BBA 75 845

SODIUM TRANSFER IN BULLFROG SMALL INTESTINE

STIMULATION BY EXOGENOUS ATP

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(Received August 6th, 1971)

SUMMARY

1. ATP (5 mM) added to the mucosal medium causes sustained increases in potential difference (PD) and short circuit current (I_{sc}) across sheets of isolated bullfrog small intestine mounted between identical Na_2SO_4 Ringer solutions at pH 7.2. Serosal ATP has no effect. The ATP response can be evoked in the presence of dinitrophenol or under N_2, is obtained during virtually maximal stimulation of PD and I_{sc} by mucosal D-glucose, D-galactose or L-valine, is not inhibited by mucosal phlorizin, but is inhibited by serosal ouabain.

2. The ATP response is relatively specific. Related phosphorylated compounds either did not change PD and I_{sc} or elicited weak, transient increases in these parameters. 5'-AMP produced apparent changes in PD and I_{sc} which were not obviously related to the ATP effect. Mucosal ADP strongly inhibited the stimulatory effect of mucosal ATP on PD and I_{sc}.

3. Radioisotope studies indicated that the stimulatory effect of mucosal ATP is due to an increased mucosal to serosal flux of Na^+, serosal to mucosal Na^+ flux remaining essentially unchanged.

4. It is suggested that the stimulatory effect of mucosal ATP on PD and I_{sc} involves a direct interaction of this compound with a component of the active Na^+-transfer system (possibly a ouabain sensitive ATP-ase) and that mucosal ATP can gain access to the interior of the epithelial cells either by simple diffusion or mediated transfer across the mucosal membrane.

INTRODUCTION

Isolated bullfrog small intestine maintained in isotonic Ringer solutions in which Cl^- is completely replaced by SO_4^{2-} shows many functional analogies to isolated mammalian small intestine. For example, under these conditions the transmural potential difference (PD) is serosal positive and short circuit current (I_{sc}) is equal to net mucosal to serosal Na^+ flux[1]. Further, addition of actively transported sugars or amino acids to the mucosal medium causes marked increases in PD and I_{sc} (ref. 2).

Abbreviations: PD, potential difference; I_{sc}, short circuit current.

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As reported for mammalian small intestine[3,4] these increases are saturable functions of the concentration of the added solute. Also the effects of sugars and amino acids are additive, *i.e.* during maximal stimulation by an actively transported sugar a further saturable increase in PD and I_{sc} can be elicited by an actively transported amino acid and *vice versa*. Finally, the response of net mucosal to serosal Na^+ transport (and its electrical correlates, PD and I_{sc}) in isolated bullfrog small intestine to N_2 and metabolic inhibitors under these conditions[5] is strongly reminiscent of the findings reported for mammalian small intestine[6].

Because of these similarities, the finding of KOHN *et al.*[7,8] that exogenous ATP causes transient increases in PD and I_{sc} in isolated rat jejunum prompted us to perform a comparable study of the effect of ATP and other high energy phosphorylated compounds on isolated bullfrog small intestine. The results of this study are reported herein. Preliminary accounts of portions of this work were given elsewhere[9,10].

METHODS

The preparation and mounting of intestinal sheets between the two sides of a divided lucite USSING chamber, together with the chamber itself, the methods used to monitor PD and I_{sc}, and the method of oxygenating and circulating the fluid in both halves of the chamber have been fully described elsewhere[1,5]. The medium used was a phosphate buffered Cl^- free Na_2SO_4 Ringer solution (pH 7.2) made isosmotic with mannitol[1]. When glucose, galactose or valine were incorporated in this medium an equivalent amount of mannitol was omitted. These solutes were used at a constant relative concentration[11] computed on the basis of the apparent Michaelis constants for their stimulatory effects on PD and I_{sc} (ref. 2). The high-energy intermediates and related compounds employed were 5'-AMP, ADP, ATP, GTP, ITP, 3'-AMP, 2'-AMP, adenosine, adenine sulfate, and sodium pyrophosphate. 5'-AMP and ADP were obtained from General Biochemicals Co., Chagrin Falls, Ohio; ATP, GTP, GDP, ITP, 3'-AMP, 2'-AMP and adenosine from Sigma Chemical Co., St. Louis, Mo.; adenine sulfate from Eastman Organic Chemicals, Rochester, N.Y., and sodium pyrophosphate from Matheson, Coleman and Bell, Cincinnati, Ohio. The inhibitors used were phlorizin, (Calbiochem, Los Angeles, Calif.), 2,4-dinitrophenol and NaF (Fisher Co., Pittsburgh, Pa.), and ouabain (this was a highly purified sample kindly given us by Dr. T. Z. Csaky). All other reagents were Analytical Reagent Grade and all solutions were made up in distilled water which had been further purified by being passed twice through a mixed-bed ion exchanger.

