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Characterization of adenosine receptors on rat ileum, ileal longitudinal muscle and muscularis mucosae

Julia Nicholls *, Susanna M.O. Hourani

Receptors and Cellular Regulation Research Group, School of Biological Sciences, University of Surrey, Guildford, Surrey GU2 5XH, UK

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Abstract

Adenosine receptors were studied in isolated rat ileum, ileal longitudinal muscle and muscularis mucosae, using a range of agonists and an antagonist. In the rat ileal longitudinal muscle adenosine receptor agonists relaxed the tissues. N^6-cyclopentyladenosine (CPA) was more potent than 5′-*N*-ethylcarboxamidoadenosine (NECA) or adenosine and 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) (1 nM) gave a 5-fold parallel shift to the right of the concentration–response curves to both CPA and NECA corresponding to an apparent pA_2 value of 9.6 suggesting that the agonists relax via adenosine A_1 receptors. In the intact ileum adenosine receptor agonists also relaxed the tissue but NECA and CPA were equipotent. DPCPX (3 nM) however inhibited responses to both CPA and NECA with dose-ratios of 8 and 15.6, corresponding to pA_2 values of 9.3 and 9.7, respectively. DPCPX (300 nM) gave a much greater shift to the right of the concentration–response curve to NECA with a dose-ratio of 769, corresponding to an apparent pA_2 of 9.4. This suggests that the agonists are acting at adenosine A_1 receptors to cause relaxation of the whole tissue. Adenosine receptor agonists contracted rat ileal muscularis mucosae with a potency order indicative of an A_1 adenosine receptor. DPCPX (3–100 nM) antagonized responses to CPA giving a linear Schild plot with a slope close to unity and a pA_2 of 8.4 suggesting an action on adenosine A_1 receptors. © 1997 Elsevier Science B.V.

Keywords: Adenosine receptor; Ileum, rat

1. Introduction

Adenosine has been shown to have pharmacological actions on a variety of isolated tissue preparations. It causes its effects via adenosine receptors (also known as P_1-purinoceptors) for which four receptors, adenosine A_1, A_{2A}, A_{2B} and A_3, have recently been cloned (Fredholm et al., 1994). The receptors can be distinguished in functional studies by the relative potency of adenosine agonists and antagonists. For the adenosine A_1 receptor N^6-substituted analogues such as N^6-cyclopentyladenosine (CPA) and N^6-phenylisopropyladenosine (R-PIA) are more potent than 5′-substituted analogues such as 5′-*N*-ethylcarboxamidoadenosine (NECA) and the A_1 selective antagonist 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) has a dissociation constant in the nanomolar range. For the adenosine A_2 receptor this agonist potency order is reversed and DPCPX has a dissociation constant in the micromolar range (Bruns, 1990; Collis and Hourani, 1993). The adenosine A_{2A} and A_{2B} receptor subtypes can be differentiated by the high potency of some C2-substituted analogues such as 2-*p*-(2-carboxyethyl)phenethylamino-5′-*N*-ethylcarboxamidoadenosine (CGS21680) at the high affinity A_{2A} subclass of adenosine A_2 receptors (Jarvis et al., 1989). At the adenosine A_3 receptor N^6-(3-iodo-benzyl)adenosine-5′-*N*-methyl uronamide (IBMECA) is a selective agonist, CPA and NECA are equipotent and a concentration of DPCPX in excess of 10 μM is required to antagonize responses (Van Galen et al., 1994; Jacobson et al., 1995).

In the gastrointestinal tract non-adrenergic non-cholinergic (NANC) inhibitory neurones are known to mediate descending inhibition, an important component of the peristaltic reflex (Abrahamsson, 1986). Adenosine triphosphate (ATP) has been proposed as a NANC transmitter in the gut (Burnstock et al., 1970) and as it is known that ATP can be rapidly dephosphorylated to adenosine by ectonucleotidases (Bailey and Hourani, 1990, 1992; Hourani et al., 1991; Nicholls et al., 1992b) it is reasonable to presume that adenosine may have important physiological actions in this tissue. Indeed, in the rat jejunum and ileum it has been shown that adenosine agonists can inhibit

* Corresponding author. Tel.: (44-1483) 259-710; Fax: (44-1483) 576-978.

intestinal fluid secretion and peristalsis (Coupar and Hancock, 1994) via adenosine A_{2B} and adenosine A_1 receptors respectively (Hancock and Coupar, 1995a,b).

