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Chronic Nicotine Administration in the Drinking Water Affects the Striatal Dopamine in Mice

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PIETILÄ, K. AND L. AHTEE. *Chronic nicotine administration in the drinking water affects the striatal dopamine in mice.* PHARMACOL BIOCHEM BEHAV **66**(1) 95–103, 2000.—Although tobacco contains a large variety of substances, its addictive properties are most probably due to the reinforcing actions of nicotine that motivates continued tobacco use. Animals and humans self-administer nicotine, a response that appears to involve the mesolimbic dopamine system and to be common to other abused drugs. The present article reviews animal models to administer nicotine chronically. We also describe a new animal model in which nicotine is given to mice in drinking water as their sole source of fluid. This treatment produced nicotine plasma concentrations comparable to or above those found in smokers. We found that mice withdrawn from nicotine were tolerant to the effects of nicotine challenge on striatal dopamine metabolism as well as on body temperature and locomotor activity. Furthermore, ^{3}H-nicotine binding in the cortex and midbrain was significantly increased in mice withdrawn from nicotine. The last part of the article will focus on the effects of this chronic nicotine treatment on striatal dopamine. Dopamine and its metabolites and locomotor activity were increased in the forenoon in mice still drinking nicotine solutions. We also report recent data in which chronic nicotine administration in the drinking water enhanced the effect of dopamine receptor agonist, quinpirole, on striatal metabolism. The animal model described appears to be a relevant method for studying the mechanisms that are thought to be involved in nicotine dependence. © 2000 Elsevier Science Inc.

Chronic nicotine Striatal dopamine metabolism Mouse Quinpirole Tolerance

INCREASING evidence suggests that nicotine is the dependence-producing constituent of tobacco (76). Nicotine has been shown to elicit drug-seeking behavior in both self-administration and place preference procedures in experimental animals (1,32,77), and nicotine alters the threshold for intracranial self-stimulation (ICSS) (15,49,61). Furthermore, the discriminative stimulus properties of nicotine are well established and can be blocked or reduced by centrally acting nicotinic antagonists (9,33). Studies with nicotine and other dependence-producing drugs have suggested that mesolimbic dopamine is central in drug-rewarding mechanisms (13,36). Evidence for tolerance to many effects of nicotine has been observed both in animals (45,46) and in humans (4,82). Furthermore, sensitization (reverse tolerance, an increase in an effect of a drug after repeated or chronic administration) has been demonstrated in locomotor activity and in dopamine overflow in the nucleus accumbens of rats treated chronically with nicotine (3,14).

Although animal models of nicotine administration are potentially useful for research on mechanisms of nicotine dependence and to screen proposed interventions to aid smoking cessation, there are currently few such models. Most often nicotine has been administered by repeated subcutaneous (SC) or intraperitoneal (IP) injections (12,80). A recently much used parenteral method for the administration of nicotine is to implant SC nicotine-releasing osmotic minipumps or reservoirs in rats and mice. In addition, nicotine has been administered to experimental animals in drinking water or by exposure of tobacco smoke.

CHRONIC NICOTINE ADMINISTRATION VIA PARENTERAL ROUTES TO EXPERIMENTAL ANIMALS

Nicotine self-administration studies have been conducted in monkeys, rats, baboons, dogs, and humans by giving nicotine intravenously (IV). In these experiments both simple and complex schedules of drug administration (fixed-interval and fixed-ratio schedules) have been used (1,21,32,80). The conditions under which nicotine can be established as a reinforcer are much more limited than those for other dependence-producing drugs (80). Nicotine seems to maintain self-adminis-

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tration behavior more effectively in fixed-interval and second-order schedules (33,71). In these studies, the reinforcing effects of nicotine were related to how the injections were arranged within a session. Nicotine seemed to be more effective when presented intermittently than when continuous availability prevailed (32). In addition, in rats almost all nicotine self-administration has been shown to occur during the night (active period) (18,38).

Tobacco smoke has been administered to mice and rats through connecting tube during 10-min sessions (24,81). Although tobacco smoke appears to sustain a sucking behavior in the experimental animals, it is difficult to assess its reinforcing effects and to which degree the smoking behavior results in the inhalation of smoke (80). However, this method can be successfully used in studying the effects of passive smoking.

The most frequently used method of chronic nicotine administration has been parenteral injections (SC or IP) of nicotine given a few times a day (50,80). With this method, the dose of nicotine administered can be carefully controlled. However, this method suffers from a number of disadvantages. The stress of daily handlings in a long-term study cannot be neglected (70), and the animals cannot self-administer nicotine during their active period. In addition, when the short elimination half-life of nicotine is taken into account, the pharmacokinetic profile of nicotine administered by injecting rather high doses a few times daily does not correspond to the much more frequent administration which characterizes cigarette smoking in humans.

