STUDIES ON THE ACTION OF HORMONES ON THE INTESTINAL TRANSPORT OF L-HISTIDINE

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Summary: Intestinal absorption of L-histidine as affected by insulin, diabetes, hydrocortisone, adrenocorticotrophic hormone (ACTH) and thyroxine has been studied. Intestinal absorption of L-histidine is not significantly increased following insulin administration. Similarly, addition of insulin *in vitro* did not change the transport activity significantly. When rats are made diabetic on administration of alloxan monohydrate, the transport activity is increased. Hydrocortisone, adrenocorticotrophic hormone and thyroxine treatment increased the absorption of L-histidine by small intestine. However, addition *in vitro* of ACTH and hydrocortisone did not change the absorption of L-histidine by small intestine. It appears that the facilitative action of these hormones is not due to their direct action of the membrane.

Key words : intestinal absorption insulin diabetes hydrocortisone adrenocorticotrophic hormone thyrosine

INTRODUCTION

Some of the hormones have been reported to control the activity of the cell by influencing the permeability of nutrients across the cell membrane (21). Lundsgaard (14) in his studies in 1939 with perfused striated muscle, was, perhaps, the first to show that insulin increases sugar uptake by a tissue. Insulin facilitates the transport of apha amino isobutyric acid into diaphragm and skeletal muscle (20) and that of glycine and proline in diaphragm (1). Therefore, control of transport may be one factor, though not the only one in insulin regulated protein synthesis. Akedo and Christensen (1) did not find stimulatory effect of insulin on the absorption of D-and L-alanine, L-serine, L-valine, L-histidine and DL-norvaline. The features of amino acid structure that make certain amino acid more sensitive than others to insulin have not been indentified as yet. Akedo and Christensen have suggested that insulin may fail to show an effect on the uptake of amino acid which has low Km for the active transport. Their generalization is based on their results with diaphragm and liver. Therefore, it is of interest to study the effect of insulin on the transport of amino acids in intestinal tissue which is an important part of digestive tract since nearly 90 per cent of food is digested and absorbed through it. Effect of diabetes was also studied with a view to relate its effect to that of insulin. Stimulation of amino acid transport into liver by hydrocortisone has been reported by Hempling and Hare (10) while Shishowa (22) showed that the rate of absorption of amino acids in the intestine of adrenalectomized rats was markedly reduced. Decreased uptake of amino acids and sugar by adrenal glands *in vivo* after hypophysectomy

and their raturn to high levels following the administration of ACTH preparations was obtained by Hechter and Lester (9). Decreased uptake of glucose and acetate across small intestine of mouse in hyperthyroidism is also reported (12). However, the data represented by Althausen (3) is contrary to these findings. Keeping these findings in view, it was of interest to study the effect of hormones on the intestinal transport of L-histidine.

MATERIALS AND METHODS

Female albino rats (weighing 100-200 *g*), obtained from Indian Drugs & Pharmaceuticals Ltd., Rishikesh, India, were used. The animals were maintained on a basal laboratory diet.

Procedures for studying the intestinal transport have been described previously by Wilson and Wiseman (23). The animals were fasted for 10-20 hrs before sacrifice. The animals were first stunned and exsanguinated by decapitation. The abdomen was opened to expose the gestrointestinal tract and a portion of small intestine from duodenum to jejunum was taken out. It was washed in cold 0.9% saline. The intestine was everted and cut into five to six pieces of equal length and sacs were prepared.

Sacs were filled with about 0.4 to 0.6 *ml* of Krebs-Ringer bicarbonate buffer containing 10 mM of L-histidine. One bubble of oxygen was also introduced in the sac. Blind sacs containing medium were incubated in 50 *ml* conical flasks containing 6 *ml* of the same oxygenated medium, in a Dubnoff incubator for 1 hr at 37°C. The shaker was operated at 60 cycles/min.

After incubation, sacs were blotted and weighed, fluid drained and empty sacs reweighed. L-histidine was analysed from serosal and muscosal fluids by the method of Macpherson (17). Amino acid transport was calculated by difference in the concentration of L-histidine in the initial and final serosal fluid and volume change and expressed as μ moles L-histidine/*g* dry weight of tissue/hr incubation at 37°C.

