UTILIZATION OF *N*-ACETYL-L-TRYPTOPHAN GIVEN INTRAVENOUSLY TO UNRESTRAINED ADULT RATS

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Summary The nutritional value of *N*-acetyl-L-tryptophan (NALT) given intravenously was examined in unrestrained adult rats. They were given solutions of 4% Vuj pattern amino acids, 24.5% glucose, vitamins and electrolytes intravenously for 10 days. They were divided into three groups; one group was then injected intravenously with complete amino acid solution (T group), one with tryptophan-free amino acid solution (TF group) and one group with complete amino acid solution but with NALT instead of tryptophan (NALT).

The biological value of the NALT group was 49.6; this value corresponded to 90% of that of the T group. Excretion of injected amino acids totalled about 2.0 to 3.0% in this experiment. The effects of NALT on carcass composition, certain blood constituents and tryptophan pyrrolase activity in the liver were also examined.

Keywords *N*-acetyl-L-tryptophan, tryptophan, intravenous nutrition, unrestrained IVH, nitrogen balance

Recently, the clinical value of injected amino acids as nutrients before and after surgery has become widely recognized. At the same time, it has been demonstrated that the utilization of ingested amino acids greatly depends on the amount of energy simultaneously supplied (1,2). Some investigators have suggested that for intravenous injection glucose is one of the best energy sources (2,3). Accordingly, it seems necessary to examine the effect of injections of nutritionally suitable ratios of amino acids, energy sources and other nutrients. Amino acid solutions are usually mixed with sugar solution just before use, although the mixing procedure is troublesome and may introduced bacterial infection. Mixing immediately prior to use is necessary because on standing a mixture of amino acids and glucose the maillard reaction may occur due to combination of the α -amino groups of amino acids with the carbonyl groups of

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glucose, and amadori compounds may be formed as unavailable forms of amino acids; moreover the solutions may become brown (4-6). Therefore, we attempted to find suitable derivatives of amino acids that would not form unavailable compounds, but that could be utilized in the body after injection. Preliminary studies on mixtures of amino acids and sugar showed that tryptophan is one of the main amino acids undergoing the reactions described above. Berg *et al.* (7) demonstrated that the N-substituted derivative of tryptophan, N-acetyl-L-tryptophan (NALT), was utilized as well as tryptophan (Trp) when given orally. A similar conclusion was reached by du Vigneaud *et al.* (8) and Ichihara and Goto (9), after subcutaneously injecting NALT into rats. Boggs *et al.* (10) recently demonstrated the nutritional values of oral N-acetyl-L-methionine. On the other hand, some investigators have suggested that N-acetyl-L-amino acids may be deacetylated not only by non-specific deacetylase in organs such as kidneys (11) but also by enzymes of gastrointestinal bacteria (12). If deacetylation is mainly due to intestinal bacteria, injected NALT should have lower nutritional value at least than oral NALT.

Therefore, in this study we examined the nutritional values of NALT when given to adult rats intravenously, using different indexes.

EXPERIMENTAL

Male Wistar strain rats weighing 300 g were given a complete diet (15% amino acid diet; Miyazaki pattern) until they weighed 330 g, and were then divided into three groups of six or seven rats each. They were given solutions of 4% Vuj pattern¹ amino acids, 24.5% glucose, vitamins and electrolytes intravenously for 10 days. For the operational procedure, a slight modification of the method of Steiger *et al.* (13) was used so that the experiments could be carried out under unrestrained conditions. With all other nutrients, one group was injected intravenously with complete amino acid solution (T group) one with tryptophan-free amino acid solution (TF group) and one group with complete amino acid solution but with NALT instead of tryptophan (NALT group). The effect of injecting all nutrients intravenously on the nutritional value of NALT was examined. The infusion rate was 2.2 ml/hr (160 ml/kg of body weight, 52.8 ml/day), and the animals were given 55.4 kcal of energy per day (as glucose plus amino acids), 2.11 g of Vuj pattern amino acid mixture and adequate vitamins and electrolytes. The details for experimental design and nutrient solutions are indicated in Fig. 1.

