Effect of Dietary Lysine and Tryptophan Supplementation on Growth Rate and Toxicity of Barbiturates and Ethanol in Rats

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Abstract □ Female Sprague–Dawley rats exhibited significantly improved growth after a 30-day feeding with lysine-supplemented rations. Supplementation with lysine and tryptophan led to improved growth in two of three experimental trials. Although increased weight gain was noted in male rats fed the supplemented rations, the results were significant in only one of three trials. The order of limiting amino acids and the supplementation level were calculated on the basis of the fasting plasma amino acid concentrations. After ethanol (6 g/kg po), animals fed either supplemented ration showed significant sleeping time reductions and significant increases in the onset of loss of the righting reflex. The ethanol LD_{50} value increased significantly only for the rats fed the ration supplemented with both amino acids. After pentobarbital (20 mg/kg po), the onset of loss of the righting reflex increased significantly in rats fed the lysine–tryptophan-supplemented ration. Effects on sleeping time and the LD_{50} were insignificant with rats fed either supplemented ration. Rats fed either supplemented ration showed significant sleeping time decreases and increases in the onset of loss of the righting reflex after hexobarbital (28 mg/kg po). No significant effect on the hexobarbital LD_{50} was observed with either supplemented ration.

Keyphrases □ Dietary supplements—effect of lysine and tryptophan on growth rate, barbiturate toxicity, ethanol toxicity, rats □ Essential amino acids—effect of dietary lysine and tryptophan supplementation on growth rate, barbiturate toxicity, ethanol toxicity □ Pentobarbital toxicity—effect of dietary lysine and tryptophan supplementation □ Hexobarbital toxicity—effect of dietary lysine and tryptophan supplementation □ Ethanol toxicity—effect of dietary lysine and tryptophan supplementation

The ability of rats to tolerate ethanol was shown to vary with the nature of their dietary protein (1). When rats were fed a diet that promoted better growth, they generally became less intoxicated as compared with rats fed a diet that was not as effective in growth promotion. Such reduced intoxication was observed after chronic administration. Among the natural protein mixtures studied (1), egg and milk proteins were more effective than those from cereal and other plant proteins. Milk protein and egg protein were equally effective in protecting the rats against ethanol toxicity. However, milk protein was considerably less effective against the inebriating effects of ethanol.

BACKGROUND

Pretreatment of mice with L-asparagine potentiated the depressant properties of ethanol and hexobarbital as measured by sleeping and immobility times (2). The LD_{50} values of intraperitoneal ethanol and hexobarbital were not affected; these values were 8.6 g/kg and 28 mg/kg, respectively.

Pretreatment of male Sprague–Dawley rats with L-tryptophan was reported to enhance the toxicities of pentobarbital, hexobarbital, and ethanol (3). The central nervous system (CNS) depressant effects of these barbiturates and ethanol also were enhanced, as shown by increased sleeping and immobility times. Ward *et al.* (4) showed that intraperitoneal or oral lysine administration 30 min prior to ethanol administration did not increase the LD_{50}. Pretreatment with intraperitoneal L-lysine significantly reduced the duration and prolonged the onset of sleeping time induced by ethanol.

Enzymatic activity in the liver was reported to decrease during fasting (5). Studies on hepatic enzyme concentrations showed a marked reduction after protein depletion. It is generally agreed that the principal ethanol oxidation site is in the liver. During fasting, the rat's ability to oxidize ethanol decreased (5). However, as soon as the weight of the animal became stationary after feeding, the rat's ability to oxidize ethanol returned to normal. The ethanol oxidation rate was proportional to the dose up to a maximum of 2.5–3 g/kg. At higher doses, the absolute rate in milligrams per kilogram of body weight per hour decreased (5).

