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Long-Term Antidepressant Treatment Restores Clonidine's Effect on Growth Hormone Secretion in a Genetic Animal Model of Depression

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AULAKH, C. S., P. MAZZOLA-POMIETTO AND D. L. MURPHY. *Long-term antidepressant treatment restores clonidine's effect on growth hormone secretion in a genetic animal model of depression.* PHARMACOL BIOCHEM BEHAV **55**(2) 265–268, 1996.—We have recently demonstrated that various doses of clonidine failed to increase growth hormone (GH) in Fawn-hooded (FH) rats (a rat strain suggested to represent a genetic model of depression). In the present study, we investigated whether long-term antidepressant treatment might normalize clonidine's effect on GH secretion in male FH rats. Long-term (16 days) treatment with the tricyclic antidepressant, imipramine (5 mg/kg/day), the 5-HT uptake inhibiting antidepressant, fluoxetine (2.5 mg/kg/day), and the noradrenergic uptake inhibiting antidepressant, desipramine (5 mg/kg/day), accentuated clonidine's effect on GH levels. On the other hand, long-term treatment with the monoamine oxidase type-A inhibiting antidepressant, clorgyline (1 mg/kg/day) and the α_2-noradrenergic antagonists, yohimbine and 1-phenylpiperazine (1 mg/kg/day, each) did not modify clonidine's effect. These findings suggest enhancement of 5-HT_{2C} receptor-mediated function following long-term treatment with uptake inhibiting antidepressants in a genetic animal model of depression. **Copyright © 1996 Elsevier Science Inc.**

Imipramine Desipramine Fluoxetine Clorgyline Fawn-hooded rat strain Yohimbine

BRAIN serotonin (5-HT) changes have been implicated in the etiology of affective illness and mode of action of antidepressant and antimanic drugs (17). 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 central serotonergic functions following long-term antidepressant treatment have been identified using behavioral, electrophysiological, and neuroendocrine paradigms as well as in measurements of 5-HT receptor densities in various brain areas (23).

We have recently demonstrated that clonidine stimulates growth hormone (GH) secretion by activation of α_2-adrenergic heteroreceptors present on 5-HT nerve terminals, which, in turn, enhance 5-HT activity via stimulation of postsynaptic 5-HT_{2C} (formerly known as 5-HT_{1C}) receptors to promote GH-releasing factor (2). Furthermore, we demonstrated that either α_2-adrenergic heteroreceptors or 5-HT_{2C} receptors or both were functionally subsensitive in the Fawn-hooded (FH) rat strain relative to the Wistar rat strain (2). In several previous reports from this laboratory, we have demonstrated impaired central serotonergic function in the FH rat strain relative to the Wistar and Sprague–Dawley (SD) rat strains (4,9,22). Recently, the FH rat strain has been suggested to represent a genetic model of depression and alcoholism (2,6,18,19).

In two recent reports from this laboratory, we have demonstrated accentuation of 5-HT_{2C} receptor-mediated anorexia following long-term lithium treatment (5) as well as restoration

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of clonidine's effect on GH secretion following lithium treatment in the FH rat strain (6). The purpose of the present study was to investigate whether long-term treatment with other antidepressants would also restore clonidine's effect on GH secretion in this genetic animal model of depression. Therefore, we studied the effects of long-term (16 days) treatment with a variety of antidepressants on clonidine's effect on GH secretion in FH rats. In addition, we also studied the effects of long-term (16 days) treatment with the α_2-noradrenergic antagonists on clonidine's effect on GH secretion in FH rats because such treatment in SD rats accentuates GH responses to clonidine (13).

METHOD

Male FH rats (Frederick Cancer Research and Development Center, Frederick, MD), weighing approximately 250 g at the beginning of the study, were used. The animals were housed three per cage in a temperature-controlled (22 ± 1°C) room with a 12 L:12 D cycle (lights on at 0700 h). The animals had free access to Purina rat chow and water at all times. Separate groups of animals were used for each treatment.

For chronic antidepressant and α_2-noradrenergic antagonist treatment studies, imipramine (5 mg/kg/day), desipramine (5 mg/kg/day), fluoxetine (2.5 mg/kg/day), clorgyline (1 mg/kg/day), yohimbine (1 mg/kg/day), 1-PP (1.0 mg/kg/day), or saline were subcutaneously administered continuously for 16 days by means of osmotic minipumps (Model 2002, Alza Corporation). The selection of doses was based on our previous work as well as the clinical dosage in the case of the antidepressants (3). The selection for duration for long-term antidepressant and α_2-noradrenergic antagonist treatments was based on the previously published literature demonstrating functional adaptational changes in both noradrenergic and serotonergic neurotransmitter mechanisms following 2–3 weeks of treatment in rats (3,13). On day 16, saline or antidepressant-treated animals were challenged with clonidine (50 μg/kg). The α_2-adrenergic antagonist-treated animals were challenged with clonidine (50 μg/kg) on day 16, 48 h after the removal of minipumps. Clonidine was injected intraperitoneally between 1015 and 1100 h. The animals were sacrificed 30 min after clonidine injection between 1045 and 1130 h.

