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Chronic Treatment With MK-801 Decreases D_2 Dopamine Receptor Function in Rat Striatum

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GANDOLFI, O. AND R. DALL'OLIO. *Chronic treatment with MK-801 decreases D_2 dopamine receptor function in rat striatum.* PHARMACOL BIOCHEM BEHAV 44(3) 683–687, 1993.—Present results show that a single treatment with dizocilpine (MK-801, 0.25 mg/kg IP) failed to modify the specific binding to D_1 or D_2 DA receptors. In contrast, repeated administrations for 3 weeks resulted in a statistically significant decrease of [^{3}H]Spiroperidol binding to cortical or striatal membranes but did not change the number or the apparent affinity of [^{3}H]MK-801 binding in well-washed cortical membranes. Consistent reduction in specific D_2 receptor mediated behavior was obtained. The data suggest that the changes in DAergic function following repeated administrations with MK-801 could be suggestive of potential therapeutic uses of negative allosteric drugs in some DA related disfunctions.

MK-801 repeated treatment [^{3}H]MK-801 binding [^{3}H]Spiroperidol binding [^{3}H]SCH 23390 binding
LY 171555-induced hypermotility

DIZOCILPINE maleate (MK-801) is a potent non competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist acting at the phencyclidine (PCP) recognition site located within the lumen of the NMDA-sensitive glutamate receptor coupled cation channel. It has been widely accepted that MK-801, similarly to PCP, induces a characteristic behavioral syndrome consisting in turning behavior, head weaving and hypermotility (11,22); in addition, this drug displays a wide spectrum of pharmacological activity including anticonvulsant (13,18) and protective (8,9) actions from ischaemic damage in stroke and head trauma.

Recently, growing interest has been focused on the involvement of glutamatergic system in the pathophysiology of Parkinson's and other neurodegenerative disorders. As repeated drug administrations are necessary in the treatment of neurodegenerative disorders and as the effects of prolonged NMDA receptor blockade have been poorly understood, this study describes behavioral and biochemical experiments aiming at elucidating the effects of repeated MK-801 administrations on its own recognition site and on specific dopamine receptor function. To pursue this goal SKF 38393 (D_1 agonist) or LY 171555 (D_2 agonist), to elicit grooming behavior or hypermotility respectively, were administered to rats submitted to repeated treatments with MK-801 or saline. For each agonist we used the lowest effective doses in inducing behavioral effects (7), so that possible changes in DAergic function produced by the prolonged blockade of NMDA receptors could be unmasked avoiding supramaximal dopaminergic stimulation.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (250–280 g, Charles River, Como, Italy) were housed in groups of four under controlled conditions of light (from 7:00 a.m. to 7:00 p.m.), temperature (22 ± 2°C) and humidity (65%) and were allowed free access to standard laboratory diet and tap water.

Drugs

MK-801 and SKF 38393 (RBI, Natick, MA), were dissolved in distilled water. LY 171555 (Lilly, Indianapolis, IN), was dissolved in saline. In radioligand binding studies, [^{3}H]MK-801, [^{3}H]SCH 23390 and [^{3}H]Spiroperidol (NEN, Boston, MA), SCH 23390 (RBI, Natick, MA), and (+)-Butaclamol (Sigma) were used.

Biochemical Procedure

[^{3}H]MK-801 binding was assayed in well-washed membranes prepared from rat frontal cortices or striata (21). In

