Hypertension and enhanced β -adrenoceptor-mediated facilitation of noradrenaline release produced by chronic blockade of adenosine receptors

¹S. Guimarães, A. Albino-Teixeira & M.Q. Paiva

Institute of Pharmacology and Therapeutics, Faculty of Medicine, 4200-Porto, Portugal

1 The study was undertaken to compare the β -adrenoceptor-mediated facilitation of noradrenaline release in the tail artery of vehicle-treated rats and of rats rendered hypertensive by chronic administration of 1,3-dipropyl-8-sulphophenylxanthine (DPSPX). Artery rings were loaded with [3 H]-noradrenaline, and five periods of electrical stimulation (1 Hz for 2 min) were applied. To eliminate the influence of prejunctional α_2 -adrenoceptors, the tissues were pre-exposed to 1 μ M phenoxybenzamine.

2 Isoprenaline caused a concentration-dependent increase of tritium overflow elicited by electrical stimulation. It was more effective in arteries from DPSPX-treated than in those from vehicle-treated rats; isoprenaline (27.8 nM) increased by 30% tritium overflow in vessels from vehicle-treated rats whereas isoprenaline (7.0 nM) produced a 30% increase in vessels from DPSPX-treated animals. Furthermore, the maximal effect of isoprenaline was a 32.6% increase in control rats but a 48.6% increase in DPSPX-treated rats.

3 These results show that the sympathetic nerve endings of the rat tail artery are endowed with prejunctional β -adrenoceptors which mediate facilitation of noradrenaline release elicited by electrical stimulation. They also suggest that adenosine receptors and β -adrenoceptors interact at the prejunctional level and that impairment of this 'talk' may lead to the development of a hypertensive state.

Keywords: Adenosine receptors; prejunctional β -adrenoceptors; hypertension; DPSPX; rat tail artery

Introduction

Many peripheral noradrenergic axons possess prejunctional β -adrenoceptors, activation of which increases the release of noradrenaline evoked by nerve stimulation (for reviews see Majewski, 1983; Misu & Kubo, 1986; Nedergaard & Abrahamson, 1990; Floras, 1992). It is still uncertain if this β -adrenoceptor-mediated facilitation of transmitter release plays a physiological role. However, a substantial body of evidence supports the involvement of prejunctional β -adrenoceptors in the development and maintenance of some kinds of hypertension (Majewski *et al.*, 1981; Kubo *et al.*, 1984; Borkowski & Quinn, 1985; Misu *et al.*, 1987).

Recently, it was shown that chronic treatment of rats with the non-selective antagonist of adenosine receptors 1,3-dipropylsulphophenylxanthine (DPSPX) leads to a hypertensive state (Albino-Teixeira *et al.*, 1991; Matias *et al.*, 1991) and alters the prejunctional effects of the selective α_2 -adrenoceptor agonist, UK-14,304 (Guimarães *et al.*, 1994). The present investigation was undertaken to compare the role of β -adrenoceptor-mediated facilitation of noradrenaline release in the tail artery of normotensive rats and of rats rendered hypertensive by long-term administration of DPSPX.

Methods

Male Wistar rats with an initial body weight of 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 three weeks. Five determinations were made each time and the means used for further calculation. On day 0, an Alzet osmotic minipump (model 2ML1; Alza, Palo Alto, CA, U.S.A.) was implanted intraperitoneally under pentobarbitone sodium anaesthesia (30 mg kg⁻¹, i.p.) for

continuous infusion for one week of DPSPX (90 μ g kg⁻¹ h⁻¹) or saline (vehicle of DPSPX). The infusion 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 the end of the infusion.

