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On the roles of dopamine D-1 vs. D-2 receptors for the hyperactivity response elicited by MK-801

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Summary. The present study was aimed at clarifying to what extent the hypermotility induced by the uncompetitive N-methyl-D-aspartate (NMDA) antagonist MK-801 depends on dopamine (DA) D-1 compared to D-2 receptor tone. The D-1 receptor antagonist SCH 23390 was found to reduce locomotion to a greater extent in MK-801-treated than in vehicle-treated mice, whereas the reverse appeared to be the case for the DA D-2 receptor antagonist raclopride. In other words, MK-801-induced hyperactivity was more readily antagonized by SCH 23390 than by raclopride and, thus, DA D-1 receptors seem to be more important than D-2 receptors for MK-801-induced hyperactivity. These results are in line with our previous observation that MK-801 generally interacts synergistically with a DA D-1 but not with a D-2 receptor agonist in monoamine-depleted mice. In view of the possible role of deficient glutamatergic neurotransmission in schizophrenia, our findings underline the importance of investigating the efficacy of selective DA D-1 antagonists in this disorder.

Keywords: NMDA receptors, DA receptors, MK-801, SCH 23390, raclopride, locomotor activity, schizophrenia.

Introduction

Previous work in the present laboratory has disclosed powerful interactions with regard to locomotor activity between N-methyl-D-aspartate (NMDA) receptor antagonists and catecholaminergic receptor agonists. Thus, a strong potentiation between NMDA antagonists, administered systemically or into the nucleus accumbens of mice, and the α_2-adrenoceptor agonist clonidine was observed. A somewhat less marked potentiation was found between the uncompetitive NMDA antagonist MK-801 (dizocilpine) and the DA D-1 receptor agonist SKF 38393 or the mixed D-1/D-2 receptor agonist apomorphine, whereas the interaction rather tended to be antagonistic when MK-801 was combined with a DA D-2 receptor agonist (Carlsson and Carlsson, 1990; Svens-

son and Carlsson, 1992; Svensson et al., 1992; cf Morelli et al., 1990; Goodwin et al., 1992). (In this paper, D-2 generally refers to the whole D-2 family, comprising the D-2, D-3 and D-4 subtypes. Likewise, D-1 refers to D-1 = D_{1A} and D-5 = D_{1B}.) These experiments were performed in monoamine-depleted mice (pretreated with reserpine and α-methyl-p-tyrosine), and thus the interaction presumably took place at the postsynaptic level. In non-depleted animals, MK-801 stimulates locomotor activity in about ten times lower dosage than in monoamine-depleted animals (Clineschmidt et al., 1982 a). One possible explanation for this dose discrepancy could be that in non-depleted animals a synergistic interaction occurs between MK-801 and endogenous DA and/or noradrenaline at the postsynaptic receptor, in analogy with the potentiation referred to above.

Clineschmidt et al. (1982 a) observed that the locomotor stimulation induced in mice by MK-801 was antagonized by the preferential DA D-2 receptor antagonist haloperidol and the selective α_1-adrenoceptor antagonist prazosin. Using the competitive NMDA antagonist AP-5 (DL-2-amino-5-phosphonopentanoic acid), injected into rat antero-dorsal striatum, Schmidt (1986) observed behavioral stimulation that could be antagonized by haloperidol or clozapine. However, it appears that a comparison between selective DA D-1 and D-2 receptor antagonists has not been made with respect to effects on MK-801-induced hyperactivity; therefore the present study was performed using the selective DA D-1 receptor antagonist SCH 23390 and the selective DA D-2 receptor antagonist raclopride. In addition, the effect of the α_2-adrenoceptor antagonist yohimbine on MK-801-induced locomotor stimulation was investigated.

Materials and methods

Animals

Male albino mice of the NMRI strain weighing 18–20 g were purchased from ALAB, Sollentuna, Sweden.

Drugs

MK-801 ([+]-5-methyl-10, 11-dihydro-5H-dibenzo-[a, d]-cyclohepten-5, 10-imine hydrogen maleate; dizocilpine; Research Biochemicals Inc, MA, U.S.A.), SCH 23390 ([S]-[+]-8-chloro-2, 3, 4, 5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol HCl; Research Biochemical Inc) and raclopride tartrate (courtesy Prof. S. Ahlenius at Astra Läkemedel AB) were dissolved in physiological saline. Yohimbine HCl (Sigma, St. Louis, U.S.A.) was dissolved in a few drops of glacial acetic acid and 5.5% glucose solution. The drugs were injected intraperitoneally, except for SCH 23390 which was given subcutaneously. Injection volumes were 10 ml/kg if not otherwise stated. The control animals were always given appropriate vehicle treatment.

