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RAPID COMMUNICATION

Enhanced Anorexic Responses to m-Chlorophenylpiperazine During Lithium Administration to Fawn-Hooded Rats

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AULAKH, C. S., J. L. HILL AND D. L. MURPHY. *Enhanced anorexic responses to m-chlorophenylpiperazine during lithium administration to Fawn-Hooded rats.* PHARMACOL BIOCHEM BEHAV **49**(3) 759-762, 1994. – In the present study, we investigated whether functional adaptational changes in the serotonergic neurotransmitter mechanisms regulating food intake following long-term lithium treatment in Fawn-Hooded rats (a rat strain suggested to represent a genetic model of depression) were different or similar to those previously observed in Wistar rats. Long-term (21-25 days) lithium treatment accentuated m-chlorophenylpiperazine (m-CPP, a 5-HT agonist) induced decreases in food intake. There was no significant difference in either brain m-CPP concentrations or hypothalamic norepinephrine, dopamine and 5-hydroxyindoleacetic acid concentrations between control and long-term lithium-treated rats following m-CPP. However, hypothalamic serotonin concentrations were significantly higher in long-term lithium-treated compared to saline-treated animals. This finding contrasts with our previous report demonstrating attenuation of m-CPP-induced anorexia in Wistar rats following similar long-term lithium treatment, and therefore suggests a differential adaptation in the serotonergic neurotransmitter mechanisms regulating food intake in a genetic animal model of depression.

m-CPP Food intake Fawn-Hooded rat Serotonin Depression

LITHIUM is widely used clinically for the treatment of acute manic illness, for prophylaxis in manic-depressive (bipolar) and unipolar depressive disorders and, in conjunction with antidepressant drugs, for the treatment of resistant depressive illness (17). Brain serotonin changes have been implicated in the etiology of affective illness and the mode of action of antidepressant and antimanic drugs (14). m-Chlorophenylpiperazine (m-CPP), a metabolite of the antidepressant drug, trazodone, is a postsynaptic 5-HT receptor agonist. Administration of m-CPP to rats produces dose-dependent reductions in food intake (5). Antagonist studies indicate that m-CPP reduces food intake by stimulation of postsynaptic 5-HT_{2C} (formerly designated 5-HT_{1C}) receptors (10). In a previous report from this laboratory, we demonstrated attenuation of m-CPP-induced decreases in food intake following long-term lithium treatment in Wistar rats (5).

In several previous reports from this laboratory, we have demonstrated impaired central serotonergic function in the Fawn-Hooded (FH) rat strain relative to the Wistar and Sprague-Dawley rat strains (2-4,19). Recently, the FH rat strain has been suggested to represent a genetic model of depression and alcoholism (2,15). The purpose of the present

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study was to investigate whether functional adaptational changes in the serotonergic neurotransmitter mechanisms regulating food intake following long-term lithium treatment in FH rats were different or similar to those previously observed in Wistar rats. Therefore, we studied the effects of short-term (3–7 days) and long-term (21–25 days) lithium treatment on m-CPP-induced decreases in food intake in FH rats. In addition, we measured the concentrations of various neurotransmitters and metabolites (norepinephrine, dopamine, serotonin, and 5-hydroxyindoleacetic acid) in the hypothalamus as well as levels of m-CPP in the rest of the brain in long-term saline and lithium-treated animals following intraperitoneal administration of m-CPP in FH rats.

