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Effects of 5-HT_{1A} and 5-HT_2 receptor agonists on the behavioral and neurochemical consequences of repeated nicotine treatment

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Abstract

This study investigated the effects of repeated daily (15 days) treatment with nicotine, alone or in combination with the 5-$HT_{1A/7}$ receptor agonist (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) or the 5-HT_2 receptor agonist (±)-2,5-dimethoxy-4-iodoamphetamine (DOI) on locomotor sensitization, mesolimbic dopamine neurochemistry and on behavioral inhibition in the rat. Acute nicotine elevated the extracellular dopamine levels in the nucleus accumbens and stimulated locomotor activity, effects that were sensitized after repeated nicotine treatment. Repeated nicotine administration also produced nicotine-induced behavioral disinhibition in the elevated plus-maze. Treatment with DOI counteracted the expression of the nicotine-induced locomotor and neurochemical sensitization, but had no effect on nicotine-induced behavioral disinhibition. Treatment with 8-OH-DPAT decreased the expression of nicotine-induced behavioral disinhibition, but had no effect on locomotor or neurochemical sensitization. Taken together, these findings suggest that the 5-HT_{1A} and the 5-HT_2 receptor subtypes are differentially involved in the effects of repeated nicotine on locomotor sensitization, behavioral inhibition and mesolimbic dopamine neurochemistry.

Keywords: Repeated nicotine treatment; 8-OH-DPAT; DOI

1. Introduction

Drugs of abuse share the ability to activate the mesolimbic dopamine system (Di Chiara and Imperato, 1988; Koob, 1992), a neural pathway that projects from the ventral tegmental area in the midbrain to the nucleus accumbens in the ventral striatum. These neurons are functionally involved in processes related to incentive motivation and associative learning (Jentsch et al., 2000; Mogenson et al., 1993). These neurons have also been implicated in processes related to reward (Engel, 1977; Koob, 1992; Wise et al., 1992) and the drug-induced activation of the mesolimbic dopamine neurons has been proposed to mediate the rewarding and addictive properties of most drugs abused by man.

Activation of postsynaptic dopamine receptors in the nucleus accumbens stimulates locomotor activity in rats (Pijnenburg and van Rossum, 1973), and, consequently, the mesolimbic dopamine activation produced by nicotine and psychostimulants is associated with elevated locomotor activity in rats (Wise and Bozarth, 1987). Repeated exposure to many of these drugs progressively enhances the drug-induced locomotor stimulation (Clarke and Kumar, 1983; Pierce and Kalivas, 1997). This behavioral sensitization is long-lasting and appears to be attributed to drug-induced pre- and postsynaptic neuroadaptive alterations. As a result of the repeated or prolonged drug exposure, both the drug-induced elevation of extracellular accumbal dopamine levels (Balfour et al., 1998; Parsons and Justice, 1993; Pierce and Kalivas, 1997) and the postsynaptic dopamine receptor function (Engel and Liljequist, 1976; Fung and Lau, 1988; Henry and White, 1991) are enhanced. Moreover, different intracellular signal transduction pathways and transcription factors in these neurons as well as in other systems are also found to be altered by repeated drug exposure (Nestler and Aghajanian, 1997). Hence, the neurobiological correlate to locomotor sensitization has been proposed to be a hypersensitive mesolimbic dopamine system (Nestler and Aghajanian, 1997; Pierce and Kalivas, 1997; Robinson and Berridge, 1993).

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The incentive-sensitization theory of addiction (Robinson and Berridge, 1993) hypothesizes that the neurobiological processes that underlie drug-induced locomotor sensitization are involved in drug-seeking and drug-taking behavior. It is proposed that the hypersensitive mesolimbic dopamine system is activated by different internal or external drug-associated (conditioned) stimuli that provide strong motivational impulses for obtaining and consuming the drug. In support of this theory, experiments have demonstrated that previous drug experience may increase subsequent self-administration of both amphetamine (Piazza et al., 1990) and cocaine (Horger et al., 1990). Moreover, the rewarding effects of psychostimulants (Lett, 1989; Shippenberg and Heidbreder, 1995) as well as the amphetamine-induced elevation of the responding for conditioned reinforcers (Taylor and Horger, 1999) are also enhanced after psychostimulant-induced sensitization. Together, the available data suggest that drug-induced sensitization of the mesolimbic dopamine neurons may contribute to the increased control of behavior exerted by drug-associated stimuli. As such, drug abuse can be viewed as a state characterized by compulsive behaviors focused on drug-seeking and intake. The incentive motivational and rewarding effects of the addictive compounds do, however, not appear to fully explain the compulsive nature of the addiction-related behaviors observed in substance abusers. Consequently, the neurobiological substrate underlying other pathological behaviors observed in drug-addiction has recently gained increased attention. These behaviors include decreased inhibitory control (Jentsch and Taylor, 1999; Olausson et al., 1999; Robbins and Everitt, 1999) and the impaired cognitive functions (Jentsch and Taylor, 1999; Robbins and Everitt, 1999) that are observed after repeated drug exposure in animals as well as in human drug addicts.

