Effect of Lysine and Diethanolamine–Rutin on Blood Levels, Withdrawal Reaction, and Acute Toxicity of Ethanol in Mice

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Abstract □ The effects of L-lysine and diethanolamine–rutin on blood ethanol levels and withdrawal convulsions following a 4-day exposure to ethanol vapors were evaluated in 30–40-g male Swiss-Webster mice. The animals were exposed in groups, along with simultaneous controls, in a 34-liter dynamic Plexiglas exposure chamber. Blood was collected by retro-orbital puncture and analyzed enzymatically for ethanol. At the end of the inhalation period, convulsions on handling were scored over 24 hr. In addition, the effects of L-lysine and diethanolamine–rutin on blood ethanol levels and on acute ethanol toxicity following oral and intraperitoneal administration were evaluated. L-Lysine lowered blood ethanol levels following inhalation or oral administration of ethanol. Diethanolamine–rutin had no effect on blood ethanol levels. Both L-lysine and diethanolamine–rutin decreased the withdrawal reaction in dependent mice. L-Lysine increased the oral LD_{50} of ethanol, while diethanolamine–rutin decreased the intraperitoneal LD_{50} of ethanol.

Keyphrases □ Lysine—effect on blood levels, withdrawal reaction, and toxicity of ethanol, mice □ Diethanolamine–rutin—effect on blood levels, withdrawal reaction, and toxicity of ethanol, mice □ Ethanol—blood levels, withdrawal reaction, and toxicity, effect of lysine and diethanolamine–rutin, mice □ Blood levels—ethanol, effect of lysine and diethanolamine–rutin, mice □ Withdrawal reactions—ethanol, effect of lysine and diethanolamine–rutin, mice □ Toxicity—ethanol, effect of lysine and diethanolamine–rutin, mice □ α-Amino acids—lysine, effect on blood levels, withdrawal reaction, and toxicity of ethanol, mice □ Alcohols—ethanol, blood levels, withdrawal reaction, and toxicity, effect of lysine and diethanolamine–rutin, mice

Depletion of amino acids was reported to be characteristic in the development of delerium tremens following chronic alcohol ingestion (1). The prophylactic use of amino acids was accompanied by a high degree of success in forestalling delerium tremens in individuals whose low amino acid levels indicated their increased susceptibility (1). Specific amino acids, such as L-glutamine and L-lysine, also were effective in reducing or preventing ethanol toxicity (2–4).

BACKGROUND

Schiller (5) investigated the effects of amino acids (as a solution of protein hydrolysates[1]) on blood ethanol levels and the rate of disappearance of ethanol from the blood. The amino acids were administered intravenously and ethanol was administered orally to chronic alcoholics free of liver impairment. Ethanol-utilization curves showed that amino acids decreased blood ethanol levels and accelerated the rate of disappearance of ethanol from the blood (5). The specific amino acids in the mixture used by Schiller (5) could not be determined. Selected amino acids were effective in decreasing blood ethanol levels in dogs when administered orally simultaneously with ethanol (6). Certain amino acids possibly impede the absorption of orally administered ethanol by formation of a stable complex (7).

A significant reduction in the depressant action of ethanol (30% v/v) was reported following a 30-min pretreatment with L-lysine (2.5 g/kg ip or po) (3). The oral administration of L-lysine and ethanol resulted in the complete elimination of hypnotic effects. Administration of L-lysine and ethanol intraperitoneally resulted in a 65% reduction in ethanol sleeping time. Also, an increase in the LD_{50} of ethanol in rats was noted following treatment with L-lysine (3).

The interaction of ethanol or acetaldehyde, its major metabolite, with various biogenic amines is a possible cause of ethanol dependence following chronic administration (8–10). The presence of acetaldehyde may lead to localized increases in the concentrations of aromatic aldehydes in tissues rich in biogenic amines (9). Furthermore, these highly reactive aldehydes condense with their parent amines to yield a morphine-like benzyltetrahydroisoquinoline alkaloid (9). This effect was demonstrated *in vitro* using rat liver homogenates (8). Such condensation products were reported for dopamine (9), epinephrine, and norepinephrine (levarterenol) (10).

