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ORIGINAL INVESTIGATION

# Acute nicotine changes dynorphin and prodynorphin mRNA in the striatum

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**Abstract**
*Rationale* Nicotine displays rewarding and aversive effects, and while dopamine has been linked with nicotine's reward, the neurotransmitter(s) involved with aversion remains speculative. The κ-dynorphinergic system has been associated with negative motivational and affective states, and whether dynorphin (Dyn) contributes to the behavioral pharmacology of nicotine is a pertinent question.
*Objective* We determined whether administration of a single dose of nicotine alters the biosynthesis of Dyn in the striatum of mice.
*Results* Nicotine free base, 1 mg/kg, sc, induced a biphasic, protracted increase of striatal Dyn, an initial rise by 1 h, which declined to control levels by 2 h, and a subsequent increase, between 6 and 12 h, lasting over 24 h. At 1 h, the nicotine effect was dose dependent, with doses ≥0.5 mg/kg inducing a response. Prodynorphin mRNA increased by 30 min for over 24 h, and in situ hybridization demonstrated elevated signal in caudate/putamen and nucleus accumbens. The nicotinic antagonist mecamylamine prevented the Dyn response, and a similar effect was observed with D1- and D2-like dopamine receptor antagonists, SCH 23390, sulpiride, and haloperidol. The glutamate NMDA receptor antagonist MK-801 reversed the nicotine-induced increase of Dyn, while the AMPA antagonist NBQX had a marginal effect.
*Conclusions* We interpret our findings to indicate that acute nicotine enhances the synthesis and release of striatal Dyn. We propose that nicotine influences Dyn primarily through dopamine release and that glutamate plays a modulatory role. A heightened dynorphinergic tone may contribute to the aversive effects of nicotine in naive animals and first-time tobacco smokers.

**Keywords** Nicotine · Dynorphin · Prodynorphin · Striatum · Acetylcholine nAChR receptors · Dopamine D1 and D2 receptors · Glutamate NMDA and AMPA receptors

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## Introduction

Opioids are thought to play an essential role in drug addiction (Hertz 1998), and a link between nicotine dependence and brain opioid systems has been suggested (Koob and Nestler 1997; Pomerleau 1998). Indeed, tobacco smoking and nicotine alter the synthesis and release of endogenous opioids (Pomerleau 1998 and discussion therein); nicotine withdrawal produces a somatic, opiate-like syndrome (Malin et al. 1992; Isola et al. 1999); opioid antagonists precipitate some of the somatic and motivational features of nicotine withdrawal (Malin et al. 1993; Ise et al. 2000; Watkins et al. 2000; unpublished observations); and opioid antagonists have been used for tobacco-smoking cessation (Ismail and el-Guebaly 1998). Notwithstanding, the role(s) of the various opioidergic systems in nicotine dependence is not fully understood, and the opioid peptide response(s) to nicotine administration are relatively unexplored.

In the striatum, central to opioid function are the medium spiny neurons who supply two major afferent projections: the direct striatonigral pathway that expresses dynorphin (Dyn) and the indirect striatopallidal pathway that expresses met-enkephalin (Met-Enk). Dynorphins and enkephalins have opposing actions on dopaminergic neurons of substantia nigra and ventral tegmental area and are components of circuits promoting negative or positive motivational and affective states, respectively (Spanagel et al. 1990, 1992; reviewed by Steiner and Gerfen 1998). Dopamine controls the synthesis of striatal Dyn and Met-Enk at the genomic level (Angulo and McEwen 1994), and consistent with their indirect dopaminergic action, psychostimulant drugs alter the content of the opioids and the expression of their precursor messenger RNA (mRNA; Trujillo et al. 1993; McGinty 2007; Shippenberg et al. 2007). Nicotine may mimic the psychostimulants' effect on striatal opioid peptides, and changes in Met-Enk content and preproenkephalin mRNA have been observed after acute and chronic nicotine administration (Pierzcala et al. 1987; Houdi et al. 1991; 1998; Dhatt et al. 1995; Wewers et al. 1999; Isola et al. 2000), as well as during nicotine withdrawal (Isola et al. 2002). Whether nicotine affects dynorphinergic function in the striatum is unclear. Contrary to reports from chronic nicotine studies evaluating prodynorphin (PD) mRNA expression (Mathieu et al. 1996; Mathieu-Kia and Besson 1998; Le Foll et al. 2003), we have reported that the synthesis and release of Dyn are enhanced in the caudate/putamen and nucleus accumbens of nicotine-dependent and withdrawn mice (Isola et al. 2008) and suggested that Dyn might contribute to the emergence of the negative affective states associated with nicotine withdrawal (Kenny and Markou 2001).

