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STIMULATION OF ELECTROLYTE SECRETION IN RABBIT COLON BY ADENOSINE

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SUMMARY

1. Serosal addition of adenosine after inhibition of adenosine deaminase with deoxycoformycin increases short-circuit current (I_{sc}) and tissue conductance of isolated epithelia of rabbit descending colon. In the presence of Cl this increase in I_{sc} results from a reversal of electrically neutral Cl absorption to rheogenic Cl secretion. When Cl is absent the stimulating effect of adenosine on I_{sc} is reduced to one-third and appears to be brought about by HCO_3 secretion. Under all conditions active Na transport remains unaltered.

2. Adenosine-induced electrolyte secretion is markedly decreased by serosal addition of furosemide and depends on the presence of Na on the serosal side of the tissue. The stoichiometry of the interaction of Na and Cl with the basolateral Cl entry mechanism appears to be 1:1. Under Na-free conditions adenosine elicits a current transient which is carried by Cl ions and which is not inhibited by furosemide. Hence this current transient seems to be brought about by rheogenic apical Cl efflux. All these findings suggest that the conductive step in transepithelial Cl secretion resides in the apical membrane. Hyperpolarization of the Na-transporting cells by luminal addition of amiloride does not enhance electrolyte secretion.

3. The site of action of adenosine is the extracellular surface of the basolateral membrane, because (a) luminal addition of adenosine is ineffective, (b) nitrobenzylmercaptopurineriboside, a blocker of cellular nucleoside uptake, augments the effect of serosal adenosine, and (c) the intracellular metabolities of adenosine do not mediate the effect. From the rank-order of potency of adenosine and its analogues 5′-*N*-ethylcarboxamide adenosine and N^6-cyclohexyladenosine it is concluded that the adenosine receptors involved in electrolyte secretion are of the R_a subtype. Theophylline partially inhibits the secretory effect.

4. The intracellular mediator of adenosine appears to be cyclic AMP and/or cyclic GMP, since the tissue levels of both compounds are rapidly elevated after addition of adenosine and both cyclic AMP and cyclic 8-bromo-GMP are able to mimic the adenosine action.

INTRODUCTION

In a recent study (Grasl, Krivanek & Turnheim, 1982) adenosine was shown to increase markedly the ATP content of isolated epithelia of rabbit descending colon,

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while the maximum transport rate of the Na pump was not altered. It was therefore concluded that under aerobic conditions epithelial ATP synthesis is not rate-limiting for Na transport. But although adenosine did not affect unidirectional or net transepithelial Na fluxes, it significantly increased transepithelial short-circuit current, I_{sc}. Hence in the presence of adenosine I_{sc} cannot be solely accounted for by active Na transport, as is the case under normal drug-free conditions (Frizzell, Koch & Schultz, 1976); rather, adenosine appears to induce rheogenic (electrogenic) transport of others ion(s).

The present paper is an attempt to elucidate this adenosine effect in rabbit descending colon. Evidence is presented that adenosine and related compounds induce furosemide-inhibitable rheogenic Cl secretion via extracellular receptors in the basolateral cell membrane. In addition, adenosine appears to elicit rheogenic HCO_3 secretion. Both cyclic AMP and cyclic GMP are candidates for the intracellular mediator of the adenosine effect.

METHODS

Segments of descending colon were obtained from white rabbits (2–3 kg) which had been killed with pentobarbitone. The outer muscle layers were stripped off by blunt dissection and the resulting 'partial mucosal strip' preparations mounted vertically in Ussing-type chambers for recording of short-circuit current (I_{sc}), open-circuit transepithelial electrical potential difference (ψ^{ms}) and epithelial conductance ($G_t = I_{sc}/\psi^{ms}$) as described previously (Frizzell *et al.* 1976). Current sufficient to clamp ψ^{ms} at ± 10 mV was used to calculate G_t when I_{sc} was low.

Standard electrolyte solution (composition in mM: 140 Na, 124 Cl, 21 HCO_3, 5·4 K, 2·4 HPO_4, 0·6 H_2PO_4, 1·2 Mg, 1·2 Ca) was used as the bathing medium unless stated otherwise. Na-free solutions were prepared by isomolar replacement of Na with choline; Cl-free solutions contained either sulphate, methylsulphate, isethionate or benzene sulphonate instead of Cl. These solutions were gassed with a mixture of 95% O_2 and 5% CO_2, resulting in a pH of 7·4. HCO_3-free solutions were buffered with Hepes–Tris (pH 7·4) and gassed with pure O_2. All electrolyte solutions contained additionally 10 mM-D-glucose. The experiments were performed at 37 °C.

Unidirectional fluxes of Na and Cl in either direction across the tissue under short-circuit conditions were determined by adding the approximate radionuclide to the solution on one side of the epithelium and measuring the steady-state rate of appearance of radioactivity on the other.

For the determination of tissue cyclic nucleotide levels epithelia were stripped from the underlying muscle layers with a glass slide and incubated at 37 °C in reaction flasks containing 50 ml electrolyte solution stirred by a gas stream of 95% O_2 and 5% CO_2. Incubation was terminated by submerging the tissues in liquid N_2 after brief blotting with filter paper. The frozen epithelia were pulverized with a percussion-ball homogenizer (Braun, Melsungen, F.R.G.) and thawed in 5% trichloroacetic acid. After centrifugation (2000 *g*, 20 min) in the cold 0·1 volume of 0·1 M-HCl was added to the supernatants which were then extracted five times with three volumes of water-saturated diethyl ether. Aliquots of the aqueous phase were dried at 50 °C under a stream of N_2, and the residues redissolved in the respective assay buffers. Cyclic AMP and cyclic GMP were then determined using commercially available kits based on competitive protein binding (Radiochemical Centre, Amersham, England). Protein was measured by the method of Lowry, Rosebrough, Farr & Randall (1951).

5′-*N*-ethylcarboxamide adenosine was a gift of Byk-Gulden Lomberg Chem. Fabr., Konstanz, F.R.G.; N^6-cyclohexyladenosine was obtained from Boehringer Mannheim, F.R.G.; nitrobenzylmercaptopurineriboside was generously supplied by Dr A. R. P. Paterson, University of Alberta, Canada; amiloride was kindly provided by Merck, Sharp & Dohme, Vienna, Austria; and deoxycoformycin by Parke Davis, Ann Arbor, Michigan, U.S.A. All other chemicals and reagents were purchased from local suppliers.

Results are expressed as the mean ± S.E. of the mean. The statistical significance of a difference between means was calculated using the *t* test. Non-linear regressions were calculated by a least-squares curve-fitting procedure.

