Biochimica et Biophysica Acta, 332 (1974) 114–121

BBA 76518

THE EFFECT OF ATP ON THE TRANSPORT OF HEXOSES AND AMINO ACIDS IN EVERTED SACS OF RAT SMALL INTESTINE

PETER T. HARDCASTLE

Department of Physiology, University of Sheffield, Sheffield, S10 2TN (Great Britain)

(Received August 15th, 1973)

SUMMARY

1. The effects of exogenous ATP (5 mM) on the transport of hexoses and amino acids in everted sacs of rat small intestine have been studied.

2. Mucosal ATP is inhibitory to net leucine, glycine and galactose transport, whether transport is expressed as mucosal transfer or final tissue concentration.

3. The inhibition of transfer was specific to the ATP molecule; ADP, AMP, adenosine or adenine producing no effect.

4. The inhibitory effect of ATP could be prevented if additional Ca^{2+} or Mg^{2+} was present in the incubation medium. The divalent cations themselves had no effect on transport.

5. ATP stimulated the efflux of leucine and galactose from preloaded preparations. The stimulatory effect was specific to ATP and could be prevented by extra Mg^{2+} in the incubation medium.

6. The mechanisms by which ATP could influence transport processes are discussed and it is suggested that the results can best be explained by an action of ATP on the passive permeability of the intestinal preparation.

INTRODUCTION

There is now a considerable body of evidence to suggest that active transport across cell membranes can be energized by ATP [1–4]. The small intestine has the capacity to actively absorb hexoses and amino acids and it is probable that the energy for these processes is derived, either directly [5,6] or indirectly [7,8] from the hydrolysis of ATP. One way to investigate whether ATP is supplying energy for transport is to study the effect of exogenous ATP on transport processes. Using this approach ATP has been shown to have the expected stimulatory effect on electrical activity [9,10] and Na^+ transport [11] in small intestine, although it has been found that ATP inhibits rather than stimulates amino acid transport [12,13].

Kohn et al. [10] and Gerencser and Armstrong [11] have both suggested that exogenous ATP plays a direct role in intestinal Na^+ transfer and the aim of the present study has been to examine whether the pumping energy for transport of hexoses and amino acids can be derived from extracellular ATP.

METHODS

Experiments were performed on white male rats of the Sheffield strain. They weighed between 230 and 250 g and were maintained on an unrestricted diet (Diet 86, Oxoid, London) with free access to water.

The preparation used was the sac of everted small intestine [14] as modified by Barry et al. [15]. Sacs were made from the middle fifth (sac III) of the combined jejunum and ileum, representing the distal jejunum. Each sac was filled initially with 1 ml of bicarbonate saline [16] (serosal fluid) and was incubated in 25 ml bicarbonate saline (mucosal fluid) for 5 min at 38 °C in equilibrium with a gas phase of O_2–CO_2 (95:5). This relatively short incubation time was chosen for two reasons: (i) The increased potential difference due to the presence of mucosal ATP lasts for only 5–6 min in rat small intestine [10]. (ii) Rat small intestine rapidly hydrolyses ATP present in the mucosal fluid, but during the first 5 min of incubation only 8.3% of the added ATP is hydrolysed [17].

The transport of D-[^{14}C]galactose, L-[^{14}C]leucine and [^{14}C]glycine was investigated since these are not significantly metabolized in the rat small intestine and they use separate carrier mechanisms [18]. Each amino acid or hexose was added to the mucosal solution only. The adenine nucleotides, nucleosides and adenine were added to the incubation medium as shown in the tables. At the end of the incubation period the final serosal fluid was collected and the gut homogenized and deproteinized. The amount of substance under investigation was determined in each fraction separately by liquid scintillation counting using a liquid scintillator for aqueous solutions [19]. The parameters used for assessing transfer were mucosal transfer, expressed as μmole/sac per 5 min and the final tissue concentration, in mM.

