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Ethanol Consumption Following Acute Fenfluramine, Fluoxetine, and Dietary Tryptophan

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LU, M.-R., G. C. WAGNER AND H. FISHER. *Ethanol consumption following fenfluramine, fluoxetine, and dietary tryptophan.* PHARMACOL BIOCHEM BEHAV 44(4) 931–937, 1993.—Male Sprague-Dawley rats fed a commercial diet with or without tryptophan supplementation (0.5% L-TRP) were treated with single IP injections of fenfluramine or fluoxetine. Rats had been water deprived prior to injection and food was removed during the period of fluid availability. They were offered, following drug or saline injection, water, a 5% ethanol solution, or an isocaloric sucrose solution (8.75%) for 1 h. Fenfluramine injection significantly reduced intake of all fluids, but its effect on ethanol was significantly greater than for water or sucrose solutions. Fluoxetine suppressed water and ethanol intake but not that of sucrose; the reduction in ethanol intake was significantly greater than for water. Ingestion of the tryptophan-supplemented diet in the absence of any drug treatment had no effect on fluid intake. However, the tryptophan supplementation significantly enhanced the reduction in ethanol intake induced by fenfluramine and fluoxetine. It appears that both fenfluramine and fluoxetine decrease ethanol intake more so than that of water or sucrose and that this effect is exacerbated by tryptophan supplementation.

Fenfluramine Fluoxetine Serotonin Ethanol Sprague-Dawley rats Sucrose solution

RECENT studies have indicated that increasing serotonin [5-hydroxytryptamine (5-HT)] in the synaptic cleft significantly attenuates ethanol intake (18,19). Animals treated with IP 5-hydroxytryptophan (the precursor of 5-HT) or intraventricular 5-HT significantly reduced their ethanol intake (13,14). In addition, 5-hydroxytryptophan selectively decreased ethanol consumption in rats with genetically high ethanol intake (1). These observations are consistent with the fact that increasing the availability of 5-HT affects other consummatory behaviors of rats, in particular food intake (6).

In this regard, it has been well established that fenfluramine (FFL), a 5-HT releaser, exerts a potent anorectic effect yet has been little studied in connection with ethanol intake. In one study, it was reported that rats given 20 mg/kg FFL reduced the percentage of fluid consumed as ethanol (7). In addition, it was previously reported that FFL alone or in conjunction with a tryptophan (TRP) supplement suppressed ethanol intake of chickens but did not alter water consumption (4). This latter report is of interest because it was also found that nor-FFL reduced the food intake of rats significantly more than water intake (20). Collectively, these data reveal that serotonergic activation results in a reasonably selective reduction in food and ethanol intake while sparing water intake.

Fluoxetine (FLU), a 5-HT uptake blocker, has also been shown to reduce ethanol intake in animals (10). A 5-mg/kg dose of FLU reduced consumption of a 10% ethanol solution by 80% in an ethanol-preferring (P) line of rats without decreasing water intake (12). In a study using a procedure similar to the one employed in the present investigation, it was reported that both FFL and FLU significantly reduced consumption of a hypertonic saline solution (1.8%) but did not affect either water or isotonic salt intake (16). Therefore, like FFL, FLU reduces ethanol consumption at doses that do not affect water intake. However, the dissociation of the anorectic actions of these compounds from their effects on ethanol intake remains problematic (5). Accordingly, one objective of the present study was to evaluate the effects of FFL and FLU on ethanol intake and further determine the treatment specificity by offering animals water and a sucrose solution isocaloric with the ethanol solution.

A second factor that was examined was the possibility that TRP supplementation of the diet might potentiate the suppressant effects of FFL and FLU on ethanol consumption. Atten-

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tion has been called to the specific value of the precursor (TRP) to its neurotransmitter (5-HT) pathway, in contrast to drugs, which "even if they are relatively specific, often impact upon multiple neuronal systems and have receptor effects unlike the endogenous neurotransmitter" (2). In this regard, it was found that dietary tryptophan supplementation was ineffective in modulating alcohol-induced intoxication (22) but caused a reversal in alcohol-induced impairment of facial recognition (21). Similarly, TRP use has been explored in combination with drugs for treatment of depression (3) and of chronic, disabling pain (17). However, with regard to the regulation of ethanol intake the use of dietary TRP in combination with drugs such as FFL or FLU seems not to have been studied.

