SHORT COMMUNICATIONS

BBA 73083

Amino acid transport in rat-liver slices during development

Incidental to studies on amino acid transport in rat-liver slices[1], we noted a tendency toward relatively high uptake of the nonmetabolizable amino acid, α-aminoisobutyric acid, in slices prepared from young animals. Higher concentrations of amino acids *in vivo* have been found in regenerating liver[2] and in fetal[3] and newborn livers[4] than in non-regenerating adult liver; similar findings with respect to the effect of age have also been made for muscle[5]. Since uptake of amino acids into the tissue is especially important in such situations, we thought it of interest to investigate the effects of age on the net transport of amino acids by liver slices.

Pregnant or male weanling rats (Holtzman) were maintained on a stock diet. At 1–80 days after birth, the livers were removed, chilled and cut with a McIlwain tissue chopper into slices 0.4 mm thick; 200 ± 10 mg were preincubated for 15 min in 3 ml of Krebs–Ringer bicarbonate buffer (pH 7.4). The slices were then transferred to fresh medium containing either 1 mM α-amino[1–^{14}C]isobutyric acid or 2 mM uniformly ^{14}C-labeled histidine and were incubated for 60 min. The slices were separated from the medium and were homogenized with 3% sulfosalicylic acid. After centrifugation of the media and homogenates, radioactivity measurements were made on the supernatant fluid. Results were calculated as the distribution ratio:

$$\text{Distribution ratio} = \frac{\text{disint./min per ml intracellular fluid}}{\text{disint./min per ml extracellular fluid}}$$

Distribution of total water between the intracellular and extracellular spaces was measured with [*carboxyl*-^{14}C]inulin. Details of these procedures have been described earlier[1].

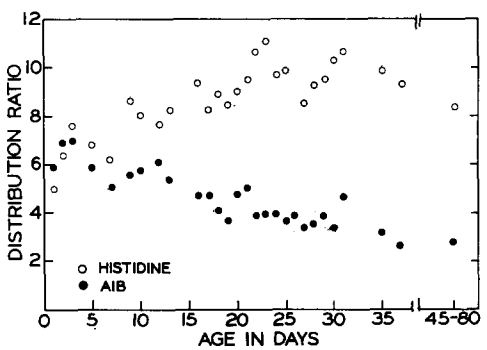

Fig. 1. Uptake of α-amino[1-^{14}C]isobutyric acid or uniformly ^{14}C-labeled histidine in rat-liver slices during development. Incubations were carried out in Krebs–Ringer bicarbonate buffer at 37°; preincubation and incubation periods were 15 min and 60 min, respectively. Initial concentrations of α-aminoisobutyric acid (AIB) and histidine were 1 and 2 mM, respectively. Averages are shown for 3–6 experiments up to 22 days, and for 3–12 experiments thereafter.

The highest distribution ratios for α-aminoisobutyric acid were found in livers taken from the youngest rats (Fig. 1). The ratios declined with increasing age so that α-aminoisobutyric acid uptake in rats 45–80 days old was about half that seen in 1-day-old rats. There appeared to be little change in transport of α-aminoisobutyric acid for approx. 10 days after weaning.

The experiments with histidine were included as an illustration of the effects of metabolism of the substrate on distribution ratios as calculated solely from radioactivity data. Fig. 1 shows that such ratios for histidine slowly increased until the animals were about 3 weeks of age. The studies also involved the simultaneous analysis of portions of the slice extracts for free histidine concentration and for the distribution of radioactivity derived from histidine (Technicon Autoanalyzer; Packard Flow Cell and Monitor). In the newborn rat, relatively minor amounts of radioactivity were found in compounds other than histidine; hence, the ratios (Fig. 1) for the youngest animals do represent primarily histidine transport. However, the amount of unaltered histidine present gradually decreased with age so that by the time the rats were 3–4 weeks old, only 20–30 % of the radioactivity in the liver slices was in this amino acid. Most of the remainder of the label was found in a single histidine derivative; its location on the amino acid chromatogram and the formation of [^{14}C]glutamate upon acid hydrolysis of the appropriate effluent fractions suggested it to be formiminoglutamic acid[6].

MAKOFF AND BALDRIDGE[7] found that histidase (EC 4.3.1.3) and urocanase activities are negligible or low in the livers of newborn rats. Although urocanase activity was found to increase slowly up to 4 weeks of age, histidase activity did not show much change until the rats were 2 weeks old. These observations support our findings that, although the total amount of histidine entering the cell increased with age, the amount remaining as unaltered histidine was actually much lower in the older rats, as compared to those 1 day of age. Based on the concentration of histidine in the tissue, rather than on the measurements of radioactivity, the distribution ratios in livers of rats above 21 days of age were about 1.5–2.0. It cannot be unequivocally concluded from the experiments shown in Fig. 1 that, similar to the results with α-aminoisobutyric acid, a decreasing uptake of histidine would be seen in aging animals if its simultaneous metabolism did not also occur. For instance, it is possible that the metabolites themselves could influence the subsequent transport of histidine.

