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EFFECT OF THEOPHYLLINE AND Na^+ ON METHIONINE INFLUX IN Na^+-DEPLETED INTESTINE

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SUMMARY

The unidirectional influx of methionine into the brush border epithelium of chicken jejunum has been studied. Tissues leached of Na^+ transport methionine from a medium devoid of Na^+ with reduced apparent affinity (K_t) and maximal flux (J_{max}). Addition of Na^+ to the medium during a 1-min incubation with substrate, or during a 30-min preincubation, restored K_t but affected J_{max} slightly. Theophylline was found to maintain J_{max} in the absence of Na^+. Essentially complete restoration of K_t and J_{max} could be attained when theophylline-treated tissue was exposed to Na^+ for 30 min. Influx from a Na^+ medium was unaffected by theophylline pretreatment in Na^+-containing buffer. K_t was increased without an effect upon J_{max} when influx was studied from choline medium following preincubation in Na^+.

Modifiers of tissue cyclic AMP levels were investigated in conjunction with theophylline. Histamine and carbachol were found to inhibit theophylline-stimulated transport. Secretin was found to stimulate influx in Na^+-leached tissue, but did not potentiate the theophylline effect. Amino acids in the incubation medium inhibited theophylline-stimulated influx, whereas preloaded lysine or methionine had no effect.

The results are interpreted in terms of a model which envisions roles for cellular and external Na^+ and for cyclic AMP in the activation and regulation of amino acid transport in intestine.

INTRODUCTION

Theophylline, a potent inhibitor of phosphodiesterase [1], has been found to have a number of effects upon various transport processes in tissues. Stimulation of amino acid transport by theophylline has been observed in rat intestine [2], pelvic bone [3] and in liver [4]. Phang and Downing [5], in contrast, have noted that theophylline inhibited amino acid uptake in fetal calvaria bone and other investigators have demonstrated a reversal of growth hormone-stimulated transport in diaphragm [6]. In mammalian intestine, theophylline was discovered to stimulate NaCl secretion, to decrease tissue conductance and to inhibit in a non-competitive fashion the coupled NaCl influx [7–11]; however, passive permeability to urea and mannitol

was not affected [11]. In addition to a possible effect of this alkaloid on cyclic AMP-mediated processes, Plagemann and Sheppard [12] have recently shown that it can inhibit nutrient transport at the cell surface in cultured tissue by a direct competitive action. Nonetheless, in a number of tisssues such as uterus [13], kidney [14], and bone [3, 5] either dibutyryl cyclic AMP, another inhibitor of phosphodiesterase, or cyclic AMP [13, 14] has been shown to stimulate amino acid transport. In calvaria [5] and kidney [14], cyclic nucleotide appeared to enhance uptake by augmenting synthesis of a Na^+-dependent transport system; similarly in liver cycloheximide was found to block cyclic AMP-stimulated transport [15].

In view of the documented effects of Na^+ in augmenting transport in a wide variety of systems [16] and the current reports on theophylline, we chose in this study to investigate the relationship between Na^+ and theophylline effects. Moreover, we have proposed a model which attempts to explain the roles of Na^+ and cyclic AMP in intestinal membrane transport.

METHODS AND MATERIALS

White Rock x Cornish male chickens, 11–15 weeks old, were starved 18 h prior to sacrifice by cervical dislocation. The jejunum was excised, opened along the mesenteric border and washed in previously gassed (O_2 : CO_2, 95 : 5, v/v) 0.9 % NaCl solution enriched with 0.3 % glucose. Jejunal segments were mounted in lucite chambers described by Schultz, et al. [17] for the determination of unidirectional influx of [^{14}C]methionine from the mucosal solution across the brush border into the absorptive epithelium. Krebs–Henseleit bicarbonate buffer of the following composition (mM); NaCl, 118; KCl, 4.7; $CaCl_2 \cdot 2H_2O$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$ 25; at pH 7.4 was used during preincubation unless otherwise indicated. NaCl and $NaHCO_3$ were replaced isotonically by choline chloride and choline bicarbonate in Na^+-free solutions, respectively. The O_2–CO_2 gas mixture was used for both aerating and stirring incubation solutions. Incubations were performed in a room maintained at 36 ± 1 °C. Influx was measured by exposing the mucosa for 1 min to Krebs–Henseleit buffer which contained [^{14}C]methionine and was expressed as nmoles transferred from the medium to the cell per cm^2 of tissue. Immediately following the removal of the incubation solution, the tissue was washed with either physiological saline (when the incubation was performed in Na^+-containing media) or with choline chloride (for incubations carried out in Na^+-depleted media). The exposed mucosal area was punched out, placed in culture tubes containing 1.5 ml of 2.5 % trichloroacetic acid and shaken for 2 h. Radioactivity in the clarified extract was determined by standard scintillation counting techniques. The mediated portion of methionine transport was determined from the difference of influx in the absence and presence of 40 mM leucine, which was a concentration of competitor sufficient to prevent access of tracer to the carrier. The uninhibitable flux was a linear function of substrate concentration to 50 mM methionine and was attributed to non-specific binding and diffusion. Data were corrected for non-mediated entry using an apparent diffusion coefficient of 4.1 nmoles $\cdot$ $cn^{-2} \cdot min^{-1} \cdot mM^{-1}$ which was the slope of the line measuring influx vs methionine concentration in the presence of leucine. Preliminary experiments showed that preincubation in the absence of Na^+, with theophylline, or with 2, 4-dinitrophenol did not detectably alter non-mediated influx. Mannitol was used

