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MK-801-induced hyperlocomotion: Differential effects of M100907, SDZ PSD 958 and raclopride

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Abstract

The influence of three selective monoamine receptor antagonists on spontaneous locomotion and on the hyperlocomotion induced by the un-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist [+]-5-methyl-10,11-dihydro-5H-dibenzo-[*a*,*d*]-cyclohepten-5,10-imine hydrogen maleate (MK-801; dizocilpine) was investigated. The selective and potent 5-hydroxytryptamine (5-HT_{2A}) receptor antagonist *R*(+)-α(2,3-dimethoxyphenyl)-1-[2(4-fluorophenyl)ethyl)]-4-piperidine-methanol (MDL100,907; M100907) displayed a clear-cut selectivity for reduction of MK-801-induced as compared to spontaneous locomotion, in that the former was dose-dependently (0.001, 0.01, 0.1 mg/kg i.p.) blocked and even totally abolished by the highest dose, while the latter was only modestly affected. Even at high doses of M100907 (up to 9 mg/kg i.p.), spontaneous locomotion was not reduced below 40% of control. The selective dopamine D_1 receptor antagonist (−)-[4*aR*,10*aR*]-1,2,3,4,4*a*,5,10,10*a*-octahydro-4-(4-chloro-2-methyl-phenyl)-1-methyl-benzo[g]quinoxaline-6-ol (SDZ PSD 958; 0.017, 0.15, 1.35 mg/kg i.p.) decreased both spontaneous and MK-801-induced locomotion with a slight preference for the latter; spontaneous locomotion was dose-dependently diminished to approx. 10% of controls (at 8 mg/kg i.p.). The dopamine D_2 receptor antagonist raclopride ([(−)-(*S*)-3,5-dichloro-*N*-((1-ethyl-2-pyrrolidinyl) methyl)-6-methoxy-salicylamide tartrate]; 0.11, 0.33, 1.0 mg/kg i.p.) reduced both MK-801-induced and spontaneous locomotion to a similar extent. An orthogonal matrix experimental design, and multiple regression, were used to evaluate the effects of several combinations of different doses of the 5-HT_{2A} receptor antagonist and the dopamine D_1 receptor antagonist. No synergistic actions on reduction of spontaneous or MK-801-induced locomotion were detected between M100907 and SDZ PSD 958. If the hyperlocomotion elicited by acutely administered MK-801 is a valid model of at least some aspects of schizophrenia, these results indicate that the 5-HT_{2A} receptor antagonist M100907 will have efficacy in treating this condition.The lack of effect on spontaneous locomotion, suggests that M100907, compared to dopamine receptor antagonists, will be less prone to induce psychomotor side-effects. Ongoing clinical studies will hopefully give the answers in the near future. © 1997 Elsevier Science B.V.

Keywords: MK-801 ([+]-5-methyl-10,11-dihydro-5H-dibenzo-[*a*,*d*]-cyclohepten-5,10-imine hydrogen maleate, dizocilpine); M100907(*R*(+)-α(2,3-dimethoxyphenyl)-1-[2(4-fluorophenyl)ethyl)]-4-piperidine-methanol, MDL100,907); Dopamine receptor antagonist; (Mouse); Locomotion; Schizophrenia

1. Introduction

Several recent hypotheses on the pathophysiology of schizophrenia (Kim et al., 1980; Kornhuber and Kornhuber, 1986; Javitt, 1987; Carlsson, 1988) include a suggestion that deficient central glutamatergic transmission is an important factor. In animals, experimental impairment of glutamatergic transmission can be produced by pharmacological intervention at the level of the NMDA receptor. The most potent and selective antagonist known to interact in an un-competitive manner with this receptor complex, is [+]-5-methyl-10,11-dihydro-5H-dibenzo-[*a*,*d*]-cyclohepten-5,10-imine hydrogen maleate (MK-801; dizocilpine; Wong et al., 1986; Javitt and Zukin, 1991). In rodents, MK-801 induces a behavioral syndrome, including hyperlocomotion, head weaving, body rolling, ataxia, reduced rearing behavior and stereotypies (Clineschmidt et al., 1982; Tricklebank et al., 1989; Liljequist et al., 1991), which has been proposed to represent an animal model of certain aspects of schizophrenia (Carlsson and Carlsson, 1990; Tiedtke et al., 1990).

Dopamine D_2 receptor antagonists given systemically inhibit the MK-801-induced hyperlocomotion (Clinesch-

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midt et al., 1982; Hoffman, 1992; Ögren and Goldstein, 1994), and likewise, the dopamine D_1 receptor antagonist SCH 23390 (Iorio et al., 1983) reduces locomotion induced by MK-801 (Ouagazzal et al., 1993). None of these studies reported how the dopamine D_1 or D_2 receptor antagonists affected vehicle-treated (non-MK-801) animals. However, Dall'Olio et al. (1992) described a 50% reduction in MK-801-induced hypermotility, using either SCH 23390 or the dopamine D_2 receptor antagonist YM 09151-2 in doses that left locomotion in saline-treated animals unaffected. Furthermore, we have found that, given in doses that were equally efficient with regard to inhibition of motor activity in saline-treated controls, SCH 23390 had a greater inhibitory effect on MK-801-induced hyperactivity than did the dopamine D_2 receptor antagonist raclopride (Martin et al., 1994).

