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Comparison of the effects of nicotine and epibatidine on the striatal extracellular dopamine

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Abstract

We compared the effects of nicotine and epibatidine on striatal extracellular dopamine and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), by microdialysis in freely moving rats. Nicotine (0.5 mg/kg) elevated dopamine in the caudate–putamen and somewhat more in the nucleus accumbens. Epibatidine at 0.3 μg/kg reduced, and at 0.6 and 1.0 μg/kg increased, dopamine in the caudate–putamen; 2.0 and 3.0 μg/kg had no effect. Accumbal dopamine epibatidine elevated only at 3.0 μg/kg. Thus, in contrast to nicotine, epibatidine increased dopamine output in the caudate–putamen at smaller doses than in the accumbens. Both epibatidine and nicotine enhanced accumbal dopamine metabolism clearly more than that in the caudate–putamen. Also epibatidine was found to elevate 5-hydroxyindoleacetic acid (5-HIAA) in the nucleus accumbens at smaller doses than in the caudate–putamen. Similarly to what has been reported concerning nicotine, the dose–response curve of epibatidine to increase the dopamine output in the caudate–putamen was bell-shaped and clearly differed from that in the accumbens. These findings indicate that the nicotinic mechanisms controlling dopamine release and metabolism in the nigrostriatal and mesolimbic dopaminergic pathways differ fundamentally.

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

1. Introduction

The discovery of the multiplicity of neuronal nicotinic acetylcholine receptors has raised hopes to develop compounds affecting neuronal nicotinic receptors, which might be of therapeutic importance in the treatment of diseases such as tobacco addiction, Parkinson's and Alzheimer's diseases, Tourette's syndrome, schizophrenia, anxiety, depression and pain (Role and Berg, 1996; Holladay et al., 1997; Lloyd and Williams, 2000). Neuronal nicotinic receptors consist of five subunits combined to form an ion channel, and in the brain of mammals, these combinations include α2–α7 and β2–β4 subunits (McGehee and Role, 1995; Clementi et al., 2000). Most common nicotinic receptors in the mammalian brain are those with α4, β2 or α7 subunits (Clarke et al., 1985; Wada et al., 1989; Cimino et al., 1992; Charpantier et al., 1998). There is evidence that nicotinic receptors composed of different subunits differ in their pharmacological properties, and also that they are differentially distributed in the brain, which makes it more complicated to develop selective ligands with desired therapeutic properties.

Nicotine stimulates dopamine release in the brain, as seen in vitro with striatal, accumbal and frontal cortical slices and synaptosomes (Westfall, 1974; Giorguieff-Chesselet et al., 1979; Sakurai et al., 1982; Rapier et al., 1988; Rowell, 1995; Whiteaker et al., 1995; Teng et al., 1997) and by indirect evidence in vivo (Nose and Takemoto, 1974; Lichtensteiger et al., 1982). Acute systemically administered nicotine has been found to facilitate dopamine output in vivo in numerous microdialysis studies (Imperato et al., 1986; Di Chiara and Imperato, 1988; Damsma et al., 1989; Benwell and Balfour, 1992; Mirza et al., 1996; Nisell et al., 1996; Benwell and Balfour, 1997; Seppa and Ahtee, 2000; Seppa et al., 2000). The mesolimbic dopamine has been found to be more sensitive to the stimulatory effects of nicotine than that in the dorsal

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striatum (Imperato et al., 1986; Di Chiara and Imperato, 1988; Benwell and Balfour, 1997; Seppa and Ahtee, 2000). (±)-Epibatidine is a nicotinic receptor agonist derived from the Ecuadorian poison frog *Epipedobates tricolor*, and it has been widely studied because of its analgesic properties (Spande et al., 1992). Epibatidine is the most potent nicotinic receptor agonist yet known, and its effects on central and peripheral functions occur at smaller doses and last longer than those of nicotine (Badio and Daly, 1994; Brioni et al., 1997; Lembeck, 1999). Epibatidine was found to release [^{3}H]dopamine in vitro from striatal slices and synaptosomes in a mecamylamine-sensitive way (Sacaan et al., 1996; Reuben et al., 2000), and to be even 150-fold more potent in stimulating dopamine release from rat striatal slices than (−)-nicotine (Sullivan et al., 1994). However, its effects on the dopamine release have not been consistently studied in vivo.

The aim of this study was to investigate the effects of epibatidine on the extracellular dopamine levels in the caudate–putamen and in the nucleus accumbens, and to compare these effects with those of nicotine. Nicotine was used at the dose, 0.5 mg/kg, which dose as regards analgesia and hypothermia is equieffective with 3.0 μg/kg of epibatidine (Sullivan et al., 1994). Also at doses 0.4–0.6 mg/kg, as found repeatedly, nicotine clearly increases the striatal dopamine output (Imperato et al., 1986; Benwell and Balfour, 1997; Seppa and Ahtee, 2000). Part of this work was presented earlier in abstract form (Janhunen et al., 2002, 2003).