In certain experiments the effect of adenine nucleotides on the efflux of L-leucine and D-galactose from sacs of small intestine was studied. The sacs were loaded with substrate during a 30-min preincubation period in bicarbonate saline containing the labelled substrate. After 30 min the preparation had accumulated significant amounts of substrate in the tissue wall. The sac was then washed to remove adhering substrate and quickly transferred to a flask containing 10 ml of bicarbonate saline containing no labelled substrate. The amount of labelled substrate appearing in the incubation solution was then estimated after a 5-min incubation period at 38 °C.

RESULTS

The presence of 5 mM ATP in the mucosal solution caused a significant inhibition ($P<0.001$) of both the mucosal transfer and the tissue concentration of all the transported substrates (Table I). These observations agree with the demonstration of an inhibitory action of ATP on amino acid transport in sacs of hamster small intestine [12] and in isolated cells of rat small intestine [13] and extends the inhibitory actions of ATP to the hexose transport system. The normal degradation products of ATP (ADP, AMP, adenosine and adenine) [20] had no significant ($P>0.1$) effect on either the mucosal transport or the tissue concentration of the substances tested.

The addition of ATP to the mucosal solution caused a reduction in the pH of the saline. Since variations in the pH of the incubation medium have been shown to affect intestinal function [21,22], the pH of the saline was adjusted to the control value by the addition of $NaHCO_3$ (10 mM). The small increase in the Na^+ concen-

TABLE I

THE EFFECT OF 5 mM EXOGENOUS ATP AND RELATED ADENINE COMPOUNDS ON THE TRANSPORT OF LEUCINE, GLYCINE AND GALACTOSE IN SAC III OF RAT SMALL INTESTINE

7.5 mM leucine, glycine or galactose was present initially in the mucosal fluid and the adenine compounds were present as shown. The initial serosal fluid was Krebs bicarbonate saline. Initial mucosal volume was 25 ml. Incubation time was 5 min at 38 °C. Values given are the mean ± S.E. with the number of experiments in parentheses.

Addition to mucosal solution	Leucine uptake		Glycine uptake		Galactose uptake	
	Tissue concentration (mM)	Mucosal transfer (μmoles/sac per 5 min)	Tissue concentration (mM)	Mucosal transfer (μmoles/sac per 5 min)	Tissue concentration (mM)	Mucosal transfer (μmoles/sac per 5 min)
Control (none)	7.07 ± 0.21	10.40 ± 0.32 (31)	7.51 ± 0.24	11.50 ± 0.50 (9)	8.93 ± 0.39	13.54 ± 0.49 (8)
5 mM ATP	4.80 ± 0.24	6.74 ± 0.33 (17)	5.51 ± 0.38	7.53 ± 0.44 (8)	5.64 ± 0.29	8.08 ± 0.45 (11)
5 mM ADP	7.43 ± 0.48	9.85 ± 0.61 (10)	7.02 ± 0.17	10.96 ± 0.11 (3)	—	—
5 mM AMP	6.40 ± 0.27	9.41 ± 0.58 (10)	7.39 ± 0.11	11.03 ± 0.50 (4)	8.70 ± 0.44	13.41 ± 0.69 (7)
5 mM Adenosine	7.03 ± 0.40	10.05 ± 0.47 (7)	—	—	10.54 ± 0.47	14.87 ± 0.63 (3)
5 mM Adenine	8.48 ± 0.66	11.19 ± 0.84 (6)	—	—	10.11 ± 0.68	14.59 ± 0.28 (3)
5 mM ATP, pH adjusted with $NaHCO_3$	4.32 ± 0.21	5.53 ± 0.16 (5)	—	—	—	—

tration of the incubation medium is thought to have no influence on amino acid transport [13]. After adjustment of the pH, mucosal ATP caused an even greater inhibition of mucosal leucine transport (47% inhibition compared with 35% inhibition with the un-neutralized ATP). All subsequent experiments with ATP were carried out with the pH adjusted to the control value.

