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Long-Term Imipramine Treatment Differentially Affects Fenfluramine-Induced Suppression of Food Intake and Locomotor Activity

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AULAKH, C. S., K. M. WOZNIAK, J. L. HILL AND D. L. MURPHY. *Long-term imipramine treatment differentially affects fenfluramine-induced suppression of food intake and locomotor activity.* PHARMACOL BIOCHEM BEHAV 31(1) 97–101, 1988.—Administration of fenfluramine to rats produced decreases in one-hour food intake and locomotor activity. Short-term (2–6 days) or long-term (21–25 days) treatment with the tricyclic antidepressant, imipramine, did not affect daily food intake, body weight gain or baseline locomotor activity when compared to saline treatment. However, long-term but not short-term imipramine treatment attenuated fenfluramine-induced decreases in one-hour food intake. On the other hand, neither short-term nor long-term imipramine treatment affected fenfluramine-induced decreases in locomotor activity. These findings demonstrate a differential effect of long-term imipramine treatment on fenfluramine-induced suppression of food intake and locomotor activity.

Food intake Locomotor activity Fenfluramine Imipramine Long-term Food-deprived

SEROTONERGIC and noradrenergic mechanisms remain the two major neurotransmitter systems implicated in the etiology of affective illness (7,35). The major neurochemical action of imipramine and other tricyclic antidepressants has been ascribed to an inhibition of serotonin and/or norepinephrine uptake at the neuronal membrane (6,17). However, the rapid action of tricyclic antidepressant drugs on reuptake is difficult to reconcile with their delayed clinical efficacy (22). Therefore, animal studies of molecular mechanisms pertinent to antidepressant efficacy have concentrated on the adaptive changes in the serotonergic and the noradrenergic neurotransmitter mechanisms following long-term antidepressant treatment.

There is a variety of evidence suggesting an inhibitory effect of the serotonergic neurotransmitter system on the expression of feeding behavior (3,23). Administration of serotonin receptor agonists suppresses food intake, while serotonin antagonists block this effect and may themselves potentiate feeding (4). Drugs which release serotonin (5-HT) from presynaptic terminals such as fenfluramine also reduce food intake (4). The anorexic effect of fenfluramine is attenuated by serotonin antagonists (10), by the 5-HT synthesis inhibitor parachlorophenylalanine (PCPA) (24), by 5-HT depletion with 5,6-dihydroxytryptamine (5,6-DHT) (9), or by raphe lesions (31). Administration of fenfluramine reduces locomotor activity in certain test situations, yet in food-deprived animals, fenfluramine reduces food intake without producing marked changes in activity (5).

The purpose of the present study was to investigate adaptational changes in the serotonergic neurotransmitter mechanisms involved in suppression of food intake and locomotor activity by using fenfluramine as a challenge agent following long-term treatment with the tricyclic antidepressant imipramine.

METHOD

Male Wistar rats weighing approximately 250 g at the beginning of the study were used. Separate groups of animals were used for locomotor and food intake studies.

Food Intake Study

The animals were housed individually and had free access to water. The animals were trained to take their daily food (Purina food pellets) from 10 a.m. to 2 p.m. for 10 days before antidepressant treatment was begun. At the end of the first hour of food access the remaining food, including the spillage, was weighed, and the difference from the original amount constituted the measure of food intake. In addition, total daily food intake (4 hr) and body weight were also recorded for each animal. The animals were divided into control and imipramine treatment groups with six rats in each

