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Behavioral and Biochemical Manifestations of Mecamylamine-Precipitated Nicotine Withdrawal in the Rat: Role of Nicotinic Receptors in the Ventral Tegmental Area

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Brain mesolimbic dopamine (DA) neurons are considered critical for the dependence-producing action of nicotine, and its stimulatory effect on behavior and DA neurotransmission appears largely mediated via nicotinic receptors (nAChRs) in the ventral tegmental area (VTA). The nAChR antagonist mecamylamine administered systemically in chronically nicotine-treated rats elicits a behavioral withdrawal syndrome concomitant with a reduced DA output in the nucleus accumbens (NAC). Here, we investigated the behavioral and biochemical consequences of intrategmental administration of mecamylamine in rats chronically infused with nicotine by means of minipumps for 14 days (9 mg/kg/day). Bilateral, intrategmental mecamylamine injections (1, 3 or 9 μg/0.5 μl/ side) dose-dependently increased abstinence signs such as gasps, teeth chatter, and reduced locomotor activity in nicotine-treated, but not in control animals. Moreover, a unilateral intrategmental injection of 9 μg mecamylamine reduced DA output in the ipsilateral NAC of chronically nicotine-treated rats, but not in control animals. Consequently, nAChRs in the VTA may be involved not only in the stimulatory effects of acute nicotine administration, but also in the withdrawal reaction following cessation of chronic nicotine treatment.
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KEY WORDS: *Dopamine; Intracerebral injections; Locomotor activity; Microdialysis; Nicotine dependence; Nucleus accumbens*

The habit of tobacco smoking is widely considered to represent a form of drug addiction to nicotine (Clarke 1990; Corrigall et al. 1992) and, in fact, constitutes the leading preventable cause of human morbidity and premature mortality in the US (US Department of Health and Human Services 1988). Although smokers are usually aware of the negative health consequences of their habit, the prognosis of successful smoking cessation is relatively poor. Thus, approximately 80% of smokers enrolled in cessation programmes fail to quit (Stitzer and Gross 1988). A nicotine withdrawal reaction, which usually emerges within 24 hrs after the last cigarette, is considered to significantly contribute to the high relapse rate during the early stages of attempted cessation (Shiffman and Jarvik 1976; Hughes et al. 1992; Killen et al. 1991; West et al. 1989), although a lack of correlation

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between withdrawal severity and probability for relapse has been reported in other studies (see Hughes et al. 1991).

In rats chronically treated with nicotine, sudden withdrawal precipitated by systemic administration of the non-competitive nicotinic acetylcholine receptor (nAChR) antagonist mecamylamine (Malin et al. 1994; Hildebrand et al. 1997), as well as spontaneous withdrawal caused by termination of a chronic nicotine infusion (Malin et al. 1992; Hildebrand et al. 1997), produces behavioral symptoms similar to those described in opiate withdrawal (see Bläsig et al. 1973). Acute as well as chronic administration of nicotine increases locomotor activity in rats (Benwell and Balfour 1992; Clarke and Kumar 1983; Stolerman et al. 1973), whereas in nicotine withdrawal locomotor activity is markedly reduced (Malin et al. 1992, 1994; Hildebrand et al. 1997).

Compelling evidence indicates that the mesolimbic dopamine (DA) system plays a pivotal role in mediating the reinforcing effects of various dependence-producing drugs and in goal-oriented behaviors elicited by natural rewards or incentive stimuli, e.g. feeding, drinking, and sexual activity, as well as in the control of locomotion (Koob 1992; Koob and Bloom 1988). This neuronal system consists of cell bodies localized in the ventral tegmental area (VTA) with axons projecting *inter alia* to limbic structures, such as the nucleus accumbens (NAC) and the amygdala (Björklund and Lindvall 1984).

The presence of nAChRs has been demonstrated both in the VTA and in several DA target areas, including the NAC (Clarke and Pert 1985; Wonnacott 1990). Hence, nicotine can affect mesolimbic DA functions at both levels. Acute systemic administration of nicotine increases neuronal activity, in particular burst firing, of DA neurons located in the VTA (Grenhoff et al. 1986; Mereu et al. 1987), as well as the release of DA in terminal areas (Imperato et al. 1986; Nisell et al. 1994, 1996). These effects are associated with behavioral correlates, such as locomotor stimulation (cf. above) and self-administration (Corrigall and Coen 1989), which can be abolished by lesions of the mesolimbic DA projection as well as by systemic administration of DA receptor antagonists (Clarke et al. 1988; Corrigall and Coen 1991). Thus, stimulation of the mesolimbic DA system appears to be of critical importance for the reinforcing, behaviorally stimulant and dependence-producing properties of nicotine (Clarke 1990; Corrigall 1991). Conversely, the behavioral withdrawal reaction precipitated by systemically administered mecamylamine in chronically nicotine-treated rats has been found to be associated with a marked reduction of DA output in the NAC (Hildebrand et al. 1998). This finding is analogous to the reported decrease in DA output in the NAC following withdrawal from chronic treatment with several other dependence-producing drugs, such as amphetamine, cocaine, ethanol and morphine (see Rossetti et al. 1992).

Experimental data strongly support the hypothesis that several behavioral effects of systemically administered nicotine that can be related to mesolimbic DA neurotransmission are executed primarily by stimulation of nAChRs in the VTA. For example, nicotine applied intrategmentally elicits a more pronounced locomotor activating effect than that of intraaccumbal administration (Reavill and Stolerman 1990; Leikola-Pelho and Jackson 1992; Goshima et al. 1996). In addition, intrategmental application of the nAChR agonist cytisine enhances locomotion, an effect that is blocked by systemic administration of either a nAChR antagonist or a dopaminergic antagonist (Museo and Wise 1990). Also the rewarding properties of nicotine appear to be executed primarily within the VTA, since blockade of nAChRs in the VTA, but not in the NAC, abolishes nicotine self-administration in rats (Corrigall et al. 1994). This notion is further strengthened by the finding that application of cytisine into the VTA can produce a conditioned place preference (Museo and Wise 1994).

Biochemically, the nicotine-induced increase in DA output in the NAC seems to be due primarily to stimulation of nAChRs located in the VTA, rather than in the NAC, since local administration of mecamylamine intrategmentally, but not into the NAC, antagonizes the systemic nicotine-induced DA release within the NAC (Nisell et al. 1994). Consequently, nAChRs located in the VTA seem crucial for the stimulatory effects of systemic nicotine on accumbal DA output, an effect which seems caused by increased neuronal activity, particularly burst firing, in VTA DA neurons (cf. above). In addition, the postsynaptic consequences, as reflected by an activation of immediate-early genes, of the enhanced DA overflow in the NAC following systemically administered nicotine (Kiba and Jayaraman 1994) can be mimicked by nicotine application into the VTA (Panagis et al. 1996). However, the putative role of nAChRs in the VTA in the above described behavioral and biochemical manifestations of the nicotine withdrawal syndrome has, as yet, not been investigated. Therefore, in the present study we examined the effects of intrategmental mecamylamine administration on the symptoms of the withdrawal reaction, locomotor activity and DA output in the NAC.

METHODS

Subjects

All experiments in the present study were performed with the consent of the local ethical committee and careful measures were taken to minimize pain or discomfort of the animals. Subjects were male Wistar rats, weighing about 280–330 g at the time of surgery, that

were housed in individual cages with *ad libitum* access to food and water in a temperature and humidity controlled room under a 12-hr light:dark cycle (lights on at 6.00 A.M.). All experiments were performed during the daylight period (8.00 A.M. to 6.00 P.M.).

Chronic Nicotine Treatment

Rats in the nicotine-treated group were under sodium pentobarbital (Apoteksbolaget, 60 mg/kg, i.p.) anaesthesia implanted subcutaneously with Alzet osmotic minipumps (model 2ML2) containing nicotine hydrogen tartrate (Sigma); nicotine was dissolved in saline and the solution pH-adjusted to 7.20–7.40. The concentration of nicotine was adjusted to compensate for differences in the body weight of the subjects, and the average-weighed rat received a dose of approximately 9 mg/kg/day nicotine hydrogen tartrate for 14 days. Before implantation, each pump was primed for 4 hrs in 37°C physiological saline. Rats in the control group were implanted with empty minipumps.

