Rewarding Properties of Methylphenidate: Sensitization by Prior Exposure to the Drug and Effects of Dopamine D1- and D2-Receptor Antagonists

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Received December 18, 2000; accepted April 30, 2001 This paper is available online at http://jpet.aspetjournals.org

ABSTRACT
In drug addiction, a sensitization phenomenon has been postulated to play a critical role. The aim of our study was to evaluate whether sensitization occurs to the rewarding properties of methylphenidate, a psychostimulant drug known to possess abuse potential, as assessed with the biased conditioned place preference method in rats. In addition, since the brain dopaminergic system is considered to be important in drug-reward, the involvement of dopamine D1- and D2-receptors both in the rewarding properties of methylphenidate and in sensitization to these properties was assessed. Conditioning with methylphenidate at doses of 1.25 to 20 mg/kg increased preference for the paired environment, whereas a dose of 0.31 mg/kg was ineffective. However, following the 7-day sensitization treatment with methylphenidate (0.62–20 mg/kg), conditioning with a dose of 0.31 mg/kg resulted in an increased preference for the paired environment, i.e., the rewarding properties of methylphenidate appeared to be sensitized. Control experiments indicated that the enhancement of preference was not due to attenuation of sensitization treatment-induced withdrawal nor to tolerance to aversive properties of methylphenidate. When conditioned with methylphenidate, D1-antagonist SCH 23390 but not D2-antagonist raclopride prevented place preference. However, when coadministered with methylphenidate during the sensitization treatment, both SCH 23390 and raclopride prevented the development of sensitization. These data indicate that the rewarding properties of methylphenidate are sensitized by prior exposure to the drug and that both D1- and D2-receptors, the latter of which possibly more specifically, appear to be involved in the development of this sensitization.

Methylphenidate (MP) is a psychostimulant drug widely used for the treatment of attention deficit hyperactivity disorder and narcolepsy. In controlled use for the treatment of attention deficit hyperactivity disorder among children and adolescents, the abuse of MP is considered to be rare, but its illicit use has been recognized by several reports indicating that MP possesses abuse potential (Rappley, 1997).

In the brain, MP increases the extracellular levels of dopamine and norepinephrine in a manner similar to cocaine and amphetamine. The mechanism of action is thought to include dopamine reuptake inhibition, yet other mechanisms may also be involved (Nomikos et al., 1990). However, unlike the two other psychostimulants, MP does not affect the serotonergic system (Koe, 1976; Segal and Kuczenski, 1999), and hence it has been used as a tool in animal experiments for characterizing dopamine-behavior relationship without a serotonin effect (e.g., Segal and Kuczenski, 1999). MP’s behavioral profile in rats, including induction of locomotor activity and stereotypes, resembles those of cocaine and amphetamine, and in behavioral tests assessing abuse potential MP has been shown to possess rewarding properties (Martin-Iverson et al., 1985; Mithani et al., 1986).

The repeated use of a psychostimulant may result in an augmented behavioral response to the subsequent administration of the drug, frequently referred to as sensitization. Determination of whether sensitization develops to the rewarding properties of drugs is critical in view of the postulated role of sensitization in the development of drug-craving and compulsive drug-seeking behavior (Robinson and Berridge, 1993). The repeated administration of cocaine and amphetamine has been shown to sensitize their rewarding properties (Lett, 1989; Shippenberg and Heidbreder, 1995; Le Pen et al., 1998; Pierre and Vezina, 1998), as well as locomotor activity (Kuribara and Uchihashi, 1993; Mattingly et al., 1994). Regarding MP, however, whether sensitization occurs to its rewarding properties has not been elucidated. When sensitization to its locomotor-stimulating effects is assessed, somewhat inconsistent results have been obtained. In some studies, locomotor activity has been reported to be

ABBREVIATIONS: MP, methylphenidate; RAC, raclopride; SAL, saline; SCH, SCH 23390; ANOVA, analysis of variance; ANCOVA, analysis of covariance.
sensitized (Shuster et al., 1982; Gaytan et al., 1997; McDougall et al., 1999), whereas in other studies no such effect was found (McNamara et al., 1993; Izenwasser et al., 1999). In fact, some authors have suggested that this lack of sensitization could be attributed to the inability of MP to affect the serotonergic system (Izenwasser et al., 1999).

The brain dopaminergic system is considered to be involved in mediating the rewarding properties of psycho-stimulants. In addition to the rewarding properties, there is evidence to suggest the involvement of at least D1-receptors in the development of sensitization to these properties. For example, the facilitation of amphetamine self-administration by prior treatment has been prevented by the D1-antagonist SCH 23390 (SCH; Pierre and Vezina, 1998). Furthermore, SCH prevented the development of sensitization to cocaine-induced place preference, whereas the D2-antagonist raclopride (RAC) was ineffective (Shippenberg and Heidbreder, 1995). Regardless of the latter finding, the conceivable involvement of D2-receptors in development of sensitization to rewarding properties of other psychostimulants should not be ignored, since D2-antagonists have prevented development of sensitization to the locomotor-stimulating effects of at least amphetamine and methamphetamine (Kuribara and Uchihashi, 1993; Meng et al., 1998). With regard to MP in particular, the role of dopaminergic mechanisms both in its rewarding properties and in the development of sensitization to these properties is unclear. Although the dopaminergic system is apparently involved in several behavioral responses to MP, various dopaminergic manipulations have failed to affect its rewarding properties. For example, MP can induce place preference in rats with 6-hydroxydopamine-induced dopaminergic lesions, as well as in dopamine-transporter knockout mice (Martin-Iverson et al., 1985; Sora et al., 1998). Concerning dopamine receptors, only the involvement of D2-receptors has been assessed; however, the D2-antagonist haloperidol did not affect MP-induced place preference, except at a dose of 1 mg/kg, which was considered to be nonspecifically high (Martin-Iverson et al., 1985; Mithani et al., 1986).

