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THE EFFECT OF AMINO ACIDS ON THE INTESTINAL TRANSPORT OF L- AND D-XYLOSE *IN VITRO*

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SUMMARY

1. The intestinal transport of D-xylose by sacs of everted small intestine of the hamster is partially inhibited by L-histidine in concentrations of 5, 10 and 20 mM but not of 2 mM.

2. 20 mM concentrations of D-histidine and L-methionine also produce inhibition.

3. The inhibition is not due to an alteration in water transport or to a change in pH of the incubating medium.

4. The rate of the intestinal transport of L-xylose is about $\frac{1}{4}$ of the rate of D-xylose transport and is unaffected by a 20 mM concentration of L-histidine.

INTRODUCTION

It has been shown (Hindmarsh, Kilby & Wiseman, 1966*a*, *b*, *c*) that actively transported L- and D-amino acids partially inhibit the absorption of actively transferred sugars. In view of the report (Duthie, Webster & Hindmarsh, 1966) that L-histidine reduces the absorption of D-xylose in man, the effect of L- and D-histidine and L-methionine on the transport of D- and L-xylose has been studied by the use of sacs of everted small intestine of the hamster. A preliminary report of some of these results has been given by Duthie *et al.* (1966).

METHODS

Sacs were made from the everted small intestine of male golden hamsters (*Mesocricetus auratus*) according to the method described by Wilson & Wiseman (1954) and Wiseman (1961). The animals weighed between 70 g and 100 g. At the end of 1 hr the sac was removed, blotted, and the contents drained into a small glass beaker which was then weighed to estimate the final fluid volume contained in the sac. The accuracy of the amount recovered from the sac was measured and found to have less than a 2 % error. Six consecutive sacs were made, using the whole of the small intestine excepting the duodenum. In all experiments L- or D-xylose was dissolved in the outer (mucosal) solution at a concentration of 16·7 mM. The amino acids were dissolved in both the outer (mucosal) and inner (serosal) solutions at initially the same concentrations, which varied from 2 to 20 mM: the monohydrochloride monohydrates of L- and D-histidine were used. After incubation of 1 hr, the concentrations of xylose in the serosal and mucosal solutions were estimated by the method

of Roe & Rice (1948), and the amount of water transported estimated by subtracting the initial from the final serosal volumes. The results are expressed as the total amounts of xylose entering the serosal fluids during incubation of 1 hr and also as the final serosal/mucosal concentration ratios.

RESULTS

L-histidine in a 20 mM concentration partially inhibited the intestinal transport of D-xylose right along the small intestine.

Water transport in sacs 1–5 was not affected by the amino acid. In sac 6 (terminal ileum) the amount of water transferred was decreased (Table1).

TABLE 1. The effect of 20 mM L-histidine on the transport of D-xylose, the final serosal/mucosal concentration ratios for D-xylose and the water transport in six consecutive sacs of everted small intestine of the hamster, means ± S.E. of mean, number of experiments in parentheses

	Sac	No histidine present	Histidine present
Serosal appearance of D-xylose in μmoles/100 mg dry weight of sac.hr	1	23·5 ± 1·5 (12)	16·4 ± 1·1 (11)
	2	25·6 ± 1·3 (14)	16·9 ± 1·3 (9)
	3	23·0 ± 1·5 (12)	14·5 ± 0·7 (10)
	4	23·2 ± 0·8 (14)	16·2 ± 0·7 (9)
	5	22·0 ± 1·1 (10)	14·4 ± 1·0 (8)
	6	19·9 ± 1·3 (8)	9·4 ± 0·9 (11)
Final serosal/mucosal concentration ratios	1	0·64 ± 0·02 (13)	0·54 ± 0·03 (9)
	2	0·60 ± 0·03 (13)	0·45 ± 0·02 (9)
	3	0·62 ± 0·02 (29)	0·45 ± 0·01 (13)
	4	0·67 ± 0·03 (16)	0·47 ± 0·02 (9)
	5	0·70 ± 0·03 (10)	0·47 ± 0·02 (8)
	6	0·59 ± 0·03 (8)	0·34 ± 0·02 (8)
Serosal water appearance in m-moles/100 mg dry weight of sac.hr	1	23·4 ± 4·1 (12)	26·0 ± 6·9 (11)
	2	38·8 ± 4·5 (14	32·2 ± 4·8 (9)
	3	36·8 ± 5·6 (12)	23·8 ± 4·3 (10)
	4	25·7 ± 3·3 (14)	24·7 ± 4·8 (9)
	5	12·1 ± 2·4 (10)	13·0 ± 1·5 (8)
	6	10·8 ± 3·1 (8)	0·4 ± 1·6 (11)

