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THE EFFECT OF ADENOSINE TRIPHOSPHATE ON THE TRANSMURAL POTENTIAL IN RAT SMALL INTESTINE

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SUMMARY

1. ATP either in the mucosal or serosal fluid caused a transient increase in the potential difference and short-circuit current across the wall of rat jejunum *in vitro*, the serosal solution becoming more positive.

2. Similar responses were observed in the ileum and colon, and in *in vivo* preparations of small intestine.

3. The response is relatively specific for ATP.

4. The transient nature of the response is not due to rapid hydrolysis of extracellular ATP.

5. High concentrations of K^+ in the mucosal medium, serosal ouabain or mucosal 2,4-dinitrophenol all inhibit the response without altering the time course.

6. Significant inhibition was not observed in the presence of ADP or in Mg^{2+}-free salines.

7. The results are consistent with an intracellular action of ATP in the epithelium to stimulate net ion transport. The results do not demonstrate whether or not extracellular ATP can act as an energy donor for an electrogenic ion pump.

8. Theophylline prolongs the time course of the response, and the involvement of the adenyl cyclase system cannot be excluded as an explanation for the findings.

INTRODUCTION

The small electrical potential which exists across the wall of the small intestine can be changed in various ways which have been discussed by Smyth (1967) as diffusion potentials, osmotically induced potentials and transfer potentials. The present paper deals with a potential occurring in the presence of adenosine triphosphate (ATP) which does not fit into any

of the above categories, and which is of interest in view of the role postulated for ATP in the transfer and metabolic activities of the cell. A preliminary account has been given by Kohn, Newey & Smyth (1967).

METHODS

Female albino rats of the Sheffield strain weighing 220–260 g were used unless otherwise stated, and before experiment were maintained on a stock diet of Oxoid cubes (Diet 86). Intestine from rabbit, guinea-pig and frog was used in a few cases. Experiments have been performed both *in vitro* and *in vivo*, in which the potential across the wall of the small or large intestine was recorded in the presence of ATP and a number of other substrates.

In vitro. In most cases the apparatus used was similar to that described by Barry, Dikstein, Matthews, Smyth & Wright (1964), in which the potential is recorded across the wall of sacs of everted intestine, one electrode being in the fluid inside the sac (serosal fluid) and the other in the fluid in which the sac is suspended (mucosal fluid). This preparation is not suitable for experiments in which the serosal fluid is altered, and for these a different procedure was used. The apparatus was that of Wiseman (1953) as modified by Smyth & Whaler (1953). However, the segment was everted, so as to maintain the polarity in the same direction as the other *in vitro* experiments. Agar/KCl bridges were inserted into the mucosal and serosal fluids. In all *in vitro* experiments the bathing medium was bicarbonate saline (Krebs & Henseleit, 1932) containing dissolved substances and gassed with 95 % O_2, 5 % CO_2. The part of the intestine used is indicated by the Roman numeral I–V as used by Barry, Matthews & Smyth (1961) in which these refer to fifths of the combined jejunum and ileum numbered from the jejunal end.

In a few control experiments sacs of everted intestine were used, and before measurement of the potential the epithelial cells were removed by gently scraping with a glass microscope slide against a large sheet of glass. On completion of the experiment the sacs were fixed on Bouin's fluid, sectioned and stained. The sections were compared with those from unscraped sacs to ascertain that the epithelial cells had been removed.

In the colon a preparation of mucous membrane with the muscle layer stripped off was frequently used as described by Parsons & Paterson (1960). The whole colon was excised and a glass rod passed through the lumen. The colon was tied to the glass rod, and a cut was made through the muscle layers at the proximal end. The muscle layers were then removed by gentle traction with the fingers. The colon, whether stripped or not, was divided into two segments, proximal and distal, corresponding approximately to the ribbed and unribbed portions.

In vivo. The technique was that described by Barry *et al.* (1964) with one electrode in the intestinal lumen and the other in the adjacent peritoneal cavity.

Short-circuit current measurements were made using the apparatus described by Barry, Smyth & Wright (1965). Current was applied for periods of 15 out of every 30 sec. In this way, changes in short-circuit current and potential could be measured. Assuming the tissue to behave as an ohmic resistance, a value of tissue resistance can be obtained from the ratio of the potential to the short circuit current.

The chemicals used were as follows: adenosine-5′-triphosphate, disodium salt (ATP) from C. F. Boehringer and Soehne or British Drug Houses; adenosine-5′-monophosphate, disodium salt (5′-AMP) and inosine-5′-triphosphate, trisodium salt (ITP), Boehringer; cytidine-5′-triphosphate, disodium salt (CTP), guanosine-5′-triphosphate, disodium salt (GTP), uridine-5′-triphosphate, trisodium salt (UTP),

creatine phosphate, disodium salt (CP), all from Schwarz Biochemicals; adenosine adenosine-3-monophosphoric acid (3′-AMP) and adenosine-5′-diphosphate, sodium salt (ADP) from Sigma Chemical Company.

