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Characterization of Absorption Process of Taurine Across Rat Small Intestine

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Abstract □ A mechanism of taurine transfer across the rat small intestine was elucidated by using the *in situ* recirculation perfusion or loop method. Taurine uptake was saturable, Km=39.9 mM, and energy dependent, and required sodium. The close structural analogues, aminomethane sulfonic acid, γ-aminobutyric acid, hypotaurine, and β-alanine, reduced significantly taurine uptake when present in 10-fold excess. The α-amino acid, glycine, did not inhibit uptake. Hence, all of these findings lead to a conclusion that a carrier-mediated transport system for taurine exists in the small intestine.

Keywords □ Mechanism, Taurine transfer, Saturable, Energy dependent, Sodium dependent, Carrier-mediated transport system.

Taurine (2-amino ethane sulfonic acid) is a structurally simple and chemically stable amino acid that is ubiquitously and abundantly distributed as a free form in many tissues of the animal kingdom[1]. In addition, it has a number of features that distinguish it from other amino acids: the acidic grouping is a sulfonate rather than a carboxylate, and the amino group is on β-carbon. It is generally believed that a substance occurring so ubiquitously and in such high concentration must have a potent physiological role, although its function in many tissues is poorly understood.

During the past several years, taurine has been found to exert its beneficial action not only as an absorption promotor toward some drugs in the gastrointestinal tract, but also as a preventive factor against aspirin-induced gastric mucosal lesions in rat[2].

The purpose of present work is to characterize the absorption process of taurine itself across the small intestine and to demonstrate whether the uptake of taurine satisfies the generally accepted criteria of a carrier mediated process, such as saturability and structural specificity. In addition, I characterized the dependence of this system on sodium ion and energy and demonstrated that it was distinct from the known transport systems for neutral α-amino acids.

EXPERIMENTAL METHODS

Materials

Aminomethane sulfonic acid (Aldrich Chemical Co. Inc., U.S.A.), and hypotaurine (Calbiochem-Behring Co., U.S.A.) were used without further purification. N-Acetyltaurine sodium salt was generous gift from Taisho Pharmaceutical Co., Japan. All other chemicals were of reagent grade from Nakarai Chemical Co., Japan.

Analytical Method

Analysis of taurine was carried out by post column derivatization as shown in Fig. 1. It was first separated by HPLC (TRI ROTAR, Japan Spectroscopic Co.) and streamed through a cation exchange column (Partisil-10 SCX), followed by reation with both *o*-phthaldehyde and 2-mercaptoethanol dissolved in pH 10.5

