Vol. 115, No. 2, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 15, 1983 Pages 756-761

CYCLIC ADENOSINE-3',5'-MONOPHOSPHATE AND FOLATE TRANSPORT IN RAT JEJUNUM

Hamid M. Said*+ and Williamson B. Strum

Department of Basic and Clinical Research, Scripps Clinic and Research Foundation, La Jolla, California 92037

Received August 11, 1983

The intestinal transport of 5-methyltetrahydrofolate and pteroylmonoglutamate was examined in everted sacs of rat jejunum exposed to compounds which increase intracellular cyclic adenosine-3', 5'-monophosphate. Adenyl cyclase stimulators (hydrocortisone and prostaglandin), phosphodiesterase inhibitors (3-isobutyl-1-methylxanthine, aminophylline and papaverine), and dibutyryl adenosine-3',5'-cyclicmonophosphate added to the mucosal medium inhibit the mucosal-to-serosal transport of physiological concentrations of 5-methyltetrahydrofalate and pteroylmonoglutamate. Transport inhibition is correlated with the ability of these agents to increase cellular cyclic adenosine-3', 5'-monophosphate . The active, carrier-mediated transport system of folate compounds is highly sensitive to the increase in cyclic adenosine-3', 5'-monophosphate level, while the diffusion system is insensitive. These data indicate that the active transport system of folates is modulated by cellular cyclic adenosine-3', 5'-monophosphate.

The intestinal transport system of 5-methyltetrahydrofolate ($5\text{-}CH_3H_4PteGlu$), pteroylmonoglutamate (PteGlu) and amethopterin is composed of two distinct processes: 1) an active, carrier-mediated system which functions at low, physiological concentrations, and 2) a diffusion system which is demonstable at high concentrations (1-10). The active system is characterized by: a) saturation kinetics with an apparent K_t of 0.3 μM for $5\text{-}CH_3H_4PteGlu$, 4 μM for PteGlu and 3.2 μM for amethopterin; b) accumulation against a concentration gradient; c) inhibition by metabolic poisons; d) inhibition by structural analogues; e) temperature-dependence; f) Na^+-dependence, and g) specificity for the jejunum. These features are

* To whom correspondence should be sent.

+ Present address: Department of Medicine, Division of Gastroenterology, University of California, Irvine, California 92717

Abbreviations used: $5\text{-}CH_3H_4PteGlu$, 5-methyltetrahydrofolate; PteGlu, pteroylmonoglutamate; cAMP, cyclic adenosine-3', 5'-monophosphate;

0006-291X/83 $1.50

profoundly pH-dependent with an optimum at pH 6 and are less detectable or absent at pH 7 and higher or pH 5 and lower (1-10). The diffusion system is characterized by: a) a linear increase in the mucosal-to-serosal transport of 5-CH_3H_4PteGlu above 1 μm and of PteGlu and amethopterin above 10 μM, b) energy-independence (1, 6-8), and c) pH-independence (1, 6-8). These features of the transport process of 5-CH_3H_4PteGlu, PteGlu and amethopterin are almost identical. 5-CH_3H_4PteGlu is unique in requiring glucose in the incubation medium and having a lower apparent K_t (7).

Cyclic adenosine-3', 5'-monophosphate (cAMP) regulates many intestinal absorption and secretion processes (11-13), yet its effect on folate transport has received limited attention (13). The quantitative demonstration of its influence on the transport of folate compounds would further our knowledge about the intestinal transport of these nutrients. The studies presented indicate that cAMP has a pronounced effect on the intestinal transport of folate compounds.

Materials and Methods:

Chemicals:

5-CH_3H_4PteGlu-barium salts (specific activity, 58 mCi/mmol) and [3',5',7,9-^{3}H] PteGlu-potassium salt (specific activity 500 mCi/mmol) purchased from Amersham/Searle Corp., Des Plaines, Ill.; prostaglandin (16,16-dimethyl PGE_2) was a gift from Dr. Mary J. Rumart (Upjohn Company, Kalamazoo, Michigan). 3-Isobutyl-1-methylxantnine, aminophylline, papaverin, hydrocortisone, dibutyryl adenosine-3',5'-cyclicmonophosphate and bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.). Cyclic adenosine-3'-5'-monophosphated determination Kit (Bochringer Mannheim, Indianapolis, Ind.). All chemicals were of the highest purity available.