Previous studies on the rat gastrointestinal tract have shown that the profile of adenosine receptors is different in different parts of the gut. In the rat duodenum we have shown that adenosine agonists relaxed intact duodenum via a mixture of adenosine A_1 and A_{2B} receptors (Nicholls et al., 1992a). The presence of adenosine A_1 receptors was also confirmed by radioligand binding experiments (Peachey et al., 1994). Further studies demonstrated that the longitudinal muscle dominated the response of the intact tissue with adenosine agonists relaxing pre-contracted longitudinal muscle by both adenosine A_1 and A_{2B} receptors. The duodenum muscularis mucosae however was shown to contract to adenosine analogues via adenosine A_{2B} receptors (Nicholls et al., 1996). This was unusual since it is generally thought that adenosine A_1 receptors mediate contraction and adenosine A_2 receptors mediate relaxation of smooth muscle (White, 1988; Kennedy, 1990; Collis and Hourani, 1993). Also the significance of the presence of two adenosine receptors mediating the same relaxant response was not clear.

More conventionally, in the rat colon adenosine agonists were shown to relax the longitudinal muscle via adenosine A_2 receptors and contract the muscularis mucosae via adenosine A_1 receptors (Bailey and Hourani, 1992; Bailey et al., 1992).

There is little current information on the effects of adenosine and its analogues on the smooth muscle layers of rat ileum. Therefore the aim of this study was to characterize fully the adenosine receptors present on the intact rat ileum, ileal longitudinal muscle and muscularis mucosae to determine the distribution of adenosine receptors throughout the gastrointestinal tract.

2. Methods

Male Wistar albino rats (200–250 g), Bantin and Kingman, were killed by cervical dislocation. The distal ileum was dissected out by cutting a 2 cm length approximately 10 cm up from the ileo-caecal valve and cleared of any connective tissue. For studies on the longitudinal muscle and muscularis mucosae the ileum was placed over a fine bore (diameter: 5 mm) pipette and a longitudinal cut made. The longitudinal muscle was separated by gently rubbing the layer with moist cotton wool leaving a thick walled tube of mucosal tissue which contains the muscularis mucosae. The tissues were mounted in 4 ml organ baths by threads tied around each end, so that in the case of the intact rat ileum and muscularis mucosae the lumens were sealed and maintained in Krebs solution of the following composition (in mM): NaCl, 118; KCl, 4.8; $MgSO_4$, 1.2; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $NaHCO_3$, 25 and glucose, 11. The Krebs solution was aerated with 95% O_2:5% CO_2 and maintained at 35–36°C and the tissues were equilibrated for at least 45 min before the addition of drugs. A resting tension of 1 g was applied to the tissues and the force was recorded isometrically with Grass FT03 transducers and displayed on Grass 79D polygraphs. All concentration–response curves were obtained non-cumulatively.

2.1. Intact ileum and ileal longitudinal muscle

Inhibitory responses were quantified by pre-contracting the tissues with carbachol (0.3 μM) before challenge with the purines. Contractions to carbachol were measured from the peak of spontaneous activity to the highest point of carbachol contraction. Relaxations were measured as the reduction in this peak height and expressed as % inhibition of carbachol contraction. For studies with DPCPX, tissues were incubated with antagonist for 30 min prior to carbachol or adenosine agonist dosing. Concentration–response curves to CPA or NECA in the presence and absence of antagonist were performed in parallel with 2 cm segments of ileum or ileal longitudinal muscle taken 10 and 12 cm from the ileo-caecal valve, respectively.

For the longitudinal muscle studies involving cromolyn, ω-conotoxin and tetrodotoxin, the tissues were incubated with drugs for 60 min prior to dosing with agonists. Inhibition of nerve-mediated transmitter release by the combination of tetrodotoxin and ω-conotoxin was confirmed by the blockade of responses to field stimulation of tissues (Grass S48 stimulator, stimulus parameters 30 s train duration, 10 Hz, 1 ms pulse width, 60 V). For studies with compound 48/80 and calcitonin gene-related peptide (CGRP) drugs were added after pre-contracting with carbachol. Where contractions to CPA were investigated CPA was added direct to tissue with no pre-contraction with carbachol.

2.2. Muscularis mucosae

For the muscularis mucosae preparation contractions were observed by adding drugs direct to the bath without pre-contracting the tissue, and contractions were expressed as % of contraction induced by KCl (35 mM). Relaxations of the muscularis mucosae were investigated by pre-contracting the tissue with carbachol (10 μM) before addition of the adenosine analogue. The dose-cycle was 10–15 min and no tachyphylaxis was observed. After concentration–response curves to CPA had been obtained, tissues were incubated for 30 min with DPCPX or vehicle and the concentration–response curves were then repeated in the presence of the antagonist or vehicle.