Nicotine displays rewarding and aversive properties in rodents (Fudala et al. 1985; Risinger and Oaks 1995; Risinger and Brown 1996; Gommans et al. 2000; Shoaib et al. 2002; Pescatore et al. 2005; Le Foll and Goldberg 2005), and first-time nicotine users report positive and negative affective effects (Foulds et al. 1997; Heishman and Henningfield 2000). While the ability of nicotine to release dopamine in the mesolimbic system has been linked with its rewarding properties (Di Chiara 2000), the neurotransmitter(s) involved with nicotine's aversion has not been established. We have presented preliminary evidence that a single dose of nicotine increases the levels of Dyn and PD mRNA in the striatum (Hadjiconstantinou et al. 2002). This finding might be of importance as stimulation of κ opioid receptors inhibits dopamine release (Di Chiara and Imperato 1988b) and produces aversion (Mucha and Hertz 1985; Pfeifer et al. 1986; Bals-Kubik et al. 1993), and the dynorphinergic system has been implicated in a number of behaviors, dysphoria, anhedonia, depression, and stress responses, relevant to drug use (Zimmer et al. 2001; McLaughlin et al. 2003; Shirayama et al. 2004; Todtenkopf et al. 2004; Carlezon et al. 2006).

We have expanded our original observations and studied the effect of a single dose of nicotine on Dyn (1–13) and PD mRNA in the caudate/putamen and nucleus accumbens of mice and the pharmacology of the response. Acute systemic administration of nicotine increases the firing rate of ventral tegmental and nigral dopaminergic neurons and enhances the release of dopamine in ventral and dorsal striatum (Grenhoff et al. 1986; Di Chiara and Imperato 1988a), responses that are modulated by glutamate NMDA receptors (Toth et al. 1992; Sziraki et al. 1998, 2002; Schilström et al. 1998; Wonnacott et al. 2000, 2005; Fu et al. 2000). Furthermore, the synthesis of Dyn in the striatum is under the tonic excitatory control of dopamine, which, predominantly, through D1 receptors, regulates the genomic expression of the precursor peptide PD (Gerfen et al. 1990, 1991; reviewed by Angulo and McEwen 1994). Accordingly, the role of dopamine and glutamate receptors in the regulation of Dyn synthesis by nicotine in the striatum was explored. Investigating how the dynorphinergic system responds to nicotine at the various stages of nicotine exposure and the pharmacological mechanisms involved may provide new insights into the neuronal substrates involved in the nicotine dependence.

## Materials and methods

*Animals* Male Swiss–Webster (Harlan) mice, 25–30 g, were used for the experiments. They were housed in our vivarium under a 12-h dark/light cycle and provided with chow and water ad libitum. The studies were conducted in accordance with the Guide for Care and Use of Laboratory Animals as adopted and promulgated by the National Institute of Health, USA, and were approved by the OSU Institutional Laboratory Animal Care and Use Committee.

