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Facilitation of noradrenaline release by adenosine A_{2A} receptors in the epididymal portion and adenosine A_{2B} receptors in the prostatic portion of the rat vas deferens

Glória Queiroz, Carmen Diniz, Jorge Gonçalves*

Laboratório de Farmacologia, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha, 164, 4050-047 Porto, Portugal

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Abstract

The adenosine-receptor modulation of noradrenaline release was compared in prostatic and epididymal portions of rat vas deferens. In both portions, tritium overflow elicited by electrical stimulation (100 pulses/8 Hz) was reduced by the adenosine A_1 receptor agonist, N^6-cyclopentyladenosine, and enhanced by the nonselective receptor agonist, 5′-*N*-ethylcarboxamidoadenosine, in the presence of the adenosine A_1 receptor antagonist, 1,3-dipropyl-8-cyclopentyl-1,3-dipropylxanthine (DPCPX; 20 and 100 nM). The adenosine A_{2A} receptor agonist, 2-*p*-(2-carboxyethyl)phenethyl-amino-5′-*N*-ethylcarboxamidoadenosine, increased tritium overflow, but only in the epididymal portion. The enhancement caused by NECA was prevented by the adenosine A_{2A} receptor antagonist, 4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo-[2,3-a][1,3,5]triazin-5-ylamino]ethyl)phenol (ZM 241385; 20 nM), in the epididymal and by the adenosine A_{2B} receptor antagonist, alloxazine (1 μM), in the prostatic portion. Inhibition of adenosine uptake enhanced tritium overflow in both portions, an effect blocked by ZM 241385 in the epididymal and by alloxazine in the prostatic portion. The results indicate that adenosine exerts an adenosine A_1 receptor-mediated inhibition, in both portions, and facilitation mediated by adenosine A_{2A} receptors in the epididymal and by A_{2B} receptors in the prostatic portion.

Keywords: Adenosine A_1 receptor; Adenosine A_{2A} receptor; Adenosine A_{2B} receptor; Noradrenaline; Vas deferens, rat; Distribution; Differential

1. Introduction

Purines (mainly ATP and adenosine) play multiple roles in postganglionic sympathetic transmission. ATP is a cotransmitter with noradrenaline and, in the vas deferens, mediates a significant component of the neurogenic contraction (see Burnstock, 1990; von Kügelgen and Starke, 1991). Adenosine, formed by degradation of ATP or released per se, modulates sympathetic transmission. Adenosine exerts its effects by activating membrane receptors, known as P1 or adenosine receptors. Four different P1 receptor subtypes have been cloned: A_1, A_{2A}, A_{2B} and A_3 receptors (Mahan et al., 1991; Reppert et al., 1991; Stehle et al., 1992; Salvatore et al., 1993), and present distinct pharmacological profiles (see Ralevic and Burnstock, 1998).

In the rat vas deferens, sympathetic transmission is modulated by prejunctional adenosine A_1 receptors that mediate inhibition of noradrenaline release (Muller and Paton, 1979) and, at least in the epididymal portion, by prejunctional adenosine A_2 receptors (likely A_{2A}) that mediate facilitation of noradrenaline release (Gonçalves and Queiroz, 1993). Synaptic transmission is also modulated by postjunctional adenosine A_1 receptors that mediate facilitation of contractile responses (Hourani and Jones, 1994) and, at least in the prostatic portion, by postjunctional adenosine A_2 receptors that mediate inhibition of contractile responses (Brownhill et al., 1996; Peachey et al., 1996). The aim of the present study was to compare the adenosine receptor-mediated modulation of noradrenaline release in the prostatic and epididymal portions of the rat vas deferens. This aim was justified since the importance of ATP as transmitter is different in the two portions of the vas deferens (Sneddon and Machaly, 1992), as differences in the adenosine receptor modulation of postjunctional responses have been observed (Brownhill et al., 1996), and a recent immunohistochemical study (Diniz et al.,

* Corresponding author. Tel.: +351-222-2078-932; fax: +351-222-078-969.