Dose-ratios were calculated from the mean EC_{30} values (concentration of agonist producing 30% of the response) in the absence and presence of antagonist. Apparent pA_2 values were calculated as the negative logarithm of the molar concentration of the antagonist divided by the dose-ratio-1.

2.3. Materials

Adenosine, NECA, carbachol, 2CADO, R-PIA, isoprenaline, cromolyn, compound 48/80 and tetrodotoxin were obtained from Sigma, UK. CPA, CGS21680, IB-MECA, DPCPX and ω-conotoxin were obtained from Research Biochemicals and CGRP was obtained from Bachem, UK. The buffer salts were of analytical grade and were obtained from BDH.

CPA (10 mM) was dissolved in 20% ethanol except for relaxation studies on the muscularis mucosae when CPA (0.1 M) was made up in 100% ethanol. DPCPX (1 mM) was dissolved in 6% aqueous dimethylsulphoxide (DMSO) containing 6 mM NaOH and isoprenaline was dissolved in acidified water. All other drugs were dissolved in distilled water.

3. Results

3.1. Intact ileum

CPA, NECA and adenosine relaxed carbachol-contracted rat ileum with an agonist potency order of NECA $\geq$ CPA > adenosine and EC_{30} values of 0.15, 0.1 and 1.9 μM, respectively (Fig. 1a and Fig. 5a). DPCPX (1 nM) gave a small shift to the right of the concentration–response curve to CPA with a dose-ratio of 2.3 corresponding to an apparent pA_2 of 9.1 (results not shown). DPCPX (3 nM) inhibited responses to CPA and NECA, giving dose-ratios of 8.0 and 15.6, corresponding to apparent pA_2 values of 9.3 and 9.7, respectively (Fig. 1b and c). DPCPX (300 nM) gave an even greater shift in the concentration–response curve to NECA and a depression of the maxima with a dose-ratio of 769, corresponding to an apparent pA_2 of 9.4 (results not shown).

No contractions to CPA (30 μM) were observed when agonist was added direct to the tissue (results not shown).

3.2. Ileal longitudinal muscle

CPA, NECA and adenosine all relaxed the carbachol-contracted rat ileal longitudinal muscle (Fig. 2a and Fig. 5b). CPA was more potent than NECA and adenosine with EC_{30} values of 0.3, 1.5 and 12.7 μM, respectively (Fig. 2a). IB-MECA (10 μM) had no relaxant effect on rat ileal longitudinal muscle (Fig. 5b).

DPCPX (1 nM) gave a parallel shift to the right of concentration–response curves to CPA and NECA with a dose-ratio of 4.8 for each agonist corresponding to an apparent pA_2 of 9.6 (Fig. 2b and c). DPCPX (3 nM) gave a much greater shift to the right of the concentration–response curve to CPA and markedly reduced the maximum response (results not shown), thus making it impossible to calculate an accurate dose-ratio. For this reason Schild analysis was not performed on this tissue. DPCPX (100 nM) completely abolished responses to CPA (0.1–10 μM) ($n = 3$, results not shown) and almost abolished responses to NECA (10 and 100 μM NECA gave 10.1 ± 1.1% and 9.4 ± 2.4% relaxation, respectively, $n = 4$, results not shown).