*Treatments* Mice were given a single dose of nicotine free base, 1 mg/kg, sc, or saline and killed at various times, 15 min–48 h, as indicated in the figures and tables. The nicotine dose was selected based on dose–response studies, where the effect of nicotine, 0.25, 0.5, 1, and 2 mg/kg, sc, on Dyn content in the striatum was evaluated at 1-h post-injection. Antagonists and appropriate vehicles were administered 30 min prior to nicotine, and animals were killed 1 or 18 h later, based on nicotine time–response studies (Fig. 1a). The following antagonist drugs were used: mecamylamine, 3 mg/kg, ip, nAChR nonselective; SCH 23990, 1 mg/kg, ip, dopamine D1-like receptor selective; sulpiride, 50 mg/kg, ip, dopamine D2-like receptor selective; haloperidol, 1 mg/kg, ip, dopamine D2/D1 receptor nonselective; MK-801, 0.5 mg/kg, ip, NMDA glutamate

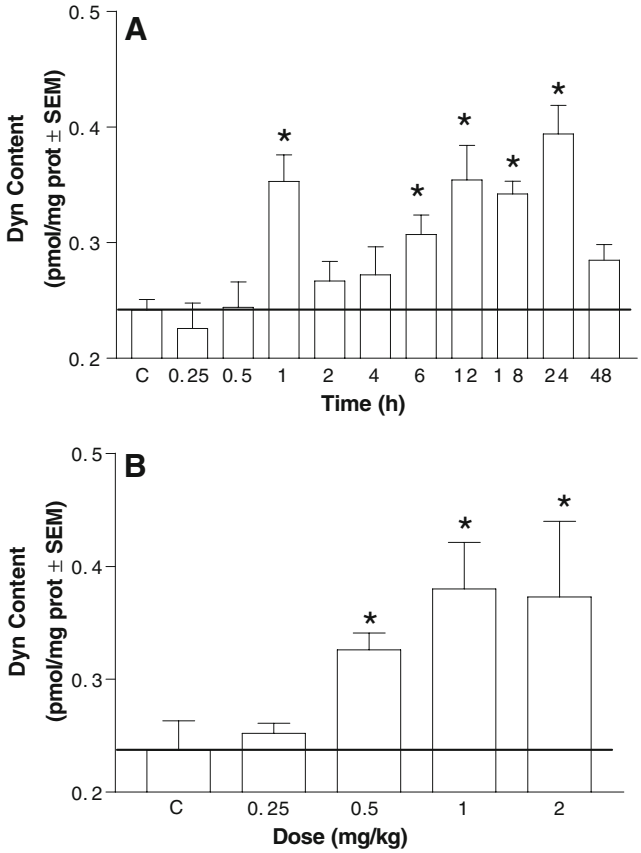


**Fig. 1** Acute administration of nicotine increases the content of Dyn in the striatum. **a** Time–response: Mice were treated with nicotine, 1 mg/kg, sc, or vehicle (Control; *C*) and killed at the indicated times post-injection. Dyn content was estimated by RIA as described in the "Materials and methods". $N$=8–12. *$P$<0.05 compared with Control. **b** Dose–response: Mice were treated with various doses of nicotine or vehicle (Control; *C*) and killed 1 h post-injection. Dyn content was estimated by RIA as described in the "Materials and methods". $N$=5. *$P$<0.05 compared with Control

receptor noncompetitive; and NBQX, 5 mg/kg, ip, AMPA glutamate receptor selective. The doses of the dopamine and glutamate antagonist drugs have been based on the literature (Hanson et al. 1987; Sivam 1989; Trujillo et al. 1990; Singh et al. 1991; Kosowski et al. 2002). At the indicated times after treatment, mice were decapitated, brains were removed, and the striatum was dissected. For some studies (Table 1), dorsal (caudate/putamen) and ventral striatum (nucleus accumbens), hypothalamus, hippocampus, olfactory tubercle, and prefrontal cortex were dissected at 1 and 18 h after nicotine. All collected tissues were frozen in liquid nitrogen and stored at −70°C until analyzed. For the in situ hybridization studies, animals were killed 3 h after nicotine administration, and whole brains were removed, frozen with pulverized dry ice, and stored at −70°C.

