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Acute Amino Acid Loads That Deplete Brain Serotonin Fail to Alter Behavior

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BROWN, C. M., P. J. FLETCHER AND D. V. COSCINA. *Acute amino acid loads that deplete brain serotonin fail to alter behavior.* PHARMACOL BIOCHEM BEHAV **59**(1) 115–121, 1998.—Acute depletion of brain serotonin (5-HT) can be induced in both rats and humans by giving an amino acid load deficient in tryptophan (TRY). Because this treatment is relatively easy to administer and short-acting, it seems well suited for studying mood and/or behavioral changes linked to aberrant 5-HT functioning in humans. To investigate the ability of a TRY-deficient amino acid load to induce behavioral changes in animals, this study measured performance on an operant schedule of differential reinforcement of low rates of responding (DRL), locomotor activity in both novel and familiar environments, and paw-lick latencies on a hot-plate. All of these measures have been found previously to be altered by impairments of the brain 5-HT system. Adult male Sprague–Dawley rats were administered by gavage an amino acid load lacking TRY, an amino acid load containing TRY, or distilled water. Three hours later, behavioral tests were conducted. Although 5-HT levels were decreased in the hippocampus (−23.3%) and 5-hydroxyindoleacetic acid (5-HIAA) levels were decreased in the striatum (−35.1%) and hippocampus (−38.5%), there were no effects of the TRY-deficient load on any of the behavioral tests. Because reliable mood-altering effects have been reported in human subjects using this method, their behavioral counterparts may be too subtle to observe in animals. Alternatively, effects observed in humans may reflect nonserotonergically mediated consequences of such tryptophan deficient amino acid loads and/or preexisting abnormalities of 5-HT or other systems in such people. © 1998 Elsevier Science Inc.

5-HT Impulsivity Open field Pain Activity Amino acids Precursor Tryptophan

BRAIN serotonin (5-hydroxytryptamine, 5-HT) is an indoleamine contained in cell bodies that originate along the midline/raphe region of the midbrain and project to various forebrain regions, including the limbic system (5). One proposed function of brain 5-HT is to control behavioral inhibition (23,38,39). In animals, reduced 5-HT functioning is associated with increased locomotor activity in a familiar environment (19), increased aggression (18,21), and impairment on measures of response inhibition (16,17,40). Altered 5-HT neurotransmission has also been linked to a number of psychiatric disorders in humans (3,13). In particular, studies measuring cerebrospinal fluid levels of the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) indicate that low 5-HT activity is associated with suicide (4), aggression (9,29), alcoholism (8,28), bulimia (27), and mood disorders (10). All of these disorders have been linked to impulsivity or lack of response inhibition (11).

In recent years a technique used to study the acute effects of reducing 5-HT function in humans involves administering amino acid mixtures that are high in amino acids which compete with tryptophan (TRY) for brain uptake but lack this precursor to 5-HT. This manipulation, which substantially lowers plasma TRY levels, has been shown in animal studies to reduce brain (32) and frontal cortex (24) 5-HT and 5-HIAA levels by between 40 and 50%. Acute TRY depletion has several potential methodological advantages for experimental purposes: it reduces brain 5-HT in a reversible manner, the same basic method can be used to study behavioral processes in both humans and animals, and the procedure is relatively nonintrusive.

Studies involving human subjects have shown that acute TRY depletion lowers mood ratings (7), produces depressive relapses in patients who had previously responded to serotonergic antidepressant treatment (12), increases aggressive be-

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havior in males who have consumed alcohol (35), and exacerbates body dysmorphic disorder (6), premenstrual syndrome (31), and autistic disorders (30). However, some authors have failed to find any effects of TRY depletion on 5-HT–mediated processes such as mood (33,34), appetite (33,34), or anxiety (22,26).

Although there have been a number of recent studies using acute TRY depletion as a method of studying the contribution of the 5-HT system to neuropsychiatric illness in humans, this procedure has not, to the best of our knowledge, been used as an investigative tool in animal subjects. In rats, acute or chronic reductions in 5-HT activity lead to impaired acquisition and/or performance of a lever-press response sustained by a schedule of differential reinforcement for low rates of responding (DRL). On this schedule, responses are reinforced only after a specified period of time has elapsed since the previous response. Thus, successful performance requires episodic inhibitory control over operant output. Given the emergent use of TRY depletion in human subjects and the link between low 5-HT and impulsivity in humans and response inhibition in animals, the present study examined the effects of TRY depletion on response inhibition using performance on a DRL schedule. The effects of TRY depletion on other behaviors previously shown to be sensitive to disruption of brain 5-HT function were also examined, namely spontaneous locomotor activity (19) and analgesia (36). In addition, brain neurochemical measurements were employed to determine the extent of 5-HT and 5-HIAA changes after TRY depletion.

