Pharmacology Biochemistry & Behavior, Vol. 12, pp. 173–179. Printed in the U.S.A.

Effects of Dietary Supplements and a Tryptophan-Free Diet on Aggressive Behavior in Rats[1]

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Received 28 September 1979

KANTAK, K. M., L. R. HEGSTRAND, J. WHITMAN AND B. EICHELMAN. *Effects of dietary supplements and a tryptophan-free diet on aggressive behavior in rats.* PHARMAC. BIOCHEM. BEHAV. 12(2) 173–179, 1980.—The effects of dietary excesses of tryptophan, histidine, tyrosine or choline and of a tryptophan-free diet were examined on shock-induced fighting, muricide and jump-flinch thresholds. Following the tryptophan-free diet, shock-induced fighting and pain sensitivity were specifically increased. The increased incidence of muricide was not specific to the lack of tryptophan in the diet. Groups of rats which were pair fed chow or had 0.15% L-tryptophan added to the tryptophan-free diet increased muricide as well. Brain 5-HT levels were 41% depleted following the tryptophan-free diet and reduced 13% with the 0.15% tryptophan supplement. In addition body weights were reduced in the three groups compared to control. None of the excess diets affected shock-induced fighting, muricide and jump-flinch thresholds. Body weights were decreased in the excess tryptophan, histidine, tyrosine and choline groups. These data indicate that the expression of different forms of aggression appears to be influenced by a tryptophan deficiency in the diet, but not by excesses of tryptophan, tyrosine, histidine and choline.

Tryptophan-free diet Shock-induced fighting Muricide Pain sensitivity

SOME evidence suggests that serotonin (5-hydroxytryptamine, 5-HT) might mediate the inhibition of aggressive behavior. Following drug treatments or lesions which lower brain 5-HT, muricide [4, 14, 18, 21, 32, 35, 38, 44] and shock-induced fighting [15, 25, 49] in rats are facilitated. Raising or restoring brain 5-HT has been shown to inhibit or reverse muricide [3, 18, 28, 38]. Since the synthesis of 5-HT depends upon tryptophan availability [55], limiting dietary tryptophan leads to depletion of brain 5-HT levels [16,17] and elevating dietary tryptophan leads to increases in 5-HT levels and metabolism [7,19]. It has recently been demonstrated that maintaining rats on a tryptophan-free diet for 4 to 6 days induces muricide in nonkiller rats and facilitates muricide in killer rats [19]. However, supplementing the tryptophan-free diet with tryptophan loads (0.5% or 2%) did not produce any behavioral changes different from control although brain 5-HT and 5-HIAA were increased. Other investigators [51] found that a 4% tryptophan load in the diet had no modulatory effect on fighting in previously isolated mice; whereas a 4% tyrosine load increased aggressiveness in mice.

It was the purpose of the present experiments to examine the effects of a 5% tryptophan load and a tryptophan-free diet on shock-induced fighting in rats. In addition to the tryptophan load, loads of tyrosine, histidine and choline were examined. These experiments were performed to determine if (1) shock induced fighting can be influenced by the diet as is muricide and isolation-induced fighting; and (2) if the dietary effects support known drug and lesion effects. Only by gathering this information from a variety of approaches for several models of aggression will the neurochemical mechanisms underlying aggression be precisely known and understood.

METHOD

Animals

Holtzman male albino rats were housed individually and had access to food and water. A continuous 12 hr light–12 hr dark cycle and constant humidity and temperature were maintained. Eighty rats, 180–200 g on arrival, were used in the first experiment to study the effects of excesses in the

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0091-3057/80/020173-07$01.20/0

TABLE 1

VALUES ARE THE MEAN ± SEM FOR SHOCK-INDUCED FIGHTING AND PAIN SENSITIVITY

	Shock-induced fighting # Attacks /50 footshocks		Muricide % Killers per group		Pain sensitivity Footshock intensity (mA)	
	Baseline days 1–3	Diet days 8–10	Baseline	Diet	Flinch	Jump
Control	11.33 ± 2.40	13.79 ± 3.75	31.25%	31.25%	0.08 ± 0.00	0.23 ± 0.05
Tryptophan	12.42 ± 3.02	17.00 ± 3.81	12.5%	31.25%	0.08 ± 0.01	0.23 ± 0.07
Histidine	10.21 ± 3.05	20.92 ± 3.36	25%	31.25%	0.08 ± 0.01	0.24 ± 0.05
Tyrosine	8.75 ± 1.91	11.08 ± 2.28	31.25%	25%	0.08 ± 0.00	0.25 ± 0.05
Choline	11.46 ± 2.99	17.33 ± 3.74	31.25%	31.25%	0.08 ± 0.00	0.26 ± 0.07