RESULTS

Effect of ATP on gut potential

If ATP at a concentration of 10^{-3} M is added to the mucosal fluid bathing Sac III a small potential change is obtained (Fig. 1), the serosal side being positive to the mucosal. There is an initial time lag of about 15 sec before any change occurs. This is probably not due to time required for mixing,

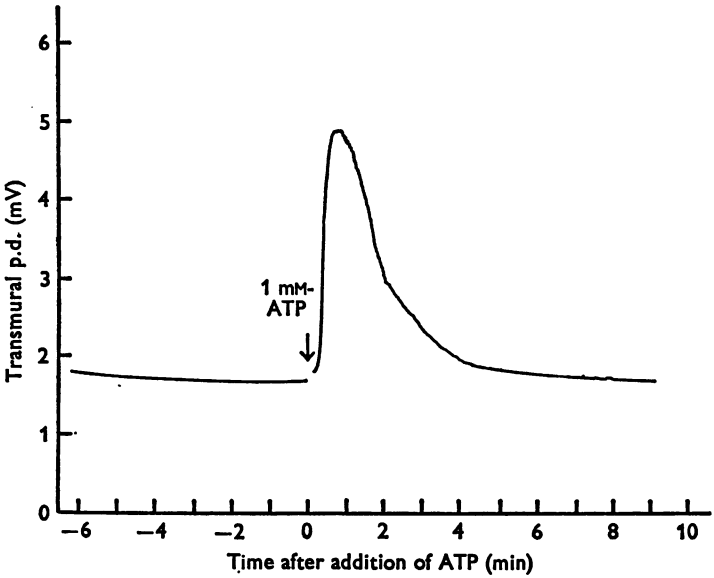

Fig. 1. Time course of the change in potential difference across the wall of rat jejunum following the addition of 1 mM-ATP to the mucosal bathing medium. In all Figures positive values for potential indicate that the serosal side is positive with respect to the mucosal side.

as the potential on addition of glucose occurs within 1 sec. After the initial delay there is a rise to a peak reached 60–70 sec after adding ATP. The maximum potential is about 2·7 mV. The potential then falls slowly and is near the original level within 6–7 min. The addition of ATP at concentration 1 mM caused a change in pH of only 0·05 pH units, so that changes in the H^+ ion concentration are unlikely to be the cause of the potential.

The effects of ATP were studied on segments representing all parts of the small and large intestine. The potential obtained was usually similar to that shown in Fig. 1 but the maximum potential varied, and the values

for different parts of the gut are included in Table 1. The greatest potential and the greatest variability are seen in Sac V. In some cases and particularly in the distal part of the ileum and colon, a somewhat different response was obtained in which there was an initial depression of potential before the rise.

The gut is less sensitive to addition of ATP to the serosal fluid and using the segment technique 1 mM-ATP caused a potential of 0·60 (± 0·15) mV compared with 2·15 (± 0·45) mV on the mucosal side. The maximum potential was about 2·5 mV in both cases.

TABLE 1. Comparison of the effects of 1 mM-ATP on transmural potential in different regions of the intestine. The p.d.s are expressed as the mean value with the S.E. of mean, the number of experiments being given in brackets. Positive values indicate that the serosal side is positive

Region	Δ p.d. (mV)
Sac I (proximal jejunum)	1·00 ± 0·20 (6)
Sac III (midgut)	2·70 ± 0·08 (80)
Sac V (distal ileum)	5·35 ± 0·65 (5)
Unstripped proximal colon	2·60 ± 0·45 (5)
Stripped proximal colon	2·60 ± 0·55 (7)
Unstripped distal colon	4·25 ± 0·65 (6)
Stripped distal colon	3·90 ± 0·45 (11)

Site of action of ATP

Since the whole intestinal wall is present, the possibility of the muscle layer as the site of the ATP effect must be considered. In the rat small intestine it is not possible to make a preparation of epithelial layer separate from the muscle to test the response of the epithelium to ATP. It is, however, possible to make a preparation of muscle layer without epithelium as described in the Methods section. The complete removal of the epithelium was confirmed histologically. No response to ATP could be obtained in such a preparation.

In the rat colon it is possible to strip off the bands of muscle and obtain a preparation of mucosa only. The results of potential measurement with this preparation are included in Table 1, and it is seen that removal of the muscle does not affect the potential, which must have its origin in the epithelial layer.

Effect of concentration

A graded response of ATP is seen over a range of concentration from 10^{-5} to 5×10^{-3} M, the time course being approximately the same in all cases. Fig. 2 shows the results for segment III, the peak response being plotted against concentration, and it is evident that 1 mM-ATP produces

a nearly maximum response. A double reciprocal plot (Lineweaver & Burk, 1934) gives values of 0·04 mM for apparent K_m and 2·8 mV for maximum p.d.

