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Effects of nicotine and epibatidine on locomotor activity and conditioned place preference in rats

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Abstract

We studied the effects of nicotine and epibatidine given s.c. acutely and repeatedly, on locomotor activity and conditioned place preference (CPP) in rats. Nicotine at 0.5 mg/kg immediately and at 0.8 mg/kg after a delay increased the locomotor activity and its locomotor stimulant effects were greatly sensitized (about fourfold) when it was given repeatedly. Acute epibatidine at 0.6 and 3.0 μg/kg increased the activity modestly after a delay. When given repeatedly epibatidine's stimulant effects, mainly those at 3.0 μg/kg, were somewhat sensitized (less than twofold). Nicotine at 0.5 and 0.8 mg/kg produced CPP in rats in a biased paradigm. Epibatidine elicited CPP at very low dose (0.1 μg/kg), but at 0.3 or 0.6 μg/kg it induced neither preference nor aversion and at the 3.0 μg/kg dose it was aversive. Both acutely and after the repeated administration, epibatidine enhanced the locomotor activity of rats clearly less than nicotine agreeing with its previously reported lesser effects on accumbal dopamine output. Thus, while nicotine elicits CPP at doses (0.5 and 0.8 mg/kg) equal to those that increase accumbal dopamine output and locomotor activity, epibatidine seems to be aversive at the dose (3.0 μg/kg) that enhances accumbal dopamine output and increases locomotor activity.

Keywords: Nicotine; Epibatidine; Locomotor activity; Conditioned place preference; Rat

1. Introduction

Similarly to other drugs of abuse, nicotine induces drug-seeking behaviour as demonstrated by self-administration and conditioned place preference (CPP) experiments in animals (Stolerman, 1991). In addition, nicotine stimulates locomotor activity in habituated rats, an effect that becomes enhanced when nicotine is given repeatedly (Benwell and Balfour, 1992; Ksir et al., 1985; O'Neill et al., 1991). This sensitized behavioural response to nicotine and other addictive drugs has been suggested to be associated with neuronal adaptations that lead to drug dependence (for reviews see Balfour et al., 2000; Robinson and Berridge, 1993). Nicotine stimulates cerebral dopaminergic transmission and particularly the stimulation of the mesolimbic/mesocortical dopaminergic pathway is thought to relate to its rewarding, reinforcing and locomotor stimulant effects (Balfour et al., 2000; Clarke et al., 1988; Corrigall et al., 1992). In addition, the aversive motivational effects of nicotine have recently been suggested to depend upon mesolimbic dopamine (DA) signalling (Laviolette and van der Kooy, 2003), although other neurotransmitters such as serotonin also play a role in these effects (File et al., 2000).

Nicotine's effects are mediated by neuronal nicotinic acetylcholine receptors (nAChRs) (Stolerman, 1991). (±)-Epibatidine is a nAChR agonist that displays higher relative affinities for α4β2, α3β2, α3β4 and α7 nAChR subtypes than nicotine (Gerzanich et al., 1995). Epibatidine acts as a partial agonist at α4β2 nAChR subtype and causes a pronounced inhibition of agonist-evoked currents at non-activating concentrations (Buisson et al., 2000). Nicotine has repeatedly been reported to preferentially elevate the DA output in the nucleus accumbens, compared to the caudate–putamen (Benwell and Balfour, 1997; Imperato et al., 1986; Seppa and Ahtee, 2000). We recently found that epibatidine, in contrast to nicotine, increased the DA output at lower doses (0.6 μg/kg) in the caudate–putamen than in the nucleus accumbens (3.0 μg/kg) (Janhunen and Ahtee, 2004). This suggests that epibatidine may be less rewarding and stimulate locomotor activity to a

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lesser extent than nicotine. Indeed, acute epibatidine increased less than nicotine the activity of rats (Reuben et al., 2000) and mice did not self-administer epibatidine, but they did self-administer nicotine (Rasmussen and Swedberg, 1998). However, the effects of epibatidine on locomotor activity have not previously been studied after repeated administration nor have its effects on the CPP been investigated.