METHOD

Subjects

Two groups of 24 male Sprague-Dawley rats (125–150 g body weight) were used, one for the FFL study and the other for the FLU study. Each of the groups was subdivided into two groups of 12 rats each, one as a "control" subgroup, which was fed a regular commercial rat diet; the other subgroup was fed the same diet with a 0.5% L-TRP supplement (the commercial diet supplied 0.3% TRP). All rats were maintained on ad lib food and water on a 12 L : 12 D cycle (light 7:00 a.m.–7:00 p.m.) in a temperature-controlled room. Rats were individually housed in standard size (24.5 × 18 × 18 cm^3) wire mesh bottom cages. Glass drinking bottles, 250 ml, with rubber stoppers and ball-bearing-tipped metal sipper tubes delivered water and ethanol and sucrose solutions. Rats were allowed to adjust to laboratory conditions and the two diets for 1 week prior to the start of the experiments.

Procedure

For each of the two groups of rats, there were three phases of study: First, an effort was made to determine a concentration of ethanol that would be consumed in amounts approximating water intake; second, a dose–response curve for the effects of presession FFL or FLU on ethanol intake was determined; finally, the 8.0-mg/kg dose of FFL or FLU that significantly reduced ethanol intake was further evaluated in these same rats offered a sucrose solution isocaloric with the ethanol.

In determining an appropriate ethanol concentration, all rats were first water deprived for 24 h and then injected IP with saline (1 ml/kg body weight), usually between 11:00 and 11:30 a.m. Next, rats, randomly, were given access to water or 3% (v/v) or 5% (v/v) ethanol solution for exactly 1 h. Each solution was given to each rat once, with 3 days intervening between each treatment. Food was removed during the drinking period only. Fluid consumption was recorded at the end of the 1-h period of fluid availability. This procedure was carried out twice, once for rats to be tested with FFL injections and again for those allotted to the FLU phase of the study. The 24-h water deprivation was employed because it led to an intake of the 5% ethanol solution in amounts that did not significantly differ from water. This 5% ethanol concentration was, therefore, chosen for subsequent drug treatment.

For the dose–response determinations, the experimental procedures were followed as described above. Rats in the FFL (24) and FLU (24) groups were randomly assigned to be injected, IP, with 0 (saline), 2, 4, or 8 mg/kg body weight of FFL or FLU, respectively. The fluids supplied after injection were either water or 5% (v/v) ethanol solution. Each dose and fluid was administered to each rat once and 3 days intervened between treatments, during which time rats had full access to their respective diet and water. The drug dosage was chosen on the basis of a plateauing effect on water intake when rats consumed the unsupplemented, control diet.

Finally, when an effective dose of FFL and FLU in relation to a decrease in ethanol consumption had been determined (8 mg/kg) the same methodology as described above was used to randomly inject FFL or FLU, IP, followed 30 min later with the availability of water, 5% ethanol, or 8.75% sucrose solutions (the sucrose solution was isocaloric with the 5% ethanol solution). All pre- and posttreatment measurements were the same as in the previous phases of this investigation and 3 days intervened between each treatment.

Statistical Analysis

SAS analysis of variance (ANOVA, repeated measures) was used to compare treatment means. Posthoc individual treatment means were compared by one-factor ANOVA and Fisher's PLSD test. The drug dose–response data were analyzed by linear regression.

RESULTS

The consumption of different ethanol solutions (0, 3, and 5%) by saline-injected rats on either the control or TRP-supplemented diets was similar for rats used for the FFL as well as the FLU experiments. The results were, therefore, combined and are presented in Fig. 1. There were no differences in the amount of fluid consumed as 3 or 5% ethanol in either the control or TRP-supplemented group; therefore, the 5% ethanol solution was selected for the next phases of these experiments. TRP supplementation, however, had a small but significant ($p = 0.04$) reducing effect on the intake of the 5% ethanol solution in comparison with the intake on the control (unsupplemented) diet.

The log response curves for water consumption of rats on both diets showed significantly linear relationships (control diet group $r = 0.60$, TRP-supplemented diet group $r = 0.65$) to dosage of FFL (Fig. 2a). The presession administration of FFL caused a significant ($p < 0.0001$) dose-dependent decrease in water consumption (Fig. 2a) with no significant ef-