Specificity of response

A number of compounds related to ATP were tested on segment III. Adenosine diphosphate (1 mM) gave a response of 0·60 ($\pm$ 0·22) mV; this may have been due to contamination with ATP. (Less than 1 % contamination would have been sufficient; Fig. 2.) Adenosine-5′-monophosphate

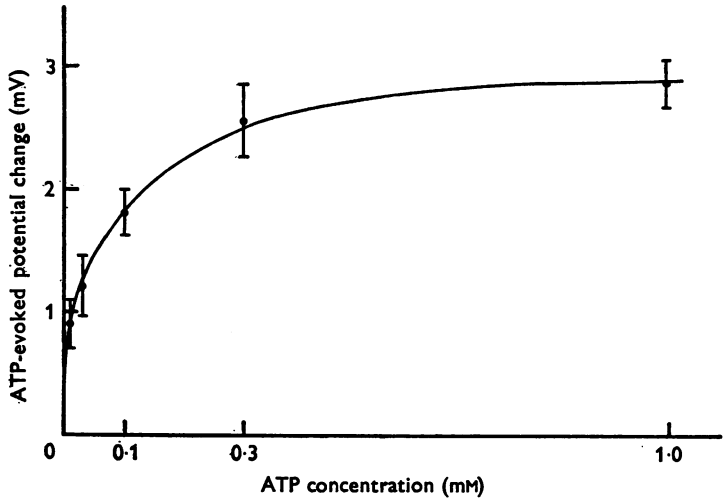

Fig. 2. Effect of ATP concentration on the maximum potential change produced.

(1 mM) and cytidine triphosphate, inosine triphosphate, uridine triphosphate and guanosine triphosphate (all 0·1 mM) gave much smaller responses than the same dose of ATP. All the responses showed the same time course as ATP itself. Adenine, adenosine, adenosine-3′-monophosphate and creatine phosphate (all 1 mM) gave no significant response. The effects of ATP were also tested in a small number of experiments using intestine of rabbit, guinea-pig or frog. All gave very similar results to those in rat so that while there is a high degree of specificity for ATP the same type of response was observed in all the species tested.

Transient nature of the response

As the potential caused by ATP lasts for such a short time, the question arises whether this is due to rapid hydrolysis of ATP or the incapacity of the tissue to continue to respond to it. The first of these questions can be answered by adding ATP to the mucosal solution bathing a sac, and after the response draining off the mucosal fluid and putting it in contact with

another sac. In this case the normal response was obtained in the second sac, thus showing that enough ATP was still available to cause a response.

An experiment was done in which ATP was added to the mucosal fluid and at the end of the response a second dose was added. The results are shown in Fig. 3 where it is seen that the response to the second application was much smaller than to the first. This Figure also shows that the addition of 10 mM glucose gave the normal glucose response.

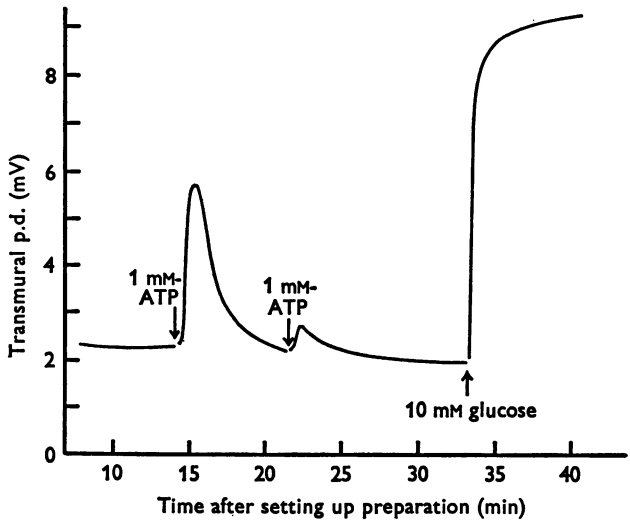

Fig. 3. Potential changes produced by repeated doses of ATP. Although the presence of ATP inhibits the response to further ATP, a normal stimulation of potential by glucose is observed.

In another experiment using a pair of sacs from the same intestine ATP was added to one sac and at the end of the response was washed out and the gut left in contact with bicarbonate saline for 35 min. A second application of ATP gave a smaller response, but, as Fig. 4 shows, this was the same as the control experiment in which ATP was added at the equivalent time after setting up the preparation. It can therefore be concluded that in this experiment the gut had recovered its capacity to respond to ATP.

In another experiment the ATP was applied only for 15 sec and the result is shown in Fig. 5. It is seen that although the response is somewhat smaller than the control experiment in which the application of ATP lasted much longer, the electrical change lasted after the tissue had been removed to an ATP-free solution, i.e. the response appears not to depend only on the presence of ATP in the mucosal fluid.

