Nitrogen retention in rats fed on diets enriched with arginine and glycine

2. Effect of diethyl ether anaesthesia on N retention

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(*Received* 2 *March* 1976 – *Accepted* 20 *May* 1976)

1. Adult rats were subjected to a brief period of diethyl ether anaesthesia and were given diets with 200 or 100 g casein/kg with or without arginine plus glycine supplementation in the post-anaesthesia period. Nitrogen retention was measured as well as liver protein content and liver and muscle transaminase activities (L-aspartate aminotransferase (GOT), (*EC* 2.6.1.1), and L-alanine aminotransferase (GPT) (*EC* 2.6.1.2)).

2. Results demonstrated that anaesthesia-stressed rats consuming the high-protein diet with supplemental arginine and glycine retained twice as much N as did rats given the diet with 200 g casein/kg alone, for the first 5 d post-anaesthesia.

3. Anaesthesia-stressed animals consuming the diets with 100 g casein/kg with or without arginine plus glycine supplementation did not differ from each other in N retention.

4. Liver protein content increased after anaesthesia in rats given the high-protein diets; liver transaminase activity increased, whereas muscle transaminase activity decreased, in animals consuming the high protein diets.

5. Possible mechanisms to account for these results are discussed.

In the previous report (Sitren & Fisher, 1977) hind-leg fracture was produced under diethyl ether anaesthesia. This paper describes the effect of diethyl ether anaesthesia on nitrogen retention and other protein-related responses in rats. The observations recorded were derived from studies on N balance in diethyl ether-stressed rats as a function of their dietary protein and amino acid intake.

EXPERIMENTAL

Three experiments were carried out with adult, male, Sprague–Dawley rats weighing approximately 300 g which had been raised on a commercial stock diet (Purina Rat Chow, Ralston Purina Co., St Louis, Mo., USA). In Expt 1, rats weighing approximately 260 g were fed on a control, high casein (200 g/kg) diet (20C) for a pre-stress period of approximately 10 d. All rats then underwent a single, 6 min exposure to diethyl ether in a closed vessel. One-half of the animals were then given an experimental, high-casein (200 g/kg) diet (20EC) which was supplemented with 20 g arginine plus 10 g glycine/kg. The complete composition of the diets, which were pair-fed in each experiment, has already been described (Sitren & Fisher, 1977).

The protocol for Expt 2 followed that of Expt 1, except that the rats weighed approximately 290 g and the diets consisted of a control, low-casein (100 g/kg) diet (10C) and an experimental, low-casein (100 g/kg) diet (10EC) supplemented with 10 g arginine plus 5 g glycine/kg.

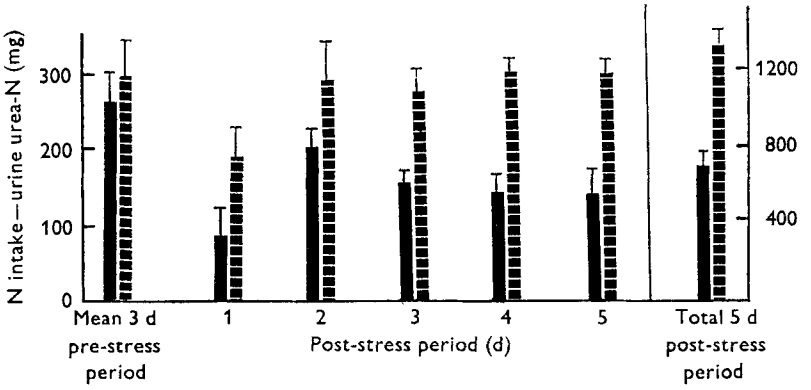

Fig. 1. Nitrogen retention of anaesthesia-stressed rats given a diet with 200 g casein/kg without (C) or with 20 g arginine and 10 g glycine/kg supplementation (EC). ▬, C-E (C diet pre- and post-stress; anaesthesia-stressed); ▬▬, C-CE-A (C diet pre- and EC diet post-stress; anaesthesia-stressed).

