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Short Communication

Relaxation of the ovine isolated iris sphincter by adenosine receptor agonists: Lack of effect of adenosine A_1 and A_2 receptor antagonists

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Abstract

The effects of adenosine receptor ligands on the tone of the ovine isolated iris sphincter were investigated, and adenosine analogues were found to relax the carbachol-contracted tissue in a concentration-dependent manner with an order of potency of 5′-*N*-ethylcarboxamidoadenosine ≥ 2-(p-(2-carboxyethyl)phenylethylamino)-5′-*N*-ethylcarboxamidoadenosine (CGS 21680) ≥ N^6-cyclopentyladenosine > adenosine, consistent with activation of an adenosine A_{2A} receptor. However, these responses were not inhibited by the non-selective adenosine A_1/A_2 receptor antagonist 8-*p*-sulphophenyltheophylline (50 μM), the selective adenosine A_{2A}/A_1 receptor antagonist *N*-[2-(dimethylamino)ethyl]-*N*-methyl-4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1H-purin-8-yl)benzenesulphonamide (PD 115,199) (0.1 μM) or the non-xanthine adenosine A_{2A} receptor antagonist (4-(2-[7-amino-2-(2-furyl) [1,2,4]-triazolo[2,3-a][l,3,5]triazin-5-yl-amino]ethyl)phenol) (ZM 241385) (0.1 μM). The relaxations cannot therefore be mediated by activation of adenosine A_1, A_{2A} or A_{2B} receptors. © 1997 Elsevier Science B.V.

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1. Introduction

Extracellular adenosine has effects on many smooth muscles and other cell types, acting via G protein-coupled receptors of which four classes, adenosine A_1, A_{2A}, A_{2B} and A_3, have been cloned and can be distinguished pharmacologically (Collis and Hourani, 1993; Fredholm et al., 1994). In general adenosine A_1 receptors mediate contraction of smooth muscle preparations and adenosine A_2 receptors mediate relaxations, while adenosine A_3 receptors occur on mast cells and cause degranulation. At adenosine A_1 receptors N^6-substituted analogues such as N^6cyclopentyladenosine (CPA) are more potent than 5′-substituted analogues such as 5′-*N*-ethylcarboxamidoadenosine (NECA), while at adenosine A_2 receptors this order is reversed, and adenosine A_{2A} and A_{2B} receptors can be distinguished by the potent agonist action of C^2-substituted analogues of NECA, such as 2-(*p*-(2-carboxyethyl)phenylethylamino)-5′-*N*-ethylcarboxamidoadenosine (CGS 21680) at the former. Some methylxanthines such as 8-*p*-sulphophenyltheophylline are antagonists at both adenosine A_1 and adenosine A_2 receptors, and there are also xanthine-based subtype-selective antagonists such as the adenosine A_1 receptor antagonist 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) and the adenosine A_{2A}/A_1 receptor antagonist *N*-[2-(dimethylamino)ethyl]-*N*-methyl-4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1H-purin-8-yl)benzenesulphonamide (PD 115,199). Recently a non-xanthine adenosine A_{2A} receptor-selective antagonist (4-(2-[7-amino-2-(2-furyl) [1,2,4]-triazolo[2,3-a][l,3,5]triazin-5-yl-amino]ethyl)phenol) (ZM 241385) has also been developed (Poucher et al., 1995). Adenosine A_3 receptors are generally resistant to these antagonists, but marked species differences exist, with adenosine A_3 receptors from the rat being highly resistant while those from the sheep are much more sensitive, particularly to xanthines with acidic phenyl substitutions at the 8 position (Linden et al., 1993; Linden, 1994).

