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Research Article

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The Effect of Inhibitors of Renal Transport on the Small Intestine *

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In the genetically determined disorders cystinuria and Hartnup disease, amino acid transport defects are present in both the small intestine and the kidney (2–4). It would seem logical that agents which alter kidney function may also affect the transporting ability of the small intestine. We have therefore studied the effect on intestinal transport (by means of *in vitro* intestinal preparations) of some of the drugs that inhibit renal tubular transport.

Methods

Everted gut sacs were made from the small intestine of male golden hamsters weighing between 80 and 130 g (5). The nonfasting animal was killed by a blow on the head. The small intestine was then isolated, washed *in situ* with pH 7.4 Krebs bicarbonate buffer, and removed by stripping off the mesentery. It was then everted, and three segments each weighing approximately 600 mg were constructed from the entire length of the bowel. The nonelectrolyte whose transport was to be studied was placed at varying concentrations in the buffer, and 1 ml of this fluid was placed inside the sac. The sac was then placed in an Erlenmeyer flask with 5 ml of buffer containing the nonelectrolyte at an identical concentration. Potential inhibitors at several concentrations were added to the buffer as indicated. After being gassed with 95% O_2 and 5% CO_2, the flasks were stoppered and placed in an oscillating water bath kept at 37° C and 50 vibrations per minute for 1 hour. Portions of the serosal and mucosal fluids were centrifuged and then analyzed for radioactivity in a liquid scintillation spectrometer.[1] Sacs were blotted and weighed.

The radioactively labeled materials L-lysine-^{14}C (UL),[2] methyl-L-methionine-^{14}C,[2] 2-uracil-^{14}C,[2] glucose-^{14}C (UL),[2] and ^{22}Na,[2] as well as L-lysine monohydrochloride,[3] were obtained commercially. Ethacrynic acid [2,3-dichloro-4-(2 methylenebutyryl)-phenoxyacetic acid],[4] probenecid,[4] and chlorothiazide [4] were used as the pure powders.

Krebs bicarbonate buffer (pH 7.4; sodium, 147 mEq per L; chloride, 127 mEq per L; H_2PO_4, 1.1 mEq per L; and HCO_3^-, 25 mEq per L) with a potassium concentration of 6.1 mEq per L was used in all experiments unless otherwise indicated. In some experiments buffer was used with a potassium concentration of 16.1 mEq per L and a sodium concentration of 135 mEq per L; this is referred to as "high potassium" buffer. In other experiments potassium was removed from the buffer, and the sodium concentration was increased to 152 mEq per L; this is referred to as "potassium-free" buffer. Anion concentrations remained unchanged in all buffers.

Nonelectrolyte transport. Nonelectrolyte transport was expressed as the ratio of mucosal-to-serosal concentration. Per cent inhibition was determined by comparing the increase in counts in the serosal medium per gram of wet tissue weight to that of an equal number of control sacs. In each experiment the absorption of six sacs from two animals in the presence of a potential inhibitor was compared to that of an equal number of control sacs. Each result represents at least two separate experiments.

Water transport. Net movement of water into the serosal compartment was determined gravimetrically. All weighings were performed in an identical fashion. The initial weight of the serosal fluid was determined before incubation by subtracting the weight of the empty sac from the weight of the filled sac. The weight of the serosal volume after incubation was determined by weighing the filled sac and reweighing after thorough drainage. The difference between these two weights was a measure of the net change in the serosal water compartment. To express this as milligram per milligram of dry tissue weight, we dried the tissue at 105° C overnight and reweighed it.

Net sodium movement. ^{22}Na was utilized in an estimate of net sodium movement by subtracting the initial total counts in the serosal compartment from the final total counts in the serosal compartment. Total counts

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[1] Picker Nuclear Corp., White Plains, N. Y.

[2] New England Nuclear Corp., Boston, Mass.

[3] Nutritional Biochemicals Corp., Cleveland, Ohio.

[4] Kindly supplied by Merck Sharp & Dohme Research Laboratories, West Point, Pa.

TABLE I

Inhibition by 5 mM ethacrynic acid of nonelectrolyte absorption against a concentration gradient by the hamster everted small intestine

	Control	Ethacrynic acid	p
5×10^{-6} M L-methionine	6.40 ± 2.56* (12)	1.37 ± 0.42 (11)	< 0.001
10^{-4} M L-lysine	3.39 ± 1.78 (12)	1.43 ± 0.26 (12)	< 0.001
10^{-3} M glucose	8.36 ± 3.40 (12)	1.36 ± 0.19 (12)	< 0.001
10^{-5} M uracil	3.49 ± 1.32 (12)	1.25 ± 0.23 (12)	< 0.001

* Mean of mucosal/serosal concentration ± standard deviation. Number in parentheses represents number of sacs.

in the serosal fluid were calculated initially from the known volume and the counts per unit volume. The total counts in the serosal compartment after incubation were determined by counting the entire inside volume in an automatic gamma counter.[5] The per cent increase in total counts at the end of the incubation period per milligram of dry tissue weight represented net movement of ^{22}Na.

