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Effect of Dietary Amino Acids on Distribution of α-Aminoisobutyrate in the Rat (40851)

JEAN K. TEWS AND ALFRED E. HARPER

Departments of Biochemistry and Nutritional Sciences, College of Agricultural and Life Sciences, University of Wisconsin-Madison, Madison, Wisconsin 53706

Alterations in α-aminoisobutyric acid (AIB) transport have been shown to occur *in vitro* in liver slices taken from rats subjected to various dietary and hormonal treatments *in vivo*. AIB transport is stimulated by consumption of a high level of dietary protein (1), by administration of glucagon (2), or by force-feeding certain amino acids or protein hydrolysates (3). Transport of AIB in liver slices from rats fed low-protein diets is normal, but the effectiveness of various transport stimuli is decreased in the protein-depleted animal (4). Recently we have found that clearance of AIB from plasma and its distribution *in vivo* between plasma and liver as well as several other tissues were altered by changes in dietary protein content and by glucagon (5). We have now carried out experiments in which we have examined the effects of force-feeding glycine or casein hydrolysate on the distribution *in vivo* of AIB between plasma and liver and other tissues from rats previously fed normal or low levels of dietary protein. Since AIB serves only as a model, primarily for the naturally occurring small neutral amino acids, we have also compared changes in AIB distribution with those for total ninhydrin-positive material.

Methods. Male rats of the Holtzman strain were obtained at 3 to 4 weeks of age. They were housed individually in metal cages with wire mesh bottoms and were kept in an animal room lighted from 0700 to 1900 hr. The rats were first fed *ad libitum* a diet containing 18% casein prepared as an agar gel (1) until they weighed about 90 g; the diet was then fed for 3 hr per day (from 0800 to 1100 hr) until the animals weighed about 100 to 105 g. At this time some of the animals were fed for 8 days meals of diets containing 6% casein; control rats continued to receive the 18% casein diet. On Day 9 rats were injected at zero time (0800 hr) with [^{14}C]AIB (New England Nuclear; specific activity = 9.9 mCi/mmole; 1 μCi/100 g rat, sc) and were fed immediately the usual 18 or 6% casein meal. Twenty-four hours after AIB administration the rats were force-fed 5 ml of either water, a suspension containing 1 g of an enzymatic hydrolysate of casein, or a solution of glycine (2 mmole). The rats were decapitated after 1½ hr and blood and tissue samples were taken for determination of radioactivity in acid-soluble supernates. Results are expressed as the distribution ratio for AIB:

$$\frac{\text{dpm/ml tissue water}}{\text{dpm/ml plasma}}.$$

Further details have been described elsewhere (5).

Total ninhydrin-positive material in suitably diluted portions of the acid-soluble supernates was also determined (6) and results were again calculated as the distribution ratio:

$$\frac{\mu\text{mole ninhydrin-positive material/ml tissue water}}{\mu\text{mole ninhydrin-positive material/ml plasma}}.$$

Statistical significance was determined by Student's *t* test.

Results. Distribution of [^{14}C] AIB. Force-feeding casein hydrolysate or glycine (compared with force-feeding water) did not alter plasma concentrations of AIB in intact rats previously fed meals containing either 18 or 6% casein and injected with AIB (Fig. 1). As with liver slices (4), the distribution ratio for AIB increased significantly in livers from the rats previously fed an adequate amount of protein and then force-fed casein hydrolysate or glycine ($P < 0.05$). The actual concentration of AIB (dpm/ml tissue water) also increased after administration of glycine ($P < 0.05$); the increase after feeding casein hydrolysate was