Breglia *et al.* (6) demonstrated that there was no significant alteration of the ethanol LD_{50} with pretreatment or with simultaneous administration of selected amino acids. On the other hand, pretreatment with L-lysine, L-arginine, L-ornithine, or glycine counteracted the CNS effects of ethanol. Thus, sleeping time was reduced significantly, the onset of ataxia was prolonged, and the number of animals losing their righting reflex decreased. Ward *et al.* (4) prevented ethanol sleep induction by pretreatment with selected amino acids. These investigators used 30% ethanol, whereas Breglia *et al.* (6), employed 37.8% ethanol. Ethanol toxicity increased in relation to both concentration and dose in one study (7).

Hexobarbital metabolism increased in animals fed a high protein diet and decreased in those fed a low protein diet (8). These effects were much greater in male animals. Stimulation of microsomal enzymes was concluded to be much greater in rats fed a high protein diet. Hexobarbital, which is metabolized primarily in the liver, was reported to exert a greater depressant effect in fasting animals due to a decrease in hepatic microsomal oxidase activity (9).

Rats fed a rancid, biotin-deficient diet were more resistant to pentobarbital toxicity. This diet increased liver weight. Thus, a larger liver appeared to explain the ability of such animals to metabolize pentobarbital more effectively. When doses were calculated on the basis of liver weight, there was no increased resistance to pentobarbital (10).

Since supplemented rations were to be used, various procedures were considered for determining which of the essential amino acids in a ration was limiting. In addition, the calculation of the supplementation level was considered. Several procedures have been proposed for predicting the limiting amino acid; the chemical score method was used for a ration (11–13). The validity of this method rests on two important assumptions: (*a*) the estimated amino acid requirements are reasonably accurate, and (*b*) the amino acids in the ration are fully available.

Two methods, the plasma amino acid (PAA) score and the plasma amino acid ratio, were proposed for predicting the limiting amino acid in rations (14). In these procedures, the fasting plasma amino acid concentrations are compared with the plasma amino acid levels taken 6 hr after a test diet is fed. The modified plasma amino acid ratio (15) and the plasma amino acid score (16) can be calculated by:

$$\text{modified PAA} = \frac{\text{mean PAA level of test group} - \text{mean fasting PAA level}}{\text{animal's amino acid requirement}} \quad \text{(Eq. 1)}$$

$$\text{PAA score} = \frac{\text{mean PAA level of test group}}{\text{mean fasting PAA level}} \times 100 \quad \text{(Eq. 2)}$$

The fasting plasma profile equivalent (FPPE) concept was used to determine the limiting essential amino acid (17). The supplementation level can be calculated by Eq. 3 (the reported amino acid requirement of the animal is not needed for the calculation):

$$\text{FPPE} = \frac{\text{millimoles of amino acid/100 g of diet}}{\text{millimoles per liter in animal's fasting plasma}} \quad \text{(Eq. 3)}$$

Thus, for example, 100 g of ration[1] contains 10.8 mmoles of lysine. There is 0.389 mmole of lysine/liter in the fasting plasma of Sprague–Dawley

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Table I—Weight Increase of Sprague–Dawley Rats after 30 Days with a Control Ration in Comparison with a Lysine- and a Lysine–Tryptophan-Supplemented Ration

