Kobe J. Med. Sci. 48, 59/62 April 2002

Phenol Red Inhibits Uptake of Phosphate by the Everted Gut Sacs of Mice

P. L MARY and J. PRAKASA RAO*
Department of Physiology, Christian Medical College, Vellore - 632 002, India

Received 11 March 2002/ Accepted 21 May 2002

Key words: intestine; phosphate; uptake; dye; inhibition

Uptake of phosphate by the everted intestinal sacs of mice was measured at various concentrations of this anion in the incubation medium. Phenol red when added to the serosal side was able to inhibit the uptake of phosphate significantly, while uptake of glucose or proline was unaffected. When para amino hippuric acid was present along with phenol red in the serosal compartment, the inhibition exerted by the dye on phosphate uptake was significantly less. These findings suggest that entry of phenol red into gut tissue from the serosal side interferes with the mucosal uptake of phosphate by the everted intestinal sacs of mice.

Many substances are absorbed by the intestinal mucosa by active and passive processes, which have been well characterized. Recently specific transport of organic ions from the blood into the intestinal lumen has been described. Prominent among such substances secreted into the intestine are quarternary ammonium compounds (13), guanidine (8), sulphanilic acid (12), fluoroquinolines (4) and phenol red (10). Studies have shown that some of these secretory transports may interfere with the absorption of specific substances indicating their possible employment to regulate blood levels of therapeutic agents (2,13). In a bid to find out if such secretory transport would interfere with absorption of essential substances, we studied the effect of phenol red on the absorption of nutrients by everted intestinal sacs of mice.

MATERIALS AND METHODS

Everted duodenal sacs were prepared from male Swiss albino mice (3 months old) obtained from the institutional animal house. The animals were maintained on commercial feed (calcium 1%, phosphate 0.6%) obtained from Gold Mohur Animal Feeds (Bangalore, India). After over night fasting, mice were killed under ether anaesthesia, and the intestine extending from the pyloric end to the ileocecal junction was excised carefully. Fat and mesenteric attachments were removed. This separated segment was immediately chilled and flushed extensively and slowly with ice cold 0.9% saline using a syringe equipped with blunt needle. Everted sacs of 6cm. length were made from the duodenum according to the method described by Wilson & Wiseman (15). The distal end of the sac was tied with a ligature (000 Ethilon Black braided nylon). A ligature was placed loosely around the proximal end.

After weighing, the empty sac was filled with 0.5 mL of the desired incubation medium (serosal compartment) using a micro syringe (Gas tight syringe 1750, Hamilton Company, USA) fitted with a blunt needle. The filled sac was slipped off the needle carefully and the loose ligature on the proximal end was tightened. After weighing, the distended sac was placed in 5 mL of the same incubation medium contained in a 25 mL Erlenmeyer flask. After gassing for 30 seconds with 100% oxygen, the flasks were tightly stoppered and incubated at 37 ˚C for 1 h in a metabolic shaker bath (Techno India Ltd, Pune, India) at a frequency of

Phone: 91-416-262603 Fax: 91-416-262788 E-mail: jprao2001@hotmail.com

100--110 shakes/min. At the end of the incubation period (60 min), the sacs were removed from the flasks, blotted and weighed again. The incubation medium contained NaCl (135mmol/L), KCl (11mmol/L) and $CaCl_2$ (0.04 mmol/L) dissolved in phosphate buffer of desired concentration (KH_2PO_4 and Na_2HPO_4) at pH 7.4. The amount of phosphate removed from the incubation medium in the flask (mucosal compartment) was characterized as 'uptake' and is expressed as μmol/gm tissue wet weight/hr. The uptake of glucose and proline were also estimated in similar fashion after incorporating these substances in the incubation medium at concentrations of 5.5 mM and 2 mM respectively. Spectrophotometric methods (3, 1, 16) were used for the estimation of phosphate, glucose and proline. Phenol red was added to the serosal compartment at the desired concentration. All the chemicals were procured from Sigma (U.S.A.).

