Concentrative and reversible character of intestinal amino acid transport

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Either \(^{14}\text{C}\)-labeled \(\alpha\)-aminoisobutyric acid or \(^{14}\text{C}\)-labeled \(\alpha\)-aminocyclopentane-carboxylic acid was injected into a rat and, after at least 24 hr, a suitable dilution of the same solution of the amino acid was introduced into an intestinal loop in situ and the change in its distribution observed by radioisotope counting. The two amino acids could be concentrated from the small intestine to establish steady-state distribution ratios of from 12 to 100, for the plasma level with respect to the level in the lumen. These values are far higher than the distribution ratios produced across excised intestine. These steady-state values were approached from either above or below, by absorption or release of the amino acid. The release was accelerated by the presence of the same or another amino acid of high transport affinity in fluid perfusing the intestinal loop, an action that tends to identify the outward migration from lumen to plasma with a transport process. The test amino acids were released into the colon also, although at slower rates, the steady-state distribution ratio lying in the neighborhood of unity. This release appears also to be a specific process, and to account for the slow fecal excretion of these difficultly metabolized amino acids.

Uphill transport of amino acids is known to occur across the intestinal wall in vitro, from a saline solution placed on the mucosal side to one on the serosal side (1-4). This is a two-way process, a similar value for the partition coefficient of an amino acid being reached whether one begins with a higher or lower partition coefficient (5). Similar behavior might be expected for the intestine in situ with reference to the distribution of amino acids between the extracellular fluid and the fluid in the intestinal lumen. A formal demonstration of this behavior has, however, been prevented by the rapidity with which amino acids are removed from the bloodstream, and the high rate at which many of them can be catabolized. The body serves as an effective sink for the amino acids, so that the full potentiality for “uphill” transport cannot easily be observed. At the same time the presence of ordinary amino acids in intestinal fluid would not establish, nor their absence disprove, the reversibility of intestinal transport, since they might be formed or destroyed by the cells with which they come in contact.

Because \(\alpha\)-aminocyclopentane-carboxylic acid (5) is almost totally resistant to metabolic modification in the animal organism, and because it is excreted exceedingly slowly, essentially constant levels in the body fluids can easily be obtained during long experimental intervals (6, 7). Its transport behavior is similar to that of normal amino acids, and resembles particularly that of methionine (6, 8, 9). Accordingly, this model amino acid presents us with an opportunity to determine whether transport across the intestine in situ is as strongly concentrative and as reversible as it is for the excised tissue.

Our results show that this test amino acid and another one, \(\alpha\)-aminoisobutyric acid, are released into the intestine and absorbed from it, so that each yields similar partition coefficients between the plasma and the intestinal lumen, whether one begins with proportionately too high or too low levels in the lumen. These partition coefficients reveal far more intense uphill absorption than has been obtained for amino acids with excised intestinal sacs.

EXPERIMENTAL METHODS

Stock solutions of \(\alpha\)-aminocyclopentanecarboxylic acid (1-1.5%), and \(\alpha\)-aminoisobutyric acid (usually 2.5%) were prepared in 0.9% NaCl. These were labeled by the inclusion of suitable quantities of our own preparations of the \(^{14}\text{C}\)-forms of these amino acids, so that the dose administered to the rat (usually 100 mg/kg body wt.) represented 10–25 \(\mu\)g/kg. For the first amino acid, \(\alpha\)-aminocyclopentanecarboxylic acid, a delay of 1–3 days

Received for publication 15 January 1963

This study was supported, in part, by National Institutes of Health Grants C-2645 (to HNC) and A-3404 (to ABH).

As a medical student, held a Dr. Henry Uriah Upjohn Memorial Fellowship during this work.
intervened before the experiments, the last 24 hr under fasting; for 8-aminoisobutyrate we waited only 24 hr (with fasting) because of the somewhat more rapid excretion of this amino acid. At the end of these intervals a suitably diluted solution of the same amino acid was introduced into the intestine of the anesthetized animal, in order to examine the direction of net migration and the position of the steady state of distribution of the amino acid.

