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TRANSPORT OF THE AMINO ACIDS, 1-AMINO CYCLOPENTANE-1-CARBOXYLIC ACID AND ε-AMINOCAPROIC ACID, ACROSS INTESTINAL MUCOSA *IN VITRO*

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Abstract—Two α-methyl substituted amino acids, 1-amino*cyclo*pentane-1-carboxylic acid (ACPC), and *iso*valine, are actively transported across everted sacs of small intestine from the rat. ACPC inhibits growth of the Walker rat carcinoma. The antifibrinolytic amino acid, ε-aminocaproic acid (EACA), was not actively transported at therapeutic concentrations. These studies are relevant to the design of pharmacologically active amino acids or their analogues which may be introduced into cells by an active process rather than by passive diffusion.

THE transport of those amino acids present in proteins,[1] and also non-protein amino acids,[2] has been studied using mammalian small intestine *in vitro*. These and other systematic studies (reviewed by Wilson[3]) of series of amino acids and their analogues have revealed that they may be transported actively, against a concentration gradient, or by passive diffusion with the concentration gradient. Since some analogues exhibit potentially useful pharmacological or therapeutic effects it is of interest to study the mode of absorption from the intestine. Such data are useful not only when the compounds are given orally but also as a parameter of the uptake of amino acids by other somatic cells. Everted sacs of small intestine from the rat are useful preparations for investigating the transport mechanism for such drugs e.g. the carcinostatic amino acid derivatives of nitrogen mustard.[4] An extension of these studies to other pharmacologically-active amino acids is presented in this communication.

The amino acids tested were ACPC, which inhibits growth of tumours[5] and protozoa,[6] and EACA, which inhibits fibrinolysis.[7]

EXPERIMENTAL

Test compounds

DL-Valine, DL-*iso*valine, DL-α-amino-*n*-butyric acid and EACA (all from Koch-Light Laboratories Ltd.) and ACPC were checked for purity as previously published.[4]

In addition to the criteria of purity used previously[4] the EACA was subjected to low-voltage paper electrophoresis[9, 10] at pH 1·9, 3·6, 4·0 and 6·0; also high-voltage paper electrophoresis[11] at pH 4·0. No ninhydrin-reacting impurities were revealed.

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Furthermore, only one ninhydrin-reacting peak was obtained when EACA was analysed on an automatic amino acid analyzer (Technicon Instruments Co. Ltd.).

General methods

Preparation of everted sacs of small intestine from male albino rats (Wistar strain) of 200–300 g body wt was carried out as previously published.[4] Analysis using ninhydrin-hydrindantin reagent followed deproteinisation of material from everted sac experiments carried out as before.[4] Transport of EACA was measured by a different method after deproteinisation. EACA transport was measured at four different concentrations during a single experiment to reduce errors due to slight variations in experimental conditions. Two sacs from a single rat were used for each different concentration.[4] L-Histidine was placed in two sacs to demonstrate active transport in this series of experiments. Histidine was measured by a spectrophotometric method.[8]

Quantitative determination of EACA using high-voltage paper electrophoresis

Electrophoresis at pH 4·0 (Pyridine/glacial acetic acid/water: 10/28/962) was carried out at 150–180 V/cm for 30 min using Whatman 3 MM paper in a commercial apparatus (Locarte and Co. Ltd.). For quantitative determinations cadmium-ninhydrin reagent was used, the colours were developed,[12] eluted and the extinctions were measured at 500 mμ using a Unicam SP 500.