Since SCH 23390 also has considerable affinity for 5-hydroxytryptamine $(5\text{-HT})_2$ receptors in vitro and in vivo (Christensen et al., 1984; Bischoff et al., 1986; Riddall, 1992), a novel selective dopamine D_1 receptor antagonist (400 fold less active at dopamine D2 receptors), (−)-[4*aR*,10*aR*]-1,2,3,4,4*a*,5,10,10*a*-octahydro-4-(4-chloro-2-methyl-phenyl)-1-methyl-benzo[*g*]quinoxaline-6-ol (SDZ PSD 958; Markstein et al., 1996) was used in the present study. In an attempt to evaluate to what extent the marked effect of SCH 23390 on MK-801-induced hyperactivity can be attributed to antagonism at each of, or an interaction between, these receptors, SDZ PSD 958 and *R*(+)-α(2,3-dimethoxyphenyl)-1-[2(4-fluorophenyl)ethyl)]-4-piperidine-methanol (MDL100,907; M100907), a highly selective 5-HT_{2A} receptor antagonist, were employed. Also, the importance of dopamine D_2 receptors was assessed using (−)-(*S*)-3,5-dichloro-*N*-((1-ethyl-2-pyrrolidinyl) methyl)-6-methoxy-salicylamide tartrate (raclopride; Köhler et al., 1985) as selective tool. Dose–response relationships were evaluated for each drug in MK-801-treated and vehicle-treated animals. Furthermore, to evaluate the interaction between SDZ PSD 958 and M100907, both drugs were administered concomitantly in various doses according to an orthogonal matrix experimental design.

2. Materials and methods

2.1. Animals

Male albino mice ($n = 242$) of the NMRI strain weighing 18–20 g were purchased from BeeKay (Sollentuna, Sweden). The experimental procedures were approved by the Animal Ethics Committee of the University of Göteborg.

2.2. Drugs

MK-801 was obtained from Research Biochemicals International (Natick, MA, USA). M100907 was generously supplied by the Marion Merrell Dow Research Institute (Cincinnati, OH, USA). Raclopride tartrate and SDZ PSD 958 were kindly provided by Professor S. Ahlenius at Astra Läkemedel AB and Dr. R. Markstein, Sandoz Pharma (Basel, Switzerland), respectively. All drugs were dissolved in physiological saline and injected by the intraperitoneal route (i.p.), except for SDZ PSD 958 which was given subcutaneously (s.c.). Injection volumes were 10 ml/kg if not otherwise stated. The control animals were always given appropriate vehicle treatment.

2.3. Locomotor registration

A single end-point, locomotor activity, was measured on one animal at a time, in eight square plexiglass cages, by means of electronic motility meters (Digiscan activity monitor RXYZM(16)TAO, Omnitech Electronics, Columbus, OH, USA).

2.4. Statistics

All statistical analyses were performed on data collected during the first 30 min period, which corresponds to the duration of the habituation phase in non-MK-801-treated animals. However, the time–response curves of all data obtained are shown in all figures except in Fig. 3.

2.4.1. ANOVA and post-hoc test

In Figs. 1 and 2 and 4–6, the locomotor activity values from each of 6 consecutive 5 min time periods were subjected to a two-factor analysis of variance (ANOVA) with repeated measures. Due to the discrepancy in variance between the group treated with the NMDA receptor antagonist and the saline-treated group, the two-factor ANOVA and the post-hoc test (Fischer's protected least significant difference; Fischer's PLSD) were both applied separately on these two groups of animals.

The four different doses (including zero) of dopamine or 5-HT receptor antagonists were considered the independent factor and time the repeated measure. The two-factor ANOVA with repeated measures discloses statistical information concerning (a) the overall effect of increasing doses, (b) the main effect over time, and (c) the interaction between the effect of increasing doses and the effect of time (i.e., evaluating if time–response curves for different doses are not shifted only in parallel, but also differ in shape). Since normal distribution was not prevailing (e.g., S.D. often $> 0.5 * \text{mean}$), the value from each 5 min period was subjected to logarithmic transformation (after addition of 1 cm to each value in order to avoid logarithms of zero). However, very similar results were obtained by statistical evaluation of non-log transformed data. When the ANOVA revealed an overall significant difference between groups, the post-hoc test was applied.

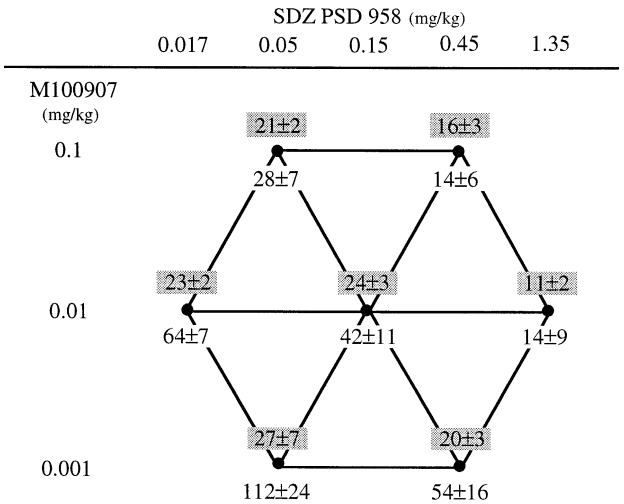
2.4.2. Multiple regression

The orthogonal matrix experimental design (see Table 1) underlying the results presented in Fig. 3a,b, was generated as proposed by Doehlert (1970). The Doehlert matrix is a form of factorial design in which there are $d2 + d + 1$ equally distributed points in space, where d is the number of independent variables in the design. In the present paper, SDZ PSD 958 and M100907 were considered the independent variables and locomotion the dependent variable.

