Pharmacology Biochemistry & Behavior, Vol. 33, pp. 265–267. Maxwell Pergamon Macmillan plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

BRIEF COMMUNICATION

Differential Effect of Clomipramine Treatment on m-Chlorophenylpiperazine-Induced Increases in Plasma Prolactin and Corticosterone in Rats

KRYSTYNA M. WOZNIAK,[1,2] CHARANJIT S. AULAKH, JAMES L. HILL AND DENNIS L. MURPHY

Section on Clinical Neuropharmacology, Laboratory of Clinical Science
National Institute of Mental Health, Bethesda, MD 20892

Received 6 June 1988

WOZNIAK, K. M., C. S. AULAKH, J. L. HILL AND D. L. MURPHY. *Differential effect of clomipramine treatment on m-chlorophenylpiperazine-induced increases in plasma prolactin and corticosterone in rats.* PHARMACOL BIOCHEM BEHAV 33(1) 265–267, 1989.—Intravenous administration of m-chlorophenylpiperazine (m-CPP, a serotonin agonist) to rats increased plasma prolactin and corticosterone concentrations. Long-term (21-day) and short-term (3-day) treatment with the tricyclic antidepressant, clomipramine, did not have any significant effect on baseline levels of either prolactin or corticosterone. Long-term but not short-term clomipramine treatment significantly potentiated m-CPP's effect on plasma prolactin. On the other hand, both long-term and short-term clomipramine treatment significantly attenuated m-CPP's effect on plasma corticosterone. These findings are consistent with other animal and clinical studies demonstrating a differential effect of antidepressant treatment on two different serotonin-mediated neuroendocrine functions.

Clomipramine Prolactin Corticosterone m-CPP Rats

SINCE a therapeutic lag exists between the initiation of antidepressant drug treatment and onset of clinical effects, animal studies of molecular mechanisms pertinent to antidepressant efficacy have concentrated on the adaptive changes in various aminergic neurotransmitter mechanisms following long-term antidepressant treatment. Adaptational changes in the serotonergic neurotransmitter system following long-term antidepressant treatment have been investigated using behavioral, electrophysiological, neuroendocrine, and biochemical paradigms (19). m-Chlorophenylpiperazine (m-CPP), a metabolite of the antidepressant trazodone, readily displaces [^{3}H]serotonin (5-HT) in binding studies, possessing greater affinity for 5-HT_1 than for 5-HT_2 sites (8,10), with a predominant selectivity for the 5-HT_{1B} receptor subtype (6,16). Administration of m-CPP to humans, monkeys, and rats produces increases in plasma prolactin and cortisol (humans and monkeys) or corticosterone (rats) (11).

The purpose of the present study was to use m-CPP as a challenge agent to explore functional adaptational changes in the serotonergic mechanisms mediating prolactin and corticosterone release following long-term treatment with the tricyclic antidepressant, clomipramine, which is a potent 5-HT uptake inhibitor (7). Similar neuroendocrine studies to date have often used 5-HT precursors as challenge agents (19). These precursors would of course ultimately result in the stimulation of all the 5-HT receptors and subtypes. The use of the more specific agent, m-CPP, could help to delineate more specifically the functional status of the serotonergic systems mediating these responses. Thus, we studied the effects of short-term (3-day) and long-term (21-day) clomipramine (a tricyclic antidepressant) treatment on m-CPP-induced increases in plasma prolactin and corticosterone in rats.

METHOD

Male Wistar rats weighing approximately 250 g at the begin-

[1]Present address: Laboratory of Clinical Studies, National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD 20892.

[2]Requests for reprints should be addressed to Dr. K. M. Wozniak, Section on Clinical Neuropharmacology, Laboratory of Clinical Science, National Institute of Mental Health, NIH Clinical Center, 10-3D41, Bethesda, MD 20892.

ning of the study were used. The animals were housed six per cage and had free access to food and water. Under halothane anesthesia, the left femoral artery and vein were cannulated (PE 50, polyethylene tubing) in each animal, and the catheters were exteriorized subcutaneously at the back of the neck. After surgery, each animal was housed individually in a clear plastic cage with food and water freely available. Saline or various doses of m-CPP were injected intravenously (11:00–11:15 a.m.) at least 48 hours after the surgery. Separate groups of animals were used for short-term and long-term antidepressant treatment studies. Saline or clomipramine (5 mg/kg/day) was subcutaneously administered continuously by means of osmotic minipumps (Alza Corporation) for 28 days; the pumps were reimplanted at 2 weeks. Both the clomipramine-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 (1.5 ml) were drawn between 11:30–11:45 a.m. in each animal 30 min 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 centrifuge tubes containing 0.5 ml of ethylenediaminetetracetic acid (EDTA). Following centrifugation, plasma samples were collected and stored at −70°C. The plasma concentrations of prolactin and corticosterone were measured by radioimmunoassays as described in a previous report from this laboratory (1).