METHOD

Subjects

A total of 48 adult male Sprague–Dawley rats were used for all experiments. Animals were purchased from the Charles River Company (St. Constant, Quebec) and were double-housed in plastic cages with free access to water at all times in a temperature (22 ± 2°C)- and humidity-controlled room with lights on from 0900 to 2100 h. Upon arrival, subjects weighed between 270 and 300 g. Rats in the DRL experiment as well as in the second phase of activity/paw-lick testing and neurochemical studies (see below) were reduced to approximately 90% of their free-feeding weights by giving each pair 40 g of food (Purina Chow pellets) per day for the remainder of the experiments. Home-cage feeding always took place at least 1 h posttest. All behavioral and neurochemical studies were conducted during midlight cycle and controlled with respect to time of day when testing took place.

Experimental Treatments

Intragastric loads. All intragastric loads were given by gavage. Following a 1-week period of daily habituation to this procedure, each subject received 10 ml/kg of either distilled water, an amino acid load lacking tryptophan (TRY−), or an amino acid load including tryptophan (TRY+). The ratio of amino acids in these mixtures (see Table 1) was the same as reported previously (12).

Drug administration. Once animals had been tested on the DRL schedule with the amino acid loads, testing was conducted with chlordiazepoxide (CDZ), which served as a positive control (37). CDZ (generously provided by Hoffmann–La Roche, Mississauga, ON) was dissolved in sterile physiological saline (0.9%). Either the saline vehicle or a dose of 10 mg/ml/kg CDZ was injected subcutaneously 30 min prior to testing.

TABLE 1
AMINO ACID FORMULA

Amino Acid	Amount (g)
l-Alanine	0.825
l-Arginine	0.735
l-Cysteine	0.405
Glycine	0.480
l-Histidine	0.480
l-Isoleucine	1.200
l-Leucine	2.030
l-Lysine HCl	1.650
l-Methionine	0.450
l-Phenylalanine	0.855
l-Proline	1.830
l-Serine	1.040
l-Threonine	1.040
l-Tyrosine	1.040
l-Valine	1.340
Total	15.40
l-Tryptophan	0.346

The mixture was dissolved in 22.5 ml of 0.25% agar–agar and sonicated for approximately 1 min to enhance solubility.

DRL Responding

Twenty-four subjects were tested in this phase. Operant testing was carried out in eight chambers measuring 28 cm long, 21 cm wide, and 21 cm high (Med Associates Inc., Georgia, VT). Each chamber contained a food pellet dispenser and a response lever that was 4.5 cm wide and 7 cm above the floor of the chamber. The center of the lever was located 6.5 cm to the left of a central food hopper positioned 3 cm from the floor of the chamber. Each chamber was illuminated by a house light and was contained within a sound-attenuating box equipped with a ventilating fan. Apparatus control and data collection were accomplished with a 386-SX IBM-type computer.

Rats were shaped to bar press for 45 mg food pellets (Noyes Formula A, Lancaster, NH). Once subjects had learned this task, they were transferred to a DRL20 schedule of reinforcement in which bar presses were reinforced only if at least 20 s had elapsed since the previous response. Any responses occurring prior to this time were not rewarded and reset the 20-s requirement. A total of 50 daily sessions were required to produce stable baseline responding.

Three hours after receiving the intragastric load (water, TRY+, or TRY−), or 30 min after receiving the injection of CDZ or its vehicle, subjects' performance was assessed by measuring the number of responses made, number of reinforcements earned, overall response efficiency (percentage of responses that resulted in the delivery of a reinforcer), and the mean interresponse time (IRT). Behavioral testing was conducted at least 3 days apart, with subjects being run as usual on the DRL20-s schedule on nondrug days. All treatments were administered to all rats in a counterbalanced order across subsets of animals. At the conclusion of these experiments, rats' body weights ranged from 450 to 500 g.