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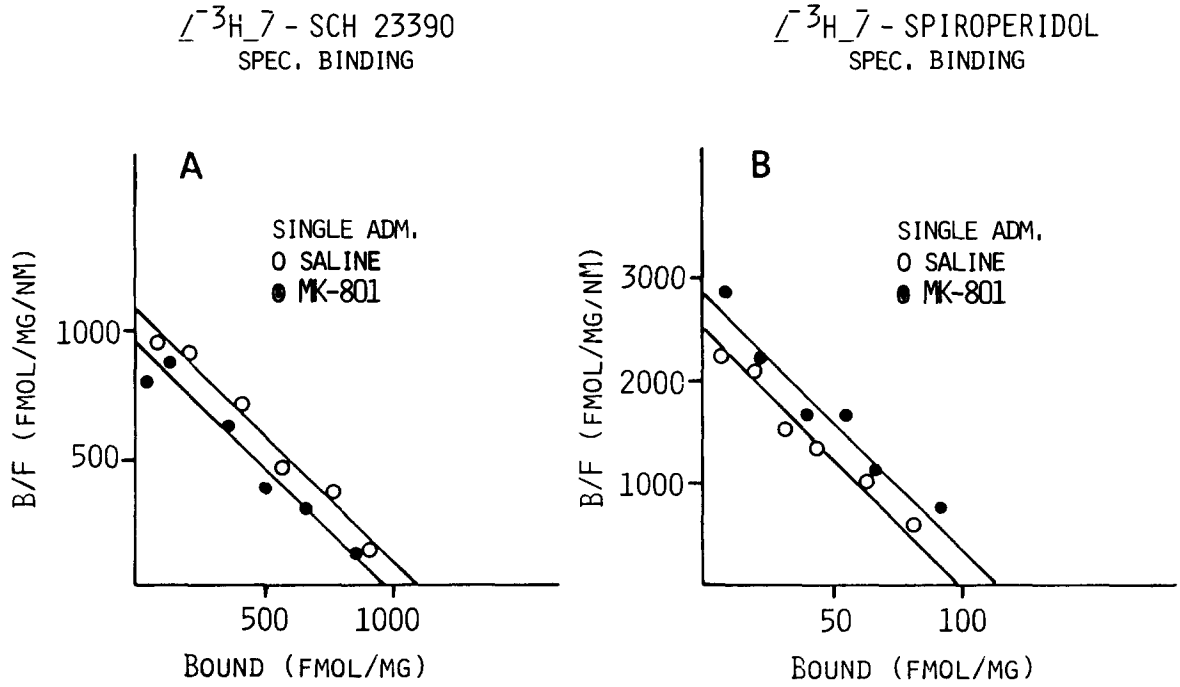

FIG. 1. Effect of a single administration of MK-801 (0.25 mg/kg IP) on [^{3}H]SCH 23390 (A) and [^{3}H]Spiroperidol (B) specific binding. Scatchard analysis of data generated from a representative experiment to CSM prepared from pooled striata. Values are the mean ± SEM from six animals with each value measured in triplicate. (A) [^{3}H]SCH 23390 (0.25–5 nM) B_{max} values (fmol/mg prot) were 1048 ± 47 and 942 ± 51 for saline and MK-801 treated rats. K_d values (nM) were 1.1 ± 0.05 and 1.1 ± 0.05, respectively. (B) [^{3}H]Spiroperidol (0.02–1 nM) B_{max} values (fmol/mg prot) were 198 ± 4 and 215 ± 6 for saline and MK-801 treated rats. K_d values (nM) were 0.08 ± 0.006 and 0.08 ± 0.005, respectively.