Alzet minipumps have been widely employed for chronic continuous administration of nicotine as a model of nicotine dependence (Epping-Jordan et al. 1998; Hildebrand et al. 1998; Malin et al. 1992). The stability of nicotine in the minipumps, as well as the achieved steady-state concentration in the plasma of the rat, have been demonstrated to be both reliable and reproducible (Benwell et al. 1994, 1995). In the present study we quantified, by means of gas chromatography, as previously described (Curvall et al. 1982), the nicotine content in rat plasma after 14 days of infusion. The mean concentration was 32 ng/ml (ranging between 30 and 37 ng/ml; $n = 6$).

In previous experiments, using Alzet 2ML1 minipumps, we assessed the nicotine content in rat plasma after seven days of infusion of the same dose as in the present experiments (unpublished observations); the mean nicotine concentration at that time was 43 ng/ml (ranging between 33 and 61 ng/ml; $n = 6$). The significantly ($p = 0.034$) lower steady-state nicotine levels after the longer treatment regimen may, in all probability, reflect an enhanced metabolic degradation and/or elimination of nicotine. The nicotine levels found in the plasma of the rats in the present study correspond to those encountered in smokers consuming 1–2 packets of cigarettes a day, i.e. 39 ng/ml (Sutherland et al. 1992).

Intracranial Surgery

Implantation of Guide Cannulae. The rats were anaesthetized with an induction dose of sodium pentobarbital (Apoteksbolaget; 60 mg/kg, i.p.) followed by, if necessary, smaller i.p. maintenance doses. Subsequently, they were mounted in a stereotaxic frame (Kopf) and implanted with guide cannulae (18 mm long, 25 gauge) into the VTA. With the incisor bar set at −3.3 mm, the coordinates were (in mm): 5.3 posterior to bregma, 0.7 lateral to the midline, and 7.2 below the brain surface (i.e. *dura mater*) according to the atlas (Paxinos and Watson 1998). The tips of the guide cannulae were located 1.0 mm above the actual injection sites. The guide cannulae were subsequently fixed to the skull with anchoring screws and dental cement. Copper wires (18 mm, 31 gauge) were inserted into the guide cannulae and, thus, prevented them from clogging. The rats that were used for microdialysis experiments were implanted with a unilateral (right side) cannula, whereas rats used for the behavioral experiments were implanted bilaterally.

Implantation of Microdialysis Probes. Immediately following the implantation of the guide cannula, as described above, each rat used for the microdialysis experiments was stereotaxically implanted in the ipsilateral (right side) NAC with a vertical concentric microdialysis probe of own production. Coordinates (in mm) were, with the incisor bar set at −3.3: AP +1.6, ML −1.2, and DV −8.2 measured from bregma. The probe was anchored and fixed to the skull with stainless steel screws and dental cement. The active surface length of the semipermeable dialysis membrane (copolymer of acrylonitrile and sodium methallyl sulfonate, inner-diameter (i.d.) = 0.24 mm; 40,000 Da molecular weight cutoff, Hospal AN69, Filtral 10) was 2.25 mm beginning approximately 0.5 mm from the tip of the probe.

Intrategmental Injections

For intrategmental drug administration, an injection cannula (19 mm long, 31-gauge) was inserted into the guide cannulae, thus protruding 1 mm into the VTA, and connected via PE50 polyethylene tubing to a 1 ml CMA microsyringe (Carnegie Medicin AB) mounted in a CMA 100 microinjection pump (Carnegie Medicin AB). A volume of 0.5 μl saline or 0.5 μl (containing 1, 3, or 9 μg) mecamylamine hydrochloride (RBI) solution was injected into the VTA of the restrained animal during approximately 1 min. The injection cannula was then left in place for an additional 1 min after the injection, in order to allow sufficient diffusion of the drug. The day prior to the experiment, during habituation, the injection cannula was inserted in the guide cannulae for 1 min without any actual injection. This insertion most likely caused a small brain tissue lesion similar to that produced by an actual injection, thus interference by putative lesion-induced effects with drug-induced effects on biochemistry or behavior on the experimental day was, in all probability, minimal.

Experimental Procedures

Different groups of rats were used for the assessment of somatic signs, measurements of locomotor activity, and microdialysis experiments, respectively.

Assessments of Somatic Signs. Nine chronically nicotine-treated and 10 control animals were used. The animal was placed in a Plexiglas cage (35 × 35 × 40 cm) where it remained for a 30 min session during which its behavior was observed. The frequency of gasps, ptosis, shakes, teeth chatter, and yawns was assessed by an observer that was unaware of the treatment that the animal had received. Ptosis, if displayed, was only counted once per minute. Hence, the maximal score for ptosis was 30. The individual categories of signs were defined prior to the experiments. The total score for abstinence signs was calculated as the sum of the scores for all individual signs of the withdrawal reaction.

Assessments of somatic signs were performed on five consecutive days. The day prior to the start of the series of observations, the animals were habituated to the test cage for 60 min. Before the onset of habituation, each rat was also restrained and received an intrategmental insertion, as outlined above. The first of the five experimental sessions occurred 10 days and the last 14 days after the beginning of nicotine infusion, or after the implantation of an empty pump. In the first (day 10) and the last (day 14) session, animals received bilateral intrategmental injections (as described above) with 0.5 μl saline and in the sessions on day 11–13 they were injected with 1, 3, or 9 μg mecamylamine in 0.5 μl saline in a random order across animals and days, as previously described in a study of morphine withdrawal (Maldonado et al. 1992). The last saline injection (day 14) was performed in order to detect tentative effects of conditioned withdrawal on behavior.

Immediately after drug administration, the animal was transferred to the Plexiglas cage and the observation commenced. The Plexiglas cage was wiped clean between animal sessions.

Measurements of Locomotor Activity. Eight chronically nicotine-treated and eight control animals were used. Motor activity was measured in a computer-assisted square open-field arena (68 × 68 × 45 cm), equipped with two rows of eight photocells, along two adjacent sides, that were placed 4 and 12.5 cm above the floor (see Ericson et al. 1991 for a detailed description). The open-field was enclosed in a dark, ventilated, and sound-attenuating box. Any movement of the animal interrupted the photobeams, which was detected and recorded by a Commodore PC20-III microcomputer.

The following quantitative variables were calculated: horizontal activity (all photobeam interruptions in the lower rows); peripheral activity (all interruptions of photobeams spaced next to the wall in the lower rows); forward locomotion (successive interruptions of photocells in the lower rows with the animal moving in the same direction); and rearing (total number of photobeam interruptions in the upper rows). The percentages of peripheral activity and forward locomotion counts to horizontal activity counts were also calculated to assess the pattern, i.e. the qualitative aspects of locomotion. Thus, the percentages of peripheral to horizontal activity (i.e. thigmotaxis; PA/HA) counts and of forward locomotion to horizontal activity (FL/HA) counts are indicative of the spatial distribution of movements and of the perseverance of forward locomotion, respectively.

Locomotor activity was assessed for 30 min on five consecutive days. The day before the first observation, the rats were habituated to the open-field for 30 min. Prior to the habituation, each rat received an intrategmental insertion as described above.

The first experimental session occurred 10 days and the last 14 days after the onset of nicotine infusion, or after implantation of an empty pump. Bilateral intrategmental injections with 0.5 μl saline on day 10 and 14 and with 1, 3, or 9 μg mecamylamine in 0.5 μl saline on days 11–13 were administered as described for the assessments of somatic signs (cf. above). Following drug administration animals were immediately placed in the thoroughly cleaned open-field arenas and the measurement begun.

Microdialysis Experiments. Nine chronically nicotine-treated and eight control animals were used. The day prior to the dialysis experiment, the rats were transported from their home cage to the room where dialysis experiments took place and habituated to the experimental setting. The animals remained there, in the same cage that would be used in the biochemical experiment the next day, for approximately 10 hrs (8.00 A.M. to 6.00 P.M.). During habituation, each rat was restrained and received an intrategmental insertion as outlined above.