E-mail address: jorge.goncalves@ff.up.pt (J. Gonçalves).

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2001) suggested differences in the distribution of prejunctional adenosine receptors along the rat vas deferens.

2. Materials and methods

2.1. Chemicals

The following drugs were used: levo-[ring-2,5,6-^{3}H]-noradrenaline, specific activity 46.8 Ci mmol^{-1}, was from DuPont NEN (Garal, Lisboa, Portugal); adenosine hemisulfate, alloxazine, 2-*p*-(2-carboxyethyl)-phenethylamino-5′-*N*-ethylcarboxamidoadenosine hydrochloride (CGS 21680), N^6-cyclopentyladenosine (CPA), 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), desipramine hydrochloride, 3,7-dimethyl-1-(2-propynyl)xanthine (DMPX), 5′-*N*-ethylcarboxamidoadenosine (NECA) and *S*-(4-nitrobenzyl)-6-thioinosine (NBTI) were from Sigma (Alcobendas, Spain); 4-(2[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]-triazin-5-ylamino]ethyl)-phenol (ZM 241385) was from Tocris (Bristol, UK). Solutions of drugs were prepared with dimethylsulphoxide and diluted with medium immediately before use. Solvent was added to the superfusion medium in parallel control experiments.

2.2. Experimental protocol

Adult male Wistar rats (290–400 g; CRIFFA, Barcelona, Spain) were used. The animals were kept under standard laboratory conditions: light/dark cycle of 12:12 h, temperature of 20–22 °C, and free access to water and pellet food. Handling and care of animals were conducted according to the EU guiding principles in animal research (86/609/EU), as adopted by Portuguese law (Portaria no. 1005/92 and no. 1131/97). Animals were killed by cervical dislocation and exsanguination. Prostatic and epididymal halves of vas deferens were dissected out and cleaned of connective tissue. Tissue preparations (prostatic and epididymal portions) were incubated in 2-ml medium containing 0.1 μM [^{3}H]noradrenaline, for 40 min at 37 °C. Individual preparations were placed in superfusion chambers between platinum electrodes and superfused with [^{3}H]noradrenaline-free medium at a rate of 1 ml min^{-1}. Successive 5-min samples of the superfusate were collected from $t=55$ min onwards ($t=0$ min being the start of superfusion). At the end of the experiments, tritium was determined in superfusate samples and in tissues by scintillation spectrometry (Beckman LS 6500, Beckman Instruments, Fullerton, USA). The medium contained (mM): NaCl 118.6, KCl 4.70, $CaCl_2$ 2.52, $MgSO_4$ 1.23, $NaHCO_3$ 25.0, glucose 10.0, ascorbic acid 0.3, and disodium EDTA 0.031, was saturated with 95% O_2:5% CO_2 and kept at 37 °C. The superfusion medium also contained desipramine (400 nM) to inhibit neuronal uptake of noradrenaline.

Five identical periods of electrical stimulation were applied (Stimulator II, Hugo Sachs Elektronik, March-Hugstetten, Germany; constant current mode; rectangular pulses; 1-ms width; current strength 50 mA; voltage drop between electrodes 18 V cm^{-1}). The first, starting at $t=30$ min (S_0) was not used for determination of tritium outflow. The subsequent periods (S_1 up to S_4), also consisting of 100 pulses at 8 Hz, started at $t=60$ min with 30-min intervals. Concentration–response curves were obtained by adding the agonist at increasing concentration 5 min before S_2, S_3 and S_4 up to the end of each stimulation period. Antagonists were added 20 min before S_2 and kept until the end of the experiment. In some experiments, 1,3-dipropyl-8-cyclopentyl-1,3-dipropylxanthine (DPCPX 20 nM or 100 nM; to block adenosine A_1 receptors) was added throughout superfusion.

