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The effects of nicotine on locomotor activity and dopamine overflow in the alcohol-preferring AA and alcohol-avoiding ANA rats

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Abstract

The aim of the study was to investigate the importance of the interaction between central dopaminergic and cholinergic mechanisms for ethanol reinforcement. This was done by comparing the effects of nicotine on locomotor activity and release of dopamine in the nucleus accumbens of the alcohol-preferring Alko Alcohol (AA) and alcohol-avoiding alko non-alcohol (ANA) rats. Nicotine was administered acutely (0.25, 0.50 or 0.75 mg/kg, s.c.) or repeatedly once daily (0.5 mg/kg, s.c.) for 8 days. An acute dose of nicotine increased locomotor activity and the extracellular levels of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) measured with in vivo microdialysis suggesting stimulation of dopamine release by nicotine. No difference in the stimulation of locomotor activity or in the increase in the extracellular concentrations of dopamine or its metabolites by nicotine was found between the rat lines. The concentrations of nicotine in the plasma were also identical. The rats treated repeatedly with nicotine showed a progressive increase in locomotion. On the challenge day, 1 week after termination of nicotine or saline injections, rats previously treated with nicotine were activated more by nicotine than saline-treated rats. This behavioral sensitization was not accompanied by an increase in the amplitude of the neurochemical response to nicotine, but the duration of the increase in the levels of DOPAC was longer in the nicotine than saline-treated animals. The increases in locomotor activity and metabolite levels were, however, similar in both rat lines. These data suggest that differences in the interaction of central dopaminergic and cholinergic mechanisms probably do not contribute to the difference in ethanol self-administration between the AA and ANA rat lines.

Keywords: Dopamine; Nicotine; Ethanol; Microdialysis; Nucleus accumben; Selected line

1. Introduction

The Alko Alcohol (AA) and Alko Non-Alcohol (ANA) rat lines have been developed by selective outbreeding for differences in voluntary ethanol consumption (Eriksson, 1968, 1969). These and other selected lines of rodents can be a useful tool in studies on the biological mechanisms of ethanol abuse because the lines should, theoretically, differ from each other only in the trait upon which selection has been applied and in traits that are related to the selected trait (Sinclair et al., 1989; Kiianmaa et al., 1992).

Self-administration of and addiction to ethanol have been linked to the reinforcement produced by it (Di Chiara, 1995; Wise, 1998; Koob et al., 1999). Central dopaminergic neurons and the mesolimbic dopamine pathway in particular have been hypothesized to be the neural substrate of the reinforcement produced by ethanol, since ethanol stimulates the release of dopamine in the nucleus accumbens and other structures innervated by the ascending dopamine neurons. Earlier studies using conventional techniques demonstrated that ethanol increases the rate of dopamine synthesis (Carlsson and Lindqvist, 1973; Kiianmaa and Tabakoff, 1984), and metabolism in various brain parts (Murphy et al., 1988; Kiianmaa et al., 1991). Similarly, data from electrophysiological studies have shown that ethanol increases the firing rate of neurons in the substantia nigra (Gessa et al., 1985). More recent studies using in vivo microdialysis or voltammetry have provided

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evidence that ethanol increases the extracellular concentrations of dopamine, the nucleus accumbens of freely moving rats, suggesting enhanced release after ethanol (Imperato and Di Chiara, 1986; Yoshimoto et al., 1991, 1992; Engel et al., 1992; Weiss et al., 1992, 1993; Kiianmaa et al., 1995).

To study the role of central dopaminergic mechanisms in ethanol reinforcement, attempts have been made to determine whether the difference in ethanol consumption between the AA and ANA lines could be related to the functions of the central dopaminergic neurons. These investigations have, however, not provided support for dopamine mediating reinforcement from ethanol in AA and ANA rats. Earlier post-mortem studies established differences in the basal levels of dopamine and its metabolites, with the alcohol-preferring AA rats showing higher levels in various brain parts than the alcohol-avoiding ANA rats (Ahtee and Eriksson, 1975; Kiianmaa and Tabakoff, 1984). The lines have, however, been shown not to differ in the effects of ethanol on the rates of synthesis and metabolism of the catecholamines (Kiianmaa et al., 1991). Microdialysis studies have shown accordingly that although ethanol dose-dependently increases the extracellular concentrations of dopamine in the nucleus accumbens of naive AA and ANA rats, no difference between the rat lines in ethanol-induced dopamine release was found, suggesting that the reinforcement from ethanol is not related to the amount of dopamine released by ethanol (Kiianmaa et al., 1995). Prior ethanol exposure in a non-contingent manner was not found to change the dopaminergic reaction to a challenge dose of ethanol either: the increase in the extracellular levels of dopamine was similar to that in naive AA and ANA rats (Nurmi et al., 1996). Contingent ethanol exposure resulted in a suppressed response rather than sensitized one. Furthermore, when extracellular dopamine was measured during oral ethanol self-administration in AA rats, no substantial changes were found (Nurmi et al., 1998). Acute or repeated treatment with ethanol did not produce any change in the locomotor activity of AA and ANA rats (Honkanen et al., 1999).