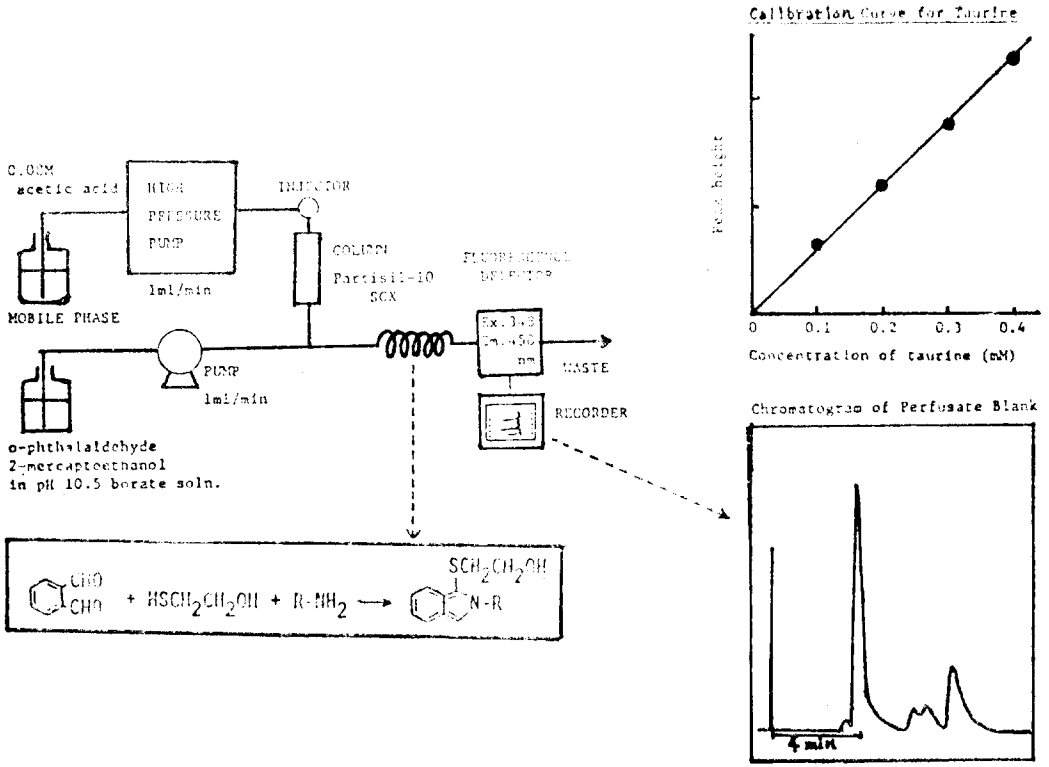

Fig. 1: System for chromatography and post column derivatization.

borate solution in advance. The reaction mixture was determined with a fluorescence detector (FP-110, Japan Spectroscopic Co.) at Ex. 348 and Em. 450 nm. The method which was newly designed in this laboratory, seemed to be suitable for the detection of taurine, since the calibration curve was linearly obtained and the chromatogram was well seperated from other materials.

Procedure of Absorption Experiments

Male Wistar albino rats weighing 160~200g were used. Animals were anesthetized with pentobarbital given intra-peritoneally, and the intestine was cannulated for *in situ* recirculation method[3], and perfused for 1 hr (pH 6.5, Na_2HPO_4-NaH_2PO_4; volume, 40ml for small intestine and 20ml for large intestine), and the absorption from the stomach or the small intestine for the study of energy dependency was examined by *in situ* loop method (pH 1.1, NaCl-HCl, stomach; pH 6.5, small intestine; volme, 4ml)[4]. The amount of drug absorbed in 1hr, was calculated by the difference in the amount of the drug between the initial and final solutions.

RESULTS AND DISCUSSION

Our previous paper[5] has found that taurine enhanced the absorption of some drugs both in the stomach and in the small intestine. Hence, a study was first undertaken to determine whether taurine itself would be absorbed in the GI tract. Taurine remaining in luminal solution after 1 hr, first reacted with both *o*-phthaldehyde and 2-mercaptoethanol and the reaction

Table I: Site specificity of taurine absorption.

Site	Blank (μmole released/hr)	% Absorbed in one hour[d]
Stomach[a]	0.46±0.07(4)	1.1±2.6(4)
Small intestine[b]	4.9±0.6 (4)	34.7±1.5(3)
Large intestine[c]	0.31±0.04(4)	3.5±0.5(4)

a) pH 1.1, loop, 4ml.
b) pH 6.5, perfusion, 40ml.
c) pH 6.5, perfusion, 20ml.
d) initial concentration of taurine=1mM.
Results are expressed as the mean±S.E.M. followed by the number of animals in parentheses.

mixture was measured fluorometrically. The blank shown in Table I indicates the amount of endogeneous taurine released in 1hr when a sham experiment was carried out, and correction was made from the experimental values. As is evident from Table I, no transfer was observed across gastric mucosa. It is noteworthy that taurine exerted its influence on the gastric absorption of some drugs, despite its negligible transfer.

Further investigation was performed both in the small intestine and in the large intestine. Taurine was considerably absorbed approximately up to 35% in the small intestine, while it was hardly absorbed in the large intestine. Although taurine uptake systems have been found to occur ubiquitously in a variety of mammalian tissues including the kidney[6,7], retina[8,9], blood platelets[10,11], Ehrlich's cell[12], and heart[13], interestingly enough, the transfer was limited to the small intestine among the three segments of GI tract. The author thus undertook to further characterize taurine transport in the small intestine.

