ELSEVIER

European Journal of Pharmacology 397 (2000) 103–111

ejp

www.elsevier.nl/locate/ejphar

Nicotine induces disinhibitory behavior in the rat after subchronic peripheral nicotinic acetylcholine receptor blockade

Mia Ericson *, Peter Olausson, Jörgen A. Engel, Bo Söderpalm

Institute of Physiology and Pharmacology, Department of Pharmacology, PO Box 431, SE-405 30 Göteborg, Sweden

Received 3 January 2000; received in revised form 25 February 2000; accepted 3 March 2000

Abstract

The present study investigated the effects of subchronic nicotine, mecamylamine and hexamethonium, alone or in combinations, on locomotor activity and behavioral inhibition. Rats were divided into groups and tested for locomotor activity after acute nicotine. The different groups received vehicle, nicotine, mecamylamine, mecamylamine + nicotine, hexamethonium (two different concentrations) and hexamethonium + nicotine injections once a day for 15 days after which they were tested for nicotine-induced locomotor activity again. Acutely, nicotine stimulated locomotor activity, and repeated daily nicotine or hexamethonium + nicotine administration sensitized the animals to this nicotine-induced locomotor stimulation (locomotor sensitization). Mecamylamine administered subchronically in combination with nicotine was able to block the induction to locomotor sensitization to nicotine. None of the nicotinic receptor antagonists induced locomotor sensitization to nicotine by themselves. In the elevated plus-maze, subchronic nicotine treatment demonstrated a nicotine-induced behavioral disinhibition, measured as an increase of time spent in and entries made into open arms. In contrast to the findings regarding locomotor sensitization, none of the antagonists counteracted the induction of this nicotine-induced behavioral disinhibition after subchronic co-treatment with nicotine. In addition, both antagonists by themselves produced a similar effect as subchronic nicotine, i.e. promoted the development of nicotine-induced disinhibitory behavior. It was concluded that the induction of locomotor sensitization to nicotine involves stimulation of central nicotinic acetylcholine receptors, whereas the development of nicotine-induced behavioral disinhibition involves blockade of peripheral nicotinic acetylcholine receptors, and that the latter, but not the former, phenomenon from a pharmacological point of view appears to be related to the increased ethanol consummatory behavior observed after subchronic nicotine administration.

Keywords: Nicotinic acetylcholine receptor; Sensitization; Disinhibition; (Rat); Locomotor activity; Plus-maze

1. Introduction

The mesocorticolimbic dopamine system, a major part of the brain reward system, originates in the ventral tegmental area and projects mainly to the nucleus accumbens and the prefrontal cortex (cf. Koob, 1992). This system is activated by drugs of abuse and is most likely involved in their reinforcing properties (Engel, 1977; Wise and Rompre, 1989), which, in turn, are believed to be connected to their addictive properties. The abuse of nicotine and ethanol is a major health problem in many countries, it is therefore urgent to further investigate the mechanisms involved in the addictive effects of these drugs.

Nicotine stimulates or depresses locomotor activity in rats depending on the dose, duration of exposure and administration procedure. Acute low doses of nicotine increase locomotor activity (e.g. Benwell and Balfour, 1992), whereas after high doses (> 0.6 mg/kg s.c.) locomotor activity is initially decreased and a stereotypic behavior may develop (Clarke and Kumar, 1983). The locomotor stimulatory effect of low doses of nicotine appears to involve activation of brain dopamine and noradrenaline systems (Lee, 1985; unpublished results from our laboratory). When nicotine (0.35 mg/kg s.c.) is administered intermittently over a period of 10–15 days sensitization to the locomotor stimulatory effects of the drug develops in habituated animals (locomotor sensitization; Ksir et al., 1985; Benwell and Balfour, 1992; Johnson et al., 1995a). The sensitization phenomenon consists of two phases, the induction of the phenomenon and its expression, which may involve overlapping but not necessarily identical neu-

* Corresponding author. Tel.: +46-31-773-3400; fax: +46-31-773-3284.

E-mail address: mia.ericson@pharm.gu.se (M. Ericson).

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00191-6

rochemical mechanisms, as exemplified by, e.g. the involvement of glucocorticoids in the induction but not the expression of nicotine-induced locomotor sensitization (Johnson et al., 1995b). Behavioral sensitization to drugs of abuse has been hypothesized to be involved in transforming the wanting of a drug into craving for the drug (Robinson and Berridge, 1993) and appears to be associated with long-lasting neurochemical changes in i.a. the mesocorticolimbic dopamine system (Kalivas and Stewart, 1991; Nestler, 1994).

Several clinical studies have demonstrated a high correlation between tobacco and alcohol use (Dreher and Fraser, 1967; Crowley et al., 1974; for a review see Bien and Burge, 1990). Thus, 90% of alcoholics smoke and alcoholism is 10 times more common among smokers than non-smokers. Moreover, an early smoking debut may be a predictive factor for future addiction to ethanol. Interestingly, in addition to the possible involvement of socioeconomic or psychological factors recent experimental work in animals suggests that this correlation may derive from pharmacodynamic similarities and interactions between nicotine and ethanol. Thus, both cross-tolerance and cross-sensitization have been observed between some aspects of nicotine and ethanol pharmacology (Burch et al., 1988; Johnson et al., 1995a; Blomqvist et al., 1996), and a series of publications from this laboratory indicates that the dopamine activating and reinforcing properties of ethanol involve a direct or indirect interaction between ethanol and nicotinic acetylcholine receptors in the ventral tegmental area (Blomqvist et al., 1992, 1996, 1997; Ericson et al., 1998). Furthermore, Blomqvist et al. (1996), and others (Pothoff et al., 1983; Gauvin et al., 1993), have demonstrated that subchronic nicotine administration increases voluntary ethanol intake in rats. It was hypothesized that this increase is due to the development of cross-sensitization between nicotine and ethanol, and, indeed, cross-sensitization as regards the mesolimbic dopamine activating effects of the drugs was reported (Blomqvist et al., 1996).

