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Anorexia and Brain Serotonin: Development of Tolerance to the Effects of Fenfluramine and Quipazine in Rats with Serotonin-Depleting lesions

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CARLTON, J. AND N. ROWLAND. *Anorexia and brain serotonin: Development of tolerance to the effects of fenfluramine and quipazine in rats with serotonin-depleting lesions.* PHARMACOL BIOCHEM BEHAV **20**(5) 739–745, 1984.—The acute and chronic effects of the "serotonergic anorectics" quipazine and dl-fenfluramine were examined in rats with substantial and specific depletions of brain 5-hydroxytryptamine (5-HT) induced by 5,7-dihydroxytryptamine (5,7-DHT). A "dessert" test which did not involve food deprivation was used to assess anorexia. Markedly increased sensitivity to the L-5-HTP-induced behavioral syndrome in 5,7-DHT-lesioned rats indicated postsynaptic 5-HT receptor supersensitivity. We found low (2 mg/kg) and intermediate (5 mg/kg) doses of fenfluramine, a putative presynaptic agent, were more effective in producing anorexia in lesion rats versus controls. A higher dose of fenfluramine (10 mg/kg) was less effective in lesion rats, suggesting that high dose and low dose fenfluramine anorexia are mediated by different mechanisms. We found quipazine, a putative 5-HT postsynaptic agonist, in a dose range of 2–10 mg/kg, to be no more effective in producing anorexia in lesion rats compared to controls. The development of tolerance to both fenfluramine and quipazine anorexia was similar for lesion and control rats showing that an intact brain 5-HT system is not necessary for tolerance. Tolerance to the "behavioral syndrome" induced by high doses of these agents developed rapidly in controls but not at all in lesion rats. This suggests that the behavioral syndrome and anorexia are independent effects of fenfluramine and quipazine. These results also challenge the popular notion that the primary anorectic action of fenfluramine is via brain serotonin.

5-Hydroxytryptamine Anorexia Fenfluramine Quipazine Tolerance Serotonin behavioral syndrome 5,7-Dihydroxytryptamine

A substantial body of experimental and clinical evidence has been accumulated in favor of a role for brain 5-hydroxytryptamine (5-HT) in the regulation of feeding behavior [3, 16, 31]. The precise role remains unclear, however it is generally believed to be inhibitory on food intake.

Fenfluramine is a popularly prescribed anorectic agent which may act via increased 5-HT release and inhibition of 5-HT uptake in brain [11, 12, 14]. Quipazine is also an anorectic agent, possibly by virtue of its direct agonist properties on 5-HT receptors [19, 24, 28]. Quipazine may also block 5-HT autoreceptors [32], thus indirectly increasing release. The pharmacological specificities of these agents have not been fully established, but both are routinely referred to as serotonergic anorectics.

Repeated administration of either fenfluramine or quipazine leads to rapid and often complete tolerance in free-feeding or schedule-fed rats [1,26]. However, cross tolerance does not develop between the two agents [1,25] due in part to the biochemical nature of fenfluramine tolerance versus the learned nature of quipazine tolerance [27].

In the present study we have examined whether prior damage to brain 5-HT neurons would influence the initial anorexia and the development of tolerance to these agents. Brain serotonin depletions were produced by the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT). 5,7-DHT, administered in combination with a catecholamine uptake blocker, selectively destroys serotonergic nerve terminals producing a permanent 5-HT depletion in most brain regions [15], a decrease in 5-HT uptake [38] and postsynaptic denervation supersensitivity [22,35].

If quipazine acts postsynaptically to produce anorexia, then its acute effects should be enhanced by the lesion; the mechanisms of tolerance should, however, be postsynaptic and intact. If fenfluramine acts presynaptically, then its effects should be diminished in lesion animals and tolerance may be retarded. Previous studies on the acute effects of

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fenfluramine in rats with brain 5-HT depletions have yielded conflicting results [5, 17, 18, 30, 34]. Lesion-to-test interval, extent of depletion or nonspecific damage to other than 5-HT systems, may account for some of these discrepancies.

We have examined the behavioral effects of acute and chronic administration of various doses of fenfluramine and quipazine in rats with 5,7-DHT-induced brain serotonin depletions. A "dessert test" which did not involve food deprivation was used to assess anorectic effects of these agents. We believe this is more relevant to human overeating than are traditional deprivation paradigms.

