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Effect of Selected Amino Acids on Ethanol Toxicity in Rats

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Abstract □ The effects of L-lysine, L-arginine, L-ornithine, and glycine on acute ethanol intoxication, and the degree of ethanol-induced CNS depression, were investigated. Amino acid treatment was shown to prolong the onset of ataxia, reduce the duration of sleeping time, and decrease the number of rats losing the righting reflex, but it did not alter the LD_{50} values in response to ethanol. The protective actions of the amino acids are attributed to the formation of an amino acid–acetaldehyde complex, although other possible interactions are discussed.

Keyphrases □ Ethanol toxicity—effect of amino acids, rats □ Amino acids—effect on ethanol toxicity, rats □ CNS depression, ethanol induced—effect of amino acids, rats □ Toxicity, ethanol—effect of amino acids, rats

The effect of amino acids on the concentration and rate of disappearance of ethanol from the blood was the subject of the investigations of Schiller *et al.* (1, 2). After simultaneous administration of 120 ml. p.o. of ethanol (50% v/v) together with an amino acid–dextrose mixture (protein hydrolysates[1]), given intravenously to chronic alcoholics without hepatic dysfunction or nutritional deficiency, ethanol utilization curves showed that the amino acid mixture significantly decreased the maximum blood ethanol levels obtained and accelerated the rate of disappearance of ethanol from the blood. The activity of the amino acid–dextrose mixture was confirmed in an *in vitro* preparation of rat liver slices incubated with ethanol; while the specific amino acids responsible for these effects could not be identified, these experiments indicated that they contain four carbons or less.

Alanine has been shown to follow the pattern reported for pyruvate in increasing the disappearance of ethanol from the blood (3, 4). This is presumed to be the result of transformation of alanine to pyruvate, a Kreb's cycle intermediate which is thought to accelerate the disappearance of ethanol by increasing its aerobic metabolism (5).

When glycine or alanine was administered orally simultaneously with 15 g. of ethanol to dogs, the maximum concentration of ethanol appearing in the blood was lower than the control group (6, 7). The author concluded that the disappearance of ethanol must take place during absorption (6) and that a stable compound of ethanol and the amino acids may be formed to account for the decrease in the concentration of ethanol in the blood (7).

The nature of dietary protein fed to rats which were given a constant volume (4.0–6.6 g./kg.) of 40% ethanol four times weekly for 16 months was found to be related to the ability to reduce the degree of inebriation and the numbers of deaths due to ethanol toxicity (8). In this study, egg protein was the most effective while a mixture of peanut meal and soy protein was the least effective in reducing the toxic and CNS depressant effects of ethanol. It was concluded that the protective effects of the dietary proteins were not related to an effect on the rate of ethanol absorption or the emptying time of the stomach but were due to differences in ethanol utilization once it was absorbed.

Jarowski and Ward (9) reported that 30 min. of pretreatment with L-tryptophan (165 mg. i.p.) before a 2-g./kg. i.p. dose of 95% ethanol in unfasted rats produced a significant potentiation of the acute depressant effects of ethanol as measured by changes in the LD_{50}, sleeping, and immobility times. The fasting essential amino acid profile of blood plasma, which has been useful in predicting the relative dietary value of proteins (10), was determined in that study and served as a basis for the use of L-tryptophan supplementation (9). In the fasting essential amino acid profile of blood plasma, the concentrations of amino acids in the plasma

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

of fasting rats indicate which essential amino acid is the limiting amino acid (present in lowest concentration) and also which amino acid would be most appropriate to supplement in an attempt to affect drug toxicity (10).

Other data originating in this laboratory showed that there was a slight elevation of the LD_{50} of ethanol after rats had been fed a diet supplemented with L-lysine and/or L-tryptophan.

Mueller *et al.* (11), using a model based on the performance of a trained rat, were able to quantify and differentiate ethanol-induced changes in the behavioral performance of rats. The effect of ethanol on the "number of shocks not terminated" was inhibited by D,L-threonine, L-methionine, and D,L-glutamine.

Hakkinen and Kulonen (12) found that simultaneous administration of 1.67 g./kg. p.o. of L-glutamine abolished the symptoms of intoxication produced by a 4.3-g./kg. dose of ethanol (33% v/v) in fasted rats. In a similar study, Forney *et al.* (13) demonstrated a significant potentiation of the depressant action of ethanol (4 g./kg. p.o.) following a 1- or 4-hr. pretreatment with 1 g./kg. s.c. of L-asparagine, as indicated by prolonged sleeping and immobility times.

Significant reduction in the depressant action of ethanol (30% v/v) after a 30-min. pretreatment with L-lysine (2.5 g./kg. i.p. or p.o.) was reported by Ward *et al.* (14). When ethanol, 3.6 g./kg., and L-lysine were given orally, there was a complete abolition of the hypnotic effect of ethanol; when both drugs were administered intraperitoneally, there was a 65% reduction in sleeping time. Furthermore, the LD_{50} of ethanol (30% v/v, intraperitoneally or orally) was slightly elevated by pretreatment with a 2.5-g./kg. dose of L-lysine.