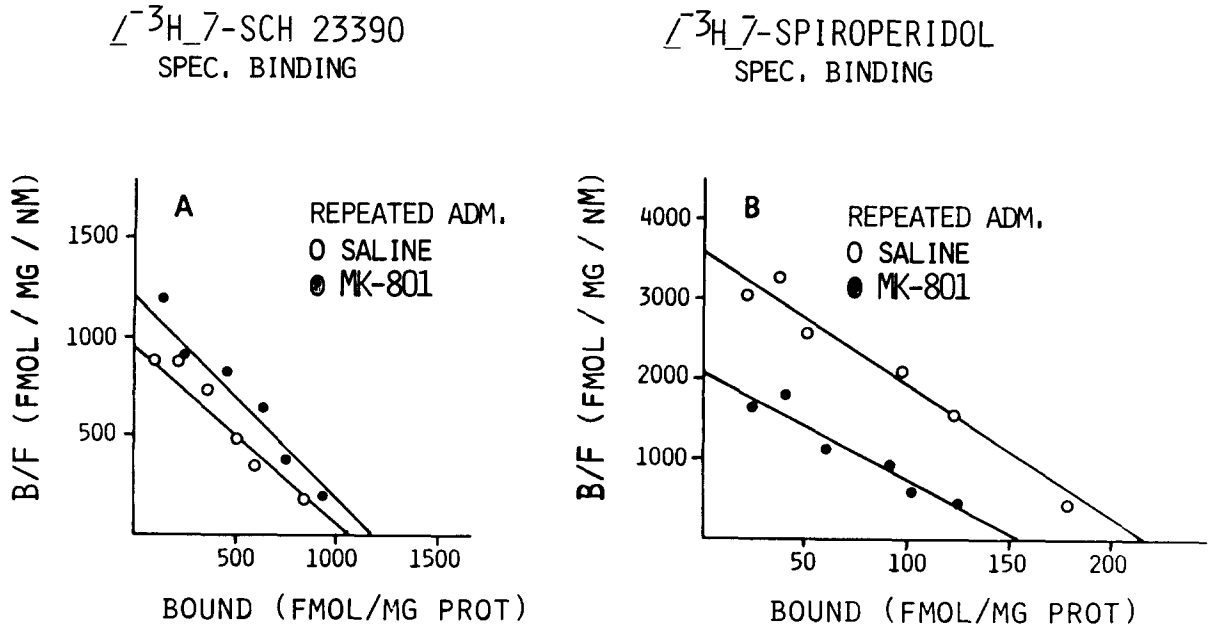

FIG. 2. Effect of repeated administration of MK-801 (0.25 mg/kg IP, daily for 21 consecutive days) on [^{3}H]SCH 23390 (A) and [^{3}H]Spiroperidol (B) specific binding. Scatchard analysis of data generated from a representative experiment to CSM prepared from pooled striata. Values are the mean ± SEM from six animals with each value measured in triplicate. A [^{3}H]SCH 23390 (0.25–5 nM) B_{max} values (fmol/mg prot) were 1040 ± 67 and 1193 ± 84 for repeated saline and MK-801 administration. K_d values (nM) were 1.1 ± 0.06 and 1.0 ± 0.08, respectively. B[^{3}H]Spiroperidol (0.02–1 nM) B_{max} values (fmol/mg prot) were 212 ± 11 and 156 ± 7* for repeated saline and MK-801 administration. K_d values (nM) were 0.06 ± 0.008 and 0.07 ± 0.009, respectively. *$p < 0.05$ (Student's t test) compared to rats repeatedly treated with saline.

brief, tissues were homogenized in 0.32 M Sucrose, the homogenates were centrifuged, pellets were resuspended and, following recentrifugation, the supernatants were pooled and centrifuged again to yield P2 pellets. After resuspension in 5 mM Tris-HCl pH 7.7 and centrifugation, the pellets were washed four times with ice-cold distilled water and the final pellets were frozen at −20°C for at least 24 h. On the day of assay, pellets were thawed at room temperature, resuspended in 5 mM Tris-HCl, washed five times by centrifugation and the resultant suspension was incubated at room temperature for 20 min and centrifuged (30,000 g, 20 min) again. The final pellets were resuspended in Tris-HCl pH 7.7 to give a protein concentration of approximately 0.7–1 mg/ml. Binding assays at equilibrium were performed by incubating [^{3}H]MK-801 (12.5 nM) with membrane preparation for 2 h at 25°C prior to filtration through Whatman GF/C filters presoaked with 0.05% polyethylenimine. Specific binding was defined as that displaced by cold MK-801 (10 μM). In saturation experiments, 10 different concentrations of [^{3}H]MK-801 (0.25–50 nM) were used.

The binding assays for [^{3}H]SCH 23390 (1) or [^{3}H]Spiroperidol (4) to D_1 or D_2 dopamine receptors, respectively, were carried out in crude synaptic membranes (CSM) prepared from rat nucleus caudateputamen (striatum) or cortex. In brief, pooled striatal tissues were homogenized in ice-cold 50 mM Tris-HCl pH 7.4, centrifuged (48,000 g, 20 min) twice and the final pellets were resuspended in 50 mM Tris-HCl buffer containing 120 mM NaCl, 5 mM KCl, 2 mM CaCl, and 1 mM MgCl. Aliquots of membrane suspensions were incubated at 37°C for 30 min with different concentrations of [^{3}H]SCH 23390 (0.05–5 nM) or [^{3}H]Spiroperidol (0.025–0.5 nM). Specific binding was determined as the difference between the total binding and the binding remaining in the presence of a specific displacer: 1 μM cold SCH 23390 or 1 μM (+)-Butaclamol for [^{3}H]SCH 23390 and [^{3}H]Spiroperidol, respectively. The incubation reactions were stopped by rapid filtration through Whatman GF/C filters presoaked in 0.05% polyethylenimine.

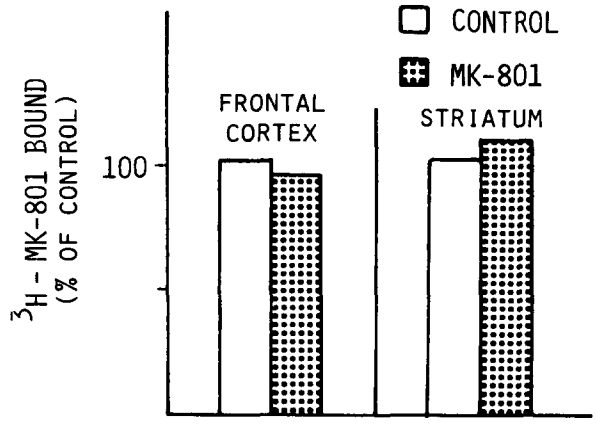

FIG. 3. [^{3}H]MK-801 specific binding in CSM prepared from the frontal cortices or striata of rats repeatedly treated with MK-801 (0.25 mg/kg IP for 3 weeks) or saline. Rats were killed 4 days after the last injection. [^{3}H]MK-801 (12.5 nM) binding was measured in CSM preparations from cortices (saline 726 ± 22 fmol/mg prot; MK-801 687 ± 19 fmol/mg prot) or striata (saline 247 ± 8 fmol/mg prot; MK-801 273 ± 9 fmol/mg prot), respectively. Values are % of the means of three experiments and varied less than 10%.