**Part 1. Perfusion experiments.** (Performed at Ann Arbor.) Female rats weighing 120-150 g (Sprague-Dawley strain; the gift of the Upjohn Company) received intraperitoneally a solution, as described above, representing 10 or 15 mg of labeled 1-aminocyclopentanecarboxylic acid. After one or more days, as indicated above, the animal was anesthetized by injection intramuscularly of 3.4 mg pentobarbital/100 g body wt. A tube for breathing was inserted into the trachea. The abdomen was opened, and either a 15- to 20-cm length of the proximal jejunum or almost the entire colon was isolated by ligation. A cannula was secured into each end, and the loop was perfused first with 0.9% NaCl and then with Krebs-Ringer medium containing 25 mM bicarbonate. The perfusion fluid, maintained at 37°C, was circulated through the loop propelled by a stream of 95% O₂, 5% CO₂ bubbled through an ascending portion of the external circuit. Exposed tissues were covered with saline-soaked gauze, and a lamp was directed at the abdomen to help maintain the tissue temperature in the normal range.

Two types of perfusion experiments were performed. In the first type, 10 min after perfusion with 40 ml of the buffered Krebs-Ringer solution had begun, an aliquot portion of the same solution of 1-aminocyclopentanecarboxylic acid used for injection was added to the perfusing fluid. The amount was selected to yield an initial distribution ratio between the blood plasma and the perfusing fluid in the range of 2-28. At 15 or 30 min intervals samples were taken of the perfusing fluid to determine the residual radioactivity, these samples being centrifuged to remove any sedimentable material. Blood was collected from the tail vein at the beginning of each experiment, and also at the end in a few cases to determine the concentration of the amino acid in the plasma. No significant change in concentration was found, confirming previous observations.

In the second type of perfusion experiment the movement of radioactivity from the body into the intestinal fluid was observed at intervals. Either no labeled amino acid was added to the perfusing fluid, or else 4.8-19.4 mM unlabeled 1-aminocyclopentanecarboxylic acid or 4-18.5 mM L-methionine was included in it.

**Part 2. Experiments with isolated loops.** (Performed at La Jolla.) In these experiments male rats of the same strain were used, weighing 370-500 g. These animals received 100 mg (20 μe) of the test amino acid per kilogram, except where indicated otherwise. After the intervals described above, the animals were anesthetized as before with pentobarbital injected subcutaneously in the scapular region. No need was encountered for an artificial breathing passage. The abdomen was opened on the midline, and a ligature placed at a selected point of the small or large intestine. Adjacent to the ligature a small cannula formed from about 2 cm of a K-32 soft polyethylene feeding tube, as used for infants, was inserted through a small cut and tied into place. A ring of small-bore rubber tubing had first been fitted over one end of the cannula to prevent its extrusion from the loop. During the insertion a length of loose-fitting copper wire was left in the cannula to stiffen it and to limit the constriction produced by ligation. About 9 ml of a solution identical to that to be placed in the loop was then washed through the loop from a syringe by way of a no. 19 needle, which fitted firmly into the cannula. The wash fluid escaped through a small opening at the opposite

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**TABLE 1. Typical experiments illustrating the observations of part I**

<table>
<thead>
<tr>
<th>Type of Exp.</th>
<th>Plasma Radioactivity</th>
<th>Observed Radioactivity of Perfusate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Init.</td>
<td>15 min</td>
</tr>
<tr>
<td>I*</td>
<td>763</td>
<td>664</td>
</tr>
<tr>
<td>I†</td>
<td>11,300</td>
<td>9,840</td>
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</tbody>
</table>

Values are expressed as counts/min ml. * Labeled 1-aminocyclopentanecarboxylic acid (10.2 mg) was injected and 6.37 mg added as a volumetric aliquot of the same solution to yield 40 ml of perfusion fluid. † Labeled amino acid (15.3 mg) was injected and 95 mg unlabeled amino acid added to the 40 ml of perfusion fluid.
end of the section of intestine to be used, into a waste receptacle. This opening was closed by an additional ligature, and the sample solution (about \(1.5\) ml) was introduced into the loop at an observed time with only gentle pressure. The cannula was then stoppered by a short length of tightly fitting copper wire. The loop was then slipped back into the abdominal cavity. When consecutive loops were to be studied a second cannula was placed in the opening serving for discharge in washing out the first loop, and so on. Otherwise a ligature served to isolate the unused portion of the intestine.