Acetaldehyde, the toxic metabolite of ethanol, was shown (15) chromatographically to combine rapidly with the amino group of certain amino acids. The rate and extent of this reaction were dependent on the nature of the amino acid and the pH of the reaction medium. Arginine reacted most rapidly, followed by glycine, lysine, and ornithine. Reactive carbonyl groups, in compounds such as acetaldehyde, are thought to combine with the undissociated amino groups of the amino acids (16). Of the essential amino acids, lysine has the greatest ratio of amino groups in undissociated form available for reaction with acetaldehyde (16).

The purpose of this investigation was to determine whether the oral administration of selected amino acids, before or concurrently with ethanol, has a protective action against the acute toxicity and CNS depression produced by ethanol in rats.

EXPERIMENTAL

Adult male and female (125–150 g.) Sprague-Dawley rats, approximately 2 months old, were employed for all experimental procedures. The animals were kept in the animal quarters for 1 week after shipment to allow them to become acclimated. During this period the animals were fed a standard laboratory animal diet and tap water *ad libitum*. On the day prior to use, the animals were inspected for disease and diseased animals were discarded.

Aqueous solutions of the following amino acids[2] were prepared just prior to use: L-lysine hydrochloride, L-arginine hydrochloride, L-ornithine hydrochloride, glycine hydrochloride (10 and 20% w/v), sodium chloride USP (0.9%), and alcohol USP (ethanol 95%). Stock solutions of ethanol in distilled water (37.8 and 50.4% by volume) were prepared and standardized by specific gravity determination according to the method of Thor *et al.* (17). The choice and use of these particular percentages of ethanol were based on two considerations: to parallel human consumption and to keep the injected volume below 6 ml. According to Gillespie and Lucas (18), administration of ethanol (60% by volume) to rats resulted in a negligible amount of damage to the gastric and intestinal mucosa. All injections, both of ethanol and amino acid solutions, were made by intubation with curved animal feeding needles. A modified "rotarod" was constructed to determine the forced coordinated motor ability of rats (19).

Twenty-four hours before all experimentation, animals were randomly chosen, caged separately (to prevent cannibalism), and deprived of food (tap water *ad libitum*). To ensure a uniform rate of absorption of the test preparations, animals were fasted for 24 hr. Each animal was used only once, and each experiment was intiated at the same time every day throughout the testing period.

The effect of selected amino acids on ethanol toxicity was ascertained by two distinct procedures, with all treatments given orally.

Seventy-Two-Hour LD_{50} as a Measure of Acute Toxicity—Acute toxicity measurements by LD_{50} determinations, for all treatments described here, were carried out with 10 fasted Sprague-Dawley rats (five males and five females per group) and a total of six groups per LD_{50} determination. Ethanol dose levels ranged from 6 to 16 g./kg. where applicable. The number dead at 72 hr. was counted and analyzed by the method of Litchfield and Wilcoxon (20). Ethanol (50.5% by volume) was used throughout this procedure.

The treatments given can be divided into three distinct series as follows.

Pretreatment with Amino Acid before Ethanol Administration—The amino acid solution was administered at two dose levels 30 min. before ethanol. The dosages of the amino acids employed were: L-lysine, 120 mg./kg. and 2.5 g./kg.; L-arginine, 140 mg./kg. and 2.31 g./kg.; glycine, 73 mg./kg. and 1.53 g./kg.; and L-ornithine, 111 mg./kg. and 2.31 g./kg. The L-arginine, L-ornithine, and glycine dose levels are equimolar to the low and high dose levels of L-lysine to which they correspond. The dose of 120 mg./kg. of L-lysine was equal to two times the minimum daily requirement for the adult rat (21); the dose of 2.5 g./kg. was recommended by results from previous work (14). Saline controls of 1.3 ml./kg., corresponding to the volume of the low doses of the amino acids, and 13.3 ml./kg., corresponding to the volume of the high doses of the amino acids, were tested simultaneously. The concentrations of the amino acid solutions (in distilled water) used for low and high dosages were 10 and 20% (w/v), respectively, to keep the volume of solution to a minimum.

Administration of Amino Acid–Ethanol Solution—The amino acid–ethanol solution was formulated to deliver the dose of amino acid and ethanol required simultaneously. The doses of the amino acids employed were equal to the low doses already described. Administration of ethanol alone served as the control for this series of experiments.

Administration of Amino Acids Alone for LD_{50} Determination—Amino acid solutions (in distilled water) were administered to demonstrate the acute toxicity of the individual amino acids. The concentrations were varied to keep the injected volume below 6 ml.

Evaluation of Degree of Intoxication after Ethanol Treatment—The degree of intoxication for all treatment groups, described below, was evaluated using 10 fasted rats (five males and five females) per group. Ethanol (37.8% by volume) was given at a dose level of 6 g./kg. p. o. either as a single or a divided dose.