ATP can form strong chelates with divalent cations [23,24] and it is possible that it could be exerting its inhibitory effect by combining with membrane-bound divalent cations. The addition of 10 mM $MgCl_2$ to the mucosal solution had no significant effect on either the mucosal transport or the tissue concentration of leucine (Table II). However, $MgCl_2$ prevented mucosal ATP from exerting any inhibitory effect on leucine transport. This effect was not specific to Mg^{2+} since 10 mM $CaCl_2$ in the mucosal solution, which did not itself influence leucine transport, also prevented the inhibition by ATP. It appears that the effect of ATP can be overcome if the divalent cation concentration of the medium is raised to a point where the majority of the divalent ion-complexing species are saturated.

TABLE II

THE EFFECT OF DIVALENT CATIONS ON THE INHIBITORY ACTION OF ATP ON LEUCINE TRANSPORT IN SAC III OF RAT SMALL INTESTINE

7.5 mM leucine was present initially in the mucosal fluid with the additions as shown. The initial serosal fluid was Krebs bicarbonate saline. Initial mucosal volume was 25 ml. Incubation time was 5 min at 38 °C. Values given are the mean ± S.E. with the number of experiments in parentheses.

Additions to mucosal solution	Leucine uptake		
	Tissue concentration (mM)	Mucosal transfer (μmoles/sac per 5 min)	*P* value (mucosal transfer)
Control	7.07 ± 0.21	10.40 ± 0.32 (31)	
5 mM ATP	4.32 ± 0.21	5.53 ± 0.16 (17)	< 0.001
10 mM $MgCl_2$	7.94 ± 0.51	9.85 ± 0.62 (3)	> 0.1
5 mM ATP + 10 mM $MgCl_2$	8.01 ± 0.45	10.13 ± 0.79 (4)	> 0.1
10 mM $CaCl_2$	8.37 ± 0.53	11.46 ± 0.48 (3)	> 0.05
5 mM ATP + 10 mM $CaCl_2$	8.34 ± 0.27	11.05 ± 0.41 (3)	> 0.1

The reduction in the net uptake of actively transferred solutes caused by ATP could be due to either an inhibition of influx into the tissue or to a stimulation of efflux from the tissue to the mucosal solution. In order to distinguish between these possibilities the effect of ATP on the efflux of leucine into the mucosal medium was studied. Table III shows that in the presence of ATP the net efflux of leucine was significantly stimulated ($P < 0.001$). The stimulatory effect appears to be specific for the ATP molecule since ADP and AMP did not significantly alter leucine efflux. The addition of $MgCl_2$ (10 mM) to the incubation solution had no significant effect on leucine movement. However, when ATP was added to the incubation medium the additional Mg^{2+} completely abolished the stimulation of leucine efflux.

ATP may be having no direct effect on the leucine efflux, but may prevent the

TABLE III

THE EFFECT OF ADENINE NUCLEOTIDES ON THE EFFLUX OF LEUCINE FROM SAC III OF RAT SMALL INTESTINE

The sacs were loaded with leucine by preincubation for 30 min at 38 °C with 7.5 mM leucine and the sacs were then reincubated for 5 min at 38 °C in 10 ml Krebs bicarbonate saline with the additions as shown. Values given are the mean ± S.E. with the number of experiments shown in parentheses. *P* values indicate the significance of the difference from the value in the absence of any mucosal addition.