Serotonin (5-HT) may have a critical role in the regulation of some of these drug-induced behaviors. Serotonin is involved in neuronal processes related to conflict behavior, inhibitory control and impulsivity (Roy and Linnoila, 1988; Söderpalm, 1990; Soubrié, 1986), but also modulates reward-related mechanisms (Cunningham et al., 1992; Olausson et al. 1999). The neural substrates for the interaction between the brain 5-HT systems and the mesolimbic dopamine system are well established (Kelland and Chiodo, 1996), and some studies have implicated serotonergic mechanisms in the development or expression of drug-induced sensitization (King et al., 1997; Olausson et al., 1999; Parsons and Justice, 1993).

The 5-HT_{1A} and 5-HT_2 receptor subtypes have previously been demonstrated to modulate the activity of the mesocorticolimbic dopamine system (Kelland and Chiodo, 1996). We have previously demonstrated that chronic treatment with the selective 5-HT reuptake inhibitor citalopram suppresses the expression of both the enhanced locomotor activity and the disinhibited behavior observed after repeated daily nicotine injections (Olausson et al., 1999). To investigate the specific 5-HT–dopamine interactions possibly underlying the effects of citalopram on nicotine-induced sensitization and disinhibition, the present study evaluated the influence of the 5-$HT_{1A/7}$ receptor agonist (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) and the 5-HT_2 receptor agonist (±)-2,5-dimethoxy-4-iodoamphetamine (DOI) on locomotor activity after acute/repeated nicotine treatment and on behavioral inhibition after repeated nicotine treatment, in male Sprague–Dawley rats. The effects of 8-OH-DPAT and DOI on the activating effects of nicotine on mesolimbic dopamine neurotransmission after repeated treatment were also evaluated using in vivo microdialysis.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats ($n = 128$), supplied by BeeKay (Sollentuna, Sweden), weighing 250–280 g at the start of the experiment were used in all tests. The rats were housed four per cage under constant cage temperature (20°C), humidity (40–50%) and controlled light–dark conditions (light on at 6 a.m. and off at 6 p.m.). The rats had free access to standard laboratory food (BeeKay Feeds) and tap water at all times. The animals were allowed to adapt to the animal department facilities for at least 1 week before the start of any experiment. The present study was conducted in a manner consistent with Swedish legislation for Animal Welfare and was approved by the Ethics Committee for Animal Experiments, Göteborg, Sweden.

2.2. Drugs

(±)-8-Hydroxy-2-(di-*n*-propylamino)tetralin HBr 0.5 mg/kg (8-OH-DPAT; Sigma, USA), (±)-2,5-dimethoxy-4-iodoamphetamine HCl 1.0 mg/kg (DOI; RBI, USA) and (−)-nicotine ditartrate 0.35 mg/kg (1.0 mg/kg of the salt; Sigma) were used. The nicotine solution was neutralized with a few grains of sodium bicarbonate. All drugs were dissolved in physiological saline (0.9% NaCl) and injected subcutaneously (s.c.) in a volume of 2 ml/kg. The 8-OH-DPAT and DOI doses are expressed as the weight of the salt.

2.3. Experimental methods

2.3.1. Locomotor activity

Locomotor activity was measured using computerized Digiscan animal activity monitors (Omnitech Electronics, USA) that were placed in eight identical sound- and light-attenuating boxes containing a weak light and a fan. The activity meter was equipped with three rows of infrared photosensors, each row consisting of 16 sensors

placed 2.5 cm apart. Two rows were placed in a 90° angle along the front and side of the floor of the cage and the third row was placed 10 cm above the floor to measure vertical activity. The activity meters were connected to an analyzer system (Omnitech Electronics) and the data were collected using LabVIEW computer software (National Instruments, USA).

According to the treatment schedule, rats were injected with the 5-HT receptor agonist (e.g. 8-OH-DPAT or DOI) or vehicle, placed in transparent plastic boxes and put into the activity meters. The animals were then allowed a 30-min habituation period, after which they were taken out, injected with nicotine or vehicle, and returned to the boxes. Locomotor activity was recorded for 60 min in 5-min bins starting 5 min after drug injection to avoid unspecific injection-induced hyperactivity. All experiments were performed between 8 a.m. and 6 p.m. in a balanced order.

2.3.2. Behavioral inhibition

To evaluate the effects of the present drug treatments on behavioral inhibition, the performance in the elevated plus-maze paradigm was investigated. The experimental apparatus consisted of a plus-formed maze with mesh-wire floor, elevated approximately 0.8 m above the ground in a semi-illuminated room. The arms of the plus-maze were 40 cm long and 10 cm wide. Two opposing arms were surrounded by 10-cm-high black walls (closed arms), while the other arms were devoid of walls (open arms). This conflict model is based on the observation that the contrast between the elevated open and closed arms in the elevated plus-maze inhibits the exploratory behavior normally displayed by rats placed in a novel environment. The exploration of open arms is thus suppressed, and in the present setting, a non-treated, normal rat spends only about 15–30% of the total arm time on open arms. Manipulations that increase the percentage of time spent on and entries made onto the open arms are therefore considered to produce behavioral disinhibition.

According to the drug treatment schedule, each animal received injections of a 5-HT receptor agonist or vehicle and was returned to the home cage. Thirty minutes later, the rat was injected with nicotine 0.35 mg/kg or vehicle and put into an unfamiliar environment (a dark box with a grid floor) for 5 min to stimulate exploratory behavior. Thereafter, the rat was placed in the center of the plus-maze facing a closed arm. Entry into one arm was defined as the animal placing all four paws into the arm. The investigator was situated 2 m from the center of the maze. After every tested animal, the maze was carefully wiped with a wet cloth. The time spent in and the number of entries made into open and closed arms were recorded during a 5-min test session, and the time spent on and number of entries made onto open arms were expressed as percentage of the total time and total entries made into both open and closed arms.

