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Short communication

Adenosine analogues relax guinea-pig taenia caeci via an adenosine A_{2B} receptor and a xanthine-resistant site

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Abstract

In this study we have sub-classified the adenosine A_2 receptor mediating relaxation in the guinea-pig taenia caecum using the adenosine A_{2A} receptor-selective agonist CGS 21680 (2-[*p*-(2-carboxyethyl)phenylamino]-5′-*N*-ethylcarboxamidoadenosine) and the adenosine A_{2A} receptor-selective antagonist ZM 241385 (4-(2-[7-amino-2-(2-furyl) [1,2,4]-triazolo[2,3-*a*][1,3,5]triazin-5-yl amino]ethyl)phenol). CGS 21680 did not elicit relaxations, and a pK_B value of 7.80 was obtained for ZM 241385 against 5′-*N*-ethylcarboxamidoadenosine suggesting the presence of adenosine A_{2B} receptors. Relaxations are also mediated via a xanthine-resistant site. In this study relaxations to the adenosine A_3 receptor agonist IB-MECA (N^6-(3-iodo-benzyl)adenosine-5′-*N*-methyluronamide) were blocked by neither 8-sulphophenyltheophylline (100 μM) nor the adenosine A_3 receptor antagonist BW-A1433 (1,3-dipropyl-8-(4-acrylate)phenylxanthine, 100 μM), suggesting that this site is not an adenosine A_3 receptor. © 1997 Elsevier Science B.V.

Keywords: Adenosine; Taenia caecum; Purinoceptor; Adenosine A_{2B} receptor; Adenosine A_3 receptor

1. Introduction

Although the presence of adenosine A_2 receptors mediating relaxation in the guinea pig taenia caecum has long been established (Burnstock et al., 1984) the receptor has not been sub-classified as an adenosine A_{2A} or adenosine A_{2B} receptor. We have therefore studied the effects of the adenosine A_{2A} receptor-selective agonist CGS 21680 (2-[*p*-(2-carboxyethyl)phenylamino]-5′-*N*-ethylcarboxamidoadenosine) which has a K_i at adenosine A_{2A} receptors in rat striatum of 15 nM, (Jacobson et al., 1995) and the adenosine A_{2A} receptor-selective antagonist ZM 241385 (4-(2-[7-amino-2-(2-furyl) [1,2,4]-triazolo[2,3-*a*][1,3,5]triazin-5-yl amino]ethyl)phenol) which has a pA_2 at adenosine A_{2A} receptors in the guinea-pig Langendorff heart of 9.02 against CGS 21680 and at adenosine A_{2B} receptors in the guinea-pig aorta of 7.06 against adenosine (Poucher et al., 1995), to determine whether the receptor is of the adenosine A_{2A} or adenosine A_{2B} receptor subtype.

There is evidence that, as in other smooth muscle tissues such as rat and frog aorta (Prentice and Hourani, 1996; Knight and Burnstock, 1996), guinea-pig trachea (Brackett and Daly, 1991) and rat coronary artery (Otley et al., 1996) relaxations to adenosine and some of its analogues in the guinea pig taenia caecum can also be mediated via a site distinct from adenosine A_1 and adenosine A_2 receptors (Prentice et al., 1995). The site shares some characteristics in common with the adenosine A_3 receptor in that it is apparently resistant to blockade by xanthines such as 8-phenyltheophylline and DPCPX (1,3-dipropyl-8-cyclopentylxanthine, Prentice et al., 1995). We have therefore also studied the effects of IB-MECA (N^6-(3-iodo-benzyl)adenosine-5′-*N*-methyluronamide) which is an agonist at adenosine A_3 receptors with a K_i of 1.1 nM (Jacobson et al., 1995), and blockade of these effects by the non-selective adenosine receptor antagonist 8-sulphophenyltheophylline and by BW-A1433 (1,3-dipropyl-8-(4-acrylate)phenylxanthine) which is an antagonist with some affinity at adenosine A_3 receptors. It is widely accepted that there are substantial species differences in the affinity of xanthines at adenosine A_3 receptors, and the reported K_i values for BW-A1433 are 15 μM at the rat adenosine A_3 receptor clone, 55 nM at the human adenosine A_3 receptor clone and 21 nM at the sheep adenosine A_3 receptor clone (see Linden, 1994). The adenosine A_3 receptor from the guinea-pig has yet to be cloned.