Additions to mucosal solution	Net efflux of leucine (μmoles/sac per 5 min)	*P* value
Control	3.17 ± 0.16 (12)	
5 mM ATP	4.59 ± 0.14 (10)	< 0.001
5 mM ADP	3.30 ± 0.07 (6)	> 0.1
5 mM AMP	3.17 ± 0.12 (4)	> 0.1
10 mM $MgCl_2$	3.45 ± 0.34 (4)	> 0.1
5 mM ATP + 10 mM $MgCl_2$	3.04 ± 0.10 (4)	> 0.1

leucine which has diffused into the incubation medium from being transported back into the tissue. This would result in the increased amount of leucine in the incubation medium. In order to examine this possibility the effects of ATP on the efflux of D-galactose were investigated (Table IV). The movement of D-galactose back into the tissue can be prevented by the presence of $5 \cdot 10^{-4}$ M phlorrhizin in the mucosal medium [25] and the presence of phlorrhizin significantly ($P < 0.001$) increased the amount of galactose appearing in the mucosal solution. ATP produced a stimulation of the amount of galactose released from the tissue, but when both ATP and phlorrhizin were present together in the incubation medium significantly more galactose appeared in the incubation solution ($P < 0.05$). This stimulatory effect was specific to ATP since AMP had no significant effect ($P > 0.05$) on the net efflux of galactose from the tissue.

TABLE IV

THE EFFECT OF ADENINE NUCLEOTIDES ON THE EFFLUX OF D-GALACTOSE FROM SAC III OF RAT SMALL INTESTINE

The sacs were loaded with D-galactose by preincubation for 30 min at 38 °C with 7.5 mM D-galactose, then the sacs were reincubated for 5 min at 38 °C in 10 ml Krebs bicarbonate saline with the additions as shown. Values given are the mean ± S.E. with the number of experiments shown in parentheses.

Additions to mucosal solution	Net efflux of D-galactose (μmoles/sac per 5 min)
Control	3.55 ± 0.30 (4)
$5 \cdot 10^{-4}$ M phlorrhizin	6.25 ± 0.18 (4)
5 mM ATP	5.62 ± 0.26 (5)
5 mM ATP + $5 \cdot 10^{-4}$ M phlorrhizin	7.39 ± 0.36 (4)
5 mM AMP	4.35 ± 0.16 (3)

These results suggest that the inhibitory action of ATP on transport processes is due to an increase in the efflux of the transported solute, rather than a decrease in its influx.

DISCUSSION

Under the conditions employed in this study, 5 mM ATP in the mucosal solution inhibited the mucosal transport of leucine, glycine and galactose. There are four possible ways in which ATP could influence intestinal transport and which would result in the inhibition of net transport observed. (1) ATP could possess structural features which would give it a high affinity for the binding sites on the carriers [12]. (2) ATP could cause pH changes at the surface of the membrane which could decrease transport [12]. (3) ATP could be inhibitory to enzyme systems important to cell metabolism and energy production and this could result in a decreased supply of energy available for active transport [13]. (4) ATP could in some way be influencing the passive permeability of the tissue either by a direct action on membrane structure or by an indirect action on cell enzyme systems influencing cell metabolism necessary to maintain structural integrity.

Since glycine, leucine and galactose are each transported by a different carrier system it is unlikely that the ATP molecule would have structural features which would give it an affinity for all three transport systems. ATP has also been shown to be inhibitory to the transport of basic amino acids [13], which use yet another transport system.

The addition of ATP to the incubation saline causes a drop in its pH and Jackson et al. [21,22] have reported that low pH affects some transport systems. The transport of leucine and glycine is stimulated by low pH, whereas galactose transport is unaffected. Since ATP was inhibitory in all cases it appears that it is not exerting its actions on transport through pH changes. In support of this view is the finding that ATP is more inhibitory to leucine transport when it has been neutralized. After neutralization any stimulation of leucine transport due to lowered pH would no longer occur and the full inhibitory effect of ATP would be observed.

