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INTERACTION OF PURINES WITH THE PYRIMIDINE TRANSPORT PROCESS OF THE SMALL INTESTINE

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Abstract—Numerous purine and purine-like compounds inhibit the active transport of uracil across the intestinal wall *in vitro*. The degree of inhibition depends mainly on structural features of the pyrimidine portion of the molecules. For example hypoxanthine, xanthine, 6-mercaptopurine, 4-oxypyrazolo(3,4-d)pyrimidine, and 7-oxypyrazolo-(4,3-d)pyrimidine are strong inhibitors; uric acid and guanine are weaker; and adenine, 3-methylxanthine, theophylline, theobromine, and caffeine have no detectable inhibitory activity. Hypoxanthine or its intestinal metabolites, or both, are readily absorbed from the small intestine of the rat *in vivo* by a saturable transport process. Absorption is blocked by uracil and thymine, suggesting that the purines and pyrimidines compete for a common transport process.

THE SMALL intestine possesses a number of active transport systems, each specific for the absorption of a particular class of natural substrates. For example there is one for monosaccharides, one or more for amino acids, and another for pyrimidines.[1] In each of these systems, structurally related substrates compete with one another for transport; thus galactose inhibits the absorption of glucose,[2] L-methionine that of L-histidine,[3, 4] and uracil that of thymine.[5]

In an earlier study of the pyrimidine transport process in the rat it was noted that the absorption of thymine was inhibited not only by certain pyrimidines but also by the purine compound, hypoxanthine.[5] In view of more recent studies *in vitro*,[6, 7] showing that the pyrimidine absorption process has a high degree of structural specificity, the earlier observation with hypoxanthine seemed surprising.

The present investigation shows that some purine and purine-like compounds interact with the pyrimidine transport system of the rat intestine *in vitro*, and that the interaction depends on structural features of the pyrimidine portion of the molecules. In addition, evidence is presented suggesting that, in the intact animal, hypoxanthine is absorbed by the same process that transports pyrimidine compounds.

METHODS AND MATERIALS

Studies with the intestine in vitro

Male Sprague-Dawley rats (125 to 140 g), fasted for 16 to 18 hr but allowed free access to water, were decapitated, and the upper part of the small intestine was removed. Sacs of everted intestine, prepared according to a method described previously,[7] were filled with 2 ml of Krebs-Henseleit solution[8] containing 1 g glucose/l. and

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various concentrations of a ^{14}C-labeled pyrimidine or purine compound. In some experiments another unlabeled purine compound was added to the solution to investigate its effect on the transport of the labeled compound. Each sac was suspended in 15 ml of the same fluid contained in a 50-ml beaker, and the beakers were shaken in a Dubnoff metabolic shaker (90 oscillations/min) at 37° in an atmosphere of 95% oxygen–5% carbon dioxide. After 1 hr the mucosal and serosal solutions were collected and the concentrations of radioactivity measured.

To estimate the degree of inhibition of uracil transport by various compounds, the serosal/mucosal concentration ratio of uracil in the presence of an inhibitor was compared with that in simultaneously incubated control preparations as described previously.[7]

Absorption studies in vivo

Male Sprague-Dawley rats (120 to 130 g), fasted as described above, were anesthetized with pentobarbital and ether. Through an abdominal incision the small intestine was cannulated at the duodenal and ileal ends, the incision closed, and 50 ml of a solution of a radioactive substance continuously circulated through the intestine for 1 hr at a rate of 1·5 ml/min.[5] The degree of absorption was calculated from the decrease in concentration of radioactivity, corrected for the change of volume, as described previously.[5]

Analytical procedures

The radioactivity of solutions was measured by the liquid counting technique of Cotlove.[9]

For the paper chromatographic identification of radioactive substances, samples were applied to sheets of Whatman No. 1 filter paper and chromatographed with either a 16% aqueous solution of ammonium bicarbonate[10] or a mixture of collidine, quinoline, and water (1:2:1·5).[11] Radioactive spots were detected on radioautograms of the chromatograms. Substances were identified by comparison with standards chromatographed on the same sheet.