MATERIALS AND METHODS

Male FH rats weighing approximately 200 g at the beginning of the study were used. The animals were housed in temperature-controlled (22 ± 1°C) room with 12-h light dark cycle (lights on 0700). Separate groups of animals were used for food intake and biochemical 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 1000 to 1400 for 10 days before lithium treatment was begun. At the end of the first hour of food access, the remaining food was weighed, and the difference from the original amount constituted the measure of food intake. After weighing, the food was put back into the cages for the next 3 hours. The animals were divided into control and lithium treatment groups with six rats in each group. For lithium treatment, the animals were given rat chow containing lithium carbonate (1.6 g/kg) for 28 days. Plasma levels of lithium in rats maintained on this diet were found to be 0.8 ± 0.07 mEq/l in our previous study (5). Each animal in both groups was challenged first with saline followed by 1.25 mg/kg and 5.0 mg/kg doses of m-CPP. Each dose was separated by 48 h during both short-term (3–7 days) and long-term (21–25 days) lithium treatment. The food intake on days between challenge days was observed to return to baseline values. m-CPP or saline was injected intraperitoneally (IP) 10 min before placing the food into the cages.

Biochemical Study

Twelve FH rats were used in this study. The animals were divided into control and lithium treatment groups with six rats in each group. For lithium treatment, the animals were given rat chow containing lithium carbonate (1.6 g/kg) for 23 days. On day 23, both control and lithium-treated animals were challenged with 2.5 mg/kg dose of m-CPP. m-CPP was injected IP and the animals were sacrificed 30 min after m-CPP injection.

The rats were sacrificed by decapitation. The brains were dissected out and the hypothalamus was removed from each brain for determinations of various neurotransmitters and their metabolites. The rest of the brain was used for determination of m-CPP levels. The concentrations of norepinephrine (NE), dopamine (DA), serotonin (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) as well as levels of m-CPP in the brain were determined by high pressure liquid chromatography (HPLC) as described in a previous report from this laboratory (4).

Drugs

m-CPP hydrochloride (Aldrich Chemical Co., WI) was dissolved in saline. Lithium carbonate (BIO-serve, Frenchtown, NJ) was given in rat chow (1.6 g/kg). Doses of m-CPP given in the text refer to the salt. The volume injected was 0.1 ml/100 g body weight.

Statistical Analysis

The effects of various doses of m-CPP on food intake were analyzed using repeated measures design analysis of variance accompanied by contrasts of main and interactive effects within the analysis of variance procedure. For comparison of brain m-CPP concentrations and hypothalamic concentrations of various neurotransmitters between control and lithium-treated animals, Student's t-test was used and a Bonferroni correction was applied because multiple t-tests were performed. Calculation procedures were those of the statistical analysis system (SAS Institute, Cary, NC).

RESULTS

Short-term lithium treatment produced significant decreases in baseline food intake, a change that disappeared during long-term lithium treatment (Fig. 1). During short-term lithium treatment (Fig. 1), there was a significant [$F(2, 20) = 54.9$, $p < 0.001$] m-CPP effect as well as a significant [$F(1, 10) = 23.1$, $p < 0.001$] lithium treatment effect. How-