To investigate whether the differences in the effects of nicotine and epibatidine on the accumbal DA release are reflected in the behavioural effects of these drugs we studied the effects of repeated nicotine and epibatidine on locomotor activity and the CPP. The 0.5 mg/kg dose of nicotine is about equieffective with the 3.0 μg/kg dose of epibatidine, as regards analgesia, hypothermia and antidiuresis (Lembeck, 1999; Sullivan et al., 1994). Previously, acute nicotine at 0.4 mg/kg and epibatidine at 3.0 μg/kg have been found to produce maximal locomotor stimulation in rats (Clarke and Kumar, 1983; Menzaghi et al., 1997). In CPP studies in rats, nicotine at the 0.1–2.0 mg/kg doses either induced place preference or aversion, or failed to alter these conditioned responses (Calcagnetti and Schechter, 1994; Clarke and Fibiger, 1987; Fudala et al., 1985; Fudala and Iwamoto, 1986; Jorenby et al., 1990; Le Foll and Goldberg, 2005). The quality of the response depends on the nicotine dose and experimental set-up (Calcagnetti and Schechter, 1994; Fudala et al., 1985; Fudala and Iwamoto, 1986; Vastola et al., 2002). Recently, Le Foll and Goldberg (2005) extensively compared the development of CPP to nicotine in rats using the unbiased and biased design and concluded that the biased design in which the drug is paired with an initially non-preferred compartment is more suitable than the unbiased design for evaluation of nicotine's rewarding effects. Thus, the biased design may also better reveal epibatidine's effects on the CPP. The studied doses of nicotine (0.5 and 0.8 mg/kg) and epibatidine (0.1, 0.3, 0.6 and 3.0 μg/kg) were chosen on the basis of our present locomotion study and previous microdialysis study (Janhunen and Ahtee, 2004).

2. Materials and methods

2.1. Subjects and drugs

Male Wistar rats (240–320 g, Harlan, The Netherlands) were housed in groups of four at an ambient temperature of 20–22 °C. A 12-h light–dark cycle was imposed with lights on at 06:00 h. The rats had free access to food pellets and tap water. The experimental design was approved by the Committee for Animal Experiments of the University of Helsinki. All experiments were conducted according to the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (European Communities Council Directive of 24 November 1986; 86/609/EEC).

(−)-Nicotine base (Fluka Chemie, Switzerland) and (±)-epibatidine hydrochloride (Sigma, MO, USA) were dissolved in saline. The pH of the nicotine solution was adjusted to 7.0–7.4 with 0.05 M HCl. All doses refer to free base.

2.2. Locomotor activity

The rats were brought daily 30 min before the experiments to a room reserved for the behavioural experiments. The experiments were conducted between 08:00 and 14:00 h. Drugs or saline was administered daily at the same time (±1 h) to each rat. The rats were individually placed in Plexiglas boxes ($43 \times 43 \times 30$ cm^3; MED Associates ENV-515, GA, USA). A computer registered the interruptions of infrared photo beams. In the locomotion studies, the rats were habituated to the boxes on two consecutive days for 120 min each and on the second day the rats were given saline (0.9% NaCl solution, 1 ml/kg s.c.) immediately before the habituation. On the following day, test day 1, the rats were given nicotine (0.5 or 0.8 mg/kg s.c.), epibatidine (0.6 or 3.0 μg/kg s.c.) or saline (1 ml/kg s.c.) and the activity was measured immediately at 5 min intervals for 120 min. The drugs were given daily on five consecutive days and the activity was measured on test days 1, 3 and 5. On days 2 and 4 the rats were given the drugs in their home cages without being exposed to the activity monitoring boxes. On test day 5, some control rats previously treated with saline were acutely given either nicotine (0.5 or 0.8 mg/kg) or epibatidine (0.6 or 3.0 μg/kg) and the rest of the control rats were given saline. To further investigate the effect of repeated epibatidine, the rats repeatedly given epibatidine (or saline as control) were given an additional challenge dose of epibatidine (or saline) on test day 7. On day 6 the rats received no treatment.

2.3. Place conditioning

The CPP apparatus consisted of two equally sized compartments ($41 \times 41 \times 28$ cm^3) that were separated by a black wall with guillotine door (MED Associates ENV-515, GA, USA). Tactile (a grid rod floor or a wire mesh floor) and visual (a white or a black layer outside the compartment walls) cues differentiated the compartments. Computer-registered interruptions of infrared photo beams were used to determine the position of rat in the apparatus and to measure the locomotor activity. White noise was used to cover background noise. Each rat was handled for 5 min on two days before experiments. The rats were brought daily 30 min before the experiments to a room reserved for the CPP experiments. The experiments were conducted between 08:00 and 15:00 h. Drugs or saline was administered daily at the same time (±1 h) to each rat. Each experiment was performed over six consecutive days and consisted of three phases: habituation (days 1 and 2, one session per day), conditioning (days 3, 4 and 5, two sessions per day) and testing (day 6, one session).