However, the values of the independent variables used for multiple regression calculations were not the actual doses used in the experiments, but rather the scaled and centered logarithm of the doses, equalling the geometrical values obtained from Table 1, considering the legs of each equilateral triangle in the hexagon to be 1.15 units long. Hence, the points (x, z) employed in multiple regression were (0.00, 0.00), (1.15, 0.00), (0.57, 1.0), (−1.15, 0.00), (−0.57, −1.00), (−0.57, 1.00) and (0.57, −1.00). In order to extend the models to include quadratic functions, the variables x^2 and z^2 were added. The fifth independent variable in the multiple regression, $x * z$, or the cross term, can indicate whether there is an interaction (e.g., synergism) at hand between the x and z variables.

The function yielded by multiple regression ($y = \beta_0 + \beta_1 x + \beta_2 z + \beta_{1,1} x^2 + \beta_{2,2} z^2 + \beta_{12} xz$) represents the best fit to the dependent variable input values, i.e., the individual locomotion values (meters/30 min) for each animal ($n = 4$–5/ group; 7 treatment groups) in an experiment. The resulting function is presented as a 3-dimensional (3-D) response surface graph (Fig. 3a,b). Furthermore, the magnitude, confidence interval and significance of the parameters (linear terms, quadratic terms, cross term) obtained, are presented in Table 2a,b.

Table 1
Orthogonal matrix experimental design



Orthogonal matrix experimental design used in Fig. 3a–b, to evaluate the interactive effects of M100907 and SDZ PSD 958 on spontaneous or MK-801-induced locomotion in mice. Shown are the 7 dose combinations used (see Section 2.4.2). The mean and SEM of the results obtained (m/30 min) for each dose combination are presented for vehicle-treated (grey upper boxes) and MK-801 treated (white lower boxes) animals. (However, note that the multiple regression in Fig. 3, Table 2 was calculated using the individual values for each animal.) Values for vehicle-treated control groups were 26 ± 4 (vehicle) and 88 ± 16 (MK-801) m/30 min.

Table 2
Statistical results from multiple regression in Fig. 3a–b

Regressor	Coefficient	C.I. (95%)	P value
(a) Multiple regression in Fig. 3a			
INTERCEPT	23		
x-term (SDZ)	−5.1	(−8.8– −1.4)	0.0085
z-term (M907)	−2.5	(−6.2– +1.2)	0.18
x^2-term (SDZ)	−5.1	(−12– +1.8)	0.14
z^2-term (M907)	−0.60	(−4.8– +3.6)	0.86
$x * z$-term (SDZ * M907)	1.2	(−5.2– +7.6)	0.70
(b) Multiple regression in Fig. 3b			
INTERCEPT	42		
x-term (SDZ)	−25.	(−38– −12)	0.0008
z-term (M907)	−31.	(44– −18)	0.0001
x^2-term (SDZ)	−2.7	(−27– +22)	0.82
z^2-term (M907)	11.	(6.6– +15)	0.36
$x * z$-term (SDZ * M907)	19.	(−3.8– +42)	0.098

The tables display the value of the intercept and, the magnitude, the 95% confidence interval and the statistical significance of the regressors (linear terms, quadratic terms, cross term) in the function obtained from multiple regression in (A) saline-treated animals (Fig. 3a.; 7 treatment groups; $n = 5$/group; ($F(5,29) = 2.59$; $P < 0.047$; $R^2 = 0.31$)) and (B) in MK-801-treated animals (Fig. 3b.; 7 treatment groups; $n = 4$/group; ($F(5,22) = 8.77$; $P < 0.0001$; $R^2 = 0.67$)). M907 = M100907.

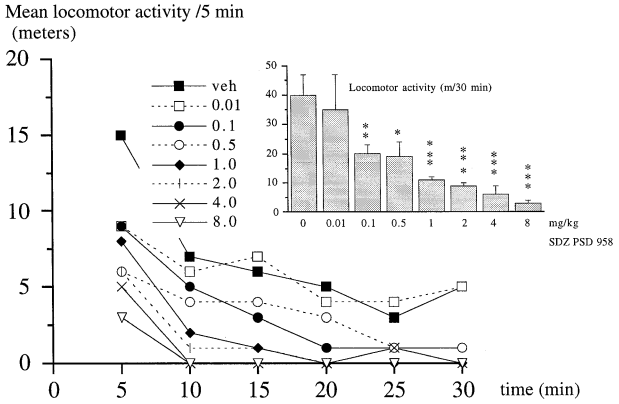


Fig. 1. Effects of SDZ PSD 958 on locomotor activity in drug-naive mice. The animals were placed in the motility meters immediately after drug administration and locomotor activity was recorded for 30 min. Shown are the mean values for each consecutive 5 min measurement period. The insert refers to the mean total meters and the S.E.M. recorded during the period 0–30 min. $N = 3$ (0.5 mg/kg), 4 (0.01, 0.1, 8 and 10 mg/kg), 5 (2 and 4 mg/kg), 9 (1 mg/kg) and 10 (vehicle). The data from two well-corresponding experiments were merged. Statistical significance according to Fischer's PLSD test: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to the saline group. Linear regression ($r = -10.6$; $R^2 = 0.536$; see Section 2.4.3) revealed a statistically significant divergence (Student's t-test, $P < 0.0001$) of the slope of the SDZ PSD 958 dose–response curve from a horizontal line.

