responses to exogenous noradrenaline (0.1-300 μM) and adenosine 5'-triphosphate (0.01-3 mM) were unaffected after DPSPX treatment, indicating prejunctional alteration of sympathetic cotransmission during DPSPX-induced hypertension.

5 Acute exposure to DPSPX (10 μM) did not modify vasoconstrictor responses to transmural nerve stimulation, thus supporting the claim that the enhancement of sympathetic neurotransmission only results from long-term DPSPX treatment.

6 The noradrenaline content of the tail arteries of DPSPX-treated (4.498 ± 0.26 ng cm^{-1}; $n=4$) was significantly greater than saline-treated (3.440 ± 0.30 ng cm^{-1}; $n=5$) animals.

7 These findings show that chronic inhibition of the actions of endogenous adenosine by DPSPX results in an elevation of systolic blood pressure accompanied by enhanced sympathetic cotransmission and enhanced noradrenaline content of the rat tail artery.

Keywords: DPSPX; induced hypertension; rat tail artery; sympathetic neurotransmission

Introduction

It is now well established that elevated blood pressure observed in human essential hypertension as well as in animal models of hypertension is associated with enhanced sympathetic nerve activity and morphological changes in the blood vessel wall (Stul *et al.*, 1983; Westfall & Meldrum, 1985). Sympathetic nerve activity is enhanced both in human hypertension (Manica *et al.*, 1993) and in the most similar animal model, spontaneously hypertensive rats (SHR; Judy *et al.*, 1976; Lundin *et al.*, 1984). The density of innervation in arteries of SHR is significantly increased (Scott & Pang, 1983) with a widespread increase in noradrenaline (NA) content in the peripheral vasculature (Head *et al.*, 1985). Morphological changes observed in SHR include thickening of the medial layer of both large and small arteries and increased wall thickness due to hypertrophy and hyperplasia of the smooth muscle cells (Owens *et al.*, 1981).

Adenosine is a potent regulator of vascular tone, exerting its effects both directly on vascular smooth muscle cells or through prejunctional modulation of perivascular sympathetic neurotransmission (Burnstock & Kennedy, 1986; Olsson & Pearson, 1990). Matias and co-workers have set up an animal model of hypertension induced by chronic administration of the adenosine antagonist, 1,3-dipropyl-8-sulphophenylxanthine (DPSPX). The authors have shown a consistent increase in systemic blood pressure accompanied by increased thickness of the media, hyperplasia and hypertrophy of smooth muscle cells in tail and mesenteric arteries of the rat (Matias *et al.*, 1991).

This study was designed to examine whether infusion of the adenosine antagonist, DPSPX, results in functional alteration and modification of neurotransmitter content associated with the elevated blood pressure and morphological modifications described by Matias and co-workers in 1991. To this effect vascular responses to transmural nerve stimulation (TNS) and co-transmitters NA and adenosine 5'-triphosphate (ATP) were evaluated in the tail artery of DPSPX- and saline-treated rats. The tissue content of NA was also measured.

Methods

DPSPX treatment

The experimental model used was as described by Matias *et al.* (1991). Alzet osmotic minipumps (model 2001; Alza, Palo Alto, CA, U.S.A.) were implanted intraperitoneally in male Wistar rats (250-300 g) for constant infusion of DPSPX (30 μg kg^{-1} h^{-1}) or saline. Seven days after surgery rats were killed by an overdose of sodium pentobarbitone. During treatment, systolic blood pressure was measured daily by the tail-cuff technique (model 8006, Ugo Basile Instruments, Comerio, Italy).

Pharmacology

Tail arteries were dissected and cleaned of excess tissue. Ring segments of 3-4 mm length were cut and mounted in 5 ml organ baths containing (mM): NaCl 133, KCl 4.7, NaH_2PO_4 1.35, $NaHCO_3$ 16.3, $MgSO_4$ 0.61, $CaCl_2$ 2.52 and glucose 7.8, gassed with 95% O_2, 5% CO_2 at 37°C. Rings were left to equilibrate for 60-90 min under a resting tension of 1 g. Isometric tension was recorded with a Grass FT03C transducer and displayed on a Grass ink-writing polygraph (model 79). TNS was achieved by passing a current between two electrodes parallel to the arterial rings.