TABLE 2

	Food intake (g) Diet days 9–14	Water intake (ml) Diet days 9–14	Body weight (g) Day 1	Day 8	Day 16
Control	27.2 ± 2.68	36.5 ± 0.85	302.6 ± 3.66	297.1 ± 3.24	337.4 ± 4.39
Tryptophan	27.9 ± 3.24	—	304.9 ± 4.88	299.8 ± 5.44	321.3 ± 5.78*
Histidine	28.0 ± 4.37	—	304.2 ± 4.93	299.9 ± 5.04	325.9 ± 7.74*
Tyrosine	28.2 ± 3.72	—	299.0 ± 4.52	296.0 ± 5.12	327.5 ± 8.49*
Choline	27.3 ± 3.40	32.7 ± 1.13	298.8 ± 5.17	296.1 ± 5.26	315.6 ± 4.68*

Values are the Mean ± SEM.
*Significantly different from control, $p<0.01$.

diet; and sixty-four rats, 275–300 g on arrival, were used in the second experiment to study the effects of a trypophan deficiency in the diet.

Apparatus

The testing chamber for Experiment 1 consisted of a 32×25.5×30.5 cm blue Plexiglas box, with one clear side for viewing. Electric shock was delivered by a power source described elsewhere [2]. For shock-induced fighting [12], footshock was 2 mA for a duration of 0.5 sec which was presented every 7.5 sec for 50 shocks. Jump-flinch thresholds for pain sensitivity were also determined as previously described [12] with shock intensities of 0.08, 0.1, 0.2, 0.3, 0.4 and 0.5 mA.

The testing chamber for Experiment 2 consisted of a 32×25.5×30.5 cm Coulbourn Instruments Model E10-10 small animal test cage. A Coulbourn Instruments solid state shocker/distributor and power supply were programmed to deliver footshock, as described above, for shock-induced fighting and jump-flinch thresholds. However, the shock intensities for jump-flinch were 0.1, 0.2, 0.3, 0.4, and 0.5 mA.

Diets

The diets for Experiment 1 consisted of powdered Purina Laboratory Chow to which 5% w/w L-tryptophan (Nutritional Biochemicals), L-histidine (Ajinomoto Co.), or L-tyrosine (Ajinomoto Co.) was added. For the choline enriched diet, 15 mg/ml of choline chloride (Eastman) were added to the drinking water [6]. Control and choline animals were given powdered chow. All food was contained in preweighed food cups. Daily food intake was recorded for control, L-tryptophan, L-histidine and L-tyrosine groups and water intake was recorded daily for the control and choline groups after initiation of the test diets.

The diets for Experiment 2 consisted of powdered Purina Laboratory Chow (CHOW) or a powdered tryptophan-free diet (DIET) from Nutritional Biochemicals composed of 17% vitamin-free casein, 69% sucrose, 10% vegetable oil, 4% salt mix, and a vitamin supplement. Because the diet was found to reduce food intake to approximately 16 g/day and has a lower than normal protein content, two additional control groups of rats were tested. In one group the powered CHOW was rationed at 16 g/day (DEPRIVED). For the other group, the tryptophan-free diet was supplemented with 0.15% tryptophan, the amount required daily by rats [30] (DIET+TRP). Thus the effects of below normal protein in the test diet could be specifically measured. All food was contained in preweighed food cups. Daily food and water intakes and body weight were recorded for all groups from the beginning of the experiment.

