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Repeated Administration of Antidepressants Enhances Agonist Affinity for Mesolimbic D_2-Receptors

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Abstract—Studies have shown an increased responsiveness of the dopaminergic system after repeated administration of a variety of antidepressant drugs. In the present study, the effect of repeated administration (twice daily for 14 days) of imipramine and mianserin on the affinity of dopamine D_2-receptors for quinpirole, a D_2-agonist, and on the quinpirole-induced locomotor hyperactivity was examined in rats. Repeated doses of imipramine and mianserin increased the affinity of quinpirole for [^{3}H]spiperone binding sites in membranes prepared from the limbic system but not the striatum. The locomotor hyperactivity induced by quinpirole was enhanced by chronic treatment with both antidepressants. The data indicate that the enhanced responsiveness of the dopaminergic system in rats, observed after chronic treatment with antidepressants, may result from an increased affinity of agonists at D_2-receptors in the mesolimbic system.

We have previously reported that antidepressant drugs given repeatedly, but not acutely, enhance the locomotor hyperactivity induced by (+)-amphetamine, apomorphine and nomifensine (Maj 1984, 1986). A similar increase in the (+)-amphetamine-induced hyperactivity has been reported by others (Spyraki & Fibiger 1981; Martin-Iverson et al 1983; Arnt et al 1984). Antidepressants given repeatedly enhance the behavioural stimulation induced by (+)-amphetamine, apomorphine or dopamine injected into the nucleus accumbens (Maj & Wedzony 1985, 1988; Maj et al 1985; Plaznik & Kostowski 1987). Heal & Green (1978) observed a potentiation of the dopamine- and dibutyryl cyclic AMP-induced locomotion when the compounds were injected into the nucleus accumbens after repeated electroconvulsive shock.

Up to the present, the detailed mechanism of such effects remain unclear. No changes have been found in presynaptic dopamine neurons, release or turnover after repeated administration of antidepressants (Willner 1983; Maj 1984). Furthermore, the binding to dopamine receptors, assessed by using [^{3}H]spiperone as a ligand, is not modified by antidepressants (Peroutka & Snyder 1980; Martin-Iverson et al 1983; Klimek & Nielsen 1987).

Since two types of dopamine receptors, D_1 and D_2, have been characterized (Kebabian & Calne 1979), it appeared worthwhile to determine which subtype of those receptors was involved in adaptive changes found after repeated administration of antidepressants. According to some data, the locomotor hyperactivity is evoked by stimulation of D_2-subtype of dopamine receptors (Seeman 1980; Starr & Starr 1986). Our preliminary experiments demonstrated that imipramine and (+)-oxaprotiline administered repeatedly increase the locomotor hyperactivity induced by quinpirole, a selective agonist of D_2-receptors (Maj et al 1989).

The aim of the present report was to obtain further information on whether repeated administration of antidepressants may modify the affinity of dopamine D_2-receptors to their agonist quinpirole (LY-171555). Hence the ability of quinpirole to displace [^{3}H]spiperone from its binding sites in two brain regions (limbic system and striatum) was determined. The effect of antidepressants on the quinpirole-induced locomotor hyperactivity was also studied. Two drugs were used: imipramine, a typical antidepressant which inhibits noradrenaline and 5-hydroxytryptamine (5-HT) uptake, and mianserin, an atypical drug without uptake-inhibiting properties.

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Materials and Methods

Male Wistar rats (200–230 g) were caged in groups of 7–8 under standard laboratory conditions on constant 12 h light/dark cycle with free access to food and water.

Impramine or mianserin were administered in a dose of 10 mg kg^{-1} p.o. once or repeatedly (twice a day for 14 days). Controls received water according to the same schedule.

Biochemical effects

Twenty four h after the last dose of drug the rats were killed and striatum and limbic system (containing olfactory tubercle, preoptic area, nucleus accumbens, septum and amygdala) dissected and frozen.

To measure the ability of quinpirole to displace [^{3}H]spiperone binding, the tissue was gently homogenized in 10 volumes (w/v) of ice-cold potassium phosphate buffer (50 mM, pH 7·4) using a Polytron homogenizer for 15 s and centrifuged at 25000 *g* for 15 min. This step was repeated twice. The final pellets were resuspended in 100 or 50 volumes (w/v) of potassium phosphate buffer (pH 7·4) containing 40 nM ketanserin to occlude the 5-HT (S_2) sites, for the striatum and limbic system, respectively.

