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Purinoceptor modulation of noradrenaline release in rat tail artery: tonic modulation mediated by inhibitory P_{2Y}- and facilitatory A_{2A}-purinoceptors

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1 The effects of analogues of adenosine and ATP on noradrenaline release elicited by electrical stimulation (5 Hz, 2700 pulses) were studied in superfused preparations of rat tail artery. The effects of purinoceptor antagonists, of adenosine deaminase and of adenosine uptake blockade were also examined. Noradrenaline was measured by h.p.l.c. electrochemical detection.

2 The A_1-adenosine receptor agonist, N^6-cyclopentyladenosine (CPA; 0.1–100 nM) reduced, whereas the A_{2A}-receptor agonist 2-*p*-(2-carboxyethyl)phenethylamino-5'-N-ethylcarboxamidoadenosine (CGS 21680; 3–30 nM) increased evoked noradrenaline overflow. These effects were antagonized by the A_1-adenosine receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; 20 nM) and the A_2-adenosine receptor antagonist, 3,7-dimethyl-1-propargylxanthine (DMPX; 100 nM), respectively. The P_{2Y}-purinoceptor agonist, 2-methylthio-ATP (1–100 μM) reduced noradrenaline overflow, an effect prevented by the P_2-purinoceptor antagonist, cibacron blue 3GA (100 μM) and suramin (100 μM).

3 Adenosine deaminase (2 u ml^{-1}), DMPX (100 nM) and inhibition of adenosine uptake with S-(*p*-nitrobenzyl)-6-thioinosine (NBTI; 50 nM) decreased evoked noradrenaline overflow. DPCPX alone did not change noradrenaline overflow but prevented the inhibition caused by NBTI. The P_{2Y}-purinoceptor antagonist, cibacron blue 3GA (100 μM) increased evoked noradrenaline overflow as did suramin, a non-selective P_2-antagonist.

4 It is concluded that, in rat tail artery, inhibitory (A_1 and P_{2Y}) and facilitatory (A_{2A}) purinoceptors are present and modulate noradrenaline release evoked by electrical stimulation. Endogenous purines tonically modulate noradrenaline release through activation of inhibitory P_{2Y} and facilitatory A_{2A} purinoceptors, whereas a tonic activation of inhibitory A_1 purinoceptors seems to be prevented by adenosine uptake.

Keywords: Rat tail artery; modulation of noradrenaline release; endogenous adenosine; A_1-adenosine receptor; A_{2A}-adenosine receptor; P_{2Y}-purinoceptor

Introduction

Adenosine is a neuromodulator both in the central and in the peripheral nervous system (see Ribeiro & Sebastião, 1991). Its ability to inhibit the release of noradrenaline has led to the proposal that adenosine serves to maintain a 'purinergic inhibitory tone on noradrenergic synaptic output' (Harms *et al.*, 1978). However, several lines of evidence suggest that the modulation by purines of neurotransmitter release is not limited to the 'classical' inhibitory effect of adenosine. Adenosine can also facilitate noradrenaline release (Wiklund *et al.*, 1989; Fuder *et al.*, 1992; Gonçalves & Queiroz, 1993; Todorov *et al.*, 1994), and adenine nucleotides such as ATP may inhibit noradrenaline release by an action on P_2-purinoceptors (von Kügelgen *et al.*, 1989; 1993; 1994a;b; Fuder & Muth, 1993). The lack of selective agonists and antagonists had limited the study of these multiple ways of purinoceptor modulation and has sometimes led to conflicting interpretations and conclusions (in rat vas deferens: Forsyth *et al.*, 1991; Kurz *et al.*, 1993; in rat tail artery: Shinozuka *et al.*, 1988; Illes *et al.*, 1989).

In the present experiments the purinoceptor modulation of noradrenaline release in the rat tail artery was examined in more detail with regard to: (i) P_1-purinoceptor mediated modulation (A_1-mediated inhibition and A_2-mediated facilitation); (ii) P_2-purinoceptor mediated modulation and (iii) the occurrence of tonic modulation mediated by these purinoceptors.