Although ethanol seems to activate the mesolimbic dopaminergic neurons directly (Gessa et al., 1985), it is possible that ethanol, because of its nonspecific nature, can modulate the functioning of the dopaminergic systems indirectly via some other neuronal system. The dopaminergic neurons have connections, for instance, with the cholinergic systems: the ventral tegmental area receives cholinergic innervation (Bolam et al., 1991), and nicotinic acetylcholine receptors are located on the cell bodies of the mesolimbic dopaminergic neurons (Clarke and Pert, 1985). Acute administration of nicotine also increases utilization of dopamine or the extracellular concentrations of dopamine in the nucleus accumbens and enhances locomotor activity (Imperato et al., 1986; Clarke et al., 1988; Nisell et al., 1995). Repeated administration of nicotine has been reported to induce sensitization of the responsiveness of dopamine transmission in the nucleus accumbens to nicotine as well as motor stimulant effects of nicotine (Benwell and Balfour, 1992; Balfour et al., 1998). Furthermore, nicotine has been shown to interact with ethanol on neurochemical and behavioral levels. The affinity of the nicotinic receptor is enhanced by ethanol (Forman et al., 1989), nicotine alters ethanol self-administration (Nadal et al., 1998; Lê et al., 2000), and blockade of ventral tegmental acetylcholine receptors antagonizes the ethanol-induced increase in the extracellular levels of dopamine in the accumbens as well as lowers ethanol intake and ethanol-induced locomotor activity (Blomqvist et al., 1996; Nadal et al., 1998; Ericson et al., 1998). Thus, it is conceivable that the interaction of dopaminergic neurons with cholinergic neuronal systems may contribute to the increase in the extracellular dopamine levels in the nucleus accumbens found after acute ethanol administration.

The present study was aimed at investigating the effects of acute or repeated administration of nicotine on the extracellular levels of dopamine in the nucleus accumbens of the alcohol-preferring AA and alcohol-avoiding ANA rats. Since nicotine-induced locomotor activity seems to depend upon the effect of nicotine on the mesolimbic dopamine pathway and since locomotor activity is sometimes interpreted as reflecting reinforcement from a drug (Carlsson et al., 1972), the effects of nicotine on the locomotor activity of the AA and ANA rats were also studied using similar paradigms.

2. Materials and methods

2.1. Animals

Naive male AA and ANA rats of generation F_{76-80} and ranging from 3 to 4 months of age were used. They were housed in individual Plexiglas ($24 \times 24 \times 30$ cm^3) cages in a colony room. The room temperature was kept at 22°C, the relative humidity at $50 \pm 5\%$, and the 12 h light/dark cycle with lights on at 0600 h. The rats had access to normal maintenance food (RM1 (E) SQC pellets from SDS, Witham, Essex, England) and tap water at all times.

All experimental procedures were conducted under protocols approved by the Institutional Animal Care and Use Committee at the National Public Health Institute and the Chief Veterinarian of the County Administrative Board.

2.2. Nicotine treatment

The rats were given nicotine acutely or repeatedly. The acute behavioral effects were studied by giving the rats nicotine 0.5 mg/kg. In the acute neurochemical studies, the animals received 0.25, 0.50 or 0.75 mg/kg. The repeated nicotine treatment consisted of eight injections of nicotine 0.5 mg/kg given on eight consecutive days. An

additional challenge dose of nicotine was given to the rats 1 week after cessation of the daily injections.

Nicotine was dissolved in saline, and the pH was adjusted to 7.4 by adding a small quantity of NaOH. The doses refer to free base. All injections were given subcutaneously in a volume of 1 ml/kg. The controls received an equal volume of saline.

2.3. Locomotor activity

Locomotor activity was measured in transparent Macrolon III cages ($18 \times 33 \times 15$ cm^3) that were placed inside a frame with seven photocells on each side of the frame (Cage Rack Activity System, San Diego Instruments, CA, USA). The photocells were located approximately 5 cm above the surface of the cage bedding. The number of interruptions of successive photocells was used as the measure of forward locomotor activity and was recorded by a computer at 10-min intervals. During testing, rats were removed from their individual home cages in the colony room and placed into the Macrolon test cages located in an adjacent experimental room. Under these conditions, the contextual cues of the testing environment were different from those of the home environment. Rats were habituated to the test cages during the first three sessions. During the last two habituation sessions, rats were first allowed to habituate to the cage for 15 min, after which they received a saline injection (1 ml/kg). After the injection, locomotor activity was monitored for 1 h. The same procedure was used for the next 8 days: rats received either saline or nicotine injections (0.5 mg/kg) and were then tested. One week after cessation of daily injections and locomotor activity monitoring, all animals received a challenge nicotine injection (0.5 mg/kg) and were tested as described above.