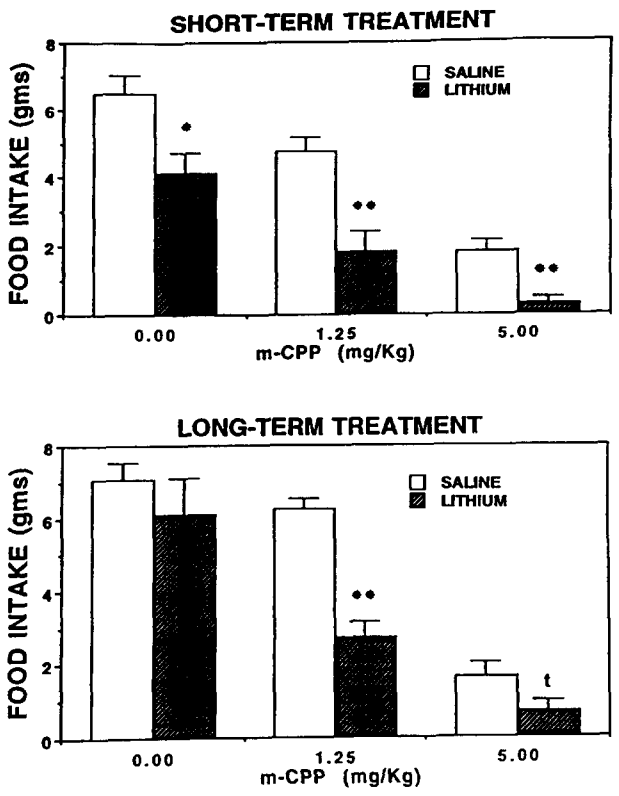

FIG. 1. Effects of various doses of m-CPP on 1-h food intake in saline-treated ($n = 6$) and lithium-treated ($n = 6$) animals. Data are expressed as means ± SEM. Values of lithium-treated animals significantly different from saline-treated animals are represented by $*p < 0.05$; $**p < 0.01$. t = trend, $p < 0.07$.

TABLE 1

BRAIN m-CPP CONCENTRATIONS AND HYPOTHALAMIC NOREPINEPHRINE (NE), DOPAMINE (DA), SEROTONIN (5-HT) AND 5-HYDROXYINDOLEACETIC ACID (5-HIAA) CONCENTRATIONS IN LONG-TERM (23 DAYS) SALINE-TREATED (n = 7) AND LITHIUM-TREATED (n = 5) FAWN-HOODED RATS FOLLOWING INTRAPERITONEAL ADMINISTRATION OF 2.5 MG/KG m-CPP.

Long-Term Treatment	m-CPP (ng/g)	Neurotransmitter Monoamines and 5-HIAA (ng/g)			
		NE	DA	5-HT	5-HIAA
Saline	2.19 ± 0.28	1510 ± 107	251 ± 17	718 ± 28	1059 ± 61
Lithium	2.41 ± 0.38	1243 ± 77	240 ± 18	895 ± 30*	989 ± 112

Values are expressed as means ± SEM. Values of long-term lithium-treated animals significantly different from saline-treated animals are represented by *$p < 0.01$.

ever, the lithium treatment × m-CPP interaction was nonsignificant.

During long-term lithium treatment (Fig. 1), there was a significant [$F(2, 20) = 88.58$, $p < 0.001$] m-CPP effect, a significant [$F(1, 10) = 12.73$, $p < 0.01$] lithium treatment effect, and a significant [$F(2, 20) = 6.47$, $p < 0.01$] m-CPP × lithium treatment interaction. Further analysis revealed that the food intake suppressant effects of m-CPP were significantly accentuated in long-term lithium-treated animals when compared to controls.

Brain m-CPP concentrations and hypothalamic NE, DA, 5-HT, and 5-HIAA concentrations in control and lithium-treated animals following administration of m-CPP are shown in Table 1. There was no significant difference between the two groups in brain m-CPP concentrations or hypothalamic NE, DA, and 5-HIAA concentrations. However, hypothalamic 5-HT concentrations were significantly higher in long-term lithium-treated animals relative to control animals (Table 1).

DISCUSSION

The demonstration of dose-related reductions in food intake in FH rats following m-CPP administration in the present study is consistent with a previous report from this laboratory in Wistar rats (5). m-CPP decreases food intake by stimulation of postsynaptic 5-HT_{2C} receptors (10). In accord with several earlier reports (5,10,19), we studied the effect of m-CPP administration on the first hour food intake only due to relatively short half-life of m-CPP (7). Moreover, we have demonstrated in a previous report from this laboratory that m-CPP administration suppresses food intake during the first hour and then, enhances food consumption during the next 3 hours due to a rebound effect in both Wistar and FH rats (19).

The present study further demonstrates that long-term lithium treatment accentuates m-CPP-induced decreases in food intake in FH rats. This finding contrasts with our previous finding in Wistar rats in which similar long-term lithium treatment attenuated m-CPP-induced decreases in food intake (5). For comparative purposes, administration of 1.25 mg/kg and 5.0 mg/kg doses of m-CPP produced 55% and 90% decreases in food intake relative to controls (dose 0), respectively, in long-term lithium-treated FH rats in the present study, whereas the same two doses produced considerably smaller decrements of 6% and 35%, respectively, in food intake in long-term lithium-treated Wistar rats (5).