For the habituation the rats were placed in the chambers with free access to both compartments (door open) for 30 (nicotine) or 60 min (epibatidine). Only rats that initially spent more time in the black compartment were included in the data (165 of 176 rats). The time that the rat spent in the non-preferred white compartment during the first 15 min on the habituation day 2 was used as the initial preference level (preconditioning time). A further nine rats, the preconditioning

times of which considerably (>70%) differed from the mean times of groups, were excluded from the data. The preconditioning times were 246±8 s (mean±SEM, $N=92$) in the first group of studies and 244±11 s ($N=64$) in the second, verifying studies. The data from the two studies were combined, because there were no differences. In the morning of the three conditioning days, the rats were given saline and immediately exposed to the black compartment (door closed). At noon, the rats were given nicotine (0.5 or 0.8 mg/kg), epibatidine (0.1, 0.3, 0.6 or 3.0 μg/kg) or saline (controls) and immediately exposed to the white (drug-paired) compartment (door closed). The conditioning time was 20 min for nicotine (Fudala et al., 1985; Fudala and Iwamoto, 1986) and 60 min for epibatidine. These conditioning times were selected on the basis of the time points of the maximal locomotor stimulant effects to ensure the full effects of the drugs. However, it should be noted that locomotor activity and CPP are most probably regulated by differing cerebral mechanisms. On day 6, preference for compartments was tested without treatments in a similar way to the testing on habituation day 2 (door open). The time spent in the drug-paired compartment during the first 15 min was measured (postconditioning time) and difference from the preconditioning time was calculated. When the change was positive, a drug was considered to induce place preference and when the change was negative, a drug was considered to induce place aversion.

2.4. Statistics

Data are expressed as means±SEM. The activity data were analysed with one- or two-way analysis of variance (ANOVA) for repeated measures (days or time points) (GraphPad Prism 3.0, GraphPad Software, CA, USA). The CPP data were analysed with one-way ANOVA. When appropriate ($p<0.05$), multiple comparisons were conducted using Student Newman Keuls post hoc test or by Student's t-test (the effect of the 3.0 μg/kg dose of epibatidine on the CPP).

3. Results

3.1. Locomotor activity

Fig. 1 shows the effects of nicotine and epibatidine on the locomotor activity during the first 60 min after their administration. Acute nicotine (Test day 1) at 0.5 mg/kg significantly increased the activity during the first 60 min, compared to

