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Nicotine and epibatidine alter differently nomifensine-elevated dopamine output in the rat dorsal and ventral striatum

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Abstract

We studied the effects of nicotine and epibatidine given in combination with dopamine uptake inhibitor, nomifensine, on striatal extracellular dopamine and its metabolites by using brain microdialysis in freely moving rats. Nomifensine (3 mg/kg) elevated extracellular dopamine in the caudate-putamen, and clearly more in the nucleus accumbens. In the caudate-putamen, nicotine (0.5 mg/kg) and epibatidine (0.6 μg/kg but not 3.0 μg/kg) enhanced nomifensine's effect on dopamine. The effect of nomifensine on accumbal dopamine was enhanced by nicotine, but inhibited by epibatidine at 0.6 μg/kg. The larger dose of epibatidine had no effect. Thus, the effects of the smaller epibatidine dose (0.6 μg/kg) on the dopamine output in the caudate-putamen but not in the accumbens resemble those of nicotine 0.5 mg/kg. Discrepancies in the effects of epibatidine and nicotine are most probably due to differences in their affinities to nicotinic receptor subtypes regulating dopamine release. Further, different responses to low concentrations of epibatidine between the brain areas suggest that there are differences in the nicotinic regulation of nigrostriatal and mesolimbic dopaminergic pathways.

Keywords: Nicotine; Epibatidine; Nomifensine; Dopamine; Nucleus accumbens; Caudate-putamen; In vivo microdialysis

1. Introduction

Cholinergic pathways are known to interact with the dopaminergic pathways in the brain and these networks are involved in several functions, such as learning, memory, attention, movements, motivation, dependence and reward (Clarke et al., 1987; Dani et al., 2001). Stimulation of nicotinic acetylcholine receptors modulates the release of various neurotransmitters, including dopamine (Klink et al., 2001; Wonnacott, 1997). A number of nicotinic receptor subtypes containing at least α4β2, α3/α6β2 or β3 subunits are suggested to mediate nicotine's effects on dopamine release (Kaiser et al., 1998; Kulak et al., 1997; Sharples et al., 2000; Wonnacott et al., 2000). Furthermore, there is evidence that nicotinic receptors composed of α7-subunits mediate effects on dopamine release indirectly by stimulating glutamate release (Kaiser and Wonnacott, 2000).

Nicotine has been repeatedly found to increase in vitro [^{3}H]dopamine release from rat brain slices and synaptosomes (e.g. Giorguieff-Chesselet et al., 1979; Rapier et al., 1988; Sakurai et al., 1982; Teng et al., 1997; Westfall, 1974) and to elevate in vivo striatal and even more accumbal concentrations of dopamine metabolites (Grenhoff and Svensson, 1988; Lichtensteiger et al., 1982; Nose and Takemoto, 1974). Also, several in vivo microdialysis studies have shown that acute systemically administered nicotine increases the dopamine output in the caudate-putamen and even more in the nucleus accumbens (Benwell and Balfour, 1997; Di Chiara and Imperato, 1988; Imperato et al., 1986; Janhunen and Ahtee, 2004; Seppa and Ahtee, 2000).

(±)-Epibatidine is a potent nicotinic receptor agonist, which binds with a high affinity at α4β2, α3β2 and α3β4 nicotinic receptor subtypes, and also at α7 subtypes with higher affinity than nicotine (Gerzanich et al., 1995; Xiao et al., 1998). Epibatidine has been reported to stimulate in vitro

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[^{3}H]dopamine release from striatal slices much more potently than (−)-nicotine (Sullivan et al., 1994). In previous microdialysis studies, we found that epibatidine altered differently the extracellular dopamine concentrations in the caudate-putamen and the nucleus accumbens, and its effects were not parallel to those of nicotine (Janhunen and Ahtee, 2004; Seppa and Ahtee, 2000). Epibatidine at the dose 0.6 μg/kg but not at 3.0 μg/kg increased the dopamine output in the caudate-putamen, whereas in the nucleus accumbens, the dopamine output increased first at the dose 3.0 μg/kg (Janhunen and Ahtee, 2004). Epibatidine has been recently reported to even decrease the extracellular dopamine concentration in the nucleus accumbens (Bednar et al., 2004).

When administered locally the dopamine uptake inhibitor, nomifensine, intensified the nicotine-induced elevation in the striatal and accumbal extracellular dopamine (Benwell and Balfour, 1992, 1997). We recently found that the dopamine uptake inhibition induced by systemic nomifensine substantially further enhanced the effects of nicotine, 0.5 mg/kg, on the dopamine output in the caudate-putamen, whereas substances inhibiting dopamine metabolism (tolcapone, selegiline) or presynaptic dopamine receptors (haloperidol) did not have such effect (Kaakkola et al., 2000; Janhunen et al., submitted for publication). Therefore, in the present experiments, we compared the effects of epibatidine and nicotine on the extracellular dopamine in the caudate-putamen and the nucleus accumbens in the presence of nomifensine to further study the possible differences in the effects of epibatidine and nicotine.

Some of this work was presented earlier in abstract form (Janhunen et al., 2002, 2003).

2. Materials and methods

Naïve male Wistar rats weighing 250–330 g in the beginning of experiments were used. The rats were housed in groups of four in an animal room lit from 6 a.m. to 6 p.m. and at the temperature of 20–23 °C. The rats were housed one per cage after the surgery and during the experiments. Food pellets (Altromin 1324, Chr. Petersen A/S, Denmark) and tap water were constantly available ad libitum. The animal experiments had an approval from the Committee for Animal Experiments of the University of Helsinki, and the chief veterinarian of the county administrative board. All experiments were conducted according to the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes".

