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Release inhibitory receptors activation favours the A_{2A}-adenosine receptor-mediated facilitation of noradrenaline release in isolated rat tail artery

[1]Paula Fresco, [1]Carmen Diniz, [1]Glória Queiroz & *,[1]Jorge Gonçalves

[1]Serviço de Farmacologia, Faculdade de Farmácia, Universidade do Porto Rua Aníbal Cunha, 164, 4050-047 Porto, Portugal

1 Interactions between A_{2A}-adenosine receptors and α_2-, A_1- and P2- release-inhibitory receptors, on the modulation of noradrenaline release were studied in isolated rat tail artery. Preparations were labelled with [^{3}H]-noradrenaline, superfused with desipramine-containing medium, and stimulated electrically (100 pulses at 5 Hz or 20 pulses at 50 Hz).

2 Blockade of α_2-autoreceptors with yohimbine (1 μM) increased tritium overflow elicited by 100 pulses at 5 Hz but not by 20 pulses at 50 Hz.

3 The selective A_{2A}-receptor agonist 2-*p*-(2-carboxyethyl)phenethylamino-5′-N-ethylcarboxamidoadenosine (CGS 21680; 1–100 nM) enhanced tritium overflow elicited by 100 pulses at 5 Hz. Yohimbine prevented the effect of CGS 21680, which was restored by the A_1-receptor agonist N^6-cyclopentyladenosine (CPA; 100 nM) or by the P2-receptor agonist 2-methylthioadenosine triphosphate (2-MeSATP; 80 μM).

4 CGS 21680 (100 nM) failed to increase tritium overflow elicited by 20 pulses at 50 Hz. The α_2-adrenoceptor agonist 5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline (UK 14304; 30 nM), the A_1-receptor agonist CPA (100 nM) or the P2-receptor agonist 2-MeSATP (80 μM) reduced tritium overflow. In the presence of these agonists CGS 21680 elicited a facilitation of tritium overflow.

5 Blockade of potassium channels with tetraethylammonium (TEA; 5 mM) increased tritium overflow elicited by 100 pulses at 5 Hz to values similar to those obtained in the presence of yohimbine but did not prevent the effect of CGS 21680 (100 nM) on tritium overflow.

6 It is concluded that, in isolated rat tail artery, the facilitation of noradrenaline release mediated by A_{2A}-adenosine receptors is favoured by activation of release inhibitory receptors.
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Keywords: A_{2A}-adenosine receptors; noradrenaline release; A_1-adenosine receptors; α_2-autoreceptors; P2-receptors; rat tail artery; facilitation of neurotransmitter release; receptor interactions

Abbreviations: CGS 21680, 2-*p*-(2-carboxyethyl)phenethylamino-5′-N-ethylcarboxamidoadenosine; CPA, N^6-cyclopentyladenosine; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; 2-MeSATP, 2-methylthioadenosine triphosphate; TEA, tetraethylammonium; UK 14304, 5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline; ZM 241385, 4-(2[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-ylamino]ethyl)phenol

Introduction

Noradrenaline release from postganglionic sympathetic nerve terminals is modulated by inhibitory α_2-autoreceptors and also by receptors for other endogenous modulators (see Starke, 1977). Adenosine is one of these modulators (Fredholm & Hedqvist, 1980) and exerts its effects through activation of membrane A_1-, A_{2A}-, A_{2B}- and A_3-adenosine receptors (see Ribeiro, 1999). A_1- and A_{2A}-adenosine receptors may coexist in the same nerve terminal (rat neuromuscular junction: Correia-de-Sá *et al*., 1991; rat vas deferens: Gonçalves & Queiroz, 1993; rat tail artery: Gonçalves & Queiroz, 1996) and mediate opposite effects on neurotransmitter release: an inhibition, mediated by A_1-receptors, and a facilitation, mediated by A_{2A}-receptors.