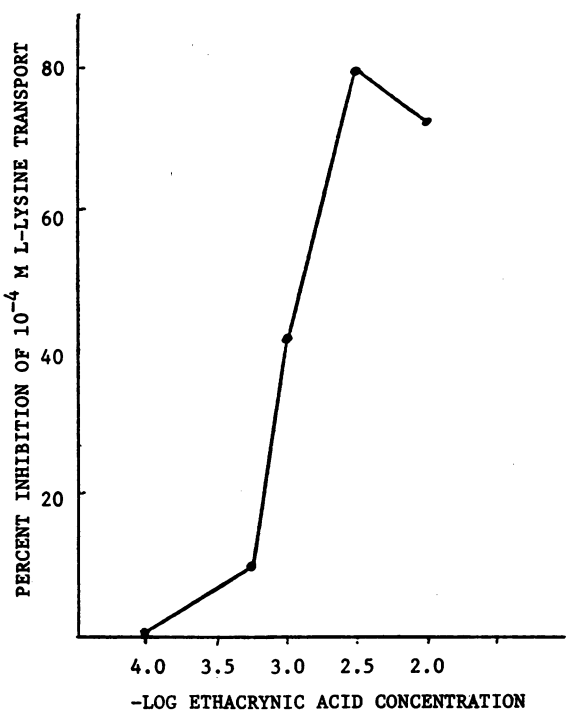

FIG. 1. EFFECT OF VARYING CONCENTRATIONS OF ETHACRYNIC ACID ON AMINO ACID ABSORPTION IN THE HAMSTER SMALL INTESTINE. The inhibition of the transport of 10^{-4} M L-lysine by ethacrynic acid was dose related between 0.5 mM and 5 mM concentration. Ten mM ethacrynic acid did not cause any further inhibition than 5 mM. The incubation procedure was as described in the text, with the concentration of ethacrynic acid being varied. Each point represents the mean of six sacs. The standard deviations were approximately 25% of the mean values.

[5] Picker Nuclear Corp., White Plains, N. Y.

Student's *t* test was applied by standard statistical methods (6).

Results

Ethacrynic acid. The absorption of 5×10^{-6} M L-methionine, 10^{-4} M L-lysine, 10^{-3} M glucose, and 10^{-5} M uracil by the hamster small intestine was markedly inhibited by 5 mM ethacrynic acid (Table I).

The effect of varying the concentration of ethacrynic acid on the inhibition of absorption of 10^{-4} M L-lysine was determined (Figure 1). Ethacrynic acid at 0.1 mM and 0.5 mM concentrations failed to inhibit absorption, but maximal inhibition was obtained at a 5 mM concentration. The inhibition of L-lysine absorption by 5 mM ethacrynic acid was similar at 10^{-3} M, 5×10^{-4} M, and 10^{-4} M concentrations of amino acid (Table II).

Water and ^{22}Na absorption were also markedly impaired in the presence of 5 mM ethacrynic acid (Tables III and IV). Inhibition of water absorption was also dependent on the dosage of ethacrynic acid. One-tenth mM ethacrynic acid had no effect; 0.5 mM acid significantly inhibited water absorption (Table III).

A study of the importance of the concentration of potassium in the medium was made. At 0.5 mM ethacrynic acid, there was no effect on L-lysine absorption with a potassium concentration of 6.1 mEq per L (Figure 1). This is in contrast to a significant inhibition in a potassium-free medium.

TABLE II

Inhibition of absorption of varying concentrations of L-lysine by 5 mM ethacrynic acid in the hamster small intestine

	% transport of controls
10^{-3} M L-lysine	16
5×10^{-4} M L-lysine	12
10^{-4} M L-lysine	19

TABLE III

*Translocation of water across the hamster everted small intestine as modified by ethacrynic acid**

	Water uptake†	p
Control	4.89 ± 2.82‡ (15)	
Ethacrynic acid		
5 mM	−0.74 ± 0.89 (6)	< 0.001
1 mM	−0.28 ± 0.96 (5)	< 0.01
0.5 mM	0.25 ± 1.20 (9)	< 0.01
0.1 mM	2.66 ± 1.74 (5)	NS

* Each sac filled with 0.5 ml Krebs-bicarbonate buffer containing 12.2 mM glucose was placed in 7.5 ml buffer. Each flask was incubated at 37° C for 60 minutes after gassing with 95% O_2–5% CO_2.