2.4. Microdialysis studies

2.4.1. Surgical procedure

The implantation of the guide cannula was made under halothane anaesthesia (3.5% for 4 min, then 1–2.5%, as required when the rat was attached to the stereotactic frame). The guide cannula was lowered to a position just above the nucleus accumbens, extending 6.5 mm below the dura, 1.5 mm lateral to the midline, and 1.7 mm anterior to the bregma (Paxinos and Watson, 1982). The cannula was fastened with dental cement anchored with three stainless steel screws to the skull. The rats were administered buprenorphine (Temgesic 0.3 mg/ml) 0.15 mg/kg, s.c. immediately after surgery and during the next days if swelling was apparent and/or normal behavior was impaired. They were allowed to recover from the surgery for at least 6 days. The rats were then accustomed to the microdialysis experimental situation. They were tethered to the counterbalancing arm for a few hours and picked up at least 3 days before the actual experiments took place.

2.4.2. Microdialysis

For nicotine administration and microdialysis, the animals were taken each day into the experimental room, with conditions as above, and placed in Plexiglass cages similar to their home cages. After the experiment, the animals were moved back to their home cages in the colony room.

The microdialysis experiments were started at 0800 h by tethering the rats and inserting a CMA/12 probe (o.d. 0.50 mm, length 2 mm, polycarbonate membrane with a 20,000-Da cutoff) into the guide cannulas. The probe was perfused with modified Ringer solution (147 mM NaCl, 3 mM KCl, 1.3 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1 mM Na_2HPO_4, pH 7.25) with a flow rate 1.4 μl/min using a CMA 100 microinjection pump (CMA Microdialysis, Stockholm, Sweden). Baseline collection was started after 180 min, with samples being collected every 10 min for 120 min. After this, the rats were given an acute injection of nicotine or saline. Dialysate samples were collected for 150 min after the injection.

For studies on the effects of repeated nicotine treatment, the rats with guide cannulas implanted received the nicotine or saline injections in the experimental room. The microdialysis experiment was conducted on day 8, following the procedures as above. On the nicotine challenge day, 1 week after termination of daily nicotine or saline injections, the probe was reinserted and all rats were given a challenge nicotine injection (0.5 mg/kg).

The samples were collected into vials containing 2 μl 1 mM glutathion in 0.15 M HCl. The samples were immediately refrigerated and analyzed by high performance liquid chromatography (HPLC) using electrochemical detection.

2.4.3. Analytical procedure

The concentrations of monoamines in the dialysate samples were analyzed by high performance liquid chromatography using electrochemical detection. The system consisted of a Hewlett Packard 1100 isocratic pump (Palo Alto, CA, USA) with degaser unit, a refrigerated autoinjector CMA/200, and an amperometric detector Intro (Antec Leyden, The Netherlands) with a glassy carbon VT-03 cell. The glassy-carbon working electrode was set to 700 mV versus Ag/AgCl reference electrode. The column was MIC 10-3-C18 (Hypersil, 100×1 mm I.D.) with particle size of 3 μm (LC-Packings, The Netherlands). The mobile phase was 10% (v/v) methanol in 100 mM phosphate buffer, 0.15 mM EDTA, and 0.22 mM sodium octylsulfonate at pH 4.17. The solution was filtered through a PVDF filter with 0.45-μm pores (Millipore, Milford, MA, USA). The flow rate of the mobile phase was 25 μl/min, and the injection volume was 14 μl. The chromatograms were acquired and processed using the Waters 820 Maxima Software, version 3.31 (Waters Association, Milford, MA, USA). The raw microdialysis data (pmol or fmol/10 min) were converted into percentages of the baseline consisting of the mean of the last three baseline samples.

2.5. Histology

After the experiment, the brains of the rats were removed and fixed in formalin. The positions of the probes were verified making frozen 100-μm coronal sections, which were stained with thionine.

2.6. Measurement of nicotine

For the determination of the concentration of nicotine in the plasma, AA and ANA rats were given a subcutaneous injection of nicotine (0.5 mg/kg), and 0.4 ml of blood was withdrawn from the tip of the tail 2–5, 30 and 60 min after the injection. The blood was mixed with 1.2 ml of sodium citrate solution (0.5%, w/v), and the plasma was separated from the blood by centrifugation at $800 \times g$ for 20 min.

The plasma concentrations of nicotine were determined by a gas chromatographic–mass spectrometric (GC–MS) method using selected ion monitoring (SIM) (Leikola-Pelho et al., 1990). GC–MS analyses were performed on an Hewlett Packard 5970 quadrupole MS coupled to a Hewlett Packard 5890 GC using an NB-54 fused silica column (15 m). The MS conditions were: ion source, 70 eV; electron multiplier voltage, 2200 V; ion source temperature, 250°C. In the GC–MS–SIM analyses, fragment ions of m/z 84 (nicotine), 98 (cotinine) and 129 (quinoline internal standard) were used.