2.3.3. In vivo microdialysis

To extract dopamine from the extracellular fluid, a microdialysis probe was implanted into the brain. The microdialysis was performed with I-shaped probes with 2-mm-long active dialysis membrane tips. The rats were anaesthetized with ketamin 50 mg/ml (Parke-Davies, Spain) and xylazin 20 mg/ml (Bayer, Germany) in a mixture of 2:1, that was intraperitoneally injected in a volume of 2 ml/kg. The microdialysis probe was then implanted in the nucleus accumbens of the brain by a stereotactic operation, using a Kopf stereotaxic instrument, and fixed to the skull using Phosphatine dental cement (Svedia Dental Industri, Sweden). The stereotactic coordinates of the probe relative to the bregma were A/P + 1.85, L/M − 1.4, V/D − 7.8 (Paxinos and Watson, 1986). To substitute for the loss of body fluids that occur due to the operation, the rats were injected with 3 ml of 0.9% NaCl (s.c.) and were then allowed to recover for at least 40 h before the start of the microdialysis experiment.

At the beginning of the experiment, the inlet of the probe was connected to a syringe perfusion pump via a swivel, and the outlet was connected to a collecting tube. The swivel allowed the animals to move freely during the experiment. The probe was then perfused with Ringer solution containing in mM: NaCl 140, $CaCl_2$ 1.2, KCl 3.0 and $MgCl_2$ 1.0. The perfusion rate during the experiment was 2 μl/min and dialysate fractions (40 μl) were collected every 20 min. The dopamine levels in the perfusate samples were determined using a standard HPLC technique with electrochemical detection.

2.4. Experimental design

2.4.1. Behavioral studies

In the studies of locomotor sensitization and behavioral inhibition, animals were randomly divided into four groups ($n = 8$). These groups received daily injections during 15 days according to the following paradigm: (1) vehicle + vehicle, (2) 5-HT receptor agonist + vehicle, (3) vehicle + nicotine and (4) 5-HT receptor agonist + nicotine. Each day, 8-OH-DPAT and DOI were administered approximately 15 min prior to the nicotine injection.

The locomotor activity after these treatments was recorded after acute (day 1) and repeated (day 15) treatment. On day 17, the effects of a nicotine challenge on locomotor activity (i.e. 48 h after the last repeated drug injection) were recorded in groups 1–3. Moreover, the nicotine-induced locomotor stimulation in the repeatedly vehicle + nicotine-treated animals (group 4) receiving acute pretreatment with the 5-HT receptor agonists was also evaluated. The following day (treatment day 18), all rats were subjected to the elevated plus-maze to evaluate the effect of the drug treatments on behavioral inhibition. An additional group ($n = 8$), not included in the locomotor activity experiment, which had received 15 days repeated

daily treatment with vehicle in the same manner as group 1, was added to the experiment to evaluate the acute effects of nicotine in the elevated plus-maze.

2.4.2. In vivo microdialysis experiments

Animals were randomly divided into four balanced groups ($n = 12$, all groups). These groups received daily injections during 15 days according to the following paradigm: (1) vehicle + vehicle, (2) 5-HT receptor agonist + vehicle, (3) vehicle + nicotine and (4) 5-HT receptor agonist + nicotine. Each day, 8-OH-DPAT and DOI were administered approximately 15 min prior to the nicotine injection.

On day 15, the animals were surgically prepared for the microdialysis experiments according to the procedures outlined above. On the testing day (day 17), the animals were connected to the perfusion equipment and allowed at least 90 min for stabilization. Perfusate samples were then collected during 80 min, or until a stable baseline (±10%) was obtained, before 8-OH-DPAT or DOI or the equivalent volume of vehicle was administered according to the treatment schedule. Two more samples were collected and 40 min after the first injection, all animals received nicotine. Perfusate samples were collected every 20 min for an additional 120 min before the animals were sacrificed and their brains removed for confirmation of the probe placement. Only animals with correctly placed probes were included in the statistical analysis of the data.

2.5. Statistics

The results from the behavioral experiments were evaluated using a one-way analysis of variance (ANOVA) with treatment as the independent factor followed by Fisher's protected least significant difference (PLSD) test. To control for unspecific effects of drug-induced locomotor stimulation, the total number of entries was included as a covariant in the analysis of measures of behavioral inhibition/disinhibition in the elevated plus-maze. The in vivo microdialysis experiments were statistically analyzed with an ANOVA for repeated measures with pretreatment and time as independent factors. Multiple comparisons were corrected for using Holm's sequential rejective multiple test procedure (Holm, 1979), a weighted improvement of the Bonferroni–Dunn correction. A probability value (P) less than 0.05 was considered statistically significant.

3. Results

3.1. Locomotor activity studies

3.1.1. Acute treatment

On treatment day 1, there was a statistically significant main effect of treatment in both the 8-OH-DPAT ($F(7,28) = 43.560$; $P \leq 0.0001$) and the DOI ($F(7,28) = 23.083$; $P \leq 0.0001$) experiments. Acute nicotine treatment stimulated locomotor activity in the habituated rats ($P \leq 0.001$; Figs. 1 and 2). Acute treatment with 8-OH-DPAT + vehicle ($P \leq 0.05$; Fig. 1) and DOI + vehicle ($P \leq 0.001$; Fig. 2) also stimulated locomotor activity. Administration of 8-OH-DPAT 30 min before injection of nicotine increased the acute nicotine-induced locomotor stimulation ($P \leq 0.0001$; Fig. 1).

