Adenosine and adenosine analogues stimulate adenosine cyclic 3', 5'-monophosphate-dependent chloride secretion in the mammalian ileum.

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Research Article

Adenosine receptors that modulate adenylate cyclase activity have been identified recently in a number of tissues. The purpose of these investigations was to determine the effect of adenosine on ion transport in rabbit ileum in vitro. Adenosine and some of its analogues were found to increase the short circuit current (Isc) and the order of potency was N-ethylcarboxamide-adenosine greater than or equal to 2-chloroadenosine greater than phenylisopropyladenosine greater than adenosine. Purine-intact adenosine analogues had no effect on Isc. The effect of adenosine on Isc was enhanced by deoxycoformycin, an adenosine deaminase inhibitor, and by dipyridamole, an adenosine uptake inhibitor. The increase in Isc induced by 2-chloroadenosine was partially reversed in a dose-dependent manner by 8-phenyltheophylline but not by theophylline or isobutylmethylxanthine. 2-Chloroadenosine increased cyclic AMP content, and stimulated net Cl secretion; these effects were partially blocked by 8-phenyltheophylline. These results suggest that there is an adenosine receptor on rabbit ileal mucosal cells that stimulates adenylate cyclase, which results in secondary active Cl secretion.

Adenosine and Adenosine Analogues Stimulate Adenosine Cyclic 3′,5′-Monophosphate-dependent Chloride Secretion in the Mammalian Ileum

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Abstract. Adenosine receptors that modulate adenylate cyclase activity have been identified recently in a number of tissues. The purpose of these investigations was to determine the effect of adenosine on ion transport in rabbit ileum in vitro. Adenosine and some of its analogues were found to increase the short circuit current (Isc) and the order of potency was *N*-ethylcarboxamide-adenosine ≥ 2-chloroadenosine > phenylisopropyladenosine > adenosine. Purine-intact adenosine analogues had no effect on Isc. The effect of adenosine on Isc was enhanced by deoxycoformycin, an adenosine deaminase inhibitor, and by dipyridamole, an adenosine uptake inhibitor. The increase in Isc induced by 2-chloroadenosine was partially reversed in a dose-dependent manner by 8-phenyltheophylline but not by theophylline or isobutylmethylxanthine. 2-Chloroadenosine increased cyclic AMP content, and stimulated net Cl secretion; these effects were partially blocked by 8-phenyltheophylline. These results suggest that there is an adenosine receptor on rabbit ileal mucosal cells that stimulates adenylate cyclase, which results in secondary active Cl secretion.

Introduction

Adenosine, which is produced by all cells, has long been recognized to have numerous metabolic effects in a variety of tissues, including coronary vasodilatation, stimulation of steroidogenesis, stimulation of platelet aggregation, and inhibition of lipolysis (1–7). These and other effects of adenosine are antagonized by methylxanthines and potentiated by adenosine uptake inhibitors, suggesting an external receptor (8–10). Additionally, adenosine analogues that are taken up poorly by cells can have potent metabolic effects (11, 12), further suggesting an external receptor.

Present evidence suggests that there are two adenosine receptors that modulate adenylate cyclase activity (Fig. 1) (8–10). One has a high affinity for adenosine, inhibits adenylate cyclase activity, and is termed the A_1 receptor by Van Calker et al. (13) and the R_i receptor by Londos et al. (8). The other class of receptors has a lower affinity for adenosine, stimulates adenylate cyclase, and has been termed the A_2 or R_a[1] receptor. A lower affinity internal "P site," which inhibits the catalytic unit of adenylate cyclase, has also been postulated (14).