2.7. Drugs and chemicals

(−)-Nicotine hydrogen tartrate, dopamine hydrochloride, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA), sodium octane sulphonate, and gluthation were purchased from Sigma (St. Louis, MO, USA). For HPLC, ultrapure reagent-grade water was obtained with a Milli-Q system (Millipore), and HPLC grade methanol was supplied by Merck (Darmstadt, Germany).

2.8. Statistics

The statistical analysis of the locomotor activity (total photocell counts during the 1-h session) and neurochemical data was performed with analyses of variance with repeated measures over time with rat lines (AA, ANA) and treatment (saline, nicotine) as the independent factors analyzed. Activity scores on the challenge day were analyzed using a two-way (rat line, treatment) analysis of variance. Post hoc analyses were performed with Newman–Keuls test where appropriate.

3. Results

3.1. Locomotor activity

The first nicotine injection day shows the effects of acute nicotine on locomotor activity in AA and ANA rats (Fig. 1). A two-way analysis of variance conducted on the activity scores revealed that nicotine increased activity significantly more than saline [$F(1,28) = 30.718$, $P < 0.001$, for treatment]. A significant main effect of rat line showed that AA rats were more activated than ANA rats [$F(1,28) = 8.818$, $P < 0.006$], but a similar line difference

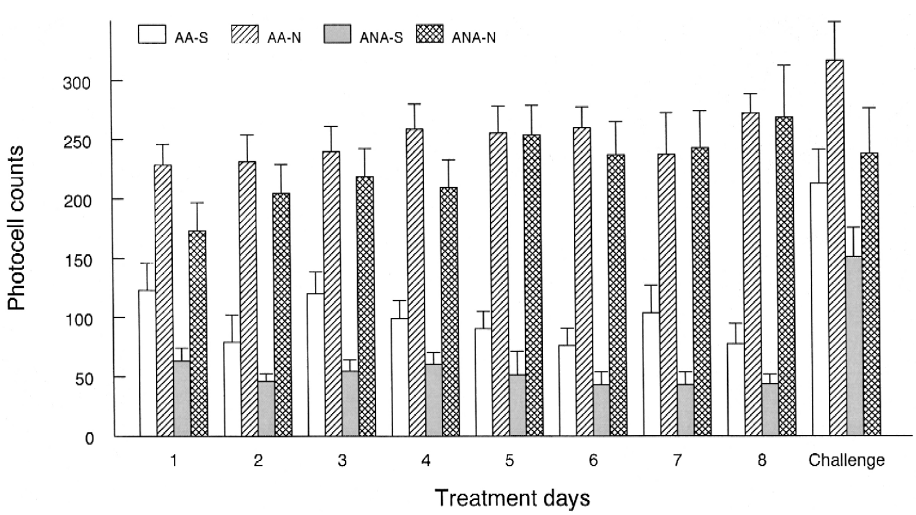

Fig. 1. The effect of repeated administration of nicotine on locomotor activity in alcohol-preferring AA (AA-N) and alcohol-avoiding ANA (ANA-N) rats. Nicotine (0.5 mg/kg, s.c.) was administered on eight consecutive days, and a challenge dose was given 1 week after termination of the nicotine injections. Controls (AA-S, ANA-S) received saline. Locomotor activity was monitored for 1 h. Mean photo cell counts ± S.E.M. of eight animals are given for each day.

was produced by saline scores, as shown by the non-significant rat line × drug interaction.

Nicotine injections activated AA and ANA rats significantly more than saline injections over the eight treatment days, as revealed by the significant main effect of drug [$F(1,28) = 103.135$, $P < 0.001$]. In order to analyze further the significant main effect of line [$F(1,28) = 4.348$, $P = 0.046$] and the day × drug interaction [$F(7,196) =$