The coadministration of an excess reactive α-amino acid and ethanol might lead to a decrease in ethanol toxicity due to the combination of acetaldehyde with the amino acid (3).

Rutin-enhanced diethanolamine mixtures protected rats from the motor effects of ethanol, without affecting blood ethanol levels, *via* a weak agonist–antagonist action (11–13).

This study was undertaken to determine the extent to which L-lysine, a reactive α-amino acid, protects against ethanol toxicity and alters the ethanol-withdrawal reaction in dependent mice. In addition, because of the possible agonist–antagonist relationship of diethanolamine to ethanol, the effects of diethanolamine–rutin on the ethanol-withdrawal reaction and acute ethanol toxicity in mice were evaluated.

L-Lysine was chosen because of previous studies (3, 4) and because: (*a*) it has one of the fastest rates of reaction with acetaldehyde (14); (*b*) L-lysine levels of 0.57, 0.98, and 1.53 mg % were reported for patients with delerium tremens, recovered patients, and normal subjects, respectively (1); (*c*) L-lysine is the only essential α-amino acid having sufficient amino groups in nonzwitterionic form to interact with acetaldehyde (15); and (*d*) treatment with L-lysine significantly increased the LD_{50} of acetaldehyde[2].

EXPERIMENTAL

Production of Ethanol Dependence—Male Swiss-Webster mice[3], 30–40 g, were divided into four treatment groups of 10 mice each. These treatment groups were housed individually in 34-liter Plexiglas exposure chambers, along with simultaneous pH controls, for 4 days. Air was introduced into the chamber at the rate of 8 liters/min. The total air flow was split between two 1-liter erlenmeyer flasks (16); one flask contained ethanol (95%, USP) and the other remained empty. The air flow was joined again just before entering the chamber.

The relationship of air flow to chamber concentration is shown in Table I. A flowmeter in each line, between the air compressor pump and the flasks, allowed for control of the chamber ethanol levels. A chamber ethanol concentration of 11–15 mg/liter was appropriate in establishing ethanol dependence in mice over 4 days. The priming dose of ethanol (17) was not necessary if the chamber was allowed to reach equilibrium before introducing the mice (18). Therefore, the air flow through the chamber was begun approximately 20 hr before introducing the mice. During the exposure period, the chamber temperature varied from 24 to 27° and the relative humidity varied from 60 to 70%.

The test animals were removed from the chamber for a short period each day for administration of: Solution A, L-lysine[4] (20% w/v) prepared in a solution of protein hydrolysates[1], 1 g/kg po, pH 5.7; Solution B, L-lysine[4] (20% w/v) as an aqueous solution, 1 g/kg po twice daily,

[1] Aminosol, Abbott Laboratories, Chicago, Ill.

[2] Unpublished data.

[3] Camm Research Institute, Wayne, N.J.

[4] As the monohydrochloride salt, Sigma Chemical Co., St. Louis, Mo.

Table I—Relationship of Air Flow Split between Ethanol and Air Flasks to Chamber Ethanol Concentration

Air Flow through Ethanol Flask, liters/min	Air Flow through Air Flask, liters/min	Chamber Concentration, mg/liter
0.75	7.25	11
0.88	7.12	13
1.10	6.90	15
1.30	6.70	18
1.50	6.50	22

pH 5.7; protein hydrolysate[1], 9 ml/kg po, pH 5.7; or diethanolamine–rutin[5], 4.5 ml/kg po, pH 7.4.