Materials

Uracil-2-^{14}C and hypoxanthine-8-^{14}C were obtained from the New England Nuclear Corp., Boston, Mass.; and xanthine-8-^{14}C and uric-2-^{14}C acid from the Volk Radiochemical Co., Chicago, Ill. Most of the unlabeled compounds were obtained from the Nutritional Biochemicals Corp., Cleveland, Ohio. 4-Oxypyrazolo(3,4-d)pyrimidine and 7-oxypyrazolo(4,3-d)pyrimidine were kindly supplied by Dr. T. L. Loo of the National Cancer Institute, Bethesda, Md.

RESULTS

Effect of purines on the active transport of uracil across the intestinal wall in vitro

A number of purine compounds inhibited the active transport of uracil, whereas others did not (Table 1). For example, hypoxanthine, xanthine, uric acid, 6-mercaptopurine, and guanine, in concentrations of 0·5 to 5·0 mM, depressed the transport of 0·02 mM uracil by 30 to 100 per cent. In contrast, adenine, 3-methylxanthine, 1,3-dimethylxanthine, 3,7-dimethylxanthine, and 1,3,7-trimethylxanthine showed no inhibitory activity in these concentrations.

TABLE 1. INHIBITION OF ACTIVE TRANSPORT OF URACIL BY VARIOUS PURINE AND PURINE-LIKE COMPOUNDS

Sacs of everted small intestine of the rat were filled with a 0·02 mM solution of uracil-2-^{14}C containing 0·5 or 5 mmoles of another compound/L. The sacs were suspended in the same solution and incubated at 37° for 1 hr. The percentage depression of uracil transport is expressed as the mean ± S.E. in 5 to 7 animals.

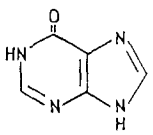
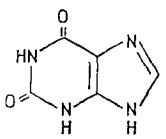
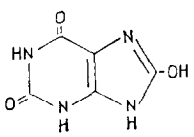
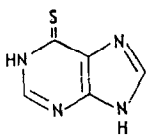
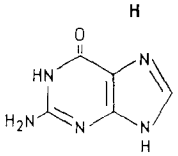
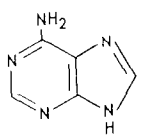
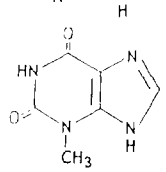
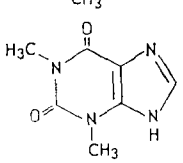
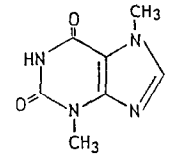
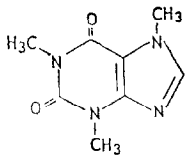
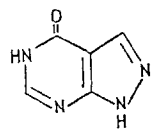
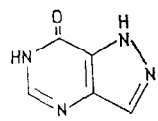
Structure	Name	Concentration 0·5 mM	Concentration 5·0 mM
		Depression of uracil transport (%)	Depression of uracil transport (%)
	Hypoxanthine	100 ± 2	100 ± 2
	Xanthine	61 ± 3	96 ± 2
	Uric acid	45 ± 3	65 ± 3
	6-Mercaptopurine		95 ± 2
	Guanine*	30 ± 3	
	Adenine	2 ± 3	0 ± 3
	3-Methylxanthine	0 ± 2	1 ± 3
	1,3-Dimethylxanthine (theophylline)	2 ± 2	0 ± 3

TABLE 1—*continued*

	3,7-Dimethylxanthine (theobromine)	0 ± 2	1 ± 2
	1,3,7-Trimethyl-xanthine (caffeine)	2 ± 2	0 ± 2
	4-Oxypyrazolo-(3,4-d)pyrimidine		90 ± 3
	7-Oxypyrazolo-(4,3-d)pyrimidine		98 ± 2

* Guanine could not be studied at a concentration of 5 mM because of its limited solubility in water.

Hypoxanthine was the strongest inhibitor, completely blocking the transport of uracil at a concentration of 0·5 mM. Xanthine and 6-mercaptopurine appeared to be weaker inhibitors, failing to block transport completely at a concentration of 5 mM, and uric acid and guanine were weaker still.