In the preliminary experiments, parameters for TNS (65 V, 0.1 ms, 4-64 Hz, for 1 s) were selected in order to activate

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both noradrenergic and purinergic components of perivascular sympathetic neurotransmission. Reproducible vascular responses were obtained with TNS of 1 s duration. No significant changes in the ratio noradrenergic/purinergic components were observed following longer periods of stimulation. Cumulative concentration-response curves to NA and ATP were established. Vascular responses were evaluated as changes in tension (g). When the effect of acute exposure to DPSPX was evaluated, TNS was obtained in the absence and in the presence of 10 μM DPSPX, after 30 min incubation with the antagonist.

Noradrenaline assay

The proximal end of the tail artery was dissected out and cleaned of excessive tissue. Segments of 3–4 cm length were rapidly frozen and stored in liquid nitrogen until assay. After measurements of weight and length, NA levels were measured by high-performance liquid chromatography with electrochemical detection. Tissue samples were homogenized in 500 μl of 0.1 M perchloric acid containing 0.4 M sodium bisulphate and 25 ng of dihydroxybenzylamine (Aldrich, Gillingham, Dorset) with a motor-driven glass homogenizer. After 2.5 min at 13000 r.p.m. centrifugation, the supernatants were subject to alumina extraction. Noradrenaline and dihydroxybenzylamine were separated on a 5 μm Spherisorb C-18 reverse-phase column (Hichrom, Reading, Berks) using a mobile phase of 0.1 M sodium dihydrogen phosphate (pH 5.0) containing 5 nM heptane sulphonate (Aldrich), 0.1 mM ethylenediaminetetraacetic acid, and 13% (v/v) methanol. Noradrenaline levels were corrected for recovery using dihydroxybenzylamine internal standard.

Drugs

(-)-Noradrenaline bitartrate, adenosine 5'-triphosphate and tetrodotoxin were obtained from Sigma (Poole, Dorset). Sodium dihydroxybenzylamine and heptane sulphonate were obtained from Aldrich (Gillingham, Dorset). Pentobarbitone from Sagatal, RMB Animal Health (Dagenham, Essex) and DPSPX from RBI (Natick, MA, U.S.A.) were also commercially obtained.

Statistical analysis

All results are expressed as means ± s.e.mean; n refers to the number of animals used. Statistical analysis was performed by Student's paired and unpaired t test as required and a value of $P<0.05$ was considered statistically significant.

Results

Systolic blood pressure

Prior to the implantation of osmotic minipumps, systolic blood pressure was 137.3 ± 2.8 ($n=11$) mmHg. Continuous infusion of DPSPX induced a significant elevation of blood pressure within the first 24 h following the implantation of minipumps, which was maintained during the course of the treatment. When the animals were killed, blood pressure was 164.0 ± 2.9 ($n=6$) mmHg in the DPSPX-treated rats and 140.0 ± 2.8 ($n=5$) mmHg in saline-control animals.

Pharmacology

In rat tail arterial rings TNS (4–64 Hz) evoked frequency-dependent contractile responses. These responses were abolished by application of tetrodotoxin, thus revealing their neural origin. The increase in contractile tension by TNS was greater in rings from DPSPX-treated animals than in saline controls (Figure 1). The responses to medium and high frequencies were significantly greater by at least 2 fold in DPSPX-compared with saline-treated rats. At 64 Hz, vascular responses were 1.75 ± 0.24 ($n=10$) and 0.79 ± 0.12 ($n=10$) g in DPSPX- and saline-treated rings, respectively (Figure 2). In the presence of 1 μM prazosin, vascular responses to TNS were attenuated, and were due to the purinergic component of perivascular sympathetic neurotransmission. In these experimental conditions, vascular responses to TNS were enhanced after DPSPX treatment at all frequencies of stimulation, and were statistically significant at 8 Hz (Figure 3a). Treatment of the preparation with α,β-methylene ATP 1 μM (in the presence of prazosin 1 μM) almost abolished vascular responses to TNS. In the same preparations, the noradrenergic component of vascular responses to TNS was calculated as the differences between contractile responses in the absence and in the presence of prazosin. In DPSPX-treated animals the noradrenergic component of perivascular sympathetic neurotransmission was higher than in control animals at 32 and 64 Hz and was statistically significant at 32 Hz (Figure 3b).