Locomotor registration

Locomotor activity was measured by means of an "M/P 40 Fc Electronic Motility Meter" (Motron Products, Stockholm) with 40 photoconductive sensors (5 rows × 8, centre-centre distance 40 mm), with one animal at a time in the motility meters.

Statistics

The experimental data was subjected to Kruskal-Wallis' analysis of variance followed by a post hoc Mann-Whitney U-test.

Results

Figure 1 shows the locomotor stimulant effect of MK-801. Unlike the animals given vehicle, the MK-801-treated animals displayed a pronounced locomotor activity during the entire observation period. The dose of 0.3 mg/kg MK-801 appeared more efficacious than 0.8 mg/kg with regard to locomotor stimulation; following the lower dose predominantly forward locomotion was observed, while the higher dose caused more of stereotypies and less forward locomotion. During the initial phase of active exploration the control animals and the 0.3 mg/kg group showed similar locomotor activities. However, whereas the usual decline in activity, due to habituation, was observed in the controls, the 0.3 mg/kg group displayed a continued high locomotor activity (cf Liljequist et al., 1991).

Figure 2 shows that both the DA D-1 receptor antagonist SCH 23390 and the DA D-2 receptor antagonist raclopride significantly reduced MK-801-induced hyperactivity. In view of the short duration of action of SCH 23390, the recording period in subsequent experiments was 10 min only, beginning 10 min after the SCH 23390 injection. Unlike the case with SCH 23390, the decrement of locomotion after injection of raclopride was of long duration and tended to be more accentuated with time. The α_2-adrenoceptor antagonist yohimbine, given in a dose previously shown to effectively antagonize hyperactivity induced by MK-801 and clonidine in monoamine-depleted mice (Carlsson and Carlsson, 1989), did not antagonize the locomotor stimulant effect of MK-801.

To enable an appropriate choice of SCH 23390 dose for investigating the

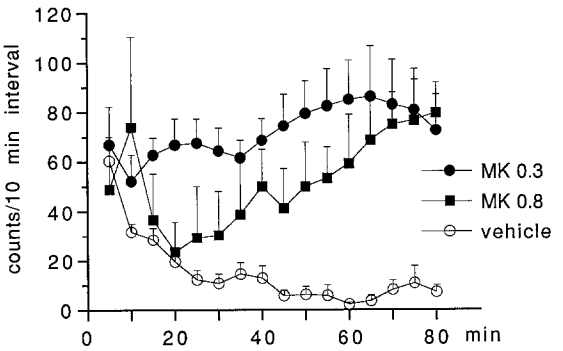

Fig. 1. Effects of MK-801 on locomotor activity in non-habituated mice. Immediately after administration of MK-801 (0.3 or 0.8 mg/kg) or vehicle the animals were placed in the motility meters and locomotor activity was recorded for 80 min. Shown are the means ± sem, n = 6 (vehicle), 5 (MK-801 0.3) and 3 (MK-801 0.8). MK-801 induced a statistically significant increase in cumulated motility counts for 80 min; MK-801 0.3 vs vehicle: $p < 0.01$, MK-801 0.8 vs vehicle: $p < 0.05$

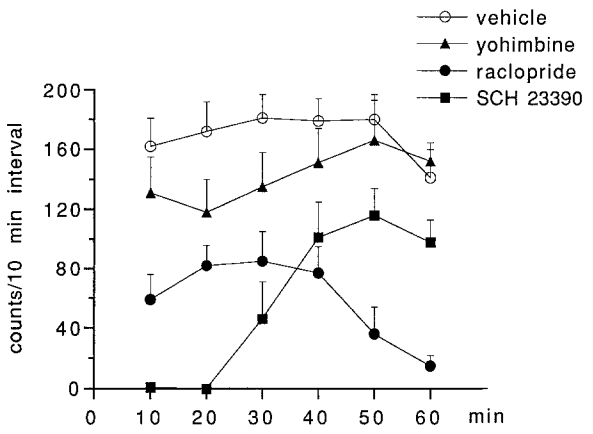

Fig. 2. Effects of various catecholamine antagonists on locomotor hyperactivity induced by MK-801. MK-801 (0.3 mg/kg) was administered 30 min, yohimbine (5 mg/kg; 5 ml/kg) and raclopride (1 mg/kg; 5 ml/kg) 20 min and SCH 23390 (0.1 mg/kg; 5 ml/kg) 10 min before the animals were placed in the motility meters. Locomotor activity was recorded for 60 min. Shown are the means ± s e m, n = 6. There was a statistically significant decrease in cumulated motility counts for 30 min after treatment with SCH 23390 ($p < 0.01$) and raclopride ($p = 0.01$)