3.1.2. Sensitization

On treatment day 15, there was also a significant main effect of treatment in both the 8-OH-DPAT ($F(3,28) = 21.155$; $P \leq 0.0001$) and the DOI ($F(3,28) = 24.495$; $P \leq 0.0001$) experiments. Further analysis demonstrated that repeated daily nicotine treatment in the vehicle + nicotine group enhanced the nicotine-induced locomotor stimulation ($P \leq 0.01$; Figs. 1 and 2). However, repeated treatment with 8-OH-DPAT + nicotine (Fig. 1) or DOI + nicotine (Fig. 2) did not significantly alter the locomotor stimulation recorded on treatment day 15 compared to that observed on treatment day 1 after administration of the same drugs. Instead, on treatment day 15, the locomotor activation produced by DOI + nicotine was lower than that observed in the vehicle + nicotine animals ($P \leq 0.001$; Fig. 2). Repeated daily treatment for 15 days did not alter the locomotor stimulatory effect of 8-OH-DPAT alone (8-OH-DPAT + vehicle group; Fig. 1), whereas tolerance developed to the stimulant effect of DOI (DOI + vehicle group; $P \leq 0.001$; Fig. 2).

Post-sensitization tests were performed on day 17, i.e. 48 h after the locomotor activity experiments on day 15. In these experiments, a statistically significant main effect of treatment was observed in both the 8-OH-DPAT ($F(3,28) = 14.682$; $P \leq 0.0001$) and the DOI ($F(3,28) = 6.608$; $P \leq 0.01$) experiments. When tested without 5-HT receptor

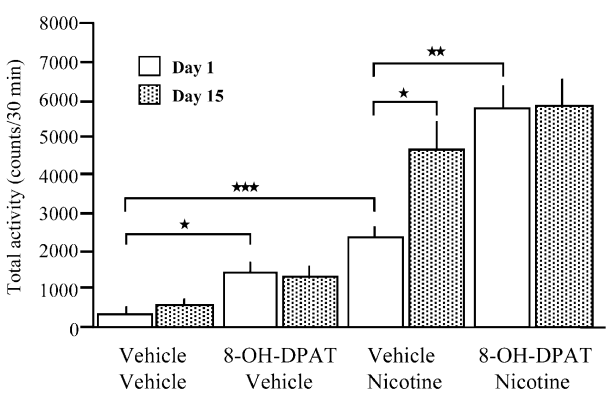

Fig. 1. The effects of acute and repeated daily treatment (15 days) with 8-OH-DPAT (0.5 mg/kg s.c.) and nicotine (0.35 mg/kg s.c.), alone or in combination, on locomotor activity recorded on treatment days 1 and 15. Shown are the means + S.E.M.; $n = 8$, all groups. Statistics: ANOVA followed by Fisher's PLSD test. Multiple comparisons were corrected for using Holm's sequential rejective test procedure. ★ $P \leq 0.05$, ★★ $P \leq 0.01$ and ★★★ $P \leq 0.001$.

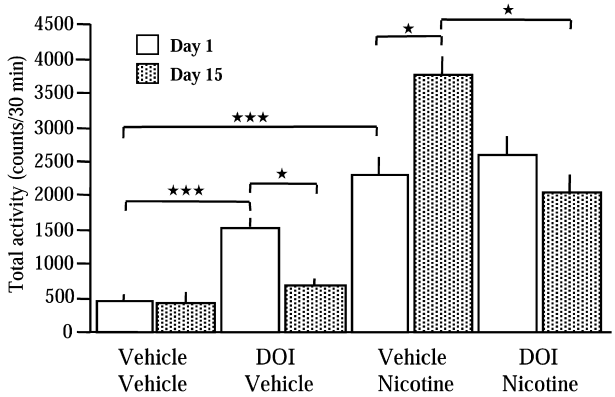

Fig. 2. The effects of acute and repeated daily treatment (15 days) with DOI (1.0 mg/kg s.c.) and nicotine (0.35 mg/kg s.c.), alone or in combination, on locomotor activity recorded on treatment days 1 and 15. Shown are the means + S.E.M.; $n = 8$, all groups. Statistics: ANOVA followed by Fisher's PLSD test. Multiple comparisons were corrected for using Holm's sequential rejective test procedure. ★ $P \leq 0.05$ and ★★★ $P \leq 0.001$.