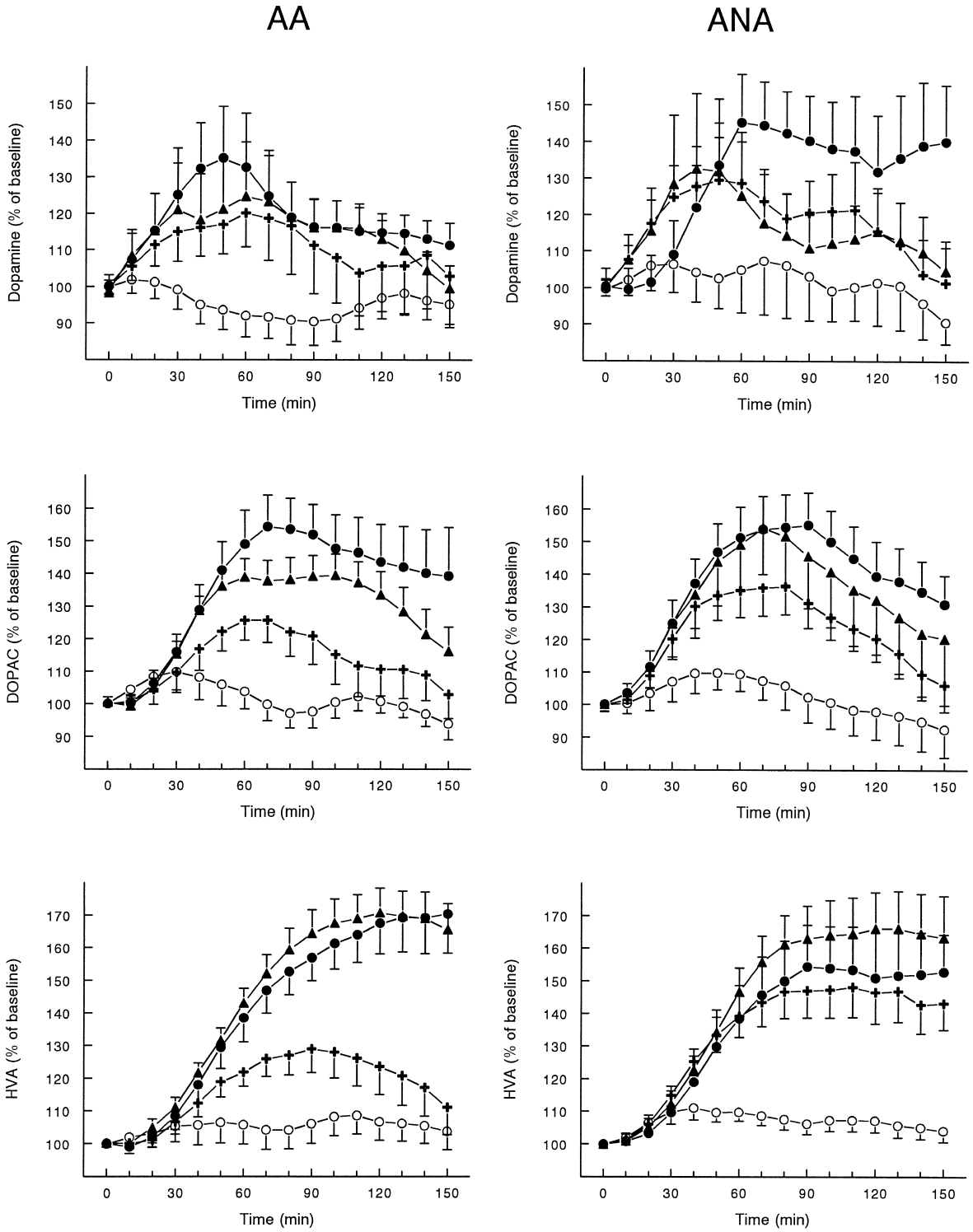

Fig. 2. The effect of an acute, subcutaneous injection of 0.25 (+), 0.5 (▲) or 0.75 (●) mg/kg of nicotine on the extracellular levels of dopamine, DOPAC and HVA in the nucleus accumbens of alcohol-preferring AA and alcohol-avoiding ANA rats. The controls (○) were injected with saline. The values are expressed as a percentage of the preinjection baseline levels. The moving average of three time points ± S.E.M. of 8–18 animals is given.

4.449, $P < 0.001$], simple effects analyses were conducted separately on the nicotine and saline groups. These analyses showed that there was no difference in nicotine-induced locomotion between AA and ANA rats during the eight injection days. However, there was a significant main effect of day [$F(7,98) = 3.250$, $P = 0.004$], indicating that daily nicotine injections progressively increased locomotor activity in AA and ANA rats. In contrast, the activity of saline-treated rats did not change over the injection days, but there was a significant line difference in activity [$F(1,14) = 9.465$, $P = 0.008$], reflecting the baseline activity difference seen during the last habituation day [$F(1,30) = 5.404$, $P < 0.027$, data not shown].

On the nicotine challenge day, 1 week after termination of daily nicotine and saline injections, all rats were tested after a challenge nicotine injection (Fig. 1). Rats that had previously received repeated nicotine injections were significantly more activated by the challenge injections than those treated with saline [main effect of treatment $F(1,28) = 9.171$, $P = 0.005$], indicating that nicotine treatment had sensitized the animals to the locomotor activating effects of nicotine. Both nicotine and saline ANA rats were lower than AA rats [$F(1,28) = 4.960$, $P = 0.034$, for rat line], but there was no rat line × drug interaction, showing that the lines did not differ in the amount of sensitization.

3.2. Microdialysis studies

An acute dose of nicotine significantly increased the extracellular levels of dopamine in the nucleus accumbens of both AA and ANA rat lines [$F(3,69) = 3.869$, $P = 0.013$, for treatment; $F(14,966) = 3.122$, $P < 0.001$, for time] (Fig. 2). The effect of nicotine did not seem to be dose dependent: all doses increased the levels of dopamine in a similar manner. The AA and ANA rats did not differ in the effect of nicotine over the 150-min period. Although the over all time × rat line × treatment interaction did not reach significance, the effect of the highest dose of nicotine (0.75 mg/kg) was longer lasting in the ANA line than in the AA line [$F(14,280) = 2.102$, $P = 0.012$, for time × rat line interaction].