The high-energy intermediates were used in a final concentration of 5 mM. Many of them were supplied as sodium salts (*e.g.*, the disodium salt of ATP) and, when this was the case, equivalent amounts of Na^+ were omitted from the medium by reducing the amount of Na_2SO_4 used. Otherwise, osmotically equivalent amounts of mannitol were omitted. Where necessary, the pH of the medium was adjusted to 7.2 with Tris. All experiments were performed at 26°.

Following excision and mounting of the tissue, I_{sc} was recorded every 5 min until a steady state was reached (at the same time PD was recorded every 10 min). This normally required from 30 to 60 min. When sufficient measurements had been made to establish the steady state PD and I_{sc}, the required constraint was placed on the system, *i.e.* the O_2 supply was changed to N_2, the Ringer solution in the chamber was

removed and replaced with one containing the solute to be tested, or a small volume (usually 50 μl) of the test solution was added directly to one or both compartments. Following this, measurement of I_{sc} and PD was continued as before.

The effect of ATP on unidirectional Na^+ fluxes was determined as follows. ^{24}Na, obtained as Na_2CO_3 in aqueous solution (Nuclear Science and Engineering Corp, Pittsburgh, Pa.) was diluted with an equal volume of 1.9 times isotonic Ringer solution and, if necessary, adjusted to pH 7.2 with 0.5 M H_2SO_4. An appropriate volume of this solution (usually 0.1–1.0 ml, depending on its activity at the beginning of the experiment) was used to replace an equal volume of Ringer solution in one half of the chamber shortly after the tissue was mounted. At the same time a small amount (about 15 μl) of ^{22}Na (NaCl in 0.5 M HCl, New England Nuclear Corp, Boston, Mass.) was added to the other half chamber. It was found by actual measurement that the small amount of HCl added did not change the pH of the Ringer solution. The preparation was allowed to reach a steady state electrically, by which time the isotopic fluxes were also in steady state[1]. After this, 0.1-ml samples were removed from both halves of the chamber at 15-min intervals and transferred to plastic counting tubes (Packard Instrument Co., Downer's Grove, Ill.). Following addition of ATP, sampling was continued until the end of the experiment. The samples were counted in a two-channel Packard Series 5000 autogamma spectrometer using the procedures and precautions outlined by QUAY AND ARMSTRONG[1]. From the results obtained, mucosal to serosal ($J_{m\to s}$) and serosal to mucosal ($J_{s\to m}$) Na^+ fluxes were computed as described by these authors.

RESULTS

Effect of ATP on PD and I_{sc}

Addition of ATP (5 mM) to the mucosal medium elicited marked increases in the PD and I_{sc} observed in oxygenated substrate free media (Fig. 1). The onset of these changes was rapid. They were sustained for long periods of time (though they sometimes showed a tendency to "tail off" gradually after 30–60 min) were completely abolished by rinsing and refilling the mucosal compartment with ATP free medium and could be elicited repeatedly in the same preparation by repeated rinsing and readditon of ATP (Fig. 1). Serosal ATP (5 mM) had no effect on PD or I_{sc}, and no effect on these parameters was observed following rinsing and refilling the serosal compartment with fresh ATP-free Ringer solution. Mucosal ATP did not alter the steady-state tissue resistance.

The effect of metabolic inhibitors and of ouabain on the increase in PD and I_{sc} elicited by mucosal ATP was also studied. Representative examples of these experiments are illustrated in Figs. 2–4. In these experiments, as in the experiment illustrated in Fig. 1, no significant changes were observed in the steady-state resistance of the tissue (*i.e* changes in PD parallelled those in I_{sc}). Therefore, only the I_{sc} values are shown.

Fig. 2 shows that serosal ouabain (2mM) inhibited I_{sc} in the presence of ATP. The time required for the onset of inhibition by ouabain (6–15 min) and the time course of inhibition by this compound were essentially the same whether ATP was present or not[5]. Conversely, addition of mucosal ATP following inhibition of PD and I_{sc} by serosal ouabain elicited only small transient increases in these parameters (Fig. 3).