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Methods

Male Wistar rats (250–320 g) were killed by cervical dislocation and exsanguination. The ventral tail artery (proximal segment, about 60 mm) was dissected out and cleaned of connective tissue. Four tissue preparations (about 15 mm each) were obtained from each artery. They were incubated at 37°C for 30 min in 20 ml vials containing medium (composition below) with 1 mM pargyline (to block monoamine oxidase). Following incubation one preparation was transferred to each of four perfusion chambers where it was held by a polypropylene mesh between platinum electrodes. The tissues were perfused continuously with medium at a constant rate of 1 ml min^{-1}. In general, one tissue was used as control (solvent) while the three others were used to test effects of drugs.

The medium used for incubation and perfusion contained (in 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, ascorbic acid 0.57 and disodium EDTA 0.027. It was saturated with 95% O_2 + 5% CO_2 and kept between 36 and 37°C. Desipramine (400 nM; to inhibit uptake$_1$), 3,4-dihydroxy-2-methyl propiophenone (U-0521; 40 μM; to inhibit catechol-*O*-methyltransferase) and yohimbine (1 μM; to prevent auto-inhibition mediated by prejunctional α_2-adrenoceptors) were added only to the perfusion medium.

A Coulbourn stimulator (Model E13-65, Coulbourn Instruments, Allentown, U.S.A.) operating in the constant current mode was used for electrical field stimulation. A total of three identical periods of stimulation were applied (5 Hz; 1 ms; 10 mA; 2700 pulses, i.e. 9 min), starting at $t=45$, 90 and 135 min (S_0, S_1 and S_2, respectively; $t=0$ was the onset of

perfusion). Only S_1 and S_2 were considered for determination of the electrically evoked noradrenaline overflow. Two consecutive 12 min samples were collected for each stimulation period: the first sample corresponded to the 12 min before onset of stimulation (basal outflow; b_1 before S_1 and b_2 before S_2) and the second sample to the 9 min of electrical stimulation plus 3 min post-stimulation. Drugs (or solvents) were added to the medium after S_1 (times as indicated in results). At the end of each experiment, tissues were removed from the organ bath, blotted with filter paper and weighed (5.3 ± 0.6 mg; $n=284$).

Perifusate was collected in ice-cooled plastic vials containing perchloric acid (0.1 M final concentration) and 18 pmol of dihydroxybenzylamine (internal standard). For determination of catecholamines, samples were processed as previously described (Soares-da-Silva, 1988). Equipment for high performance liquid chromatography (h.p.l.c) with electrochemical detection, mobile phase and analysis of chromatograms was as previously described (Queiroz *et al.*, 1995).

The evoked overflow of noradrenaline was estimated by subtracting the basal outflow (b_1 or b_2) from the total outflow during and 3 min after S_1 or S_2. Outflow and evoked overflow of noradrenaline are expressed as pmol mg^{-1} tissue. Ratios of noradrenaline outflow in b_2 and in b_1 (b_2/b_1) and of noradrenaline overflow evoked by S_2 and by S_1 (S_2/S_1) were calculated for further evaluation of effects of drugs (or solvents) on basal outflow and on electrically evoked overflow, respectively. S_2/S_1 ratios obtained in individual experiments with a test compound were calculated as a percentage of the mean S_2/S_1 ratio in the appropriate control group.

