Effect of Certain Nonsteroid Antirheumatic Drugs on Active Amino Acid Transport Across the Small Intestine

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Indomethacin (5 m*M*), phenylbutazone (5 m*M*), and salicylate (15 m*M*) prevent the active transport of the amino acid, L-tryptophan, across the small intestine of the rat. Aspirin (15 m*M*) appears to inhibit somewhat, but does not prevent, this process. Salicylate (15 m*M*) significantly inhibits the active transport of L-tryptophan across the hamster small intestine; aspirin (15 m*M*) has no inhibitory effect. These results are consistent with the relative effectiveness of these drugs as uncouplers of oxidative phosphorylation.

SALICYLATE and certain other nonsteroid anti-inflammatory drugs are potent uncouplers of oxidative phosphorylation (1). They may, therefore, be expected to interfere with the energy supply necessary for active intestinal transport processes (2, 3). Smith (3) has shown that 5 m*M* salicylate inhibits the active transport of glucose and water across the small intestine of the rat, and Quastel (4) has recently reported that 10 m*M* salicylate inhibits the active transport of phenylalanine by the guinea pig intestine. Since the oral administration of salicylate and other anti-inflammatory agents can yield appreciable concentrations of these drugs in intestinal fluids, it is desirable to assess the effect of these substances on active intestinal transport processes. It has been shown that modification of the phenolic group of salicylate causes a loss in uncoupling activity (5), and it became of interest therefore to determine if an inhibitory effect of salicylate may be reduced or circumvented totally by using aspirin. The present communication deals with the effects of salicylate, indomethacin, and phenylbutazone on the active intestinal transport of the neutral amino acid, L-tryptophan, with particular emphasis on the relative effects of salicylate and aspirin.

EXPERIMENTAL

Absorption of L-tryptophan across the rat small intestine was studied by the cannulated everted intestine method developed by Crane and Wilson (6). Male Sprague-Dawley rats, weighing about 250 Gm., were fasted overnight, but had access to drinking water at all times. The small intestine was removed under ether anesthesia and rinsed with Ringer's solution. The intestine was then sleeved onto a glass rod and everted carefully. Two segments of 10-cm. length (when stretched slightly by attaching an 8-Gm. weight and suspending the segment vertically) were obtained, distal ends were tied, and proximal ends were attached to the cannula of the apparatus described by Crane and Wilson (6). An equal number of first (from the proximal end) and second intestinal segments were used in each experiment. These segments will be referred to as proximal and distal segments, respectively. Each everted and cannulated intestine segment was suspended in 45 ml. of Krebs–Ringer–bicarbonate solution (pH 7.4) containing 2 m*M* L-tryptophan maintained at 37° and gassed continuously with a mixture of 95% oxygen and 5% carbon dioxide. The serosal (inner) solution also consisted of 2 ml. of Krebs–Ringer–bicarbonate solution containing 2 m*M* L-tryptophan. Drugs were added to both the mucosal and serosal solutions in equal specified concentrations. After 1 hr. of incubation, aliquots of both solutions were assayed. One of the two segments of intestine obtained from each rat was used for a drug experiment, while the other segment served as a control. Each set of drug and control experiments was carried out with an equal number of proximal and distal segments, except when an odd number of experiments were conducted.

Absorption of L-tryptophan across the hamster small intestine was studied by the everted sac method described by Wilson and DeCarlo (7). Three segments of small intestine, each approximately 6 cm. long, were removed under ether anesthesia from male golden hamsters weighing 100–150 Gm. Everted sacs were prepared and filled with 1 ml. of Krebs–Ringer–bicarbonate solution (pH 7.4) containing 2 m*M* L-tryptophan. The sacs were placed in 15 ml. of the same solution in 50-ml. capacity conical flasks. Both mucosal and serosal solutions contained drugs in equal specified concentrations. The flasks were flushed with a mixture of 95% oxygen and 5% carbon dioxide,

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TABLE I—ACTIVE TRANSPORT OF L-TRYPTOPHAN ACROSS THE SMALL INTESTINE OF THE RAT[a]

Mean Concn. Ratio,[b] Serosal/Mucosal			
All Expt.		Successful Expt.[c]	
Proximal Segments	Distal Segments	Proximal Segments	Distal Segments
1.31 (*13*)	1.40 (*13*)	1.43 (*9*)	1.54 (*9*)
All Segments		All Segments	
1.36 (*26*)		1.48 (*18*)	

[a] Cannulated everted segments of rat small intestine were incubated for 1 hr. at 37° in oxygenated Krebs–Ringer–bicarbonate solution (pH 7.4) containing 2 m*M* L-tryptophan. [b] Number of experiments listed in parentheses. [c] Concentration ratio of "controls" ≥1.20.

sealed, placed in a 37° water bath, and agitated moderately by means of a reciprocating shaker. Aliquots of the mucosal and serosal solutions were obtained after 1 hr. of incubation and were assayed.