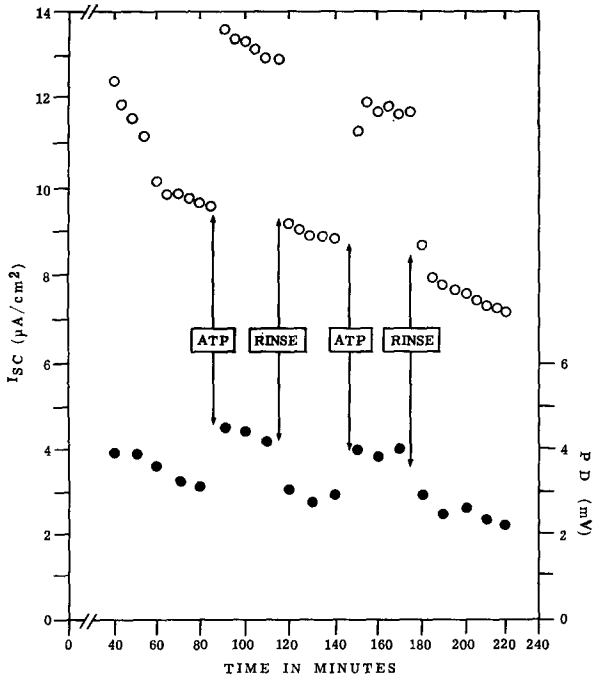

Fig. 1. Effect of 5 mM mucosal ATP on PD (●) and I_{sc} (○) in substrate-free medium.

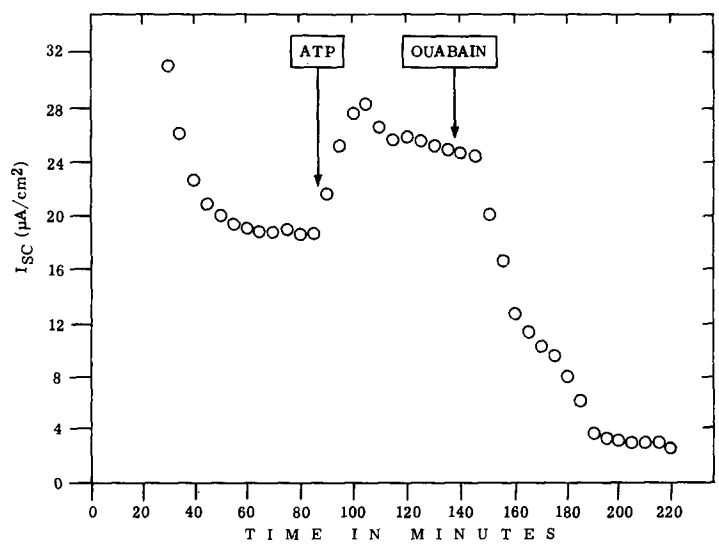

Fig. 2. Effect of 2 mM serosal ouabain on I_{sc} following addition of 5 mM mucosal ATP.

On the other hand, sustained increases in PD and I_{sc} were obtained following addition of ATP to the mucosal fluid during inhibition by anoxia (Fig. 4), anoxia *plus* F^-, or dinitrophenol. Fig. 4 also shows that, under these conditions, the ATP

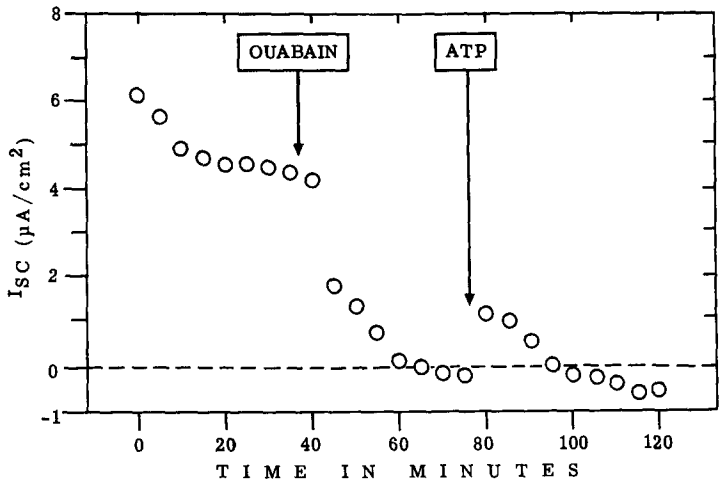

Fig. 3. Effect of 5 mM ATP on I_{sc} following inhibition by 2 mM serosal ouabain.