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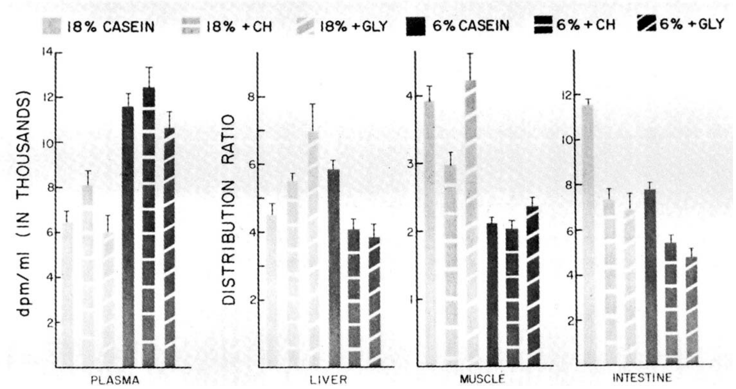

FIG. 1. AIB uptake *in vivo* into tissues of meal-fed rats: effect of low-protein meals on the response to one force-fed meal of casein hydrolysate or glycine. Rats were previously fed 18 or 6% casein meals as described under Methods. [^{14}C]AIB was administered and the accustomed meal was fed; 24 hr later the rats were force-fed a test meal of either water (5 ml), casein hydrolysate (CH; 5 ml containing 1 g), or glycine (5 ml containing 2 mmole) and killed 1.5 hr thereafter. Results are expressed as the distribution ratio (except for plasma). Bars show SEM; five rats per group, except four rats force-fed CH in 18% casein group.

not significant ($P < 0.1$). These agents had no stimulatory effect on AIB uptake by liver in the low-protein group; in fact, the distribution ratios were significantly lower than those obtained when water alone was fed ($P < 0.01$ for either casein hydrolysate or glycine). In these rats glycine caused a significant fall in AIB concentration in liver ($P < 0.001$); the decrease after casein hydrolysate was not significant ($P < 0.1$). Liver was the only tissue in which such divergent responses to the stimuli were observed in the adequate and low-protein groups.

The results for skeletal muscle (Fig. 1) were quite similar to those for heart (not shown). Feeding glycine had no significant effect on the distribution ratio for AIB in skeletal muscle or heart regardless of the protein content of the diet, whereas intubation of casein hydrolysate decreased the ratio in muscle only in the group previously fed the adequate protein diet ($P < 0.02$); the same trend in heart was not significant. Similar patterns for brain (not shown) were seen in the two groups; glycine was again without effect while feeding casein hydrolysate significantly decreased the distribution ratio for AIB in both the 6 ($P < 0.05$) and 18% ($P < 0.001$) groups.

The effects on intestine (Fig. 1) and kidney (not shown) also were similar to each other, with the overall pattern differing from those for the other tissues (except for liver in the low-protein group). Glycine and casein hydrolysate significantly decreased the ratios for AIB in both the low-protein and control groups ($P < 0.001$ or 0.01 in all cases when compared with the appropriate water-fed control). AIB concentrations (dpm/ml tissue H_2O) were also decreased in intestine and kidney of both groups of rats fed glycine ($P < 0.001$ or 0.01); feeding casein hydrolysate decreased AIB content in both kidney and intestine in the 6% group but not in intestine from rats receiving 18% casein ($P < 0.01$ for kidney; 0.05 for intestine in 6% group).

Amino nitrogen changes. In order to determine whether diet-induced changes in AIB distribution *in vivo* reflect similar changes in distribution of the naturally occurring amino acids and related compounds, total ninhydrin-positive materials were analyzed in plasma and other tissues. Distribution ratios calculated on the basis of ninhydrin-positive material in plasma and tissues were higher than those for AIB in all tissues except kidney. This is illustrated in Table I in which the ratios of the distribution ratios for ninhydrin-positive material to those for AIB (relative distribution ratios) are presented.