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The iris sphincter receives both autonomic and sensory innervation, and adenosine has been reported in the rabbit iris to inhibit the response to cholinergic nerve stimulation via prejunctional adenosine A_1 receptors but also to enhance cholinergic responses via postjunctional adenosine A_1 receptors, as well as enhancing sensory non-adrenergic noncholinergic responses (see Hall et al., 1993b) via prejunctional adenosine A_2 receptors (Gustafsson and Wiklund, 1986). In the isolated rat iris prejunctional adenosine A_1 receptors have also been reported to inhibit, and adenosine A_2 receptors to enhance, noradrenaline release from sympathetic nerves (Fuder et al., 1992). However, the effect of adenosine directly on the iris sphincter smooth muscle has not been previously investigated in any species. This present study was designed to investigate the effects of adenosine on the smooth muscle of the ovine iris sphincter and to determine the receptor types involved.

2. Materials and methods

Ovine eyes were freshly obtained from a local slaughter house and transported to the University of Surrey in pre-oxygenated Krebs solution (composition in mM: NaCl, 118; KCl, 4.7; $NaHCO_3$, 25; glucose, 11; $MgSO_4 \cdot 7H_2O$, 0.45; KH_2PO_4, 1.2; and $CaCl2 \cdot 2H_2O$, 2.5). This work was carried out before the introduction in the UK of the Heads of Sheep and Goats Order 1996, which effectively now precludes the supply of this tissue. The outer membrane of the eye was pierced and the posterior section, vitreous humour and lens of the eye were discarded. The iris sphincter muscle was carefully dissected away from the remainder of the tissue, threads were tied to either side of the ring of muscle and the whole tissue was mounted vertically in a 3.5 ml organ bath filled with Krebs solution, gassed with 95% O_2 and 5% CO_2 and maintained at 37°C, essentially as described previously (Hall et al., 1993b). An initial resting tension of 1.5 g was applied and the preparations were then allowed to stabilise for 60 min, and responses were recorded and displayed using a Grass FT03 force displacement transducer and a Grass 79 polygraph.

The tissues were precontracted with a submaximal concentration of carbachol (1 μM), and challenged at the start of the experiment with a test dose of NECA (100 μM). A tissue was considered viable for experimentation if a sustained contraction was maintained with carbachol and a subsequent relaxation observed with NECA. Approximately 70% of the preparations responded to carbachol and of these approximately 85% subsequently responded to the test dose of NECA. If no stable and measurable responses were obtained the tissue was discarded, but if viable the preparations were washed several times and equilibrated for 60 min in the presence or absence (control tissues) of an appropriate antagonist. Carbachol (1 μM) was then re-added and a cumulative dose–response curve was then constructed for a single agonist in each preparation, responses to each concentration of agonist being allowed to reach a plateau (approximately 10 min) prior to the subsequent addition of agonist. Values are expressed as mean ± s.e.m. of the percentage reversal of the contractile response to carbachol (1 μM).

The following drugs were used: NECA and carbachol from Sigma, UK; CGS 21680 and 8-*p*-sulphophenyltheophylline from Research Biochemicals International (RBI), USA; PD 115199, kindly donated by Dr. M.G. Collis of Pfizer Central Research, Sandwich, Kent; ZM 241385, kindly donated by Dr. S. Poucher, ZENECA pharmaceuticals, Mereside, Alderley Park, Macclesfield, Cheshire. NECA, 8-*p*-sulphophenyltheophylline and carbachol were dissolved, in distilled water. ZM 241385 and PD115199 were dissolved in 20% and 50% dimethyl sulphoxide (DMSO) respectively. Further dilutions were made with distilled water. At the concentrations used DMSO had no effect on the relaxatory responses to NECA.