Transmitter overflow

Vehicle- or DPSPX-treated animals were anaesthetized (see above). A segment of the proximal portion of the ventral tail artery, about 6 cm long, was dissected free and placed in aerated (95% O₂; 5% CO₂) modified Krebs-Henseleit medium (Guimarães & Osswald, 1969) of the following composition (mM): NaCl, 118.6, KCl, 4.70, CaCl₂ 2.52, KH₂PO₄ 1.18, MgSO₄ 1.23, NaHCO₃ 25.0, glucose 10.0. To avoid autoxidation of [3 H]-noradrenaline, EDTA 0.027 mM and ascorbic acid 0.57 mM were added to the medium. The segment of the vessel was cut into four small rings which were preincubated for 30 min in medium containing 1 mM pargyline (to inhibit monoamine oxidase), 40 μ M hydrocortisone and 40 μ M U-0521 (3,4-dihydroxy-2-methylpropiophenone) (to inhibit extraneuronal removal of noradrenaline) (Guimarães *et al.*, 1978). Hydrocortisone and U-0521 were also kept in the medium for the remainder of the experiment. After preincubation, the vessel segments were exposed for 60 min to [3 H]-noradrenaline (0.2 μ M). Thereafter they were mounted in 2 ml glass chambers between two platinum electrodes and perfused with amine-free medium (aerated and at 37°C). From $t = 110$ min ($t = 0$ min being the onset of the perfusion) the perfusion fluid was collected continuously as 5 min samples. Transmural electrical stimulation (1 Hz, 2 ms, 100 V, for 2 min; Stimulator II X, Hugo Sachs Elektronik, March-Hugstetten, Germany) was applied at min 120 (S₁), 160 (S₂), 200 (S₃), 240 (S₄) and 280 (S₅).

In most experiments, the vessel rings were exposed to 1 μ M phenoxybenzamine, which was present in the fluid during a 45 min period starting at the beginning of the perfusion.

¹ Author for correspondence.

Isoprenaline was added to the perfusion fluid 15 min before S_3 , S_4 and S_5 in concentrations increasing by half-log increments. In the experiments in which antagonism against isoprenaline was studied, the antagonist (atenolol or the compound ICI-118,551) was added to the perfusion fluid 30 min before S_1 and kept for the remainder of the experiment; pA_2 values were calculated according to the method of Van Rossum (1963) from the equation: $pA_2 = pA_x + \log(x - 1)$ in which x represents the factor or the shift of the concentration-response curve to the right and pA_x the negative logarithm of the molar concentration of the antagonist which caused this shift.

For the calculation of the overflow induced by electrical stimulation, those 5 min samples were taken into account in which the outflow of tritium exceeded that in the last pre-stimulation control sample; usually this applied to the 3 or 4 samples collected during and after stimulation. The spontaneous outflow measured in the last pre-stimulation sample was assumed to represent the spontaneous outflow in subsequent samples; it was subtracted from the outflow determined in stimulation and post-stimulation samples.

Fractional 3H -efflux min^{-1} was calculated by dividing 3H -efflux in each 5 min sample by tritium present in the tissue at the onset of the respective collection period and by 5. The fractional release per shock (FR) was calculated by dividing evoked tritium overflow by tritium present in the tissue at the beginning of the stimulation period and by the number of shocks. Drug effects are expressed as percentage of the FR of tritium evoked by S_3 (or S_4 or S_5) over that evoked by S_2 . Each result was corrected for time-dependent changes as determined in parallel drug-free control experiments.

Radioactivity was measured by liquid scintillation counting (liquid scintillation counter 1209 Rackbeta, LKB Wallac, Turku, Finland) in 2 ml aliquots of perfusate or 0.5 ml of tissue extract + 1.5 ml of Krebs-Henseleit solution. The extraction was made in 3 ml of 0.1 M perchloric acid during 18 h.

Statistics

Results are expressed as arithmetic means $\pm$ s.e. mean. One-way analysis of variance was used to test differences between unpaired results. A probability level of 0.05 or less was considered statistically significant.