† Expressed as the increase in the serosal compartment (in milligrams) per milligram dry tissue weight.

‡ Mean ± standard deviation. Number in parentheses represents number of sacs.

The mucosal-serosal concentration ratio in a potassium-free buffer without ethacrynic acid was 3.72 ± 0.91 (not significantly different from that in 6.1 mEq per L potassium medium shown in Table I), and in the presence of 0.5 mM ethacrynic acid it was 2.34 ± 0.52 ($p < 0.001$). An increased potassium concentration decreased the inhibition of 10^{-4} M L-lysine absorption by 5 mM ethacrynic acid; in Krebs-bicarbonate buffer (K = 6.1 mEq per L), L-lysine absorption was inhibited 80% by 5 mM ethacrynic acid, but in a high potassium buffer the inhibition by 5 mM ethacrynic acid was reduced to 62%.

Chlorothiazide. Two mM chlorothiazide did not significantly inhibit intestinal transport of L-methionine, L-lysine, glucose, or uracil (Table V). Water and ^{22}Na absorption were impaired in the presence of 2 mM chlorothiazide (Tables IV and VI).

Probenecid. The intestinal transport of L-methionine and L-lysine was significantly inhibited by

TABLE IV

*Net absorption of ^{22}Na across the everted hamster small intestine as modified by ethacrynic acid, chlorothiazide, and probenecid**

	Net ^{22}Na absorption†	p
Control (9)	0.42 ± 0.40‡	
Ethacrynic acid 5 mM (6)	−0.45 ± 0.14	< 0.001
Probenecid 5 mM (7)	0.24 ± 0.27	NS
Chlorothiazide 2 mM (5)	−0.11 ± 0.16	< 0.02

* Details of incubation procedure are as described in Table III except that an equal amount of ^{22}Na was added to both mucosal and serosal media.

† Net ^{22}Na movement expressed as increase in counts in total serosal medium at the end of the incubation period per initial total counts in serosal medium per milligram dry tissue weight.

‡ Mean ± standard deviation.

TABLE V

*Nonelectrolyte absorption across the everted hamster small intestine in the presence of 2 mM chlorothiazide**

	Control	Chlorothiazide	p
5×10^{-6} M L-methionine	5.49 ± 0.72* (24)	5.01 ± 1.85 (24)	NS
10^{-4} M L-lysine	2.96 ± 1.29 (17)	3.24 ± 1.28 (18)	NS
10^{-3} M glucose	3.29 ± 2.33 (18)	3.46 ± 2.32 (18)	NS
10^{-5} M uracil	3.84 ± 2.19 (12)	4.96 ± 1.09 (11)	NS

* Mean of mucosal/serosal concentration ± standard deviation. Number in parentheses represents number of sacs.

5 mM probenecid (Table VII). The inhibition of L-lysine and L-methionine transport by probenecid was dose related (Table VIII). Water and ^{22}Na absorption in the hamster small intestine was not impaired by the presence of 5 mM probenecid (Tables IV and VI). The transport of glucose and uracil was decreased, but not significantly, by 5 mM probenecid.

Discussion

Ethacrynic acid is a potent diuretic agent that inhibits sodium and water reabsorption in the ascending limb of the loop of Henle (7). Ethacrynic acid has been demonstrated to inhibit renal sodium-potassium—dependent ATPase (8, 9) and to inhibit both sodium efflux from erythrocytes (10, 11) and sodium movement across the toad bladder (12).

In the present study, ethacrynic acid also inhibited transport of sodium in sacs made from the small intestine of the golden hamster. The occurrence of this inhibition appears straightforward, although the mechanism (whether due to inhibition of ATPase or another phenomenon) is uncertain. Ouabain and oligomycin, drugs that inhibit sodium-potassium ATPase and the "sodium pump," also inhibit amino acid and sugar transport (13, 14). Ethacrynic acid inhibited nonelectrolyte transport in a dose-related manner, prob-

TABLE VI

*Net absorption of water as modified by chlorothiazide and probenecid across the everted hamster small intestine**

	Net water uptake	p
Controls	4.89 ± 2.82† (15)	
Probenecid 5 mM	3.62 ± 2.31 (7)	NS
Chlorothiazide 2 mM	0.79 ± 0.75 (14)	< 0.001

* See Table III for details of incubation procedure.