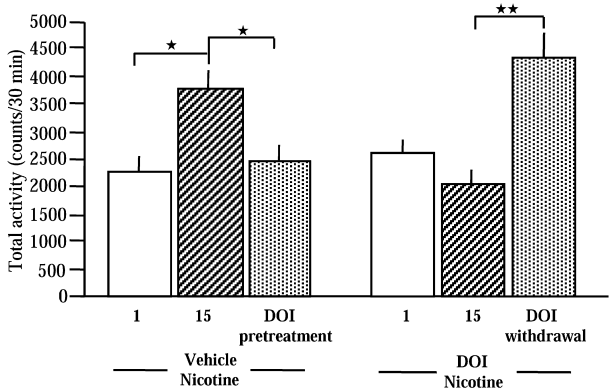

Fig. 4. The effects of acute DOI (1.0 mg/kg s.c.) pretreatment in rats repeatedly treated with vehicle + nicotine for 15 days and DOI withdrawal (−48 h) in animals repeatedly treated with DOI + nicotine for 15 days, on nicotine (0.35 mg/kg s.c.)-induced locomotor activity recorded post-treatment on day 17. Shown are the means + S.E.M.; $n = 8$, all groups. The data from days 1 and 15 were also shown in Fig. 3. Statistics: ANOVA followed by Fisher's PLSD test. Multiple comparisons were corrected for using Holm's sequential rejective test procedure. ★ $P \leq 0.05$ and ★★ $P \leq 0.01$.

agonists on board, the nicotine-induced locomotor stimulation was greater in the 8-OH-DPAT + nicotine ($P \leq 0.05$; Fig. 3) and the DOI + nicotine ($P \leq 0.001$; Fig. 4) pretreated rats than in drug-naive vehicle + vehicle-treated rats receiving an acute injection with nicotine. Withdrawal of 8-OH-DPAT decreased the nicotine-induced locomotor activity compared to that observed in the same animals after combined treatment with 8-OH-DPAT + nicotine on treatment day 15 ($P \leq 0.01$; Fig. 3). Moreover, acute challenge with 8-OH-DPAT potentiated ($P \leq 0.01$; Fig. 3), while DOI challenge decreased ($P \leq 0.05$; Fig. 4) the locomotor stimulatory effects of nicotine in the vehicle + nicotine-treated animals.

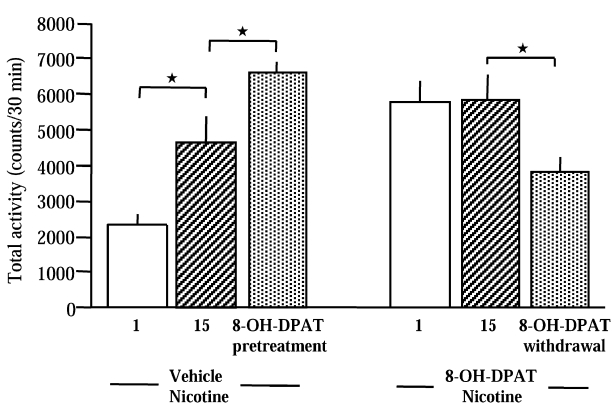

Fig. 3. The effects of acute 8-OH-DPAT (0.5 mg/kg s.c.) pretreatment in rats repeatedly treated with vehicle + nicotine (1.0 mg/kg s.c.) for 15 days, and of 8-OH-DPAT withdrawal (−48 h) in animals repeatedly treated with 8-OH-DPAT + nicotine for 15 days, on nicotine-induced locomotor activity recorded post-treatment on day 17. Shown are the means + S.E.M.; $n = 8$, all groups. The data from days 1 and 15 were also shown in Fig. 1. Statistics: ANOVA followed by Fisher's PLSD test. Multiple comparisons were corrected for using Holm's sequential rejective test procedure. ★ $P \leq 0.05$, ★★ $P \leq 0.01$ and ★★★ $P \leq 0.001$.

3.2. The elevated plus-maze

All animals were tested in the elevated plus-maze on day 18, i.e. after repeated drug treatment, and the experimental findings outlined below are described in Table 1. In this experiment, there was a main effect of treatment on the percentage of time spent on open arms ($F(6,97) =$

Table 1
Effects of 8-OH-DPAT and DOI on nicotine-induced behavioral disinhibition

Treatment	Percentage of time (open)	Percentage of entries (open)	Total entries
Vehicle + vehicle	24.8 ± 1.7	32.5 ± 1.7	12.2 ± 0.8
Vehicle + nicotine (acute)	19.0 ± 1.6	27.2 ± 1.8	9.5 ± 0.7[a]
Vehicle + nicotine (repeated)	39.5 ± 2.6[b]	42.9 ± 2.0[b]	17.4 ± 0.8[b]
8-OH-DPAT + vehicle	29.0 ± 6.7	37.2 ± 8.6	14.0 ± 2.2
8-OH-DPAT + nicotine	25.2 ± 6.6[c]	26.3 ± 7.2[c]	20.4 ± 3.3[a]
DOI + vehicle	32.9 ± 7.8	35.7 ± 4.6	10.1 ± 1.3
DOI + nicotine	32.7 ± 3.8[a]	39.3 ± 3.0	13.9 ± 1.1[c]

The effects of repeated daily treatment with vehicle + vehicle, vehicle + nicotine, 8-OH-DPAT + vehicle, 8-OH-DPAT + nicotine, DOI + vehicle or DOI + nicotine on behavioral inhibition in the elevated plus-maze after an acute nicotine (0.35 mg/kg s.c.; 5 min) injection. Shown are the means + S.E.M.; $n = 8$, all groups. Statistics: one-way ANOVA followed by Fisher's PLSD test. The total number of entries was included as a covariant in the analysis of the measures of behavioral inhibition/disinhibition. Multiple comparisons were corrected for using Holm's sequential rejective test procedure.

[a] $P \leq 0.05$ compared to vehicle + vehicle.
[b] $P \leq 0.001$ compared to vehicle + vehicle.
[c] $P \leq 0.05$ compared to repeated nicotine.