The statistical analysis, Student's *t* test, of intoxication was based on the following neurological parameters:

1. *Ataxia*—This was defined as the inability to maintain position by using four-leg motor coordination for one revolution of the modified "rotarod." Each animal was subjected to this test individually throughout the experimental period, unless unconscious (19). A normal rat can maintain its equilibrium for an indefinite period (19), and a short training period of three to four trials was added to ensure uniform response (22).

2. *Sleeping Time*—This was defined as the total elapsed time from the initial loss of righting reflex to its return without remission (13). The righting reflex was said to be lost when the animal was placed on its back and did not immediately right itself fully.

The treatments given can be divided into four distinct series as follows.

[2] All amino acids were water-soluble monohydrochloride salts, Sigma grade, Sigma Chemical Co., St. Louis, Mo.

Single-Dose Administration of Ethanol with Amino Acid Pretreatment—Amino acid solutions were administered at two dose levels 30 min. before the ethanol dose of 6 g./kg. The doses of amino acids and associated controls were equal to the low and high doses previously described.

Single-Dose Administration of Amino Acid–Ethanol Solution—The amino acid–ethanol solution was formulated to deliver the required dose of amino acid and 6 g./kg. of ethanol simultaneously. The dosages of amino acids employed were equal to the low doses already described. The administration of 6 g./kg. ethanol alone served as the control group for these experiments.

Serial Administration of Ethanol with Amino Acid Pretreatment—Ethanol was administered in three equally divided doses, 2.0 g./kg. per dose (total of 6 g./kg.), at 15-min. intervals. The amino acid solutions were administered at two dose levels 30 min. before the first ethanol dose. The doses of amino acids and associated controls were equal to the low and high doses previously described.

Serial Administration of Amino Acid–Ethanol Solution—The amino acid–ethanol solution used was formulated to deliver the required dose of amino acid and ethanol simultaneously (6 g./kg. total) in three equally divided doses at 15-min. intervals. The doses of amino acids employed were equal to the low doses already described. The administration of ethanol in divided doses served as the control for these experiments.

Evaluation of Toxicity of Amino Acids—Amino acid solutions were administered at two dose levels, as previously described, with no ethanol treatment. The concentrations of the amino acid solutions used for the low and high dosages were 10 and 20% (w/v), respectively. The rats were then subjected to the same neurological tests for signs of pharmacological effect.

pH Controls for Amino Acid Pretreatment—In this series of experiments, hydrochloric acid buffer solution[3] (pH 1.2) and phosphate buffer solution[3] (pH 5.8), which were equivalent to the pH of the corresponding amino acid solutions (20% w/v), were given at the same dose level as the saline (volume) controls (13.3 ml./kg.) for the high dose amino acid pretreatment before the single and divided ethanol doses. Amino acid solutions (20% w/v) were found to have the following pH values: glycine hydrochloride, 1.2; L-lysine hydrochloride, 5.8; L-arginine hydrochloride, 5.7; and L-ornithine, 5.8. The buffer solutions were administered 30 min. prior to the single or divided ethanol dose (6 g./kg.), after which the degree of intoxication was determined as previously described.

RESULTS

Determinations of Acute Toxicity—There was no significant alteration of the LD_{50} for ethanol (50.4% by volume) with either pretreatment or simultaneous administration of the amino acids. For the concentration and route of administration of ethanol used in this study, the LD_{50} values obtained (Tables I and II) are in agreement with a previously published LD_{50} range following ethanol administration (23). In chronological order (earliest first), the following signs of ethanol intoxication were observed in all groups after ethanol administration: ataxia, decreased muscle tonus, bradypnea, decreased spontaneous activity, hind-leg paralysis, loss of righting reflex, loss of corneal reflex, loss of pinna reflex, and death. The rapidity, intensity, and number of these signs of acute intoxication to which the animals responded were dose related. When an animal recovered, the process was very gradual with the animal reversing the above-mentioned sequence. The sequence described is similar to observations made by other investigators (8, 24).

When rats were treated with amino acids alone, all of the amino acids were found to be without pharmacological effect at the doses employed for pretreatment or simultaneous administration in the ethanol LD_{50} determinations. At high dosages of the amino acids, some signs of toxicity did appear such as ataxia, dyspnea, decreased muscle tonus, and loss of righting reflex. Toxicity signs were first observed for each amino acid at the following doses: glycine hydrochloride 2.5 g./kg.; L-lysine hydrochloride, 8 g./kg.; L-ornithine hydrochloride, 9 g./kg.; and L-arginine hydrochloride, 11 g./kg. When the LD_{50} values for the four amino acids used in this study were calculated by the method of Litchfield and Wilcoxon (20), the results were as follows (g./kg.): L-lysine hydrochloride, 10.13 ± 0.97; L-arginine hydrochloride, 12.40 ± 0.61; L-ornithine hydrochloride, 10.27 ± 0.070; and glycine hydrochloride, 3.34 ± 0.65.