We have then measured regional levels of 5-HT, norepinephrine (NE) and dopamine (DA). These measures provide an estimate of the extent of 5-HT depletion and account for possible nonspecific effects.

METHOD

Male Long Evans rats, initially weighing 200–300 grams, were housed individually in hanging wire cages and maintained under standard laboratory conditions (24°C; 12/12 light/dark cycle) with ad lib Purina chow pellets and water.

Surgical Procedures

Intracerebroventricular (IVT) injections of 200 μg 5,7-DHT (dose expressed as free base) in a volume of 20 μl vehicle (0.9% NaCl and 0.1% L-ascorbic acid) were administered to rats anesthetized with 2.5 ml/kg Equithesin. Forty rats received a first injection of 200 μg 5,7-DHT into the left lateral ventricle. Seven days later, a second, and identical injection was given into the right ventricle. Thirty minutes prior to sterotaxic surgery, animals were pretreated with desmethylimipramine (DMI; 25 mg/kg, SC) to prevent uptake of the neurotoxin into noradrenergic neurons [15]. Control rats (n=48) received vehicle injections or were unoperated.

Pretest: 5-HT Behavioral Syndrome

At 20, 36 and 43 days after the second surgery, rats were tested for sensitivity to L-5-HTP-induced "behavioral syndrome." This syndrome is a behavioral model for the study of functional activation of central 5-HT systems [20,33].

Rats were pretreated with clorgyline (0.05 mg/kg, SC) a type A monoamine oxidase (MAO) inhibitor. Thirty minutes later, they were injected (IP) with one of three doses of L-5-HTP. Five, 10 and 25 mg/kg L-5-HTP were administered in varying order to groups of rats on test days. Each rat received each of the three doses on separate test days in random order. A group of lesion and control rats (n=24) were also tested with 0.05 mg/kg clorgyline followed by saline injection.

Animals were closely observed for one hour following L-5-HTP injection. Nine behaviors observed in association with the 5-HT behavioral syndrome were scored at 20 minute intervals. Splayed hind limbs, head weaving, forepaw treading, "wet dog" shakes, ejaculation, straub tail, crawling, hunching and rigidity were scored as absent (0); present (1) or intense (2). The first test for each rat was conducted in a 12×24 in. Plexiglas cage. Subsequent tests were performed in home cages. No marked effects of this environmental change were evident.

Baseline "Dessert" Intake

On the basis of their cumulative scores on the three tests of L-5-HTP-induced behavioral syndrome, lesion rats were assigned to one of eight matched groups. Control rats were randomly assigned to these groups. There were five lesion and six control rats per group.

All animals were then allowed one hour daily (2–3 p.m.) access to a sweetened milk solution consisting of 100 grams powdered milk and 100 grams sucrose per liter solution. This milk solution was offered in a graduated cylinder with a drinking spout. Chow and water were continuously available. Thirty minutes prior to milk presentation, animals were injected with 1 ml/kg isotonic saline (IP). Following ten days adaptation to this procedure, milk intake was measured to the nearest milliliter on four consecutive days. Each animal's mean intake for this period was taken as a baseline measure.

Dissections for Monoamine Analysis

Following this determination of baseline milk intake, the rats in one group were killed by decapitation and their brains were rapidly removed. Dissection were performed on ice and completed within three minutes. Cortex, hippocampus, hypothalamus, striatum and brain stem tissue samples were stored at −80°C for subsequent monoamine analysis. Brainstem samples included pons and medulla. An oblique cut was made from the caudal border of the inferior colliculus to the caudal border of the mamillary bodies. The dissection procedure was performed between 12 noon and 2 p.m. on the day corresponding to the first day of anorectic drug treatment for the remaining animals. This was 57 days after the second IVT injection of 5,7-DHT or vehicle.

Anorectic Drug Treatment

The remaining animals made up seven treatment groups in which acute and chronic anorectic effects of various doses of fenfluramine and quipazine were tested. The groups were as follows: (1) 2 mg/kg fenfluramine; (2) 5 mg/kg fenfluramine; (3) 10 mg/kg fenfluramine; (4) 2 mg/kg quipazine; (5) 5 mg/kg quipazine; (6) 10 mg/kg quipazine and (7) 1 ml/kg saline. Drug concentrations were such that injected volume was 1 ml/kg body weight. Drugs were administered (IP) 30 minutes prior to milk presentation for 14 consecutive days.