2. Materials and methods

Male Wistar rats (250–330 g) were housed in groups of four before surgery, and singly following surgery at a constant humidity and at an ambient temperature of 20–23 °C. A 12-h light–dark cycle was imposed with lights on at 6 a.m. Food pellets (Altromin 1324, Chr. Petersen, Denmark) and tap water were available throughout the experiment ad libitum. The experimental design was approved by 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

Rats were anaesthetised with halothane (3.5% induction, 2% maintenance) and placed in a stereotaxic apparatus (Stoelting, IL, USA). The skull was exposed, and small holes were drilled to insert a microdialysis guide cannula, which was secured to the skull with three stainless-steel screws and zinc polycarboxylate cement (Aqualox; VOCO, Germany). In the first series of experiments in the caudate–putamen (experiment A studying the effects of nicotine and epibatidine at doses 0.6, 2.0 and 3.0 μg/kg), we used a microdialysis probe with 4.00 mm membrane (BAS MD-2200, 0.32 o.d., Bioanalytical Systems, IN, USA), and therefore, the coordinates relative to the bregma and dura in the insertion of a guide cannula (BAS MD-2250; Bioanalytical Systems) above the caudate–putamen were A/P +1.0, L/M +2.7 and D/V −2.0 (Paxinos and Watson, 1986). In the second series of experiments in the caudate–putamen (experiment B studying the effects of epibatidine at doses 0.1, 0.3 and 1.0 μg/kg), we used a BAS microdialysis probe with 2.00 mm membrane and the depth coordinate was D/V −4.0. Thus, final locations of the tips of the microdialysis probes were in both experiments D/V −6.0 for the caudate–putamen. In the nucleus accumbens, a BAS microdialysis probe with 2.00 mm membrane was used in all the experiments, and the coordinates were A/P +1.7, L/M +1.4 and D/V −6.3, respectively. Thus, the final locations of the tips of the probes were D/V −8.3 for the nucleus accumbens. After the experiments, the rats were decapitated, their brains were rapidly taken out and frozen on dry ice (−80 °C). The positions of the probes in the caudate–putamen and nucleus accumbens were examined microscopically from 100-μm coronal sections stained with thionine.

Rats were allowed to recover from the surgery for 5–7 days in individual transparent test cages (30 × 30 × 40 cm), and then moved 1 day prior to the experiments to the experiment room. Microdialysis experiments were performed in freely moving animals. On the experimental day, between 7 and 8 a.m., a microdialysis probe was inserted into the cannula and the inputs of the probes were connected to a microperfusion pump (Harvard Apparatus, MA, USA), which 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 probe at a flow rate of 2 μl/min. Ringer solution was then infused for 3–3.5 h before the baseline samples were collected to obtain stable basal extracellular levels of dopamine. The microdialysate samples (30 μl) were collected every 15 min. When a stable outflow was shown by four consecutive samples of dopamine, rats were given saline (0.9% NaCl solution, 3 ml/kg) i.p. and 30 min later nicotine (0.5 mg/kg) or epibatidine (0.6, 1.0, 2.0 or 3.0 μg/kg) s.c. Control rats received a second saline injection (1 ml/kg) s.c. The dialysates were collected for 4 h after the administration of nicotinic agonists. The stress caused by the i.p. saline injection and handling of the rats was not found to alter the extracellular dopamine levels. In part of experiments, when the rats were given epibatidine, 0.1 or 0.3 μg/kg, or saline (1 ml/kg) s.c. after four stable consecutive samples, the dialysates were collected for 2.5 h after the injection. Microdialysate levels of dopamine, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) were analysed immediately.

2.2. Analysis of dialysate samples

The high-performance liquid chromatography (HPLC) with electrochemical detection system consisted of an ESA Coulochem II detector (ESA, MA, USA) equipped with a model 5014B microdialysis cell, a Pharmacia LKB model 2248 HPLC pump (Pharmacia LKB, Sweden) and a model LP-21 pulse damper (Scientific Systems, PA, USA). The column (Spherisorb ODS2, 3 μm, 4.6 × 100 mm) was kept at 40 °C with a CROCO CIL column heater (Cluzeau Info-Labo, France). 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 CMA/200 auto-injector (CMA/Microdialysis, Stockholm, Sweden) was utilized for injecting 20 μl of the microdialysate sample into the HPLC system. Dopamine was reduced with an amperometric detector (potential −100 mV) after having been oxidized with a coulometric detector (+300 mV). The chromatograms were processed with a Shimadzu C-R5A chromatointegrator (Shimadzu, Kyoto, Japan). Values were not corrected for in vitro probe recovery efficiency, which was approximately 25% for dopamine and 9–12% for its metabolites and 5-HIAA. The average concentration of the first four stable dialysis samples (<20% variation) was determined as baseline and defined as 100%. To minimize variability between individual rats, data are expressed as percent changes of the baseline values.

2.3. Drugs

Nicotine solution was prepared by diluting (−)-nicotine base (Fluka Chemie, Buchs, Switzerland) with saline (0.9% NaCl solution) and by adjusting pH to 7.0–7.4 with 0.05 M HCl. Epibatidine hydrochloride (Sigma, MO, USA) was dissolved in saline. Nicotine and epibatidine were administered subcutaneously in a volume of 1 ml/kg and all doses refer to base forms. Altogether, three epibatidine batches and stock solutions with different concentrations (12, 30 and 50 μg/ml free base) were used in these experiments. Stock solutions were frozen in small aliquots and stored at the temperature of −80 °C. An aliquot was diluted with saline to required concentration (0.1–3.0 μg/ml free base) on each experimental day. We analysed different stock solutions by using ultraviolet-spectrometry and thin-layer chromatography (TLC Silica gel 60 F254, 0.2 mm, Merck) to ensure that the stability of epibatidine was not affected by the freezing, defrosting or dilution procedures. One stock solution analysed was defrosted after 8 months of storage at −80 °C and another was prepared immediately before analysis. In ultraviolet-spectrometry, the maximal absorbance occurred at the wavelength of 270 nm, and the absorbances of the stock solutions were comparable (0.59 and 0.52, respectively). Neither did thin-layer chromatography reveal any breaking down of epibatidine in the stock solutions. These verifications were carried out because epibatidine's effects in our present experiment occurred at a smaller dose than in our previous experiment (Seppa and Ahtee, 2000) when, furthermore, the rats tolerated the dose 10 μg/kg, whereas in the present experiments already the 3.0 μg/kg dose induced clear tremor and respiratory difficulties. Due to its high potency, even a slight variation in the epibatidine batches or stock solutions can lead to a meaningful variation in the actual doses given to the rats. To eliminate this possibility in the present study, we used several epibatidine batches and stock solutions in all the treatment groups, and furthermore, as described above, we controlled the concentrations of epibatidine in solutions.