2.1. Microdialysis in freely moving rats

The rats were anaesthetised with halothane (3.5% during induction, 2% maintenance) and placed in a stereotaxic apparatus (Stoelting, IL, U.S.A.). The guide cannula (BAS MD-2250; Bioanalytical Systems, IN, U.S.A.) was inserted through a small hole in the skull above the caudate-putamen (A/P+1.0, L/M±2.7, D/V−2.0) or the nucleus accumbens (A/P+1.7, L/M±1.4, D/V−6.3) using coordinates relative to the bregma and dura according to Paxinos and Watson (1986). The guide cannula was secured to the skull with three stainless steel screws and zinc polycarboxylate cement (Aqualox; VOCO, Germany). After the surgery, tramadol (1.0 mg/kg s.c.) was given for postoperative pain. The rats were allowed to recover for 5–7 days in transparent Plexiglas test cages (30×30×40 cm^3) and then moved one day before experiments to the experiment room. On the experimental day between 7 and 8 a.m., a microdialysis probe (BAS MD-2200, 0.32 O.D., 4.0 mm membrane for the caudate-putamen, 2.0 mm membrane for the nucleus accumbens, Bioanalytical Systems, IN, U.S.A.) was inserted into the cannula, and the final coordinates of the tips of probes were D/V−6.0 for the caudate-putamen and D/V−8.3 for the nucleus accumbens. In the experiments, microperfusion pump (Harvard Apparatus, MA, U.S.A.) delivered modified Ringer solution (147 mM NaCl, 1.2 mM $CaCl_2$, 2.7 mM KCl, 1.0 mM $MgCl_2$ and 0.04 mM ascorbic acid) through the probes at flow rate of 2 μl/min, and in order to obtain stable basal extracellular levels of dopamine, Ringer solution was infused for 3–3.5 h. The microdialysate samples (30 μl) were then collected every 15 min, and when a stable outflow was shown by four consecutive samples of dopamine, the rats were given nomifensine (3 mg/kg, 3 ml/kg) i.p. and 30 min later nicotine (0.5 mg/kg), epibatidine (0.6 or 3.0 μg/kg) or saline (1 ml/kg) s.c. The dialysates were collected for 4 h after the second drug injection. After the experiments, the rats were decapitated; their brains were rapidly taken out and frozen on dry ice (−80 °C). The positions of probes in the caudate-putamen and the nucleus accumbens were examined microscopically from 100-μm coronal sections stained with thionine.

2.2. Analysis of dialysate samples

Microdialysate levels of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were analysed immediately with high performance liquid chromatography (HPLC) with electrochemical detection. The HPLC system used for the determination consisted of a detector (Coulochem II, ESA, MA, U.S.A.) equipped with a model 5014B microdialysis cell and a model 2248 HPLC pump (Pharmacia LKB, Sweden). The column (Spherisorb ODS2, 3 μm, 4.6×100 mm, Waters, U.S.A.) was kept at 40 °C. The mobile phase was 0.1 M NaH_2PO_4 buffer (pH 4.0 adjusted with 1.0 mM citric acid), 0.6–0.8 mM octane sulphonic acid, 10–15% (v/v) methanol and 1.2 mM EDTA. The flow rate of the mobile phase was 1.0 ml/min. A total of 20 μl of the dialysis sample was injected onto the column with an autoinjector (CMA/200,

CMA Microdialysis, Sweden). Dopamine was reduced with an amperometric detector (potential—100 mV) after being oxidized with a coulometric detector (+300 mV). The chromatograms were processed with a chromatointegrator (Shimadzu C-R5A, Japan). Values were not corrected for in vitro probe recovery efficiency that was approximately 25% for dopamine and 9–12% for its metabolites. The average concentration of the first four stable samples (<20% variation) was determined as baseline and defined as 100%.

2.3. Drugs

Nicotine solution was prepared by diluting (−)-nicotine base (Fluka Chemie Ag, Switzerland) with saline (0.9% NaCl solution) and adjusting pH to 7.0–7.4 with 0.05 M HCl. (±)-Epibatidine hydrochloride (Sigma Chemical, MO, U.S.A.) was dissolved in saline. Nicotine and epibatidine were given subcutaneously in a volume of 1 ml/kg. Nomifensine maleate (Sigma Chemical, MO, U.S.A.) was dissolved in sterile water by warming it up in a hot water bath and by shaking vigorously. Nomifensine was administered intraperitoneally in a volume of 3 ml/kg. All drug doses are expressed as free base.

2.4. Data analysis

All data are expressed as means±standard errors of the means (S.E.M.). The data were analysed with one-, two- or three-way analysis of variance (ANOVA) for repeated measures (time periods) (Statview 5.0, SAS Institute, U.S.A.). The main factors were the first injection (saline/nomifensine), the second injection (saline/nicotine/epibatidine) and the dialysed brain area (caudate-putamen/nucleus accumbens). When appropriate ($P<0.05$), multiple comparisons were conducted using the contrast analysis with Bonferroni levels.

3. Results

Only data from the rats with probes correctly implanted in the caudate-putamen or in the nucleus accumbens were included in the results. All of the probes inserted in the caudate-putamen and 90% of the probes inserted in the nucleus accumbens were accurately implanted. The locations of the probes in the nucleus accumbens were similar to those illustrated in our previous publication (Janhunen and Ahtee, 2004). Almost all the probes in the nucleus accumbens perfused both the shell and core; some probes were more in the shell and some more in the core, but all in all, it is likely that most of the dopamine collected in the samples originate both from the shell and the core. The data on saline-pretreated rats were reported earlier (Janhunen and Ahtee, 2004).

3.1. The extracellular levels of dopamine and its metabolites in the caudate-putamen

The basal extracellular levels of dopamine, DOPAC and homovanillic acid (HVA) in dialysates of the caudate-putamen were 47.9±10.7 fmol, 17.4±1.4 pmol and 11.1±1.0 pmol (means±S.E.M. per 20 μl, $n=27$), respectively. There were no significant differences in the basal levels between the treatment groups, and the values were similar to those in the saline-pretreated rats reported by Janhunen and Ahtee (2004).