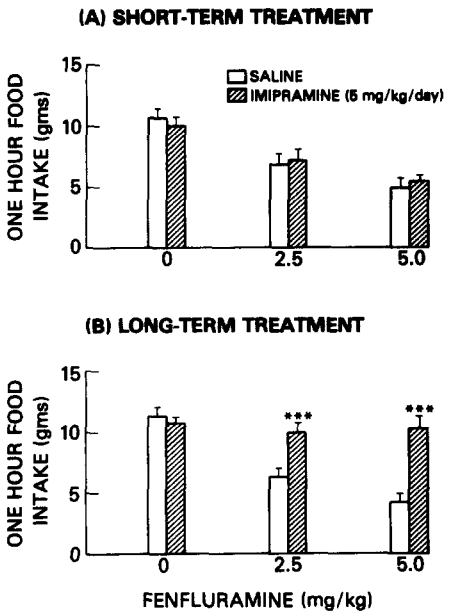

FIG. 1. Effect of short-term (2–6 days) or long-term (21–25 days) imipramine (5 mg/kg/day) treatment on fenfluramine-induced suppression of food intake in rats. Values are expressed as means± S.E.M. Values of imipramine-treated animals (n=6) significantly different from saline-treated animals (n=6) are represented by ***$p<0.001$.

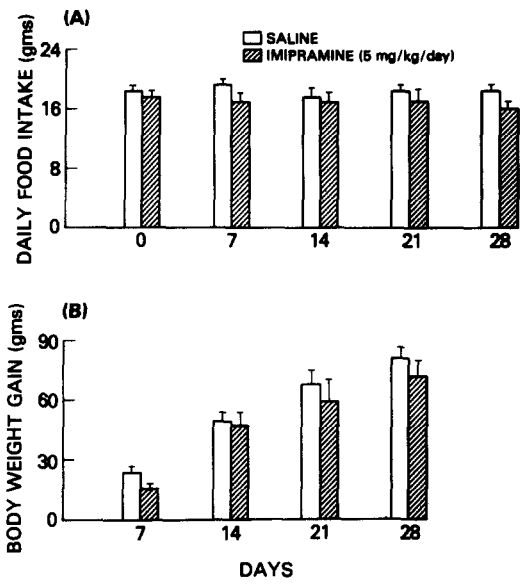

FIG. 2. Effect of imipramine (5 mg/kg/day) treatment on daily (4 hours) food intake (A) and body weight gain (B) in rats. Values are expressed as means±S.E.M. There was no significant difference between saline-treated (n=6) and imipramine-treated (n=6) animals.

group. Imipramine hydrochloride (5 mg/kg/day) or saline was subcutaneously administered continuously by means of osmotic minipumps (Alza Corporation) for 28 days; the pumps were reimplanted at two weeks.

Each animal in both groups was challenged first with saline followed by 2.5 and 5.0 mg/kg doses of d,l-fenfluramine hydrochloride, respectively, with each dose separated by 72 hr during both short-term (2–6 days) and long-term (21–25 days) antidepressant drug treatment. Fenfluramine was injected IP 10 min before placing the food cups into the cages. Food intake on days in between drug days was observed to return to baseline.

Locomotor Study

The animals were housed six per cage and had free access to food and water. Imipramine hydrochloride (5 mg/kg/day) or saline was administered subcutaneously and continuously by means of osmotic minipumps (Alza Corp.) for 28 days. The pumps were reimplanted at two weeks. Each animal in both groups was challenged first with saline followed by 2.5 and 5.0 mg/kg doses of d,l-fenfluramine hydrochloride, respectively, with each dose separated by at least 72 hr during both short-term (2–6 days) and long-term (21–25 days) antidepressant drug treatment. Fenfluramine was dissolved in saline and injected intraperitoneally (IP) 15 minutes before the animals were placed in the activity boxes for locomotor assessment.

Locomotor activity of individual rats was recorded daily for a period of 30 min at the same time of the day in the same test cages (Coulbourn Instruments, 30×25×29 cm), each equipped with five photocell detectors which were located parallel to each other 6 cm apart and 2 cm above the grid floor. The test cage was enclosed in a sound-proof cubicle with a house light and a fan attached on the back side, and a small window for observation of the animal in the front. Interruptions of the photocell beams were recorded automatically by digital counters. Baseline activity was recorded for 7 days for all the animals before the start of antidepressant drug treatment.

Statistics

Statistical analysis of main effects and interactions was conducted using repeated measures analysis of variance (GLM procedure, SAS Institute, Cary, NC), accompanied by a priori designed contrasts. One-way analysis of variance and *t*-tests were used to help characterize significant interactive effects.