2.3. Data evaluation

The outflow of tritium was expressed as fraction of the tissue tritium content at the start of the respective collection period (fractional rate of outflow, min^{-1}). Effects of drugs on basal tritium outflow were estimated from the values of b_n/b_1 and expressed as percentages of the mean ratio obtained in the appropriate control; b_n was the fractional rate of outflow in the 5-min period before S_2, S_3 and S_4 (b_2, b_3 and b_4, respectively) and b_1, the fractional rate of outflow in the 5-min period before S_1. The electrically evoked overflow of tritium was calculated as the difference between ‘‘total tritium outflow during the 10 min period after start of stimulation’’ and the estimated ‘‘basal outflow’’, and expressed as percentage of the tissue tritium content at the time of stimulation. Effects of drugs added after S_1 on electrically evoked overflow were evaluated as ratios of the overflow elicited by S_2, S_3 and S_4 (S_n) and the overflow elicited by S_1 (S_n/S_1). S_n/S_1 values obtained in individual experiments in which a test compound A was added after S_1 were calculated as percentages of the respective mean ratio in the appropriate control group (solvent instead of A). When the interaction of A, added after S_1, and drug B, added either after S_1 or at the beginning of superfusion, was studied, the ‘‘appropriate control’’ was a group in which B alone was used (von Kügelgen et al., 1995).

Results are presented as means ± S.E.M.; n is the number of experiments. The effect of drugs on both basal tritium outflow and evoked tritium overflow was tested for significance using one-way analysis of variance (ANOVA) followed by Dunnett’s test. P values lower than 0.05 were taken to indicate significant differences.

3. Results

3.1. General observations

Basal tritium outflow and electrically evoked tritium overflow from prostatic and epididymal portions of rat vas deferens are shown in Table 1. When the selective adenosine A_1 receptor antagonist, DPCPX (Lohse et al., 1987), was

Table 1
Basal tritium outflow (b_1) and electrically evoked tritium overflow (S_1) from prostatic and epididymal portions of rat vas deferens

Tissue/drugs throughout	Basal tritium outflow (% of tissue tritium min^{-1})	Evoked tritium overflow (% of tissue tritium)
Prostatic		
Solvent	0.094 ± 0.002 (194)	0.194 ± 0.004 (194)
DPCPX (20 nM)	0.118 ± 0.009 (38)	0.181 ± 0.008 (38)
DPCPX (100 nM)	0.107 ± 0.005 (38)	0.216 ± 0.014 (38)
Epididymal		
Solvent	0.121 ± 0.002 (196)	0.220 ± 0.006 (196)
DPCPX (20 nM)	0.116 ± 0.004 (37)	0.225 ± 0.014 (37)
DPCPX (100 nM)	0.132 ± 0.006 (37)	0.228 ± 0.012 (37)

After pre-incubation with [^{3}H]noradrenaline, tissue preparations were superfused with medium containing the drugs indicated (in addition to desipramine 400 nM that was always present throughout superfusion). S_1 was applied after 60 min of superfusion and consisted of 100 pulses/8 Hz; b_1 refers to the 5-min period immediately before S_1. Values are means ± S.E.M. for (n) tissue preparations.

present throughout superfusion, basal tritium outflow and evoked tritium overflow were not different from those observed in experiments with desipramine alone.

Basal outflow and evoked tritium overflow remained constant throughout the experiment with b_n/b_1 and S_n/S_1 values close to unity in both portions (not shown). The basal tritium outflow was not changed by the adenosine receptor agonists or antagonists added after S_1. However, the adenosine uptake blocker *S*-(4-nitrobenzyl)-6-thioinosine (NBTI; 5 μM) increased basal outflow to 130 ± 5 % (n = 18; P < 0.01) and identically in both portions of rat vas deferens.