The microdialysis experiments were performed in freely moving rats 14 days after the beginning of nicotine infusion, or after the implantation of an empty pump, and approximately 48 hrs after intracranial surgery. The dialysis probes were perfused with a physiological salt solution (Apoteksbolaget) containing 147 mM NaCl, 3.0 mM KCl, 1.3 mM $CaCl_2$, 1.0 mM $MgCl_2$, and 1.0 mM sodium phosphate (pH 7.4) at a rate of 2.5 μl/min by means of a microperfusion pump (Harvard Apparatus). A sample of the perfusate was collected, loaded directly into the sample loop of the injector (Valco Instruments Co.) and automatically injected into the analytical system every 15 min. The loading and injecting modes of the injector were directed by a computer using the Turbochrom 4 software (Perkin Elmer). The extracellular concentrations of DA, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA) were determined by high-performance liquid chromatography with electrochemical detection (HPLC-ED) as previously described (Nisell et al. 1994). The mobile phase was delivered by an HPLC pump (Pharmacia LKB HPLC-pump 2150) at 0.8 ml/min.

Electrochemical detection was accomplished using a coulometric detector (esa Coulochem II) connected with a conditioning cell (esa model 5021) and with a high sensitivity analytical cell (esa model 5011) that allowed amine detection by the sequential oxidation and reduction of the microdialysis samples. Chromatograms were simultaneously collected on a two-pen chart recorder (Kipp and Zonnen).

On the day of the experiment, after a stable outflow of DA, DOPAC, HVA, and 5-HIAA had been established, the animals received first an intrategmental injection of 0.5 μl saline (0.9% NaCl) followed 2 hrs later (i.e. after eight 15-min samples) by an injection of 0.5 μl saline containing 9 μg mecamylamine hydrochloride. The experiment was ended 4 hrs later (i.e. sixteen 15-min samples). At the end of the experiment, a few randomly chosen animals were perfused in the NAC with either a Ca^{2+}-free perfusion solution or with 2 μM tetrodotoxin in the perfusion solution to confirm that the DA assessed was indeed of neuronal origin. In all cases, DA levels decreased substantially, i.e. more than 75% below basal values.

Histological Examination

Rats were killed upon termination of the experiment and their brains were subsequently sliced (50 μm) on a microtome and stained with neutral red in order to allow microscopical verification of cannula and probe (microdialysis experiments) placement. Only the data from rats with cannulae and probes located within the VTA and NAC, respectively, according to the stereotaxic atlas (Paxinos and Watson 1998), were used for subsequent calculations. Representative photomicrographs for the cannula and microdialysis probe placement in the VTA and NAC, respectively, have previously been published (see Panagis et al. 1996; Nisell et al. 1994). In the few nicotine-treated rats that were excluded due to cannula placement outside of the VTA, there were no effects of the mecamylamine injections on any of the behavioral or biochemical parameters studied.

Data Analysis

All statistics were performed using the Statistica software suite (StatSoft Inc.). The basal values of DA and its metabolites in the two treatment groups were evaluated by Student's *t*-test. Raw data were used for the statistical evaluation of the behavioral measurements after the two saline injections and injection of the three doses of mecamylamine, whereas the biochemical data were calculated as percent changes from baseline levels according to the following scheme: Firstly, the average of the two samples preceding the saline injection was defined as 100%; this baseline was then used for the subsequent eight samples. The sample labeled B1 in the figures is the last sample before the saline injection. Secondly, the average of the two samples immediately preceding the mecamylamine injection was defined as 100% and used as a baseline for the following sixteen post-mecamylamine samples. The sample labeled B2 in the figures is the last sample before the mecamylamine injection. Thus, the biochemical data were evaluated in two sets, i.e. one set included the results of the last baseline sample together with the subsequent eight samples after saline injection, whereas the second set included data of the last sample before and all samples after mecamylamine injection. This design was used to compensate for the increases that were seen in the dialysate levels of DA and its metabolites following the saline injection. Statistical significance was determined by using two-way (treatment × time) analysis of variance (ANOVA) with one repeated-measures variable (time; within measurements) or one-way ANOVA with one repeated-measures variable (time), when only a significant time effect was revealed with the two-way ANOVA. The ANOVA was followed by Newman-Keuls post-hoc test, when appropriate. Student's *t*-test was employed for analysis of between group differences, when appropriate, i.e. when only a significant treatment effect was revealed with the ANOVA. *p*-values of less than 0.05 were considered to be significant.

RESULTS

Effects of Intrategmental Mecamylamine Administration on the Somatic Signs of the Nicotine Withdrawal Reaction

Statistical evaluation of the number of overall or individual categories of signs showed no significant differences between the groups treated with nicotine ($n = 9$) and control animals ($n = 10$) in the first or in the last session, i.e. after the intrategmental saline injections. However, analysis of the occurrence of gasps following intrategmental mecamylamine showed a significant time [$F(4,68) = 7.52$, $p < .001$] and interaction [$F(4,68) = 3.31$, $p < .05$] effect, whereas the treatment effect did not reach statistical significance. Post-hoc analysis revealed that nicotine-treated rats displayed significantly more gasps, as compared to baseline (intrategmental saline), after intrategmental injection of 3 μg ($p < .01$) or 9 μg ($p < .001$) mecamylamine. Compared with controls, the nicotine-treated group showed significantly more gasps both after injections of 3 μg ($p < .05$) and 9 μg ($p < .001$) mecamylamine (Figure 1a).

Similarly, statistical analysis of teeth chatter scores showed significant time [$F(4,68) = 6.90$, $p < .001$] and interaction [$F(4,68) = 4.64$, $p < .01$] effects, whereas the treatment effect did not reach statistical significance. Post-hoc analysis revealed that nicotine-treated rats dis-