The following drugs were used: adenosine deaminase type IV, cibacron blue 3GA, S-(*p*-nitrobenzyl)-6-thioinosine (NBTI), pargyline hydrochloride, tetrodotoxin, yohimbine hydrochloride (Sigma, St Louis, U.S.A.), 2-*p*-(2-carboxyethyl) phenethylamino-5'-*N*-ethylcarboxamidoadenosine hydrochloride (CGS 21680), N^6-cyclopentyl-adenosine (CPA), 3,7-dimethyl-1-propargylxanthine (DMPX), 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), 2-methylthio-ATP tetrasodium salt, suramin tetrasodium salt (Research Biochemicals, Natick, U.S.A.), desipramine hydrochloride (Ciba-Geigy, Basel, Switzerland), 3,4-dihydroxy-2-methyl propiophenone (U-0521; Upjohn, Kalamazoo, U.S.A.). Adenosine deaminase was supplied as a suspension (in 3.2 M $(NH_4)_2SO_4$, 0.01 potassium phosphate, pH 6.0). Tetrodotoxin was dissolved in 0.02 M acetic acid. Solutions of other drugs were prepared with either distilled water or dimethylsulphoxide. Drugs were diluted with medium immediately before use. Solvents were added to the perfusion medium in parallel control experiments and none of the solvents used influenced basal or evoked overflow of noradrenaline.

Results are given as arithmetic means ± s.e.mean and *n* represents the number of tissue preparations. Differences between means were tested for significance by Student's unpaired *t* test. $P<0.05$ was taken to be statistically significant.

Results

Basal outflow of noradrenaline in the 12 min just prior to S_1 (b_1) was 0.5 ± 0.1 pmol mg^{-1} tissue ($n=284$). In control conditions the basal outflow of noradrenaline prior to S_2 (b_2) was not different: the b_2/b_1 ratio was 1.03 ± 0.02 ($n=54$). None of the drugs used influenced the basal outflow of noradrenaline (data not shown).

Electrical field stimulation (5 Hz, 2700 pulses) elicited an increase in noradrenaline outflow: in the 12 min during and after S_1 it increased to 5.9 ± 0.3 pmol mg^{-1} ($n=284$). This value corresponded to about 11 times the basal outflow and to an overflow of 5.4 ± 0.3 pmol mg^{-1} ($n=284$).

In control conditions the overflow of noradrenaline evoked by electrical field stimulation decreased from S_1 to S_2 and this decrease was similar for all the solvents used: the S_2/S_1 ratio of control experiments was 0.73 ± 0.02 ($n=54$).

Noradrenaline overflow elicited by electrical field stimulation, but not basal outflow, was abolished by 1 μM tetrodotoxin (S_2/S_1 ratio of experiments in which tetrodotoxin was added 20 min before S_2 was 0.04 ± 0.03; $n=4$).

Purinoceptor agonists and antagonists

To determine if prejunctional P_1-purinoceptors were modulating noradrenaline release, the effects of the selective A_1 receptor agonist, CPA and the A_{2A} receptor agonist, CGS 21680 were investigated. The agonists were added 3 min before S_2.

CPA (0.1 – 100 nM) decreased noradrenaline overflow elicited by electrical stimulation in a concentration-dependent manner (Figure 1). The maximal inhibitory effect was obtained with 100 nM and consisted in a reduction by about 70%; 1000 nM CPA decreased noradrenaline overflow by only $42 \pm 8\%$ ($n=3$; not shown). The concentration of CPA that reduced the evoked overflow of noradrenaline by 50%, (estimated from Figure 1) was about 7 nM.

The effect of CPA was also investigated in the presence of the A_1-adenosine receptor antagonist DPCPX. DPCPX (20 nM) was added 33 min before S_2 and CPA as above. DPCPX alone did not change noradrenaline overflow elicited by electrical stimulation (Table 1) but abolished the effect of CPA. A tendency for an increase in noradrenaline overflow with 100 nM CPA was observed in the presence of DPCPX (Figure 1).

The A_{2A}-adenosine receptor agonist CGS 21680 increased the noradrenaline overflow elicited by electrical stimulation in a concentration-dependent manner (Figure 2). The maximal effect was obtained with 100 nM CGS 21680 and consisted in a 58% increase; 300 nM CGS 21680 increased noradrenaline overflow by only $32 \pm 10\%$ ($n=3$; not shown).

