Dietary amino acid analogs alter activities of some amino acid-metabolizing enzymes in rat liver

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Dietary amino acid analogs often strongly and selectively modify rat tissue amino acid profiles. These effects are accompanied by altered activities of several enzymes of amino acid catabolism. In a preliminary study, activities of hepatic serine dehydratase (SDH), glutamate-pyruvate (GPT), GABA and tyrosine (TAT) aminotransferases, and ornithine carbamoyltransferase (OCT) were high in rats fed diets containing a mixture (3%) of analogs (norleucine, norvaline, α-aminophenylacetate, and α-aminooctanoate); pyruvate kinase (PK) was low. Raising the protein content of the diet often lessened the effect. Analog effects on SDH, GPT, and PK occurred within 2 days, but not within 4 hr. After adaptation to a 50% protein diet that raised SDH and GPT and lowered PK, rats were fed a 6% protein diet with or without the analogs; the normal decline in initially high SDH and GPT was slowed within 4 days in rats fed the analogs, whereas the normal increase in PK induced by a low protein diet was strongly blocked by day 1. In rats fed a 6% amino acid diet, dietary norleucine stimulated branched-chain ketoacid dehydrogenase (BCKAD) activity about 290%, had lesser effects on SDH, GPT, and OCT, and did not alter PK, TAT, or branched-chain aminotransferase; norvaline stimulated only BCKAD (85%). In rats fed an 8% amino acid mixture limiting in leucine, SDH activity was stimulated (up to 800%), depending on norleucine level in the diet, and was lessened by added dietary leucine; similar, but less striking patterns occurred for GPT. If all indispensable amino acids were fed in adequate amounts, SDH and GPT were not stimulated by norleucine. Overall, analog effects were usually more prominent in rats fed suboptimal diets.

Keywords: amino acid analog; diet; enzyme activity; liver; norleucine; norvaline

Introduction

Many enzymes involved in amino acid metabolism are known to be stimulated by various dietary treatments, especially by raising protein content of the diet.[1–4] More recently, we observed a modest increase of at least 100% in activity of hepatic 4-aminobutyrate-2-oxoglutarate aminotransferase (GABA-T) after rats were adapted to a diet containing 60% casein.[5] Incidental to later studies on effects of dietary amino acid analogs,[6] we noted an even larger increase in activity of this enzyme if the diet also contained a mixture of amino acid analogs. A subsequent preliminary experiment (described below) demonstrated that this stimulatory effect of amino acid analogs also occurred for certain other amino acid-metabolizing enzymes.

We have now performed a more detailed survey to investigate further these findings. The studies have also been expanded to include analyses of pyruvate kinase (PK), an enzyme known to be repressed by feeding a high protein diet.[4] The results provide further information concerning the effects in the intact rat of several analogs of the neutral amino acids.

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Materials and methods

In these studies enzyme analyses were generally performed on liver samples obtained in experiments primarily designed to observe the effects of various dietary amino acid analogs on feeding behavior, growth, and tissue amino acid

profiles.[6-9]* Most analyses utilized frozen samples maintained either in liquid nitrogen or in a −70°C freezer; except for a preliminary study, PK and branched-chain ketoacid dehydrogenase (BCKAD) activities were determined in fresh tissue.

Animal treatment

Young male rats of the Sprague-Dawley strain were used in these studies (King Animal Laboratories, Oregon, WI or Harlan Sprague-Dawley, Madison, WI USA). Protocols were approved by the Research Animal Resources Center of the University of Wisconsin. Initial body weights generally ranged from 60–80 g. The animals were housed individually in cages with wire-mesh bottoms in an animal room maintained at about 24°C and lighted from 0700 to 1900 hr. They received protein- or amino acid-based diets without or with amino acid analogs, added singly or as mixtures.

In the initial experiment, enzyme assays were done on pooled samples to determine if the modest increases previously found to be induced by analogs in GABA-T activity† were unique to this enzyme, or if other amino acid-metabolizing enzymes were also affected. Rats were fed diets containing 6, 15, 25, or 50% protein from lactalbumin ad libitum; the diets also contained corn oil, 5 g; mineral mixture,[10] 5 g; vitamin mixture,[10] 0.5 g; choline chloride, 0.2 g; and equal amounts of cornstarch and glucose monohydrate to make a total of 100 g. After 7 days each group was divided; half of the rats continued to receive the above diets and the other half received these diets containing 3% of an equimolar mixture of four amino acid analogs (l-norvaline, l-norleucine, dl-α-aminophenylacetate and dl-α-aminooctanoate). After 10 days the rats were decapitated and samples were taken and frozen for later analysis.[6]

Experiment 2 was performed to determine the effect of time on enzyme responses to the dietary analogs. Rats were adapted to a diet containing 15% protein from lactalbumin; half of the animals were then switched to this diet containing 3% of an equimolar mixture of analogs. Other ingredients were included as described in Experiment 1. Liver samples were taken on days 0 (at end of adaptation period), 1, 2, 4, and 7. Fresh liver preparations were used for analysis of PK, branched-chain aminotransferase (BCAA-T), and BCKAD activities; other assays were performed later on frozen samples.