A recent alternative method for the chronic administration of nicotine has been the use of SC implanted nicotine-releasing osmotic minipumps. The commercial (11,23,51,70) or self-made nicotine-releasing pellets and reservoirs (42, 50,78) have been used successfully in both rats and mice. The ease of use of the nicotine-releasing minipumps, the excellent reproducibility from animal to animal and the lack of adverse effects due to excessive nicotine doses with this route have probably made this the method of choice in delivering nicotine to rats (51,70). After SC administration, nicotine is absorbed quickly and almost completely. In mice, self-made nicotine releasing reservoirs induced plasma nicotine concentrations of 200 to 840 ng/ml during the first week of treatment. After that the concentrations decreased to a steady-state level of 50 to 125 ng/ml (42). Commercial nicotine-releasing osmostic minipumps induced more stable plasma nicotine concentrations in rats comparable to those found in smokers (11, 23,51). Nicotine has also been administered by IV infusion to mice via the jugular vein either as constant infusion or as successive boluses (45,63). The constant infusion does not mimic the normal smoking situation in smokers, because of the absence of the high nicotine peaks found in the plasma of smokers (68).

Nicotine has also been administered chronically to experimental animals with repeated IP injections or infusions (31,55,78). By using this route nicotine passes the liver before entering the circulation. Thus the blood concentrations after IP administration of nicotine and the amounts of nicotine transported to different target organs have been shown to be influenced by the fast metabolism of nicotine in the liver (79).

CHRONIC ORAL NICOTINE ADMINISTRATION TO EXPERIMENTAL ANIMALS

Nicotine has been administered orally to experimental animals via liquid diets or in drinking fluids. In addition, nicotine has been administered by forced oral administration or schedule-induced polydipsia, in which food delivery in animals with reduced body weight is associated with increased fluid intake (38). In most studies employing the oral route, nicotine has been given ad libitum by adding it in the drinking water (2,35,54,56,62,67,78).

It has been especially difficult to get rats to drink nicotine-containing solutions (41,51). The reason for the failure of attempts to induce addiction to oral nicotine in rats has been explained by their aversion to the taste of nicotine (41,51,52). In addition, after oral administration very slow absorption through the gastrointestinal tract under acidic conditions is suggested to lead to a failure to reach pharmacologically active blood nicotine concentrations (2,41). Due to the rapid metabolism in the liver, the oral administration of nicotine can be expected to result in a relatively lower percentage of nicotine and a higher percentage of its metabolites as compared with the parenteral route of administration.

CHRONIC NICOTINE ADMINISTRATION IN THE DRINKING WATER TO MICE

In our animal model, nicotine is administered to mice in their drinking water as the sole source of fluid with gradually increasing concentrations (50 to 500 μg/ml) during the 7-week period (56). Nicotine administered to mice orally in the drinking water does not alter the body weight gain or fluid intake of the mice until after 2 weeks of administration when the drinking fluid contains 200 μg/ml nicotine (Fig. 1). A longer treatment with a further elevation of the nicotine concentration reduces the rate of gain in body weight. The amount of decrease in fluid intake is directly proportional to the nicotine concentration in the drinking water. After 7-week treatment when the mice are withdrawn by replacing the nicotine solutions with tap water, the fluid intake increases significantly in nicotine-treated mice as compared with control mice. The increase in fluid intake returns to the level of control mice within 1 week, and the body weights increase to the level of the control mice within 24 h (Fig. 1). The hypodipsia found might be due to the bitter taste of the nicotine solutions and/or to the antidiuretic actions of nicotine (44). Nicotine is known to be a powerful stimulant of antidiuretic hormone secretion release in rats and humans and thus reduces the output of urine (10). As the small rodents maintain their fluid balance very effectively it is possible that the chronically nicotine-treated mice reduce their fluid intake to compensate for the reduced fluid output. Due to the reduced fluid intake the estimated daily nicotine dose remains at about the same level from the third week onward (Fig. 1). This is also reflected by the about similar concentrations of plasma nicotine and cotinine at weeks 2, 4, and 7 (56).

Smokers tend to regulate their tobacco intake to maintain their plasma nicotine within an individually determined concentration range (37). In humans, some circadian changes in nicotine clearances have been found to exist that are suggested to influence cigarette smoking behavior (27). In animal studies, nicotine has been administered in doses to induce the same nicotine concentration in the plasma as that found in smokers. It has been argued that by using different routes of administration the pharmacokinetics of nicotine does not correlate with the smoking situation in humans.