In the present study, the aims were to evaluate whether sensitization occurs to the rewarding properties of MP as assessed in the conditioned place preference method and to evaluate the roles of D1- and D2-receptors, using the respective antagonists SCH and RAC, both in the rewarding properties of MP and in the development of sensitization to these properties.

**Materials and Methods**

**Animals**

Adult male Han:Wistar rats weighing 200 to 300 g were used in the study. The rats were obtained from Harlan Nederland B.V. (Horst, The Netherlands), at least 1 week before the experiments, and they were housed two per Macrolon III-type cage (18 × 33 × 15 cm) in a temperature-controlled room (22 ± 2°C) with a light cycle of 12 h. The lights were on from 8:00 AM to 8:00 PM, during which time all experiments were conducted. The animals had free access to standard laboratory chow and tap water, except in the conditioned taste aversion test (see **Conditioned Taste Aversion**). The animal experiments were approved by the local institutional animals care and use committee and the chief veterinarian of the county administrative board, and they were conducted according to the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes".

**Drugs**

MP hydrochloride (donated by Novartis Pharma AG, Basel, Switzerland), SCH hydrochloride (D 654, Sigma/RBI, Natick, MA), RAC l-tartrate (R 121, Sigma/RBI), yohimbine hydrochloride (Y 100, Sigma/RBI), and lithium hydrochloride (L 0505, Sigma, St. Louis, MO) were dissolved in saline (SAL; 0.9% NaCl). All the drugs were administered intraperitoneally at a volume of 1 ml/kg, except that RAC was given subcutaneously and in the conditioned taste aversion test MP, lithium, and SAL were given at a volume of 8 ml/kg. Drug doses were calculated as free base.

**Conditioned Place Preference**

**Apparatus.** The conditioned place preference test was conducted in eight identical rectangular boxes (60 × 30 × 45 cm) made of polyvinylchloride. Each box was divided into two compartments of equal size by a separating wall with a guillotine door (8 × 6 cm). Both compartments were covered with loose-fitting transparent plastic lids. One compartment was black with a smooth floor and small drops of 10% acetic acid added in both back corners, and the other was white with wire mesh on the floor and no acetic acid added. Thus, the compartments differed in three modalities: visual, tactile, and olfactory. The boxes were placed in a quiet dim room with white noise present for masking external sounds.

**The Rewarding Properties of MP as Assessed by the Conditioned Place Preference Method.** The dose-response profile of MP in the place preference test was first assessed, and the highest dose that was clearly ineffective was chosen as a reference dose for the sensitization experiment. The biased place preference procedure used consisted of three phases:

1. **Preconditioning Phase (Days 1–3):** The guillotine door of the box was open, and equal numbers of rats were placed in the black or white compartment and allowed to explore both compartments freely for 15 min (900 s). On the 3rd day, the times spent in both compartments were measured with a stopwatch. According to this preconditioning time, the rats were assigned to treatment groups with the less preferred compartment serving as the drug-paired compartment. If the preconditioning time for a rat was less than 200 s in either compartment, the rat was excluded from further testing. Further testing was discontinued in approximately 15% of the rats, following this criterion. In each treatment group, approximately half of the rats were assigned to the black compartment as the drug-paired compartment, while the other half were assigned to the white compartment as the drug-paired compartment (± 1 rat when n ≤ 10; ± 2 rats when n > 10).

2. **Conditioning Phase (Days 4–6):** During the conditioning phase, the rats experienced two conditioning sessions each day. In the first session, the rats received a SAL injection and were immediately confined to the SAL-paired compartment opposite the drug-paired compartment for 40 min. After an interval of at least 90 min, the second session of the day was begun; the rats received MP at different doses (0.31, 0.62, 1.25, 5, or 20 mg/kg) or SAL and were immediately confined to the drug-paired compartment for 40 min. The procedure of two conditioning sessions per day with this order of conditioning has been used previously to obtain results comparable with those obtained with more standard procedures (Calcagnetti and Schechter, 1992; Haile et al., 2001). During this phase the guillotine door was closed.

3. **Postconditioning Phase (Day 7):** The guillotine door was opened, the rats placed in the drug-paired compartment, and the times they spent in the compartments measured for 15 min. The shifts in preference for the drug-paired compartment induced by conditioning (postconditioning time – preconditioning time) served as a measure of reward.
Sensitization to the Rewarding Properties of MP. In the sensitization experiment the reference dose, which was the highest ineffective dose in the place preference test, was tested after a 7-day sensitization treatment with daily injections of MP at different doses to determine whether it would now induce place preference, i.e., whether prior exposure would sensitize the rewarding properties of MP. During the sensitization treatment, the rats received MP (0.31, 0.62, 1.25, 5, or 20 mg/kg) or SAL in their home cages once daily for 7 consecutive days. On the final day at least 3 h before the last sensitization injection, the place preference procedure as described above was commenced; the rats were allowed to freely explore the compartments of the box for 15 min. On 2 subsequent days (days 2–3 of the place preference test), the preconditioning phase was continued with no injections and on the last day the preconditioning time of the rats was measured. During the conditioning phase (days 4–6), the rats received SAL in the SAL-paired compartment and MP (the reference dose of 0.31 mg/kg, chosen on the basis of the results in the MP-induced place preference experiments) or SAL in the drug-paired compartment. Thus, the interval between the sensitization and conditioning phases was 3 days, as there is evidence that, at least with cocaine injected once daily for 5 days, this may yield the greatest sensitization (Shippenberg and Heidbreder, 1995). Finally, on the last day (day 7) the postconditioning times were measured for 15 min.