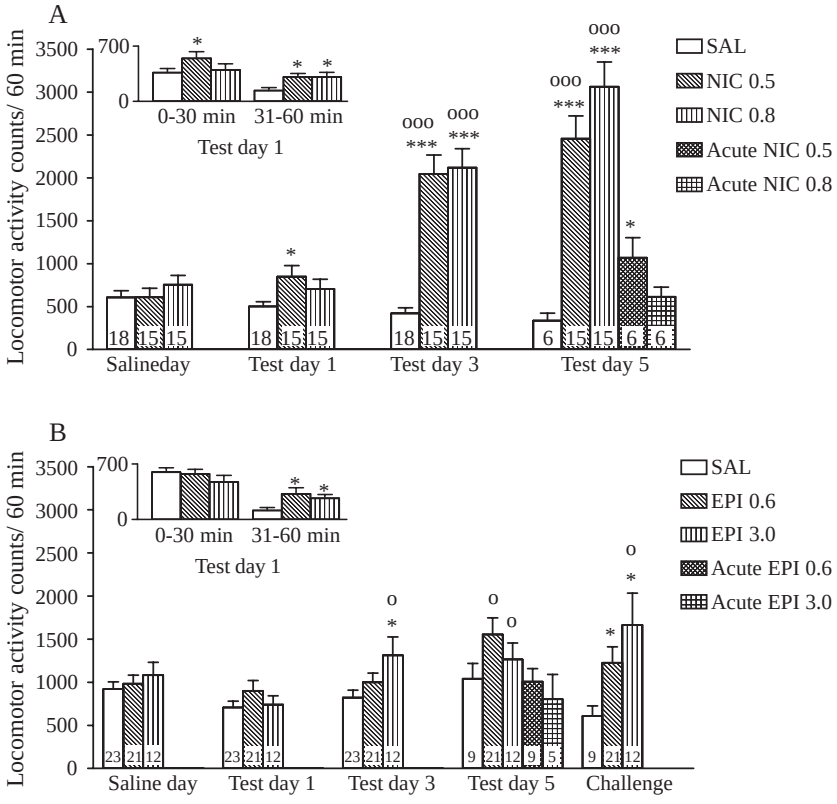

Fig. 1. Effects of daily nicotine (Panel A) and epibatidine (Panel B) treatments on locomotor activity in rats. Nicotine (NIC, 0.5 or 0.8 mg/kg) or epibatidine (EPI, 0.6 or 3.0 μg/kg) was given s.c. daily on five consecutive days immediately before the 60 min measurement period. The locomotor activity was measured on the first (Test day 1), the third (Test day 3) and the fifth day (Test day 5), and in the epibatidine experiments also after a further challenge dose on the seventh day (Challenge). Control rats received saline (SAL) s.c. on corresponding days. Saline day presents the second habituation day when locomotor activities of all six groups of rats were measured during the first 60 min after a saline injection. On test day 5, some of the saline control rats were acutely given nicotine or epibatidine, the effects of which are shown by the fourth and fifth columns giving the results on test day 5. The inserts show the locomotor activities during 0–30 min and 31–60 min on test day 1 after administration of nicotine, epibatidine or saline. All data are expressed as means±SEM. The numbers in the columns give the numbers of animals per each group. Student Newman Keuls post hoc test after ANOVA: *$p<0.05$, ***$p<0.001$ vs. corresponding SAL controls, $^{O}p<0.05$, $^{OOO}p<0.001$ vs. Test day 1.

saline [Fig. 1A; $F(2,45)=3.3$, $p=0.0125$, post hoc $p<0.05$]. The 0.8 mg/kg dose increased the activity only during the second 30 min (Fig. 1A Insert), and thus the increase during the 60 min period failed to reach significance. During the second test hour nicotine significantly increased the locomotor activity at both doses [mean counts/61–120 min±SEM: saline: 164±46, 0.5 mg/kg: 626±82, 0.8 mg/kg: 901±186, $N=15$–18; $F(2,45)=11.2$, $p=0.0001$, post hoc $p<0.01$ for NIC 0.5, $p<0.001$ for NIC 0.8]. On test days 3 and 5, one-way ANOVA revealed significant treatment-effects [Fig. 1A; day 3: $F(2,45)=31.7$, $p<0.0001$, day 5: $F(2,33)=13.5$, $p<0.0001$] and nicotine at both doses significantly increased the locomotor activity during the first 60 min (post hoc $p<0.001$). When some of the control rats repeatedly treated with saline were acutely given nicotine on test day 5, its effects on the activity were similar to those found after acute nicotine on test day 1. The 0.5 mg/kg dose increased the activity significantly during both hours, but 0.8 mg/kg only during the second hour [Fig. 1A; 0–60 min: $F(2,15)=5.5$, $p=0.0163$, post hoc $p<0.05$ for NIC 0.5; mean counts/61–120 min±SEM: saline: 95±59, 0.5 mg/kg: 449±88, 0.8 mg/kg: 565±74, $N=6$; $F(2,15)=10.8$, $p=0.0013$, post hoc $p<0.01$ for both doses]. Two-way ANOVA for repeated measures revealed significant treatment-effect [$F(2,33)=13.1$, $p<0.0001$] and treatment × day(1, day(1, 3 and 5)-interaction [$F(4,66)=8.9$, $p<0.0001$]. Thus, the rats repeatedly given nicotine 0.5 or 0.8 mg/kg were significantly more active on test days 3 and 5 than on test day 1, compared to the controls (Fig. 1A; post hoc $p<0.001$).

Epibatidine's effect on the locomotor activity lasted for about 60 min after both acute and repeated administration. Fig. 2 shows that the activity of the control rats was transiently increased during the first 10 min. On test day 1, epibatidine 3.0 μg/kg significantly inhibited this increase [Fig. 2; $F(2,53)=4.7$, $p=0.0138$, post hoc $p<0.05$]. Although acute epibatidine did not significantly alter the activity during the first 30 min or the total 60 min activity, one-way ANOVA revealed that both epibatidine doses significantly increased the activity during 31–60 min [Fig. 1B Insert; $F(2,53)=3.9$, $p=0.0253$, post hoc $p<0.05$]. However, on test day 5 when acute epibatidine was given to the control rats previously treated with saline such stimulation was not seen (data not shown).

Figs. 1B and 2 show that the stimulant effects of both epibatidine doses were enhanced after the repeated administration; the effects of the 3.0 μg/kg dose more markedly than those of 0.6 μg/kg. According to one-way ANOVA, the rats given repeated epibatidine 0.6 μg/kg were on the challenge day significantly more active than the corresponding controls [$F(2,39)=3.4$, $p=0.0455$, post hoc $p<0.05$]. The 3.0 μg/kg dose of epibatidine significantly increased the locomotor activity on test day 3 [$F(1,33)=6.4$, $p=0.0167$] and on the challenge day [$F(2,39)=3.4$, $p=0.0455$, post hoc $p<0.05$].