Two-way ANOVA for repeated measures revealed that nomifensine 3 mg/kg significantly elevated the extracellular dopamine level in the caudate-putamen when compared with the rats pretreated with saline [Fig. 1; pretreatment $F(1,45)=49.1$, $P<0.0001$]. The maximal elevation to 223% of the baseline (=100%) was found at 60 min after the nomifensine administration, and the dopamine level returned to the baseline level 2 h later. According to two-way ANOVA, there were no significant pretreatment (saline or nomifensine)×treatment (saline, nicotine, epibatidine 0.6 or 3.0 μg/kg)-interactions on the dopamine level in the caudate-putamen [$F(3,45)=1.7$, $P>0.10$]. Nicotine 0.5 mg/kg elevated the dopamine level of the nomifensine-pretreated rats significantly [Fig. 1; treatment $F(3,23)=4.9$, $P=0.0086$; contrast analysis $P<0.0001$]. The maximal peak (322% of the baseline) occurred at 75 min after the administration of nomifensine, and the dopamine level stayed elevated throughout the whole 4-h experiment. Also, epibatidine 0.6 μg/kg significantly elevated (maximally to 264% of the baseline) throughout the 4-h experiment the dopamine level in the caudate-putamen of the nomifensine-pretreated rats (contrast analysis $P<0.0001$). However, epibatidine at the dose 3.0 μg/kg was not found to alter the effect of nomifensine ($P>0.10$).

Nomifensine significantly decreased the extracellular level of DOPAC (maximally by 20%) but not that of homovanillic acid in the caudate-putamen [Fig. 1; pretreatment $F(1,45)=8.2$, $P=0.0064$ for DOPAC]. The non-significant elevation of DOPAC induced by epibatidine 3.0 μg/kg in the saline-pretreated rats was counteracted by nomifensine pretreatment [$F(1,13)=5.4$, $P=0.0367$], but otherwise, the metabolite levels in rats given nicotine or epibatidine in combination with nomifensine did not differ from those in rats given nomifensine only (Fig. 1).

3.2. The extracellular levels of dopamine and its metabolites in the nucleus accumbens

The basal extracellular levels of dopamine, DOPAC and homovanillic acid (HVA) in dialysates in the nucleus accumbens were 16.3±2.7 fmol, 6.6±0.7 pmol and 3.1±0.3 pmol (means±S.E.M. per 20 μl, $n=27$), respectively. The basal levels did not significantly differ between

