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Inhibition of amino acid uptake by ATP in isolated intestinal epithelial cells

SHELDON REISER and PHILIP A. CHRISTIANSEN

Gastrointestinal Research Laboratory and Departments of Medicine and Biochemistry, Indiana University School of Medicine and Veterans Administration Hospital, Indianapolis, Ind. 46202 (U.S.A.)

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SUMMARY

The presence of 2 mM ATP in the incubation media inhibited the uptake of 1 mM leucine by isolated intestinal epithelial cells after incubation periods as short as 0.5 min. Inhibition was maximum after 5 min (73.4%) and diminished to 41.4% after 15 min. ATP also inhibited the active uptake of lysine, alanine, valine, and isoleucine. The inhibition was not produced by ADP, 5'-AMP, cyclic 3',5'-AMP, GTP, or inorganic phosphate. Preincubation of the cells with 2 mM ATP inhibited the subsequent uptake of amino acids from ATP-free media. These results suggest an interaction of ATP with an easily accessible site on the cell membrane resulting in an inhibition of a process involved in energizing amino acid uptake.

In a previous study describing the properties of leucine uptake by isolated intestinal epithelial cells, it was found that 2 mM ATP when added to the incubation medium inhibited leucine uptake by about 40%[1]. In view of the role of ATP in energizing monovalent cation transport[2,3] and the synergistic relationship between sodium transport and amino acid transport in intestine[4], an inhibitory effect of ATP on leucine uptake is unexpected. This study represents a confirmation and an extension of the original observation and presents results which define the nature of the ATP inhibition of amino acid uptake.

Wistar strain, male rats weighing 180–260 g were used as a source of the isolated epithelial cells. The animals were fed on a standard diet and watered *ad libitum* but deprived of food 4–8 h prior to sacrifice. The methodology used to prepare the isolated intestinal epithelial cells has been described in detail[1]. The only modification employed was the filtering of the cell suspension through a single layer of gauze prior to the final collection step. To measure amino acid uptake, 0.3 ml of the cells representing an average of 6.40 mg protein were added to 5 ml of an oxygenated Krebs–Ringer Tris buffer (pH 7.4) containing 118 mM NaCl, 25 mM Tris–HCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4 and ^{14}C-radioactive and nonradioactive L-amino acid to a final

concentration of 1 mM. In addition disodium ATP, disodium ADP, monosodium 5'-AMP, cyclic 3',5'- AMP (all Sigma Chemical Company) or disodium GTP (PL Biochemicals, Inc.) were added to the incubation media as indicated in the text to give a medium concentration of 2 mM. When necessary, the pH of the incubation media containing the nucleotides was adjusted to 7.4 with NaOH. The maximum concentration of NaOH added to the media to obtain the desired pH at no time exceeded 3 mM. The small increase in the sodium concentration of the incubation media containing the nucleotides would not be expected to influence amino acid uptake[1]. The reaction mixture was incubated at 37° with shaking for the desired time after which the reaction was terminated by pouring the contents of the reaction mixture into a graduated centrifuge tube in an ice bath, and the cells were centrifuged in the cold at 275 × *g* for 2 min. The cells were then washed and centrifuged 3 additional times with 5 ml cold Krebs–Ringer Tris buffer. The uptake of the amino acids by the cells was determined as previously described[1] and expressed as the intracellular accumulation of the amino acids in the cell water.

Table I shows the effect of 2 mM ATP on the uptake of 1 mM L-leucine by isolated intestinal epithelial cells as a function of time. A significant 35% inhibition of leucine uptake was observed at incubation periods as short as 0.5 min. Since a molecule as highly charged as ATP would be expected to have difficulty entering the cell after incubation periods as short as 0.5 min, it appears that the site of ATP action is at an easily accessible portion of the epithelial cell membrane. The inhibition reached a maximum level of 73.4% at 5 min and then diminished to 41.4% after 15 min. The level of inhibition at 15 min corresponds to the 40% inhibition of the preferential uptake of L-leucine by 2 mM ATP after 15 min reported previously[1]. The time response of the ATP inhibition of leucine uptake is generally similar to the ATP-mediated transient increase in the potential difference (serosal side becoming more positive) and short-circuit current across rat intestine *in vitro*[5].