The dependence of absorption on the concentration of taurine in the medium is demonstrated in Fig. 2. Taurine absorption was measured at the concentration of 1, 2, 10, and 75mM, res-

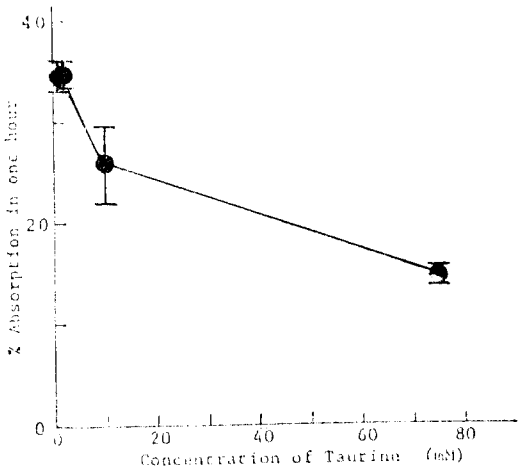

Fig. 2: Concentration dependency of taurine absorption.
Results are expressed as the mean±S.E.M..

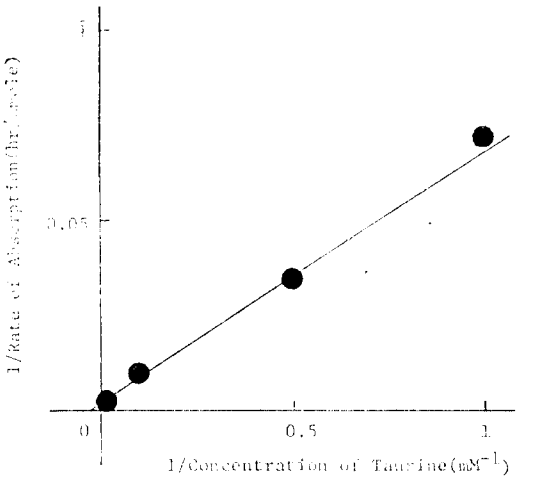

Fig. 3: Lineweaver-Burk plot for taurine absorption.

pectively, The resultant absorption showed a saturable process at higher concentrations. Further, a least square linear regression analysis of a double reciprocal plot of the data obtained from Fig. 2, gave values for Km of 39.9mM and Vmax of 571.4 μmole/hr, as shown in Fig.3 This estimation certainly suggests that taurine transfer is mediated by a low-affinity transport system, which has been exhibited in platelets.[11]

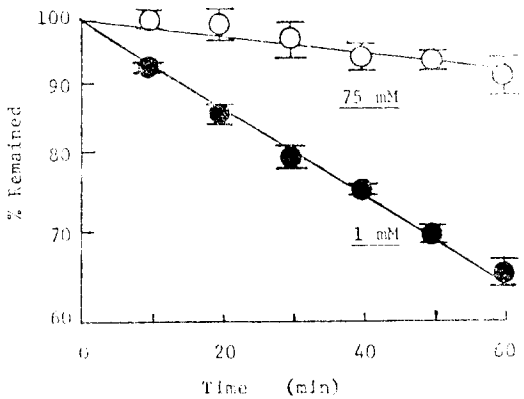

Fig. 4: Time course of taurine absorption. Results are expressed as the mean±S.E.M..

Fig. 4. shows the time course of taurine absorption, which was measured at 10 min intervals over 1 hr. The absorption was linear at 1 mM during the period, but not at 75mM. Zero order kinetics observed at 75mM is considered to be due to the saturability of transport system, which has been frequently observed in the carrier-mediated transport processes[14,15]. Furthermore, $HgCl_2$, which is well known as a-SH blocking agent, was treated for 5 min before the recirculating perfusion. As is evident from Table II, the absorption was markedly inhibited by 52%. This result implies that some protein appeared to be involved in taurine transfer. In order to further confirm the involvement of protein, structurally related compounds or sugars were tested for their ability to inhibit taurine absorption. The recirculating perfusion was carried out both in the presence of 1mM taurine and 10mM inhibitor. As shown in Table III, compounds structurally similar to taurine such as aminomethane sulfonic acid or γ-aminobutyric acid (variations in carbon chain length), hypotaurine (difference in the oxidation state of sulfur moiety), and β-alanine, were all relatively good competitive inhibitors. These results suggest that a mechanism for taurine transport in the rat small intestine is separate and distinct from known neutral α-amino acid transport process[16] based on the different affect of α-amino acid (glycine) and β-amino acid (β-alanine) on taurine uptake. Besides, taurine absorption was significantly reduced by the presence of glycylglycine, L-glutamic acid, or L-lysine, all

Table II: Effect of pretreatment with $HgCl_2$ on intestinal absorption of taurine.