The effect of CGS 21680 was also investigated in the presence of the A_2-adenosine receptor antagonist, DMPX. DMPX (100 nM) was added 33 min before S_2 and CGS 21680 as above. DMPX (100 nM) decreased noradrenaline overflow elicited by electrical stimulation by about 45% (Table 1). Furthermore, DMPX (100 nM) attenuated the facilitation caused by CGS 21680 (Figure 2).

The effect of the P_2-purinoceptor agonist, 2-methylthio-ATP was investigated in order to look for a modulation of noradrenaline release mediated by P_{2Y}-receptors. 2-Methylthio-ATP (added 3 min before S_2) decreased noradrenaline overflow elicited by electrical stimulation in a concentration-

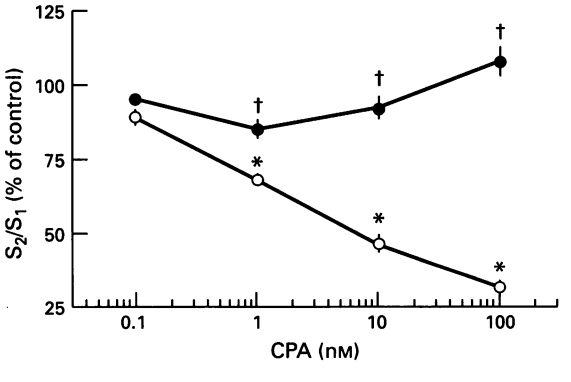

Figure 1 Effect of N^6-cyclopentyladenosine (CPA) on the electrically evoked noradrenaline overflow in the absence (○) and in the presence of 20 nM 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; ●). Tissues were stimulated 3 times at 5 Hz, 2700 pulses (S_0-S_2). The agonist was added 3 min and the antagonist 33 min before S_2 and kept for the remainder of the experiment. S_2/S_1 values are expressed as a percentage of the respective average control S_2/S_1 ratio (solvent for ○; antagonist + solvent for ●). Each point is the mean ± s.e.mean of 5–6 experiments. Significant differences from control $*P<0.05$; from agonist alone; $\dagger P<0.05$.

Table 1 Effects of purinoceptor antagonists, NBTI and adenosine deaminase on electrically evoked noradrenaline overflow

Drugs	S_2/S_1 (% of control)	n
DPCPX (20 nM)	106 ± 5	29
DMPX (100 nM)	55 ± 6*	18
Cibacron blue 3GA (100 μM)	140 ± 7*	9
Suramin (100 μM)	122 ± 8*	6
Adenosine deaminase (2 u ml^{-1})	68 ± 7*	9
NBTI (50 nM)	73 ± 3*	6
NBTI (50 nM) + DPCPX (20 nM)	101 ± 7†	6

Tissues were stimulated 3 times at 5 Hz, 2700 pulses (S_0-S_2). The drugs indicated, or their solvent, were added 33 min (30 min for adenosine deaminase) before S_2. S_2/S_1 values are expressed as a percentage of the respective average control (solvent). DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; DPMX, 3,7-dimethyl-1-propargylxanthine, NBTI, S-(*p*-nitrobenzyl)-6-thioinosine. Means ± s.e.mean of *n* experiments.
Significant differences: from control (*$P<0.05$); from NBTI alone (†$P<0.05$).