However, in a recent study subchronic intermittent administration of the tertiary nicotinic receptor antagonist mecamylamine or the quaternary nicotinic receptor antagonist hexamethonium, alone or in combination with nicotine, increased voluntary ethanol intake and preference to the same extent as did nicotine by itself (Ericson et al., 2000). Based on these and other findings it was hypothesized that blockade of peripheral nicotinic receptors rather than stimulation of central nicotinic receptors is involved in nicotine-induced enhancement of ethanol consumption. Since nicotine-induced locomotor sensitization has been suggested to involve central rather than peripheral nicotinic receptors (Clarke and Kumar, 1983), these findings may argue against the idea that the cross-sensitization phenomenon (between nicotine and ethanol) may involve different levels of interaction depending on the pharmacological effect studied.

It has recently been demonstrated that subchronic intermittent administration of nicotine to rats is associated with the development of a nicotine-induced disinhibitory behavior, as measured in Montgomery's elevated plus-maze (Olausson et al., 1999). This disinhibition was counteracted by concomitant subchronic treatment with citalopram, a selective serotonin reuptake inhibitor, indicating that the phenomenon may involve serotonin. The nicotine-induced disinhibition in this study was interpreted to reflect increased impulsivity rather than anxiolysis, since, based on other findings in the literature, it appeared more likely that subchronic citalopram would counteract an impulsive act than an anxiolytic effect (cf. Olausson et al., 1999, for discussion). Interestingly, increased impulsivity, signs of a deficient serotonergic system, and enhanced ethanol consumption appear to be linked in the rat (see e.g. LeMarquand et al., 1994). Thus, there is a possibility that this particular disinhibitory effect after subchronic nicotine is more closely related to the enhanced ethanol consumption observed after such a treatment than is the development of nicotine induced locomotor sensitization outlined above, and, in turn, that these two nicotine-induced effects may at least partly involve different mechanisms.

The present experiments were performed in order to investigate whether the induction of nicotine-induced locomotor sensitization and/or of nicotine-induced behavioral disinhibition involve subchronic intermittent stimulation or blockade of central or peripheral nicotinic receptors.

2. Materials and methods

2.1. Animals

Male Wistar rats, weighing approximately 240 g were supplied by Beekay (Stockholm, Sweden). Upon arrival in the laboratory, the animals were housed in groups of 4 per cage ($55 \times 35 \times 20$ cm) at constant room temperature (22°C) and humidity (65%) for 1 week in order to adapt to the novel environment. The animals were kept under artificial light–dark conditions (light on at 7:00 a.m. and off at 7:00 p.m.) and had free access to ''rat and mouse standard feed'' (Beekay Feeds) and tap water.

2.2. Experiment procedure

The animals were divided into eight groups and treated subchronically once a day for 15 days with: (1) vehicle (s.c.) + vehicle (i.p.), (2) nicotine (0.35 mg/kg s.c.) + vehicle (i.p.), (3) vehicle (s.c.) + mecamylamine (4 mg/kg i.p.), (4) vehicle (s.c.) + hexamethonium (10 mg/kg i.p.), (5) vehicle (s.c.) + hexamethonium (20 mg/kg i.p.), (6) nicotine (0.35 mg/kg s.c.) + mecamylamine (4 mg/kg i.p.), (7) nicotine (0.35 mg/kg s.c.) + hexamethonium (10 mg/kg, i.p.) and (8) nicotine (0.35 mg/kg s.c.) + hexamethonium (20 mg/kg i.p.). The i.p. injections was administered approximately 5 min before the s.c. injection.

2.3. Locomotor activity

Locomotor activity was measured using activity meters (Digiscan animal activity monitor, model RZYCCM Tao, Omnitech Electronics, Columbus, OH, 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 two rows of infrared photo sensors, each row consisting of 16 sensors placed 2.5 cm apart. The rows were placed in a 90° angle along the front and side of the floor of the cage. The activity meters were connected to an analyzer system (Omnitech Electronics, Columbus, OH, USA) and the data was collected using an Apple Macintosh Quadra 650 and the computer software LabVIEW (National Instruments, Austin, TX, USA).

On day 0 the animals were tested for locomotor activity. After 30 min of habituation all groups except the vehicle group received nicotine (0.35 mg/kg s.c.) and the vehicle group received a saline injection. Locomotor activity was recorded for 60 min. Fifteen days later the animals were tested again for locomotor activity; after 30 min of habituation all groups were injected with nicotine (0.35 mg/kg s.c.) and tested for 60 min.

2.4. Studies of behavioral inhibition

To evaluate the effects of the present drug treatments on behavioral inhibition, the performance in Montgomery's elevated plus-maze was studied. The experimental apparatus consisted of a plus-formed maze with mesh-wire floor, elevated approximately 0.75 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). The contrast between the elevated plus-maze open and closed arms inhibits the exploratory behavior displayed by rats placed in a novel environment. The exploration of open arms is thus suppressed, and, consequently, a non-treated, normal rat spends only about 15–25% of the total arm time on open arms.

The day after the locomotor activity test the animals were allowed to habituate for 1 h to the testing room before the start of the plus-maze experiment. All of the animals received an injection of nicotine (0.35 mg/kg s.c.) and, in order to stimulate the spontaneous exploratory behavior, were then put into an unfamiliar environment (a dark box with a grid floor for 5 min; see also Pellow et al., 1985), after which they were 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. The time and number of entries made into open arms were expressed as percent of the total time and total entries made into both open and closed arms.

