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Interaction between adenosine A_{2B}-receptors and α_2-adrenoceptors on the modulation of noradrenaline release in the rat vas deferens: Possible involvement of a group 2 adenylyl cyclase isoform

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Abstract

In the prostatic portion of rat vas deferens, activation of adenosine A_{2B}-receptors, β_2-adrenoceptors, adenylyl cyclase or protein kinase A caused a facilitation of noradrenaline release. Blockade of α_2-adrenoceptors with yohimbine (1 μM) attenuated the facilitation mediated by adenosine A_{2B}-receptors and by direct activation of adenylyl cyclase with forskolin but not that mediated by β_2-adrenoceptors or by direct activation of protein kinase A with 8-bromoadenosine-3′,5′-cyclicAMP.

The adenosine A_{2B}- and the β_2-adrenoceptor-mediated facilitation was prevented by the adenylyl cyclase inhibitors, 2′,5′-dideoxyadenosine (3 μM) and 9-cyclopentyladenine (100 μM), at concentrations that also attenuated the release enhancing effect of forskolin, but were not changed by the phospholipase C inhibitor 1-[6-[((17β)-3-methoxyestra-1,3,5[10]-trien-17-yl)amino]hexyl]-1H-pyrrole-2,5-dione (U-73122, 1 μM). Facilitation of noradrenaline release mediated by adenosine A_{2B}-receptors was also attenuated by activation of protein kinase C with the phorbol ester 12-myristate 13-acetate (1 μM) and by inhibition of Gβγ subunits with an anti-βγ peptide; facilitation mediated by β_2-adrenoceptors was mainly attenuated by the calmodulin inhibitor calmidazolium (10 μM) and by the calmodulin kinase II inhibitor (*N*-[2-[*N*-(4-chlorocinnamyl)-*N*-methylaminomethyl]phenyl]-*N*-(2-hydroxyethyl)-4-methoxybenzene-sulfonamide phosphate (KN-93, 5 μM).

The results suggest that adenosine A_{2B}- but not β_2-adrenoceptor-mediated facilitation of noradrenaline release is enhanced by an ongoing activation of α_2-adrenoceptors. They further suggest that adenosine A_{2B}-receptors and β_2-adrenoceptors are coupled to distinct adenylyl cyclase isoforms what may explain the different influence of α_2-adrenoceptor signalling pathway on the facilitatory effects mediated by the two adenylyl cyclase coupled receptors.

Keywords: Adenosine A_{2B}-receptors; β_2-adrenoceptors; Adenylyl cyclase; Noradrenaline release; $G_{i/o}$-protein coupled receptors; Receptor interactions

Abbreviations: 2′,5′-dd-Ado, 2′,5′-dideoxyadenosine; 8-Br-cAMP, 8-bromoadenosine-3′,5′-cyclicAMP; 9-CP-Ade, 9-(cyclopentyl)-adenine; AC, adenylyl cyclase; ADA, adenosine deaminase; CaM, calmodulin; 1,9-dd-FOSK, 1,9-dideoxyforskolin; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; ICI 118,551, (±)-1-[2,3-(dihydro-7-methyl-1H-inden-4-yl)oxyl]-3-[(1-methylethyl)amino]-2-butanol hydrochloride; KN-92, (2-[*N*-(4′-methoxybenzenesulfonyl)]amino-*N*-(4′-chloro-phenyl)-2-propenyl-*N*-methylbenzylamine phosphate; KN-93, (*N*-[2-[*N*-(4-chlorocinnamyl)-*N*-methylaminomethyl]phenyl]-*N*-(2-hydroxyethyl)-4-methoxybenzenesulfonamide phosphate; NBTI, S-(4-nitrobenzyl)-6-thioinosine; NECA, 5′-(*N*-ethylcarboxamido)adenosine; NEM, N-ethylmaleimide; PMA, phorbol 12-myristate 13-acetate; PKC, protein kinase C; PKA, protein kinase A; Rp-cAMPS, Rp-adenosine-3′,5′-cyclic-monophosphorothioate; RO 32-0432, bisindolylmaleimide XI; U-73122, 1-[6-[((17β)-3-methoxyestra-1,3,5[10]-trien-17-yl)amino]hexyl]-1H-pyrrole-2,5-dione; U-73343, 1-[6-[((17β)-3-methoxyestra-1,3,5[10]-trien-17-yl)amino]hexyl]-2,5-pyrrolidinedione

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The α_2-adrenoceptors are the main $G_{i/o}$-protein coupled autoreceptors involved on the inhibition of noradrenaline release (Starke et al., 1989) but the noradrenergic nerve terminals also possess heteroreceptors by which other transmitters and mediators influence noradrenaline release (Boehm and Kubista, 2002). The α_2-adrenoceptors play a key regulatory role on synaptic transmission at the postganglionic sympathetic nerve terminals not only due to its direct effects on the modulation of sympathetic transmitters release (von Kügelgen et al., 1994), but also by modulating effects mediated by other heteroreceptors.