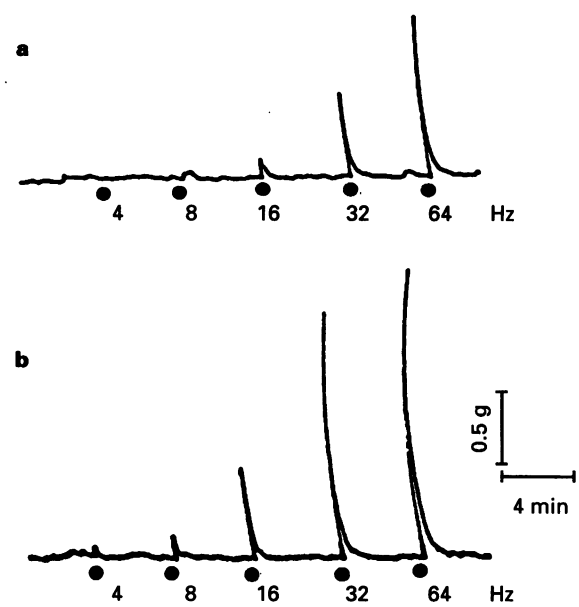

Figure 1 Recording of a typical experiment showing responses to transmural nerve stimulation (65 V, 0.1 ms, 4–64 Hz, for 1 s) in rat tail arteries taken from saline-treated controls (a) and DPSPX-treated (b) animals.

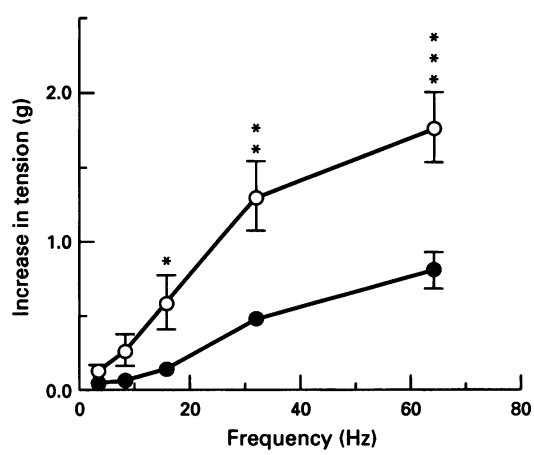

Figure 2 Frequency-response curves to transmural nerve stimulation (65 V, 0.1 ms, 4–64 Hz, for 1 s) in rat tail arteries taken from saline-treated controls (●, $n=10$) and DPSPX-treated (○, $n=10$) animals. *$P<0.05$, **$P<0.02$ and ***$P<0.001$.