ATP has been demonstrated to be inhibitory to several enzymes [26,27]. By inhibiting enzymes necessary for metabolism exogenous ATP could reduce the amount of energy available in the cell for transport processes. It can clearly be seen how a decreased energy supply could inhibit net transport if the decrease in transport was due to a reduced influx. However, since the inhibition of transport appears to be due, at least in part, to a stimulation of efflux, it is necessary to explain how decreased energy supplies could stimulate efflux. One possibility is that the decreased energy supply could limit the amount of Na^+ pumped out of the cell allowing intracellular Na^+ to accumulate. The reduced Na^+ gradient would allow more accumulated substrate to move out from the cell as well as inhibit the influx [28]. Although the inhibitory actions of ATP on non-electrolyte transport could be explained in terms of its inhibiting energizing processes, this would not directly account for the stimulatory effect of ATP on electrical activity and Na^+ transport.

An increase in the passive permeability of the mucosal membrane by ATP could result in an inhibition of net transport. Under control conditions transported substrate accumulates within the epithelial cells and some of this will diffuse back

into the mucosal solution down its concentration gradient. If ATP increases membrane permeability, without affecting the transport systems at the luminal pole of the cell, this would result in an inhibition of net mucosal transport. Mg^{2+} and Ca^{2+} regulate the permeability of the mucosal surface of the intestine [29,30]. These divalent cations can form strong chelates with ATP [24,31,32] and it is possible that ATP in the mucosal solution could bring about an increase in mucosal permeability by removing divalent cations from the membrane. Such an increase in permeability would explain both the inhibitory effects of exogenous ATP on net intestinal transport and the stimulatory effects on net efflux from preloaded sacs. The actions of ATP are specific to the ATP molecule and this supports the suggestion that ATP exerts its effect by the removal of divalent cations from the membrane since AMP has little ability to chelate divalent cations and adenosine and adenine no ability [23]. ADP has some chelating ability, although less than ATP [32], but has no effect on transport, possibly due to its instability in solution [20], the ADP being hydrolysed before significant amounts of divalent cations can be chelated.

In support of the suggestion that divalent cations are directly involved in the action of ATP is the observation that the addition of Ca^{2+} or Mg^{2+} to the incubation medium prevented both the inhibition of mucosal transport and the stimulation of efflux caused by ATP (Tables II and III). This does not necessarily indicate that both these ions were acting by restoring membrane permeability. The additional metal ions could have opposed the inhibition by chelating with the nucleotide, saturating its chelating capacity. In this form the ATP would no longer be able to increase membrane permeability since it would have lost its ability to chelate any of the membrane-bound divalent cations. The implication of Ca^{2+} involvement in the inhibitory action of ATP on membrane transport is further substantiated by the finding that lack of Ca^{2+} stimulates the efflux of hexoses from rabbit renal cortex slices [33].

It is not necessary to explain the effect of ATP on intestinal Na^+ transport and electrical activity in terms of a direct interaction with the $(Na^+–K^+)$-dependent ATPase. ATP could, by chelating divalent cations, increase the permeability of the mucosal membrane and thus allow more Na^+ to move down its electrochemical gradient into the cell. The resultant rise in intracellular Na^+ concentration would stimulate the electrogenic Na^+ pump, leading to the increase in the potential difference and Na^+ transport observed. This view is supported by the finding that the chelating agent EDTA causes a transient increase in the rat transintestinal potential similar to that caused by ATP [34]. In addition, it has been shown that ouabain inhibits both the effects of ATP and EDTA [11,34] and this is a further indication that ATP is not exerting its effects by a direct interaction with the $(Na^+–K^+)$-dependent ATPase.

There have been many investigations into the effect of ATP on membrane transport in tissues other than the small intestine and in the majority of cases the effects of ATP have been explained in terms of an alteration in membrane permeability [35,36]. Although it is suggested that ATP inhibits intestinal transport by an increase in membrane permeability, the way in which ATP brings about the permeability change is not fully understood. ATP could act directly on the membrane due to its ability to chelate divalent metal ions but is also possible that ATP could affect enzyme systems necessary to maintain cellular structural integrity.