Experiment 3 was designed to test the effect of analogs on restoration of enzyme activity to control levels following changes in activity resulting from adaptation to a high protein diet. Rats were first adapted for 4 days to a lactalbumin diet containing 15% protein before receiving a lactalbumin diet containing 50% protein for 10 days. The animals then received either a 6% protein (lactalbumin) diet or this low protein diet containing 3% of the amino acid analog mixture described in experiment 1. After 9 days, the analog-supplemented diet was removed and the remaining rats received only the 6% protein diet. Samples for enzyme analysis were taken after the adaptation to the 15 and 50% protein diet (day 0), on days 1, 2, 3, 4, 7, and 9 of feeding the analog-supplemented diet, and on day 2 after analogs were removed from the low protein diet (day 11 after first administering the analogs).

Experiment 4 involved analyses of livers taken from rats fed diets based on a complete amino acid mixture without or with the addition of the individual branched-chain amino acid isomers norvaline and norleucine for 10 days. Rats received a valine-limiting, 1-amino acid diet without and with additions of the analogs and extra valine.* The control diet contained the indispensable amino acids (IAA) at 75% of their requirements for growth of the rat,[11] except valine at 50% of its requirement, with additional dispensable amino acids to make a total of 6% of the diet. Dietary amino acid concentrations were: arginine·HCl, 0.45%; histidine, 0.23%; isoleucine, 0.38%; leucine, 0.56%; lysine·HCl, 0.53%; methionine, 0.45%; phenylalanine, 0.60%; threonine, 0.38%; tryptophan, 0.11%; valine, 0.30%; glutamic acid, 0.96%; aspartic acid, alanine, serine, tyrosine, proline and cystine, 0.096% each; glycine, 0.64%; and asparagine, 0.16%. Sodium acetate, in amounts equimolar to the added amino acid hydrochlorides, was also included. Certain diets contained an additional 0.60% valine, thereby raising its level to 150% of its requirement for growth. Norvaline (1% of the diet) and equimolar norleucine (1.12%) were added to the diets as desired. Remaining diet ingredients were added as described for experiment 1.

Experiment 5 involved a series of studies in which rats were fed for 10 days amino acid diets containing leucine in amounts limiting for growth. Control diets contained leucine at 65% of its requirement; the other IAA were present at either 75% (experiment 5A) or 125% (experiment 5B) of their requirements for growth.[9] Dispensable amino acids were included so that total 1-amino acid content of the diet was 8%. Experimental diets contained varying amounts of norleucine and extra leucine (up to 150% of its requirement) to obtain information concerning dose responses to these amino acids. Other diet ingredients were added as in experiment 1.

Enzyme assays

Measurements of enzyme activity were carried out with standard published procedures. These included assays for glutamate-pyruvate aminotransferase[12] (GPT, E.C. 2.6.1.15); serine dehydratase[13] (SDH, E.C. 4.2.1.13); PK[14] (E.C. 2.7.1.40); tyrosine aminotransferase[15] (TAT, E.C. 2.6.1.5); ornithine carbamoyltransferase[16] (OCT, E.C. 2.1.3.3); GABA-T[5,17] (E.C. 2.6.1.19); total (fully activated) BCKAD[18] (E.C. 1.2.4.4); and BCAA-T[18] (E.C. 2.6.1.42).

Assays of GABA-T, BCKAD, and BCAA-T were performed on whole homogenates; other enzymes were analyzed in high speed supernates. Preliminary tests showed that assays were not affected by presence of the analogs. Protein concentrations in liver homogenates were determined by the method of Lowry et al.[19] with bovine serum albumin as the standard.