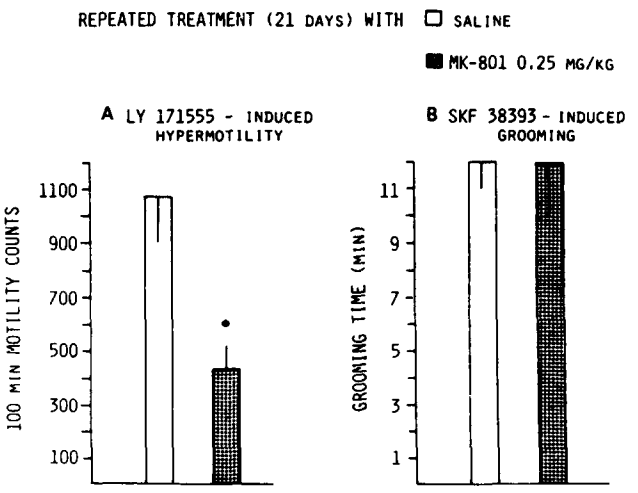

FIG. 4. Behavioral responses of rats (n = 8 per group) challenged 4 days after repeated treatment (21 days) with saline or MK-801 (0.25 mg/kg IP, daily). (A) Hypermotility induced by LY 171555 (0.3 mg/kg IP): mean motility counts ± SEM (20 to 120 min after injection). (B) Grooming induced by SKF 38393 (10 mg/kg IP): mean time (min) ± SEM of grooming during a 1-h observation period. *$p < 0.05$ when compared to the respective control group (Dunnet's t test after ANOVA).

[^{3}H]MK-801-, [^{3}H]SCH 23390-, or [^{3}H]Spiroperidol-specific binding were run either in CSM prepared from rats acutely treated with MK-801 (0.25 mg/kg IP, 1 or 24 h before) either in rats treated with repeated administration of MK-801 (0.25 mg/kg IP, daily for 21 consecutive days) and killed 4 days after the last administration.

A least-square curve fitting program LIGAND (15) was used in computer analysis of radioligand binding data generated from pooled striata or cortices from six animals. The kinetic characteristics of the specific bindings were analyzed according to the method of Scatchard (19). Proteins were measured using bovine serum albumin as internal standard (12).

Behavioral Procedure

Different groups of rats were treated IP daily for 21 consecutive days with saline or 0.25 mg/kg MK-801. Following a washout period of 4 days, they were challenged with LY 171555 (0.3 mg/kg IP) or SKF 38393 (10 mg/kg IP) and observed for:

a) LY 171555-induced hypermotility: After 1 h habituation period in actometric cages (6), the rats were injected with the D_2 dopamine receptor agonist (0.3 mg/kg IP) and their motility counts were recorded for 2 h.
b) SKF 38393-induced grooming: The rats were allowed to explore the experimental cage for 60 min before receiving the D_1 dopamine receptor agonist (10 mg/kg IP). Immediately after the drug administration, observers unaware of the treatment recorded the total time (min) of grooming episodes performed by animals in 1 h.

Statistical Analysis

Motility counts were analyzed by means of ANOVA followed by single comparisons of the means (Dunnet's t test). Radioligand binding data were analyzed by Student's t-test.

RESULTS

Figure 1 shows that a single administration of MK-801 (0.25 mg/kg IP 60 min before sacrifice) did not modify the kinetic characteristics of [^{3}H]SCH 23390 (part A) or [^{3}H]Spiroperidol (part B) binding to D_1 or D_2 receptors, respectively, in striatal CSM. Moreover, in CSM prepared from the n. striatum or the n. accumbens of rats treated with the same dose of MK-801 24 h before sacrifice, the characteristics of D_1 and D_2 receptors were not changed (not shown). We were unable to study the characteristics of D_2 receptors in rat cerebral cortex since in this area [^{3}H]Spiroperidol labels $5HT_2$ recognition sites.

