

Stimulation of Erythropoietin Secretion by Single Amino Acids (40300)

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Protein deprivation in rats results in a decrease of the amount of erythropoietin (Ep) produced in response to hypoxic stimulation (1). This effect is rapidly reversed if the protein deprived animals are fed a single protein meal consisting of albumin or hemoglobin just prior to or shortly after the onset of the hypoxic stimulus (2). The study detailed below provides evidence that in protein deprived rats erythropoietin secretion can be stimulated by individual amino acids.

Materials and Methods. Female Sprague-Dawley rats weighing 100–200 g were used. A special diet in pellet form containing less than 0.5% protein ("protein-free"), but otherwise nutritionally complete, was purchased from Purina-Ralston Co., Missouri. Solutions of single amino acids in 3 cc of distilled water were given with a blunt-ended #18 needle inserted per os into the rat stomach. To dissolve the amino acids, the pH of the solution had to be changed at times from moderately acid (pH 3.0) to strongly alkaline (pH 9.0). However, in each individual experiment, the pH of the water fed to control rats was adjusted to that of the test amino acid solutions. This difference in the pH of the various solutions fed to the rats was not found to affect their plasma Ep levels.

Erythropoietin production was stimulated by placing the animals in a hypobaric chamber and exposing them to 0.5 atmosphere for 7 hours. Immediately afterwards, rats were exsanguinated by cardiocentesis and the plasma obtained from each experimental group (4–5 rats) was pooled and assayed for determination of Ep levels in posthypoxic polycythemic mice by the method of Gordon and Weintraub (3) (6–8 assay mice each received 0.5 ml of pooled plasma). It should be noted that minor variations in the spring mechanism of the regulatory valve of our hypobaric chamber result in some inconsistency in the chamber pressure from experi-

ment to experiment. Therefore, a control group of rats fed water adjusted to the pH of the amino acid preparations were always included in each experimental trial. The statistical significance of the differences was determined by the Student's *t* test.

Results. Effect of single L-amino acids (Table I). Rats fed a protein-free diet for 6 days were fed 100 mg of an L-amino acid and were immediately afterwards exposed to hypoxia. Table I shows the mean plasma Ep levels of rats fed various amino acid solutions. The data indicate that L-methionine, L-cystine and L-leucine produced the most intense and consistent stimulation of Ep production ($P < 0.001$). L-Tyrosine and L-asparagine produced a small but still significant ($P < 0.05$) increase in plasma Ep levels whereas the rest of the amino acids had no significant effect ($P > 0.05$).

Effect of various doses of L-amino acids (Tables II and III). Protein-deprived rats fed from 25 to 100 mg L-methionine or L-cystine prior to hypoxia had significantly higher plasma Ep levels compared to the control group. Increasing the amount fed to 800 mg did not produce a further increase in Ep production and may have been inhibitory. When histidine or glycine was fed in doses of 10 to 400 mg per rat, no increase in the posthypoxic plasma Ep levels was detected.

Discussion. Decreased Ep production occurs in the presence of protein deficiency (1). This decrease has been related by some to the depression of basal metabolism associated with starvation (4). We have recently demonstrated that a single feeding of protein (hemoglobin or albumin) to protein deprived rats produces an immediate enhancement of Ep production which is dose related and which does not correlate with changes in the oxygen consumption of the animals (2). We concluded that the production of erythropoietin depends not only on oxygen supply vs

TABLE I. EFFECT OF FEEDING A SINGLE L-AMINO ACID ON POSTHYPOXIC PLASMA Ep LEVELS OF PROTEIN DEPRIVED RATS.