In addition, each animal received pyrazole (34 mg/kg ip) for the maintenance of stable blood ethanol levels (19, 20). On Day 5, the animals were removed from the ethanol environment, and convulsions on handling were evaluated according to the method of Goldstein and Pal (19) over 24 hr.

pH Controls for Amino Acid and Diethanolamine–Rutin Treatments—A pH 5.7 phosphate buffer solution was administered orally to control groups exposed to ethanol vapors simultaneously with amino acid treatment groups. The control buffer was administered in volumes equivalent to the various amino acid treatments. Another pH 7.4 phosphate buffer was administered to the control group exposed simultaneously with the diethanolamine–rutin treatment group in a volume equivalent to that of the treatment. Each animal in the control groups received pyrazole, 34 mg/kg ip. The control groups were removed from the ethanol environment, and their withdrawal scores were evaluated simultaneously with the treatment groups (19).

Environmental Control—Ten animals were introduced into the exposure chamber and kept there for 4 days. The air flow was maintained at 8 liters/min. Ethanol, however, was not introduced into the chamber. The animals were removed for short periods each day for intubation. On Day 5, the animals were removed from the chamber, and convulsions on handling were evaluated (19).

Evaluation of Blood Ethanol Levels Resulting from Inhalation of Ethanol Vapors—Blood ethanol levels were measured enzymatically. Each day during the exposure period, three animals from the treated and control groups were randomly selected for blood sampling. The blood samples were obtained by retro-orbital puncture (21–23) before the administration of any treatment or control.

A volume of 0.25 ml of blood was collected from each animal, deproteinated, and added to the enzymatic reaction mixture[6] (24). After 30 min at room temperature, the fluorescence[7] of the mixture was measured at an absorption maximum of 340 nm and an emission maximum of 458 nm. This method is based on the natural fluorescence of the reduced diphosphopyridine nucleotide formed, since ethanol is metabolized by alcohol dehydrogenase to acetaldehyde (24). The reaction is pushed toward completion by the addition of semicarbazide (25). On Day 5, five animals from each group were chosen randomly for the blood ethanol analysis.

Determination of Chamber Ethanol Concentration—The chamber ethanol concentration also was determined enzymatically (24). Each day, a 1-ml sample of chamber air was withdrawn using a gastight syringe[8]. The sample was then injected into the headspace of a vial containing 3.5 ml of the enzymatic reaction mixture[6] (25). After 30 min at room temperature, the fluorescence[7] of the reaction mixture was measured (24). The sampling port was positioned to facilitate sampling at the respiratory level of the test animals (26). The chamber ethanol concentrations resulting from various rates of flow are shown in Table I.

Evaluation of Ethanol Intoxication—Before each exposure period, the animals were trained to maintain their balance on a rotating roller (10 rpm) for 1 min (27). This simple test was used each morning during the exposure period, before any treatment was administered, as an indication of the level of intoxication, tolerance, and loss of muscle control due to ethanol inhalation.

Effect of Amino Acids and Diethanolamine–Rutin on Acute Blood Ethanol Levels—Male Swiss-Webster mice, 30–40 g, were divided into 12 treatment groups of six mice each, with appropriate pH controls. The treatments were administered, as previously indicated, for 4 days. One hour after the last treatment on Day 4, ethanol was administered, 3 g/kg ip or 6 g/kg po. One treatment group received 6 g/kg po simultaneously with the last treatment. Blood was sampled 0.5 or 1 hr after the intraperitoneal or oral administration of ethanol, respectively. Blood was obtained by retro-orbital puncture (21–23) and analyzed enzymatically for ethanol (24, 25).

[5] Prepared according to Blum *et al.* (13).
[6] Sigma Chemical Co., St. Louis, Mo.
[7] Spectrofluorometer model 430, G. K. Turner Associates, Palo Alto, Calif.
[8] Precision Sampling Corp., Baton Rouge, La.

Table II—Mean Time on Rotating Roller during Ethanol Inhalation

Treatment	Mean Time, min ± *SD*	*p* Level[a] (Relative to Drug Control)	*p* Level[a] (Relative to Environmental Control)
Solution A	0.75 ± 0.17	NS	<0.05
Solution B	0.64 ± 0.22	NS	<0.01
Control	0.49 ± 0.29	—	<0.01
Protein hydrolysates	0.83 ± 0.13	NS	<0.05
Control	0.68 ± 0.21	—	<0.02
Diethanolamine–rutin	0.85 ± 0.14	NS	<0.05
Control	0.64 ± 0.24	—	<0.01
Environmental control	1.00 ± 0.00	—	—

[a] For significance, $p < 0.05$.