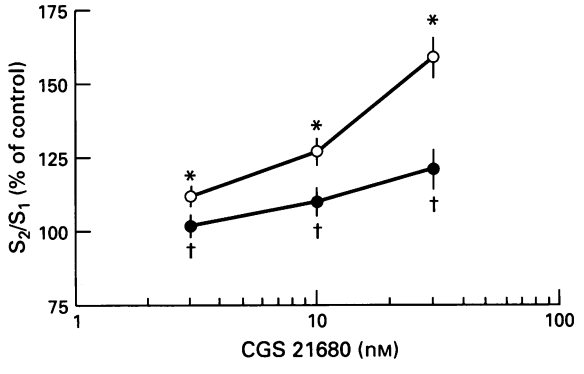

Figure 2 Effect of 2-*p*-(2-carbonylethyl)-phenethylamino-5′-*N*-ethylcarboxaminoadenosine (CGS 21680) on the electrically evoked noradrenaline overflow in the absence (○) and in the presence of 100 nM 3,7-dimethyl-1-propargylxanthine (DMPX; ●). Tissues were stimulated 3 times at 5 Hz, 2700 pulses (S_0-S_2). The agonist was added 3 min and the antagonist 33 min before S_2 and kept for the remainder of the experiment. S_2/S_1 values are expressed as a percentage of the respective average control S_2/S_1 ratio (solvent for ○; antagonist + solvent for ●). Each point is the mean ± s.e.mean of 6 experiments. Significant differences from control *$P<0.05$; from agonist alone; †$P<0.05$.

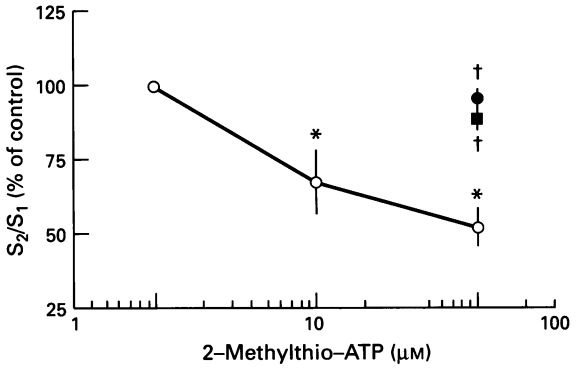

Figure 3 Effect of 2-methylthio-ATP on the electrically evoked noradrenaline overflow in the absence of antagonists (○), in the presence of 100 μM cibacron blue 3GA (●) or in the presence of 100 μM suramin (■). Tissues were stimulated 3 times at 5 Hz, 2700 pulses (S_0-S_2). The agonist was added 3 min and the antagonists 33 min before S_2 and kept for the remainder of the experiment. S_2/S_1 values are expressed as a percentage of the respective average control S_2/S_1 ratio (solvent for ○; antagonist + solvent for ●). Each point is the mean ± s.e.mean of 6 experiments. Significant differences from control *$P<0.05$; from agonist alone; †$P<0.05$.

dependent manner (Figure 3). At the maximal concentration used (100 μM) 2-methylthio-ATP decreased noradrenaline overflow by about 49% (Figure 3).

The effect of 2-methylthio-ATP (100 μM) was then investigated in the presence of the P_2-purinoceptor antagonists, cibacron blue 3GA (100 μM) and suramin (100 μM). Both cibacron blue 3GA (100 μM) and suramin (100 μM) increased noradrenaline overflow elicited by electrical stimulation (by 40 and 22%, respectively; Table 1) and both attenuated the inhibition of noradrenaline overflow caused by 2-methylthio-ATP (Figure 3).

It should be noted that the antagonisms shown in Figures 2 and 3 are not simply the addition of opposite effects of agonists and antagonists: effects (S_2/S_1) of agonists in the presence of antagonists in the figures are expressed as % of effects (S_2/S_1) observed in the presence of antagonists alone.

Endogenous adenosine

Adenosine deaminase (which deaminates adenosine to inosine) and NBTI (which inhibits adenosine uptake) were used in order to investigate how changes in endogenous adenosine levels influenced noradrenaline overflow evoked by electrical stimulation.

Adenosine deaminase (2 u ml^{-1}) was added 20 min before S_2 and reduced noradrenaline overflow by about 32% (Table 1). NBTI (50 nM) was added 33 min before S_2 and reduced noradrenaline overflow by about 27% (Table 1). The effect of NBTI was not concentration-dependent: 50 μM NBTI reduced noradrenaline overflow by 21 ± 4% ($n=3$; not shown). The effect of NBTI was also studied in the presence of DPCPX. DPCPX and NBTI were added before S_2 (times as above). DPCPX (20 nM) alone did not change noradrenaline overflow, as in the preceding section (see Table 1), but prevented the inhibition caused by 50 nM NBTI (Table 1).