Ration	Average Starting Weight, g	Average Finishing Weight, g	Average Weight Gained ± *SD*, g	Weight Gained ± *SD*, %	Control *versus* Supplemented Rations (*p* Value)
			Males		
Experiment 1					
Control	86.1	204.9	118.8 ± 30.7	138 ± 36	—
Control + lysine	92.3	248.0	155.7 ± 28.1	169 ± 30	0.05
Control + lysine + tryptophan	80.4	220.0	139.6 ± 28.4	174 ± 35	<0.05
Experiment 2					
Control	94.7	227.9	133.2 ± 27.5	141 ± 29	—
Control + lysine	85.6	212.3	126.7 ± 31.6	148 ± 37	NS[a]
Control + lysine + tryptophan	93.5	231.5	138.0 ± 26.4	148 ± 28	NS
Experiment 3					
Control	131.6	221.5	89.9 ± 19.2	68 ± 15	—
Control + lysine	135.0	234.4	99.4 ± 18.4	74 ± 14	NS
Control + lysine + tryptophan	148.3	227.3	79.0 ± 17.1	53 ± 12	—
			Females		
Experiment 4					
Control	88.3	177.4	89.1 ± 19.3	101 ± 22	—
Control + lysine	80.9	184.3	103.4 ± 19.5	128 ± 24	<0.02
Control + lysine + tryptophan	86.9	180.5	93.6 ± 18.5	108 ± 21	NS
Experiment 5					
Control	114.2	196.1	81.9 ± 18.2	72 ± 16	—
Control + lysine	87.1	179.9	92.8 ± 19.2	107 ± 22	<0.001
Control + lysine + tryptophan	100.3	200.0	99.7 ± 17.1	99 ± 17	<0.01
Experiment 6					
Control	127.7	190.4	62.7 ± 17.7	50 ± 14	—
Control + lysine	125.7	218.5	92.8 ± 19.6	74 ± 16	<0.01
Control + lysine + tryptophan	120.5	206.1	85.6 ± 19.6	71 ± 16	<0.01

[a] Not significant; $p > 0.05$ (Student t test).

rats. Consequently, in the ration used, the fasting plasma profile equivalent of lysine is 27 (10.8/0.389). Since this value is the smallest fasting plasma profile equivalent value for the essential amino acids, lysine is considered to be limiting. The value for tryptophan is 29, and it is the next limiting amino acid.

The objective of this investigation was to determine the effect of lysine- and lysine–tryptophan-supplemented diets on the growth rate and on ethanol and barbiturate toxicity in Sprague–Dawley rats.

EXPERIMENTAL

Three groups of 10 Sprague–Dawley rats were fed three diets concurrently. The first group received the control ration[1]. The second group received the control ration supplemented with L-lysine monohydrochloride[2] (0.142 g added to 100 g of ration). This supplementation level made lysine and tryptophan equally limiting. The third group received the control ration supplemented with L-lysine monohydrochloride (0.995 g) and L-tryptophan[2] (0.132 g). In the latter supplemented ration, lysine, tryptophan, and threonine (the third limiting amino acid) were equally limiting.

The rats were fed for 30 days. Each day, a fresh 20-g portion of ration was placed in the feeding container. Water was provided *ad libitum*.

The diets were prepared as follows. The ration (1362 g) and povidone 400[3] (13.62 g) were placed in a mixer. A solution prepared by dissolving 1.93 g of L-lysine monohydrochloride in 1 liter of distilled water was distributed uniformly throughout the ration by paddle agitation. Ethanol (700 ml) was added to guard against mold growth during the drying step. The wet mass was passed through a No. 1 sieve. The granules were tray dried in an air-circulating oven at room temperature. In the preparation of the lysine–tryptophan-supplemented diet, 13.55 g of L-lysine monohydrochloride and 1.8 g of L-tryptophan were added to 1362 g of ration. The control diet was treated similarly in that distilled water and ethanol were added. The use of povidone 400 in the three rations favored the formation of homogeneous blends.

The effect on the growth rate was determined after 30 days by comparing the weight increase of the two test groups with that of the control group.

At the end of the 30-day feeding trial, the rats weighed between 175 and 200 g. These rats were used to determine the effect of the diet on the LD_{50}, loss of the righting reflex, and sleeping time after oral administration of ethanol, hexobarbital[4], and pentobarbital[4].

The LD_{50} values were determined by the Litchfield and Wilcoxon (18) method. The rats were fasted for 24 hr before being used in the LD_{50} determinations.

Sleeping time was defined as the total time elapsed from the initial loss of the righting reflex to its return without remission. Loss of the righting reflex was defined as the inability of the animal to right itself immediately when placed on its back.