We found that the mice drank mainly during the night as the plasma concentrations of nicotine and cotinine reached their peak during nighttime (58). Our findings agree with those of Kita et al. (35) who found that the drinking behavior of rats with nicotine added to drinking water did not differ from that of the controls. As nocturnal animals, the water consumption of rats was at its highest at the end of the light period and in the early morning. Furthermore, the drinking

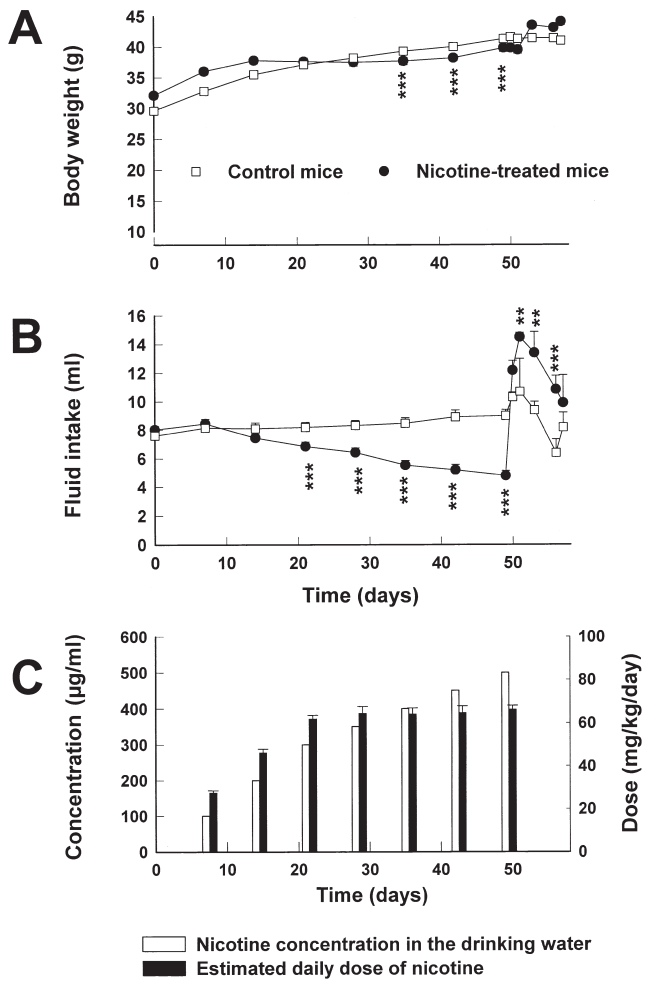

FIG. 1. Effects of chronic nicotine administration in the drinking water and withdrawal on body weight (A) and on fluid intake (B) in the control mice (open squares) and in the nicotine-treated mice (closed circles). Graph C (redrawn from original data given in ref. 56) gives the concentration of nicotine in the drinking water (open columns) and the estimated daily dose ± SEM (filled columns). 5-week-old male NMRI mice weighing 26–27 g were divided in groups of 6 to 8 per cage, at an ambient temperature of 20 to 22°C, and were given either nicotine-containing tap water or plain tap water as the sole source of fluid for 7 weeks. Lights were on from 0600 to 1800 h. Standardized laboratory food was available ad libitum. The drinking fluid was changed daily. The concentration of nicotine in the drinking fluid was gradually increased in amounts of 50 µg/ml at 3 to 4 day intervals to 350 µg/ml at 3 weeks, and after this at 7-day intervals to 500 µg/ml. At the end of the 7-week experiment, the mice were withdrawn from chronic nicotine by replacing the nicotine solution with tap water. Body weights and fluid intake were recorded weekly during the chronic administration and daily during the withdrawal period. Individual fluid intake (mg/kg/day) was estimated by averaging the amount consumed by the 6 to 8 mice present in each cage. The body weight and fluid intake data are expressed as means ± SEM of 14 mice. One-way analysis of variance (ANOVA) for repeated measures was used for analyses of differences in fluid intake and body weight followed by Student's *t*-tests. $^{**}p<0.01$, $^{***}p<0.001$ compared with the control mice.

behavior was found to correlate with the ambulatory activity of the animals (35).

In smokers, the afternoon blood nicotine concentrations have been found to vary from 2.5 to 50 ng/ml depending on the inhalation technique (1,4). The blood cotinine concentrations, the main metabolite of nicotine, were measured to vary between 10 to 900 ng/ml in smokers (average of 250 to 300 ng/ml) (5). In our mice, the plasma concentrations of nicotine (peak 114 ± 25 ng/ml at 0500 h; trough 33 ± 14 ng/ml at 2100 h) as well as the concentrations its main metabolite cotinine (peak 3233 ± 497 ng/ml at 0500 h; trough 923 ± 323 ng/ml at 2100 h) were found to be similar to and higher than those reported in heavy smokers suggesting that nicotine was consumed in amounts high enough to activate and/or desensitize the nicotinic acetylcholine receptors (nAChRs). Indeed, our studies on striatal dopamine indicate that at night when plasma nicotine concentrations were at their peak the nAChRs might be desensitized.