In vivo response

Some experiments were performed with the *in vivo* preparation described by Barry *et al.* (1964). The results are similar in many respects to those *in vitro*, but with doses of ATP greater than 2 mM the p.d. did not return to the initial level (as it did *in vitro*) until the lumen was again bathed with an ATP-free solution.

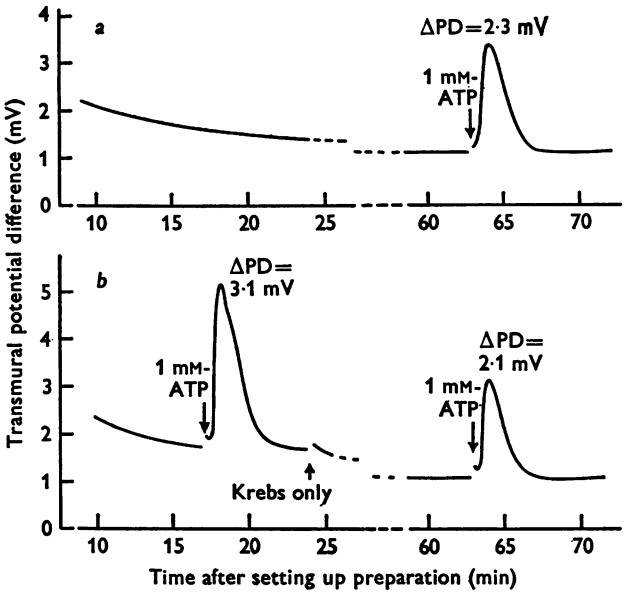

Fig. 4. The effect of repeated doses of ATP, with an intermediate ATP-free period. The response to ATP more than 1 hr after setting up the preparation is very similar in the control (*a*) and in the sac which had previously responded to ATP (*b*). The sacs were made from adjacent pieces from Sac III of the same intestine.

The largest response is found in the distal ileum, and the maximum response *in vivo* is similar to that *in vitro* for the equivalent region of the intestine. The variability in size of response is greater than *in vitro*. No significant differences were observed between experiments using 0·9% NaCl (gassed with pure O_2) or bicarbonate saline (gassed with 95% O_2, 5% CO_2) as the luminal fluid.

Effect of ADP on the ATP response

A group of experiments was performed in which the effect of the presence of 1 mM-ADP was examined on the response to 0·1 mM-ATP. No change in the time course was observed and the fall in the ATP response, from 1·36 (± 0·48) to 0·82 (± 0·35) mV, was not significant. Since a second

application of ATP gives a smaller response, any decrease in the response to ATP in the presence of ADP could be due to contamination of the ADP with ATP.

Effect of inhibitors

A number of inhibitors known to affect transport processes in the intestine have been tested for their effects on the ATP-induced potential. Except in the case of ouabain these were added to the mucosal fluid.

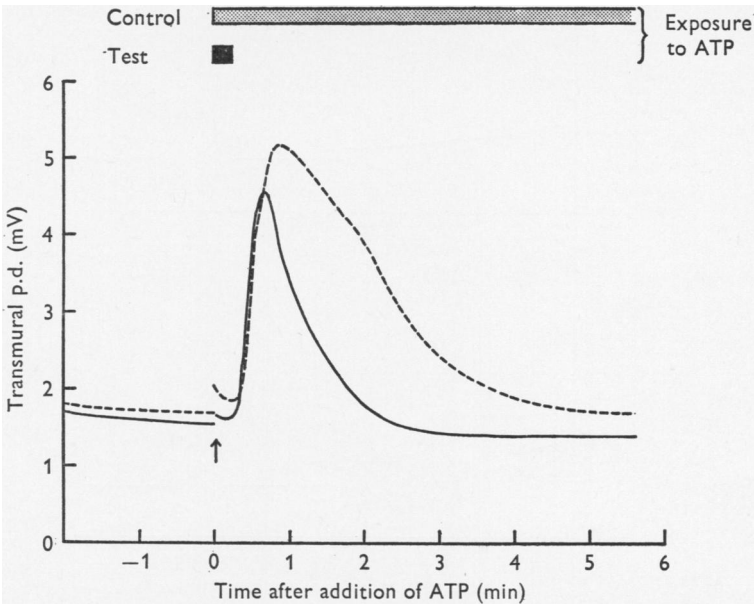

Fig. 5. Effect of a 15 sec exposure to 1 mM-ATP compared with the effect of sustained exposure. The continuous line shows the result of the 15 sec exposure compared with a control sac from the same intestine (interrupted line). The bars above show the time of exposure to ATP on the same scale.