RESULTS

Effects of adenosine on I_{sc}, G_t and ion fluxes

Typical examples of original tracings of the adenosine effect on I_{sc} across isolated epithelia of rabbit descending colon are illustrated in Fig. 1. Adenosine was added in a final concentration of 0·5 mM either to the luminal or the serosal solution in the absence or presence of 1 μM-deoxycoformycin, a potent inhibitor of adenosine deaminase (for references see Daly, 1982). Clearly, adenosine stimulated I_{sc}, but only

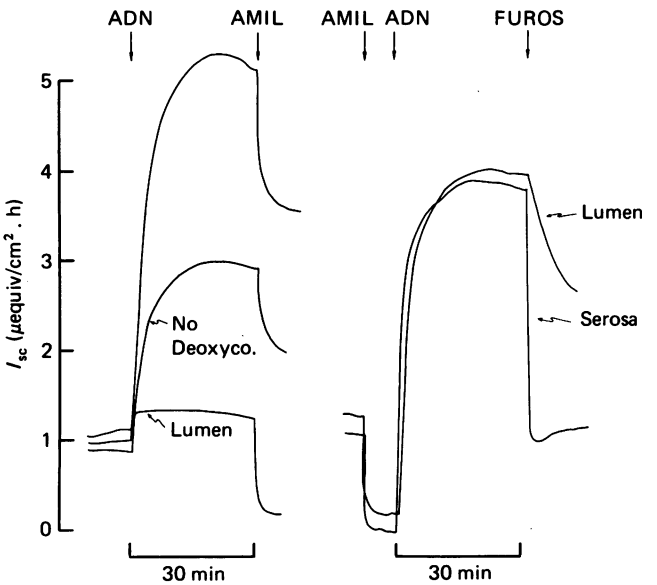

Fig. 1. Effect of 0·5 mM-adenosine on I_{sc}. Adenosine (ADN) was added to the serosal solution except in the experiment marked 'Lumen', in which it was added to the luminal solution. All tissues were pre-treated with 1 μM-deoxycoformycin except the one marked 'No deoxyco.'. Amiloride (AMIL) at 0·1 mM was added to the luminal solution and 1 mM-furosemide (FUROS) to either the luminal or the serosal solution as indicated.

when added to the solution bathing the serosal surface of the tissue. Pre-treatment with 1 μM-deoxycoformycin, which by itself does not alter I_{sc}, potentiated the effect of serosal adenosine. Hence all further experiments involving adenosine were performed in the presence of this adenosine deaminase inhibitor.

The stimulated I_{sc} was only partially inhibitable by luminal addition of 0·1 mM-amiloride. The decrease in I_{sc} induced by amiloride was more or less equal to the I_{sc} before addition of adenosine.

The stimulating effect of adenosine on I_{sc} was also observed after the initial I_{sc} had been reduced to values close to zero by amiloride. Serosal addition of 1 mM-furosemide, a so-called loop diuretic, rapidly inhibited the adenosine-induced current. Furosemide in the luminal solution caused a retarded and less pronounced inhibitory effect (Fig. 1).

In order to elucidate the ionic basis of the increase in I_{sc} caused by adenosine, the unidirectional transepithelial fluxes of labelled Na and Cl were determined before (control) and after serosal addition of adenosine. The results of this study are

compiled in Table 1. Under control conditions there was net absorption of both Na and Cl. Since I_{sc} in rabbit colon can be attributed entirely to net Na transport, net Cl transport must be electrically neutral, i.e. it must be compensated by a 'residual' ion flux of equal magnitude (Frizzell *et al.* 1976). In the presence of adenosine net Cl absorption was reversed to net Cl secretion. This effect was brought about by a

TABLE 1. Effects of serosal addition of 1 mM-adenosine on transepithelial Na and Cl fluxes, short-circuit current (I_{sc}) and conductance (G_t) in rabbit colon

	Control	With adenosine
J^{Na}_{ms} (9)	2·86±0·37	3·40±0·34*
J^{Na}_{sm} (9)	1·31±0·10	1·62±0·07*
J^{Na}_{net} (9)	1·55±0·39	1·77±0·33
J^{Cl}_{ms} (9)	4·63±0·37	4·30±0·31
J^{Cl}_{sm} (9)	3·30±0·14	6·52±0·35*
J^{Cl}_{net} (9)	1·32±0·32	−2·22±0·31*
J^{r}_{net} (9)	1·44±0·36	0·22±0·09*
I_{sc} (18)	1·67±0·33	4·21±0·18*
G_t (18)	5·12±0·26	7·25±0·32*

J^{i}_{ms} and J^{i}_{sm} are the unidirectional fluxes of the designated ions in the direction lumen to serosa and serosa to lumen, respectively. $J^{i}_{net} = J^{i}_{ms} - J^{i}_{sm}$. The residual ion flux (J^{r}_{net}) is calculated from $I_{sc} - J^{Na}_{net} + J^{Cl}_{net}$. All fluxes and I_{sc} are given in units of μequiv/cm².h, G_t in mS/cm². Numbers of experiments are given in parentheses.

* $P < 0·01$ with respect to the control value.

marked increase in the unidirectional flux of Cl in the direction serosa to lumen, while the Cl flux in the reverse direction was not significantly changed. Net Na transport was unaffected. In the presence of adenosine the sum of the net Na and Cl fluxes, 4·0±0·3 μequiv/cm².h, was approximately equal to the simultaneously measured I_{sc} of 4·2±0·2 μequiv/cm².h, indicating that the calculated residual ion flux is close to zero under these conditions (Table 1).

The adenosine-induced net Cl secretion is associated with an increase in electrical conductance of the tissue (Table 1).

Structure–activity and concentration–response relations

The purine nucleotides 5′-AMP and ATP were as effective as adenosine in enhancing I_{sc} when added in equimolar concentrations to the serosal solution (Table 2). In order to prevent degradation of 5′-AMP to adenosine the tissues were pre-treated with 10 μM-α,β-methyleneadenosine-5′-diphosphate, a potent inhibitor of 5′-nucleotidase (Bruns, 1980). The effect of ATP, on the other hand, was examined in the presence of an ATP-regenerating system consisting of 1 mM-phosphoenolpyruvate and 20 μg/ml pyruvate kinase. The increase in I_{sc} caused by ATP was partially inhibited by pre-treatment with 1 u./ml adenosine deaminase (data not shown). Hence, the intrinsic activity of ATP itself may be relatively low; rather, the ATP effect seems to be mediated, at least in part, by local conversion to adenosine.