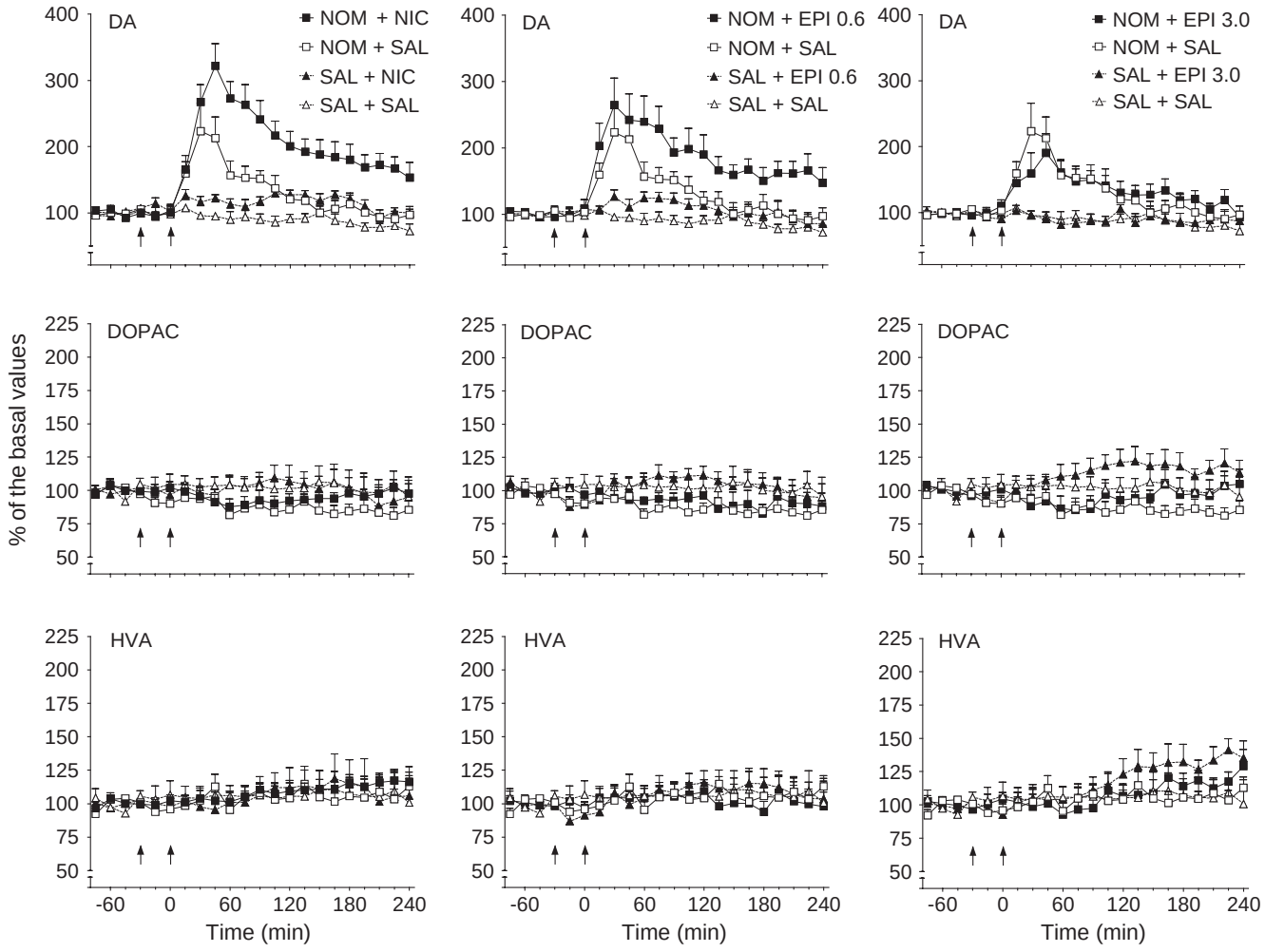

Fig. 1. The effects of nicotine and epibatidine on the levels of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the caudate-putamen dialysates. Rats were given nomifensine (NOM, 3 mg/kg) i.p. (first arrow) after collecting four baseline samples and 30 min later nicotine (NIC, 0.5 mg/kg), epibatidine (EPI, 0.6 or 3.0 μg/kg) or saline (SAL) s.c. (second arrow). Dashed lines present an earlier published data (Janhunen and Ahtee, 2004) from rats that were given nicotine (0.5 mg/kg) or epibatidine (0.6 or 3.0 μg/kg) after saline pretreatment. The results (means±S.E.M.) are expressed as percentages of the four consecutive samples collected before injections (n=6–8). It is to be noted that the scale of the ordinates differs between the DA and its metabolites. Contrast analysis after analysis of variance for repeated measures, 0–240 min, revealed the following statistically significant interactions: DA, pretreatment NOM vs. SAL (P<0.0001), in the nomifensine-pretreated rats: NIC vs. SAL (P<0.0001), EPI 0.6 vs. SAL (P<0.0001), NIC/EPI 0.6 vs. EPI 3.0 (P<0.0001); DOPAC, pretreatment NOM vs. SAL (P<0.0001).

the treatment groups, and the values were similar to those in the saline-pretreated rats reported by Janhunen and Ahtee (2004).

Two-way ANOVA for repeated measures revealed that nomifensine 3 mg/kg significantly elevated the accumbal extracellular dopamine level when compared with that of the saline-pretreated rats [Fig. 2; pretreatment $F(1,47)=71.7$, $P<0.0001$]. Nomifensine maximally elevated the extracellular dopamine level to 403% of the baseline (=100%) at 75 min after the administration whereupon the elevated dopamine level gradually declined so that it was still clearly elevated (145% of the baseline) at the end of experiment. Furthermore, nomifensine elevated the dopamine level more in the nucleus accumbens than in the caudate-putamen, and three-way ANOVA revealed a significant brain nucleus (accumbens or caudate-putamen)-effect [$F(1,92)=10.6$, $P=0.0016$] and a significant brain nucleus×pretreatment-interaction [$F(1,92)=10.4$, $P=0.0017$].

The nicotinic receptor agonists had a significant treatment-effect [$F(3,47)=4.4$, $P=0.0080$] and a significant pretreatment×treatment-interaction [$F(3,47)=2.8$, $P=0.0479$] on the accumbal dopamine level during the first 90 min after their administration (Fig. 2). In the nomifensine-pretreated rats, nicotine 0.5 mg/kg further elevated the dopamine level maximally to 533% of the baseline at 45 min after the administration of nicotine, and the increase was significantly higher than that induced by nomifensine alone during the time period 0–120 min [treatment $F(3,23)=3.22$, $P=0.0416$; contrast analysis $P=0.0014$]. The dopamine levels in the rats treated with nomifensine+nicotine gradually decreased to the same level as after nomifensine alone within approximately 150 min. In contrast to nicotine, the smaller