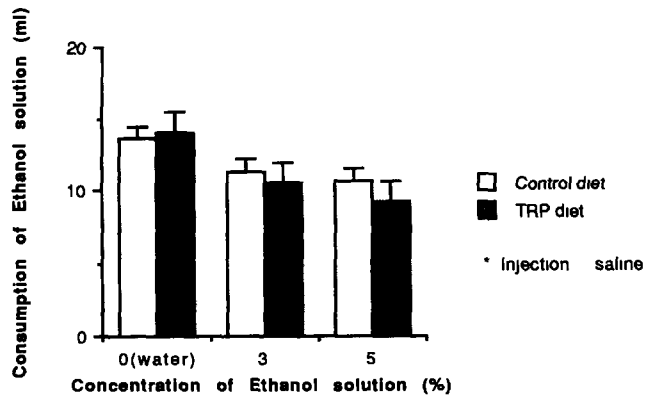
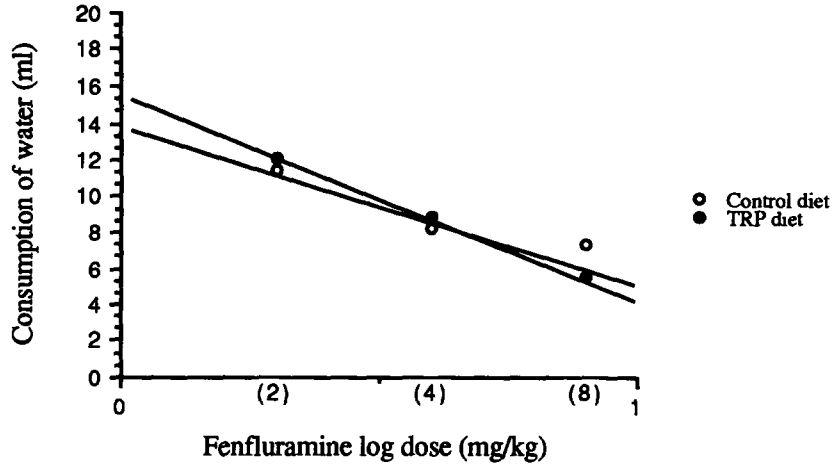


FIG. 1. Consumption of different ethanol concentrations by saline-injected rats.



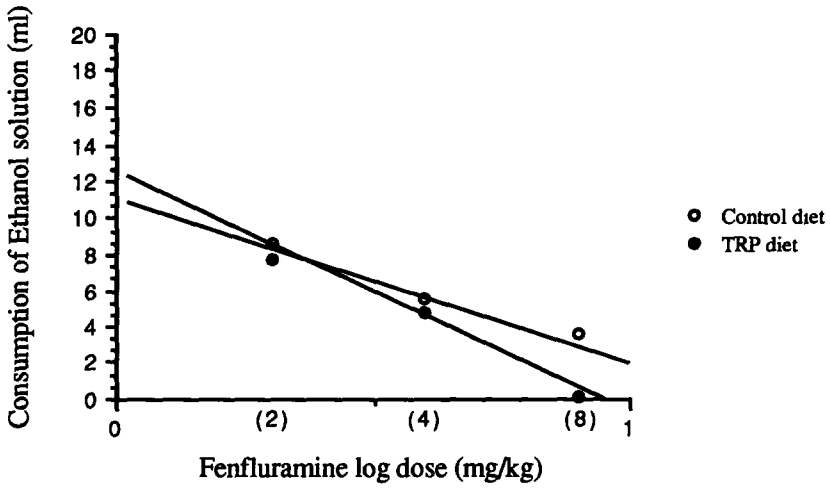


FIG. 2. (a) Fitted, linear regression lines between log dose fenfluramine and water consumption. Numbers in brackets are the administered doses. Regression data: control group, $y = 13.6 - 8.0\times$; $p = 0.0001$; $r = 0.602$; SEM = 2.7; TRP group, $y = 15.4 - 10.8\times$; $p = 0.0001$; $r = 0.647$; SEM = 3.2. (b) Fitted, linear regression lines between log dose fenfluramine and consumption by rats of a 5% ethanol solution. Numbers in brackets are the administered doses. Regression data: control group, $y = 10.8 - 8.5\times$; $p = 0.0002$; $r = 0.587$; SEM = 2.9; TRP group, $y = 12.1 - 12.8\times$; $p = 0.0001$; $r = 0.871$; SEM = 1.8.

fect of diet or dose × diet interaction. Posthoc analysis revealed that rats in the TRP-supplemented group consumed significantly ($p < 0.05$) less water following administration of 8 mg/kg FFL as compared to saline or 2 mg/kg.