Inosine and adenine were totally ineffective in causing electrolyte secretion (Table 2). Similarly, pre-treatment with 1 u./ml adenosine deaminase almost completely abolished the adenosine effect on I_{sc} (data not shown).

TABLE 2. Effects of serosal addition of various purine compounds (0·5 mM) on I_{sc}

		ΔI_{sc} (μequiv/cm^2 . h)
Adenosine	(23)	2·49 ± 0·20
5′-AMP*	(5)	2·56 ± 0·70
ATP†	(8)	2·24 ± 0·17
Inosine	(7)	0·04 ± 0·02
Adenine	(6)	−0·08 ± 0·04

Numbers of experiments are given in parentheses.
* After pre-treatment with 10 μM-α,β-methyleneadenosine-5′-diphosphate.
† After pre-treatment with 1 mM-phosphoenolpyruvate and 20 μg/ml pyruvate kinase.

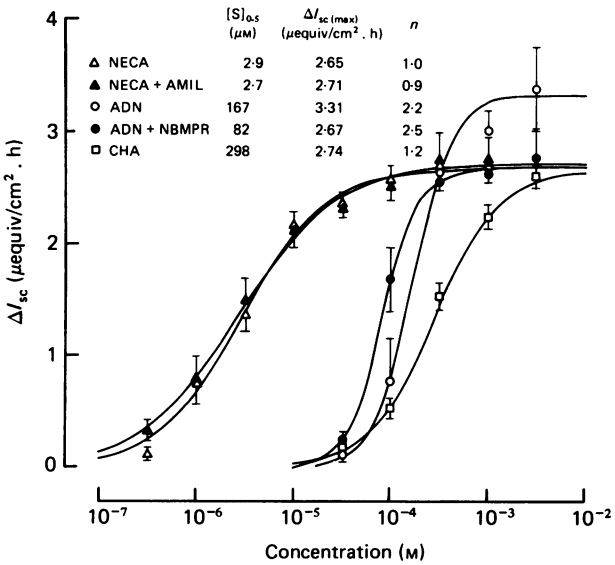

Fig.2. Cumulative concentration–response relations of the stimulating effect of adenosine, NECA or CHA on I_{sc}. Also shown are the effects of adenosine (ADN) in the presence of 10 μM-NBMPR in the serosal solution and those of NECA in the presence of 0·1 mM-amiloride (AMIL) in the luminal solution. ΔI_{sc} is the change in I_{sc} caused by the various agents. The individual curves were obtained by non-linear regression analysis of the experimental data underlying the Hill equation. In this manner $[S]_{0.5}$ (the concentration at which the increase in I_{sc} is half-maximal), $\Delta I_{sc\,(max)}$ (the maximum effect on I_{sc}) and n (the Hill coefficient) were calculated. These parameters are given in the inset. The high Hill coefficients of the adenosine concentration–response curves presumably are a consequence of incomplete inhibition of adenosine deaminase by deoxycoformycin.

Effects comparable to that produced by adenosine were seen using the adenosine-deaminase-insensitive analogues NECA (5′-*N*-ethylcarboxamide adenosine) and CHA (N^6-cyclohexyladenosine) (Fig. 2). The concentration of adenosine needed to produce half-maximal effects was less than that for CHA and two orders of magnitude greater than for NECA. Similar effects with NECA were produced in the presence of amiloride. Maximal effects from all compounds were similar. The presence of NBMPR (nitrobenzylmercaptopurineriboside), an inhibitor of cellular adenosine uptake

(Paterson, 1979), decreased slightly the concentration of adenosine needed to produce half-maximal effects. Possible interpretations of these results are discussed later.

The increments in I_{sc} elicited by the individual concentrations of adenosine and its analogues were accompanied by proportional increases in the electrical conductance of the tissue, G_t. The resulting linear relation between the changes in I_{sc} and G_t is

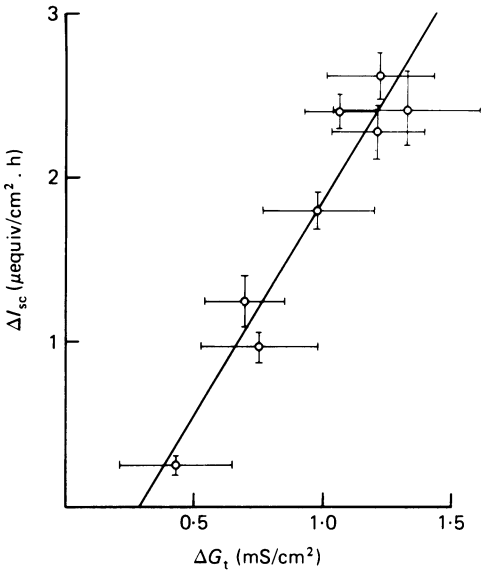

Fig. 3. Relation between the changes in I_{sc} (ΔI_{sc}) elicited by stepwise increases in NECA concentration and the corresponding changes in conductance (G). E, which was calculated from the slope of this relation as 70 mV, represents the over-all electromotive force for electrolyte secretion.

illustrated for the case of NECA in Fig. 3. The linearity of the relation suggests ohmic properties of the conductive pathway induced by NECA. Hence the magnitude of the increase in I_{sc} appears to be primarily determined by the conductance of this route, whereas the equivalent electromotive force which drives the secretory flux remains essentially constant at least over the range of currents observed. The electromotive force for electrolyte secretion, E, was approximately 70 mV, as estimated from the slope of the relation between ΔI_{sc} and ΔG_t.

Inhibition of the adenosine effect by theophylline and furosemide

Theophylline elicited an increase in I_{sc} which was much smaller than that of adenosine (Fig. 4). When adenosine was added after theophylline, I_{sc} was stimulated to a much lesser extent than with adenosine alone. Hence, pre-treatment with 10 mM-theophylline partially antagonized the effect of 1 mM-adenosine.

