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Lack of Specific Effects of Selective D_1 and D_2 Dopamine Antagonists vs. Risperidone on Morphine-Induced Hyperactivity

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RODRIGUEZ-ARIAS, M., I. BROSETA, M. A. AGUILAR AND J. MIÑARRO. *Lack of specific effects of selective D_1 and D_2 dopamine antagonists on morphine-induced hyperactivity.* PHARMACOL BIOCHEM BEHAV **66**(1) 189–197, 2000.—In the present study, three different dopamine antagonists were challenged in order to counteract hyperactivity induced by 50 mg/kg of morphine. A wide range of doses of morphine (50, 25, 12.5, 6.25, or 3.12 mg/kg) were evaluated on spontaneous locomotor activity. A significant increase was observed only with the two higher doses tested (25 and 50 mg/kg). No decrease was found with any of the doses used at any period of time. After analyzing doses of SCH 23390 (0.5, 0.1, and 0.05 mg/kg), raclopride (0.5, 0.25, and 0.125 mg/kg) and risperidone (0.1, 0.05, and 0.025 mg/kg) administered alone, only the 0.5 mg/kg dose of SCH 23390 decreased locomotor activity. The three compounds counteracted morphine-induced hyperactivity, but with SCH 23390 it was only achieved with the dose of 0.5 mg/kg, which also decreased spontaneous locomotor activity and induced catalepsy. On the other hand, raclopride and risperidone neutralized morphine-induced hyperactivity at doses that did not affect locomotor activity, although the former induced catalepsy when administered with morphine. It is concluded that although the blockade of D_1 and D_2 DA receptors decreases morphine-induced hyperactivity, this action is not specific, contrary to the action of risperidone, which counteracts this hyperactivity without any other motor effects.

SCH 23390 Raclopride Risperidone Hyperactivity Morphine

IN mice, morphine administration produces different behavioral effects on locomotor activity, depending on the dose used. It has been observed that with a range of doses from 3 to 10 mg/kg, morphine has elicited an initial behavioral depression, followed in the second hour by hyperlocomotion (38). However, other authors have found only hyperactivity with doses between 10 and 40 mg/kg (10–12,23,25,27).

It is well know that mesolimbic dopaminergic neurons are necessary for the expression of the increase in locomotor activity induced by opioids. An injection of morphine either in the ventral tegmental area or the nucleus accumbens produces, depending on the dose used, behavioral activation, or an initial inhibition of activity, followed by desinhibition (6,8). The increase in locomotor activity in mice caused by opioids may reflect an enhancement of dopaminergic neurotransmission (16). Morphine administration produces an increase in the transmission preferentially of the mesolimbic system (9). Low doses produce an increase of DA release in the accumbens at the same time as the behavioral stimulation (9,11,12). These results are in line with the hypothesis that μ-agonists stimulate locomotor activity through an activation of DA transmission (37).

Previous studies have pointed out that lesions with 6-OHDA of the dopaminergic system (28) or the blockade of dopaminergic receptors can prevent the morphine-induced hyperactivity. Apomorphine, at doses that presumably produce presynaptic inhibition of dopaminergic neurotransmission by activating autoreceptors, and the D_2 antagonist spiperone, inhibited the morphine-induced enhancement of locomotor activity at doses that did not produce significant motor impairment (16). In other studies, the blockade of the D_2 dopaminergic receptor by haloperidol (0.2 mg/kg) signifi-

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cantly antagonised the effects of morphine on locomotor activity, but lower doses were not efficient (24). However, diverse studies have provided other results, for instance, Vaccarino and coworkers (39) have observed that the dose of the DA antagonist α-flupenthixol necessary to interrupt amphetamine-induced locomotion, failed to prevent hyperactivity induced by morphine. In a more recent study, the selective D_2 antagonist eticlopride failed to block morphine-induced ambulation (19).

On the other hand, the activation of D_1 receptors seems to play an important role in the expression of morphine-induced hyperlocomotion because the administration of the selective D_1 antagonist SCH 23390 reduced this behavior (12,19,23). Moreover, the increase in locomotor activity induced by the D_1 agonist SKF 38393 was completely antagonised by pretreatment with SCH 23390 (12).

Risperidone, a benzisoxazol derivative, is an antipsychotic drug that combines potent serotoninergic 5-HT_2 and DA D_2 receptor antagonism (13,18). It also has affinity for the α1, α2-adrenergic and histamine H1 receptors (30). Risperidone differs from other typical and atypical antipsychotics by its more pronounced predominance of 5-HT_{2A} vs. D_2 receptor occupancy (35).

To our knowledge, no study has been conducted to study the ability of the mixed DA antagonist risperidone to counteract morphine-induced hyperactivity. With the aim of further clarifying the role of DA in this effect of morphine, risperidone and two selective DA antagonists, the D_1 antagonist SCH 23390 and the D_2 antagonist raclopride, were challenged in this study. In the first experiment, a wide range of doses of morphine, and three doses of these neuroleptics were analysed to elucidate their effect on spontaneous locomotor activity. Finally, in the second experiment these antagonists were administered to counteract the hyperactivity induced by 50 mg/kg of morphine.

METHOD

Subjects

In this study, 260 albino male mice of the OF1 strain, which were acquired in CRIFFA (Barcelona), aged 42 days when arriving at the laboratory, were housed under standard conditions: constant temperatures (21°C), a reversed light schedule (with lights on: 0730–1930 h) and food and water available ad lib except during the behavioral tests. Animals were housed in groups of 10 in transparent plastic cages (22 × 38 cm) where they stayed during a period of 1 month before the start of the experiments. The animals in the present study have been used in accordanc with national, regional, and local laws and regulations. The procedures used are equivalent to those recommended by the documents "Policy on Human Care and Use of Laboratory Animals" (USPHS) and "Guide for the Care and Use of Laboratory Animals" (NIH), both from the USA.

Drug Procedure

Morphine hydrochloride (Laboratories Alcaliber, Toledo, Spain), SCH 23390 (Shering Ploug, Madrid, Spain), raclopride (Astra Laboratories, Södertälje, Sweden), risperidone (Jannsen Farmaceutica, Beerse, Belgium), and physiological saline (NaCl 0.9%) were used in this experiment. Drugs were diluted in physiological saline (0.1 mg/ml), except for risperidone, which was dissolved in physiological saline plus tartaric acid, and administered IP. The injection volume was proportional to the weight of the mouse (1 ml of dissolution per 100 g of weight). The neuroleptics were administered at the same time of morphine, using two different syringes.

In the first experiment, animals were allocated to 15 groups. Five groups of experimental animals received morphine 50, 25, 12.5, 6.25, or 3.12 mg/kg (M50, M25, M12.5, M6.25, and M3.12), three groups received 0.5, 0.1, and 0.05 mg/kg of SCH 23390 (SCH 0.5, SCH 0.1, and SCH 0.05), three groups received raclopride 0.5, 0.25, and 0.125 mg/kg (R 0.5, R 0.25, and R 0.125), and another three groups received risperidone 0.1, 0.05, and 0.025 mg/kg (Rp 0.1, Rp 0.05, and Rp 0.025). The control group received physiological saline.