2.5. Drugs

Nicotine ditartrate salt ([−]-1-methyl-2-[pyridyl]pyrrolidine di-[+]tartrate salt purchased from Sigma) was dissolved in 0.9% NaCl and administered (s.c.) in volumes of 2.0 ml/kg. The dose of nicotine is expressed as free base throughout. Nicotine was always neutralized with a few grains of sodiumbicarbonate. Mecamylamine HCl (2-[methylamino]isocamphane hydrochloride purchased from Sigma), a tertiary nAChR antagonist, was dissolved in 0.9% NaCl and administered i.p. in volumes of 2 ml/kg. Hexamethonium Cl (hexane-1,6-bis[trimethyl-ammonium

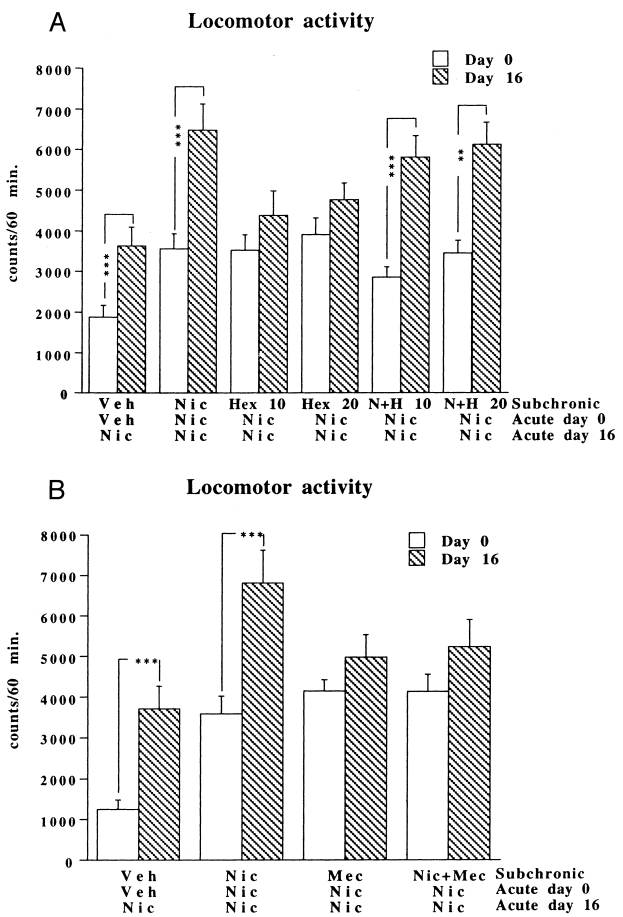

Fig. 1. (A) Effect of nicotine (0.35 mg/kg s.c.) on locomotor activity in Wistar rats treated once a day for 15 days with nicotine (0.35 mg/kg s.c.), hexamethonium (10 mg/kg i.p.), hexamethonium (20 mg/kg i.p.), nicotine + hexamethonium (10 mg/kg), nicotine + hexamethonium (10 mg/kg) or vehicle. On day 0 the vehicle group received an acute saline injection. Shown are means ± S.E.M.; $n = 15–16$. Statistics: Wilcoxons signed rank test $^{**}P \leq 0.01$, $^{***}P \leq 0.001$. (B) Effect of nicotine (0.35 mg/kg s.c.) on locomotor activity in Wistar rats treated once a day for 15 days with nicotine (0.35 mg/kg s.c.), mecamylamine (4 mg/kg i.p.), nicotine + mecamylamine or vehicle. On day 0 the vehicle group received an acute saline injection. Shown are means ± S.E.M.; $n = 15–16$. Statistics: Wilcoxons signed rank test $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

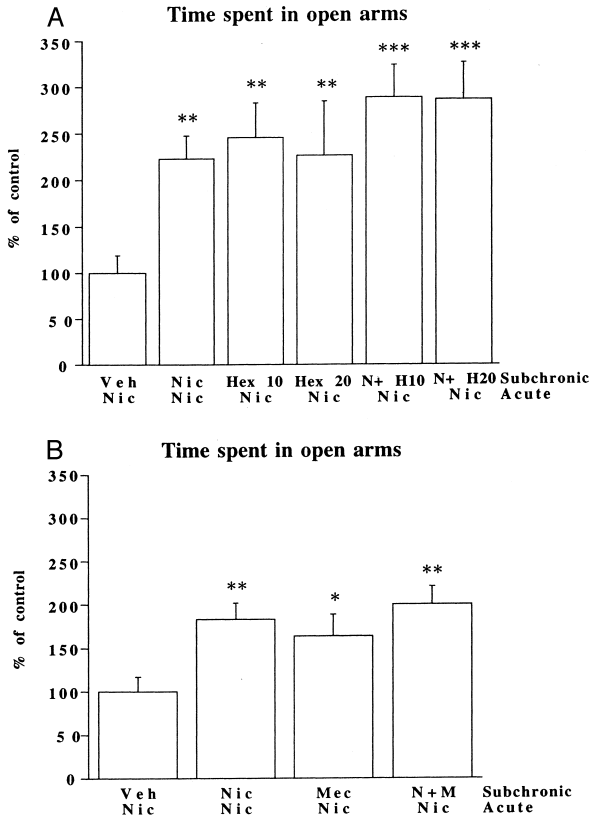

Fig. 2. (A) Effect of acute nicotine on the percent of time spent on open arms in the elevated plus-maze in Wistar rats previously treated once a day for 15 days with nicotine (0.35 mg/kg s.c.), hexamethonium (10 mg/kg i.p.), hexamethonium (20 mg/kg i.p.), nicotine + hexamethonium (10 mg/kg), nicotine + hexamethonium (10 mg/kg) or vehicle. Shown are means ± S.E.M.; $n = 15–16$. Statistics: ANOVA followed by Fishers PLSD $^{**}P \leq 0.01$, $^{***}P \leq 0.001$. (B) Effect of acute nicotine on the percent of time spent on open arms in the elevated plus-maze in Wistar rats previously treated once a day for 15 days with nicotine (0.35 mg/kg s.c.), mecamylamine (4 mg/kg i.p.), nicotine + mecamylamine or vehicle. On day 0 the vehicle group received an acute saline injection. Shown are means ± S.E.M.; $n = 15–16$. Statistics: ANOVA followed by Fishers PLSD $^{*}P \leq 0.05$, $^{**}P \leq 0.01$.

chloride] purchased from Sigma), a quaternary nicotinic receptors antagonist, was dissolved in 0.9% NaCl and administered subcutaneously (s.c.) in volumes of 2 ml/kg. Vehicle animals were given 0.9% NaCl in the same volumes.