For FLU-dosed rats, water consumption was also significantly linearly correlated (control diet group $r = 0.72$, TRP-supplemented group $r = 0.62$) to FLU dose on both diets (Fig. 3a). There was no significant difference in water consumption between rats on the control vs. those on the TRP-supplemented diet. Posthoc analysis showed a significant ($p < 0.05$) reduction in water consumption by rats in both diet groups administered the 8-mg/kg dose of FLU as compared to saline or 2 mg/kg FLU.

Rats on the TRP-supplemented diet showed a significantly ($p = 0.0034$) lower intake of ethanol solution in comparison with those on the control diet (Fig. 2b). The TRP-supplemented diet group at the 8-mg/kg dosage of FFL drank almost no ethanol solution at all (only 2.2% that of rats on the control diet).

Rats administered FLU also significantly ($p < 0.0001$) decreased their ethanol consumption on the control diet; however, there was no linear relationship with dosage of FLU. Rats on the TRP-supplemented diet drank significantly ($p = 0.0017$) less ethanol solution than control-fed rats receiving the same dosage of FLU (Fig. 3b). The linearity of the relationship between dosage of FLU and ethanol solution con-

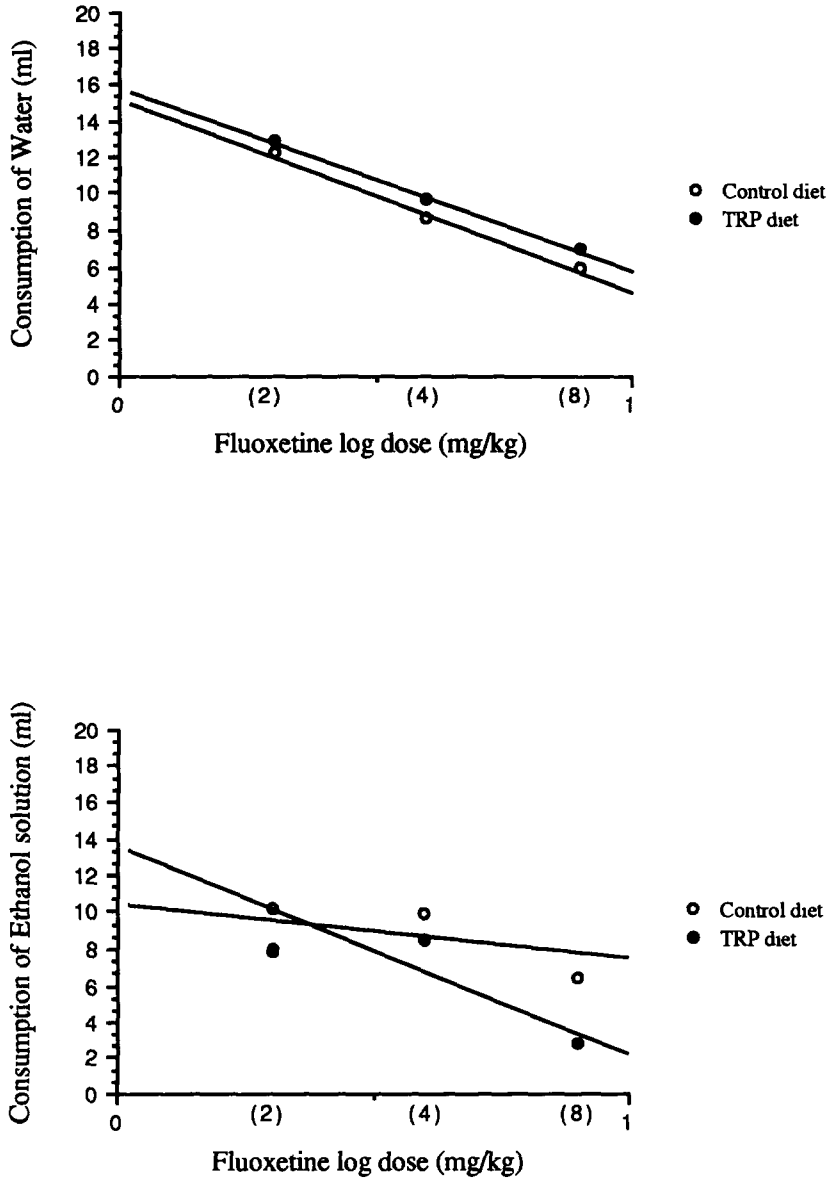


FIG. 3. (a) Fitted, linear regression lines between log dose fluoxetine and water consumption. Numbers in brackets are the administered doses. Regression data: control group, $y = 15.2 - 10.5\times$; $p = 0.0001$; $r = 0.723$; SEM = 2.5; TRP group, $y = 15.8 - 9.8\times$; $p = 0.0001$; $r = 0.625$; SEM = 3.1. (b) Fitted, linear regression lines between log dose fluoxetine and consumption by rats of a 5% ethanol solution. Numbers in brackets are the administered doses. Regression data: control group, $y = 9.6 - 2.6\times$; $p = 0.158$; $r = 0.24$; SEM = 2.6; TRP group, $y = 11.4 - 8.3\times$; $p = 0.0001$; $r = 0.623$; SEM = 2.6.