After preparation and filling of the several loops to be studied and careful replacement within the abdominal cavity, the incision was covered with gauze moistened with \(0.9\) % NaCl, and the animal maintained for 2 hr as described in Part 1. Serial samples were taken from the loops at 30-min intervals with \(1\)-ml tuberculin syringes fitted with no. 19 needles. The exposed portion of the cannula was washed with \(0.9\) % NaCl and blotted dry before being opened for insertion of the needle. The syringe was then filled to about \(0.2\) ml and the contents expelled again into the loop two or three times to secure good sampling. During later samplings it was necessary to bring the distal end of the loop to a superior position to make the sample accessible to the cannula. Jejunal loops could be sampled only from one to three times before the fluid was absorbed.

At the end of the experiment, the chest was opened and blood was taken by cardiac puncture. In two cases blood was taken from the tail at the beginning of the experiment, to determine the rate of decline of the plasma \(\alpha\)-aminoisobutyric acid level. The change during the 2 hr was about 5\%, confirming earlier observations. The value for the plasma concentration observed at the end of the experiment was therefore applied in calculating concentration ratios.

**Analyses.** Centrifuged samples of the perfusion fluid (\(0.200\) ml) or of the loop contents (\(0.200\) ml or \(0.100\) ml + \(0.1\) ml water) were diluted with \(3.0\) ml absolute alcohol and then with \(6.8\) ml of a scintillation phosphor solution in toluene (6). The serum was deproteinized by treatment with \(4\) vol \(10\) % trichloroacetic acid; \(0.200\) ml of the filtrate likewise was treated with ethanol and the phosphor solutions. Disintegrations were counted for three to five 10-min intervals with the Packard Tri-Carb liquid-scintillation spectrometer. Potassium was determined with a Baird flame photometer, and bicarbonate by the Van Slyke-Neill technique, micropipettes being used to measure the small samples available.

**RESULTS AND DISCUSSION**

*Part 1.* Our experiments of type I began conservatively with the plasma radioactivity set at two to five times the initial level in the perfusion fluid; for example, the plasma level was \(0.6\) mm in \(\alpha\)-aminocyclopentanecarboxylic acid and the perfusion fluid was \(0.3\) mm. Because at each test we found the level in the perfusing fluid dropping steadily, we set the value of the initial distribution ratio higher and higher. This increase was obtained partly by elevating the level in the body fluids to a maximum of \(1.4\) mm by increasing the dose to \(15\) mg, and partly by lowering the level in the perfusion fluid, since a further increase in the injected dose might well lead to a toxic action \((6,10)\). Table 1 illustrates results obtained in such experiments.

In each case, as represented by the type I experiment...
The plasma and perfusate to values as low as amino acid radioactivity from the body. This release into the intestine brought the distribution ratio between any added amino acid, the perfusion fluid slowly gained results found in experiments of type 11. In the absence of had no measurable effect on the release of the radio-

accelerated by the presence of 4.8-19.4 mM levels of the carboxylic acid into the perfusion fluid did not appear to represent simple diffusion nor an erosion of cells from the same unlabeled amino acid in the perfusing fluid (Fig. 2). This behavior has been confirmed repeatedly with both amino acids. 25, 30, 60, 90, 120

The rather slow release of 1-aminocyclopentanecarboxylic acid into the perfusing fluid did not appear to represent simple diffusion nor an erosion of cells from the intestinal surface, followed by their extraction, because the release of the labeled amino acid could be greatly accelerated by the presence of 4.8-19.4 mM levels of the same unlabeled amino acid in the perfusing fluid (Fig. 9). A similar effect was produced by adding 18 mM L-methionine. A trial of 20 mM α-N-dimethylaminoisobutyric acid, an amino acid obstructed structurally so as to eliminate its transport affinity (11), showed that it had no measurable effect on the release of the radio-

active amino acid into the perfusing fluid. This action of added amino acids of appropriate structure is presumed to represent an acceleration of exchange between the two compartments, a widespread phenomenon in biological transport (see references and discussion in (9)). It also tends to identify the release of labeled 1-aminocyclopentanecarboxylic acid with a specific chemical process, presumably a transport process.