2.6. Statistics

The elevated plus-maze data were analyzed using a factorial analysis of variance (ANOVA) followed by Fisher's protected least significant difference (PLSD). The locomotor activity data were analyzed using a Wilcoxon's signed rank test. A probability value (P) less than 0.05 was considered statistically significant. All values are expressed as means ± S.E.M.

3. Results

3.1. Locomotor activity

Acute nicotine (0.35 mg/kg s.c.) challenge on day 16 significantly increased locomotor activity in the nicotine ($P < 0.001$), the nicotine + hexamethonium (10 mg/kg) ($P < 0.001$) and the nicotine + hexamethonium (20 mg/kg) ($P = 0.003$) treated groups, as compared to day 0. Animals receiving the nicotinic receptor antagonists alone or nicotine + mecamylamine did not significantly alter their locomotor activity response to nicotine after the subchronic treatment. Animals in the vehicle treated group, that received a saline injection day 0 and a nicotine injection day 16, increased their locomotor activity significantly ($P < 0.001$) to approximately the same level as did animals in the nicotine treated group day 0 (Fig. 1A and B).

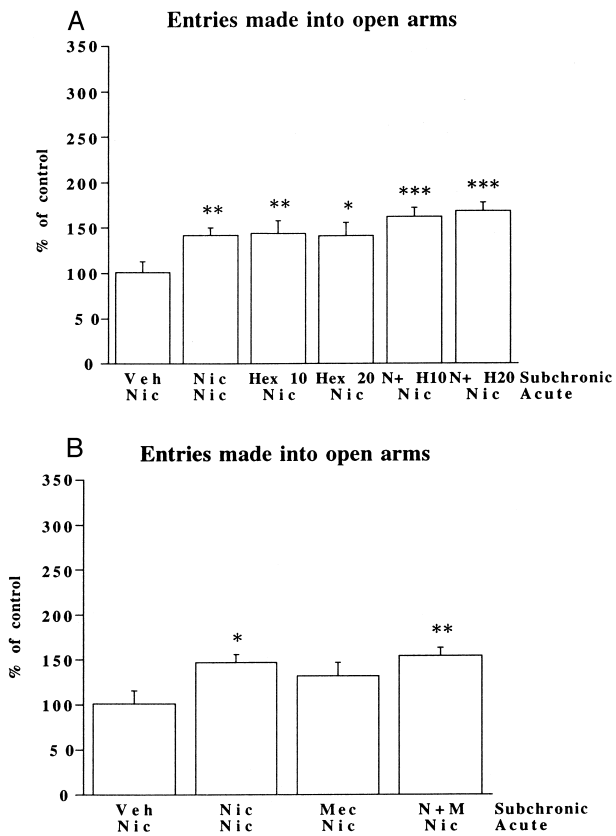

Fig. 3. (A) Effect of acute nicotine on entries made into open arms in the elevated plus-maze in Wistar rats previously treated once a day for 15 days with nicotine (0.35 mg/kg s.c.), hexamethonium (10 mg/kg i.p.), hexamethonium (20 mg/kg i.p.), nicotine + hexamethonium (10 mg/kg), nicotine + hexamethonium (10 mg/kg) or vehicle. Shown are means ± S.E.M.; $n = 15–16$. Statistics: ANOVA followed by Fishers PLSD $^{**}P \leq 0.01$, $^{***}P \leq 0.001$. (B) Effect of acute nicotine on entries made into open arms in the elevated plus-maze in Wistar rats previously treated once a day for 15 days with nicotine (0.35 mg/kg s.c.), mecamylamine (4 mg/kg i.p.), nicotine + mecamylamine or vehicle. On day 0 the vehicle group received an acute saline injection. Shown are means ± S.E.M.; $n = 15–16$. Statistics: ANOVA followed by Fishers PLSD $^{*}P \leq 0.05$, $^{**}P \leq 0.01$.

3.2. Elevated plus-maze

The acute injection of nicotine (0.35 mg/kg s.c) significantly increased the percentage time spent on the open arms (Fig. 2A; $P = 0.007$, Fig. 2B; $P = 0.006$) in animals subchronically pre-treated with nicotine, as compared to the vehicle pre-treated animals. Also rats subchronically pre-treated with the centrally acting nicotinic receptor antagonist mecamylamine (4 mg/kg i.p.) or with the combination of mecamylamine and nicotine increased their time spent in the open arms ($P = 0.03$; $P = 0.01$) after acute nicotine (Fig. 2A). Animals subchronically treated with hexamethonium (10 and 20 mg/kg) significantly increased their time spent in open arms on the plus-maze after acute nicotine ($P = 0.003$, $P = 0.01$) (Fig. 2B). The same applied to the animals that received subchronic injections of nicotine together with hexamethonium ($P < 0.001$ and $P < 0.001$) (Fig. 2B).