Analytical assay. The nitrogen balance was examined during the last 3 days of the experiment, and urine was collected in dilute sulfuric acid solution.

At the end of the experiment, the tubing was removed under ether anesthesia and the final body weight was measured. The animals were bled by heart puncture, and the hematocrit and the hemoglobin content were measured by high

¹ For details, see the legend of Fig. 1.

Fig. 1. Experimental design and nutritional instructions. Injection rate: 2.2 ml/hr (160 ml/kg). Complete solution (composition): 24.5% glucose, 4.0% Vuj pattern amino acids (Arg·HCl, 0.80; His·HCl·H₂O, 0.40; Ile, 0.55; Leu, 1.23; Met, 0.71; Phe, 0.87; Tyr, 0.54; Trp, 0.18; Val, 0.61; Lys·HCl, 1.86; Gly, 1.00) and appropriate amounts of vitamins (B₁, B₂, B₆, C, niacin, pantothenate-Ca, A, D₂) and electrolytes (Na⁺, K⁺, Mg²⁺, Cl⁻, HPO₄²⁻, C₃H₅O₃⁻, HSO₃⁻). Energy content: 1.05 kcal/ml (55.4 kcal/day). Amino acid content: 0.04 g/ml (2.105 g/day). Osmolarity: 2,000 mOsm/liter. pH: 7.0 to 6.5. Abbreviations: i.v., intravenous injection; T, tryptophan; TF, tryptophan-free; NALT, N-acetyl-L-tryptophan.

centrifugation and the cyanmethemoglobin method, respectively. The plasma was rapidly separated at 4°C for measurement of protein, tryptophan, urea, and alanine and aspartate transaminase activity. The liver was removed and weighed and its tryptophan pyrrolase activity was determined by the method of Knox *et al.* (14). The nitrogen content of the urine and feces was measured by the Kjeldahl method. From these results, the nitrogen balance and biological value were calculated. Urea, ammonia and indole derivatives in the urine were also measured. Transaminase, urea, and ammonia, tryptophan and indole derivatives were measured by the 2,4-dinitrophenylhydrazine method (15) and the indophenol method (16) and the method of Denckla and Dewey (17) and Fischel (18), respectively.

The water, crude fat and crude protein content of the carcass was measured. For these estimations the carcass was frozen chopped up and dried at 100 to 105°C. Crude fat and protein in the dried carcass were measured by hot ether extraction and Kjeldahl method, respectively.

RESULTS

Weight gain

Results of weight gain and food consumption are shown in Table 1. When all nutrients were given intravenously, the weight gains in the T, TF and NALT groups were 0.90 ± 0.30 , -1.75 ± 1.09 and 1.89 ± 0.44 g/day, respectively. The value for the NALT group was about twice that of the T group, although total food intake was the same as in the T group; this difference was significant.

Table 1. Effect of intravenous injection of *N*-acetyl-L-tryptophan on weight gain and food consumption.

		Body weight				Food intake (g/day)
		Initial (g)	Final (g)	Period (days)	Gain (g/day)	
T	(7) ^a	332.4 ± 2.1 ^b	341.4 ± 4.3	10	0.90 ± 0.30	15.0 ^c
TF	(6)	332.8 ± 3.9	315.0 ± 9.5	10	-1.75 ± 1.09***	15.0
NALT	(7)	330.9 ± 1.6	349.7 ± 4.5	10	1.89 ± 0.44***	15.0

^a Figures in parentheses indicate numbers of rats. ^b Values are means ± SD. ^c Total amounts of Vuj pattern amino acids and glucose given intravenously in the experimental period. *** Significantly different from the value for the T group at $p < 0.001$. For abbreviations of groups, see the text.

Nitrogen balance and biological values

The results are shown in Table 2. The nitrogen balances of groups T, TF and NALT were $+108.6 \pm 37.7$, -46.1 ± 37.8 and $+89.8 \pm 19.8$ mgN/day, respectively. Although growth of the NALT group was significantly higher than that of the T group, N balance and biological value in this group were slightly, but not significantly, lower than in the T group; the biological value was 55.6 in the T group and 49.6 in the NALT group, when NALT was injected with sufficient energy source, and thus the utilization of NALT was 90% of that of tryptophan.