Recently, Forrest and collaborators (15–17) demonstrated that methylxanthine-sensitive adenosine receptors mediate chloride secretion and cyclic AMP accumulation in the elasmobranch rectal gland. Spinowitz and Zandunaisky (18) previously found that chloride transport in the amphibian cornea is increased by adenosine. Because secondary active chloride transport is common to several epithelia, including amphibian cornea, elasmobranch rectal gland, and mammalian intestine (19), we studied the effects of adenosine on ion transport in the rabbit ileum. Our studies provide the first evidence for adenosine-mediated cyclic AMP-dependent chloride transport in a mammalian epithelium.

Methods

Nonfasting male New Zealand white rabbits weighing 2–3 kg were killed with an intravenous air bolus and the terminal ileum was

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1. *Abbreviations used in this paper:* Ado, adenosine; 2-Cl-Ado, 2-chloroadenosine; GMP, guanosine monophosphate; IBMX, isobutylmethylxanthine; Isc, short circuit current; NECA, *N*-ethylcarboxamideadenosine; PIA, phenylisopropyladenosine; 8-PT, 8-phenyltheophylline; R_a and R_i, adenosine receptors that stimulate and inhibit adenylate cyclase, respectively.

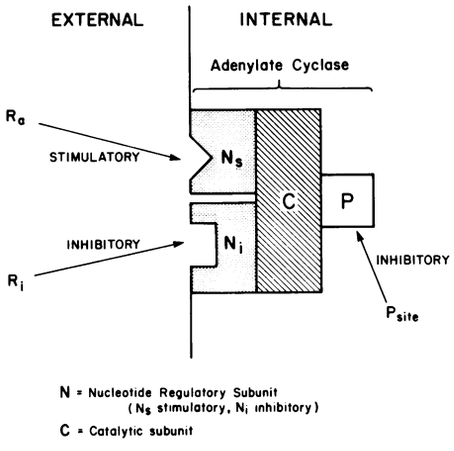

Figure 1. Proposed schema for adenosine receptors.

removed and placed in Ringer's solution at room temperature and gassed with 95% O_2/5% CO_2. The ileum was placed over a pipette and the serosal and muscular layers were removed by making an incision into these layers, then stripping them off with tweezers. The mucosa was then opened, cut into ~3-cm lengths and mounted in Lucite chambers with a surface area of 1.13 cm^2 and attached to mucosal and serosal reservoirs containing identical volumes of Ringer's solution (millimolar): Na, 140; K, 5.2; Ca, 1.2; Mg, 1.2; Cl, 119.8; HCO_3, 25; HPO_4, 2.4; H_2PO_4, 0.4; pH 7.4, when gassed with 95% O_2 and 5% CO_2 at 37°C. Glucose (10 mM) was present in the serosal bathing solution and 10 mM mannitol was present in the mucosal solution.

The spontaneous potential difference across the mucosal was measured by calomel half cells in 3 M KCl, monitored by a potentiometer, and nullified (short circuited) by an automatic voltage clamp throughout the experiment. The current required to nullify the spontaneous potential difference across the mucosa is referred to as the short circuit current (Isc). Tissue conductance was determined every 3 min by clamping to −3 mV for 1 s, measuring the current necessary for this clamp, and calculating conductance according to Ohm's law.

Unidirectional Na and Cl fluxes were determined by using ^{22}Na and ^{36}Cl oppositely directed on adjacent tissue pairs. Tissue pairs were discarded if conductance differed by more than 25%. Isotope was added at least 30 min before beginning flux studies.

Cyclic AMP and cyclic guanosine monophosphate (GMP) assays. A short segment of ileum was rinsed, drawn over a pipette, and the serosal and muscle layers were stripped off as described previously. The remaining mucosal layer was opened lengthwise and pinned down in a dissection dish containing Ringer's solution with 10 mM glucose and gassed with 95% O_2/5% CO_2. Four 6-mm diam pieces were obtained with a skin biopsy punch. Tissues for protein determination, by the method of Lowry et al. (20), were dissolved in 0.1 N NaOH. Reactions were started by transferring a piece of tissue to an assay tube containing 0.5 ml Ringer's solution with 10 mM glucose, gassed with 95% O_2/5% CO_2, stoppered, and incubated in a shaking water bath at 37°C. Reactions were stopped by adding 0.5 ml cold 12% trichloroacetic acid and placing the tubes in an ice bath.