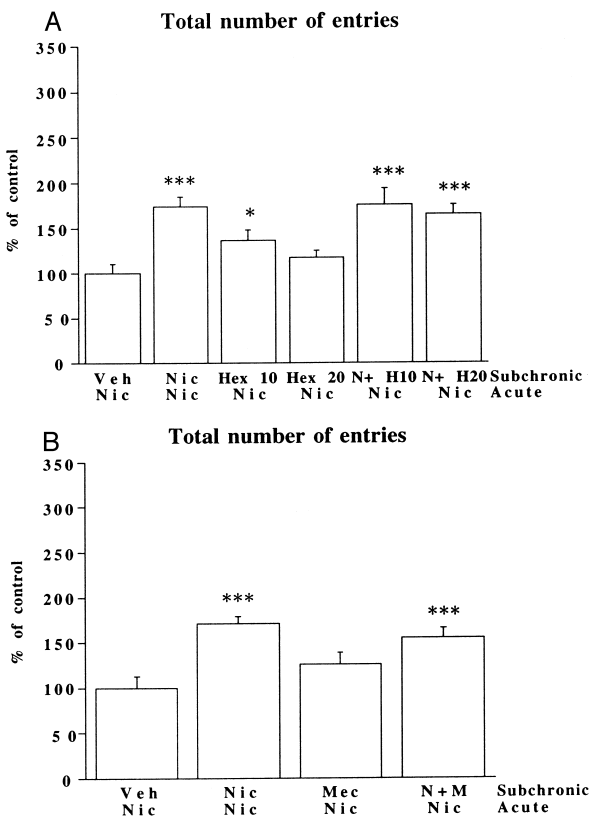

Fig. 4. (A) Effect of acute nicotine on the total number of entries made into any arm in the elevated plus-maze in Wistar rats previously treated once a day for 15 days with nicotine (0.35 mg/kg s.c.), hexamethonium (10 mg/kg i.p.), hexamethonium (20 mg/kg i.p.), nicotine + hexamethonium (10 mg/kg), nicotine + hexamethonium (10 mg/kg) or vehicle. Shown are means ± S.E.M.; $n = 15–16$. Statistics: ANOVA followed by Fishers PLSD $^{*}P \leq 0.05$, $^{***}P \leq 0.001$. (B) Effect of acute nicotine on the total number of entries made into any arm in the elevated plus-maze in Wistar rats previously treated once a day for 15 days with nicotine (0.35 mg/kg s.c.), mecamylamine (4 mg/kg i.p.), nicotine + mecamylamine or vehicle. On day 0 the vehicle group received an acute saline injection. Shown are means ± S.E.M.; $n = 15–16$. Statistics: ANOVA followed by Fishers PLSD $^{***}P \leq 0.001$.

After acute nicotine the percentage of entries made into the open arms was significantly increased in the rats subchronically treated with nicotine, hexamethonium and the combination of nicotine and the antagonists, as compared to vehicle (nicotine (Fig. 3A) $P = 0.01$, nicotine (Fig. 3B) $P = 0.006$, hexamethonium 10 $P = 0.009$, hexamethonium 20 $P = 0.02$, nicotine + mecamylamine $P = 0.004$, nicotine + hexamethonium 10 $P = 0.002$ and nicotine + hexamethonium $P < 0.001$). In animals receiving mecamylamine alone there was only a tendency ($P = 0.08$) for an enhancement of the percentage of entries made into the open arms after nicotine challenge.

The total number of entries made into both open and closed arms after nicotine challenge was significantly increased after most subchronic treatments (nicotine (Fig. 4A) $P < 0.001$, nicotine (Fig. 4B) $P < 0.001$, hexamethonium 10 $P = 0.03$, nicotine + mecamylamine $P = 0.001$, nicotine + hexamethonium 10 $P < 0.001$ and nicotine + hexamethonium $P = 0.001$) as compared to vehicle. However, subchronic treatment with mecamylamine alone or hexamethonium 20 mg/kg failed to increase the total number of entries made into both open and closed arms after nicotine challenge as compared to vehicle.

4. Discussion

Supporting previous studies nicotine (0.35 mg/kg, s.c.) increased locomotor activity in rats that had been habituated to the recording chambers and this effect was significantly enhanced after subchronic nicotine administration (Ksir et al., 1985; Benwell and Balfour, 1992; Johnson et al., 1995a). Thus, nicotine-induced locomotor sensitization developed. Subchronic treatment with the tertiary nicotinic receptor antagonist mecamylamine together with nicotine prevented the induction of locomotor sensitization to nicotine, whereas subchronic treatment with the antagonist alone did not alter the locomotor activity response after nicotine challenge. Subchronic treatment with the quaternary nicotinic receptor antagonist hexamethonium (10 or 20 mg/kg) together with nicotine failed to block the induction of nicotine-induced locomotor sensitization and also did not affect the locomotor response after nicotine challenge. The vehicle group, that on the first test day received a saline injection and after 15 days of repeated saline injections received an acute nicotine challenge, displayed a locomotor activity score almost identical to that observed in the nicotine group on day 0.

Since only the blood brain barrier penetrating nicotinic receptor antagonist (mecamylamine) prevented the subchronic nicotine effect, these results indicate that the induction of locomotor sensitization to nicotine after repeated systemic administration of the drug is due to an intermittent stimulation of central nicotinic receptors. These

results are in line with previous reports showing that intermittent intracerebral application of nicotine produces locomotor sensitization to the drug (Kita et al., 1992; Panagis et al., 1996). In addition, Suemaru et al. (1997) found that mecamylamine blocked the induction of sensitization to nicotine-induced tail-tremor. By means of a similar experimental approach as the presently used, that is co-treatment with the above antagonists, it has previously been demonstrated that also the expression of the locomotor sensitized response to nicotine most likely is due to activation of central nicotinic receptors (Clarke and Kumar, 1983). Taken together, it appears that both the induction and the expression of nicotine-induced locomotor sensitization involve stimulation of central nicotinic receptors.

Since evidence supports the contention that acute systemic nicotine produces its mesolimbic dopamine activating effect, and hence probably at least part of its locomotor stimulatory effect (Benwell and Balfour, 1992), through activation of nicotinic receptors in the ventral tegmental area, but not in the nucleus accumbens (Nisell et al., 1994), it is tempting to speculate that this population of central nicotinic receptors is involved also in the induction and the expression of locomotor sensitization to the drug. This issue has, however, not yet been properly addressed experimentally. Thus, even though intermittent intracerebral application of nicotine in the ventral tegmental area (Panagis et al., 1996; Kita et al., 1992) or in the nucleus accumbens (Kita et al., 1992) appears to produce locomotor sensitization to nicotine, these experiments did not take into consideration the probably considerable intracerebral spread of this highly lipophilic substance. Experiments with systemic nicotine injections and concomitant injections of preferably hydrophilic anatagonists (e.g. hexamethonium) at different locations in the brain have, to our knowledge, not yet been performed to address this question.