In contrast, repeated administration of MK-801 (0.25 mg/kg IP for 21 consecutive days) decreased [^{3}H]Spiroperidol binding in striatal membranes of rat sacrificed following a washout period of 4 days (Fig. 2, part B). Scatchard analysis revealed that the reduction of [^{3}H]Spiroperidol binding resulted from a decrease (28%) in receptor density (B_{max} = 212 ± 11 for saline; B_{max} = 156 ± 7 for MK-801) with no change in apparent affinity (K_d = 0.06 ± 0.008 for saline; K_d = 0.07 ± 0.009 for MK-801). The figure (part A) shows also that repeated administration of MK-801 had no effect on the kinetic characteristics of striatal D_1 dopamine receptors. In spite of the decrease in D_2 receptor number upon repeated MK-801 administration, no significant changes in [^{3}H]MK-801 specific binding were observed in well washed membranes prepared either from cerebral cortices or striata of rats sacrificed following a washout period of four days (Fig. 3). Moreover, when the saturation isotherm of this specific binding was analyzed by the method of Scatchard, neither maximum number or affinity of [^{3}H]MK-801 receptors were changed (not shown).

Figure 4 shows the influence of repeated administration of MK-801 on specific DA mediated behaviors. In spite of motility counts during the habituation period that were not changed (saline = 535 ± 30 vs. MK-801 = 498 ± 45), a significant decrease in the hypermotility induced by the D_2 agonist LY 171555 (0.3 mg/kg IP) was detected 4 days following repeated treatments with MK-801 (part A). In contrast the same treatment failed to modify the grooming behavior induced by the specific D_1 receptor agonist SKF 38393 (10 mg/kg IP) (part B).

DISCUSSION

Our previous observations showed that specific dopamine receptor antagonists, at doses devoid of any behavioral effect per se, efficiently block MK-801-induced hypermotility (5), suggesting that NMDA-sensitive glutamate receptors could be regulated at least in part by dopaminergic mechanisms. Other results showing that single treatments with MK-801 failed to modify either DA and metabolite contents in any brain areas (11) and the characteristics of specific [^{3}H]SCH 23390 and [^{3}H]Spiroperidol bindings (present data), cast some doubts on the hypothesis of functional interactions between dopaminergic and glutamatergic systems in rat CNS. These results prompted us to undertake repeated administration of MK-801, aiming to verify whether adaptive changes due to chronic treatments with drugs acting at modulatory sites could at least in part explain the biochemical mechanisms underlying such interaction.

The results of this paper show that similar to single treatment, repeated administration of MK-801 failed to cause any significant changes in the frequency of the NMDA-sensitive cation channel opening, confirming other results obtained with a different non competitive antagonist (PCP), where no differences in [^{3}H]TCP binding were observed either in mouse brain 48 h after twice daily injections for 21 days (10) or in rats infused using SC osmotic minipumps for 10 days (16). At variance, other groups observed significant increases in the apparent number of [^{3}H]TCP binding following chronic infusion of PCP in rat (14). However, while several lines of experimental evidence indicate that the biochemical properties of neurotransmitter receptors may be altered following chronic treatment with agonists (down regulation) or antagonists (supersensitivity) specifically interacting with their own recognition sites, repeated administration of drugs acting on allosteric sites produced equivocal results.

The decrease in the number of striatal D_2 receptors in a behavioral correlate that, although not strictly dependent to the same cerebral area, is related to D_2 receptor activation, while leaving D_1 receptors unchanged deserve some comments.

It is possible that the decrease in D_2 binding and in LY 171555-induced hyperactivity may be related to various effects of MK-801 on dopaminergic function; however, conflicting data have been reported regarding the modulatory role of noncompetitive NMDA antagonists on DA release and uptake (2,3,17,20). The presynaptic hypothesis, furthermore, does not explain why D_1 receptors (where DA and DA agonists more efficiently bind) were not modified by the treatments.

Because of the long washout period these changes are probably not related to the presence of MK-801 (or one metabolite) on receptors at the time of binding assay, since it has been reported that upon MK-801 (2 mg/kg IP) administration, the decline in plasma concentrations was apparently biphasic with a $T_{1/2}$ = 1.9 h and the time course of MK-801 in brain paralleled that in plasma with a comparable elimination time $T_{1/2}$ = 2.05 h (23).

All these results, taken together to our observation that in vitro the addition of MK-801 decreased the apparent affinity of [^{3}H]Spiroperidol binding at concentrations higher than 5 × 10^{-4} M, suggest that a direct interaction of MK-801 on DA binding sites is unlikely.

In summary, this study suggests that, although the exact mechanism is still unknown, a potential therapeutic use of negative allosteric drugs of NMDA receptor complex is possible since upon chronic treatment they are able to modulate dopaminergic function.

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