Phlorrhizin was without effect at a concentration (2×10^{-4} M) at which it is a powerful inhibitor of hexose transfer and the transfer potentials. Ouabain in the serosal fluid gave a 50% inhibition at a concentration ($1–2 \times 10^{-3}$ M) which did not reduce the endogenous potential (i.e. the potential in the absence of other solutes in the bicarbonate saline). Mucosal ouabain produced a detectable effect only at concentrations greater than 10^{-2} M. A 50% inhibition is caused by 2×10^{-5} M 2,4-dinitrophenol. Sodium fluoride was without effect in concentrations (5×10^{-3} M) at which it was shown by Detheridge, Matthews & Smyth (1966) to inhibit glucose dependent fluid transfer. Fluoroacetate (10^{-2} M) inhibited the ATP potential by about 50% but the result is complicated by the fact that fluoroacetate itself produced a transient potential change not unlike the ATP response.

Changes in ionic environment

The effects were studied by alteration of the saline in various ways. In these experiments normal bicarbonate saline was used in washing out and removing the intestine, and in making the sacs. In setting up the sacs for electrical measurement the modified bicarbonate solutions were used for both mucosal and serosal solutions except where otherwise stated. It appeared, in fact, that all the changes found could be produced by changing the mucosal solutions only, and this was done in some experiments as described.

TABLE 2. Transmural p.d. changes in Sac III rat small intestine in response to 1 mM-ATP when bicarbonate salines are used in which all the NaCl is replaced by the solute indicated. PD is the maximum p.d. change observed. The changes are expressed as the mean value with the S.E. of mean, the number of experiments being given in brackets. (N.B. the basal p.d. levels in salines where NaCl is replaced by mannitol were relatively unstable.) Positive values indicate that the serosa becomes more positive

Solute replacing NaCl	PD (mV)
KCl	$-0{\cdot}20 \pm 0{\cdot}05$ (4)
Choline chloride	$6{\cdot}05 \pm 0{\cdot}90$ (3)
Mannitol	$6{\cdot}30 \pm 0{\cdot}75$ (6)
(No replacement)	$2{\cdot}70 \pm 0{\cdot}08$ (80)

Magnesium. The replacement of magnesium sulphate in the saline by sodium sulphate had no effect on the ATP response.

Sodium and chloride. The effects of these ions were studied by replacing the NaCl in the saline with KCl, choline chloride or mannitol, the normal amount of $NaHCO_3$ was however present. The results are seen in Table 2. Replacement of NaCl with choline chloride and mannitol caused similar effects, i.e. a substantial increase in the ATP potential. Replacement of NaCl by KCl abolished the potential increase completely, and indeed a small potential in the opposite direction was produced by ATP. In two experiments where KCl replaced NaCl in the mucosal medium only, PD was 0·20 and 0·15 mV.

To study this effect of K^+ further a series of experiments was done in which NaCl was substituted to a varied extent with KCl. Fig. 6 shows that there is an approximately linear relation between log $[K^+]$ and the ATP potential, except at very low K^+ concentrations. Experiments in which KCl was omitted from the solution by substitution with NaCl gave ATP potentials which did not differ from those in normal bicarbonate solutions.

Effect of hexoses

The effect was tested of a number of hexoses on the ATP potential and mannitol was also used as an osmotic control. The hexose was present in the mucosal fluid in concentrations of either 2 mM or 28 mM and in the serosal solution at 222 mM. ATP was not added until the p.d. was steady and for at least 15 min after addition of the hexose. It must be remembered

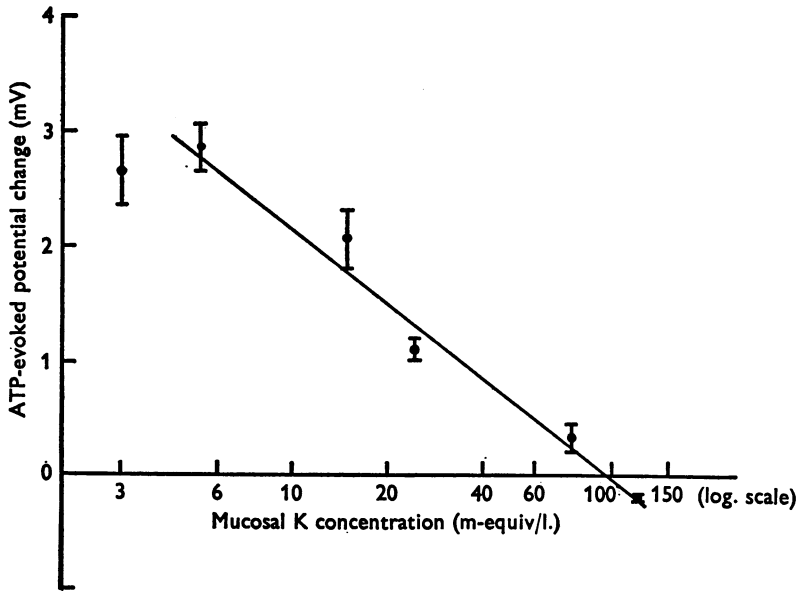

Fig. 6. The effect of mucosal K^+ concentration on the change in transmural p.d. produced by addition of 1 mM-ATP to the medium bathing the mucosal face of sacs of rat mid-intestine. The mean and S.E. of mean is shown for each K^+ concentration.

that the transferable hexoses (glucose and galactose) themselves cause a sustained increase in potential, and the results in Table 3 record the additional increases caused by ATP.