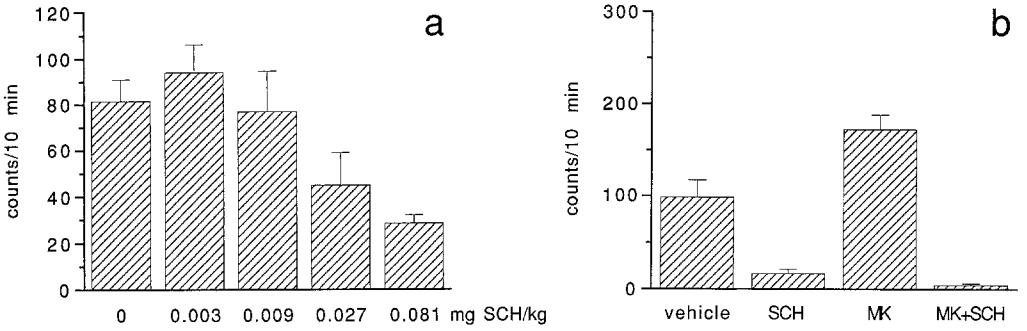

Fig. 3. a Effects of various doses of SCH 23390 on locomotor activity in non-habituated mice. Five min after administration of SCH 23390 the animals were placed in the motility meters and locomotor activity was recorded for 10 min. Shown are the means ± s e m, n = 4. There was a statistically significant correlation between dose of SCH 23390 and the motility counts of the individual animals ($r = -0.75$, $p < 0.001$). **b** Effects of SCH 23390 (0.05 mg/kg) on the locomotor hyperactivity induced by MK-801. MK-801 (0.3 mg/kg) was administered 30 min and SCH 23390 10 min before the animals were placed in the motility meters, where locomotor activity was recorded for 10 min. Shown are the means ± s e m, n = 5–6

importance of the DA D-1 receptor for the locomotor stimulation produced by MK-801, a dose-response study was conducted in non-habituated mice. There was a negative correlation between SCH 23390 dose and number of motility counts (Fig. 3 a). Based on this dose-response curve 0.05 mg/kg of SCH 23390 was chosen in the interaction experiment with MK-801, shown in Fig. 3 b.

SCH 23390 reduced the locomotor activity of animals given MK-801 to 2% of MK-801-treated controls. Animals treated with SCH 23390 only showed a reduction in locomotion to 17% compared to mice receiving vehicle only. A comparison between the motor activity of the animals receiving SCH 23390, and those receiving both SCH 23390 and MK-801 revealed a statistically significant difference ($p < 0.05$). This difference is remarkable in view of the fact that the activity was higher in the MK-801-treated group than in the vehicle group; to take into account the large differences in baseline activity between the two control groups, the motility count for each animal in the groups receiving SCH 23390 or SCH 23390 plus MK-801 was divided by the mean of the corresponding control group. A statistically significant difference was obtained between these two groups of quotients ($p < 0.01$).

The importance of the DA D-2 receptor for the behavioral stimulation produced by MK-801 was examined in parallel with the investigation of the role of the D-1 receptor. In Fig. 4 a are shown the effects of various doses of raclopride, a selective DA D-2 receptor antagonist (Köhler et al., 1985), on locomotor activity of non-habituated mice. A dose-dependent decrease was observed, but this decrease seemed to level out at about 30 counts/10 min, i.e., a level similar to that induced by the highest dose of SCH 23390.

Figure 4 b shows that 1 mg/kg of raclopride reduced the locomotor activity of animals receiving vehicle to 32% of controls, whereas the activity of mice given MK-801 plus raclopride merely was reduced to 56% of MK-801-treated controls. The difference between these percentage figures was not statistically significant.