Each animal's daily milk intake was measured at 30 and again at 60 minutes. These data were subsequently combined and only the total one hour intakes are presented since any effects present at 30 minutes were also observed at one hour. The absolute intake in milliliters was then transformed to percent of baseline for each animal. The group means are the average percent intake for lesion or control animals in each drug treatment group.

Drugs

Drugs used in this experiment were as follows: dl-fenfluramine hydrochloride (a gift from A. H. Robins); quipazine maleate (Miles); 5,7-dihydroxytryptamine creatine sulfate (Sigma); clorgyline (a gift from May and Baker) and desmethylimipramine hydrochloride (a gift from Merrill). Drug doses are expressed as weight of the salt.

Monoamine Analysis

Regional concentrations of NE, DA and 5-HT were analyzed by high performance liquid chromatography with electrochemical detection. Individual tissue samples were weighed, then homogenized (Polytron) in approximately 20 volumes of cold 0.2 N $HClO_4$+2.5×10^{-5}M EDTA. Homogenates were centrifuged at 10,000 g at 2° for 10 minutes. The

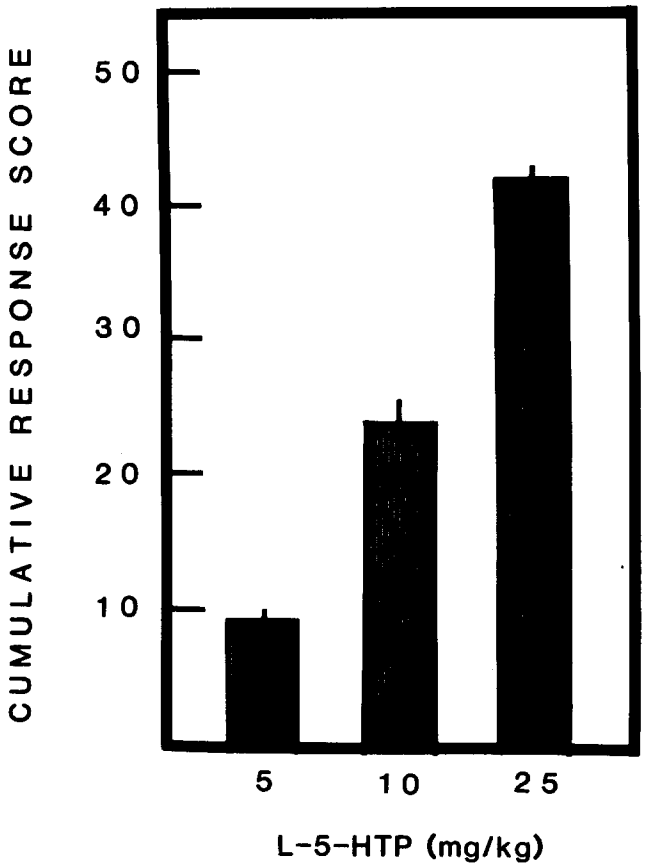

FIG. 1. Intensity of the behavioral syndrome induced by various doses of L-5-HTP in conjunction with 0.05 mg/kg clorgyline in 5,7-DHT-treated rats. Shown are mean (±SEM) cumulative scores for nine behaviors. Behaviors were scored as : absent (0); present (1) or intense (2) at three observation periods at 20 minute intervals. The maximum possible cumulative score is 54.