Effect of Amino Acids and Diethanolamine–Rutin on 24- and 72-hr LD_{50} of Ethanol—Male Swiss-Webster mice, 30–40 g, were divided into groups of 10 and treated as previously described for 4 days. One hour after the last treatment on Day 4, ethanol was administered (40% w/v) in graded doses of 1–5 g/kg ip and 5–11 g/kg po. The LD_{50} was calculated from the number dead at 24 and 72 hr by the log-probit method of Miller and Tainter (28).

Effect of Various Treatments and Controls on Weights of Mice Exposed to Ethanol Vapor—The subjects in the treatment and control groups were weighed each day. The mean weight of each group on Day 1 was taken as the base for comparison. Weight loss during the exposure period was recorded as percent of the starting weight.

RESULTS

No difference was found between treatment and control groups in their ability to maintain balance on a rotating roller (Table II). However, each treatment group maintained their balance for a longer time than the corresponding control.

None of the treatments used decreased blood ethanol levels after intraperitoneal administration (Table III). In those groups receiving ethanol orally, only the Solution A and Solution B treatments decreased the blood ethanol levels. This result was true for pretreatment as well as simultaneous administration (Table III).

Among the groups exposed to the inhalation of ethanol vapor, only the Solution A group showed a decrease in the daily mean blood ethanol levels (Table IV). However, both the Solution A and Solution B groups had blood ethanol levels below those of controls on Days 4 and 5 (Table IV).

The evaluation of convulsions on handling (19) during the withdrawal period showed that Solution A and diethanolamine–rutin groups had reduced mean withdrawal scores (Table V). The peak withdrawal score, the mean of the three highest consecutive scores, was reduced in the Solution A, Solution B, and diethanolamine–rutin groups (Table V). All treatment and control groups had peak withdrawal scores greater than the environmental control (Table V, $p < 0.001$). Peak withdrawal development, the mean of all scores up to the highest mean score for each group, was reduced in only the Solution A and diethanolamine–rutin groups (Table V).

The 24-hr oral LD_{50} of ethanol (40% w/v) was increased by Solutions A and B and protein hydrolysate (Table VI). The 72-hr oral LD_{50} of ethanol was increased only by Solutions A and B (Table VI). None of the various amino acid treatments affected either the 24- or 72-hr intraperitoneal LD_{50} of ethanol (Table VII).

The diethanolamine–rutin treatment did not affect the 24- or 72-hr oral LD_{50} of ethanol, nor did it affect the 24-hr intraperitoneal LD_{50} (Tables VI and VII). However, diethanolamine–rutin did decrease the 72-hr intraperitoneal LD_{50} of ethanol (Table VII).

Since weight loss can indicate toxicity, the mean percent weight

Table III—Effect of Amino Acids and Diethanolamine–Rutin on Blood Ethanol Levels[a]

Treatment	Blood Ethanol Levels, mg/ml ± *SD*					
	Pretreatment				Simultaneous	
	Intraperitoneal[b]	*p* Level[a]	Oral[c]	*p* Level[a]	Oral[d]	*p* Level[a]
Solution A	4.16 ± 0.57	>0.05	6.35 ± 1.03	<0.05	3.60 ± 0.58	<0.001
Control	4.06 ± 0.43	—	7.39 ± 0.42	—	6.98 ± 0.29	—
Solution B	4.67 ± 0.55	>0.05	6.93 ± 0.66	<0.02	5.20 ± 0.82	<0.02
Control	4.82 ± 0.48	—	7.63 ± 0.26	—	7.35 ± 1.56	—
Protein hydrolysate	3.70 ± 0.51	>0.05	5.25 ± 0.59	>0.05	5.98 ± 0.90	>0.05
Control	3.98 ± 0.48	—	5.82 ± 0.41	—	5.86 ± 0.38	—
Diethanolamine–rutin	4.10 ± 0.55	>0.05	6.29 ± 0.75	>0.05	7.72 ± 1.33	>0.05
Control	4.17 ± 0.41	—	5.85 ± 0.43	—	7.50 ± 0.93	—