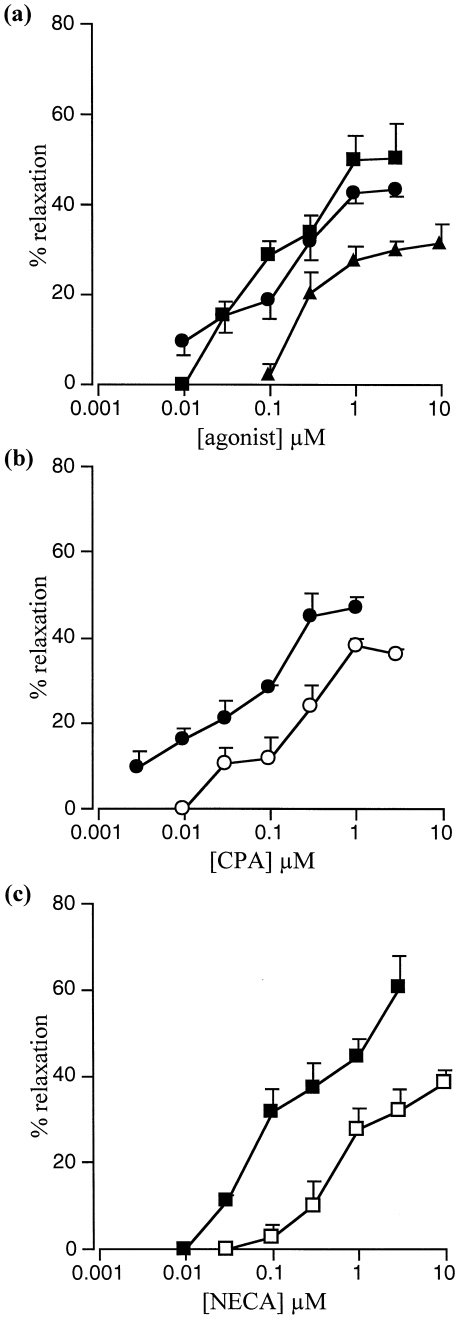

Fig. 1. Effects of adenosine receptor ligands on rat intact ileum. (a) Relaxations induced by CPA (●), NECA (■) and adenosine (□); (b) effect of CPA in the absence (closed symbols) and presence (open symbols) of DPCPX (3 nM); (c) effect of NECA in the absence (closed symbols) and presence (open symbols) of DPCPX (3 nM). Each point is the mean with S.E.M. of at least four determinations. For abbreviations see text.

It was observed that on both isolated ileum and ileal

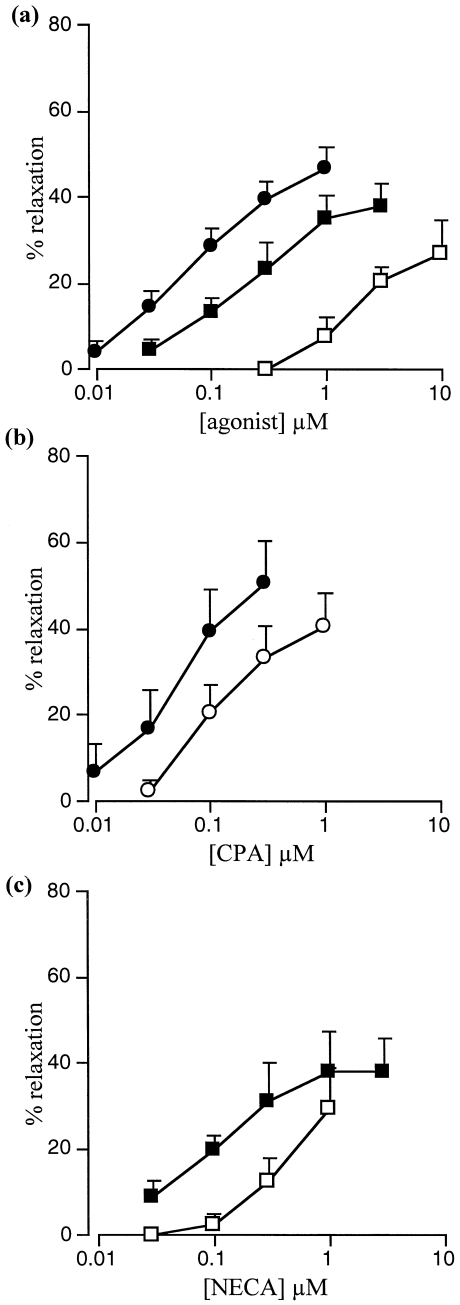

Fig. 2. Effects of adenosine receptor ligands on rat ileal longitudinal muscle. (a) Relaxations induced by CPA (●), NECA (■) and adenosine (□); (b) effect of CPA in the absence (closed symbols) and presence (open symbols) of DPCPX (1 nM); (c) effect of NECA in the absence (closed symbols) and presence (open symbols) of DPCPX (1 nM). Each point is the mean with S.E.M. of at least four determinations. For abbreviations see text.

longitudinal muscle, relaxant responses to agonists started to wane after only 4 doses of agonist. Further relaxations could not be obtained even after tissues had been allowed to recover for 60 min. Therefore full concentration–response curves as shown in Figs. 1 and 2 are a compilation of many separate experiments. Repetitive dosing with isoprenaline (0.1 μM), a β-receptor agonist, gave a greater

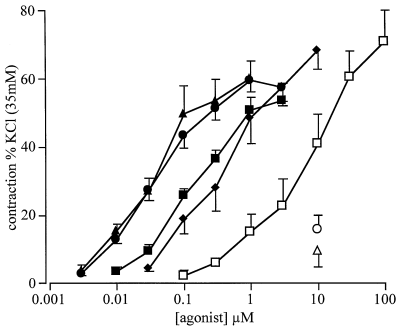

Fig. 3. Contraction of rat ileal muscularis mucosae induced by CPA (●), R-PIA (▲), NECA (■), 2CADO (♦), adenosine (□), CGS21680 (○) and IB-MECA (△). Each point is the mean with S.E.M. of at least four determinations. For abbreviations see text.