3. Results

Adenosine and its analogues relaxed the carbachol-contracted ovine iris sphincter with an order of potency of NECA ≥ CGS 21680 ≥ CPA > adenosine (Fig. 1). The log concentration–response curves were rather shallow and did not plateau so no accurate EC_{50} values could be obtained, but by linear regression analysis of the rising part of the individual log concentration–response curves the means and s.e.m. of the negative log of the concentrations required to give 40% reversal of the carbachol contraction (EC_{40}; approximately half the response to the highest concentration of NECA tested) were calculated to be 5.38 ± 0.21, 5.08 ± 0.62, 4.81 ± 0.13 and 3.01 ± 0.25, respectively, which correspond to EC_{40} values of 4.20, 8.33, 15.3 and 977 μM, respectively. Responses to NECA, CGS 21680 and CPA were not inhibited by PD 115199 (0.1 μM) (Fig. 2a–c), responses to CGS 21680 were not inhibited by ZM 241385 (0.1 μM) (Fig. 2d) and responses

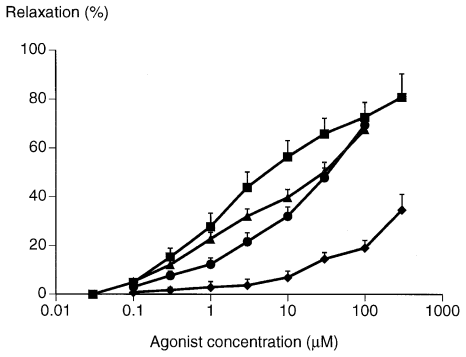

Fig. 1. Relaxation of the ovine iris sphincter by NECA (■), CGS 21680 (▲), CPA (●) and adenosine (♦). Responses are expressed as % reversal of the contraction induced by carbachol (0.1 μM), and are the mean ± s.e.m. of at least 6 determinations. For abbreviations see text.

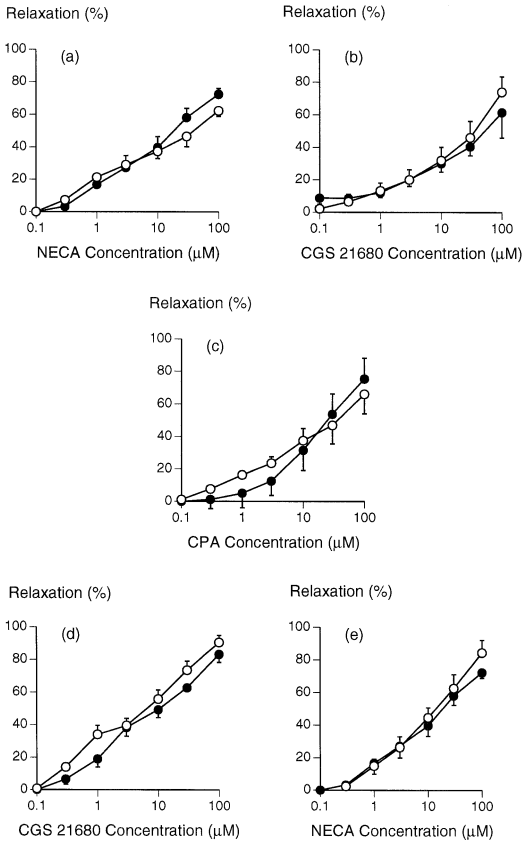

Fig. 2. Relaxation of the ovine iris sphincter by adenosine analogues alone (●) or in the presence of antagonist (○). (a) NECA ± PD 115199 (0.1 μM); (b) CGS 21680 ± PD 115199 (0.1 μM); (c) CPA ± PD 115199 (0.1 μM); (d) CGS 21680 ± ZM 214385; (e) NECA ± 8-*p*-sulphophenyltheophylline (50 μM). Responses are expressed as % reversal of the contraction induced by carbachol (0.1 μM), and are the mean ± s.e.m. of at least 4 determinations. For abbreviations see text.

to NECA were not inhibited by 8-*p*-sulphophenyltheophylline (50 μM) (Fig. 2e).