Locomotor Activity

A separate group of 24 rats was tested in this phase. Activity tests were conducted in four Plexiglas activity chambers

(Med Associates Inc., Georgia, VT) measuring 40 cm long, 40 cm wide, and 28 cm high. Ambulatory (horizontal) movement was detected by two arrays of 16 infrared beams, while a third array positioned 10 cm above the floor detected vertical movement.

Two phases of testing were conducted. The first consisted of rats being randomly assigned to one of three groups of eight, each receiving either water, TRY+, or TRY− and then tested 3 h later. This constituted a test of activity in a novel environment. The second phase took place after 4 weeks of adaptation to food restriction as described for the DRL study. It consisted of the same subjects being placed in the test apparatus undrugged for 30 min per day for 15 days. Following this, rats were rank ordered on their baseline activity levels, assigned to one of the same three treatment groups equated for basal activity, and tested following one of the three gavage conditions. This constituted a test of activity under familiar conditions. Each test session lasted 30 min.

Paw-lick latencies were tested by placing each subject on a hot plate analgesiometer (Omnitech Electronics, Columbus, OH) that measured 28 cm long, 28 cm wide, and 18 cm high. The temperature of the metal floor was held at 54°C. Paw-lick latencies were timed to the nearest 1/100th s. The timer was activated when a rat was placed on the heated surface and deactivated when any one paw was lifted and licked. The paw-lick tests were conducted immediately after each locomotor activity test. The data from two animals' first test days were omitted due to experimenter error.

Neurochemistry

To approximate the neurochemical state of rats during the behavioral test phases, the 24 subjects used in the locomotor activity experiment were divided into three new groups of eight equated for activity and given one of the three intragastric loads while still under the food restriction schedule. Three hours later, each rat was sacrificed by decapitation, then their brains removed and placed on a cold plate. Frontal cortex, hippocampus, and striatum were dissected free from the rest of the brain, frozen, and then stored at −70°C for later HPLC analyses. At the conclusion of these experiments, rats' body weights ranged from 450 to 550 g.

5-HT, norepinephrine, dopamine, 5-HIAA, homovanillic acid, and DOPAC were extracted from the tissues harvested in 0.1 N perchloric acid, containing 2 mM sodium bisulphite as an antioxidant, then were analyzed using high-performance liquid chromatography (HPLC) with electrochemical detection. The analytical equipment consisted of a Waters 600 multisolvent pump, a Highchrom 250 × 4.6 mm column with ODS2 5 m packing material, an ESA Coulochem 5100A detector with 5011 analytical cell, a TSP AS3000 refrigerated autosampler, and a Spectra Physics SP4290 integrator. The aqueous mobile phase consisted of 0.1 M sodium acetate, 0.74 M glacial acetic acid, 0.13 mM EDTA, 4% methanol, and octane sulphonate (130 mg/l). Two 100 ml samples of diluted brain area extracts (3:1 to 40:1 depending on the area) were sequentially injected; the first was the pure brain extract, the second a 50:50 mixture of brain extract and monoamine standard cocktail. Measurement of integrated peak areas was used to quantitate both the standard and the sample monoamine concentrations (ng/mg of brain tissue).

Statistical Analyses

Dependent measures from the DRL experiments were subjected to separate one-way repeated measures analyses of variance (ANOVAs) for the TRY depletion experiment, and to paired t-tests for the CDZ experiment. Further analysis of the interresponse times was done using separate two-way ANOVAs with treatment (TRY depletion study) or dose (CDZ study) and time as factors. Any post hoc comparisons were made using Dunnett's test.

Dependent measures for the activity tests were subjected to repeated measures two-way ANOVAs with environment and treatment as factors. Separate two-way repeated measures ANOVAs were conducted for both activity measures under novel and habituated environment tests using treatment and time as factors.

The results from the hot-plate tests were analyzed using one-way ANOVAs, with paw-lick latencies as the dependent variables. Neurochemical analyses were carried out using separate one-way ANOVAs, with post hoc comparisons made using Tukey's test.