In Expt 3, rats weighing 300–350 g were given either the 20C or the 20EC diets throughout the experimental period. Groups of five or six animals were killed at 1, 3, or 7 d after the administration of diethyl ether.

On Expts 1 and 2, urine was collected from the first 3 d before anaesthesia stress and for the next 5 d post-stress. Preliminary analysis revealed that a highly significant ($P < 0{\cdot}001$) correlation existed between urinary urea-N and total urinary N in both the pre- and post-stress periods (Sitren & Fisher, 1977). Therefore, N retention was calculated as the difference between N intake and urinary urea-N. In Expt 3, liver protein concentration was measured and both liver and skeletal muscle transaminase activity (L-aspartate aminotransferase (GOT) (*EC* 2.6.1.1) and L-alanine aminotransferase (GPT) (*EC* 2.6.1.2) was determined.

The methods used for preparing tissue homogenates and procedures for analytical determinations have been reported (Sitren & Fisher, 1977).

Throughout the text the following code was used to describe the treatment groups: C-A, C diet pre-and post-stress; animals were anaesthesia-stressed; C-EC-A, C diet pre- and EC diet post-stress; animals were anaesthesia-stressed.

RESULTS AND DISCUSSION

N retention values from rats in Expt 1 are presented in Fig. 1. On the first day following the administration of diethyl ether, N retention decreased significantly ($P < 0{\cdot}05$) compared with the mean 3 d pre-stress period for rats consuming either the 20C or the 20EC diet, although the decrease was more pronounced for rats given the 20C diet. N retention of rats receiving the 20EC diet was greater than that of those receiving the 20C diet for all 5 d following anaesthesia stress. The totals showed that animals given the 20EC diet retained twice as much N as did those consuming the 20C diet for the 5 d post-anaesthesia period. It was previously shown in unstressed rats that N retention was similar if they were given either the 20C or the 20EC diet (Sitren & Fisher, 1977).

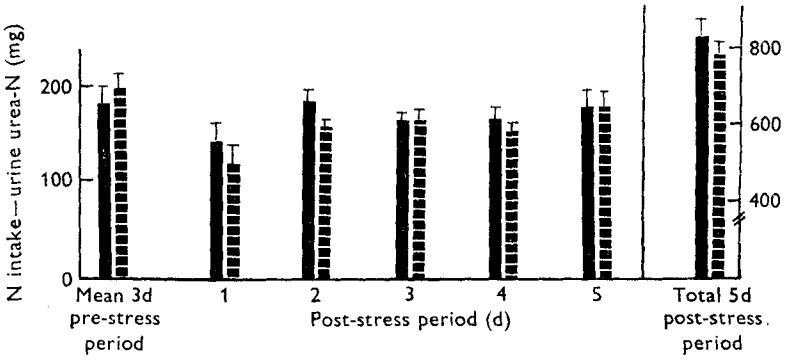

Fig. 2. Nitrogen retention of anaesthesia-stressed rats given a diet with 100 g casein/kg without (C) or with 10 g arginine and 5 g glycine supplementation (EC). ▬, C-A (C diet pre- and post-stress; anaesthesia-stressed); ▮▮▮▮, C-EC-A (C diet pre- and EC diet post-stress; anaesthesia-stressed).

Table 1. *Total liver protein content of anaesthesia-stressed rats given a diet with 200 g casein/kg without (C) or with 20 g arginine and 10 g glycine/kg supplementation (EC)*

		Liver			
		Weight (g/kg body-wt)		Protein (mg/g wet tissue)	
Stress	Day post-stress	Mean	SE	Mean	SE
		Diet C			
None	–	35·8	0·8	111	4
Anaesthesia	1	35·8	0·6	130	4*
Anaesthesia	3	34·6	1·0	151	7*
Anaesthesia	7	36·2	0·3	124	5*
		Diet EC			
None	–	35·9	1·0	115	2
Anaesthesia	1	35·2	0·5	134	9*
Anaesthesia	3	34·0	1·2	132	8*
Anaesthesia	7	35·1	0·6	122	2

* Values significantly different from the unstressed group given the same diet: $P < 0.05$.

