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Different Effects of Dopamine Antagonists on Spontaneous and NMDA-Induced Motor Activity in Mice

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GIMÉNEZ-LLORT, L., E. MARTÍNEZ, AND S. FERRÉ. *Different effects of dopamine antagonists on spontaneous and NMDA-induced motor activity in mice.* PHARMACOL BIOCHEM BEHAV **56**(3) 549–553, 1997—The spontaneous motor activity of mice exposed to a new environment is characterized by an initial hyperactivity (explorarory period) followed by low levels of motor activity (habituation period). High doses of the dopamine D_1 receptor antagonist SCH 23390 (1 mg/kg SC) and the dopamine D_2 receptor antagonist raclopride (1 mg/kg SC) partially decreased motor activity during the exploratory period and did not modify motor activity during the habituation period or after reserpinization (5 mg/kg SC 20 h before motor activity recording). The systemic administration of a subconvulsant dose of *N*-methyl-D-aspartate (NMDA) (75 mg/kg IP) decreased motor activity during the exploratory period and increased motor activity during the habituation period. Both SCH 23390 and raclopride partially counteracted the NMDA-induced motor activation. Neither SCH 23390 nor raclopride counteracted the NMDA-induced motor activation in reserpinized mice. On the contrary, raclopride was found to potentiate the NMDA-induced motor activation in reserpinized animals. The present results suggest the existence of dopamine-dependent and dopamine-independent mechanisms involved in the motor activating effects of NMDA.

NMDA Dopamine D_1 receptor Dopamine D_2 receptor Reserpine Motor activity

MOST experimental studies about the behavioural effects induced by the systemic administration of excitatory amino acid receptor agonists have been related with convulsant activity, which is associated with excitotoxicity (29). We have shown that low non-convulsant systemic doses of NMDA induce very pronounced motor effects in rodents. The administration of subconvulsant doses of NMDA in mice exposed to a new environment induces a bifasic effect on motor activity, with an initial motor depression followed by motor activation (12,15,16). Based on some experimental findings we have hypothesized that the motor depressant and stimulant effects of NMDA involve different mechanisms and different brain structures.

The neurotransmitter dopamine and the neuromodulator adenosine influence motor activity by acting on dopamine and adenosine receptors localized in the striatum (9). Stimulation of dopamine receptors stimulates and their blockade inhibits motor activity. Conversely, stimulation of adenosine receptors inhibits and their blockade stimulates motor activity. The motor effects of adenosine receptor agonists and adenosine antagonists seem to be mainly mediated by specific antagonistic interactions between striatal adenosine and dopamine receptors (9–11,13). Adenosine mediates the motor depressant effect of NMDA, since it is counteracted by the previous administration of low doses of the adenosine antagonist theophylline (15). In fact, the stimulation of central NMDA receptors has been shown to increase the extracellular concentrations of adenosine in the brain, including the striatum (5,17,24,25). Therefore the NMDA receptor-induced striatal adenosine release is the most probable mechanism responsible for the NMDA-mediated motor depression.

NMDA has also been shown to induce dopamine release in the striatum (18,19,21,28). Furthermore, an increase in motor activity has been obtained after the local administration of

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NMDA in the striatum, mainly the nucleus accumbens, in the hippocampus and in the substantia innominata (7,22,26,30). The motor activating properties of NMDA have therefore been suggested to be related to its dopamine-releasing effects (7,28). However, we have also shown that NMDA induces motor activation in reserpinized mice (12,15) and that the non-selective dopamine antagonist haloperidol does not counteract that effect (15).Therefore, we have postulated the existence of another mechanism responsible for the motor activating effects of NMDA, which would take place in brain structures other than the striatum. In fact, we have recently found that the systemic administration of a subconvulsant dose of NMDA induces *c-fos* mRNA expression in the hippocampus, amygdala, hipothalamus, cortex and bed nucleus of the stria terminalis, but not in the striatum (14). In the present work, by using selective antagonists for dopamine D_1 and D_2 receptors in reserpinized and non-reserpinzed animals, we wanted to analize with more detail the contribution of dopamine-dependent and dopamine-independent mechanisms in the elicitation of NMDA-induced motor activation.

METHODS

Animals

Male mice of the OF1 strain, weighing 25–31 g were used. The animals were adjusted to a room with a 12-h light/dark cycle and 22 ± 2°C. They had free access to food and water up to the time of measurement of motor activity. The mice were used only once.