In Montgomery's elevated plus-maze, rats that had been subchronically treated with saline and that were acutely challenged with nicotine spent most of their time on, and made most of their entries into the closed arms. These rats thus demonstrated an inhibited exploratory behavior towards the open arms as also been previously demonstrated in rats treated with acute vehicle (Olausson et al., 1999). In line with previous results from this group (Olausson et al., 1999), nicotine challenge to Wistar rats subchronically pretreated with nicotine for 15 days significantly increased the percentage time spent on as well as the percentage number of entries made into open arms. Thus, subchronic nicotine treatment disclosed a nicotine-induced disinhibitory behavior towards the open arms. Interestingly, also after subchronic treatment with the nicotinic receptor antagonists hexamethonium or mecamylamine, alone or in combination with nicotine, the rats developed a nicotine-induced disinhibitory behavior to the same extent, or more, than animals receiving subchronic pre-treatment with nicotine alone. The percentage of entries made into the open arms largely followed the same pattern as the percentage time spent on the open arms, although pre-treatment with mecamylamine by itself failed to significantly increase the percentage of open-arm entries.

Vale and Balfour (1989) have previously demonstrated an increase in the total number of entries made on the elevated plus-maze after chronic administration of nicotine. The present study supports these findings. In line with the results obtained in the locomotor activity studies this effect was not blocked by co-pretreatment with hexamethonium. However, in contrast to the locomotor activity experiments above, co-pretreatment with mecamylamine failed to prevent the subchronic nicotine effect. This finding may be difficult to interpret, but it should be noted that in the locomotor activity studies, but not in the plus-maze studies, the animals had been habituated to the recording chambers for 30 min. In the former study, a stimulatory behavior is thus evoked in animals that are at rest, whereas in the latter a drug effect imposed on an active, exploratory behavior is studied. Moreover, a previous study (Olausson et al., 1999) demonstrated that the degree of nicotine-induced locomotor sensitization did not correlate with the total number of entries into the plus-maze arms. This would then indicate that the intermittent nicotine treatment induces two parallel phenomena with different neurochemical mechanisms.

Whereas, as discussed above, the induction of locomotor sensitization to nicotine appears to involve intermittent stimulation of central nicotinic receptors, this appears not to hold true for the induction of nicotine-induced behavioral disinhibition. In that case neither hexamethonium nor mecamylamine, nor the combination of mecamylamine and nicotine, would have been expected to produce an effect identical to that induced by subchronic nicotine treatment. These results indicate that the inductions of locomotor sensitization to nicotine and nicotine-induced behavioral disinhibition involve different neurochemical mechanisms. Moreover, since the same pre-treatments that induced nicotine-induced behavioral disinhibition also enhanced ethanol consumption in a previous study (Ericson et al., 2000), the

Table 1
Effects of subchronic treatment of nicotine, mecamylamine, hexamethonium or the combination of nicotine and the antagonists on nicotine-induced sensitization to the locomotor stimulatory effects, nicotine-induced disinhibition (plus-maze) or increased ethanol consumption in low- and medium-preferring animals. (+) denotes effect of treatment and 0 denotes no effect of treatment

	Veh	Nic	Mec	Mec + Nic	Hex	Hex + Nic
Behavioral (locomotor) sensitization	0	+	0	0	0	+
Increased ethanol consumption	0	+	+	+	+	+
Behavioral disinhibition	0	+	+	+	+	+

two behaviors may be mechanistically related (Table 1). The similarities are not so evident, however, with respect to the induction of locomotor sensitization to nicotine (Table 1). Indeed, in a previous study we failed to find a correlation between the acute locomotor stimulatory response to nicotine or the development of sensitization to this effect of nicotine and the ensuing enhancement of ethanol intake or preference (Blomqvist et al., 1996).

In our study regarding the enhancement of ethanol consumption after the various nicotinic drugs here used we arrived at the conclusion that the common denominator between the drugs used is that they all produce an intermittent blockade of peripheral nicotinic receptors. This holds true probably also for nicotine after the dose here used (Taylor, 1996). It was further speculated that an intermittent ganglionic blockade could produce autonomic adaptations that then may contribute to the behavior observed, and, indeed, some support was obtained for that an increased activity at peripheral muscarinic receptors may be involved in expressing the enhanced ethanol consummatory behavior (Ericson et al., 2000). Studies are now underway to investigate whether peripheral muscarinic receptors may be involved in expressing also the disinhibitory behavior observed here.

Since the doses of hexamethonium used in this and in our previous study (Ericson et al., 2000) are rather high, it could be argued that some amount of drug would pass the blood brain barrier, and hence that the hypothesis of central contra peripheral nicotinic receptors involvement was not adequately tested. However, since a clear differentiation was observed between the results obtained with respect to the two behaviors studied, this possibility appears highly unlikely. If hexamethonium would have passed the blood–brain barrier the results obtained would have indicated that central intermittent nicotinic receptors blockade induces the disinhibitory behavior, whereas, at the same time, hexamethonium, contrary to mecamylamine, would have failed to block the induction of nicotine-induced locomotor sensitization. This would have been a contradiction. Also, literature findings argue against this possibility. Although local perfusion of hexamethonium in the ventral tegmental area blocked ethanol induced dopamine overflow in the nucleus accumbens, systemically administered hexamethonium (10 mg/kg) did not, whereas systemic mecamylamine (1 mg/kg) did (Blomqvist et al., 1997). Furthermore, hexamethonium (3 mg/kg) pre-treatment did not at all alter nicotine self-administration whereas mecamylamine pre-treatment dose-dependently did (1 mg/kg and 3 mg/kg; Corrigal and Cohen, 1989).