Effects of D1- and D2-Receptor Blockade on the Rewarding Properties of MP. The experiments were conducted as described above with a modified injection schedule. During the conditioning phase, in the first session of the day, the rats were given two injections of SAL at an appropriate interval, and after the second injection they were immediately confined to the SAL-paired compartment. In the second session, the rats were injected first with either SCH (0.05, 0.1, or 0.2 mg/kg), RAC (0.2, 0.4, or 0.8 mg/kg), or SAL. After an appropriate interval, the rats were given a second injection of MP (5 mg/kg) or SAL, followed immediately by confinement in the drug-paired compartment. The intervals were 40 min for the SCH-treated groups and 10 min for the RAC-treated groups. According to previous studies, the doses of SCH and RAC with the intervals used should be behaviorally relevant (Agmo et al., 1993; Mattingly et al., 1994; Hoffman and Donovan, 1995; Millan et al., 1998; Pierre and Vezina, 1998). Particularly, the possible affinity of SCH for serotonin-2-receptors appears to be insignificant at these doses (Bischoff et al., 1986). The dose of MP was chosen, based on results in the present and previous studies (Martin-Iverson et al., 1985; Mithani et al., 1986). When the effect of SCH and RAC alone was studied, the antagonists were assigned to the preferred compartment as the drug-paired compartment, since there is evidence that SCH may possess some aversive properties (Tzschentke, 1998). Furthermore, in separate experiments the effects of two higher doses of SCH (0.1 and 0.2 mg/kg) and RAC (0.4 and 0.8 mg/kg) alone were also associated with the less preferred compartment.

Effects of D1- and D2-Receptor Blockade on Sensitization to the Rewarding Properties of MP. During the sensitization treatment, the rats were injected with SCH (0.1 or 0.2 mg/kg), RAC (0.2 or 0.4 mg/kg), or SAL. After an interval of 40 min for the SCH-treated groups and 10 min for the RAC-treated groups, MP (5 mg/kg) or SAL was administered. Otherwise, the experiments were conducted as described under Sensitization to the Rewarding Properties of MP.

Assessment of Withdrawal Symptoms

It is possible that place preference induced by MP following the 7-day sensitization treatment would reflect the alleviation of sensitization treatment-induced withdrawal symptoms rather than a true sensitization phenomenon. Therefore, to assess the magnitude of withdrawal symptoms, the rats were first injected with MP (5 or 20 mg/kg) or SAL once per day for 7 consecutive days, similar to the 7-day sensitization treatment in the place preference experiments, after which weight change (Planeta et al., 1994), food consumption (Krauchi et al., 1984), anxiety-like behavior (Costall et al., 1989), and changes in motor activity (Pulvirenti and Koob, 1993) were measured.

Changes in Weight and Food Consumption. Weight change and food consumption were measured daily for 3 days after the last injection. Daily weight change was calculated as the difference from the weight measured on the preceding day. Food consumption was assessed as the amount of food consumed per two rats, since the rats were housed in pairs, both of which always received the same treatment.

Anxiety-Like Behavior. On the 3rd day after the last injection (corresponding to the beginning of the conditioning phase in the place preference experiments), anxiety-like behavior was measured by using the black-white box test modified from Timothy et al. (1999). The boxes used in the black-white box test were similar to those used in the place preference experiments, except that neither wire mesh was placed on the floor nor was acetic acid added. Instead, the black compartment was illuminated with a 20-W red bulb and the white compartment with a 10-W white bulb covered with a thin sheet of paper; both bulbs were placed approximately 25 cm above the box with a dark cardboard sheet between them, preventing light from illuminating the opposite compartment. Thus, the compartments differed only in the magnitude of luminousness. Each rat was tested once by placing it gently in the middle of the black compartment, facing away from the doorway. The following parameters were measured with a stopwatch for 8 min (480 s): time to enter the white compartment for the first time, total time spent in the white compartment, activity both in the black and white compartments (number of crosses over midline), and number of crosses between the black and white compartments. Prior to conducting the experiments with withdrawal-experiencing rats, the validity of the black-white box test used here was tested in a separate experiment by administering the known anxiogenic drug yohimbine (2.5 mg/kg) or SAL to rats 30 min before placing in the test box.

Motor Activity. After the black-white box test, the motor activity of the rat was measured for 40 min in a test cage (transparent Macrolon III-type cage) by means of a computer-controlled photo-beam activity system (San Diego Instruments, San Diego, CA). Both ambulatory and rearing activities were recorded as interruptions of photo-beams at heights of 5 and 12.5 cm from the cage floor, respectively.