† Mean ± standard deviation. Number in parentheses represents number of sacs.

TABLE VII

*Nonelectrolyte absorption across the everted hamster small intestine in the presence of 5 mM probenecid**

	Control	Probenecid	p
5×10^{-6} M L-methionine	5.77 ± 3.28* (12)	2.66 ± 1.26 (12)	< 0.01
10^{-4} M L-lysine	2.86 ± 1.10 (12)	1.79 ± 0.23 (12)	< 0.01
10^{-3} M glucose	4.59 ± 2.79 (23)	3.26 ± 1.68 (24)	NS
10^{-5} M uracil	3.77 ± 2.18 (17)	3.03 ± 1.51 (18)	NS

* Mean of mucosal/serosal concentration ± standard deviation. Number in parentheses represents number of sacs.

ably secondary to the inhibition of sodium transport (we have preliminary data that the inhibition is noncompetitive, since it is not overcome by increasing the concentration of the nonelectrolyte). Whether ethacrynic acid also acts as a metabolic inhibitor in the intestine is unknown. After a 100-mg dose of ethacrynic acid, if the compound were dissolved in 5 L of blood, the concentration would be approximately 10^{-4} mole per L. The actual quantity bound to the kidney or gut *in vivo* is as yet unknown. The action of ethacrynic acid is clearly related to the potassium concentration of the medium. At low potassium concentrations ethacrynic acid is more effective as an inhibitor; its action is decreased by increasing the potassium concentration. Similar results are seen in studies of sodium efflux in human erythrocytes (10). As a possible explanation of this phenomenon Hoffman has suggested that potassium is bound to the site of ethacrynic acid action (15).

Chlorothiazide probably inhibits reabsorption of sodium in the distal tubules and also interferes with the transport of uric acid (16). However, its ability to inhibit ATPase has not been demonstrated (8). Two mM chlorothiazide and 0.5 mM ethacrynic acid fail to inhibit nonelectrolyte transport, but do inhibit water transport. This apparent dissociation of sodium transport as reflected by water transport and amino acid transport may be related to the linear relationship between sodium and amino acid transport at sodium concentrations below 100 mEq per L. Two mM chlorothiazide and 0.5 mM ethacrynic acid may not inhibit sodium transport sufficiently to inhibit amino acid transport.

Probenecid is an established inhibitor of renal tubular transport that can block either reabsorption or secretion of several organic compounds such as penicillin and uric acid (17). Therefore, that it can also inhibit intestinal transport is not unexpected. Its action on amino acid transport seems to be specific, since probenecid does not inhibit water and sodium transport.

In the present study we have demonstrated the ability of several drugs, whose primary and perhaps sole function had been thought to be on the renal tubules, to inhibit intestinal absorption. This strengthens the evidence that there are functional similarities in the transport systems in the kidney and the small intestine. Morphologically the kidney and the small intestine have at least one similarity. Microvilli are characteristic of the small intestine and many other absorptive surfaces and are also found in the proximal tubules of the kidney (18). More recently, the site of amino acid reabsorption in the kidney has also been localized to the proximal tubules (19). A further similarity is that defects in amino acid transport systems have been found in the small intestine as well as in the kidney in the genetically determined diseases, cystinuria and Hartnup disease (2–4).

These observations may have clinical pertinence to the cachexia seen in some patients with chronic congestive heart failure. In addition to the malabsorption usually attributed to tissue anoxia (20), the cardiorenal drugs might also contribute to cardiac cachexia by inhibiting intestinal absorption. The diarrhea that infrequently occurs during ethacrynic acid therapy (21–23) may be a consequence of inhibition of ATPase activity and sodium transport as has been postulated for the cathartic action of cascara and other vegetable cathartics (24) and in the diarrhea of cholera (25).

TABLE VIII

Inhibition of amino acid absorption by varying concentrations of probenecid in the hamster small intestine

	% transport of controls	
	L-Lysine	L-Methionine
Probenecid 0.5 mM	113	99
Probenecid 5.0 mM	62	47

Summary

Nonelectrolyte transport has been studied in everted hamster small intestinal sacs. Ethacrynic acid, a diuretic agent, significantly inhibited amino acid, glucose, and pyrimidine transport. Water and sodium transport was impaired by both ethacrynic acid and chlorothiazide. Probenecid inhibited amino acid transport only.

We suggest that these observations provide further evidence of functional similarities between the kidney and the small intestine. The relationship of cardiorenal drugs to intestinal absorption was discussed.

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