The stimulatory effect of adenosine on I_{sc} was not only inhibited by theophylline but also by furosemide, as shown in Fig. 1. This inhibitory effect of furosemide was assessed more closely by performing a cumulative concentration–response study with this diuretic. Fig. 5 represents an example of such an experiment. The serosal

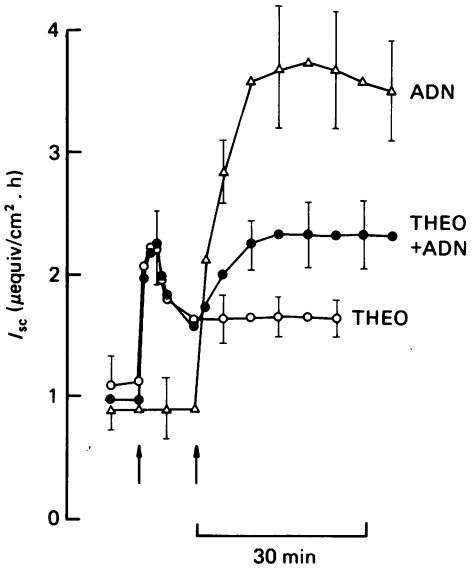

Fig. 4. Inhibition of the stimulating effect of adenosine on I_{sc} by theophylline. In the experiments marked THEO 10 mM-theophylline was added to the serosal solution at the first arrow; in the experiments marked ADN 0·5 mM-adenosine was added to the serosal solution at the second arrow; in the experiments marked THEO + ADN adenosine was added after theophylline.

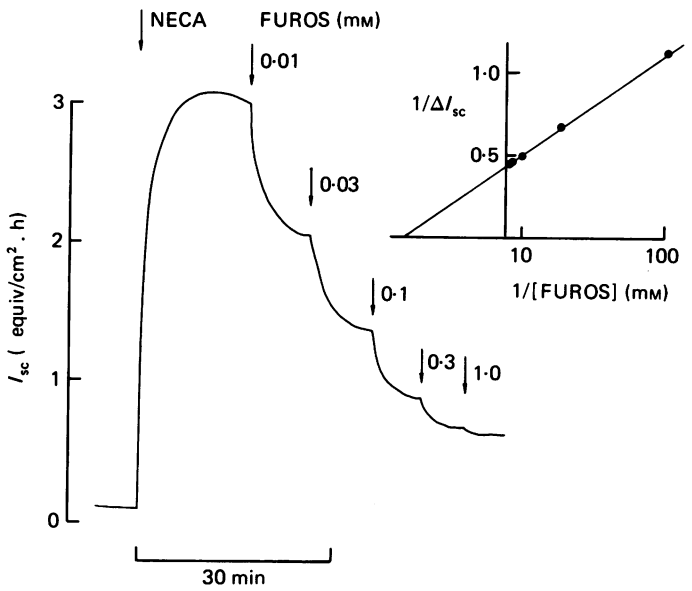

Fig. 5. Furosemide inhibition of 0·5 mM-NECA-induced I_{sc}. Active Na transport was inhibited by pre-treatment with 0·1 mM-amiloride in the luminal solution. The inset gives a double-reciprocal plot of the furosemide (FUROS) effect. ΔI_{sc} denotes the change in I_{sc} induced by the individual furosemide concentrations.

furosemide concentration at which the inhibitory effect was half-maximal averaged 20 ± 4 μM.

Ion-replacement studies

The ionic requirements for the stimulatory effect of adenosine on I_{sc} were tested by using bathing solutions in which Na, Cl or HCO_3 had been replaced by appropriate ions. According to the ion flux measurements given in Table 1 adenosine increases I_{sc} by causing Cl secretion. Hence omission of Cl from the bathing solution should abolish the adenosine effect on I_{sc}. But although adenosine stimulated I_{sc} to a much

TABLE 3. Dependence of the stimulation of I_{sc} (μequiv/cm$_2$.h) by 0·5 mM-adenosine on the ionic composition (in mM) of the bathing solutions

[Na]	[Cl]	[HCO_3]	ΔI_{sc}
140	124	21	2·86 ± 0·17* (15)
140	0	21	0·89 ± 0·26* (18)
140	0	0†	0·07 ± 0·01 (15)
140	124	0†	2·66 ± 0·27* (10)
0	124	21	0·50 ± 0·11* (12)
0	124	0†	0·11 ± 0·02 (5)
0	0	21	0·18 ± 0·04* (6)

ΔI_{sc} is the change in I_{sc} induced by adenosine. The replacement ions for Na, Cl and HCO_3 respectively are given in the Methods section. The tissues were bathed by identical solutions on both sides (symmetric incubation conditions). Numbers of experiments are given in parentheses.

* $P < 0·05$.

† After pre-treatment with 0·1 mM-acetazolamide.

smaller extent in a Cl-free solution, there was a significant Cl-independent increase in I_{sc} of approximately 0·9 μequiv/cm^2.h (Table 3). This phenomenon was not dependent on the type of replacement anion used (isethionate, sulphate, methylsulphate, benzene sulphonate), and hence it appears highly unlikely that these structurally diverse anions are all substrates for the rheogenic secretory process induced by adenosine. However, the stimulatory effect on I_{sc} was totally abolished when both Cl and HCO_3 were absent and endogenous H_2CO_3 production was blocked by 0·1 mM-acetazolamide.

The Cl-independent component of the adenosine effect on I_{sc} seems to be small at high Cl concentrations because (a) in standard electrolyte solution the sum of the net Na and Cl fluxes agreed well with I_{sc} (Table 1), and (b) the adenosine effect on I_{sc} in a solution containing Na and Cl was not significantly altered when HCO_3 was omitted (Table 3). Hence Cl and the Cl-independent current may share at least part of a common saturable pathway.

Replacing Na by choline in a solution containing Cl and HCO_3 resulted in a marked reduction of the adenosine effect on I_{sc} (Table 3). The small Na-independent increase in I_{sc} was decreased to values indistinguishable from zero when both Na and HCO_3 were absent.

The adenosine effects given in Table 3 are the steady-state values. Examples of the time course of the adenosine-induced changes in I_{sc} in the presence of bathing solutions of differing ionic composition are illustrated in Fig. 6. When 0·5 mM-adenosine

was added to the serosal side of epithelia incubated for at least 30 min in a Na-free solution, there was a rapid surge in I_{sc} which reached a maximum 1–2 min after addition of adenosine. Then I_{sc} declined in a quasi-exponential manner with a half-time of approximately 1·4 min to reach a steady state which was dependent on the presence of HCO_3 (Fig. 6*A*). This transient change in I_{sc} after the addition of adenosine was not inhibitable by 1 mM-furosemide. The overshoot in I_{sc} clearly required the presence of Cl: in a Cl-free bathing solution adenosine caused a continuous rise in I_{sc} until the steady state was reached (Fig. 6*B*). It is therefore concluded that the current transient in the absence of Na is carried by Cl ions. Additionally, Fig. 6*B* shows that the Cl-independent effect of adenosine on I_{sc} is small in the absence of Na and partly inhibitable by furosemide.