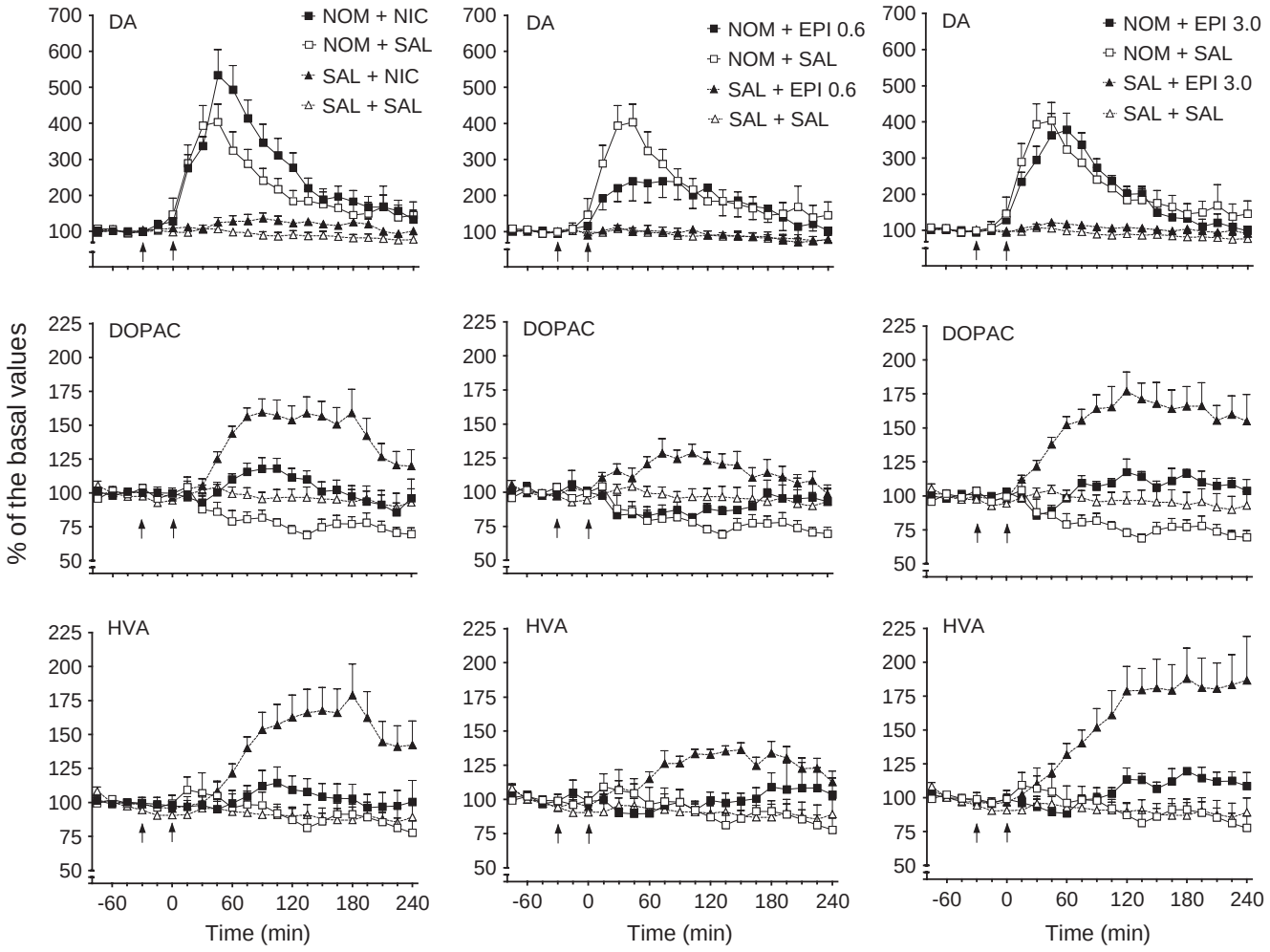

Fig. 2. The effects of nicotine and epibatidine on the levels of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the nucleus accumbens dialysates. Rats were given nomifensine (NOM, 3 mg/kg) i.p. (first arrow) after collecting four baseline samples and 30 min later nicotine (NIC, 0.5 mg/kg), epibatidine (EPI, 0.6 or 3.0 μg/kg) or saline (SAL) s.c. (second arrow). Dashed lines present an earlier published data (Janhunen and Ahtee, 2004) from rats that were given nicotine (0.5 mg/kg) or epibatidine (0.6 or 3.0 μg/kg) after saline pretreatment. The results (means±S.E.M.) are expressed as percentages of the four consecutive samples collected before injections (n=6–7). It is to be noted that the scale of the ordinates differs between the DA and its metabolites. Contrast analysis after analysis of variance for repeated measures (time) revealed the following statistically significant interactions: DA and DOPAC, pretreatments NOM vs. SAL (P<0.0001); in the nomifensine-pretreated rats: DA during 0–120 min, NIC vs. SAL (P=0.0014), EPI 0.6 vs. SAL (P=0.0063), NIC vs. both EPI doses (P<0.001), EPI 0.6 vs. EPI 3.0 (P=0.0066); DOPAC during 0–240 min: NIC vs. SAL (P<0.0001), both EPI doses vs. SAL (P<0.0001), NIC vs. EPI 0.6 (P<0.0001), EPI 3.0 vs. EPI 0.6 (P<0.0001).

epibatidine dose (0.6 μg/kg) significantly decreased the nomifensine-induced increase in the dopamine level during the time period 0–120 min after the administration of epibatidine when compared with the level after nomifensine alone (contrast analysis P=0.0063). The peak dopamine level was about 30% lower than that after nomifensine alone. The larger epibatidine dose (3.0 μg/kg) did not significantly alter the nomifensine-induced increase in the accumbal dopamine level (P>0.10). Thus, due to the opposite effects of nicotine and epibatidine in the nomifensine-pretreated rats, the dopamine level in the nucleus accumbens was found to be significantly higher after nicotine than after either dose of epibatidine studied (contrast analysis P<0.0001).

Nomifensine decreased the extracellular DOPAC (maximally by 30%) but not homovanillic acid level in the nucleus accumbens. According to two-way ANOVA, the DOPAC level was significantly decreased when compared with the saline-pretreated rats [Fig. 2; pretreatment $F(1,47)$=46.0, P<0.0001]. Furthermore, nomifensine decreased the DOPAC more in the nucleus accumbens than in the caudate-putamen, and three-way ANOVA revealed a significant brain nucleus-effect [$F(1,92)$=12.5, P=0.0006] and a significant brain nucleus×pretreatment-interaction [$F(1,92)$=8.4, P=0.0047].

In the nomifensine-pretreated rats, nicotine significantly increased DOPAC [maximally by 40%; treatment $F(3,23)$=6.12, P=0.0032; contrast analysis P<0.0001] but not homovanillic acid (P>0.10) from the levels after nomifensine alone (Fig. 2). Also, epibatidine reversed the decrease of DOPAC concentration induced by nomifensine.

As in the saline-pretreated rats, the effect of epibatidine was dose-dependent so that the maximal increases of DOPAC and homovanillic acid were 45% (DOPAC) and 32% (HVA, $P>0.10$) after epibatidine 3.0 μg/kg and less after epibatidine 0.6 μg/kg when compared with the levels after nomifensine alone (at both doses: $P<0.0001$ for DOPAC).

4. Discussion

Nicotine increased the extracellular dopamine both in the caudate-putamen and the nucleus accumbens of the nomifensine-pretreated rats, whereas the smaller dose of epibatidine studied (0.6 μg/kg) enhanced the nomifensine-induced elevation in the caudate-putamen but reduced it in the nucleus accumbens. The larger dose of epibatidine (3.0 μg/kg) did not alter the dopamine in either brain area studied. Thus, the effects of nicotine and epibatidine on the extracellular dopamine concentration differed between the caudate-putamen and the nucleus accumbens. Previously, we reported that in the saline-pretreated rats the effects of nicotine (0.5 mg/kg) and epibatidine (0.6 μg/kg) were similar in the caudate-putamen but differed from each other in the nucleus accumbens (Janhunen and Ahtee, 2004). As will be discussed below, these differences revealed by the two nicotinic receptor agonists with different affinities to nicotinic receptor subtypes are most probably due to differences in the dopamine turnover as well as in the nicotinic regulation between the two brain areas studied.

