Indirect Effects of Adenosine Triphosphate on Chloride Secretion in Mammalian Colon

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Summary. The effects of adenosine triphosphate (ATP) on short-circuit current (SCC) in rat colonic epithelium are described. ATP caused a large increase in inward-going current and was considerably more potent in this respect than ADP, AMP or adenosine. The response to ATP was sided, there being only minor effects when the nucleotide was added to the apical side of the tissue. The effects of ATP were not modified by the cyclooxygenase inhibitor, indomethacin, eliminating eicosanoid formation as a mechanism. The effects of ATP were potentiated by theophylline and not blocked by α,β-methylene ATP. The data are consistent with the effect being dependent on the activation of adenylate cyclase, but it has not been possible to classify the receptors into P1 or P2 categories. Using inhibitors of NaCl cotransport (piretanide), carbonic anhydrase (acetazolamide), and chloride channels (diphenylamine-2-carboxylate), it was concluded that the SCC response to ATP was due to chloride secretion with, perhaps, a minor contribution from bicarbonate. Flux measurements with $^{22}$Na and $^{36}$Cl confirmed this view, there being approximate equivalence of chloride secretion with the SCC responses. Additionally, flux measurements revealed an inhibition of electroneutral NaCl absorption in response to ATP. The effects of ATP were antagonized by tetrodotoxin (TTX), greater than 50% inhibition being achieved with 10 nm TTX. This result suggests that ATP does not act directly on receptors in the epithelial cells but rather on neuronal elements in the lamina propria. It will be necessary to re-examine other secretagogues for indirect effects of this kind and to search for the final effector neurotransmitter which evokes secretion.

Key Words  ATP · ADP · AMP · adenosine · tetrodotoxin · chloride secretion · intramural plexus · rat colon epithelium

Introduction

The effects of adenine nucleosides and nucleotides on transepithelial ion transport in the mammalian gut have been reported previously (Kohn, Newey & Smyth, 1970; Grasl & Turnheim, 1984). While our results do not differ in many respects from the earlier reports, we have discovered an important major difference. We have found that tetrodotoxin can virtually abolish the effects of ATP on electrogenic chloride secretion. The data are consistent with the view that the effects of the nucleotide are indirect, via neural elements in the intramural plexus. This view contrasts with a direct action on epithelial cells reported previously. There are a great variety of secretagogues that stimulate chloride secretion in the mammalian gut, and it will be necessary to re-examine many of these to see if they too act indirectly. It is conceivable that many secretagogues act through a final, common, neuroeffector mechanism.

Materials and Methods

All experiments were made with the isolated colonic epithelium of male rats (Sprague Dawley). From each colon two pieces of tissue, about 1 cm long, were taken 4 to 5 cm from the most caudal Peyer's patch. These were opened, washed in Krebs Henseleit (K-H) solution, and pinned to a dissecting tray with the mucosa downwards. The tissues were bathed on each side with 20 ml K-H solution, gassed with 95% O$_2$:5% CO$_2$ and maintained at 37°C. The pH was at 7.4. The tissues were short circuited using a standard, previously described, methodology (Cuthbert & Margolius, 1982).

The K-H solution had the following composition (in mM): NaCl 117, KCl 4.7, CaCl$_2$ 2.5, MgSO$_4$ 1.2, NaHCO$_3$ 24.8, K$_2$HPO$_4$ 1.2, and glucose 11.1. All drugs used were obtained from normal commercial sources, dissolved in distilled water, and added in small volume (maximal 200 μl) to the solution bathing the tissue. The exception was diphenylamine-2-carboxylate, which was a gift from Dr. R. Greger. This material was dissolved in a small volume of 0.1 NaOH to form the sodium salt. There was no change in pH when added to the fluid bathing the tissue.

Transepithelial flux studies were made with either $^{22}$Na or $^{36}$Cl. Paired preparations were used, and a trace of isotope (2–4 μCi) was added to the apical bathing solution of one preparation and the basolateral side of the other. Around 45 min was allowed for the isotope to achieve a constant specific activity within the tissue. Five samples (each 1 ml) were collected from the trans side at 20-min intervals. Smaller samples (100 μl) were taken...
Fig. 1. Effects of adenosine analogues on SCC in isolated rat colon. In each experiment the epithelial area was 0.6 cm². The values of SCC increase (given in μEq) were measured during 10 min following addition of the drug. All drugs were added to solutions bathing the basolateral side of the tissue. (a) Concentration response curve for ATP. Each value shows the mean ± SE for thirteen separate preparations. Each concentration of ATP was applied once to each preparation, with washing and restoration of basal SCC between each application. (b) Partial concentration response curves for ATP, ADP, AMP and adenosine. A single preparation was exposed to the two concentrations of ATP and the two concentrations of one of the other analogues. After each response the drug was washed away and basal SCC restored before further drug exposure occurred. Mean ± SE values are given for 16 (ATP), 4 (ADP), 4 (AMP) and 8 (adenosine) separate values. The responses for ATP were significantly greater (P < 0.001) at both concentrations than the corresponding responses to the other analogues.

Results

Effects of Adenosine Analogues on Epithelial Transport

The concentration response relationship for ATP on the colon was investigated by using four concentrations of the nucleotide, applied in a randomized order to the basolateral side of the tissue, in thirteen separate experiments. At concentrations above 0.5 mM the SCC response was maintained, while at lower concentrations the SCC usually declined from a peak value to give a sustained plateau. In every instance the response was terminated after 10 min and the area under the curve integrated to give the total charge transfer, calculated as μeq of univalent ion. The values of charge transfer were used to construct the concentration-response curve shown in Fig. 1a.

The activity of ATP relative to that of ADP, AMP and adenosine was investigated in a separate set of experiments using only two concentrations of each compound. In every instance ATP was compared with one of the other compounds in the same preparation and at the same concentration. The results of these measurements are given in Fig. 1b. It is clear that ADP, AMP and adenosine are relatively inactive compared to ATP. Adenosine appears to have the least activity, while ADP and AMP have similar activity.

The foregoing data were obtained by application of ATP and its analogues to the basolateral side of the tissue. As it is not uncommon for epithelia to show asymmetry in response to drugs (Cuthbert, 1984), the sidedness of the response was investigated. The experimental protocol can be seen by reference to Fig. 2. Paired preparations taken from adjacent pieces of colon were prepared and exposed to two concentrations (300 μM and 1 mM) of ATP on