Conditioned Taste Aversion

Instead of sensitization to the rewarding properties, it is also possible that after the sensitization treatment MP would induce place preference due to sensitization treatment-induced tolerance to the aversive properties of the reference dose (0.31 mg/kg) used in the place preference experiments. That is, without the sensitization treatment, conditioning with MP at the reference dose would not induce place preference because the rewarding and aversive properties have canceled each other out. Instead, following the sensitization treatment, the reference dose would induce place preference due to the development of tolerance to its aversive properties. Therefore, the aversive properties of MP at this dose were assessed in a conditioned taste aversion test (Nachman and Ashe, 1973). If the reference dose per se did not possess aversive properties, enhancement of place preference would probably not result from tolerance to, i.e., attenuation of, the aversive properties of the dose.

The rats were housed in pairs with laboratory chow freely available. During the experiment, daily drinking was restricted to two sessions: 1) Habituation/pairing/test session (15 min, from 8:15 AM to 8:30 AM): the rats were placed singly in separate Macrolon III-type cages; both bulbs were placed approximately 25 cm above the box with a dark cardboard sheet between them, preventing light from illuminating the opposite compartment. Thus, the compartments differed only in the magnitude of luminousness. Each rat was tested once by placing it gently in the middle of the black compartment, facing away from the doorway. The following parameters were measured with a stopwatch for 8 min (480 s): time to enter the white compartment for the first time, total time spent in the white compartment, activity both in the black and white compartments (number of crosses over midline), and number of crosses between the black and white compartments. Prior to conducting the experiments with withdrawal-experiencing rats, the validity of the black-white box test used here was tested in a separate experiment by administering the known anxiogenic drug yohimbine (2.5 mg/kg) or SAL to rats 30 min before placing in the test box.

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rats were allowed to drink water freely in their home cages with no injections or fluid measurements. Prior to beginning the experiment, the bottles were removed from the home cages at 6:00 PM the previous evening. During the preconditioning phase (days 1-3) in the habituation sessions, the bottle was filled with water. During this phase, no injections were given. The rats were assigned to treatment groups according to water consumption in the habituation session of the 3rd day. On the subsequent day, the 1st day of the conditioning phase (days 4-6) was begun; in the pairing session the bottle was filled with saccharin solution, and after the session the rat was injected with MP (0.31 mg/kg, the reference dose used in the place preference experiments), a known aversive drug lithium (8.2 mg/kg corresponding to approximately 51 mg/kg or 0.15 M of lithium chloride; Nachman and Ashe, 1973), or SAL. This saccharin-drug pairing was repeated for the next 2 days. During the following postconditioning phase (day 7) in the test session, two bottles, 1 filled with water and the other with saccharin solution, were placed in the test cages, and fluid consumption in both bottles was separately measured. The saccharin preference ratio in the test session was calculated with the following equation: saccharin solution consumption/ (saccharin solution consumption + water consumption) and was taken as a measure of taste aversion.

Statistical Analysis

In the place preference experiments, the shift in preference for the drug-paired compartment induced by conditioning served as a measure of reward. Statistical analysis was performed using one-way ANCOVA (dependent factor: shift in preference; independent factor: drug doses; covariant: preconditioning time). In the assessment of withdrawal symptoms, weight change, food consumption, and motor activities were analyzed using ANOVA for repeated measures (dependent factor: weight change, food consumption, or motor activity counts; independent factor: drug doses; repeated measure: time), while the parameters of anxiety-like behavior in the black-white box test were assessed with one-way ANOVA (dependent factor: time to enter the white compartment for the first time, total time in the white compartment, line crossings in the white compartment, line crossings in the black compartment, or crossings between the compartments; independent factor: drug doses). In the conditioned taste aversion test, the saccharin preference ratio in the test session was taken as a measure of taste aversion, which was analyzed using the two-sample t-test. Elsewhere, paired and two-sample t tests were used as appropriate. Post hoc comparisons were made using Bonferroni’s test adjusted for appropriate number of comparisons. All data are expressed as mean ± S.E. unless otherwise stated.

Results

The Rewarding Properties of MP as Assessed by the Conditioned Place Preference Method

As shown in Fig. 1, MP increased preference for the drug-paired compartment dose dependently. There was a statistically significant difference between the conditioning doses [F(5,43) = 7.292, p < 0.001, one-way ANCOVA (p = 0.093 for covariant)]. In the post hoc comparison, the doses of 1.25, 5, and 20 mg/kg differed from the SAL group. A dose of 0.62 mg/kg showed some tendency to increase preference, but a dose of 0.31 mg/kg was clearly ineffective. Therefore, this dose was chosen as the reference dose for the sensitization experiment.

Sensitization to the Rewarding Properties of MP

As evident in Fig. 2, after the 7-day sensitization treatment with SAL, conditioning with MP at a reference dose of 0.31 mg/kg caused no shift in preference. Instead, following the sensitization treatment with MP at different doses, conditioning with the reference dose resulted in a significant increase in preference for the drug-paired compartment, i.e., prior exposure to the drug appeared to sensitize the rewarding properties of MP. There was a statistically significant difference between the groups given SAL or MP at different doses during the sensitization treatment [F(5,65) = 4.360, p = 0.002, one-way ANCOVA (p = 0.03 for covariant)]. In the post hoc comparison, the doses of 0.62, 1.25, 5, and 20 mg/kg differed from the SAL group. When a separate group of rats was sensitized with MP at a dose of 20 mg/kg and conditioned with SAL, no shift in preference was observed in comparison with either the pre- and postconditioning times [t(5) = 0.045, p = 0.966, paired t test], or to the group conditioned with SAL without sensitization (Fig. 2) [F(1,12) = 0.456, p = 0.512, one-way ANCOVA (p = 0.205 for covariant)].