Procedures

Experiment 1. Following 2 weeks adaptation to their home cages, rats in each group (n=16) were weighed, paired and subjected to 3 days of shock-induced fighting (Days 1–3, Baseline SIF). On the fourth day rats were tested for muricide by placing a mouse into the home cages at 9 a.m. Observations were made at 4 p.m. and again at 9 a.m. the following day for suriving and dead mice. Diets were started on the fifth day and were continued for the remainder of the experiment. On Day 8 animals were weighed again and pairs were fought for the next three days (Diet SIF). A retest for muricide occurred on Day 12 and jump-flinch thresholds (n=8) were obtained on Day 14. At the end of the experiment, Day 16, rats were again weighed.

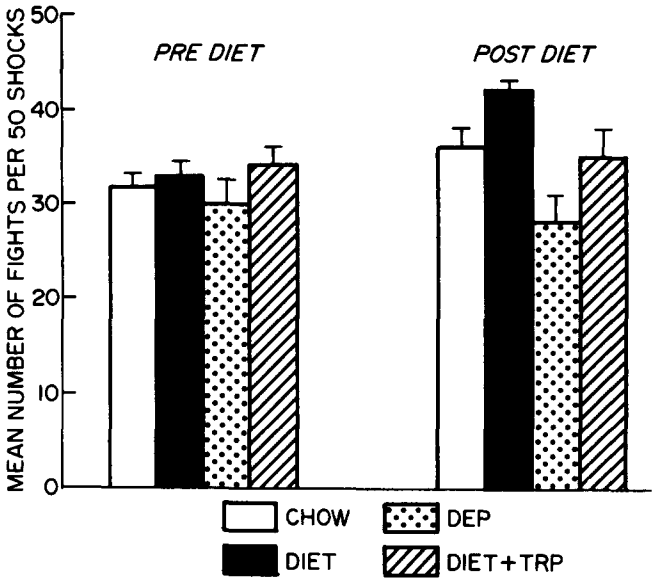

FIG. 1. Mean ± SEM number of fights per 50 footshocks prior to (Days 8–10, pre-diet) and following initiation of the different diets (Days 23–25, post-diet) for the ad lib chow (CHOW), tryptophan-free diet (DIET), food deprived to 16 g/day (DEP), and tryptophan-free diet+0.15% tryptophan (DIET+TRP) groups.

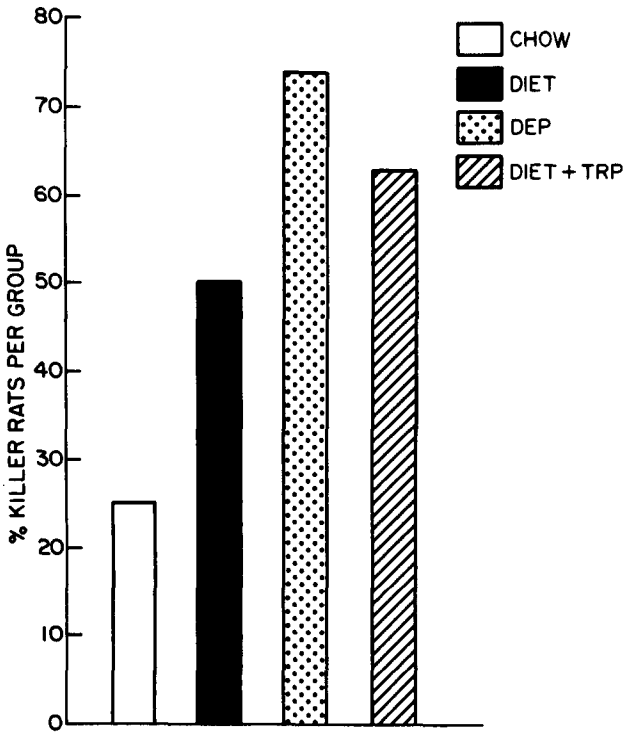

FIG. 2. Percent killer rats in the CHOW, DIET, DEP and DIET+TRP groups eight days after the initiation of the different diets (Experimental Day 21).

Experiment 2. Following 1 week adaptation to their home cages and powdered CHOW, rats from each group (n=16) were paired and subjected to 3 days of SIF. The different diets were started on Day 14 and were continued for the remainder of the experiment. Eight days after the initiation of the diets, rats were tested for muricide as described in Experiment 1. Two days following the muricide test pairs of rats were fought again for 3 days (DIET SIF). Jump-flinch thresholds (n=8) were obtained two days following Diet SIF. At the end of the experiment, Day 29, rats were decapitated and brains were removed and assayed for serotonin using a modification of the radioenzymatic assay of Saavedra, *et al.* [48].