4.196; $P \leq 0.001$), the percentage of entries made onto open arms ($F(6,97) = 2.889$; $P \leq 0.05$), and on the total number of entries made onto any arm ($F(6,97) = 9.910$; $P \leq 0.0001$). Acute nicotine decreased the total number of entries made in any arm during the test session ($P \leq 0.05$). Repeated treatment with vehicle + nicotine increased the percentage of time spent on ($P \leq 0.0001$), the entries made onto ($P \leq 0.001$) open arms, and the total number of arm entries ($P \leq 0.001$) made during the test period, compared to vehicle + vehicle-treated rats receiving vehicle or acute nicotine.

Treatment with 8-OH-DPAT + vehicle or DOI + vehicle did not influence the behavior in the elevated plus-maze on day 18 in animals previously exposed to these drugs for 15 days. However, the percentage of time spent on ($P \leq 0.01$) and entries made onto ($P \leq 0.0001$) open arms of the elevated plus-maze were significantly lower in the 8-OH-DPAT + nicotine group than that observed in the vehicle + nicotine rats. The measures of behavioral inhibition did not differ between the vehicle + nicotine and the DOI + nicotine groups, but the total number of entries was decreased in the animals treated with DOI + nicotine ($P \leq 0.05$).

3.3. In vivo microdialysis

The basal levels of dopamine in the dialysate from the nucleus accumbens in this experiment were 2.21 ± 0.38 nM. In the microdialysis experiments, there was a main effect of pretreatment ($F(3,31) = 2.97$; $P \leq 0.05$). There was also a significant Treatment × Time interaction ($F(15,160) = 2.665$; $P \leq 0.01$). Analysis demonstrated that nicotine increased the accumbal dopamine output in all experimental groups ($P \leq 0.001$; Figs. 5 and 6), whereas injection of vehicle (e.g. 0.9% NaCl) had no effect. The nicotine-induced dopamine elevation was significantly greater in animals repeatedly treated with vehicle + nicotine ($P \leq 0.05$; Figs. 5 and 6) or 8-OH-DPAT + nicotine ($P \leq 0.05$; Fig. 5) compared to controls previously treated with vehicle + vehicle. There was no statistically significant difference between nicotine-induced elevation of the accumbal dopamine output in rats repeatedly treated with vehicle + vehicle and those treated with DOI + nicotine ($P = 0.77$; Fig. 6).

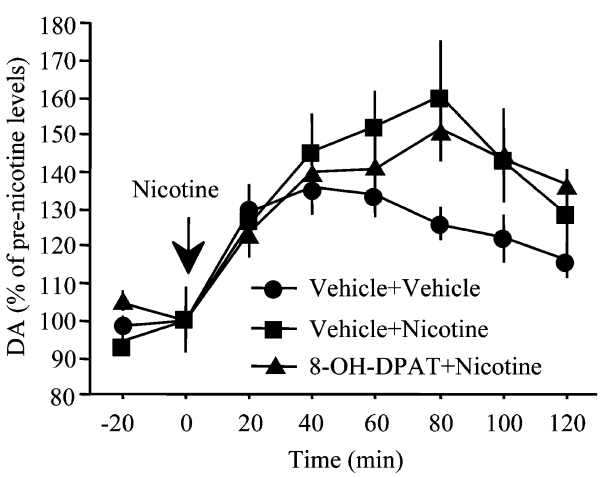

Fig. 5. Effect of nicotine challenge on the dopamine output in the nucleus accumbens after repeated daily treatment with vehicle + vehicle, vehicle + nicotine and 8-OH-DPAT + nicotine. Animals received 8-OH-DPAT (0.5 mg/kg s.c.) or vehicle 40 min before an injection of nicotine (0.35 mg/kg s.c.). Shown are the means ± S.E.M.; $n = 9–11$, all groups. Statistics: ANOVA followed by Fisher's PLSD test. Acute nicotine increased the dopamine levels in the nucleus accumbens compared to the pre-drug baseline ($P \leq 0.001$). Repeated treatment with nicotine alone ($P \leq 0.001$), or in combination with 8-OH-DPAT ($P \leq 0.05$), augmented the nicotine-induced dopamine output.

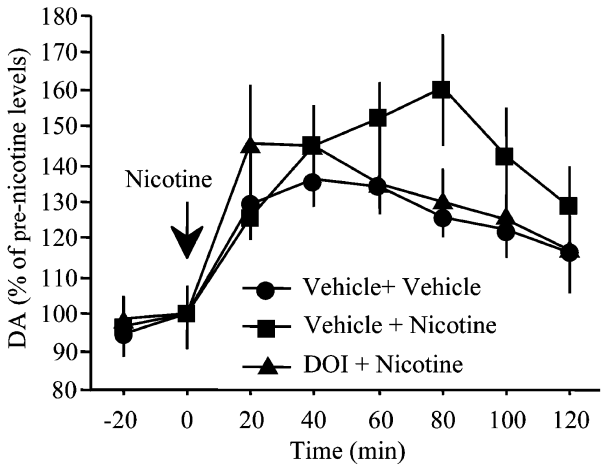

Fig. 6. Effect of nicotine on the dopamine output in the nucleus accumbens after repeated daily treatment with vehicle + vehicle, vehicle + nicotine or DOI + nicotine on the dopamine output. Animals received DOI (1.0 mg/kg s.c.) or vehicle 40 min before an injection of nicotine (0.35 mg/kg s.c.). Shown are the means ± S.E.M.; $n = 8–11$, all groups. Statistics: ANOVA followed by Fisher's PLSD test. Acute nicotine increased the dopamine levels in the nucleus accumbens compared to the pre-drug baseline ($P \leq 0.001$). Repeated treatment with nicotine alone, but not in combination with DOI, augmented the nicotine-induced dopamine output.