RESULTS

Food Intake

Administration of fenfluramine produced significant, $F(2,18)=84.25$, $p<0.001$, decreases in 1 hour food intake in both the saline-treated and imipramine-treated animals. There was also a significant, $F(1,9)=6.47$, $p<0.05$, imipramine treatment effect, significant, $F(1,9)=8.22$, $p<0.05$, short- vs. long-term imipramine effect as well as significant, $F(2,18)=5.39$, $p<0.05$, term × dose × treatment interaction. Further analysis revealed that during short-term (Fig. 1A) imipramine treatment, there was no significant difference between the two groups. However, after long-term (Fig. 1B) imipramine treatment, the suppressant effect of fenfluramine on 1-hour food intake was significantly ($p<0.001$) attenuated in imipramine-treated animals compared to saline-treated animals. During 28 days of imipramine or saline treatment there was no significant difference between the two groups with regard to daily baseline food intake, $F(1,10)=1.62$, $p>0.05$ (Fig. 2A) or body weight gain, $F(1,9)=0.05$, $p>0.05$ (Fig. 2B).

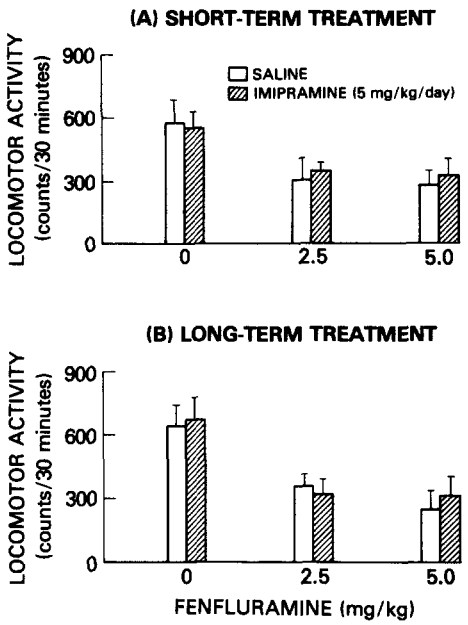

FIG. 3. Effect of short-term (2–6 days) or long-term (21–25 days) imipramine (5 mg/kg/day) treatment on fenfluramine-induced suppression of locomotor activity in rats. Values are expressed as means±S.E.M. There was no significant difference between saline-treated (n=6) and imipramine-treated (n=6) animals.

Locomotor Activity

Short-term (Fig. 3A) or long-term (Fig. 3B) imipramine treatment did not affect baseline locomotor activity. Administration of fenfluramine produced significant, $F(2,20)=11.62$, $p<0.01$, decreases in locomotor activity in both the saline-treated controls and imipramine-treated animals. However, there was neither a significant imipramine treatment, $F(1,10)=0.07$, $p>0.05$, effect nor a significant, $F(1,10)=0.22, p>0.05$, short- vs. long-term imipramine effect.

DISCUSSION

The demonstration of decreased food intake following fenfluramine administration in the present study is consistent with a variety of evidence suggesting an inhibitory effect of the serotonergic system on suppression of feeding behavior (3). Fenfluramine reduces food intake by an action on central serotonergic mechanisms since the peripherally acting 5-HT antagonist, xylamidine (11), does not attenuate it (16). Furthermore, microinjection of fenfluramine into the hypothalamic paraventricular nucleus (PVN) also suppresses food intake (36). The medial PVN is known to have an important function in the control of eating behavior (27). Autoradiographic studies have demonstrated the existence of serotonergic receptors in the medial hypothalamus (29). Recently, Curzon *et al.* (13) demonstrated that 5-HT agonists produce anorexia by stimulation of postsynaptic 5-HT_{1B} receptors. This is also consistent with the dose-dependent reductions in food intake following m-chlorophenylpiperazine (m-CPP) administration (32) which has been shown to have high affinity for 5-HT_{1B} receptors (34) in radioligand studies.