Transport studies and cAMP determinations:

Male albino Sprague-Dawley rats weighing 180 to 220 g were sacrificed by a blow to the head followed by immediate cervical dislocation. The abdomen was opened and the jejunum, starting 14 cm from the pylorus, was removed, washed with ice cold buffer and divided into four equal segments of 8 cm. Everted sacs were then prepared and 0.5 ml of the phosphate buffer used for incubation at pH 6 was introduced to there serosal compartment. (The buffer medium contained: 110 mM NaH_2PO_4, 35.2 mM NaCl, 5.5 mM KCl, 1.8 mM $MgSO_4$, 20 mM glucose and 3 mg/ml sodium ascorbate). The sacs were incubated for 30 min, which in prior study showed to be within the linear rate of transport (5), at 37°C in 25 ml Erlenmeyer flasks containing 10 ml of continuously oxygenated (5% CO_2 in O_2) incubation medium. At the end of incubation, sacs were removed, washed and the serosal content was drained and analyzed for radioactivity. For cAMP determination the mucosal layer of the jejunum, after the transport study was performed, was removed by scraping using a microscope slide and was suspended in 10 ml of ice cold 0.9% NaCl solution to which 5 ml of 10% trichloroacetic acid was added. The tissue was homogenized and the supernatant was recovered by centrifugation for 20 min at 4°C (16,000 x g). Four ml of the supernatant was taken, acidified with 4.3

ml of 2 M HCl and extracted 4 times with water-saturated ether to remove trichloroacetic acid. After being lyophilized, each sample was resuspended in 1 ml of 0.05 M sodium acetate buffer pH 4.3 and assayed for cAMP using the isotope dilution method of Gilman (14) modified by inclusion of ammonium sulfate precipitation as described by Illiano et al (15). Protein content of the precipitate was assayed by biuret reaction with bovine serum albumin as the standard (16). The in vitro tissue preparation was judged to be viable as it supported a potential difference of 9.1 mV $\pm$ 0.5 for 30 min and transported 0.02 mM L-leucine against concentration gradient (serosal/mucosal concentration ratio of 1.90 $\pm$ 0.20 was recorded after 30 min incubation) (5,7). All transport determinations were performed in triplicate and the results were expressed as a percentage relative to simultaneous untreated controls. cAMP determination was performed in duplicate and the result expressed as pmoles/mg protein.

Results and discussion:

The addition of adenyl cyclase stimulators and phosphodiesterase inhibitors to the mucosal medium of jejunal everted sacs markedly inhibits the mucosal-to-serosal transport of 5-CH_3H_4PteGlu and PteGlu and increases cAMP content of the intestinal mucosa compared to untreated controls (Table 1). The inhibitory effect of prostaglandin-E_2 on the transport of 5-CH_3H_4PteGlu increases as the concentration of the inhibitor in the mucosal medium increases and was associated with a progressive increase in mucosal cAMP content. A combination of either 0.5 mM papaverine and 5 mM 3-isobutyl-1-methylxanthine, or 1 mM hydrocortisone and 5 mM 3-isobutyl-1-methylxanthine added to the mucosal medium causes greater inhibition in the transport of 5-CH_3H_4PteGlu and PteGlu than either compound alone. Dibutyryl cAMP in the mucosal medium also inhibits the mucosal-to-serosal transport of 5-CH_3H_4PteGlu and PteGlu and the inhibition is dependent on the inhibitor concentration (Table 1). The inhibitory effect of dibutyryl cAMP cannot be attributed to the presence of butyrate impurities, since 0.1 and 1 mM butyric acid shows no effect on the transport process. The inhibitory effect of adenyl cyclase stimulators and phosphodiesterase inhibitors, which was associated with an increase in mucosal tissue cAMP content, and the inhibitory effect of dibutyryl cyclic AMP on the transport rate of 5-CH_3H_4PteGlu and PteGlu suggest that the inhibition in the transport of folate compounds is mediated in part through an increase in cellular cAMP.

