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Long-Term Imipramine Treatment Potentiates m-Chlorophenylpiperazine-Induced Changes in Prolactin but not Corticosterone or Growth Hormone Levels in Rats

CHARANJIT S. AULAKH,[1] MARCUS HAASS,* JOSEPH ZOHAR, KRYSTYNA M. WOZNIAK, JAMES L. HILL AND DENNIS L. MURPHY

Laboratory of Clinical Science, National Institute of Mental Health NIH Clinical Center, 10/3D41, Bethesda, MD 20892
**Department of Cardiology, University of Heidelberg, Federal Republic of Germany*

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AULAKH, C. S., M. HAASS, J. ZOHAR, K. M. WOZNIAK, J. L. HILL AND D. L. MURPHY. *Long-term imipramine treatment potentiates m-chlorophenylpiperazine-induced changes in prolactin but not corticosterone or growth hormone levels in rats.* PHARMACOL BIOCHEM BEHAV **32**(1) 37–42, 1989.—Intravenous administration of m-chlorophenylpiperazine (m-CPP, a selective 5-HT agonist) to rats produced increases in plasma prolactin and corticosterone and a decrease in plasma growth hormone concentrations. Long-term but not short-term imipramine treatment potentiated m-CPP's effect on plasma prolactin, but not its effects on corticosterone or growth hormone. Short-term or long-term imipramine treatment did not produce significant changes in baseline levels of prolactin, corticosterone or growth hormone. These findings are compatible with development of functional supersensitivity of 5-HT receptors mediating prolactin release. Lack of potentiation of m-CPP's effects on corticosterone and growth hormone following long-term imipramine treatment suggests either differential regulation of these hormones by serotonergic and possibly other mechanisms, or different 5-HT receptor subtypes mediating the release of these hormones. Alternatively, adaptive changes in other aminergic neurotransmitter mechanisms such as the noradrenergic system may account for the differential effect of long-term imipramine treatment on m-CPP-induced neuroendocrine changes.

m-CPP Imipramine Prolactin Corticosterone Growth hormone Long-term

DUE to the therapeutic lag between the initiation of antidepressant treatment and onset of clinical effects, animal studies of molecular mechanisms pertinent to antidepressant efficacy have concentrated on the adaptive changes in the various aminergic neurotransmitter mechanisms following long-term antidepressant treatment. Adaptive changes in the serotonergic mechanisms following long-term antidepressant treatment have been studied using behavioral, electrophysiological and neuroendocrine paradigms as well as changes in 5-HT receptor densities in various brain areas (54). Among behavioral paradigms, the serotonin behavioral syndrome in rats and head twitch response in mice have been widely used (54). However, both of these behaviors are hindbrain mediated (6,13) and are of uncertain relevance to antidepressant mechanisms. Most of the electrophysiological studies have used serotonin itself, while neuroendocrine studies have used serotonin precursors such as 5-hydroxytryptophan (5-HTP) and tryptophan as challenge agents (54). In recent years, radioligand studies have demonstrated the existence of 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, and 5-HT_2 receptors (22) in the rat brain. Furthermore, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OHDPAT) has been shown to have higher affinity for 5-HT_{1A} receptors (34).

m-Chlorophenylpiperazine (m-CPP), a metabolite of the antidepressant trazodone, has been shown to have high affinity for 5-HT receptors in radioligand studies (44). However, there is controversy regarding the reported affinities of piperazine-type serotonin agonists such as m-CPP and m-trifluoromethylphenylpiperazine (TFMPP) for 5-HT_{1A} and 5-HT_{1B} sites. In rat brain, some studies have reported

[1]Requests for reprints should be addressed to Dr. Charanjit Aulakh, Laboratory of Clinical Science, NIMH, NIH Clinical Center, 10/3D41, 9000 Rockville Pike, Bethesda, MD 20892.