2.4. Data analysis

The data were analysed with two-way (treatment × time interaction) analysis of variance (ANOVA) for repeated measures (time) (Statview 5.0, SAS Institute, USA). When appropriate ($P<0.05$), multiple comparisons between treatments were conducted using the contrast analysis with Bonferroni levels.

3. Results

Only the data from rats that had correctly positioned dialysis probes in the caudate–putamen or nucleus accumbens were included in the results. All the probes inserted in the caudate–putamen and about 90% of the probes inserted in the nucleus accumbens were accurately implanted. Fig. 1 shows the representative placements of the properly implanted probes in the nucleus accumbens; the overlapping probe locations are not shown. Almost all the probes in the nucleus accumbens perfused both the shell and the core. However, some probes were to a larger extent in the shell and some in the core, and the distances of the tips of the probes from the olfactory tubercle varied somewhat. However, the proportion of the active membrane exposed to the tissue outside the nucleus accumbens was so small that it is likely that most of the dopamine collected in the samples originates both from the shell and the core.

3.1. The extracellular levels of dopamine and its metabolites in the caudate–putamen and the nucleus accumbens

The baseline extracellular levels of dopamine, DOPAC and HVA in 20-μl dialysates of the caudate–putamen were 47.6 ± 9.5 fmol, 18.3 ± 1.5 pmol and 12.2 ± 1.1 pmol (means ± S.E.M., $n=33$) in experiment A, and 31.7 ± 3.5 fmol, 17.1 ± 1.3 pmol, and 10.1 ± 0.7 pmol ($n=25$) in experiment B, respectively. The lengths of the microdialysis probe membranes differed between experiments A and B (see Section 2.1). Between the treatment groups within each

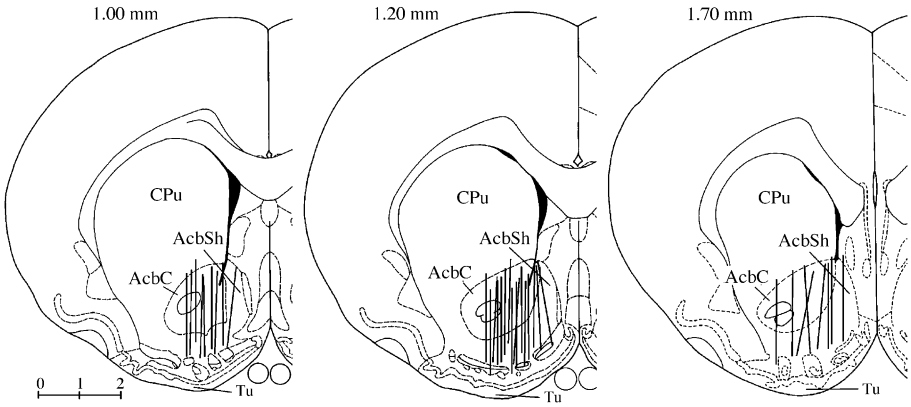

Fig. 1. Representative locations of microdialysis probes in the nucleus accumbens. The overlapping locations of probes are not shown in the figure, and therefore the quantity of probe placements is only indicative. Numbers at the top mark the distance (mm) from bregma (Paxinos and Watson, 1986). The scale bar is in millimeters. The lines correspond to the location of active membrane area of the microdialysis probes. CPu = caudate–putamen, AcbC = nucleus accumbens core, AcbSh = nucleus accumbens shell, Tu = olfactory tubercle.

experiment, no significant differences in basal levels were found.

The baseline levels of accumbal dopamine, DOPAC and homovanillic acid in 20-μl dialysates were 17.6 ± 1.3 fmol, 6.9 ± 0.4 pmol and 3.2 ± 0.2 pmol (means ± S.E.M., $n = 60$), respectively. Between the treatment groups, no significant differences in basal levels were found.

3.2. The effect of nicotine on dopamine and its metabolites

Nicotine 0.5 mg/kg significantly elevated the extracellular dopamine levels in the caudate–putamen when compared with the controls given saline [Fig. 2; time 0–240 min, F treatment (2,16) = 3.799, $P = 0.0447$]. The maximal increase of the baseline values (= 100%) was 29% at 105