[3] See "The United States Pharmacopeia," 17th rev., Mack Publishing Co., Easton, Pa., 1965, p. 913.

Table I—Effect on Amino Acid Pretreatment on Acute Ethanol Toxicity in Rats

Drug Treatment	Dose	LD_{50} of Ethanol[a], g./kg.
L-Lysine hydrochloride	120 mg./kg.	9.50 ± 0.78
L-Lysine hydrochloride	2.50 g./kg.	11.20 ± 1.86
L-Arginine hydrochloride	140 mg./kg.	9.70 ± 0.61
L-Arginine hydrochloride	2.88 g./kg.	10.00 ± 1.67
L-Ornithine hydrochloride	111 mg./kg.	10.21 ± 1.30
L-Ornithine hydrochloride	2.31 g./kg.	10.05 ± 1.41
Glycine hydrochloride	73 mg./kg.	8.40 ± 1.12
Glycine hydrochloride	1.53 g./kg.	9.20 ± 0.80
Saline control	1.3 ml./kg.	10.00 ± 0.83
Saline control	13.3 ml./kg.	10.15 ± 1.16

[a] Dead animals per group counted at 72 hr. after drug administration. No difference in mortality between males and females was noted.

Degree of Intoxication—Any delaying of the onset of ataxia and/or shortening of the subsequent sleeping time by an agent can be seen as a protective action from the effects of acute ethanol intoxication (14, 22). The term used to express the delay or prolongation of the onset of ataxia was "protection factor," and that used to express the shortening of sleeping time was "percentage of reduction of sleeping time."

The mean time for onset of ataxia for different groups (Tables III–VI) was compared by the number of times the elapsed time for the onset of ataxia of the test group was greater than the elapsed time for the onset of ataxia of the control group (protection factor) (Tables VII and VIII). For the purpose of this comparison, only significant ($p < 0.05$) changes due to amino acid treatment were considered.

Pretreatment with all of the amino acids at the higher dosage level, when ethanol was administered as a single dose, resulted in significant prolongation of the onset of ataxia, with L-lysine prolonging it most, followed by glycine, L-arginine, and L-ornithine. The single-dose administration of the L-arginine–ethanol solution showed a sevenfold increase in the onset of ataxia. The responses for all amino acid treatments with serial administration were similar, with the exception of the glycine–ethanol solution treatment. The protection factors were higher for the high dose pretreatment than for the low dose pretreatment of amino acids, but protective factors for the low dose simultaneous administration of L-lysine and L-arginine were higher than either the low or high pretreatment dose of L-lysine or L-arginine (Table VIII). The protective factor was usually indicative of the subsequent degree of depression due to acute ethanol intoxication.

Significant reduction in the sleeping time and a decrease in the number of rats responding characterized the groups receiving the high dose pretreatment with amino acids when ethanol was administered as a single or divided dose (Tables IX–XII). There was almost a complete abolition of the sleeping time after glycine (1.53 g./kg.) pretreatment with single-dose administration of ethanol. The other high dose pretreatments with amino acids showed large and uniform responses in the reduction of sleeping time

Table II—Effect of Simultaneous Administration of Amino Acid–Ethanol Solution on Acute Ethanol Toxicity

Drug Treatment	Dose of Amino Acids, mg./kg.	LD_{50} of Ethanol[a], g./kg.
L-Lysine hydrochloride	120	10.15 ± 2.05
L-Arginine hydrochloride	140	10.05 ± 1.32
L-Ornithine hydrochloride	111	10.05 ± 0.96
Glycine hydrochloride	73	10.10 ± 1.42
Ethanol control	—	10.10 ± 1.09

[a] Dead animals per group counted at 72 hr. after drug administration. No difference in mortality between males and females was noted.

Table III—Onset of Ataxia with Amino Acid Pretreatment before Single-Dose Administration of Ethanol

Treatment	Dose	Mean Onset[a] of Ataxia, min.	*p* Value[b]
L-Lysine hydrochloride	120 mg./kg.	5.05 ± 0.18	0.5
L-Lysine hydrochloride	2.50 g./kg.	74.76 ± 7.85	0.001
L-Arginine hydrochloride	140 mg./kg.	8.23 ± 3.27	0.2
L-Arginine hydrochloride	2.88 g./kg.	30.98 ± 5.29	0.0001
L-Ornithine hydrochloride	111 mg./kg.	13.25 ± 6.05	0.2
L-Ornithine hydrochloride	2.31 g./kg.	23.49 ± 6.44	0.02
Glycine hydrochloride	73 mg./kg.	3.56 ± 0.23	0.3
Glycine hydrochloride	1.53 g./kg.	68.61 ± 10.06	0.001
Saline	1.3 ml./kg.	3.06 ± 0.32	—
Saline	13.3 ml./kg.	6.47 ± 1.50	—

[a] Onset of ataxia values (min.) ± *SE*. [b] *p* value < 0.05 was significant.