Several studies have provided evidence that the effects mediated by adenosine receptors influence or are influenced by the activation of other adenosine receptor subtypes or by receptors for other modulators (see Schlicker & Göthert, 1998; Ribeiro, 1999; Sebastião & Ribeiro, 2000). For instance, in rat hippocampal slices, activation of A_1-receptors attenuates the inhibition of noradrenaline release mediated by α_2-autoreceptors and by κ-opioid receptors (Limberger *et al*., 1988) whereas activation of α_2-autoreceptors attenuates the inhibition of noradrenaline release mediated by A_1-receptors (Allgaier *et al*., 1987; Limberger *et al*., 1988; Bucher *et al*., 1992). The facilitatory A_2-receptors also seem to be involved in receptor-interactions. At motor nerve terminals, activation of A_2-receptors potentiates the CGRP elicited facilitation of acetylcholine release, an effect opposite to that caused by activation of A_1-receptors (Correia-de-Sá & Ribeiro, 1994). Because the rat tail artery is also endowed with prejunctional A_{2A}-adenosine receptors (Gonçalves & Queiroz, 1996), the aim of the present study was to investigate whether, as described for the A_1-receptor-mediated inhibition, the A_{2A}-adenosine receptor-mediated facilitation of noradrenaline

*Author for correspondence; E-mail: jorge.goncalves@ff.up.pt

release can be influenced by activation of release inhibitory receptors, namely α_2-autoreceptors.

A preliminary account of this work has been presented previously (Diniz *et al*., 1999).

Methods

Adult male Wistar rats (290–400 g; Instituto Gulbenkian de Ciência, Oeiras, Portugal and CRIFFA, Barcelona, Spain) were sacrificed by cervical dislocation and exsanguination. The ventral tail artery was dissected out and cleaned of connective tissue. Eight tissue preparations (5–8 mg) were obtained from each artery. These 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 ($t=0$ being the onset 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, U.S.A.).

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 dissodium EDTA 0.031; it was saturated with 95% O_2–5% CO_2 and kept at 37°C. Desipramine (400 nM; to inhibit neuronal uptake of noradrenaline) and, in some experiments, yohimbine (1 μM; to block α_2-autoreceptors) were added throughout superfusion.

Up to five 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) and consisting of 100 pulses at 5 Hz, was not used for determination of tritium outflow. The subsequent periods (S_1 up to S_4), consisting of either 100 pulses at 5 Hz or 20 pulses at 50 Hz, started at $t=60$ min with 30 min intervals, unless otherwise stated.

Concentration-response curves for the A_{2A}-adenosine receptor agonist 2-*p*-(2-carboxyethyl)phenethylamino-5′-N-ethylcarboxamidoadenosine (CGS 21680; Jarvis *et al*., 1989; Lupica *et al*., 1990) were obtained in experiments in which five stimulation periods (S_0 to S_4) were applied. The agonist was added, at increasing concentrations, 5 min before S_2, S_3 to S_4 and kept until the end of the stimulation period. In all other experiments only three stimulation periods (S_0 and S_2) were applied. CGS 21680 and the other agonists tested were added 5 min before S_2, as previously; antagonists were added 20 min before S_2 (unless stated otherwise).

The outflow of tritium was expressed as the fraction of tissue content at the onset of the respective collection period (fractional rate of outflow; min^{-1}). Effects of drugs on basal tritium outflow were estimated by the values b_n/b_1 and expressed as the percentage 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 and after stimulation' minus 'basal outflow', and expressed as percentage of the tissue tritium content at the time of stimulation. Effects of drugs that were added after S_1 on electrically evoked overflow were evaluated as ratios of the overflow elicited by S_2, S_3 and S_4 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 a percentage of the respective mean ratio in the appropriate control group (solvent instead of A). When the interaction of A, added after S_1, and a drug B either added 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 expressed as mean ± s.e.mean; *n* denotes the number of tissue preparations. Differences between means were tested for significance using the unpaired Student's *t*-test or one-way ANOVA followed by Tukey's multiple comparison test. A *P* value lower than 0.05 was taken to indicate significant differences.

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); 5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline tartrate (UK 14304), 2-*p*-(2-carboxyethyl)phenethylamino-5′-N-ethylcarboxamidoadenosine hydrochloride (CGS 21680), N^6-cyclopentyladenosine (CPA), 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), desipramine hydrochloride, tetraethylammonium chloride monohydrate (TEA), yohimbine hydrochloride were from Sigma and 2-methylthioadenosine triphosphate tetrasodium (2-MeSATP) was from RBI (Sigma Aldrich, 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, U.K.).