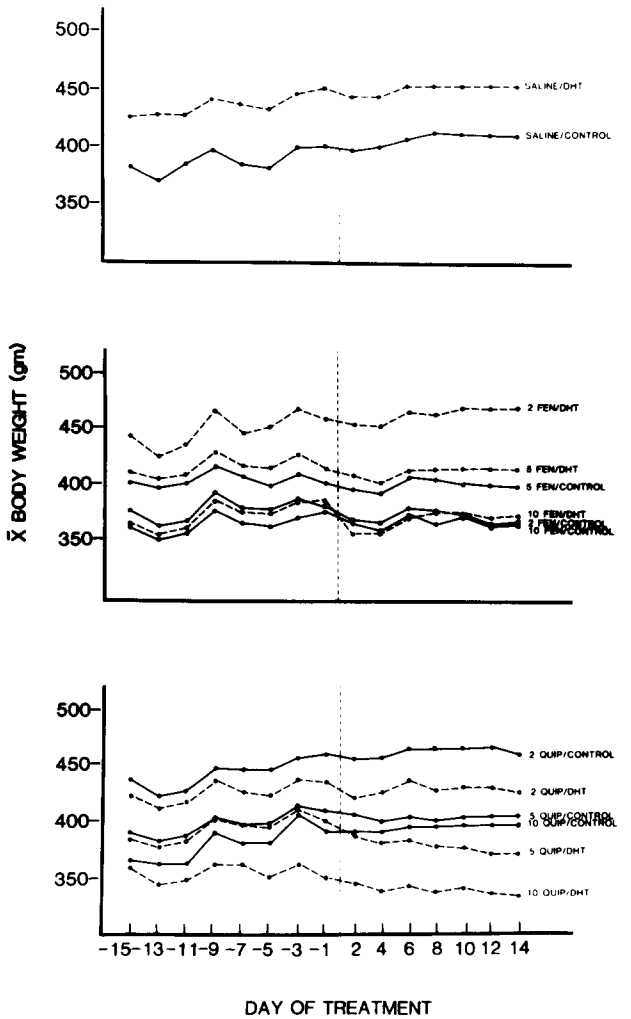

FIG. 2. Group mean body weights over a 4 week period beginning 2 weeks before initiation of treatment regimen through the final day of treatment (Day 14).

supernatant was decanted and stored a maximum of 12 hours on ice until analysis was performed.

Chromatography was performed using a Waters M-45 pump, a μBondpak C-18 reversed phase column and a Bioanalytical Systems model LC-4B electrochemical detector with a glassy carbon electrode. An applied potential of +0.9 V was maintained between the working electrode and an Ag/AgCl reference electrode. Peak areas were used for concentration analysis and were determined automatically by a Perkin Elmer Sigma 15 Data Station. Tissue concentrations were computed from peak areas of 10^{-6} standard solutions.

The mobile phase consisted of a mixture of 84 parts 0.1 M NaH_2PO_4 and 16 parts methanol with 2.6×10^{-3} M octyl sodium sulfate, 1.0×10^{-4} M EDTA and 2.5×10^{-4} M Et_3N. The pH was adjusted to 3.35 with 85% H_3PO_4. This eluant allowed for simultaneous determination of the compounds of interest [36].

RESULTS

L-5-HTP-Induced Behavioral Syndrome

The results are shown in Fig. 1. The 5,7-DHT-treated rats showed a dose-related increase in intensity/duration of the behavioral syndrome, $F(2,123)=102.83$, $p<0.0001$. Even at the lowest dose of L-5-HTP virtually every animal showed activation. In contrast, no control rat showed the syndrome even at 25 mg/kg L-5-HTP. Clorgyline alone had no effect on observed behaviors of lesion or control rats.

The ability of 5-HTP to elicit the syndrome at low doses is behavioral evidence that the lesion rats had substantial depletions of brain serotonin and resultant supersensitivity of receptors mediating the syndrome [22, 33, 35].

"Dessert" Test

Baseline milk intake was similar for lesion (26±1.1 ml) and control rats (25±1.4 ml). Furthermore, mean weight change during the time between surgery and initiation of the anorectic drug regimen was not significantly different for lesion (15.2±2.6 grams) versus control rats (21.3±4.5 grams). Group mean body weights over a 4 week period beginning 2 weeks before initiation of anorectic drug treatment are shown in Fig. 2.

Acute effects of anorectic agents are summarized in Fig. 3. Clearly, the anorectic potencies of fenfluramine and