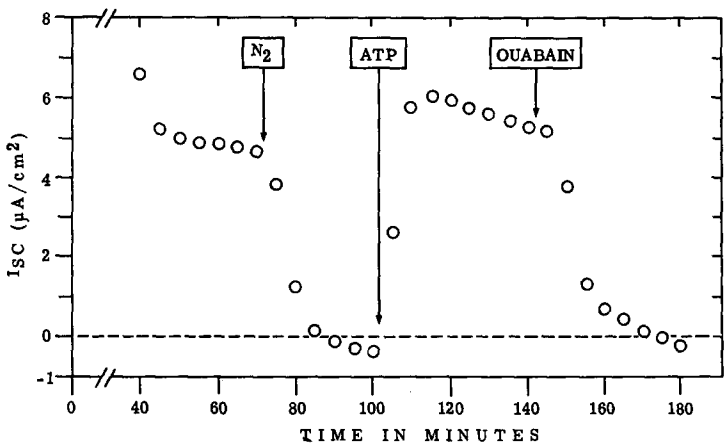

Fig. 4. Stimulation of I_{sc} by 5 mM ATP under anoxic conditions and inhibition of ATP elicited I_{sc} by 2 mM serosal ouabain.

induced current was inhibited by serosal ouabain. The same was true for the corresponding ATP stimulated PD.

Specificity of the response of PD and I_{sc} to ATP

A number of compounds related to ATP were tested (in 5 mM concentration) for their effects on PD and I_{sc}. ITP, GDP, 3'-AMP, 2'-AMP, adenosine, adenine and pyrophosphate were without effect whether added to the mucosal or the serosal solution. GTP added to the mucosal solution elicited small increases in PD and I_{sc} which remained stable for about 30 min.

ADP added to the mucosal solution elicited small transient increases in PD and I_{sc}, *e.g.* in the experiment in which this effect was most noticeable PD increased from 4.3 to 5.3 mV in 10 min following addition of ADP. During the same time I_{sc} increased

from 13.7 to 15.6 μA/cm^2. Both parameters had decreased to control values 30 min after ADP addition. To check the possibility that this transient increase was due to contamination of the ADP by ATP, the former was tested chromatographically for purity. A negligible amount only (25 nM/mM) of ATP was detected*.

Serosal ADP had no effect on PD and I_{sc}. However, 5 mM mucosal ADP rapidly and completely abolished the electrical changes induced by 5 mM mucosal ATP (Fig. 5).

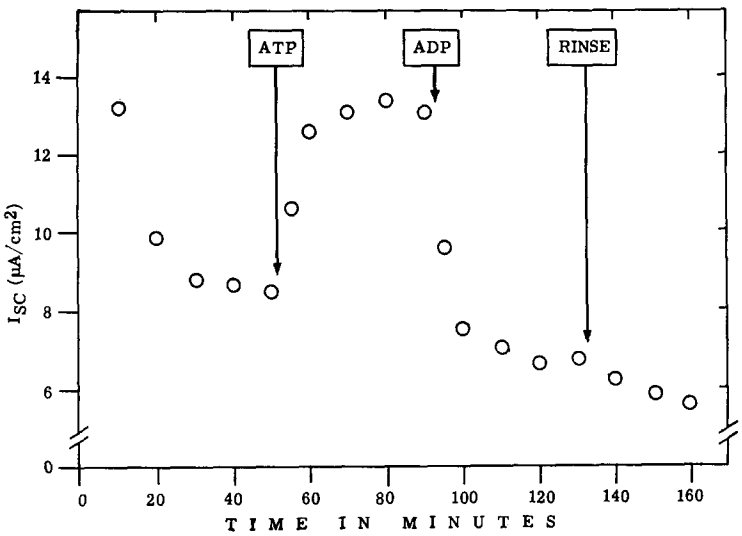

Fig. 5. Effect of 5 mM mucosal ADP on I_{sc} stimulated by 5 mM mucosal ATP.

When 5'-AMP (5 mM) was added to the mucosal solution PD and apparent I_{sc} were reversed (*i.e.* PD became serosal negative and a current consistent with a net mucosal to serosal flow of anions was obtained when the PD was adjusted to zero). Addition of 5'-AMP to the serosal medium increased the (serosal positive) PD and the apparent I_{sc}. Both the mucosal and serosal effects of 5'-AMP were immediately abolished by rinsing and replacing with fresh AMP-free medium. Simultaneous addition of equal amounts of 5'-AMP to both media had little effect on PD or I_{sc}. With tissues mounted between identical Tris sulfate-Ringer solutions (in which steady state PD and I_{sc} are virtually zero in most cases) a negative PD and I_{sc} could be elicited by adding 5'-AMP to the mucosal medium.