RESULTS

As shown in Table Ⅰ phenol red inhibited only the uptake of phosphate. The uptake of either glucose or L – proline was not affected. When phenol red concentration was increased progressively (Table Ⅱ) on the serosal side, inhibition exerted on the phosphate uptake was also increased in a similar manner. Presence of para amino hippuric acid along with the dye on the serosal side led to a partial loss of the inhibitory effect of phenol red. Para amino hippuric acid alone did not show any effect on phosphate uptake.

Table Ⅰ : Effect of phenol red on uptake of substrates by everted intestinal sacs of mice.

Substrate & concentrations in the medium	Uptake (micromoles per gm tissue weight per hour)	
	Control	Test
Phosphate 2mM	9.2±0.12	3.1±0.3*
Phosphate 3mM	12.7±0.12	5.2±0.05*
Phosphate 5mM	16.5±0.14	6.9±0.11*
Phosphate 8mM	20.0±0.32	7.6±0.11*
Phosphate 10mM	20.5±0.25	7.7±0.14*
Glucose 5mM	35.5±0.62	33.5±0.52
L-Proline 2mM	13.3±0.15	12.7±0.21

All the values are expressed as mean ± SEM of six experiments. The sacs in the test groups contained phenol red on the serosal side at a concentration of 84.6 μM. The values marked * are significantly less than the controls ($p<0.001$).

DISCUSSION

Uptakes of glucose and phosphate reported in this paper agree well with the values obtained in the previous studies (6, 7). Phenol red inhibited the phosphate uptake by everted gut sacs of mice. This effect of phenol red seems to be specific since uptakes of glucose and proline were not interfered with. Leakage of fluid, interference with metabolism or inadequate oxygenation are also likely to yield similar results. However these factors would affect the uptakes of all the substrates studied since they are all active, energy requiring processes mediated by sodium dependent carrier mechanisms (11). Although the intestinal wall is made up of four layers- mucosa, sub- mucosa, muscle and serosa, it is the mucosal layer that acts as the critical barrier to the transportation of substances from either intestinal luminal or serosal side (2). Blood borne substances gain access to the sub-mucosa of intestinal wall through capillaries but need to cross the mucosal barrier akin to the substances

placed on the serosal side of the everted gut sac. They enter the luminal side making use of specific systems of transportation situated in the basolateral and apical membranes of

Table II : Effects of Phenol red and Para amino hippuric acid on phosphate uptake.

Substance and concentration (μM)	Uptake of Phosphate (micromoles/gm wet wt/hr)	Relative to Control (%)
None	9.8±0.42[b]	100
Phenol red 2.82	7.8±0.34	80
Phenol red 28.2	4.7±0.32	48
Phenol red 84.6	3.3±0.25[*]	34
Phenol red 282	2.4±0.26	24
Phenol red 84.6 & Para amino hippuric acid 282	5.6±0.14	60
Para amino hippuric acid 282	9.2±0.19[b]	94

Each value represents mean ± SEM of six experiments. Value marked * is significantly different ($p<0.01$) from each of the other values. Values with same superscript do not differ significantly from one another. The phosphate concentration in medium in all the experiments was kept at 2mM. Substances were added to the serosal compartment only.

enterocytes. Existence of such systems in the renal tubular cells is well documented. Para-amino hippuric acid is known to share the same transport system used by phenol red in the renal tubular cell where it competitively inhibits the secretion of phenol red from the blood into the renal tubular lumen (14). Results obtained by Saitoh et al with phenol red and para amino hippuric acid (10) suggest the existence of organic anion secretory transport from serosal to mucosal compartments of the intestine, in a manner similar to the renal tubule. In our study, when phenol red and para amino hippuric acid were placed together in the serosal compartment, the inhibitory action of phenol red on the phosphate uptake is reduced significantly. This may be due to reduced entry of phenol red into the enterocyte caused by para amino hippuric acid. Promoting the entry by raising the concentration of the dye has led to a greater inhibition of phosphate uptake in our study. This clearly indicates that the entry of the dye into the critical mucosal layer in sufficient quantity and not its mere presence in the serosal compartment, is responsible for the inhibition of phosphate uptake. The exact mechanism of this inhibition is not clear from our study. However, since para-amino hippuric acid by itself is not able to curtail the phosphate uptake, it is likely that the entry of the dye into the intestine and its inhibition of uptake of phosphate occur at separate sites.