TABLE 1

EFFECTS OF SALINE, SHORT-TERM (2–4 DAYS) OR LONG-TERM (21–23 DAYS) IMIPRAMINE (5 mg/kg/DAY) TREATMENT ON m-CPP-INDUCED INCREASES IN PLASMA PROLACTIN (ng/ml) LEVELS

	Plasma Prolactin Concentrations (ng/ml)		
m-CPP (mg/kg)	Saline	Short-Term Imipramine	Long-Term Imipramine
0	7.24 ± 1.1 (7)	9.50 ± 2.9 (7)	4.7 ± 0.8 (9)
1.25	35.87 ± 5.6 (7)	39.3 ± 3.0 (7)	76.6 ± 13.1 (7)*†
2.5	60.21 ± 8.9 (6)	73.7 ± 23.0 (6)	178.5 ± 27.3 (6)*†

Values are expressed as means ± S.E.M. Values of long-term imipramine-treated animals significantly different from saline or short-term imipramine-treated animals are represented by $*p<0.01$ and $\dagger p<0.01$, respectively.

TFMPP and m-CPP to have a 30–70-fold preference for binding to 5-HT_{1B} sites (2,44), whereas another study reported m-CPP to have almost equal affinity for 5-HT_{1A} and 5-HT_{1B} sites (21). In bovine brain, TFMPP was shown to have a 4–5-fold preference for 5-HT_{1A} sites (38). In human cortex, both TFMPP and m-CPP were shown to have little preference for either site (48). Administration of m-CPP to humans (35) and monkeys (1) produces increases in plasma prolactin and cortisol. In rats, m-CPP administration produces increases in plasma prolactin (42), corticosterone (16) and a decrease in growth hormone levels (3). m-CPP-induced prolactin secretion is mediated by postsynaptic 5-HT receptors since it is prevented by the serotonergic receptor antagonist metergoline and moreover, is potentiated in 5,7-dihydroxytryptamine- (5,7-DHT) lesioned animals (42). By using m-CPP as a challenge agent, the present study investigated adaptive changes in the serotonergic neurotransmitter mechanisms involved in the secretion of various pituitary hormones following long-term treatment with the tricyclic antidepressant imipramine in rats.

METHOD

Male Wistar rats weighing approximately 250 g at the beginning of the study were used. The animals were housed six per cage and had free access to food and water. The animals were housed in a room with automatic light control which provide 12 hr light (7 a.m.–7 p.m.) and 12 hr dark each day. Under halothane anesthesia, left femoral artery and vein were cannulated (PE50, polyethylene tubing) in each animal, and the catheters were exteriorized subcutaneously at the back of the neck (20). For protection of the exteriorized cannulas, the animals were put into special jackets (Harvard Instruments) allowing the animals to freely move in their individual cage without twisting or damaging the catheter. After surgery, each animal was housed individually in a clear plastic cage with food and water freely available. Both the arterial and venous cannulae were flushed every day with heparinized saline to prevent blood clotting. Saline or various doses of m-CPP were injected intravenously (11–11:15 a.m.) at least 48 hours after the surgery. Separate groups of animals were used for short-term and long-term antidepressant study. In the antidepressant study, 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 osmotic minipump was 2.5 cm long with a mean pumping rate of 0.49 μl/hr and a mean fill volume of 193 μl. We demonstrated in a previous report from this laboratory that similar long-term imipramine treatment (5 mg/kg/day for 21 days) does not have any significant effect on body weight gain as compared to saline-treated animals (4). Both the imipramine-treated and saline-treated animals were challenged first with saline, followed by 1.25 and 2.5 mg/kg doses of m-CPP, respectively, with each dose separated by 24 hours during both short-term (2–4 days) and long-term (21–23 days) antidepressant treatment. Blood samples (2.0 ml) were drawn between 11:30–11:45 a.m. in each animal 30 minutes after saline or m-CPP injection. After each drawing, blood volume was restored by infusing the appropriate quantity of blood obtained from the naive donor animals. Blood was collected in heparinized tubes. Following centrifugation, plasma samples were collected. The plasma concentrations of prolactin, corticosterone and growth hormone were measured by radioimmunoassays as described elsewhere (15, 37, 51). The prolactin levels were measured using a double antibody radioimmunoassay (JDN-RIA) which consists of radioiodinated LER-1382-82 and an antiserum prepared in rabbits to LER-1223-DI (37). To avoid possible interassay variation, all samples were assayed within the same radioimmunoassay for each hormone. Since there was no significant difference between short-term and long-term saline-treated animals in response to the m-CPP challenge for either hormone, we have included in the paper only the values obtained from long-term saline-treated animals in order to reduce the number of tables. During the course of the study, none of the animals died after m-CPP treatment, surgery, or chronic imipramine administration. However, in a few animals, the arterial cannulae became blocked and we therefore could not draw blood from those animals.

