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Inhibition of the intestinal transport of uracil by hexoses and amino acids

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Various hexoses and amino acids were tested as potential inhibitors of the active mucosal to serosal transport of uracil across the everted rat jejunum. Uracil transport displayed Michaelis-Menten type kinetics with a V_{max} of 10.4 ± 0.2 $\mu mol \cdot g^{-1} \cdot h^{-1}$ and an apparent K_m of 0.047 ± 0.002 mM (means ± S.D.). Scilliroside, an inhibitor of the basolateral $(Na^+ + K^+)$-ATPase, dose-dependently inhibited the transport of uracil consistent with the Na^+ dependency of uracil transport. Thymine was a full competitive inhibitor ($K_i = 0.021 \pm 0.002$ mM) of uracil transport. All actively transported substances tested including L-phenylalanine, L-leucine, D-galactose, D-glucose, and 3-*O*-methylglucose inhibited the transport of uracil. In contrast, L-glucose and fructose, substances which are not actively transported, were without effect on uracil transport. Further studies with D-galactose indicated that it acts as a partial noncompetitive inhibitor ($K_i = 6.0 \pm 1.4$ mM) of uracil transport. This K_i is in good agreement with the apparent K_t (5.8 ± 1.1 mM) for D-galactose transport. Phlorizin (0.1 mM), an inhibitor of galactose transport, blocked the inhibitory effect of galactose on uracil transport. In the ileum D-galactose had no effect on uracil transport but thymine caused the same degree of inhibition as in the jejunum. The results demonstrate that heterologous inhibition is a more general phenomenon than had previously been realized.

Introduction

Several hypotheses have been proposed to explain the mutual inhibition in transport between hexoses and amino acids [1–7], but they have all been ruled out with the exception of the allosteric interaction- or *cis*-hypothesis [4,8] and the accelerated efflux- or *trans*-hypothesis [5–7,9,10]. Both hypotheses agree that the inhibition is localized at the brush-border membrane. According to the *cis*-hypothesis binding of the inhibitor with its binding site at the outer brush-border membrane involves an allosteric effect on the functionally related binding site of the substrate. This allosteric effect is thought to inhibit the translocation of the substrate across the apical membrane. In contrast, the *trans*-hypothesis suggests that the passage of the inhibitor across the brush-border membrane is necessary for inhibition. As a consequence of the sodium-coupled influx of the inhibitor the electrochemical potential gradient for sodium is diminished and therefore the influx of the concerned substrate is decreased and the back flux increased. If the mutual inhibitory effects of hexose and amino acids are in some way due to the sodium-dependent nature of their transport one might predict a heterologous interaction between the transport of hexoses and amino acids and other substrates transported by a sodium-dependent mechanism. Uracil is reported to be actively transported [11] by a sodium-dependent system [12]. The purpose of this study was to determine

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whether uracil transport could be inhibited by hexoses and amino acids.

Materials and Methods

Animals

Male Sprague-Dawley rats (220–250 g) were used. The animals had free access to water but were deprived of food 18 h prior to the experiment.

Tissue preparation

Preparation and incubation of everted sacs were carried out according to the method of Parsons and Paterson [13] with modifications [14]. Animals were anesthetized with ethyl ether and opened by a midline incision. Three segments of the upper jejunum of 8 cm length were prepared. Segments were placed in gassed buffer solution, rinsed, everted and mounted on glass cannulae. A 2 g weight was attached to the closed end of the sac. For ileum experiments three sacs were prepared according to the same protocol from an ileum segment.