4. Discussion

The order of potency of adenosine analogues in relaxing the ovine iris sphincter is indicative of activation of an adenosine A_{2A} receptor, with NECA being more potent than CPA and CGS 21680 being similar in potency to NECA. A relaxant response is also typical of both adenosine A_2 receptor subtypes, and consistent with their known ability to stimulate adenylate cyclase (Collis and Hourani, 1993; Fredholm et al., 1994). However, use of antagonists did not support the presence of an adenosine A_{2A} receptor in this tissue. 8-*p*-Sulphophenyltheophylline at a concentration of 50 μM would be expected to antagonise responses mediated by adenosine A_1, A_{2A} and A_{2B} receptors, as K_D values in the low micromolar range are commonly obtained for this compound at each of these receptors (for review see Jacobson, 1990; Fredholm et al., 1994). However, it had no effect on responses to NECA in this tissue. In addition, the adenosine A_1/A_{2A} receptor antagonist PD 115199 at a concentration of 0.1 μM did not antagonise responses to NECA (a nonselective agonist), CGS 21680 (a selective adenosine A_{2A} receptor agonist) or CPA (which preferentially activates adenosine A_1 receptors). This antagonist has been reported to have a K_i of 15.5 nM at adenosine A_{2A} receptors and 14 nM at adenosine A_1 receptors (Bruns et al., 1987) and although originally thought to bind only weakly to adenosine A_{2B} receptors has more recently been shown to antagonise responses mediated via adenosine A_{2B} receptors in NIH 3T3 cell membranes with a K_B of 160 nM (Brackett and Daly, 1995). At a concentration of 0.1 μM it would therefore certainly be expected to inhibit responses mediated via adenosine A_{2A} or A_1 receptors, and the fact that no inhibition was seen here argues against the presence of an adenosine A_{2A} receptor in this tissue. This was confirmed by the lack of inhibition by the adenosine A_{2A} receptor-selective antagonist ZM 241385 of the relaxations caused by the adenosine A_{2A} receptor-selective agonist CGS 21680. This antagonist has been reported to have a pA_2 value of 8.57 as an inhibitor of responses induced by NECA in the guinea-pig Langendorff heart (Poucher et al., 1995), so at a concentration of 0.1 μM a significant inhibition of responses mediated by adenosine A_{2A} receptors would be expected. These data apparently preclude the relaxant adenosine receptor in this tissue from being of the adenosine A_{2A} receptor type, although as the ovine adenosine A_{2A} receptor has not been cloned or characterized it remains a theoretical possibility that the receptor could be a species homologue of the adenosine A_{2A} receptor that has the structure–activity relationships for agonists typical of this subtype but a very low affinity for the antagonists used here, as has been reported for some other receptor types (see Hall et al., 1993a)

It appears therefore that the relaxant responses induced by adenosine receptor agonists in the ovine iris sphincter are not mediated by adenosine A_1, A_{2A} or A_{2B} receptors, in spite of the fact that the structure–activity relationships for the agonists are indicative of interaction with an adenosine A_{2A} receptor. The lack of effect of antagonists could indicate that the responses are mediated via adenosine A_3 receptors, since in the rat these responses are generally regarded as being resistant to the commonly-used antagonists. However, no direct functional responses in any isolated tissue preparations via adenosine A_3 receptors have been reported, although transient responses via release of mediators from mast cells have been shown (e.g. Doyle et al., 1994). We have also failed to find responses via adenosine A_3 receptors even in tissues in which we have observed xanthine-resistant relaxations (Lewis et al., 1994; Prentice et al., 1996; Prentice and Hourani, 1996, 1997). In addition, this is an ovine tissue, and the ovine adenosine A_3 receptor has been cloned and partially char-

acterized, and shown to be insensitive to CPA which had a K_i value in excess of 100 μM (Linden et al., 1993), whereas in this study CPA was only about four-fold less potent than NECA. Further, although the antagonists used here have not been tested against the ovine adenosine A_3 receptor, based on its sensitivity to xanthines with an acidic phenyl substitution at the 8 position it has been stated that 8-*p*-sulphophenyltheophylline is likely to be an effective antagonist (Linden et al., 1993; Linden, 1994). It is therefore extremely unlikely that the observed responses are mediated via adenosine A_3 receptors, and the receptor involved in mediating relaxation in the ovine iris sphincter remains unknown.

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