In the present study, m-CPP pharmacokinetic factors do not seem to play any role since brain m-CPP levels were not significantly different in control and long-term lithium-treated FH rats following m-CPP administration. On the other hand, 5-HT levels in the hypothalamus were significantly higher in long-term lithium-treated animals and might possibly contribute to the observed accentuation of m-CPP's effects on food intake. In addition to acting as a direct 5-HT_{2C} agonist (10), m-CPP could also act, in part, indireclty by releasing 5-HT, an effect demonstrated in hypothalamic slices studied in vitro (16).

Alternatively, 5-HT_{2C} receptors may become supersensitive in FH rats following long-term lithium treatment. However, long-term lithium treatment has been reported to decrease postsynaptic 5-HT_1 and 5-HT_2 receptor densities (8) and the activity of the synthetic enzyme, tryptophan hydroxylase (11). On the other hand, it is of note that long-term (3 weeks) but not short-term (5 days) lithium increased 5-HT stimulated cyclic adenosine monophosphate (cAMP) formation despite decreased 5-HT_1 receptor binding (8). Unfortunately, the effect of repeated lithium administration on brain 5-HT_{2C} receptors has apparently not yet been assessed; however, as long-term lithium treatment has been shown to increase the availability of inositol triphosphate (IP_3) for cholinergic receptor stimulation in rat brain (12), and as IP_3 is also the second messenger for the 5-HT_{2C} receptor, such a change could contribute to the enhanced 5-HT_{2C} responses observed in the present study.

The demonstration of a differential effect of long-term lithium treatment in the FH rat strain vs. the Wistar rat strain is very intriguing. Recently, we have observed potentiation of m-CPP-induced hyperthermia in FH rats following long-term treatment with the tricyclic antidepressants, imipramine and clomipramine (our unpublished observations), whereas the same treatment produced attenuation of m-CPP-induced hyperthermia in Wistar rats (20). The FH rat strain is also functionally subsensitive to the hyperthermic effect of m-CPP relative to the Wistar rat strain (13). Furthermore, m-CPP-induced hyperthermia has recently been suggested to be mediated by stimulation of 5-HT_{2C} receptors (13). There are numerous reports in the literature suggesting that the hypothalamus is involved in the regulation of both food intake and temperature. However, we did not observe any difference in mesulergine-labeled 5-HT_{2C} receptor density in the hypothalamus between the Wistar and the FH rat strains (9). Therefore, it is tempting to speculate that the functional subsensitive responses in the FH rat strain may be due to changes in the postreceptor signal-transducing mechanisms, and, furthermore, the effect of long-term lithium treatment in FH vs. Wistar rats may also be due to a differential effect of long-term lithium treatment on postreceptor signal-transducing

mechanisms between these two strains. Only further experimentation will clarify this phenomenon.

It is of interest to note that like depressed patients, the FH rat strain has higher baseline levels of corticosterone (4), reduced platelet 5-HT uptake (1), and also manifests functional subsensitivity to 5-HT agonists (3,4,19). Recently, the FH rat strain has been suggested to represent a genetic model of depression and alcoholism (2,15). In depressed patients, long-term treatment with the tricyclic antidepressant, clomipramine, has recently been reported to potentiate fenfluramine-induced increases in plasma prolactin (18). In another study, long-term lithium treatment enhanced 5-HT mediated neuroendocrine responses in tricyclic resistant depressed patients (6). The demonstration of enhancement of serotonergic function following lithium treatment observed in a genetic animal model of depression suggests that these effects of lithium may be responsible for correcting as yet unspecified abnormalities of 5-HT function, which may be involved in the pathogenesis of depression.

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