as an osmotic control in the inhibition experiments which employed high concentrations of amino acids.

All chemicals were reagent grade except where otherwise noted. L-[^{14}C]methionine was obtained from New England Nuclear Corp. (Boston, Mass.).

RESULTS

Effects of Na^+ and theophylline on methionine influx

Table I summarizes the effects of theophylline and Na^+ on the maximal flux and the apparent Michaelis constant, K_t, for methionine influx in Na^+-depleted intestine. A concentration of 7 mM theophylline was found to produce maximal effects (Fig. 1) and was used in all experiments requiring theophylline pretreatment. The kinetic parameters typically obtained under Na^+-replete conditions are given in Expt 1 in which the intestine was exposed for 30 min to normal Krebs buffer followed by a 1-min exposure to tracer methionine in the same buffer. In the second experiment the tissue was depleted of Na^+ and influx was measured from Na^+-free choline-substituted buffer. The results were a marked decrease in J_{max} and a 4-fold increase in K_t. Expt 3 revealed that incubating the tissue in normal Na^+ buffer following Na^+

TABLE I

EFFECTS OF THEOPHYLLINE AND Na^+ ON J_{max} AND K_t OF METHIONINE INFLUX IN Na^+-DEPLETED INTESTINE

This table summarizes the kinetic constants obtained from Lineweaver–Burk plots. The curves were fitted with the aid of a weighted least squares analysis with J^4 used as the weighting factor [37]. Intestinal segments were mounted in lucite chambers in which only the mucosal surface was exposed to either Krebs–Henseleit buffer (Na^+) or choline-substituted Krebs–Henseleit buffer (no Na^+). Each preincubation period was 30 min; incubation with tracer methionine was for 1 min. Methionine concentration in the incubation solutions ranged from 0.17 to 20 mM. Theophylline was added to the preincubation solutions as indicated at a concentration of 7 mM. Glucose (0.3 %) was added to all solutions. For other details see text.

Experiment	Treatment			J_{max} nmoles · cm^{-2} · min^{-1}	K_t (mM)
	First preincubation	Second preincubation	Incubation		
1	Na^+	None	Na^+	94	1.7
2	No Na^+	None	No Na^+	35	7.0
3	No Na^+	None	Na^+	50	2.2
4	No Na^+	Na^+	Na^+	50	1.6
5	No Na^+ theophylline	None	No Na^+	94	12.0
6	No Na^+, theophylline	None	Na^+	94	4.4
7	No Na^+, theophylline	Na^+	Na^+	94	2.4
8	Na^+, theophylline	None	Na^+	94	1.9
9	Na^+	None	No Na^+	94	4.1

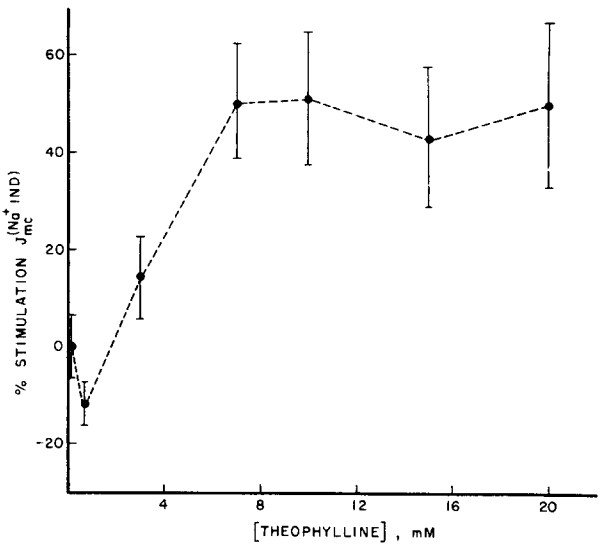

Fig. 1. Dose response curve for theophylline stimulation of methionine influx. Percent stimulation represents stimulation of the total flux in the absence of Na^+, uncorrected for diffusion. Methionine concentration was 0.056 mM. Each point is the mean ±S.E. of 8 determinations on tissues from 4 animals.