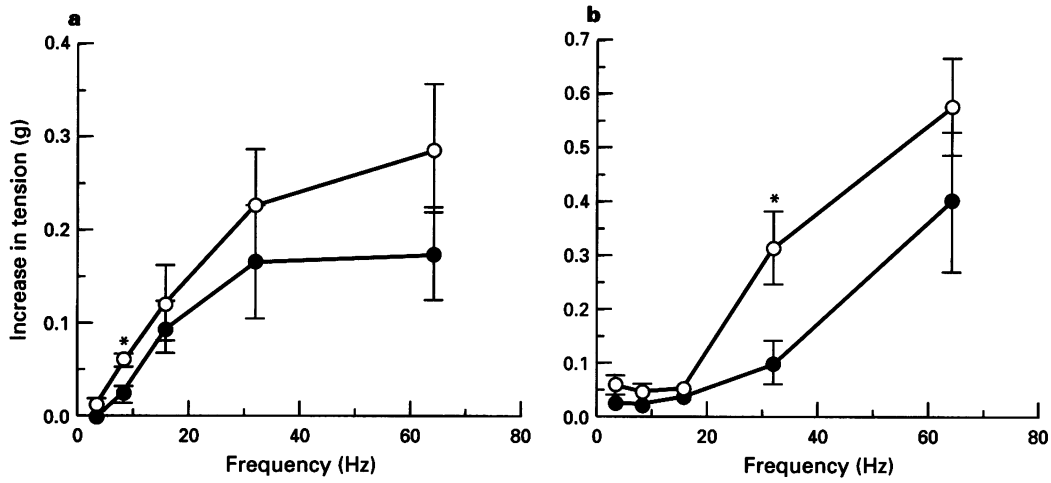

Figure 3 Frequency-response curves to transmural nerve stimulation (TNS, 65 V, 0.1 ms, 4–64 Hz, for 1 s) in rat tail arteries taken from saline-treated controls (●, $n=6$) and DPSPX-treated (○, $n=6$) animals. (a) Purinergic component of vascular response to TNS. (b) Noradrenergic component of vascular response to TNS. *$P<0.05$.

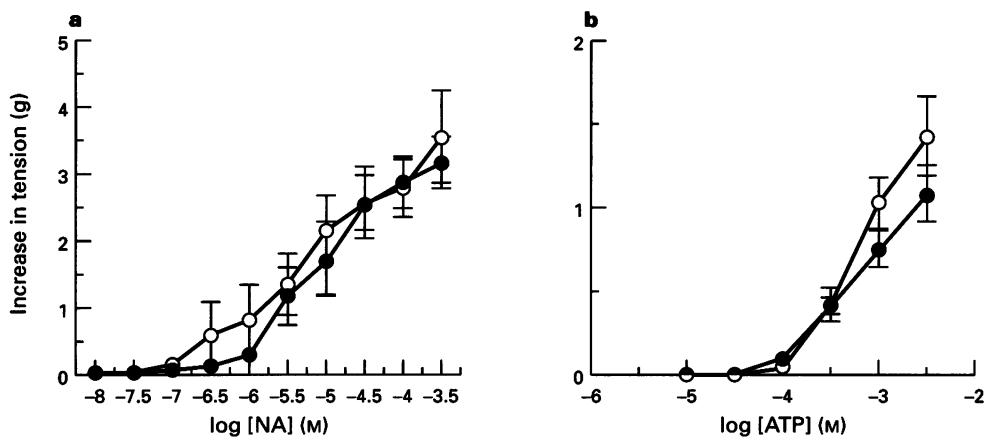

Figure 4 Concentration-response curves showing vasoconstrictor responses to (a) noradrenaline (NA) and (b) adenosine 5'-triphosphate (ATP) of rat tail arteries taken from saline-treated controls (●, $n=5$) and DPSPX-treated (○, $n=5$) animals.

Application of exogenous NA (0.1–300 μM) evoked concentration-dependent contractile responses which were similar in both groups of animals (Figure 4a). Similarly, vascular responses to ATP (0.01–3 mM) did not significantly differ in arterial segments from DPSPX-treated and saline control animals (Figure 4b). The tension developed by application of 120 mM KCl at the end of each experiment was 1.66±0.14 ($n=5$) g in DPSPX-treated rings and 1.62±0.12 ($n=5$) g in control preparations.

The effect of acute exposure to DPSPX was evaluated in a group of untreated animals. In the presence of 10 μM DPSPX vascular responses to TNS were not modified (Figure 5). At 64 Hz, the vasoconstrictor response to TNS was 1.33±0.16 ($n=4$) and 1.20±0.11 ($n=4$) g in the absence and presence of DPSPX, respectively (Figure 5).

Noradrenaline assay

The NA content of the proximal tail artery was significantly increased compared to levels in control arteries after 7 days DPSPX treatment, as shown in Figure 6.