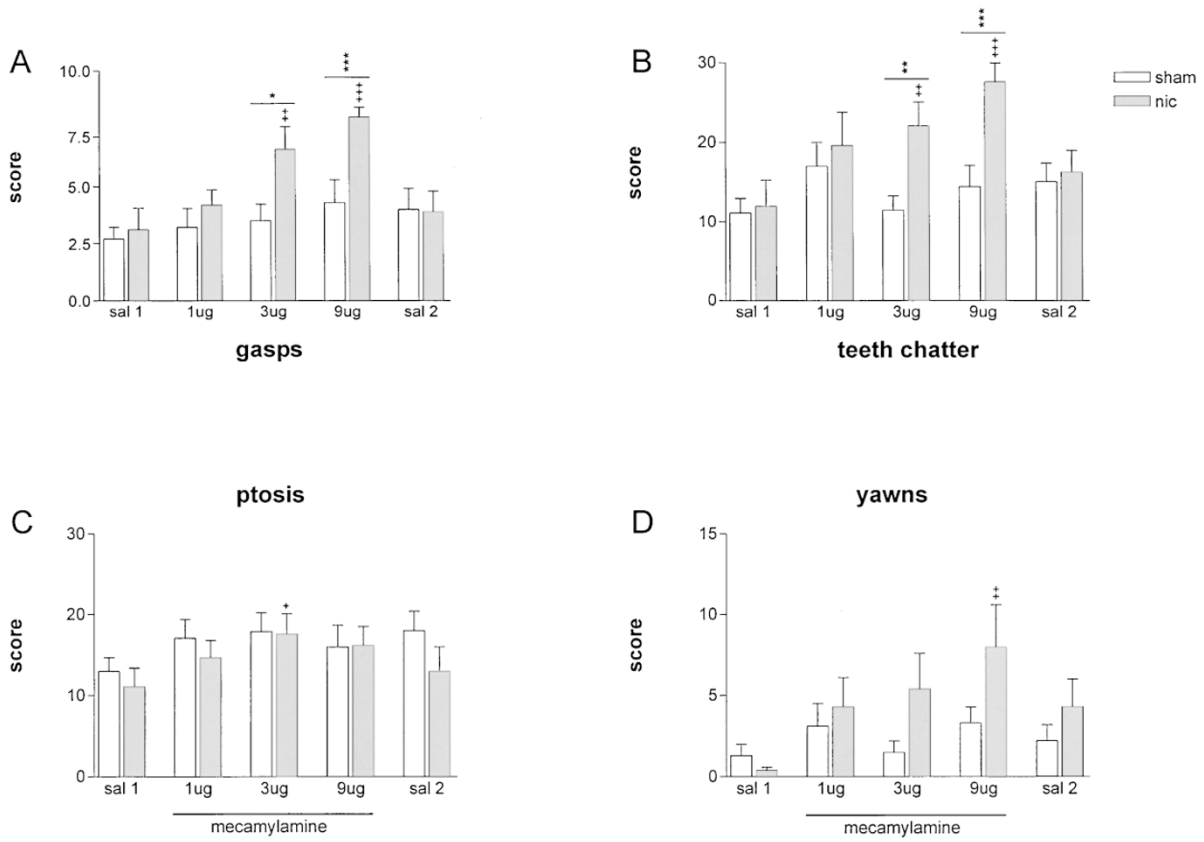

Figure 1. Individual categories of signs of the nicotine abstinence syndrome including **(A)** gasps, **(B)** teeth chatter, **(C)** ptosis, and **(D)** yawns following bilateral intrategmental injections of 0.5 μl saline (sal 1 and sal 2) or three different doses of mecamylamine (1, 3, or 9 μg) in 0.5 μl saline in a group treated chronically with nicotine, i.e. 9 mg/kg/day for 14 days, (n = 9; nic; solid bars) and in a group carrying empty pumps (n = 10; sham; open bars). Data are presented as mean (+ S.E.M.) score over 30 min following drug administration. Crosses indicate within group differences from the respective baseline score (sal 1) and stars between group (treatment) differences. $^{+}$,* = $p < .05$; $^{++}$,** = $p < .01$; and $^{+++}$,*** = $p < .001$.

played a significantly higher teeth chatter score, as compared to the score at baseline, after injection of 3 μg ($p < 0.01$) or 9 μg ($p < .001$) mecamylamine. Animals in the nicotine-treated group demonstrated significantly more teeth chatter, as compared with controls, both after injections of 3 μg ($p < .01$) and 9 μg ($p < .001$) mecamylamine (Figure 1b).

Moreover, statistical analysis of the ptosis and yawns scores showed significant time effects, whereas neither treatment nor interaction effects reached statistical significance. One-way ANOVAs [$F(4,32) = 2.72$, $p < .05$] and [$F(4,32) = 3.38$, $p < .05$] for ptosis and yawns, respectively and post-hoc analysis revealed a significantly increased ptosis score ($p < .05$) following injection with 3 μg mecamylamine, as well as a significant increase in yawns ($p < .01$) following injection with 9 μg mecamylamine in the nicotine-treated group as compared to its baseline scores (Figures 1c and d). No statistically significant effects on the frequency of shakes were detected.

Effects of Intrategmental Mecamylamine Administration on Locomotor Activity

Statistical evaluation showed no significant differences in the various parameters of locomotor activity between animals treated chronically with nicotine (n = 8) and control animals (n = 8) in the first or in the last session, i.e. after the intrategmental saline injections. Intrategmental injection of mecamylamine affected several measures of locomotor activity in a dose-dependent manner in the nicotine-treated group, but not in the nicotine-naive group (Figures 2 and 3).

Specifically, statistical evaluation of horizontal activity counts following intrategmental mecamylamine showed significant treatment [$F(1,14) = 13.25$, $p < .01$], time [$F(4,56) = 5.23$, $p < .01$], and interaction [$F(4,56) = 5.45$, $p < .001$] effects. Post-hoc analysis revealed reduced horizontal activity in the nicotine-treated rats, as compared to baseline, after intrategmental injection of 1 μg ($p < .01$), 3 μg ($p < .01$), or 9 μg ($p < .001$) mecamylamine. Compared with controls, the nicotine-treated

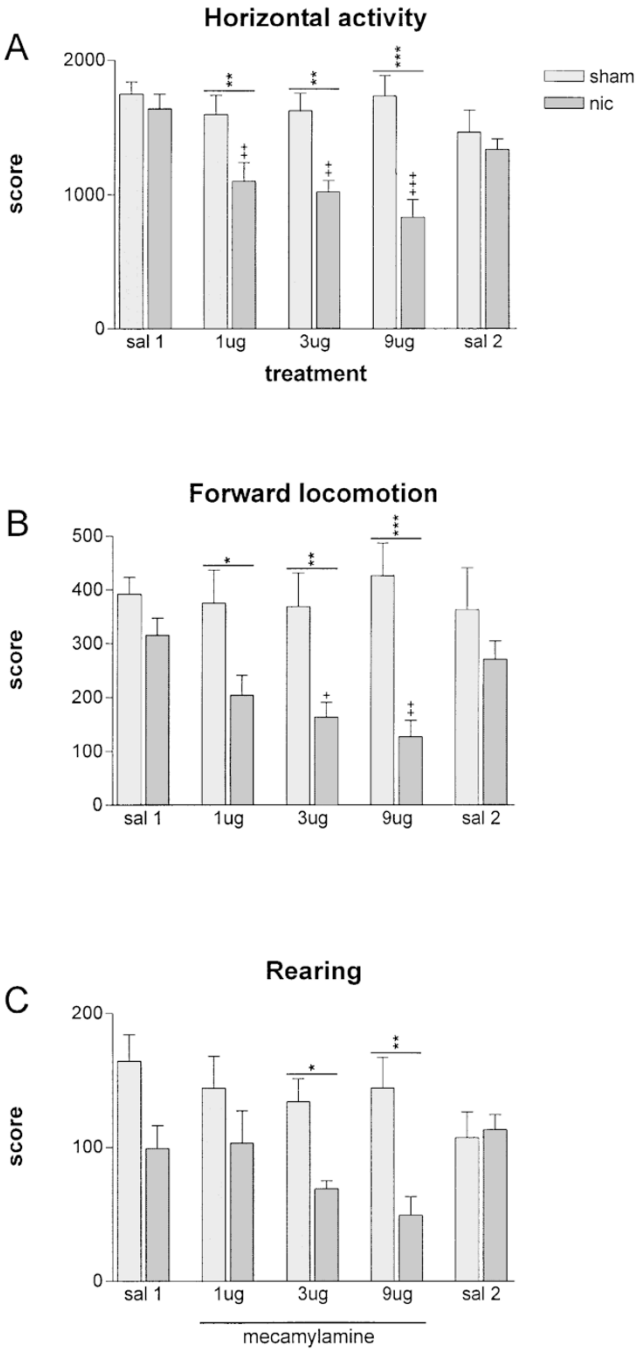

Figure 2. Effects of bilateral intrategmental injections of 0.5 μl saline (sal 1 and sal 2) or various doses of mecamylamine (1, 3, or 9 μg) in 0.5 μl saline on locomotor activity as assessed by **(A)** horizontal activity, **(B)** forward locomotion, and **(C)** rearing measurements in animals treated chronically with nicotine, i.e. 9 mg/kg/day for 14 days, (n = 8; nic; solid bars) and in animals implanted with empty pumps (n = 8; sham; open bars). Data are presented as mean (+ S.E.M.) photobeam interruptions over 30 min following drug administration. Crosses indicate within group differences from the respective baseline score (sal 1) and stars between group (treatment) differences. $^{+}$,* = $p < .05$; $^{++}$,** = $p < .01$; and $^{+++}$,*** = $p < .001$.

group showed significantly less horizontal activity after all doses of mecamylamine ($p < .01$–$.001$) (Figure 2a). In addition, statistical analysis of forward locomotion counts showed a significant treatment [$F(1,14) = 11.02$, $p < .01$] and interaction [$F(4,56) = 3.09$, $p < .05$] effect, whereas, the time effect did not reach statistical significance. Post-hoc analysis revealed that forward locomotion was significantly decreased in the nicotine-treated rats, as compared to baseline, after injection of 3 μg ($p < .05$) or 9 μg ($p < .01$) mecamylamine. Compared with controls, the nicotine-treated group showed significantly less forward locomotion after all doses of mecamylamine ($p < .05$–$.001$), (Figure 2b).