TABLE 1

EFFECT OF ADENYL CYCLASE STIMULATORS AND PHOSPHODIESTERASE INHIBITORS ON MUCOSAL-TO-SEROSAL TRANSPORT OF 5-CH_3H_4PteGlu AND PteGlu AND JEJUNAL MUCOSAL cAMP

Compound	Concentration (mM)	% Inhibition		Mucosal cAMP (pmole/mg protein)
		5-CH_3H_4PteGlu	PteGlu	
Control		0	0	3.2
Adenyl Cyclase Stimulators				
Hydrocortisone	1	65	69	6.0
Prostaglandin (PGE_2)	0.005	23		4.7
	0.05	48		5.3
	0.5	59		6.0
Phosphodiesterase Inhibitors				
3-isobutyl-1-methylxanthine	5	68	70	7.3
Papaverine	0.5	67	71	5.7
Aminophylline	10	54	60	7.7
Papaverine + 3-isobutyl-1-methylxanthine	0.5 5	76	75	9.0
Hydrocortisone + 3-isobutyl-1-methylxanthine	1 5	80	76	
Dibutyryl cAMP	1	40	32	
	10	73	62	
Butyric acid	0.1	0	0	
	1	0	0	

Rat jejunal everted sacs were incubated in phosphate buffer pH 6 for 30 min at 37°C. 5-CH_3H_4PteGlu (0.1 μM) or PteGlu (1 μM) was added to mucosal medium at the outset of the incubation.

Rat jejunum, which accumulates low concentrations of 5-CH_3H_4PteGlu and PteGlu against a concentration gradient, loses this capability when 5 mM 3-isobutyl-1-methylxanthine, 0.5 mM papaverine, 1 mM hydrocortisone or 10 mM aminophylline are added to the mucosal medium (Table 2).

The transport of low concentrations of 5-CH_3H_4PteGlu, which occurs via an active carrier-mediated system (8,10), is inhibited by 5 mM

TABLE 2

EFFECT OF ADENYL CYCLASE STIMULATORS AND PHOSPHODIESTERASE INHIBITORS ON THE ACTIVE ACCUMULATION OF 5-CH_3H_4PteGlu AND PteGlu AGAINST A CONCENTRATION GRADIENT

Compound	Conc. (mM)	*S/M Ratio	
		5-CH_3H_4PteGlu	PteGlu
Control		1.50 ± 0.11	1.51 ± 0.30
Hydrocortisone	1	0.92 ± 0.05	1.02 ± 0.12
3-isobutyl-1-methylxanthine	5	0.92 ± 0.02	0.98 ± 0.02
Papaverine	0.5	0.89 ± 0.01	0.86 ± 0.03
Aminophylline	10	1.01 ± 0.04	1.03 ± 0.06

Rat jejunal everted sacs were incubated in phosphate buffer pH 6 for 30 min at 37°C. Equal concentrations (0.1 μM) of 5-CH_3H_4PteGlu or PteGlu were added to the mucosal and the serosal media at the onset of the incubation.

*Mucosal/serosal concentration ratio after 30 min incubation.

3-isobutyl-1-methylxanthine while the transport of high concentrations of 5-CH_3H_4PteGlu, which occur via passive diffusion (8,10), is not affected (69, 42 and 15 percent inhibition in the transport of 0.1, 1 and 10 μM 5-CH_3H_4PteGlu was observed respectively when 5 mM 3-isobutyl-1-methylxanthine was added to the mucosal medium).

Inhibition of the transport of folate compounds by cAMP is not unique, as many intestinal absorption, as well as secretion, processes are regulated by cAMP. cAMP decreases sodium absorption and increases chloride secretion in the intestine (11). Absorption of neutral and dibasic amino acids in rat jejunum is enhanced by elevation of mucosal cAMP (12). In other non-intestinal systems including L1210 mouse leukemia cells (17), normal human hematopoietic cells and human leukemia cells (18), elevated cAMP levels decrease the transport of the folate analogue amethopterin.

Our studies suggest that cAMP modulates the active, carrier-mediated transport system of 5-CH_3H4PteGlu and PteGlu in rat jeunum and that an inverse relation between jejunal mucosal cAMP and the transport of these

folates exist. The mechanism by which cAMP affects intestinal folate transport is not known. Activation of a protein kinase that catalyses the ATP-dependent phosphorylation of the transport protein(s) leading to a decrease in the affinity of the transporting protein for its substrate, and/or changes in the mobility of the carrier across the membrane have been suggested as putative mechanisms in leukemia cells (17).

Acknowledgement: This work was supported by research grant CA 17809 from the National Cancer Institute, National Institute of Health and special grants from the Bockus International Society of Gastroenterology and the Scripps Clinic Gastroenterology Research Fund.

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