Recoveries of cyclic AMP and cyclic GMP were determined by addition of 0.1 pmol (4,000 cpm) ^3H-cyclic AMP or 0.2 pmol (4,000 cpm) ^3H-cyclic GMP to each tube. Tissues were homogenized with a Polytron (Beckman Instruments Inc., Fullerton, CA), centrifuged at 2,500 *g* for 15 min at 4°C, and the supernatant was saved. 2.1 g of tri-*n*-octylamine was brought up to 10 ml with freon 11 and 1 ml was added to each tube of supernatant, vortexed for 30 s, and centrifuged at 1,000 *g* for 5 min at 4°C. Duplicate 90-μl samples from the aqueous (top) layer were added to 10 μl of 0.5 M sodium acetate buffer. Cyclic AMP and cyclic GMP were determined by radioimmunoassay (21) with kits provided by New England Nuclear (Boston, MA). Samples for cyclic GMP were acetylated by adding 5 μl of acetylating reagent (prepared by mixing 1 vol of acetic anhydride with 2 vol of triethylamine immediately before use) and vortexing immediately (22). Heat-stabile enterotoxin purified from *Escherichia coli* strain 431 was kindly provided by Donald C. Robertson, Department of Microbiology, University of Kansas.

Results

The effect of adenosine and adenosine analogues on Isc is illustrated in Fig. 2. Phenylisopropyladenosine (PIA) had only a small effect and adenosine (Ado) increased the Isc only at one mM. 2-Chloroadenosine (2-Cl-Ado) and *N*-ethylcarboxamideadenosine (NECA) caused the greatest increase in Isc and NECA was potent in the micromolar range. Two adenosine analogues, 2'-deoxyadenosine and 9-B-D-arabinofuranosyladenine, had no effect on the Isc (data not shown).

The relative impotency of adenosine seen in Fig. 2 might be due to either its catabolism by adenosine deaminase or to its uptake by the tissue or both. To test for these possibilities, we determined the effect of the adenosine deaminase inhibitor, deoxycoformycin (23), and the adenosine uptake inhibitor, dipyridamole (24–26), on adenosine-induced increase in the Isc (Fig. 3). Both of these compounds markedly increased the

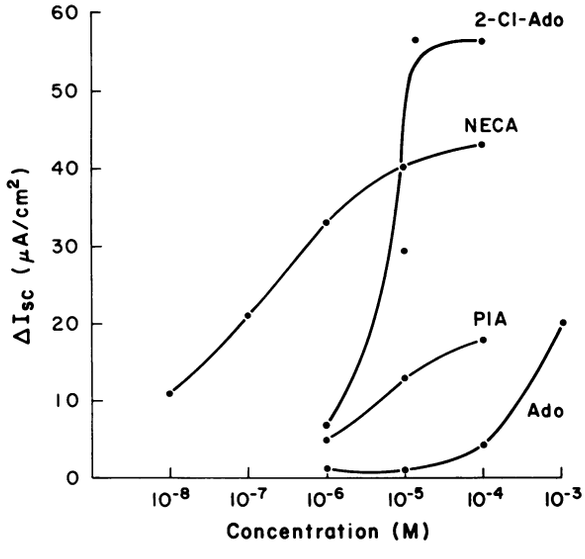

Figure 2. Effect of adenosine and adenosine analogues on the Isc. The peak increase in the Isc in the 15 min after serosal addition of the analogue is shown. Each point is the mean of at least three tissues from different animals.