The occurrence of interactions between α_2-autoreceptors and inhibitory heteroreceptors has been described in noradrenergic neurones both in the central nervous system and in peripheral tissues and, in general, activation of α_2-adrenoceptors decreases the effect mediated by other $G_{i/o}$-protein coupled receptors (Schlicker and Göthert, 1998). The site of interaction is, in most cases, the G-protein to which both types of receptors are coupled (Hertting et al., 1990). Interaction between α_2-autoreceptors and prejunctional facilitatory receptors has also been extensively reported in different sympathetically innervated tissues where ongoing α_2-autoinhibition has been shown to enhance facilitation of noradrenaline release mediated by adenosine A_{2A} (Fresco et al., 2002; Queiroz et al., 2003), angiotensin AT_1 and bradykinin B_2 receptors (Cox et al., 2000; Mota and Guimarães, 2003; Trendelenburg et al., 2003) and to attenuate the facilitation mediated by β_2-adrenoceptors (Majewski and Rand, 1981; Johnston and Majewski, 1986; Queiroz et al., 2003).

The interaction between α_2-adrenoceptors and facilitatory receptors seems to take place at some step of the transduction pathways. Angiotensin AT_1 and bradykinin B_2 receptors, that require an ongoing inhibition mediated by α_2-adrenoceptors to facilitate noradrenaline release, are coupled to the $G_{q/11}$–phospholipase C–protein kinase C (PKC) pathway. It has been proposed that facilitation of noradrenaline release mediated by these receptors is caused by a PKC-mediated disruption of the $G_{i/o}$-pathway to which α_2-adrenoceptors are coupled, by phosphorylation of the $G_{i/o}$-proteins (Katada et al., 1985), of the α_2-adrenoceptor itself (Liang et al., 1998) or of the N-type Ca^{2+}-channel (Hamid et al., 1999). The facilitation mediated by β_2-adrenoceptors, which are coupled to the G_s–adenylyl cyclase (AC)–protein kinase A (PKA) pathway, is attenuated by an ongoing activation of α_2-adrenoceptors what may be explained by opposite actions of αi and αs subunits of G-proteins on the AC activity (Johnston and Majewski, 1986).

In the prostatic portion of rat vas deferens, a sympathetically innervated tissue, noradrenaline release is enhanced by activation of adenosine A_{2B}-receptors which are coupled to the AC–PKA pathway and, subsequently, to PKC, both pathways contributing to the release enhancing effects (Queiroz et al., 2004). In this preparation, sympathetic nerve terminals are also endowed with prejunctional inhibitory α_2-adrenoceptors. The aim of the present study was to investigate the influence of α_2-adrenoceptors on the facilitation of noradrenaline release mediated by adenosine A_{2B}-receptors and the mechanisms involved on such interaction.

1. Experimental procedures

1.1. Preparation and experimental conditions

Adult male Wistar rats (250–300 g; IBMC, Porto, Portugal) were kept at a constant temperature (21 °C) and a regular light (06.30–19.30 h)/dark (19.30–06.30 h) cycle, with food and water ad libitum. Animal handling and experiments with animals were conducted according to the guidelines of the European Communities Council Directive (86/609/EEC). Animals were killed after stunning followed by exsanguination. The vasa deferentia were dissected out, cleaned of connective tissue and divided in portions of about 18 mg weight. Only tissue preparations from prostatic portions were used. Tissue preparations were incubated and superfused, at 37 °C, with gassed (95% O_2 and 5% CO_2) Krebs solution of the following composition (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. A Ca^{2+}-free solution was prepared by equimolar replacement of $CaCl_2$ with NaCl.

1.2. Experimental protocol

The vas deferens has been widely used as a model to study the postganglionic sympathetic transmission. The procedures used to label tissue preparations with [^{3}H]-noradrenaline and to estimate changes on electrically evoked tritium overflow as an indicator of changes on neuronal noradrenaline release, have been previously described (e.g. Queiroz et al., 2003, 2004). Briefly, tissue preparations of the prostatic portion of rat vas deferens were pre-incubated in 2 ml of Krebs solution containing [^{3}H]-noradrenaline (0.1 μM; specific activity of 53.0 Ci mmol^{-1}) for 40 min. Tissue preparations were then transferred to superfusion chambers where they were held between platinum electrodes 7 mm apart by a polypropylene mesh, and superfused with [^{3}H]-noradrenaline free medium at a constant flow rate of 1 ml min^{-1}. A stimulator (Hugo Sachs Elektronic, Type 215, March-Hungstetten, Germany), operating in the constant current mode, was used for electrical field stimulation with square wave pulses (1 ms width; 50 mA current strength; voltage drop of 18 V per chamber). The stimulation periods consisted of 100 pulses at 8 Hz (100 p/8 Hz) or 20 pulses at 50 Hz (20 p/50 Hz). A primer stimulation period, applied at $t = 30$ min ($t = 0$ min being the onset of superfusion) was not used for determination of tritium overflow. Subsequent stimulation periods were applied at $t = 60$ min (S_1), $t = 90$ min (S_2),