Relationship of ATP induced electrical responses to changes in PD and I_{sc} evoked by actively transported sugars and amino acids

In some respects the ATP induced increases in PD and I_{sc} reported herein resemble the increases in these parameters observed in the presence of actively transported sugars and amino acids[2]. In both cases these increases are rapid, well sustained, are only obtained when the solute is added to the mucosal medium, and are quickly reversed by rinsing and refilling the mucosal compartment with solute-free medium.

* We are indebted to Dr. S. Reiser for this analysis.

These points of resemblance raised the possibility that the enhancing effect of ATP on PD and I_{sc} might share a common mechanism with the effects produced by actively transported sugars and amino acids. Since it is well known that the enhancing effects of sugars and amino acids on PD and I_{sc} in small intestine are linked to the transport of these solutes across the mucosal membrane[12] which is, in mammalian intestine at least, markedly dependent on an adequate mucosal O_2 supply[13], the fact

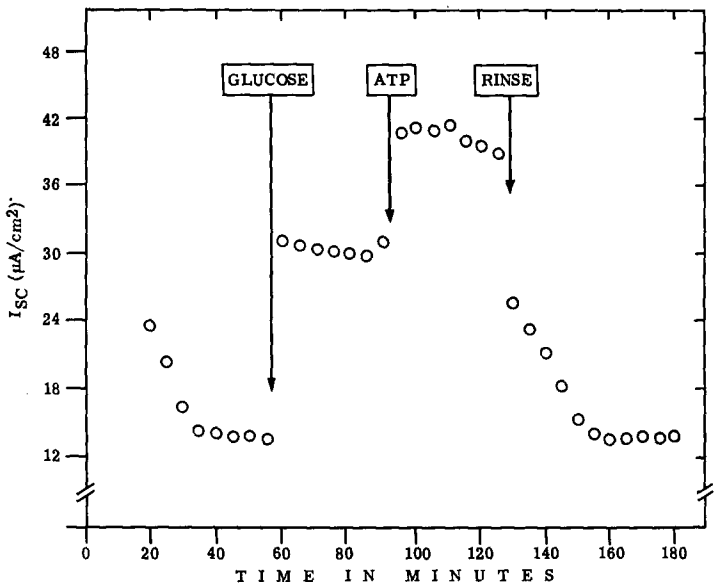

Fig. 6. Additive effects of 11 mM mucosal glucose and 5 mM mucosal ATP on I_{sc}.

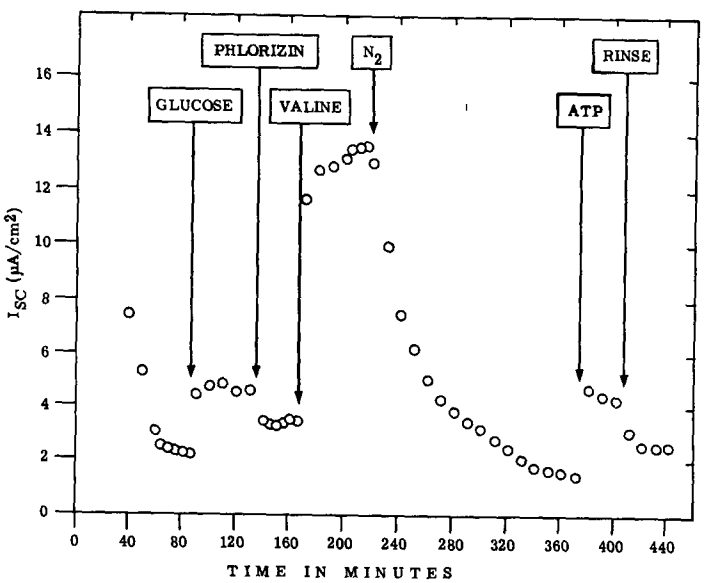

Fig. 7. Differentiation between effects of glucose, valine and ATP on I_{sc}.

that, in the present studies, the response of PD and I_{sc} to ATP could be elicited in the presence of anoxia or metabolic inhibitors seemed to argue against this supposition. In an attempt to obtain more definitive evidence concerning possible interrelationships between the effects of actively transported organic solutes and those of ATP, a further series of experiments was performed.

First, it was found that following virtually maximal stimulation of PD and I_{sc} by mucosal glucose (11 mM) a further increase in these parameters could be obtained by addition of ATP to the mucosal medium (Fig. 6). Similar results were obtained with 65 mM galactose and 20 mM valine. It was further found with glucose and galactose that, following complete inhibition of the hexose evoked PD and I_{sc} with 0.1 mM phlorizin, a typical response of both these parameters to mucosal ATP could be elicited. Finally, an experiment was designed which permitted differentiation between the effects of glucose, those of valine, and those of ATP in the same preparation. The results are shown in Fig. 7. In this experiment steady-state PD and I_{sc} were first stimulated by addition of glucose, the glucose response was then inhibited by addition of phlorizin. Following this a second increase in PD and I_{sc} was induced by adding valine. The valine evoked increase together with any residual effect of glucose were then completely inhibited by N_2. Under these conditions, addition of ATP to the mucosal medium elicited a typical response in the transmural electrical parameters of the tissue.