All subsequent experiments were performed using sacs 3 and 4 only and the results pooled, each group consisting of at least six animals. The transport of D-xylose was inhibited not only by a 20 mM concentration of L-histidine; 5 and 10 mM concentrations had a similar effect although a 2 mM concentration had not (Table 2).

Water transport was not affected by these smaller concentrations of the amino acid. D-Histidine and L-methionine in 20 mM concentrations produced a similar inhibition of D-xylose transport without affecting water transport (Table 3).

L-histidine in a 20 mM concentration had no effect on the intestinal transport of L-xylose or of water (Table 4).

TABLE 2. The effect of various concentrations of L-histidine on the transport of D-xylose, the final serosal/mucosal concentration ratios for D-xylose and the water transport in sacs of everted small intestine of the hamster, sacs 3–4 pooled, means ± S.E. of mean, number of experiments in parentheses

	No histidine present	2 mM L-histidine present	5 mM L-histidine present	10 mM L-histidine present	20 mM L-histidine present
Serosal appearance of D-xylose μmoles/100 mg dry weight of sac.hr	21·8 ± 0·6 (45)	18·9 ± 0·9 (13)	15·7 ± 0·7 (12)	14·7 ± 0·5 (12)	15·3 ± 0·5 (29)
Final serosal/mucosal concentration ratios	0·64 ± 0·02 (45)	0·62 ± 0·02 (12)	0·53 ± 0·02 (12)	0·51 ± 0·02 (12)	0·46 ± 0·01 (23)
Serosal water appearance m-moles/100 mg dry weight of sac.hr	29·0 ± 2·3 (45)	39·9 ± 4·5 (12)	29·5 ± 3·4 (12)	29·1 ± 3·2 (12)	23·4 ± 2·5 (29)

TABLE 3. The effect of 20 mM concentrations of various amino acids on the transport of D-xylose, the final serosal/mucosal concentration ratios for D-xylose and the water transport in sacs of everted small intestine of the hamster, sacs 3–4 pooled, means ± S.E. of mean, number of experiments in parentheses

	No histidine present	20 mM L-histidine present	20 mM D-histidine present	20 mM L-methionine present
Serosal appearance of D-xylose μmoles/100 mg dry weight of sac.hr	21·8 ± 0·6 (45)	15·3 ± 0·5 (29)	14·8 ± 0·6 (15)	15·9 ± 0·4 (25)
Final serosal/mucosal concentration ratios	0·64 ± 0·02 (45)	0·46 ± 0·01 (23)	0·55 ± 0·02 (15)	0·49 ± 0·02 (20)
Serosal water appearance m-moles/100 mg dry weight of sac.hr	29·0 ± 2·3 (45)	23·4 ± 2·5 (29)	24·7 ± 3·0 (15)	24·6 ± 2·1 (25)

TABLE 4. The effect of 20 mM-L-histidine on the transport of L-xylose, the final serosal/mucosal concentration ratios for L-xylose and the water transport in sacs of everted small intestine of the hamster, sacs 3–4 pooled, means ± S.E. of mean, number of experiments in parentheses

	No histidine present	20 mM L-histidine present
Serosal appearance of L-xylose μmoles/100 mg dry weight of sac.hr	5·0 ± 0·3 (11)	5·2 ± 0·8 (16)
Final serosal/mucosal concentration ratios	0·20 ± 0·03 (11)	0·17 ± 0·01 (16)
Serosal water appearance m-moles/100 mg dry weight of sac.hr	16·7 ± 3·1 (11)	22·7 ± 3·1 (16)