Quinpirole hydrochloride in concentrations ranging from 10^{-9} to 10^{-3}M and [^{3}H]spiperone (0·2 nM; spec. act. 20·2 Ci mmol^{-1} NEN Chemicals) were used in the displacement studies. Dissociation constants were obtained by using the iterative non-linear least squares curve-fitting program LIGAND (Munson & Rodbard 1980).

Behavioural effects

Twenty four h after the last dose of antidepressant, the rats were injected with quinpirole in a dose of 0·3 mg kg^{-1} s.c. After 30 min, the animals were individually placed in the photoresistor actometers (two light beams) and their locomotor activity (horizontal movements) was measured for 2 h. Groups consisted of 9–10 animals each.

Drugs

The drugs used were: imipramine hydrochloride (Polfa), ketanserin (Janssen Pharmaceutica), mianserin hydrochloride (Organon), quinpirole hydrochloride (Lilly). All drugs were dissolved in distilled water.

Results

Biochemical effects

Competition studies showed that the ability of quinpirole to inhibit the binding of [^{3}H]spiperone to limbic system dopamine D_2-receptors occured with two distinct computer fitted, dissociation constants, $KD_1 = 0{\cdot}094 \pm 0{\cdot}001$ μM and $KD_2 = 16 \pm 0{\cdot}9$ μM in the control membranes (Fig. 1). The D_2-(high) and D_2-(low) affinity states constitutes 47 and 53% of the total number of dopamine receptors measured. Repeated treatment with imipramine and mianserin significantly enhanced the ability of quinpirole to displace [^{3}H]spiperone binding. This result was seen as a shift to the left, the change in slope of the competitive curve after imipramine pretreatment resulting in values of $KD_1 = 0{\cdot}0015 \pm 0{\cdot}0001$ μM, $KD_2 = 1{\cdot}28 \pm 0{\cdot}09$ μM and $KD_3 = 188 \pm 11{\cdot}2$ μM for the slopes, indicating an increase in the affinity of the agonist to its receptor (Fig. 1). After repeated treatment with mianserin, quinpirole displaced [^{3}H]spiperone also to give three dissociation constants, $KD_1 = 0{\cdot}0018 \pm 0{\cdot}0001$ μM, $KD_2 = 0{\cdot}65 \pm 0{\cdot}06$ μM and $KD_3 = 35{\cdot}8 \pm 3{\cdot}13$ μM. As is shown in Fig. 2, [^{3}H]spiperone binds to two distinct sites of D_2-receptors in the striatal membrane preparation. When competitive curves for inhibition by quinpirole of [^{3}H]spiperone binding to striatal membranes were obtained, neither imipramine nor mianserin changes the KD_1 or KD_2 values significantly (Fig. 2).

A single dose of imipramine or mianserin did not influence the ability of quinpirole to compete with [^{3}H]spiperone for its binding sites (data not shown) in the striatum and limbic system.

Behavioural effects

Quinpirole in a dose of 0·3 mg kg^{-1} s.c. significantly enhanced the locomotor activity of control rats (Fig. 3). Imipramine and mianserin administered to rats repeatedly (10 mg kg^{-1} p.o., twice daily for 14 days) had no effect on spontaneous locomotor activity. On the other hand, the quinpirole-induced hyperactivity was increased by repeated administration of imipramine and mianserin (Fig. 3).

Twenty four h after a single dose of either drug, the spontaneous locomotor activity and quinpirole-induced hyperactivity were not modified (data not shown).

Discussion

Our biochemical data indicate that imipramine and mianserin, given repeatedly, increase the ability of quinpirole and dopamine D_2-receptor agonist, to displace [^{3}H]spiperone from its binding sites in the limbic system membranes. It results in a shift to higher affinity for both D_2-(high) and D_2-(low) states of dopamine receptors. This is not the case, however, with the striatal membranes.