Drugs

The following were used: ($\pm$)-atenolol hydrochloride (ICI, Macclesfield, UK); cocaine hydrochloride (Uquipa, Lisboa, Portugal); DPSPX(1,3-dipropyl-8-sulphophenylxanthine; Research Biochemicals, Natick, U.S.A.); hydrocortisone 21-hemisuccinate sodium (Sigma, St Louis, MO, U.S.A.); ($\pm$)-ICI-118,551 hydrochloride (erythro-DL 1 (7-methylindan-4-yloxy)-isopropylaminobutan-2-ol); (–)-isoprenaline bitartrate (Sigma); [3H]-7-(–)-noradrenaline(18.2–21.1 Ci mmol^{-1} , New England Nuclear, Dreieich, Germany); pargyline hydrochloride (Sigma); U-0521 (3,4-dihydroxy-2-methylpropiphenone; Upjohn Kalamazoo, MI, U.S.A.).

Results

The mean weight of vehicle-treated rats and DPSPX-treated rats was not significantly different: 312 ± 18 and 298 ± 20 g, ($n = 7$), respectively. The systolic and diastolic blood pressures of vehicle-treated rats were 138 ± 4 and 104 ± 3 mmHg ($n = 7$), respectively, while those of DPSPX-treated rats were 164 ± 5 and 130 ± 4 mmHg ($n = 6$), respectively ($P < 0.05$).

Some experiments were carried out on tail arteries from either vehicle-treated or DPSPX-treated rats in the absence of phenoxybenzamine. In these experiments, in which the fractional release of tritium evoked by S_2 was $7.1 \pm 1.1 \times 10^{-5}$ ($n = 5$), isoprenaline (3.3–100 nM) caused concentration-

dependent increases of tritium overflow, the maximal increase reaching $33.9 \pm 3.2\%$.

To eliminate the influence of prejunctional α_2 -auto-receptors, the tissues were pre-exposed to phenoxybenzamine (1 μM) in those experiments designed to compare the effect of the β -adrenoceptor agonist, isoprenaline, in the tail arteries from vehicle-treated and DPSPX-treated rats. In arteries from vehicle-treated rats exposed to phenoxybenzamine, the fractional release of tritium at S_2 was $43.3 \pm 2.7 \times 10^{-5}$ ($n = 5$; compare with smaller release above). In arteries from DPSPX-treated rats exposed to phenoxybenzamine the fractional release of tritium evoked by S_2 was $40.6 \pm 3.1 \times 10^{-5}$ ($n = 6$), not significantly different from vehicle-treated rats.

In the tissues pre-exposed to phenoxybenzamine, both from vehicle-treated (Figure 1) and from DPSPX-treated rats, isoprenaline caused a concentration-dependent enhancement of tritium overflow evoked by electrical stimulation. However, as shown in Figure 2, the concentration of isoprenaline required to increase tritium overflow by 30% was lower in the arteries from DPSPX-treated than in those from vehicle-treated rats: 7.0 nM isoprenaline in the arteries from DPSPX-treated rats were equipotent with 27.8 nM in the arteries from vehicle-treated animals. Furthermore, the maximal enhancement of tritium overflow evoked by electrical stimulation caused by isoprenaline was larger in the tissues from DPSPX-treated than in tissues from vehicle-treated rats: $48.6 \pm 5.3\%$ and $32.6 \pm 2.7\%$ ($n = 6$), respectively ($P < 0.05$).

In some experiments on arteries from vehicle-treated rats, the acute influence of DPSPX on the effect of isoprenaline was tested. DPSPX, 30 min before S_1 , did not change the effect of isoprenaline (not shown).

The antagonist action of atenolol and ICI-118,551 against isoprenaline-induced facilitation was studied on rings obtained from vehicle-treated rats pre-exposed to phenoxybenzamine. Atenolol and ICI-118,551 did not change the overflow of tritium elicited by S_2 . ICI-118,551 (1–10 nM) was a potent antagonist against isoprenaline. Atenolol (0.01–0.3 μM) did not shift the concentration-response curve of isoprenaline ($n = 4$).