depletion resulted in restoration of K_t with only a slight increase in J_{max}. Furthermore, experiments were performed in which methionine influx was measured from solutions containing 20, 40, 60 or 80 mM Na^+ following preincubation in choline buffer. The kinetic parameters obtained under these conditions did not differ significantly from those found in Expt 3 (data not shown). Longer exposure to Na^+ following Na^+-depletion did not alter significantly K_t or J_{max} (Expt 4). These data indicate that the removal of Na^+ from this preparation resulted in a loss of transport capability (as noted by a decrease in J_{max}) which could not be restored by the addition of Na^+, although the effects upon K_t could be reversed by Na^+.

When the jejunum was pretreated with choline buffer, containing theophylline, and then incubated with substrate in the absence of Na^+ (Expt 5), J_{max} was maintained at the level found in Expt 1. These results are in contrast to those reported in Expt 2, and in addition, demonstrate that theophylline pretreatment increased K_t relative to the Na^+-depleted case. In Expt 6, pretreatment with theophylline was followed by incubation with substrate in normal Na^+ buffer. Na^+ did not potentiate the effects of theophylline on J_{max} in accordance with the results in Expt 3; however, K_t was decreased relative to Expt 5. When the initial preincubation with theophylline was followed by a second preincubation in normal Na^+ buffer, the kinetics of influx from a Na^+ medium were virtually the same as those determined without prior Na^+ depletion (Expt 7). In order to ascertain whether the decrease in K_t seen in Expt 7 was due to a prolonged exposure to Na^+ rather than to a loss of theophylline from the tissue into the Na^+-containing medium, the same experiment was performed with the exception that theophylline was added to the second preincubation medium. Under these conditions no significant change was noted in either K_t or J_{max} (data not

shown). These observations allow for the interpretation that prolonged exposure to Na^+ was essential for full restoration of K_t in the theophylline-treated tissue.

The observation that theophylline pretreatment in the presence of Na^+ (Expt 8) did not cause a potentiation of influx when compared to Expt 1 indicates that the actions of these modifiers on J_{max} are not independent. Furthermore, these data support the premise that theophylline functions in maintaining J_{max} under conditions of Na^+ depletion. Experiments were also performed in which preincubation in normal Na^+ buffer was followed by measurement of influx from a choline medium (Expt 9). The tissue was rapidly washed twice in choline buffer to remove adherent Na^+ and then exposed to tracer. In this treatment the J_{max} was unchanged compared with Expt 1, whereas K_t was increased by a factor of two. These findings implicate Na^+ as having an intracellular role in maintaining J_{max}.

Influence of various agents on theophylline-stimulated transport

The mechanism of theophylline stimulation might be to enhance glucose transport and (or) metabolism with the resultant increase in ATP production leading to an augmented energization of methionine influx. This possibility was ruled out by the results of Fig. 2 which show glucose to have no effect on influx.

In preliminary experiments 0.3 mM 2,4-dinitrophenol was found to essentially abolish Na^+-independent influx. Fig. 2 shows that 2,4-dinitrophenol inhibited a portion of theophylline-stimulated transport. The flux not subject to inhibition under these conditions was comparable in magnitude to the difference between the theophylline-stimulated control flux and the Na^+-independent control flux. The Na^+-indepen-

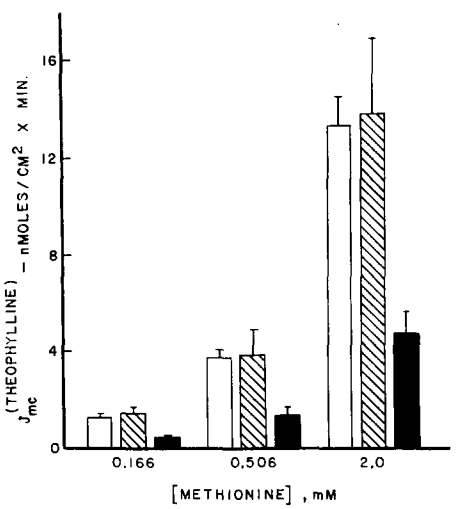

Fig. 2. Effects of dinitrophenol and glucose on theophylline-stimulated methionine influx. Each value represents the mean ±S.E. of 8–24 determinations on tissues from 4–12 animals. Intestinal segments were mounted in lucite chambers and preincubated in the absence of Na^+ with 7 mM theophylline and with (□) or without 0.3% glucose (▩) or with 0.3 mM 2,4-dinitrophenol (■). Glucose was not used in the dinitrophenol experiments. Influx in the presence of dinitrophenol and theophylline was statistically different from influx in the presence of theophylline alone at $P<0.001$ for all concentrations of methionine.