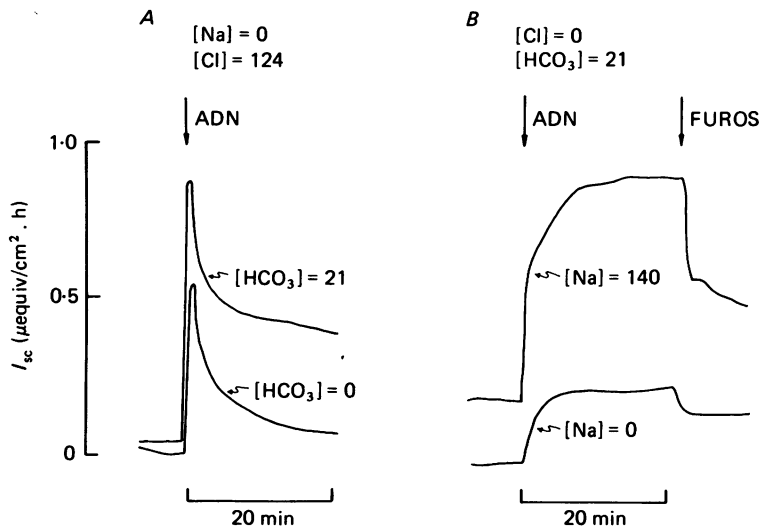

Fig. 6. Time course of the changes in I_{sc} caused by 0·5 mM-adenosine in the presence of different bathing solutions. The ionic compositions (mM) of the bathing solutions used in the individual experiments are given in the Figure. In the experiment with [Na] at 140 mM, active Na transport was inhibited by pre-treatment with 0·1 mM-amiloride. Note that the transient in I_{sc} after addition of adenosine (ADN) was observed only in the presence of Cl. FUROS, furosemide.

The dependence of electrolyte secretion on Cl and Na was further characterized by performing concentration–response studies with both ions. For this purpose 1 mM-adenosine was added to the serosal solution after inhibition of active Na transport by amiloride, and when the adenosine effect on I_{sc} had stabilized either the Na concentration, [Na], or the Cl concentration, [Cl], was decreased or increased in a step-wise manner on both sides of the tissue. The changes in steady-state I_{sc} resulting from this manoeuvre are illustrated in Fig. 7. Again Cl-independent and Na-independent components of the adenosine effect on I_{sc} were observed. As [Na] was enhanced the adenosine effect, ΔI_{sc}, also increased in a manner resembling saturation kinetics. When this relation is analysed according to the Hill equation using non-linear regression analysis, a half-saturation constant for Na of approxi-

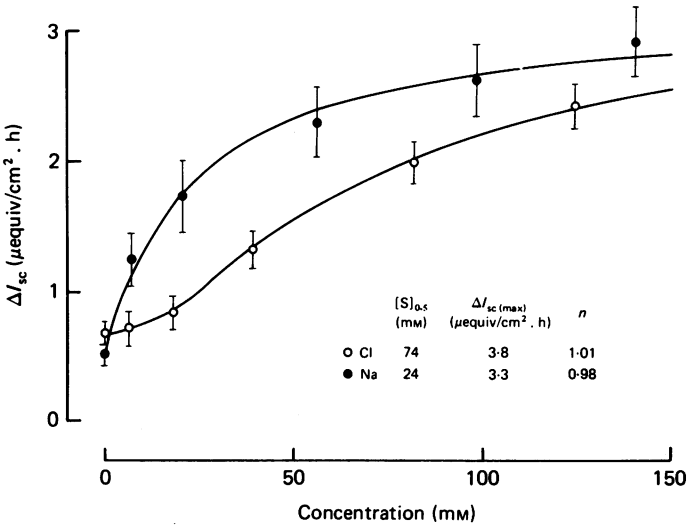

Fig. 7. Dependence of the stimulatory effect of 1 mM-adenosine on I_{sc} on the Cl and Na concentrations in the bathing solution. For the concentration–response study of Cl the Na concentration was fixed at 140 mM; for the concentration–response study of Na the Cl concentration was fixed at 124 mM. The data were fitted by use of the Hill equation; the resulting parameters $[S]_{0.5}$ (the concentration of Cl or Na at which the effect is half-maximal), $\Delta I_{sc(max)}$ (the maximum change in I_{sc}) and n (the Hill coefficient of the interaction of Cl or Na with the transport process) are given in the inset. In the case of Cl only the four highest concentrations were used for this analysis.

TABLE 4. Dependence of the stimulation of I_{sc} by 0·5 mM-adenosine on the presence of Na in the solution bathing the luminal or serosal surface of the tissue (asymmetric incubation conditions)

[Na] (mM)		ΔI_{sc}
Luminal	Serosal	(μequiv/cm². h)
0	140	3·15±0·29 (5)
140	0	2·96±0·56 (4)
140+0·1 mM-amiloride	0	0·98±0·09 (4)

ΔI_{sc} is the change in I_{sc} induced by adenosine. Na-free solutions were prepared by isomolar replacement with choline. [Cl] was 124 mM and $[HCO_3]$ 21 mM on both sides of the tissue. Numbers of experiments are given in parentheses.

mately 24 mM, a maximum transport capacity of the secretory mechanism of 3·3 μequiv/cm². h and a Hill coefficient of 0·98 can be derived.

The relation between ΔI_{sc} and [Cl] departed from simple saturation kinetics at low values of [Cl]. This type of relation is to be expected when a Cl-independent current decreases in a saturating fashion as [Cl] increases. We have therefore fitted the parameters of the Hill equation only to the data points at the four highest Cl concentrations. Under these restrictions a half-saturation constant for Cl of approximately 74 mM, a maximum transport capacity of the secretory mechanism of 3·8 μequiv/cm². h and a Hill coefficient of 1·01 were obtained.

From the evidence presented above it is clear that Na is a necessary cofactor for electrolyte secretion. In order to examine on which side of the epithelium this

interaction occurs, tissues were incubated in Ussing chambers with asymmetric bathing solutions: [Cl] was 124 mM on both sides, but Na was replaced by choline either on the luminal or on the serosal side of the tissue. Luminal omission of Na did not affect electrolyte secretion, but surprisingly stimulation of I_{sc} was also observed when the serosal solution was nominally Na-free (Table 4). This finding may be a result of the unstirred layer: the Na concentration on the extracellular surface of the basolateral cell membrane may exceed that in the serosal bulk solution, since the epithelium actively transports Na from the luminal to the serosal side. Indeed, inhibition of active Na transport by luminal addition of 0·1 mM-amiloride reduced the adenosine effect to approximately 1 μequiv/cm^2.h. From these results it may therefore be concluded that transepithelial Cl secretion is dependent on the presence of Na on the serosal side of the tissue.