The rats were sacrificed by decapitation, and trunk blood was collected in centrifuge tubes containing 0.5 ml of ethylenediaminetetraacetic acid. Following centrifugation, plasma samples were collected and stored at −70°C. The plasma concentrations of GH were measured by radioimmunoassay as described elsewhere (12).

Drugs

The drugs, clonidine HCl, clorgyline HCl, imipramine HCl, yohimbine HCl (Research Biochemicals, Inc., Natick, MA), l-phenylpiperazine (1-PP) dihydrochloride (Aldrich Chemical Company, Milwaukee, WI), fluoxetine HCl (Eli Lilly and Company, Cincinnati, OH), and desipramine HCl (Sigma Chemical Company, St. Louis, MO), were used in the study. Fluoxetine was dissolved in 100% dimethylsulfoxide (DMSO). All other drugs were dissolved in 0.9% saline. In the text, all the drug doses given refer to the salt.

Data Analysis

The effect of clonidine on GH following chronic treatment with antidepressants or α_2-noradrenergic antagonists was examined using a one-way analysis of variance accompanied by single degree of freedom contrasts established a priori in which the control group was compared with each of the other treatments. All data are reported as means ± SEM.

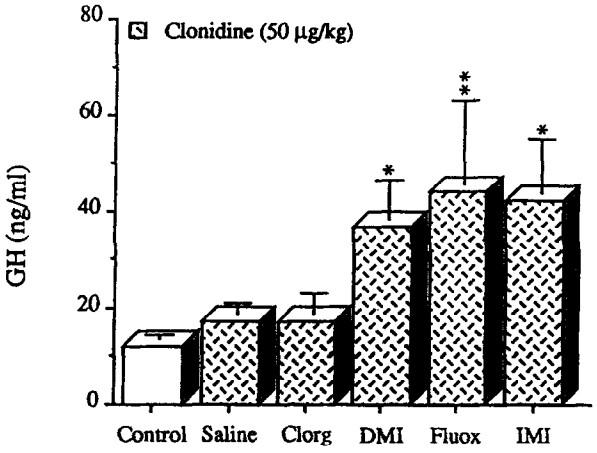

FIG. 1. Effects of chronic imipramine (IMI), fluoxetine (Fluox), desipramine (DMI), clorgyline (Clorg), or saline treatment on clonidine's effect on growth hormone (GH) levels in FH rats. Values are expressed as means ± SEM from at least six animals. *$p < 0.05$, **$p < 0.01$, significantly different from control animals.

RESULTS

The effects of chronic treatment with saline, imipramine, desipramine, fluoxetine, and clorgyline on clonidine-induced increases in GH levels are shown in Fig. 1. Analysis of variance showed an overall significant, $F(5, 38) = 2.69$, $p < 0.05$, treatment effect. Further analysis revealed that clonidine administration produced significant increases in GH levels only in imipramine-treated, desipramine-treated, and fluoxetine-treated animals relative to control animals (Fig. 1).

The effects of chronic treatment with the α_2-noradrenergic antagonists, yohimbine and 1-PP, on clonidine-induced increases in GH levels are shown in Fig. 2. Analysis of variance showed an overall nonsignificant, $F(3, 28) = 1.79$, $p > 0.05$, treatment effect.

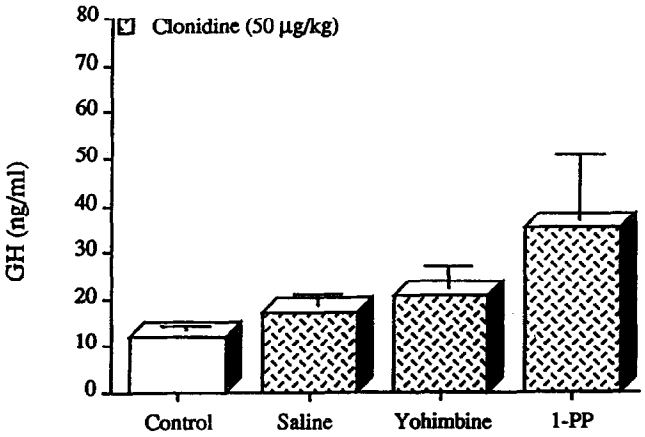

FIG. 2. Effects of chronic yohimbine, 1-PP, or saline treatment on clonidine's effect on growth hormone levels in FH rats. Values are expressed as means ± SEM from eight animals. There was no significant difference between control animals and any of the other treatments.