Table V—Onset of Ataxia with Amino Acid Pretreatment before Serial Administration of Ethanol

Treatment	Dose	Mean Onset[a] of Ataxia, min.	*p* Value[b]
L-Lysine hydrochloride	120 mg./kg.	25.10 ± 4.89	0.05
L-Lysine hydrochloride	2.50 g./kg.	56.40 ± 4.81	0.001
L-Arginine hydrochloride	140 mg./kg.	26.76 ± 1.70	0.001
L-Arginine hydrochloride	2.88 g./kg.	66.32 ± 9.46	0.001
L-Ornithine hydrochloride	111 mg./kg.	19.06 ± 1.58	0.01
L-Ornithine hydrochloride	2.31 g./kg.	57.50 ± 11.20	0.01
Glycine hydrochloride	73 mg./kg.	34.67 ± 3.78	0.001
Glycine hydrochloride	1.53 g./kg.	89.82 ± 6.71	0.001
Saline	1.3 ml./kg.	12.34 ± 1.54	—
Saline	13.3 ml./kg.	19.13 ± 1.90	—

[a] Onset of ataxia values (min.) ± *SE*. [b] *p* value < 0.05 was significant.

whether ethanol was administered as a single or divided dose. L-Arginine was the only amino acid which, when given (140 mg./kg.) before a single dose of ethanol as a low dose, resulted in a significant reduction of sleeping time. However, this reduction was not apparent in the low dose pretreatment of arginine when the ethanol was administered serially. L-Lysine and L-arginine were the only amino acids that had a significant reduction in sleeping time when given simultaneously with serial administration of ethanol (Table VIII). Thus, significant antagonism of the intoxicating effects was found upon treatment with L-lysine (120 mg./kg.) and L-arginine (140 mg./kg.), but these results were more variable than the results obtained with the respective high doses of these amino acids.

The amino acid controls for each amino acid administered demonstrated that no acute toxicological effects in this experimentation were due to the administration of amino acids alone.

Saline (volume) controls were used as the basis for comparison for amino acid pretreatment in all tables. The pH controls were found not to be significantly different from the saline (volume) controls in the degree of intoxication produced by 6 g./kg. of ethanol.

DISCUSSION

In the LD_{50} determinations, amino acid treatment did not demonstrate any antagonism to the acute toxic effects of ethanol. This lack of protective action of the amino acids could be due to the inhibition of the absorption of amino acids with an increasing concentration of ethanol present in the GI tract (25) or to the rapid CNS effects of ethanol on vital cortical centers due to rapid absorption (26). Since amino acid pretreatment was not effective in elevating the LD_{50} of ethanol, it seems apparent that the absence of antagonism was probably due to the rapid absorption of large doses of ethanol which could mask any detoxicant actions of the amino acids.

Ward *et al.* (14) reported a slight elevation of the oral LD_{50} of ethanol (30% by volume) after pretreatment with L-lysine hydrochloride (2.5 g./kg. orally). In the present investigation, the percentage of ethanol used for all LD_{50} determinations was 50.4% by volume, which is known to be more toxic (27) than ethanol (30% by volume) used in the former report (14). Thus, amino acid treatment appears to be somewhat limited in preventing the acute toxic effects of ethanol.

After experimentation with dogs using simultaneous oral administration of either glycine (8.0 g./kg.) or alanine (3.0 g./kg.) and ethanol (5.5 g./kg.), Widmark (6) concluded that an amino acid–ethanol complex is formed with the subsequent reduction of ethanol absorption. The formation of this complex would explain the low peak of ethanol blood levels and the rapid disappearance of ethanol from the blood after amino acid treatment observed in his study.

After simultaneous oral administration of glycine (0.5 g./kg.) and ethanol (1.0 g./kg.) to rats, Haggard and Greenberg (28) also reported the same reductions in blood ethanol concentration (6). These authors concluded that glycine caused a prolonged retention of ethanol in the stomach and that this delay in absorption resulted in the metabolism of ethanol almost as quickly as it was absorbed.

In these investigations (6, 28), no determinations of the degree of ethanol intoxication were made; but because the depressant effects of ethanol are related to blood ethanol levels (26), a reduction in CNS depression would be an expected result with the alterations in blood ethanol concentration described.

In the present investigation, pretreatment with L-lysine (2.50 g./kg.), L-arginine (2.88 g./kg.), L-ornithine (2.31 g./kg.), and

Table IV—Onset of Ataxia after Singe-Dose Administration of Amino Acid–Ethanol Solution

Treatment	Dose of Amino Acid, mg./kg.	Mean Onset[a] of Ataxia, min.	*p* Value[b]
L-Lysine hydrochloride	120	4.92 ± 0.72	0.2
L-Arginine hydrochloride	140	23.16 ± 7.60	0.02
L-Ornithine hydrochloride	111	3.77 ± 0.34	0.2
Glycine hydrochloride	73	2.62 ± 0.22	0.1
Ethanol control	—	3.19 ± 0.20	—

[a] Onset of ataxia values (min.) ± *SE*. [b] *p* value < 0.05 was significant.