DISCUSSION

It is clear from these experiments that the partial inhibition of the intestinal transport of monosaccharides by amino acids, in this case L- and D-histidine and L-methionine, includes the sugar D-xylose. This observation is not unexpected in view of the clinical observations which initiated this study (Duthie *et al.* 1966). Furthermore, the general rule

suggested by Hindmarsh *et al.* (1966*c*) that actively transported amino acids partially inhibit the intestinal transfer of actively transferred sugars has been obeyed in this case, as there is now abundant evidence that the absorption of D-xylose involves some active process (Crane, 1960; Salomon, Allums & Smith, 1961; Csaky & Lassen, 1964; Alvarado, 1964; Csaky & Ho, 1965; Alvarado, 1966*a*, *b*).

The intestinal transport of L-xylose was not affected by the presence of 20 mM L-histidine and its transfer rate was approximately $\frac{1}{4}$ as fast as that of D-xylose. Larson, Blatherwick, Bradshaw, Ewing & Sawyer (1940) also showed a slower transfer rate for L-xylose when comparing these two sugars, although Kohn, Dawes & Duke (1965) did not. These observations of a slower transfer rate of L-xylose and that it is unaffected by L-histidine suggest that it is not actively transferred by the hamster small intestine.

The pH of the incubation medium decreased from 7·3 to 7·1 when 20 mM concentrations of L- and D-histidine were used but not when lesser concentrations of L-histidine or 20 mM concentrations of L-methionine were used. It seems extremely unlikely, however, that this alteration in pH, when it occurred, affected the absorption of the sugar, as the pH changes which seem to influence sugar transport are much greater than those encountered in the present experiments (Goldenberg & Cummins, 1963). In addition, inhibition occurred in those experiments in which there was no pH change.

The inhibition of sugar transport in the above experiments was not due, in sacs 1–5 at least, to a primary inhibition of water transport since, in sacs 1–5, the amount of water transport was not significantly altered. Nor is it likely that it was due to a primary inhibition of sodium transport, because experimental results from the perfused rat ileum have shown that when sodium transport is reduced, water transport is similarly affected (Curran & Solomon, 1957).

It has already been shown (Hindmarsh *et al.* 1966*c*) that the inhibition occurs at or near the luminal border of the cell and that it is unlikely to be due to a competition of the energy supplying the intracellular accumulation process for sugars.

By exclusion the evidence suggests that the inhibition may occur at the entry of D-xylose into the epithelial cell. If we assume that this entry occurs by means of a membrane carrier common to D-xylose and other actively transported sugar (Salomon *et al.* 1961), one of several effects might be involved. Inhibition may be due to competition for an identical binding site on the membrane carrier. This is unlikely because of the dissimilarity of the molecular structure of amino acids and D-xylose and because the accumulation of D-xylose by hamster small intestinal epithelial cells (Alvarado, 1964, 1966*a*) and the intestinal absorption of

D-xylose by rats *in vivo* (Csaky & Ho, 1965) are reduced by 10^{-4} M phlorrhizin, whereas the intestinal transport of actively transported amino acids is not affected by similar concentrations of phlorrhizin (Fridhandler & Quastel, 1955; Newey & Smyth, 1964).

Inhibition may be allosteric if D-xylose and the amino acids are carried on different binding sites on the same membrane carrier.

This mechanism has been suggested by Alvarado (1966*b*) to account for the partial inhibition by various actively transported sugars of the uptake by hamster intestinal epithelial cells of some actively transferred amino acids.

Finally, Alvarado (1964, 1966*a*) has shown that the uptake of D-xylose by hamster intestinal epithelial cells is sodium-dependent, so it seems likely that it may enter the cell by means of a sodium-dependent carrier, as postulated by Crane (1965) for other sugars.

As the intestinal transport of actively transported amino acids is similarly sodium-dependent (Csaky, 1963; Rosenberg, Coleman & Rosenberg, 1965) it seems likely that the amino acids we used, namely L- and D-histidine and L-methionine, may also enter the cell by means of a sodium-dependent carrier. Thus the partial inhibition of the intestinal transport of D-xylose which we observed may have resulted from competition between D-xylose and the amino acids for available sodium although different carriers may have been used to enter the cell.