3.2. Modulation of tritium overflow by adenosine A_1 receptors

The selective adenosine A_1 receptor agonist, N^6-cyclopentyladenosine (CPA; 0.001–1 μM), and the nonselective adenosine receptor agonist, 5′-*N*-ethylcarboxamidoadenosine (NECA; 0.03–1 μM), inhibited the evoked overflow of tritium in a concentration-dependent manner in both prostatic and epididymal portions (Fig. 1). The effect of CPA was antagonised, and the effect of NECA reversed, in both portions, by the selective adenosine A_1 receptor antagonist, DPCPX (20 nM; Fig. 1).

3.3. Modulation of tritium overflow by adenosine A_2 receptors

The selective adenosine A_{2A} receptor agonist, 2-*p*-(2-carboxyethyl)phenethylamino-5′-*N*-ethylcarboxamidoadenosine (CGS 21680; 0.01-1 μM; Lupica et al., 1990) increased the evoked overflow of tritium in a concentration-dependent manner. However, this was only observed in the epididymal portion and not in the prostatic portion of rat vas deferens (Fig. 2). The facilitatory effect of CGS 21680 was prevented by the selective A_{2A} receptor antagonist, 4-

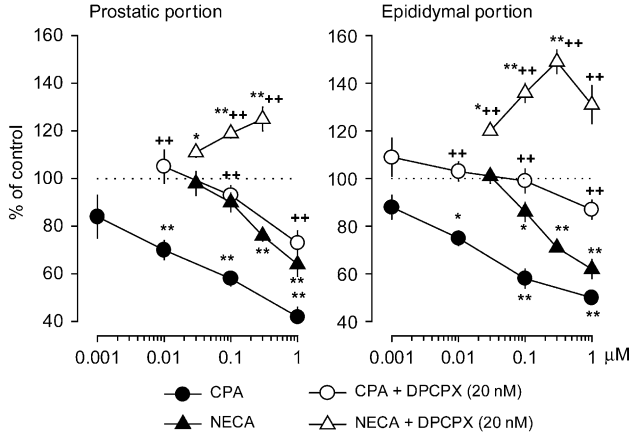

Fig. 1. Effects of CPA and NECA on the evoked tritium overflow from prostatic and epididymal portions of rat vas deferens in the absence (filled symbols) and in the presence of 20 nM DPCPX (open symbols). DPCPX was added 20 min before S_2 and kept throughout. Tissues were electrically stimulated with 5 trains of 100 pulses/8 Hz (S_0–S_4). CPA and NECA were added 5 min before S_n (S_2, S_3, S_4) at increasing concentrations. For evaluation of the effects of drugs on the electrically evoked tritium overflow, S_n/S_1 ratios obtained in the presence of agonists were expressed as percentages of the corresponding average control S_n/S_1 value. Ordinates, tritium overflow expressed as percentage of the respective control. Abscissas, concentration of the adenosine receptor agonists. Values are means ± S.E.M. from 4–12 experiments. Significant differences from respective control, $*P<0.05$ and $**P<0.01$; from the effect of agonist alone, $^{++}P<0.01$.