Two-way ANOVA for repeated measures did not reveal any significant treatment-effects or treatment × day(1, 3 and 5)-interactions for epibatidine during the five test days, but one-way ANOVA revealed that the rats given epibatidine 0.6 μg/kg were more active on test day 5 than on test days 1 and 3

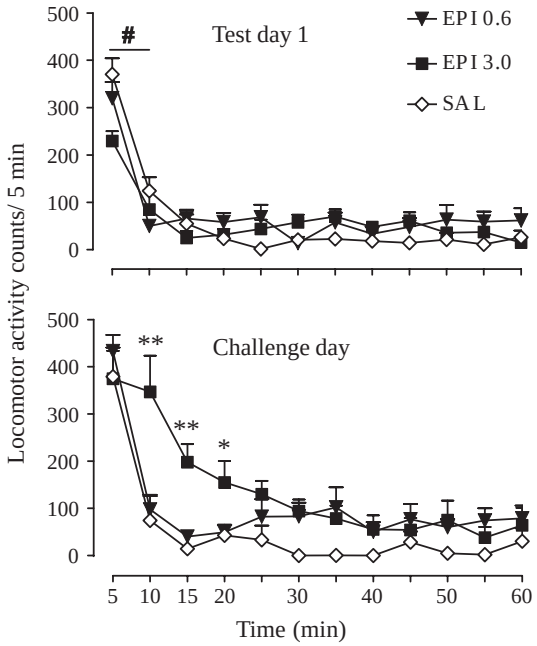

Fig. 2. Effects of acute epibatidine on locomotor activity of naïve rats (Test day 1) and after repeated treatment (Challenge). Epibatidine (EPI, 0.6 or 3.0 μg/kg) or saline (SAL) was given s.c. daily on five consecutive days and as a further challenge dose on the seventh day. On day 6 the rats received no treatment. Given are the locomotor activities at 5 min intervals during the 60 min on the first day (Test day 1) and on the seventh day (Challenge day) (means±SEM, $N=12$–23). Student Newman Keuls post hoc test after ANOVA: $^{*}p<0.05$, $^{**}p<0.01$ EPI 3.0 vs. SAL/EPI 0.6; the horizontal line indicates 0–10 min data, $^{\#}p<0.05$ EPI 3.0 vs. SAL.

[$F(2,60)=6.0$, $p=0.0043$, post hoc $p<0.05$]. The rats given epibatidine 3.0 μg/kg were more active on test days 3 and 5 than on test day 1 [$F(2,33)=3.3$, $p=0.0489$, post hoc $p<0.05$]. On the challenge day, two-way ANOVA revealed that there was a significant treatment-effect [$F(1, 38)=5.8$, $p=0.0207$] and treatment × day(Test day 1 and Challenge)-interaction [$F(1, 38)=4.5$, $p=0.0400$], and the locomotor activity of the rats repeatedly treated with epibatidine 3.0 μg/kg was significantly enhanced from that on test day 1, compared to the controls.

As shown in Fig. 2, the enhanced stimulant effect of epibatidine 3.0 μg/kg on the challenge day was mainly due to a change during the first 30 min, while 0.6 μg/kg increased the activity during the second 30 min. Thus, the 3.0 μg/kg dose significantly increased the activity during 6–20 min, compared to the 0.6 μg/kg dose and saline [6–20 min: $F(2,39)=8.3$, $p=0.0009$, post hoc $p<0.05$].

3.2. Place conditioning

In the CPP studies, nicotine at 0.5 and 0.8 mg/kg significantly increased the time spent in the drug-paired compartment [Fig. 3; $F(2,53)=5.7$, $p=0.0058$, post hoc $p<0.01$ for NIC 0.5, $p<0.05$ for NIC 0.8]. Epibatidine significantly increased the time spent in the drug-paired compartment at the lowest dose studied, 0.1 μg/kg (63±31 s, mean±SEM, $N=24$), but not anymore at the higher doses studied [Fig. 3; $F(4,101)=5.7$, $p=0.0004$, post hoc $p<0.05$

for EPI 0.1]. After conditioning, the control rats given saline spent less time in the initially non-preferred compartment than during the habituation (nicotine exp.: −44±22 s, mean±SEM, $N=21$; epibatidine exp.: −41±22 s, $N=24$). Epibatidine induced at 0.3, 0.6 and 3.0 μg/kg a dose-dependent decrease in the time spent in the drug-paired compartment (Fig. 3). The highest dose, 3.0 μg/kg, decreased the time even more than saline in the control rats, and indeed, Student's *t*-test revealed a significant difference between the control rats and the rats given epibatidine 3.0 μg/kg ($p=0.031$). When compared to the rats given epibatidine 0.1 μg/kg, the rats given epibatidine 3.0 μg/kg spent significantly less time in the drug-paired compartment (post hoc $p<0.001$).

