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STRUCTURAL SPECIFICITY OF THE PYRIMIDINE TRANSPORT PROCESS OF THE SMALL INTESTINE

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Abstract—To investigate the structure-activity relationship in the active intestinal transfer of pyrimidines, various pyrimidine analogues were tested as inhibitors of the transport of uracil across the rat intestinal wall *in vitro*. Strong inhibition was seen with simple derivatives of uracil having a substituent replacing a hydrogen atom at carbon number 5 or 6. Changes in almost any other portion of the uracil molecule resulted in compounds of either weak or insignificant inhibitory activity.

TWO PYRIMIDINES that occur naturally, uracil and 5-methyluracil (thymine), and two foreign pyrimidines, 5-fluorouracil and 5-bromouracil, have been shown to cross the intestinal epithelium by active transport as well as by passive diffusion.[1-3] Active transport is the predominant mode of absorption at low concentrations of the compounds, whereas passive diffusion predominates at high concentrations at which the active process is saturated.

Absorption studies with the intact rat and with the isolated intestinal wall *in vitro* have indicated that uracil and the 5-substituted compounds mentioned above are transported at similar rates and that each competitively inhibits the transport of the other.[1-3] These observations suggest that the substituent at the 5-position of the uracil molecule is of little importance in determining affinity for the transport process and raise the question of which portions of the molecule do have significant influence in this regard.

In the present study the structural specificity of the pyrimidine transport process is explored, and the structural requirements are shown to be rather exacting.

METHODS AND MATERIALS

Experimental procedure

Male Sprague–Dawley rats (120–135 g), fasted for 16–18 hr, but allowed free access to water, were killed by decapitation, and the upper part of the small intestine was removed. Sacs of everted intestine were prepared according to the method of Wilson and Wiseman,[4] except that the sacs were considerably longer (20 cm) and were filled with a relatively small volume of fluid which did not distend the intestinal wall.[3] Krebs–Henseleit solution,[5] 2 ml, containing 1 g glucose/l, 0·02 mmole uracil-2-^{14}C/l, and either 0·5 or 5·0 mmoles/l of another pyrimidine compound (one being

tested for inhibitory activity) was placed in each intestinal sac, and the sac was suspended in 15 ml of the same fluid contained in a 50-ml beaker. The beakers were shaken in a Dubnoff metabolic shaker (90 oscillations/min) at 37 °C in an atmosphere of 95% oxygen–5% carbon dioxide. After 1 hr the mucosal and serosal solutions were collected, and the concentration of uracil was estimated from the radioactivity of the solutions.

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. The ratios obtained in the absence of inhibitors ranged from 3·5 to 4·0.

It has been shown previously that complete inhibition of uracil transport results in serosal/mucosal ratios of 0·8–0·9, the uneven distribution of the pyrimidine being a consequence of a dilution of the serosal solution by water transferred from the mucosal solution.[3] Accordingly, in the present study a ratio of less than 0·9, obtained in the presence of an inhibitor, was taken as 100 per cent depression of active uracil transport.

Analytical procedure

Uracil-2-^{14}C (New England Nuclear Corp., Boston, Mass.), cytosine-2-^{14}C (Nichem Inc., Bethesda, Md), and barbituric-2-^{14}C acid (Volk Radiochemical Co., Chicago, Ill.) were measured by the liquid counting technique of Cotlove.[6]

Chemical structures

Some of the compounds studied exhibit keto-enol tautomerism in aqueous solution. The structures shown in Table 1 represent the tautomeric forms thought to exist in solutions of pH 7·4, and are based largely on the spectrophotometric determinations of Shugar and Fox[7] and Marshall and Walker.[8] The resonance hybrid structure shown for barbituric acid is that proposed by Fox and Shugar.[9]

Most of the compounds listed in Table 1 were obtained from the Nutritional Biochemicals Corp., Cleveland, Ohio. 5-Bromouracil and 2-amino-4,6-dioxy-5-methylpyrimidine were obtained from the California Corp. for Biochemical Research, Los Angeles, Calif.; and isocytosine from the Aldrich Chemical Co., Inc., Milwaukee, Wis. 5-Fluorouracil was kindly supplied by Hoffmann-La Roche Inc., Nutley, N.J.