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2. Materials and methods

Lengths of taenia caecum (1–1.5 cm) dissected from male guinea-pigs (350–400 g) were suspended in 3.5 ml organ baths containing modified Krebs solution maintained at 33°C and constantly bubbled with 95% O_2/5% CO_2 (mM composition: NaCl 120, KCl 5.9, $MgCl_2$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 15.4 and glucose 11). Preparations were washed then allowed to equilibrate for approximately 60 min under an initial resting tension of 1 g. Responses were subsequently measured isometrically by Grass FT03 force displacement transducers and displayed on a Grass polygraph (model 79). In preliminary experiments concentration-response (E/[A]) curves to the muscarinic agonist carbachol were constructed (data not shown) and a concentration corresponding to approximately 80% of the maximum response (0.1 μM) was chosen to contract tissues prior to construction of relaxant E/[A] curves to adenosine receptor ligands. Preparations were challenged with this concentration of carbachol to check their viability then washed, incubated for 60 min in the absence or presence of antagonist, challenged again with 0.1 μM carbachol and, when the response had attained a plateau, relaxant agonist E/[A] curves were constructed by cumulative dosing. Relaxant responses were expressed as percentage decrease in carbachol contraction and only one curve was obtained per preparation.

Since E/[A] curves to NECA (5′-*N*-ethylcarboxamidoadenosine) were biphasic with a second relaxant phase apparent at concentrations above 100 μM (data not shown) only data up to this concentration were analysed to allow fitting of the NECA E/[A] curves to the Hill equation (see Prentice et al., 1995). This allowed estimates of midpoint slope parameter, midpoint location ($\log[A]_{50}$) and upper asymptote to be obtained. The pK_B value for ZM 241385 was estimated by fitting the individual $\log[A]_{50}$ values obtained in the absence and presence of the antagonist to a derivation of the Schild equation as described previously (Black et al., 1985). Since it was not possible to fit IB-MECA E/[A] curves to the Hill equation, estimates of the concentration giving 40% relaxation of the carbachol contraction (pEC_{40}) were obtained by regression analysis of the linear portion of the E/[A] curve (3–30 μM IB-MECA). The 40% relaxation point was chosen as this was approximately half of the maximal response produced by NECA.

CGS 21680 and 8-sulphophenyltheophylline were obtained from Research Biochemicals International (Natick, MA, USA). Carbachol was obtained from Sigma (Poole, UK). ZM 241385 was kindly provided by Dr. S. Poucher (ZENECA Pharmaceuticals, Macclesfield, UK), BW-A1433 by the Wellcome Research Laboratories (Beckenham, UK) and IB-MECA by Dr. K. Jacobson (National Institutes of Health, Bethesda, MD, USA). All drugs were made up at a stock concentration of 10 mM and stored frozen. CGS 21680 was made up in 7% ethanol, and 8-sulphophenyltheophylline, NECA and carbachol in distilled water. IB-MECA was made up in 100% dimethyl sulphoxide (DMSO), ZM 241385 in 20% DMSO and BW-A1433 in 25% ethanol plus 30 mM NaOH. Further dilutions were freshly made up in distilled water. For the agonists, the vehicles alone had no effect at levels equivalent to the highest concentration of agonist used, and for the antagonists the control E/[A] curves were carried out in the presence of vehicle at a level equivalent to the highest concentration of antagonist used.