Moreover, statistical analysis of rearing showed a significant treatment [$F(1,14) = 9.78$, $p < .01$] and interaction [$F(4,56) = 2.91$, $p < .05$] effect, whereas, the time effect did not reach statistical significance. Post-hoc analysis revealed a tendency towards a decreased rearing score in the nicotine-treated group following the two higher doses of mecamylamine ($p = .18$ and $p = .07$ for the 3 μg and 9 μg doses, respectively). Compared with controls, however, the nicotine-treated group showed significantly less rearing both after injections of 3 μg ($p < .05$) and 9 μg ($p < .01$) mecamylamine (Figure 2c).

Statistical analysis of the ratio FL/HA, i.e. forward locomotion to horizontal activity, showed a significant treatment [$F(1,14) = 10.19$, $p < .01$] and interaction [$F(4,56) = 3.02$, $p < .05$] effect, whereas, the time effect did not reach statistical significance. Post-hoc analysis revealed that FL/HA was significantly reduced in the nicotine-treated rats, as compared to baseline, only after injection of the highest dose, i.e., 9 μg ($p < .01$) mecamylamine, although there was a tendency also with the 3 μg dose ($p = .09$). Compared with controls, the nicotine-treated group showed a significantly reduced FL/HA-ratio after injections of 3 μg ($p < .01$) and 9 μg ($p < .001$) mecamylamine (Figure 3a).

Finally, statistical analysis of the ratio PA/HA, i.e. peripheral to horizontal activity, showed a significant interaction [$F(4,56) = 3.55$, $p < .05$] effect, whereas neither the treatment nor the time effect did reach statistical significance. Post-hoc analysis revealed that PA/HA was significantly increased in the nicotine-treated rats, as compared to controls following the 9 μg dose of mecamylamine ($p < .05$) (Figure 3b).

Effects of Intrategmental Mecamylamine Administration on Extracellular Concentrations of DA, DOPAC, HVA, and 5-HIAA in the NAC

Basal dialysate concentrations of DA, DOPAC, HVA, and 5-HIAA, expressed as fmol/min (mean ± S.E.M.), were 3.02 ± 0.75, 866 ± 75, 328 ± 48, and 613 ± 116 in

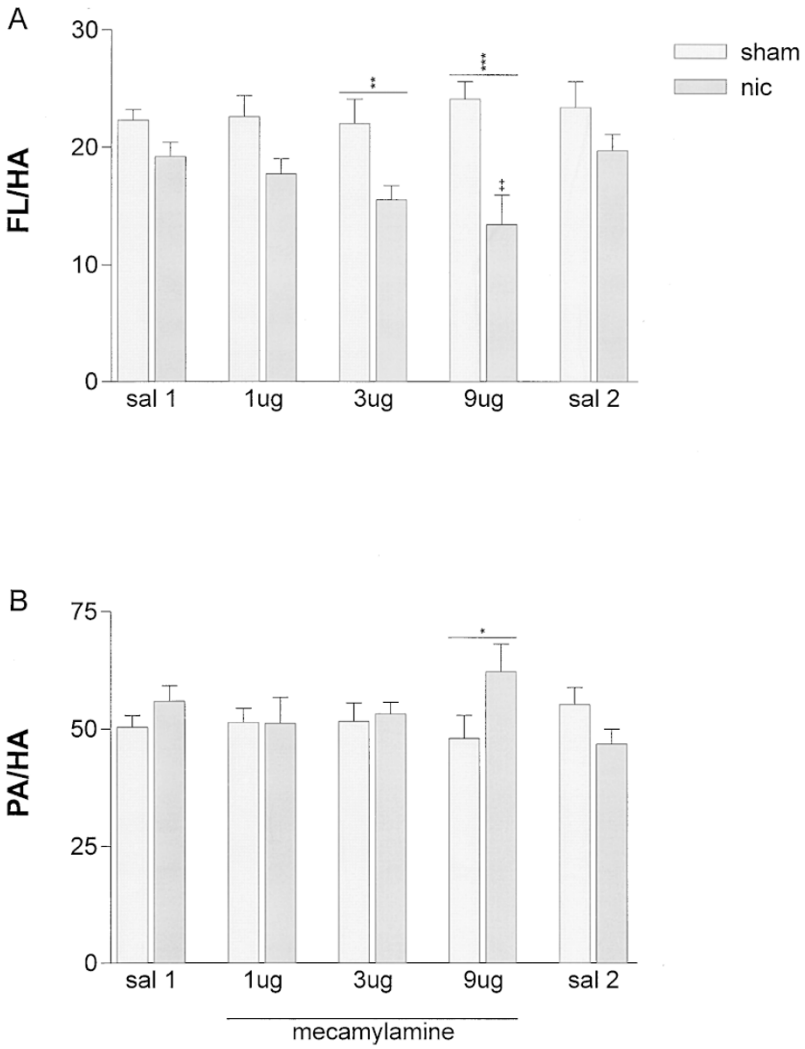

Figure 3. Effects of bilateral intrategmental injections of 0.5 μl saline (sal 1 and sal 2) or different doses of mecamylamine (1, 3, or 9 μg) in 0.5 μl saline on locomotor activity as assessed by **(A)** the ratio of forward locomotion to horizontal activity (FL/HA) counts and **(B)** the ratio of peripheral to horizontal activity (PA/HA) counts in a group treated chronically with nicotine, i.e. 9 mg/kg/day for 14 days, (n = 8; nic; solid bars) and in a group carrying empty pumps (n = 8; sham; open bars). Data are presented as mean (+ S.E.M.) of the ratio of the FL/HA and PA/HA photobeam interruptions over 30 min following drug administration. Crosses indicate within group differences from the respective baseline score (sal 1) and stars between group (treatment) differences. * = $p < .05$; $^{++}$,** = $p < .01$; and *** = $p < .001$.

the nicotine-treated group (n = 9), and 2.51 ± 0.53, 855 ± 107, 329 ± 53, and 1052 ± 156 in the nicotine-naive group (n = 8), respectively. Statistical analysis revealed that the basal levels of DA, DOPAC, or HVA did not significantly differ between the nicotine-naive and the nicotine-treated group, whereas the basal concentration of 5-HIAA was significantly lower in the nicotine-treated group ($p < .05$).

In nicotine-treated, but not in nicotine-naive, animals a significant increase in DA (maximal increase: 16%) outflow was observed following saline injection. Statistical analysis showed a significant time effect, whereas both treatment and interaction effects failed to reach statistical significance. One-way ANOVA [$F(8,64) = 2.81$, $p < .01$] and post-hoc analysis revealed that the increase in DA outflow following saline injection was significant ($p < .05$) (Figure 4) during the first 45 min of the postinjection period, i.e. in samples 1–3.

The DA output in animals treated with nicotine, but not in controls, was reduced following mecamylamine injection (maximal reduction: 25% in sample 7). Statistical evaluation of the data showed significant treatment [$F(1,15) = 11.28$, $p < .01$] and time [$F(16,240) = 6.18$, $p < .001$] effects, whereas the interaction effect did not reach statistical significance. One-way ANOVA [$F(16,128) = 5.49$, $p < .001$] and post-hoc analysis revealed that mecamylamine significantly decreased DA outflow during the 45–210 min postinjection period, i.e. in samples 4–14 ($p < .05$–$.001$) compared to the pre-mecamylamine baseline. Student's t-test revealed that DA output was reduced during the first 15 min as well as during the 30–165 min postinjection period, i.e. in samples 1 and 3–11 ($p < .05$–$.001$) as compared to the control group (Figure 4).