Motor Activity Recording

The motor activity was recorded with a video-computerized system (Videotrack 512, View Point, Lyon) by using a subtraction image analysis. The system was set to measure any kind of motor activity (locomotion, rearing, intense grooming, jumps) and to avoid monitoring of very small movements (breathing, non-intense grooming, tremor). Four open field cages (35.5 × 35.5 × 35.5) were simultaneously registered in a soundproof, temperature-controlled (22 ± 2°C) experimental room, which was uniformly illuminated with two incandescent lamps (100 W) located 2 m above the floor. The motor activity of three equally treated mice (n =1), placed simultaneously in the same motor cage, was recorded to decrease the variability of the results (2). Motor activity was recorded immediately after the animals, either reserpinized (one 1-h period of observation) or non-reserpinized (two 1-h periods of observation), were placed in the open-field cages without any acclimatization period.

Drugs

Reserpine (Sigma, St. Louis, MO) was dissolved in a drop of glacial acetic acid which was made up to volume with 5.5% glucose. *N*-methyl-D-aspartic acid (Sigma) was dissolved in 5.5% glucose and adjusted to pH 7.4 with NaOH. R(-)-SCH 23390 hydrochloride (Sigma) and raclopride tartrate (Astra, Södertälje) were dissolved in 5.5% glucose. Reserpine (5 mg/kg SC) was administered 20 h and SCH 23390 (1 mg/kg SC) and raclopride (1mg/kg SC) were administered 15 min prior to motor activity recording. NMDA (75 mg/kg IP) was administered just before motor activity recording. The volume of injection was always 10 ml/kg.

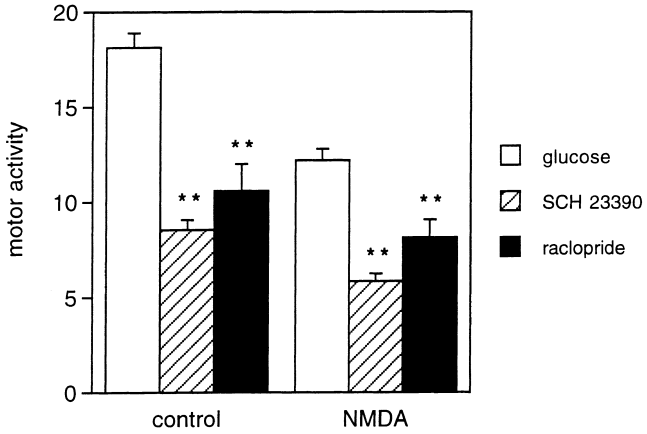

FIG. 1. Means ± SEM of all 10 min transformed data per three mice (n = 1) from the first 1 h period of observation (exploratory period) of non-reserpinized mice (n = 4–5/group). For control and NMDA-treated animals, **significantly different (ANOVA, $p < 0.01$) compared to the respective glucose-treated group.

Statistical Analysis

All values (amount of time in seconds) registered per 10 min were transformed (square root of (counts + 0.5)) (2) and analyzed by the summary measures method (20), by using the mean of all the transformed data per 3 mice (n = 1) as the summary statistic and by using a bifactorial analysis of variance (ANOVA) followed by post-hoc one-way ANOVA with Fishers PLSD test to analyze differences among groups.

RESULTS

Effects of the Dopamine Receptor Antagonists SCH 23390 and Raclopride on Spontaneous and NMDA-Induced Motor Activity in Non-Reserpinized Mice

The effects of NMDA (NMDA factor, with the two levels, NMDA and control) and the dopamine receptor antagonists SCH 23390 and raclopride (antagonist factor, with the three levels, glucose, SCH 23390 and raclopride) during the first and second h of motor activity recording were separately analyzed (for the first and second h periods of observation). During the first hour NMDA induced a significant decrease (F = 28.6, $p < 0.0001$) of motor activity (Fig. 1). SCH 23390 and raclopride induced a significant decrease of motor activity compared with the glucose-treated groups (one-way ANOVA with Fishers PLSD: $p < 0.01$ in both cases) with or without the administration of NMDA (significant effect of the antagonist factor: $F = 47.3, p < 0.0001$; no significant interaction between the NMDA and the antagonist factors: F = 2.6) (Fig. 1).

During the second h of motor activity recording NMDA induced a significant increase in motor activity ($F = 38.2, p <$ 0.0001) (Fig. 2). A statistical significance was obtained with the antagonist factor ($F = 4.4, p < 0.05$) and with the interaction between the NMDA and the antagonist factors ($F = 0.05, p <$ 0.05). By using post-hoc comparisons (one-way ANOVA with Fishers PLSD), a significantly lower motor activity was found in the SCH 23390 and raclopride groups compared to the glucose group ($p < 0.01$ and 0.05, respectively), but only when treated with NMDA (Fig. 2).

