Pharmacology Biochemistry & Behavior, Vol. 39, pp. 769–771. Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

RAPID COMMUNICATION

Down-Regulation of Dopamine$_1$ (D_1) Receptors by Chronic Imipramine Is Species-Specific

GABRIEL NOWAK,[1] PHIL SKOLNICK AND IAN A. PAUL

Laboratory of Neuroscience, National Institute of Diabetes, Digestive, and Kidney Diseases
National Institutes of Health, Bethesda, MD 20892

Received 3 April 1991

NOWAK, G., P. SKOLNICK AND I. A. PAUL. *Down-regulation of dopamine$_1$ (D_1) receptors by chronic imipramine is species-specific.* PHARMACOL BIOCHEM BEHAV **39**(3) 769–771, 1991.—Chronic treatment with imipramine (15 mg/kg, twice daily for 14 days) induces a down-regulation of limbic D_1 receptors in rats but not in mice. In this mouse strain, both chronic and acute imipramine treatment have been shown to produce clear behavioral effects in the forced swim test. While the data presented here are consistent with previously reported findings in rats, they demonstrate that the down-regulation of D_1 receptors by chronic antidepressant treatment is species-specific. This phenomenon indicates that D_1 receptor down-regulation is not critical to the therapeutic mechanism of action of antidepressants.

D_1 receptors Dopamine Imipramine Antidepressants Mice Rats Species-specificity Limbic system Corpus striatum

CHRONIC, but not acute treatment of rats with imipramine results in a down-regulation of D_1 receptors in both limbic structures and corpus striatum without affecting dopamine$_2$ (D_2) receptors in these areas (3,6). Moreover, stimulation of dopamine D_2 receptors has been reported to reduce immobility in a behavioral screen for antidepressant drug activity, the forced swim test (1). It has further been argued that dopaminergic neurotransmission exerts a permissive role in the behavioral response to antidepressants in the forced swim test (2). Thus both neurochemical (3,6) and behavioral (1,2) studies have implicated dopaminergic pathways in the antidepressant actions of imipramine [reviewed in (7)].

During the course of studies comparing the effects of imipramine to other putative antidepressants in mice (8), we attempted to replicate the effects of chronic imipramine treatment on dopamine receptors in this species. We now report that chronic imipramine treatment is without effect on dopamine receptors in mice, but as previously reported, down-regulates D_1 but not D_2 receptors in rat limbic homogenates (3,6).

METHOD

Animals and Treatment

Male, NIH Swiss mice (20–25 g) and Sprague-Dawley rats (200–250 g, Taconic Farms) were housed in groups of 8–12 (mice) or 4–6 (rats) on a 12-h light-dark cycle (lights on from 0700 to 1900) with free access to food and water. The animals were injected twice daily (IP) for 14 days with either 0.9% saline or 15 mg/kg imipramine in a volume of 0.1 ml (mouse) or 0.5 ml (rat).

Drugs and Reagents

Imipramine was obtained from Sigma (St. Louis, MO). [^{3}H]SCH-23390 was obtained from Amersham (Arlington Heights, IL) and [^{3}H]spiperone from NEN-Dupont (Wilmington, DE). Hydrofluor was obtained from National Research Diagnostics (Mannville, NJ). All other drugs and reagents were obtained from RBI (Natick, MA).

Tissue Preparation

Twenty-four h after the last injection, the animals were sacrificed by decapitation. Limbic structures (containing the olfactory tubercle, preoptic area, nucleus accumbens, septum, amygdala and overlying limbic cortex) and striatum were dissected and placed immediately on solid CO_2 (6). The tissue was stored at −70°C until assayed using the method of Hyttel and Arnt (5). Briefly, the tissue was homogenized in 50 vol. (w/v) of an ice-cold 50 mM potassium phosphate buffer, pH 7.4, using a Brinkmann Polytron. The homogenates were centrifuged at 25,000 × g for 10 min, pellets were rehomogenized in another portion of buffer and then centrifuged. The final pellets were re-

[1]Requests for reprints should be addressed to Dr. Gabriel Nowak, Laboratory of Neuroscience, NIDDK/NIH, Building 8, Room 111, Bethesda, MD 20892.

suspended in 350 volumes (limbic system, w/v) or 1000 volumes (striatum) of buffer.

Protein determinations were made using the bicinchoninic acid method with kits supplied by Pierce (Rockford, IL).