The composition and method of preparation of Krebs–Ringer–bicarbonate solution are described elsewhere (8). Calcium chloride and magnesium sulfate had to be omitted in the indomethacin experiments due to the formation of precipitates; these salts were also excluded from the solution used in the corresponding control experiments. The drugs used in this study and their respective sources were: 2,4-dinitrophenol (Mann Research Laboratories); indomethacin (Merck Sharp and Dohme); phenylbutazone (Geigy Pharmaceuticals); aspirin (Mallinckrodt); sodium salicylate (Fisher Scientific Co.).

Tryptophan was determined by procedure *A* of the method of Spies and Chambers (9). Dinitrophenol, indomethacin, and phenylbutazone interfered with the tryptophan assay and had to be removed by extraction. For this purpose, an aliquot of the solution to be assayed was diluted with water to 3 ml., 0.5 ml. of 3 *N* hydrochloric acid was added, and the solution was extracted with 20 ml. of ethylene dichloride. The salicylate content of aspirin solutions was determined immediately after completion of the experiments by the method of Brodie *et al.* (10).

RESULTS AND DISCUSSION

The active intestinal transport of L-tryptophan has been studied in detail by Spencer and Samiy (11). This amino acid is transported by the monoamino-monocarboxylic acid transport system, which is responsible for the active transport of at least 14 amino acids (12). In the presently described investigation, L-tryptophan absorption was studied initially by the cannulated everted rat intestine method, using the occurrence of absorption against a concentration gradient (as evidenced by the magnitude of serosal/mucosal concentration ratios) as the criterion of active transport. The results of the experiments with L-tryptophan alone are shown in Table I. Some of the experiments did not yield serosal/mucosal concentration ratios significantly greater than unity; this appeared to be due to technical difficulties. However, there was an excellent correlation between the results obtained with the proximal and the distal intestine segments of any one animal. Using a serosal/mucosal concentration ratio of 1.2 as an arbitrary minimum value to establish active transport, only one out of 13 pairs of intestinal segments yielded dissimilar results in that one segment showed active transport while the other did not. Of the 13 pairs of experiments, nine pairs were designated as "successful" since the segments predesignated as the "control" (*i.e.*, either the proximal or the distal segment of each pair) yielded a serosal/mucosal tryptophan concentration ratio greater than 1.2.

The effects of various drugs on the active transport of L-tryptophan across the rat small intestine are shown in Table II. The results are listed both as the average of all experiments and as the average of the "successful" experiments. A 1 m*M* concentration of the classical uncoupling agent 2,4-dinitrophenol completely abolished active transport of L-tryptophan. Five millimolar indomethacin and 5 m*M* phenylbutazone, respectively, had the same effect. Fifteen millimolar salicylate prevented the active transport of L-tryptophan, while the same concentration of aspirin inhibited, but did not prevent, the active transport process.

To obtain more definitive data concerning the relative inhibitory effect of salicylate and aspirin on the active transport of L-tryptophan, additional experiments were carried out using the small intestine of hamsters. It has been pointed out recently that the hamster is a much more satisfactory experimental animal for active transport studies than is the rat (7). The results obtained were consistent with this statement since all the experiments were "successful" in that each control

TABLE II—EFFECT OF VARIOUS DRUGS ON ACTIVE TRANSPORT OF L-TRYPTOPHAN ACROSS THE SMALL INTESTINE OF THE RAT[a]

Drug	Concn., m*M*	Mean Tryptophan Concn. Ratio,[b] Serosal/Mucosal — All Expt. — With Drug	All Expt. — Without Drug	Successful Expt. — With Drug	Successful Expt. — Without Drug	Statistical Significance[c] of Difference, Drug *vs.* No Drug — All Expt.	Successful Expt.
2,4-Dinitrophenol	1	0.96 (*7*)	1.21 (*7*)	0.98 (*3*)	1.40 (*3*)	+	+
Indomethacin	5	0.92 (*6*)	1.41 (*6*)	0.93 (*4*)	1.56 (*4*)	+	+
Phenylbutazone	5	0.98 (*8*)	1.27 (*8*)	1.00 (*4*)	1.42 (*4*)	+	+
Phenylbutazone	10	1.01 (*5*)	1.26 (*5*)	1.00 (*4*)	1.30 (*4*)	+	+
Aspirin	10	1.16 (*16*)	1.26 (*16*)	1.23 (*7*)	1.47 (*7*)	−	−
Aspirin	15	1.19 (*13*)	1.27 (*13*)	1.20 (*7*)	1.42 (*7*)	−	+
Sod. salicylate	5	1.10 (*8*)	1.25 (*8*)	1.17 (*4*)	1.41 (*4*)	−	−
Sod. salicylate	10	1.16 (*11*)	1.35 (*11*)	1.24 (*9*)	1.40 (*9*)	+	−
Sod. salicylate	15	1.02 (*17*)	1.23 (*17*)	1.05 (*8*)	1.36 (*8*)	+	+

[a] Cannulated everted segments of rat small intestine were incubated for 1 hr. at 37° in oxygenated Krebs–Ringer–bicarbonate solution (pH 7.4) containing 2 m*M* L-tryptophan. [b] Number of experiments listed in parentheses. [c] Student's *t* test, significant differences $p < 0.05$.

segment yielded serosal/mucosal tryptophan concentration ratios appreciably greater than 1.2. The effects of salicylate and aspirin on L-tryptophan transport across the hamster small intestine are listed in Table III. Fifteen millimolar salicylate significantly inhibited the active transport of L-tryptophan, while the same concentration of aspirin had no inhibitory effect.