TABLE II

EFFECTS OF VARIOUS MODIFIERS OF CELLULAR CYCLIC AMP LEVELS ON L-METHIONINE INFLUX

Intestinal segments were preincubated in choline-substituted Krebs–Henseleit buffer with or without the indicated additions. Influx was measured in 1 min from choline buffer containing 0.056 mM methionine. Values are the mean ± S.E. of 16 determinations on tissues from 8 animals. The data were not corrected for diffusion. Significance of difference was determined by a paired-difference test.

Modifier	Influx, J (nmoles · cm^{-2} · min^{-1})			
	Control	Modifier	Modifier + theophylline (7 mM)	Theophylline (7 mM)
45 mM carbachol	0.536 ± 0.029	0.557 ± 0.037	0.666 ± 0.042*	0.787 ± 0.058
1 mM histamine	0.424 ± 0.040	0.419 ± 0.023	0.505 ± 0.040*	0.583 ± 0.051
0.36 unit/ml secretin	0.452 ± 0.022	0.523 ± 0.046**	0.689 ± 0.058	0.685 ± 0.048
1.6 units/ml secretin	0.504 ± 0.032	0.696 ± 0.061***	0.807 ± 0.074	0.724 ± 0.063
4.1 mM dibutyryl cyclic AMP†	0.785 ± 0.077††	0.775 ± 0.049††	1.030 ± 0.096	0.861 ± 0.075†††
1 mM imidazole	0.485 ± 0.036	0.466 ± 0.033	0.645 ± 0.065	0.670 ± 0.056
0.1 mM norepinephrine	0.469 ± 0.023	0.452 ± 0.034	0.657 ± 0.037	0.684 ± 0.047

* Versus theophylline-stimulated influx (column 4), $P < 0.05$.
** Versus control, $P < 0.05$.
*** Versus control, $P < 0.02$.
† All preincubation solutions contained 4.1 mM Na^+.
†† Versus column 3, $P < 0.01$.
††† Versus column 3, $P < 0.02$.

dent control values were 1.07 ± 0.17; 2.10 ± 0.31; 6.70 ± 1.2 nmoles · cm^{-2} · min^{-1} for 0.166; 0.506; 2.0 mM methionine, respectively.

The various agents listed in Table II were selected on the basis of their modifying effects on cellular cyclic AMP levels. Carbachol (carbamyl choline chloride) had no effect on the control flux but significantly decreased theophylline-stimulated influx. Similar results were obtained with histamine, although this amine was found to be more potent than carbachol in reducing influx in the presence of theophylline. The duodenal hormone secretin at a concentration of 0.36 unit/ml incubation solution brought about a slight but significant stimulation of influx in the absence of theophylline. At 1.6 units/ml the magnitude of the secretin potentiation was equivalent to that of theophylline. At neither concentration did secretin increase the influx above that caused by theophylline alone when it was used in combination with the alkaloid. The secretin used in these experiments was a commercial preparation (Sigma Chemical Co., St. Louis) known to contain as much as 30% pancreozymin. The possibility exists therefore that the effects may be attributable, at least in part, to the presence of pancreozymin. The interpretation of the results with dibutyryl cyclic AMP was

complicated by the use of the Na^+ salt of this nucleotide which necessitated the addition of 4.1 mM NaCl to all preincubation treatments. Dibutyryl cyclic AMP in combination with theophylline increased influx above the control. However, neither agent alone stimulated influx above the control flux which was substantially greater than that obtained in the absence of Na^+. Thus the stimulatory effect of Na^+ may have masked the normal theophylline potentiation (which appeared to be present, although the data were not significantly different from the control) and possibly that of dibutyryl cyclic AMP. The presence of imidazole or norepinephrine in the preincubation solution was without effect on either the Na^+ independent or the theophylline-stimulated flux.

Specificity of methionine influx

Figs 3a and 3b show the relationship between the inhibition of the Na^+-independent influx and the inhibition of the flux measured in the presence of Na^+ or theophylline by a series of amino acids. The data suggest that the specificity of the transport sites was the same with or without Na^+ or theophylline being present. The correlation coefficient was 0.72 and 0.83 for the data presented in Fig. 3a and Fig. 3b, respectively. Lysine was found to be considerably more effective in reducing influx in the absence of Na^+ than in its presence. A possible explanation for this behavior may be that in the absence of Na^+ the lysine ε-amino group can bind more favorably to a site on the methionine carrier which is normally occupied by Na^+. In the presence of medium Na^+ the lysine interaction would be diminished through competition with Na^+ [18]. Lysine was also found to be an unusually potent inhibitor of the theophylline-stimulated flux.