REFERENCES

ALVARADO, F. (1964). D-xylose, a substrate for the process of sugar active transport by the small intestine. *Experientia*, **20**, 302–307.

ALVARADO, F. (1966*a*). D-xylose active transport in the hamster small intestine. *Biochim. biophys. Acta* **112**, 292–306.

ALVARADO, F. (1966*b*). Transport of sugars and amino acids in the intestine, evidence of a common carrier. *Science, N.Y.* **151**, 1010–1013.

CRANE, R. K. (1960). Studies on the mechanism of the intestinal absorption of sugars, mutual inhibition in vitro between some actively transported sugars. *Biochim. biophys. Acta* **45**, 477–482.

CRANE, R. K. (1965). Sodium dependent transport in the intestine and other animal tissues. *Fedn Proc.* **24**, 1000–1006.

CSAKY, T. Z. (1963). A possible link between active transport of electrolytes and non electrolytes. *Fedn Proc.* **22**, 3–7.

CSAKY, T. Z. & HO, P. M. (1965). Intestinal transport of D-xylose. *Proc. Soc. exp. Biol. Med.* **120**, 403–408.

CSAKY, T. Z. & LASSEN, U. V. (1964). Active intestinal transport of D-xylose. *Biochim. biophys. Acta* **82**, 215–217.

CURRAN, P. F. & SOLOMON, A. K. (1957). Ion and water fluxes in the ileum of rats. *J. gen. Physiol.* **41**, 143–168.

DUTHIE, H. L., WEBSTER, J. H. H. & HINDMARSH, J. T. (1966). Inhibition of D-xylose absorption by L-histidine. *Gut*, **7**, 104, Abstract.

FRIDHANDLER, L. & QUASTEL, J. H. (1955). Absorption of amino acids from isolated surviving intestine. *Archs Biochem. Biophys.* **56**, 424–440.

GOLDEBERG, J. & CUMMINS, A. J. (1963). The effect of pH on the absorption rate of glucose in the small intestine of humans. *Gastroenterology* **45**, 189–195.

HINDMARSH, J. T., KILBY, D. & WISEMAN, G. (1966*a*). Inhibition by L-histidine of intestinal active transport of D-glucose. *J. Physiol.* **182**, 52–53*P*.

HINDMARSH, J. T., KILBY, D. & WISEMAN, G. (1966*b*). Effect of amino acids on intestinal sugar transport. *J. Physiol.* **183**, 78*P*.

HINDMARSH, J. T., KILBY, D. & WISEMAN, G. (1966*c*). Effect of amino acids on sugar absorption. *J. Physiol.* **186**, 166–174.

KOHN, P., DAWES, E. D. & DUKE, J. W. (1965). Absorption of carbohydrates from the intestine of the rat. *Biochim. biophys. Acta* **107**, 358–362.

LARSON, H. W., BLATHERWICK, N. R., BRADSHAW, P. J., EWING, M. E. & SAWYER, S. D. (1940). The metabolism of L-xylose. *J. biol. Chem.* **136**, 1–7.

NEWEY, H. & SMYTH, D. H. (1964). Effects of sugars on the intestinal transfer of amino acids. *Nature, Lond.* **202**, 400–401.

ROE, J. H. & RICE, E. W. (1948). A photometric method for the determination of free pentoses in animal tissues. *J. biol. Chem.* **173**, 507–512.

ROSENBERG, I. H., COLEMAN, A. L. & ROSENBERG, L. E. (1965). The role of sodium ion in the transport of amino acids by the intestine. *Biochim. biophys. Acta* **102**, 161–171.

SALOMON, L. L., ALLUMS, J. A. & SMITH, D. E. (1961). Possible carrier mechanisms for the intestinal transport of D-xylose. *Biochem. biophys. Res. Commun.* **4**, 123–126.

WILSON, T. H. & WISEMAN, G. (1954). The use of sacs of everted small intestine for the study of the transference of substances from the mucosal to the serosal surface. *J. Physiol.* **123**, 116–125.

WISEMAN, G. (1961). Methods of study of intestinal absorption and metabolism. III. Sac of everted intestine technic for study of intestinal absorption in vitro. *Meth. med. Res.* **9**, 287–292.