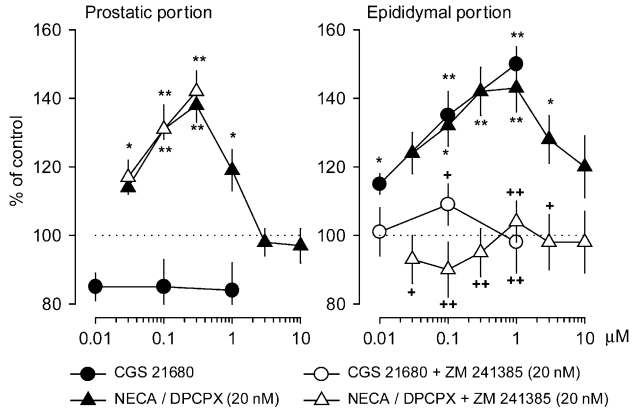

Fig. 2. Effect of CGS 21680 and NECA (in the presence of 20 nM DPCPX) on tritium overflow from prostatic and epididymal portions of rat vas deferens, in the absence (filled symbols) and in the presence of 20 nM ZM 241385 (open symbols). DPCPX was added at the beginning of superfusion and kept throughout; ZM 241385 was added 20 min before S_2 and kept throughout. Tissues were electrically stimulated with 5 trains of 100 pulses/8 Hz (S_0–S_4). CGS 21680 and NECA were added 5 min before S_n (S_2, S_3, S_4) at increasing concentrations. For evaluation of the effects of drugs on the electrically evoked tritium overflow, S_n/S_1 ratios obtained in the presence of agonists were expressed as percentages of the corresponding average control S_n/S_1 value. Ordinates, tritium overflow expressed as percentage of the respective control. Abscissas, concentration of the adenosine receptor agonists. Values are means ± S.E.M. from 4–8 experiments. Significant differences from respective control, $*P<0.05$ and $**P<0.01$; from the effect of CGS 21680 alone or NECA in the presence of DPCPX alone, $^{+}P<0.05$ and $^{++}P<0.01$.

(2-[7-amino-2-(2-furyl)[1,2,4]triazolo-[2,3-a][1,3,5]triazin-5-ylamino]ethyl)phenol (ZM 241385; 20 nM; Poucher et al., 1995). NECA (0.03–10 μM), in the presence of DPCPX (20 nM), increased tritium overflow in both portions (Fig. 2). The facilitatory effect of NECA was antagonised by ZM 241385 (20 nM), again only in the epididymal portion.

A putative involvement of adenosine A_{2B} receptors in the increase of tritium overflow caused by NECA was investigated further. NECA was tested in higher concentrations because it was shown that adenosine A_{2B} receptors are preferentially activated by NECA in the high micromolar range (Daly et al., 1983; Bruns et al., 1986). To reduce the effect of activation of the A_1 receptors by this higher range of NECA concentration, the concentration of DPCPX was increased to 100 nM. Under these conditions, and as before, NECA increased tritium overflow in both portions (Fig. 3). In the epididymal portion, this enhancement was antagonised by ZM 241385 (20 nM) but was not changed by the adenosine A_{2B} receptor antagonist, alloxazine (1 μM; Brackett and Daly, 1994). In the prostatic portion, the increase in tritium overflow caused by NECA was antagonised by alloxazine (1 μM) but not by ZM 241385 (Fig. 3).

3.4. Effects of P1 antagonists and NBTI on tritium overflow

In order to investigate if the adenosine receptors are tonically activated by endogenous adenosine, the effects of adenosine receptor antagonists were tested. When tested alone, neither DPCPX (20 nM), ZM 241385 (20 nM) nor the nonselective adenosine A_2 receptor antagonist, 3,7-dimethyl-1-(2-propynyl)xanthine (DMPX; 1 μM; Daly et