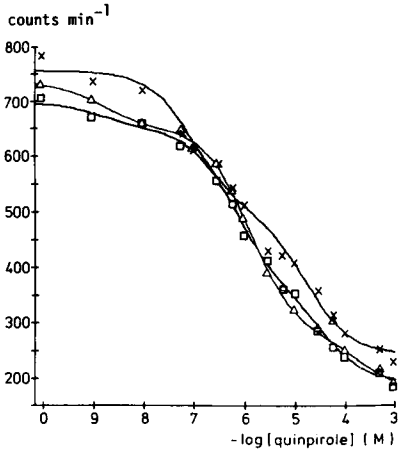

FIG. 1. Competition of quinpirole for [^{3}H]spiperone binding sites in the limbic system membranes of rats repeatedly treated with imipramine [IMI] and mianserin (MIA). Estimated dissociation constants (μM) by using the computer program LIGAND were:

Saline	×——×	$KD_1 = 0{\cdot}094 \pm 0{\cdot}001$ (46·7%); $KD_2 = 16 \pm 0{\cdot}9$ (53·3%) ($P < 0{\cdot}001$)
Imipramine	△——△	$KD_1 = 0{\cdot}0015 \pm 0{\cdot}0001$ (12·7%); $KD_2 = 1{\cdot}28 \pm 0{\cdot}09$ (66·2%) ($P < 0{\cdot}05$); $KD_3 = 188 \pm 11{\cdot}2$ (21·1%) ($P < 0{\cdot}01$)
Mianserin	□——□	$KD_1 = 0{\cdot}0018 \pm 0{\cdot}0001$ (8·8%); $KD_2 = 0{\cdot}65 \pm 0{\cdot}06$ (60·8%) ($P < 0{\cdot}001$); $KD_3 = 35{\cdot}8 \pm 3{\cdot}13$ (30·4%) ($P < 0{\cdot}01$)

The experiments were repeated three times with similar results.

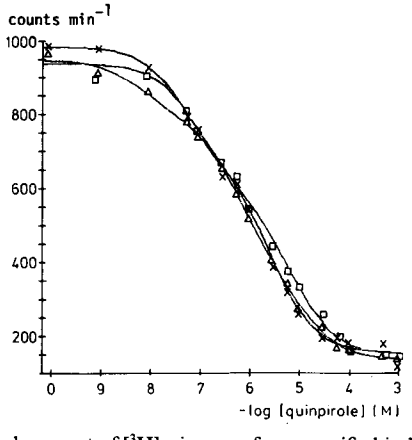

FIG. 2. Displacement of [^{3}H]spiperone from specific binding sites in the striatal membranes of rats repeatedly treated with imipramine or mianserin, by increasing concentrations (M) of quinpirole. Dissociation constants (μM) were estimated by using LIGAND,

Saline	×——×	$KD_1 = 0{\cdot}050 \pm 0{\cdot}005$ (40·7%); $KD_2 = 2{\cdot}72 \pm 0{\cdot}25$ (59·3%) ($P < 0{\cdot}001$)
Imipramine	△——△	$KD_1 = 0{\cdot}056 \pm 0{\cdot}005$ (40·7); $KD_2 = 3{\cdot}39 \pm 0{\cdot}29$ (59·3%) ($P < 0{\cdot}001$)
Mianserin	□——□	$KD_1 = 0{\cdot}104 \pm 0{\cdot}01$ (47·3%); $KD_2 = 6{\cdot}58 \pm 0{\cdot}05$ (52·7%) ($P < 0{\cdot}001$)

The experiments were repeated three times with similar results.

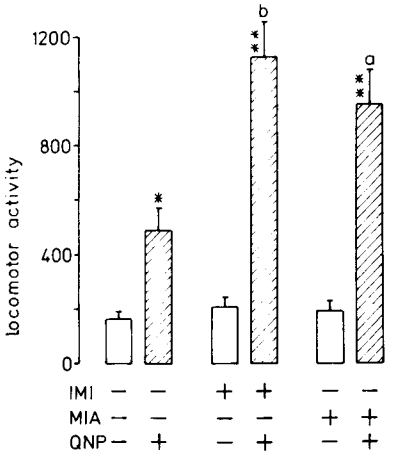

FIG. 3. Effect of imipramine (IMI) and mianserin (MIA) administered repeatedly on locomotor hyperactivity induced by quinpirole (QNP), 0·3 mg kg^{-1} s.c. given to rats 24 h after their last dose. Data represent means (± s.e.m.) of 9–10 animals per group. The statistical significance was evaluated by ANOVA followed by Duncan test; * $P < 0.05$, ** $P < 0.01$ – vs vehicle group; a-$P < 0.02$, b-$P < 0.01$ – vs quinpirole group.