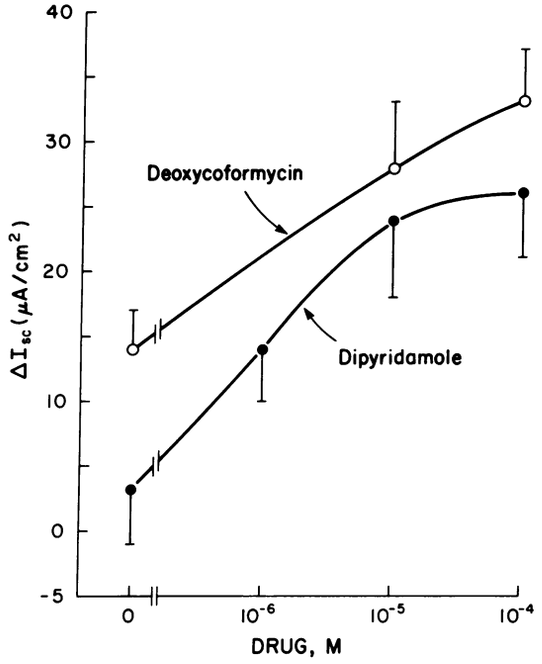

Figure 3. Modulation of the adenosine effect on Isc. Tissues were pretreated for 5–6 min with either deoxycoformycin (○) or dipyrimadole (●) and then 100 μM (●) or 1 mM adenosine (○) added to the serosal solution. The peak increase in Isc in the 15 min after addition of adenosine is shown. Each point is mean±SE of at least five tissues from at least three different animals.

potency of adenosine. The effect of the methylxanthine, 8-phenyltheophylline (8-PT) on 2-Cl-Ado-induced increase in the Isc is illustrated in Fig. 4. 8-PT reversed the effect of 2-Cl-Ado on Isc in a dose-dependent manner.

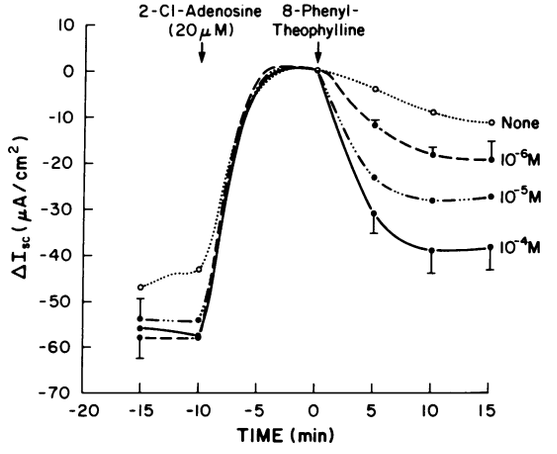

Figure 4. The effect of 8-phenyltheophylline on 2-chloroadenosine-induced increase in Isc. The results shown are the mean±SE (where shown) of at least six tissues from four different animals.

The effect of theophylline and isobutylmethylxanthine (IBMX) on 2-Cl-Ado-induced increase in the Isc is illustrated in Fig. 5. Whereas 8-PT reversed the effect of 2-Cl-Ado on Isc, theophylline and IBMX either had no effect or caused a further increase in Isc.

The effects of 2-Cl-Ado and 8-PT on Na and Cl fluxes are shown in Table I. Compared with control tissues, 2-Cl-Ado stimulated net Cl secretion by increasing serosal-to-mucosal Cl movement. Mucosal-to-serosal Cl movement was decreased to a similar extent but was not significantly different from control tissues. Although mucosal-to-serosal Na movement was decreased, net Na movement was unaffected. Tissue conductance was decreased and the residual flux, J_R, was