Solutions of drugs were prepared with either distilled water or dimethylsulphoxide and diluted with medium immediately before use. Solvents were added to the superfusion medium in parallel control experiments.

Results

In the absence of drugs (except desipramine 400 nM, present in the superfusion medium of all experiments), the fractional rate of outflow immediately before S_1 (b_1) was 0.126 ± 0.006% min^{-1} ($n=154$) and remained almost constant throughout the experiment with b_2/b_1, b_3/b_1 and b_4/b_1 of 0.86 ± 0.02, 0.85 ± 0.03 and 0.82 ± 0.03, respectively ($n=14$–30). The fractional rate of outflow was not different when yohimbine (1 μM) was added throughout (0.129 ± 0.004% min^{-1}; $n=78$). None of the drugs used (except for TEA; see below) or their solvents altered the basal tritium outflow (not shown).

Effect of CGS 21680 on tritium overflow evoked by trains of 100 pulses at 5 Hz

Stimulation with trains of 100 pulses at 5 Hz caused an overflow of tritium. In the absence of other drugs, the S_1 value was 0.520 ± 0.029% ($n=138$) of the tritium content of the tissue and remained almost constant throughout the experiment with S_2/S_1, S_3/S_1 and S_4/S_1 values of 0.91 ± 0.04, 0.86 ± 0.04 and 0.88 ± 0.04 ($n=52$), respectively. Yohimbine

(1 μM), when introduced at the beginning of the superfusion and kept throughout, increased tritium overflow (S_1 values of $1.799 \pm 0.059\%$ of the tissue tritium content; $n=77$) which remained constant throughout the experiment (S_2/S_1, S_3/S_1 and S_4/S_1 values of 0.97 ± 0.03, 0.97 ± 0.03 and 0.93 ± 0.04, respectively; $n=31$). The higher tritium overflow observed in the presence of 1 μM yohimbine indicated that a marked 2-autoinhibition of noradrenaline release was occurring. This was confirmed in experiments where yohimbine, introduced 20 min before S_2, increased tritium overflow (S_2/S_1 value of $346 \pm 22\%$; $n=6$; $P<0.05$).

In the absence of yohimbine, the selective A_{2A}-adenosine receptor agonist CGS 21680 enhanced the evoked overflow of tritium in a concentration dependent manner (Figure 1). The facilitatory effect of CGS 21680 was blocked not only by the A_{2A}-adenosine-receptor antagonist ZM 241385 (20 nM; Poucher *et al*., 1995), but also by the α_2-adrenoceptor antagonist yohimbine (Figure 1).

In the presence of yohimbine (1 μM), either the A_1-adenosine receptor agonist CPA (100 nM) or the P2-receptor agonist 2-MeSATP (80 μM), when added 5 min before S_2, reduced the evoked overflow of tritium: S_2/S_1 values of $70 \pm 4\%$ ($n=9$) and $73 \pm 12\%$ ($n=6$), respectively ($P<0.05$). CGS 21680 (100 nM) that, under these experimental conditions, did not modify tritium overflow ($S_2/S_1 = 100 \pm 9\%$; $n=8$), increased tritium overflow when tested in the presence of CPA (100 nM) or 2-MeSATP (80 μM): S_2/S_1 values of $151 \pm 24\%$ ($n=9$) and $121 \pm 5\%$ ($n=10$), respectively ($P<0.05$).

Effect of CGS 21680 on tritium overflow evoked by trains of 20 pulses at 50 Hz

Stimulation with trains of 20 pulses at 50 Hz caused an overflow of tritium. In the absence of other drugs, the S_1 value was $0.362 \pm 0.025\%$ ($n=22$) of the tritium content of the tissue and remained constant when a third period of stimulation was applied (S_2/S_1 values of 1.06 ± 0.06; $n=22$). Stimulation with this brief train of pulses elicited a tritium overflow under α_2-autoinhibition-poor conditions: yohimbine (1 μM), when introduced 20 min before S_2, did not increase the evoked overflow of tritium ($S_2/S_1 = 92 \pm 11\%$; $n=7$).