Enzyme activities are expressed as μmol product formed/min·g liver protein. Statistical analysis was performed by analysis of variance (ANOVA); where significant differences occurred the least significant difference test was employed to determine differences among groups ($P < 0.05$). Most data underwent log transformation to lesson heterogeneity of variance.[20]

Results

Experiment 1

This preliminary study with pooled samples suggested that inclusion in the diet of a mixture of amino acid

*Tews, J. K. (1984). Food intake, growth and tissue valine content of rats fed a valine-limiting diet containing norleucine or norvaline. *Fed. Proc.* **43,** 300 (abs)

†Unpublished results, J. K. Tews and J. J. Repa

analogs (norvaline, norleucine, α-aminophenylacetate, and α-aminooctanoate) resulted in altered activities of several hepatic enzymes *(Table 1)*. The extent of these effects also appeared to depend on the protein content of the diet, a factor long known to modify activities of numerous hepatic enzymes.[1–5]

Consistent with earlier reports, we found *(Table 1)* that feeding a 50% protein diet induced only modest increases of about 40 and 110% in mean activities of GABA-T and OCT, respectively, whereas effects on GPT and TAT were somewhat more pronounced (increases of about 145 and 290%). The most striking changes occurred with SDH; its mean activity was raised by more than 170 fold. Conversely, mean activity of PK in livers of rats fed the 50% protein diet was only 17% of that of the 6% protein control group. Differences in responses to graded dietary protein content were most apparent for SDH and PK.

Addition of the analogs to the various diets raised mean values for GABA-T activity 30–80% above those of the corresponding control groups *(Table 1)*. SDH activity was raised by about 1400% when the analogs were added to the 6% protein diet, but by less than 30% when the diets also contained 50% protein. Analog-associated increases over mean control values for GPT, TAT, and OCT were about 170, 60, and 75%, respectively, in livers of rats fed the 6% protein diet, and about 30, 95, and 40%, respectively, in rats receiving the 50% protein diet. In contrast, mean hepatic PK activities in rats fed the analogs decreased to 40 or 80% of the corresponding control values of rats fed the 6 or 50% protein diet. In this experiment, values for PK serve only as a guide; measurements were made on frozen livers, a treatment that tends to lower PK activity (observed during initial tests of the assay procedure).

In an extension of this study, rats were trained to consume daily 3-hour meals of either the 6 or 50% protein diet.[6] After 8–10 days, each of these groups was divided into four groups (four rats each); they were then fed a single meal of the 6 or 50% protein diet, each without or with an added 3% of the analog mixture. Liver samples were taken at zero time (the time of the accustomed meal) and 4 hr after the meal was first fed. Activities of the enzymes of *Table 1* were not altered within 4 hr after feeding a single meal of the analog-containing diets (results not shown); PK activity was not measured.

Experiment 2

This study showed that altered SDH, GPT, and PK activities associated with feeding the analog mixture in a 15% protein diet generally became apparent only after 2 days of exposure to the diet *(Figure 1)*. SDH activity in control livers was maintained at generally similar levels throughout the 7-day study *(Figure 1A)*, whereas activity was significantly higher for the analog-treated groups than for the control rats starting on day 2 after the diets were first fed. Although mean values for the treated rats gradually increased with time, absolute values for days 2, 4, and 7 of analog treatment did not differ statistically.

GPT activities for the control groups were essentially constant during the 7-day feeding period *(Figure 1B)*. Enzyme activity in the analog-treated groups was only moderately stimulated by 60–65% during the experimental period.

PK activities were consistently higher for the control than for the analog groups on all but day 1 of the treatment *(Figure 1C)*. Values for PK on days 4 and 7 were not lower than that on day 2; in each case PK activity was about 45–50% of the corresponding control value.

No significant differences between the control and treatment groups were observed for BCKAD, TAT, or BCAA-T (results not shown). OCT activity tended to be high in the analog-treated group after day 2, but because of variability within groups, differences were significant ($P < 0.05$) only on day 7 (410 ± 36 versus the control value of 267 ± 15 μmol/min·g liver protein; other results not shown).

Table 1 Hepatic enzyme activities after adaptation of rats to diets containing different levels of protein without and with added amino acid analogs

Treatment	Enzyme activity (μmol product/min·g liver protein)					
	GABA-T	SDH	GPT	TAT	OCT	PK
6% protein	6.3	0.57	84.8	3.4	427	266
6% protein + 3% AAA	8.3	8.3	230	5.5	751	108
15% protein	6.0	4.8	83.7	3.4	—	181
15% protein + 3% AAA	9.8	35.6	159	7.2	—	102
25% protein	6.6	39.2	102	5.2	—	122
25% protein + 3% AAA	8.5	73.6	191	8.4	—	65.8
50% protein	8.9	96.8	209	13.1	891	46.4
50% protein + 3% AAA	15.7	123	272	25.5	1240	37.2

Diets contained lactalbumin as the protein source; AAA, equimolar mixture of amino acid analogs (norleucine, norvaline, α-aminophenylacetate, and α-aminooctanoate). Diets were fed for 10 days. Assays were done on pooled samples (n = 4 livers per sample). Omitted values for OCT were unreliable.