Disinhibited behavior in the elevated plus-maze is commonly interpreted as an alleviation of anxiety (Pellow, 1986; Pellow et al., 1985). However, it has also been argued that behavioral disinhibition in experimental animal models which invoke conflict situations, like the elevated plus-maze, may rather reflect a loss of impulse control (Soubrié, 1986), with or without a concomitant alleviation of anxiety. Interestingly, a connection between impulsivity and self-administration of addictive compounds has repeatedly been observed in animal experiments, and impulsivity was recently advanced as a major component in the behaviors underlying the compulsive drug seeking and drug intake observed in drug abuse (Jentsch and Taylor, 1999). In animal studies, manipulations which increase impulsivity, such as serotonin depletion, also increase the self-administration of various drugs, including ethanol (cf. Engel et al., 1992; Roberts et al., 1994; LeMarquand et al., 1994). Moreover, Poulos et al. (1995) have demonstrated that the propensity of rats to consume ethanol correlates to their impulsivity. Thus, even though the elevated plus-maze has not been validated as a model for impulsivity it is possible that the behavioral disinhibition observed in this model after repeated treatment with nicotine (present study; Olausson et al., 1999), nicotinic receptor antagonists (present study) and amphetamine (Olausson et al., 2000) is involved in the elevated ethanol intake observed after these same treatments (Blomqvist et al., 1996; Fahlke et al., 1995; Ericson et al., 1998). Further investigations will be made to establish whether the increased disinhibited behavior after subchronic treatment with nicotine and/or nicotinic receptor antagonist antagonists correlates to the increased ethanol intake produced by the same treatments.

In conclusion, subchronic administration of nicotine produces locomotor sensitization and, concomitantly, promotes the development of a nicotine-induced disinhibitory behavior in the elevated plus-maze. Experiments applying co-administration of blood–brain barrier or non-blood brain barrier penetrating nicotinic receptors antagonists, or administration of these compounds alone, revealed that the induction of locomotor sensitization to nicotine may involve intermittent stimulation of central nicotinic receptors, whereas the development of nicotine-induced disinhibition instead most likely is due to intermittent blockade of peripheral nicotinic receptors. The data obtained further indicate that the development of nicotine-induced disinhibition, but not the induction of locomotor sensitization, from a pharmacological point of view appears to be related to the increased ethanol consummatory behavior observed after subchronic nicotine administration. The tentative peripheral mechanisms involved in the development of a disinhibitory behavior associated with increased ethanol consumption remain to be established, but, if elucidated, could provide clues to the development of novel pharmacological therapies against alcohol abuse.

Acknowledgements

This study was financially supported by grants from the Swedish Medical Research Council (no 4247 and no 11583), the Swedish Alcohol Monopoly Foundation for Alcohol Research, Swedish Society for Medical Research, Swedish Match, Swedish Society of Medicine, Orion

Pharma neurology, Lundbecks Fond för Psykofarmakologisk Forskning, Wilhelm och Martina Lundgrens vetenskapsfond, Clas Groschinskys minnesfond, Stiftelsen Lars Hiertas minne, Fredrik och Ingrid Thurings Stiftelse and National Institute on Drug Abuse Grant (IR 01 DA 10765-03).

References

Benwell, M.E.M., Balfour, D.J.K., 1992. The effects of acute and repeated nicotine treatment on nucleus accumbens dopamine and locomotor activity. Br. J. Pharmacol. 105, 849–856.

Bien, T.H., Burge, R., 1990. Smoking and drinking: a review of the litterature. Int. J. Addict. 25, 1429–1454.

Blomqvist, O., Söderpalm, B., Engel, J.A., 1992. Ethanol-induced locomotor activity: involvement of central nicotinic acetylcholine receptors? Brain Res. Bull. 29, 173–178.

Blomqvist, O., Ericson, M., Johnson, D.H., Engel, J.A., Söderpalm, B., 1996. Volutary ethanol intake in the rat: effects of nicotinic acetylcholine receptor blockade and nicotine sensitization. Eur. J. Pharmacol. 314, 257–267.

Blomqvist, O., Ericson, M., Engel, J.A., Söderpalm, B., 1997. Accumbal dopamine-overflow after ethanol; localization of the antagonizing effect of mecamylamine. Eur. J. Pharmacol. 334 (2–3), 149–156.

Burch, J.B., de Fiebre, M., Marks, M.J., Collins, A.C., 1988. Chronic ethanol or nicotine treatment results in partial cross-tolerance between these agents. Psychpharmacology 95, 452–458.

Clarke, P.B.S., Kumar, R., 1983. The effects of nicotine on locomotor activity in non-tolerant and tolerant rats. Br. J. Pharmacol. 78, 329–337.

Corrigal, W.A., Cohen, K.M., 1989. Nicotine maintains robust self-administration in rats on a limited-access schedule. Psychopharmacology 99, 473–478.

Crowley, T.J., Chesluk, D., Dilts, S., Hart, R., 1974. Drug and alcohol abuse among psychiatric admissions; a multidrug clinical–toxicological study. Arch. Gen. Psychiatry 30, 13–20.

Dreher, K.F., Fraser, J.G., 1967. Smoking habits of alcohol outpatients. Int. J. Addict. 2, 259–268.

Engel, J.A., 1977. Neurochemical aspects of the euphoria induced by dependence-producing drugs. In: Ideström, C.M. (Ed.), Recent Advances in the Study of Acoholism. Exerpta Medica, Amsterdam, pp. 16–22.

Engel, J.A., Enerbäck, C., Fahlke, C., Hulthe, P., Hård, E., Johannessen, K., Svensson, L., Söderpalm, B., 1992. Serotonergic and dopaminergic involvement in ethanol intake. In: Naranjo, C.A., Sellers, E. (Eds.), Novel Pharmacological Interventions for Alcoholism. Springer-Verlag, New York, pp. 68–82.

Ericson, M., Blomqvist, O., Engel, J.A., Söderpalm, B., 1998. Voluntary ethanol intake in the rat and the associated accumbal dopamine overflow is blocked by ventral tegmental mecamylamine. Eur. J. Pharmacol. 358, 189–196.