The two fluxes across the perfused section of small intestine were estimated, using the release of radioactivity into the perfusate (relative to the level in the plasma) in the second type of experiment as a first approximation of the efflux; and then using this value to correct the rate of uptake (relative to level in the perfusate) observed in the type I experiment. The new influx value was then used to correct the initial efflux estimate, and the result used in turn to correct the first estimate of influx. In this way an approximate value for the flux ratio, for 20 cm of jejunum, of go was calculated (inward, toward the plasma 10 µmoles/hr for each millimole per liter of perfusate; outward, into the lumen 0.2 µmoles/hr for each millimole per liter of plasma). Hence the steady-state distribution should find the plasma level at roughly 90 times the perfusate level; that is, at a plasma level of 1 mM the perfusion fluid should contain about 0.01 mM 1-aminocyclopentanecarboxylic acid. This approximate value of go lies between the distribution ratios of 43 and 176, found in the first and second type of experiments, respectively.

Perfusion of a 20-cm length of the colon also showed a release of 1-aminocyclopentanecarboxylic acid into the perfusion fluid, although at only 0.03 µmoles/hr, for
every millimole per liter in the plasma, a rate slower than that shown by jejunal loops. This release was, however, accelerated to 0.12 μmoles/liter by the presence of 18 μm methionine in the perfusion fluid.

Part 2. With this information in hand we were able to establish, in isolated intestinal loops, levels of L-amino-cyclopentanecarboxylic acid near the steady state between absorption and secretion. Such tests approximately confirmed the distribution ratio predicted above from the flux values. The same kind of result could also be obtained for α-aminoisobutyric acid, as is illustrated in Fig. 3. Under these conditions the steady-state distribution ratios between the plasma and the fluid in the lumen for both jejunum and ileum were generally in the range of 12–50, whereas that for the colon was very low and not clearly above unity.

In connection with these experiments we investigated the approximate potassium and bicarbonate levels of fluids characteristically reached at each of three levels of the rat intestine. For bicarbonate these values in milliequivalents per liter were approximately as follows: proximal jejunum 2; terminal ileum 75–120; colon 90. These results resemble those reported by Parsons (12). For the potassium ion, fluid introduced into jejunal and ileal loops tended to reach 6–7 mEq/liter during 2 hr, the colon 25–30 mEq/liter (cf. 13). The solutions selected for the amino acid studies had the following initial compositions: for jejunal loops, 10 mM each in K+ and HCO3−; for ileal loops 10 mM in K+ and 75 mM in HCO3−; for colon, 20 mM in K+ and 25 mM in HCO3−.

To show more clearly the reversibility of intestinal transport, three successive ileal loops, each about 8 cm in length, were filled with the same medium containing the test amino acid at three different levels, one certainly above and one certainly below the steady state level. The terminal 2 cm of ileum was avoided in preparing these loops. Figure 4 shows a typical result, among several similar ones, with 1-amino-cyclopentanecarboxylic acid. In this case the observed distribution ratios are plotted on the left-hand vertical scale, and the actual concentrations in the loop contents, in micromoles per liter, appear at the right.

To show that this phenomenon is nearly independent of the absolute level of the amino acid, experiments were made with α-aminoisobutyric acid, administered to each of three rats at doses of 20, 100, and 500 mg/kg body wt.; in each case using about the same amount of radioactive tracer. In this way serum levels of 0.11, 0.51, and 1.3 μmoles α-aminoisobutyric acid/liter were obtained. The tendency for solutions in ileal loops to gain or lose amino acid and to approach somewhat similar distribution ratios is shown in Fig. 5. The values obtained for the steady-state distribution ratio for α-aminoisobutyric acid have ranged from 12 to 50; those for 1-amino-cyclopentanecarboxylic acid from 50 to 250. We do not know why some experimental preparations showed substantially higher distribution ratios than others. The lower ratios, in the ranges 12–25 with α-aminoisobutyric acid and 60–100 with 1-amino-cyclopentanecarboxylic acid, were typical. The values for the jejunum were sometimes higher and sometimes lower than those for the ileum. The use of the ileum is technically advantageous because of the easy accessibility of sufficient lengths and because of the slower absorption of water.