TABLE 5. Tissue content of cAMP and cGMP (pmol/mg protein) under control conditions and after 25 min of exposure to stimulators or inhibitors of electrolyte secretion

	cAMP	cGMP
Control	6·1 ± 0·6 (36)	0·4 ± 0·1 (14)
Adenosine (1 mM)	9·1 ± 1·9* (8)	0·7 ± 0·1* (6)
NECA (0·5 mM)	10·1 ± 1·1* (22)	0·8 ± 0·1* (14)
Theophylline (10 mM)	11·3 ± 1·6* (16)	1·9 ± 0·5* (6)
Furosemide (1 mM)	6·2 ± 0·6 (4)	0·5 ± 0·1 (5)
Furosemide + adenosine (both 1 mM)	10·2 ± 2·1* (4)	0·9 ± 0·1* (5)

Numbers of experiments are given in paretheses.
* $P < 0·05$ with respect to the control value.

Adenosine-induced changes in tissue cyclic nucleotide levels

Many intestinal secretory states are associated with elevated epithelial cyclic AMP (cAMP) and cyclic GMP (cGMP) levels (Field, Graf, Laird & Smith, 1978; Field, 1979). We therefore measured both cAMP and cGMP contents of isolated epithelia of rabbit descending colon which had been incubated in flasks containing standard electrolyte solution under control conditions or after exposure for 25 min to agents which stimulate or inhibit electrolyte secretion (Table 5). Adenosine, NECA and theophylline all caused statistically significant increases in cAMP levels. It is noteworthy that theophylline elicited at least as high an increase in cAMP as did adenosine or NECA, although the phosphodiesterase inhibitor caused a much smaller increase in I_{sc} than did adenosine or NECA and markedly inhibited the adenosine response (see Fig. 4). The stimulating effect of NECA on epithelial cAMP content was not dependent on the presence of Na (data not shown).

Both adenosine and NECA also increased tissue cGMP content; however, the highest increase in cGMP was again caused by theophylline. Furosemide inhibited neither the adenosine-induced increase in tissue cAMP nor that in cGMP content.

The onset of the increase in both cyclic nucleotides was equally rapid, steady-state levels being reached by 2 min after addition of NECA; the steady state of electrolyte secretion, however, was only reached after about 15 min (Fig. 8*A*).

The concentration–response relation of the stimulatory effect of NECA on tissue cyclic nucleotide levels is illustrated in Fig. 8*B*. Both cAMP and cGMP exhibited a

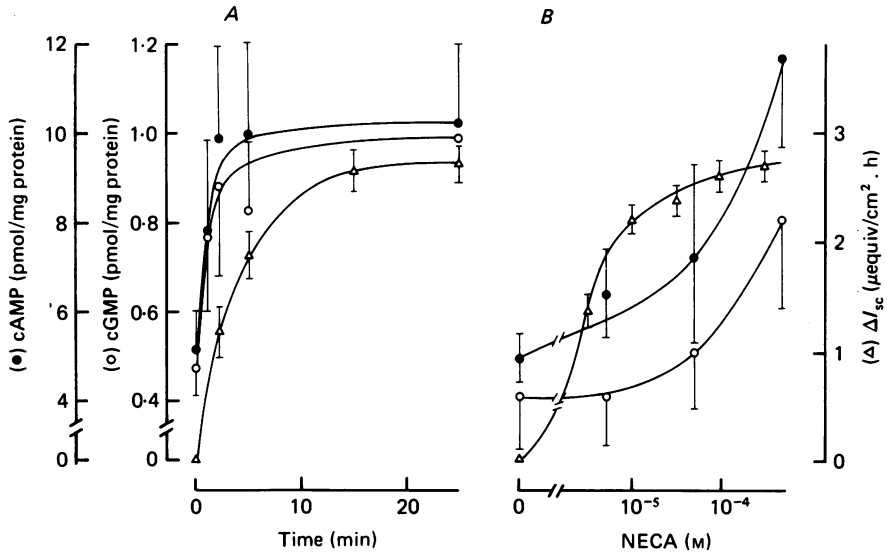

Fig. 8. Time-dependence (*A*) and concentration-dependence (*B*) of the effects of NECA on I_{sc} and the tissue levels of cAMP and cGMP. In *A*, 0·5 mM-NECA was added at time 0. Note that the maximum changes in epithelial cyclic nucleotide levels precede those in ΔI_{sc} and that the concentration-dependence of the changes in cyclic nucleotide levels does not correspond with the concentration–response curve of the increase in ΔI_{sc}.

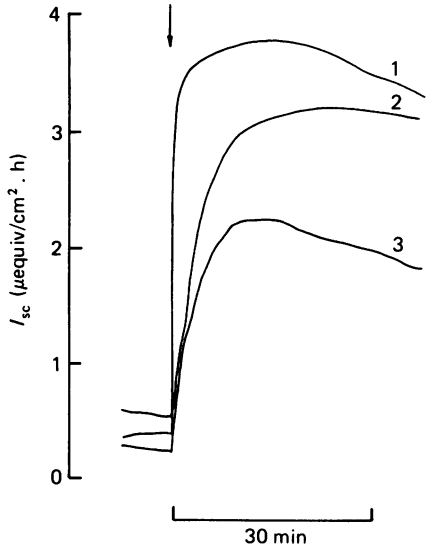

Fig. 9. Effects of (1) 0·5 mM-NECA, (2) 2·3 mM-8-Br-cGMP and (3) 7·5 mM-cAMP (added at the arrow) on I_{sc}. Tissues were pre-treated with 0·1 mM-amiloride to inhibit active Na transport.

similar dependence on the concentration of NECA, the effects at 5×10^{-4} M-NECA seeming to be far from maximal. There was a marked discrepancy between the concentration–response curves of NECA with respect to the stimulatory effect on I_{sc} and the effect on tissue cyclic nucleotide levels, the NECA effect on electrolyte secretion saturating at lower concentrations than that on the tissue content of cyclic nucleotides.

8-Bromo-cGMP (8-Br-cGMP), a lipophilic and presumably more readily penetrating cGMP analogue, was at least as potent a secretagogue as cAMP (Fig. 9). The effect of 8-Br-cGMP was also inhibitable by serosal addition of furosemide.