CGS 21680 (up to 100 nM), when added 5 min before S_2, failed to increase the tritium overflow elicited by trains of 20 pulses at 50 Hz (S_2/S_1 value of $98 \pm 5\%$; $n=17$). However, the selective α_2-adrenoceptor agonist UK 14304 (30 nM), which decreased the evoked overflow of tritium (S_2/S_1 value of $65 \pm 6\%$; $n=12$; $P<0.05$), restored the ability of CGS 21680 to enhance tritium overflow under these stimulation conditions (Figure 2). The A_1-adenosine receptor agonist CPA (100 nM) or the P2-receptor agonist 2-MeSATP (80 μM), when added 5 min before S_2 also reduced tritium overflow: S_2/S_1 values of $55 \pm 5\%$ ($n=8$) and $32 \pm 11\%$ ($n=6$), respectively ($P<0.05$) and restored the facilitatory effect of CGS 21680 (Figure 2).

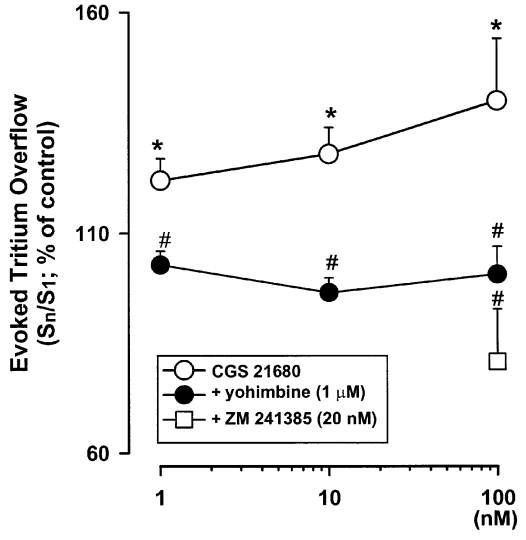

Figure 1 Effect of the A_{2A}-adenosine receptor agonist CGS 21680 on the evoked tritium overflow from isolated rat tail artery in the absence of the α_2-adrenoceptor antagonist yohimbine (α_2-autoinhibition rich conditions: open circles, CGS 21680 alone; open squares, CGS 21680 in the presence of the A_{2A}-receptor antagonist ZM 241385) and in the presence of 1 μM yohimbine (α_2-autoinhibition poor conditions; filled circles). S_1 to S_n (S_2, S_3 and S_4) are the overflows elicited by trains of 100 pulses at 5 Hz (30 min intervals between trains). CGS 21680 was added, at increasing concentrations, 5 min before S_2, S_3 and S_4; ZM 241385 was added 20 min before S_4. When indicated, yohimbine was present throughout. *Ordinate*, S_n/S_1 values obtained in individual tissue preparations and expressed as percentage of the corresponding average control S_n/S_1 value. Values are means $\pm$ s.e.mean from 7–20 tissue preparations. Significant differences from the appropriate control: $*P<0.05$ (unpaired Student's *t*-test); from CGS 21680 alone: $\#P<0.05$ (ANOVA followed by Tukey's *t*-test).

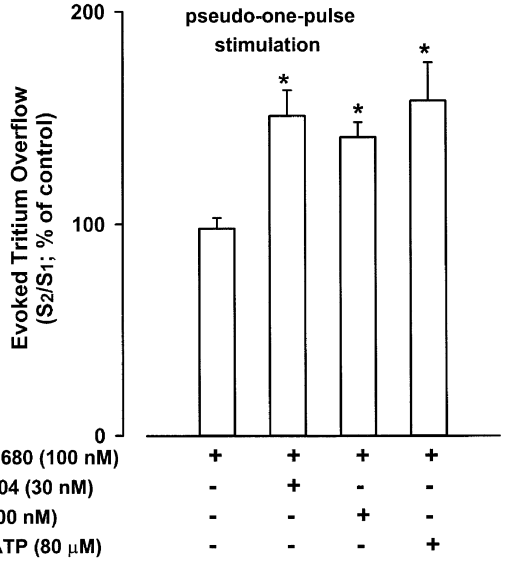

Figure 2 Effect of the A_{2A}-adenosine receptor agonist CGS 21680 on the evoked tritium overflow from isolated rat tail artery in α_2-autoinhibition poor conditions. S_1 and S_2 are the overflows elicited by trains of 20 pulses at 50 Hz (pseudo-one-pulse stimulation; 30 min intervals between trains). CGS 21680 and/or UK 14304 (an α_2-adrenoceptor agonist), CPA (an A_1-receptor agonist) or 2-MeSATP (P2-receptor agonist) were added 5 min before S_2. When tested alone, UK 14304, CPA and 2-MeSATP reduced tritium overflow (S_2/S_1 values between 32 and 65%, see text). *Ordinate*, S_2/S_1 values obtained in individual tissue preparations and expressed as percentage of the corresponding average control S_2/S_1 value. Values are means $\pm$ s.e.mean from 7–12 tissue preparations. Significant differences from the appropriate control: $*P<0.05$ (unpaired Student's *t*-test).