Sacs were brought into chambers filled with 35 ml of the standard incubation medium and different concentrations of cold and ^{14}C-labelled uracil and the inhibitors. The standard incubation medium was pH 7.4, 285 mosM and contained 100 mmol Na_2SO_4, 25 mmol $NaHCO_3$, 4.8 mmol KCl, 2.6 mmol $CaCl_2$, 1.3 mmol KH_2PO_4, 1.9 mmol $MgSO_4$ per liter. All the chemicals used were commercial samples of chemically pure grade. [2-^{14}C]Uracil (54.3 mCi/mmol; purity > 98%) was purchased from New England Nuclear. The everted sacs and cannulae were filled from a tared syringe with fluid of the same composition as the mucosal bath medium, so that uracil was present initially in both the mucosal and serosal fluids at equal concentrations. The cannulae were filled to establish a serosal to mucosal hydrostatic pressure gradient of 5 cm water. Bridges reported [14] that at this initial hydrostatic pressure gradient mucosal to serosal transfer across the rat jejunum was maximal. During the incubation, which was carried out at 37°C, the mucosal bathing solution was gassed and stirred by a gas mixture of 95% O_2 and 5% CO_2. Net transport was measured over a 60-min period. Control experiments indicated that uracil transport was linear over this time. Subsequently, the sacs were removed and the serosal fluid carefully collected. After removal from the cannulae, the sacs were dried at 95°C for 24 h. The sacs generally had a final length of 5–6 cm and a dry weight of 30–50 mg. Samples of 100 μl of the serosal fluid were mixed with 7 ml of a commonly used scintillation fluid and counted in a Philips liquid scintillation counter.

The net transport of uracil was calculated by subtracting the amount (concentration times volume) after a 15-min preincubation period ($t_{15\,min}$) from the final amount at $t_{75\,min}$ (incubation time = $t_{75\,min}$ minus $t_{15\,min}$ = 60 min). Data were normalized to tissue dry weight and expressed as μmol uracil · (g dry gut)$^{-1}$ · h^{-1}.

Statistics

Each data point is represented by six measurements and given as the mean ± standard error of the mean (S.E.) unless otherwise stated. Significances of differences were tested by the two-tailed unpaired Student's *t*-test. Data were fitted to the Michaelis-Menten-equation by a nonlinear regression BASIC program for microcomputers [15]. 'NONLIN' [16] was used to fit the data to more complex equations. A *F*-test was performed to determine the appropriate kinetic model [17].

Results

Net transport of uracil across the everted sac as a function of the uracil concentration is shown in Fig. 1. At low concentrations of uracil a 5-fold increase in the serosal concentration over the mucosal concentration was achieved confirming the active nature of uracil transport. At high uracil concentrations saturation of the transport system was observed. The kinetics of uracil net transport could be described by the Michaelis-Menten equation. The kinetic constants obtained when data were fitted to the Michaelis-Menten equation were: $V_{max} = 10.4 \pm 0.2$ μmol · g^{-1} · h^{-1} and $K_t = 0.047 \pm 0.002$ mM (means ± S.D.). As many other active solute transport systems, the uracil transport mechanism has been shown to be sodium-dependent [12]. The sodium-dependence of the uracil transport mechanism was tested in this study with the use of scilliroside, a potent inhibitor of the

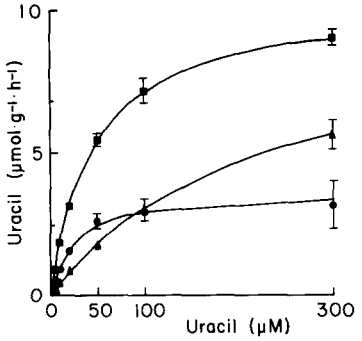

Fig. 1. Active transport of uracil as a function of uracil concentration in the absence (■) or presence of 0.1 mM thymine (▲) or 10 mM D-galactose (●). The solid lines are the calculated curves, when data are fitted to the Michaelis-Menten equation. Points are given as the mean values ± S.E. for at least six determinations.

basolateral ($Na^+ + K^+$)-ATPase [18]. When added to the serosal solution, scilliroside, 0.1 and 0.5 mM, reduced the transport of uracil by 60 ± 6% and by 87 ± 3%, respectively.

Inhibition of uracil transport by sugars and amino acids is shown in Table I. L-Phenylalanine and L-leucine both inhibited the transport or uracil significantly. The aldo-hexoses D-galactose, D-glucose and 3-*O*-methylglucose all caused a significant inhibition of the transport of uracil. In contrast L-glucose and fructose, a keto-hexose, did not significantly alter the transport of uracil. To examine further the nature of the inhibition of uracil transport by such heterologous substances the effects of D-galactose on uracil transport were examined in detail. For comparison the effects of thymine, a chemically homologous substance, were studied in parallel.