An increase in DOPAC outflow (maximal increase: 13%) was observed in nicotine-naive, but not in nicotine-treated, animals following saline injection. Statistical analysis showed a significant time effect, whereas both treatment and interaction effects failed to reach statistical significance. One-way ANOVA [$F(8,56) =$

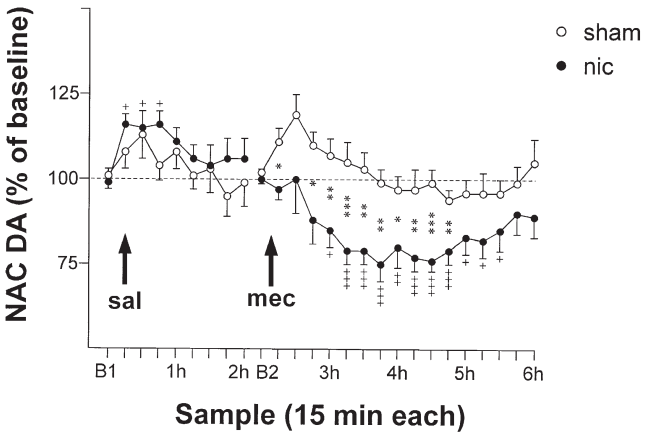

Figure 4. Temporal changes of extracellular concentrations of dopamine (DA) in the nucleus accumbens after ipsilateral intrategmental injection (as indicated by the arrows) of 0.5 μl saline (sal) and 9 μg mecamylamine (mec) in 0.5 μl saline in a group treated chronically with nicotine, i.e. 9 mg/kg/day for 14 days, (n = 9; nic; solid circles) and in a group carrying empty pumps (n = 8; sham; open circles). B1 indicates baseline 1, which is the sample immediately preceding the saline injection (100% in the figure is the average of the two samples preceding the saline injection). B2 indicates baseline 2 which is the sample immediately preceding the mecamylamine injection (100% is defined as the average of the two samples preceding the mecamylamine injection). Data are thus presented as mean (±S.E.M.) extracellular concentrations of DA expressed as percentages of the baselines B1 and B2, respectively. Crosses indicate within group differences from the respective baseline samples and stars between group (treatment) differences. $^{+}$,* = $p < .05$; $^{++}$,** = $p < .01$; and $^{+++}$, *** = $p < .001$.

2.80, $p < 0.05$] and post-hoc analysis revealed that the increase in DOPAC outflow following saline injection was significant during the 15–60 min postinjection period, i.e. in samples 2–4 ($p < 0.05$) (Figure 5a).

Mecamylamine injection in animals treated with nicotine resulted in a transient increase in DOPAC outflow (maximal increase: 18%). Moreover, DOPAC levels in the nicotine-treated group were lower than baseline (maximal decrease: 16%; sample 12) from the sixth sample following mecamylamine injection and throughout the observation period, although statistical significance was not attained. Statistical evaluation of the data showed a significant time [F(16,240) = 9.33, $p < .001$] and interaction [F(16,240) = 2.14, $p < .01$] effect, whereas the treatment effect did not reach statistical significance. One-way ANOVA [F(16,128) = 7.65, $p < .001$] and post-hoc analysis revealed that the increase in DOPAC outflow was significant during the 15–45 min postinjection period only, i.e., in samples 2 and 3 ($p < .05$–.01) as compared to the pre-mecamylamine baseline. Student's t-test showed that DOPAC levels were significantly lower in the nicotine-treated group, as compared to the nicotine-naive group, during the 90–120 min postinjection period, i.e. in samples 7 and 8 following mecamylamine injection ($p < .05$) (Figure 5a).

Moreover, in both nicotine-treated and nicotine-naive animals an increase in HVA outflow (maximal increase: 14% in both groups) was observed following saline injection (Figure 5b). Statistical evaluation of the data showed a significant time effect, whereas the treatment and interaction effects did not reach statistical significance. One-way ANOVAs [F(8,64) = 2.84, $p < .01$] and [F(8,56) = 3.66, $p < .01$]for the nicotine-treated and nicotine-naive groups, respectively and post-hoc analysis revealed that the increase in HVA outflow following saline injection was significant during the 45–90 min (i.e. in samples 4–6; $p < .05$–.01) and 45–60 min (i.e. in sample 4; $p < .01$) postinjection period for the nicotine-treated and the nicotine-naive groups, respectively.

Similarly to DA, mecamylamine injection in nicotine-treated animals resulted in a significant decrease in HVA outflow (maximal reduction: 21%). Statistical analysis showed a significant time [F(16,240) = 16.19, $p < .001$] and interaction [F(16,240) = 3.04, $p < .001$] effect, whereas the treatment effect did not reach statistical significance. One-way ANOVA [F(16,128) = 19.12, $p < .001$] and post-hoc analysis revealed that the reduction in HVA outflow was significant during the 90–240 min postinjection period, i.e. in samples 7–16 ($p < .01$–0.001) as compared to the pre-mecamylamine baseline. Student's t-test showed that HVA levels were significantly lower in the nicotine-treated group, as compared to the nicotine-naive group, during the 90–225 min postinjection period, i.e. in samples 7–15 following mecamylamine injection ($p < .05$–.01) (Figure 5b).

The 5-HIAA levels following saline injection were significantly higher in the nicotine-treated group, as compared to the nicotine-naive group. Statistical analysis showed a significant treatment effect, whereas both time and interaction effects failed to reach statistical significance. Student's t-test revealed that this between-group difference was significant during the first 45 min, i.e. in samples 1–3, of the postinjection period ($p < .05$–.001) (Figure 5c).

Similarly to DA and HVA, mecamylamine injection in nicotine-treated animals resulted in a significant decrease in 5-HIAA outflow (maximal reduction: 10%). Statistical analysis showed a significant time effect, whereas both treatment and interaction effects failed to reach statistical significance. One-way ANOVA [F(16,128) = 12.57, $p < .001$] and post-hoc analysis revealed that the reduction in 5-HIAA outflow was significant during the 90–240 min postinjection period, i.e. in samples 7–16 ($p < .05$–.001) (Figure 5c).

Mecamylamine injection in the nicotine-naive control animals did not significantly alter the levels of DOPAC, HVA, or 5-HIAA (Figures 5a–c).

Although the behavior of the animals in the microdialysis experiments was not systematically examined