In addition to increased preference, separate analysis revealed that after the sensitization treatment at a dose of 5 mg/kg, conditioning with the reference dose is able to induce a true place preference for the drug-paired compartment, i.e., during the postconditioning phase the rats spent more time in the drug- than in the SAL-paired compartment (Fig. 3). First, because in our biased method some rats showed a substantial aversion to the drug-paired compartment during the preconditioning phase, these rats may have spent less time in the drug- than in the saline-paired compartment during the postconditioning phase, despite a significantly increased shift in preference for the drug-paired compartment. Therefore, according to the preconfining time (average of 375 s) the rats that showed only a mild initial aversion to the drug-paired compartment (preconditioning time >375 s) were included in the analysis. Second, to gain statistical power, all rats receiving sensitization treatment with MP at a dose of 5 mg/kg alone or with SAL were pooled (see Materials and Methods). As shown in Fig. 3, after sensitization treatment with MP at a dose of 5 mg/kg and conditioning at the reference dose, the rats (preconditioning time = 414 ± 3 s) spent significantly more time in the drug- than in the SAL-paired compartment [t(20) = 4.724, p < 0.001, paired t test]. In contrast, after sensitization treatment with SAL and conditioning at the reference dose, the rats (preconditioning time = 407 ± 11 s) spent more time in the SAL than in the drug-paired compartment [t(5) = -2.817, p < 0.037, paired t test]. Thus, after sensitization treatment with MP at a dose of 5 mg/kg, conditioning at the reference dose was able to induce a true place preference, at least in rats that had only a mild initial aversion to the drug-paired compartment.

Effects of D1- and D2-Receptor Blockade on the Rewarding Properties of MP

The effects of SCH and RAC on MP-induced increase in preference for the drug-paired compartment are shown in Fig. 4. MP-induced increase in preference was attenuated by SCH (Fig. 4A): there was a statistically significant difference between the groups conditioned with SAL or SCH at different doses in combination with MP (5 mg/kg) [F(3,40) = 4.751, p = 0.006, one-way ANCOVA (p = 0.386 for covariant)]. In the post hoc comparison, the dose of 0.2 mg/kg differed from the SAL group. Unlike SCH, RAC failed to affect MP-induced increase in preference (Fig. 4B) [F(3,28) = 0.467, p = 0.708, one-way ANCOVA (p = 0.002 for covariant)]. When conditioned alone in the preferred compartment, neither SCH nor
RAC was able to induce shift in preference when tested with one-way ANCOVA (Fig. 4, C and D) [SCH-conditioned: $F(3,19) = 0.119, p = 0.948$ (with covariant); RAC-conditioned: $F(3,19) = 0.28, p = 0.839$ (with covariant)]. When conditioned alone in the less preferred compartment, SCH did not induce shift in preference (Fig. 4E) [$F(2,17) = 1.364, p = 0.282$, one-way ANCOVA ($p = 0.376$ for covariants)]. Instead, RAC increased preference for the drug-paired compartment (Fig. 4F) [$F(2,24) = 3.469, p = 0.048$, one-way ANCOVA ($p = 0.186$ for covariants)]. In the post hoc comparison, the dose of 0.8 mg/kg appeared to differ from the SAL group ($p = 0.05$, Bonferroni’s test).

**Effects of D1- and D2-Receptor Blockade on Sensitization to the Rewarding Properties of MP**

The effects of SCH and RAC on the development of sensitization to the rewarding properties of MP are shown in Fig. 5. SCH prevented sensitization to the rewarding properties of MP (Fig. 5A) after the 7-day sensitization treatment with SAL combined with MP (5 mg/kg), conditioning with MP at a reference dose of 0.31 mg/kg increased preference for the drug-paired compartment, whereas after the sensitization treatment with SCH at different doses combined with MP (5 mg/kg), conditioning with the reference dose failed to do so. There was a statistically significant difference between the groups given SAL or SCH at different doses in combination with MP during the sensitization treatment [$F(2,33) = 3.875, p = 0.031$, one-way ANCOVA ($p = 0.774$ for covariants)]. In the post hoc comparison, the dose of 0.2 mg/kg differed from the SAL group. Similarly, RAC prevented sensitization to the rewarding properties of MP (Fig. 5B). There was a statistically significant difference between the groups given SAL or RAC at different doses in combination with MP during the sensitization treatment [$F(2,29) = 7.189, p = 0.003$, one-way ANCOVA ($p = 0.046$ for covariants)]. In the post hoc comparison, the dose of 0.4 mg/kg differed from the SAL group.

After sensitization with SCH at a dose of 0.2 mg/kg or RAC at a dose of 0.4 mg/kg alone, conditioning with SAL induced no shift in preference (Fig. 5, C and D) in comparison between the pre- and postconditioning times tested with the paired t test [SCH-sensitized: $t(7) = 0.528, p = 0.614$; RAC-sensitized: $t(7) = 0.442, p = 0.672$], or in comparison with the group conditioned with SAL without sensitization and tested with the one-way ANCOVA [SCH-sensitized: $F(1,16) = 0.033, p = 0.857$ (with covariant); RAC-sensitized: $F(1,16) = 0.096, p = 0.76$ (with covariant)]. Similarly, after sensitization with SCH or RAC alone, conditioning with MP at a dose of 0.31 mg/kg induced no shift in preference (Fig. 5, C and D) in comparison between the pre- and postconditioning times tested with the paired t test [SCH-sensitized: $t(5) = 0.492, p = 0.614$; RAC-sensitized: $t(5) = 0.096, p = 0.92$], or in comparison with the group sensitized with SAL and conditioned with MP at the dose of 0.31 mg/kg tested with the one-way ANCOVA [SCH-sensitized: $F(1,15) = 0.062, p = 0.807$ (with covariant); RAC-sensitized: $F(1,15) = 0.025, p = 0.877$ (with covariant)].