Administration of fenfluramine also produced decreases in locomotor activity in the present study. It is well known that the nucleus accumbens and the mesolimbic projections it receives from the midbrain are involved in the initiation of locomotor activity (30). Microinjections of dopamine (DA) itself or DA agonists into the nucleus accumbens stimulate locomotor activity (30). However, the nucleus accumbens also receives serotonergic projections from the raphe nuclei (19). Lesioning of raphe nuclei produces hyperactivity which is attenuated by 5-HT administration into the nucleus accumbens (12). An inhibitory role of the serotonergic system on locomotor activity is further supported by the finding that DA-stimulated locomotor activity is attenuated by injecting 5-HT into the nucleus accumbens through the same cannula (12). In a previous report from this laboratory, we demonstrated dose-dependent decreases in locomotor activity in rats following m-CPP administration (2). Based on radioligand studies showing m-CPP to have higher affinity for 5-HT_{1B} receptors (34), it is tempting to speculate that m-CPP and possibly fenfluramine may also reduce locomotor activity by stimulating 5-HT_{1B} receptors. However, further studies with 5-HT antagonists of various 5-HT receptor subtypes will be needed to clarify this question. It is possible that decreased locomotor activity induced by fenfluramine may be responsible for fenfluramine-induced anorexia. However, it seems unlikely since in food-deprived rats, fenfluramine has been shown to reduce food intake without producing marked changes in activity (5).

Attenuation of fenfluramine's effect on food intake following long-term but not short-term imipramine treatment suggests either functional subsensitivity of a 5-HT receptor subtype involved in suppression of food intake or marked inhibition of the 5-HT uptake system following long-term imipramine treatment. The former hypothesis seems unlikely since long-term imipramine treatment actually produces functional supersensitivity of 5-HT_{1B} receptors involved in suppression of food intake, locomotor activity (2) and stimulation of prolactin release (1). Other investigators have demonstrated enhanced 5-HT-induced sleep in young chicks following chronic administration of imipramine, desimipramine, amitryptyline and mianserin (26). In electrophysiological studies, chronic administration of tricyclic antidepressants such as imipramine, desipramine and clomipramine potentiates the inhibitory response of forebrain neurones to iontophoretically-administered 5-HT (14).

Biochemical studies have shown that fenfluramine has to be taken up by the 5-HT uptake mechanism into the presynaptic neuron in order to produce release or depletion of serotonin since pretreatment with 5-HT uptake inhibitors of the tricyclic type, such as clomipramine but not imipramine or desipramine, prevents the decrease in 5-HT caused by fenfluramine (20). It is noteworthy that several investigators have reported that acute pretreatment with clomipramine, unlike imipramine, attenuated fenfluramine-induced anorexia (10, 15, 18, 20, 25). In another study, only long-term administration of imipramine inhibited uptake of intraventricularly administered [^{3}H]5-HT into synaptosomal fractions (33). This would be consistent with the failure of fenfluramine to reduce food intake in long-term imipramine-treated animals in the present study. Attenuation of food intake but not of the locomotor suppressant effect of fenfluramine by long-term imipramine treatment suggests that these changes are not due to imipramine's effect on the metabolism of fenfluramine and furthermore, that a decrease in locomotor activity is not always related to a decrease in food intake.

The demonstration of a differential effect of an antidepressant on two different 5-HT mediated functions in the present study is consistent with a variety of clinical and animal studies. Thus, long-term treatment with tricyclic antidepressants potentiates L-tryptophan-induced increases in prolactin (8) but attenuates 5-hydroxytryptophan-induced increases in cortisol (28) in depressed patients. In another study, long-term lithium treatment potentiated L-tryptophan-induced increases in prolactin but not growth hormone in normal subjects (21). In a recent study from our laboratory, long-term clomipramine treatment was shown to attenuate the behavioral and temperature but not the neuroendocrine responses to the serotonin agonist m-CPP in obsessive compulsive patients (37). In animal studies, three-day imipramine treatment potentiated m-CPP's effect on food intake but not on locomotor activity (2). These findings suggest that adaptational consequences of chronic treatment with various antidepressants are not equal throughout the brain and depend more specifically on changes induced within the brain areas influencing that particular paradigm. In summary, this study demonstrates a differential effect of long-term imipramine treatment on fenfluramine-induced suppression of food intake and locomotor activity.

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