Effect of CGS 21680 under conditions leading to high tritium overflow levels

The overflow of tritium from preparations stimulated with 100 pulses at 5 Hz was markedly enhanced by yohimbine (see above). In order to test whether yohimbine prevents the effects of CGS 21680 by increasing tritium overflow so markedly that makes difficult to envisage further increases, the effect of CGS 21680 was investigated in the presence of the non-selective K^+-channel blocker TEA (Cook, 1988).

TEA (5 mM) caused a marked increase on basal outflow which stabilized only after 25 min incubation: b_2/b_1 value of $139 \pm 3\%$ ($n=8$). Therefore, stimulation periods (100 pulses at 5 Hz) were applied with 45 min intervals. TEA (5 mM), when introduced 10 min after S_1 (35 min before S_2), increased the evoked tritium overflow (S_2/S_1 value of $417 \pm 68\%$; $n=8$). Yohimbine (1 μM), when introduced 35 min before S_2, increased tritium overflow (S_2/S_1 value of $328 \pm 30\%$; $n=4$; not different from that elicited by TEA alone). When yohimbine was combined with TEA, it potentiated the increase on tritium overflow caused by TEA alone (S_2/S_1 value of $774 \pm 164\%$; $n=4$; $P<0.05$, from the increase caused by TEA or yohimbine alone). Contrasting with the effect of CGS 21680 on tritium overflow in the presence of yohimbine, CGS 21680 significantly enhanced tritium overflow in the presence of TEA (Figure 3).

Effect of α_2-autoinhibition on the tonic effects mediated by adenosine receptors

The effects of selective A_1- and A_{2A}-adenosine receptor antagonists on tritium overflow elicited by trains of 100 pulses at 5 Hz were tested in the absence and in the presence of yohimbine in order to investigate if a putative modulation exerted by endogenous adenosine is influenced by the α_2-autoinhibition.

The A_1-adenosine receptor antagonist DPCPX (20 nM) did not change tritium overflow either in the absence or in the presence of 1 μM yohimbine, (Figure 4). However, yohimbine influenced the effects of the A_1-adenosine receptor agonist N^6-cyclopentyladenosine (CPA; 100 nM) which reduced tritium overflow less markedly in the absence ($S_2/S_1 = 83 \pm 9\%$; $n=9$) than in the presence of 1 μM yohimbine ($S_2/S_1 = 58 \pm 4\%$; $n=12$; $P<0.05$).

The A_{2A}-adenosine receptor antagonist ZM 241385 (20 nM) caused, *per se*, an unexpected increase on tritium overflow (S_2/S_1 value of $148 \pm 6\%$; $n=6$) which did not occur when ZM 241385 was tested in the presence of 1 μM yohimbine (Figure 4).

Discussion

The electrically evoked tritium overflow from tissue preparations of rat tail artery preincubated with [^{3}H]-noradrenaline was assumed to reflect action potential-evoked neuronal noradrenaline release and drug-induced changes in evoked tritium overflow assumed to reflect changes in neuronal release. This is consistent with the established release-inhibitory effects of α_2-adrenoceptor, A_1-adenosine and P2-receptor agonists observed in this preparation (Illes *et al*., 1989; Gonçalves & Queiroz, 1996) and with the release