The quinpirole competition curves showed a biphasic shape in the control membranes. These two curves are suggested to be the result of quinpirole interaction with D_2-(high) and D_2-(low) affinity states, as has been described by Creese et al (1984) and Grigoriadis & Seeman (1984) on the basis of dopamine receptors affinity for agonist such as dopamine. Seeman & Grigoriadis (1987) have also found that in the rat striatum [^{3}H]spiperone binds to two populations of sites recognized by a dopamine agonist ADTN (b-amino-5,6,7,8-tetrahydro-2,3-napthalenediol). In the rat nucleus accumbens [^{3}H]spiperone labels two binding sites (Beart & McDonald 1982).

Our data analysed by the computer curve-fitting program LIGAND showed that after repeated administration of imipramine or mianserin, [^{3}H]spiperone binds to three classes of sites in the limbic system membranes. The third site has very low affinity for the agonist quinpirole. It could be supposed that this third site is 5-hydroxytryptaminergic in nature, but in our method, ketanserin (40 nM) was included in the assay. It may be that this concentration of ketanserin was not sufficient to occlude all the 5-HT (S_2) receptors and some binding to this site occurred.

Earlier results did not show any changes in the characteristics of [^{3}H]spiperone binding to dopamine receptors after chronic administration of antidepressants (see: introduction). A number of studies suggest that the functionally relevant receptor changes correlate with differences in the agonist competition for ^{3}H-antagonist binding sites better than with changes in the binding parameters of these ^{3}H-agonists (Wessels et al 1979; Menkes et al 1983; De Ceballos et al 1985; Hall & Sallemark 1987).

De Ceballos et al (1985) reported an enhanced ability of dopamine receptors after a prolonged prenatal exposure to chlorimipramine, iprindole, mianserin and nomifensine. The Scatchard analysis showed no changes in the characteristics of [^{3}H]spiperone binding.

There are some difference between our results obtained in the striatum and those reported by De Ceballos et al (1985). These may be due to the fact that the latter authors administered antidepressants to pregnant rats and studied the affinity of D_2-receptors in newborn pups; furthermore, they used dopamine, and not quinpirole, as a displacer.

Our behavioural results confirm previous reports (e.g. Tsuruta et al 1981; Fuller & Hemrick-Luecke 1985) that quinpirole increases the locomotor activity of rats. The quinpirole-induced hyperactivity is potentiated by repeated, but not acute, administration of imipramine and mianserin. Similar results were obtained previously in a study (Maj et al 1989) after prolonged administration of imipramine and (+)-oxaprotiline, when quinpirole was given 2 or 48 h after the last dose of antidepressant.

We have found that antidepressants given repeatedly increase the affinity of an agonist for dopamine D_2-receptors in the limbic system, but not in the striatum. This finding is in line with the observations that repeated administration of antidepressants potentiates the locomotor hyperactivity induced by dopamine agonists, but not stereotypy (Delini-Stula & Vassout 1979; Maj 1984; Spyraki & Fibiger 1981) regarded as a striatum-mediated effect.

We have previously reported that different antidepressants given repeatedly decrease the density of dopamine $_1$-receptors, labelled with [^{3}H]SCH 23390 (*R*(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol) a D_1-antagonist, in the limbic system and in the striatum (Klimek & Nielsen 1987). Preliminary results indicate that the affinity of SKF 38393 (2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1-H-3-benzazepine), a D_1-receptor agonist, is not changed by repeated doses of antidepressants (unpublished).

Antidepressants given repeatedly probably enhance the responsiveness of dopamine mesolimbic system, to dopamine and its agonists (see: introduction). The present data allow us to suppose that the enhanced responsiveness results from an increase in the affinity of dopamine D_2-receptors for their agonists.

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