Changes in amino-N and indole derivatives in urine

The results are shown in Table 2. The amounts of indole-N and amino-N

Table 2. Effect of *N*-acetyl-L-tryptophan administration on nitrogen balance, biological value and urinary excretion of amino-N and indole derivatives.

		Nitrogen balance ^a (mgN/day)	Biological value ^a	Urinary excretion			
				amino-N (mgN/day)	(%)	Indole derivatives (mgN/day)	(%)
T	(7) ^b	108.6 ± 37.7 ^c	55.6 ± 12.4	7.3 ± 1.3	(2.4) ^d	0.66 ± 0.37	(11.1) ^d
TF	(6)	−46.1 ± 37.8***	8.3 ± 7.3***	10.3 ± 3.5*	(3.4)	0.74 ± 0.27	
NALT	(7)	89.8 ± 39.8	49.6 ± 13.2	7.4 ± 1.4	(2.4)	0.47 ± 0.16	(7.9)

^a Values were calculated from average values in the last 3 days of the experiment.

^b Figures in parentheses indicate numbers of rats. ^c Values are means ± SD. ^d Figures in parentheses show values as percentages of dose. * *** Significantly different from the value for the T group at $p < 0.05$ and $p < 0.001$, respectively. For abbreviations of group, see the text.

excreted were 0.66 mg and 7.30 mg in the T group, 0.74 mg and 10.3 mg in the TF group and 0.47 mg and 7.4 mgN/day in the NALT group; thus in all groups, only 2 to 3% of the injected amino acids was excreted in the urine. There were no significant differences between groups T and NALT.

Changes in blood constituents

Data on the hemoglobin, the hematocrit, plasma protein, urea, transaminase activity and plasma tryptophan in the groups are shown in Table 3. In the NALT group, only plasma protein and urea N showed significant changes, but values remained within the normal range. In the TF group the values for plasma tryptophan and protein were very low (highly significant, $p < 0.001$) in comparison with those in other two groups.

Table 3. Effect of *N*-acetyl-L-tryptophan administration on blood constituents.

	T (7) ^b	TF (6)	NALT (7)
Hematocrit	44.1 ± 2.8 ^c	44.0 ± 1.1	42.7 ± 2.8
Hemoglobin (g/100 ml)	14.3 ± 1.2	14.5 ± 0.6	13.5 ± 1.0
Plasma protein (g/100 ml)	5.51 ± 0.21	4.53 ± 0.31***	5.83 ± 0.22*
Plasma tryptophan ^a (μg/ml)	26.3 ± 4.7	6.9 ± 2.1***	26.7 ± 6.5
Urea N (mgN/100 ml)	7.3 ± 0.8	12.3 ± 0.8***	6.0 ± 1.1*
Asp transaminase (Karmen units)	92 ± 55	149 ± 7**	84 ± 27
Ala transaminase (Karmen units)	11 ± 4	16 ± 7	15 ± 8

^a Total amounts (free form plus bound form). ^b Figures in parentheses indicate numbers of rats. ^c Values are means ± SD. ***, **, * Significantly different from the value for the T group at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. For abbreviations of groups, see the text.

Induction of tryptophan pyrrolase activity in the liver

The results are shown in Fig. 2. When NALT was injected with a sufficient supply of other nutrients, the activity of this enzyme was induced to 80% of that in the T group; there was no significant difference between values in the T group and NALT group. These results indicate that NALT injected with sufficient amounts of other nutrients was converted to tryptophan in the tissues, because tryptophan pyrrolase was not induced by NALT.

Carcass analysis

The results of carcass analyses are shown in Table 4. Values for body fat were higher, and values for body water lower, in the NALT group and TF group than in the T group; the differences between values in the T and TF groups were statistically significant. The value for body protein was significantly lower in the NALT group than in the T group; this was not due to a decrease in the total amount of body protein but to an increase in body fat.