REFERENCES

1 Skou, J. C. (1957) Biochim. Biophys. Acta 23, 394–401
2 Hoffman, J. F. (1962) Circulation 26, 1202–1213
3 Whittam, R. and Ager, M. E. (1965) Biochem. J. 97, 214–227
4 Sen, A. K., Tobin, T. and Post, R. L. (1969) J. Biol. Chem. 244, 6596–6604
5 Csaky, T. Z. (1963) Fed. Proc. 22, 3–7
6 Kimmich, G. A. (1970) Biochemistry 9, 3669–3677
7 Crane, R. K. (1965) Fed. Proc. 24, 1000–1006
8 Schultz, S. G. and Zalusky, R. (1964) J. Gen. Physiol. 47, 1043–1059
9 Lindemann, B. and Curran, P. F. (1962) Proc. Int. Congr. Physiol. Sci. II, 610
10 Kohn, P. G., Newey, H. and Smyth, D. H. (1970) J. Physiol. London 208, 203–220
11 Gerencser, G. A. and Armstrong, W. McD. (1972) Biochim. Biophys. Acta 255, 663–674
12 McConnell, K. P. and Cho, G. J. (1967) Am. J. Physiol. 213, 150–156
13 Reiser, S. and Christiansen, P. A. (1971) Biochim. Biophys. Acta 233, 480–484
14 Wilson, T. H. and Wiseman, G. (1954) J. Physiol. London 123, 116–125
15 Barry, B. A., Matthews, J. and Smyth, D. H. (1961) J. Physiol. London 157, 279–288
16 Krebs, H. A. and Henseleit, K. (1932) Hoppe-Seyler's Z. Physiol. Chem. 210, 33–66
17 Hardcastle, P. T. (1969) Ph.D. Thesis, Sheffield
18 Newey, H. and Smyth, D. H. (1964) J. Physiol. London 170, 328–343
19 Bray, G. A. (1960) Anal. Biochem. 1, 279–285
20 Chaudry, I. H. and Gould, M. K. (1970) Biochim. Biophys. Acta 196, 320–326
21 Jackson, M. J., Levin, R. J. and Thompson, E. A. (1968) J. Physiol. London 197, 16–17P
22 Jackson, M. J., Levin, R. J. and Thompson, E. A. (1969) J. Physiol. London 201, 38–39P
23 Martell, A. E. and Schwarzenbach, G. (1956) Helv. Chim. Acta 39, 653–661
24 Hammes, G. G. and Levison, S. A. (1964) Biochemistry 3, 1504–1506
25 Newey, H., Parsons, B. J. and Smyth, D. H. (1957) J. Physiol. London 139, 21–22P
26 Reynard, A. M., Hass, L. F., Jacobson, D. D. and Boyer, P. D. (1961) J. Biol. Chem. 236, 2277–2283
27 Shepherd, D. and Garland, P. B. (1966) Biochem. Biophys. Res. Commun. 22, 89–93
28 Hajjar, J. J., Lamont, A. S. and Curran, P. F. (1970) J. Gen. Physiol. 55, 277–296
29 Tidball, C. S. (1964) Am. J. Physiol. 206, 243–246
30 Cassidy, M. M. and Tidball, C. S. (1967) J. Cell. Biol. 32, 685–698
31 Taqui Khan, M. M. and Martell, A. E. (1962) J. Phys. Chem. 66, 10–15
32 Walaas, E. (1958) Acta Chem. Scand. 12, 528–536
33 Kleinzeller, A. (1970) Biochim. Biophys. Acta 211, 277–292
34 Hardcastle, P. T. and Eggenton, J. (1971) Biochim. Biophys. Acta 241, 930–933
35 Karjalainen, E. (1968) in Regulatory functions of Biological membranes (Jarnefelt, J., ed.), pp. 107–115, Elsevier, Amsterdam
36 Stewart, C. C., Gasic, G. and Hempling, H. G. (1969) J. Cell. Physiol. 73, 125–131