Drugs

Imipramine hydrochloride (Sigma Chemical Co., St. Louis, MO) and m-CPP hydrochloride (Aldrich Chemical Co., Milwaukee, WI) were all dissolved in saline. Doses of drugs given in the text refer to the salt. The volume injected was 0.1 ml/100 g of body weight.

TABLE 2

EFFECTS OF SALINE, SHORT-TERM (2–4 DAYS) OR LONG-TERM (21–23 DAYS) IMIPRAMINE (5 mg/kg/DAY) TREATMENT ON m-CPP-INDUCED INCREASES IN PLASMA CORTICOSTERONE (μg/dl) LEVELS

	Plasma Corticosterone Concentrations (μg/dl)		
m-CPP (mg/kg)	Saline	Short-Term Imipramine	Long-Term Imipramine
0	5.53 ± 1.0 (7)	6.0 ± 0.8 (7)	4.3 ± 0.9 (8)
1.25	23.4 ± 1.9 (9)	22.3 ± 2.0 (6)	19.7 ± 2.5 (7)
2.5	25.3 ± 0.8 (8)	20.7 ± 3.2 (4)	21.6 ± 2.3 (8)

Values are expressed as means ± S.E.M. There was no significant difference between saline-treated and imipramine-treated animals.

TABLE 3

EFFECTS OF SALINE, SHORT-TERM (2–4 DAYS) OR LONG-TERM (21–23 DAYS) IMIPRAMINE (5 mg/kg/DAY) TREATMENT ON m-CPP-INDUCED DECREASES IN PLASMA GROWTH HORMONE (ng/ml) LEVELS

	Plasma Growth Hormone Concentrations (ng/ml)		
m-CPP (mg/kg)	Saline	Short-Term Imipramine	Long-Term Imipramine
0	82.3 ± 16.3 (7)	82.8 ± 33.5 (6)	76.4 ± 15.4 (7)
1.25	32.3 ± 8.1 (9)	28.7 ± 3.4 (6)	28.3 ± 9.6 (6)
2.5	17.8 ± 3.4 (8)	15.4 ± 5.1 (6)	25.9 ± 7.8 (6)

Values are expressed as means ± S.E.M. There was no significant difference between saline-treated and imipramine-treated animals.

Statistics

These data were analyzed using a variety of analysis of variance techniques. Because most of the measurements were made more than once on the same animal, repeated measures analysis of variance were used. Significant effects were further characterized by one-way analysis of variance at each level of the repeated factor accompanied by a priori designed contrasts. To evaluate the contribution of those animals in which arterial cannulae got blocked, two-way and one-way analysis of variance across and within treatment combinations which paralleled the repeated measures analyses were carried out.

RESULTS

Short-term (3-day) or long-term (21-day) imipramine treatment did not produce significant changes in baseline levels of prolactin (Table 1), corticosterone (Table 2) or growth hormone (Table 3) as compared to saline treatment. Administration of m-CPP produced overall significant drug effect on prolactin, $F(2,8)=25.2$, $p<0.001$ (Table 1), corticosterone, $F(2,14)=73.4$, $p<0.001$ (Table 2), and growth hormone, $F(2,14)=14.9$, $p<0.01$ (Table 3). However, m-CPP administration produced dose-related changes only in prolactin [0.0 vs. 1.25: $F(1,4)=36.2$, $p<0.001$; 1.25 vs. 2.5: $F(1,4)=11.4$, $p<0.05$] and not in corticosterone [0.0 vs. 1.25: $F(1,7)=87.01$, $p<0.001$; 1.25 vs. 2.5: $F(1,7)=0.13$, $p>0.05$] and growth hormone [0.0 vs. 1.25: $F(1,7)=12.9$, $p<0.01$; 1.25 vs. 2.5: $F(1,7)=3.75$, $p>0.05$].