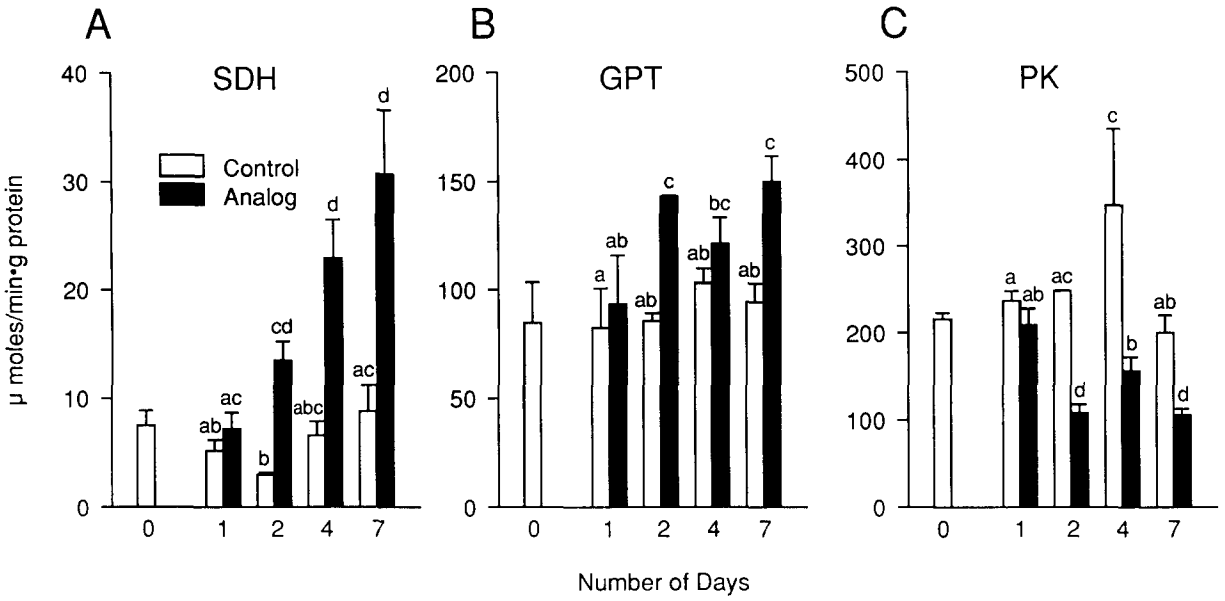

Figure 1 Activities of hepatic SDH, GPT, and PK with time after feeding diets containing 15% protein from lactalbumin without or with added amino acid analogs. The analog mixture (equimolar in norvaline, norleucine, α-aminophenylacetate, and α-aminooctanoate) was included at 3% of the diet. Different letters above bars indicate significant differences among treatments (ANOVA followed by the least significant difference test).

Experiment 3

This study demonstrated that when rats were fed a 6% protein diet after their adaptation to a 50% protein diet, the inclusion of analogs in the low protein diet clearly altered the extent to which adapted enzymes (SDH, GPT, and PK) reverted to levels observed before the high protein diet was first fed *(Figure 2)*.

SDH activity increased approximately 1750% 10 days after rats were switched from a 15% to a 50% protein diet *(Figure 2A)*. During the first 3 days after the protein-adapted rats were switched to a 6% protein diet without or with 3% of the analog mixture, declines in SDH activity from the high, protein-adapted value were similar, regardless of analog presence in the diet. However, by day 4, enzyme activities in the analog-supplemented rats were at least twice those in the animals receiving the 6% protein diet alone. On day 7 values for the analog group remained at levels observed on day 4, whereas SDH activity in the control group had declined further. By day 9 of feeding the 6% protein diet, control activity was 5% of that in the analog group. Two days after removal of analogs from the 6% protein diet (day 11), SDH activity in this group was half that found on day 9 of treatment with the analogs. Because no analog-supplemented rats were available for testing on day 11, it is not certain that the further decline in enzyme activity on day 11 was due solely to removal of the analogs from the diet.

Patterns of responses of hepatic GPT activity to the dietary sequence were often similar to those for SDH, but the effects were less striking *(Figure 2B)*. Switching the rats from a 15 to 50% protein diet raised GPT activity 250%. There was a statistically insignificant decline in activity on the first day after the rats were