Agreeing with earlier findings (Carboni et al., 1989; Cass et al., 1993), acute nomifensine in our experiment increased the dopamine concentration preferentially in the nucleus accumbens. We found that accumbal dopamine clearance was slow and about half of that in the caudate-putamen (13 fmol and 30 fmol per h after the maximal peak, respectively). In line with this, nomifensine reduced significantly more the DOPAC concentration in the nucleus accumbens than in the caudate-putamen. Another dopamine uptake inhibitor, cocaine, has been shown to elevate the extracellular dopamine more in the nucleus accumbens than in the caudate-putamen (Carboni et al., 1989; Cass et al., 1993). These differences may be due to structural and functional differences in these two dopaminergic nerve terminal areas. Indeed, the density of the dopamine uptake sites in the nucleus accumbens has been found to be only half or one third of that in the caudate-putamen (Hoffman and Gerhardt, 1998; Marshall et al., 1990). Furthermore, Andén et al. (1983) reported that synthesis rate of dopamine is about 50% higher in the nucleus accumbens than in the more dorsal parts of striatum. Thus, the preferential effect of nomifensine in the accumbens might result on one hand from faster synthesis and on the other hand from reduced transport of dopamine into the neuron. When nomifensine prevents dopamine's access to intraneuronal monoamine oxidase, increased extracellular level of dopamine and reduced level of DOPAC follow.

The nomifensine-elevated dopamine level in the caudate-putamen was significantly further increased by nicotine and by the dose 0.6 μg/kg of epibatidine but not by the dose 3.0 μg/kg of epibatidine. The dose–response curve for nicotine on the dopamine output in the caudate-putamen has been earlier reported to be bell-shaped (Benwell and Balfour, 1997), and our experiments show that epibatidine induces a corresponding bell-shaped dose–response curve not only alone but also in combination with nomifensine. This suggests that at larger doses epibatidine desensitizes the nicotinic receptor subtypes mediating its effects on the dopamine output in the caudate-putamen.

In the nucleus accumbens, nicotine 0.5 mg/kg increased basal (Janhunen and Ahtee, 2004) and nomifensine-induced (present study) extracellular dopamine level by 36–32% as compared with the corresponding control. This finding is in line with the earlier study by Benwell and Balfour (1992). Nicotine also enhanced the elevation of the accumbal dopamine produced by the dopamine uptake inhibitors, cocaine and methylphenidate (Gerasimov et al., 2000). In contrast to nicotine, epibatidine at 0.6 μg/kg clearly reduced the nomifensine-induced elevation of accumbal dopamine but it had no significant effect at the dose 3.0 μg/kg. We reported recently that epibatidine increased the accumbal dopamine output first at the dose 3.0 μg/kg in the saline-pretreated rats (Janhunen and Ahtee, 2004). Bednar et al. (2004) found that epibatidine decreased the accumbal extracellular dopamine in Sprague–Dawley rats, which were studied already 17–22 h after the implantation of guide cannula. It is possible that in the presence of elevated extracellular dopamine, induced by nomifensine or by implantation of the probe, epibatidine's effects on the mechanisms inhibiting the accumbal dopamine release are emphasized. As discussed above, the nucleus accumbens is more sensitive to nomifensine than the caudate-putamen. Thus, nomifensine by elevating the extracellular dopamine causes a hyperpolarization of dopaminergic neurons and reduces their firing rates (Mercuri et al., 1992). Recently, the effects of nicotine on dopamine release have been reported to depend on the firing rate of the dopaminergic neurons so that desensitization of nicotinic receptors inhibits dopamine release at low firing rates but accelerating the firing rate attenuates this effect (Rice and Cragg, 2004; Zhang and Sulzer, 2004). Therefore, nomifensine could be expected to shift the effects of nicotinic receptor agonists to low frequency effects, i.e. depression of dopamine release. This might explain why the low dose of epibatidine, which probably desensitized nicotinic receptors (Buisson et al., 2000), was found to decrease the dopamine output in the presence of nomifensine.

The inhibitory effect of epibatidine at 0.6 μg/kg on the accumbal dopamine output in our present study was characteristic for the nucleus accumbens and was not found in the caudate-putamen. This suggests that there are differences in the nicotinic regulation of the mesolimbic and nigrostriatal dopaminergic pathways. Neither nicotine nor

epibatidine elevated DOPAC or homovanillic acid in the caudate-putamen. In contrast, in the nucleus accumbens, both nicotine and epibatidine elevated DOPAC and homovanillic acid in the saline-pretreated rats as well as counteracted the nomifensine-induced decrease of DOPAC. These findings show another difference in the effects of nicotinic receptor agonists on the turnover and metabolism of dopamine between the nucleus accumbens and the caudate-putamen. These differences are probably related to structural and/or physiological differences in the two brain areas as discussed above. Also, the mesolimbic dopaminergic pathway may contain a different set of nicotinic receptor subtypes than the nigrostriatal dopaminergic pathway. Indeed, α7 nicotinic receptors are more highly expressed in the ventral tegmental area than in the substantia nigra (Wooltorton et al., 2003), and they are suggested to be preferentially involved in the accumbal dopamine release whereas those containing α3/α6 subunits mediate effects on the dopamine release in the caudate-putamen (Kaiser et al., 1998; Kulak et al., 1997; Schilstrom et al., 1998). Thus, the different affinities of epibatidine and nicotine to various nicotinic receptor subtypes as well as regional variations in the expression of these receptors are most probably involved in our findings that epibatidine's effects on the accumbal dopamine release differ from those of nicotine.

The larger epibatidine dose (3.0 μg/kg) did not alter the nomifensine-elevated extracellular dopamine but elevated the metabolites even more than nicotine. Thus, it most probably enhanced dopamine release, which effect counteracted epibatidine's decreasing effect on the extracellular dopamine. Such a conclusion is supported by the study of Bednar et al. (2004) in which the epibatidine dose that decreased the accumbal extracellular dopamine did not significantly elevate DOPAC. All in all, these findings suggest that epibatidine acts on the accumbal dopamine via several mechanisms.