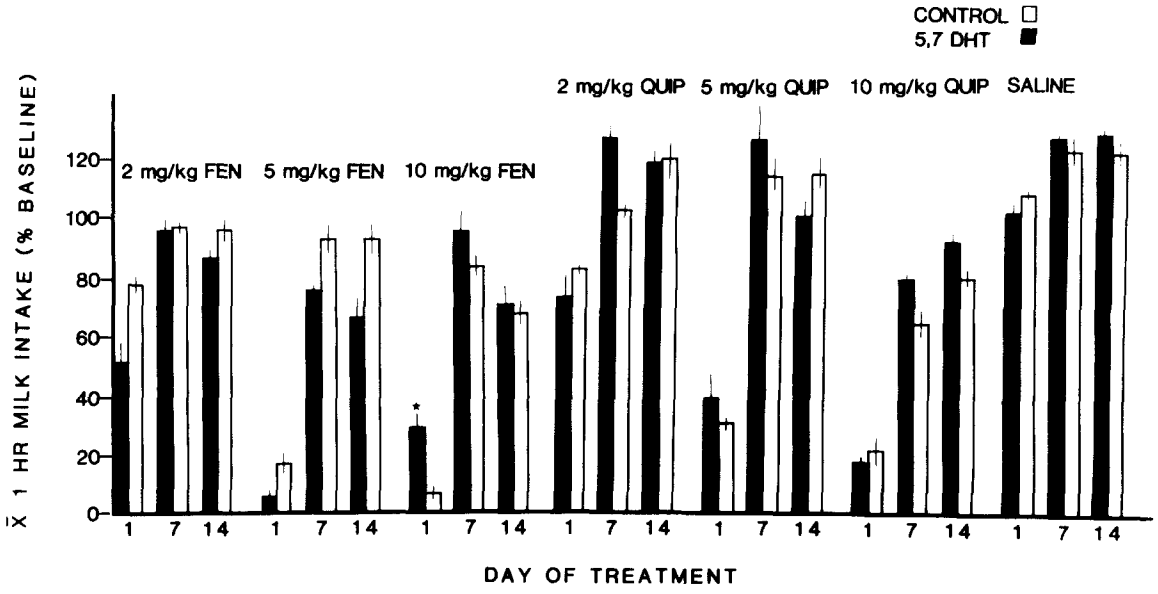

FIG. 3. Milk intake expressed as mean percentage (±SEM) of baseline for the various groups on days 1, 7 and 14 of treatment. Intake on day 1 of treatment was significantly below baseline in all groups (2 tail t's $p<0.05$). Only one lesion vs. control comparison was significant ($^*p<0.05$). Intake for lesion and control groups was not significantly different from baseline on days 7 and 14.

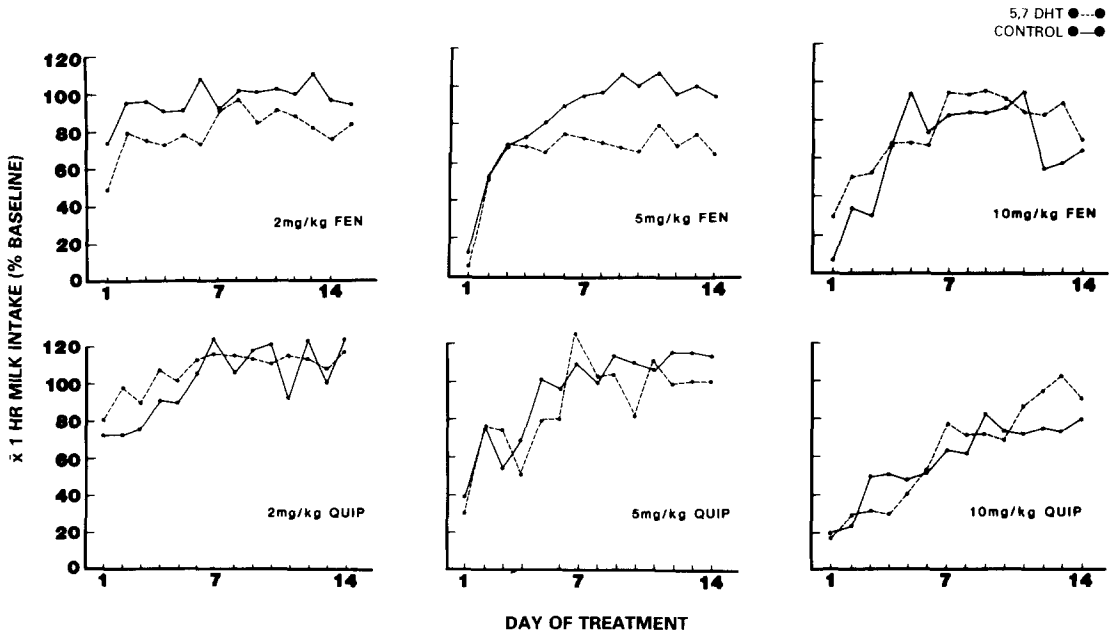

FIG. 4. Development of tolerance to fenfluramine- and quipazine-induced anorexia in lesion and control rats. Shown are mean milk intakes, expressed as percentage of baseline, on days 1 through 14 of drug treatment.