The absorption of methionine by chicken jejunum appears to occur via a process shared with other aliphatic amino acids, glycine and proline. A second, though relatively minor route of entry not shared with glycine and proline, is also evident

TABLE III

EFFECTS OF PRELOADED LYSINE OR METHIONINE ON Na^+-INDEPENDENT AND THEOPHYLLINE-STIMULATED INFLUX

Jejunal segments were preincubated for 30 min in a choline-substituted Krebs–Henseleit buffer with or without theophylline. Segments were exposed to 10 mM methionine or lysine as indicated during the last 15 min of the preincubation period. Influx was measured from choline buffer containing 0.056 mM methionine. The number of observations was equal to 16 for the lysine preloading experiment and 32 for the methionine preloading experiment. Values represent the mean ±S.E. The data were not corrected for diffusion.

Preloaded amino acid	Influx, J (nmoles · cm^{-2} · min^{-1})			
	Control	Preloaded amino acid	Preloaded amino acid theophylline	Theophylline
Lysine	0.659±0.045	0.625±0.037	0.999±0.082	0.990±0.084
Methionine	0.621±0.032	0.728±0.040*	0.920±0.052	0.838±0.038

* Versus control, $P < 0.01$. Significance of difference was determined using a paired-difference test.

[19]. The specificity data indicate that the stimulatory effects of theophylline reside at the carrier level and that the same carrier(s) are most likely involved which normally transport in the presence of Na^+.

Effect of preloaded amino acid on influx

Table III summarizes the effects of preloaded amino acids on the theophylline-stimulated and the Na^+-independent fluxes of methionine. Preloaded lysine had no influence upon the influx irrespective of the presence or absence of theophylline. On the other hand, preloaded methionine appeared to slightly augment the Na^+-independent influx; however, it did not potentiate the the theophylline-stimulated flux. Thus the mechanism of the theophylline effect does not seem to involve either homo- or heteroexchange diffusion.

DISCUSSION

Kinzie and coworkers [2] observed that theophylline stimulated transport of neutral and basic amino acids in rat jejunum. The effect was seen as a decrease in K_t with no change in maximal uptake from a Na^+-containing medium. In contrast, we found an enhancement of the maximal flux and a slight increase in K_t under conditions of Na^+ depletion. According to Kinzie et al. [2] this enhancement in the rat was positively correlated with tissue cyclic AMP levels which could be maintained at about 95 % of those present in fresh tissue. Without theophylline, cyclic AMP concentrations fell rapidly during a period of incubation in vitro.

Dibutyryl cyclic AMP was shown to enhance neutral amino acid accumulation in the rat jejunum [2]. In our system this nucleotide potentiated the effects of a low concentration of Na^+ only when theophylline was also present during the preincubation. Theophylline alone appeared to potentiate the action of Na^+ in these experiments. In an effort to explain these results we must consider that with even small amounts of Na^+ present, the system may be near full activation as measured by J_{max}. We are limited therefore by our ability to experimentally determine small additional potentiations above the effects of Na^+.

Secretin was found to stimulate influx in our system without potentiation of the effect of theophylline. In rat pancreas and in isolated fat cells this hormone was shown to increase cyclic AMP levels [20, 21]. In addition to these findings, both cholera toxin, an agent that increases intestinal cyclic AMP levels through its action on adenylate cyclase [22], and secretin affect water and ion fluxes in a similar manner [23]. Moreover, the toxin was observed to enhance leucine transport in rat intestine [2]. These findings suggest that secretin may also act upon adenylate cyclase to increase cyclic AMP levels in intestine and thereby influence transport. The action of histamine in our study to reduce theophylline-stimulated transport was consistent with the findings that this amine activates phosphodiesterase in tissue homogenates [24, 25]. Carbachol, which was found to reduce tissue cyclic AMP levels in rat pancreas [20], also inhibited the theophylline-stimulated influx in chicken jejunum. The known effects of theophylline and dibutyryl cyclic AMP to inhibit phosphodiesterase activity and the evidence presented above on the other effectors of cyclic AMP levels suggest that the theophylline-stimulated influx in chicken jejunum is mediated by cyclic AMP.