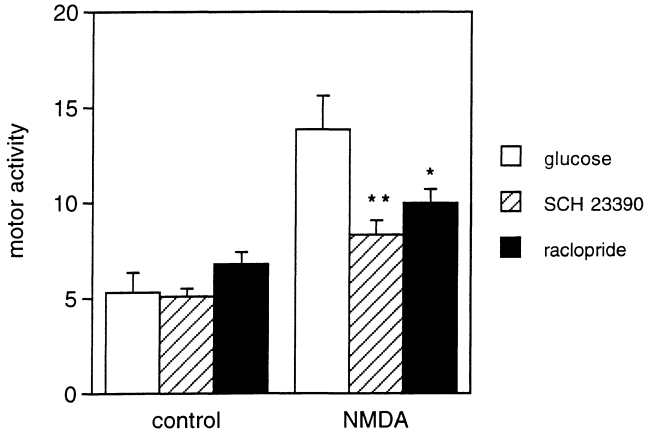

FIG. 2. Means ± SEM of all 10 min transformed data per three mice ($n = 1$) from the second 1 h period of observation (habituation period) of non-reserpinized mice (n = 4–5/group). For control and NMDA-treated animals, * and **significantly different (ANOVA, $p < 0.05$ and $p < 0.01$, respectively) compared to the respective glucose-treated group.

Effects of the Dopamine Receptor Antagonists SCH 23390 and Raclopride on Spontaneous and NMDA-Induced Motor Activity in Reserpinized Mice

NMDA induced a significant increase in motor activity ($F = 54.6$, $p < 0.0001$) (Fig. 3). A statistical significance was also obtained for the antagonist factor ($F = 4.9$, $p < 0.01$) and for the interaction between the NMDA and the antagonist factors ($F = 4.1$, $p < 0.05$). By using post-hoc comparisons (one-way ANOVA with Fishers PLSD), a significantly higher motor activity was found in the raclopride group compared to the glucose group ($p < 0.01$), but only when treated with NMDA (Fig. 3).

DISCUSSION

The spontaneous motor activity of mice exposed to a new environment is characterized by an initial hyperactivity during the first hour, which mostly reflects exploratory activity (exploratory period), followed by low levels of motor activity during the second h (habituation period) (12,15). In the present work the initial motor activation (first h of exposure to the new environment) was partially counteracted by the previous administration of a high dose of the selective dopamine D_1 receptor antagonist SCH 23390 or the selective dopamine D_2 receptor antagonist raclopride. On the other hand, no effect of either dopamine receptor antagonist was observed during the second hour of spontaneous motor activity or after reserpinization. The lack of effect of SCH 23390 and raclopride during the habituation period can not be attributed to their kinetic properties, since they could still counteract the motor activation observed during the second hour after the systemic administration of NMDA (see below). Van den Boss et al. (3) found similar results in rats treated with raclopride (directly infused in the nucleus accumbens). Those authors found that high doses of raclopride were only able to reduce the initial exploratory activity associated with the exposure to a new environment. All these results suggest that dopamine neurotransmission mediated by both dopamine D_1 and D_2 receptors is specially involved in the initial motor activity associated with the exposure to a new environment, such as exploratory activity.

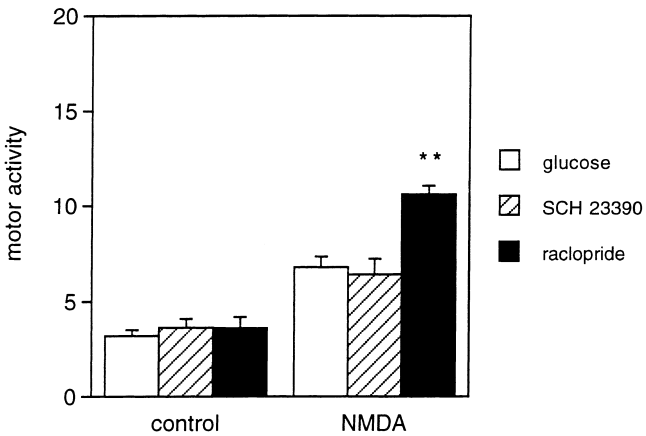

FIG. 3. Means ± SEM of all 10 min transformed data per three mice ($n = 1$) from the first 1 h period of observation of reserpinized mice (n = 9–10/group). For control and NMDA-treated animals, **significantly different (ANOVA, $p < 0.01$) compared to the respective glucose-treated group.