The three concurrent feeding trials (control and two supplemented rations) were conducted six times. Three such studies each were carried out with male and female rats. The results represent averaged data points derived from 10 animals.

RESULTS AND DISCUSSION

The female rats showed significantly improved growth after the 30-day feeding trial with the lysine-supplemented rations (Table I). Supplementation with lysine and tryptophan led to improved growth in two of three feeding trials. Although increased weight gain was noted in male rats fed the supplemented rations, the results were significant in only one of three trials.

On the basis of the fasting plasma concentrations, lysine was calculated to be the first limiting essential amino acid in the commercial diet[1]. The second limiting amino acid in this ration was tryptophan. However, on the basis of whole egg protein, the first limiting essential amino acid in

[1] Rockland Farms mouse/rat diet.
[2] Sigma Chemical Co., St. Louis, Mo.
[3] Polyvinylpyrrolidone 400, Sigma Chemical Co., St. Louis, Mo.
[4] Merck and Co., Rahway, N.J.

Table II—Ethanol-Induced Toxicity and CNS Depression in Sprague–Dawley Rats Fed Lysine- and Lysine–Tryptophan-Supplemented Rations (Ethanol, 6 g/kg po)

Parameter	Male	Female	Combined Results	*p* Value
Control diet				
Sleeping time, min ± *SD*	273.0 ± 19.9	285.5 ± 18.5	279.2 ± 18.8	—
Loss of righting reflex, min ± *SD*	4.0 ± 1.4	4.3 ± 0.75	4.3 ± 1.1	—
LD_{50}, g/kg (range)	8.3 (7.54–9.13)	8.4 (7.6–9.2)	8.23 (7.88–8.53)	—
Control diet + lysine				
Sleeping time, min ± *SD*	230.0 ± 38.9	225.0 ± 46.9	227.5 ± 42.9	0.05
Loss of righting reflex, min ± *SD*	6.9 ± 1.9	6.0 ± 1.4	6.25 ± 1.7	0.05
LD_{50}, g/kg (range)	9.2 (8.37–10.12)	8.9 (7.9–9.3)	9.01 (8.53–9.54)	NS[a]
Control diet + lysine and tryptophan				
Sleeping time, min ± *SD*	190.0 ± 23.1	180.0 ± 20.2	185.0 ± 20.2	0.02
Loss of righting reflex, min ± *SD*	7.4 ± 2.3	7.6 ± 1.9	7.25 ± 2.1	0.03
LD_{50}, g/kg (range)	9.3 (8.68–9.85)	10.5 (8.9–12.6)	9.8 (9.3–10.09)	0.05

[a] Not significant.

Table III—Pentobarbital-Induced Toxicity and CNS Depression in Sprague–Dawley Rats Fed Lysine- and Lysine–Tryptophan-Supplemented Rations (Pentobarbital, 20 mg/kg po)

Parameter	Male	Female	Combined Results	*p* Value
Control diet				
Sleeping time, min ± *SD*	104.9 ± 6.5	106.7 ± 6.9	105.8 ± 6.7	—
Loss of righting reflex, min ± *SD*	7.4 ± 3.4	9.4 ± 6.3	8.4 ± 4.8	—
LD_{50}, mg/kg (range)	66.7 (55.9–77.8)	62.4 (51.2–74.4)	65.5 (58.0–76.1)	—
Control diet + lysine				
Sleeping time, min ± *SD*	92.8 ± 5.7	94.2 ± 5.9	93.5 ± 5.8	0.50
Loss of righting reflex, min ± *SD*	9.0 ± 3.4	10.4 ± 5.9	9.7 ± 4.7	0.50
LD_{50}, mg/kg (range)	68.8 (52.3–88.4)	65.8 (54.1–81.2)	67.8 (58.0–76.1)	0.50
Control diet + lysine and tryptophan				
Sleeping time, min ± *SD*	85.3 ± 5.0	87.3 ± 5.6	86.3 ± 5.3	0.10
Loss of righting reflex, min ± *SD*	16.9 ± 10.1	18.5 ± 11.5	17.7 ± 10.8	0.05
LD_{50}, mg/kg (range)	75.9 (68.2–82.5)	75.6 (68.2–82.5)	75.7 (68.2–82.5)	0.30