For prolactin (Table 1), there was a significant, $F(1,4)=8.41$, $p<0.05$, treatment effect as well as a significant, $F(2,8)=7.10$, $p<0.05$, drug × treatment interaction. Further analysis revealed that values of long-term imipramine-treated animals differed significantly ($p<0.01$) from both the saline and short-term imipramine-treated animals. There was no significant difference between the saline-treated and short-term imipramine-treated animals. For corticosterone (Table 2), there was neither a significant, $F(1,7)=3.57$, $p>0.05$, treatment effect nor a significant, $F(2,14)=1.14$, $p>0.05$, drug × treatment interaction. Similarly, for growth hormone (Table 3), there was neither a significant, $F(1,7)=0.05$, $p>0.05$, treatment effect nor a significant, $F(2,14)=0.6$, $p>0.05$, drug × treatment interaction.

DISCUSSION

The present study demonstrates that intravenous administration of m-CPP, a 5-HT agonist, stimulates secretion of prolactin and corticosterone while inhibiting secretion of growth hormone. This is consistent with earlier reports suggesting a stimulatory role for a serotonergic neurotransmitter mechanism in the secretion of prolactin (31) and corticosterone (17). m-CPP-induced prolactin release is mediated by postsynaptic 5-HT receptors since it is attenuated by the serotonin receptor antagonist metergoline (3,42). Furthermore, serotonin depletion by 5,7-DHT has been shown to enhance the prolactin releasing effect of m-CPP, suggesting development of supersensitivity of central 5-HT receptors

involved in prolactin release (42). Serotonin-induced prolactin release has been suggested to be due to an action on prolactin releasing factor neurons in the hypothalamus (11,29). Serotonin does not have direct effect on the pituitary lactotroph (27).

Suppression of growth hormone secretion by m-CPP observed in the present study is consistent with earlier reports suggesting an inhibitory role of serotonergic neurotransmitter mechanism in the secretion of growth hormone (28,36). On the other hand, Smythe *et al.* (45) have reported a facilitatory role for the serotonergic mechanisms in the secretion of growth hormone. Growth hormone secretion in the rat is under the control of both growth hormone releasing factor (GHRF) and somatostatin. GHRF stimulates growth hormone release while somatostatin inhibits it (26). One possible explanation for the decreased growth hormone release observed in the present study may be a direct stimulation of somatostatin release by m-CPP. Alternatively, m-CPP might release somatostatin indirectly via CRF. Serotonin stimulates CRF release in vitro (23) and CRF has been shown to stimulate somatostatin release (39).

Potentiation of m-CPP's effect on plasma prolactin following long-term but not short-term imipramine treatment suggests development of functional supersensitivity of 5-HT receptors involved in prolactin secretion. This is consistent with previous neuroendocrine studies in both rodents (32) and humans (9) which demonstrated potentiation of prolactin releasing effect of 5-HT precursors following long-term administration of tricyclic antidepressant drugs. In behavioral studies, we have previously demonstrated potentiation of m-CPP-induced suppression of food intake and locomotor behavior following long-term imipramine treatment (4). Other investigators have demonstrated enhanced serotonin-induced sleep in young chicks (24) and increased hyperthermic responses to m-CPP in rats (53) following long-term antidepressant treatment. Similarly, electrophysiological studies also demonstrate an increase in the inhibitory response of forebrain neurons to iontophoretic serotonin following chronic administration of tricyclic antidepressant drugs such as imipramine, desipramine, and clomipramine (14).