TOLERANCE TO NICOTINE IN MICE TREATED CHRONICALLY WITH NICOTINE IN THE DRINKING WATER

It is known already from the studies by Langley (39) that tolerance may develop to the effects elicited by nicotine. Tolerance can be defined as a state in which a challenge dose following repeated administration of a drug produces a smaller effect than before, or alternatively a state in which increased amounts of a drug are required to achieve the effect observed with the first dose. Chronic administration of nicotine has been shown to induce tolerance to its various acute effects in rats and mice such as the locomotor depressant actions of nicotine (45,46,4,60) and the decrease in body temperature (16,45,46,59). Tolerance has been shown during abstinence from chronic nicotine administration by using various routes of administration to rats and mice (14,16,31,45,46,47,59,60). Tolerance occurred even when nicotine was administered only 1 to 3 times weekly (14).

Table 1 shows that mice withdrawn from nicotine in the drinking water were tolerant to nicotine challenge. We found tolerance to nicotine's hypothermic and locomotor activity depressing effects (Table 1) (60) as well as to nicotine-induced elevation of striatal dopamine metabolism after 7-week chronic nicotine administration (59). The hypothermic effect of nicotine, but not its effects on locomotor activity (Table 1) or striatal dopamine (Pietilä et al., unpublished), was reduced already after 4-week nicotine administration.

In numerous studies using different routes of administration (reviewed by 85), chronic nicotine treatment has been shown to increase the number of nicotinic binding sites in various brain areas. The loss of function resulting from long-term desensitization or inactivation of the receptor during chronic nicotine treatment has been suggested to lead to up-regulation of nAChRs as an adaptive response (73). The receptor up-regulation has been found to result from increased density of the receptors (B_{max}) leaving the affinity (K_d) unchanged (85). It has been found that simultaneously with development of tolerance the binding of ^{3}H-nicotine increases in the brain of rodents (16,45,46,72).

As shown in Table 2 also we (60) found that the binding of ^{3}H-nicotine to cortex and midbrain membranes of mice withdrawn from 4- or 7-week nicotine treatment was elevated. The binding of ^{3}H-nicotine to cortical membranes of our mice was the larger the longer the nicotine treatment had lasted, and the greatest increase by 116% was found at 24 h after 7-week nicotine treatment (Table 2). The binding in the midbrain was elevated less than in the cortex and to about the same degree (by about 40%) both after 4- and 7-week nicotine treatments. No alterations were found in the cerebellar ^{3}H-nicotine binding or after 2-week nicotine administration in the drinking water (60). Our experiments suggest that pro-

TABLE 1

THE EFFECT OF ACUTE NICOTINE CHALLENGE ON BODY TEMPERATURE AND LOCOMOTOR ACTIVITY IN CONTROL MICE AND IN MICE TREATED WITH ORAL NICOTINE FOR 4 OR 7 WEEKS

Chronic/Acute Treatment	Control	4 Week/0 h	4 Week/24 h	7 Week/ 0 h	7 Week/24 h
Body temperature, ΔT rect, °C					
Saline	−0.4 ± 0.1	−0.1 ± 0.1	0. ± 0.1	−0.4 ± 0.1	−0.4 ± 0.1
Nicotine	−4.5 ± 0.2	−2.1 ± 0.8**	−3.0 ± 0.5**	−2.0 ± 0.7**	−2.4 ± 0.3***
Locomotor activity, counts/60 min					
Saline	829 ± 87	970 ± 124	1152 ± 88	1085 ± 222	1340 ± 104
Nicotine	452 ± 65°°°	639 ± 75°°	682 ± 95°°°	849 ± 98°	1381 ± 173**