RESULTS

DRL Responding

Figures 1 and 2 summarize the results from the DRL experiments. A one-way repeated measures ANOVA (see Fig. 1) indicated that, in comparison to the water control, both types of amino acid loads produced small but significantly reliable decreases in the number of responses made [TRY− =

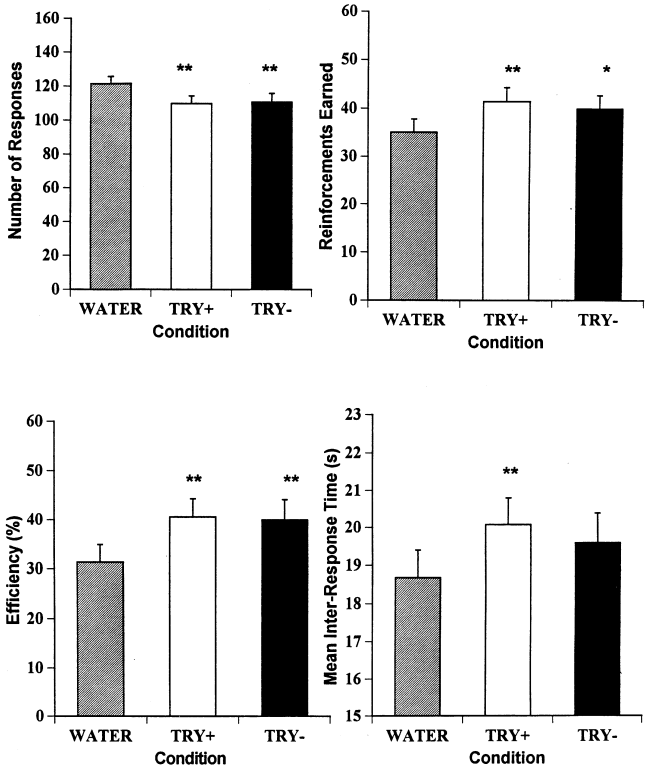

FIG. 1. The effects of water, a balanced amino acid load (TRY+), or a load lacking tryptophan (TRY−) on DRL performance. In comparison to water control, both types of amino acid load significantly decreased the number of responses made (upper left), increased the number of reinforcements earned (upper right), increased the overall efficiency (lower left), and raised the mean interresponse time (lower right). $*p < 0.001$ (Dunnett's test), $*p < 0.01$.

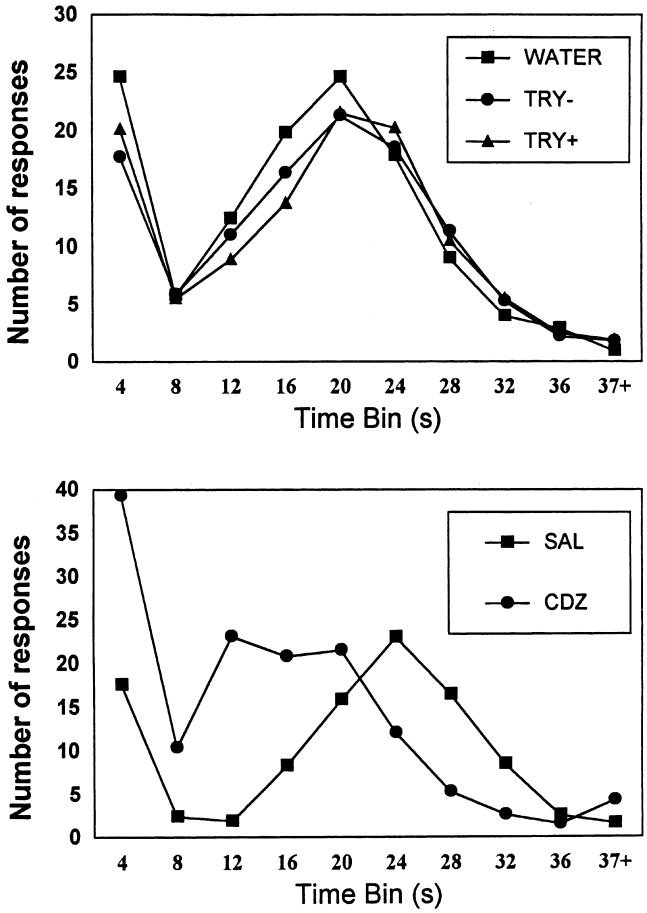

FIG. 2. Frequency distribution of interresponse times for DRL performance for amino acid experiment (upper panel) and CDZ (lower panel).