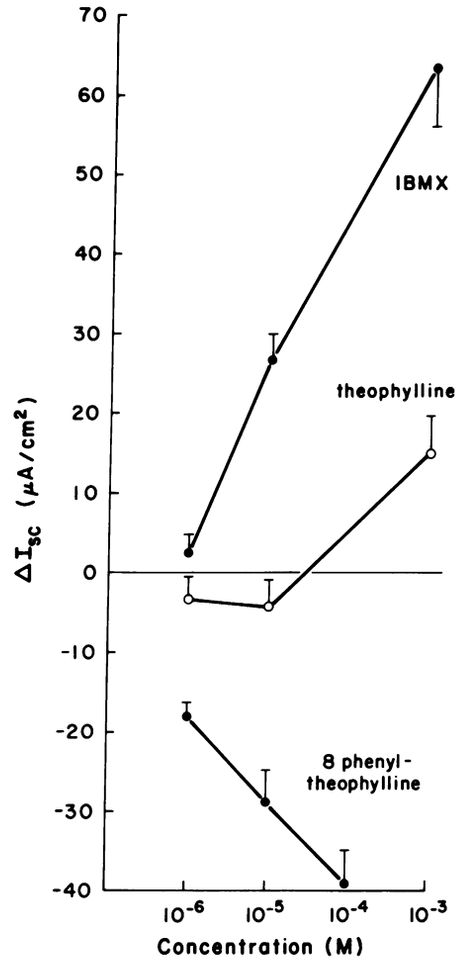

Figure 5. The effect of methylxanthines on 2-chloroadenosine-induced increase in Isc. The zero line is the Isc 10 min after the addition of 20 μM 2-chloroadenosine and the results shown are the peak change in Isc in the 15 min after addition of drug. Each point represents the mean±SE of at least three tissues from at least three different animals.

Table I. Effect of 2-Cl-Ado and 8-PT on Ion Flux

	Na flux			Cl flux					
	MS	SM	NET	MS	SM	NET	Isc	G	J_R
Control									
Period 1	9.9±1.3	10.1±0.8	−0.2±0.7	6.7±0.8	6.6±0.5	0.1±0.8	1.0±0.4	20.5±2.1	1.3±0.6
Period 2	11.1±1.2	11.3±0.9	−0.2±0.7	7.1±0.9	6.8±0.6	0.3±0.9	1.0±0.5	23.0±2.2	1.5±0.8
Δ	1.3±0.5	1.2±0.6	0.1±0.6	0.4±0.7	0.2±0.3	0.2±0.7	0.0±0.2	2.5±0.4	0.2±1.0
P^1	<0.05	NS	NS	NS	NS	NS	NS	<0.001	NS
2-Cl-Ado									
Period 1	9.9±1.3	10.1±1.1	−0.1±0.7	7.7±0.9	6.6±0.5	1.1±0.7	0.7±0.4	20.0±2.1	1.9±0.6
Period 2	8.2±1.2	9.6±1.2	−1.4±0.6	6.4±1.1	8.1±0.7	−1.8±0.8	2.1±0.4	17.9±2.3	1.8±0.7
Δ	−1.7±0.6	−0.4±0.7	−1.3±0.8	−1.3±0.5	1.5±0.4	−2.9±0.6	1.4±0.2	−2.2±0.8	−0.1±0.8
P^1	<0.025	NS	NS	<0.05	<0.005	<0.001	<0.001	<0.05	NS
P^2	<0.005	NS	NS	NS	<0.01	<0.005	<0.001	<0.001	NS
8-PT									
Period 1	10.1±1.3	10.1±0.9	0.1±0.8	7.1±0.9	6.4±0.6	0.7±0.8	1.0±0.4	20.2±2.0	1.6±0.8
Period 2	11.2±1.3	11.0±1.1	0.2±1.1	6.8±1.0	6.5±0.6	0.3±0.8	1.3±0.4	22.6±2.4	1.4±1.1
Δ	1.1±0.7	1.0±0.6	0.1±0.7	−0.3±0.4	0.1±0.5	−0.4±0.7	0.3±0.2	2.3±0.7	−0.2±1.0
P^1	NS	NS	NS	NS	NS	NS	NS	<0.01	NS
P^2	NS	NS	NS	NS	NS	NS	NS	NS	NS
8-PT + 2-Cl-Ado									
Period 1	10.2±1.2	9.7±1.0	0.5±0.4	8.4±1.0	6.9±0.6	1.5±0.8	0.8±0.4	20.9±2.0	1.7±0.6
Period 2	9.7±1.1	10.3±1.1	−0.6±0.6	6.9±0.9	6.9±0.7	−0.0±1.1	1.8±0.5	20.2±2.2	2.4±0.7
Δ	−0.5±0.6	0.6±0.9	−1.2±0.8	−1.5±0.6	0.0±0.5	−1.5±0.8	1.0±0.2	−0.8±0.9	0.7±0.8
P^1	NS	NS	NS	<0.05	NS	NS	<0.005	NS	NS
P^2	<0.05	NS	NS	NS	NS	NS	<0.005	<0.005	NS