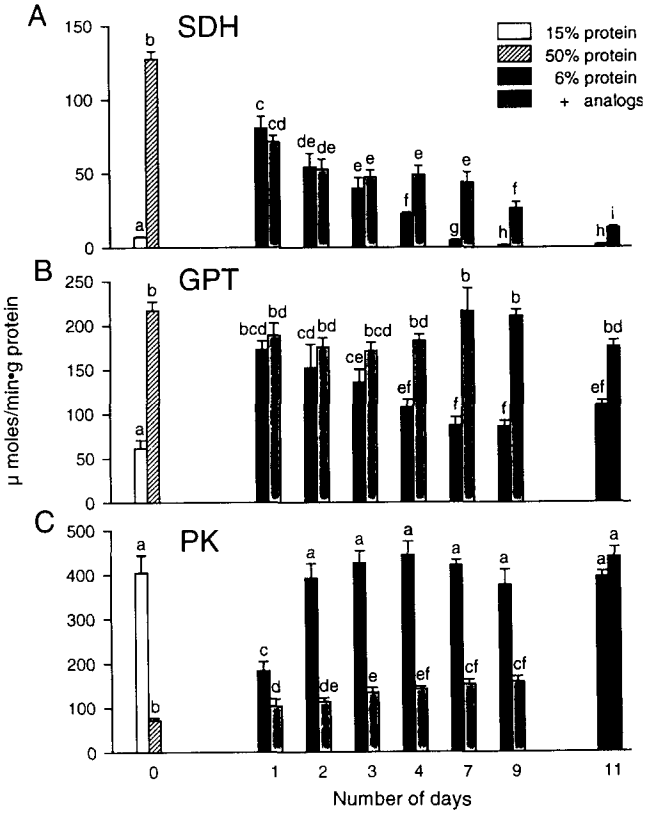

Figure 2 Activities of hepatic SDH, GPT, and PK after adaptation of rats to a diet containing 50% protein from lactalbumin, and subsequent change in activities with time after consumption of a 6% protein diet without or with added amino acid analogs (equimolar mixture of norvaline, norleucine, α-aminophenylacetate, and α-aminooctanoate). Different letters above bars indicate significant differences among treatments (ANOVA followed by the least significant difference test). Right half of bars on day 11 shows values 2 days after analogs were removed from the 6% protein diet.

fed the 6% protein diet, regardless of the presence of the amino acid analogs in the diet. For rats fed the 6% protein diet, mean enzyme activity continued to decline gradually so that, from days 2–9, values were significantly lower than that observed after adaptation to the 50% protein diet; however, absolute values remained essentially constant from days 4–11. Inclusion of the analogs in the low protein diet appeared to prevent the decline in GPT activity seen in the control groups, as observed values never differed statistically from that obtained after adaptation to the high protein diet. Beginning with day 4, activities in the analog-treated rats were consistently higher than in rats receiving the 6% protein diet. Two days after removal of the analogs from the diet (day 11), enzyme activity remained similar to that found for the analog-treated groups on days 1–9.

Hepatic PK activity of rats adapted to the 50% protein diet was less than 20% of activity in rats fed the 15% protein diet *(Figure 2C)*. Refeeding a 6% protein diet for 1 day resulted in a 160% increase in PK activity. By day 2, activity had increased to the level seen in rats fed the 15% protein diet; further change did not occur during the remainder of the experimental period. Inclusion of the analogs in the low protein diet was highly effective in interfering with this restoration of enzyme activity; by day 1, activity in the analog-treated rats was significantly higher than after feeding the high protein diet, but was only 60% of the increased value observed in the group switched to the 6% protein diet alone. Small but significant increases in activities of the analog groups occurred during days 2–9, ranging from 29–42% of the mean values for the corresponding control, 6% protein groups. However, within 2 days after removal of the analogs from the diet, enzyme activity increased to that observed on days 2–9 for rats fed only the low protein diet.

These results could have been compromised by the fact that the analogs depress food intake of young rats fed a low protein diet.[6] Therefore, a control experiment was also performed in which rats previously adapted to the 50% protein diet then consumed the control, 6% protein diet either ad libitum or in amounts restricted to those consumed by rats fed this diet containing the analogs. The results (not shown) indicated that SDH and GPT activities were not affected by restricted food intake over a 7-day period. In two separate experiments, PK activity was little affected on days 1, 7, or 9 of food restriction, but did tend to be low on days 2 and 4. Values ranged from 93% of the ad libitum control on day 1, to 85%, 73%, and 98% on days 2, 4, and 7, respectively. These depressed enzyme levels were still well above those obtained for the rats receiving the 6% protein diet containing the analogs as shown in *Figure 2C:* values were 57% of the analog-free control on day 1, and 29%, 32%, and 36% on days 2, 4, and 7, respectively. Thus, the analogs were clearly effective in reducing the restoration of PK activity in rats switched from a high to a low protein diet, despite the accompanying more minor effects of reduced food intake alone.

Experiment 4

This study showed that enzyme activities were also affected by adding individual analogs to a valine-limiting diet containing 6% amino acids rather than intact protein. After 10 days of dietary treatment, norvaline, an isomer of valine, stimulated total BCKAD activity and OCT activities by 85 and 35%, respectively (treatment C, *Table 2*). There were no significant effects on SDH, GPT *(Table 2)*, PK, TAT, or BCAA-T (results not shown).