	% Absorbed in one hour	% Inhibition
Sham	26.1±2.1(3)	—
$HgCl_2$-treated	12.6±1.6(4)	51.7

*Pretreatment with 1mM $HgCl_2$ for 5 min.
Results are expressed as the mean±S.E.M. followed by the number of animals in parentheses.

Table III: Effect of amino acids, sugars, or a dipeptide on intestinal absorption of taurine.

Adjuvant	% Absorbed in one hour	Statistical significance
None	34.7±1.5(3)	—
Aminomethane sulfonic acid	21.2±3.0(6)	$p<0.05$
Hypotaurine	18.9±1.8(4)	<0.01
N-Acetyltaurine	29.2±1.9(3)	NS
Isethionic acid	31.8±2.7(3)	NS
L-Cysteine	28.4±3.8(3)	NS
Glycine	29.8±1.5(4)	NS
β-Alanine	22.9±3.5(4)	NS
γ-Aminobutyric acid	15.3±2.0(5)	<0.001
L-Lysine	22.3±3.4(3)	<0.05
L-Glutamic acid	26.7±2.1(4)	<0.05
D-Glucose	18.0±1.0(3)	<0.001
D-Fructose	32.3±2.7(3)	NS
Glycylglycine	27.2±1.2(6)	<0.01

Concentrations of taurine and adjuvants were 1 and 10mM.
Results are expressed as the mean±S.E.M. followed by the number of experiments in parentheses. NS; not significant, compared with each control.

Table IV: Effect of metabolic inhibitor on intestinal absorption of taurine.

Inhibitor	% Absorbed in 10 min	Statistical significance(p)
None	46.5±1.6(4)	
1.0mM DNP	37.4±2.4(4)	0.05

Concentration of taurine=1mM.
Results are expressed as the mean±S.E.M. followed by the number of animals in parentheses.

structurally different from taurine. These interesting findings might be interpreted by the assumption that the taurine transport system seems to be, at least in part, overlapped with those of the dipeptide and the acidic or the basic amino acid.

In agreement with previous reports from studies of taurine uptake in kidney[6,7] retina,[8,9] and platelets,[10] sodium was found to be required for maximal rates of transport, as shown in Fig. 4. Osmolarity was held constant by replacing NaCl with mannitol. The complete substitution of NaCl with mannitol caused to reduce signtificantly the transfer.

In connection, energy requirement for the taurine uptake was also demonstrated, with applying loop method. Table III shows that the metabolic inhibitor, 2, 4-dinitrophenol, decreased the uptake by 80% from that of the control,

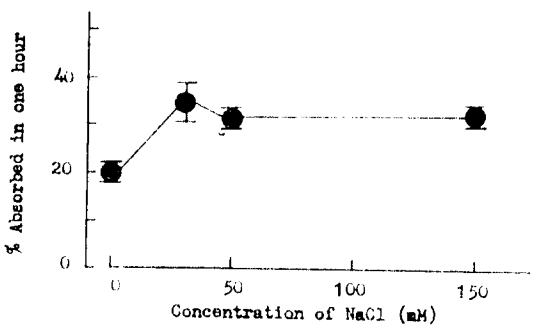

Fig. 5: Sodium ion dependency of taurine absorption. Results are expressed as the mean±S.E.M..

$p<0.05$. This result is a characteristic of taurine transport in the rat small intestine shared with other tissues[8,9,10,12].

Taken together, a conclusion can be drawn that a carrier-mediated transport system for taurine appeared to exist in the small intestine based on the demonstration of saturability, structural selectivity, sodium ion-and energy dependency of the uptake process.

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