2.4.3. Linear regression

The data describing total locomotor activity (0–30 min) in Figs. 4–6 were further analyzed. For each treatment group (saline or MK-801), the slope of the decline in total locomotor activity was fitted by means of linear regression, utilizing data on each individual animal's total locomotor activity, after the saline groups (dose = zero) had been excluded and the dose levels had been subjected to logarithmic transformation. The statistical significance of the difference between the respective slopes in each treatment group, and between each slope and a horizontal straight line, was determined by means of Student's *t*-tests. Formulas used to obtain the standard error for the difference between the means were obtained from a statistical textbook (Altman, 1991). Graphs are not shown; results are presented as '(regression coefficient; R^2-value; *p*-value)'. Corresponding operations were performed on the data in Figs. 1 and 2, respectively, testing each slope against a horizontal straight line.

3. Results

3.1. Effects of SDZ PSD 958 and M100907 on spontaneous locomotion in mice

The dose-dependent effects of SDZ PSD 958 (0.01–8 mg/kg s.c.), are shown in Fig. 1. The two-factor ANOVA revealed an overall statistical significant difference between treatment groups ($F(7,37) = 14.26$; $P < 0.0001$) and a main significant effect of time ($F(5,185) = 77.28$; $P <$ 0.0001). There was also a significant interaction between dose and time ($F(35,185) = 1.802$; $P < 0.0001$). Fischer's PLSD test indicated that animals treated with doses of 0.1 mg/kg, or higher, of SDZ PSD 958 showed a statistically significant decrease in locomotor activity as compared to saline-treated controls. Animals receiving the highest dose (8 mg/kg) displayed approx. 10% of the locomotion in the saline-treated group.

In the case of M100907 (Fig. 2; cf., Fig. 5 for lower doses), the two-factor ANOVA did not detect any main effect of dose ($F(5,25) = 2.32$; $P < 0.073$) but a significant main effect of time ($F(5,125) = 106$; $P < 0.0001$). Unlike for SDZ PSD 958, the curve levelled out at approximately 45% of saline-treated controls. Higher doses than 9 mg/kg were not given, as this was the limit of solubility for M100907, when injected at 10 ml/kg.

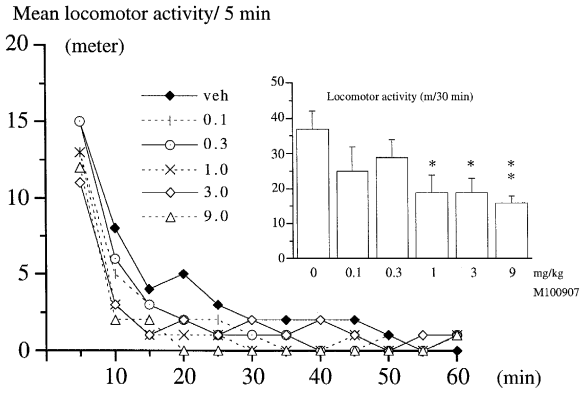


Fig. 2. Effects of M100907 on locomotor activity in drug-naive mice. The animals were placed in the motility meters immediately after drug administration and locomotor activity was recorded for 60 min. $N = 4$ (9 mg/kg), 5 (1 and 3 mg/kg) or 6 (0.1 and 0.3 mg/kg). Shown are the mean values for each consecutive 5 min measurement period. The insert refers to the mean total meters and the S.E.M. recorded during the period 0–30 min. Statistical significance according to Fischer's PLSD test: * $P < 0.05$, ** $P < 0.01$, compared to the saline group. Linear regression ($r = -5.96$; $R^2 = 0.125$; see Section 2.4.3) indicated no statistically significant divergence (Student's *t*-test, $p = 0.076$) of the slope of the M100907 dose–response curve from a horizontal line.

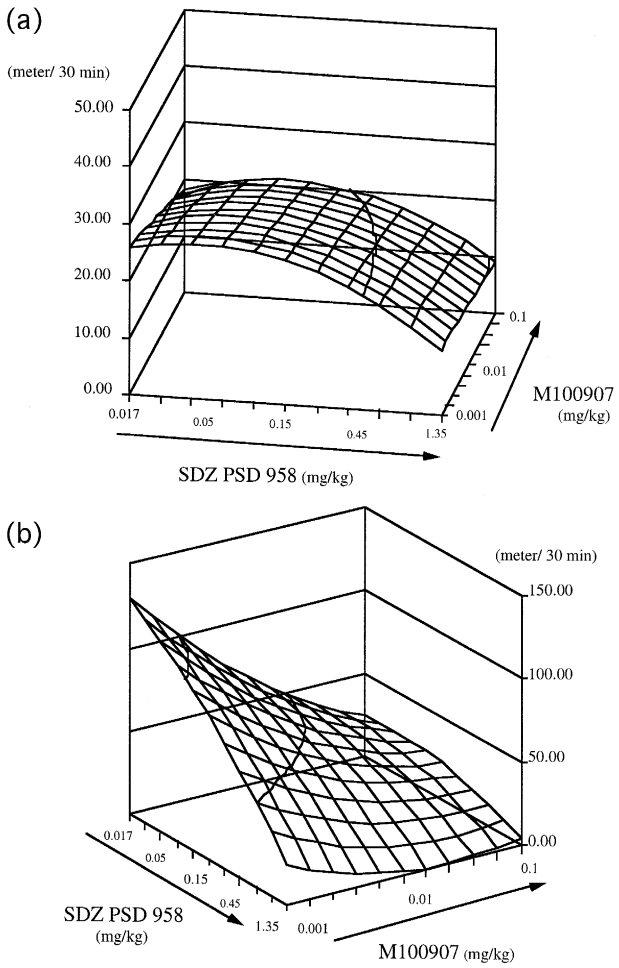


Fig. 3. Concomitant administration of M100907 and SDZ PSD 958 in seven dose combinations according to an orthogonal matrix experimental design (see Table 1). All mice also received an injection of saline (A), or an injection of MK-801 (B; 0.3 mg/kg i.p.), and were immediately placed in the motility meters where locomotor activity was recorded for 30 min. The response surface was obtained by use of multiple regression (see Section 2.4.2); the regression parameters are presented in Table 2.