DISCUSSION

The present study demonstrates that clonidine administration produced significant increases in GH levels in long-term imipramine-treated, desipramine-treated, and fluoxetine-treated but not saline-treated FH rats. Clonidine stimulates GH secretion by activation of α_2-adrenergic heteroreceptors present on 5-HT nerve terminals, which, in turn, enhance 5-HT activity via stimulation of postsynaptic 5-HT_{2C} receptors to promote GH-releasing factor (2). These findings suggest the development of functional supersensitivity of either α_2-adrenergic heteroreceptors or 5-HT_{2C} receptors following long-term treatment with imipramine, desipramine, and fluoxetine. The contribution of pharmacokinetic factors in enhancing clonidine's effect on GH secretion seems unlikely because two to four times higher doses (100 μg and 200 μg/kg) of clonidine did not elicit GH secretion in FH rats (2).

The demonstration of an accentuation of clonidine's effect on GH secretion in FH rats following long-term imipramine and fluoxetine treatment in the present study contrasts with our previous findings in Wistar rats in which clonidine-induced GH secretion was significantly attenuated following similar long-term treatment with imipramine and fluoxetine (3). Other investigators have also reported attenuation of clonidine-induced GH secretion following long-term treatment with imipramine in Sprague–Dawley rats (13). Long-term clorgyline treatment did not modify clonidine's effect on GH secretion in Wistar rats (3) as well as in depressed patients (21). The demonstration of a differential effect of long-term antidepressant treatment in the FH rat strain vs. the Wistar rat strain is very intriguing. Recently, we have demonstrated accentuation of m-chlorophenylpiperazine (m-CPP)-induced hypophagia in FH rats following long-term lithium treatment (5), whereas the same treatment produced attenuation of m-CPP-induced hypophagia in Wistar rats (10). Furthermore, long-term treatment with the tricyclic antidepressants, imipramine, and clomipramine, accentuated m-CPP-induced hyperthermia in FH rats (8), whereas the same treatment produced attenuation of m-CPP-induced hyperthermia in Wistar rats (24). The FH rat strain is also functionally subsensitive to the hypophagic (22) and hyperthermic (16) effects of m-CPP relative to the Wistar rat strain. Furthermore, m-CPP-induced hypophagia (7,15) and hyperthermia (16) are also mediated by stimulation of 5-HT_{2C} receptors.

One possible explanation for the enhanced effect of clonidine on GH secretion my be that 5-HT_{2C} receptors become supersensitive in FH rats following long-term treatment with imipramine, desipramine, and fluoxetine. The effects of long-term treatment with these antidepressants on brain 5-HT_{2C} receptor density have apparently not yet been assessed in FH rats. In Wistar rats, [^{3}H]mesulergine-labeled 5-HT_{2C} receptor density in various brain areas was not modified by long-term treatment with imipramine and clomipramine, whereas clorgyline treatment significantly reduced [^{3}H]mesulergine binding (B_{max} values) in both the hypothalamus and striatum compared to saline-treated animals (14). Therefore, it is possible that the differential effects of antidepressants in FH vs. Wistar rats may be due to a differential effect of long-term antidepressant treatment on either 5-HT_{2C} receptor density or postreceptor signal-transducing mechanisms between these two rat strains. Only further experimentation will clarify this phenomenon.

Another possible explanation for the enhanced effect of clonidine on GH secretion in FH rats may be that α_2-adrenergic heteroreceptors present on 5-HT nerve terminals become supersensitive following long-term antidepressant treatment. However, this possibility seems unlikely because chronic administration of α_2-adrenergic antagonists failed to restore clonidine's effect on GH secretion in FH rats in the present study. Chronic (14 days) treatment with yohimbine has been shown to potentiate clonidine-induced GH secretion in Sprague–Dawley rats (13). Moreover, attenuation of clonidine-induced GH release following long-term treatment with 5-HT uptake inhibiting antidepressants in Wistar rats has been suggested to be due to decreased sensitivity of α_2-heteroreceptors present on 5-HT nerve terminals (3).

Finally, it is of interest to note that like depressed patients, the FH rat strain has higher baseline levels of corticosterone (9), reduced platelet 5-HT uptake (1), exhibit high degrees of immobility in the forced swim test (19) and also manifests functional subsensitivity to many 5-HT agonists (2,4,9,22). In depressed patients, long-term treatment with the tricyclic antidepressant, clomipramine, potentiated fenfluramine-induced increases in plasma prolactin (20). In another study, long-term lithium treatment enhanced 5-HT–mediated neuroendocrine responses in tricyclic-resistant depressed patients (11). Thus, the demonstration of enhancement of 5-HT_{2C} receptor-mediated functions following long-term treatment with the tricyclic antidepressants in the present study and a previous study (8) as well as with lithium (5) observed in a genetic animal model of depression suggests that these effects of tricyclic antidepressants and 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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