3. Results

No relaxant responses were obtained to CGS 21680 at concentrations below 10 μM (Fig. 1a) whereas relaxant

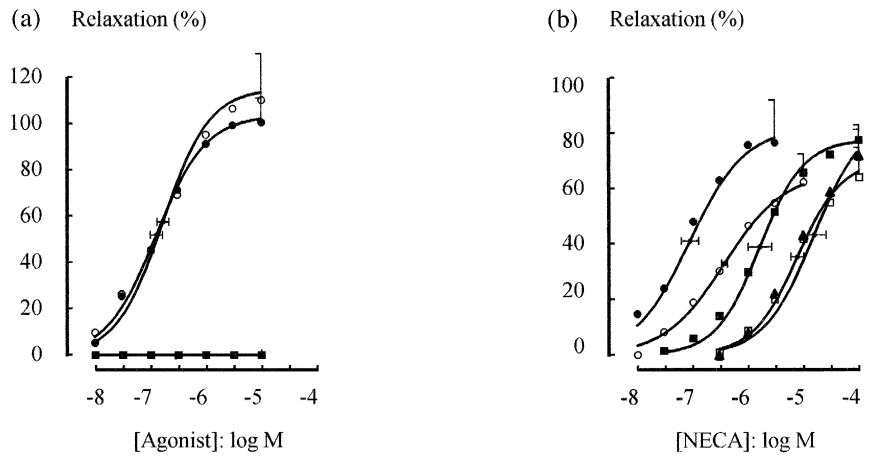

Fig. 1. Concentration-response curves to (a) CGS 21690 (■) and NECA in the absence (●) and presence (○) of 1 μM CGS 21680 and (b) NECA in the absence (●) and presence of ZM 241385 0.1 μM (○), 0.3 μM (■), 1 μM (□), and 3 μM (▲). Data points are average responses (% relaxation of carbachol-induced contraction), and the lines through the data were generated by use of the average logistic fitting parameters. Average $p[A]_{50}$ and asymptote values are marked together with their associated S.E.M., $n = 3–6$.

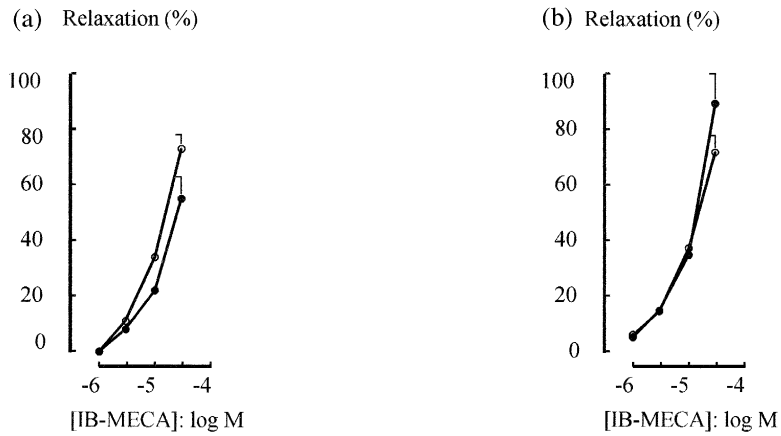

Fig. 2. Concentration–response curves to IB-MECA (a) in the absence (●) and presence of 100 μM 8-sulphophenyltheophylline (○) and (b) in the absence (●) and presence of 100 μM BWA 1433 (○). Data points are average responses (% relaxation of carbachol-induced contraction), $n = 3–4$.