−9%, TRY+ = −9.5%; $F(2, 46) = 7.799, p < 0.001$] while increasing the number of reinforcements earned [TRY− = +14%, TRY+ = +18%; $F(2, 46) = 5.654$, $p < 0.006$], the overall efficiency of responding [TRY− = +28%, TRY+ = +30%; $F(2, 46) = 6.949$, $p < 0.002$], and the mean IRT [TRY− = +5%, TRY+ = +8%; $F(2, 46) = 4.12$, $p = 0.023$]. The latter measure was calculated after omitting all responses that occurred during the first 4 s following a reinforcement. However, there was no effect of the TRY− mixture when compared with TRY+ on any of these measures.

By comparison, the administration of CDZ (10 mg/kg) impaired overall DRL performance substantially (data not graphed) by increasing the number of responses made by 40% [saline = 97.9 ± 4.2; CDZ = 140.6 ± 11.4; $t(8) = -3.678, p < 0.008$], reducing by over 50% the number of reinforcements earned [saline = 52.9 ± 2.2; CDZ = 26.4 ± 3.2; $t(8) = -6.662, p < 0.001$], decreasing by about two-thirds the overall efficiency of responding [saline = 51.5% ± 6.8; CDZ = 20.5% ± 4.1; $t(8) = 4.046, p < 0.005$], and decreasing by almost one-quarter the mean IRT [saline = 22.1 ± 0.6; CDZ = 17.5 ± 1.0; $t(8) = 4.623, p < 0.002$].

Frequency distributions for both the amino acid and the CDZ experiments are summarized in Fig. 2. For the amino acid experiment, a two-way repeated measures ANOVA revealed overall main effects of treatment, $F(2, 46) = 7.695, p < 0.001$, time, $F(9, 207) = 29.933$, $p < 0.001$, and a significant treatment × time interaction, $F(18, 414) = 3.424$, $p < 0.001$. These results revealed that both amino acid conditions lowered the number of responses made in the short (0–4 s) time bin as well as the number of responses in the 12–16 and 16–20-s time bins. In the positive control CDZ experiment there were main effects of treatment, $F(1, 7) = 13.545$, $p < 0.008$, time, $F(9, 63) = 18.604, p < 0.001$, and for the treatment × time interaction, $F(9, 63) = 11.151$, $p < 0.001$. This confirmed that CDZ increased the number of responses made in the 0–4 s time bin, plus shifted the entire frequency distribution to the left.

Locomotor Activity

Novel environment. Results from the locomotor activity tests when the environment was novel are summarized in Fig. 3. A two-way repeated-measures ANOVA on ambulatory counts revealed a significant main effect of treatment, $F(1, 22) = 4.964$, $p < 0.036$, with the water group being significantly more active than both amino acid groups, and a significant effect of time, $F(5, 110) = 2.388, p = 0.043$. However, the treatment × time interaction was not significant, $F(5, 110) = 0.187$, $p = 0.967$. For vertical counts there was a significant main ef-

