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INTENSIFIED GRADIENTS FOR ENDOGENOUS AMINO ACID SUBSTRATES FOR TRANSPORT SYSTEM *L* ON INJECTING A SPECIFIC COMPETITOR FOR THAT SYSTEM

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Summary

The injection into the rat of 8.1 mmoles of aminoendo(±)-2-aminobicycloheptane-2-carboxylic acid per kg body weight intensified in 2 hr the gradients of several System *L* substrates characteristically maintained by the liver with respect to the blood plasma. The gradients of amino acids predominantly transported by systems other than *L* were not, with the exception of proline, significantly influenced. We interpret this effect on System *L* substrates as supporting the principal service of System *L* in net cellular exodus of these amino acids, although other factors in the effects are not necessarily excluded.

In our earlier tests by administering non-metabolizable analogs on the distribution of the amino acids in the rat (1), we had expected perturbations of only one kind. We predicted decreases, not increases, to arise through the competitive action of the artificial amino acid on the ability of tissues to maintain normal concentrations of certain groups of amino acids in relation to their plasma levels. And those were in general the results we observed (1). But our protocols reveal retrospectively an opposite effect which we have now come to understand and expect, namely intensified hepatic gradients of certain amino acids 2 hr after injecting the norbornane amino acid "BCH" (the aminoendo(±) isomer of 2-aminobicyclo-(2,2,1)heptane-2-carboxylic acids). On the basis of experiments with Ehrlich ascites tumor cells, we have concluded that the amino acids transported especially by System *L* nevertheless undergo their net concentrative uptake mainly by the steeply uphill System *A*. Nevertheless these amino acids maintain only moderate intracellular:extracellular gradients we believe because in the physiological context System *L* comes to serve mainly for their net exodus (2). Accordingly, an effect of excess BCH as an apparently specific System *L* inhibitor not to perturb the distribution of the amino acids transported mainly by System *A*, but instead to intensify the gradients maintained for a group of amino acids with longer, branched sidechains of low polarity, becomes logical and the first good evidence for *in vivo* operation of the cycle, net cellular entrance by *A* and net exodus by *L*.

Methods

The norbornane amino acid, our own preparation (3,4) was injected intraperitoneally, 8.1 mmoles per kg body weight, into male Sprague-Dawley rats weighing 250 to 350 g. Food had been withheld 18 to 20 hr before the injection, and the liver removed, killing the animal. The tissues were analyzed as described previously (1), applying automated ion-exchange chromatography for the amino acids. Control rats received an injection of isotonic NaCl in the corresponding volume. The conditions of adrenalectomy were as described previously (1).

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Experimental Results

Fig. 1 shows that BCH injection produced in 2 hr no significant changes in the distribution ratio between liver and plasma for aspartate, glutamate, threonine, serine, the sum of asparagine plus glutamine, glycine, tyrosine, phenylalanine, lysine or histidine. The amino acids strongly affected were alanine, valine, methionine, isoleucine, leucine, ornithine and proline. Their gradients were in all cases raised. We find immediately logical the inclusion of the first 5 of the latter group of amino acids, all with apolar sidechains and showing substantial BCH-inhibitable, Na^+-independent uptake in isolated rat hepatocytes, i.e. uptake by System *L* (5,6). Proportional transport of alanine by System *L* into hepatocytes can exceed that by System *A* (Table II, ref. 7). The observation that phenylalanine and tyrosine escape the effect does not agree with the role accorded System *L* for these two aromatic amino acids in various other cells (see ref. 6). Therefore this result has set us to looking for a contribution by a possible aromatic transport system which may have been heretofore overlooked. Evidence for independently regulated intestinal absorption of these two amino acids has been reported (8). The sensitivity of the ornithine distribution could arise from the circumstance that the cationic amino acid transport system can scarcely be detected in isolated hepatocytes (M. F. White, doctoral thesis in preparation), a circumstance that might leave much of the *in vitro* uptake of ornithine to System *L*, for which ornithine can become a substrate by deprotonation to a dipolar form, as reported earlier for the Ehrlich cell (9,10). The present finding may mean that System *L* serves more for hepatic exodus than uptake of ornithine in the fasting rat. The position of proline in the list of responsive amino acids remains unexplained, but might be a result of its generation from ornithine at a rate sufficient to cause accumulation. These paradoxes mean that some of the effects may have alternative explanations. We plan to turn to a homologous metabolism-resistant model substrate for System *L* that fails to stimulate insulin release.