Table 1 summarizes results from Ref. 59 and 60 as well as includes some new unpublished data. Nicotine was administered chronically by giving to male NMRI mice nicotine-containing tap water as the sole source of drinking fluid, see legend to Fig. 1. After 4- or 7-week chronic nicotine treatment, part of the mice were withdrawn for 24 h by replacing the nicotine solution with tap water. Mice still drinking nicotine containing tap water, mice withdrawn for 24 h and control mice were challenged acutely with nicotine (0.5 or 1 mg/kg) or 0.9% NaCl solution SC. In the table are given the mean differences of rectal temperatures (ΔT rect, °C) ± SEM measured before and at 30 min after the nicotine challenge (1 mg/kg) by inserting an electric thermocouple, Ellab Instruments, Copenhagen, Denmark, 1 cm in the rectum. In locomotor activity experiments, the mice were given a challenge dose of 0.5 mg/kg on nicotine or saline, and were placed one at a time in 18 × 33 × 15 cm cages immediately after the acute injections to measure locomotor activity for 60 min. Horizontal interruptions of photocell beams (40 photocells per cage) were registered by a computerized counter. The mice were habituated for 60 min to the test situation 24 h before the experiment. The acute challenge experiments were performed at an ambient temperature of 20° to 24°C between 1000 h and 1200 h. The effect of nicotine challenge dose on rectal temperatures was analyzed by two-way ANOVA for repeated measurements followed by Tukey's post hoc comparisons. The locomotor activity data was analyzed by two-way ANOVA followed by Tukey's post hoc comparisons. The asterisks show the significant differences (**$p < 0.01$, ***$p < 0.001$) in the effects of acute nicotine on mice treated with nicotine in the drinking water for 4 or 7 weeks as compared with the control mice given nicotine for the first time. °$p < 0.05$, °°$p < 0.01$, and °°°$p < 0.001$ indicate the effect of an acute nicotine challenge on locomotor activity of mice as compared with the corresponding mice given saline acutely.

found changes occur in the cerebral nicotine receptors during the treatment of mice with nicotine in the drinking water. The findings may reflect regional differences in the existence of different subtypes of nicotinic receptors and their sensitivity to nicotine. The differences in onset and offset of tolerance have suggested the involvement of different nicotinic receptor subtypes with different desensitizing properties and/or location on different neural pathways in mediating these effects (19).

NICOTINE'S EFFECTS ON DOPAMINE RELEASE AND METABOLISM IN THE STRIATA OF MICE DURING CHRONIC NICOTINE TREATMENT

Nicotine exerts its effects in the brain by interacting with neuronal nicotinic acetylcholine receptors (nAChRs). These receptors are located on both somatodendritic and nerve terminal membranes and thus, may influence neurotransmitter secretion at synapses (3).

As cerebral dopamine (DA) systems are widely believed to be central to nicotine's role in tobacco smoking (36,77), we have focused our studies on the effects of nicotine on DA systems by estimating the striatal concentrations of DA and its main metabolites. The main metabolites of DA are homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC), and also a small amount of 3-methoxytyramine is formed. Released DA is converted to DOPAC by intraneuronal monoamine oxidase (MAO) after re-uptake into the nerve terminal. In the rat brain, about 90% of DA is catabolized to DOPAC (83). DOPAC is suggested to be a poor indicator of the release of DA, whereas the striatal concentration of DOPAC seems to be an index of DA synthesis, because a substantial amount of DOPAC is derived from a newly formed pool of DA (86). Released DA is also converted to HVA through the action of catechol-*O*-methyltransferase (COMT) and MAO (75), and HVA is considered to indicate the sum of DA synthesis, metabolism and release (83,84).

TABLE 2

PERCENTAGE CHANGES INDUCED BY CHRONIC NICOTINE ADMINISTRATION IN THE DRINKING WATER TO MICE IN BINDING OF ^{3}H-NICOTINE IN THE CORTEX AND MIDBRAIN.

		Percentage increases in ^{3}H-nicotine binding		
		Withdrawal time		
Brain area	Length of chronic treatment	24 h	48 h	72 h
Cortex	4 weeks	19%	55%**	65%***
	7 weeks	116%***	72%***	66%***
Midbrain	4 weeks	45%	40%*	46%***
	7 weeks	35%	38%**	32%

Membrane preparations were made from cortex and midbrain tissue of mice withdrawn from chronic nicotine in the drinking water as well as from the corresponding tissues of control mice. Nicotine was administered chronically by giving male NMRI mice nicotine-containing tap water as the sole source of drinking fluid, see legend to Fig. 1. After 4- or 7-week chronic nicotine treatment, the mice were withdrawn by replacing the nicotine solution with tap water. Membranes were prepared as described in Ref. 40 resulting in synaptosomal P_2 fractions of final protein concentrations of 0.15 to 0.30 mg protein/100 μl. Binding of ^{3}H-nicotine to cerebral membranes was measured as described in Ref. 87. The assays were performed by incubating aliquots (100 μl) of the P_2 fractions with 5 nM (−)-^{3}H-nicotine in 50 mM Tris-HCl buffer (pH 8.0) for 40 min at 0-4°C. After incubation, the samples were filtered under vacuum by a Brandell M-24 Cell Harvester. The filters were washed and the radioactivity trapped on the filters was counted. The specific binding of ^{3}H-nicotine (fmol/mg protein) was calculated by subtracting the values for nonspecific binding in the presence of 10^{-4} M unlabelled nicotine from the total binding. Data were analysed using the GraphPad Prism 2.0 program. The percentage increases give changes from the control (^{3}H-nicotine binding in the cortex and midbrain membranes of control mice). The asterisks show significant increases (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$) of ^{3}H-nicotine binding in nicotine-treated mice as compared with those in brains of control mice. Modified from original data given in Ref. 60.