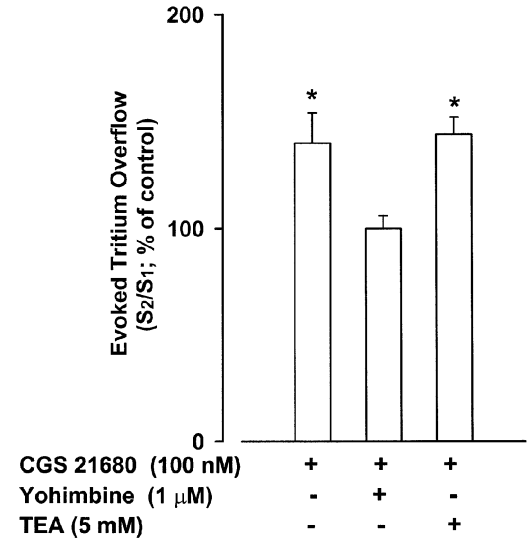

Figure 3 Effect of the A_{2A}-adenosine receptor agonist CGS 21680 on the evoked tritium overflow from isolated rat tail artery in the absence or in the presence of yohimbine or tetraethylammonium (TEA). S_1 and S_2 are the overflows elicited by trains of 100 pulses at 5 Hz (45 min intervals between trains). Yohimbine (1 μM) or TEA (5 mM) were added 35 min and CGS 21680 (100 nM) 5 min before S_2. S_2 value (% of tritium content) in the absence of drugs was $0.480 \pm 0.077\%$ ($n=12$), in the presence of yohimbine was $1.823 \pm 0.013\%$ ($n=4$) and in the presence of TEA was $2.022 \pm 0.340\%$ ($n=8$). *Ordinate*, S_2/S_1 values obtained in individual tissue preparations and expressed as percentage of the corresponding average control S_2/S_1 value. Values are means $\pm$ s.e.mean from 6–8 tissue preparations. Significant differences from the appropriate control: $^*P<0.05$ (unpaired Student's *t*-test).

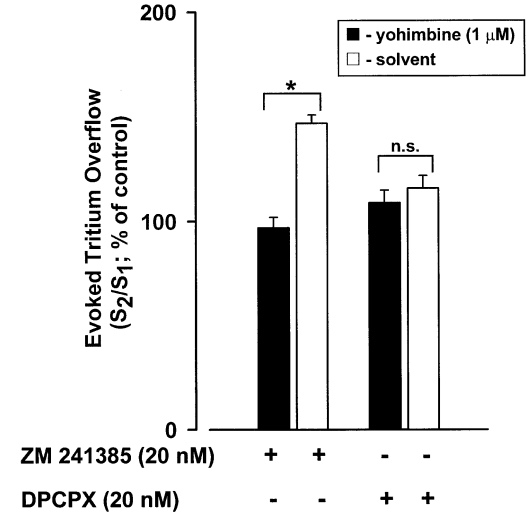

Figure 4 Effect of the A_{2A}-adenosine receptor antagonist ZM 241385 and of the A_1-adenosine receptor antagonist DPCPX on the evoked tritium overflows from isolated rat tail artery in the absence (solvent; open bars) or in the presence of the α_2-adrenoceptor antagonist yohimbine (filled bars). S_1 and S_2 are the overflows elicited by trains of 100 pulses at 5 Hz (30 min intervals between trains). When indicated, yohimbine (1 μM) was added throughout the superfusion; ZM 241385 or DPCPX were added 20 min before S_2. *Ordinate*, S_2/S_1 values obtained in individual tissue preparations and expressed as percentage of the corresponding average control S_2/S_1 value. Values are means $\pm$ s.e.mean from 8–15 tissue preparations. Significant differences from the effect in the presence of yohimbine: $^*P<0.05$; n.s., non significant (unpaired Student's *t*-test).

desinibiting effects of α_2-adrenoceptor antagonists observed in the present experimental conditions.