In the second experiment, animals were allocated to 11 groups. The experimental groups received 50 mg/kg of morphine plus the following drugs: 0.5, 0.1, and 0.05 mg/kg of SCH 23390 (M50+SCH 0.5, M50+SCH 0.1 and M50+SCH 0.05); raclopride 0.5, 0.25, and 0.125 mg/kg (M50+R 0.5, M50+R 0.25, and M50+R 0.125); risperidone 0.1, 0.05, and 0.025 mg/kg (M50+Rp 0.1, M50+Rp 0.05, and M50+Rp 0.025); and saline (M50+saline). Control animals received two injections of physiological saline.

Apparatus

For the measurement of spontaneous locomotor activity shown by the animals an actimeter was used (ACTISYSTEM II, Panlab S.L., Barcelona). The actimeter has four sensory plates 35 × 35 cm (pb 46603), an interfase (pb 40035) and a computer (Olivetti PCS 286) with the DAS 16 programme (v. 1.0.). The four sensory plates register the activity of the animals by means of an electromagnetic system and the DAS program allows the acquisition and storage of the data from the sensory plates.

Procedure

After the adaptation period in the laboratory, animals were divided into groups of 10. Immediately after the drug administration, animals were placed onto the sensory plates for a period of 60 min. The computer registered the activity each 15 min, i.e., at 15, 30, 45, and 60 min after the drug administration.

Catalepsy Test

Fourteen groups of animals were treated with saline (Sal); 50 mg/kg of morphine (M50); 0.5 or 0.1 mg/kg of SCH 23390 (SCH 0.5 and SCH 0.1); 50 mg/kg of morphine plus 0.5 or 0.1 mg/kg of SCH 23390 (M50+SCH 0.5 and M50+SCH 0.1); 0.5 or 0.25 mg/kg of raclopride (raclopride 0.5 and raclopride 0.25); 50 mg/kg of morphine plus 0.5 or 0.25 mg/kg of raclopride (M50+raclopride 0.5 and M50+raclopride 0.25); 0.1 or 0.05 mg/kg of risperidone (risperidone 0.5 and risperidone 0.05); 50 mg/kg of morphine plus 0.5 or 0.05 mg/kg of risperidone (M50+risperidone 0.5 and M50+risperidone 0.05). The animals underwent a test of catalepsy by means of the bar test: an aluminium bar of 5 mm in diameter was placed 4 cm above the floor, animal's forepaw were gently put on the bar, and the time it took for the animal to place at least one paw on the floor was ,measured with a maximum time of observation of 60 s. Successive behavioral evaluations of catalepsy were carried out 15, 30, and 45 min after drug administration. Between determinations, mice were kept in their home cages.

Statistical Analyses

All the data were analyzed by an analysis of variance (ANOVA) with two factors, one between (treatment) and

one within (time of recording the data), with four levels (15, 30, 45, and 60 min), except three levels in the catalepsy test (15, 30, and 45 min). Post hoc comparisons (Newman–Keuls) were subsequently carried out to ascertain pair-wise differences between means. Analysis of simple main effects for interactions was also conducted. One-way ANOVA was carried out when significant interactions were found.

RESULTS

First Experiment: Effects of Morphine, SCH 23390, Raclopride, and Risperidone on Locomotor Activity

Morphine. ANOVA revealed that treatment, $F(5, 54) = 8.527$, $p < 0.0001$, had a significant effect, but time of recording, $F(3, 15) = 2.204$, $p < 0.089$, did not influence this measure. Interaction, $F(15, 162) = 4.778$, $p < 0.0001$, was also significant (see Fig. 1).

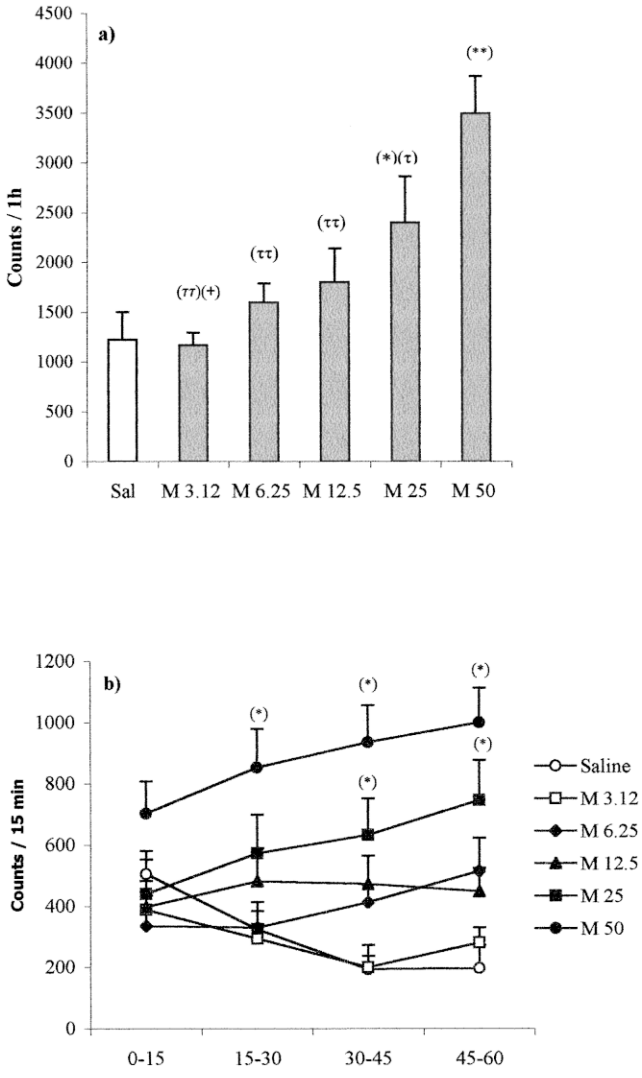

FIG. 1. Means (±SEM) of locomotor activity shown for six groups of mice receiving saline or five different doses of morphine (3.12, 6.25, 12.5, 25, and 50 mg/kg). (a) Total activity counts per hour. $**p < 0.01$, $*p < 0.05$ with respect to saline, $\tau\tau p < 0.01$, $\tau p < 0.05$ with respect to M 50; $+p < 0.05$ with respect to M 25. (b) Counts per 15 min during the first hour after injection. $*p < 0.01$ with respect to saline.

Newman–Keuls post hoc analysis of treatment showed that locomotor activity was higher in the animals receiving 50 mg/kg with respect to the other groups ($p < 0.01$). Also, those receiving 25 mg/kg presented a significant increase when compared with the control and those in the 3.12 mg/kg groups ($p < 0.001$).

Simple effects showed that time of recording was significant in all the groups ($p < 0.05$) except in the 12.5 and 3.12 mg/kg groups. On the other hand, treatment was significant at all the time points ($p < 0.03$).

An ANOVA was carried out in each moment of recording with one between factor with six levels (treatment), resulting significant in all periods of time. At 15 min the 50 mg/kg dose of morphine was significantly higher than the 6.25 mg/kg dose ($p < 0.05$). At 30 min, the high dose of morphine showed a significant increase with respect to the other five groups ($p < 0.01$ with saline, M6.25, and M3.12, and $p < 0.05$ with respect to M12.5 and M25). At 45 and 60 min, the 50 mg/kg of morphine was significantly higher than saline, M12.5, M6.25, and M3.12 ($p < 0.01$), and likewise, the 25 mg/kg dose of morphine was significantly higher than saline and M3.12 ($p < 0.01$).