(approximately 70%) relaxation of the pre-contracted ileal longitudinal muscle and was reproducible over a 3-h period with a dose-cycle of 10 min (results not shown). The nature of the fade of the relaxant response to adenosine agonists was therefore investigated further using a variety of pharmacological tools.

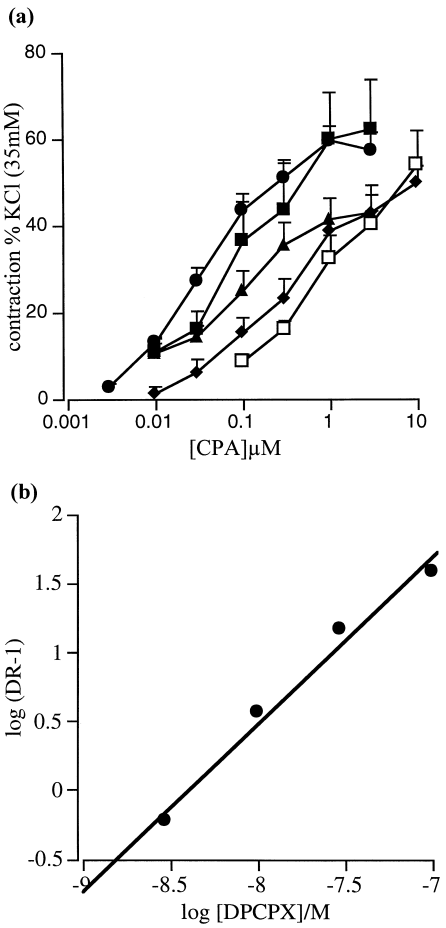

Fig. 4. (a) Contractions of the rat ileal muscularis mucosae induced by CPA in the absence (●) or presence of DPCPX 3 nM (■), 10 nM (▲), 30 nM (♦) and 100 nM (□). Each point is the mean with S.E.M. of at least four determinations. For abbreviations see text. (b) Schild plot of the data shown in (a).

Compound 48/80 (2 and 10 μg/ml), a mast cell degranulator, had no effect on pre-contracted rat ileal longitudinal muscle and cromolyn (10 μM), a mast cell stabiliser, did not affect relaxations to CPA (results not shown).