quipazine are broadly similar in control and lesion rats. An overall ANOVA showed significant effects of surgery, $F(1,13)=10.61$, $p<0.01$, treatment, $F(6,13)=42.23$, $p<0.0001$, and the surgery × treatment interaction, $F(6,13)=2.42$, $p<0.05$. Specific t-test comparisons revealed some interesting lesion effects. Compared to nonlesion controls, 5,7-DHT-treated rats showed greater anorexia in response to 2 and 5 mg/kg fenfluramine ($0.07>p>0.05$). Compared to controls, the 5,7-DHT-treated rats showed significantly decreased anorexia in response to 10 mg/kg fenfluramine ($p<0.05$). There were no significant differences between 5,7-DHT-treated rats and controls in response to 2, 5 or 10 mg/kg quipazine. Milk intake for both lesion and control rats in the saline treatment group was similar throughout the experiment.

During the "dessert" test sessions, the animals were observed informally. There were no obvious differences in the pattern of milk consumption for lesion versus control rats. Rats began drinking milk immediately upon presentation, consuming most of their daily intake within the first 30 minutes. Decreases in milk intake were manifested in earlier cessation of drinking rather than increased latency to drink.

On the first day of anorectic drug administration, control rats treated with the high doses of fenfluramine and quipazine exhibited mild behavioral activation similar to the 5-HT "behavioral syndrome." By the second day of treatment, however, these behaviors were no longer apparent. The 5,7-DHT-treated rats displayed similar behavioral activation in response to 5 and 10 mg/kg fenfluramine and quipazine. These observations were informal and unscored, but the effects in the lesion rats did not appear to diminish during the 14 day treatment period. The fact that drinking began immediately and that full anorectic tolerance developed despite the ongoing syndrome, suggest that these behaviors do not mutually interfere.

Anorectic Tolerance

Figure 4 summarizes the time course of the development of tolerance to fenfluramine and quipazine-induced anorexia. By day 7, milk intake had restabilized and, with the exceptions of 5 and 10 mg/kg fenfluramine and 10 mg/kg quipazine, was back to baseline level ($p>0.05$ versus predrug baseline). On day 14, milk intake of control rats receiving the highest dose of fenfluramine and quipazine remained significantly below baseline. Milk intake of 5,7-DHT-treated rats in all anorectic drug treatment groups was not significantly different from baseline at this time.

Monoamine Analysis

Table 1 shows the extent of regional depletions of central 5-HT. Values expressed in Table 1 are the means determined from individual tissue samples. Regional depletions of 5-HT in 5,7-DHT-treated rats ranged from 49% depletion in striatum to 88% in cortex. Regional levels of NE and DA were similar in lesion and control animals indicating that our surgical procedures did not impair central catecholamine systems.

TABLE 1

CONCENTRATIONS OF SEROTONIN IN CONTROL AND 5,7-DHT-TREATED RATS IN VARIOUS BRAIN REGIONS

Region	Control	5,7-DHT	(%)
Cortex	40.4 ± 3.4	4.8 ± 0.3	(12%)†
Brainstem	30.8 ± 2.2	3.9 ± 0.8	(13%)†
Hippocampus	13.7 ± 2.0	2.0 ± 0.2	(15%)†
Hypothalamus	29.5 ± 6.0	9.0 ± 0.7	(30%)*
Striatum	10.6 ± 2.5	5.4 ± 1.4	(51%)

‡Shown are M ± SEM concentrations expressed as 10^{-10} moles/gram tissue.

*$p<0.01$, †$p<0.001$ lesion group less than control.

DISCUSSION

The results of this study do not confirm predictions based on the presumed mechanisms of quipazine and fenfluramine anorexia. It has been hypothesized that quipazine produces anorexia via its effects as a direct postsynaptic 5-HT receptor agonist [19, 24, 28]. We therefore predicted an increase in anorectic efficacy of quipazine in rats with brain 5-HT depletions and subsequent denervation supersensitivity of 5-HT postsynaptic receptors. We found no significant differences in anorectic efficacy of acute or chronic quipazine in 5,7-DHT-treated rats versus controls.