Although the results are not very clear cut, certain tentative conclusions may be drawn. From the mucosal side all the hexoses are inhibitory with the exception of galactose at low concentrations, i.e. all the metabolized hexoses are inhibitory; galactose is actively transported but only metabolized to a slight extent. From the serosal side, only fructose inhibits to a significantly greater extent than the osmotic control. Mannose, which is better metabolized than fructose, is not inhibitory.

Short-circuit current and tissue resistance

As has been pointed out by Ginzburg & Hogg (1967), the measurement of short-circuit current has limited value in situations where transient changes in active transport or passive membrane permeability are occurring. Recognizing this, the results obtained must be interpreted with caution. However, in every case the short-circuit current and potential increased in parallel, and significant changes in tissue resistance were not observed. Fig. 7 shows the results of one such experiment.

TABLE 3. The effect of hexoses in the mucosal or serosal bathing medium on the maximum p.d. change produced in Sac III of rat small intestine by 1 mM-ATP. The p.d.s are expressed as the mean value with the S.E. of mean, the number of experiments being given in brackets

Substrate	Mucosal/serosal	Response
2 mM glucose	Mucosal	Very variable/ always below control
2 mM galactose	Mucosal	2·45 ± 0·15 (5)
2 mM mannose	Mucosal	1·55 ± 0·25 (6)
2 mM fructose	Mucosal	1·00 ± 0·25 (6)
28 mM glucose	Mucosal	1·05 ± 0·20 (12)
28 mM galactose	Mucosal	0·88 ± 0·11 (9)
28 mM fructose	Mucosal	1·00 ± 0·20 (8)
28 mM mannitol	Mucosal	2·65 ± 0·25 (8)
222 mM glucose	Serosal	2·00 ± 0·45 (5)
222 mM galactose	Serosal	1·80 ± 0·25 (4)
222 mM fructose	Serosal	0·50 ± 0·09 (8)
222 mM mannose	Serosal	1·70 ± 0·30 (8)
222 mM mannitol	Serosal	2·05 ± 0·25 (11)

Theophylline

In six experiments the response to 1 mM-ATP was tested in the presence of 2 mM theophylline in the mucosal medium. Control sacs from an adjacent segment of intestine were used. Theophylline itself causes a marked and very variable stimulation of potential. In some cases this was well maintained, in others the potential fell to a new steady level. When the potential was steady the ATP response was obtained. In every case the ATP response was greatly prolonged, the time to reach the peak increase being doubled and the decay also being slightly slower. There was also a stimulation of the size of the response although this was by less than 30% in all but one experiment. Fig. 8 shows the results of a typical experiment.

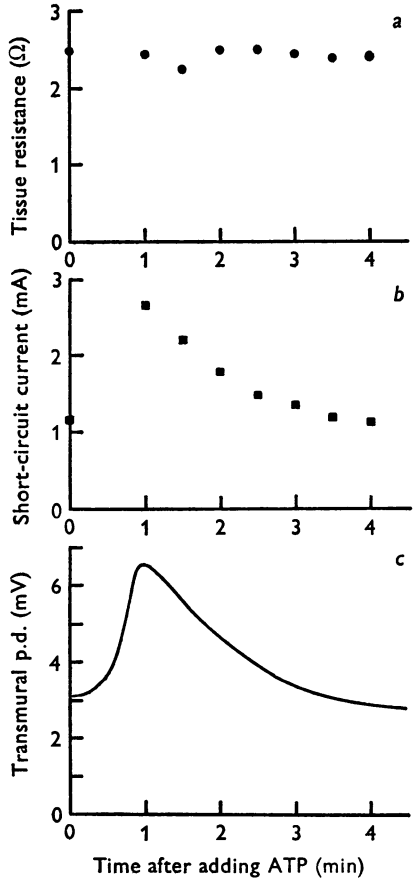

Fig. 7. Effect of 1 mM-ATP on (*a*) tissue resistance, (*b*) short-circuit current and (*c*) transmural potential difference across a piece of rat small intestine (Sac III). The three records were obtained simultaneously from the same piece of intestine.

DISCUSSION

In a previous paper (Kohn *et al.* 1967) we reported a stimulation of the potential difference across the small intestine by ATP, and we have now examined this effect in more detail. No response is given by sacs of small intestine when the epithelial cells have been removed by gentle scraping. On the other hand, preparations of colon from which the muscle layers have been stripped respond to ATP in a way indistinguishable from the intact preparation. Thus the effect does not appear to be associated with the muscle cells. Although no direct evidence for entry of ATP is presented the similarity of the response to mucosal and serosal ATP is consistent with an intracellular site of action. The higher 'apparent K_m' for serosal

ATP is in agreement with the findings for other substrates to which the epithelial cells are known to be permeable. High serosal concentrations are always necessary to achieve adequate tissue concentrations.