Nicotine enhances DA release from midbrain slices and synaptosomal preparations in vitro (25,85). On the other hand, studies using in vitro repetitive stimulation by nicotine have resulted in attenuation of nicotine-induced ^{3}H-DA release from rat striatal synaptosomes, which was suggested to reflect desensitization or tachyphylaxis of nAChRs (47,66). Acute administration of (−)-nicotine systemically is also known to enhance DA metabolism and turnover in the striata of mice and rats in vivo (30,42,64). In addition, local application of nicotine via a microdialysis probe to the caudate-putamen, the nucleus accumbens and the frontal cortex increased dose-dependently DA release in these brain areas of rats (48). In vivo microdialysis (6,20,34) studies as well as postmortem estimations (26) have shown that systemically administered nicotine releases DA preferentially from the nucleus accumbens, whereas higher concentrations of the drug are required in order to increase nigrostriatal DA release.

Chronic nicotine treatment with repeated intermittent nicotine injections for 5 to 15 days (6) or with osmotic minipumps releasing nicotine continuously for 5 to 21 days (23) neither affected nicotine's acute effects on DA nor altered release of DA in caudate/putamen in rats. However, repetitive injections of nicotine evoked sensitization of the mesoaccumbal DA overflow in microdialysis experiments (7). Furthermore, during chronic infusion of nicotine by minipumps at a small dose the stimulatory effects of acute nicotine challenge on DA overflow in rat nucleus accumbens were abolished suggesting desensitization of nAChRs (8), whereas at 2 to 7 days after removal of the minipumps a sensitized mesoaccumbal DA response to nicotine was seen (8). In another experiment, systemic nicotine increased DA release in the prefrontal cortex of rats, and this effect was enhanced by repeated SC nicotine treatment for 12 days, but the stimulatory effect of nicotine on accumbal DA release was not altered by this repeated treatment (53). The differential responses between brain areas may be due to the fact that the effects of nicotine on DA release are mediated by nAChRs of different subtypes with different locations and functional roles (25,47). Indeed, different subtypes of nAChRs, for example those containing α3, α4, or α7 subtypes, probably differ regarding the concentration of nicotine required to induce receptor desensitization and tolerance (22). Also the length of nicotine exposure required to induce changes in nAChRs may differ between various subtypes (43).

When nicotine was administered SC twice daily for 9 days no tolerance towards its effect on striatal DA metabolism occurred in mice (74). However, we have reported (59) that after long enough nicotine treatment there is tolerance towards nicotine's enhancing effects on DA metabolism. In our control mice an acute nicotine dose of 1 mg/kg SC increased striatal concentrations of DOPAC and HVA (59), which findings agree with our previous findings in mice (30,42). In mice treated for 7 weeks with nicotine in the drinking water followed by 24 h withdrawal both these effects of nicotine were abolished (59). Thus our results show that tolerance can be induced towards the effects of acute nicotine on striatal DA metabolism by administering nicotine in the drinking water for 7 weeks to mice. Nicotinic receptors are characterised by their ability to desensitize in the continuous presence of the agonist. However, in our mice the effects of nicotine on striatal DA metabolism remained abolished even when no nicotine or cotinine was detected in the plasma of the mice (56,59). Thus our findings suggest that after 7-week chronic nicotine treatment the nAChR function in the brain of the mice was longlastingly inactivated. Such a long-lasting effect could be distinguished in in vitro experiments from reversible receptor desensitization, and was caused by prolonged treatment or high concentrations of nicotine (65). Recently, we found also in rats in vivo evidence for longlasting inactivation of those nicotinic receptors which regulate limbic DA metabolism (69).