The kinetics of uracil transport were measured in the presence of the concerned inhibitor at a fixed concentration (10 mM D-galactose, 0.1 mM thymine). Fig. 1 illustrates that thymine as well as D-galactose inhibited the transport of uracil at all examined uracil concentrations. The inhibitory strength of thymine decreased with increasing uracil concentration, whereas D-galactose had an almost constant inhibitory effect. Michaelian behavior of uracil transport was not altered in the presence of the inhibitors, so that the kinetic constants could be determined by a nonlinear regression of the data to the Michaelis-Menten equation. The results indicated different types of inhibition for thymine and D-galactose (Table II). Thymine solely increased the K_t (0.23 ± 0.01 mM; mean ± S.D.) without having a significant effect on the V_{max}. In contrast, D-galactose decreased the V_{max} considerably (3.5 ± 0.2 $\mu mol \cdot g^{-1} \cdot h^{-1}$; mean ± S.D.) without having an inhibitory effect on the apparent K_t. Thus, thymine showed the characteristics of a competitive inhibitor and D-galactose that of a noncompetitive inhibitor.

To determine the maximal degree of inhibition attainable by thymine and D-galactose, the inhibitor concentrations were varied for two fixed uracil concentrations (0.01 and 0.3 mM). The results are shown in Fig. 2. The inhibitory effects of thymine

TABLE I

INHIBITION OF URACIL TRANSPORT BY DIFFERENT HEXOSES AND AMINO ACIDS

Active transport of uracil at 0.3 mM uracil across everted rat small intestine was measured in the presence of 20 mM mannitol (controls) or 20 mM of different hexoses and amino acids. Data represent the mean inhibition ± S.E. compared to the controls for at least six measurements.

Inhibitor	Inhibition (%)	*P*
D-Galactose	70 ± 4	0.0005
3-*O*-Methylglucose	35 ± 7	0.005
D-Glucose	59 ± 3	0.0005
L-Glucose	0 ± 13	not signif.
L-Fructose	3 ± 9	not signif.
L-Phenylalanine	65 ± 4	0.0005
L-Leucine	38 ± 5	0.0005

TABLE II

KINETIC PARAMETERS OF URACIL TRANSPORT IN THE ABSENCE OR PRESENCE OF A FIXED INHIBITOR CONCENTRATION

The active transport of uracil was measured across rat small intestine in the absence or in the presence of thymine at 0.1 mM or D-galactose at 10 mM, respectively. Kinetic parameters and their standard deviations (S.D.) were obtained by subjecting experiment data to nonlinear regression to the Michaelis-Menten equation.

Inhibitor	V_{max} ($\mu mol \cdot g^{-1} \cdot h^{-1}$)	app. K_t (mM)
None	10.4 ± 0.2	0.047 ± 0.002
Thymine	9.9 ± 0.2	0.23 ± 0.01
D-Galactose	3.5 ± 0.2	0.023 ± 0.004

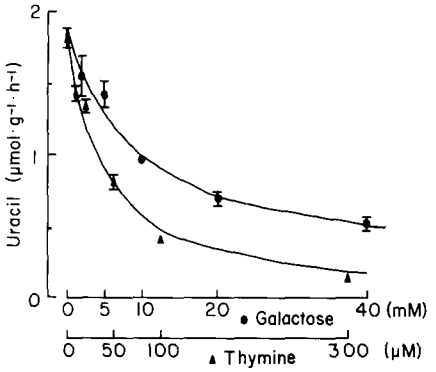

Fig. 2. Inhibition of uracil transport at 0.01 mM uracil by different concentrations of D-galactose and tymine. The solid lines are the calculated curves when data are fitted to the equations for competitive or partial noncompetitive inhibition. Points represent the mean values ± S.E. for six measurements.

were markedly greater at 0.01 mM uracil than at 0.3 mM. For example, thymine at 0.1 mM reduced uracil transport at 0.01 mM by 76 ± 2% and at 0.3 mM uracil only by 40 ± 5%. For both uracil concentrations, inhibition by thymine increased with the thymine concentration. At a high thymine concentration (0.3 mM) and a low uracil concentration (0.01 mM), the transport of uracil was nearly completely blocked (93%). As theoretically expected, the pattern of thymine effects on uracil transport indicate that it acts as a fully competitive type inhibitor.