The constancy of the time course of the ATP response is very striking. Factors which alter the magnitude of the response do not appear to affect the time course. The continued ability of the tissue to respond to actively transported hexoses suggests that the transient nature of the response is

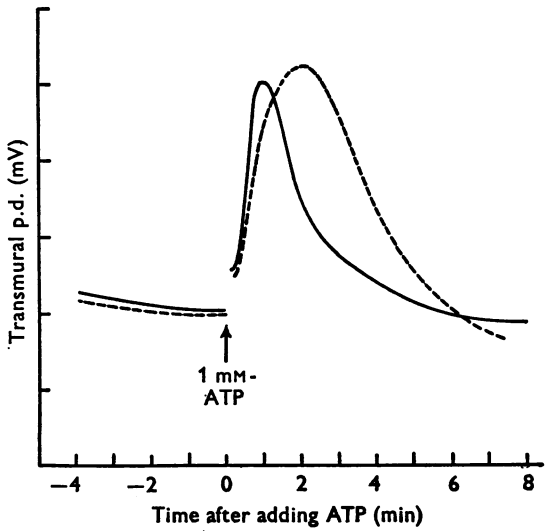

Fig. 8. Effect of 2 mM mucosal theophylline on the change in transmural p.d. across a sac of rat mid-intestine when 1 mM-ATP is added to the mucosal medium. The interrupted line is the test response, the continuous line the control response from an adjacent piece of the same intestine. Since theophylline itself stimulates the p.d., the response has been vertically transposed to superimpose the two responses. The ordinate indicates units of 1 mV but not absolute values.

not due to tissue damage. We have also shown that it does not represent complete utilization of the ATP. It seems probable that the falling away of the initial response and the greatly reduced response to a second application of ATP are closely related. The observed time course is consistent with the initiation of two separate responses, one stimulatory, the other inhibitory. The situation is analogous to an action potential but with a time scale of minutes rather than milliseconds. The second, inhibitory response seems to persist as long as ATP is present in the bathing medium. The inhibitory response could represent formation of high levels of ADP at the pump site, despite the failure to demonstrate direct inhibition by ADP. Certainly one could expect 2,4-DNP to raise tissue levels of ADP

when oxidative phosphorylation is inhibited. This is consistent with the observed inhibition of the ATP response by this compound.

The experiments performed in the presence of mucosal hexoses are puzzling. At least at low concentrations (2 mM), the metabolizable hexoses are more inhibitory than the actively transported galactose. At 28 mM all the hexoses tested were inhibitory. Even more surprising is the finding that with 222 mM serosal hexoses, only fructose is inhibitory, even though serosal mannose is a better source of metabolic energy than fructose. We can see no explanation for these findings.

The changes observed do not appear to fall into any of the categories of potential change described hitherto. Both transfer potentials and osmotically induced potentials are maintained for long periods of time, the limiting factor usually being the viability of the tissue. In contrast, the ATP potential is transient even though the tissue is still viable. Osmotically induced potentials are small and in the opposite direction when solutes are present on the mucosal side. Actively transported substances only affect the potential from the serosal side when present in very high concentrations (at least 100 mM). In contrast, although somewhat higher serosal than mucosal concentrations of ATP are required to produce the maximum response, 5 mM serosal ATP and 1 mM mucosal ATP produce changes which are indistinguishable in time course, direction and magnitude. A similar argument rules out the possibility that the observed effect is a diffusion potential caused by a difference in ionic concentrations between the solution in contact with the two sides of the gut.

Any change in transmural potential could result from changes in either the active or passive movements of ions in the system. Our results indicate an increase in short-circuit current without a change in tissue resistance. This might represent a direct stimulation of the electrogenic sodium pump at the serosal pole of the epithelial cells. Alternatively, a temporary increase in the mucosal permeability to Na^+ would allow more sodium to enter the cell. This in turn could stimulate the pump resulting in an increase in short-circuit current and potential without necessarily producing a detectable decrease in tissue resistance. Effects of this type have been postulated to explain the effects of hormones on short-circuit current and potential in amphibian skin and urinary bladder (Leaf, 1965; Orloff & Handler, 1967).

However, transient passive movement of ions could also give rise to an apparent increase in short-circuit current (Ginzburg & Hogg, 1967). Two pieces of evidence make this explanation unlikely in the present case. Removal of ATP does not result in a reversed potential and short-circuit current. If the electrical effects represent changes in tissue ionic concentrations caused by alterations of membrane permeability and passive

ionic movement, removal of ATP should produce electrical changes of opposite sign whilst the original ionic balance is restored. On the other hand, no such reverse response would be expected if ATP alters, directly or indirectly, the rate of operation of the electrogenic sodium pump.