[a] For significance, $p < 0.05$. [b] Ethanol, 3 g/kg ip, 1 hr following pretreatment; blood sampled 0.5 hr after ethanol. [c] Ethanol, 6 g/kg po, 1 hr following pretreatment; blood sampled 1 hr after ethanol. [d] Ethanol, 6 g/kg po, simultaneously with treatment; blood sampled in 1 hr.

Table IV—Effect of Amino Acids and Diethanolamine–Rutin on Blood Ethanol Levels Produced by Inhalation of Ethanol Vapors for 4 Days[a]

Treatment	Day 2, mg/ml	Day 3, mg/ml	Day 4, mg/ml	*p* Level[b]	Day 5, mg/ml	*p* Level[b]	5-Day Mean, mg/ml	*p* Level[a]
Solution A	1.70 ± 0.40	1.28 ± 0.52	1.40 ± 0.12	<0.05	1.46 ± 0.68	<0.02	1.46 ± 0.18	<0.05
Solution B	1.90 ± 0.55	1.33 ± 0.25	1.27 ± 0.12	<0.05	1.85 ± 0.48	<0.05	1.58 ± 0.33	>0.05
Control	2.00 ± 0.11	1.76 ± 0.57	2.40 ± 0.16	—	2.95 ± 1.03	—	2.28 ± 0.52	—
Protein hydrolysate	1.98 ± 0.35	1.42 ± 0.17	2.18 ± 0.37	>0.05	2.85 ± 0.67	>0.05	2.11 ± 0.51	>0.05
Control	2.04 ± 0.19	1.75 ± 0.12	2.55 ± 0.42	—	3.08 ± 0.26	—	2.35 ± 0.51	—
Diethanolamine–rutin	1.66 ± 0.23	1.87 ± 0.52	2.18 ± 0.27	>0.05	2.68 ± 0.38	>0.05	2.10 ± 0.37	>0.05
Control	1.66 ± 0.21	1.75 ± 0.31	2.14 ± 0.22	—	2.87 ± 0.39	—	2.11 ± 0.45	—

[a] Chamber concentration was 11–15 mg/liter. [b] For significance, $p < 0.05$.

loss of each treatment group was compared with the appropriate control (Table VIII). Only the diethanolamine–rutin group showed a lower mean percent weight loss. The weight loss in the diethanolamine–rutin group did not differ significantly from that resulting from pyrazole treatment alone. As expected, all groups showed weight loss when compared to the environmental control.

When the Solution A and Solution B treatments were compared, the Solution A group demonstrated a lower peak withdrawal score (0.95 ± 0.09 and 1.32 ± 0.12, respectively; $p < 0.02$). Also, simultaneous administration of ethanol (40% w/v) and amino acids orally resulted in lower blood ethanol levels for the Solution A group (3.60 ± 0.58 and 5.20 ± 0.82, respectively; $p < 0.01$). No difference in blood ethanol levels established by inhalation, nor in any other parameters except those mentioned, was noted between the Solution A and Solution B groups (Table IV).

DISCUSSION

Physical dependence on ethanol is rapidly established in rodents by inhalation techniques (16, 17, 19). To maintain adequate and stable blood ethanol levels, pyrazole, an inhibitor of alcohol dehydrogenase (20, 29), was necessary (16, 17, 19). A dose of 34 mg/kg ip (0.5 mmole/kg), rather than 68 mg/kg ip (1 mmole/kg) as previously reported (17, 19), was adequate (30). A 96-hr exposure at an ethanol concentration of 11–15 mg/liter produced a satisfactory, postalcohol, hyperexcitability reaction in mice. Beginning the air flow approximately 20 hr before introducing the animals to the chamber allowed for the elimination of a priming dose of ethanol (18).