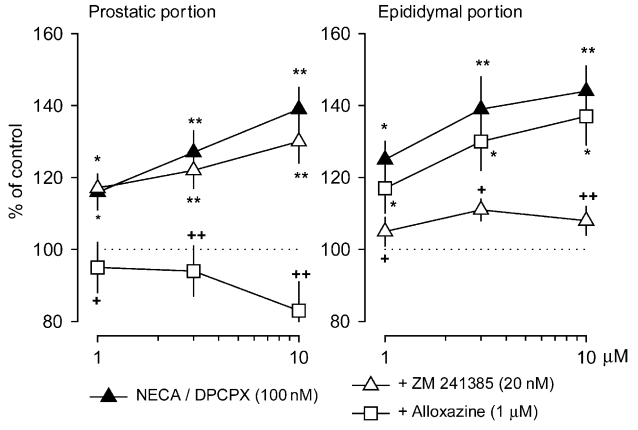

Fig. 3. Effects of NECA (in the presence of 100 nM DPCPX) on tritium overflow from prostatic and epididymal portions of rat vas deferens in the absence (filled symbols) and in the presence of 20 nM ZM 241385 or 1 μM alloxazine (open symbols). DPCPX was added at the beginning of superfusion and kept throughout; ZM 241385 and alloxazine were added 20 min before S_2 and kept throughout. Tissues were electrically stimulated with 5 trains of 100 pulses/8 Hz (S_0–S_4). NECA was added 5 min before S_n (S_2, S_3, S_4) at increasing concentrations. For evaluation of the effects of NECA on the electrically evoked tritium overflow, S_n/S_1 ratios obtained in the presence of NECA were expressed as percentages of the corresponding average control S_n/S_1 value. Ordinates, tritium overflow expressed as percentage of the respective control. Abscissas, concentration of NECA. Values are means ± S.E.M. from 5–9 experiments. Significant differences from respective control, $^{*}P<0.05$ and $^{**}P<0.01$; from the effect of NECA in the presence of DPCPX alone, $^{+}P<0.05$ and $^{++}P<0.01$.

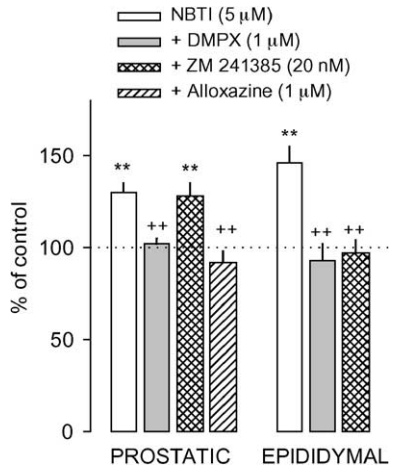

Fig. 4. Effects of inhibition of adenosine uptake by 5 μM NBTI on tritium overflow from prostatic and epididymal portions of rat vas deferens in the absence and in the presence of 1 μM DMPX, 20 nM ZM241385 or 1 μM alloxazine. NBTI, DMPX, ZM 241385 and alloxazine were added 20 min before S_2 and kept throughout. Tissues were electrically stimulated with 3 trains of 100 pulses/8 Hz (S_0–S_2). For evaluation of the effects of drugs on the electrically evoked tritium overflow, S_2/S_1 ratios obtained in the presence of drugs were expressed as percentages of the corresponding average control S_2/S_1 value. Ordinates, tritium overflow expressed as percentage of the respective control. Values are means ± S.E.M. from 5–13 experiments. Significant differences from respective control, $^{**}P<0.01$; from the effect of NBTI alone, $^{++}P<0.01$.