In parallel with its effect on dopamine, nicotine increased the extracellular levels of DOPAC in a similar manner in the nucleus accumbens of both rat lines [$F(3,70) = 8.746$, $P < 0.001$, for treatment; $F(14,980) = 22.400$, $P < 0.001$, for time] (Fig. 2). In contrast to dopamine, this effect seemed to be dose dependent, since there was a significant difference between nicotine-treated groups in the effect of nicotine [$F(2,57) = 3.398$, $P = 0.04$, for treatment].

The extracellular concentrations of HVA were also significantly increased in the nucleus accumbens of AA and ANA rats [$F(3,91) = 14.979$, $P < 0.001$, for treatment; $F(14,1274) = 6.611$, $P < 0.001$, for time] (Fig. 2). A significant main effect of treatment was found between the nicotine-treated groups suggesting dose dependence in the effect of nicotine, but this seemed to be due to the lower response in the AA line after the lowest dose of nicotine. Although the overall time × rat line × treatment interaction did not reach significance [$F(14,1274) = 1.344$, $P = 0.072$], the HVA levels remained elevated longer in the ANA rats than in the AA rats following the lowest

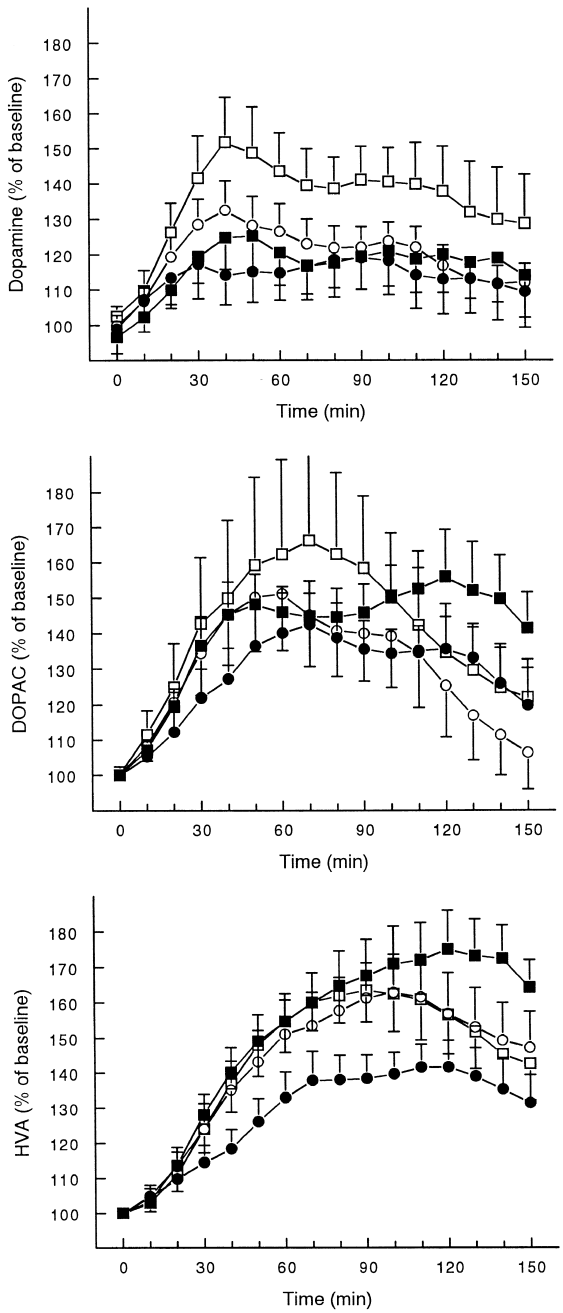

Fig. 3. The effect of repeated, once-daily administration of nicotine (0.5 mg/kg, s.c.) for 8 days on the extracellular levels of dopamine, DOPAC and HVA in the nucleus accumbens of alcohol-preferring AA (■) and alcohol-avoiding ANA (●) rats. The AA (□) and ANA (○) controls were injected with saline on days 1–7, but received nicotine (0.5 mg/kg, s.c.) on day 8. The values are expressed as a percentage of the preinjection baseline levels. The moving average of three time points ± S.E.M. of 7–11 animals is given.

dose (0.25 mg/kg) of nicotine [$F(14,280) = 2.228$, $P = 0.007$, for time × rat line interaction].

The rats treated repeatedly with nicotine (0.5 mg/kg) showed a significant increase in the concentration of extracellular dopamine [$F(14,448) = 2.796$, $P = 0.001$, for time] when measured on day 8 after an injection of nicotine (0.5 mg/kg) (Fig. 3). No difference was seen between the rats pretreated with nicotine or saline, or between the two rat lines in the effect of nicotine. The levels of DOPAC were also significantly increased by nicotine [$F(14,364) = 9.479$, $P < 0.001$, for time] and there also was a significant time × treatment interaction [$F(14,364) = 3.972$, $P < 0.001$] suggesting that the effect of nicotine on the extracellular levels of DOPAC was longer lasting and sensitized the animals receiving nicotine repeatedly. The concentrations of HVA were also elevated after nicotine administration [$F(14,532) = 29.107$, $P < 0.001$, for time], but the interaction was not significant.