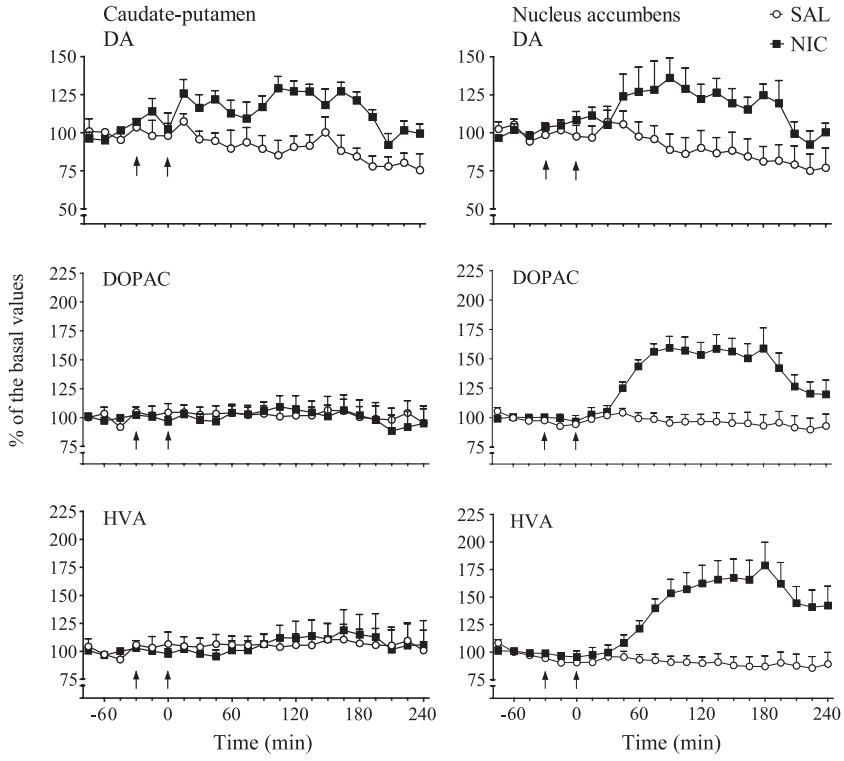

Fig. 2. The effects of nicotine (NIC, 0.5 mg/kg) on the levels of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the caudate–putamen and nucleus accumbens dialysates. Rats were given saline i.p. (first arrow) after collecting the four baseline samples and 30 min later nicotine or saline (SAL) s.c. (second arrow). 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 with repeated measures, 0–240 min, revealed the following statistically significant interactions for the caudate–putamen: DA: NIC vs. SAL ($P < 0.0001$); and for the nucleus accumbens: DA, DOPAC and HVA: NIC vs. SAL ($P < 0.0001$).

min after nicotine administration. Nicotine did not significantly alter the extracellular DOPAC or homovanillic acid levels in the caudate–putamen (Fig. 2).

Nicotine 0.5 mg/kg also significantly elevated the extracellular dopamine levels in the nucleus accumbens when compared with the saline controls [Fig. 2; time 30–240 min, F treatment (2,19) = 3.669, P = 0.0450]. Nicotine maximally increased the dopamine levels by 36% at 90 min after its administration. As shown in Fig. 2 nicotine also significantly elevated the extracellular DOPAC and homovanillic acid levels in the nucleus accumbens [time 0–240 min, DOPAC F treatment (4,30) = 7.652, P = 0.0002, HVA F treatment (4,30) = 4.859, P = 0.0038, respectively].

3.3. The effect of epibatidine in the caudate–putamen

Initially, the effects of epibatidine were examined using the dose 3.0 μg/kg, which, as described in the Introduction, is equipotent with nicotine 0.5 mg/kg. As this dose induced in the rats tremor and enhanced their respiratory rate, we did not study the effects of larger doses. In the first series of experiments shown in Fig. 3, the effects of epibatidine 0.6, 2.0 and 3.0 μg/kg on the extracellular dopamine in the caudate–putamen were studied. The 0.6 μg/kg dose of epibatidine was found to significantly elevate the dopamine levels when compared with the saline controls [Fig. 3; time 0–240 min, F treatment (2,16) = 3.799, P = 0.0447]. The dopamine levels were maximally increased by 26% at 30 min after epibatidine administration. However, epibatidine at the two larger doses studied did not significantly alter the dopamine levels from those of the saline controls (Fig. 3; P>0.10).

To further study the finding that epibatidine 0.6 μg/kg elevated the dopamine levels in the caudate–putamen epibatidine was given at doses 0.1 and 0.3 μg/kg (Fig. 4), and at 1.0 μg/kg in a separate longer-lasting experiment (Fig. 5). The small doses, 0.1 and 0.3 μg/kg, tended to decrease the dopamine levels, and the decrease by the dose 0.3 μg/kg reached significance during the time period 105–150 min when compared with the saline controls [Fig. 4; F treatment (1,11) = 5.945, P = 0.0329]. To verify the elevating effect of epibatidine at the dose 0.6 μg/kg we studied also epibatidine at the dose 1.0 μg/kg, which indeed significantly increased the dopamine levels in the caudate–putamen during the time period 15–105 min when compared with the saline controls [Fig. 5; F treatment (1,11) = 5.035,