Figure 5 shows the results of an experiment where the interaction of MK-

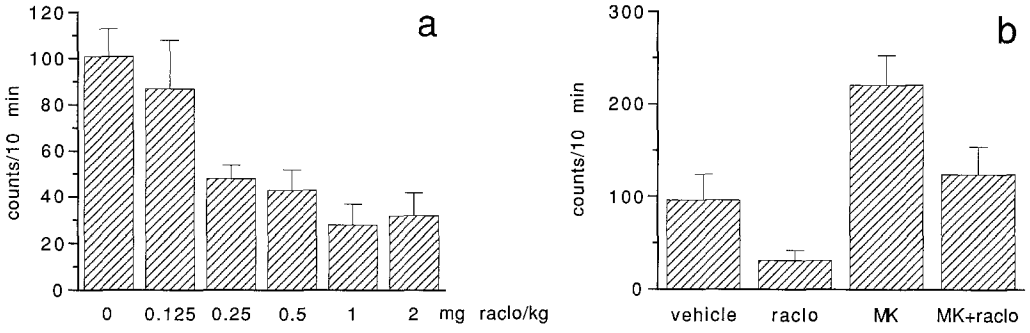

Fig. 4. a Effects of various doses of raclopride on locomotor activity in non-habituated mice. Twenty min after administration of raclopride the animals were placed in the motility meters and locomotor activity was recorded for 10 min. Shown are the means ± s e m, n = 4–5. There was a statistically significant correlation between dose of raclopride and the motility counts of the individual animals ($r = -0.71$, $p < 0.001$). **b** Effects of raclopride (1 mg/kg) on the locomotor hyperactivity induced by MK-801. MK-801 (0.3 mg/kg) was administered 30 min and raclopride (1 mg/kg) 20 min before the animals were placed in the motility meters, where locomotor activity was recorded for 10 min. Shown are the means ± s e m, n = 5

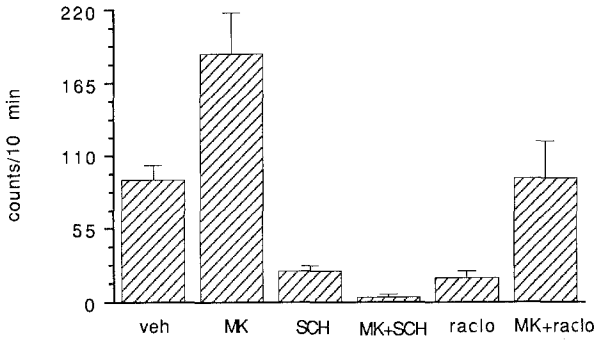

Fig. 5. Effects of 0.07 mg/kg of SCH 23390 or 5 mg/kg of raclopride on the locomotor hyperactivity induced by MK-801. MK-801 (0.3 mg/kg) was administered 40 min, raclopride 20 min and SCH 23390 10 min before the animals were placed in the motility meters, where locomotor activity was recorded for 10 min. Shown are the means ± s e m, n = 7

801 with either raclopride or SCH 23390 was investigated simultaneously. Administration of SCH 23390 (0.07 mg/kg) alone or raclopride (5 mg/kg) alone reduced the locomotor activity to 25 and 20% of saline-treated controls, respectively. Injection of the same doses to animals pretreated with MK-801 (0.3 mg/kg) resulted in a reduction of locomotor activity to 2% of MK-801-treated controls in the case of SCH 23390 and 50% of MK-801-treated controls in the case of raclopride. There was a significant difference between these latter groups receiving MK-801 in combination with either DA antagonist ($p < 0.001$).

Another interesting observation from this last experiment is that MK-801 can both potentiate and attenuate the effects of a DA receptor blocker, depending on whether the interaction is with the D-1 or the D-2 antagonist. Thus, in agreement with the experiment shown in Fig. 3 b, MK-801 produced a further reduction of locomotor activity in animals receiving SCH 23390 (MK-801 + SCH 23390 vs SCH 23390; $p < 0.01$); on the contrary, MK-801 counteracted the suppression of locomotion produced by raclopride (MK-801 + raclopride vs raclopride: $p < 0.05$).

Discussion

The main finding of the present study is that the DA D-1 receptor antagonist SCH 23390 more effectively than the D-2 receptor antagonist raclopride counteracts MK-801-induced hyperactivity.