Norleucine, an isomer of leucine and isoleucine, significantly raised activity of SDH, GPT, OCT, and BCKAD (treatment E, *Table 2*), but had no effect on PK, TAT, or BCAA-T (not shown). BCKAD activity increased almost 300% when rats received diets containing norleucine or norleucine plus norvaline (treatment G, *Table 2*). Corresponding increases in GPT activity were about 80%, and SDH increased about 100–400%. Increases in OCT were about 50–60%. Raising the valine content of the diet (treatments B, D, F, and H) did not significantly alter responses of the enzymes to the analogs.

Table 2 Hepatic enzyme activities after adaptation of rats to a 6% amino acid diet without or with added norvaline or norleucine

Treatment	Enzyme activity (μmol product/min·g liver protein)			
	BCKAD	SDH	GPT	OCT
A Control	1.61 ± 0.37^{a}	0.20 ± 0.04^{a}	53.5 ± 10.2^{a}	181 ± 35^{a}
B A + 0.6% Val	1.61 ± 0.34^{a}	$0.25 \pm 0.03^{a,b}$	48.1 ± 6.6^{a}	$202 \pm 25^{a,b,c}$
C A + 1% Norval	2.97 ± 0.40^{b}	0.15 ± 0.05^{a}	$75.0 \pm 9.8^{a,b}$	$245 \pm 24^{c,d}$
D C + 0.6% Val	3.09 ± 0.74^{b}	0.20 ± 0.02^{a}	$68.5 \pm 9.2^{a,b}$	$197 \pm 21^{a,b}$
E A + 1.12% Norleu	$6.30 \pm 0.26^{c,d}$	0.41 ± 0.05^{b}	$95.7 \pm 12.2^{b,c}$	$272 \pm 27^{c,d}$
F E + 0.6% Val	$4.26 \pm 0.58^{b,c}$	0.47 ± 0.10^{b}	85.6 ± 12.9^{b}	$257 \pm 24^{b,c}$
G A + Norval + Norleu	$5.86 \pm 0.66^{c,d}$	1.02 ± 0.33^{c}	$97.5 \pm 13.0^{b,c}$	292 ± 26^{d}
H G + 0.6% Val	9.21 ± 1.77^{d}	1.09 ± 0.34^{c}	122 ± 4.0^{c}	292 ± 20^{d}
$P <$	0.0001	0.0014	0.0001	0.0133

IAA, except valine, were fed at 65% of their requirements for growth; valine was included in the diets at either 50% or 150% of its requirement. Diets were fed for 10 days; $n = 6$, except three assays for BCKAD of two pooled samples each. Different superscripts indicate significant differences among treatments (ANOVA, followed by the least significant difference test).

Experiment 5

The ability of dietary norleucine to stimulate SDH or GPT activity was clearly affected by the proportions of amino acids in the diets *(Table 3)*. For example, in experiment 5A, in which the diets contained leucine at 65% of its requirement and all other IAA at 75% of their requirements for maximal growth of the rat, SDH activity increased 150, 370, and 800% when norleucine was also included at 0.2, 0.5, and 1.12% of the diet, respectively (treatments C, E and G versus treatment A). Conversely, when only leucine was limiting for growth (experiment 5B), norleucine at 0.2% of the diet was ineffective, and even at 1.12% of the diet, stimulated SDH activity by less than 200%. When leucine was increased in experiment 5B to 150% of its requirement (so that all IAA were present in amounts more than adequate for growth), dietary norleucine no longer stimulated SDH activity at any of the tested levels (treatments D, F, and H). However, when the other IAA were fed at only 75% of their requirement (experiment 5A), additional dietary leucine prevented a statistically significant increase in SDH activity only when the lower levels of norleucine were included in the diet; leucine only partially prevented the stimulation when the diet also contained 1.12% norleucine (experiment 5A, treatments G and H).

Induction of hepatic GPT activity also depended on the dietary level of norleucine, although effects were less striking than with SDH. GPT activity significantly increased by 30, 80 and 120% when the leucine-limiting diet contained 0.2, 0.5, and 1.12% norleucine, respectively, with other IAA at 75% of their requirements *(Table 3,* experiment 5A). Again, additional dietary leucine prevented this stimulation in all but the group receiving 1.12% norleucine. When IAA were included in the leucine-limiting diet at 125% of their requirement, stimulation of GPT activity by norleucine again depended on the dietary level of the analog (experiment 5B); the extent of stimulation tended to be slightly less than that observed with IAA at 75% of requirements. The effects were prevented by feeding extra leucine.