The present study confirms previous observations (Gonçalves & Queiroz, 1996; Mota *et al*., 2000) indicating the presence of release-facilitatory A_{2A}-adenosine receptors in the rat tail artery. However, in the present experimental conditions, the selective A_{2A}-receptor agonist CGS 21680 enhanced noradrenaline release only when α_2-autoinhibition was occurring: blockade of α_2-autoreceptors with yohimbine prevented the A_{2A}-mediated facilitation of noradrenaline release caused by CGS 21680. This observation is in agreement with results published recently (Mota *et al*., 2000). An A_{2A}-receptor-mediated facilitation was previously observed even in the presence of yohimbine (Gonçalves & Queiroz, 1996). However, in this study noradrenaline release was estimated by measuring overflow of endogenous noradrenaline and, therefore, a long train of stimulation (2700 pulses) had to be used. The effects of CGS 21680 in the absence of yohimbine were not investigated and the possibility that the facilitatory effect of CGS 21680 would be greater in yohimbine-free medium cannot be excluded. The fact that CGS 21680 was still able to cause facilitation of noradrenaline in the presence of yohimbine may also be due to the occurrence of P2-autoinhibition under that experimental conditions: revealed by the P2-antagonist Reactive Blue 2 (Gonçalves & Queiroz, 1996): or due to a higher degree of α_2-autoinhibition attained with the long train of pulses, not totally blocked with the concentration of yohimbine used. The possibility that an increase in the train length may strengthen the autoinhibition is compatible with the observation that tritium overflow elicited by a long train of pulses decreases along the train (Driessen *et al*., 1994). In the present study it was not feasible to estimate drug effects based on tritium overflow elicited by 2700 pulses because tritium overflow was markedly reduced along the experiments, with S_2/S_1 values lower than 0.2 (not shown).

A possible explanation for the failure of CGS 21680 to facilitate noradrenaline release in the presence of yohimbine could be that the levels of noradrenaline released under the present experimental conditions are so high that further increases would become difficult to envisage. However, the following observations argue against this hypothesis: (*i*) TEA enhances noradrenaline release by a mechanism that does not involve α_2-adrenoceptor-mediated autoinhibition (Kirpekar *et al*., 1976) and, by opposition to what happened in the presence of yohimbine, the A_{2A}-receptor-mediated facilitation of noradrenaline release was still observed when noradrenaline release was raised by TEA; (*ii*) by using a short train of high frequency stimulation it is possible to elicit noradrenaline release under α_2-autoinhibition poor conditions (pseudo-one-pulse; brain slices: Singer, 1988; postganglionic sympathetic nerve terminals: Trendelenburg *et al*., 1997). Under this α_2-autoinhibition poor conditions, the A_{2A}-adenosine receptor agonist CGS 21680 failed to increase noradrenaline release although it occurred when α_2-autoreceptors were activated by an exogenous agonist or when other release inhibitory receptors were activated.

Another tentative hypothesis could be that yohimbine is acting as an antagonist at A_{2A}-adenosine receptors. The following observations argue against this hypothesis. In the rat vas deferens, CGS 21680 causes an A_{2A}-adenosine-receptor-mediated inhibition of contractions to exogenous noradrenaline, and this effect of CGS 21680 was not changed by 1 μM yohimbine (Diniz & Gonçalves, unpublished observation). Furthermore, in other receptor systems, interactions of this kind have been revealed irrespective of the α_2-antagonist used (phentolamine: Majewski & Rand, 1981; Johnston & Majewski, 1986; Costa & Majewski, 1988; Cox *et al*., 2000; rauwolscine: Bucher *et al*., 1992; yohimbine: Allgaier *et al*., 1987; Limberger *et al*., 1988).

In summary, the present study provides evidence that the occurrence of an A_{2A}-adenosine receptor-mediated facilitation of noradrenaline release is neither dependent on the levels of the released noradrenaline nor on the presence of yohimbine *per se*: it occurs either with low (100 pulses, 5 Hz, yohimbine absent) or with high (100 pulses, 5 Hz, TEA present) noradrenaline released levels, but not in α_2-autoinhibition poor conditions, irrespective of release noradrenaline levels being high (100 pulses, 5 Hz, yohimbine present) or low (20 pulses, 50 Hz, yohimbine absent).

In our view, the most likely hypothesis is that the inhibition by the α_2-adrenoceptor antagonist of an effect mediated by A_{2A}-adenosine receptors is due to the occurrence of an interaction between α_2-autoreceptors and A_{2A}-receptors in a way that activation of α_2-autoreceptors would favour the A_{2A}-receptor-mediated facilitation of noradrenaline release. Interactions between α_2-autoreceptors and other release-inhibitory receptors have been previously described both at central and peripheral nervous systems (see Schlicker & Göthert, 1998). Interactions between α_2-autoreceptors and release-facilitatory receptors had also been investigated but these led to conflicting results. The release-facilitatory effect of angiotensin II was reported to be increased (Costa & Majewski, 1988), decreased (Cox *et al*., 2000) or not influenced (Mota *et al*., 2000) by blockade of α_2-autoreceptors. Furthermore, it was also reported that the release-facilitatory effect mediated by β-adrenoceptors was increased (Majewski & Rand, 1981; Johnston & Majewski, 1986) or not influenced (Cox *et al*., 2000; Mota *et al*., 2000) by blockade of α_2-autoreceptors. The reasons for these differences are not understood but may be the use of different tissue preparations and/or different stimulation parameters.