Fig. 2 shows the N retention of diethyl ether-stressed rats consuming either the 10C or the 10EC diet after exposure. No differences in N retention were observed during the post-stress period. Thus, the addition of extra arginine and glycine to a diet with 100 g casein/kg (10EC) exerted no benefit on N retention of anaesthesia-stressed rats, whereas supplements of arginine and glycine to a diet with 200 g casein/kg (20EC) led to significant improvement in N retention.

Information concerning the influence of anaesthesia on protein metabolism is limited. Tilstone & Cuthbertson (1970) have reported that administration of diethyl ether anaesthesia to rats altered the liver polysome profile by increasing the proportions of monomers and dimers. In a preliminary study of N balance in rats, Flint & Richardson (1974) showed that sodium pentobarbitone, given orally, led to increased excretion of urinary N.

Table 2. *Specific activity (m-units†/mg protein) of liver and muscle* L-*aspartate aminotransferase (EC* 2.6.1.1) *(GOT) of anaesthesia-stressed rats given a diet with* 200 *g casein/kg without (C) or with* 20 *g arginine and* 10 *g glycine/kg supplementation (EC)*

Stress	Day post-stress	Liver Mean	Liver SE	Muscle Mean	Muscle SE
		Diet C			
None	–	615	45	1704	75
Anaesthesia	1	1032	41*	1326	21*
Anaesthesia	3	1278	81*	1217	192*
Anaesthesia	7	1189	89*	1191	141*
		Diet EC			
None	–	537	88	1736	54
Anaesthesia	1	868	129*	1449	84
Anaesthesia	3	992	76*	1396	137*
Anaesthesia	7	1133	58*	930	180*

* Values significantly different from the unstressed group given the same diet: $P < 0.05$.
† One unit of enzyme activity is that amount that will catalyse the transformation of 1 μmole of substrate/min under the conditions of the assay (see Sitren & Fisher (1977)).

Table 3. *Specific activity (m-units†/mg protein) of liver and muscle* L-*alanine aminotransferase (EC* 2.6.1.2) *(GPT) of anaesthesia-stressed rats given a diet with* 200 *g casein/kg without (C) or with* 20 *g arginine and* 10 *g glycine/kg supplementation (EC)*

Stress	Day post-stress	Liver Mean	Liver SE	Muscle Mean	Muscle SE
		Diet C			
None	–	367	43	235	8
Anaesthesia	1	341	24	204	9
Anaesthesia	3	489	45*	148	14*
Anaesthesia	7	430	46	194	19
		Diet EC			
None	–	341	38	222	14
Anaesthesia	1	286	28	189	8
Anaesthesia	3	379	44	173	18
Anaesthesia	7	411	9	149	23*

* Values significantly different from the unstressed group given the same diet: $P < 0.05$.
† One unit of enzyme activity is that amount that will catalyse the transformation of 1 μmole of substrate/min under the conditions of the assay (see Sitren & Fisher, 1977).

Total liver weight and protein content for the rats in Expt 3 are shown in Table 1. The administration of diethyl ether had no effect on liver weight of rats given either the 20C or the 20EC diet. However, significant changes in liver protein were recorded. Anaesthesia-stressed rats consuming the C diet had significantly increased liver protein on days 1, 3 and 7 after stressing.

Anaesthesia-stressed animals given the EC diet showed significantly higher liver protein levels on days 1 and 3 but by day 7 the value was no longer significantly different from that of unstressed rats.