A further study[9] demonstrated that when rats consumed diets containing the IAA at 125% of their requirements, addition of only 0.26% leucine (to 100% of its requirement) was sufficient to completely prevent the increase in SDH and GPT activities induced by adding 1.12% norleucine to the diet (as in *Table 3,* experiment 5B, treatment G).

Livers of rats consuming norleucine, but not norvaline, tended to be small and to have higher protein concentrations than did livers of rats eating the control diets ($P < 0.0001$, based on ANOVA for eight treatment groups each for experiments 5A or 5B). For example, in experiment 5A livers of rats fed the control diet (treatment A) weighed 4.87 ± 0.15 g, whereas livers of rats fed the diet containing 1.12% norleucine (treatment G) weighed 3.95 ± 0.14 g. A similar pattern was observed in experiment 5B: 4.26 ± 0.21 g (treatment A) versus 3.05 ± 0.17 g (treatment G). Corresponding liver protein concentrations in experiment 5A were 16.0 ± 0.3% (treatment A) and 18.5 ± 0.4% (treatment G); values for experiment 5B were 14.1 ± 0.2% (treatment A) and 16.7 ± 0.6% (treatment G). Inclusion of extra leucine in the norleucine-supplemented diets frequently raised liver weights to those observed for the norleucine-free groups B in experiments 5A and 5B. Liver protein concentrations tended to be low in rats fed norleucine plus extra leucine, although not always as low as in treatment groups B.

Discussion

Hepatic enzymes involved in amino acid catabolism have long been known to be affected by dietary conditions. In particular, activities of a number of enzymes can be stimulated to varying degrees during adaptation of rats to diets containing high levels of protein.[1-4,21] In contrast, activities of certain enzymes associated with carbohydrate metabolism are decreased after adaptation to a high protein diet.[4,22] The results of the present survey are consistent with these early observations, as we found increases in activities of GABA-T, SDH, GPT, TAT, and OCT, whereas PK activity

Table 3 Effects of norleucine on hepatic SDH or GPT in rats fed amino acid mixtures containing different proportions of amino acids

	Enzyme activities (μmol product/min·g liver protein)			
	Experiment 5A		Experiment 5B	
Treatment	SDH	GPT	SDH	GPT
A Control	0.57 ± 0.03[a]	51.5 ± 4.0[a]	0.49 ± 0.04[a]	85.2 ± 9.4[a]
B A + 0.64% Leu	0.79 ± 0.06[a]	60.9 ± 3.7[a,b]	0.51 ± 0.04[a]	87.3 ± 14.0[a]
C A + 0.2% Norleu	1.42 ± 0.37[b]	66.1 ± 3.9[b]	0.61 ± 0.07[a]	92.0 ± 13.5[a]
D C + 0.64% Leu	0.87 ± 0.18[a,b]	63.5 ± 2.8[b]	0.51 ± 0.07[a]	97.4 ± 15.3[a]
E A + 0.5% Norleu	2.68 ± 0.57[c]	92.9 ± 4.4[c]	1.05 ± 0.27[b,c]	130 ± 9.9[b,c]
F A + 0.64% Leu	1.05 ± 0.29[a,b]	68.9 ± 3.2[b]	0.47 ± 0.05[a]	91.3 ± 7.2[a]
G A + 1.12% Norleu	5.15 ± 0.85[d]	112 ± 4.5[d]	1.38 ± 0.43[c]	151 ± 10.2[c]
H G + 0.64% Leu	2.25 ± 0.38[c]	83.2 ± 3.7[c]	0.63 ± 0.06[a,b]	101 ± 3.3[a,b]
$P <$	0.0001	0.0001	0.0001	0.0001

Experiment 5A: The control diet contained leucine at 65% of its requirement (treatment A); added leucine raised its level to 150% of requirement (treatment B). Other IAA were included at 75% of requirements (control diet 1, experiement 1).[9] Experiment 5B: As in Experiment 5A, except other IAA were present at 125% of requirements (control diet 2, experiment 4).[9] Diets were fed for 10 days; $n = 6$. Different superscripts indicate significant differences among treatments (ANOVA, followed by the least significant difference test).

was low in livers of young rats adapted to a 50% protein diet.