SCH 23390. ANOVA revealed that treatment, $F(3, 36) = 3.519$, $p < 0.0246$, and time of recording, $F(3, 108) = 33.067$, $p < 0.0001$, had a significant effect, but not so interaction, $F(9, 108) = 1.524$, $p < 0.1485$ (see Fig. 2).

Newman–Keuls post hoc analysis of treatment showed differences between the SCH 0.5 and SCH 0.05 groups. Time of recording was significantly higher ($p < 0.01$) in the first measure (15 min) compared with the other three time points.

Raclopride. ANOVA revealed that treatment, $F(3, 36) = 0.342$, $p < 0.7948$, was nonsignificant, but time of recording, $F(3, 108) = 16.628$, $p < 0.0001$, and interaction, $F(9, 108) = 2.799$, $p < 0.0054$, were significant (see Fig. 3).

Newman–Keuls post hoc analysis of time showed that the first measure (15 min) was significantly higher ($p < 0.01$) compared with the other three time points. Furthermore, the recording at 30 min was significantly higher ($p < 0.01$) than the last measure (60 min).

Simple effects revealed that time of recording was significant only with the saline and the groups receiving the lowest dose of raclopride ($p < 0.001$).The effect of treatment was not significant in any group.

Risperidone. ANOVA revealed that treatment, $F(3, 36) = 0.605$, $p < 0.6158$, was nonsignificant but time of recording, $F(3, 108) = 38.866$, $p < 0.0001$, had a significant effect. Interaction, $F(9, 108) = 0.722$, $p < 0.6878$, was not significant (see Fig. 4).

Newman–Keuls post hoc analysis of time showed that the first measure (15 min) was significantly higher ($p < 0.01$) compared with the other three times of recording.

Second Experiment: Effects of Dopaminergic Antagonists on Morphine-Induced Hyperactivity

SCH 23390. ANOVA revealed that treatment, $F(4, 45) = 4.379$, $p < 0.0045$, and time of recording, $F(3, 135) = 6.434$, $p < 0.0004$, had a significant effect. Interaction, $F(12, 135) = 8.933$, $p < 0.0001$, was also significant (see Fig. 5).

Newman–Keuls post hoc analysis of treatment showed differences between the saline and SCH 0.05 groups ($p < 0.05$). Time was significantly higher in the last measure (60 min) compared with the other three time points ($p < 0.01$ with respect to 15 and 30 min, $p < 0.05$ with respect to 45 min).

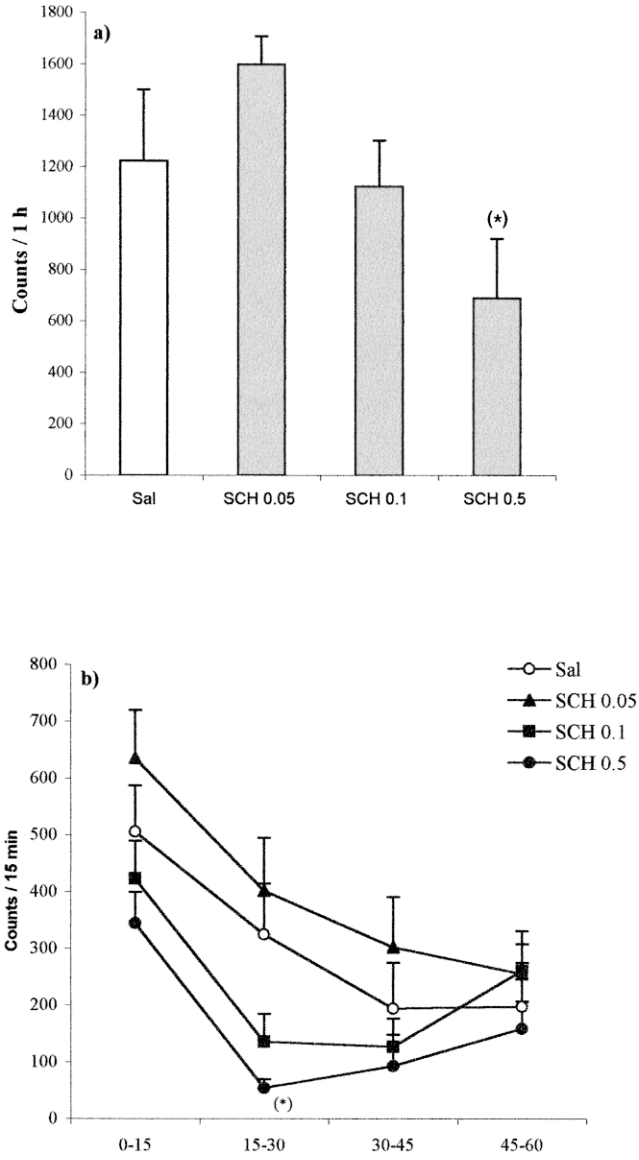

FIG. 2. Means (±SEM) of locomotor activity shown for four groups of mice receiving saline or three different doses of SCH 23390 (0.05, 0.1, and 0.5 mg/kg). (a) Total activity counts per hour. *$p < 0.05$ with respect to SCH 0.05 group. (b) Counts per 15 min during the first hour after injection. *$p < 0.05$ with respect to saline.

Simple effects showed that treatment was significant in every time point except in the 15-min test ($p < 0.002$). The moment was significant in each treatment ($p < 0.01$).

An ANOVA was carried out in each moment of the recording with one between factor with five levels (treatment), which resulted as significant ($p < 0.001$) in each moment except in the first measurement (15 min). At 30 and 45 min the saline and the M50+SCH 0.5 groups showed a significant decrease with respect to the other three groups ($p < 0.05$). At 60 min, only the saline group was significantly lower than M50+saline, SCH 0.1, and SCH 0.05 groups ($p < 0.01$).

Raclopride. ANOVA revealed that treatment, $F(4, 45) = 8.441$, $p < 0.0001$, time of recording, $F(3, 135) = 26.645$, $p < 0.0001$, and interaction, $F(12, 135) = 17.162$, $p < 0.0001$, were all significant (see Fig. 6).