**Withdrawal Symptoms**

The results of the experiments assessing withdrawal symptoms following the 7-day treatment with MP at doses of 5 and 20 mg/kg are summarized in Fig. 6.

**Changes in Weight and Food Consumption.** Daily weight change and food consumption (of two rats in one cage) of MP-treated rats did not differ from those of SAL-treated rats.
rats (Fig. 6, A and B). ANOVA for repeated measures showed no difference between the treatments in weight change \([F(2,21) = 2.214, p = 0.314]\) or food consumption \([F(2,9) = 2.499, p = 0.137]\). Instead, there was a significant effect of day on weight change \([F(2,42) = 6.511, p = 0.003]\) and on food consumption \([F(2,18) = 15.379, p < 0.001]\). However, no significant treatment \(\times\) day interaction was found \(\text{[weight change: } F(4,42) = 0.824, p = 0.518; \text{food consumption: } F(4,18) = 0.441, p = 0.777]\), showing that the changes across the days were similar in the groups treated with SAL or MP. In particular, on the 3rd day of withdrawal (corresponding to the beginning of the conditioning phase in the place preference experiments) there was no difference between the treatments, when tested with the one-way ANOVA, in weight change \([F(2,21) = 1.632, p = 0.219]\) or food consumption \([F(2,9) = 0.154, p = 0.859]\).

**Anxiety-Like Behavior.** Anxiety-like behavior measured for 8 min (480 s) was assessed with two parameters of the black-white box test: the time to enter the white compartment for the first time and the total time spent in the white compartment. In the SAL-treated rats, the average times were as follows: 16 s (range: 6–30 s) for the time to enter the white compartment and 174 s (range: 42–265 s) for the total time in the white compartment. The parameters of black-white box test were calculated as percentages of SAL-treated rats tested daily.

As expected, anxiogenic yohimbine at a dose of 2.5 mg/kg used as a positive control showed anxiety-like behavior in the test. In rats administered with yohimbine, the time to enter the white compartment was increased \([254 \pm 62\% \text{ of the SAL group}, \(t(7.2) = -2.505, p = 0.04; \text{two-sample } t\text{ test, } n = 8]\), and the total time in the white compartment was decreased \([59 \pm 13\% \text{ of the SAL group}, \(t(14) = 2.291, p = 0.038, \text{two-sample } t\text{ test, } n = 8]\). The motor activity of yohimbine-treated rats in the compartments, when tested with the two-sample \(t\text{ test}, \) did not differ from that of the SAL-treated rats (white compartment: 99 ± 11%; black compartment: 92 ± 9% of the SAL group), but the number of intercompartmental crosses was decreased \([56 \pm 4\% \text{ of the SAL group}, \(t(13.2) = 6.72, p < 0.001, n = 8]\), possibly reflecting reluctance to enter the opposite compartment.

As shown in Fig. 6C, the 7-day treatment with MP at a dose of 5 mg/kg caused no anxiety-like behavior on the 3rd day of withdrawal (corresponding to the beginning of the conditioning phase in the place preference experiments). Instead, treatment with a dose of 20 mg/kg showed some anxiety-like behavior in the test. There was a significant difference between the treatments in the time to enter the white compartment \([F(2,21) = 4.633, p = 0.022, \text{one-way ANOVA}]\). In the post hoc comparison, the dose of 20 mg/kg differed from the SAL group. However, in comparison of the total time spent in the white compartment, only a trend between the treatments was seen \([F(2,21) = 2.942, p = 0.075, \text{one-way ANOVA}]\). Motor activity in the compartments and the number of intercompartmental crosses were similar in all the treatment groups.

**Motor Activity.** On the 3rd day of withdrawal, both ambulatory activity (Fig. 6D) and rearing activity (data not shown) were similar in the treatment groups. ANOVA for repeated measures showed only a significant effect of time on...
ambulatory activity [$F(7,147) = 95.556, p < 0.001$] and on rearing activity [$F(7,147) = 125.299, p < 0.001$], but there was neither a significant difference between the treatments [ambulatory activity: $F(2,21) = 0.372, p = 0.694$; rearing activity: $F(2,21) = 2.81, p = 0.083$] nor a significant treatment × time interaction [ambulatory activity: $F(14,147) = 0.976, p = 0.481$; rearing activity: $F(14,147) = 0.87, p = 0.592$].

**Conditioned Taste Aversion**

Lithium at a dose of 8.2 mg/kg when used as a positive control, but not MP at a reference dose of 0.31 mg/kg, induced taste aversion. There was no difference in water consumption between the treatment groups before the conditioning phase (8.8 ± 0.8 ml for SAL-, 8.3 ± 0.6 ml for MP-, and 8.9 ± 0.5 ml for lithium-treated rats). As shown in Fig. 7, after the conditioning phase there was a significant difference in the saccharin preference ratio between rats conditioned with lithium or SAL ($t(13.6) = -4.177, p = 0.001$, two-sample t test), but not between rats conditioned with MP or SAL ($t(7.7) = 0.995, p = 0.35$, two-sample t test).