Statistical Analyses

Data from shock-induced fighting, jump-flinch thresholds, brain 5-HT levels, food and water intakes and body weight were evaluated by analyses of variance. Where appropriate, simple main effects tests were performed and the Neuman-Keuls procedure was used for all post hoc testing. Fisher exact probability tests were done for the muricide data. Water intake in Experiment 1 was analyzed by a *t*-test.

RESULTS

Experiment 1

Shock-induced fighting, muricide, flinch-jump thresholds. Analysis of the mean number of fights for 3 days revealed no significant supplementary diet effects on shock-induced fighting. All groups fought the same amount during baseline SIF and during diet SIF. There was a significant days effect, $F(1,35)=30.19$, $p<0.01$, indicating that all groups increased their fighting during the diet SIF period (Table 1).

Analysis of the mouse killing data indicated no significant differences in the incidence of muricide either within groups or between groups. These data are presented in Table 1 as the percentage of mouse killers in each group.

Pain sensitivity, as measured by jump-flinch thresholds, was not affected by any of the supplementary diets used. These data are also presented in Table 1.

Food, water, body weight. Analyses of these data revealed no significant differences in food intake among diet groups or in water intake between control and choline animals. However, analysis of body weight indicated significant differences for days, $F(2,150)=178.33$, $p<0.001$; and for groups×days, $F(8,150)=3.63$, $p<0.001$. Further testing revealed that the body weights in the choline, histidine, tryptophan and tyrosine groups were significantly less than the control group on Day 16, $p<0.01$. Body weights among groups did not differ on Day 1 or on Day 8. These data are presented in Table 2.

Experiment 2

Shock-induced fighting, muricide, flinch-jump thresholds. Analysis of the mean number of fights for 3 days revealed significant differences between groups, $F(3,28)=3.44$, $p<0.05$; days, $F(1,28)=20.25$, $p<0.001$; and groups ×days, $F(3,28)=10.05$, $p<0.001$. Further testing revealed that groups did not differ on baseline SIF, but did differ on diet SIF. DIET rats (tryptophan deficient) fought significantly more compared to CHOW, DEPRIVED, and DIET+TRP, $p<0.01$. DEPRIVED rats fought significantly less than all other groups, $p<0.01$. The CHOW and DIET+TRP groups did not differ. These results are presented in Fig. 1.

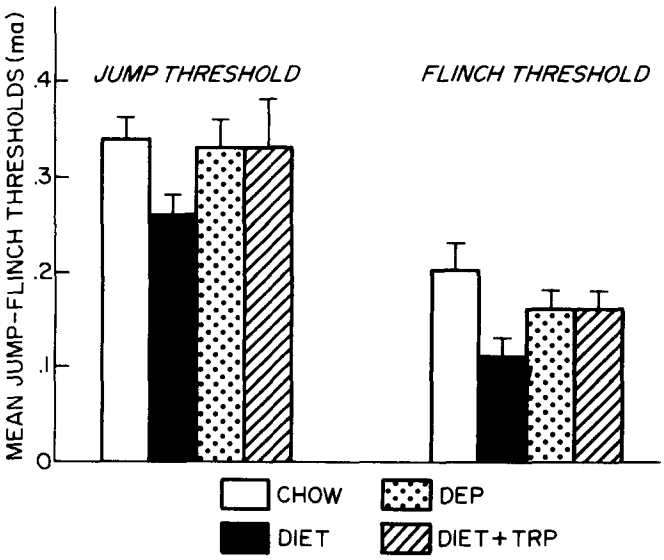

FIG. 3. Mean ± SEM jump and flinch thresholds in the CHOW, DIET, DEP and DIET+TRP groups sixteen days after the initiation of the different diets (Experimental Day 27).

Analysis of the mouse killing data indicated an increased incidence in muricide in the DIET ($p=0.10$). DEPRIVED ($p=0.006$), and DIET+TRP ($p=0.03$) groups compared to CHOW. These data are presented in Fig. 2 as the percentage of mouse killers in each group.