RESULTS

Separation of EACA from other amino acids

EACA was poorly separated from methionine, phenylalanine, leucine and *iso*-leucine by two-dimensional paper chromatography in *n*-butanol/acetic acid/water (120:30:50) and 80% (w/v) phenol/ethanol/water/sp.gr. 0·88 ammonia (150:40:10:1). From the experiments with buffers at various pH values pH 4·0 was found to give the best separation of EACA, from representative amino acids, using low-voltage electrophoresis. Similarly, with the high-voltage method, pH 4·0 gave good separations of EACA from the amino acids likely to be encountered in extracts of mammalian tissues (Fig. 1). Therefore, pH 4·0 buffer was used throughout for the quantitative determination of EACA using high-voltage electrophoresis. Calibration curves of aliquots of EACA eluted from paper as the cadmium-ninhydrin colours, before or after electrophoresis, gave very similar results. Hence there was little or no loss of EACA on electrophoresis. In common with other ω-amino acids EACA has a poorer colour yield with ninhydrin than most α-amino acids. The plot of EACA concentration against $E_{500m\mu}$ of the cadmium-ninhydrin colour was linear from 0 to 50 μg after electrophoresis.

Calculation of results from everted-sac experiments

The concentration ratio was calculated as the fraction:

$$\frac{\text{Concentration of compound on the serosal side}}{\text{Concentration of compound on the mucosal side}}$$

Theoretically, a ratio greater than 1·0 indicates active transport against a concentration difference. From the results obtained (Table 1) a concentration ratio exceeding about 1·1 was taken to indicate active transport. The rates of transference were cal-

culated by subtracting the amount originally put into the sac from that found within the sac by analysis at the end of the experiment.

ACPC, *iso*valine and valine were actively transported but not α-amino-*n*-butyric acid (Table 1). Also EACA was not actively transported over a concentration range

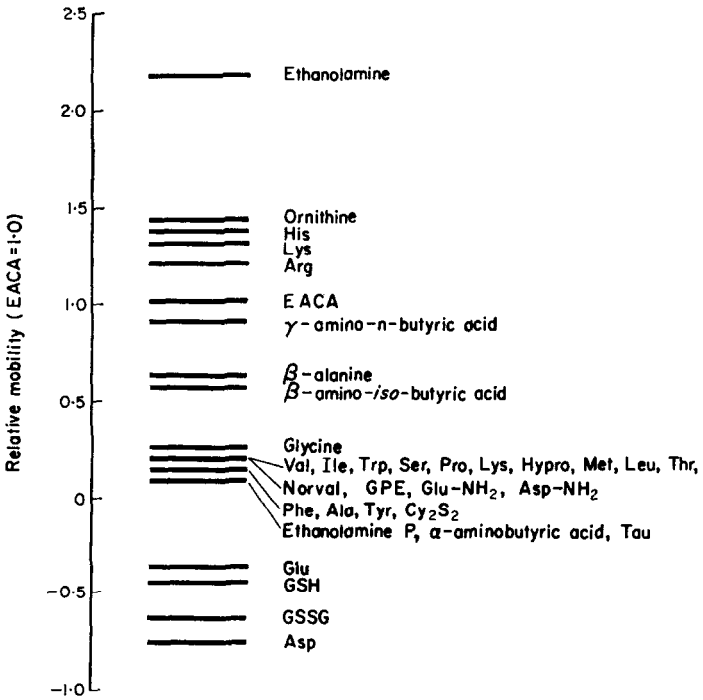

FIG. 1.

of 2–20 mM (Table 2). The potassium content and the water content of everted sacs at the end of an experiment suggested that neither EACA[4] nor the other test compounds used (Table 1) were toxic to the intestinal tissue under these experimental conditions.

DISCUSSION

Two compounds lacking an α-hydrogen atom, namely *iso*valine and ACPA, were both actively transported (Table 1). Active transport of the latter compound, and also

TABLE 1.