Discussion

Under the conditions of the present experiments the changes in evoked noradrenaline overflow caused by purinoceptor agonists and antagonists cannot be ascribed to an interference with the inactivation of noradrenaline, since monoamine oxidase, catechol-*O*-methyl transferase and neuronal uptake of noradrenaline were blocked. Thus, overflow of noradrenaline evoked by electrical stimulation can be interpreted as noradrenaline release. α_2-Adrenoceptors were also blocked, so any interference of auto-inhibition mediated by prejunctional α_2-autoreceptors with the effect of purinoceptor agonists and

antagonists was avoided (see Enero & Saidman, 1977; Allgaier *et al.*, 1987; Limberger *et al.*, 1988; Guimarães *et al.*, 1994).

CPA reduced noradrenaline release evoked by electrical stimulation with an $EC_{50\%}$ of about 7 nM (see Figure 1). CPA is a highly selective A_1 agonist and this concentration is only 10 times higher than the K_i value at A_1-adenosine receptors (Moos *et al.*, 1985; Williams *et al.*, 1986). This result confirms previous observations that adenosine analogues inhibit noradrenaline release in rat tail artery (Shinozuka *et al.*, 1988; 1990; Illes *et al.*, 1989). The effect of CPA was antagonized by 20 nM DPCPX, a selective antagonist of A_1 receptors; the concentration is about 30 fold its K_i for A_1-adrenosine receptors (Sebastião *et al.*, 1990). Thus, the inhibitory effect of CPA on noradrenaline release seems to be mediated via A_1-adenosine receptors, in agreement with results obtained by Illes *et al.* (1989) who used **R**-PIA as a selective A_1-adenosine agonist.

The P_{2Y}-purinoceptor-selective agonist 2-methylthio-ATP inhibited noradrenaline release and this effect was antagonized by cibacron blue 3GA, an isomer of reactive blue 2, also P_{2Y}-selective (Shirahase *et al.*, 1991; Boland *et al.*, 1992; von Kügelgen *et al.*, 1994b) and inactive at A_1-purinoceptors (Tschöpl *et al.*, 1992; von Kügelgen *et al.*, 1994b). The effect of 2-methylthio-ATP was in addition antagonized by suramin, a P_2-antagonist also inactive at A_1-purinoceptors (von Kügelgen *et al.*, 1994b). These results suggest that, in rat tail artery, noradrenaline release may be inhibited through activation of P_2-purinoceptors, probably of the P_{2Y} subtype. Release-inhibiting P_2-purinoceptors have previously been demonstrated at the postganglionic sympathetic axons of mouse and rat vas deferens (von Kügelgen *et al.*, 1989; 1993; 1994a; Kurz *et al.*, 1993), rat iris (Fuder & Muth, 1993), atria (von Kügelgen *et al.*, 1995) and brain cortex (von Kügelgen *et al.*, 1994b) and at cultured chick sympathetic neurones (Allgaier *et al.*, 1994). Results obtained in guinea-pig saphenous artery (Fujioka & Cheung, 1987) and in rat tail artery (present study) suggest that release-inhibiting P_2-purinoceptors are also present in vascular tissue.

In a previous study it was concluded that adenosine and adenine nucleotides inhibit release of noradrenaline in rat tail artery via one common type of purinoceptor(s) (P_3) 'which possess some of the characteristics of a P_1-receptor (...) and some characteristics of a P_2-receptor...' (Shinozuka *et al.*, 1988). Our results suggest that adenosine inhibits noradrenaline release via P_1-purinoceptors (A_1) whereas adenine nucleotides inhibit noradrenaline release via P_2-purinoceptors (P_{2Y}) in rat tail artery.