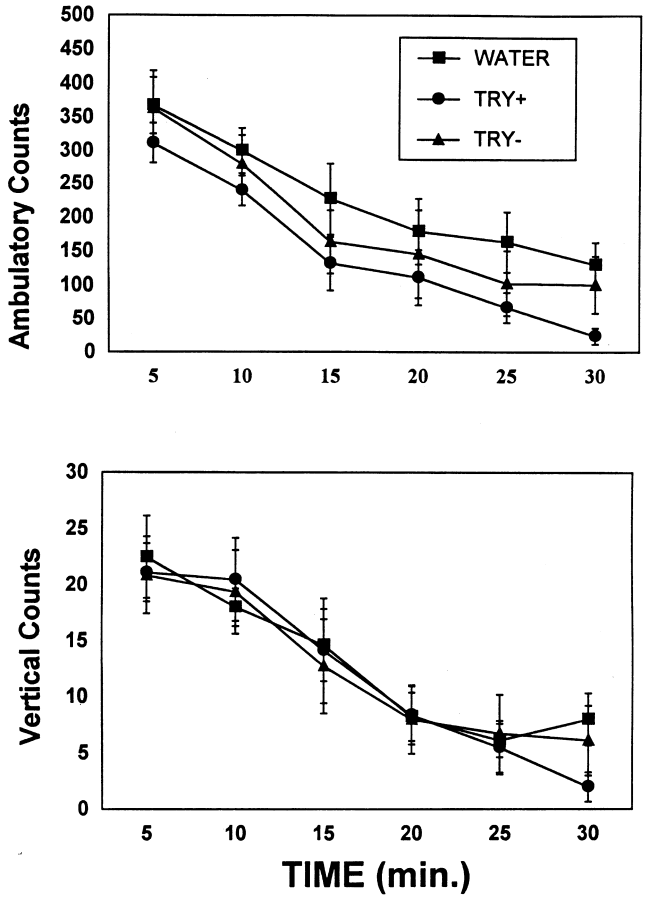

FIG. 3. The effects of water, a balanced amino acid load (TRY+), or a load lacking tryptophan (TRY−) on locomotor activity in a novel environment. There was a significant effect of time for both ambulatory (upper panel) and vertical counts (lower panel). There was also a significant effect of treatment for ambulatory counts, with the water group being significantly more active than both amino acid groups across all time bins.

fect of time, $F(5, 110) = 2.763$, $p = 0.022$, but no treatment, $F(1, 22) = 0.109$, $p = 0.745$, or treatment × time interaction, $F(5, 110) = 0.704$, $p = 0.621$.

Familiar environment. The effects of amino acid loads on locomotor activity in a familiar environment are summarized in Fig. 4. A two-way repeated measures ANOVA was performed to determine if repeated exposure to the activity test resulted in a reduced level of movement. This test revealed a significant interaction between environment and time for both ambulatory, $F(5, 225) = 8.310$, $p < 0.001$, and vertical, $F(5, 225) = 9.689$, $p < 0.001$, counts in the predicted direction of less activity on both measures when tested in the familiar environment. Amino acid treatment produced no effects on activity levels under this familiarity condition. However, there was a significant main effect of time for ambulatory counts, $F(5, 115) = 30.947$, $p < 0.001$, and for vertical counts, $F(5, 115) = 29.264$, $p < 0.001$.

Paw-Lick Latencies

There were no significant differences among the three groups [TRY− = 7.62 ± 1.0 s; TRY+ = 6.12 ± 0.4 s; water = 5.88 ± 0.3 s; $F(2, 43) = 2.086$, $p = 0.137$] (data not graphed).

Neurochemistry

The results from the HPLC analyses are summarized in Fig. 5. There was a significant effect of treatment on striatal 5-HIAA, $F(2, 21) = 4.656$, $p = 0.021$, and hippocampal 5-HIAA ($F(2, 21) = 7.312$, $p < 0.004$, levels, with the TRY− group being significantly lower than water control (Tukey's test). As well, hippocampal 5-HT was decreased, $F(2, 21) = 4.393$, $p < 0.025$, in the TRY− group when compared to the TRY+ group. In the frontal cortex, there was a significant effect of treatment on 5-HT levels, $F(2, 21) = 14.459$, $p < 0.001$. Using Tukey's posthoc test, it was determined that the TRY+ group was significantly higher in 5-HT content than both the water (+45%) and TRY− (+52.7%) groups; however, the difference between water control and the TRY− group was not significant (−31.4%)($p = 0.094$). There were no significant differences among the groups on frontal cortex 5-HIAA levels. When 5-HIAA/5-HT ratios were computed for each region, no significant differences were found across the three treatments (data not shown). In addition, levels of dopamine, norepinephrine, and their metabolites were not significantly altered by any of these treatments (data not shown).