The treatment given to various groups of animals is as follows:

Group A, control (untreated); Group B, insulin treated (0.4 *I.U./kg* given intramuscularly daily for 6 days); Group G, insulin *in vitro* (added 0.2 I.U. of insulin/100 *ml* in the medium); Group C, diabetic (injected intraperitoneally 30 *mg* of alloxan/100 g body weight); Group D, hydrocortisone treated (2.5 *mg* was given intramuscularly daily for 6 days); Group E, ACTH treated (1.0 I.U./100 *g* given intramuscularly for 10 days); Group F, Thyroxine treated (0.5 *mg/kg* given intramuscularly daily for 6 days); Group H, hydrocortisone *in vitro* (added 83.4 *mg*/100 *ml* in the medium), Group J, thyroxine *in vitro* (added 3.34 *mg*/100 *ml* of the medium).

Effectiveness of the doses were confirmed by measuring liver glycogen level in case of Insulin (7), hydrocortisone (13) and thyroxine (6) and muscle glycogen level in ACTH (5) treatment. Extent of diabetes was recorded by measuring urine glucose level with Benedicts reagent.

RESULTS AND DISCUSSION

The results presented in Table I show that insulin treatment has no significant effect on the transport of L-histidine. Similar effect of insulin is further noted by adding insulin in the medium itself (Table II). These results on L-histidine transport through intestinal tissue under insulin treatment appear to be similar to those obtained in other tissues like liver and diaphragm (1,20). Similarly, transport of methionine has been found to be stimulated both in diaphragm (1) and intestinal tissue (16). Thus, the transport system of an amino acid may be the same in various tissues. Results on the lack of insulin stimulation of L-histidine transport may indicate a lower Km for L-histidine as compared to that for methionine for active transport, as suggested by Akedo and Christensen (1).

TABLE I : *In vivo* effect of hormones and alloxan diabetes on the transport of L-histidine by intestinal sacs in rats. (Results, Mean ± S.E. are expressed in μ moles of L-histidine transported/g dry wt. of sac/hr incubation at 37°C).

	Treatment	*No. of sacs*	*Transport activity*	*'t' values at 5% level between*
A	(Control)	17	28.9 ± 1.76	
B	(Insulin)	13	30.0 ± 2.88	A & B = 0.33
C	(Alloxan)	17	50.4 ± 2.80	A & C = 6.30*
D	(Hydrocortisone)	15	89.9 ± 3.87	A & D = 9.35*
E	(ACTH)	17	51.0 ± 2.90	A & E = 6.31*
F	(Thyroxine)	20	52.4 ± 1.87	A & F = 4.45*

*Significant

TABLE II: *In vitro* effect of hormones on the transport of L-histidine by intestinal sacs in rats. (Results, mean ± S.E. are expressed in μ mole of L-histidine transported/g dry wt. of sac/hr incubation at 37°C).

	Treatment	*No. of sacs*	*Transport activity*	*'t' value at 5% level between*
A	(Control)	17	28.9 ± 1.76	
G	(Insulin)	6	27.9 ± 3.01	A & G = 0.27
H	(Hydrocortisone)	6	31.8 ± 4.77	A & H = 0.67
I	(ACTH)	6	34.5 ± 2.91	A & I = 1.56
J	(Thyroxine)	7	22.3 ± 1.95	A & J = 2.95*

*Significant.

When the rats were made diabetic by administration of alloxan, the intestinal transport of L-histidine was increased. This may not be related to non-utilization of sugar in diabetic condition since methionine transport has been stimulated both in insulin treatment (16) as well as in

diabetic condition (15). Transport of L-histidine under diabetic condition is again an active transport as it is abolished by dinitrophenol treatment (15) and diabetic condition did not increase the absorption of D-glucose which is absorbed passively (19). The increase in amino acid transport across the intestinal wall of alloxan diabetic animal has been postulated as due to the conformational changes in the transport system. Similar results have been reported by Crane (4). He reported increased sugar transport in intestinal strips taken from alloxan diabetic rats which was not reduced to normal by addition of insulin *in vitro* or treatment of diabetic animal with insulin.