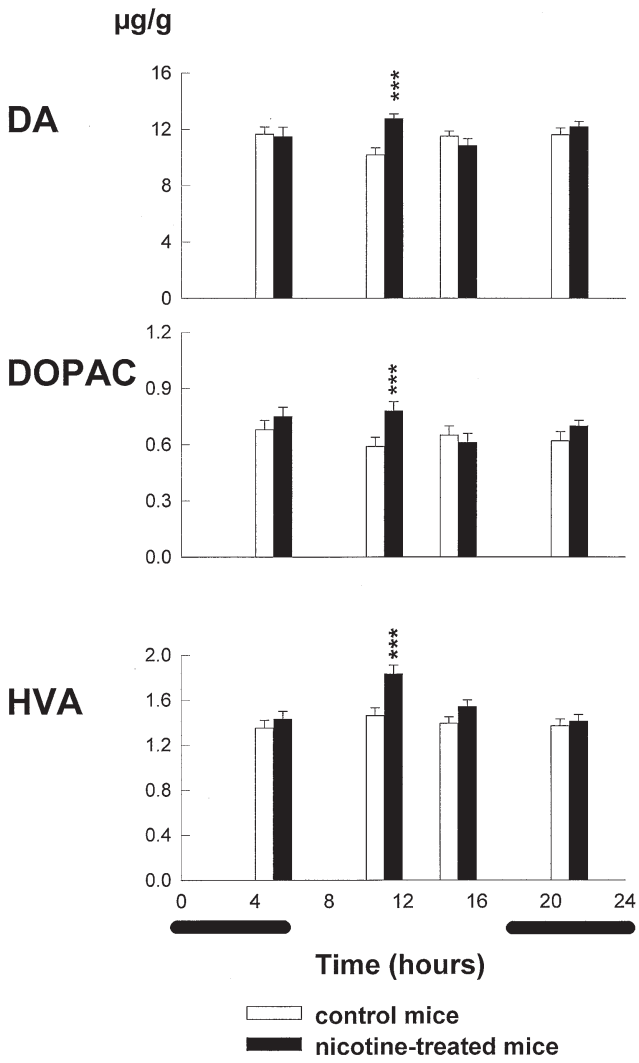

FIG. 2. The effect of chronic nicotine administration in the drinking water for 7 weeks on striatal concentrations of dopamine (DA) and its metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in control mice (open columns) and in nicotine-treated mice (closed columns) on the 50th day of chronic administration of nicotine in the drinking water. After 7 weeks, the nicotine-treated and control mice were killed by decapitation and their striata were dissected at 0500, 1100, 1500, or 2100 h. The concentrations of DA, DOPAC and HVA were measured using high-performance liquid chromatography (HPLC) as described in Ref. 29. The tissue extracts were purified by gel chromatography and the final separation of the compounds was achieved by using C_{18} reverse phase (Spherisorb ODS 5 μm) columns (25 cm, 5 mm ID) connected to the HPLC equipment. An electrochemical detector with a rotating disc working electrode was used. The statistical analysis was carried out by two-way ANOVA. The independent factors were the time of day (four levels) and the treatment (two levels). The significant effects were further analyzed by comparing appropriate cell means with linear contrasts. Results given are means ± SEM from 17 to 20 mice kept under a 12-h cycle of light (0600 to 1800 h) and dark (1800 to 0600 as shown by horizontal black bars). ***$p<0.001$ compared with the corresponding time in control mice. Modified from original data given in Ref. 58.

After 7-week nicotine administration, the striatal concentrations of DA, DOPAC, and HVA were significantly elevated at

1100 h in the nicotine-treated mice as compared with control mice, indicating an elevated DA turnover in the forenoon (Fig. 2) (58). Interestingly, we recently demonstrated that locomotor activity of mice treated with nicotine in the drinking water (the locomotor activity counts/60 min: 1661 ± 125, $n = 8$) was significantly increased on the 50th day of chronic treatment as compared with control mice drinking tap water (1313 ± 133, $n = 8$, $p < 0.05$) (28). This is to our knowledge the first time when nicotine was found to induce behavioral stimulation in mice. In combination with our previous finding of elevated striatal DA, DOPAC, and HVA concentrations (58) these findings suggest that the cerebral dopaminergic systems might be important in the mediation of the nicotine-induced locomotor hyperactivity in mice as they have been shown to be of importance in rats (13).

We also estimated the plasma nicotine and cotinine concentrations at various times during the day. In contrast to DA turnover, the plasma nicotine and cotinine concentrations were at their highest (114 ng/ml) at nighttime, when mice are active, and at their lowest (33 ng/ml) at the beginning of the dark period. The finding that DOPAC and HVA (Fig. 2) were not elevated in the nicotine-treated mice at 0500 h, when plasma nicotine and cotinine concentrations were at their highest, might indicate that nicotine indeed was consumed in amounts which induces desensitization of nAChRs regulating dopaminergic receptors. It has been repeatedly found that chronic nicotine treatment attenuates nicotine's effects on DA metabolism probably by altering receptor conformation and inducing desensitization (8,69). On the other hand, the significantly but modestly increased concentrations of DA and its metabolites at noon might reflect sensitization of nAChRs regulating DA systems. The rather small increase indicates that only part of the striatal DA neurons responded with sensitization. It is to be noted that in our experiments the brain area dissected as striatum contained the dorsal (caudate/putamen) as well as the ventral striatum (nucleus accumbens). As discussed above there is evidence that mesoaccumbal DA responses but not those in the caudate/putamen in rats are sensitized to chronic nicotine (3).