Other preparations *in vitro* showing a decline in net amino acid transport with increasing age include kidney[8,9], intestine [10,11] and diaphragm[12]. Concentration by liver and other tissues of α-aminoisobutyric acid injected into intact rats has also been reported to be higher in young than in adult rats[13]. All these observations are consistent with our results indicating that the transport of α-aminoisobutyric acid by rat liver slices is also high in newborn rats and slowly declines thereafter. The results suggest, but do not prove, that the transport of histidine undergoes a similar change with age and that in the rat post-weaning, the rate of metabolism of an amino acid is an important factor influencing its total uptake by the liver.

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1 J. K. TEWS AND A. E. HARPER, *Biochim. Biophys. Acta*, 183 (1969) 601.
2 H. N. CHRISTENSEN, J. T. ROTHWELL, R. A. SEARS AND J. A. STREICHER, *J. Biol. Chem.*, 175 (1948) 101.
3 W. L. RYAN AND M. J. CARVER, *Nature*, 212 (1966) 292.
4 S. S. OJA, A. J. UUSITALO, M. L. VAHVELAINEN AND R. S. PIHA, *Brain Res.*, 11 (1968) 655.
5 H. N. CHRISTENSEN AND J. A. STREICHER, *J. Biol. Chem.*, 175 (1958) 95.
6 J. D. HERBERT, *Comp. Biochem. Physiol.*, 24 (1968) 229.
7 R. MAKOFF AND R. C. BALDRIDGE, *Biochim. Biophys. Acta*, 90 (1964) 282.
8 W. A. WEBBER AND J. A. CAIRNS, *Can. J. Physiol. Pharm.*, 46 (1968) 165.
9 W. A. WEBBER, *Can. J. Physiol. Pharm.*, 46 (1968) 765.
10 M. NING, S. REISER AND P. A. CHRISTIANSEN, *Proc. Soc. Exptl. Biol. Med.*, 129 (1968) 799.
11 B. STATES AND S. SEGAL, *Biochim. Biophys. Acta*, 163 (1968) 154.
12 L. J. ELSAS, I. ALBRECHT AND L. E. ROSENBERG, *J. Biol. Chem.*, 243 (1968) 1846.
13 T. R. RIGGS AND L. M. WALKER, *J. Biol. Chem.*, 233 (1958) 132.

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Stereochemical specificity of neutral amino acid transfer systems in rat small intestine

It is now well established that there are two carriers involved in the intestinal transfer of neutral amino acids[1–4]. These can conveniently be called the sarcosine carrier and the methionine carrier, since sarcosine is transported mainly by one carrier and methionine by the other. A 'betaine carrier' has been described in the hamster small intestine[5], and, although this has not been shown to be involved in amino acid transfer, it may be equivalent to what we have termed the sarcosine carrier. The specificity of these carriers in relation to length of carbon chain and position of the amino group has recently been discussed[6], and the present work explores the stereochemical specificity.

Experiments were carried out with sacs of everted rat intestine prepared from middle fifth of the combined jejunum and ileum. The sacs contained 1 ml of bicarbonate saline[7] and were suspended in 25 ml of bicarbonate saline containing 28 mM glucose and 1 mM [*Me*-^{14}C]methionine or [*carboxy*-^{14}C]sarcosine. The transfer of methionine and of sarcosine was studied in the presence of a number of other amino acids and related substances.

The results are shown in Table I. In the case of those amino acids which inhibit methionine transfer, the L-enantiomorphs inhibit considerably more than the D-enantiomorphs, in confirmation of previous work[8–11]. In contrast, the L- and D-enantiomorphs have nearly equal effects on sarcosine transfer. In particular D-alanine, D-proline and hydroxy-D-proline inhibit sarcosine at least as much as do the corresponding L-enantiomorphs. It is also seen that both enantiomorphs of azetidine (with a four-membered ring) inhibit sarcosine transfer, but pipecolic acid (with a six-membered ring) has less effect. In this respect it is of interest that L-azetidine, but not pipecolic acid inhibits incorporation of proline during protein synthesis in *Escherichia coli* (ref. 12). However, other workers[13] have observed a large inhibition of sarcosine by DL-pipecolic acid in hamster intestine. These authors also stated that L-proline transfer is not