DISCUSSION

Adenosine increases short-circuit current (I_{sc}) and tissue conductance (G_t) of rabbit descending colon by reversing electrically neutral net Cl absorption to rheogenic net Cl secretion. The effect of adenosine resembles that of many other intestinal secretagogues such as gastrointestinal hormones, cholera toxin and *Escherichia coli* enterotoxins, prostaglandins, theophylline, exogenous cAMP, bile salts (see Field, 1974, 1979) and possibly certain laxatives (Gaginella & Bass, 1978) that stimulate fluid and electrolyte secretion in mammalian small and large intestine. Stimulation of rheogenic Cl transport by adenosine does not seem to be confined to colonic epithelium: recently Spinowitz & Zadunaisky (1979) reported that adenosine stimulates I_{sc} in frog cornea and that this current is due to increased net Cl transport.

Whereas in frog cornea adenosine had no effect on I_{sc} under Cl-free conditions (Spinowitz & Zadunaisky, 1979), it elicits a Cl-independent increase of I_{sc} in rabbit colon. The stimulation of I_{sc} under Cl-free conditions seems to result from rheogenic HCO_3 secretion, since this effect was totally abolished when both Cl and HCO_3 were omitted from the bathing solutions and carbonic anhydrase was inhibited by acetazolamine. Rheogenic HCO_3 secretion induced by agents known to elevate cell cAMP levels has been demonstrated in mammalian gall bladder (Stewart, Goetz & Heintze, 1982), mammalian and amphibian duodenum (Flemström & Garner, 1982), and amphibian urinary bladder (Ehrenspeck, 1982). In rabbit ileum the I_{sc} response to theophylline or cAMP is reduced by only 75% when Cl is replaced by SO_4, but when both HCO_3 and Cl are replaced the stimulating effect on I_{sc} is totally abolished (Field, Plotkin & Silen, 1968). This type of ionic dependence of the secretagogue effect is identical to the one observed in the present investigation. A Cl-independent component of the I_{sc} increase induced by prostaglandin E_1 in canine tracheal epithelium is also apparent from the results of Frizzell, Welsh & Smith (1981), the magnitude of this effect being almost 30% of the response observed in the presence of Cl.

Site of action of adenosine

Cellular uptake of adenosine does not precede the onset of electrolyte secretion, rather the site of action is clearly the extracellular surface of the basolateral cell membrane. The evidence for this conclusion is: (1) adenosine is effective only when added to the solution bathing the serosal side of the tissue; (2) the adenosine response is augmented by NBMPR, a potent inhibitor of carrier-mediated nucleoside uptake

into cells; (3) NECA, which is more potent on a molar basis than adenosine in eliciting electrolyte secretion, has a much lower affinity for the transport system for cellular nucleoside uptake than adenosine (Turnheim, Plank & Kolassa, 1978); (4) subseqent to cellular uptake adenosine is rapidly metabolized either by deamination or phosphorylation so that it is restricted to the extracellular space (reviewed by Fox & Kelly, 1978). Inosine (the deaminated adenosine metabolite) and adenine are totally ineffective. Phosphorylation to purine nucleotides is also not necessary for the response, since NECA, which is not a substrate for adenosine kinase, is a stronger agonist then adenosine. Hence the fact that intact adenosine rather than its deaminated or phosphorylated metabolites evokes electrolyte secretion additionally substantiates the extracellular location of the adenosine receptors in rabbit colon.

An extracellular site of action has also been established for the many other effects of adenosine in different organs (for reviews see Schwabe, 1981; Daly, 1982). Analogous to the responses mediated by extracellular adenosine receptors in other organs, theophylline inhibits adenosine-induced electrolyte secretion at least partially. From the rank-order of potency of NECA, adenosine and CHA the adenosine receptors involved in colonic electrolyte secretion can be classified as R_a-receptors according to the definition introduced by Londos, Cooper & Wolff (1980).

Cyclic nucleotides as intracellular mediators of the adenosine effect

As are other procedures that result in epithelial Cl secretion (Field, 1974, 1979), adenosine-induced electrolyte secretion in rabbit colon is associated with an increase in tissue cAMP content. Activation of a membrane-bound adenylate cyclase is characteristic for R_a-receptor agonists (Schwabe, 1981; Daly, 1982). However, there is no simple correlation between the increase in cAMP and electrolyte secretion: (1) theophylline caused at least as high an increase in tissue cAMP as adenosine or NECA, but had a much smaller stimulating effect on electrolyte secretion and even partially inhibited the adenosine action; and (2) there was a marked discrepancy between the concentration–response relations of NECA with respect to stimulation of electrolyte secretion and enhancement of tissue cAMP. This apparent lack of parallel changes in electrolyte secretion and cAMP levels suggests that not all of the epithelial cAMP is coupled to transport or, in other words, that there is compartmentalization of epithelial cAMP. Sheerin & Field (1977) have previously reported findings which indicate that there is an epithelial cAMP pool unrelated to electrolyte secretion.

Mediation of rheogenic Cl secretion by elevated intracellular cAMP is consistent with the conventional model for this secretory process (Field, 1979; Frizzell, Field & Schultz, 1979). But according to the present results colonic electrolyte secretion evoked by adenosine, NECA or theophylline is also associated with a significant increase in epithelial cGMP levels. The increase in cGMP after addition of NECA is as rapid as that in cAMP: a plateau of elevated cGMP levels is reached within 2 min, which precedes the maximum I_{sc} response. There is evidence that the cGMP system is predominantly localized in the apical region of intestinal cells (i.e. close to or in the membrane in which conductance changes during electrolyte secretion occur), whereas the cAMP system resides primarily at the opposite pole of the cell (De Jonge, 1976; Walling, Mircheff, Van Os & Wright, 1978). Addition of 8-Br-cGMP is able to mimic the effect of adenosine on electrolyte transport. Hence, cGMP is as plausible a candidate as cAMP for the intracellular mediator of the adenosine effect.

Cell model for adenosine-induced electrolyte secretion

The properties of rheogenic Cl secretion in rabbit colon observed after serosal addition of adenosine may be summarized as follows: Although active transepithelial Na transport is not affected, Cl secretion is dependent on the presence of Na in the serosal solution and inhibitable by serosal addition of furosemide, which blocks NaCl co-transport in many tissues. Na appears to stimulate basolateral Cl entry since the increase in epithelial content of cyclic nucleotides and the apical efflux of Cl were also observed in Na-free bathing solutions. The Hill coefficients of both the Na and Cl dependence of the stimulatory adenosine effect on I_{sc} are indistinguishable from 1, suggesting that one Na ion and one Cl ion interact with the basolateral entry mechanism. Since basolateral Cl entry seems to be electrically neutral, the conductive step in transepithelial rheogenic Cl secretion must reside in the luminal membrane. Consistent with conductive apical Cl exit is the Cl-dependent current transient observed after addition of adenosine to Na-free bathing solution, as discussed below. All these findings are in agreement with the cell model for rheogenic Cl secretion that is applicable to a wide variety of epithelia (Frizzell *et al.* 1979). According to this model the entry of Na and Cl across the basolateral membrane is obligatorily coupled and driven by the chemical potential gradient for Na across this barrier, which is maintained by the basolateral ouabain-inhibitable Na pump. This combination of coupled NaCl entry and active Na extrusion leads to an accumulation of Cl in the cell interior above electrochemical equilibrium. Transepithelial rheogenic Cl secretion results when the Cl conductance of the apical membrane is increased by procedures that elevate intracellular cAMP levels. Hence, the mechanism of apical Cl exit is simply electrodiffusion.