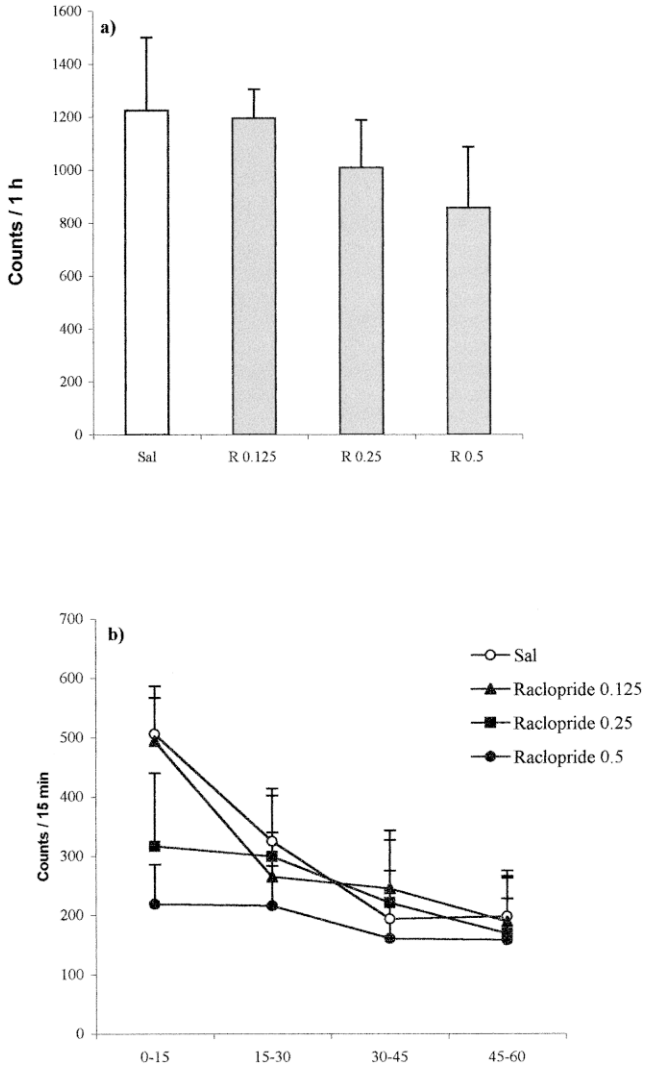

FIG. 3. Means (±SEM) of locomotor activity shown for four groups of mice receiving saline or three different doses of raclopride (0.125, 0.25, and 0.5 mg/kg). (a) Total activity counts per hour. (b) Counts per 15 min during the first hour after injection.

Newman–Keuls analysis of treatment indicated that the M50+saline group presented a significant increase with respect to the other three groups ($p < 0.05$ with respect to M50+R 0.125 group and $p < 0.01$ with the other three groups). The M50+R 0.125 group also presented a significant increase ($p < 0.05$) with respect to the M50+R 0.5 group. The post hoc analysis of the time showed that the last measure (60 min) was significantly higher ($p < 0.01$) compared with the other three times of recording. Moreover, the 45-min recording was significantly higher ($p < 0.01$) than the 15- and 30-min measures.

Simple effects showed that the moment of the measurement was significant with all the treatments ($p < 0.001$). Likewise, Treatment was significant at all time points.

An ANOVA was made for each time of recording with one between factor with four levels (treatment), resulting as significant in all of the times recorded. At 15 min, the saline and M50+saline groups were significantly higher ($p < 0.01$)

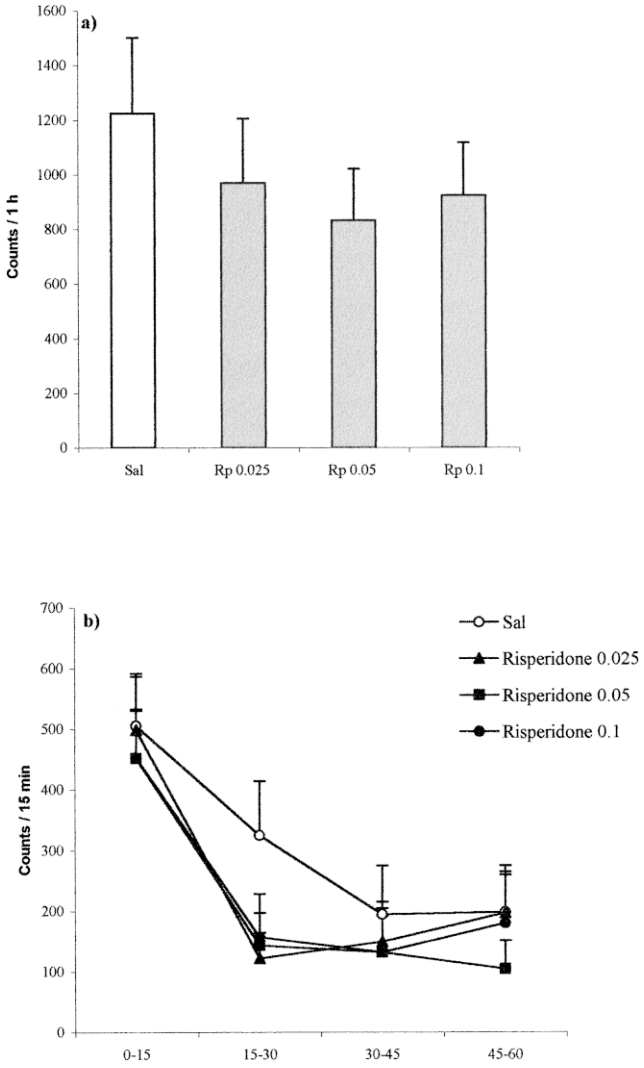

FIG. 4. Means (±SEM) of locomotor activity shown for four groups of mice receiving saline or three different doses of risperidone (0.025, 0.05, and 0.1 mg/kg). (a) Total activity counts per hour. (b) Counts per 15 min during the first hour after injection.

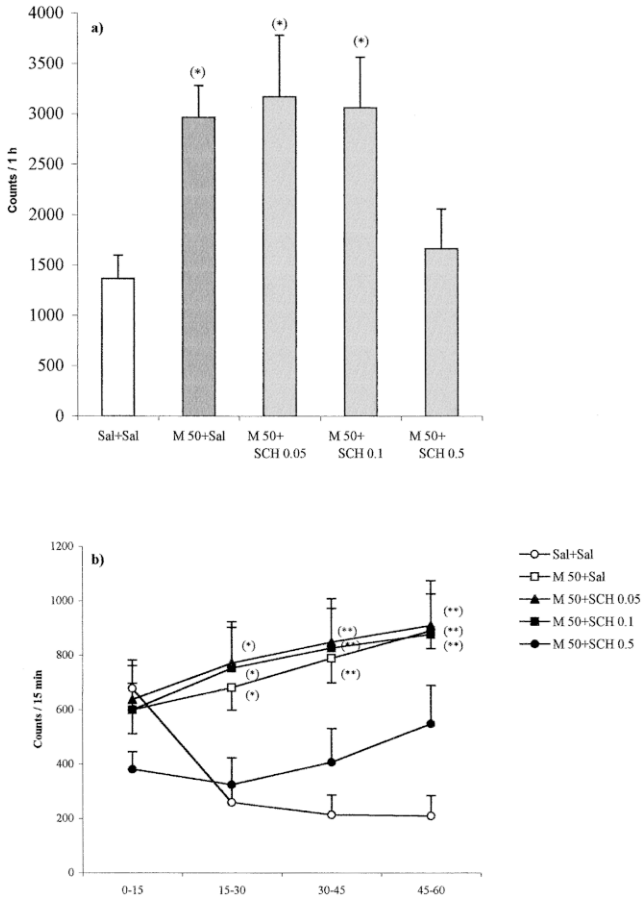

FIG. 5. Means (±SEM) of locomotor activity shown for five groups of mice receiving saline, morphine 50 mg/kg plus saline or morphine 50 mg/kg plus three different doses of SCH 23390 (0.05, 0.1, and 0.5 mg/kg). (a) Total activity counts per hour. *$p < 0.05$ with respect to saline group. (b) Counts per 15 min during the first hour after injection. *$p < 0.05$, **$p < 0.01$ with respect to saline.

than the other three groups. The M50+R 0.125 group was also significantly higher ($p < 0.05$) than the M50+R 0.5 group. At 30 min, the M50+saline group showed a significant increase with respect to the other four groups ($p < 0.05$ for M50+R0.125, and $p < 0.01$ with respect to the rest of the groups). At 45 min, the M50+saline group was significantly higher ($p < 0.01$) than saline and M50+R0.5. In this measure, the saline was significantly lower ($p < 0.05$) than the M50+R0.25 and M50+R0.125. In the last time point, the saline and the M50+R0.5 groups were significantly lower than the other three experimental groups ($p < 0.01$ in the case of saline comparisons and M50+R0.5 with respect to M50+saline, and $p < p < 0.05$ in M50+R0.5 with respect to M50+R0.25 and M50+R0.125).