Na and Cl flux, Isc, and the residual flux (J_R), are expressed as $\mu eq/cm^2 \cdot h$. Conductance (G) is expressed as mS/cm^2. After an initial 15-min flux period in all four groups, dimethyl sulfoxide (DMSO) (27 μl) was added to the control pair and the 2-Cl-Ado pair, and 100 μM 8-PT (final concentration) in 27 μl DMSO was added to the 8-PT pair and the 8-PT plus 2-Cl-Ado pair. 5 min later 20 μM 2-Cl-Ado was added to the 2-Cl-Ado tissue pair and the 8-PT plus 2-Cl-Ado tissue pair. No additions were made to the other two pairs. 10 min later a second 15-min flux period was determined. All additions were made to the serosal side. The data shown is the mean±SE of nine tissue pairs. P^1 values shown were obtained with the paired *t* test and represent differences between the two flux periods. P^2 values shown were obtained with the unpaired *t* test and represent differences between the control and experimental pairs. NS indicates a *P*-value of >0.05. MS, mucosal to serosal; SM, serosal to mucosal.

unaffected. 8-PT produced no significant changes in ion flux or electrical parameters. When 8-PT was added to the tissue 5 min before 2-Cl-Ado, the effect of 2-Cl-Ado was blunted, but not completely abolished. In fact, the change in net Cl secretion induced by 2-Cl-Ado (Δ = 2.85±0.056 μeq/cm^2·h) was not significantly different from the change induced by 8-PT followed by 2-Cl-Ado (Δ = 1.49±0.81 μeq/cm^2·h, $P < 0.2$), and a similar lack of effect was seen for Isc.

To determine if 8-PT could significantly inhibit 2-Cl-Ado-induced Cl secretion, we reduced the concentration of 2-Cl-Ado, thus establishing a more favorable antagonist/agonist ratio. As illustrated in Fig. 6, 10 μM 2-Cl-Ado stimulated net Cl secretion (2.0±0.4 μeq/cm^2·h), an effect totally abolished by 100 μM 8-PT. 100 μM 8-PT also significantly inhibited the increase in Isc induced by 10 μM 2-Cl-Ado.

The effects of 2-Cl-Ado on tissue cyclic nucleotide accumulation are shown in Table II. 2-Cl-Ado increased tissue cyclic AMP content with time, compared with control tissues. 8-PT had no effect on cyclic AMP content but significantly inhibited the increase in cyclic AMP induced by 2-Cl-Ado. 2-Cl-Ado, 8-PT, and the combination had no effect on cyclic GMP. For the cyclic GMP experiments, heat-stabile *E. coli* enterotoxin served as a positive control.