Although the properties of adenosine-induced Cl secretion in rabbit colon are in basic agreement with the conventional model for Cl secretion, a few modifications are suggested. One possible modification concerns the intracellular mediator for electrolyte secretion as discussed above, another the anion-selectivity of the conductive pathway induced by the secretory stimuli in the apical membrane. The results of the ion-replacement studies reported here, and of an earlier investigation in rabbit ileum (Field *et al.* 1968), indicate that not only the apical conductance for Cl but also that for HCO_3 may be increased in response to secretagogues. Since the intracellular HCO_3 activity is at least 2·6 times higher than the predicted equilibrium value (Frizzell *et al.* 1979), any increase in apical HCO_3 conductance will result in HCO_3 secretion. However, rheogenic HCO_3 secretion was apparent only in the absence of Cl, and hence HCO_3 and Cl may compete for exit through the same adenosine-induced conductive pathway in the apical membrane.

The adenosine-induced increase in I_{sc} was linearly related to changes in G_t. Hence E, the over-all electromotive force for electrolyte secretion, appears to be constant, the rate of secretion being determined by the conductance of the secretory pathway. E was approximately 70 mV in rabbit colon, whereas E_{Na}, the electromotive force for active transepithelial Na transport, was determined in this tissue to be approximately 110 mV (Schultz, Frizzell & Nellans, 1977). The fact that E of the secretory pathway is lower than E_{Na} is consistent with the secondary active nature of adenosine-induced electrolyte secretion, although interpretation of the term E is fraught with difficulties. But the observation that E remains constant over a wide

range of conductance changes of the apical membrane raises questions concerning the regulation of the basolateral coupled NaCl influx mechanism. E of the transepithelial secretory pathway is the sum of E^m and E^s, the electromotive forces across the apical and basolateral membranes, respectively. According to the conventional model for Cl secretion E^m is the electrochemical potential gradient for Cl across the apical membrane; hence an increase in apical Cl conductance and Cl efflux is expected to decrease the electrical and chemical potential difference across the apical membrane and consequently E, if basolateral Cl entry is unchanged. The fact that E remains essentially constant despite large variations in apical membrane conductance may therefore be considered to represent suggestive evidence that secretagogues stimulate not only apical Cl efflux but additionally basolateral Cl entry. Although this notion is admittedly speculative and has to be tested directly, it is supported by the finding of Frizzell and co-workers (1981) that intracellular Cl activity in shark rectal gland was not significantly decreased when Cl secretion was induced by cAMP and theophylline.

An implicit although not explicitly stated consequence of the conventional model of Cl secretion is that the Cl permeability for basolateral exit is very low. The current transient evoked by adenosine when the tissue is bathed in Na-free solution (see Fig. 6) is in excellent agreement with this assumption. The transient was clearly brought about by apical Cl exit since (1) it was not observed in a Cl-free solution, and (2) basolateral Cl entry was most probably very low because the bathing solution was Na-free and the transient was not attenuated by furosemide in the serosal solution.

Rabbit descending colon absorbs not only Na but also Cl, as is clear from the flux data given in Table 1. Active transepithelial Cl absorption, which is an electrically neutral process, is not Na-dependent, i.e. Cl absorption in Na-free bathing solution is equal to Cl absorption in the presence of Na (Frizzell *et al.* 1976). The transient in apical Cl exit observed after addition of adenosine to Na-free bathing solution indicates that the intracellular transport pool for Cl secretion is distinct from that for Cl absorption, because in Na-free bathing solution the Cl accumulated in the intracellular space would dissipate via the basolateral Cl transfer mechanism involved in Cl absorption if a common intracellular Cl pool existed.

Let us finally address the question of which cells are responsible for electrolyte secretion in rabbit colon. According to the model described for Cl secretion, manoeuvres that hyperpolarize the apical cell membrane should increase Cl secretion by augmenting the driving force for apical Cl exit. However, the secretory effect of NECA was identical in the absence and presence of amiloride (see Fig. 2), a substance, which is known to increase the electrical potential difference across the apical membrane of Na-transporting cells by blocking electrogenic apical Na entry (Schultz *et al.* 1977). This observation suggests that the Na-transporting cells, which are the superficial cells in rabbit colon, are not involved in electrolyte secretion. Hence electrolyte secretion would have to originate from the crypts, as proposed earlier by Field (1979) who noted that cAMP-stimulated anion secretion is observed in epithelia rich in crypts (such as rabbit colon) but not in those that do not contain crypts (such as rabbit gall bladder or flounder intestine). Welsh & Frizzell (1980) have provided morphological evidence that colonic electrolyte and fluid secretion induced by serosal addition of prostaglandin E_2 arises from the crypts. Restriction of electrolyte

secretion to only a fraction of the epithelial cells is also consistent with the observations of the present study that (1) the intracellular transport pool for Cl secretion is distinct from that for Cl absorption, and (2) there is compartmentalization of epithelial cyclic nucleotides, i.e. only a fraction of the tissue content of cyclic nucleotides appears to be involved in electrolyte secretion.

In conclusion it can be said that colonic electrolyte secretion has to be added to the long list of effects elicited by adenosine (reviewed by Daly, 1982). Hence adenosine may not only be responsible for the intrinsic regulation of intestinal blood flow (Granger, Valleau, Parker & Lane, 1978; Walus, Fondacaro & Jacobson, 1981) but also play a pathophysiological role in certain diarrhoeal states. Indeed, preliminary experiments have shown that adenosine causes electrolyte secretion in isolated human colonic mucosa as it does in rabbit colon (M. Grasl & K. Turnheim, unpublished). Purinergic nerves with ATP or adenosine as neurotransmitters have been reported to be abundant in the intestine (Burnstock, 1979). Therefore it appears worth while to give closer attention to the possibility of clinically relevant colonic secretion of fluid and electrolytes mediated by adenosine or other purine compounds.

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