Risperidone. An ANOVA revealed that treatment, $F(4, 45) = 6.071$, $p < 0.0005$, and the time of recording, $F(3, 135) = 10.660$, $p < 0.0001$, had a significant effect. Interaction, $F(12, 135) = 9.042$, $p < 0.0001$, was also significant (see Fig. 7).

Newman–Keuls analysis of treatment showed that the M50+saline group presented a significant increase with respect to the saline, M50+Rp0.1 and M50+Rp0.05 groups ($p < 0.01$). The post hoc analysis of time indicated that the first (15 min) and the last measure (60 min) were significantly higher ($p < 0.01$ in the comparisons of 15 min and 60 min with respect to 30 min, and $p < 0.05$ in 60 min compared with 45 min).

Simple effects showed that time was significant ($p < 0.001$) in all except at 15 min. Similarly, treatment was significant in all the moments of the measure except in the M50+Rp 0.125 group.

An ANOVA was made in each time of recording with one between factor with four levels (treatment), which resulted significant in all the time points, except at 15 min. At 30 min, the M50+saline group showed a significant increase ($p < 0.01$) with respect to the saline, M50+Rp 0.1 and M50+Rp 0.05 groups. At 45 and 60 min, the M50+saline group was significantly higher than saline, M50+Rp 0.1, M50+Rp 0.05 ($p < 0.01$) and M50+Rp 0.025 ($p < 0.05$) groups. In this measure, the M50+Rp 0.025 was significantly higher ($p < 0.05$) than the saline and M50+Rp 0.1 groups.

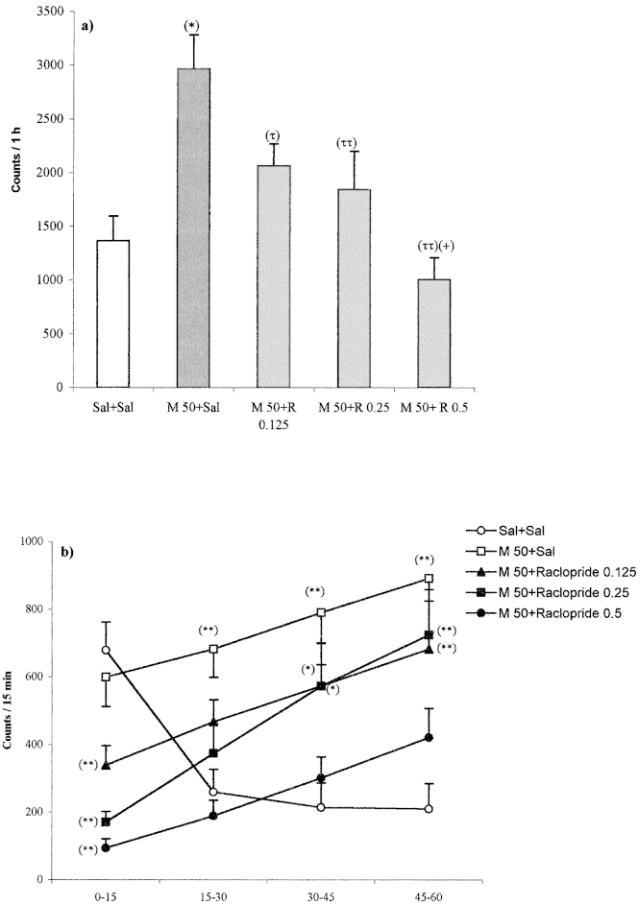

FIG. 6. Means (±SEM) of locomotor activity shown for five groups of mice receiving saline, morphine 50 mg/kg plus saline or morphine 50 mg/kg plus three different doses of raclopride (0.125, 0.25, and 0.5 mg/kg). (a) Total activity counts per hour. $*p < 0.01$ with respect to saline group; $\tau p < 0.05$, $\tau\tau p < 0.01$ with respect to M 50 + Sal; $+p < 0.05$ with respect to M 50 + raclopride 0.125. (b) Counts per 15 min during the first hour after injection. $*p < 0.05$, $**p < 0.01$ with respect to saline.

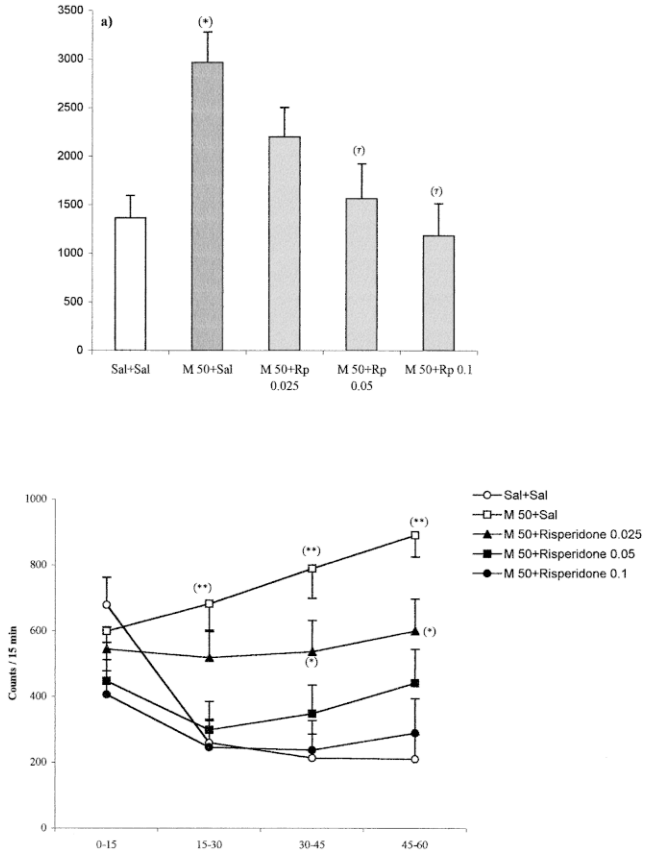

FIG. 7. Means (±SEM) of locomotor activity shown for five groups of mice receiving saline, morphine 50 mg/kg plus saline, or morphine 50 mg/kg plus three different doses risperidone (0.025, 0.05, and 0.1 mg/kg). (a) Total activity counts per hour. $*p < 0.01$ with respect to saline group; $\tau p < 0.01$ with respect to M 50 + Sal. (b) Counts per 15 min during the first hour after injection. $*p < 0.05$, $**p < 0.01$ with respect to saline.

Catalepsy Test

Treatment with risperidone alone or plus 50 mg/kg of morphine did not show a significant effect in the catalepsy test. Conversely, ANOVA revealed that treatment with SCH 23390, $F(5, 42) = 6.882$, $p < 0.0001$, had a significant effect. Newman–Keuls post hoc analysis showed that the groups that received M50+SCH 0.5 and M50+SCH 0.1 differed significantly from the saline and the M50 groups ($p < 0.01$). In addition, administration of either doses of SCH 23390 alone, also presented higher scores of catalepsy than saline and M50 groups ($p < 0.05$) (see Fig. 8). Finally, ANOVA revealed that treatment with raclopride plus morphine, $F(5, 42) = 4.160$, $p < 0.0037$, had a significant effect. Newman–Keuls post hoc analysis showed that the groups that received M50+raclopride 0.5 and M50+raclopride 0.25 differed significantly from the saline group ($p < 0.05$) (see Fig. 9).