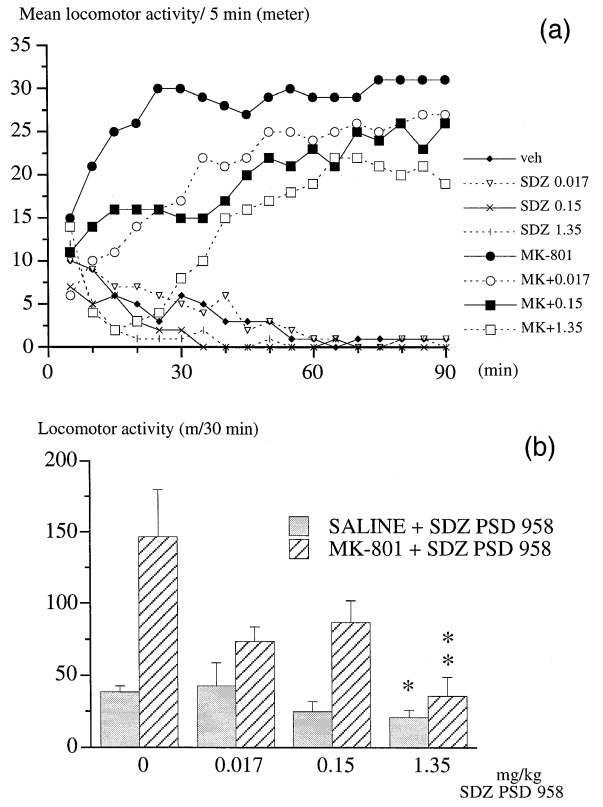


Fig. 4. Effects of SDZ PSD 958 (SDZ) given to mice during concomitant administration of either saline or MK-801 (MK; 0.3 mg/kg). The animals were placed in the motility meters immediately after drug administration and locomotor activity was recorded for 90 min. Shown are (a) the mean values for each consecutive 5 min measurement period and (b) the mean total meters and the S.E.M. recorded during the period 0–30 min. $N = 4$ animals/dose. Statistical significance according to Fischer's PLSD test: ** $P < 0.01$, compared to the MK-801 group. Linear regression (see Section 2.4.3) revealed no statistically significant difference between the slopes of the SDZ PSD 958 dose–response curves in the presence or absence of MK-801.

3.2. Effects of co-administration of SDZ PSD 958 with M100907 in saline- or MK-801-treated mice

The dopamine D_1 receptor antagonist and the 5-HT_{2A} receptor antagonist were administered simultaneously in seven different dose combinations (see Table 1) to saline-treated (Fig. 3a) or MK-801-treated (Fig. 3b) mice. In order to cover a large dose interval of both antagonists, while minimizing the number of treatment groups, an orthogonal matrix design was applied (see Section 2.4.2 for details).

SDZ PSD 958 displayed a statistically significant negative linear term (x-term) — i.e., an inhibitory effect on locomotion both in saline- (Table 2a, Fig. 3a) and in MK-801-treated (Table 2b, Fig. 3b) animals. The magnitude of this negative linear term was approximately five times larger in the MK-801-treated animals. M100907, on the other hand, had no significant effect in saline-treated animals (Table 2a, Fig. 3a). However, in MK-801-treated animals (Table 2b, Fig. 3b), the negative linear term for M100907 (z-term) increased about twelve-fold and was statistically highly significant.

The quadratic terms for the receptor antagonists (x^2 and z^2) were not statistically significant in either of the two experiments. This was true also for the cross-term ($x * z$).

3.3. Effects of either SDZ PSD 958, M100907 or raclopride, in saline- or MK-801-treated mice

The response to MK-801 was similar in Figs. 4–6. The time–response curves for MK-801-treated controls show an initial phase of rapid increase in locomotion, which lasts for approximately 15–20 min, before it levels out at 25–30 m/5 min interval.

No significant ($F(3,12) = 2.683$; $P < 0.094$) main effect of increasing doses of SDZ PSD 958 was found in saline-treated animals (Fig. 4). A highly significant main