sumption for the TRP-supplemented group was significant ($p = 0.0001, r = 0.62$).

Because 8 mg/kg FFL caused a significant decrease in ethanol consumption while having less of an effect on water intake, it was chosen for the critical next series of experiments. At this dose, rats behaved completely normally, moved about the cage, and showed no signs of vasodilation previously noted by us after higher dosages (8). As shown in Fig. 4a and Table 1, following saline injection, the 8.75% sucrose solution was consumed in significantly ($p = 0.0001$) greater amount by rats on the control diet than was water or the ethanol solution. Rats on the TRP-supplemented diet consumed significantly ($p = 0.0001$) more water and sucrose solution than ethanol solution after saline injection.

In general, FFL injection caused a significant reduction in the consumption of all these fluids, $F(1, 23) = 52.55$; $p < 0.0001$, compared to saline injection. Posthoc analysis showed, however, that FFL, in both diet treatments, significantly reduced ethanol consumption ($p < 0.05$) below that of either water or sucrose solution. Sucrose solution was con-

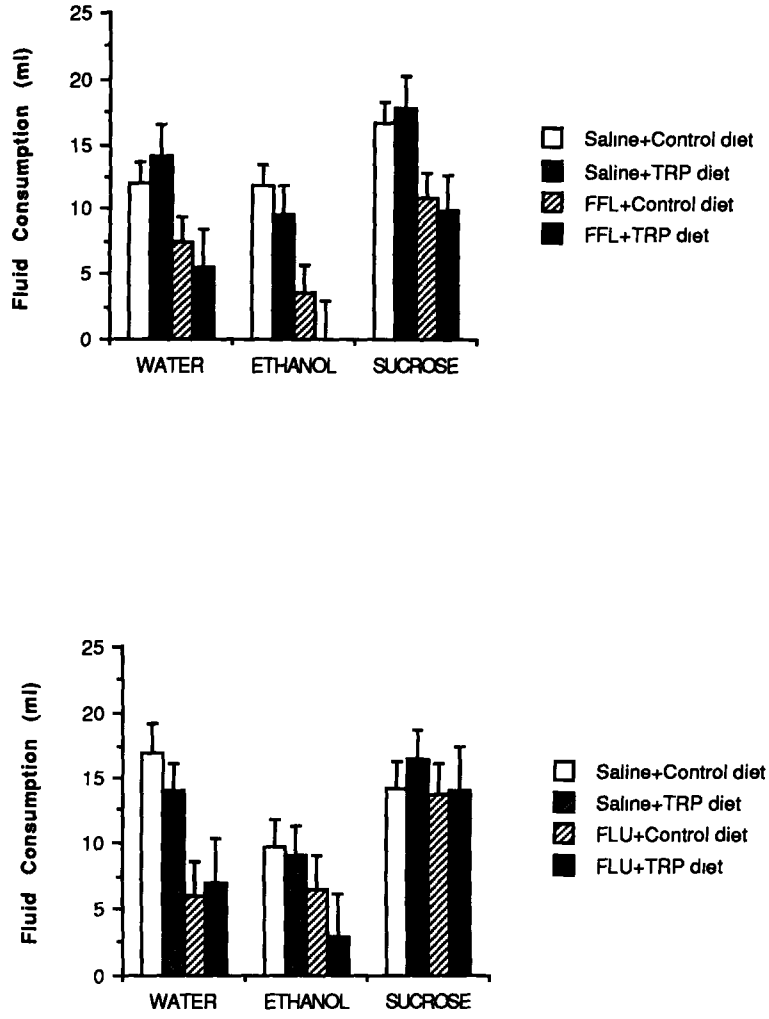


FIG. 4. (a) Fluid consumption (water, 5% ethanol, and 8.75% sucrose solutions) of rats injected, IP, with saline or 8 mg/kg fenfluramine and fed a commercial diet with or without tryptophan supplementation. (b) Fluid consumption (water, 5% ethanol, and 8.75% sucrose solutions) of rats injected, IP, with saline or 8 mg/kg fluoxetine and fed a diet with or without tryptophan supplementation.

sumed in significantly ($p < 0.05$) greater amount following FFL injection than was water, again, irrespective of diet treatment. FFL injection of rats fed the TRP supplement resulted in a highly significantly ($p = 0.0015$) greater suppression of ethanol intake in comparison with rats on the unsupplemented control diet.