In agreement with previously reported data, the systemic administration of a subconvulsant dose of NMDA decreased motor activity during the exploratory period and increased motor activity during the habituation period (12,15). The decrease in exploratory activity induced by NMDA seems to be adenosine-mediated, since low doses of the adenosine receptor antagonist theophylline counteract such effect (15). In fact, adenosine agonists induce motor depression by antagonizing the effects of dopamine receptor stimulation in the striatum (9–11,13) and NMDA receptor stimulation induces adenosine release (5,17,24,25).

The administration of SCH 23390 or raclopride significantly decreased the NMDA-mediated induced motor activation during the habituation period. Since the dopamine antagonists did not modify the spontaneous motor activity during the same period in the control group, the present results demonstrate the involvement of dopamine in the NMDA-induced motor activation in non-reserpinized mice. Both dopamine D_1 and D_2 receptors are implicated and, therefore, the mechanism of action might well be the already described NMDA-induced dopamine release (18,19,21,28). These results suggest that in the non-reserpinized animal NMDA can influence motor activity by its adenosine- and dopamine-releasing properties. Therefore, the outcome will depend on the level of dopamine neurotransmission, most probably in the striatum. The adenosine-mediated motor inhibition would be observed under conditions of high stimulation of dopamine receptors, such as the exploratory period. On the other hand, the dopamine-dependent motor stimulation, would be seen under conditions of low dopamine receptor stimulation, such as the habituation period.

As previously reported (12,15), NMDA induced motor activation in reserpinized mice. However, in contrast to the results obtained in non-reserpinized animals, neither SCH 23390 nor raclopride were able to counteract the NMDA-induced motor activation in reserpinized mice. In fact, raclopride caused a clear potentiation of the effect of NMDA. Therefore, these results support the existence of two mechanisms of action responsible for the motor activating effects of NMDA: (1) a dopamine-dependent mechanism, which can be observed in non-reserpinized mice under low levels of

dopamine receptor stimulation; (2) a dopamine-independent mechanism, which is only apparent after dopamine depletion, in reserpinized animals. This dopamine-independent mechanisms seems to be modulated by a strong dopamine D_2-mediated tonic inhibition. The significant effect of raclopride in the reserpinized animal suggests that some dopamine remaining after reserpinization is still able to antagonize NMDA-induced motor effects. In fact, it has been previously reported that the dose of reserpine used in the present experiments does not completely deplete dopamine in the brain 24 h after its administration (27).

An antagonistic dopamine D_2-NMDA interaction has already been described in the striatum. By using patch-clamp techniques in rat and cat striatal slices, Cepeda et al. (4) have shown that the dopamine D_2 receptor agonist quinpirole attenuated the NMDA-evoked neuronal activation. By using *in vivo* microdialysis, Morari et al. (21) found that raclopride enhanced the NMDA-induced GABA release in the rat striatum. However, a striatal antagonistic dopamine D_2-NMDA interaction can not explain the present behavioural results, since blockade of striatal D_2 receptors induces motor depression and catalepsy (3,23). Therefore, as previously suggested (15), the present results indicate that most probably the dopamine-independent NMDA-induced motor activation originates in brain structures other than the striatum.

We have recently performed some *in situ* hybridization studies on *c-fos* mRNA expression in the brain of reserpinized and non-reserpinized mice after the systemic administration of a non-convulsant dose of NMDA (the same animal strain and the same doses of reserpine and NMDA than those used in the present experiments) to unravel the brain structures involved in the NMDA-induced motor activation (14). In non-reserpinized animals the NMDA-induced *c-fos* mRNA expression was predominant in the dentate gyrus and in the medial mammillary nucleus and less pronounced in other hippocampal areas, cortical areas, bed nucleus of the stria terminalis and posterior amydaloid nuclei. No NMDA-induced *c-fos* mRNA expression was found in the striatum. Reserpinization induced a significant potentiation of the NMDA-induced *c-fos* mRNA expression in those brain areas, which suggested the existence of a strong and selective amine-dependent modulation of NMDA neurotransmission (14). The present results suggest that dopamine, by acting on dopamine D_2 receptors, can be responsible for such modulation. The hippocampus might well be the brain structure which mediates the dopamine-independent NMDA-induced motor activity, since the hippocampal administration of NMDA induces motor activation (30). Furthermore, a tonic dopaminergic input to the hippocampus, mediated by dopamine D_2 receptors, has already been described and it has been suggested to function physiologically to prevent epileptogenesis (1,8), where NMDA receptors are considered to play a very distinct role (6).

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