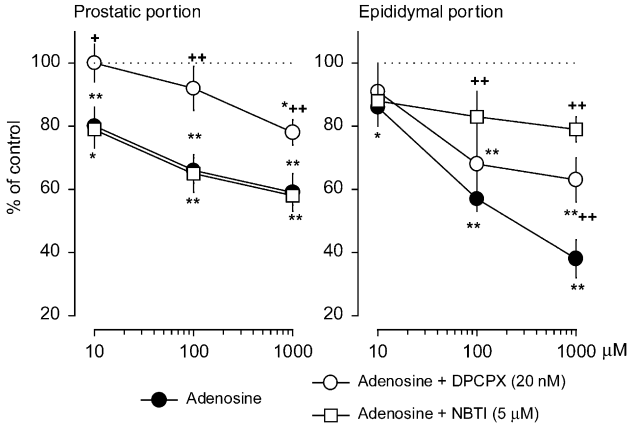

Fig. 5. Effect of adenosine on tritium overflow from prostatic and epididymal portions of rat vas deferens in the absence (filled symbol) and in the presence of 20 nM DPCPX or 5 μM NBTI (open symbols). DPCPX and NBTI were added 20 min before S_2 and kept throughout. Tissues were electrically stimulated with 5 trains of 100 pulses/8 Hz (S_0–S_4). Adenosine was added 5 min before S_n (S_2, S_3, S_4) at increasing concentrations. For evaluation of the effects of adenosine on the electrically evoked tritium overflow, S_n/S_1 ratios obtained in the presence of adenosine were expressed as percentages of the corresponding average control S_n/S_1 value. Ordinates, tritium overflow expressed as percentage of the respective control. Abscissas, concentration of adenosine. Values are means ± S.E.M. from 5–14 experiments. Significant differences from respective control, $^{*}P<0.05$ and $^{**}P<0.01$; from the effect of adenosine alone, $^{+}P<0.05$ and $^{++}P<0.01$.

al., 1986), nor the adenosine A_{2B} receptor antagonist, alloxazine (1 μM; tested only in the prostatic portion), changed tritium overflow in a significant manner in either prostatic or epididymal portions (not shown).

In an attempt to increase extracellular levels of endogenous adenosine, adenosine uptake was inhibited with NBTI (Paterson et al., 1977; Thorn and Jarvis, 1996). NBTI (5 μM) enhanced tritium overflow in both prostatic and epididymal portions (Fig. 4). The enhancement of tritium overflow caused by NBTI was, in both portions, prevented by the adenosine A_2 receptor antagonist, DMPX (1 μM); in the epididymal portion it was also prevented by ZM 241385 (20 nM), whereas in the prostatic portion it was prevented by alloxazine (1 μM) but not by ZM 241385 (see Fig. 4).

Exogenous adenosine (10–1000 μM) caused a different pattern of effects. It caused a concentration-dependent inhibition of tritium overflow in both portions (Fig. 5) that was antagonised by DPCPX (20 nM). The effect of exogenous adenosine was also prevented by NBTI (5 μM), but only in the epididymal portion (Fig. 5).

4. Discussion

The electrically evoked tritium overflow from tissue preparations of rat vas deferens pre-incubated with [^{3}H]noradrenaline was assumed to reflect action potential-evoked neuronal release of noradrenaline. Therefore, changes in evoked tritium overflow were assumed to reflect changes in neuronal release of noradrenaline and effects of adenosine receptor agonists and antagonists, and of NBTI assumed to be mediated by adenosine receptors (likely prejunctional).

In accordance with previous findings (Clanachan et al., 1977; Wakade and Wakade, 1978; Muller and Paton, 1979; Kurz et al., 1993; Gonçalves and Queiroz, 1993), noradrenaline release was inhibited by the selective adenosine A_1 receptor agonist, CPA, and by the nonselective adenosine receptor agonist, NECA, effects that were antagonised by a selective A_1 receptor antagonist (DPCPX), confirming the occurrence of an adenosine A_1 receptor-mediated inhibition of noradrenaline release in the rat vas deferens, in both prostatic and epididymal portions (see Brownhill et al., 1996).

Recently, an immunohistochemical study indicated the existence of adenosine A_1 receptors in both the prostatic and the epididymal portion of the rat vas deferens that were co-localised with the sympathetic nerves (Diniz et al., 2001). The observation of an adenosine A_1 receptor-mediated inhibition of noradrenaline release in both portions of the rat vas deferens provides functional evidence that these receptors may be located in the sympathetic nerves. This study also indicated co-localisation with sympathetic nerves of adenosine A_{2B} receptors in the prostatic portion, and of A_{2A} and A_{2B} receptors in the epididymal portion (Diniz et al., 2001). The occurrence of an adenosine A_{2A} receptor-mediated facilitation of noradrenaline release in the epididymal portion previously observed (Gonçalves and Queiroz, 1993) and confirmed in the present study, provides functional evidence for a prejunctional localisation of A_{2A} receptors in the epididymal portion of the rat vas deferens. In the prostatic portion, we did not observe any adenosine A_{2A} receptor-mediated facilitation of noradrenaline release: the selective adenosine A_{2A} receptor agonist, CGS 21680, failed to increase noradrenaline release and the increase in release caused by NECA in the presence of DPCPX was not changed by the selective adenosine A_{2A} receptor antagonist, ZM 241385.