To consider these results in terms of a structure–activity relationship, it is appropriate to compare the pyrimidine portion of the purine molecules with the uracil molecule. It can be seen in Fig. 1 that xanthine is in essence a uracil molecule substituted in the 5- and 6-positions; the same is true of uric acid. Since previous work with

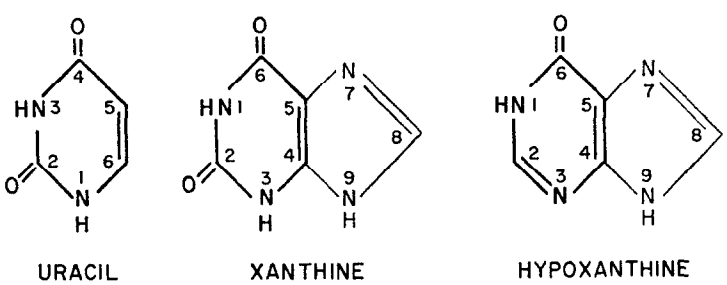


FIG. 1. Chemical structures of uracil, xanthine, and hypoxanthine.

pyrimidines has shown that a variety of substituents can replace hydrogen at the 5- and 6-positions without drastically lowering the affinity of the molecule for the transport system,[7] it is not surprising that xanthine and uric acid have inhibitory activity.

The weak inhibitory activity or lack of activity seen with guanine, adenine, and the N-methylated xanthines is likewise understandable in terms of the structure of the pyrimidine portion of their molecule. For example, guanine and adenine, with an amino group replacing an oxygen, resemble the amino-substituted pyrimidines, cytosine and *iso*cytosine, compounds that are weak inhibitors of the transport of uracil.[7] Moreover, the various N-methylated xanthines resemble roughly the pyrimidine compound, 1,3-dimethyluracil, a substance devoid of inhibitory activity.[7]

The strong inhibitory action of hypoxanthine, on the other hand, is somewhat surprising since this compound lacks one of the oxygen groups of uracil (the one in the 2-position; see Fig. 1). Previous studies with pyrimidines had suggested that this group was important in determining affinity for the transport process, inasmuch as its replacement by a sulfur or amino group resulted in a drastic or complete loss of inhibitory activity.[7] The present results with hypoxanthine indicate that this oxygen group is not an absolute requirement for interaction with the transport process, a view supported by the high degree of inhibitory activity seen with 6-mercaptopurine, 4-oxypyrazolo(3,4-d)pyrimidine, and 7-oxypyrazolo(4,3-d)pyrimidine, compounds which, like hypoxanthine, have a hydrogen atom in place of oxygen in the 2-position (Table 1).

TABLE 2. ABSORPTION OF HYPOXANTHINE AND ITS INTESTINAL METABOLITES FROM THE RAT SMALL INTESTINE *in vivo*

Fifty ml of a solution of hypoxanthine-8-^{14}C was continuously circulated through the small intestine of anesthetized rats and the extent of absorption of radioactivity measured after 1 hr.

Number of animals	Initial concentration of ^{14}C-labeled hypoxanthine (mM)	Percentage of radioactivity absorbed (mean ± range)
8	0·1	47 ± 4
4	0·5	35 ± 4
5	5·0	10 ± 1

Absorption of hypoxanthine from the small intestine in vivo

The intestinal absorption of most naturally occurring purine bases is difficult to study because the compounds are metabolized by intestinal enzymes. For example in studies with the rat and hamster intestine *in vitro*, Wilson and Wilson[12] have shown that both guanine and hypoxanthine are converted to xanthine, and that xanthine is converted to uric acid. Moreover, similar results have been obtained in the present study with hypoxanthine-8-^{14}C *in vivo*. For instance, when solutions of the compound (0·1 to 5·0 mM) were circulated through the rat intestine for 1 hr and then subjected to paper chromatography with two different solvent systems, radioautograms of the chromatograms revealed a spot with the Rf value of hypoxanthine, a spot with the Rf value of uric acid, and a pale, elongated spot covering a range of Rf values which included that of xanthine.