Table II. Effect of 2-Cl-Ado and 8-PT on Cyclic Nucleotide Levels

	Time (min)		
	0	10	20
A Control	12.6±1.4 (12)	9.8±1.2 (15)	9.4±0.8 (9)
2-Cl-Ado	11.0±1.2 (15)	16.8±2.1 (15)	20.0±2.4 (9)
P	NS	<0.01	<0.001
B Control	—	12.2±1.3 (5)	—
8-PT	—	10.7±1.7 (5)	—
2-Cl-Ado	—	17.4±1.2 (5)	—
8-PT + 2-Cl-Ado	—	13.6±0.6 (4)	$P < 0.05$
C Control	0.40±0.07	0.29±0.06	0.32±0.05
2-Cl-Ado	0.48±0.01	0.40±0.10	0.43±0.06
8-PT	0.44±0.08	0.34±0.06	0.30±0.02
E. coli	0.61±0.16	2.00±0.24	1.80±0.09

Cyclic nucleotide content is expressed as picomoles per milligram protein. The results shown are the mean±SE of the number of tissues shown in parentheses, except in *C*, where four tissues were in each group (except the 20-min control, n = 3). In *A* and *B*, cyclic AMP is shown and in *C*, cyclic GMP. The concentrations used were: 2-Cl-Ado, 20 μM; 8-PT, 100 μM; heat-stabile *E. coli* enterotoxin; 0.2 μg/ml. 0.5 μl dimethyl sulfoxide was added to all tissues. In *A*, *P* is the difference between control and 2-Cl-Ado tissues. In *B*, *P* is the difference between 8-PT and 8-PT plus 2-Cl-Ado tissues. *P* values were obtained with the unpaired *t* test.

Discussion

Considerable evidence has been generated in the past few years suggesting a regulatory role for adenosine in cell-mediated processes, similar to neurohumoral agents (8–10, 14). There is no evidence, however, that adenosine is either a neurotransmitter or a humoral agent. Adenosine appears to be more like the prostaglandins, which are released from the cell membrane to work on the same and surrounding cells (paracrine effect). Adenosine differs from the prostaglandins in that it is derived from cytosolic precursors rather than the cell membrane. Two external receptors for adenosine have been identified, one that stimulates (R_a) and one that inhibits (R_i) (8–10, 13, 18) adenylate cyclase. The order of potency of adenosine analogues on the R_a receptor is NECA > Ado > PIA and on the R_i receptor is PIA > Ado > NECA (8).

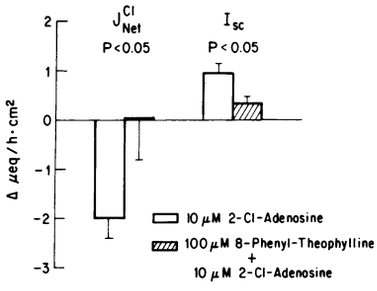

Figure 6. The effect of 2-chloroadenosine and 8-phenyltheophylline on Na and Cl flux and Isc. After an initial minute flux period, 27 μl of dimethyl sulfoxide was added to the 2-Cl-Ado tissue pair and 100 μM 8-PT in 27 μl dimethyl sulfoxide was added to the 8-PT plus 2-Cl-Ado tissue pair. 5 min later 10 μM 2-Cl-Ado was added to both tissue pairs. 10 min later a second 15-min flux period was obtained. The results shown represent the difference between the two flux periods and were obtained from eight tissue pairs. The open bar represents tissues exposed to 10 μM 2-chloroadenosine and the hatched bar tissues exposed to 100 μM 8-phenyltheophylline, then 10 μM 2-chloroadenosine. The *P*-values shown were obtained with the unpaired *t* test.

The order of potency we found (in increasing the Isc) was NECA > PIA > Ado, suggestive of an R_a type receptor. If rabbit ileum is rich in the enzyme that catabolizes adenosine, adenosine deaminase, then the relative impotency of adenosine on the Isc may be due to rapid breakdown. The effect of adenosine on the Isc was enhanced by deoxycoformycin, an adenosine deaminase inhibitor, consistent with this hypothesis. Dipyridamole, an adenosine uptake inhibitor (24–26) also increased the effect of adenosine, suggesting that rapid uptake of adenosine, was also contributing to its relative impotency. This latter observation must be interpreted with caution, however, since dipyridamole can also inhibit phosphodiesterase activity (27). Neither deoxycoformycin nor dipyradimole added alone to the serosal bathing solution, had an effect on the Isc (data not shown). 2-Cl-Ado, an analogue poorly taken up by cells and resistent to adenosine deaminase (28) had a potent effect on the Isc (Fig. 2), further suggesting that the relative impotency of adenosine is due to uptake by the tissue.