RESULTS AND DISCUSSION

A number of pyrimidine analogues inhibited the active transport of uracil, but the degrees of inhibition varied widely. The strongest inhibitors were simple derivatives of uracil with a substituent replacing a hydrogen atom at the 5- or 6-position. Weaker inhibitors, as well as compounds devoid of inhibitory activity, were generally those in which the group at the 1-, 2-, 3-, or 4-position of the uracil molecule had been replaced (Table 1).

Uracil derivatives substituted at the number 5 or 6 position

The active transfer of 0·02 mM uracil was completely inhibited by 0·5 or 5 mM 5-methyluracil, 5-fluorouracil, 5-bromouracil, or 5-aminouracil. Strong inhibition was also seen with 6-methyluracil and 6-aminouracil, but these compounds were somewhat less active than the 5-substituted compounds.

TABLE 1. INHIBITION OF ACTIVE TRANSPORT OF URACIL BY VARIOUS PYRIMIDINE 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/l of another pyrimidine. The sacs were suspended in the same solution and incubated at 37 °C for 1 hr. The percentage depression of uracil transport is expressed as the mean ± the range of values in 5 to 8 animals.

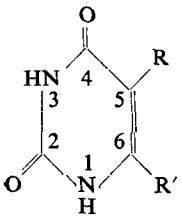
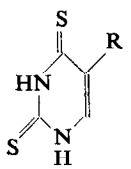
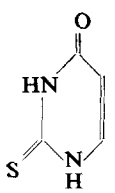
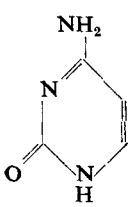
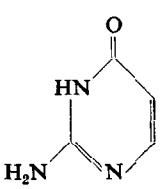
Structure			Name	Concentration	
				0·5 mM	5·0 mM
				% Depression of uracil transport	% Depression of uracil transport
	R = CH_3	R′ = H	5-Methyluracil	100	100
	R = F	R′ = H	5-Fluorouracil	100	100
	R = Br	R′ = H	5-Bromouracil	97 ± 5	100
	R = NH_2	R′ = H	5-Aminouracil		100
	R = H	R′ = CH_3	6-Methyluracil	59 ± 8	95 ± 6
	R = H	R′ = NH_2	6-Aminouracil		93 ± 4
	R = H		2,4-Dithiouracil	3 ± 4	67 ± 8
	R = CH_3		2,4-Dithiothymine	4 ± 3	52 ± 5
			2-Thiouracil	0 ± 4	3 ± 4
			Cytosine	3 ± 4	75 ± 5
			Isocytosine	2 ± 4	0 ± 3

TABLE 1.—*continued.*

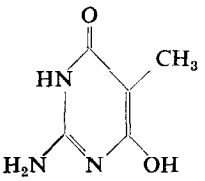
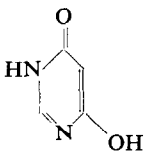
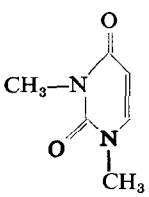
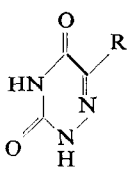
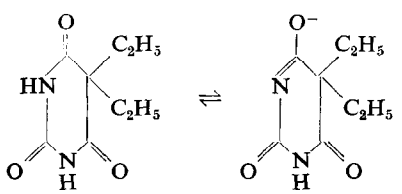
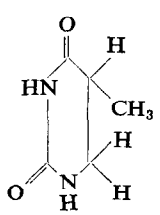
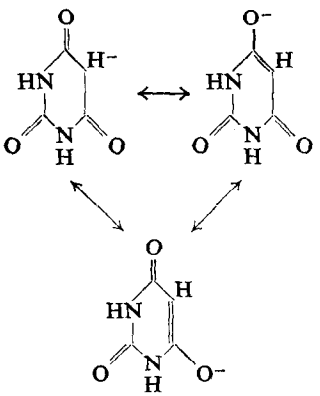
Structure		Name	Concentration	
			0·5 mM	5·0 mM
			% Depression of uracil transport	% Depression of uracil transport
		2-Amino-4,6-dioxy-5-methylpyrimidine	0 ± 5	38 ± 5
		4,6-Dioxypyrimidine	2 ± 3	41 ± 4
		1,3-Dimethyluracil	0 ± 3	0 ± 4
	R = H	6-Azauracil	3 ± 3	37 ± 3
	$R = CH_3$	6-Azathymine	1 ± 3	28 ± 4
		Barbital	0 ± 4	2 ± 3
		Dihydrothymine	6 ± 4	73 ± 5