Despite the instability of purines in the intestinal tract, it was possible to obtain some information about the absorption of hypoxanthine and its metabolites by measuring the absorption of radioactivity from solutions of various concentrations of ^{14}C-labeled hypoxanthine. For example when a 0·1 mM solution was circulated through the

rat intestine for 1 hr, 47 per cent of the radioactivity was absorbed; but at elevated concentrations such as 0·5 and 5·0 mM, the extent of absorption was reduced to values of 35 per cent and 10 per cent respectively (Table 2). The decline in the proportion of radioactivity absorbed as the concentration of the purine was increased suggested that hypoxanthine or its metabolites, or both, are absorbed by a saturable process. Evidence that this process is either identical with or linked in some way with the process responsible for the active absorption of pyrimidines was supplied by the depressant effect of uracil and thymine on the rate of absorption of the purines. For instance, when the concentration of labeled hypoxanthine was 0·1 mM, either of the pyrimidines (5 mM) lowered the proportion of radioactivity absorbed from control values of 44 to 50 per cent to values of 9 to 11 per cent (uracil, 3 animals; thymine, 3 animals).

Failure to find evidence of active transport of purines across the intestinal wall in vitro

To determine whether hypoxanthine and its metabolites are actively transported across the intestinal wall *in vitro*, solutions of the ^{14}C-labeled compound were incubated with sacs of everted rat intestine, and the distribution of radioactivity across the gut wall was measured after 1 hr. As shown in Table 3, whether the initial concentration of the purine was 0·002 or 0·03 mM, the final serosal/mucosal concentration ratio of radioactivity was 0·90. Similar results were obtained with 0·03 mM xanthine-8-^{14}C and uric-2-^{14}C acid, the final serosal/mucosal ratios of radioactivity being 0·97 and 0·94 respectively (Table 3).

TABLE 3. DISTRIBUTION OF RADIOACTIVITY ACROSS THE INTESTINAL WALL *in vitro* AFTER INCUBATION WITH ^{14}C-LABELED PURINES

Sacs of everted small intestine of the rat were filled with a solution of a ^{14}C-labeled purine compound, suspended in the same solution, and incubated aerobically at 37° for 1 hr. Distribution ratios of radioactivity are given as the mean value for 4 animals ± the range of values.

Compound	Initial concentration in mucosal and serosal solutions (mM)	Ratio $\frac{\text{cpm/ml serosal solution}}{\text{cpm/ml mucosal solution}}$ after 1 hr of incubation
Hypoxanthine-8-^{14}C	0·002	0·90 ± 0·06
	0·030	0·90 ± 0·05
Xanthine-8-^{14}C	0·030	0·97 ± 0·04
Uric-2-^{14}C acid	0·030	0·94 ± 0·05

Previous work with everted sacs of intestine has shown that if a substance is not actively transferred, its serosal/mucosal ratio will be less than 1·0, sometimes as low as 0·8, because of the net movement of water into the serosal solution.[6] Accordingly, the ratios of 0·90 to 0·97 obtained in the present study give no evidence of active transport, but transport cannot be ruled out in the case of hypoxanthine and xanthine, because chromatograms of the mucosal and serosal solutions revealed that a large proportion of the two compounds had been converted to uric acid during the experiment.

CONCLUSIONS

Several lines of evidence suggest that certain purines and pyrimidines compete for a common transport system in the small intestine. First of all, in the living animal,

thymine and uracil interfere with the intestinal absorption of hypoxanthine (or its metabolites). Second, it has been shown previously[5] that hypoxanthine interferes with the active absorption of thymine. In addition, hypoxanthine as well as a variety of purine and purine-like compounds interferes with the active transport of uracil across the intestinal wall *in vitro*. And finally, the degree to which various purines interact with the pyrimidine transport process depends mainly on structural characteristics of the pyrimidine portion of their molecule.

It is not altogether clear from experiments in the living animal whether hypoxanthine is absorbed predominantly as the unchanged molecule or in the form of its metabolites. On the other hand there is little doubt that hypoxanthine possesses affinity for the transport system since it is a much stronger inhibitor of uracil transport than are its metabolites, xanthine and uric acid.

Although hypoxanthine is metabolized to a considerable degree during prolonged exposure to the intestine *in vitro*, it is possible that in the living animal a significant proportion of the administered compound escapes destruction in the gut because of rapid transport into the blood stream.

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