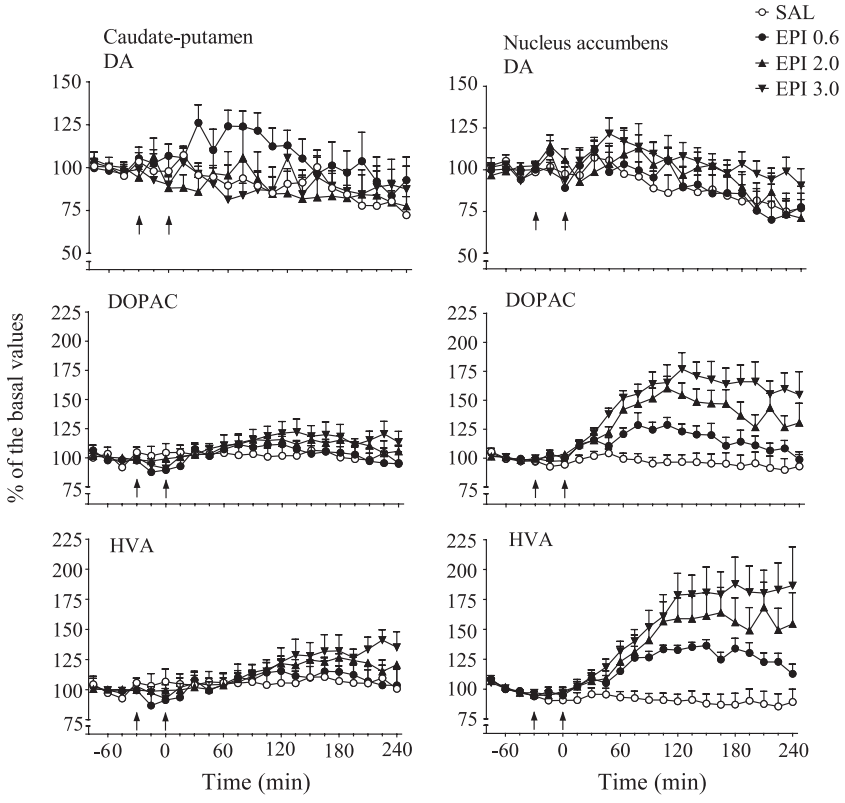

Fig. 3. The effects of epibatidine (EPI, 0.6, 2.0 or 3.0 μg/kg) on the levels of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the caudate–putamen and nucleus accumbens dialysates. Rats were given saline i.p. (first arrow) after collecting the four baseline samples and 30 min later epibatidine or saline (SAL) s.c. (second arrow). 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 with repeated measures, 0–240 min, revealed the following statistically significant interactions for the caudate–putamen: DA: EPI 0.6 vs. SAL (P < 0.0001); and for the nucleus accumbens: DA: EPI 3.0 vs. SAL (time 30–240 min, P < 0.0001), DOPAC and HVA: all the EPI doses vs. SAL (P < 0.0001), EPI 2.0 vs. EPI 0.6 (P < 0.001), EPI 3.0 vs. EPI 0.6/2.0 (P < 0.010).

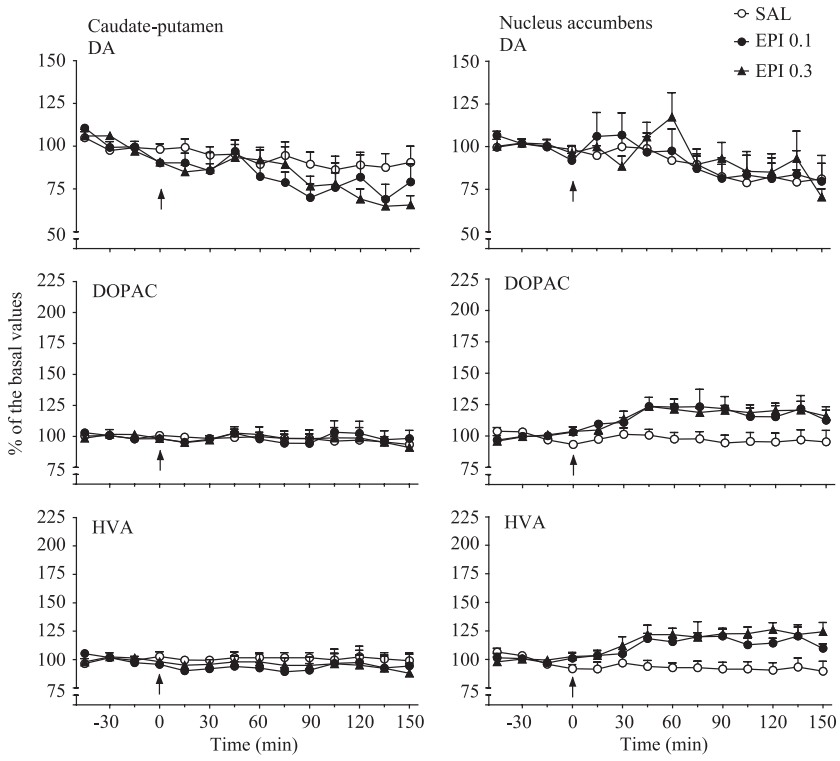

Fig. 4. The effects of epibatidine (EPI, 0.1 or 0.3 μg/kg) on the levels of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the caudate–putamen and nucleus accumbens dialysates. Rats were given epibatidine or saline (SAL) s.c. (arrow) after collecting the four baseline samples. The results (means ± S.E.M.) are expressed as percentages of the four consecutive samples collected before injections ($n = 5–7$). It is to be noted that the scale of the ordinates differs between the DA and its metabolites. Contrast analysis after analysis of variance with repeated measures, 0–150 min, revealed the following statistically significant interactions for the caudate–putamen: DA: EPI 0.3 vs. SAL (time 105–150 min, $P = 0.0002$); and for the nucleus accumbens: DOPAC and HVA: both EPI doses vs. SAL ($P < 0.0001$).

$P = 0.0464$]. The dopamine levels were maximally increased by 23% at 75 min after epibatidine administration.

None of the epibatidine doses studied significantly altered the DOPAC or homovanillic acid levels in the caudate–putamen (Figs. 3, 4 and 5; $P > 0.10$). The dose 3.0 μg/kg maximally produced an increase of 22% in the DOPAC levels and 41% in the homovanillic acid levels (Fig. 3; $P > 0.10$).

3.4. The effect of epibatidine in the nucleus accumbens

The large dose of epibatidine (3.0 μg/kg) significantly elevated the extracellular dopamine levels in the nucleus accumbens when compared with the saline controls [Fig. 3; time 30–240 min, F treatment $(2,19) = 3.669$, $P = 0.0450$]. The maximal increase was approximately 22% from the baseline at 45 min after epibatidine administration. The smaller doses of epibatidine (0.1, 0.3, 0.6, 1.0 or 2.0 μg/kg) did not significantly alter the accumbal dopamine levels (Figs. 3, 4 and 5). Nicotine was found to elevate the accumbal dopamine levels significantly more than any of the studied epibatidine doses including the dose 3.0 μg/kg (contrast analysis, $P = 0.0004$).