Ericson, M., Engel, J.A., Söderpalm, B., 2000. Peripheral involvement in nicotine-induced enhancement of ethanol intake. Alcohol, in press.

Fahlke, C., Hard, E., Eriksson, C.J., Engel, J.A., Hansen, S., 1995. Amphetamine-induced hyperactivity: differences between rats with high or low preference for alcohol. Alcohol 12 (4), 363–367.

Gauvin, D.V., Moore, K.R., Holloway, F.A., 1993. Do rat strain differences in ethanol consumption reflect differences in ethanol sensitivity or the preparedness to learn. Alcohol 10 (1), 37–43.

Jentsch, J.D., Taylor, J.R., 1999. Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behavior by reward-related stimuli. Psychopharmacology (Berl.) 146 (4), 373–390.

Johnson, D.H., Blomqvist, O., Engel, J.A., Söderpalm, B., 1995a. Subchronic intermittent nicotine treatment enhances ethanol-induced locomotor stimulation and dopamine turnover in mice. Behav. Pharmacol. 6, 203–207.

Johnson, D.H., Svensson, A.I., Engel, J.A., Söderpalm, B., 1995b. Induction but not expression of behavioral sensitization to nicotine in the rat is dependent on glucocorticoids. Eur. J. Pharmacol. 276, 155–164.

Kalivas, P.W., Stewart, J., 1991. Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain Res. Rev. 16, 223–244.

Kita, T., Okamoto, M., Nakashima, T., 1992. Nicotine-induced sensitization to ambulatory stimulant effect produced by daily administration into the ventral tegmental area and the nucleus accumbens in rats. Life Sci. 50 (8), 583–590.

Koob, G.F., 1992. Neuronal mechanisms of drug reinforcement. In: Kalivas, P.W., Samson, H.H. (Eds.), The Neurobiology of Drug and Alcohol Addiction. The New York Academy of Science, New York, pp. 171–191.

Ksir, C., Hakan, R., Hall, J., Kellar, K.J., 1985. Exposure to nicotine enhances the behavioral stimulant effect of nicotine and increases binding of [^{3}H]acetylcholine to nicotinic receptors. Neuropharmacology 24, 527–532.

Lee, E.H., 1985. Effects of nicotine on exploratory behavior in rats: correlation with regional monoamine levels. Behav Brain Res. 17 (1), 59–66.

LeMarquand, D., Pihl, R.O., Benkelfat, C., 1994. Serotonine and alcohole intake, abuse and dependence: findings of animal studies. Biol. Psychiatry 36, 395–421.

Nestler, E.J., 1994. Molecular neurobiology of drug addiction. Neuropsychopharmacology 11, 77–87.

Nisell, M., Nomikos, G.G., Svensson, T.H., 1994. Systemic nicotine-induced dopamine release in the rat nucleus accumbens is regulated by nicotinic receptors in the ventral tegmental area. Synapse 16, 36–44.

Olausson, P., Engel, J.A., Söderpalm, B., 1999. Behavioral sensitization to nicotine is associated with behavioral disinhibition; counteraction with citalopram. Psychopharmacology 142, 111–119.

Olausson, P., Engel, J.A., Söderpalm, B., 2000. Effect of serotonergic mechanisms on behavioral sensitization and disinhibition associated with repeated amphetamine treatment. Pharmacol. Biochem. Behav., in Press.

Panagis, G., Nisell, M., Nomikos, G.G., Chergui, K., Svensson, T.H., 1996. Nicotine injections into the ventral tegmental area increase locomotion and Fos-like immunoreactivity in the nucleus accumbens of the rat. Brain Res. 730, 133–142.

Pellow, S., 1986. Anxiolytic and anxiogenic drug effects in a novel test of anxiety: are exploratory models of anxiety in rodents valid?. Methods Find. Exp. Clin. Pharmacol. 8, 557–565.

Pellow, S., Chopin, P., File, S.E., Briley, M., 1985. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Methods 14, 149–167.

Pothoff, A.D., Ellison, G., Nelson, L., 1983. Ethanol intake increases during continous administration of amphetamine and nicotine, but not several other drugs. Pharmacol. Biochem. Behav. 18, 489–493.

Poulos, C., Le, A., Parker, J., 1995. Impulsivity predicts individual suceptibility to high levels of alcohol self-administration. Behav. Pharmcol. 6, 809–814.

Roberts, D.C., Loh, E.A., Baker, G.B., Vickers, G., 1994. Lesions of central serotonin systems affect responding on a progressive ratio schedule reinforced either by intravenous cocaine or by food. Pharmacol. Biochem. Behav. 49, 177–182.

Robinson, T.E., Berridge, K.C., 1993. The neural basis of drugcraving: an incentive-sensitization theory of addiction. Brain Res. Rev. 18, 247–291.

Soubrié, P., 1986. Reconciling the role of central serotonin neurons in human and animal behavior. Behav. Brain Sci. 9, 319–364.

Suemaru, K., Kawasaki, H., Oishi, R., Gomita, Y., Tanizaki, Y., 1997. Role of central nicotinic and β-adrenergic receptors in the onset and further development of tail-tremor induced by repeated nicotine ad-

ministration to rats. Naunyn-Schmiedeberg's Arch. Pharmacol. 355, 571–575.

Taylor, P., 1996. Agents acting at the neuromuscular junction and autonomic ganglia. In: Hardman, J.G., Goodman, G.A., Limbird, L.E. (Eds.), Goodman and Gilman's The Pharmacological Basis of Therapeutics. 9th edn. McGraw-Hill, New York, pp. 177–198.

Vale, A.L., Balfour, D.J., 1989. Aversive environmental stimuli as a factor in the psychostimulant response to nicotine. Pharmacol. Biochem. Behav. 32, 857–860.

Wise, R.A., Rompre, P.-P., 1989. Brain dopamine and reward. Ann. Rev. Psychol. 40, 191–225.