DISCUSSION

The results of this study clearly demonstrate that risperidone plays an important role in morphine-induced hyperactivity. At doses that do not affect spontaneous locomotor activity, this mixed antagonist firmly counteracts morphine-induced hyperactivity. The selective DA D_1 antagonist, SCH 23390 only significantly decreases this kind of hyperactivity at doses that produce a concomitant decrease in spontaneous locomotor activity. The blockade of the DA D_2 receptor with raclopride also seems to counteract this hyperactivity in a nonspecific way, as the dose necessary to achieve it produces a cataleptic effect in the animals.

Morphine administration produces a significant increase in locomotor activity only with the two higher doses tested (25 and 50 mg/kg), and no decrease has been observed at any time point. These results are not in concordance with Székely and co-workers (38) who found an initial behavioral depression followed by a hyperactive phase with a range of doses of morphine between 3 to 10 mg/kg. Nevertheless, we have not found any significant modification of locomotor activity with these doses. Our results support the hypothesis that morphine in mice produces an increase in locomotion without a preceding sedative phase in agreement with other authors (10–12,23,27).

With respect to the action of neuroleptics on locomotor activity, at the doses tested no salient effects are observed.

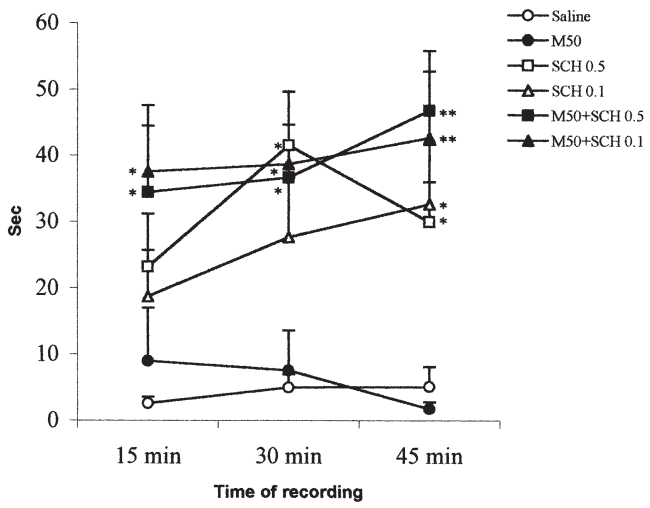

FIG. 8. Means (±SEM) of seconds shown in the catalepsy test for four groups of mice receiving saline, morphine 50 mg/kg, SCH 23390 0.5 mg/kg, SCH 23390 0.1 mg/kg, morphine 50 mg/kg plus SCH 23390 0.5 mg/kg or morphine 50 mg/kg plus SCH 23390 0.1 mg/kg. $^{**}p < 0.01$, $^{*}p < 0.05$ with respect to the saline group.

The results on a whole show that the dopamine antagonists used do not decrease motor behaviors in general. Only SCH 23390 significantly decreases locomotor activity with the dose of 0.5 mg/kg in the second period tested (15–30 min). Equally, an increase in catalepsy has been observed with the two doses higher. In prior studies in the field of aggression carried out in our laboratory, we have observed that the three doses of SCH 23390 tested decreased aggression but produced high scores of immobility (32). Thus, D_1 dopamine receptors seem to play an important role in spontaneous locomotor activity as well as catalepsy.

The other neuroleptics fail to reduce locomotor activity with any of the doses tested. Although it has been shown that

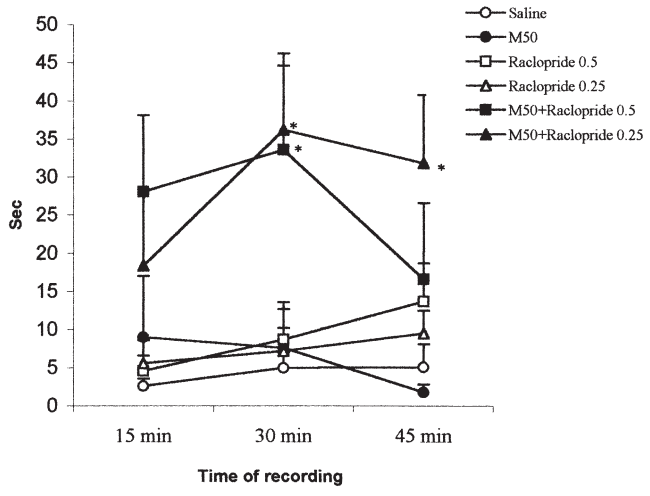

FIG. 9. Means (± SEM) of seconds shown in the catalepsy test for four groups of mice receiving saline, morphine 50 mg/kg, raclopride 0.5 mg/kg, raclopride 0.25 mg/kg, morphine 50 mg/kg plus raclopride 0.5 mg/kg, or morphine 50 mg/kg plus raclopride 0.25 mg/kg. $^{*}p < 0.05$ with respect to the saline group.

raclopride produces motor effects (with doses from 0.05 to 0.2 mg/kg), using a reaction time task (3), in other studies, it does not affect locomotor activity but reduces the hyperactivity induced by *d*-amphetamine, phencyclidine, or diazepam, and also efficiently decreases aggression without increasing immobility or affecting other motor behaviors (2,22). Our results are in accordance with these latter findings. Although risperidone is considered to be an atypical neuroleptic, it has reduced locomotor activity in prior studies (4,7). Aguilar and co-workers (1), using the conditioned avoidance response, found that risperidone (0.1 to 1 mg/kg) increases the number of nonresponses and reduces locomotor activity. In agreement with these findings, in a more recent study using an ethological analysis (32), doses of risperidone from 0.01 to 0.1 mg/kg have produced elevated scores of immobility. However, in the present study, no impairment of locomotor activity is observed. These findings are similar to those found with the typical neuroleptic haloperidol, which does not affect spontaneous locomotor activity with doses from 0.075 to 0.1 mg/kg (25) but increases immobility when it is evaluated in a social context (31).

In the second experiment, when we evaluated the ability of dopamine antagonists to counteract the morphine-induced hyperactivity, we found that the three dopamine antagonists are capable of decreasing this hyperlocomotion. With regard to SCH 23390, only the highest dose blocks this effect of morphine. It must be considered that this dose (0.5 mg/kg) administered alone produces a significant decrease in spontaneous locomotor activity as well as catalepsy. The selective D_1 antagonist has already been seen to possess the ability of counteracting morphine-induced hyperactivity. Doses of 0.1 or 0.25 mg/kg of the D_1 blocker antagonize the behavioral stimulation induced by 10 mg/kg of morphine (15,19). At these doses, SCH 23390 also appears to decrease locomotor activity when administered on its own, although this effect did not reach statistical significance. In a more recent work, 0.05 mg/kg has been enough to counteract hyperactivity produced by 20 mg/kg of morphine (10). Obviously, a superior degree in the blockade of the D_1 receptors would be necessary to abolish the motor effect of 50 mg/kg of morphine. In this work, although an effect on morphine-induced hyperactivity has been observed, the dose needed to produce it decreases spontaneous locomotor activity and induces catalepsy with or without morphine. Thus, although the highest SCH 23390 dose counteracted morphine-induced hyperactivity, we cannot conclude that it is a specific effect.