Statistics

Data were analyzed by repeated measures analysis of variance (GLM Procedure, SAS Institute, Cary, NC). Significant effects were further characterized by one-way analysis of variance at each level of the repeated factor accompanied by a priori designed contrasts.

RESULTS

Compared to saline treatment, short-term (3-day) and long-term (21-day) clomipramine treatment did not produce significant changes in baseline levels of either prolactin (Fig. 1) or corticosterone (Fig. 2). Administration of m-CPP produced an overall significant drug effect on prolactin, $F(2,28)=73.02$, $p<0.001$, and corticosterone, $F(2,24)=40.38$, $p<0.001$. However, m-CPP administration produced dose-related changes in prolactin [0.0 vs. 1.25: $F(1,14)=48.97$, $p<0.001$; 1.25 vs. 2.5: $F(1,14)=30.10$, $p<0.001$] and not in corticosterone [1.25 vs. 2.5: $F(1,12)=1.7$, $p>0.05$].

For prolactin (Fig. 1), there was a significant, $F(2,14)=4.48$, $p<0.05$, clomipramine treatment effect as well as a significant, $F(4,28)=2.99$, $p<0.05$, m-CPP drug × treatment interaction. Further analysis revealed that values of long-term clomipramine-treated animals differed significantly ($p<0.05$) from both the saline-treated and short-term clomipramine-treated animals. There was no significant difference between the saline-treated and short-term clomipramine-treated animals. For corticosterone (Fig. 2), there was a significant, $F(2,11)=5.23$, $p<0.05$, clomipramine treatment effect as well as a significant, $F(4,22)=4.95$, $p<0.01$, m-CPP drug × treatment interaction. Further analysis revealed that the m-CPP-induced corticosterone increase was significantly blunted in both the short-term and long-term clomipramine-treated animals relative to saline-treated animals. There was no significant difference between the short-term and long-term clomipramine-treated animals.

DISCUSSION

The demonstration of enhanced secretion of prolactin and corticosterone following intravenous administration of m-CPP in the present study is consistent with earlier reports from this and other laboratories (1,15). m-CPP-induced prolactin release is mediated by stimulation of postsynaptic 5-HT receptors since it is attenuated by the 5-HT receptor antagonist, metergoline, and potentiated in 5,7-dihydroxytryptamine (5,7-DHT)-lesioned animals (15). Potentiation of m-CPP's effect on plasma prolactin following long-term but not short-term clomipramine treatment in the present study suggests development of functional supersensitivity of the 5-HT receptors involved in prolactin secretion. This finding is consistent with previous neuroendocrine studies both in rodents (12) and humans (2) which demonstrated potentiation of the prolactin-releasing effect of 5-HT precursors following long-term administration of tricyclic antidepressant drugs. Similarly,

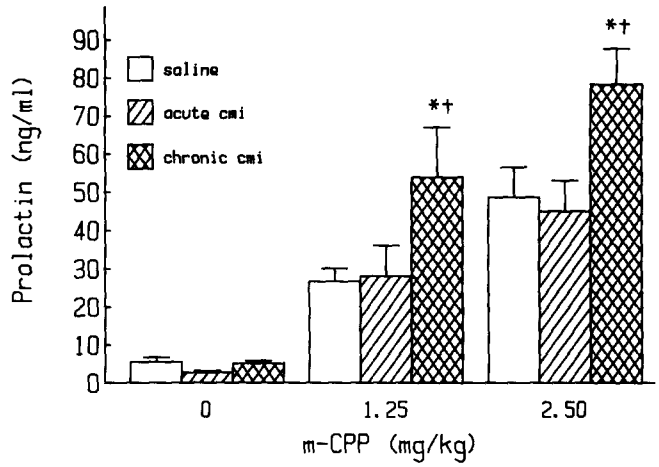

FIG. 1. The effect of saline, short-term (2–4 days) and long-term (21–23 days) clomipramine treatment on m-CPP-induced increases in plasma prolactin (ng/ml) levels (30 min after injection). Values of five to six animals are expressed as means ± S.E.M. Values of long-term clomipramine-treated animals significantly different from saline-treated and short-term clomipramine-treated animals are represented by *$p<0.05$ and †$p<0.05$, respectively.