The sensory neuropeptide CGRP (0.1 μM) relaxed

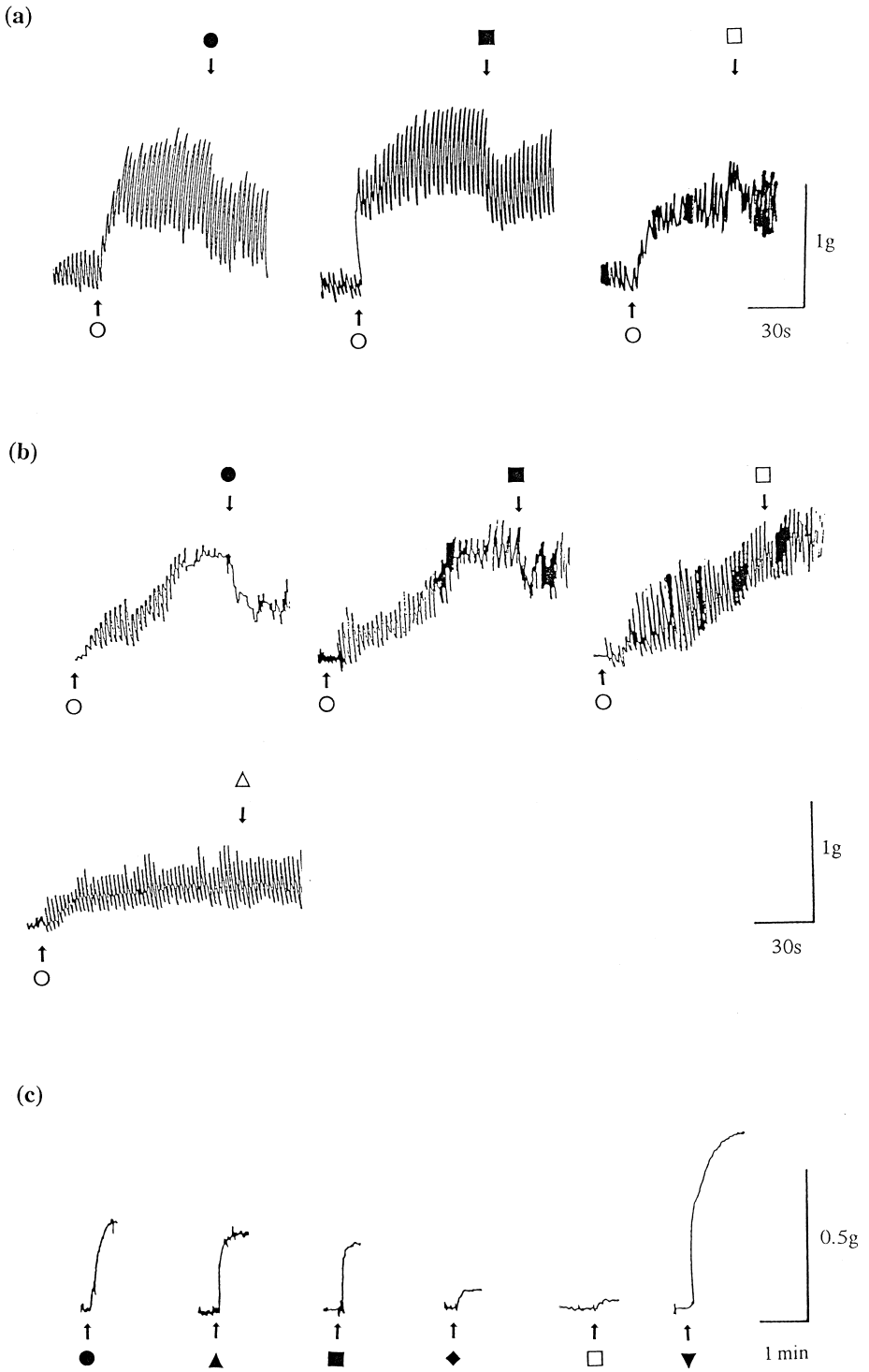

Fig. 5. Representative traces showing responses of adenosine and its analogues. (a) Relaxation of pre-contracted (0.3 μM carbachol (○)) intact rat ileum by 0.3 μM CPA (●), NECA (■) and adenosine (□); (b) relaxation of pre-contracted (0.3 μM carbachol (○)) rat ileal longitudinal muscle by 0.3 μM CPA (●), NECA (▲) and adenosine (□) and 10 μM IB-MECA (△); (c) contraction of rat ileal muscularis mucosae by 0.3 μM CPA (●), R-PIA (▲), NECA (■), 2CADO (♦) and adenosine (□) and 35 mM KCl (▼).

pre-contracted ileal longitudinal muscle (results not shown) however the neurone blocking drugs, ω-conotoxin (1 μM) and tetrodotoxin (1 μM) did not affect relaxations to CPA but did abolish responses to field stimulation (results not shown).

CPA was never observed to contract the tissue either with or without carbachol precontraction even at a concentration of 30 μM (results not shown).

3.3. Muscularis mucosae

Adenosine, NECA, CPA, R-PIA and 2CADO all contracted the rat ileal muscularis mucosae and the order of potency was R-PIA > CPA > NECA > 2CADO > adenosine with EC30 values of 0.01, 0.07, 0.18, 0.38 and 5.76 μM, respectively (Fig. 3 and Fig. 5c). CGS21680 (10 μM) and IB-MECA (10 μM) gave weak contractions of the tissue (Fig. 3). DPCPX (3–100 nM) gave parallel shifts to the right of the concentration–response curves to CPA (Fig. 4a) and Schild analysis of DPCPX versus CPA gave a linear plot ($r = 0.99$) with a slope of 1.19, corresponding to a pA_2 of 8.4 (Fig. 4b). DPCPX (1 μM) almost abolished contractions to both CPA and NECA, while vehicle alone, at a concentration equivalent to 1 μM DPCPX, had no effect on responses to CPA (results not shown).

In the absence of DPCPX, neither CPA nor NECA nor adenosine relaxed precontracted muscularis mucosae (results not shown). In the presence of 1 μM DPCPX concentrations of CPA and NECA up to 10 μM had no relaxant effect but NECA (30 μM–1 mM) gave small relaxations of the pre-contracted tissue (100 μM and 1 mM giving a $15.2 \pm 5.4\%$ and $22.5 \pm 6.4\%$ relaxation, $n = 4$, results not shown) and CPA (100 μM) also gave a $10 \pm 4.0\%$ relaxation, $n = 4$ (results not shown). Concentrations of CPA above 100 μM could not be used because at these concentrations vehicle alone had a substantial relaxant effect.