Attempts to monitor the effects of a challenge injection of nicotine by reinserting the probe 1 week after termination of repeated nicotine or saline injections were only partly successful. The effect of nicotine on the concentration of extracellular dopamine appeared to follow the same pattern as seen on day 8, but it did not reach significance in the limited material (Fig. 4). Consequently, an enhanced response to nicotine could not be demonstrated.

The basal extracellular levels of dopamine in the nucleus accumbens of AA and ANA rats were 10.7 ± 1.5 and 11.9 ± 2.8 fmol/14 μl, respectively. The values for DOPAC were 3.16 ± 0.32 and 3.28 ± 0.29 pmol/14 μl, and for HVA, 1.25 ± 0.86 and 1.41 ± 0.11 pmol/14 μl, respectively.

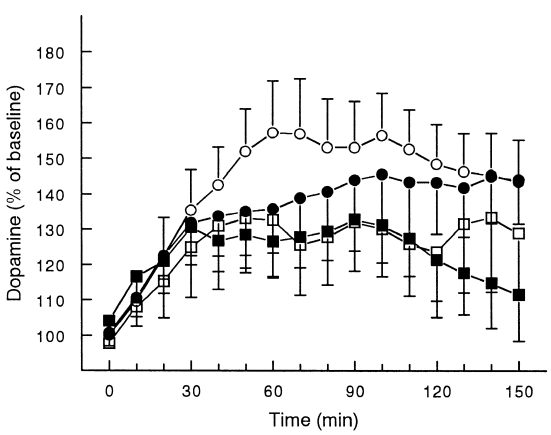

Fig. 4. The effect of a challenge dose of nicotine (0.5 mg/kg, s.c.) given 1 week after termination of the repeated, once-daily administration of nicotine (0.5 mg/kg, s.c.) for 8 days on the extracellular levels of dopamine in the nucleus accumbens of alcohol-preferring AA (■) and alcohol-avoiding ANA (●) rats. The AA (□) and ANA (○) controls were injected with saline on days 1–7, but received nicotine (0.5 mg/kg, s.c.) on day 8 and on the challenge day. The values are expressed as a percentage of the preinjection baseline levels. The moving average of three time points ± S.E.M. of four to seven animals is given.

Table 1
The concentration of nicotine (ng/ml) in the plasma of alcohol-preferring AA and alcohol-avoiding ANA rats after subcutaneous administration of nicotine 0.5 mg/kg

Rat line	2–5 min	10 min	30 min	60 min
AA	83 ± 23	126 ± 10	180 ± 60	116 ± 19
ANA	104 ± 14	160 ± 13	131 ± 19	86 ± 6

The values (means ± S.E.M.) are given in ng/ml plasma, $n = 6–15$.

3.3. Nicotine concentrations in plasma

The time courses of plasma nicotine concentrations after an acute subcutaneous injection of nicotine (0.5 mg/kg) are shown in Table 1. There were no significant differences in nicotine concentrations between the two rat lines.

4. Discussion

Evidence was obtained for both behavioral and neurochemical stimulation after an acute dose of nicotine as well as for behavioral sensitization after repeated administration of nicotine for 8 days in alcohol-preferring AA and alcohol-avoiding ANA rats.

Acute administration of nicotine (0.5 mg/kg) increased locomotor activity in both AA and ANA rats. This is in agreement with previous reports where administration of nicotine (0.4–0.6 mg/kg) was followed by an increase in locomotor activity (Imperato et al., 1986; Benwell and Balfour, 1992; Nisell et al., 1996; Reid et al., 1998). Evidence for locomotor sensitization was found after repeated nicotine treatment. A progressive increase in locomotion was seen in the rats during the 8-day nicotine pretreatment, and the nicotine challenge dose increased locomotor activity more in nicotine than saline animals. These findings are in line with the studies by Nisell et al. (1996) and by Reid et al. (1998), where rats pretreated for 12 (0.5 mg/kg) and 15 days (0.6 mg/kg), respectively, showed behavioral sensitization. In the studies by Benwell and Balfour (1992) and by Reid et al. (1996), 5-day pretreatment with nicotine was, however, sufficient for the demonstration of a sensitized behavioral response.

Acute administration of nicotine in doses of 0.25–0.75 mg/kg significantly increased the extracellular levels of dopamine as well as those of DOPAC and HVA in the nucleus accumbens of both alcohol-preferring AA and alcohol-avoiding ANA rats, suggesting that nicotine increased release of dopamine in the nucleus accumbens. These data are in accord with previous studies using microdialysis showing that acute administration of nicotine increases extracellular levels of accumbal dopamine (Bassareo et al., 1996; Reid et al., 1996, 1998; Nisell et al., 1997; Benwell and Balfour, 1997; Balfour et al., 1998),

dopamine and DOPAC (Damsma et al., 1989; Benwell and Balfour, 1992), and dopamine, DOPAC and HVA (Imperato et al., 1986; Nisell et al., 1996).