**Discussion**

In the present study, MP induced place preference in agreement with previous studies (Martin-Iverson et al., 1985; Mithani et al., 1986), but in these studies no dose-response profile was fully assessed. Our results show that conditioning with doses of 1.25 to 20 mg/kg induced place preference, whereas a dose of 0.62 mg/kg caused only a trend to preference, and conditioning with a dose of 0.31 mg/kg was completely ineffective. As expected, after the 7-day sensitization treatment with SAL, conditioning with MP at a reference dose of 0.31 mg/kg was unable to cause a shift in preference. Instead, after the sensitization treatment with MP at doses of 0.62 to 20 mg/kg, conditioning with the reference dose significantly increased preference for the drug-paired compartment, i.e., the prior exposure appeared to sensitize the rewarding properties of MP. In particular, after the sensitization treatment at a dose of 5 mg/kg and conditioning at the reference dose, the rats that had only a mild initial aversion to the drug-paired compartment spent more time in it than in the saline-paired compartment, indicating that after the sensitization treatment the reference dose can be truly rewarding instead of merely reducing aversion. Thus, given the postulated role of sensitization in the development of pathologically strong drug craving (Robinson and Berridge, 1993), repeated administration can be suggested to enhance the incentive motivational properties and abuse potential of MP. In line with our results, prior exposure has also been shown to sensitize the rewarding properties of cocaine, amphetamine, and morphine when assessed in the conditioned place preference method (Lett, 1989; Shippenberg and Heidbreder, 1995; Le Pen et al., 1998). Furthermore, a recent finding suggests that exposure to MP during adolescence enhances the acquisition of cocaine self-administration in adult rats (Brandon et al., 2000). Our results are also parallel with the findings that repeated administration of MP results in sensitization to its locomotor-stimulating effects (Shuster et al., 1982; Gaytan et al., 1997; McDougall et al., 1999), although this may not be as decided a phenomenon as with other psychostimulants (McNamara et al., 1993; Izenwasser et al., 1999).

It should be noted that during the preconditioning phase, the rats sensitized with MP may have experienced the conditioning apparatus differently from the rats sensitized with SAL. In all the treatment groups, approximately equal numbers of rats were assigned to both compartments as the drug-paired compartment, thus balancing any possible bias between them. Furthermore, when the rats were sensitized with MP at a dose of 20 mg/kg and conditioned only with SAL, neither place preference nor aversion was observed. Thus, it is unlikely that the observed sensitization phenomenon could result merely from nonspecific differences between the groups in experiencing the conditioning apparatus during the preconditioning phase.

The brain dopaminergic system is considered to be essential in the rewarding properties of abused drugs. In the present study, MP-induced place preference was prevented by administering the D1-receptor antagonist SCH during the conditioning phase, as previously observed with other psychostimulants (Tzschentke, 1998). Typically, in the place preference method multiple drug injections during the conditioning phase are required, e.g., three in our method, and hence some degree of sensitization may be involved. However, it is important to note that SCH can prevent psychostimulant-induced place preference even in a single conditioning trial, thereby excluding the interference of sensitization (Bardo et al., 1999). Unlike SCH, administration of RAC did not affect place preference induced by MP. This finding extends those of earlier studies, in which an-
other D2-receptor antagonist, haloperidol, was shown to be ineffective (Martin-Iverson et al., 1985; Mithani et al., 1986). The result obtained with the highest dose of RAC (0.8 mg/kg), however, should be interpreted with caution, since this dose alone increased preference for the drug-paired compartment. A similar finding was previously obtained with another D2-antagonist, metoclopramide, which was speculated to result from drug-induced disturbance of habituation in the paired environment leading to novelty-induced place preference (Hoffman and Beninger, 1988), yet in that study metoclopramide at similar doses was able to block amphetamine-induced place preference. Nevertheless, the two other doses of RAC (0.2 and 0.4 mg/kg) used in the present study should be behaviorally effective: at these doses or below, RAC blocked the motor-stimulating effects of the D2/D3-agonist quinpirole, amphetamine, cocaine, and MP (Millan et al., 1998), or place preference induced by amphetamine (Hoffman and Donovan, 1995) and drinking water (Ågmo et al., 1993). Several lines of evidence indicate that the brain dopaminergic system, in interplay with other neurotransmitters, is also intricately linked to sensitization to the effects of psychostimulants. Although detailed mechanisms are not completely understood, it has been suggested that in the ventral tegmental area, D1-receptor activation by drug-induced somatodendritic dopamine release and a decrease in inhibitory D2-autoreceptor function by repeated drug administration