Epibatidine at all doses studied significantly elevated the extracellular accumbal DOPAC and homovanillic acid levels when compared with the controls [Fig. 3; epibatidine at doses 0.6, 2.0 and 3.0 μg/kg: time 0–240 min, DOPAC F treatment $(4,30) = 7.652$, $P = 0.0002$, HVA F treatment $(4,30) = 4.859$, $P = 0.0038$, Fig. 4; epibatidine at doses 0.1 and 0.3 μg/kg: time 0–150 min, DOPAC F treatment $(2,15) = 4.535$, $P = 0.0288$, HVA F treatment $(2,15) = 7.805$, $P = 0.0048$, Fig. 5; epibatidine at the dose 1.0 μg/kg: time 0–240 min, DOPAC F treatment $(1,12) = 12.555$, $P = 0.0040$, HVA F treatment $(1,12) = 9.752$, $P = 0.0088$, respectively]. As shown in Figs. 3, 4 and 5, the effects of epibatidine on the DOPAC and homovanillic acid levels were dose-dependent. Furthermore, the increases of accumbal DOPAC and homovanillic acid induced by epibatidine 3.0 μg/kg lasted longer and were therefore significantly larger than those induced by nicotine 0.5 mg/kg (contrast analysis, $P < 0.01$).

3.5. The extracellular levels of 5-HIAA in the caudate–putamen and the nucleus accumbens

In the caudate–putamen, the baseline extracellular levels of 5-HIAA in 20-μl dialysates were 3.6 ± 0.3 pmol in experiment A (means ± S.E.M., $n = 33$) and 3.0 ± 0.2 pmol in experiment B ($n = 25$), and in the nucleus accumbens, the level was 2.3 ± 0.1 pmol (means ± S.E.M., $n = 60$). Be-

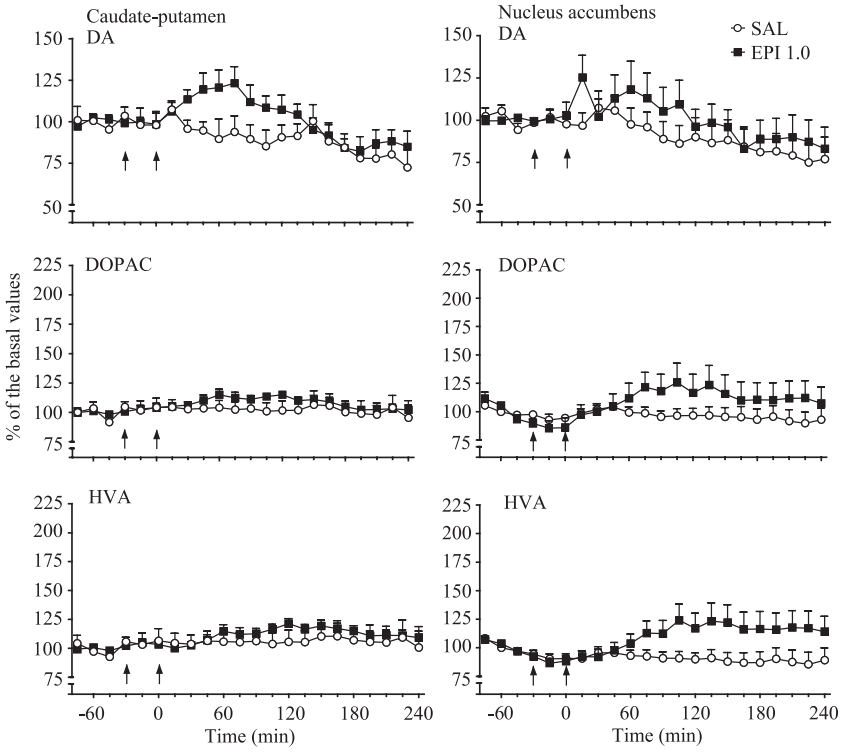

Fig. 5. The effects of epibatidine (EPI, 1.0 μg/kg) on the levels of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the caudate–putamen and nucleus accumbens dialysates. Rats were given saline i.p. (first arrow) after collecting the four baseline samples and 30 min later epibatidine or saline (SAL) s.c. (second arrow). 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 with repeated measures, 0–240 min, revealed the following statistically significant interactions for the caudate–putamen: DA: EPI 1.0 vs. SAL (time 15–105 min, $P < 0.0001$); and for the nucleus accumbens: DOPAC and HVA: EPI 1.0 vs. SAL ($P < 0.0001$).

tween the treatment groups within experiments, no significant differences in basal levels were found.

Nicotine altered the extracellular 5-HIAA levels neither in the caudate–putamen nor in the nucleus accumbens (data not shown, $P > 0.10$). Epibatidine at the dose 3.0 μg/kg, but not at smaller doses, significantly (by about 30% from the baseline) elevated the 5-HIAA levels in the caudate–putamen during the time period 75–150 min [F treatment $(1,11) = 5.833$, $P = 0.0343$]. In the nucleus accumbens, epibatidine at all doses except 1.0 μg/kg, significantly increased the extracellular 5-HIAA levels when compared with the controls [data not shown; epibatidine at doses 0.6, 2.0 and 3.0 μg/kg: time 0–240 min, F treatment $(4,30) = 3.486$, $P = 0.0188$; at doses 0.1 and 0.3 μg/kg: time 0–150 min, F treatment $(2,15) = 14.103$, $P = 0.0004$; at the dose 1.0 μg/kg: $P > 0.10$]. The maximum elevations of the accumbal 5 HIAA by epibatidine 0.1, 0.3, 0.6 and 2.0 μg/kg were 24%, 17%, 25% and 13% as compared with the baseline, respectively. Epibatidine produced the largest increase in the accumbal 5-HIAA levels at the dose 3.0 μg/kg, which elevated the 5-HIAA levels by 37–50% from the baseline at 120 min after the epibatidine administration and the 5-HIAA levels remained elevated till the end of the experiment.