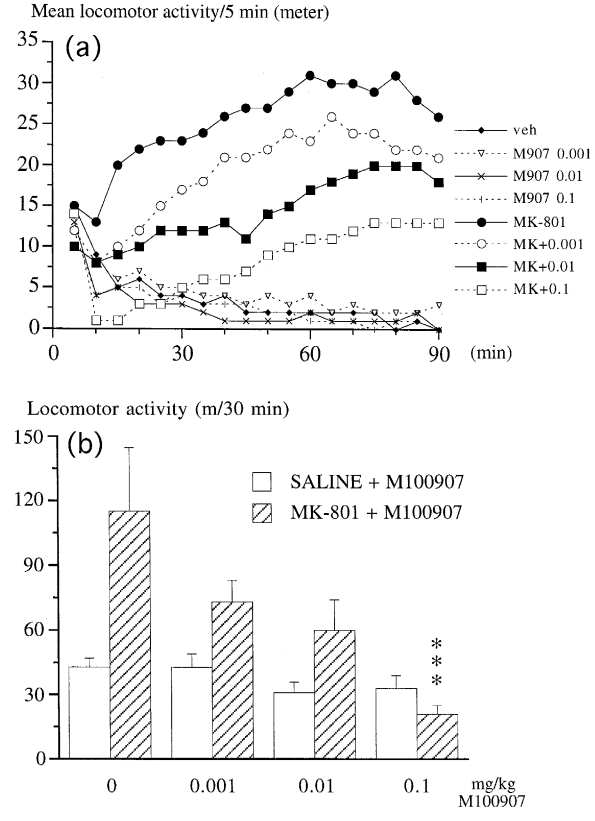


Fig. 5. Effects of M100907 (M907) given to mice during concomitant administration of either saline or MK-801 (MK; 0.3 mg/kg). The animals were placed in the motility meters immediately after drug administration and locomotor activity was recorded for 90 min. Shown are (a) the mean values for each consecutive 5 min measurement period and (b) the mean total meters and the S.E.M. recorded during the period 0–30 min. $N = 4$ animals/dose. Statistical significance according to Fischer's PLSD test: *** $P < 0.001$, compared to the saline group. Linear regression (see Section 2.4.3) revealed a statistically significant difference (Student's t-test, $P < 0.02$) between the slopes of the M100907 dose–response curves in the presence or absence of MK-801.

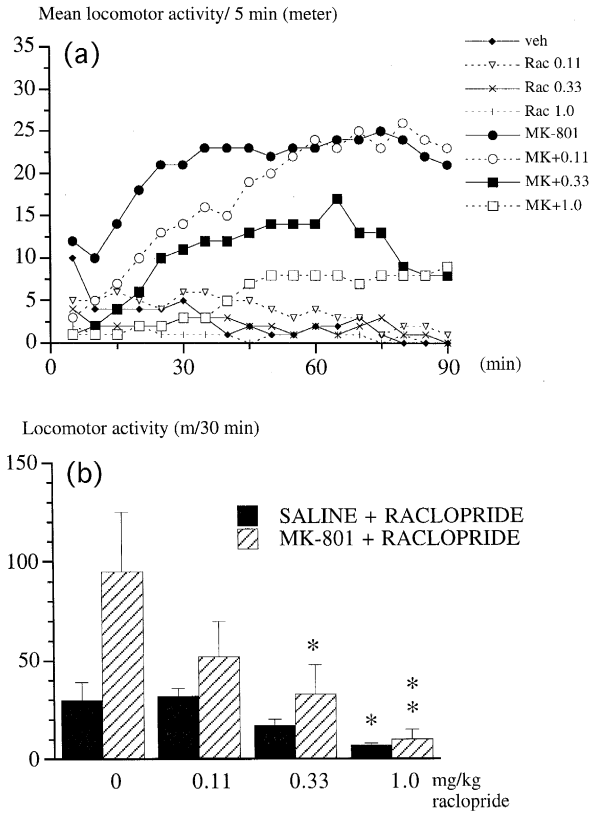


Fig. 6. Effects of raclopride (Rac) given to mice during concomitant administration of either saline or MK-801 (MK; 0.3 mg/kg). The animals were placed in the motility meters immediately after drug administration and locomotor activity was recorded for 90 min. Shown are (a) the mean values for each consecutive 5 min measurement period and (b) the mean total meters and the S.E.M. recorded during the period 0–30 min. $N = 4$ animals/dose. Statistical significance according to Fischer's PLSD test: $^{*}P < 0.05$ and $^{**}P < 0.01$, compared to the saline or MK-801 group. Linear regression (see Section 2.4.3) revealed no statistically significant difference between the slopes of the raclopride dose–response curves in the presence or absence of MK-801.

effect of time was detected ($F(5,60) = 6.28$; $P < 0.0001$). When given to MK-801-treated animals, SDZ PSD 958 produced significant main effects of both dose ($F(3,12) = 6.74$; $P < 0.0065$) and time ($F(5,60) = 4.53$; $P < 0.0014$). Furthermore, a significant interaction between dose and time ($F(15,60) = 6.88$; $P < 0.0001$) was at hand in MK-801-treated animals. Fischer's PLSD test revealed that the highest dose of SDZ PSD 958 had a significant effect in MK-801 treated mice. The slopes of the decline in locomotor activity for the two treatment groups (saline and MK-801), as calculated by linear regression, were not significantly different, either from a horizontal line, or from each other.

Fig. 5 shows the effects of the 5-HT_{2A} receptor antagonist M100907, in saline- or MK-801-treated animals. Whilst only a main effect of time ($F(5,60) = 9.25$; $P < 0.0001$) was detectable in the saline-treated animals, highly significant main effects of both dose ($F(3,12) = 13.19$; $P < 0.0004$) and time ($F(5,60) = 12.81$; $P < 0.0001$) were produced in MK-801-treated animals. A significant interaction between dose and time ($F(15,60) = 2.60$; $P < 0.0045$), was present in the latter group. Fischer's PLSD test revealed that the group of MK-801-treated animals receiving the highest dose of M100907, significantly differed from MK-801-treated controls. Furthermore, linear regression pointed out a difference ($t = 3.11$, $P < 0.01$) between saline- and MK-801-treated animals, regarding the slopes of the decline in locomotion following increasing doses of M100907. The slope produced by M100907 in the MK-801-treated animals also differed from a horizontal line ($r = -26.2$; $R^2 = 0.56$; $p = 0.0051$).