On the other hand, a very interesting result is noticed with respect to raclopride. Although this drug administered alone does not affect locomotor activity at any dose tested, the dose of 0.5 mg/kg is capable of firmly counteracting the hyperlocomotion induced by morphine. There are no homogeneous results regarding the effect of the D_2 blockade on morphine-induced locomotion. Although in some works using haloperidol the blockade of the D_2 receptors has counteracted this kind of locomotion, in another more recent study the selective D_2 blocker eticlopride fails to prevent the acute locomotor stimulant effects of 10 mg/kg of morphine (10,19). Another relevant result regarding raclopride is observed in the first 15 min of administration. The three doses of the D_2 antagonist significantly reduce locomotor activity with respect to control and morphine 50 groups. In the catalepsy study, a significant increase has been observed in the animals treated with M50+raclopride 0.5 as well as M50+raclorpide 0.25, these results being similar to those observed previously with SCH 23390. This cataleptic effect has been previously ob-

served with other DA antagonists such as haloperidol (24), or more recently with the DA release inhibitor CGS 10746B (unpublished data). This could represent the action of morphine on other opiate receptors different from those situated in the ventral tegmental area, which could be responsible for this cataleptic effect (21). Only when the dopaminergic system is blocked can the action of morphine in these receptors be expressed and the cataleptic effect observed. Thus, some of the antimorphine effects of SCH 23390 and raclopride are nonspecific, and probably mediated by catalepsy produced by inactivation of the nigrostriatal system because it has been postulated that neuroleptic catalepsy results from the blockade of dopamine receptors in the striatum (5). On the other hand, it is well accepted that the activation of the mesolimbic dopamine system may be involved in the expression of morphine-induced hyperlocomotion in mice (12). We can conclude that, although the highest raclopride dose is capable of counteracting morphine-induced hyperactivity, this action is not specific either.

Few behavioral studies have been conducted with the new neuroleptic risperidone, and to our knowledge, none that explore its capacity to counteract morphine effects. The mixed antagonist is capable of completely counteracting morphine-induced hyperactivity with the doses of 0.1 and 0.05 mg/kg, and also the lower dose (0.025 mg/kg) significantly decreases this hyperactivity, although not to control levels. However, conversely to raclopride or SCH 23390, no cataleptic effects are observed with either of the risperidone doses administered. The particular blockade profile of this neuroleptic, acting on DA D_2 receptors and in addition on the serotonergic system (5-HT_2 receptor), gives it a singular effect. Risperidone blocks 5-HT_2 receptors at doses that are about 10 times lower than those required for the blockade of central D_2 receptors, similar to clozapine, and occupies central D_2 receptors in terminal regions more gradually than haloperidol (26). Biochemical, electrophysiological, and behavioral data indicate that important functional interactions occur between brain serotoninergic and dopaminergic systems. The serotoninergic system inhibits dopaminergic function at the level of the origin of the DA system in the midbrain as well as at the terminal dopaminergic fields in the forebrain [for review, see (20)]. Treatment with a selective serotonin reuptake inhibitor (Fluoxetine) attenuates the locomotor activating effect of acute morphine treatment and blocks the sensitization response to the morphine challenge. Fluoxetine may enhance the morphine-induced increase in 5-HT activity, and this may function to inhibit opiate-induced DA activation and opiate-induced behaviors, possibly via action 5-HT at 5-$HT_{1/2}$ receptors (36). Because serotonin exerts an inhibitory influence on the DA system, treatments that increase serotonergic transmission could enhance the motor effects of DA antagonists. Conversely, manipulations that inhibit serotoninergic function (raphe lesions, 5-HT_{1A} autoreceptor agonists, or 5-HT_2 antagonists) would be expected to desinhibit the DA system and thus enhance DA-mediated locomotor behavior. For example, administration of 5-HT_{1A} antagonists induce mild behavioral activation in rats, which is probably mediated indirectly via DA systems (17). In addition, the blockade of 5-HT_2 receptors increases the activity of dopaminergic nigrostriatal neurons in the presence of D_2 blockade (34).Thus, the effects of the DA D_2 receptor blockade such as a decrease in locomotor activity, catalepsy, and extrapyramidal symptoms could be attenuated by the concurrent blockade of 5-HT_2. Risperidone, as clozapine and other atypical antipsychotics presents this combined 5-HT_2/D_2 antagonism that may contribute to its limbic selectivity with higher therapeutic efficacy and lower extrapyramidal side effects (14). The synergistic effects associated with combined 5-HT_2/D_2 antagonism may result in a more efficient control of interacting serotoninergic and dopaminergic stimuli than that obtained with conventional neuroleptics (26).

In conclusion, risperidone efficiently counteracts morphine-induced hyperactivity, without any other locomotor or cataleptic effect. Conversely, although the blockade of D_1 and DA D_2 receptors decreases this effect of morphine, this action is not specific because a decrease in locomotor activity or catalepsy is observed.

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REFERENCES

1. Aguilar, M. A.; Rodríguez-Arias, M.; Marí-Sanmillán, M. I.; Miñarro, J.: Effects of risperidone on conditioned avoidance responding in male mice. Behav. Pharmacol. 8:669–676; 1997.
2. Aguilar, M. A.; Miñarro, J.; Pérez-Iranzo, N.; Simón, V. M.: Behavioral profile of raclopride in agonistic encounters between male mice. Pharmacol. Biochem. Behav. 47:753–756; 1994.
3. Amalric, M.; Berhow, M.; Koob, G. F.: Selective effects of low-dose D_2 dopamine receptor antagonism in a reaction-time task in rats. Neuropsychopharmacology 8:195–200; 1993.
4. Arnt, J.: Differential effects of classical and newer antipsychotics on the hypermotility induced by two dose levels of *d*-amphetamine. Eur. J. Pharmacol. 283:55–62; 1995.
5. Arnt, J.; Skarsfeldt, T.; Hyttel, J.: Differentiation of classical and novel antipsychotics using animals models. Int. Clin. Psychopharmacol. 12:S9–S17; 1997.
6. Bauco, P.; Wang, Y.; Wise, R. A.: Lack of sensitization or tolerance to the facilitating effect of ventral tegmental area morphine on lateral hypothalamic brain stimulation reward. Brain Res. 617:303–308; 1993.
7. Casey, D. E.: Behavioral effects of sertindole, risperidone, clozapine and haloperidol in cebus monkeys. Psychopharmacology (Berlin) 124:134–140; 1996.
8. Cunningham, S. T.; Kelley, A. E.: Opiate infusion into the nucleus accumbens: Contrasting effects on motor activity and responding for conditioned reward. Brain Res. 588:104–114; 1992.
9. Di Chiara, G.; Imperato, A.: Opposite effects of and opiate agonists on dopamine release in the nucleus accumbens and in the dorsal caudate of freely moving rats. J. Pharmacol. Exp. Ther. 244:1067–1080; 1988.
10. Dogrul, A.; Yesilyurt, O.: Effects of $Ca^{+}+$ channel blockers on apomorphine, bromocriptine and morphine-induced locomotor activity in mice. Eur. J. Pharmacol. 364:175–182; 1999.
11. Funada, M.; Suzuki, T.; Narita, M.; Misawa, M.; Nagase, H.: Modification of morphine-induced locomotor activity by pertussis toxin: Biochemical and behavioral studies in mice. Brain Res. 619:163–172; 1993.
12. Funada, M.; Suzuki, T.; Misawa, M.: The role of dopamine D1 receptors in morphine-induced hyperlocomotion in mice. Neurosci. Lett. 169:1–4; 1994.