TABLE I

TIME COURSE OF THE INHIBITION OF 1 mM LEUCINE UPTAKE BY 2 mM ATP IN ISOLATED INTESTINAL EPITHELIAL CELLS

Isolated intestinal epithelial cells were incubated for the indicated times in a Krebs–Ringer Tris medium containing either 1 mM L-leucine or 1 mM L-leucine + 2 mM ATP. Each value represents the mean *plus* and *minus* S.E. from 6 determinations. The percent inhibition values were obtained by dividing the average uptake of leucine in the presence of ATP by the average uptake of leucine in the absence of ATP, subtracting the resulting value from 1 and then multiplying by 100. A paired-difference *t* test was used to obtain the probability values.

Time (min)	*Leucine uptake (mM)*			
	No ATP	*2 mM ATP*	*% ATP Inhibition*	*P*
0.5	0.653 ± 0.056	0.424 ± 0.044	35.1	<0.001
1	1.067 ± 0.060	0.587 ± 0.058	45.0	<0.001
2	1.824 ± 0.091	0.767 ± 0.082	57.9	<0.001
5	2.843 ± 0.214	0.756 ± 0.096	73.4	<0.001
15	2.468 ± 0.233	1.447 ± 0.123	41.4	<0.01

Table II illustrates the specificity of the inhibition both with respect to nucleotide and amino acid. The inhibition of amino acid uptake was completely specific for ATP. The inhibition could not be attributed to cell damage since microscopic examination revealed that cells incubated with 2 mM ATP appeared morphologically identical to cells incubated without ATP[1]. The normal products of ATP reactions such as ADP, 5′-AMP, and inorganic phosphate produced no inhibition. Pyrophosphate produced a 25% inhibition of leucine uptake but this was attributed to the pyrophosphate-induced partial precipitation of calcium and magnesium from the incubation medium[1]. The possibility that ATP acts through the rapid formation of cyclic 3′,5′-AMP by the adenyl cyclase enzyme system which is believed to be situated in the plasma membrane of cells[6] must be excluded since no inhibition of leucine uptake by cyclic AMP could be demonstrated at concentrations as high as 5 mM. The addition of 1 mM theophylline to the medium to inhibit the possible breakdown of the cyclic AMP[7] revealed that theophylline itself inhibited leucine uptake. The possibility that ATP acts through the chelation of a cation necessary for amino acid uptake is improbable since GTP failed to inhibit amino acid uptake[8]. In contrast to the absolute specificity for ATP, the inhibition was shown by every amino acid tested (Table II). In general, the uptake of the amino acids in the presence of ATP approached the 1 mM concentration initially present in the incubation medium suggesting an effect on the energy system required for the active uptake of amino acids. This contention is strengthened by the finding that the ATP inhibition of basic and neutral amino acid transport is of the same magnitude thereby eliminating a specific action of ATP on one of the structurally specific carriers.

Table III shows that an inhibition of amino acid uptake about equal in magnitude to that produced by the presence of 2 mM ATP in the incubation medium, could be produced by preincubation of the cells in Krebs–Ringer Tris buffer containing 2 mM ATP followed by incubation of the washed cells in ATP-free medium. The increase in the potential difference across intact intestine due to ATP was also noted after the tissue had been placed in an ATP-free solution[5]. Preincubation of the cells in isotonic KCl containing 2 mM ATP completely eliminated the subsequent inhibition of leucine uptake. This finding further eliminates the possibility that ATP inhibition is due to cell damage. The ATP-mediated increase in potential difference found in intact rat intestine was similarly eliminated by high concentrations of K^+ (ref. 5).

The similarity in the properties of the ATP inhibition of amino acid uptake in isolated cells and the ATP-mediated increase in the potential difference and short-circuit current in intact intestine[5] suggests that these events may be related. Since increases in the potential difference and short-circuit current of intestine incubated in physiological buffer are usually attributed to an increase of Na^+ movement from mucosal to serosal medium, a stimulation of sodium transport by extracellular ATP is suggested. However, such an increase in the movement of Na^+ across the intestine would be expected to accelerate rather than inhibit amino acid uptake[4]. A continuation of this line of reasoning would necessitate the assumption that the action of ATP dissociates the active transport of amino acids from the downhill movement of Na^+ into the intestine[9]. This assumption is highly suspect without more definitive information regarding the nature of the ion movements responsible for the ATP-induced electrical changes and more conclusive evidence relating the two ATP effects. Assuming that there is no relationship between the ATP inhibition of

TABLE II

EFFECT OF NUCLEOTIDES ON THE UPTAKE OF AMINO ACIDS BY ISOLATED INTESTINAL EPITHELIAL CELLS

Isolated intestinal epithelial cells were incubated for 5 min in a Krebs–Ringer Tris medium containing 1 mM of the indicated amino acids in the absence (controls) and presence of 2 mM of the various nucleotides. Each value represents the mean *plus* and *minus* S.E. from the number of determinations shown in the parentheses. The percent of control values were obtained by dividing the average uptake of the amino acids in the presence of the nucleotide by the average amino acid uptake from an equal number of controls run concurrently. A paired-difference *t* test was used to obtain the probability values. A *P* of 0.05 or less was considered significant and values significantly different from their corresponding controls are italicized.