Discussion

The results show that the sympathetic nerve endings of the rat tail artery are endowed with prejunctional β -adreno-

Figure 1 Tritium overflow evoked by electrical stimulation (1 Hz, for 2 min) from rat tail arteries, pre-exposed to phenoxybenzamine (1 μM), from vehicle-treated rats. (a) Control experiment; (b) in the presence of increasing concentrations of isoprenaline, from min 185 onwards. The Figure shows a typical experiment in which 10, 33 and 100 nM isoprenaline were used. In other experiments, isoprenaline was used in concentrations of 3.3, 10 and 33 nM.

Figure 2 Effect of isoprenaline on tritium overflow evoked by electrical stimulation (1 Hz, for 2 min) from rat tail arteries, pre-exposed to phenoxybenzamine (1 μ M), from vehicle-treated (O) and DPSPX-treated (●) rats. In some experiments S_3 , S_4 and S_5 were applied in the presence of 3.3, 10 and 33 nM isoprenaline, respectively, while in other experiments S_3 , S_4 and S_5 were applied in the presence of 10, 33 and 100 nM isoprenaline, respectively. Shown is the percentage increase in fractional release of tritium as compared to S_2 , corrected for any change occurring in experiments without isoprenaline. *Significantly different from vehicle-treated rats ($n = 6$; $P < 0.05$).

ceptors which mediate facilitation of the noradrenaline release evoked by electrical stimulation. The maximal enhancement of noradrenaline release caused by isoprenaline was about 33%. The effect of isoprenaline was not changed by pretreatment with phenoxybenzamine, which *per se* increased noradrenaline release by 6.1 times. This indicates that the noradrenaline reaching the biophase does not stimulate prejunctional β -adrenoceptors in this tissue. That noradrenaline released from sympathetic nerve varicosities activates prejunctional β -adrenoceptors to facilitate the subsequent release of noradrenaline is a very unlikely possibility because in the vast majority of the tissues, prejunctional β -adrenoceptors belong to the β_2 -subtype for which the affinity of noradrenaline is low (Lands *et al.*, 1967; Guimarães *et al.*, 1978; Guimarães & Paiva, 1981a,b; Dahlöf, 1981). The results obtained in the present study indicate that in the rat tail artery, the prejunctional β -adrenoceptors are also β_2 since ICI-118,551 was a very potent antagonist against isoprenaline, while atenolol had no effect in concentrations up to 0.3 μ M.

References

- ALBINO-TEIXEIRA, A., MATIAS, A., POLÓNIA, J. & AZEVEDO, I. (1991). Blockade of adenosine receptors causes hypertension and cardiovascular structural changes in the rat. *J. Hypertens.*, **9**, (Suppl.6), S196–S197.
- ALLGAIER, C., HERTTING, G. & KUGELGEN, O. (1987). The adenosine receptor-mediated inhibition of noradrenaline release possibly involves a N-protein and is increased by α_2 -autoreceptor blockade. *Br. J. Pharmacol.*, **90**, 403–412.
- BORKOWSKI, K.R. (1991). Effects of adrenal demedullation and adrenaline on hypertension development and vascular reactivity in young spontaneously hypertensive rats. *J. Auton. Pharmacol.*, **11**, 1–14.
- BORKOWSKI, K.R. & QUINN, P. (1985). Adrenaline and the development of spontaneous hypertension in rats. *J. Auton. Pharmacol.*, **5**, 89–100.