In the present study, the effects of epibatidine and nicotine on the dopamine output were similar in the caudate-putamen but differed in the nucleus accumbens. Discrepancies in the effects of epibatidine and nicotine are most probably related to their different affinities to nicotinic receptor subtypes regulating the release of dopamine and of other neurotransmitters that, in turn, regulate dopamine release. Also, we found marked differences in the responses of the ventral and dorsal striatal dopamine output to a low concentration of epibatidine in the presence of nomifensine. Thus, it seems that modulation of dopamine transmission with substances like nomifensine may modify also the effects of nicotinic receptor agonists on the accumbal extracellular dopamine. The differences we found between the nucleus accumbens and the caudate-putamen may be due to their different structural and physiological properties, including regulation of dopamine turnover and sets of nicotinic receptor subtypes located in these areas. In addition, our results suggest that the nicotinic mechanisms regulating the nigrostriatal and mesolimbic dopaminergic pathways differ, which supports the view that it is possible to affect these two pathways selectively with nicotinic compounds. This might be of benefit in the treatment of brain diseases, such as Parkinson's disease, and tobacco and drug addictions.

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References

Andén, N.-E., Grabowska-Andén, M., Lindgren, S., Thornstrom, U., 1983. Synthesis rate of dopamine: difference between corpus striatum and limbic system as a possible explanation of variations in reactions to drugs. Naunyn-Schmiedeberg's Arch. Pharmacol. 323, 193–198.

Bednar, I., Friberg, L., Nordberg, A., 2004. Modulation of dopamine release by the nicotinic agonist epibatidine in the frontal cortex and the nucleus accumbens of naive and chronic nicotine treated rats. Neurochem. Int. 45, 1049–1055.

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

Benwell, M.E., Balfour, D.J., 1997. Regional variation in the effects of nicotine on catecholamine overflow in rat brain. Eur. J. Pharmacol. 325, 13–20.

Buisson, B., Vallejo, Y.F., Green, W.N., Bertrand, D., 2000. The unusual nature of epibatidine responses at the alpha4beta2 nicotinic acetylcholine receptor. Neuropharmacology 39, 2561–2569.

Carboni, E., Imperato, A., Perezzani, L., Di Chiara, G., 1989. Amphetamine, cocaine, phencyclidine and nomifensine increase extracellular dopamine concentrations preferentially in the nucleus accumbens of freely moving rats. Neuroscience 28, 653–661.

Cass, W.A., Zahniser, N.R., Flach, K.A., Gerhardt, G.A., 1993. Clearance of exogenous dopamine in rat dorsal striatum and nucleus accumbens: role of metabolism and effects of locally applied uptake inhibitors. J. Neurochem. 61, 2269–2278.

Clarke, P.B., Hommer, D.W., Pert, A., Skirboll, L.R., 1987. Innervation of substantia nigra neurons by cholinergic afferents from pedunculopontine nucleus in the rat: neuroanatomical and electrophysiological evidence. Neuroscience 23, 1011–1019.

Dani, J.A., Ji, D., Zhou, F.M., 2001. Synaptic plasticity and nicotine addiction. Neuron 31, 349–352.

Di Chiara, G., Imperato, A., 1988. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc. Natl. Acad. Sci. U. S. A. 85, 5274–5278.

Gerasimov, M.R., Franceschi, M., Volkow, N.D., Rice, O., Schiffer, W.K., Dewey, S.L., 2000. Synergistic interactions between nicotine and cocaine or methylphenidate depend on the dose of dopamine transporter inhibitor. Synapse 38, 432–437.

Gerzanich, V., Peng, X., Wang, F., Wells, G., Anand, R., Fletcher, S., Lindstrom, J., 1995. Comparative pharmacology of epibatidine: a potent agonist for neuronal nicotinic acetylcholine receptors. Mol. Pharmacol. 48, 774–782.

Giorguieff-Chesselet, M.F., Kemel, M.L., Wandscheer, D., Glowinski, J., 1979. Regulation of dopamine release by presynaptic nicotinic receptors