Analysis of variance of jump thresholds was not statistically different among groups. Analysis of flinch thresholds indicated significant differences among groups, $F(3,28)=3.35$, $p<0.05$. Further testing revealed a significant decrease in flinch threshold for the DIET group compared to CHOW, $p<0.05$. The DEPRIVED and DIET+TRP groups did not differ from CHOW. These data are presented in Fig. 3.

Food, water, body weight. Analysis of food intake revealed significant differences between groups, $F(3,60)=34.86$, $p<0.001$; days, $F(1,60)=185.58$, $p<0.001$; and groups×days, $F(3,60)=31.18$, $p<0.001$. Further testing indicated that groups did not differ in food intake before the initiation of the diets, but did differ subsequent to the initiation of the diets. DIET and DEPRIVED groups ate significantly less than CHOW and DIET+TRP groups, $p<0.01$. The CHOW and DIET+TRP groups did not differ nor did the DIET and DEPRIVED groups. These data are presented in Table 3.

Analysis of water intake revealed significant differences between groups, $F(3,60)=7.07$, $p<0.001$; days, $F(1,60)=120.81$, $p<0.001$; and groups×days, $F(3,60)=13.72$, $p<0.001$. Further testing indicated that groups did not differ in water intake before the initiation of the diets, but did differ subsequent to the initiation of the diets. DIET, DEPRIVED, and DIET+TRP groups drank significantly less than the CHOW group, $p<0.01$, and did not differ among themselves. These data are presented in Table 3.

Analysis of body weight on Days 1, 13 and 26 revealed significant differences between groups, $F(3,60)=16.17$, $p<0.001$; days, $F(2,120)=782.12$, $p<0.001$; and groups ×days, $F(6,120)=89.92$, $p<0.001$. Subsequent testing indicated that groups did not differ on Days 1 and 13, but did differ on Day 26. On Day 26 all groups were significantly different from each other, $p<0.01$. The DIET and DEPRIVED groups significantly lost weight compared to Day 13, $p<0.01$; whereas the CHOW and DIET+TRP groups significantly gained weight compared to Day 13, $p<0.01$. However, body weight in the DIET+TRP group was at a reduced level compared to CHOW, $p<0.01$. These data are presented in Table 3.

Brain 5-HT. Analysis of these data indicated significant differences between groups, $F(3,60)=38.71$, $p<0.001$. Further testing revealed that the DIET group had less whole brain 5-HT than all other groups, $p<0.01$. In addition there was a small but significant decrease in brain 5-HT for the DIET+TRP group compared to both CHOW and DEPRIVED, $p<0.01$. There were no differences between CHOW and DEPRIVED. These data are presented in Fig. 4.

DISCUSSION

These data indicate that the diets containing 5% excesses of L-tryptophan, L-histidine and L-tyrosine, or choline enriched drinking water have little effect on the levels of shock-induced fighting or the incidence of muricide in rats. Others have also reported the lack of a modulatory effect of an excess tryptophan diet on muricidal behavior in rats [19]. It is evident that these diets constituted an excess because body weights were at a reduced level. Such an effect is known to occur following disproportionate amino acid diets [22,43]. In addition, external signs of tyrosine toxicity were evident [22,42]. Paw lesions and loss of patches of body fur were noted in more than 50% of the animals fed the 5% tyrosine diet.

Excess tryptophan [7,19], histidine [36], tyrosine [5] and choline [6] do increase the metabolism of serotonin, histamine, catecholamines and acetylcholine, respectively. When these excesses are administered through the diet, the increases occur within three days. There is pharmacological

TABLE 3

	Food intake (g)		Water intake (ml)		Body weight (g)		
	Baseline days 5–7	Diet days 17–19	Baseline days 5–7	Diet days 17–19	Day 1	Day 13	Day 26
Chow	25.9 ± 0.56	24.7 ± 0.52	35.7 ± 1.13	35.6 ± 1.14	286.4 ± 1.85	346.1 ± 3.87	386.4 ± 4.77
Diet	26.2 ± 0.58	15.8 ± 0.99*	35.7 ± 1.14	24.4 ± 3.22*	285.9 ± 1.98	350.5 ± 2.78	299.8 ± 2.45*
Deprived	25.1 ± 0.55	16.0 ± 0.00*	36.0 ± 0.87	21.3 ± 0.86*	280.5 ± 1.92	348.9 ± 3.89	317.3 ± 4.86*
Diet + TRP	27.1 ± 0.68	24.9 ± 0.91	37.5 ± 1.20	23.5 ± 1.35*	281.8 ± 3.02	341.2 ± 5.16	359.9 ± 6.38*

Values are the Mean ± SEM.
*Significantly different from chow, $p<0.01$.