The rotating roller test was used to evaluate the protection afforded by the various treatments against the muscular incoordination produced by ethanol (27) and to serve as an index of tolerance production. Since tolerance has been described as a homeostatic adjustment (31), a decrease followed by a gradual increase in the ability to stay on the rotating roller (10 rpm) would be expected. In fact, a rapid decline in balancing ability occurred during the first 2 days of ethanol inhalation. This decline was followed by a more gradual increase in balancing ability. Even though the mean time on the rotating roller was not significantly longer in the treated than control groups, the treated groups were consistent in maintaining balance for longer periods. This increase indicates a decrease in the intensity of ethanol effects in these groups.

Various amino acids hasten the disappearance of ethanol from the blood (5), resulting in lower blood ethanol levels. The Solution A and Solution B groups showed a decrease in blood ethanol levels following oral ethanol administration. This effect was seen with both the pretreatment with amino acids and the simultaneous administration of amino acids and ethanol. The protein hydrolysate treatment had no effect on blood ethanol levels. This result indicates the necessity of excess L-lysine (3). It was not expected that the diethanolamine–rutin treatment would affect blood ethanol levels by any route, or sequence, of administration[2], and no effect was found.

When ethanol was administered *via* inhalation, the Solution A group showed lower mean blood ethanol levels. The other treatments did not affect the mean blood ethanol levels. However, both the Solution A and the Solution B treatments lowered blood ethanol levels on Days 4 and 5. The rate of ethanol disappearance from the blood was increased by L-lysine[2] and by amino acids in general (5). An in-

Table V—Withdrawal Scores after 4-Day Exposure to Ethanol Vapors

Treatment	Mean Withdrawal Score ± *SD*	*p* Level[a]	Peak Withdrawal Score ± *SD*	*p* Level[a]	Peak Withdrawal Development ± *SD*	*p* Level[a]
Solution A	0.73 ± 0.27	<0.005	0.95 ± 0.09	<0.001	0.72 ± 0.31	<0.02
Solution B	0.94 ± 0.36	>0.05	1.32 ± 0.12	<0.001	0.98 ± 0.44	>0.05
Control	1.38 ± 0.58	—	1.94 ± 0.05	—	1.39 ± 0.68	—
Protein hydrolysate	0.78 ± 0.31	>0.05	1.08 ± 0.08	>0.05	0.84 ± 0.43	>0.05
Control	0.99 ± 0.36	—	1.22 ± 0.03	—	0.99 ± 0.42	—
Diethanolamine–rutin	0.73 ± 0.25	<0.05	1.00 ± 0.00	<0.001	0.79 ± 0.28	<0.05
Control	1.10 ± 0.39	—	1.48 ± 0.03	—	1.17 ± 0.43	—

[a] For significance, $p < 0.05$.

Table VI—Effect of Amino Acids and Diethanolamine–Rutin on the Acute Oral Toxicity of Ethanol

Treatment	24-hr LD_{50}, mg/kg ± SD	Confidence Limits (95%)	p Level[a]	72-hr LD_{50}, mg/kg ± SD	Confidence Limits (95%)	p Level[a]
Solution A	9500 ± 628.98	9026.17–9973.83	<0.05	8986 ± 592.61	8539.57–9432.43	<0.05
Solution B	9126 ± 215.67	8963.53–9288.47	<0.05	8746 ± 204.76	8591.75–8900.25	<0.05
Protein hydrolysate	9221 ± 448.25	8883.32–9558.68	<0.05	8127 ± 414.26	7814.92–8439.08	>0.05
Diethanolamine–rutin	7757 ± 864.57	7105.69–8408.31	>0.05	7747 ± 720.68	7214.09–8299.91	>0.05
Control	7484 ± 472.29	7128.21–7839.79	—	7484 ± 472.29	7128.21–7839.79	—

[a] For significance, $p < 0.05$.