System *L* of the rat liver has so far shown no obvious point of difference from the corresponding system as originally described in the Ehrlich cell (6). BCH shows a rapid uptake, with no apparent Na^+-dependent component when initial rates are approached (5). BCH inhibits the uptake by the hepatocyte of the various amino acids otherwise known to be transported by System *L*, and not Na^+-dependent components of amino acid uptake (5). Exchange by this system is as in most other cases lively (unpublished result, R. Garcia-Cañero, this laboratory). The uptake of BCH by the liver of the intact rat (11) or by the perfused liver (12) is not stimulated by insulin or glucagon. Various experiments have shown System *L* of hepatocytes likewise insensitive to these hormones (e.g. 13). BCH uptake has not been found to be stimulated by amino acid starvation, nor does BCH repress transport by System *A* (14).

The administration of BCH does stimulate the pancreatic release of insulin in the rat (15-19) (also in other species), a discovery made in the course of the present research, and one which in fact drew our attention away from immediate efforts to understand the results reported here. This action now appears to arise from a stimulation of glutamate dehydrogenase by BCH (19). It is conceivable that an elevation of the plasma insulin could have stimulated somewhat the hepatic uptake of the amino acids listed between threonine and alanine in Fig. 1, i.e., via System *A*. Such a hypothetical effect could have masked possible minor inhibition of the tissue retention of some of these amino acids by BCH, and intensified the gradients maintained for proline and alanine. Since System *L* is not stimulated by insulin, however, insulin release seems unlikely to have been a factor in the intensified gradients for valine, methionine, isoleucine, leucine and ornithine. Because we recognized that adrenal cortical steroid release after BCH injection might occur to modify amino acid distribution secondarily, we confirmed that the characteristic effects of BCH injection were also obtained in adrenalectomized rats (Fig. 1). The BCH effects tended