FIG. 4. Mean ± SEM μg/g whole brain 5-HT in the CHOW, DIET, DEP and DIET+TRP groups eighteen days after the initiation of the different diets (Experimental Day 29).

evidence that each of these putative neurotransmitter systems has a modulatory effect on rat aggression [1, 13, 18, 29, 34, 38, 46, 49, 52]. Therefore one might have expected an alteration in aggressive behavior in this study. The lack of behavioral effects might be related to the ability of these neurotransmitter precursors to be intraneuronally metabolized without the transmitter being available at the post-synaptic receptor. Such neurochemical effects are thought to occur following tryptophan loads [20]. Subsequent to tryptophan loads, no behavioral effects are usually observed [19, 20, 24, 33, 50, 51, 54]. However, whether or not loads of all these neurotransmitter precursors can be intraneuronally metabolized without the transmitter being available at the post-synaptic receptor remains to be demonstrated.

In contrast, the results from Experiment 2 indicate that a diet lacking in tryptophan specifically increases shock-induced irritable fighting in adult male rats. When the below normal levels of protein and the food deprivation inducing effects of the diet are controlled, either no changes in SIF or decreases in SIF are observed, respectively. Thus the specificity for the lack of tryptophan in the diet and depleted 5-HT levels are demonstrated. However, it cannot unequivocally be stated that the depleted 5-HT levels resulting from the tryptophan-free diet increased aggression per se. As others have shown for a low tryptophan diet [37], an increased pain sensitivity to the shock, as evidenced by a decreased flinch threshold, was demonstrated for the tryptophan-free diet. Consequently animals may have fought more simply because pain sensitivity was increased. Changes in pain sensitivity, however, are not necessarily a prerequisite for changes in SIF when modifying the serotonergic system [24,25]. Alternately, since reactivity can be confounded with sensitivity in a flinch-jump test, the tryptophan-free diet may have increased reactivity to the shock rather than sensitivity to increase the irritable fighting behavior. Serotonin depletion either by drugs [10], raphe lesions [9] or a tryptophan-free diet [53] have been shown to increase the acoustic startle response which is considered to be a measure of reactivity. For the muricide test which is a non-aversive test for aggression, DIET animals did increase the incidence of mouse-killing by 100%. However, in contrast with other reports [19], these effects were not specific to the lack of tryptophan in the diet. DEPRIVED and DIET+TRP animals increased muricide as well. The discrepancy might be related to strain differences (Long-Evans vs Holtzman); length of deprivation (4 days vs 8 days); or procedural differences (induction in known nonkillers vs unknown killing tendencies). The fact that DIET, DEPRIVED and DIET+TRP groups increased the incidence of muricide might reflect increases in predation associated with dietary need. It has been demonstrated that length and amount of food deprivation can affect the incidence of muricide [31,47]. Interestingly, all but the fur and tail of killed mice was devoured by these three groups. In contrast the mice killed by the CHOW group were left intact except for the head. Clearly, the increased incidence of muricide in this study is not correlated entirely with 5-HT depletion; whereas the increase in shock-induced fighting appears to be. It is known that food deprivation [8, 26, 27, 45] and low tryptophan or protein in the diet [11, 16, 39, 40, 41, 56] can affect the uptake of the amino acids which compete with tryptophan for entry into the brain (tyrosine, phenylalanine, leucine, isoleucine, and valine) as well as affect the uptake of tryptophan. Thus the changes in aggressive behavior produced by these diets could be related to factors other than 5-HT level, dietary need, or pain sensitivity.

In summary, shock-induced fighting is influenced by a tryptophan deficiency in the diet, but not by 5% excesses of tryptophan, tyrosine and histidine, or choline enriched drinking water. These data support and extend the hypothesis that serotonin depletion facilitates aggression in rats.

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