The results for FLU were in general similar, with certain exceptions, to those for FFL (Fig. 4b and Table 1). As was the case for FFL, the 8-mg/kg dose was also chosen as an appropriate concentration for FLU that would suppress ethanol intake. Following saline injection (Fig. 4b), regardless of diet, water and sucrose solution intake was similar and significantly ($p < 0.0001$) higher than ethanol intake. FLU injection caused a reduction in consumption, $F(1, 23) = 116.18$; $p < 0.0001$, of water and ethanol but not of sucrose solution. TRP supplementation significantly ($p = 0.0002$) exacerbated the FLU effect on ethanol consumption, producing a decrease to a level approximately half that noted for the unsupplemented control diet group of rats.

Posthoc analysis (Fisher's PLSD and Scheffe's F-test) of ethanol intake comparing rats fed the control diet and injected with saline to those injected with FLU showed a highly significant decrease in intake due to FLU ($p = 0.0009$). Similarly, the ethanol consumption on the TRP-supplemented diet was also significantly decreased by FLU injections ($p = 0.0001$).

DISCUSSION

These results confirm earlier reports that acute administration of FFL and FLU, among other serotonergic agonists, suppress ethanol, water, and other fluid intakes (5-7,11,16,18) although the action on ethanol was more pronounced, primarily in the presence of tryptophan, than for water or sucrose solutions. It has been suggested that the anorectic properties

TABLE 1

PERCENT CHANGE IN FLUID INTAKE FOLLOWING FFL OR FLU COMPARED TO SALINE INJECTIONS

Diet	Drug (8.0 mg/kg)	Change in Fluid Intake (%) Water	Ethanol (5%)	Sucrose (8.75%)
Control	FFL	−41.2*[a] (±7.4)	−69.0[b] (±8.1)	−35.2[a] (±7.4)
	FLU	−61.8[a] (±6.4)	−33.4[b] (±5.7)	−2.8[c] (±0.1)
TRP	FFL	−58.0[a] (±9.5)	−99.0[b] (±0.7)	−42.3[a] (±7.0)
	FLU	−48.3[a] (±6.3)	−68.0[b] (±3.8)	−14.0[c] (±0.1)

*Values with different superscripts within each row are significantly different from each other, $p < 0.05$.

of FFL and FLU may account for their ability to suppress ethanol intake (15). Based upon the present study, however, it would appear that this hypothesis does not fully account for the reduced ethanol consumption. Even when FFL produced a decrease in sucrose solution intake, rats still consumed as many calories from sucrose as they did from ethanol following saline injections. Further, there was no reduction in sucrose intake following FLU administration. Finally, sedation was not apparent following administration of either drug and the slopes of the dose-response curves were dissimilar, an effect that precluded drug comparisons.

The present studies extend prior knowledge in the area of suppression of ethanol intake by serotonin agonists by showing that a dietary TRP supplement, which exerted only a small effect when administered alone, significantly exacerbated the ethanol intake suppressant effect of acute FFL and FLU in water-deprived rats. In the case of FLU, the reduction in ethanol consumption was more selective because the consumption of an isocaloric sucrose solution was not affected by the drug/TRP treatment.

In the absence of neurochemical analyses of the brains of these rats, we conjecture that TRP increased synthesis of 5-HT that was stored, thereby providing a greater synaptic cleft concentration following drug treatment. Alternatively, concentrations of TRP bound to a plasma albumin might have been increased and served as a source of 5-HT as drug treatment depleted the stores. Time considerations between drug injection and the measurement of ethanol consumption make this latter suggestion unlikely. It would be of interest also to determine if this potentiation by tryptophan of FFL and FLU's effect on ethanol intake were due to central vs. peripheral effects and if it would be maintained over repeated administrations. Finally, the findings reported in the present study were based upon a single, random test day for all animals. We have, however, repeated the main treatment with other groups of rats with similar results. In addition, the reduction in ethanol intake due to FFL and FLU, and exacerbated by dietary TRP, has been noted in chickens (9).

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