Table VII—Effect of Amino Acids and Diethanolamine–Rutin on the Acute Intraperitoneal Toxicity of Ethanol

Treatment	24-hr LD_{50}, mg/kg ± SD	Confidence Limits (95%)	p Level[a]	72-hr LD_{50}, mg/kg ± SD	Confidence Limits (95%)	p Level[a]
Solution A	3742 ± 437.18	3412.66–4071.30	>0.05	3241 ± 358.92	2970.61–3511.39	>0.05
Solution B	3295 ± 309.27	3062.02–3527.98	>0.05	3000 ± 317.97	2760.47–3239.54	>0.05
Protein hydrolysate	3323 ± 532.18	2840.09–3641.91	>0.05	3000 ± 414.57	2687.69–3312.31	>0.05
Diethanolamine–rutin	3241 ± 532.18	2840.09–3641.91	>0.05	1732 ± 484.86	1366.74–2097.26	<0.05
Control	3241 ± 510.87	2856.14–3625.86	—	2876 ± 540.28	2695.91–3056.69	—

[a] For significance, $p < 0.05$.

crease in the rate of ethanol disappearance from the blood, developing over time, could account for the lower blood ethanol levels on the last 2 days of inhalation. This finding could also explain the lack of effect on blood ethanol levels following acute intraperitoneal administration of ethanol. The diethanolamine–rutin and protein hydrolysate treatments did not affect blood ethanol levels.

Differences in blood ethanol levels did not exist between Solutions A and B following ethanol inhalation or intraperitoneal or oral administration following a 1-hr pretreatment with the amino acid solutions. The lower blood ethanol level in the Solution A group following simultaneous oral administration with ethanol could be attributed to the additional reactive α-amino acids in the treatment.

Both Solutions A and B were expected to increase significantly the 24- and 72-hr oral LD_{50} of ethanol but not the 24- and 72-hr intraperitoneal LD_{50}. This was the case, but there was no difference between the two groups.

The diethanolamine–rutin treatment did not affect the 24- or 72-hr oral LD_{50} nor the 24-hr intraperitoneal LD_{50} of ethanol. However, the 72-hr intraperitoneal LD_{50} of ethanol was decreased by diethanolamine–rutin. Diethanolamine–rutin is a weak ethanol agonist, itself a central nervous system (CNS) depressant[2].

The protein hydrolysate treatment increased the 24-hr oral LD_{50} of ethanol. It did not, however, protect beyond this point. This result could be attributed to insufficient reactive α-amino acids, particularly L-lysine.

The acute and chronic effects of ethanol have been attributed to the augmentation, by ethanol and acetaldehyde, of tetrahydroisoquinoline alkaloid formation from biogenic amines (8, 9). It has been proposed that an increase in the steady-state level of one or more biogenic aldehydes, due to competitive inhibition of NAD-linked aldehyde dehydrogenase by acetaldehyde (9), could be responsible for the CNS effects related to ethanol (32).

L-Lysine decreases blood ethanol levels and accelerates its rate of disappearance from the blood. It also increases the LD_{50} of acetaldehyde (13) and forms a complex with acetaldehyde (3, 11). The formation of this complex could block, to some degree, the ability of acetaldehyde to inhibit NAD-linked aldehyde dehydrogenase, thereby decreasing the formation of tetrahydroisoquinoline derivatives and the pharmacological effects of ethanol. L-Lysine decreased ethanol sleeping time in rats (3). A decrease in alkaloid formation *per se* or in rate of formation could account for the decrease in the severity of the postalcohol hyperexcitability seen in dependent mice.

The Solution A treatment resulted in a decrease in mean withdrawal scores, peak withdrawal scores, and peak withdrawal development. The Solution B treatment resulted in a significant decrease in only the peak withdrawal, while treatment with protein hydrolysate had no effect. The Solution A treatment resulted in a lower peak withdrawal than did Solution B. This effect could be due to the additional reactive α-amino acids in the Solution A treatment.