4. Discussion

Acute nicotine administration elevates the extracellular levels of dopamine in the nucleus accumbens (Benwell and Balfour, 1992; Imperato et al., 1986) and stimulates dopamine-dependent locomotor activity in rats habituated to the testing environment (Clarke, 1990; Louis and Clarke, 1998). In the present experiments, acute nicotine stimulated locomotor activity and repeated daily nicotine treatment produced sensitization to the nicotine-induced locomotor response, findings in line with the previous reports (Benwell and Balfour, 1992; Ksir et al., 1987; Olausson et al., 1999). The expression of drug-induced locomotor sensitization has been proposed to involve an increased nicotine-induced elevation of extracellular accumbal dopamine

levels (Balfour et al., 1998; Benwell and Balfour, 1992; Cadoni and Di Chiara, 2000; but see Nisell et al., 1996) increasing the stimulation of postsynaptic dopamine receptors in the nucleus accumbens. This view was supported by the present experiments since repeated daily nicotine treatment increased the nicotine-induced elevation of the extracellular dopamine levels in the nucleus accumbens. The recent report by Cadoni and Di Chiara (2000) has indicated that the nicotine-induced neurochemical sensitization may vary in the different regional compartments of the nucleus accumbens (i.e. the core and the shell), and the response to nicotine was sensitized in the core, but not in the shell. In this study, the microdialysis probes were placed in close vicinity to the border between the core and shell regions of the nucleus accumbens, and the results obtained in the present experiments could therefore reflect alterations in any or both of the subregions.

Interestingly, treatment with 8-OH-DPAT + nicotine or DOI + nicotine did not potentiate the locomotor response after repeated daily exposure, possibly indicating that these 5-HT receptor agonists counteracted the induction and/or the expression of sensitization to the locomotor stimulatory properties of nicotine. This issue was further addressed in the post-sensitization experiments, and when tested 48 h after the last administration of the 5-HT receptor agonists, sensitized nicotine-induced locomotor stimulation was observed also in rats repeatedly treated with 8-OH-DPAT + nicotine or DOI + nicotine suggesting that treatment with the 5-HT receptor agonists did not affect the induction of nicotine sensitization. Furthermore, a single acute challenge with DOI before the administration of nicotine counteracted the expression of sensitization in vehicle + nicotine-treated animals. On the contrary, an acute injection of 8-OH-DPAT increased the nicotine-induced locomotor activity in sensitized vehicle + nicotine animals, similar to the potentiation of nicotine-induced locomotor stimulation produced by 8-OH-DPAT on treatment day 1. Taken together, these results may suggest that DOI, but not 8-OH-DPAT, counteracts the expression of nicotine-induced locomotor sensitization. These results corroborate our previous findings demonstrating that chronic citalopram treatment prevents the expression, but not the induction, of nicotine sensitization (Olausson et al., 1999).

The proposed relationship between locomotor sensitization and the elevated accumbal dopamine output observed in animals repeatedly exposed to nicotine suggested that DOI may attenuate the expression of nicotine sensitization by influencing the sensitized nicotine-induced elevation of the accumbal dopamine output. This notion was supported by the current microdialysis experiments in which repeated treatment with DOI + nicotine for 15 days did not produce sensitization to the effects on mesolimbic dopamine activity, whereas such sensitization was observed both after repeated vehicle + nicotine and 8-OH-DPAT + nicotine treatment. Available reports have suggested that systemic administration of 5-HT_2 receptor antagonists enhances the electrophysiological activity of the mesolimbic dopamine cells (North and Uchimura, 1989; Ugedo et al., 1989), indicating a tonic inhibition of the activity in these neurons mediated by 5-HT_2 receptors. Thus, it appears possible that the DOI-induced attenuation of the sensitized nicotine-induced response on the nucleus accumbens dopamine output may derive from the possible inhibitory influence of DOI on mesolimbic dopamine activity.

When tested in the elevated plus-maze, the nicotine-sensitized rats spent more time and made more entries on the open arms than animals receiving vehicle or acute nicotine, supporting our previous observations (Olausson et al., 1999). Since the normal behavior in the elevated plus-maze is inhibited (see Section 2.3), the increased exploration of the open arms reflects a nicotine-induced behavioral disinhibition in the nicotine-sensitized animals. Acute nicotine administration has previously been observed to produce a similar behavioral disinhibition in mice (Brioni et al., 1993), but in the present study, acute administration of nicotine did not alter the measures of behavioral inhibition in the elevated plus-maze. A lack of effect has also been reported after acute, repeated (6 days) and continuous nicotine exposure in rats (Balfour et al., 1986; Benwell et al., 1994).