To further investigate the dopaminergic systems during chronic nicotine treatment, we studied the effect of mixed D_2/D_3-dopamine receptor agonist quinpirole (LY171555) on striatal DA metabolism in mice treated with nicotine in the drinking water for 7 weeks. There are studies indicating that quinpirole in small doses acts selectively at DA autoreceptors, which presynaptically control the release and metabolism of DA (57). In control mice quinpirole at the dose of 0.1 mg/kg but not at the dose of 0.03 mg/kg SC decreased striatal HVA, and neither dose affected DOPAC concentration. As shown in Fig. 3, the effect of quinpirole was enhanced in nicotine-treated mice so that already the smaller quinpirole dose of 0.03 mg/kg SC decreased the striatal concentrations of HVA and the larger dose of 0.1 mg/kg decreased that of DOPAC. These findings indicate that during chronic nicotine treatment the sensitivity of the presynaptic DA receptors regulating the release and metabolism of DA in the striatum is enhanced. In combination with our find-

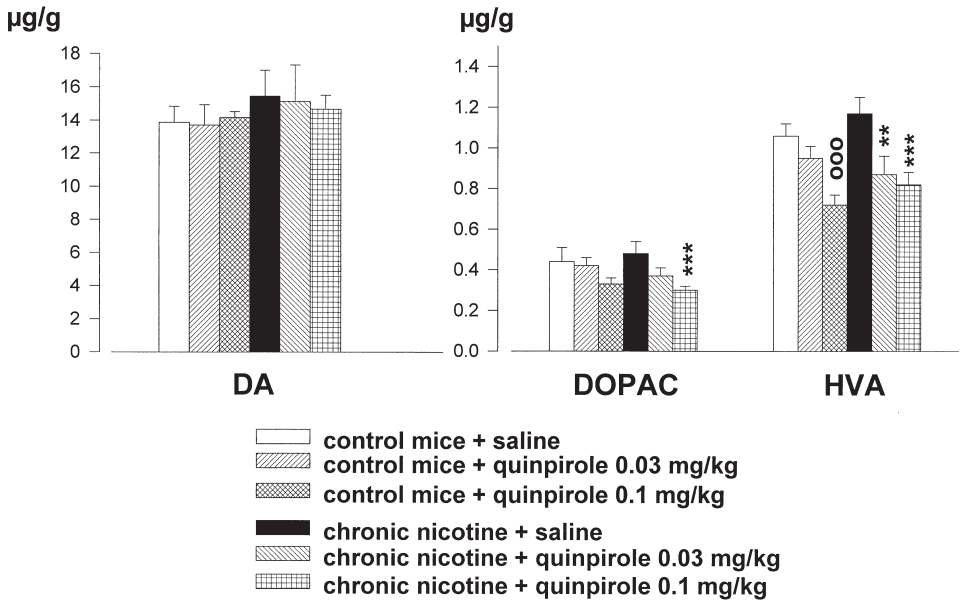

FIG. 3. The effect of acute quinpirole administration on striatal dopamine (DA) metabolism in mice treated chronically with nicotine in the drinking water for 7 weeks as well as in control mice. Nicotine was administered chronically by giving to male NMRI mice nicotine-containing tap water as the sole source of drinking fluid, see legend to Fig. 1. On the 50th day of chronic treatment, the nicotine-treated and control mice were mice were given SC either quinpirole (0.03 or 0.1 mg/kg) or 0.9% NaCl (saline) solution. After 60 min, the mice were killed by decapitation and their striata were dissected. The acute challenge experiments were performed at an ambient temperature of 20 to 24°C between 1000 and 1200 h. The striatal concentrations of dopamine (DA) and its metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were analyzed as described in legend to Fig. 2. The statistical analysis was carried out by two-way ANOVA. The significant effects were further analysed by comparing appropriate cell means with linear contrasts. The colums give the results as means ± SEM from 6 to 7 mice. $^{ooo}p < 0.001$ as compared with control mice given saline SC acutely; $^{**}p < 0.01$, $^{***}p < 0.001$ as compared with nicotine-treated mice given saline SC acutely.

ings that during chronic nicotine treatment nicotine's effects on striatal DA metabolism as well as on locomotor activity are enhanced (28,58) these findings suggest that chronic nicotine treatment indeed alters the functioning of striatal DA system.

CONCLUSIONS

Administration of nicotine in the drinking water to mice as the sole source of fluid for several weeks can be used for studying the mechanisms underlying the nicotine-induced changes in the brain and behavior as it mimics the daily variation in nicotine intake in smokers. Further, our findings suggest that the mice receive enough nicotine from drinking water during the chronic experiment to induce changes which are thought to be involved in nicotine dependence. Finally, the present results support the suggestions that nicotine's effects on DA metabolism are critical for its stimulating and thus, most probably also for its reinforcing effect.

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