Treatment with diethanolamine–rutin resulted in a reduction in mean withdrawal, peak withdrawal, and peak withdrawal development scores. However, no effect was seen on blood ethanol levels. Preliminary studies indicated a decreased rate of ethanol elimination following administration of diethanolamine–rutin[2].

Diethanolamine–rutin was proposed as a weak agonist–antagonist to ethanol[2]. However, the characteristic signs of ethanol withdrawal did not appear immediately after the administration of diethanolamine–rutin to dependent mice. Therefore, diethanolamine–rutin probably reduces the postalcohol hyperexcitability in mice by some other mechanism.

Chronic ethanol administration produced an increase in brain γ-aminobutyrate (33). Its inhibitory effects were implicated in the regulation of the depressant actions of ethanol (34, 35). A significant reduction in brain γ-aminobutyrate was reported to coincide with the maximum intensity of ethanol withdrawal scores in mice (30). The reduction in brain γ-aminobutyrate renders the subject more susceptible to postalcohol hyperexcitability (30). Since diethanolamine–rutin is also a CNS depressant[2], it too may increase brain γ-

Table VIII—Weight Loss of Animals Exposed to Ethanol Vapors for 4 Days

Treatment	Mean Weight Loss ± SD	p Level[a] (Relative to Drug Control)	p Level[a] (Relative to Pyrazole Control)	p Level[a] (Relative to Environmental Control)
Solution A	8.26 ± 1.74	>0.05	<0.01	<0.001
Solution B	10.59 ± 2.93	>0.05	<0.01	<0.001
Control	8.74 ± 2.49	—	<0.01	<0.001
Protein hydrolysate	5.68 ± 1.69	>0.05	<0.05	<0.001
Control	5.68 ± 2.85	—	>0.05	<0.01
Diethanolamine–rutin	5.37 ± 1.58	<0.05	>0.05	<0.001
Control	9.48 ± 2.76	—	<0.05	<0.001
Pyrazole control	3.26 ± 1.11	—	—	<0.001
Environmental control	0.00 ± 0.00	—	—	—

[a] For significance, $p < 0.05$.

aminobutyrate. In addition, its effect in slowing ethanol elimination from the blood[2] may cause a more gradual decline in brain γ-aminobutyrate during ethanol withdrawal, decreasing the intensity of the characteristic hyperexcitability.

Treatment with diethanolamine–rutin produced a peak withdrawal response at 5 hr, which remained constant for 3 hr. None of the other treatment groups showed a similar plateau in withdrawal responses. Possibly, brain γ-aminobutyrate was prevented from falling to levels low enough for maximal hyperexcitability to occur. Studies are in progress to determine this possibility.

SUMMARY AND CONCLUSIONS

The reactive α-amino acid, L-lysine, lowered blood ethanol levels when ethanol was administered orally or by inhalation. This decrease in blood ethanol could be the result of poor absorption or increased elimination. The withdrawal reaction, following ethanol inhalation in mice, was also reduced by L-lysine. L-Lysine possibly interferes with an acetaldehyde-mediated alkaloid formation from biogenic amines, thereby decreasing the degree of dependence on ethanol. The presence of additional reactive α-amino acids somewhat enhanced the effects of L-lysine.

Diethanolamine–rutin also reduced the ethanol withdrawal reaction in mice but had no effect on blood ethanol levels. It may prevent the fall in brain γ-aminobutyrate reported to accompany ethanol withdrawal convulsions by slowing the rate of ethanol elimination from the blood.

A relationship may exist between the formation of tetrahydroisoquinoline alkaloids and the increase in brain γ-aminobutyrate seen in ethanol-dependent mice. The increase in brain γ-aminobutyrate during ethanol administration and its subsequent decrease during withdrawal may cause postalcohol hyperexcitability in mice (30). The amino acid treatments seem to interfere with the acetaldehyde-dependent formation of these alkaloids. Diethanolamine–rutin seems to exert its effect by decreasing the rate of ethanol elimination, which then might prevent a significant decrease in brain γ-aminobutyrate during ethanol withdrawal.

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