A single experiment indicated that the steady state reached with α-aminoisobutyric acid across the ileum was much the same whether the initial K+ concentration in the loops was 0, 10, or 50 mEq/liter, even though at 30 min the K+ levels (3.4, 6.2, and 27.6) were still materially different. The use in ileal loops of initial HCO3− levels of 2 mEq/liter (rather than 75) tended to a more irregular approach to the steady state. The use of a high initial
bicarbonate level was technically advantageous also because it slowed the absorption of water.

The levels of test amino acid reached for a given ileal loop (as illustrated in Figs. 4, 5) showed better constancy with time than was observed at any given time along three successive loops of ileum. Accordingly we made a single test of another method of showing the reversibility of intestinal transport (Fig. 6). After a steady state had been established by secretion into a loop initially containing less than a steady-state level of \( \alpha \)-aminoisobutyric acid, the \( \alpha \)-aminoisobutyric acid content of the loop was increased by injection through the cannula, followed by gentle mixing by drawing fluid into the syringe and expelling it repeatedly into the loop. As Fig. 6 shows, the level in the loop was soon returned to much the same steady state by absorption.

Results on loops of ascending colon are summarized in Fig. 7. When the initial level in the lumen was less than one-fourth that in the plasma, secretion into the colon caused conspicuous changes in the level. But when the colonic levels were at about the plasma levels (lower 4 curves), changes in the amino acid concentration were very slow, and failed to give a clear-cut picture of the position of the steady-state ratio, whether above or below unity. Presumably this position could be determined more accurately by using lower concentrations of the test amino acid throughout, so as to avoid overloading the transport capacity. One experiment at 0.13 mM \( \alpha \)-aminoisobutyric acid (as marked in Fig. 7) showed that the plasma at the steady state tends to have less than twice the level in the lumen. The downward trend of the lower two curves does not prove, however, that the test amino acid is secreted uphill into the colon; this result could arise from the more rapid withdrawal of water than of the amino acid from the lumen.

Since both of these amino acids are released into the colon, we came to wonder why the fecal excretion of \( \alpha \)-aminoisobutyric acid has been found “very low” (14), whereas the fecal route is the dominant one for \( \alpha \)-aminocyclopentanecarboxylic acid (7, 15). Therefore we collected 24-hr fecal samples from two rats that had received 20 and 100 mg of labeled \( \alpha \)-aminoisobutyric acid. These animals were placed briefly every few hours in a bell jar containing ether vapor to secure uncontaminated fecal samples. They excreted 0.21 and 0.26% of the dose by this route, whereas 1.5% of a dose of \( \alpha \)-aminocyclopentanecarboxylic acid is excreted in the feces in 1 day. Hence the difference between the two substances in this respect is only quantitative.

We may assume that the natural amino acids are also slowly released into the colon, and that this release serves a significant role in the nutrition of the organisms multiplying there. Such losses probably have little quantitative importance to the host as long as the volume of fluid excreted by the large intestine remains small.

The steady-state ratio of \( \alpha \)-aminocyclopentanecarboxylic acid across the everted intestinal sac has, in contrast to the above results, been evaluated at about 2.7 for the jejunum in Krebs Ringer bicarbonate medium, and at about 4.0 for ileum in Krebs-Ringer phosphate medium (5). For \( \alpha \)-aminoisobutyric acid the corresponding ratios are only 1.0 and 2.3, respectively (5). Although this contrast speaks for itself in evaluation of the relative performance of the excised preparation, the usefulness of the latter cannot be questioned.

The technical assistance of Jane Clifford at Ann Arbor and of Josephine Reading at La Jolla is gratefully acknowledged.

ADDENDUM

In analogy to these results a slow release of 3-O-methylglucose from the body of the rat into the ileal and colonic lumen has also been observed. This release is accelerated by the presence of glucose in the intestine. Distribution ratios, serum level to ileal level, in the range 5 to 10 were obtained in the absence of added glucose (unpublished results in collaboration with Dr. Seymour Gray).

REFERENCES