One criterion for external adenosine receptors is that they

are inhibited by methylxanthines (the P site is not) (10). 8-PT reversed the effect of 2-Cl-Ado on Isc (Fig. 4), blocked the effect of 2-Cl-Ado on net Cl secretion (Fig. 6) and inhibited the increase in cyclic AMP induced by 2-Cl-Ado (Table II). Theophylline had no effect at lower concentrations and augmented the effect of 2-Cl-Ado at 1 mM (Fig. 5). IBMX augmented the effect of 2-Cl-Ado on Isc at all concentrations.

The effect of a methylxanthine on adenosine-induced increase in Isc will depend on the ratio of its K_i for the adenosine receptor to its K_i for phosphodiesterase. A methylxanthine that is a poor receptor antagonist but potent phosphodiesterase inhibitor would be expected to augment the adenosine-induced increase in Isc. Smellie et al. (29) have characterized the ability of various methylxanthines to inhibit adenosine-induced accumulation of cyclic AMP and to inhibit phosphodiesterase activity in guinea pig brain slices. They found that 8-PT was 10 times more potent than IBMX or theophylline at inhibiting adenosine but that 8-PT was a poor phosphodiesterase inhibitor. IBMX and theophylline were equally potent at inhibiting adenosine, but IBMX was much more potent than theophylline at inhibiting phosphodiesterase. These observations by Smellie et al. (29) are entirely consistent with the effects of these three methylxanthines on adenosine-induced increase in Isc observed in our study (Fig. 5). It should also be noted that 8-PT had no effect on tissue cyclic AMP content at the highest concentration used in our experiments (Table II).

2-Cl-Ado increased tissue cyclic AMP content (Table II) and, as would be expected of an agent that increases cyclic AMP content (30), stimulated net Cl secretion (Table I). The change in net Cl movement minus the change in net Na movement (1.6 $\mu eq/cm^2 \cdot h$) is equal to the change in the Isc (1.4 $\mu eq/cm^2 \cdot h$), thus, adenosine-induced increase in Isc in this tissue reflects Cl secretion.

Adenosine has not been previously found to have significant effects on Isc or potential difference in the intestine (30–32). This may have been due to failure to prevent adenosine catabolism by adenosine deaminase since this enzyme may be present in high activity in the intestine (33). ATP has been found to alter ion transport in guinea pig, rat, bullfrog, and rabbit small intestine (30–32, 34) and in dispersed enterocytes from chicken small intestine (35). ATP increased cyclic AMP levels in dispersed enterocytes from guinea pig small intestine 20-fold, whereas adenosine in similar concentrations had no effect (32).

These observations that ATP has similar effects on ion transport as adenosine and increases cyclic AMP content raises the possibility that ATP interacts with the adenosine receptor, either directly or after its catabolism to adenosine. It seems unlikely that the effect of ATP is due to its catabolism to adenosine, as one would expect equimolar concentrations of adenosine to have at least a similar effect as ATP and this has not been observed. Clarification of whether two receptors, one for adenosine and one for ATP, occur on the same cell, will require further study.

In summary, we have demonstrated that adenosine and some of its analogues increase cyclic AMP levels and stimulate Cl secretion in rabbit ileum, and these effects are inhibited by 8-PT. These observations are consistent with the presence of an R_a or A_2 adenosine receptor on mammalian ileal enterocytes that activates adenylate cyclase.

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