3.3. Locomotor activity during the place conditioning

When the locomotor activities of the rats in the drug-paired compartment on the conditioning days were analysed by one-way ANOVA, they resembled the activities measured in the locomotion studies described above. Nicotine significantly increased the activity on days 1 and 3 at the 0.5 mg/kg dose, compared to the control rats given saline [Table 1; day 1: $F(2,53)=5.9$, $p=0.0049$, post hoc $p<0.01$; day 3: $F(2,53)=11.2$, $p<0.0001$, post hoc, $p<0.001$]. The acute 0.8 mg/kg dose of nicotine did not alter the activity during the first 20 min on the conditioning day 1, but increased it significantly on the day 3 (post hoc $p<0.05$). Epibatidine at 0.1, 0.3 or 0.6 μg/kg did not alter the activity on any of the conditioning days (Table 1). However, the 3.0 μg/kg dose of epibatidine significantly increased the activity on conditioning day 3 [Table 1; $F(4,93)=6.2$, $p=0.0002$, post hoc $p<0.001$].

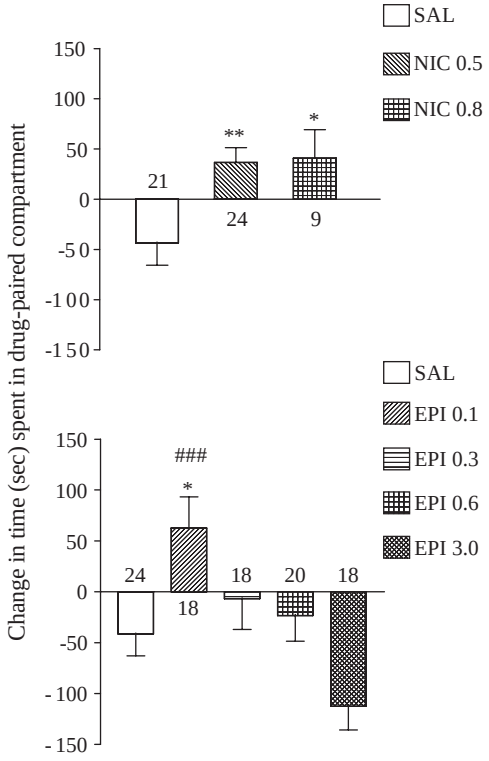

Fig. 3. Effects of nicotine and epibatidine on the place conditioning in rats. The figure presents the changes in the preconditioning and postconditioning times (in seconds) spent in the drug-paired compartment during 0–15 min. During conditioning, the rats were given nicotine (NIC, 0.5 or 0.8 mg/kg) or epibatidine (EPI, 0.1, 0.3, 0.6 or 3.0 μg/kg) s.c. on three consecutive days. The control rats were given saline (SAL) s.c. The columns show means±SEM. The numbers next to the columns give numbers of animals per each treatment group. Student Newman Keuls post hoc test after ANOVA: $*p<0.05$, $**p<0.01$ vs. SAL controls, $^{###}p<0.001$ vs. EPI 3.0.

Table 1
The effects of nicotine (NIC, 0.5 or 0.8 mg/kg), epibatidine (EPI, 0.1, 0.3, 0.6 or 3.0 μg/kg) and saline (SAL) on the locomotor activity of rats in the drug-paired compartment during the first 20 min on the first and third conditioning day

	Conditioning day 1	Conditioning day 3
SAL 1	236±34	178±30
NIC 0.5	474±74**	550±73***
NIC 0.8	182±59	416±54*
SAL 2	233±32	193±28
EPI 0.1	361±49	207±35
EPI 0.3	284±35	205±29
EPI 0.6	256±49	175±24
EPI 3.0	276±43	388±35***

Given are means±SEM, $N=9–24$. Student Newman Keuls post hoc test after ANOVA: $*p<0.05$, $**p<0.01$, $***p<0.001$ vs. corresponding SAL controls. SAL 1 includes the saline controls in the nicotine experiments and SAL 2 includes the saline controls in the epibatidine experiments.