Table IV—Hexobarbital-Induced Toxicity and CNS Depression in Sprague–Dawley Rats Fed Lysine- and Lysine–Tryptophan-Supplemented Rations (Hexobarbital, 28 mg/kg po)

Parameter	Male	Female	Combined Results	*p* Value
Control diet				
Sleeping time, min ± *SD*	63.1 ± 13.7	72.0 ± 14.1	67.5 ± 13.9	—
Loss of righting reflex, min ± *SD*	16.0 ± 6.9	17.0 ± 6.9	16.5 ± 6.6	—
LD_{50}, mg/kg (range)	37.0 (32.1–42.5)	31.5 (27.4–36.3)	33.5 (29.1–39.4)	—
Control diet + lysine				
Sleeping time, min ± *SD*	43.1 ± 5.6	49.4 ± 13.9	46.7 ± 9.7	0.01
Loss of righting reflex, min ± *SD*	30.0 ± 16.8	29.1 ± 18.4	29.5 ± 17.1	0.01
LD_{50}, mg/kg (range)	37.5 (32.1–43.1)	32.0 (28.6–39.4)	34.75 (30.6–41.3)	0.50
Control diet + lysine and tryptophan				
Sleeping time, min ± *SD*	39.0 ± 5.0	40.2 ± 8.0	39.6 ± 6.5	0.005
Loss of righting reflex, min ± *SD*	32.0 ± 11.1	31.4 ± 9.3	31.7 ± 10.2	0.01
LD_{50}, mg/kg (range)	38.5 (32.1–44.9)	37.0 (31.1–42.9)	37.75 (32.3–43.3)	0.50

the commercial ration was methionine followed by tryptophan. The improved growth rates observed in rats fed the lysine-supplemented rations provide support for the fasting plasma profile concept.

A significant increase in the ethanol LD_{50} was shown by rats fed the lysine–tryptophan-supplemented ration (Table II). This reduction in toxicity can probably be attributed to an improvement in the biological value of the commercial ration by supplementation. As mentioned earlier (1), proteins that were more effective in growth promotion also were more effective in reducing ethanol toxicity and in counteracting its inebriating effects. Consequently, it was not unexpected that rats fed either supplemented ration would show significant sleeping time reductions and significant increases in time before loss of the righting reflex after ethanol administration.

Rats fed the supplemented rations showed no LD_{50} increase for pentobarbital (Table III) or for hexobarbital (Table IV). On the other hand, significant sleeping time reductions and increases in time before loss of the righting reflex were observed after hexobarbital administration in rats being fed either supplemented ration. In addition, rats fed either supplemented ration exhibited significant increases in time before loss of the righting reflex after pentobarbital administration. However, an

insignificant effect on sleeping time was observed.

Thus, strategic supplementation of a ration with essential amino acids to improve its biological value offers promise in reducing the toxicity of ethanol and selected barbiturates as well as in mitigating the CNS effects associated with such compounds.

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Metabolic Effects of a New Hypolipidemic Agent, Ciprofibrate

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Abstract □ Ciprofibrate, a new orally effective hypolipidemic agent like clofibrate, suppressed tyloxapol-induced hypercholesterolemia in rats. Ciprofibrate at 10 mg/kg was effective. Clofibrate required a dosage of 180 mg/kg to suppress the tyloxapol effect. Norepinephrine-induced free fatty acid release was inhibited by clofibrate in rats in accordance with earlier findings. Ciprofibrate and lifibrate differed from clofibrate in that, at hypocholesterolemically effective doses, neither inhibited the hormone-sensitive lipase *in vivo*.