Raclopride (Fig. 6) exhibited a main effect of dose both in the presence ($F(3,12) = 4.39$; $P = 0.026$) and absence ($F(3,12) = 4.79$; $P = 0.020$) of the NMDA receptor antagonist. A main effect of time ($F(5,60) = 5.64$; $P < 0.0003$) was seen in MK-801-treated animals. The dopamine receptor antagonist decreased locomotion significantly in both the saline and MK-801 treated groups, according to Fischer's PLSD test. Likewise, linear regression showed a significant divergence from a horizontal line in both saline- ($r = -25.9$; $R^2 = 0.77$; $p = 0.0002$) and MK-801- ($r = -43.8$; $R^2 = 0.33$; $p = 0.050$) treated animals, but no significant difference between the two slopes was found.

4. Discussion

4.1. Effects of M100907

The main finding of this study was that low doses of the selective 5-HT_{2A} receptor antagonist M100907 were highly effective in counteracting the hyperlocomotion elicited by the un-competitive NMDA receptor antagonist MK-801. A dose of 0.1 mg/kg M100907 totally abolished the MK-801-induced hyperlocomotion, while it had no significant effect on locomotion in saline-treated mice (Fig. 5b). No apparent increase in the ataxia present in MK-801-treated mice was seen after addition of M100907.

The pronounced inhibition of MK-801-induced hyperlocomotion is probably caused by antagonism at 5-HT_{2A} receptors, since M100907 displays a high selectivity for this receptor subtype (Dudley et al., 1990; Schmidt et al., 1995; Kehne et al., 1996). Furthermore, the effect was seen at low doses, i.e., doses that did not significantly affect the locomotion of normal mice. This marked preference of M100907 for reduction of MK-801-induced locomotion as compared to spontaneous locomotion was not observed for the dopamine D_1 or the dopamine D_2 receptor antagonist.

In normal mice M100907 reduced spontaneous activity, but only at doses producing an ex vivo occupancy exceeding 95% at 5-HT_{2A}-receptors (approximately > 1 mg/kg in rat; Dr. S. Sorensen, personal communication). The maximum reduction was approximately 55%. This may be of clinical importance. It would suggest, even in substan-

tial overdosage, a low liability to side effects regarding psychomotor functions, including extrapyramidal symptoms (EPS) and sedation (cf. Kehne et al., 1996). Thus, M100907 may offer advantages over both classical neuroleptics, which produces EPS, and clozapine which causes marked sedation. It also suggests that intact 5-HT_{2A} receptor tone is not a prerequisite for intact psychomotor function in normal mice.

Data describing effects of 5-HT_2 receptor antagonists on NMDA receptor antagonist induced perturbations in vivo are sparse in the literature. However, in contrast to the present findings, it was stated that M100907 was incapable of reversing MK-801-induced hyperlocomotion in rats, while phencyclidine induced behaviors were antagonized with high efficacy by low doses of this 5-HT_{2A} receptor antagonist (Maurel Remy et al., 1995). At the moment we have no explanation for this discrepancy; in our laboratory, MK-801-induced hyperlocomotion in rats is indeed diminished by M100907 (Waters et al., 1995).

Published reports on the effect of ritanserin, a 5-$HT_{2A/2C}$ receptor antagonist (Leysen et al., 1985; Hoyer et al., 1994) on MK-801-induced behaviors are generally negative. Löscher and Hönack (1992) described a lack of effect of ritanserin (5 mg/kg) dissolved in 50% ethanol on MK-801-induced hyperlocomotion in female rats. However, the interpretability of these data is limited since the vehicle itself reduced hyperlocomotion by 30%. Furthermore, Hiramatsu et al. (1989) reported on unpublished observations revealing no effect of ritanserin on MK-801-induced stereotypies. In contrast, Carlsson (1995) showed a significant reduction of MK-801 hyperlocomotion in mice by 10 mg/kg ritanserin.

Immunocytochemical (Morilak et al., 1994) and lesioning (Francis et al., 1992) studies suggest that the localization of 5-HT_{2A} receptors in various parts of the cortex is on GABAergic interneurons. This is in line with electrophysiological studies (Marek and Aghajanian, 1994) showing that stimulation of 5-HT_{2A} receptors can mediate excitation of cortical GABAergic interneurons, which could result in an inhibition of glutamatergic pyramidal cells. Thus, in schizophrenics, M100907 would perhaps via inhibition of cortical GABAergic interneurons, be able to strengthen the hypothetically hypofunctioning glutamatergic neuronal pathways. Interestingly, a decrease in 5-HT_2-receptor density in prefrontal cortex of schizophrenics has been reported (Laruelle et al., 1993). If this is regarded as a compensatory (feedback-loop mediated?) down-regulation of 5-HT_2 receptors, aiming to counteract a primary defect in glutamatergic neurons, M100907 would perhaps be able to further strengthen this mechanism and thus exert a beneficial effect.

M100907 is presently evaluated in clinical trials as a potentially 'atypical' treatment for schizophrenia. Obviously, the results from these trials will be of considerable value for the validation of the present animal models for this disease, including the MK-801 model used here.

4.2. Effects of SDZ PSD 958

The dopamine D_1 receptor antagonist effectively decreased both spontaneous and MK-801-induced locomotion (Figs. 1 and 4). A certain preference for reduction of MK-801-induced behavior seems to be present in Fig. 4b; the failure of linear regression to detect such a selectivity could be due to the non-linear dose–response, or due to the fact that the zero-dose was not included in these calculations (see Section 2.4.3). The present findings with SDZ PSD 958 largely confirms earlier results where SCH 23390 has been shown to counteract MK-801 induced hyperlocomotion (see Section 1).