Following the suggestion that fenfluramine produces anorexia by virtue of its effects on presynaptic central 5-HT neurons [11, 12, 14], we predicted that fenfluramine anorexia would be diminished in 5,7-DHT-treated rats. However, our 5,7-DHT-treated rats showed increased anorexia in response to 2 and 5 mg/kg fenfluramine and decreased anorexia to 10 mg/kg of this agent. These data confirm and extend previous observations of increased anorexia to fenfluramine following large, selective 5-HT depletions ([17], Rowland, unpublished 1981). These results seriously question the popular notion that the primary anorectic actions of fenfluramine and quipazine are via brain 5-HT.

Tolerance to the anorectic effects of fenfluramine and quipazine developed similarly in lesion and control rats showing that tolerance to these effects does not require an intact brain 5-HT system. Based on informal observations, tolerance to the 5-HT behavioral syndrome appears to develop rapidly in controls while not at all in lesion rats. These results suggest that the behavioral syndrome and anorexia are independent effects of fenfluramine and quipazine.

Development of tolerance to the anorectic effects of quipazine and fenfluramine were similar for lesion and control animals. Development of tolerance to fenfluramine and quipazine in intact rats does not appear to be via identical mechanisms since cross tolerance does not develop between the two agents [1,25]. Furthermore, tolerance to quipazine anorexia is at least partially learned while tolerance to fenfluramine is not [27].

The results of our biochemical analyses, as well as our 5-HT behavioral syndrome tests, indicate substantial depletions of central 5-HT. These depletions are very likely to have reduced the amount of releasable 5-HT. For example, administration of 5 mg/kg fenfluramine to rats with 75% depletions of brain 5-HT induced by p-chlorophenylalanine did not further reduce 5-HT [8]. Furthermore, in vivo electrovoltammetry studies have been reported in which only 35–45% depletions of brain 5-HT prevented stimulation- and drug-induced release of 5-HT [21]. It thus seems likely that fenfluramine would have released much less 5-HT in lesion rats, yet it produced greater anorexia, arguing against a

presynaptic mediation of fenfluramine, at least at low doses. However, there is an additional degree of uncertainty in this argument because postsynaptic receptor supersensitivity might increase the effectiveness of the released 5-HT to an indeterminate extent.

While it is well established that fenfluramine affects central 5-HT transmission, the basis of fenfluramine anorexia is not conclusive. In vitro, fenfluramine is a competitive inhibitor of 5-HT membrane uptake mechanisms and releases 5-HT from intraneuronal stores. High doses initially release 5-HT, then deplete stores, possibly leading to a functional deficit. Repeated high doses may lead to long lasting, irreversible depletions in 5-HT concentration and other markers of 5-HT neurons [9].

Fenfluramine does not appear to bind to central 5-HT receptors as evidenced by its failure to displace ^{3}H-5HT [13,37]. However, norfenfluramine, an active metabolite of fenfluramine [7,12], displaces ^{3}H-5HT in rat brain preparations [13]. Central accumulation of norfenfluramine in animals treated with fenfluramine [13] may at least partially explain the inability of central 5-HT depletions to block fenfluramine anorexia [4]. Another possible explanation is that fenfluramine may decrease brain NE in animals with central 5-HT depletions [8].

Peripheral effects of fenfluramine which may be implicated in anorexia include increased glucose utilization, increased lipolysis (see [16]) and delayed gastric emptying time (LeDourac, cited in [2], [6]; Rowland and Carlton, unpublished, 1983). Delayed gastric clearance may be crucial to fenfluramine-induced anorexia in nondeprived animals.

The present study does not support the simple hypothesis that drug-induced anorexia is due to increased postsynaptic 5-HT receptor activation. The data are compatible with several alternatives. One is that both fenfluramine and quipazine act via peripheral metabolic effects and that their effects on feeding are only slightly modulated by brain 5-HT. A second possibility is that mechanisms in series with central 5-HT compensate for lesion and/or drug effects. A third possibility is that mechanisms in parallel with central 5-HT can be engaged when the 5-HT system is damaged. In any event, the neurochemical regulation of appetite clearly involves more than just 5-HT.

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