Force-feeding casein hydrolysate de-

TABLE I. RELATIVE DISTRIBUTION RATIOS FOR NINHYDRIN-POSITIVE MATERIAL AND AIB

Tissue and diet	Experimental treatment		
	H_2O	CH	Glycine
Liver			
18% Casein	3.50 ± 0.15	1.48 ± 0.10*	1.51 ± 0.20*
6% Casein	2.30 ± 0.12	1.99 ± 0.11	2.31 ± 0.18
Muscle			
18% Casein	3.87 ± 0.60	2.24 ± 0.16***	2.39 ± 0.33
6% Casein	4.93 ± 0.61	2.51 ± 0.14**	2.33 ± 0.23**
Brain			
18% Casein	6.85 ± 0.56	3.84 ± 0.29**	3.41 ± 0.35*
6% Casein	5.74 ± 0.46	3.11 ± 0.23*	2.38 ± 0.23*
Heart			
18% Casein	2.55 ± 0.34	1.28 ± 0.10**	1.16 ± 0.18**
6% Casein	3.17 ± 0.26	1.48 ± 0.13*	1.36 ± 0.19*
Intestine			
18% Casein	1.13 ± 0.07	1.38 ± 0.08***	1.33 ± 0.20
6% Casein	1.56 ± 0.10	1.95 ± 0.17	1.60 ± 0.04
Kidney			
18% Casein	0.40 ± 0.06	0.49 ± 0.03	0.50 ± 0.09
6% Casein	0.45 ± 0.04	0.57 ± 0.08	0.88 ± 0.06*

Note. Relative distribution ratio: distribution ratio for ninhydrin-positive material/distribution ratio for AIB.

* $P < 0.001$ when compared with corresponding water-treated rats.

** $P < 0.01$.

*** $P < 0.05$.

creased the relative distribution ratios in all tissues but intestine and kidney (Table I). The significantly lower relative distribution ratio in livers from the 18% casein group resulted from a lower distribution ratio for ninhydrin-positive material as well as from an increase in the ratio for AIB. Lower relative distribution ratios in muscle, brain, and heart from rats fed either 18 or 6% casein usually resulted from a sometimes large decrease in the ratio for ninhydrin-positive material along with smaller or no decreases in the ratio for AIB. The relative distribution ratios were increased or unchanged in intestine or kidney because the distribution ratios for AIB were significantly lower in these tissues. In general, force-feeding glycine similarly lowered the mean relative distribution ratios in liver (not in the 6% group), muscle, brain, and heart. Again, the lack of change or an actual increase in the relative distribution ratios for intestine and kidney reflected the large decrease induced by casein hydrolysate or glycine in the distribution ratio for AIB in these two tissues. Regardless of which material was force-fed, the decreased distribution ratios for ninhydrin-positive materials resulted from proportionately larger increases in ninhydrin-positive material in the plasma (Table II) than in the tissues.

These results imply that dietary modifications of AIB transport do not necessarily reflect similar changes in total amino acid transport; instead, AIB uptake may be more indicative of transport of the small neutral amino acids.

The changes in the relative distribution ratios after feeding casein hydrolysate or glycine were more extensive than those found in rats treated with glucagon. In these

TABLE II. NINHYDRIN-POSITIVE MATERIAL IN PLASMA

Treatment	18% Casein	6% Casein
Force-fed:		
H_2O	4.36 ± 0.14[a]	4.99 ± 0.24
CH	10.64 ± 0.23*	11.80 ± 0.73*
Glycine	9.16 ± 1.02**	12.44 ± 1.23*
Injected:		
Diluent	5.06 ± 0.24	4.51 ± 0.27
Glucagon	3.23 ± 0.21*	2.96 ± 0.29**

Note. Comparisons for statistical significance are with water- or diluent treated rats. Symbols are as in Table I.