4. Discussion

This study shows that adenosine agonists relax carbachol-contracted rat ileum and ileal longitudinal muscle and contract the muscularis mucosae demonstrating that the nature of the response to adenosine agonists is the same throughout the gastrointestinal tract. As for the rat duodenum it appears that the longitudinal muscle dominates the response of the whole tissue (Nicholls et al., 1996) since relaxations were qualitatively similar in the two preparations and in the intact ileum no contractions were observed even to the most potent contractile agonist CPA.

For the ileal longitudinal muscle the potency order of the agonists was typical for that of an adenosine A_1 receptor with CPA > NECA > adenosine. The presence of a relaxant adenosine A_1 receptor was confirmed with DPCPX (1 nM) which inhibited, to the same extent, responses to both CPA and NECA with an apparent pA_2 of 9.6. DPCPX (100 nM) almost totally abolished responses to CPA and NECA suggesting that there is no adenosine A_2 receptor component to the response to NECA. This is different to the rat duodenum where a mixture of relaxant adenosine A_1 and A_2 receptors exists and where relaxations to NECA were still apparent in the presence of μM DPCPX (Nicholls et al., 1996).

Unexpectedly in the intact ileum the potency of both NECA and adenosine was enhanced when compared to the ileal longitudinal muscle resulting in NECA and CPA being almost equipotent. The reason for this difference in agonist potency order between the two preparations is not known and might suggest the unmasking of an adenosine A_2 receptor mediated response in the intact tissue. However the same phenomenon was seen in the rat duodenum, where studies with DPCPX clearly demonstrated the presence of two adenosine receptor types in both the longitudinal muscle and the intact tissue (Nicholls et al., 1992a, 1996). In addition, the results here do not support the presence of adenosine A_2 receptors in the intact ileum as DPCPX (3 nM) inhibited responses to both CPA and NECA in a very similar fashion with dose-ratios of 8 and 15.6, corresponding to apparent pA_2 values of 9.3 and 9.7, respectively, appropriate for an adenosine A_1 receptor. DPCPX (300 nM) gave a much greater inhibition of the concentration–response curve to NECA with a dose-ratio of 769, corresponding to an apparent pA_2 of 9.4. These results are clearly indicative of an adenosine A_1 receptor mediated response with no evidence for the presence of adenosine A_2 receptors and are similar to the findings by Hancock and Coupar when investigating the inhibitory effects of adenosine agonists on peristalsis in rat ileum. They showed that DPCPX (10 nM) antagonized responses to CPA and NECA with apparent pA_2 values of 9.37 and 9.5, respectively (Hancock and Coupar, 1995b).

The reason for the lower potency of adenosine and NECA in the longitudinal muscle compared to the intact ileum is therefore not clear. It is known that in the rat small intestine adenosine deaminase, the enzyme which degrades adenosine to inosine, is very rapid (Franco et al., 1988). Also in the rat colon it has been shown that the metabolism of nucleotides by the longitudinal muscle is extremely rapid and is considerably faster than that in the muscularis mucosae in spite of the smaller size of the longitudinal muscle (Bailey and Hourani, 1990, 1992). A possible explanation for adenosine being less potent in the ileal longitudinal muscle is that it is degraded more rapidly than in the intact tissue, since a larger surface area would be accessible to the drugs when the longitudinal muscle is set up in isolation. However, as NECA is thought to be metabolically stable (Stein and Somani, 1975) this is unlikely to explain why this analogue is also less potent in the longitudinal muscle than in the intact ileum.

Interestingly, on both the rat ileum and ileal longitudinal muscle responses to adenosine agonists started to wane, usually after only 4 applications of agonist. This

was never observed in the rat duodenum (Nicholls et al., 1992a, 1996) nor rat colon (Bailey and Hourani, 1992) where adenosine agonists gave consistent relaxations (up to 80% relaxation of carbachol contraction) over a similar time-course. The reason for the failing response is unclear. Contractions to carbachol were unchanged throughout the experiment and the β-agonist, isoprenaline (0.1 μM) gave a much greater relaxation which was reproducible over a 3-h period suggesting that the lack of response is related to adenosine receptors. We therefore decided to investigate the relaxant adenosine responses on the longitudinal muscle more fully.