Hydrocortisone treatment (Table I) led to the increased transport of L-histidine in the intestinal tissue. The facilitative action of hydrocortisone on the translocation of L-histidine in intestinal tissue resembles that of glucose and alpha-amino isobutyric acid in different tissues. However, hydrocortisone may not have a general stimulatory effect as Mahmood *et al* (16) reported no significant effect of hydrocortisone on the translocation of methionine in the intestinal tissue. Hydrocortisone when added into the medium (Table II) has not affected the absorption of L-histidine. However, *in vivo* effects show that it increased the transport of the amino acid by nearly 300% This effect of hydrocortisone on L-histidine transport would suggest that hydrocortisone does not affect the memberane per se. ACTH administration also led to increased transport of L-histidine through small intestine. This effect is suggestive of its action mediated through the stimulated output of hydrocortisone by adrenal cortex, since hydrocortisone in the present study has been found to stimulate histidine transport significantly. These findings are in confirmation of those of Hechter and Lester (9) who found decreased uptake of amino acids and sugars by adrenal glands *in vivo* after hypophysectomy of experimental animals and their return to high levels following the administration of ACTH preparations. Observations on the effect of ACTH added *in vitro* (Table II) on transport of L-histidine show the absorption of this amino acid to be unaffected, These results strengthen the hypothesis that the effect of ACTH on intestinal transport of L-histidine is mediated through adrenal cortex. The action of ACTH on stimulation of transport of all the amino acids may not generally be mediated through the adrenal cortex since hydrocortisone has been found not to influence the methionine transport significantly (16), while its absorption was significantly increased under ACTH treatment (16). The data in Table I show that transport of L-histidine is significantly increased when the rats are treated with thyroxine. However, the findings of Holliday *et al* (12) with glucose and of Mahmood *et al* (16) with methionine are contrary to these findings. Thus, the effect of thyroxine on intestinal transport of nutrients rather than being general could be more specific. Thyroxine when added into the medium (Table II) was inhibitory to the absorption of L-histidine. Thyroxine is an uncoupling agent of oxidative phosphorylation (11). Thus, inhibition of transport of L-histidine with added thyroxine may in part be due to a deficiency of ATP, as the amino acid transport is energy dependent. This differential effect of thyroxine may be due to the high concentration of thyroxine *in situ* under *in vitro* conditions as compared to that available under *in vivo* conditions.

Hydrocortisone, ACTH and thyroxine when injected in the body, stimulated the absorption of L-histidine, while hydrocortisone and ACTH when added in the medium did not have any

effect on the transport system. It would seem that these hormones do not modify the membrane structure nor do they bring about any conformational changes in the substrate (L-histidine). Their stimulating effect under *in vivo* studies may be mediated through their effect on enzymes involved in the transport system. When the above results are compared with those of others, it is evident that the hormones may have a generalized effect in different tissues on the transport of a particular nutrient. However, they may specifically influence different nutrients. The range of specificity may further be narrowed particularly in the case of amino acids. Thus, involvement of different permeases or transport system may be expected to participate in the transport of various nutrients, more specifically the amino acids. Till 1972, no definite mechanism for the transport system was known. However, in 1973, Meister (18) proposed a cyclic mechanism which could be responsible for the transport of amino acids across the membrane in kidney and brain, and named this cycle as gamma-glutamyl cycle. According to Meister the first reaction in the transport of amino acids is a binding of amino acid to gamma-glutamyl residue of glutathione, catalyzed by glutamyl transpeptidase.

Gamma-glutamyl cysteinylglycine + amino acid ⟶
(glutathione)

Gamma-glutamyl-amino acid + Cysteinyl glycine

All the amino acids except proline were found to be active substrates in this reaction. Histochemical studies (2,8) have also shown that gamma-glutamyl transpeptidase of the intestine is localized in the epical portions of the epithelial cell covering the jejunal villi. Thus, Meister pointed out that all the amino acids except proline may be transported by this mechanism. However, our studies when compared with those of others have indicated that the effect of hormones on amino acid transport is dependant on individual specificity of amino acids. Hence, investigations on mechanism of action of hormones on transport of amino acids and studies on hormonal control on various enzymes of gammaglutamyl cycle of transport of amino acids would be of considerable interest.

ACKNOWLEDGEMENTS

We thank the Indian Council of Agricultural Research for providing research fellowship to one of the authors (VKK) during the course of which this study was conducted.

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