Fig. 2. Effect of *N*-acetyl-L-tryptophan administration on liver tryptophan pyrrolase activity. Activity is expressed in μ moles kynurenine produced per hr per mg protein. Columns with vertical bars indicate means $\pm$ SD. Figures in parentheses indicate numbers of rats. Animals were injected intravenously with complete amino acid solution (T), tryptophan-free amino acid solution (TF) or complete solution with *N*-acetyl-L-tryptophan (NALT) in place of tryptophan, with adequate energy supply and vitamins and electrolytes. ** Significantly different from the value for the T group at $p < 0.01$.

Table 4. Effect of *N*-acetyl-L-tryptophan administration on carcass composition.

		Water	Crude fat	Crude protein ^a
T	(7) ^b	63.16 $\pm$ 2.08 ^c	13.73 $\pm$ 3.31	21.24 $\pm$ 0.56
TF	(6)	58.72 $\pm$ 1.18**	18.99 $\pm$ 2.20**	20.36 $\pm$ 1.30
NALT	(7)	61.76 $\pm$ 1.24	15.53 $\pm$ 1.75	19.58 $\pm$ 0.24***

^a Kjeldahl N $\times$ 6.25. ^b Figures in parentheses indicate numbers of rats. ^c Values are means $\pm$ SD and are expressed as percentages. **,*** Significantly different from the value for the T group at $p < 0.01$ and $p < 0.001$, respectively. For abbreviations of groups, see the text.

DISCUSSION

When NALT with all nutrients were given intravenously, the weight gain was greater in the NALT group than in the T group, although the nitrogen balance and the biological values in the two groups were similar. The apparent utilization of NALT estimated from the weight gain exceeded the true value estimated from the N balance and biological value. These results suggest that in the NALT group accumulation of body fat and/or water was abnormal. Consistent with result of N balance and biological value, data from carcass analyses showed that the percentage of protein was significantly reduced in the NALT group and that although the amount of body water was less in the NALT group than in the T

group, the amount of body fat was greater. Accordingly, the greater weight gain in the NALT group was mainly due to deposition of body fat. This was not due to a decrease in the total amount of body protein but to an increase in body fat. These observations were supported by the findings in plasma protein and hematocrit.

In preliminary examination in which all nutrients were given orally, we observed almost the same findings as in this study. They indicate that the administration of NALT in place of tryptophan, irrespective of the feeding patterns, results in stimulated deposition of body fat in adult rats. Consequently, the nutritional significance for greater deposition of body fat in the NALT group must be confirmed by further study.

It is well known that tryptophan pyrrolase is induced by dietary tryptophan, but not by NALT (19). However, the ingested NALT is deacetylated by intestinal bacteria (12) and/or by non-specific deacetylase in tissues such as the kidneys (11). Accordingly, NALT injected intravenously should be deacetylated by non-specific deacetylase in organs, even if not in the gastrointestinal tract. In the present study the activity of liver tryptophan pyrrolase in the NALT group was induced to 200% of that in the TF group, but 80% of that in the T group, whereas there was no significant difference between values in the T group and NALT group. On the other hand, when NALT was injected intravenously its biological value was 90% of that of T group and somewhat high deposition of body fat was observed. These findings may suggest that under the present experimental conditions the conversion of NALT to Trp in the tissues was not complete. However, urinary amino-N excretion was 2 to 3% of the amount injected. This value was somewhat higher than the value (1%) reported by Ikeda (2) who gave almost the same concentrations of glucose and amino acids to growing dogs, but they were similar to results on total parenteral nutrition of men obtained by Adachi (20). Moreover, use of NALT in place of tryptophan did not result in abnormalities in blood properties such as those seen in the TF group.

Many years ago, Berg *et al.* (7) reported that when given orally, *N*-acetyl-L-tryptophan could be used as efficiently as L-tryptophan. du Vigneaud *et al.* (8) and Ichihara and Goto (9) reached a similar conclusion from experiments in which NALT was injected subcutaneously into rats in three or four divided doses. Rose *et al.* (21) and Baldwin and Berg (22) confirmed this conclusion in nitrogen balance studies in humans. With this background, we also reached almost the same conclusion with total intravenous nutrition using NALT accompanied by all other nutrients in unrestrained adult rats.

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