Although a slight stimulation of influx by preloaded methionine was noted in chicken intestine, preloading had no effect on the theophylline-stimulated influx. Therefore, the mode of action of this alkaloid does not appear to involve exchange diffusion. This conclusion is also supported by the studies with preloaded lysine. The unusual effectiveness of lysine in reducing theophylline-stimulated influx therefore cannot be explained by its interaction with a heteroexchange process. These observations coupled with the specificity data presented in Fig. 3 and the finding that dinitrophenol completely abolished influx in the absence of Na^+ seem to rule out the possibility that theophylline is stimulating transport sites which are characteristically Na^+ independent. This conclusion is supported by reports in the literature which indicate that Na^+-independent transport systems are characterized by exhibiting homo- and heteroexchange diffusion [26, 27], specificities which differ from those found for Na^+-dependent processes [28], and insensitivity to the presence of metabolic inhibitors [28].

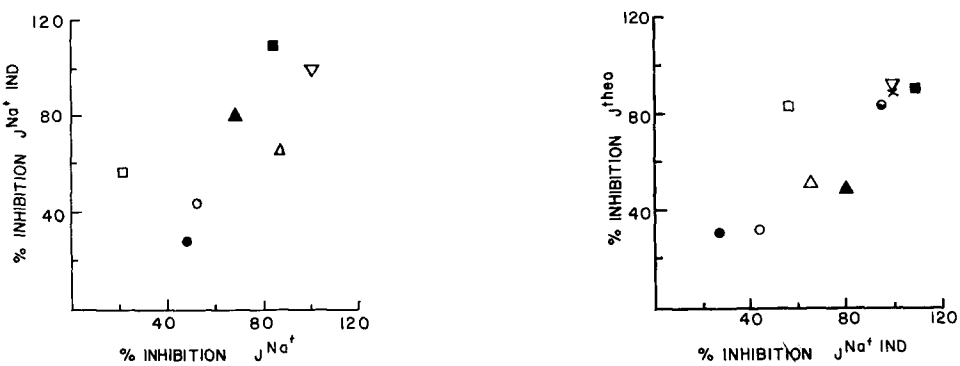

Fig. 3. (a) Comparative inhibitory effectiveness of various amino acids on methionine influx in the absence (J_{Na+IND}) and presence (J_{Na+}) of Na^+. Intestinal segments were preincubated for 30 min in either Na^+ buffer or choline buffer. Influx was measured in 1 min from the respective buffers containing 0.056 mM methionine. ●, 100 mM proline; ○, 100 mM glycine; □, 10 mM lysine; △, 50 mM phenylalanine; ▲, 40 mM tryptophan; ■, 120 mM alanine; ▽, 40 mM leucine. (b) Comparative inhibitory effectiveness of various amino acids on methionine influx in the absence of Na^+ (J_{Na+IND}) and presence of theophylline (J_{theo}). Intestinal segments were preincubated for 30 min in choline buffer with or without 7 mM theophylline. Influx was measured in 1 min from choline buffer containing 0.056 mM methionine. ●, 100 mM proline; ○, 100 mM glycine; □, 10 mM lysine; △, 50 mM phenylalanine; ▲, 40 mM tryptophan; ■, 120 mM alanine; ▽, 40 mM leucine; ◒, 60 mM valine; X, 60 mM isoleucine.

Kinetic models describing the role of extracellular Na^+ in transport processes has been extensively reviewed by Schultz and Curran [16]. Recently Kimmich [29] has proposed a model describing a functional role for intracellular Na^+ in the activation and regulation of solute transport. Neither kinetic models nor Kimmich's hypothesis can be exclusively considered as explanations for the action of Na^+ on methionine influx across chicken brush border membrane. This conclusion is based upon the observations that (1) intracellular Na^+ appears to be required for transport apart from its function as a cosubstrate; (2) influx in the absence of Na^+ is dependent upon oxidative metabolism as an energy source; (3) cyclic AMP is implicated as a

mediator of influx as demonstrated in our work and in the rat intestine [2]. Instead we feel that observations reported in this paper are consistent with the concept that Na^+ exerts a dual influence on methionine influx. External Na^+ is considered to be an activator of influx by enhancing the affinity of the transport sites for methionine as proposed by Crane [30] and Schultz and Curran [16]. Evidence for this interpretation is given in Table I (Expts 3 and 4) in which medium Na^+ functions mainly to decrease K_t while having little effect on J_{max}. Expt 9 also supports this view since in the absence of external Na^+ K_t was found to be increased. The discrepancy between the K_t values reported in Expt 9 and in Expt 2 may be attributable to activation of carrier affinity by a small amount of Na^+ trapped in the unstirred layers. Moreover our experiments demonstrated that 20 mM Na^+ in the bathing medium was sufficient to restore K_t after Na^+ depletion. The second role of Na^+ appears to be in supporting the maximal flux, but this action is considered to be an intracellular function. This concept is supported by the observations that J_{max} was decreased by the removal of tissue Na^+, whereas this parameter was unchanged when tissues bathed in Na^+ buffer were incubated with tracer in choline medium.