SCH 23390 caused a significantly stronger inhibition of locomotor activity in mice pretreated with the NMDA receptor antagonist MK-801 than in animals pretreated with vehicle. Inspection of the animals revealed that practically all animals receiving MK-801 in combination with SCH 23390 displayed a period of complete immobility, lasting 10 to 15 minutes, though with a somewhat variable time of onset. This observation is in harmony with our previous finding that the combined treatment with MK-801 and the DA D-1 agonist SKF 38393, given to monoamine-depleted mice, results in a marked potentiation in terms

of locomotor stimulation. As mentioned above, this interaction probably takes place at the postsynaptic level and may well be exerted through the phosphoprotein DARPP-32, whose phosphorylation is enhanced by DA D-1 receptors and inhibited by NMDA receptors, as indicated by observations on striatal slices (Girault et al., 1990; Halpain et al., 1990). However, since SCH 23390 also acts as an antagonist on 5-HT_2 receptors (Bischoff et al., 1986), the possible role of 5-HT_2 receptors in this context needs to be clarified in future experiments.

The DA D-2 antagonist raclopride was less effective than SCH 23390 in attenuating MK-801-induced hyperactivity. This finding, too, is in harmony with our previous observation that combining MK-801 with DA D-2 receptor agonists in monoamine-depleted mice at most results in a very slight potentiation, and then only under conditions of a low baseline activity. At a high baseline activity this drug combination rather results in reduced motility, compared to treatment with the DA D-2 receptor agonist alone (Svensson et al., 1992).

It is interesting to note that when MK-801 was combined with SCH 23390 a further reduction of locomotion occurred compared to when SCH 23390 was given alone; on the contrary, when MK-801 was combined with raclopride, the locomotor inhibition induced by raclopride was partly overcome. Thus, MK-801 interacts with a DA D-1 vs. a D-2 antagonist in a qualitatively different manner.

In a recent study it was shown that MK-801-induced locomotion and sniffing stereotypies in *rats*, were partially blocked by haloperidol (Tiedtke et al., 1990), a DA antagonist with adrenoceptor antagonistic properties (Hyttel et al., 1985), suggesting involvement of a dopaminergic and/or an adrenergic mechanism. Early observations of Clineschmidt et al. (1982 a, b) also indicate an involvement of a catecholaminergic mechanism, since both the locomotor stimulation and anticonvulsant activity of MK-801 in mice were partially blocked by haloperidol or the α_1-adrenergic antagonist prazosin. Furthermore, MK-801-induced ipsiversive turning in 6-OH-DA-lesioned rats was antagonized by the α_1-adrenergic antagonists aceperone, azapetine and prazosin as well as by the noradrenaline synthesis inhibitor FLA-63 (Martin and Papp, 1984).

The α_2-adrenoceptor antagonist yohimbine, given in a dose previously shown to effectively antagonize hyperactivity induced by MK-801 and clonidine in monoamine-depleted mice (Carlsson and Carlsson, 1989), did not antagonize the locomotor stimulant effect of MK-801 in the present study. This observation is not in harmony with our previous observation that MK-801 interacts in a synergistic manner with the α_2-adrenoceptor agonist clonidine to produce a pronounced locomotor stimulation in monoamine-depleted mice. Obviously more work is needed to obtain a clear picture of the interaction between NMDA and α_2-adrenergic receptors. The possibility of a presynaptic action of yohimbine in the present study, resulting in enhanced noradrenaline release and stimulation of α_1 and β receptors, confounds the interpretation.

The observations dealing with the effect of MK-801 on motility show some interesting similarities with the behavioral actions of dopaminergic agonists. In

both cases a marked locomotor activity occurs at a time when the control animals have become habituated to their surroundings, resulting in reduced exploratory activity. In other words, both types of agent seem to retard the process of habituation. This phenomenon is of particular interest in view of the fact that a failure of habituation is also observed in schizophrenia (Geyer et al., 1990). As is generally recognized, both NMDA antagonists and DA agonists are psychotogenic and may mimic schizophrenic symptomatology (Angrist, 1987). Another similarity between the two types of agents is that they both cause stereotypies when given in higher dosage. This phenomenon may indicate a failing integrative capacity of the brain, when exposed to an excessive sensory input, and may thus serve as a rough model of psychosis.

In summary, the present study indicates that DA D-1 receptors are more important than D-2 receptors for MK-801-induced hyperactivity. These results are in agreement with our previous observation that MK-801 generally interacts synergistically with a DA D-1 but not with a D-2 receptor agonist in monoamine-depleted mice. In view of the possible role of deficient glutamatergic neurotransmission in schizophrenia, our findings underline the importance of investigating the efficacy of selective DA D-1 antagonists in this disorder.

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