The inhibitory effect of D-galactose also depended on the concentration of this hexose. Increasing the D-galactose concentration caused a greater inhibitory effect. In contrast to thymine, however, uracil transport could not be blocked totally even at a high D-galactose concentration (40 mM). The highest observed inhibition by D-galactose was 72%. The degree of inhibition was, unlike thymine, quite independent of the uracil concentration as indicated by the following: 40 mM Dgalactose inhibited uracil transport at 0.01 mM by 69 ± 5% and at 0.3 mM by 72 ± 2%. Thus, the inhibitory effects of D-galactose differ in several points from those of thymine.

A nonlinear regression of the data to the equations for the main types of inhibition [19] was performed and provided the following results: thymine acts as a fully competitive inhibitor, type Ia [19], with an apparent inhibitor constant K_i = 0.021 ± 0.002 mM (mean ± S.D.). Thus, thymine has a considerably higher affinity to the carrier compared to uracil. D-galactose, however, is a partially noncompetitive inhibitor, type IIb [19], with a K_i of 6.0 ± 1.4 mM (mean ± S.D.).

The K_i for D-galactose inhibition of uracil transport was very similar to values reported for the K_t of galactose mucosal uptake [4,20]. To clarify, whether also under our experimental conditions such a relationship exists, the kinetics of the active transport of D-galactose across rat small intestine were investigated. D-Galactose net transport across the everted sac displayed Michaelis-Menten-type kinetics. The value of the apparent affinity constant, K_t, for D-galactose transport was 5.8 ± 1.1 mM (mean ± S.D.) and is not significantly different from the K_i of 6.0 mM for the inhibition of galactose on uracil transport. This equality points out, that there is a relationship between the active transport of D-galactose and its inhibitory effects on uracil transport.

To further support this hypothesis, the inhibition of uracil transport by D-galactose was studied under conditions, which prevented the sugar's own active transport. In a first experiment phlorizin, a potent inhibitor of hexose transport, was used. Phlorizin almost completely abolished the inhibition of uracil transport by D-galactose. Whereas D-galactose at 20 mM inhibited the transport of uracil at 0.3 mM uracil by 60%, the same concentration of the hexose in the presence of 0.1 mM phlorizin caused only an insignificant inhibition of 6%. Phlorizin by itself (at 0.1 mM) had no effect on uracil transport (at 0.3 mM uracil).

TABLE III

INHIBITION OF URACIL TRANSPORT BY D-GALACTOSE AND THYMINE IN RAT JEJUNUM AND ILEUM

Active transport of uracil at 0.05 mM was measured across everted rat jejunum and ileum for 60 min at 37°C in the absence and in the presence of D-galactose at 10 mM or thymine at 0.1 mM, respectively. Data represent means ± S.E. for six measurements.

Inhibitor	Uracil transport (μmol·g^{-1}·h^{-1})	
	jejunum	ileum
None	5.3 ± 0.3	4.7 ± 0.7
D-Galactose	2.6 ± 0.3	4.0 ± 0.6
Thymine	1.7 ± 0.2	1.2 ± 0.2

A further experiment was performed with rat distal ileum, the ileum of the rat can actively transport uracil, but does not actively transport D-galactose (data not shown). In contrast to the inhibition of uracil transport by D-galactose in the jejunum, in the ileum uracil transport was not significantly affected by D-galactose (Table III). Thymine, however, inhibited the transport of uracil in the ileum to a similar degree (73%) as seen in the jejunum (67%).