In the present study, m-CPP administration did not produce dose-related changes in either corticosterone or growth hormone, and furthermore, long-term imipramine treatment did not alter the corticosterone or GH responses to m-CPP. One possible explanation for this differential effect on prolactin vs. corticosterone and growth hormone may be that administration of a lower dose of m-CPP might have produced a nearly maximum effect on corticosterone and growth hormone but not on prolactin in the saline-treated animals. This would also explain why long-term imipramine treatment did not potentiate m-CPP's effect on corticosterone and growth hormone. However, it is noteworthy that intraperitoneal administration of higher doses (10 and 20 mg/kg) of m-CPP have been shown to increase plasma corticosterone levels up to 41.3 and 59.9 μg/dl, respectively (16,41). Therefore, it is unlikely that a ceiling effect was achieved in the present study for corticosterone with our low (1.25 and 2.5 mg/kg) doses of m-CPP. On the other hand, there are a variety of clinical and animal studies demonstrating a differential effect of long-term antidepressant treatment on 5-HT agonist-induced changes in plasma prolactin vs. corticosterone or growth hormone. Thus, long-term treatment with tricyclic antidepressants potentiates L-tryptophan-induced increases in prolactin (10), but attenuates 5-hydroxytryptophan-induced increase in cortisol (33) in depressed patients. Similarly, long-term lithium treatment potentiates l-tryptophan-induced increases in prolactin but not growth hormone in normal subjects (18). Furthermore, pretreatment with ritanserin (a selective 5-HT_2 antagonist) potentiates l-tryptophan-induced increases in prolactin but not growth hormone in normal subjects (8). In rats, long-term clorgyline treatment attenuates m-CPP's effect on prolactin but not on corticosterone or growth hormone, whereas short-term clorgyline treatment attenuates m-CPP's effect on corticosterone but not on prolactin or growth hormone (5). In another study, repeated electroconvulsive shock (ECS) treatment potentiated 5-hydroxytryptophan's effect on corticosterone (46) but not on prolactin (32).

There is evidence that in rat brain, functionally distinct subtypes of 5-HT receptor mediate certain specific behaviors (19,46). Recently, neuroendocrine studies have also shown that in rats, certain serotonin agonists release prolactin by stimulation of 5-HT_{1B} (3,52) and corticosterone by stimulation of 5-HT_{1A} and 5-HT_2 receptors (25,52). Potentiation of m-CPP's effects on prolactin (present study) and food intake (4) following long-term imipramine treatment is compatible with development of functional supersensitivity of 5-HT_{1B} receptors mediating anorexia and prolactin release. Serotonin agonists produce anorexia by stimulation of postsynaptic 5-HT_{1B} receptors (12).

In addition to being a receptor agonist, m-CPP has also been shown to release endogenous stores of hypothalamic 5-HT in vitro (40). This could explain m-CPP's effects on corticosterone and growth hormone since 5-HT would stimulate all postsynaptic 5-HT receptors. It is of related interest that long-term administration of tricyclic antidepressant drugs does not modify the 5-HT agonist-induced serotonin behavioral syndrome in rats (30). The serotonin behavioral syndrome in rats is mediated by activation of postsynaptic 5-HT_{1A} receptors (50). Also, long-term treatment with the tricyclic antidepressant drugs, including imipramine, causes either no change or a down-regulation of 5-HT_2 receptor density in the rat brain (54). This would explain a lack of potentiation of m-CPP's effect on corticosterone following long-term imipramine treatment in the present study since 5-HT_{1A} and 5-HT_2 receptors mediate corticosterone release (25,52). Alternatively, adaptive changes in other aminergic neurotransmitter mechanisms such as those of the noradrenergic system (47) following long-term imipramine treatment may be responsible for lack of potentiation of m-CPP's effects on corticosterone and growth hormone.

In summary, enhanced net sensitivity to m-CPP following long-term imipramine treatment observed in the present study is of particular interest since m-CPP is a metabolite of the antidepressant, trazodone (7) and may contribute to the pharmacologic and therapeutic effects of trazodone (16,43). The present study also indicates that 5-HT agonist-induced changes in prolactin levels may be a better neuroendocrine measure for assessing serotonergic function following long-term antidepressant treatment.

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