The administration of diethyl ether anaesthesia has been shown to be a potent

stimulus in increasing blood corticosteroids (Moore, 1957; Thoren, 1974). Matsuda, Duyck, Kendall & Greer (1964) demonstrated that adrenal secretion was increased in rats anaesthetized with diethyl ether and could not be further elevated by the trauma of leg fracture. The effect of glucocorticoids on liver protein has been studied. Goodlad & Munro (1959) and Munro (1966) have shown that liver N is increased in rats given cortisone. Corticosteroids are also associated with increased gluconeogenesis and increased degradation of skeletal muscle (Nocenti, 1968).

It is possible that elevated blood corticosteroids induced by diethyl ether anaesthesia were responsible for the observed changes in liver protein.

The specific activities of liver and muscle transaminase for Expt 3 are presented in Tables 2 and 3. In the liver of anaesthesia-stressed rats given diet C, GOT increased significantly the first day after stressing and remained elevated on days 3 and 7. In muscle tissue of anaesthesia-stressed rats given diet C, GOT activity immediately decreased and continued to decrease on days 3 and 7, by which time it was 30% lower than that of unstressed rats.

Muscle GOT activity of rats given diet EC changed differently: the decrease recorded for anaesthesia-stressed rats was not significant until the third day following the administration of diethyl ether. On day 7 the activity in anaesthesia-stressed rats given diet EC remained at a significantly lower level.

Changes in GPT activity (Table 3) were not as great as were those found in GOT activity. In rats given diet C, liver GPT increased on day 3 only, whereas no significant changes were noted in anaesthesia-stressed rats given diet EC.

Diethyl ether given to animals given diet C led to a significant fall in muscle GPT on day 3, whereas anaesthesia-stressed rats receiving diet EC showed a significant decrease on day 7 only.

In general, liver GOT activity increased after stressing in rats given either diet. Liver GPT rose significantly on the third day after stressing in rats given diet C, while the activity showed no change in animals given diet EC. Muscle transaminase activity showed a general decrease following stress in rats given diet C or diet EC.

Liver enzyme activities are affected by the stress of diethyl ether anaesthesia. Lumbers, Threlfall & Stoner (1969) found that the administration of diethyl ether anaesthesia led to decreased activity of acetyl-CoA in rat liver, whereas increased activity was observed when sodium pentobarbitone was given. Katona (1975) reported that a short period of diethyl ether anaesthesia resulted in an elevation of galactosyl transferase activity, a marked decrease in thiamine phosphate pyrophosphorylase (*EC* 2.5.1.3) activity, a decreased arylsulphatase B (*EC* 3.1.6.1) activity, and a highly significant increase in the activity of alkaline phosphatase (*EC* 3.1.3.1), in liver tissue of rats.

The effects of anaesthesia on carbohydrate metabolism have also been studied. Aynsley-Green, Biebuyck & Alberti (1973) found that plasma insulin levels increased in rats anaesthetized with diethyl ether and that these animals exhibited impaired glucose tolerance following an intravenous glucose load. These authors stated that the effects of anaesthesia in the whole animal are the result of a complex interaction of enzymatic, hormonal, cardiovascular and neurological mechanisms.

CONCLUSIONS

Administration of diethyl ether anaesthesia without associated surgery may be considered a stress severe enough to induce a significant reduction in N retention. N retention of anaesthesia-stressed rats was improved by feeding a diet with 200 g/kg casein with 20 g arginine and 10 g glycine/kg supplementation.

A diet providing only 100 g casein/kg and supplemented with arginine and glycine did not improve N retention of anaesthesia-stressed rats. Thus, the biological value of a high-protein diet only, when fed under stress conditions, was enhanced by arginine and glycine supplementation. In relation to protein metabolism and stress, the reduced N retention of anaesthesia-stressed rats suggests that, in surgical intervention, the use of anaesthesia alone should be considered sufficiently traumatic and should, therefore, be accounted for in planning the protein nutrition of the patient.

Paper of the Journal Series, New Jersey Agricultural Experiment Station; supported in part by US Public Health Service Grant AG 160-01.

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Printed in Great Britain