4. Discussion

Our experiments were carried out using habituated rats. Previous experiments have demonstrated that the locomotor activity of habituated rats is stimulated by nicotine and epibatidine (Clarke and Kumar, 1983; Menzaghi et al., 1997). As reported previously (Benwell and Balfour, 1992; Ksir et al., 1985; O'Neill et al., 1991), in the present study acute nicotine increased the activity at 0.5 mg/kg immediately and at 0.8 mg/kg after half-an-hour a delay up to 2 h. Repeated exposure to saline injections and the test apparatus did not alter nicotine's acute stimulant effects on test day 5. Consistent with a previous study (Reuben et al., 2000), acute epibatidine stimulated the activity to a lesser extent than acute nicotine. Epibatidine at 3.0 μg/kg depressed the activity during the first 10 min, but at 0.6 and 3.0 μg/kg it increased the activity during 31–60 min after administration. These findings agree with earlier studies in which acute epibatidine (3.0 μg/kg) depressed the activity of habituated rats for 10–15 min, but increased it between 30 and 40 min after its administration (Menzaghi et al., 1997; Reuben et al., 2000; Sacaan et al., 1996).

Repeated administration of nicotine (0.5 and 0.8 mg/kg) enhanced its locomotor stimulant effects. This psychomotor sensitization has been suggested to be due to enhanced mesolimbic dopaminergic transmission (Balfour et al., 2000; Benwell and Balfour, 1992). Also epibatidine's stimulant effects were enhanced after repeated administration, particularly those at the 3.0 μg/kg dose. Indeed, the initial depressant effect of the 3.0 μg/kg dose was attenuated after repeated administration. However, repeated epibatidine did not produce such a clear and rapid sensitization as nicotine and it stimulated the activity less than nicotine. The sensitization was more

pronounced at 3.0 μg/kg than at 0.6 μg/kg, which finding is consistent with our previous findings that epibatidine elevated the accumbal DA output at the 3.0 μg/kg dose, but not at 0.6 μg/kg (Janhunen and Ahtee, 2004). In contrast, the 0.6 μg/kg dose of epibatidine, but not that of 3.0 μg/kg, elevated DA output in the caudate–putamen (Janhunen and Ahtee, 2004). Thus, epibatidine-induced enhancement of locomotor activity appears to relate to increased mesolimbic dopaminergic transmission, as suggested previously for nicotine (Balfour et al., 2000; Benwell and Balfour, 1992; Clarke et al., 1988).

In the present CPP paradigm, nicotine (0.5 and 0.8 mg/kg) increased the time spent in the drug-paired compartment, which suggests that it induced place preference. These findings agree with previous studies, in which nicotine induced CPP in the biased design (Calcagnetti and Schechter, 1994; Fudala et al., 1985; Fudala and Iwamoto, 1986; Le Foll and Goldberg, 2005; Vastola et al., 2002). Nicotine can induce either place preference or aversion depending on experimental set-up (for details see Calcagnetti and Schechter, 1994; Clarke and Fibiger, 1987; Fudala and Iwamoto, 1987; Jorenby et al., 1990; Le Foll and Goldberg, 2005). However, there is some debate about the biased design. So instead of reflecting the rewarding effects of the drug, the drug-induced shift of preference may reflect other effects of the drug such as its ability to reduce aversive, anxiogenic properties of the initially non-preferred compartment. This possibility of reduced aversion, rather than increased preference also remains in our present results. Nicotine has been found to elicit anxiolytic effects on rats at low doses (up to 0.1 mg/kg i.p.) and anxiogenic effects at high doses (0.5–1.0 mg/kg i.p.) under conditions that generate moderate level of anxiety and thus resemble conditions in the present study (File et al., 1998). Thus, the effects of nicotine at 0.5 and 0.8 mg/kg on the CPP in our study most probably did not result from its anxiolytic properties.

In the present study, epibatidine induced CPP at 0.1 μg/kg, but not at 0.3, 0.6 or 3.0 μg/kg. Epibatidine has been reported to lack anxiolytic activity in mice (Sullivan et al., 1994), which suggests that the conditioning effect of the 0.1 μg/kg dose in the present study might be due to epibatidine's rewarding and reinforcing effects. Epibatidine's rewarding effects have not previously been studied using the CPP. However, mice did not self-administer epibatidine at doses 0.25–1.25 μg/kg (i.v.), although they self-administered nicotine (up to 0.075 mg/kg i.v.), which suggests that epibatidine's rewarding effects differ from those of nicotine (Rasmussen and Swedberg, 1998). The mesolimbic dopaminergic pathway is thought to mediate the motivational effects of nicotine (Corrigall et al., 1992), but also projections from tegmental pedunculopontine nucleus appear to play an important role in nicotine reward signalling in the ventral tegmental area (Laviolette et al., 2002). In our previous microdialysis study, epibatidine at 0.1, 0.3 or 0.6 μg/kg did not alter accumbal DA output, although it slightly, but significantly elevated the DA metabolites and the serotonin metabolite 5-hydroxyindoleacetic acid (Janhunen and Ahtee, 2004). Thus, in the present study the epibatidine-induced CPP occurred transiently only at a dose that was far lower than the doses that elevate accumbal DA output (Janhunen and Ahtee, 2004) and increasing the dose to such ones that elevate striatal DA output (Janhunen and Ahtee, 2004) attenuated these effects. Furthermore, the 0.1 μg/kg dose of epibatidine that elicited CPP was not found to increase locomotor activity during the conditioning period, and thus, epibatidine's effect does not seem to relate to stimulation of locomotor activity or to be due to increased tone of mesolimbic dopaminergic pathway. Interestingly, nicotine has been suggested to elicit self-administration at such low doses that fail to stimulate mesoaccumbens DA (Chiamulera et al., 1996). Indeed, other neurotransmitters such as endogenous opioids may play a role in the acquisition of reinforcement by nAChR agonists (Pomerleau, 1998) and thus in the conditioning effects of the 0.1 μg/kg dose of epibatidine.