The relative inhibitory effectiveness of the drugs tested is consistent with their relative effectiveness as uncouplers of oxidative phosphorylation. Indomethacin and phenylbutazone are approximately equipotent but have greater activity than salicylate in uncoupling oxidative phosphorylation in rat liver mitochondria (1). The cytostatic effect of these agents, as determined by their inhibition of cellular proliferation of Ehrlich ascites in suspension cultures, is of the order: indomethacin > phenylbutazone > sodium salicylate > aspirin, while their inhibitory effect on glucosamine-6-phosphate synthesis in liver homogenates is phenylbutazone > indomethacin > sodium salicylate > aspirin (13). Aspirin has practically no inhibitory effect in the latter system. Similarly, up to 1.24 m*M* aspirin has no significant effect on the oxygen uptake of rat cerebral cortex slices, while salicylate concentrations as low as 0.06 m*M* have a significant effect (14). Brostaff *et al.* have shown that modification of the phenolic hydroxyl group of salicylate (*i.e.*, alteration of its position on the benzene ring, removal, methylation, or substitution) causes a loss of uncoupling activity (5). While the rapid *in vivo* hydrolysis of aspirin to the potent uncoupler salicylic acid has made it difficult to determine the uncoupling activity of aspirin as such, it is reasonable to expect that aspirin either has no or very little uncoupling activity. The results of the present study are consistent with this assumption in that aspirin has less inhibitory effect than salicylate on the active transport of L-tryptophan across the small intestine of the rat. Further, 15 m*M* aspirin does not inhibit L-tryptophan transport across the hamster small intestine, while the same concentration of salicylate has a pronounced inhibitory effect.

There was significant hydrolysis of aspirin to salicylic acid during the absorption experiments but, at least with hamster small intestine, the concentration of salicylate did not become high enough to reach inhibitory levels (Table IV). Aspirin hydrolysis appears to be due mainly to the presence of esterase(s) in the intestinal epithelium, since hydrolysis was appreciably reduced in the absence of intestinal segments or when the intestine had been placed in boiling water to destroy the enzymes. The presence of aspirin hydrolyzing esterase(s) in the intestinal epithelium, as well as in the gastric mucosa (15), is of considerable biopharmaceutic interest because it raises the possibility of appreciable aspirin hydrolysis during gastrointestinal absorption. In this connection it is significant that nonspecific esterase is present in all regions of the human gastrointestinal tract (16). Due to the limited capacity of enzyme systems located in the gastrointestinal mucosa, the extent of aspirin hydrolysis is probably more pronounced when the drug is administered in slowly absorbed form.

One can only speculate as to the clinical implications of the amino acid transport inhibiting effects observed in this study. Patients requiring anti-inflammatory therapy with salicylate, indomethacin, or phenylbutazone may be in nutritional deficiency for many reasons other than impaired absorption. On the other hand, the chronicity of anti-inflammatory drug therapy makes additional and more detailed studies of these effects highly desirable. A next logical step is to determine if the inhibitory effect of these anti-inflammatory agents depends on their presence in intestinal fluids, or if it persists for an appreciable time after exposure of the intestine to these drugs. This is presently being investigated and will be the subject of a future communication.

TABLE III—EFFECT OF ASPIRIN AND SALICYLATE ON ACTIVE TRANSPORT OF L-TRYPTOPHAN ACROSS THE SMALL INTESTINE OF THE HAMSTER[a]

	Mean Tryptophan Concn. Ratio, Serosal/Mucosal			
Drug	First Segment[b] (5 Sacs)	Second Segment (5 Sacs)	Third Segment (5 Sacs)	All Segments (15 Sacs)
None	1.42	1.46	1.42	1.43 ± 0.14[c]
15 m*M* Aspirin	1.36	1.66	1.66	1.56 ± 0.34
15 m*M* Sod. salicylate	1.18	1.16	1.11	1.15 ± 0.08[d]

[a] Everted sacs of small intestine from the golden hamster were incubated for 1 hr. at 37° in oxygenated Krebs–Ringer–bicarbonate solution (pH 7.4) containing 2 m*M* L-tryptophan. [b] Numbered from the pyloric end. [c] Standard deviation. [d] This value differs significantly ($p < 0.01$) from the control and the aspirin values.

TABLE IV—HYDROLYSIS OF ASPIRIN BY SMALL INTESTINE OF THE HAMSTER DURING 1-hr. INCUBATION AT pH 7.4 AND 37°

Conditions	No. of Expt.	Hydrolysis of Aspirin, %
Standard[a]	15	17.4 ± 2.1[c]
No intestine, no tryptophan	15	4.9 ± 0.4
Boiled intestine[b]	3	7.1
No intestine	5	6.3

[a] As described in *Footnote a* of Table III. [b] Intestine was immersed in boiling water for 5 min. [c] Standard deviation.

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