Radioligand Binding

For single point determinations of D_1 receptor binding, 0.2–0.3 nM [^{3}H]SCH-23390 (spec.act. 76 Ci/mmol) was added to 1 ml aliquots of membrane suspension (0.15–0.25 mg protein). Nonspecific binding was defined in the presence of 5 μM cis(Z)flupentixol with a final assay volume of 1.2 ml. In saturation experiments, 0.06 to 2.0 nM [^{3}H]SCH-23390 was used. All assays were performed in duplicate and were incubated for 60 min at 30°C.

For single point determinations of D_2 receptor binding, 0.1–0.2 nM [^{3}H]spiperone (spec.act. 23 Ci/mmol) was added to 2 ml aliquots of membranes suspension. Nonspecific binding was defined in the presence of 5 μM (+)butaclamol with a final volume of 2.2 ml. In saturation experiments, 0.03 to 1.0 nM [^{3}H]spiperone were used. To exclude the binding of [^{3}H]spiperone to $5HT_2$ receptors, all the tubes contained 100 nM mianserin. The samples were incubated for 15 min at 37°C.

All assays were terminated by rapid filtration over glass fiber filters (Whatman GF/C) using a Brandel MB-48R manifold. The filters were then washed 3 times with 5 ml ice-cold buffer, dried and placed in scintillation vials with 4 ml of Hydrofluor liquid scintillation cocktail. Radioactivity was measured in a Beckman LS-5801 liquid scintillation counter with 50% efficiency.

For saturation binding analyses, B_{MAX} and K_D values were calculated using the Gauss-Marquardt method with an iterative curve fitting routine (GraphPAD/InPlot, San Diego, CA). In all cases, goodness of fit was $r^2>.95$. Data were deemed significant when $p<0.05$ using Student's *t*-test.

RESULTS

Chronic imipramine treatment resulted in a statistically significant ($p<0.02$) reduction in the specific binding of [^{3}H]SCH-23390 to D_1 receptors in rat limbic homogenates (Fig. 1). The magnitude of this reduction (18%) is consistent with previously reported values (3,6). Saturation analysis of [^{3}H]SCH-23390 binding (0.06–2.0 nM) demonstrated that this effect was consequent to a reduction in B_{MAX} (saline, 421 ± 10 fmol/mg protein; imipramine, 355 ± 18 fmol/mg protein, $p<0.05$) with no change in K_D (saline, 0.29 ± 0.07 nM; imipramine, 0.33 ± 0.10 nM) (Fig. 2). [^{3}H]SCH-23390 binding to D_1 receptors in mouse limbic homogenates was unaffected by imipramine treatment (Fig. 1). Likewise, specific [^{3}H]SCH-23390 binding to mouse striatal homogenates was unaffected by chronic imipramine (saline, 577 ± 87 fmol/mg protein; imipramine, 595 ± 86 fmol/mg protein). Consistent with previous findings (6), [^{3}H]spiperone binding to D_2 receptors was unaffected by chronic imipramine in either rats or mice (data not shown).

DISCUSSION

These data demonstrate that the down-regulation of D_1 receptors by imipramine (3,6) is species-specific, manifest in rats, but not in mice. The dose of imipramine employed in the present experiments is sufficient to induce significant behavioral effects in both the forced swim and tail-suspension tests in this mouse strain (8). Moreover, this dose of imipramine induced a significant down-regulation of β-adrenergic receptors in cortical homogenates from these subjects (−19%, $p<0.05$) suggesting that pharmacokinetic factors cannot account for this species-specificity. Further, these data indicate that down-regulation of D_1 receptors in these brain regions is not required for the behavioral action of imipramine in the mouse forced swim test.

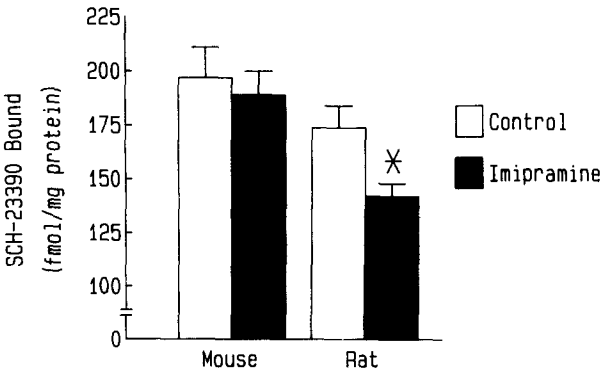

FIG. 1. Effect of chronic imipramine on D_1 dopamine receptors in limbic homogenates. Values are the mean ± SEM of 6–8 animals/group. *$p<0.02$ (two-tailed Student's *t*-test).