Table VI—Onset of Ataxia after Serial Administration of Amino Acid–Ethanol Solution

Treatment	Dose of Amino Acid, mg./kg.	Mean Onset[a] of Ataxia, min.	*p* Value[b]
L-Lysine hydrochloride	120	39.07 ± 3.85	0.001
L-Arginine hydrochloride	140	52.63 ± 7.21	0.001
L-Ornithine hydrochloride	111	24.14 ± 2.54	0.02
Glycine hydrochloride	73	18.47 ± 3.04	0.2
Ethanol control	—	11.98 ± 3.49	—

[a] Onset of ataxia values (min.) ± *SE*. [b] *p* value < 0.05 was significant.

Table VII—Effect of Amino Acid on Ethanol-Induced Ataxia and Sleeping Time with Single-Dose Administration of Ethanol

Amino Acid	Dose	Protection Factor[a]	Percentage Reduction of Sleeping Time[b]
L-Lysine hydrochloride	120 mg./kg.[c]	NS[d]	NS[d]
L-Lysine hydrochloride	2.50 g./kg.[c]	11.55	60.05
L-Lysine hydrochloride	120 mg./kg.[e]	NS	NS
L-Arginine hydrochloride	140 mg./kg.[c]	NS	58.27
L-Arginine hydrochloride	2.88 g./kg.[c]	4.79	43.72
L-Arginine hydrochloride	140 mg./kg.[e]	7.26	NS
L-Ornithine hydrochloride	111 mg./kg.[c]	NS	NS
L-Ornithine hydrochloride	2.31 g./kg.[c]	3.63	51.51
L-Ornithine hydrochloride	111 mg./kg.[e]	NS	NS
Glycine hydrochloride	73 mg./kg.[c]	NS	NS
Glycine hydrochloride	1.53 g./kg.[c]	10.62	98.96
Glycine hydrochloride	73 mg./kg.[e]	NS	NS

[a] Protection factor is the number of times onset of ataxia time for test group is greater than onset of ataxia for control group. [b] Percentage reduction of sleeping time = 100% − percentage of control sleeping time. [c] Amino acid pretreatment. [d] NS = not significant because p value > 0.05. [e] Simultaneous administration.

Table VIII—Effect of Amino Acids on Ethanol-Induced Ataxia and Sleeping Time with Serial-Dose Administration of Ethanol

Amino Acid	Dose	Protection Factor[a]	Percentage Reduction of Sleeping Time[b]
L-Lysine hydrochloride	120 mg./kg.[c]	2.03	NS[d]
L-Lysine hydrochloride	2.50 g./kg.[c]	2.95	55.38
L-Lysine hydrochloride	120 mg./kg.[e]	3.26	11.08
L-Arginine hydrochloride	140 mg./kg.[c]	2.17	NS
L-Arginine hydrochloride	2.88 g./kg.[c]	3.47	56.17
L-Arginine hydrochloride	140 mg./kg.[e]	4.39	39.22
L-Ornithine hydrochloride	111 mg./kg.[c]	1.54	NS
L-Ornithine hydrochloride	2.31 g./kg.[c]	3.01	61.15
L-Ornithine hydrochloride	111 mg./kg.[e]	2.02	NS
Glycine hydrochloride	73 mg./kg.[c]	2.81	NS
Glycine hydrochloride	1.53 g./kg.[c]	4.70	64.57
Glycine hydrochloride	73 mg./kg.[e]	NS	NS

[a] Protection factor is the number of times onset of ataxia time for test group is greater than onset of ataxia for control group. [b] Percentage reduction of sleeping time = 100% − percentage of control sleeping time. [c] Amino acid pretreatment. [d] NS = not significant because p value > 0.05. [e] Simultaneous administration.

glycine (1.53 g./kg.) before the single and serial administration of an ethanol dose (6 g./kg.) resulted in a significant reduction in sleeping time, a prolongation of the onset of ataxia, and a decrease in the number of animals losing the righting reflex. Glycine demonstrated the greatest reduction in sleeping time (98%), while the other three amino acids reduced the sleeping time 50–60%. Ward *et al.* (14), on the other hand, demonstrated a complete prevention of sleeping time with L-lysine pretreatment (2.5 g./kg. p.o.) before a single intoxicating dose of ethanol (4.3 g./kg. p.o.). If a comparison were made between their results and those reported in this investigation, it would seem that there was less antagonism to ethanol intoxication in the latter report. But this might not be so, because the toxicity of ethanol is known to increase with an increase in the concentration and/or dose of ethanol (27).

Schiller *et al.* (1) demonstrated, in humans, that there is a decrease in maximum ethanol blood levels and an increase in the disappearance of ethanol from the blood after simultaneous administration of ethanol orally and an amino acid mixture intravenously. Thus, by separation of the routes of administration of amino acids and ethanol, it becomes apparent that there might be a direct systemic reduction of ethanol blood levels rather than a reduced absorption of ethanol in response to amino acids. This direct antagonism could take the form of either an increase in the metabolism of ethanol by amino acids or a complex formation between amino acids and ethanol or one of its metabolites. Several investigations (1, 6, 28) showed that ethanol metabolism is unaffected by amino acids, so the latter explanation seems most plausible.