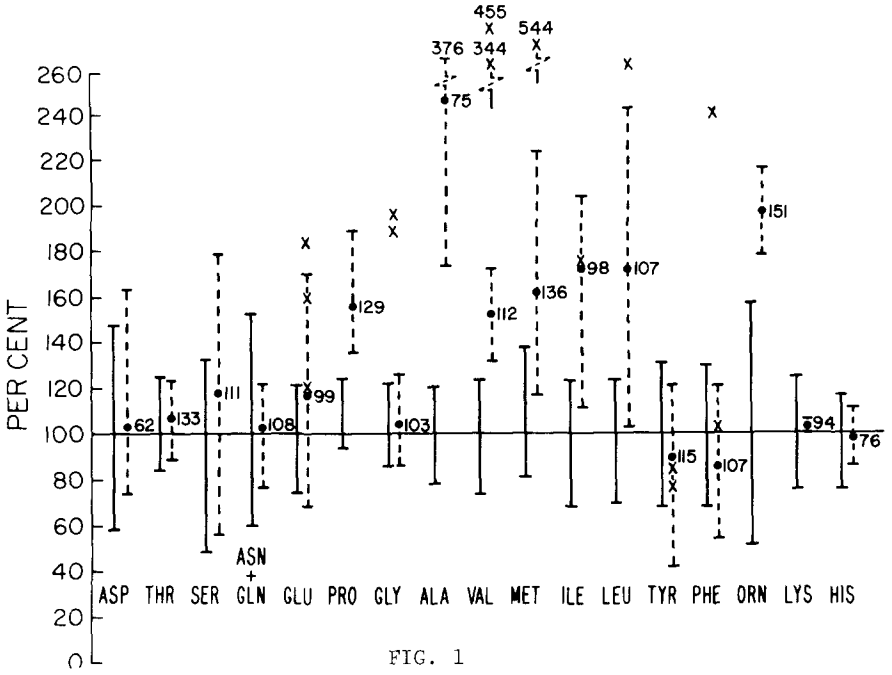

FIG. 1

Effect of intraperitoneal BCH on the distribution of amino acids between the liver and the plasma. The points -, •, and x represent by their position relative to the ordinate scale the percent of the control distribution ratio for the designated amino acid observed in an experimental rat, as in our preceding study (1). The vertical solid lines represent the range for the gradients of a given amino acid found for three or more control rats, the mean value having been normalized to 100% for each amino acid. The points • represent the mean ratios observed for the intact BCH-treated rats, four animals in each case. The vertical dashed lines show the range through which these results varied among the animals. The number adjoining the point, •, represents the mean absolute hepatic concentration (not the gradient) found for that amino acid, as percent of the mean control concentration. Control amino acid levels, mmol/kg liver cell water ± standard deviation, 5 rats, from which other concentrations can be reconstructed: Asp 2.54 ± 0.63; Thr, 048 ± 0.07; Ser 0.67 ± 0.19; Asn + Gln 1.39 ± 0.61; Glu 1.56 ± 0.61; Pro 0.24 ± 0.065; Gly 3.84 ± 0.61; Ala 2.29 ± 0.43; Val 0.42 ± 0.115; Met 0.16 ± 0.035; Ile 0.27 ± 0.07; Leu 0.50 ± 0.15; Tyr 0.15 ± 0.05; Phe 0.19 ± 0.06; Orn 0.093 ± 0.035; Lys 1.06 ± 0.18; His 0.69 ± 0.13. The mean BCH levels in mmoles/liter water were 10.1, liver cells, and 7.1, plasma. Five mmoles BCH/kg body weight also increased the liver to plasma distribution ratio for alanine, valine, isoleucine and leucine, whereas a 1 mmole dose produced only questionable increases. The points, x, represent the gradients of the amino acid produced by 8.1 mmoles BCH in two adrenalectomized rats, compared with two adrenalectomized controls. The four numbers at the top, center, represent some gradients, as percent of controls, which rose beyond the ordinate scale. We can illustrate the foregoing interpretation of the figure using valine, approximately at the center of the figure. The gradient for this amino acid has been elevated significantly by a mean of 53%, this increase being due more to a fall in plasma valine than to a rise of hepatic valine (the latter by an average of 12%). In our interpretation, for a compartment relatively as large as the hepatic one, one expects modifications of uphill transport to be reflected more by plasma changes than by changes in the liver level. The hepatic valine gradient was increased even more in two adrenalectomized animals. Interpretation for the other amino acids follows the same lines as this example.

rather to be strengthened, as though they might have been opposed to a degree by adrenal cortical activity in the intact animals. The BCH-induced increase in the gradient for glycine, associated mainly with a decline in its plasma level and seen only in the two adrenalectomized rats, remains unexplained.

Support for the concept that amino acid distribution can be selectively modified by metabolism-resistant transport analogs comes from the consistent action in the reverse direction produced by injection of 2-aminoisobutyric acid in the same study (1), which effect came to be restricted to the notable System *A* substrates when the more specific System *A* substrate, 2-(methylamino)isobutyr ic acid (5,20) was substituted for its close analog lacking the N-methyl group. An incidental question raised by those earlier results becomes steadily more interesting: Accepting that the facilities for regulation of cellular amino acid levels are conspicuous and vital (21,22), we wonder how the young growing rat can tolerate as well as it does an approximate aggregate halving on 3 weeks AIB feeding, of its hepatic amino acid levels or gradients (1).

The above results show how unwise it would be to ignore the role of transport systems other than *L* in considering the distribution of the so-called "System *L* substrates". When System *L* is largely blocked, we may see how large a part is played in their distribution by other agencies, presumably System *A* in particular. The possibility developed here that amino acid gradients may be intensified, rather than decreased, by interference with a transport system probably also deserves increased attention.

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