Long-term pretreatment of the rats with DPSPX enhanced the facilitatory effect of isoprenaline. There was not only a shift of the concentration-response curve of isoprenaline to the left, but also an increase in its maximal effect. These changes are compatible with an upregulation of the prejunctional β -adrenoceptors or some change at the level of G proteins. Long-term blockade of adenosine receptors in the rat by DPSPX also leads to an increase in the sensitivity of the sympathetic axons of the tail artery to the selective α_2 -adrenoceptor agonist UK-14,304 (Guimarães *et al.*, 1994). Those experiments had suggested a 'crosstalk' between adenosine and α_2 -adrenoceptors at the prejunctional level, an interaction already observed in different tissues, both peripherally and centrally (see Enero & Saidman, 1977; Allgaier *et al.*, 1987; Limberger *et al.*, 1988). The present data indicate an analogous 'crosstalk' in which β -adrenoceptors participate since the blockade of adenosine receptors *in vivo* enhanced β -adrenoceptor-mediated responses to isoprenaline *in vitro*.

In recent experiments carried out on rat hearts, Eikenburg & Apparsundaram (1992) showed that the facilitatory influence of salbutamol on tritium overflow elicited by electrical stimulation was increased in the presence of adenosine antagonists, suggesting that endogenous adenosine inhibits β -adrenoceptor-mediated facilitation of noradrenaline release. As the present results show, in the rat tail artery *in vitro* addition of DPSPX did not change the β -adrenoceptor-mediated facilitation of noradrenaline release indicating that no 'crosstalk' between adenosine receptors and β -adrenoceptors occurs *in vitro*.

The existence of a link between prejunctional β -adrenoceptor-mediated facilitation of noradrenaline release and the development of some hypertensive states is widely accepted. For example, infusion or depot implantation of adrenaline can cause facilitation of noradrenaline release and long-lasting hypertensive states at a time at which adrenaline plasma levels had returned to pre-infusion (or depot implantation) levels (Majewski *et al.*, 1981; Johnson *et al.*, 1983; Coppes *et al.*, 1994). In spontaneously hypertensive rats (SHR), Kubo *et al.* (1984) showed that facilitation of neurotransmission mediated by prejunctional β -adrenoceptors is enhanced in prehypertensive states. Furthermore, when SHR were adrenalectomized at a pre-hypertensive age, the development of hypertension was attenuated but could be restored by adrenaline depot implants; this pro-hypertensive effect of adrenaline was abolished by β_2 -adrenoceptor blockade (Borkowski & Quinn, 1985; Borkowski, 1991).

The present results show a new example for this relationship between prejunctional β -adrenoceptors and hypertension: chronic blockade of adenosine receptors (1) leads to a hypertensive state and (2) enhances prejunctional β -adrenoceptor-mediated facilitation of noradrenaline release, suggesting that both adenosine and β -adrenoceptors may be involved in the development of the hypertensive state.

- COPPES, R.P., BROUWER, F., FREIE, I., SMIT, J. & ZAAGSMA, J. (1994). Sustained prejunctional facilitation of noradrenergic neurotransmission by adrenaline as a co-transmitter in the portal vein of freely moving rats. *Br. J. Pharmacol.*, **113**, 342–344.
- DAHLÖF, C. (1981). Studies on β -adrenoceptor mediated facilitation of sympathetic neurotransmission. *Acta Physiol. Scand.*, (Suppl.), **500**, 1–147.
- EIKENBURG, D.C. & APPARSUNDARAM, S. (1992). Endogenous adenosine inhibits β -adrenoceptor facilitation of norepinephrine release in the heart nerve preparation. *Abstracts. Stichting 7th Int. Catecholamine Symposium* (Amsterdam), p.81.
- ENERO, M.A. & SAIDMAN, B.Q. (1977). Possible feed-back inhibition of noradrenaline release by purine compounds. *Naunyn-Schmied Arch. Pharmacol.*, **297**, 39–46.