*Dynorphin estimation* Dyn (1–13) content was estimated by RIA. In brief, tissues were heated at 95°C in 1 M acetic acid for 15 min, cooled to room temperature, and homogenized using a cell disrupter. After centrifugation, 10,000×$g$ for 20 min, the supernatant was lyophilized and stored at −70°C. Lyophilized tissues were reconstituted in RIA buffer containing the following: 0.1 M phosphate buffer, pH 6.0, 50 mM NaCl, 5 mM EDTA, 0.025% thiomerosal, 0.1% gelatin, and 0.1% Triton X-100. Samples and Dyn (1–13) standards were incubated with Dyn (1–13) antibodies (1:2,000; Peninsula Lab.), $^{125}$I-Dyn (1–13; 7,000–9,000 cpm; Peninsula Lab.), and 0.5% bovine γ-globulin at 4°C for 18–24 h. Bound antigen was separated by adding 0.5 ml of assay buffer containing 1.6% charcoal and 0.16% dextran T-70. Following centrifugation, 1,500×$g$, radioactivity in the supernatant was counted with a γ-spectrometer. Protein concentration in tissue samples was determined using the bicinchoninic acid method, with bovine albumin as standard (Isola et al. 2008).

## PD mRNA estimation

*Northern blot* Total RNA was isolated from brain tissues with Trizol (In-Vitrogen). The RNA, 20 μg, was separated by denaturing agarose gel electrophoresis and transferred to a Hybond nuclei acid transfer membrane (Amersham). A $^{32}$P-labeled antisense cRNA probe was prepared from a linearized rat PD cDNA (gift from Michael J. Iadarola, NIH, Bethesda, MD, USA) with SP6RNA polymerase and hybridized overnight with the blots at 55°C. After washing, blots were then rehybridized overnight with a β-actin DNA probe (American Type Culture Collection) and $^{32}$P-labeled by random priming to correct for differences in RNA yield among samples. The optical density of signal on the X-ray film was determined by image analysis (Universal Imaging, MetaMorph). The estimated optical density value for each band was corrected by the corresponding β-actin and data expressed as percent of the control value on the same blot (Isola et al. 2008).

**Table 1** Brain regional distribution of the nicotine effect on Dyn

| Brain region | Dyn (pmol/mg prot±SEM) | | |
|---|---|---|---|
| | Vehicle | Nicotine | |
| | | 1 h | 18 h |
| Caudate/putamen | 0.18±0.01 | 0.29±0.02* | 0.25±0.02* |
| Nucleus accumbens | 0.47±0.02 | 0.62±0.06* | 0.60±0.06* |
| Olfactory tubercle | 0.50±0.01 | 0.57±0.05 | 0.57±0.06 |
| Hippocampus | 0.19±0.02 | 0.35±0.03* | 0.17±0.02 |
| Frontal cortex | 0.12±0.01 | 0.14±0.01 | 0.12±0.01 |
| Hypothalamus | 5.8±0.6 | 8.6±0.3* | 4.6±0.04 |

Mice were treated with nicotine, 1 mg/kg, sc, or vehicle (Control) and killed 1 or 18 h later. Dyn was estimated by RIA as described in the "Materials and methods". $N$=8–12.
*$P$<0.05 compared with respective Control.