Similarly, administration of various mixtures of natural amino acids can stimulate enzyme activity.[22–25] Individual amino acids have also been found to raise activity of various enzymes. For example, intubation of tryptophan alone moderately induces threonine dehydratase (presumably equivalent to SDH)[23]; feeding glycine, tyrosine, or the BCAA raises TAT activity,[26] and dietary glycine or glycine plus methionine or arginine raises SDH and GPT activities.[27] BCKAD activity is increased by feeding diets containing a variety of amino acids.[18,21] Conversely, phosphoglycerate dehydrogenase activity is low in rats fed complete amino acid mixtures.[22]

More pertinent to the present work are studies showing the effects of amino acid analogs on activities of various enzymes other than those we have studied. However, most observations have been made in vitro with little information available concerning treatments in vivo. For example, the nonmetabolizable analog β-2-aminobicyclo[2,2,2]heptane-2-carboxylic acid (BCH) is well known as an insulin secretion stimulus. This effect apparently is dependent on allosteric activation by BCH (or norvaline, but not norleucine) of pancreatic islet glutamate dehydrogenase.[28] Similar effects on bovine liver glutamate dehydrogenase have also been reported.[29] BCH also activates hepatic mitochondrial glutaminase.[30] Hepatic phenylalanine hydroxylase can be activated by a variety of factors including its substrate, phenylalanine, and less so by norleucine and methionine.[31]

Our results provide new information concerning effects of dietary amino acid analogs on hepatic enzyme activities in vivo. Thus, feeding a mixture of norvaline, norleucine, α-aminophenylacetate, and α-aminooctanoate or norleucine alone often increased activities of SDH, GPT, GABA-T, TAT, and OCT; PK activity was low when rats were fed the mixture. BCKAD activity generally increased in rats fed norvaline or norleucine. These results were often similar to those observed after feeding a high protein diet, except that the analog effects were sometimes less striking. Chideckel and Edwards[32] reported that hepatic and renal activities of ornithine decarboxylase were high after starved rats were injected i.p. with the nonmetabolizable analogs, α-aminoisobutyric acid or cycloleucine.

Our survey does not identify the means whereby analogs affect enzyme activity; however, observations by other workers suggest various possibilities. Intubation of amino acid mixtures can increase the rate of synthesis of enzyme protein,[33] although it seems unlikely that analogs would induce such an effect. Studies in vitro have shown that amino acids may also suppress protein degradation.[34,35] Leucine antagonists such as the analogs norleucine, norvaline, d-norleucine, and 1-allo-isoleucine were as effective as leucine in preventing protein degradation in hepatocytes.[35] Other analogs also interfere with hepatic protein degradation.[36] These observations suggest that the analog-induced increases in activities we observed for several enzymes could be related to decreases in the normal degradation or turnover rates of the enzyme proteins. In particular, this conclusion is supported by the finding that after SDH and GPT activities were raised by adaptation of rats to a high protein diet, restoration to low, control levels upon feeding a low protein diet was slowed if analogs were also consumed.

The incorporation of analogs into various proteins can have subsequent effects on post-translational modifications and protein processing.[37,38] Incorporation of amino acid analogs into various proteins may increase[39,40] or reduce[39,41] subsequent degradation rates, depending on the incorporated analog or the protein in question.

Enzyme activity may also be affected by analogs because of activation via binding to a regulatory site (allosteric activation), by altering mitochondrial organization, or by affecting the phosphorylated state of the enzyme.[18,21,28,30,31,42] Our observations in a limited study suggest that dietary norvaline or norleucine may increase the amount of active (dephosphorylated) BCKAD complex present in livers of these animals; in rats fed a diet containing 12% of an amino acid mixture without or with added norvaline (1% of diet) or norleucine (1.12%) for 10 days, percentage active complex in livers of control, norvaline-, or norleucine-treated rats was 46 ± 2%, 62 ± 8%, or 74 ± 5%, respectively (n = 2 or 3). A greater degree of activation also accompanies consumption of a diet adequate in protein content.[18,21,43]

A more remote possibility is suggested by studies in which a norleucine,leucine:2-oxoglutarate aminotransferase was induced in *Candida* grown on l-norleucine and l-lysine as the only sources of nitrogen.[44] As norleucine is known to be metabolized by the rat,[6] dietary norleucine might induce an increase in the normal rate of its subsequent degradation.

These studies show that under conditions known to affect food intake, growth and tissue amino acid profiles of rats,[6–9*] consumption of various amino acid analogs also was accompanied by clear changes in activities of certain hepatic enzymes. Of those measured, the strongest effects were on SDH and PK. In fact, serine and threonine concentrations† were 40% of control values in livers of norleucine-fed rats in which SDH activity increased 800% (*Table 3*, experiment 5A, treatment G). Analog effects on enzymes were often most evident when rats received diets that were limiting in a single IAA, or were inadequate in protein content, or both. Replenishing the individual IAA (e.g., leucine) or feeding a high protein diet frequently lessened or eliminated the effects of the analog(s). It is not clear why analog effects often seemed more prominent in rats fed suboptimal diets.

*Tews, J. K. (1984). Food intake, growth and tissue valine content of rats fed a valine-limiting diet containing norleucine or norvaline. *Fed. Proc.* **43,** 300 (abs)

†Unpublished results, J. K. Tews and J. J. Repa

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