13. Hall, H.; Halldin, C.; Nyberg, S.; Farde, L.; Sedvall, G.: Effect of clozapine and risperidone on 5-HT_2 and D2 dopamine receptor binding in the post-mortem human brain. An autoradiographic study. Eur. Neuropsychopharmacol. 5:19–25; 1995.
14. Hertel, P.; Nomikos, G. G.; Iurlo, M.; Svensson, T. H.: Risperidone: Regional effects in vivo on release and metabolism of dopamine and serotonin in the rat brain. Psychopharmacology (Berlin) 124:74–86; 1996.
15. Imperato, A.; Obinu, M. C.; Casu, M. A.; Mascia, M. S.; Carta, G.; Gessa, G. L.: Chronic morphine increases hippocampal acetylcholine release: Possible relevance in drug dependence. Eur. J. Pharmacol. 302:21–26; 1996.
16. Iwamoto, E. T.: Locomotor activity and antinociception after putative and opioid receptor agonists in the rat: Influence of dopaminergic agonists and antagonists. J. Pharmacol. Exp. Ther. 217:451–460; 1981.
17. Jackson, D. M.; Wallsten, C. E.; Jerning, E.; Hu, P.-S.; Deveney A. M.: Two selective 5-HT_{1a} receptor antagonists, WAY-100 635 and NDL-249, stimulate locomotion in rats acclimatised to their environment and alter their behaviour: A behavioural analysis. Psychopharmacology (Berlin) 139:300–310; 1998.
18. Janssen, P. A. J.; Niemegeers, C. J. E.; Awouters, F.; Schellekens, K. H. L.; Megens, A. A. H. P.; Meert, T. F.: Pharmacology of risperidone (R 64 766), a new antypsychotic with serotonin-S2 and dopamine-D2 antagonistic properties. J. Pharmacol. Exp. Ther. 244:685–693; 1988.
19. Jeziorski, M.; White, F. J.: Dopamine receptor antagonists prevent expression, but not development of morphine sensitization. Eur. J. Pharmacol. 275:235–244; 1995.
20. Kapur, S.; Remington, G.: Serotonin–dopamine interaction and its relevance to schizophrenla. Am. J. Psychiatry 153:466–476; 1996.
21. Kiritsy-Roy, J. A.; Standish S. M.; Terry L. C.: Dopamine D1 and D2 receptor antagonists potentiate analgesic and motor effects of morphine. Pharmacol. Biochem. Behav. 32:717–721; 1989.
22. Lapin, I. P.; Rogawski, M. A.: Effects of D1 and D2 dopamine receptor antagonists and catecholamine depleting agents on the locomotor stimulation induced by dizocilpine in mice. Behav. Brain Res. 70:145–151; 1995.
23. Longoni, R.; Spina, L.; Di Chiara G.: Dopaminergic D1 receptors: Essential role in morphine-induced hypermotility. Psychopharmacology (Berlin) 93:401–402; 1987.
24. Magnus-Ellenbroek, B.; Havemann-Reinecke, U.: Morphine-induced hyperactivity in rats—A rebound effect? Naunyn Schmiedebergs Arch. Pharmacol. 347:635–642; 1993.
25. Manzanedo, C.; Aguilar, M. A.; Miñarro, J.: The effects of dopamine D2 and D3 antagonists on spontaneous motor activity and morphine-induced hyperactivity in male mice. Psychopharmacology (Berlin) 143:82–88; 1999.
26. Megens, A. A. H. P.; Awouters, F. H. L.; Schotte, A.; Meert, T. F.; Dugovic, C.; Niemegeers C. J. E.; Leysen, J. E.: Survey of the pharmacodinamics of the new antipsychotic risperidone. Psychopharmacology (Berlin) 114:9–23; 1994.
27. Narita, M.; Suzuki, T.; Funada, M.; Misawa, M.; Nagase, H.: Involvement of δ-opioid receptors in the effects of morphine on locomotor activity and the mesolimbic dopaminergic system in mice. Psychopharmacology (Berlin) 111:423–426; 1993.
28. Narita, M.; Takahashi, Y.; Takamori, K.; Funada, M.; Suzuki, T.; Misawa, M.; Nagase, H.: Effects of k-agonist on the antinoception and locomotor enhancing action induced by morphine in mice. Jpn. J. Pharmacol. 62:15–24; 1993.
29. Navarro, J. F.; Muñoz, A.; Miñarro, J.; Simón, V. M.: Absence of sensitisation to haloperidol in catalepsy of male mice. Med. Sci. Res. 20:381–382; 1992.
30. Pickar, D.: Prospects for pharmacotherapy of schizophrenia. Lancet 345:557–562; 1995.
31. Rodríguez-Arias, M.; Miñarro, J.; Simón, V. M.: Interaction of morphine and haloperidol on agonistic and motor behaviors of male mice. Pharmacol. Biochem. Behav. 58:153–158; 1997.
32. Rodríguez-Arias, M.; Miñarro, J.; Aguilar, M. A.; Pinazo, J.; Simón, V. M: Effects of risperidone and SCH 23390 on isolation-induced aggression in male mice. Eur. Neuropsychopharmacol. 8:95–103; 1998.
33. Rodriguez-Arias, M.; Felip, C. M.; Broseta, I.; Miñarro, J.: The dopamine D3 antagonist U-99194[a] maleate increases social behaviors of isolation-induced aggressive male mice. Psychopharmacology (Berlin) 144:90–94; 1999.
34. Saller, C. F.; Czupryna, M. J.; Salama, A. L.: 5-HR2 receptor blockade by Id 169,369 and other 5.HT2 antagonists modulates the effects of D-2 dopamine receptor blockade. J. Pharmacol. Exp. Ther. 253:1162–1170; 1990.
35. Schotte, A.; Janssen P. F. M.; Gommeren W.; Luyten, W. H. M. L.; Van Gompel P.; Lesage A. S.; De Loore, L.; Leysen, J. E.: Risperidone compared with new and reference antipsychotic drugs: In vitro and in vivo receptor binding. Psychopharmacology (Berlin) 124:57–73; 1996.
36. Sills, T. L.; Fletcher, P. J.: Fluoxetine attenuated morphine-induced locomotion and blocks morphine-sensitization. Eur. J. Pharmacol. 377:161–164; 1997.
37. Spanagel, R.; Herz, A.; Bals-Kubik, R.; Shippenberg, T. S.: β-Endorphin-induced locomotor stimulation and reinforcement are associated with an increase in dopamine release in the nucleus accumbens. Psychopharmacology (Berkin) 104:51–56; 1991.
38. Székely, J. I.; Miglécz, E.; Rónai, A. Z.: Biphasic effects of a potent analogue (D-Met2,Pro5)-enkephalinamide and morphine on locomotor activity in mice. Psychopharmacology (Berlin) 71:299–301; 1980.
39. Vaccarino, F. J.; Amalric, M.; Swerdlow, N.R.; Koob, G. F.: Blockade of amphetamine but not opiate induced locomotion following antagonism of dopamine function in the rat. Pharmacol. Biochem. Behav. 24:61–65; 1986.