The scheme shown in Fig. 4 provides a working model to account for the data presented in this paper. Cyclic AMP is envisioned to play a central role in the regulation of amino acid transport in the intestine by activating a Na^+-sensitive protein kinase. This enzyme in turn phosphorylates a membrane element, a hypothetical transport carrier or some intermediate, and by this process enhances translocation of the carrier through the membrane. This hypothesis is supported by the findings that cyclic AMP caused phosphorylation of mitochondrial and lysosomal membranes of rat liver [31] as well as phosphorylation of membrane proteins in human erythrocytes ghosts [32]. As a result of phosphorylation, mitochondrial permeability may be altered to allow for increased pyruvate uptake [31]. Alternatively, dephosphorylation of a specific membrane protein in the toad bladder has been suggested as a mechanism by which cyclic AMP regulates Na^+ and (or) water transport [33]. The action of intracellular Na^+ directly on influx is to inhibit the association of the cyclic AMP-binding regulatory (R · cAMP) and the cyclic AMP-independent catalytic (C) subunit as demonstrated by Corbin et al. [34] for the effects of NaCl on protein kinase

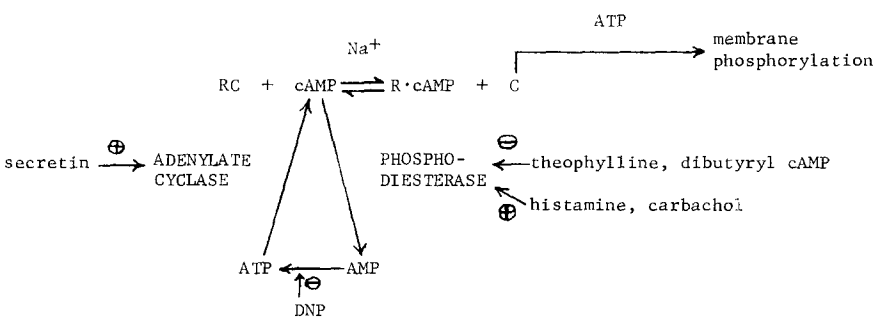

Fig. 4. Working model for the role of cellular Na^+ and Cyclic AMP in the transport of methionine. Symbols are defined as follows: RC, inactive protein kinase; R · cAMP, cyclic AMP binding regulatory subunit complex; C, cyclic AMP-independent catalytic subunit; DNP, 2,4-dinitrophenol; ⊕, activation of enzyme activity; ⊖, inhibition of enzyme activity. For other details see text.

in rat adipose tissue. These workers showed that NaCl addition decreased the requirements of this enzyme for cyclic AMP, although the salt did not affect the maximal activity when enough cyclic AMP was available (cf. Fig. 12 of ref. 34). The latter condition presumably would prevail in chicken intestine treated with theophylline during the course of Na^+ depletion. The removal of Na^+ from our system has been found to reduce the maximal flux, which cannot be restored by a subsequent exposure to either Na^+ or theophylline. In terms of the model, these observations may be explained as follows: Na^+ depletion causes a decrease in ATP production or in some other manner reduces cyclic AMP levels. The diminished levels of cyclic AMP, even in the presence of added Na^+ (or theophylline), only minimally activate protein kinase (cf. Fig. 12 of ref. 34). The ability of dinitrophenol to abolish transport in the absence of Na^+ is consistent with the concept that cyclic AMP and ATP are the primary regulators of carrier permeability. This premise is also supported by the observations that theophylline promotes influx even in the presence of dinitrophenol. Under the latter conditions there are apparently sufficient levels of cyclic AMP and ATP to sustain membrane phosphorylation despite the fact that dinitrophenol may prevent further ATP production via oxidative phosphorylation. Moreover, ATP from other sources than oxidative phosphorylation can possibly provide energy under these conditions. In this respect, theophylline may mediate cyclic AMP-induced mobilization of energy as exemplified by the cyclic AMP-induced increase in glycogenolysis observed in rat liver [35]. Potashner and Johnstone [36] have shown, in fact, that added energy sources such as glutamine and glutamate could sustain methionine transport in the presence of dinitrophenol in the Ehrlich cell. Theophylline may also repress diversion of endogenous ATP through the cyclic AMP pathway and thereby increase somewhat the supply of ATP for phosphorylation. While the model represents a working hypothesis to account for the observations reported in this paper, further studies must include determination of cellular levels of cyclic AMP and ATP and measurement of protein kinase activity.