Westerfeld and Schulman (29) suggested that: ". . . the chemically reactive acetaldehyde could combine with amino groups (of amino acids, *in vivo*) and alter some biochemical function." Beck *et al.* (30) also suggested that acetaldehyde may react (*in vivo*) with amino acids and demonstrated that these reaction products produce cortical stimulant activity in rabbits.

Robert and Penaranda (15) demonstrated that acetaldehyde, the primary metabolite of ethanol, combines rapidly *in vitro* with amino acids. The combination of acetaldehyde and arginine is the most rapid, followed by glycine, lysine, and ornithine.

It is unknown whether acetaldehyde contributes to the CNS depression due to ethanol intoxication, but there are indications that acetaldehyde potentiates the CNS actions of ethanol (31). Therefore, the combination of acetaldehyde with amino acids could reduce the degree of intoxication produced by ethanol.

Pretreatment with relatively high doses of amino acids, as done in the present investigation, would be expected to increase the concentration of circulating free amino acids available for combination with acetaldehyde and would be the reason why all the amino acids (in this investigation) were effective agents in reducing the depressant

Table IX—Sleeping Time with Amino Acid Pretreatment before Single-Dose Administration of Ethanol

Treatment	Dose	Mean Sleeping Time[a], min.	p Value[b]	Percentage of Rats Responding
L-Lysine hydrochloride	120 mg./kg.	179.32 ± 53.7	0.3	80
L-Lysine hydrochloride	2.50 g./kg.	159.87 ± 40.8	0.001	80
L-Arginine hydrochloride	140 mg./kg.	116.56 ± 48.3	0.05	50
L-Arginine hydrochloride	2.88 g./kg.	224.10 ± 50.3	0.01	70
L-Ornithine hydrochloride	111 mg./kg.	118.20 ± 58.7	0.1	40
L-Ornithine hydrochloride	2.31 g./kg.	193.77 ± 46.1	0.001	70
Glycine hydrochloride	73 mg./kg.	293.15 ± 16.9	0.9	90
Glycine hydrochloride	1.53 g./kg.	4.12 ± 3.2	0.001	20
Saline	1.3 ml./kg.	278.03 ± 58.7	—	80
Saline	13.3 ml./kg.	398.98 ± 17.0	—	100

[a] Sleeping time values (min.) ± *SE*. [b] p value < 0.05 was significant.

Table X—Effect of Amino Acid Pretreatment on Sleeping Times Produced by Serial Administration of Ethanol

Treatment	Dose	Mean Sleeping Time[a], min.	*p* Value[b]	Percentage of Rats Responding
L-Lysine hydrochloride	120 mg./kg.	299.51 ± 42.2	0.7	90
L-Lysine hydrochloride	2.50 g./kg.	170.05 ± 42.5	0.001	70
L-Arginine hydrochloride	140 mg./kg.	293.39 ± 30.9	0.6	100
L-Arginine hydrochloride	2.88 g./kg.	167.89 ± 51.6	0.01	80
L-Ornithine hydrochloride	111 mg./kg.	284.26 ± 51.0	0.6	90
L-Ornithine hydrochloride	2.31 g./kg.	148.29 ± 43.2	0.001	70
Glycine hydrochloride	73 mg./kg.	301.35 ± 56.2	0.8	90
Glycine hydrochloride	1.53 g./kg.	135.39 ± 39.1	0.001	80
Saline	1.3 ml./kg.	322.7 ± 37.3	—	100
Saline	13.3 ml./kg.	381.32 ± 30.3	—	100

[a] Mean sleeping time (min.) ± *SE*. [b] *p* value < 0.05 was significant.

effects of ethanol. L-Arginine, when given at its low dose (140 mg./kg.) by either mode of administration (pretreatment or simultaneous), was the only amino acid used in the present investigation that demonstrated significant protective activity against ethanol intoxication. In the studies of Robert and Penaranda (15), arginine reacted most rapidly with acetaldehyde. Thus, the protective effect of L-arginine may well be dependent on its reaction rate with acetaldehyde. This dependency of amino acids for their protective activity on the rate of reaction with acetaldehyde is not reflected when the high doses of amino acids are used for pretreatment and may be due to a change in the relative reaction rates of the amino acids with acetaldehyde produced by the increased concentrations of circulating free amino acids.

The chronic toxicity of ethanol has also been shown to be ameliorated by the administration of amino acids. Clinically, amino acids have been used, with much success, to treat the following conditions related to chronic ethanol ingestion: delirium tremens (32), nutritional deficiencies (33), and cirrhosis of the liver (34).

Although no toxicity was observed at the amino acid doses used in this study, an excess of amino acids may be toxic; the accumulation of ammonia, urea, and other nitrogen acceptors follows excess amino acid ingestion, especially glycine (35, 36).