Effect of ATP on Na^+ fluxes

The increases in PD and I_{sc} elicited by mucosal ATP are indicative of an enhanced net mucosal to serosal transfer of positive charge in the presence of this compound. The increases induced in these parameters by actively transported sugars and amino acids under similar conditions have been accounted for in terms of an increased net mucosal to serosal movement of Na^+ (ref. 1). Therefore, in the present study, the effect of mucosal ATP on the unidirectional mucosal to serosal ($J_{m\to s}$) and serosal to mucosal ($J_{s\to m}$) fluxes of Na^+ was investigated directly by iostopic methods.

In this series of experiments, four were completed in which a sufficiently large and well sustained response of I_{sc} to ATP was obtained to permit a reasonably unequivocal differentiation between the steady-state unidirectional Na^+ fluxes before and after addition of this compound. The results of these experiments can be summarized as follows. In three of them steady-state I_{sc} was extremely low before ATP addition. Correspondingly, no significant difference between $J_{m\to s}$ and $J_{s\to m}$ in nequiv/cm^2 per min (39.0 $\pm$ 5.5 S.E. and 41.6 $\pm$ 3.7 S.E.) for the group was observed during this time. Following addition of ATP the average values in the same units ($\pm$ S.E.) were 50.9 $\pm$ 4.8 for $J_{m\to s}$ and 44.7 $\pm$ 2.9 for $J_{s\to m}$. The average increase in $J_{m\to s}$, 11.9 $\pm$ 0.9 nequiv/cm^2 per min differed significantly from zero ($P < 0.05$). The corresponding increase in $J_{s\to m}$, 3.2 $\pm$ 0.8 nequiv/cm^2 per min did not differ significantly from zero. The mean increase in net mucosal to serosal Na^+ transfer was 6.3 $\pm$ 2.1 nequiv./cm^2 per min, the concomitant increase in I_{sc} being 4.7 $\pm$ 1.3. There was no significant difference ($P > 0.4$) between these values.

DISCUSSION

The response of the PD and I_{sc} across isolated bullfrog intestine to exogenous ATP observed in this investigation shows certain points of resemblance to the results

reported by KOHN *et al.*[7,8] for the effect of this compound on these parameters inthe small intestine of the rat, but has in addition several novel aspects. First, as reported by KOHN *et al.*[8], the response appears to be relatively specific for ATP. Second, in the present study, PD and I_{sc} increased in parallel, as in the experiments of KOHN *et al.*[8]. Third, in both cases the effect of ATP appears to depend primarily on the availability of this compound to the luminal surface of the intestine, and, in our experiments, appears to depend exclusively on the presence of ATP in the mucosal solution. Fourth, in both preparations the ATP response is not inhibited by mucosal phlorizin in a concentration sufficient to inhibit active transfer of hexoses, suggesting that it is mediated by a different mechanism from that implicated in the well known hexose evoked increases in PD and I_{sc}. Our experiments (*e.g.* Fig. 7) further indicate that the ATP response also occurs by a different mechanism from that involved in the stimulation of PD and I_{sc} by actively transported amino acids. A final point of similarity between our results and those of KOHN *et al.*[8] is the fact that in both cases the response of the tissue to ATP is inhibited by serosal ouabain.

Three major differences between our results and those of KOHN *et al.*[7,8] are immediately apparent. First, in our experiments, the ATP elicited increase in PD and I_{sc} is sustained, not transient, whereas in rat intestine the response to ATP usually peaked 60–70 sec after addition of ATP and PD and I_{sc} returned to control values within 6–7 min of this time. Second, in bullfrog intestine, unlike rat intestine, the ATP response appeared to be essentially unaffected by metabolic inhibition (Fig. 7). Third, in our experiments, mucosal ADP in a 1:1 molar ratio to mucosal ATP, rapidly and completely abolished the stimulatory effect of the latter on PD and I_{sc} (Fig. 5), whereas KOHN *et al.*[8] found no inhibition with a mucosal ADP/ATP ratio of 10:1. It seems pertinent to ask whether some explanation, other than the frequently invoked "species difference" can be offered at present for these discrepancies.