The pharmacological characterisation of responses mediated by adenosine A_{2B} receptors is more difficult because of the lack of selective agonists and antagonists (Daly, 2000). It is using ‘‘negative evidence’’, that is, based on (i) the reduced activity of CGS 21680, (ii) the activity of the nonselective adenosine receptor agonist, NECA, in the presence of selective A_1 receptor antagonists, or (iii) the ability of the slightly selective A_{2B} receptor antagonist alloxazine, to inhibit responses to NECA, that adenosine A_{2B} receptor-mediated responses have been identified (Alexander et al., 1996; Klots, 2000). In accordance with these criteria, the results obtained in the present study are compatible with the occurrence of an adenosine A_{2B} receptor-mediated facilitation of noradrenaline release in the prostatic but not in the epididymal portion of the rat vas deferens. In the prostatic portion, CGS 21680 failed to increase noradrenaline release but NECA in the presence of DPCPX increased release, an effect that was not influenced by ZM 241385 and was antagonised by alloxazine. According to these functional data, the adenosine A_{2B} receptors observed in the prostatic portion co-localised with sympathetic nerves (Diniz et al., 2001) may correspond to prejunctional adenosine A_{2B} receptors. In the epididymal portion all the ‘‘negative evidence’’ failed to indicate the presence of adenosine A_{2B} receptors involved in the modulation of noradrenaline release. The failure to detect an adenosine A_{2B} receptor-mediated facilitation in this portion may result from difficulties of characterising adenosine A_{2B} receptors, specially when A_{2A} receptors are also present, due to the lack of selective tools for adenosine A_{2B} receptors. Another possibility is that the adenosine A_{2B} receptors that in the immunohistochemical study showed to be located close to sympathetic nerves may not be prejunctional or not involved in the modulation of noradrenaline release.

Under the present experimental conditions, we did not observe tonic activation of adenosine receptors. However, the adenosine uptake inhibitor, NBTI, caused an increase in noradrenaline release, in both prostatic and epididymal portions, suggesting that it was favouring accumulation of endogenous adenosine and activation of adenosine A_2 receptors: the A_{2B} receptors in the prostatic and the A_{2A} receptors in the epididymal portion. In support of this hypothesis is the observation that the effect of NBTI was prevented by DMPX in both portions, by alloxazine in the prostatic portion, and by ZM 241385 in the epididymal portion. Interestingly, when exogenous adenosine was

tested, the predominant effect was the A_1 receptor-mediated inhibition of noradrenaline release, suggesting that exogenous adenosine activates adenosine A_1 receptors, preferentially. In the epididymal portion, inhibition of adenosine uptake blocked the inhibitory effect of exogenous adenosine, probably by favouring the adenosine A_{2A} receptor-mediated facilitation. In the prostatic portion no such effect was observed, probably because the adenosine uptake system is not influencing the access of exogenous adenosine to the A_{2B} receptors biophase (contrasting to what was observed with endogenous adenosine). An alternative explanation is that the maximal adenosine A_{2B} receptor-mediated facilitation was already occurring in the presence of NBTI, making it more difficult to envisage further increases when exogenous adenosine was added afterwards.

In conclusion, the present study demonstrated that adenosine exerts a dual and opposite modulation of noradrenaline release in the rat vas deferens, although the receptors involved may be different in the two portions. In both, inhibition of noradrenaline release seems to be mediated by adenosine A_1 receptors, whereas facilitation of noradrenaline release seems to be mediated by adenosine A_{2A} receptors in the epididymal but by A_{2B} receptors in the prostatic portion.

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