Nucleotide 2 mM	*Leucine uptake*		*Lysine uptake*		*Alanine uptake*		*Valine uptake*	
	mM/5 min	*% Control*	*mM/5 min*	*% Control*	*mM/5 min*	*% Control*	*mM/5 min*	*% Control*
None; Control (26)	2.781 ± 0.088		2.330 ± 0.086		4.587 ± 0.252		1.992 ± 0.079	
ATP (6)	*0.921 ± 0.121*	*31.3*	*0.956 ± 0.104*	*41.2*	*1.330 ± 0.159*	*30.5*	*1.143 ± 0.115*	*58.7*
ADP (4)	3.052 ± 0.221	105.3	2.650 ± 0.072	99.9	4.847 ± 0.318	96.3	2.330 ± 0.248	97.2
5′-AMP (4)	2.754 ± 0.149	104.0	2.496 ± 0.284	98.1	4.559 ± 0.339	96.2	1.903 ± 0.168	108.6
Cyclic 3′,5′-AMP (4)	2.750 ± 0.150	99.5	2.263 ± 0.151	99.1	4.242 ± 0.228	90.3	2.128 ± 0.154	103.0
GTP (4)	2.602 ± 0.161	107.9	1.947 ± 0.090	99.4	4.335 ± 0.196	97.2	1.894 ± 0.277	110.2
Na_3PO_4 (4)	2.656 ± 0.060	90.3	2.187 ± 0.156	98.1	4.403 ± 0.327	101.2	1.803 ± 0.096	87.5

TABLE III

EFFECT OF PREINCUBATION OF ISOLATED INTESTINAL EPITHELIAL CELLS IN 2 mM ATP ON THE SUBSEQUENT UPTAKE OF AMINO ACIDS

Isolated intestinal epithelial cells were preincubated for 5 min in either a Krebs–Ringer Tris buffer or Krebs–Ring Tris buffer + 2 mM ATP. The cells were then washed once in cold 4° Krebs–Tris and reincubated for 5 min in a Krebs–Ringer Tris medium containing 1 mM of the indicated L-amino acids. Each value represents the mean ± S.E from 6 determinations. A paired-difference *t* test was used to obtain the probability values.

Amino Acid (1 mM)	*Amino Acid Uptake (mM/5 min)*			
	Krebs–Ringer Tris buffer Preincubated	*Krebs–Ringer Tris buffer + 2 mM ATP Preincubated*	*% ATP Inhibition*	*P*
Leucine	2.402 ± 0.325	1.013 ± 0.237	57.8	<0.01
Lysine	2.121 ± 0.120	0.833 ± 0.135	61.7	<0.001
Alanine	3.706 ± 0.296	1.389 ± 0.419	62.5	<0.001
Valine	1.892 ± 0.229	1.002 ± 0.181	47.1	<0.02
Isoleucine	1.651 ± 0.192	1.016 ± 0.208	38.5	<0.02

amino acid uptake and the ATP-induced electrical changes noted in intact intestine, then the present results are best explained by a rapid, K^+-sensitive, interaction of ATP with an easily accessible site on the epithelial cell membrane resulting in an inhibition of a process involved in energizing active amino acid uptake.

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REFERENCES

1 S. Reiser and P.A. Christiansen, *Biochim. Biophys. Acta*, 225 (1971) 123.
2 S. Fahn, G.J. Koval and R.W. Albers, *J. Biol. Chem.*, 241 (1966) 1882.
3 A.K. Sen, T. Tobin and R.L. Post, *J. Biol. Chem.*, 244 (1969) 6596.
4 P.F. Curran, *Physiologist*, 11 (1968) 3.
5 P.G. Kohn, H. Newey and D.H. Smyth, *J. Physiol.*, 208 (1970) 203.
6 T.W. Rall and E.W. Sutherland, *Cold Spring Harbor Symp. Quant. Biol.*, 26 (1961) 347.
7 R.W. Butcher and E.W. Sutherland, *J. Biol. Chem.*, 237 (1962) 1244.
8 E. Walaas, *Acta Chem. Scand.*, 12 (1958) 528.
9 G.A. Kimmich, *Biochemistry*, 19 (1970) 3669.