At the outset, it is of interest to note that in our experiments ATP was routinely employed in a concentration of 5 mM whereas KOHN *et al.*[7,8] usually used 1 mM ATP. In preliminary experiments we found that, in bullfrog small intestine, 1 mM mucosal ATP elicited a relatively small and transient increase in PD and I_{sc}. This was, however, much slower than the response reported with rat intestine[8] requiring 8–10 min to peak following addition of ATP and having a total duration of about 30 min. At the time we attributed the transience of the response to 1 mM ATP to loss by hydrolysis of this compound from the mucosal medium but KOHN *et al.*[8] have presented evidence that this may not be the case. An alternative explanation is that the small intestine exhibits two discrete responses to mucosal ATP, a transient response at lower concentrations and a sustained response at higher concentrations. In support of this contention one may cite the observation of KOHN *et al.*[8] with the *in vivo* rat intestine that luminal ATP in concentrations greater than 2 mM evoked an increase in PD which did not return to the control level until the lumen was again bathed with ATP free solution, a situation which is essentially similar to our *in vitro* results (*e.g.* Fig. 1). Thus, although KOHN *et al.*[8] reported that their *in vitro* experiments showed a graded response (with a virtually unaltered time course) of PD to ATP over the concentration range 0.01–5 mM, their results do not, in our opinion, exclude the possibility of a second concentration dependent response of the transmural electrical characteristics of small intestine to this compound.

This concept of a sustained response of PD and I_{sc} to ATP which is mediated

by a different mechanism than that involved in the transient responses of these parameters to this compound[8], though it requires further experimental substantiation, offers a tentative explanation at least for the remaining discrepancies noted above, *i.e.* our ability, under the conditions of our experiments, to evoke a marked response of PD and I_{sc} to ATP in the presence of metabolic inhibitors, and the inhibitory effect of mucosal ADP.

The Na^+ flux data obtained in the present investigation are consistent with the hypothesis that the increment in I_{sc} elicited by mucosal ATP can be fully accounted for in terms of an enhanced mucosal to serosal net Na^+ transfer under these conditions. The average net mucosal to serosal flux found in four experiments did not differ significantly from the corresponding I_{sc}. The results of these experiments further indicate that the major effect of ATP is on $J_{m\to s}$ rather than on $J_{s\to m}$. In this connection it is of interest that KOHN *et al.*[14] failed to find any effect of ATP on Na^+ transfer, but did observe some effects of this compound on K^+, Ca^{2+} and Cl^- transport. However, since these authors did not state whether their results were obtained under open circuit or short circuit conditions, the significance of these findings is difficult to assess.

It should be emphasized, however, that while our data support the contention that the ATP driven I_{sc} is entirely (or at least predominantly) a Na^+ current, they cannot be regarded as providing rigorous proof of this conclusion. Because of the relatively small number of observations involved and, because the observed increment in I_{sc} (about 6 nequiv/cm^2 per min) was small compared to $J_{m\to s}$ and $J_{s\to m}$ (these were of the order of 45 to 55 nequiv/cm^2 per min) the power of statistical comparisons such as that between the increment in I_{sc} and the average net mucosal to serosal Na^+ transfer is low. The difficulties attendant upon a rigorous statistical demonstration of the identity between I_{sc} and net Na^+ transfer even under more favorable circumstances than those involved in the present study have been pointed out by QUAY AND ARMSTRONG[1] and it is clear that such proof would require a much more extensive set of observations than those presented herein. In summary, the results of our Na^+ flux experiments, while not providing absolute proof that the ATP elicited I_{sc} is a Na^+ current, justify the adoption of this conclusion as a working hypothesis.

On this basis a tentative model for the mechanism of the ATP effect can be offered. It would appear that this effect, though similar in nature to the enhancing effect of actively transported sugars and amino acids on PD and I_{sc} in that it involves an increase in net mucosal to serosal Na^+ transfer, is mechanistically different from the action of these solutes. This conclusion is supported by the fact that mucosal ATP elicited a characteristic increase in PD and I_{sc} during virtually maximal stimulation of these parameters by glucose (Fig. 6), galactose or valine, that the ATP effect is obtained when active sugar transport is blocked by phlorizin and that the response of PD and I_{sc} to actively transported sugars and amino acids was dependent on the presence of O_2 whereas their response to ATP was not (Figs. 4 and 7)*.