Compound (20 mM)	Number of sacs	Concentration ratio (Mean ± S.D.)	Uptake μmole/100 mg. fat-free dry wt./h (Mean ± S.D.)	Water content g/100g fat-free fresh tissue (Mean)	Potassium content mEquiv/kg fat-free dry tissue (Mean)
1-Amino*cyclo*pentane-1-carboxylic acid	6	1·43 ± 0·03	8·95 ± 1·40	88·9	321
DL-α-Amino-*n*-butyric acid	6	1·11 ± 0·5	1·90 ± 0·63	88·7	419
DL-*Iso*valine	6	1·66 ± 0·26	14·20 ± 1·52	89·7	356
DL-valine	6	1·30 ± 0·10	4·62 ± 2·42	89·0	356

of α-amino*iso*butyric acid, has been reported previously with small intestine from the rat.[13] These results all indicate that the α-hydrogen atom is not required for attachment of an amino acid to some cellular receptor acting as a carrier.

Replacement of the α-hydrogen atom by a methyl group stimulated the rate of uptake by intestinal mucosa. For example, the rate of transference and the concentration

TABLE 2.

Compound	Concentration (mM)	Concentration ratio (Mean)	Water content g/100 g fat-free fresh tissue (Mean)
L-Histidine	5	1·40	88·2
EACA	2	0·79	89·5
EACA	5	0·58	89·1
EACA	10	0·89	98·9
EACA	20	0·95	88·9

ratio developed for DL-*iso*valine were much greater than those observed for DL-α-amino-*n*-butyric acid (Table 1). DL-Valine, lacking an α-methyl group, was poorly transported compared with DL-*iso*valine (Table 1) although both molecules have the same number of carbon atoms. The effect of substituting methyl and ethyl groups respectively, on the α-carbon atom, has also been studied in everted small intestine of the rat. L-*Iso*valine was more rapidly transported than D-*iso*valine[14].

With everted sacs of hamster intestine also, the possession of an α-hydrogen atom was not a necessary condition for active transport of an L-amino acid. However, in contrast to the present study, the replacement of this hydrogen atom by a methyl group was reported to reduce the rate of transport with the following pairs of compounds: glycine and D-alanine; L-alanine and β-amino*iso*butyric acid: L-methionine and L-α-methylmethionine; L-tyrosine and L-α-methyltyrosine.[15] If, however, the results are considered for the pair glycine and the L-isomer of α-methylglycine, i.e. alanine, the presence of the α-methyl group has stimulated the rate of uptake.

Conversely, substitution of the α-hydrogen atom by a methyl group enhanced uptake of the amino acids by Ehrlich ascites tumour cells.[16] If certain α-methyl substituted amino acids showed a significantly greater uptake by neoplastic cells, compared with normal mammalian tissues, then this difference could be of importance in the design of carcinostatic drugs. ACPC may well be 'pumped' into malignant cells to achieve a high concentration within the cells. Experimentally ACPC is well concentrated by Ehrlich ascites tumour cells, slices of brain cortex[17] and pancreas[18] but not by slices of liver or kidney cortex from rats.[17] Furthermore, the mechanism of the antitumour action of ACPC may probably be attributed to its effect in preventing the combination of valine with transfer RNA.[19]

Transport of EACA

Previous experiments *in vitro* suggested that EACA was not transported actively.[2] These experiments were carried out at unphysiologically high concentrations (20 m*M*) for analytical purposes. It has been shown that some amino acids, for example tryptophan, while exhibiting active transport at low concentrations also show inhibition of

this transport at higher concentrations of the order of 20 mM.[20] This phenomenon may be analogous to that of substrate inhibition of an enzyme. Hence, if the transport of such compounds is only tested at this high concentration it may appear that they only transport by the passive process. Conversely, if a compound does show active transport at this relatively high concentration then presumably transport will also be active at lower concentrations.

Since EACA does not show active transport across the intestinal mucosa in the concentration range of 2–20 m*M* (Table 2) it seems likely that, when used therapeutically by the oral route, it is absorbed into the blood stream by passive diffusion. The lower concentration tested is very near to the blood plasma levels achieved therapeutically,[21, 22] when up to 36 g of EACA are given daily in certain fibrinolytic diseases.[22]

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