Fig. 4. Effects of dopamine D1-antagonist SCH (A) and D2-antagonist RAC (B) on the rewarding properties of MP; effects of SCH (C) and RAC (D) alone when associated with the preferred compartment; effects of SCH (E) and RAC (F) alone when associated with the less preferred compartment. The data shown represent time (mean ± S.E.) spent in the drug-paired compartment during the 15-min measurements before and after the conditioning phase (preconditioning and postconditioning times, respectively). During the conditioning phase, the effects of SCH, RAC, and SAL in combination with MP, or SCH and RAC alone [referred to as conditioning drug(s)] were associated with the drug-paired compartment in daily 40-min sessions for 3 consecutive days. Numbers within the Preconditioning time columns represent the number of rats in the group. **p < 0.01 and *p = 0.05 when compared with the rats conditioned with SAL alone, or SAL and MP at a dose of 5 mg/kg (Bonferroni’s test).
are involved in the development of sensitization (Kalivas and Stewart, 1991; Henry et al., 1998). Accordingly, sensitization to locomotor-stimulating effects of psychostimulants can be modulated by dopamine-receptor antagonists (Kuribara and Uchihashi, 1993; Meng et al., 1998). In the present study, both SCH and RAC combined with MP during the 7-day sensitization treatment prevented the enhancement of place preference, which gives evidence that both D1- and D2-receptors are involved in the development of sensitization to MP's rewarding properties. Particularly, D2-receptors appeared to be more specifically involved in the sensitization processes, as RAC failed to affect MP-induced place preference when given during the conditioning phase. In partial contrast to our results, Shippenberg and Heidbreder (1995) showed that D1-blockade with SCH, but not D2-blockade with RAC, prevented sensitization to cocaine-induced place preference. Taken together, it appears that D1-blockade can efficiently prevent sensitization to the rewarding properties of different psychostimulants. The role of D2-receptors is less well documented, but the present finding indicates their involvement in sensitization to the rewarding properties of MP.

Acute administration of psychostimulants such as cocaine and amphetamine is known to increase brain serotonergic neurotransmission, which may play a role in the development of sensitization to their effects. For example, sensitization to motor-stimulating effects of cocaine can be inhibited by ondansetron, a serotonin-3-receptor antagonist (King et al., 1997). Unlike cocaine and amphetamine, however, MP does not affect the serotonergic system (Koe, 1976; Segal and Kuczenski, 1999). Regardless of this, prior exposure sensitized the rewarding properties of MP, which is in line with a recent finding that repeated administration of GBR 12783, a specific dopamine-uptake blocker, sensitizes its own rewarding properties, as well as those of cocaine (Le Pen et al., 1998). Thus, it appears that activation of the serotonergic...
Fig. 6. The withdrawal symptoms weight change (A) \((n = 8)\), food consumption (B) \((n = 4)\), anxiety-like behavior (C) \((n = 8)\), and changes in ambulatory activity (D) \((n = 8)\) induced by 7-day treatment with daily injections of MP [5 mg/kg (●), 20 mg/kg (▲)], or SAL (□). Daily weight change and mean food consumption of two rats in the cage were measured for 3 consecutive days after the last injection. Anxiety-like behavior in the black-white box test and motor activity in an automated activity cage were assessed on the 3rd day after the last injection. All the data are represented as mean ± S.E. *\(p < 0.05\) when compared with the SAL treatment (Bonferroni’s test).
system is not a prerequisite for, although it may modulate, the development of sensitization to drug-induced reward.

Instead of a sensitization phenomenon, it is possible that the 7-day sensitization treatment with MP induced withdrawal symptoms that were attenuated by MP administered during the conditioning phase, resulting in the enhancement of place preference. Unlike ethanol or morphine, however, psychostimulants do not induce aversive physical withdrawal symptoms. Instead, psychostimulant-withdrawal has been associated with rebound feeding (Kräuchi et al., 1984), weight gain (Planeta et al., 1994), hyperactivity (Pulvirenti and Koob, 1993), and anxiety-like behavior (Costall et al., 1989). When evaluated with these parameters, the 7-day treatment with MP at a dose of 5 mg/kg induced no withdrawal, which suggests that the enhancement of place preference could not solely be explained as the attenuation of withdrawal. Another alternative explanation for the enhancement of place preference is sensitization treatment-induced tolerance to the aversive properties of MP. When assessed in the conditioned taste aversion test, however, MP at the reference dose possessed no aversive properties, and hence tolerance to these properties is unlikely to be responsible for the enhancement of place preference.

In conclusion, the results of the present study show that the rewarding properties of MP are sensitized by prior exposure to the drug. Given the postulated role of the sensitization phenomenon in the development of drug craving (Robinson and Berridge, 1993), repeated intake of MP can be speculated to progressively increase the incentive motivational properties and abuse potential of MP. Furthermore, both the D1-antagonist SCH and D2-antagonist RAC prevented the development of this sensitization, whereas only the D1-antagonist was able to directly attenuate the rewarding properties of MP. It appears that both D1- and D2-receptors, the latter of which possibly more specifically, are involved in the sensitization processes of the rewarding properties of MP.

References


Calcegnotti DJ and Schechter MD (1992) Reducing the time needed to conduct conditioned place preference testing. Prog Neuropsychopharmacol Biol Psychiatry 16:969–976.


Fig. 7. MP at the reference dose does not induce conditioned taste aversion (n = 8). The data shown represent the saccharin preference ratio (mean ± S.E.) in the test session. During the conditioning phase, the effects of MP, lithium, or SAL (referred to as the conditioning drugs) were associated with 0.2% saccharin solution for 3 consecutive days. **p < 0.01 when compared with the rats conditioned with SAL (two-sample t test).

Fig. 8. Methamphetamine (MP) at the reference dose does not induce conditioned taste aversion (mean ± 95% C.I.) in the test session. The data shown represent the saccharin preference ratio in the test session. During the conditioning phase, the effects of MP, lithium, or SAL (referred to as the conditioning drugs) were associated with 0.2% saccharin solution for 3 consecutive days. **p < 0.01 when compared with the rats conditioned with SAL (two-sample t test).

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