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REFERENCES

1 Sutherland, E. W., Robison, G. A. and Butcher, R. W. (1968) Circulation 37, 279–306
2 Kinzie, J. L., Ferrendelli, J. A. and Alpers, D. H. (1973) J. Biol. Chem. 248, 7018–7024
3 Adamson, L. F. (1970) Biochim. Biophys. Acta 201, 446–452
4 Harrison, L. I. and Christensen, H. N. (1971) Biochem. Biophys. Res. Commun. 43, 119–125
5 Phang, J. M. and Downing, S. J. (1973) Am. J. Physiol. 224, 191–196
6 Rillema, J. A., Kostyo, J. L. and Gimpel, L. P. (1973) Biochim. Biophys. Acta 297, 527–539
7 Powell, D. W., Binder, H. J. and Curran, P. F. (1972) Am. J. Physiol. 223, 531–537
8 Fromm, D. (1973) Am. J. Physiol. 224, 110–116
9 Al-Awqati, Q., Cameron, J. L. and Greenough, W. B. (1973) Am. J. Physiol. 224, 818–823
10 Nellans, H. N., Frizzell, R. A. and Schultz, S. G. (1973) Am. J. Physiol. 225, 467–475

11 Frizzell, R. A., Nellans, H. N., Rose, R. C., Markscheid-Kaspi, L. and Schultz, S. G. (1973) Am. J. Physiol. 224, 328–337
12 Plagemann, P. G. W. and Sheppard, J. R. (1974) Biochem. Biophys. Res. Commun. 56, 869–875
13 Griffin, D. M. and Szego, C. M. (1968) Life Sci. 7, 1017–1023
14 Weiss, I. W., Morgan, K. and Phang, J. M. (1972) J. Biol. Chem. 247, 760–764
15 Tews, J. K., Woodcock, N. A. and Harper, A. E. (1970) J. Biol. Chem. 245, 3026–3032
16 Schultz, S. G. and Curran, P. F. (1970) Physiol. Rev. 50, 637–718
17 Schultz, S. G., Curran, P. F., Chez, R. A. and Fuisz, R. E. (1967) J. Gen. Physiol. 50, 1241–1260
18 Thomas, E. L. and Christensen, H. N. (1970) Biochem. Biophys. Res. Commun. 40, 277–283
19 Burrill, P., Sattelmeyer, P. and Lerner, J. (1974) Fed. Proc. 33, 410
20 Heisler, S., Grondin, G. and Forget, G. (1974) Life Sci. 14, 631–639
21 Butcher, R. W. and Carlson, L. A. (1970) Acta Physiol. Scand. 79, 559–563
22 Field, M. (1971) New Engl. J. Med. 284, 1137–1144
23 Moritz, M., Finkelstein, G., Meshkinpour, H., Fingergut, J. and Lorber, S. H. (1973) Gastroenterology 64, 76–80
24 Butcher, R. W. and Sutherland, E. W. (1962) J. Biol. Chem. 237, 1244–1250
25 Honeyman, T. and Goodman, H. M. (1971) Fed. Proc. 30, 435
26 Christensen, H. N. (1969) in Advances in Enzymology (Nord, F. F., ed.), pp. 1–20, Interscience, New York
27 Reiser, S. and Christiansen, P. A. (1973) Biochem. Biophys. Acta 307, 223–233
28 Potashner, S. and Johnstone, R. M. (1970) Biochim. Biophys. Acta 203, 445–456
29 Kimmich, G. A. (1970) Biochemistry 9, 3669–3677
30 Crane, R. K. (1965) Fed. Proc. 24, 1000–1006
31 Zahlten, R. N., Hochberg, A. A., Stratman, F. W. and Lardy, H. A. (1972) Proc. Natl. Acad. Sci. U.S. 69, 800–804
32 Guthrow, C. E., Allen, J. E. and Rasmussen, H. (1972) J. Biol. Chem. 247, 8145–8153
33 DeLorenzo, R. J., Walton, K. G., Curran, P. F. and Greengard, P. (1973) Proc. Natl. Acad. Sci. U.S. 70, 880–884
34 Corbin, J. D., Soderling, T. R. and Park, C. R. (1973) J. Biol. Chem. 248, 1913–1921
35 Friedmann, N. (1972) Biochim. Biophys. Acta 274, 214–225
36 Potashner, S. J. and Johnstone, R. M. (1971) Biochim. Biophys. Acta 233, 91–103
37 Cleland, W. W. (1967) in Advances in Enzymology (Nord, F. F., ed.), pp. 1–32, Interscience, New York