It is known that solutions of ATP are strongly acidic. However, bicarbonate saline has a powerful buffering action and the dissolving of sufficient ATP to produce a 1 mM solution lowers the pH by about 0·05 units only. ATP also has a powerful chelating action, but the other nucleoside triphosphates have a similar chelating power (Walaas, 1958) and the specificity of the response to ATP makes a generalized chelating activity an unlikely explanation. These arguments do not rule out a localized chelation effect involving specific binding of ATP.

Two other actions of ATP deserve more serious consideration. ATP is the principle substrate for the Na^+, K^+-exchange pump. It is also the precursor of cyclic 3′,5′-AMP and the mode of action of a number of hormones has been proposed to be activation of adenyl cyclase, the enzyme which catalyses the conversion of ATP to cyclic AMP. Evidence for the involvement of the Na^+-pump relies on the results with ouabain which is known to inhibit the Na^+, K^+-ATPase system and the electrogenic Na^+-pump (Schatzmann, 1953; Skou, 1964). Moreover, it is only effective on the side towards which Na^+ is being pumped (Dunham & Glynn, 1961). In our experiments ouabain was a far more effective inhibitor from the serosal side despite the permeability barrier provided by the muscle layers and lamina propria. The concentration of ouabain giving 50 % inhibition of the ATP response was $1–2 \times 10^{-3}$ M, a surprisingly high figure. However, it has been shown that the Na^+, K^+-ATPase of rat intestine is relatively insensitive to ouabain (Berg & Szerkeczes, 1966).

The apparent K_m for the ATP effect was 4×10^{-5} M. A recent study on the Na^+, K^+-ATPase system of rat intestine has provided a value for the K_m of 10^{-4} M (Quigley & Gotterer, 1969). Although the similarity of these values is of interest, strong conclusions should not be drawn from a comparison of them. Two features of the ATP response do not correspond to the known properties of the ATPase system. We have failed to demonstrate a significant depression of the response in Mg^{2+}-free saline although there is an absolute requirement for Mg^{2+} by the ATPase system. However, the tissue pool of Mg^{2+} may be sufficient under these conditions to satisfy this requirement. High levels of ADP have been proposed by Caldwell to inhibit the Na^+ pump in the squid axon (cited by Hodgkin, 1965). In our experiments, a mucosal ATP/ADP ratio of 0·1 failed to inhibit the response, but the intracellular ratio under these conditions is unknown. Reduction of $[Na^+]$ in the bicarbonate saline to 25 m-equiv/l. doubles the potential. However, Schultz, Curran & Wright (1967) have shown that

under these conditions, tissue resistance is also doubled. Thus, reduction of Na^+ to 25 m-equiv/l. has no significant effect on the ATP-induced ion movement.

Formation of cyclic 3′,5′-AMP is supposed to increase membrane permeability and Rasmussen & Tenenhouse (1968) have argued that the effect may depend on conversion (at fixed sites in the membrane) of the strongly chelating ATP to cyclic 3′,5′-AMP which is an extremely weak chelator. The consequent release of Ca^{2+} causes an increase in membrane permeability. In this connexion, the experiments of Field, Plotkin & Silen (1968) on rabbit ileum are of some interest. They showed that theophylline and cyclic AMP produced sustained increases in short-circuit current but vasopressin produced only a transient response (even though it is supposed to act via activation of adenyl cyclase). Theophylline is supposed to inhibit phosphodiesterase, the enzyme responsible for breaking down cyclic 3′,5′-AMP. In the present experiments theophylline itself stimulated the potential and delayed, but did not prevent, the decay of the ATP response.

Field *et al.* were not able to explain the vasopressin effects except in rather general terms, but their results with modified bathing media suggested that theophylline and cyclic AMP stimulate active secretion of chloride and bicarbonate in the rabbit ileum. None of our findings exclude the possibility of a similar action for ATP. The strong dependence of mucosal K^+ concentration does not enable a clear choice to be made between the ATPase and adenyl cyclase systems. The K^+ ion is of great importance in the ATPase system and replacement of intracellular Na^+ by K^+ would be expected to stop the pump. Equally, in a number of tissues where the adenyl cyclase system operates, high extracellular K^+ is of importance, and may stimulate the formation of cyclic AMP (Rasmussen & Tenenhouse, 1968).

In summary, the small and large intestine respond to the presence of ATP in the bathing medium, both *in vitro* and *in vivo*, by a transient increase in potential and short-circuit current. The response appears to represent an action in the epithelial layer at a site accessible to both the mucosal and serosal bathing media. It is suggested that the response is mediated by a stimulation of active ion movement but this may result from a prior increase in passive membrane permeability.

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