4. Discussion

We studied the effects of epibatidine, a nicotinic agonist, the affinity of which to nicotinic receptors somewhat differs from that of nicotine (as described below), on striatal dopamine output and metabolism. We found that epibatidine resembled nicotine in that its dose–response curve to increase the dopamine output in the caudate–putamen was bell-shaped like that of nicotine (Benwell and Balfour, 1997). Similarly to nicotine, epibatidine was found to enhance dopamine metabolism preferentially in the nucleus accumbens as compared with its effect in the caudate–putamen. However, in contrast to nicotine, which enhances the dopamine output from the nucleus accumbens at smaller doses than from the caudate–putamen (Benwell and Balfour, 1997), epibatidine enhanced the output of dopamine at smaller doses from the caudate–putamen than from the nucleus accumbens.

In the present study, the dopamine output in the caudate–putamen responded to epibatidine in a bell-shaped manner; only the doses 0.6 and 1.0 μg/kg but not the larger doses (2.0 and 3.0 μg/kg) increased the dopamine output in the caudate–putamen. Interestingly, Benwell and Balfour (1997) reported that nicotine induced a bell-shaped response

in the dopamine output in the caudate–putamen; a clear increase in the dopamine output occurring at the dose 0.4 mg/kg but not at the doses 0.1 and 0.8 mg/kg. Such bell-shaped dose–response curves might result from dose-related desensitization of nicotinic receptors mediating this effect (Haikala et al., 1986; Haikala and Ahtee, 1988; Benwell et al., 1995). The bell-shaped dose–response curve also agrees with the previous reports that nicotine in large enough doses in rodents induces catalepsy (Zetler, 1968, 1971; Costall and Naylor, 1973), a behaviour associated with the reduced activity of the nigrostriatal pathway. Interestingly, epibatidine at the smallest doses studied, 0.1 and 0.3 μg/kg, somewhat reduced the dopamine output. One possible explanation for this effect is differential alteration of the release of neurotransmitters, such as γ-aminobutyric acid (GABA) and glutamate that, in turn, control the activity of dopaminergic neurons. The releases of GABA and glutamate are known to be regulated by presynaptic nicotinic receptors (Wonnacott, 1997).

As regards the nucleus accumbens, nicotine is known to increase the dopamine output in this area at smaller doses than in the caudate–putamen (for references, see Introduction). Thus, Benwell and Balfour (1997) reported that in the nucleus accumbens, acute nicotine at doses 0.1, 0.4 and 0.8 mg/kg increased the dopamine overflow about similarly so that already the smallest dose tested, which had no effect on the dopamine output in the caudate–putamen, evoked a near maximal response in the accumbal dopamine output. Furthermore, the accumbal response did not fade by increasing the dose; indeed, increasing the dose prolonged the duration of the response to nicotine (Benwell and Balfour, 1997). In contrast, epibatidine in our study elevated the dopamine output only at the largest dose studied, 3.0 μg/kg, which dose is three to five times larger than the dose which enhanced the dopamine output in the caudate–putamen. This finding that is also in line with our previous experiments (Seppa and Ahtee, 2000) in which epibatidine enhanced the dopamine output in the caudate–putamen at smaller doses than in the nucleus accumbens. Unfortunately, a direct comparison between our present and previous results cannot be made, because in our previous study, the epibatidine used for reasons unknown to us was weaker than the epibatidine used in the present experiments (see Section 2.3). All in all, these findings suggest that there are differences between the nicotinic receptors mediating the effects of epibatidine and nicotine on the dopamine output in the nucleus accumbens. Furthermore, the effects of nicotine and epibatidine on the accumbal dopamine clearly differ from their effects on the nigrostriatal dopamine. As described below, such differences most probably result from differences in the distribution and activation/desensitization properties of nicotinic receptors composed of different subunits.

Epibatidine at all doses studied (0.1–3.0 μg/kg) in a dose-dependent manner as well as nicotine (0.5 mg/kg) increased the accumbal levels of dopamine metabolites, DOPAC and homovanillic acid, but neither compound significantly altered the DOPAC and homovanillic acid levels in the caudate–putamen. The dose 3.0 μg/kg of epibatidine maximally increased the accumbal metabolites by 88%, and indeed, the tendency of the large epibatidine dose (3.0 μg/kg) to increase the DOPAC and homovanillic acid levels in the caudate–putamen could partly be due to the diffusion from the ventral striatum (nucleus accumbens). Previously, it has been consistently found that nicotine preferentially elevates the dopamine metabolites, DOPAC and homovanillic acid, in the nucleus accumbens as compared with its effect in the dorsal striatum (Imperato et al., 1986; Grenhoff and Svensson, 1988; Seppa and Ahtee, 2000). Our results indicate that epibatidine similarly to nicotine preferentially enhances dopamine metabolism in the nucleus accumbens as compared with that in the dorsal striatum. On the other hand, the effects of nicotine and epibatidine on the dopamine output did not correlate with their effects on the dopamine metabolism. These data indicate that the nicotinic receptors mediating epibatidine's and nicotine's effects on the dopamine output differ from those mediating their effects on the dopamine metabolism both in the caudate–putamen and in the nucleus accumbens. We have earlier suggested this with nicotine in the caudate–putamen (Leikola-Pelho et al., 1990; Seppa and Ahtee, 2000; Seppa et al., 2000). The enhancement of dopamine metabolism seems to be linked to the activation of nicotinic receptors in the somatodendritic region because locally applied nicotine failed to increase the extracellular concentrations of DOPAC and homovanillic acid in the striatum and nucleus accumbens (Toth et al., 1992; Marshall et al., 1997). Thus, the effects that nicotinic agonists have on dopamine metabolism indicate a preferential activation of dopamine cells located in the ventral tegmental area as compared with those in the substantia nigra with enhanced synthesis and degradation of dopamine following the neuronal activation. On the other hand, the changes found in dopamine output might more closely reflect direct effects of the nicotinic agonists on the nerve terminal areas.