4.3. Effects of raclopride

It is well known that dopamine D_2 receptor antagonists counteract MK-801-induced locomotion (see Section 1) and this was confirmed in this study. Raclopride dose-dependently and very effectively counteracted the MK-801 hyperlocomotion, as well as spontaneous locomotion. Similar results have been reported from experiments in rats (Svensson et al., 1995) and in mice (Martin et al., 1994), where raclopride even displayed a preferential inhibition of spontaneous locomotor activity as compared to MK-801-induced hyperlocomotion.

4.4. Lack of synergism between 5-HT_{2A} receptor antagonism and dopamine D_1 receptor antagonism

The interactions between dopamine and 5-HT that can be demonstrated are generally very complex, due to the vast amount of receptor subtypes involved. Furthermore, the interplay between 5-HT_2 and dopamine receptors is less well characterized than that between dopamine and 5-HT_1 receptors (see Jackson and Westlind Danielsson, 1994), partly because of a long-lasting lack of selective receptor agonists/antagonists. Lately, increasing attention has been paid to the relation between these receptors since antagonism on 5-HT_2 receptors is thought to contribute to the 'atypical' profile of clozapine (Meltzer, 1994).

In Fig. 3 an orthogonal matrix experimental design was used (see Section 2.4.2.) in order to investigate if there is an interaction between M100907 and SDZ PSD 958 on spontaneous or MK-801-induced locomotion. This experimental design enables investigators to resolve mutual influence of (in this case) two drugs on each others' effects, in rather large dose intervals of both drugs, using a minimal number of animals. An orthogonal matrix design involves less bias as compared to experiments where interactions between drugs are sought for by changing one variable at a time.

Fig. 3a shows that SDZ PSD 958 exerts a small but significant effect. M100907 did not affect spontaneous locomotion significantly. Furthermore, there was no sign of synergism between the two receptor antagonists in

Table 3
Summary of results

	Multiple regression		ANOVA		Linear regression			
	SAL	MK	SAL	MK	SAL [d]	SAL [e]	MK	MK − SAL [f]
SDZ PSD 958	b		n.s.			c	n.s	
		b		b			n.s.	n.s.
M100907	n.s.		n.s.		n.s.	n.s		
		c		c			c	b
Raclopride	—		a		—	c		
		—		a			a	n.s.

Taken together, multiple regression, ANOVA and linear regression show that M100907 compared to the other drugs, displays a marked preference for reduction of MK-801-induced as compared to spontaneous locomotion.
n.s. = no statistically significant difference detected.
[a] $p < 0.05$.
[b] $p < 0.01$.
[c] $p < 0.001$
[d] Results presented in Figs. 1 and 2.
[e] Results presented in Figs. 4–6.
[f] Difference between the slopes obtained (Figs. 4–6) by linear regression in MK-801-induced and spontaneous locomotion.

normal mice (i.e., the cross-term, $x * z$, was not significant). When a corresponding experiment was performed in animals treated with MK-801 (Fig. 3b), the negative influence on locomotion by the receptor antagonists was increased substantially, especially for the 5-HT_{2A} receptor antagonist. In addition, the cross-term (which is a parameter describing the interaction) almost reached statistical significance, but surprisingly this regressor had a positive sign, which would imply an antagonistic interaction between the receptor antagonists (each drug would diminish the effect of the other). The presence of a true antagonistic interaction seems unlikely; an alternative explanation is that the positive cross-term obtained is a result of a purely experimental constraint inherent in the design used. When the values of locomotion in the higher doses of the receptor antagonists are approaching zero meters, the steep slope of the response surface will be forced to flatten out, which will induce a 'twist' in the surface that can only be described mathematically by an increased positive cross-term. This implies that in order to make possible the detection of a potential synergism between the dopamine D_1 and 5-HT_{2A} receptor antagonists, on MK-801 hyperlocomotion with the present experimental design, lower doses of both of the drugs would have been desired. Another way around this problem, which is presently being evaluated in our laboratory, is to fit sigmoid, rather than linear and squared mathematical functions, to the original data.

However, both the interaction surfaces and the dose–response curves show strong effects of both M100907 and SDZ PSD 958 on MK-801-induced hyperlocomotion; thus the selective effect of SCH 23390 on MK-801-induced hyperlocomotion (see Section 1) might be due to a combined antagonism at 5-HT_{2A} and dopamine D_1 receptors, even in the absence of synergism.

In conclusion, this study has shown that the 5-HT_{2A} receptor antagonist M100907 displays a high degree of selectivity, with regard to inhibition of MK-801-induced, as opposed to spontaneous, locomotion (Table 3). Such a selectivity could not be detected for the dopamine D_2 receptor antagonist raclopride or the dopamine D_1 receptor antagonist SDZ PSD 958, although a slight preference towards inhibition of MK-801-induced hyperlocomotion could not be ruled out for the dopamine D_1 antagonist (Table 3). Contrasting the marked effects of SDZ PSD 958 and raclopride on spontaneous locomotion, M100907 was not capable of reducing locomotor activity beyond 45% of control levels. Hence, intact 5-HT_{2A}-receptor tone is not a prerequisite for spontaneous locomotion. No synergism between 5-HT_{2A} and dopamine D_1 or D_2 receptor blockade was detected, either in saline-treated or MK-801-treated animals.

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