Discussion

In the present study, vasoconstrictions evoked by TNS in arterial rings were sensitive to antagonism of the α-adrenoceptor by prazosin, desensitization of the P_{2X}-purinoceptor by α,β-methylene ATP, and were completely abolished by tetrodotoxin, which describes classical sympathetic co-transmission utilising NA and ATP in the rat tail artery (Sneddon &

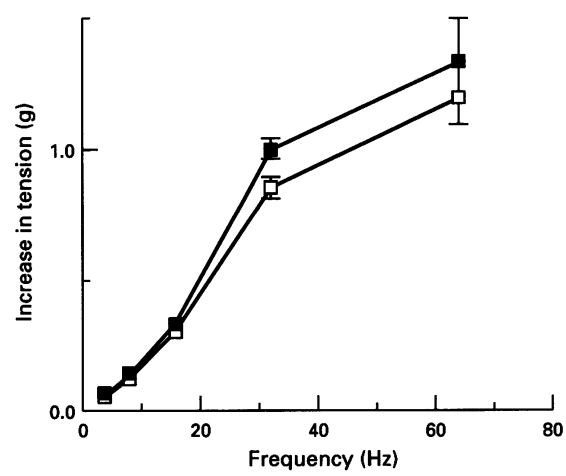

Figure 5 Frequency-response curves to transmural nerve stimulation (TNS, 65 V, 0.1 ms, 4–64 Hz, for 1 s) in rat tail arteries in absence (●, $n=4$) and in presence (○, $n=4$) of DPSPX 10 μM.

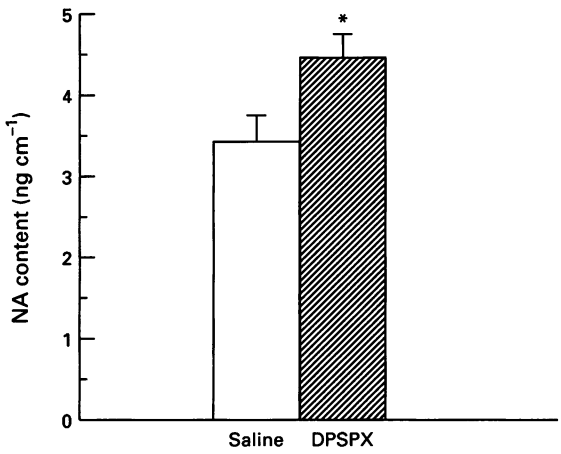

Figure 6 Tissue content (ng cm^{-1}) of noradrenaline (NA) in rat tail arteries taken from saline-treated control (open column, $n=5$) and DPSPX-treated (solid column, $n=4$) animals. *$P<0.05$.

Burnstock, 1984; Bao, 1993). Contractile responses to TNS were significantly higher after DPSPX treatment; in contrast, responses of vascular smooth muscle to the exogenous sympathetic co-transmitters NA and ATP did not differ between DPSPX-treated and control animals, thus indicating that pre- rather than post-junctional modifications account for the enhanced neurotransmission seen in the DPSPX-treated animals. The contractile responses to KCl were also unaffected after DPSPX treatment, further supporting the view that long-term adenosine antagonism by DPSPX may have no effect on post-junctional mechanisms involving sympathetic transmission. When noradrenergic and purinergic components of perivascular sympathetic neurotransmission were evaluated separately, both appeared to be enhanced following DPSPX treatment. In the presence of the α-adrenoceptor blocker, prazosin, which unmasked the purinergic component of perivascular neurotransmission, the enhancement of neurotransmission was more pronounced at lower frequencies of stimulation. In contrast, the noradrenergic component of sympathetic neurotransmission was significantly enhanced at a higher frequency of stimulation. These findings might be explained by the fact that in some blood vessels the purinergic contribution to sympathetic neurotransmission is more pronounced at lower and the noradrenergic contribution at higher frequencies of stimulation (Evans & Cunnane, 1992).