Presynaptic nicotinic receptors are suggested to modulate the release of serotonin (5-hydroxytryptamine, 5-HT) (Wonnacott, 1997). Nicotine has been reported to elevate in vivo the 5-HT levels in the cortex and pontine nucleus, but not in the striatum, and the effects are thought to result from altered glutamate release (Toth et al., 1992). Nicotine and epibatidine were also found to increase in vitro the [^{3}H]5-HT release from rat striatal slices and synaptosomes (Yu and Wecker, 1994; Reuben and Clarke, 2000). However, the evidence for nicotine's direct effect on 5-HT release or for location of nicotinic receptors on the 5-HT nerve terminals is somewhat controversial (for a review, see Seth et al., 2002). The α4β2 nicotinic receptors have been suggested to exist on striatal afferents originating from the raphe nuclei (Schwartz et al., 1984), and the α4 subunits have been recently found to co-localize with 5-HT$_3$ serotonin receptors on the nerve endings in the rat striatum indicating a direct action of nicotine on 5-HT release (Nayak et al., 2000), but

there are also contrary findings (Pradhan et al., 2002). In the present study, epibatidine at the large dose (3.0 μg/kg) elevated 5-HT metabolite, 5-HIAA, in the caudate–putamen, and at all the doses studied in the nucleus accumbens. Thus, epibatidine elevated the 5-HIAA levels at the doses that were also found to increase the dopamine metabolism, which suggests that the effects of epibatidine on the dopamine metabolism and on the serotonin metabolism could be linked. Nicotine was neither found to alter 5-HIAA in the caudate–putamen nor in the nucleus accumbens, which agrees with earlier reports (Damsma et al., 1989; Mirza et al., 1996). These data thus suggest that epibatidine affects the serotonergic–dopaminergic network differently from nicotine, an effect giving further support to the suggestion that the actions of these two agonists are mediated differently by nicotinic receptors.

The most prominent nicotinic receptor subtypes in the dopaminergic pathways contain α3, α4, α6, α7, β2 and β3 subunits (Wada et al., 1989; Le Novere et al., 1996; Kulak et al., 1997; Charpantier et al., 1998; Kaiser et al., 1998; Sorenson et al., 1998; Sharples et al., 2000; Klink et al., 2001; Zoli et al., 2002). Nicotine is known to stimulate dopamine release via at least α4β2, α3/α6β2 and β3 subunit containing nicotinic receptors (Kulak et al., 1997; Kaiser et al., 1998; Sharples et al., 2000; Wonnacott et al., 2000; Mogg et al., 2002; Cui et al., 2003). Furthermore, nicotine stimulates dopamine release by stimulating glutamate release via nicotinic receptors comprised of the α7 subunits (Kaiser and Wonnacott, 2000). Nicotine has been reported to have a high binding affinity at the α4β2 nicotinic receptor subtype and about 1000-fold lower affinity at the α7 subtype (Gerzanich et al., 1995; Gotti et al., 1997; Hahn et al., 2003). Epibatidine has an extremely high binding affinity at the α4β2 nicotinic receptor subtypes and somewhat lower affinity at the α3β2 and α3β4 subtypes (Gerzanich et al., 1995; Xiao et al., 1998; Hahn et al., 2003). Its binding affinity has been reported to be even 10 000-fold lower at the α7 than at the α4β2 nicotinic receptor subtypes. Epibatidine has been found to stay longer and/or more tightly bound to the α4β2 subtype than nicotine (Buisson et al., 2000). The differential affinities of epibatidine and nicotine at the nicotinic receptors mediating the release of transmitters could partially explain the differences seen in the present study. Indeed, recent evidence indicates that there are more α7 subunit containing nicotinic receptors in the cell body areas of the limbic dopaminergic neurons than in the somatodendritic areas of the nigrostriatal dopaminergic neurons (Wooltorton et al., 2003). Interesting is also that the α7 subunit containing nicotinic receptors were less susceptible to desensitization by low (≤100 nM) nicotine concentrations than the receptors containing β2 subunits, which furthermore, were equally distributed between these two brain areas.

In conclusion, we found that the dose–response curve of epibatidine to increase the dopamine output in the caudate–putamen was bell-shaped and similar to that of nicotine as reported earlier (Benwell and Balfour, 1997), whereas in the nucleus accumbens epibatidine similarly to nicotine (Benwell and Balfour, 1997) clearly increased dopamine output at concentrations which did not anymore increase it in the caudate–putamen. These differences suggest that the nicotinic receptors regulating dopamine output in the caudate–putamen are desensitized at smaller concentrations of nicotinic agonists than those in the nucleus accumbens. Secondly, similarly to nicotine, epibatidine was found to enhance dopamine metabolism preferentially in the nucleus accumbens as compared with its effect in the caudate–putamen suggesting that the effects of nicotinic agonists on the dopamine metabolism are mediated by mechanisms different from those involved in their effects on the dopamine output. Thirdly, in contrast to nicotine, which enhances the dopamine output from the nucleus accumbens at smaller doses than from the caudate–putamen (Benwell and Balfour, 1997), epibatidine enhanced the output of dopamine at smaller doses from the caudate–putamen than from the nucleus accumbens suggesting that different nicotinic receptors are involved in the mediation of the effects of epibatidine and nicotine on the dopamine output in the caudate–putamen and in the nucleus accumbens. All in all, our findings suggest that there are fundamental differences between the nicotinic mechanisms controlling dopamine release and metabolism in the nigrostriatal and mesolimbic dopaminergic pathways, which thus indicates that it is possible to develop nicotinic compounds selectively acting on either pathway. Such compounds could be beneficial, for example, in tobacco addiction.

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