Table XI—Sleeping Time after Serial Administration of Amino Acid–Ethanol Solution

Treatment	Dose of Amino Acid, mg./kg.	Mean Sleeping Time[a], min.	*p* Value[b]	Percentage of Rats Responding
L-Lysine hydrochloride	120	297.02 ± 49.9	0.05	90
L-Arginine hydrochloride	140	203.92 ± 35.2	0.01	100
L-Ornithine hydrochloride	111	336.2 ± 34.0	0.9	100
Glycine hydrochloride	73	325.09 ± 33.0	0.9	100
Ethanol control	—	334.01 ± 24.3	—	100

[a] Mean sleeping time values (min.) ± *SE*. [b] *p* value < 0.05 was significant.

Table XII—Sleeping Time after Single-Dose Administration of Amino Acid–Ethanol Solution

Treatment	Dose of Amino Acid, mg./kg.	Mean Sleeping Time[a], min.	*p* Value[b]	Percentage of Rats Responding
L-Lysine hydrochloride	120	223.88 ± 37.7	0.3	90
L-Arginine hydrochloride	140	315.79 ± 58.1	0.9	90
L-Ornithine hydrochloride	111	320.94 ± 41.4	0.8	90
Glycine hydrochloride	73	283.76 ± 54.3	0.9	100
Ethanol control	—	300.95 ± 48.0	—	90

[a] Mean sleeping time values (min.) ± *SE*. [b] *p* value < 0.05 was significant.

Small volumes of saline or buffer solutions used as controls for amino acid pretreatment had no effect on acute ethanol toxicity; this was expected because the absorption of ethanol takes place by simple diffusion with no active processes involved (37).

CONCLUSIONS

Within the framework of this experimental design, amino acid treatment demonstrates a definite antagonism of the intoxicating effects of ethanol. The nature of the interaction between amino acids and ethanol intoxication appears to be related to alterations in ethanol blood levels. Although there is no proof for the formation of an amino acid–acetaldehyde complex *in vivo*, the present study indicates that this may be the possible mechanism by which pretreatment or simultaneous oral administration of amino acids reduces the toxic CNS depressant effects of ethanol.

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Caffeine Complexes with Low Water Solubility: Synthesis and Dissolution Rates of 1:1 and 1:2 Caffeine–Gentisic Acid Complexes

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Abstract □ The syntheses of 1:1 and 1:2 molecular complexes of caffeine with gentisic acid are described, and their rates of dissolution are reported and compared with that of caffeine. Both complexes were less soluble in water than caffeine, and their rates of dissolution in 0.1 *N* hydrochloric acid and in a phosphate buffer at pH 7.5 were less than that of caffeine. These complexes thus present a potentially useful way of formulating caffeine in dosage forms such as chewable tablets that are intended to linger in the mouth. Such dosage forms would only release caffeine slowly and should, consequently, have an improved taste factor over ones containing pure caffeine. The rates of dissolution of the complexes were close to those predicted by equations that take into account both the diffusional and chemical equilibrium processes occurring. These equations are shown to be useful in the selection of a complex to achieve a specific dissolution rate.

Keyphrases □ Caffeine complexes with gentisic acid—synthesis, characterization, solubility, dissolution rate, compared to caffeine dissolution rate □ Dissolution rates of caffeine–gentisic acid complexes—determination, compared to caffeine dissolution rate □ Complexes, caffeine–gentisic acid—synthesis, characterization, solubility, dissolution rate □ Tablets, chewable, potential—caffeine–gentisic acid complexes

Molecular complexes of drugs with other chemicals have frequently been proposed[1] for inclusion in dosage forms to enhance the solubility, chemical stability, and absorption characteristics of the drugs. The present report describes the results of a search for molecular complexes of caffeine that would dissolve less rapidly in aqueous solutions than caffeine. The complexes that were prepared and studied had the stoichiometry of 1 : 1 and 1 : 2 caffeine–gentisic acid. It is believed that caffeine complexes that dissolve less rapidly in water than caffeine provide a useful alternative means of formulating caffeine in chewable tablets and other dosage forms which linger in the mouth. Such dosage forms should have an enhanced taste factor over ones containing pure caffeine, because their caffeine would be released more slowly and, consequently, the intensity of the extremely bitter taste produced by caffeine should be reduced.

The principles involved in this mechanism of taste masking are similar to those involved in the use of ion-exchange absorbates (2) to mask taste.

It has long been recognized that caffeine and other xanthines form molecular complexes with organic acids and organic acid anions (3). The latter type of complex is generally more soluble than the xanthine (soluble complex), while the former is commonly less soluble (insoluble complex). The present report concerns the properties of two insoluble complexes.

EXPERIMENTAL

Chemicals—Caffeine was recrystallized from water, dried under vacuum at 80°, and stored in a desiccator (m.p. 238–238.5°). Gentisic acid was boiled in an aqueous suspension of charcoal and

[1] This subject is reviewed in *Reference 1*.