Potentiation of sympathetic neurotransmission after long-term DPSPX treatment observed in this study has also been described in SHR (Westfall & Meldrum, 1985; Head, 1989). Vidal and coworkers also showed that in the tail artery of SHR the contribution of purinergic mechanisms to perivascular sympathetic neurotransmission is exacerbated, compared to control Wistar Kyoto (WKY) rats (Vidal *et al.*, 1986). There are conflicting reports concerning the changes that occur in the response of the SHR tail artery to exogenous NA during hypertension; no change (Cassis *et al.*, 1982; Dalziel *et al.*, 1989), reduction (Aidulis *et al.*, 1990) and elevation (Medgett *et al.*, 1984) of responses to exogenous NA have all been reported. Similarly Muir & Wardle (1989) reported that responses to ATP do not change in SHR compared to WKY rats. However responses to α,β-methylene ATP (a stable analogue of ATP), were reported unaltered (Guild *et al.*, 1992) or increased (Dalziel *et al.*, 1989) in SHR compared to WKY control animals. As a consequence of these observations both pre- and post-junctional changes of perivascular sympathetic neurotransmission appear to be associated with spontaneous hypertension in the SHR model.

The pharmacological evidence from this study indicates that chronic treatment with DPSPX affects sympathetic neurotransmission at the prejunctional level. This is clearly indicated by a lack of effect by DPSPX treatment on vascular responses to exogenous neurotransmitters NA and ATP. Furthermore, potentiation of perivascular sympathetic neurotransmission observed during DPSPX-induced hypertension is specific to chronic administration of the adenosine antagonist. Acute exposure to DPSPX, at concentrations actively antagonizing adenosine actions (Persson *et al.*, 1991) did not affect the vasoconstrictor response to TNS. Therefore, the possible alteration of perivascular sympathetic innervation associated with DPSPX-induced hypertension resembles neuronal modifications observed in an established animal model of hypertension, such as SHR.

The finding of a significant increase in the NA content of tail arteries after long-term DPSPX treatment is in line with pharmacological observations and further support the hypothesis of neuronal changes associated with DPSPX-induced hypertension. The elevated NA content observed after DPSPX treatment could be due to an increase in the number of sympathetic nerves or to an alteration in their transmitter synthesis and storage. Indeed increased NA content of rat tail artery as well as a variety of other blood vessels has been well documented in SHR animals (Cassis *et al.*, 1985; Head *et al.*, 1985; Donohue *et al.*, 1988). In the tail artery of SHR, increase in the density of sympathetic innervation and NA content (Cassis *et al.*, 1985) as well as enhancement of release and reuptake of NA are established (see Cheung, 1989).

Adenosine is known to act prejunctionally via the A_1 subclass of the P_1-purinoceptor to inhibit NA release and attenuation of this effect has been implicated in sympathetic neurotransmission in SHR (Kubo & Su, 1983; Jackson, 1987). Further, lack of direct dilatation of vascular smooth muscle by endogenous adenosine acting on A_2-receptors and the consequent narrowing of the lumen may also contribute to the increase in blood pressure induced by DPSPX treatment. Evidence from sympathetic denervation studies suggests that adenosine has trophic actions on vascular smooth muscle (see Azevedo & Osswald, 1992), which may account for thickening of the vessel wall and thus elevation of blood pressure induced by DPSPX treatment (Matias *et al.*, 1991).

Thus the results of this study comprising elevation of systemic blood pressure and functional changes, in addition to morphological modifications described by Matias and coworkers (1991) due to long-term adenosine antagonism, closely resemble the abnormalities described in SHR and are consistent with the view that adenosine plays a role in hypertension by altering perivascular sympathetic neurotransmission.

In conclusion, our results indicate that chronic DPSPX treatment causes elevation of systemic blood pressure associated with enhancement of sympathetic neurotransmission. This enhancement occurs at the pre-junctional level and involves both purinergic and noradrenergic components of sympathetic neurotransmission. The rise in NA content of the rat tail artery further indicates the possible alteration of innervation density, enzymatic activity or storage mechanisms in sympathetic perivascular nerves after chronic adenosine antagonism.

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