[a] Results are in μmole/ml.

studies rats were treated as described under Methods except that glucagon (2 mg/kg, sc) was administered instead of casein hydrolysate or glycine; the animals were killed 1 hr thereafter. Previous work has shown that glucagon increased the distribution ratio for AIB in all tissues except kidney, primarily as a result of a decrease in the plasma content of this nonmetabolizable amino acid (5); AIB concentration in liver also increased, consistent with observations that glucagon stimulates transport of A System amino acids (7). Liver was also the only tissue in which significant differences between the relative distribution ratios were observed after treatment of the animals with diluent or glucagon. With this one exception, tissue distribution of ninhydrin-positive materials and of AIB were similar after injection of glucagon. The relative distribution ratios for liver for rats previously fed 18% casein and then treated with either diluent or glucagon were, respectively, 3.19 ± 0.30 and 1.40 ± 0.14 ($P < 0.001$) as compared with 2.73 ± 0.18 and 1.86 ± 0.20 ($P < 0.02$) for rats fed 6% casein. The ratio was lower for livers from glucagon-treated rats primarily because there was a somewhat larger increase in the distribution ratio for AIB than for ninhydrin-positive material. Glucagon treatment decreased the amount of ninhydrin-positive material in plasma (Table II), as it did the amount of AIB (5).

We have also analyzed by ion-exchange chromatography (Technicon autoanalyzer) the free amino acid content of a few of the plasma, liver, and muscle samples from animals treated with glucagon (one to three samples per treatment). Distribution ratios between tissue and plasma were calculated for each amino acid; the results suggest a greater stimulation by glucagon of the net uptake of the small neutral amino acids (threonine, serine, and alanine) than of the large neutral, acidic or basic amino acids. These observations are consistent with numerous studies *in vitro* (7) showing that hormonal stimulation of transport is more or less restricted to the neutral amino acids (including AIB) of transport system A described by Christensen (8).

Discussion. These studies provide further evidence to show that the distribution *in vivo* of AIB in various tissues of the rat can be modified by dietary treatments. We previously found that AIB distribution may be altered especially in plasma, liver, muscle and heart by prior changes in dietary protein or by feeding a complete meal at the time AIB is administered (5). The present experiments show that force-feeding glycine or an amino acid–peptide mixture also can alter AIB distribution, especially in liver, intestine, and kidney.

It is not clear why force-feeding casein hydrolysate or glycine to rats previously injected with AIB had such disparate effects on distribution of the model amino acid in different tissues. A possible explanation might be related to competition between the dietary amino acids and AIB for transport. Force-feeding casein hydrolysate in particular would expose intestine and liver to the highest initial concentrations of the dietary amino acids. The concomitant large increase in total ninhydrin-positive materials in plasma (Table II) would include elevated levels of small neutral amino acids which are especially inhibitory of AIB transport in a variety of tissues (8). High plasma levels of specific amino acids could interfere with accumulation or redistribution of AIB in the tissues; either influx or efflux could be altered in the presence of competing amino acids. However, the fact that the distribution of AIB was so dissimilar in livers from the normal and low-protein groups suggests that some factor(s) other than increased plasma amino acid content is also important for manifestation of the effects of dietary amino acids on transport in liver. Possibilities include modification by dietary protein of hormonal effects on transport, or of the transport protein(s) itself, or in the capacity of the liver to metabolize amino acids.

Summary. Effects of dietary amino acids on tissue distribution of α-aminoisobutyrate (AIB) and of total ninhydrin-positive material have been examined in rats previously fed adequate or low amounts of protein. Intubation of casein hydrolysate or of glycine in rats receiving adequate protein increased the distribution ratio for AIB (the amount of amino acid in the tissue relative

to that in plasma) in liver, whereas the ratios were decreased in rats receiving a low-protein diet. Ratios after feeding casein hydrolysate were generally decreased in skeletal muscle, heart and brain regardless of prior protein intake; glycine was without effect. Ratios were decreased by both glycine and casein hydrolysate in intestine and kidney. The relative distribution ratio (distribution ratio for ninhydrin-positive material/distribution ratio for AIB) was generally decreased by casein hydrolysate and glycine in all tissues but intestine and kidney. Glucagon altered this ratio only in liver. The results suggest that factors affecting distribution of AIB do not always alter distribution of total ninhydrin-positive material similarly.

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