Our studies reveal the possibility of inhibition of absorption of an essential inorganic anion by the transport of a specific organic anion in the reverse direction. Arsenic poisoning due to drinking of contaminated water is assuming epidemic proportions in India and Bangladesh (9). In the form arsenate, this substance shares the same uptake process of phosphate (5). Further experimentation is needed to explore whether phenol red can inhibit arsenate uptake.

ACKNOWLEDGEMENTS

The financial assistance of the Indian Council of Medical Research and the constructive criticism of Professor G. Wilfred are gratefully acknowledged.

REFERENCES

1. **Asatoor, A.M. and King, E.J.** 1954. Simplified colorimetric blood sugar method. Biochem. J. **56**:xliv.
2. **Aungst, B.J. and Saitoh, H.** 1996. Intestinal absorption barriers and transport mechanisms including secretory transport, for a cyclic peptide, fibrinogen antagonist. Pharm. Res. **13**:114-119.
3. **Chen, P.S., Toribara, T.Y., and Warner, H.** 1956. Micro-determination of phosphorous. Anal. Chem. **28**:1756-1758.
4. **Griffiths, N.M., Hirst, B.H., and Simmons, N.L.** 1994. Active intestinal secretion of fluoroquinoline antibacterials ciprofloxacin, norfloxacin and pefloxacin, a common secretory pathway?. J. Pharmacol. Exp. Ther **269**:496-502.
5. **Hoffman, N., Thees, M., and Kinne, R.** 1976. Phosphate transport by isolated renal brush-border vesicles. Pflugers Arch. **362**:147-156.
6. **Mary, P.L. and Prakasa Rao, J.** 1989. Effect of phosphate on water and glucose transport in mouse everted gut sacs. Quart. J. Exp. Physiol. **74**:363-365.
7. **Mary, P.L. and Prakasa Rao, J.** 1993.Role of sodium-potassium ATPase in phosphate transport by the everted sacs of mouse intestine. Clin. Exp. Physiol & Pharmacol. **20**:149-153.
8. **Miyamoto, Y., Ganapathy, V., and Leibach, F.H.** 1988. Transport of guanidine in rabbit intestinal brush border membrane vesicles. Am. J. Physiol. **255**:G85-G92.
9. **Roy Chowdhury, T., Mondal, B.K., Samanta, G., Basu, G.K., Chowdhury, P.P., Chandar, C.R., and Karan, N.K.** 1997. Arnsenic exposure and health effects. (eds Abernathy C.O., Calderon R.L. and Chappell W.R.) Chapman and Hall, New York, **9**:91-111.
10. **Saitoh, H., Gerard, C., and Aungst, B. J.** 1996. The secretory intestinal transport of some beta- lactam antibiotics and anionic compounds: A mechanism contributing to poor oral absorption, J. Pharmacol. Exp. Ther. **278**:205-11.
11. **Sanford, P.A.** 1982. *In*: Digestive system Physiology (Edward Arnold Ltd, 41, Bedford Square, London). 81-102. The small intestine.
12. **Sund, R.B. and Lauterbach, F.** 1978. Intestinal secretion of sulphanilic acid by the isolated mucosa of guinea pig jejunum. Acta. Pharmacol. Toxicol. **43**:331-338.
13. **Turnheim, K. and Lauterbach, F.** 1980. Interaction between intestinal absorption and secretion of monoquaternary ammonium compounds in guinea pigs - a concept for the absorption kinetics of organic cations. J. Pharmacol Exp. Ther. **212**:418-424.
14. **Ullrich, K.J. and Rumich, G.** 1988. Contraluminal transport system in the proximal renal tubule involved in secretion of organic anions. Am. J. Physiol. **254**:F453-462.
15. **Wilson, T.H. and Wiseman, G.** 1954. The use of sacs of everted small intestine for the study of transference of substances from the mucosal to the serosal surface. J. Physiol. **123**:116-125.
16. **Wren, J.J. and Wiggal, P.H.** 1965. An improved colorimetric method for the determination of proline in the presence of other ninhydrin positive compounds. Biochem. J. **94**:216-220.