5-Hydroxytryptamine (5-HT) has been shown to relax rat ileal longitudinal muscle although relaxant responses to 5-HT are often very variable between tissues (McLean et al., 1995). On the intact ileum we showed that NECA and CPA were virtually equipotent and we therefore investigated the possibility of the agonists inducing mast cell degranulation via adenosine A_3 receptors and the concomitant release of 5-HT. Our studies showed however, that the potent adenosine A_3 receptor agonist IB-MECA did not relax the rat ileal longitudinal muscle even at a concentration of 10 μM. In addition, the mast cell degranulator, compound 48/80 (10 μg/ml), also had no effect on carbachol-contracted rat ileal longitudinal muscle and the mast cell stabiliser cromolyn (10 μm) did not block relaxations to CPA. These results would suggest that CPA and NECA are not acting via adenosine A_3 receptors to release 5-HT.

We also investigated the possibility of adenosine inducing neurogenic release either from autonomic nerves or sensory nerves as it has been shown in the pre-contracted rat duodenum and guinea-pig ileal longitudinal muscle that CGRP (a sensory neuropeptide) can cause relaxation (Maggi et al., 1987; Bartho et al., 1987). We found that CGRP relaxed ileal longitudinal muscle but that tetrodotoxin (to block autonomic nerves) and ω-conotoxin (to block sensory nerves) (1 μM), whilst totally abolishing the field stimulated response of relaxation followed by contraction had no effect on relaxations to CPA. This would suggest that the agonist is not acting to cause neuronal transmitter release.

The loss of response could also have been caused by an opposing contractile response at higher concentrations of agonist nullifying the relaxations seen. CPA however even at a concentration of 30 μM was never observed to contract the longitudinal muscle or intact ileum, and after responses to high concentrations of agonists had failed no relaxations were then observed even to low doses of agonist.

For the rat ileal muscularis mucosae adenosine agonists contracted the tissue with an agonist potency order of R-PIA $\geq$ CPA > NECA > 2CADO > adenosine. DPCPX (3–100 nM) gave increasing rightward shifts in the concentration–response curve to CPA which resulted in a Schild plot slope close to unity and a pA_2 of 8.4 suggesting that the contractile adenosine receptors are of the A_1 subtype. Although the pA_2 value here is rather lower than that found for the longitudinal muscle or the intact ileum, there is no evidence for the presence of an adenosine A_2 component of the contractions as responses to both CPA and NECA were almost abolished by DPCPX (1 μM). These results are consistent with the findings on rat colon muscularis mucosae in which contractions are also via adenosine A_1 receptors (Bailey et al., 1992). On rat duodenum muscularis mucosae however adenosine receptor agonists contracted via adenosine A_{2B} receptors (Nicholls et al., 1996) which suggests that there is a change in the contractile adenosine receptor from A_{2B} to A_1 along the length of the gastrointestinal tract. The physiological significance for this change in receptor type is not known.

When the tone of the ileal muscularis mucosae was raised with carbachol (10 μM) no relaxant response to CPA, NECA or adenosine (100 μM) was observed suggesting that the potency order for the agonists on this tissue is not complicated by an opposing relaxation. In the presence of 1 μM DPCPX (a concentration sufficient to block totally adenosine A_1 contractions) concentrations of CPA and NECA up to 10 μM again did not relax the tissues however higher concentrations did give small relaxations of the tissue. Since relaxations were only observed on the muscularis mucosae in the presence of DPCPX and only at concentrations of agonists higher than those used to construct contractile concentration–response curves, it would not be expected to complicate studies with any of the agonists tested in the absence or presence of antagonist. Similarly, CPA did not contract the longitudinal muscle nor the intact ileum, suggesting that the agonist potency order on these tissues is not complicated by an opposing contraction.

In conclusion, we have clearly shown that adenosine agonists relax the intact rat ileum and ileal longitudinal muscle and contract the ileal muscularis mucosae via adenosine A_1 receptors. Adenosine agonists consistently relax the longitudinal muscle throughout the gut and it appears that the longitudinal muscle dominates the response of the intact tissue. However there is a change in adenosine receptor type mediating the relaxant response from A_1/A_{2B} in the duodenum (Nicholls et al., 1996), A_1 in the ileum to A_2 in the colon (Bailey and Hourani, 1992). In the rat muscularis mucosae adenosine agonists contract the tissue and again the adenosine receptor type mediating this response changes along the gut from A_{2B} in the duodenum (Nicholls et al., 1996) to more conventionally A_1 in the ileum and colon (Bailey et al., 1992).

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