The effect of nicotine on the extracellular levels of dopamine did not appear to be dose-dependent in the present study: all the three doses (0.25, 0.50 and 0.75 mg/kg) increased accumbal dopamine in a similar manner. In contrast, this did not seem to be true in the cases of DOPAC and HVA, where significant differences between the effects of different doses were found. Benwell and Balfour (1997) did suggest that the duration of the response to nicotine rather than the response is dose-dependent. Adding nomifensine in the Ringer solution, they found that nicotine increased the levels of dopamine measured extracellularly in the nucleus accumbens at all the doses of 0.1, 0.4 or 0.8 mg/kg tested in a rather similar extent. Lack of dose–response relationship was also seen in their earlier study in the levels of DOPAC and HVA after administration of 0.1 or 0.4 mg/kg of nicotine although dopamine was not affected (Benwell and Balfour, 1992). Bassareo et al. (1996), however, reported a clear dose–response relationship after giving rats 0.3 or 0.6 mg/kg of nicotine.

The rats treated repeatedly with nicotine (0.5 mg/kg) showed a significant increase in the levels of dopamine, DOPAC, and HVA in the nucleus accumbens on the eighth day of treatment. No evidence was obtained for neurochemical sensitization to nicotine in terms of the amplitude of the response. The duration of the increase in the levels of DOPAC was, however, longer in the nicotine than saline animals suggesting a sensitized response in the animals receiving nicotine repeatedly.

Attempts to monitor the effects of a challenge injection by reinserting the probe 1 week later failed to give further information on the sensitization aspect. Previous studies have produced somewhat contradictory results indicating the complexity of the issue. A sensitized release of dopamine in the nucleus accumbens to nicotine has been found in rats after 5 or 15 daily injections of nicotine (0.4 mg/kg) (Reid et al., 1996, 1998; Cadoni and Di Chiara, 1999), but such a response was not found in accumbal dopamine or DOPAC by either Nisell et al. (1996) or Damsma et al. (1989) in rather similar studies in animals receiving 12 daily injections of nicotine (0.35 or 0.5 mg/kg).

The behavioral data did not provide any evidence for a differential sensitivity of the two rat lines to nicotine. The lines also did not differ in the amplitude of the nicotine-induced increase in extracellular dopamine. The only thing suggesting higher sensitivity of one line to the effect of nicotine was the finding that the dopamine levels remained elevated longer in the ANA rats after the highest dose of nicotine (0.75 mg/kg). Furthermore, a similar trend was seen in the concentrations of HVA. Since there were no differences in the concentrations of nicotine in the plasma of the AA and ANA rats, it is unlikely that the findings can be explained in terms of differential pharmacokinetics of nicotine among the lines. Longer dopamine release in the ANA line that receives less reinforcement from ethanol seems contrary to the hypothesis of dopamine mediating ethanol reinforcement.

Nicotine thus augmented the release of dopamine in the nucleus accumbens of the AA and ANA rats, but there was no further change in responsiveness as a result of repeated administration of nicotine excluding the prolongation of the increase in the levels of DOPAC.

Since nicotinic agents and ethanol have been found to cause changes in dopaminergic functions and ethanol-related behaviors via interaction, this is a tempting target of speculation. For instance, nicotinic acetylcholine receptor agonists and antagonists can modify ethanol self-administration (Pothoff et al., 1983; Gauvin et al., 1993; Blomqvist et al., 1996; Katner et al., 1997; Nadal et al., 1998; Lê et al., 2000) as well as ethanol-induced changes in locomotor activity (Blomqvist et al., 1992) and extracellular dopamine in the nucleus accumbens (Blomqvist et al., 1993; Ericson et al., 1998). Furthermore, ethanol increases the affinity of the nicotinic acetylcholine receptor for agonists (Collins, 1995). Since the rat lines have been selected for differential voluntary ethanol consumption, the present findings according to the basic hypothesis suggest that there are no differences in the interaction of the cholinergic and dopaminergic systems of rat lines that result in differential ethanol reinforcement and contribute to the difference in ethanol self-administration between the lines.

In conclusion, nicotine increased locomotor activity and increased extracellular dopamine concentrations in the nucleus accumbens of alcohol-preferring AA and alcohol-avoiding ANA rats. Repeated administration of nicotine augmented the behavioral and neurochemical effects. Dose-dependence was seen in the amplitude of the increase in DOPAC but not dopamine levels. This was augmented in the alcohol-avoiding ANA rats suggesting that there are differences in the interaction of the cholinergic and dopaminergic systems of rat lines, but they are not in the direction expected from the hypothesis that dopamine mediates the reinforcement from ethanol.

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