Keyphrases □ Ciprofibrate—hypolipidemic effects, effect on hormone-sensitive lipase, compared to clofibrate and lifibrate, rats □ Anticholesterolemic agents—ciprofibrate, effect on serum lipid levels, hormone-sensitive lipase, compared to clofibrate and lifibrate, rats

Evidence that hyperlipidemia is a major risk factor in coronary artery disease (1) has directed interest toward agents that can correct the lipid abnormality. One such agent is clofibrate, 2-(4-chlorophenoxy)-2-methylpropanoic acid ethyl ester, which serves as a model reference. Related agents are being sought that are more effective against hypercholesterolemia as well as against all types of hyperlipoproteinemia.

A previous report (2) presented evidence that ciprofibrate, 2-[4-(2,2-dichlorocyclopropyl)phenoxy]-2-methylpropanoic acid, is at least 100-fold as hypolipidemic in butter fat, cholesterol-fed hyperlipidemic rats as is clofibrate. In this discussion, additional comparisons between ciprofibrate and clofibrate are presented.

EXPERIMENTAL

Tyloxapol[1]-Induced Hypercholesterolemia—The tyloxapol treatment procedure followed that of Garattini *et al.* (3) and of Kariya *et al.* (4). Young adult Sprague–Dawley rats[2], averaging ~300 g each, were maintained on laboratory feed *ad libitum*. Based on preliminary trials, the test agents were given by intubation at one of the following dosages: ciprofibrate at 10 or 22.5 mg/kg/day or clofibrate at 90 or 180 mg/kg/day, each for 4 days. On the 4th day, the rats were each given tyloxapol at 200 mg/kg iv. Anticholesterolemic effects were evaluated on the basis of cholesterol levels in blood samples taken by cardiac puncture 20 hr later.

For analysis, the blood samples were centrifuged, and the serum was separated. Serum cholesterol was estimated colorimetrically according to the Liebermann–Burchard procedure, using a *p*-toluenesulfonic acid catalyst after Turner and Eales (5).

Inhibition of Hormone-Sensitive Lipase—Two studies were conducted in which the hypolipidemic agents were evaulated for their ability to inhibit the hormone-sensitive lipase. In one, clofibrate was compared with ciprofibrate. In the other, clofibrate was compared with lifibrate, bis(4-chlorophenoxy)acetic acid 1-methyl-4-piperidinyl ester (6).

Young adult Sprague–Dawley strain rats[2], averaging ~250–300 g each, were allocated into groups of nine. They were given one test agent at the following dosages: clofibrate at 150 mg/kg, ciprofibrate at 15 mg/kg, or lifibrate at 40 mg/kg. The test agents were given by intubation in a 1% aqueous gum tragacanth suspension (10 ml/kg) before *l*-norepinephrine (as the bitartrate). The vasoconstrictor effects of norepinephrine were inhibited with 100 mg of phenoxybenzamine/kg sc, 1 hr before norepinephrine was given.

Table I—Ciprofibrate and Clofibrate Effectiveness in Protecting Rats against Tyloxapol Hypercholesterolemia (Means ± *SE*)

Test Compound	Intragastric Dosage, mg/kg/day × 4	Tyloxapol[a]	*n*	Serum Cholesterol[b], mg/100 ml
None	None	−	12	75.2 ± 3.2
None	None	+	10	198.7 ± 16.3
Ciprofibrate	10	+	8	80.9 ± 7.9
	22.5		8	73.5 ± 5.7
Clofibrate	90	+	8	132.4 ± 23.7
	180		7	69.7 ± 4.4

[a] At 200 mg/kg iv on the 4th day of medication. [b] Blood taken 20 hr after tyloxapol.

[1] Triton WR-1339 or Triton A-20.
[2] Charles River.

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