Regional differences in the potency of antidepressants to inhibit dopamine reuptake have been reported, with IC_{50} values in rat limbic structures 1–2 orders of magnitude lower than in striatum (4). These data have led DeMontis et al. (4) to suggest that the regionally selective blockade of dopamine reuptake sites by antidepressants is responsible for the regional specificity in the down-regulation of D_1 receptors and desensitization of dopamine-stimulated adenylate cyclase activity observed following chronic antidepressant administration. Thus, one possible explanation for the inability of imipramine to down-regulate mouse limbic D_1 receptors may be that imipramine is a less potent or efficacious inhibitor of dopamine reuptake in mice than in rats.

It has previously been suggested that D_1 receptor down-regulation may be related to the therapeutic mechanism of action of antidepressant drugs (3,6). This hypothesis was based on the D_1 receptor down-regulating properties of several classes of antidepressants. However, the species specificity of D_1 receptor down-regulation in response to chronic imipramine treatment reported here raises important questions regarding the clinical relevance of this phenomenon.

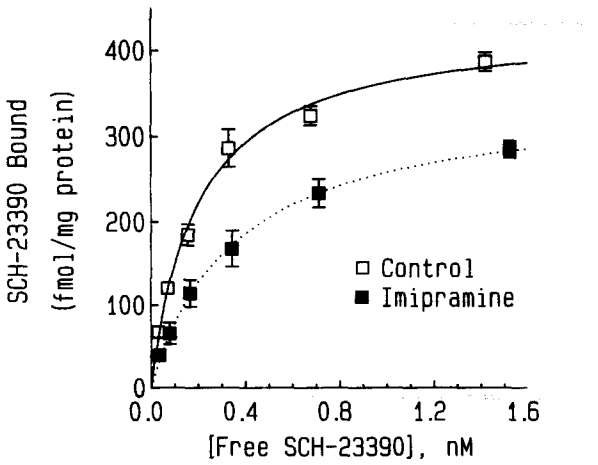

FIG. 2. Effect of chronic imipramine treatment on saturation binding of [^{3}H]SCH-23390 to rat limbic homogenates. Values are the mean ± SEM of 6–8 animals/group.

REFERENCES

1. Borsini, F.; Lecci, A.; Mancinelli, A.; D'Aranno, V.; Meli, A. Stimulation of dopamine D-2 but not D-1 receptors reduces immobility time of rats in the forced swimming test: Implications for antidepressant activity. Eur. J. Pharmacol. 148:301–307; 1988.
2. Cervo, L.; Grignaschi, G.; Samanin, R. The role of the mesolimbic dopaminergic system in the desipramine effect in the forced swimming test. Eur. J. Pharmacol. 178:129–133; 1990.
3. DeMontis, G. M.; Devoto, P.; Gessa, G. L.; Meloni, D.; Porcella, A.; Saba, P.; Serra, G.; Tagliamonte, A. Chronic imipramine reduces [^{3}H]SCH-23390 binding and DA-sensitive adenylate cyclase in the limbic system. Eur. J. Pharmacol. 167:299–303; 1989.
4. DeMontis, G. M.; Devoto, P.; Gessa, G.L.; Meloni, D.; Porcella, A.; Saba, P.; Serra, G.; Tagliamonte, A. Central dopaminergic transmission is selectively increased in the limbic system of rats chronically exposed to antidepressants. Eur. J. Pharmacol. 180:31–35; 1990.
5. Hyttel, J.; Arnt, J. Characterization of binding of [^{3}H]SCH-23390 to dopamine D-1 receptors. Correlation to other D-1 and D-2 measures and effect of selective lesions. J. Neural Transm. 68:171–189; 1987.
6. Klimek, V.; Nielsen, M. Chronic treatment with antidepressants decreases the number of [^{3}H]SCH-23390 binding sites in the rat striatum and limbic system. Eur. J. Pharmacol. 139:163–169; 1987.
7. Muscat, R.; Sampson, D.; Willner, P. Dopaminergic mechanism of imipramine action in an animal model of depression. Biol. Psychiatry 28:223–230; 1990.
8. Trullas, R.; Skolnick, R. Functional antagonists at the NMDA receptor complex exhibit antidepressant actions. Eur. J. Pharmacol. 185:1–10; 1990.