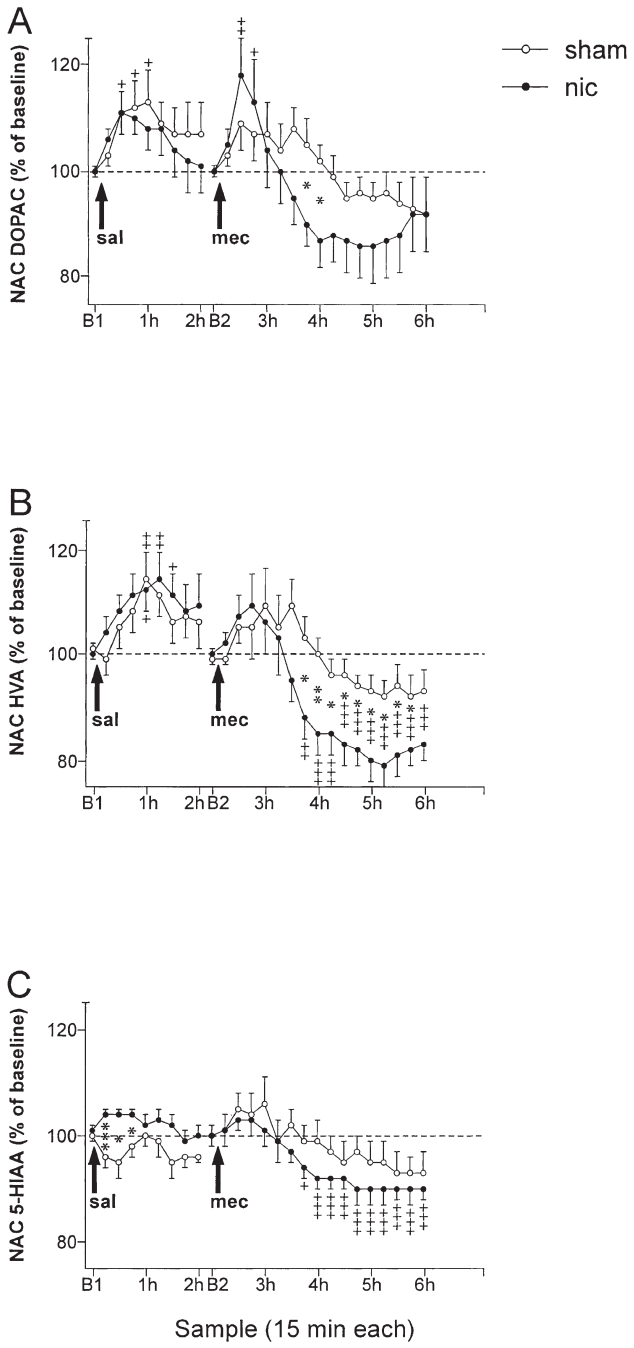

Figure 5. Temporal changes of extracellular concentrations of **(A)** dihydroxyphenylacetic acid (DOPAC), **(B)** homovanillic acid (HVA), and **(C)** 5-hydroxyindoleacetic acid (5-HIAA) in the nucleus accumbens after ipsilateral intrategmental injection (as indicated by the arrows) of 0.5 μl saline (sal) and 9 μg mecamylamine (mec) in 0.5 μl saline in animals infused chronically with nicotine, i.e. 9 mg/kg/day for 14 days, (n = 9; nic; solid circles) and in animals implanted with empty pumps (n = 8; sham; open circles). B1 indicates baseline 1, which is the sample immediately preceding the saline injection (100% in the figure is the average of the two samples preceding the saline injection). B2 indicates baseline 2 which is the sample immediately preceding the mecamylamine injection (100% is defined as the average of the two samples preceding the mecamylamine injection). Data are thus presented as mean (±S.E.M.) extracellular concentrations of DOPAC, HVA, or 5-HIAA expressed as percentages of the baselines B1 and B2, respectively. Crosses indicate within group differences from the respective baseline samples and stars between group (treatment) differences. $^{+}$,* = $p < .05$; $^{++}$,** = $p < .01$; and $^{+++}$,*** = $p < .001$.

following intrategmental mecamylamine injections it clearly appeared that the nicotine-treated animals displayed more withdrawal signs in comparison to the nicotine-naive control animals, as described above.

DISCUSSION

The major finding of the present study is that in chronically nicotine-treated rats blockade of nAChRs within the VTA, by means of local application of mecamylamine, elicits withdrawal signs such as gasps, teeth chatter, and hypolocomotion as well as a concurrent reduction in DA output in the ipsilateral NAC. These data suggest that blockade of nAChRs at the level of the VTA may contribute to the previously reported behavioral and biochemical sequelae of precipitated nicotine withdrawal following systemically administered mecamylamine (cf. Introduction), although the putative role in this regard of nAChRs on the DA nerve terminals, e.g. within the NAC, remains to be elucidated.

As has been previously demonstrated in several laboratories (Malin et al. 1994; Hildebrand et al. 1997; Epping-Jordan et al. 1998), a nicotine withdrawal reaction involving physical signs can readily be precipitated with a systemic injection of mecamylamine in rats chronically treated with nicotine. Generally, our present results add support to the notion that the somatic signs of the nicotine withdrawal reaction, precipitated by systemic mecamylamine, are largely mediated by central nAChRs. Indeed, previous data show that most individual categories of signs (i.e. gasps, teeth chatter, wet dog shakes, ptosis, and yawns) can be elicited by intracerebroventricular (ICV) administration of the competitive nAChR-antagonist dihydro-β-erythroidine (Malin et al. 1998) as well as by ICV administration of hexamethonium, a nAChR-antagonist unable to penetrate the blood brain barrier (Malin et al. 1997). There is, however, also evidence for a role of peripheral nAChRs in producing some of the components of the withdrawal reaction, since a significant increase in the withdrawal score has been reported also following systemic challenge with the peripheral nAChR-antagonist chlorisondamine (Hildebrand et al. 1997).

In the present study, bilateral intrategmental mecamylamine injections dose-dependently and significantly increased the number of several abstinence

signs, such as gasps and teeth chatter in the nicotine-treated rats in comparison with the control animals. However, there were no effects on wet dog shakes and only minor increases in yawns and ptosis, signs that are characteristic for the nicotine withdrawal reaction precipitated by a systemic mecamylamine injection. Thus, blockade of nAChRs in the VTA elicits some, but not all, physical signs of the complete nicotine withdrawal reaction. Interestingly, gasps were displayed in nicotine-treated rats both after nicotine withdrawal alone and after systemic, ICV or intrategmental administration of nAChR antagonists. Gasps have not, to our knowledge, been reported in other drug withdrawal reactions.

In contrast to gasps, yawns as well as teeth chatter can be elicited by systemic administration of cholinergic drugs, such as pilocarpine and physostigmine, which are considered to act essentially via muscarinic acetylcholine receptors (mAChR) in brain (Ushijima et al. 1984), but not by nicotine (Urba-Holmgren et al. 1977). Moreover, yawning can be elicited by low doses of DA D_2- and/or D_3-receptor agonists, e.g. apomorphine, which probably preferentially act on presynaptic autoreceptors that inhibit DA release, and it can be blocked by cholinergic as well as dopaminergic receptor antagonists (Ushijima et al. 1985; Yamada and Furukawa 1980). In addition, yawning can be enhanced by the DA depleting agent reserpine (Yamada and Furukawa 1980; Serra et al. 1986). Thus, also dopaminergic inhibition may cause yawning behavior. This contention is consonant with the decrease in mesolimbic DA activity in nicotine withdrawal reported in the present and previous (Hildebrand et al. 1998) studies from our laboratory. Other data, however, report a lack of correlation between changes in NAC DA output and yawning behavior elicited by DA agonists, DA synthesis inhibitors, or DA storage blockers (Ståhle 1987, 1992). In these studies, however, the experiments were performed almost immediately following implantation of the microdialysis probe and changes in extracellular DA may well have been blunted by the acute tissue trauma (Westerink 1995). Since stress increases yawning in the rat (Tufik et al. 1995) and some anxiolytic drugs may attenuate apomorphine-induced yawning (Simon et al. 1992; Conceicao and Frussa-Filho 1993), the occurrence of yawns might alternatively reflect a stressful and/or anxiogenic effect of nicotine withdrawal in rats.

The signs rated in nicotine abstinent rats closely resemble those occurring in rats subjected to naloxone-precipitated opiate withdrawal (Bläsig et al. 1973). The expression of the physical signs of the opiate withdrawal reaction has been demonstrated to depend on several brain regions (Maldonado et al. 1992; Baumeister et al. 1989). The same principle may apply also to the signs of the nicotine withdrawal reaction, since in the present study, investigating the specific contributions of the VTA, there were prominent dose-dependent increases only in gasps and teeth chatter. Consequently, other signs encountered in both the complete nicotine withdrawal reaction and in the opiate withdrawal reaction, such as ptosis and wet dog shakes might in nicotine withdrawn rats be elicited in other brain circuitries, e.g. the locus coeruleus, as has been shown in opiate withdrawn rats (Maldonado et al. 1992). Interestingly, whereas the mesolimbic DA system clearly is important for the mediation of the motivational effects of morphine, the physical signs of the opiate withdrawal reaction could not be effectively precipitated by naloxone or methylnaloxonium administration in either the VTA or the NAC (Maldonado et al. 1992; Baumeister et al. 1989). Thus, the rewarding and aversive stimulus properties of morphine on one hand (Stinus et al. 1990; Bozarth and Wise 1984; Phillips and LePiane 1980; Koob et al. 1989) and the physical signs presented in its withdrawal on the other (Maldonado et al. 1992; Baumeister et al. 1989) seem to be effectuated in different brain regions. In contrast, in the case of nicotine, the VTA seems to be involved both in the mediation of its rewarding effect and in at least some of the physical signs encountered in the withdrawal reaction.