- FLORAS, J.S. (1992). Epinephrine and the genesis of hypertension. *Hypertension*, **19**, 1–18.
- GUIMARÃES, S., BRANDÃO, F. & PAIVA, M.Q. (1978). A study of the adrenoceptor-mediated feedback mechanisms by using adrenaline as a false transmitter. *Naunyn-Schmied Arch. Pharmacol.*, **305**, 185–188.
- GUIMARÃES, S. & OSSWALD, W. (1969). Adrenergic receptors in the veins of the dog. *Eur. J. Pharmacol.*, **5**, 133–140.
- GUIMARÃES, S. & PAIVA, M.Q. (1981a). Two distinct adrenoceptor-biophases in the vasculature: one for α - and the other for β -agonists. *Naunyn-Schmied Arch. Pharmacol.*, **316**, 195–199.
- GUIMARÃES, S. & PAIVA, M.Q. (1981b). Two different biophases for adrenaline released by electrical stimulation or tyramine from the sympathetic nerve endings of the dog saphenous vein. *Naunyn-Schmied Arch. Pharmacol.*, **316**, 200–204.
- GUIMARÃES, S., PAIVA, M.Q., MOURA, D., VAZ-DA-SILVA, M.J. & ALBINO-TEIXEIRA, A. (1994). Long-term administration of 1,3-dipropyl-8-sulphophenylxanthine (DPSPX) alters α_2 -adrenoceptor-mediated effects at the pre- but not at the postjunctional level. *Naunyn-Schmied Arch. Pharmacol.*, **350**, 692–695.
- JOHNSON, M.D., GRIGNOLO, A., KUHN, C.M. & SCHANDBERG, S.M. (1983). Hypertension and cardiovascular hypertrophy during chronic catecholamine infusion in rats. *Life Sci.*, **33**, 169–180.
- KUBO, T., KUWAHARA, M. & MISU, Y. (1984). Effect of isoproterenol on vascular adrenergic neurotransmission in prehypertensive and hypertensive spontaneously hypertensive rats. *Jpn. J. Pharmacol.*, **36**, 419–421.
- LANDS, A.M., ARNOLD, A., MCAULIFF, J.P., LUDUENA, F.P. & BROWN, T.G. (1967). Differentiation of receptor systems activated by sympathomimetic amines. *Nature*, **214**, 597–598.
- LIMBERGER, N., SPÄTH, L. & STARKE, K. (1988). Presynaptic α_2 -adrenoceptor, opioid κ -receptor and adenosine A_1 -receptor interaction on noradrenaline release in rabbit brain cortex. *Naunyn-Schmied Arch. Pharmacol.*, **338**, 53–61.
- MAJEWSKI, H. (1983). Modulation of norepinephrine release by activation of presynaptic β -adrenoceptors. *J. Auton. Pharmacol.*, **3**, 47–60.
- MAJEWSKI, H., TUNG, L.-H. & RAND, M.J. (1981). Adrenaline induced hypertension in rats. *J. Cardiovasc. Pharmacol.*, **3**, 179–185.
- MATIAS, A., ALBINO-TEIXEIRA, A., POLÓNIA, J. & AZEVEDO, I. (1991). Long-term administration of 1,3-dipropyl-8-sulphophenylxanthine causes arterial hypertension. *Eur. J. Pharmacol.*, **193**, 101–104.
- MISU, Y.M. & KUBO, T. (1986). Presynaptic β -adrenoceptors. *Med. Res. Rev.*, **612**, 197–225.
- MISU, Y., KUWAHARA, M. & KUBO, T. (1987). Some relevance of presynaptic β -adrenoceptors to development of hypertension in spontaneously hypertensive rats. *Arch. Int. Pharmacodyn.*, **287**, 299–308.
- NEDERGAARD, O.A. & ABRAHAMSEN, J. (1990). Modulation of noradrenaline release by activation of presynaptic β -adrenoceptors in the cardiovascular system. *Ann. N.Y. Acad. Sci.*, **604**, 508–544.
- VAN ROSSUM, J.M. (1963). Cumulative dose-response curves. Technique for the making of dose-response curves in isolated organs and the evaluation of drug parameters. *Arch. Int. Pharmacodyn.*, **143**, 299–330.

(Received November 30, 1994

Revised January 9, 1995

Accepted January 12, 1995)