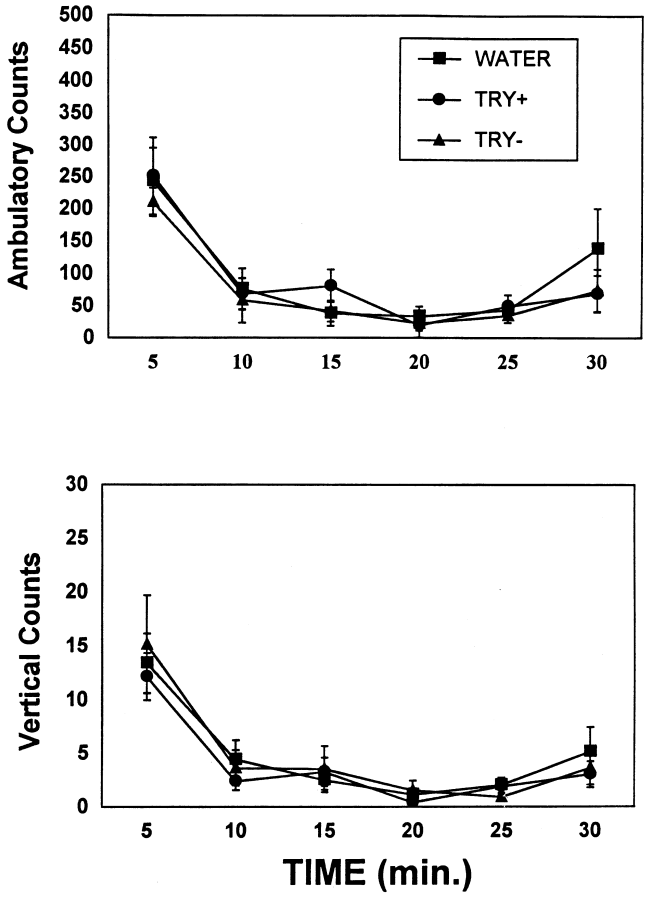

FIG. 4. The effects of water, a balanced amino acid load (TRY+), or a load lacking tryptophan (TRY−) on locomotor activity in a familiar environment. There was a significant effect of time for ambulatory counts (upper panel) and vertical counts (lower panel); however, there were no differences among the groups.

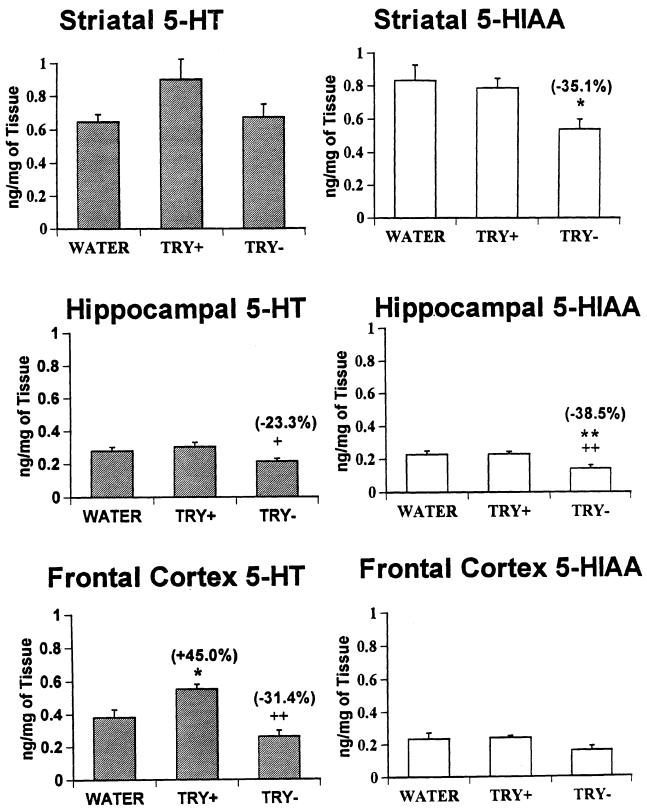

FIG 5. Striatal, hippocampal, and frontal cortex 5-HT and 5-HIAA content 3 h after receiving an intragastric load of either water, balanced amino acids (TRY+), or a load lacking tryptophan (TRY−). Numbers in parentheses indicate percentage difference from water condition. **Significantly different from water control ($p < 0.01$); *significantly different from water control ($p < 0.05$); ++significantly different from TRY+ condition ($p < 0.01$); +significantly different from TRY+ condition ($p < 0.05$).

DISCUSSION

A number of studies involving human subjects have described mood-lowering and/or symptom-exacerbating effects of acute TRY depletion (6,12,30,31,35). These effects were accompanied by decreases in plasma TRY availability, and are thought to arise from reduced 5-HT synthesis in the brain. In the present study involving rats, TRY depletion was found to reduce hippocampal 5-HT levels as well as levels of 5-HIAA in the hippocampus and striatum. Despite this evidence for diminished 5-HT functioning, TRY depletion did not alter DRL responding, locomotor activity, or analgesia in rats.