in rat striatal slices: effect of nicotine in a low concentration. Life Sci. 25, 1257–1262.
Grenhoff, J., Svensson, T.H., 1988. Selective stimulation of limbic dopamine activity by nicotine. Acta Physiol. Scand. 133, 595–596.
Hoffman, A.F., Gerhardt, G.A., 1998. In vivo electrochemical studies of dopamine clearance in the rat substantia nigra: effects of locally applied uptake inhibitors and unilateral 6-hydroxydopamine lesions. J. Neurochem. 70, 179–189.
Imperato, A., Mulas, A., Di Chiara, G., 1986. Nicotine preferentially stimulates dopamine release in the limbic system of freely moving rats. Eur. J. Pharmacol. 132, 337–338.
Janhunen, S., Ahtee, L., 2004. Comparison of the effects of nicotine and epibatidine on the striatal extracellular dopamine. Eur. J. Pharmacol. 494, 167–177.
Janhunen, S., Tuominen, R.K., Piepponen, T.P., Ahtee, L., 2002. The effects of cholinergic nicotinic receptor agonists on accumbal dopamine efflux differ in freely moving rats. Pharmacologist 44 (Suppl. 1), A246.
Janhunen, S., Tuominen, R.K., Piepponen, T.P., Ahtee, L., 2003. Acute epibatidine inhibits whereas nicotine potentiates accumbal dopamine efflux enhanced by dopamine uptake inhibition. Eur. J. Pharm. Sci. 19 (Suppl. 1), S28.
Janhunen, S., Mielikäinen, P., Paldánius, P., Tuominen, R.K., Ahtee, L., Kaakkola, S., submitted for publication. The effect of nicotine in combination with various dopaminergic drugs on the nigrostriatal dopamine in rats.
Kaakkola, S.K., Tuominen, R.K., Mielikäinen, P., Paldanius, P., Ahtee, L., 2000. Effects of nomifensine and tolcapone on nicotine-induced dopamine release in rat striatum. Abstr.-Soc. Neurosci. 26, 900.
Kaiser, S., Wonnacott, S., 2000. Alpha-bungarotoxin-sensitive nicotinic receptors indirectly modulate 3H dopamine release in rat striatal slices via glutamate release. Mol. Pharmacol. 58, 312–318.
Kaiser, S.A., Soliakov, L., Harvey, S.C., Luetje, C.W., Wonnacott, S., 1998. Differential inhibition by alpha-conotoxin-MII of the nicotinic stimulation of 3H-dopamine release from rat striatal synaptosomes and slices. J. Neurochem. 70, 1069–1076.
Klink, R., de Kerchove d'Exaerde, A., Zoli, M., Changeux, J.P., 2001. Molecular and physiological diversity of nicotinic acetylcholine receptors in the midbrain dopaminergic nuclei. J. Neurosci. 21, 1452–1463.
Kulak, J.M., Nguyen, T.A., Olivera, B.M., McIntosh, J.M., 1997. Alpha-conotoxin MII blocks nicotine-stimulated dopamine release in rat striatal synaptosomes. J. Neurosci. 17, 5263–5270.
Lichtensteiger, W., Hefti, F., Felix, D., Huwyler, T., Melamed, E., Schlumpf, M., 1982. Stimulation of nigrostriatal dopamine neurones by nicotine. Neuropharmacology 21, 963–968.
Marshall, J.F., O'Dell, S.J., Navarrete, R., Rosenstein, A.J., 1990. Dopamine high-affinity transport site topography in rat brain: major differences between dorsal and ventral striatum. Neuroscience 37, 11–21.
Mercuri, N.B., Calabresi, P., Bernardi, G., 1992. The electrophysiological actions of dopamine and dopaminergic drugs on neurons of the substantia nigra pars compacta and ventral tegmental area. Life Sci. 51, 711–718.
Nose, T., Takemoto, H., 1974. Effect of oxotremorine on homovanillic acid concentration in the striatum of the rat. Eur. J. Pharmacol. 25, 51–55.
Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates. Academic Press, San Diego, CA.
Rapier, C., Lunt, G.G., Wonnacott, S., 1988. Stereoselective nicotine-induced release of dopamine from striatal synaptosomes: concentration dependence and repetitive stimulation. J. Neurochem. 50, 1123–1130.
Rice, M.E., Cragg, S.J., 2004. Nicotine amplifies reward-related dopamine signals in striatum. Nat. Neurosci. 7, 583–584.
Sakurai, Y., Takano, Y., Kohjimoto, Y., Honda, K., Kamiya, H.O., 1982. Enhancement of [3H]dopamine release and its [3H]metabolites in rat striatum by nicotinic drugs. Brain Res. 242, 99–106.
Schilstrom, B., Svensson, H.M., Svensson, T.H., Nomikos, G.G., 1998. Nicotine and food induced dopamine release in the nucleus accumbens of the rat: putative role of alpha7 nicotinic receptors in the ventral tegmental area. Neuroscience 85, 1005–1009.
Seppa, T., Ahtee, L., 2000. Comparison of the effects of epibatidine and nicotine on the output of dopamine in the dorsal and ventral striatum of freely-moving rats. Naunyn-Schmiedeberg's Arch. Pharmacol. 362, 444–447.
Sharples, C.G., Kaiser, S., Soliakov, L., Marks, M.J., Collins, A.C., Washburn, M., Wright, E., Spencer, J.A., Gallagher, T., Whiteaker, P., Wonnacott, S., 2000. UB-165: a novel nicotinic agonist with subtype selectivity implicates the alpha4beta2* subtype in the modulation of dopamine release from rat striatal synaptosomes. J. Neurosci. 20, 2783–2791.
Sullivan, J.P., Decker, M.W., Brioni, J.D., Donnelly-Roberts, D., Anderson, D.J., Bannon, A.W., Kang, C.H., Adams, P., Piattoni-Kaplan, M., Buckley, M.J., Gopalakrishnan, M., Williams, M., Arneric, S.P., 1994. (+/−)-Epibatidine elicits a diversity of in vitro and in vivo effects mediated by nicotinic acetylcholine receptors. J. Pharmacol. Exp. Ther. 271, 624–631.
Teng, L., Crooks, P.A., Sonsalla, P.K., Dwoskin, L.P., 1997. Lobeline and nicotine evoke 3H overflow from rat striatal slices preloaded with [3H]dopamine: differential inhibition of synaptosomal and vesicular [3H]dopamine uptake. J. Pharmacol. Exp. Ther. 280, 1432–1444.
Westfall, T.C., 1974. Effect of nicotine and other drugs on the release of 3H-norepinephrine and 3H-dopamine from rat brain slices. Neuropharmacology 13, 693–700.
Wonnacott, S., 1997. Presynaptic nicotinic ACh receptors. Trends Neurosci. 20, 92–98.
Wonnacott, S., Kaiser, S., Mogg, A., Soliakov, L., Jones, I.W., 2000. Presynaptic nicotinic receptors modulating dopamine release in the rat striatum. Eur. J. Pharmacol. 393, 51–58.
Wooltorton, J.R., Pidoplichko, V.I., Broide, R.S., Dani, J.A., 2003. Differential desensitization and distribution of nicotinic acetylcholine receptor subtypes in midbrain dopamine areas. J. Neurosci. 23, 3176–3185.
Xiao, Y., Meyer, E.L., Thompson, J.M., Surin, A., Wroblewski, J., Kellar, K.J., 1998. Rat alpha3/beta4 subtype of neuronal nicotinic acetylcholine receptor stably expressed in a transfected cell line: pharmacology of ligand binding and function. Mol. Pharmacol. 54, 322–333.
Zhang, H., Sulzer, D., 2004. Frequency-dependent modulation of dopamine release by nicotine. Nat. Neurosci. 7, 581–582.