CGS 21680 (a selective A_{2A} adenosine receptor agonist; Jarvis *et al.*, 1989; Lupica *et al.*, 1990) increased noradrenaline release evoked by electrical stimulation, an effect antagonized by DMPX (a selective antagonist at A_2 receptors; Daly *et al.*, 1986; see also Gonçalves & Queiroz, 1993). These results suggest the presence of facilitatory A_2-adenosine receptors (probably of A_{2A} subtype) in rat tail artery modulating noradrenaline release. Evidence for a facilitatory effect of adenosine on noradrenaline release has been shown in guinea-pig (pulmonary artery; Wiklund *et al.*, 1989), rabbit (ear artery: Zhang *et al.*, 1989; Ishii *et al.*, 1993; saphenous artery: Todorov *et al.*, 1994; hippocampus: Rensing *et al.*, 1993) and rat (iris: Fuder *et al.*, 1992l vas deferens: Gonçalves & Queiroz, 1993).

It has been proposed that the facilitatory effect of adenosine and adenine nucleotides was mediated by a facilitatory subtype of the P_3 receptor (Ishii *et al.*, 1993; Todorov *et al.*, 1994). However, our results with CGS 21680, and its interaction with DMPX, can be fully explained by an A_2-mediated effect.

The results obtained with purinoceptor antagonists and with adenosine deaminase are compatible with the hypothesis that, in rat tail artery, endogenous purines exert a dual and opposite modulation on noradrenaline release: a P_{2Y}-mediated inhibition and an A_{2A}-mediated facilitation. The P_{2Y}-mediated inhibition became apparent by the increase of noradrenaline release caused by the P_2-receptor antagonists (cibacron blue 3GA and suramin); the A_{2A}-mediated facilitation became apparent by the decrease in noradrenaline release caused by the A_2-adenosine receptor antagonist, DMPX as well as by adenosine deaminase. Evidence for a tonic facilitation by adenosine of noradrenaline release was also obtained in rat iris (Fuder *et al.*, 1992) and vas deferens (Gonçalves & Queiroz, 1993).

A_1 receptors have an affinity for adenosine about three orders of magnitude higher than A_2 receptors (see page 781 of Olsson & Pearson, 1990). In the rat tail artery A_1-adenosine receptors are present (assumption based on the inhibitory effect of CPA), endogenous adenosine is available (assumption based on the effect of adenosine deaminase) and yet, in spite of the tonic facilitation mediated by A_{2A}-adenosine receptors, no tonic inhibition mediated by A_1-receptor was detected. A possible explanation for this unexpected finding may be the location of the A_1 receptors close to the adenosine carrier. The carrier, by preventing adenosine accumulation in the biophase of the A_1-adenosine receptor might then prevent endogenous adenosine from exerting a tonic inhibition mediated by A_1-receptors. In agreement with this hypothesis is the observation of an A_1-mediated inhibition of noradrenaline release when the adenosine carrier was blocked.

Two observations support the existence of a tonic inhibition of noradrenaline release mediated by P_2-purinoceptors: (i) cibacron blue 3GA, which preferentially blocks the P_{2Y}-subtype, increased noradrenaline release when given at a concentration that blocked the response to 2-methylthio-ATP and (ii) the same was true for suramin, a non selective P_2-antagonist. In all likelihood the increase of noradrenaline release obtained with P_2-antagonists is due to a tonic inhibition mediated by P_{2Y}-purinoceptors, similar to that reported in mouse and rat vas deferens (von Kügelgen *et al.*, 1993).

In conclusion, our results suggest that inhibitory (A_1 and P_{2Y}) and facilitatory (A_{2A}) purinoceptors are present in the rat tail artery modulating noradrenaline release evoked by electrical stimulation. Endogenous purines tonically modulate noradrenaline release through activation of both inhibitory (P_{2Y}) and facilitatory (A_{2A}) purinoceptors, whereas activation of inhibitory A_1 purinoceptors seems to be prevented by the activity of the adenosine carrier.

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