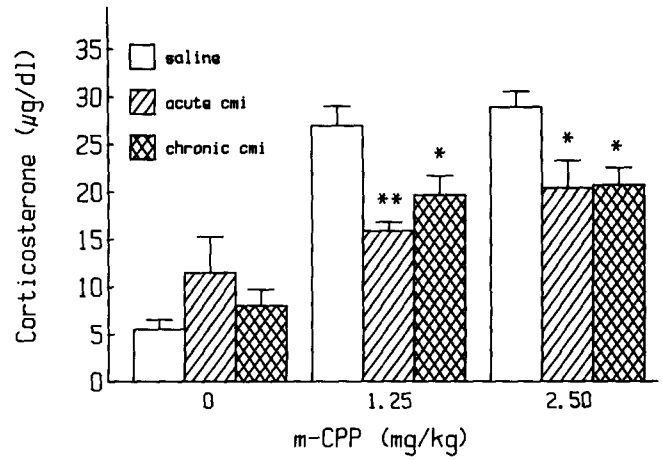

FIG. 2. The effects of saline, short-term (2–4 days) and long-term (21–23 days) clomipramine treatment on m-CPP-induced increases in plasma corticosterone (μg/dl) levels (30 min after injection). Values of five to six animals are expressed as means ± S.E.M. Values of short-term and long-term clomipramine-treated animals significantly different from saline-treated animals are represented by *$p<0.05$; **$p<0.01$.

several behavioral and electrophysiological studies have also demonstrated enhanced sensitivity of postsynaptic 5-HT receptors following long-term administration of tricyclic antidepressant drugs (19). However, evidence from radioligand binding studies shows chronic treatment with tricyclic antidepressants to down-regulate 5-HT_2 receptor sites, with little or no effect on 5-HT_1 sites (14,19). Although this is in apparent contradiction with the above, radioligand binding studies investigating the effects of antidepressant treatment on specific 5-HT receptor subtypes are lacking at this time. In addition, it is not yet clear which 5-HT receptors mediate the prolactin and corticosterone release (18). Nevertheless, it should also be remembered that decreases in receptor number need not imply a functional subsensitivity (14).

Unlike prolactin, m-CPP-induced increases in corticosterone were found to be significantly blunted in both short-term and long-term clomipramine-treated animals. One possible explanation may be that potentiation of noradrenergic function by the desmethyl metabolite of clomipramine is responsible for this observed attenuated corticosterone response. Desmethylclomipramine is a potent noradrenaline uptake inhibitor (9) and there is some evidence that the secretion of adrenocorticotropic hormone (ACTH) is influenced by an excitatory mechanism involving α-adrenoceptors and an inhibitory mechanism involving β-adrenoceptors (4). Alternatively, there may be either a differential regulation of these two hormones by the serotonergic mechanisms or indeed different 5-HT receptor subtypes may be mediating different neuroendocrine functions (18).

There are a variety of clinical and animal studies demonstrating a differential effect of long-term antidepressant treatment on 5-HT agonist-induced neuroendocrine changes. Thus, long-term treatment with tricyclic antidepressants potentiates L-tryptophan-induced increases in prolactin (3), but attenuates 5-hydroxytryptophan (5-HTP)-induced increases in cortisol (13) in depressed patients. In normal subjects, long-term lithium treatment potentiates L-tryptophan-induced increases in prolactin but not growth hormone (5). In rats, long-term clorgyline treatment attenuates m-CPP's effect on prolactin but not on corticosterone, whereas short-term clorgyline treatment attenuates m-CPP's effect on corticosterone but not on prolactin (1). In another study, repeated electroconvulsive shock treatment potentiated 5-HTP's effect on corticosterone (17) but not on prolactin (12). These findings together suggest that adaptational consequences of long-term 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.