Disinhibited behavior in the elevated plus-maze is often considered to reflect an alleviation of anxiety (Pellow et al., 1985; Pellow, 1986). However, behavioral disinhibition in animal models that invoke conflict situations, like the elevated plus-maze, may also reflect a loss of impulse control (Soubrié, 1986), especially when observed in animals with low 5-HT neurotransmission (Söderpalm and Svensson, 1999; Soubrié, 1986). Thus, together with our previous observations (Olausson et al., 1999), the present data may suggest that repeated nicotine treatment lowers inhibitory control. This notion is strongly supported by clinical findings that using several different measures have demonstrated that smokers display a disinhibited/impulsive personality (Bickel et al., 1999; Mitchell, 1999; von Knorring and Oreland, 1985).

Interestingly, the sensitized vehicle + nicotine rats also spent more time on, and made more entries onto the open arms of the elevated plus-maze than animals repeatedly treated with 8-OH-DPAT + nicotine. On the contrary, treatment with DOI + nicotine did not significantly alter the measures of nicotine-induced behavioral disinhibition. These results suggest that daily co-treatment with 8-OH-DPAT counteracts the expression of the behavioral disinhibition normally observed in nicotine-sensitized rats. Since nicotine-induced behavioral disinhibition is counteracted also by chronic citalopram (Olausson et al., 1999), it seems plausible that this effect is mediated via increased stimulation of postsynaptic 5-HT_{1A} receptors. Given the antagonistic effects of citalopram and 8-OH-DPAT, it is possible that the low impulse control observed after repeated nicotine treatment may rely on a nicotine-induced reduction of brain 5-HT neurotransmission (see above).

Interestingly, repeated nicotine exposure has been demonstrated to decrease the tissue levels of 5-HT in some brain regions (Benwell and Balfour, 1982; Kirch et al., 1987; Olausson et al., 2001). The relationship between 5-HT and impulsive behavior as well as drug intake has been described, and manipulations that attenuate brain 5-HT neurotransmission both increase impulsive behavior (Roy and Linnoila, 1988; Soubrié, 1986) and elevate the intake of various drugs of abuse (Engel et al., 1992; Roberts et al., 1994). Moreover, a high liability of rats to consume ethanol has been reported to be associated with increased impulsivity (Poulos et al., 1995). Thus, the disinhibitory effect produced by repeated nicotine (Olausson et al., 1999) or amphetamine (Olausson et al., 2000) exposure could be involved in the increased cocaine (Horger et al., 1992) and ethanol (Blomqvist et al., 1996; Fahlke et al., 1994) intake observed after such treatments.

The total number of entries made on the elevated plus-maze was also increased after repeated nicotine treatment. It has been argued that such drug-induced locomotor stimulation could result in non-specific disinhibition towards the elevated open arms, suggesting that the behavioral disinhibition observed in the nicotine-sensitized rats is but a consequence of the nicotine-induced locomotor stimulation. However, several studies have failed to observe a positive correlation between locomotor activity and the measures of behavioral inhibition in the elevated plus-maze (File et al., 1993; Ouagazzal et al., 1999; Pellow et al., 1985; Söderpalm et al., 1989). Moreover, since 8-OH-DPAT decreased the nicotine-induced disinhibition but not the total number of entries in the elevated plus-maze, whereas the opposite was true for DOI, the present results also indicate that the locomotor activity and behavioral inhibition observed in the elevated plus-maze are controlled by dissociable neural mechanisms. It should, however, be noted that the total number of entries was included as a covariable in the present statistical analysis of the elevated plus-maze results to control for possible unspecific drug-induced locomotor effects.

The current results provide further evidence that repeated daily nicotine treatment is associated with both locomotor sensitization and behavioral disinhibition, and that the expression of these behaviors can be modulated by specific agonists at 5-HT receptor subtypes. The dissociated effects of the 5-HT receptor agonists 8-OH-DPAT and DOI on sensitized nicotine-induced stimulation of mesolimbic dopamine function, locomotor activity and behavioral disinhibition support the notion that the expression of the locomotor sensitization and the behavioral disinhibition observed after repeated exposure to nicotine (Olausson et al., 1999) and amphetamine (Olausson et al., 2000) are mediated via separate neurochemical processes. Since both behavioral sensitization and disinhibition may be involved in the development and expression of addictive behaviors, drugs that block the expression of these phenomena could prove helpful in a wider treatment program for drug abuse. Indeed, some studies indicate that citalopram may reduce ethanol intake in large-scale consumers (for review, see LeMarquand et al. 1994). A Cochrane review has also concluded that some antidepressant agents (bupropion and nortriptyline) may aid smoking cessation (Hughes et al., 2000). However, although there are some indications that also selective 5-HT reuptake inhibitors facilitate smoking cessation, treatment with citalopram alone failed to lower the number of cigarettes smoked in heavy drinkers not motivated to quit smoking (Sellers et al. 1987). Given the strong behavioral manifestations of the smoking habit, it appears unlikely that treatment with a single serotonergic compound aimed at reducing craving and restoring inhibitory control will be sufficient to totally suppress the expression of all addictive behaviors in non-motivated individuals. Instead, a treatment program designed to address several of the components underlying smoking, including pharmacological treatments that reduce craving and disinhibitory behaviors, may have a significantly higher rate of success. Indeed, the selective serotonin reuptake inhibitor paroxetine was recently demonstrated to increase abstinence rates over placebo when used in combination with a transdermal nicotine substitution therapy (Killen et al., 2000). Further studies on the effect of 5-HT activity enhancing drugs, e.g. citalopram, as one part of a smoking cessation treatment program are therefore warranted.

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