In the present study, intrategmental injection of mecamylamine dose-dependently reduced horizontal activity, forward locomotion and rearing in nicotine-treated, but not in control animals. Previous work has demonstrated a markedly reduced locomotor activity not only in withdrawal precipitated by systemic mecamylamine (Malin et al. 1994), but also in spontaneous nicotine withdrawal 10 hrs (Malin et al. 1992), 24 hrs (Fung et al. 1996), as well as at 40 hrs (Hildebrand et al. 1997) following termination of a chronic nicotine infusion. These findings are in consonance with the hypolocomotion observed during withdrawal from other central stimulants, such as amphetamine (Paulson et al. 1991; Pulvirenti and Koob 1993) and cocaine (Fung and Richard 1994; Neisewander et al. 1996). Locomotor activity in morphine withdrawal, when assessed by photobeam interruptions in an open field, is in fact also reduced (van der Laan and de Groot 1988; van der Laan et al. 1991), although other aspects of motor behavior, e.g. jumping and rearing, are markedly increased (see e.g. Bläsig et al. 1973). Thus, reduced locomotor activity in rats appears as a common manifestation of withdrawal from several dependence-producing drugs, although abstinence from some drugs, such as ethanol and benzodiazepines, instead may elicit hyperlocomotion and even seizures (Majchrowicz 1975; Mehta and Ticku 1993). In all probability, the reduced locomotor activity may reflect a reduced DA output in the NAC, a biochemical manifestation of the withdrawal reactions that, in turn, may have bearing on clinical symptoms in smoking cessation, such as dysphoria, depression as well as impaired drive and motivation (cf. Introduction).

The present results demonstrate specifically that mecamylamine injection into the VTA in nicotine-treated rats significantly influences both the pattern of locomotion and the spatial distribution of movements as reflected in the ratios of forward locomotion to horizontal activity (FL/HA; i.e. perseverance of locomotion) and peripheral to horizontal activity (PA/HA; i.e. thigmotaxis) counts, respectively. Thus, there was a dose-dependent decrease in FL/HA and, with the highest dose of mecamylamine, an increase in PA/HA. Thigmotaxis, i.e. the tendency of an animal to stay close to walls or perimeters of an environment, may constitute a part of the innate defensive repertoire of the animal as well as a measure of anxiety, since anxiogenic agents increase and, conversely, anxiolytic agents reduce thigmotaxis (Treit and Fundytus 1989; Simon et al. 1994). Consequently, our findings suggest that blockade of nAChRs in the VTA and the associated reduction in DA output in mesolimbic target areas, such as the NAC (cf. below) and the central nucleus of amygdala (Panagis et al. in preparation), may contribute not only to the disrupting effect on the pattern of locomotion, but also to the anxiogenic effects (cf. above) of nicotine withdrawal.

In the present study, intrategmental application of mecamylamine in nicotine-treated rats reduced DA output in the ipsilateral NAC by about 25% within 60 min and this effect lasted approximately 3 hrs before returning to baseline levels. Thus, the reduced DA output following systemically given mecamylamine to nicotine-treated rats (Hildebrand et al. 1998), may result in large part from blockade of nAChRs located in the VTA, although it can not be excluded that local application of mecamylamine also into other brain regions, such as the NAC, in chronically nicotine-treated rats might cause a similar, or even greater, reduction in mesolimbic DA output. The reduced accumbal DA output may well underlie the reported decrease in brain reward function, as assessed by elevations in intracranial self-stimulation (ICSS) brain reward thresholds, reported in nicotine withdrawal (Epping-Jordan et al. 1998). Conversely, the rewarding effects of nicotine can be attenuated not only with mecamylamine, but also with dopamine antagonists, as previously demonstrated in ICSS (Ivanova and Greenshaw 1997) and self-administration (Corrigall and Coen 1991) paradigms. Tentatively, the dysfunction of mesolimbic DA systems may also have bearing on motivational deficits, such as the decreased responding for food reward reported in nicotine withdrawn rats (Corrigall et al. 1989).

The reduction in accumbal DA output, as well as associated behavioral effects (e.g. hypolocomotion), might possibly be related to decreased neuronal activity of VTA DA neurons, a phenomenon that has been observed in electrophysiological studies of withdrawal from chronic treatment with cocaine (Ackerman and White 1992), morphine (Diana et al. 1995), or ethanol (Diana et al. 1993) in rats. However, preliminary data from our laboratory indicate no significant overall change in VTA DA neuronal activity in animals treated chronically with nicotine and challenged with systemic mecamylamine, although in some cells there was an increase in firing and in others a decrease, whereas DA cells in control animals did not respond to mecamylamine (Hildebrand et al., in preparation). These recordings were, however, made during the first 15–20 min following i.v. mecamylamine administration and, since the reduction in NAC DA output following systemic (Hildebrand et al. 1998) or intrategmental mecamylamine begins after 30–45 min following mecamylamine administration, the data are not directly comparable. Moreover, a recent study suggests that chronic continuous nicotine treatment leads to a decreased firing rate of VTA DA cells and that, in fact, an increased firing rate may occur in the nicotine withdrawn state (Rasmussen and Czachura 1995). The animals in those experiments were, however, subjected to spontaneous rather than precipitated nicotine withdrawal and recording sites were apparently not histologically verified. In addition, the purported increase was significant only when compared to the firing rate observed during chronic treatment with, in fact, a rather high dose of nicotine (6 mg/kg/day nicotine base), but not when compared to the firing rate in control animals. The electrophysiological experiments studied the VTA DA cell firing in anaestethized rats, whereas our dialysis data are from awake, freely-moving animals, which further impairs direct comparisons. Thus, additional studies are needed to determine if putative alterations in VTA DA cell firing contribute to the nicotine withdrawal induced decrease in NAC DA output.

An issue that warrants careful consideration is to what extent the neurobiological consequences of precipitated nicotine withdrawal, i.e. nAChR-blockade by mecamylamine, in chronically nicotine-treated rats are representative of the effects produced by spontaneous nicotine withdrawal, i.e. mere cessation of a chronic nicotine infusion. Several findings from studies of spontaneous nicotine withdrawal are in line with our results using mecamylamine-precipitated withdrawal. For example, a reduction of NAC-DA tissue levels after 24 hrs of spontaneous withdrawal from a 14-day nicotine infusion (Fung et al. 1996) has been reported. Moreover, elevated ICSS brain reward thresholds (cf. above), have been reported both following spontaneous nicotine withdrawal and withrawal precipitated by systemic injections of the nAChR-antagonists dihydro-β-erythroidine (Epping-Jordan et al. 1998) or mecamylamine (S.S. Watkins, personal communication). Analogously, it has previously been demonstrated that the reduced locomotor activity as well as the increase in other rated behaviors occurs in precipitated as well as in spontaneous

withdrawal (cf. above). In addition, the behavioral and biochemical features of the opiate withdrawal reaction are virtually identical in spontaneous and naloxone-precipitated withdrawal (Acquas and DiChiara 1992; Bassareo et al. 1995; Rossetti et al. 1992). Thus, the assumption that the biochemical and behavioral sequelae of spontaneous nicotine withdrawal should closely correspond to the sequelae of mecamylamine-precipitated withdrawal seems generally justified.

In summary, the present study demonstrates that specific blockade of nAChRs in the VTA in chronically nicotine-treated rats precipitates several signs of the nicotine withdrawal reaction and reduces NAC DA output, similarly to the effects of systemic administration of mecamylamine. Hence, a significant role of nAChRs in the VTA in the mecamylamine-precipitated nicotine withdrawal reaction is suggested. The behavioral consequences of spontaneous withdrawal from chronic nicotine treatment displays fundamental similarities with those of mecamylamine-precipitated withdrawal. Thus, a reduced NAC DA output may well also contribute to the spontaneous nicotine withdrawal reaction. If nicotine withdrawal in man also results in a reduction in accumbal DA, it may significantly contribute to symptoms frequently reported in attempted smoking cessation, such as dysphoria, depression as well as impaired motivation or drive.

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