There are a number of factors that might explain the lack of behavioral effects observed in the present study. Although the amino acid mixtures used were in the same ratio as amino acids used in humans [e.g., (12)], there might be species differences with respect to the metabolism and distribution of the mixture that affected our results. Another possible reason for the lack of behavioral effects might be the time at which subjects were tested, which was 3 h after ingestion of the mixture. This time course was chosen based on the results of previous studies (20,32) using a similar amino acid concoction, showing reduced brain 5-HT (−27 to −41%) and 5-HIAA (−40 to −48%) levels that attained maximal levels at 2 h after treatment and lasted for 8 h. 5-HT and 5-HIAA levels measured in dialysate of frontal cortex tissue of freely moving rats has also been found to be decreased by 30 to 40% at 3 h postgavage (24). The results of the present study approximate the reductions found by the above authors.

Previous studies have reported that lowering 5-HT activity impairs responding on a DRL schedule (16,17,40,41), increases locomotor activity (19), and produces hyperalgesia (36). These effects have been observed following experimental manipulations such as central injections of 5,7-dihydroxytryptamine (5,7-DHT) or peripheral injections of parachlorophenylalanine, which typically produce large reductions in brain 5-HT levels. The TRY− mixture used here induced only modest reductions in 5-HT and 5-HIAA levels (−23.3% and −35.1 to −38.5%, respectively). Thus, it is possible that the lack of behavioral effects observed simply reflect an insufficient reduction in 5-HT neuronal activity. To the extent that differences in 5-HIAA/5-HT ratios reflect changes in endogenous indoleamine turnover, our inability to detect treatment effects on this measure in any brain region can be seen as supporting this conclusion.

Of particular interest was the fact that DRL performance was not affected by TRY depletion. This stands in contrast to the recent findings of others [e.g. (16,41)] who have reported that more substantial interference with 5-HT functioning impaired DRL performance. The lack of effect by TRY depletion here cannot be attributed to an insensitivity of the DRL schedule used because CDZ was effective in producing a dramatic impairment in DRL performance. Previous studies demonstrating impairments in DRL performance utilized midbrain raphe injections of 8-hydroxy-2-(di-n-propyl-amino) tetralin, which are likely to have reduced 5-HT and 5-HIAA levels 50% or more (25), or 5,7-DHT lesions, resulting in greater than 90% depletion of 5-HT (40). In the present studies, TRY depletion did not reduce brain 5-HT levels to this extent. Therefore, this procedure may not be sufficient to produce a disruption of overt behavior. In fact, the processes that are affected may be so subtle or covert that they can only be discovered when attitudinal/self-report measures are employed, as has been the case when human subjects have been tested (6,12,30,31,35).

The fact that both amino acid loads increased the efficiency of performance on the DRL schedule may reflect a partial satiety mechanism induced by either amino acid load acting to mimic a meal. This would be in agreement with the findings (1) that prefeeding food-deprived animals increased efficiency on a DRL schedule of reinforcement. These results could also be seen as agreeing with findings that both abstinent bulimics and normal females ate significantly less after receiving either TRY− or TRY+ amino acid loads compared to water control (33). Such an explanation could also account for the decreased locomotor activity of both amino acid groups in a novel environment when compared to water control, as feeding is also known to be capable of reducing locomotor activity (2).

In summary, the results of these experiments stand in apparent contrast to some studies conducted with humans wherein tryptophan deficient amino acid loads evoked mood-lowering or symptom-exacerbating effects (6,12,30,31,35). Because these studies in humans have confirmed that such amino acid loads reliably lowered plasma tryptophan levels, it was reasonable to expect that such effects were due, at least in part, to suppression of brain serotonin release (14,15). However, it is important to keep in mind that such investigations did not directly measure levels of brain serotonin as was possible in our animal work. Indeed, it was because of this quantitative capability in the present series of studies that we were able to show that a comparable TRY-depleting technique that produces reliable deficits in brain 5-HT levels is not associated with behavioral abnormalities. However, because of species and methodological differences between the studies reported here vs. those conducted in humans, we are not totally surprised that apparent concordance was not obtained. As a result, the present work cautions against the potential utility of using animals to probe the brain mechanisms by which this particular model of acute 5-HT depletion operates in humans.

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