* Some interaction between the transient response of PD and I_{sc} in rat intestine to ATP and the response of these parameters to actively transported sugars and amino acids may be inferred from the fact that hexoses partially inhibited the ATP response[8] (though the inhibition appeared to be more closely related to the metabolic utility of the sugar than to its transport characteristics) and that ATP induces a transient inhibition of amino acid uptake by isolated rat intestinal epithelial cells[15]. In our opinion, these observations underline the necessity for further investigation of the possibility that there may be at least two different responses of the transmural electrical parameters of small intestine to ATP.

On the basis of our present results we would like to suggest that the simplest explanation for the effect of ATP on PD and I_{sc} involves a direct interaction between exogenous (mucosal) ATP and a component of the active Na^+ transfer system, possibly a (Na^+-K^+)-dependent ATPase, which is implicated in the outward pumping of Na^+ across the lateral serosal membrane of the epithelial cells (the localization of the ATP effect in the epithelial cell layer has been convincingly demonstrated by KOHN *et al.*[8]). This suggests that the epithelial cells are, to some extent at least, permeable to ATP since, in other systems, stimulation of uphill Na^+ transport by high-energy phosphate compounds clearly involves an intracellular site of action for these agents[16]. Such permeability could be either a simple diffusion or a more specific mediated transfer and need not necessarily be very great since, of the relatively large amount of ATP present in the mucosal medium, it is not known how much is actually required in the region of the pump sites to stimulate Na^+ transfer. The idea that the mucosal membrane of the epithelial cells may be permeable to ATP has been invoked by KOHN *et al.*[8] and offers a ready explanation of our observation that the response of PD and I_{sc} to ATP is unaffected by metabolic inhibitors but is sensitive to ouabain, a well known inhibitor of membrane bound ATPase[17], and of the inhibition of the ATP response by ADP. The latter could, either by competing with ATP for entry into the cells, or by entering in parallel to ATP, result in an ADP/ATP ratio in the neighborhood of the pump sites unfavorable to pump activity[16].

A second possible route by which ATP might gain access to the epithelial cells may also be considered. There is a growing body of evidence in the literature that extracellular pathways play a significant role in transepithelial transport of water and solutes by intestine and other tissues[18,19]. Recently, a model based on the existence of such a transepithelial "shunt" pathway was developed in this laboratory to account for the effects of actively transported sugars and amino acids on mucosal and serosal epithelial membrane potentials in bullfrog small intestine (see refs. 20 and 21)*. According to this model ATP could gain access to the intercellular spaces from the lumen by an extracellular pathway and enter the cells *via* the lateral/serosal membrane rather than through the luminal membrane. Although we cannot, on the basis of our results, exclude this possibility, it is difficult to reconcile with our observation that serosal ATP did not affect PD or I_{sc} and that mucosal ouabain apparently cannot gain access to the site of its inhibitory action. The results of KOHN *et al.*[8] also seem to imply a differential permeability to ATP of the mucosal and serosal membranes of rat intestine cells. In view of the results obtained by these workers, as well as those reported in this paper, a direct investigation of the permeability of intestinal epithelial cells to ATP and related compounds is clearly desirable.

An alternative explanation, that the stimulating effect of ATP on PD and I_{sc} is a result of the chelating action of this compound[23], is considered unlikely by KOHN *et al.*[8]. Our results support their conclusion. Other nucleoside triphosphates (*e.g.* GTP) also have a powerful chelating effect but have relatively little effect on PD and I_{sc}. In addition, we performed a series of experiments in which the control

* Shortly afterwards, ROSE AND SCHULTZ[22] submitted for publication a paper in which a virtually identical model was proposed to explain their findings on the effect of these solutes on the electrical potential profile of isolated rabbit ileum.

medium containing 1.8 mM Ca^{2+} was replaced by a medium containing 7.2 mM Ca^{2+} and found no change in the enhancing effect of ATP on PD and I_{sc}.

Finally, our results with 5'-AMP may be considered briefly. We have no definitive explanation for these at present ,but they could be consistent with a diffusion of 5'-AMP anions across the intestinal wall as a whole in either the mucosal to serosal or serosal to mucosal direction (in which case they might traverse the epithelial cell layer *via* the extracellular route discussed above). If this is so, our failure to observe similar diffusion artifacts in the presence of 2'-AMP or 3'-AMP is admittedly puzzling and it would seem that the effect of 5'-AMP on PD and I_{sc} should be further investigated.

ACKNOWLEDGMENTS

This work was supported by United States Public Health Service Grant AM 12715 and by facilities provided by U.S. Public Health Service Grant HE 06308 G. A. Gerencser was supported by a predoctoral fellowship (U.S. Public Health Service Grant 00773) and the above work forms part of a thesis submitted by him to Indiana University in partial fulfillment of the University's Ph. D. requirements.

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