REFERENCES

1. Aulakh, C. S.; Zohar, J.; Wozniak, K. M.; Hill, J. L.; Murphy, D. L. Clorgyline treatment differentially affects m-chlorophenylpiperazine-induced neuroendocrine changes. Eur. J. Pharmacol. 150:239–246; 1988.
2. Charney, D. S.; Heninger, G. R. Monoamine receptor sensitivity and depression: Clinical studies of antidepressant effects on serotonin and noradrenergic function. Psychopharmacol. Bull. 19:490–495; 1983.
3. Charney, D. S.; Heninger, G. R.; Sternberg, D. E. Serotonin function and mechanism of action of antidepressant treatment. Arch. Gen. Psychiatry 41:359–365; 1984.
4. Checkley, S. A. Neuroendocrine tests of monoamine function in man: A review of basic theory and its application to the study of depressive illness. Psychol. Med. 10:35–53; 1980.
5. Glue, P. M.; Cowen, P. J.; Nutt, D. J.; Kolakowska, T.; Grahme-Smith, D. G. The effect of lithium on 5-HT-mediated neuroendocrine responses and platelet 5-HT receptors. Psychopharmacology (Berlin) 90:398–402; 1986.
6. Hamon, M.; Cossery, J. M.; Spampinato, U.; Gozlan, H. Are there selective ligands for 5-HT_{1A} and 5-HT_{1B} receptor binding sites in brain? Trends Pharmacol. Sci. 7:336–337; 1986.
7. Horn, A. S.; Trace, R. C. Structure activity relations for the inhibition of 5-hydroxytryptamine uptake by tricyclic antidepressants with synaptosomes from serotonergic neurones in rat brain homogenates. Br. J. Pharmacol. 51:399–403; 1974.
8. Invernizzi, R.; Cotecchia, S.; DeBlasi, A.; Mennini, T.; Patuccini, R.; Samanin, R. Effects of m-chlorophenylpiperazine on receptor binding and brain metabolism of monoamines in rats. Neurochem. Int. 3:239–244; 1981.
9. Laakman, G.; Gugath, M.; Kuss, H. J.; Zygan, K. Comparison of growth hormone and prolactin stimulation induced by clomipramine and desimipramine in man in connection with clomipramine metabolism. Psychopharmacology (Berlin) 82:62–67; 1984.
10. Martin, L. L.; Sanders-Bush, E. Comparison of the pharmacological characteristics of $5HT_1$ and $5HT_2$ binding sites with those of serotonin autoreceptors which modulate serotonin release. Naunyn Schmiedebergs Arch. Pharmacol. 321:165–170; 1982.
11. Mellow, A. M.; Aulakh, C. S.; Murphy, D. L. Neuroendocrine effects of serotonergic agents: Applications to the study of central serotonin function in humans. In: Rech, R. H.; Gudelsky, G. A., eds. 5-HT agonists as psychoactive drugs. Ann Arbor, MI: NPP Books; 1988:259–262.
12. Meltzer, H. Y.; Simonovic, M.; Sturgeon, R. D.; Fang, V. S. Effect of antidepressants, lithium, and electroconvulsive shock treatment on rat serum prolactin levels. Acta Psychiatr. Scand. 290(Suppl. 63): 100–121; 1981.
13. Meltzer, H. Y.; Lowry, M.; Robertson, A.; Goodnick, P.; Perline, R. Effect of 5-hydroxytryptophan on serum cortisol levels in major affective disorders. Arch. Gen. Psychiatry 41:391–397; 1984.
14. Peroutka, S. J.; Snyder, S. H. Long term antidepressant treatment decreases spiroperidol-labeled serotonin receptor binding. Science 210:88–90; 1980.
15. Quattrone, A.; Schettini, G.; Annunziato, L.; Direnzo, G. Pharmacological evidence of supersensitivity of central serotonergic receptors involved in the control of prolactin secretion. Eur. J. Pharmacol. 76:9–13; 1981.
16. Sills, M. A.; Wolfe, B. B.; Frazer, A. Determination of selective and non-selective compounds for the 5-HT_{1A} and 5-HT_{1B} receptor subtypes in rat frontal cortex. J. Pharmacol. Exp. Ther. 231:480–487; 1984.
17. Steiner, J. A.; Grahame-Smith, D. G. The effect of repeated electroconvulsive shock on corticosterone responses to centrally acting pharmacological stimuli in the male rat. Psychopharmacology (Berlin) 71:205–212; 1980.
18. van de Kar, L. D.; Lorens, S. A.; Urban, J. H.; Kunimoto, K.; Bethea, C. L. Comparison of the effects of serotonin (5-HT_{1A} and $_{1B}$) agonists and 5-HT_2 antagonist on renin, corticosterone and prolactin secretion. Fed. Proc. 3783; 1987.
19. Willner, P. Antidepressants and serotonergic neurotransmission: An integrative review. Psychopharmacology (Berlin) 85:387–404; 1985.