Discussion

The results of this study with the rat jejunum and ileum confirm, as first described by Schanker and Tocco [11], that uracil is actively transported by the small intestine, Csaky [12], using the isolated frog small intestine, demonstrated that the transport of uracil requires sodium in the incubation solution and stated that the transport of uracil is a sodium-dependent process. Scilliroside, a potent inhibitor of the basolateral $(Na^+ + K^+)$-ATPase, dose-dependently inhibited the net transport of uracil across the everted rat jejunum. These results further support the hypothesis that uracil is transported by a sodium-dependent mechanism. Thus the transport of uracil by the small intestine is characterized by the same typical properties as the transport of many hexoses and amino acids. The mutual inhibition in transport between hexoses and amino acids has been repeatedly reported [1,4,7,21,22]. The purpose of this study was to determine whether the transport of uracil could be inhibited by such chemically heterologous substances as hexoses and amino acids.

Net uracil transport across the everted sac of rat jejunum displayed excellent Michaelis-Menten type kinetics (Fig. 1). Furthermore, the inhibition of net uracil transport by thymine and D-galactose could be described by the equations for a fully competitive inhibition and a partially noncompetitive inhibition, type IIb [19], respectively. In each case the experimental data points were very close to the theoretical curves. However, the kinetic constants obtained should be viewed with some degree of caution. As repeatedly pointed out by Munck [23,24] and others [25] the net transmural movement of a transported solute is a complex function of unidirectional flows across both the mucosal and serosal membranes and the values of the V_{max} and K_t obtained may not be related to any single step.

All of the hexoses and amino acids examined with the exception of L-glucose and fructose caused an inhibition in the transport of uracil. The degree of inhibition differed between the individual inhibitors, but was comparable for hexoses and amino acids. For example, nearly the same degree of inhibition was achieved with D-galactose and L-phenylalanine and with 3-*O*-methylglucose and L-leucine, respectively (see Table I). All of these inhibitory substances are known to cross the brush-border membrane by a secondarily sodium-dependent facilitated diffusion mechanism [26]. In contrast, L-glucose and fructose were without effect on uracil transport. L-Glucose, the enantiomorph of D-glucose, is not accumulated by the intestine nor does it inhibit the absorption of D-glucose [27]. D-Glucose inhibition of uracil transport is therefore stereoselective. The ketohexose fructose is also not actively transported and is thought to enter the cell by a sodium-independent facilitated diffusion mechanism [28]. Thus, those substances causing an inhibition in uracil transport while lacking any similarity in chemical structure have in common at least one property, namely they are all transported by a sodium-dependent mechanism.

To compare further the mutual inhibition in transport between hexoses and amino acids with their inhibitory effects on uracil transport the effects of D-galactose were studied in detail. In addition the effects of thymine were studied in parallel. The results with thymine demonstrated that it acts as a fully competitive inhibitor of uracil transport confirming earlier results of Schanker and Tocco [29]. The same type of inhibitory action has been described for hexose-hexose inhibition and for amino acid-amino acid inhibition. Results from such studies have lead to the definition of structural requirements that a substrate must meet to be transported by a given type of carrier. Since each of the carrier types has rather different structural requirements, direct interactions at the substrate binding site between such chemically different substances as hexoses, amino acids and purines would not be expected. Rather, if such heterologous substances are to

cause a mutual inhibition in transport, then something other than a fully competitive type of inhibition is expected.

The results of this study demonstrate that D-galactose acts as a partial noncompetitive inhibitor of uracil transport. This type of inhibition agrees with that previously reported for the mutual inhibition in transport between hexoses and amino acids. Saunders and Isselbacher [3] and Reiser and Christiansen [30] concluded that D-galactose acts as a noncompetitive or partially noncompetitive inhibitor, respectively, of amino acid transport. These observations support the possibility that the mutual inhibition between such heterologous substances may be explained by a common mechanism. Two hypotheses, the allosteric interaction- or *cis*-hypothesis [4,8] and the accelerated efflux- or *trans*-hypothesis [5–7,9,10] are presently under consideration as explanations of these mutual inhibitory interactions. The results reported here neither exclude nor overwhelmingly support either of these hypotheses. The results do, however, demonstrate that heterologous inhibition is a more general phenomenon than had previously been realized.

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