$E/[A]$ curves to NECA were obtained in carbachol pre-contracted preparations, although these were biphasic with a second relaxant phase apparent at concentrations above 100 μM (data not shown). Fitting of the NECA data for concentrations below 100 μM to the Hill equation allowed estimation of a $p[A]_{50}$ value for this agonist of 6.90 ± 0.11 (Fig. 1). There was no significant right-shift of $E/[A]$ curves to NECA in the presence of 1 μM CGS 21680 ($p[A]_{50}$ in the presence of CGS 21680 $= 6.78 \pm 0.11$, $P > 0.05$, Fig. 1a). $E/[A]$ curves to NECA were however right-shifted in a manner consistent with simple competition by ZM 241385 (0.1–3 μM, Fig. 1b). A Schild plot slope not significantly different from unity was obtained (1.14 ± 0.12, $P > 0.05$) along with a pK_B value of 7.80 ± 0.14. Relaxant $E/[A]$ curves to IB-MECA were obtained which were not blocked by 8-sulphophenyltheophylline (100 μM, pEC_{40} in the absence and presence of 8-sulphophenyltheophylline $= 4.72 \pm 0.13$ and 4.98 ± 0.05, respectively, $P > 0.05$, Fig. 2a). This concentration of 8-sulphophenyltheophylline right-shifted NECA $E/[A]$ curves approximately 100-fold (data not shown) which is consistent with the reported pK_B value for this antagonist against NECA in the taenia caecum of 5.56 (Piper and Hollingsworth, 1995). However, the relaxant $E/[A]$ curves to IB-MECA were also not blocked by BW-A1433 (100 μM, pEC_{40} in the absence and presence of BW-A1433 $= 5.06 \pm 0.08$ and 5.01 ± 0.07, respectively, $P > 0.05$, Fig. 2b).

4. Discussion

The absence of relaxant responses elicited by CGS 21680 in this study suggested that the adenosine A_2 receptor mediating relaxations in the taenia caecum was not of the adenosine A_{2A} receptor subtype. CGS 21680 has been shown to behave as a partial agonist in some systems such as the rat aorta (Prentice and Hourani, 1996), therefore CGS 21680 was used as a potential antagonist at 1 μM, a concentration that should right-shift adenosine A_{2A} receptor-mediated responses approximately 70-fold on the basis of its reported adenosine A_{2A} receptor affinity (Jacobson et al., 1995). There was no significant shift of the NECA $E/[A]$ curves in the presence of this concentration of CGS 21680 confirming the presence of adenosine A_{2B} receptors rather than adenosine A_{2A} receptors. This was further confirmed by the pK_B estimate for ZM 241385 of 7.8 which is more consistent with the reported adenosine A_{2B} receptor affinity for this compound ($pA_2 = 7.06$ against adenosine in the guinea-pig aorta) than with its reported adenosine A_{2A} receptor affinity ($pA_2 = 9.02$ against CGS 21680 in the guinea-pig Langendorff heart) (Poucher et al., 1995). The presence of adenosine A_{2B} receptors in this preparation is consistent with other studies of visceral smooth muscle since adenosine A_{2B} receptors have been proposed to mediate relaxations in tissues such as rat duodenum and urinary bladder (Nicholls et al., 1992) and rat vas deferens (Major et al., 1989; Hourani et al., 1993). Indeed, Stehle et al. (1992) have reported high levels of adenosine A_{2B} receptor mRNA expressed in the intestine, caecum and urinary bladder of the rat.

The presence of a 'xanthine-resistant site' mediating relaxations in this tissue has been previously reported (Prentice et al., 1995), and the finding in this study that $E/[A]$ curves to NECA were biphasic is consistent with these previous observations suggesting that this agonist activates two sites. Since the cloned adenosine A_3 receptors are also resistant to blockade by all but a few xanthines (Jacobson et al., 1995), we investigated the activity in this tissue of the adenosine A_3 receptor agonist IB-MECA, and of BW-A1433 which is one of the few xanthine antagonists with reported adenosine A_3 receptor affinity (Jacobson et al., 1995). Although IB-MECA did elicit relaxant responses which were resistant to blockade by 8-sulphophenyltheophylline (100 μM), these responses were also resistant to blockade by 100 μM BW-A1433, a

concentration which should right-shift adenosine A_3 receptor-mediated responses approximately 8-fold on the basis of its lowest reported adenosine A_3 receptor affinity. These data indicate that the 'xanthine-resistant site' is unlikely to be a functional correlate of the adenosine A_3 receptor.

In conclusion relaxant responses to adenosine analogues in the guinea-pig taenia caecum can be mediated via adenosine A_{2B} receptors and an additional xanthine-resistant site which is unlikely to be an adenosine A_3 receptor.

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