Our experiments also provide evidences that activation of other release inhibitory receptors, such as the A_1- or the P2-receptors, also favour the A_{2A}-receptor-mediated facilitation of noradrenaline release, at least in conditions in which α_2-autoinhibition is not operating. To our knowledge, the present is the first study to demonstrate that the A_{2A}-adenosine receptor-mediated facilitation of noradrenaline release is favoured by activation of release inhibitory receptors. Nevertheless, previous observations can be interpreted according to the occurrence of such an interaction. One of the first studies on interactions between adenosine and other receptors in the brain showed that the α-adrenoceptor-mediated increase in cyclic AMP levels required the presence of endogenous adenosine (Sattin *et al*., 1975). Since none of the α_1- and α_2-receptor subtypes known is positively coupled to adenylate cyclase whereas the A_2-adenosine receptors are, the explanation may be that it was the adenosine receptor-mediated increase in cyclic AMP that was potentiated by an α-adrenoceptor activation and not the opposite, as tentatively proposed.

Receptor interactions may be mutual (see Limberger *et al*., 1988). We did not study in detail the influence of A_{2A}-receptor agonists on the α_2-autoinhibition. However, the

surprising observation that blockade of the A_{2A}-adenosine receptor increases noradrenaline release, an effect not observed when α_2-autoreceptors were blocked may indicate that the blockade of A_{2A}-receptors may also disturb the α_2-autoinhibition.

Interactions between adenosine A_2-receptors and receptors for other transmitters have been recently reviewed (see Ribeiro, 1999; Sebastião & Ribeiro, 2000). The present study presents evidence that activation of α_2-autoreceptors and other release inhibitory receptors favours the A_{2A}-receptor-mediated facilitation of noradrenaline release. The molecular mechanism involved on this interaction is not known. In mouse atria, an interaction involving α_2-autoreceptors and angiotensin or bradykinin receptors was explained by an interaction between $G_{q/11}$-protein, to which AT_1- and B_2-receptors are coupled, and $G_{i/o}$-protein, to which α_2-autoreceptors are coupled (Cox *et al*., 2000). A_{2A}-adenosine receptors are considered to be coupled to G_s (Marala & Mustafa, 1993) and the mechanism described above was shown not to be applicable to G_s coupled receptors such as the β-adrenoceptors (Cox *et al*., 2000). However, A_{2A}-receptors may be promiscuous in their interaction with G proteins and be coupled to transducing mechanisms other than G_s ($G_{i/o}$; Cunha *et al*., 1999; or the G_s-like protein, G_{olf}; Kull *et al*., 2000). Furthermore, A_{2A}-receptors not only activate adenylate cyclase but also protein kinase C (Gubitz *et al*., 1996; Sheldon *et al*., 1996), phospholipase C/inositol triphosphate/calmodulin and calmodulin kinase II pathway (Wirkner *et al*., 2000), a serine/threonine protein phosphatase (Revan *et al*., 1996) or the mitogen-activated protein kinase cascade (Sexl *et al*., 1997). Facing the multiple coupling possibilities of A_{2A}-adenosine receptors we cannot discard the possibility that, in rat tail artery, A_{2A}-receptors may be coupled to transducing systems other than G_s and the occurrence of an interaction by a mechanism similar to that proposed for $G_{q/11}$ coupled receptors (Cox *et al*., 2000; see also Selbie & Hill, 1998) cannot be excluded.

In conclusion, the present study confirms the presence of adenosine A_{2A}-receptors in the isolated rat tail artery mediating a facilitation of noradrenaline release and describes an interaction between A_{2A}-adenosine receptors and release inhibitory receptors in a way that an inhibition mediated by α_2-, A_1- or P2-receptors favours the A_{2A}-receptor-mediated facilitation of noradrenaline release.

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