Amino acid fed (100 mg)	% 59Fe uptake into RBC's of assay mice (mean \pm 1 SEM)	
	Control (H ₂ O) group	AA group
Alanine (3)	2.83 \pm 0.65	3.94 \pm 1.00
Arginine (3)	2.83 \pm 0.66	4.04 \pm 1.27
Valine (3)	4.75 \pm 1.10	5.97 \pm 1.70
Serine (4)	4.32 \pm 0.79	3.00 \pm 0.98
Methionine (7)	3.08 \pm 0.33	11.00 \pm 1.19 ^a
Cystine (4)	2.79 \pm 0.73	10.10 \pm 0.60 ^a
Tyrosine (3)	1.96 \pm 0.16	4.74 \pm 0.98 ^b
Tryptophane (2)	2.55 \pm 0.87	1.61 \pm 0.18
Phenylalanine (2)	2.86 \pm 0.56	2.41 \pm 0.28
Leucine (5)	2.06 \pm 0.12	4.52 \pm 0.44 ^a
Isoleucine (2)	2.27 \pm 0.03	4.29 \pm 1.11
Histidine (4)	3.16 \pm 0.55	3.34 \pm 0.32
Asparagine (4)	2.18 \pm 0.07	4.84 \pm 0.51 ^b
Glycine (6)	3.98 \pm 1.01	5.95 \pm 1.58
Lysine (4)	3.76 \pm 1.12	4.29 \pm 1.38
Glutamic acid (4)	3.56 \pm 1.25	6.99 \pm 2.10
Aspartic acid (4)	3.56 \pm 1.25	5.62 \pm 1.53
Threonine (4)	4.12 \pm 1.00	6.90 \pm 1.99
Proline (5)	4.93 \pm 1.17	8.77 \pm 2.92
Cysteine (6)	3.50 \pm 0.69	5.34 \pm 0.99

Numbers in parentheses signify the number of experimental trials conducted.

^a $P < 0.001$.

^b $P < 0.05$.

TABLE II. EFFECT OF FEEDING VARIOUS DOSES OF L-METHIONINE AND L-CYSTINE ON POSTHYPOXIC PLASMA Ep LEVELS OF PROTEIN DEPRIVED RATS.

Amount fed	% 59Fe Incorporation into RBC's of assay mice (mean \pm 1 SEM)	
	L-Methionine	L-Cystine
H ₂ O	2.09 \pm 0.25	2.74 \pm 0.23
25 mg	8.55 \pm 4.61	4.20 \pm 1.41
50 mg	8.41 \pm 3.39	7.00 \pm 2.05
75 mg	10.02 \pm 3.90	4.91 \pm 1.07
100 mg	10.43 \pm 1.96	5.79 \pm 0.23
H ₂ O	3.68 \pm 0.74	1.50 \pm 0.21
100 mg	12.67 \pm 1.05	9.19 \pm 0.94
400 mg	12.15 \pm 1.09	6.56 \pm 1.41
800 mg	5.70 \pm 0.27	5.41 \pm 0.32

demand of the Ep producing sites, but also on the continuous supply of amino acids (2). The present experiments were done to determine whether individual amino acids were important for the biosynthesis of erythropoietin as occurs with other polypeptide hormones (insulin, growth hormone) (5). Only three amino acids, (methionine, leucine and cystine) had a significant effect in raising the

TABLE III. EFFECT OF FEEDING VARIOUS DOSES OF L-HISTIDINE OR L-GLYCINE ON POSTHYPOXIC PLASMA Ep LEVELS OF PROTEIN DEPRIVED RATS.

Amount fed	% 59Fe Incorporation into RBC's of assay mice (mean \pm 1 SEM)	
	L-Histidine	L-Glycine
H ₂ O	4.11 \pm 0.47	3.18 \pm 0.27
10 mg	4.00 \pm 0.60	3.66 \pm 0.23
50 mg	2.51 \pm 0.58	4.63 \pm 0.56
100 mg	4.24 \pm 0.50	4.51 \pm 0.75
400 mg	3.32 \pm 0.40	2.18 \pm 0.21

plasma Ep levels of the protein deprived rats. Other amino acids had minimal or no effect. There is a parallel to this finding in the studies which show a great variation in the ability of single amino acids to stimulate secretion of insulin or growth hormone (6, 7).

Some amino acids which had no effect at the 100 mg dose level were tested at smaller doses to determine whether their dose response curves were maximal at the lower levels. The results were negative. The data also suggest that higher doses of cystine and methionine may be inhibitory. We have no explanation for this possibility, although large doses of amino acids may suppress the transport of other amino acids across cell boundaries (8).

Summary. Protein deficiency in rats results in decreased ability to produce erythropoietin after hypoxic stimulation. This defect can be reversed by a single protein feeding at the time of exposure to hypoxia. The present experiments show that feeding of methionine, leucine or cystine also corrected the defect in erythropoietin production. These amino acids may serve to signal the adequacy of protein reserves and permit the synthesis of erythropoietin. Other single amino acids had minimal or no effect.

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