TABLE 1.—*continued.*

Structure	Name	Concentration	
		0·5 mM	5·0 mM
		% Depression of uracil transport	% Depression of uracil transport
	Barbituric acid	4 ± 3	45 ± 4

Uracil derivatives substituted at the number 2 *or* 4 *position*

Replacement of one or both of the oxygens of uracil with various substituents resulted in a marked loss of inhibitory activity; for example, 2,4-dithiouracil, 2,4-dithiothymine, 2-amino-4,6-dioxy-5-methylpyrimidine, cytosine (4-aminouracil), and 4,6-dioxypyrimidine showed moderate or weak inhibitory activity; and 2-thiouracil and isocytosine (2-aminouracil) showed no activity.

Other derivatives of uracil

Substitution of methyl groups for the hydrogen atoms at the 1- and 3-positions of uracil (1,3-dimethyluracil) resulted in a complete loss of inhibitory activity.

Alteration of the structure of the pyrimidine ring, as in 6-azauracil and 6-azathymine, resulted in compounds of weak activity.

Saturation of the pyrimidine ring, as in dihydrothymine and barbital, resulted in a marked loss of inhibitory activity.

One or more of the following structural changes might account for the weak activity of barbituric acid: (1) at physiological pH values the substituents at the 4-, 5-, and 6-positions of the compound appear to be involved in a resonance hybrid structure[9] (see Table 1); (2) there is an oxygen function in place of a hydrogen atom at the number 6 position; and (3) at pH 7·4 barbituric acid (pK_a = 3·9) exists as an anion, whereas uracil (pK_a = 9·5) exists to the extent of about 99 per cent as an uncharged molecule.[7, 9]

Transport of pyrimidines against a concentration gradient

Previous studies have shown that 5-methyluracil, 5-bromouracil, and 5-fluorouracil—compounds that can block completely the transport of uracil—are themselves actively transferred across the intestinal epithelium.[1–3] Thus, when incubated aerobically at a concentration of 0·02 mM with sacs of everted rat intestine, these

compounds, as well as uracil, attain serosal concentrations that are three to five times greater than are their mucosal concentrations. The question arose whether weak inhibitors of uracil transport, like the strong inhibitors, are capable of being actively transferred. Accordingly two of them, cytosine-2-^{14}C and barbituric-2-^{14}C acid, were incubated with the rat intestine under the conditions described above, except that the concentration of the compounds was lowered to 0·01 mM. Cytosine attained a serosal mucosal concentration ratio of 0·82 (range, $\pm$0·06 in 11 animals) and barbituric acid a ratio of 0·85 ($\pm$0·05 in 6 animals). Since, in the absence of active transport, ratios of 0·8 to 0·9 result from the net movement of water into the serosal solution,[3] the data indicate no significant transport for these pyrimidines.

There are several possible explanations for the failure of cytosine and barbituric acid to be actively transferred, even though they can inhibit the transport of uracil. For instance, if it is assumed that the transport system consists of a series of chemical reactions, the compounds might react at the first stage of the process but lack the structural characteristics required to react at subsequent stages. On the other hand, the compounds might bind to a site adjacent to the "transport site" and inhibit uracil transport sterically, or might act in a nonspecific way by interfering with cell metabolism.

CONCLUSION

The structural requirements for interaction of pyrimidine compounds with the uracil transport process are rather exacting. Thus, for a high degree of activity, a compound must bear a close resemblance to the uracil molecule, with ketonic oxygen functions at carbons number 2 and 4, hydrogen atoms at carbons number 1 and 3, and a ring structure and degree of saturation the same as that of uracil. The only modifications of the uracil molecule that do not result in a great loss of activity appear to be the substitution of a small chemical group for a hydrogen atom at carbon number 5 or 6.

In contrast to these stringent requirements, considerable variation in structure is allowable for a relatively low level of activity. But even among these compounds activity can be abolished by what appear to be minor structural changes. For example, although moderate activity is seen when both of the oxygen functions of uracil are replaced by sulfur, activity is lost when only one of the oxygens is replaced (2-thiouracil). Moreover, substituting an amino group for the oxygen at the number 2 carbon of uracil (isocytosine) abolishes activity, but part of the activity can be restored by adding substituents at the 5- and 6-positions of this molecule (2-amino-4,6-dioxy-5-methylpyrimidine).

The structure-activity relationship for the intestinal transport of pyrimidines is highly complicated and, at present, gives no indication of possible biochemical reactions that might be associated with the transport process.

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