In the present study, the 3.0 μg/kg dose of epibatidine elicited even aversive effects in rats. Strong attention-generating events such as aversive stimuli may activate DA neurons (Schultz, 2000), and indeed, nicotine's aversive motivational effects have been suggested to depend upon mesolimbic DA signalling (Laviolette and van der Kooy, 2003). The 3.0 μg/kg dose of epibatidine has been found to increase accumbal DA output (Janhunen and Ahtee, 2004), which supports the view that the aversive effects of higher doses of epibatidine may be related to the stimulation of mesolimbic DA neurons. Further support for the idea that increased accumbal DA release also underlies the stimulation of locomotor activity gives our finding that the 3.0 μg/kg dose of epibatidine that induced conditioned place aversion also increased the locomotor activity during the conditioning session when it was repeatedly given to rats.

As described above, the effects of nicotine and epibatidine on the locomotor activity as well as on the place conditioning differ to some degree. Furthermore, we previously reported that in contrast to nicotine, which preferentially enhances accumbal DA output as compared with dorsal striatal DA output, epibatidine enhances accumbal DA output at doses which are 3–5 times larger than the doses that enhance DA output in the dorsal striatum (Janhunen and Ahtee, 2004). The differences between nicotine and epibatidine may be due to their different affinities for the nAChR subtypes that mediate effects on neurotransmitters that regulate locomotor activity and motivation. Nicotine has a high binding affinity for α4β2 nAChR subtype and about 1000-fold lower affinity for α7 subtype. On the other hand, epibatidine has an extremely high affinity for α4β2 and even 10 000-fold lower affinity for α7 nAChR than for α4β2 (Gerzanich et al., 1995; Hahn et al., 2003). The β2-containing nAChRs have been suggested to be involved in the mediation of nicotine's stimulant and reinforcing effects (Picciotto et al., 1998) as well as in the regulation of striatal DA release (Salminen et al., 2005). Interestingly, epibatidine, in contrast to nicotine, acts as a partial agonist at α4β2 nAChR subtype and causes at non-activating concentrations a pronounced inhibition of agonist-evoked currents (Buisson et al., 2000). Thus, it is tempting to speculate that the different activation/desensitization properties of epibatidine and nicotine on nAChR subtypes such as those containing β2 subunit might

explain the present findings that epibatidine stimulates locomotor activity less than nicotine and that epibatidine, in contrast to nicotine, is not reinforcing as assessed by CPP at the doses that increase accumbal DA output and stimulate locomotor activity.

The present findings on the effects of repeated epibatidine on locomotor activity and CPP are novel. Epibatidine both acutely and repeatedly stimulated the activity clearly less than nicotine. This agrees with epibatidine's modest effect on accumbal DA, compared to nicotine (Janhunen and Ahtee, 2004; Seppa and Ahtee, 2000). Furthermore, epibatidine's stimulant effect was sensitized particularly at the repeated 3.0 μg/kg dose, the dose which elevates accumbal DA output (Janhunen and Ahtee, 2004). These findings agree with the suggested role of mesolimbic DA in the mediation of locomotor activity and its sensitization. In addition, epibatidine appears to be to some extent rewarding and reinforcing as assessed by the CPP. Epibatidine elicited place preference at a low dose, but in contrast to nicotine, increasing the dose to such that increased accumbal DA output and stimulated locomotor activity eliminated this effect and even induced aversive effects. The differences between epibatidine and nicotine are probably due to their different affinities for nAChR subtypes that mediate their effects on neurotransmitters involved in the regulation of locomotor stimulant and motivational effects. Although the locomotor stimulant effects of epibatidine seem to be connected with its effects on the mesolimbic DA system, that system seems to be only involved in epibatidine's aversive effects but not in the rewarding actions.

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