*In situ hybridization* Frozen brains were sectioned on a cryostat and coronal sections, 12 μm, and were thaw-mounted onto SuperfrostR*/Plus slides, dried, and stored at −70°C. Prior to use, slides were warmed to room temperature; immersion-fixed in 4% paraformaldehyde and PBS, pH 7.2, for 10 min; rinsed; incubated in 0.25% acetic acid anhydride and 0.1 trietholamine, pH 8.0; dehydrated, and air-dried. For the hybridization, a 47-base oligoprobe corresponding to the 5′-GTT GTC CCA CTT CAG CTT GGG GCG AAT GCG CCG CAG GAA GCC CCC AT-3 nucleotides of the PD DNA was 3′-end-labeled with [$^{35}$S]dATP by terminal deoxynucleotidyl transferase. For the in situ hybridization, 50 μl of hybridization solution (300 mM NaCl, 20 mM Tris-HCl, pH 7.5, 5 mM EDTA, 50% formamide, 5% dextran sulfate), containing $10^5$ cpm of probe, 0.1 M dithiothreitol, and 0.36 μg of yeast tRNA, was applied on each slide followed by incubation at 37°C for 24 h. Slides were washed, dehydrated, dried, and apposed on X-ray film. Analysis of the hybridization signal was performed by quantitative image analysis (Universal Imaging Corporation, MetaMorph), and data were calculated as nanocurie per gram based on $^{14}$C standards run in parallel and expressed as percent of respective control. Brain regions of interest were identified based on anatomical coordinates (Paxinos and Franklin 2001) and hybridization signals estimated in the rostral (bregma 1.54–0.86 mm) and caudal (bregma −0.10 to −0.82 mm) aspects of caudate/putamen, as well as the rostral pole (bregma 1.94–1.70 mm), shell and core (bregma 1.54–0.86 mm) of nucleus accumbens (Isola et al. 2008).

*Statistical analysis* The Dyn content data were analyzed by a parametric one-way analysis of variance, and post hoc comparisons were conducted using the Dunnett or the Student–Newman–Keuls multiple comparisons test. A Student's *t* test was used to compare two independent groups of values. The PD mRNA data were analyzed by the non-parametric Kruskal–Wallis test followed by the Dunn's multiple comparisons test, and the Mann–Whitney test was used to compare two independent groups of values. Statistical analysis was performed using GraphPad Instat and NCSS Version 07.1.10 software, and a level of $P<0.05$ was accepted as statistically significant.

## Results

### A single dose of nicotine increases Dyn content and PD mRNA in the striatum

A single injection of nicotine induced a protracted increase of Dyn content in the striatum (Fig. 1a; $F(10–100)=5.948$, $P<0.0001$), which appeared to be biphasic. At 1-h post-nicotine, the content of Dyn was increased, but it declined to near control levels between 2 and 4 h. Subsequently, the peptide rose again by 6 h, reached maximal levels at 12–24 h, and approached control levels by 48 h. When studied at 1 h, the effect of nicotine on striatal Dyn content was dose dependent, with 0.25 mg/kg of nicotine free base being ineffective and 1–2 mg inducing a maximal response (Fig. 1b: $F(4–26)=3.250$.

The regional distribution of the Dyn response was investigated at an early, 1 h, and late, 18 h, time point following the administration of a single dose of nicotine, 1 mg/kg. The Dyn content was increased in the caudate/putamen and nucleus accumbens at both times examined, and the magnitude of the response was about 61–40% over control for the caudate/putamen and 32–28% over control values for the nucleus accumbens (Table 1). With regard to the other brain regions examined, there was an increase of the opioid in the hypothalamus and hippocampus 1 h post-nicotine only, and no significant changes were observed in frontal cortex and olfactory tubercle (Table 1).

The increase in Dyn content in the striatum was accompanied by a rise in the levels of PD mRNA evaluated by Northern blot (Fig. 2; KW=46.002, $P=0.001$). The PD mRNA increased as early as 30 min after nicotine, reached a maximum of two-fold increase between 1 and 6 h, and started declining after 6 h approaching control values by 48 h. In situ hybridization was employed to determine the regional distribution of the PD mRNA change in the striatum 3 h after the acute nicotine dosing (Fig. 3). Quantitative analysis demonstrated elevated PD mRNA levels in the rostral (Fig. 3e and Table 3; 98% over control values) and caudal (Fig. 3f and Table 3; 91% over control values) aspects of caudate/putamen, which was equally distributed between the lateral and medial regions of the nucleus (Fig. 3e and

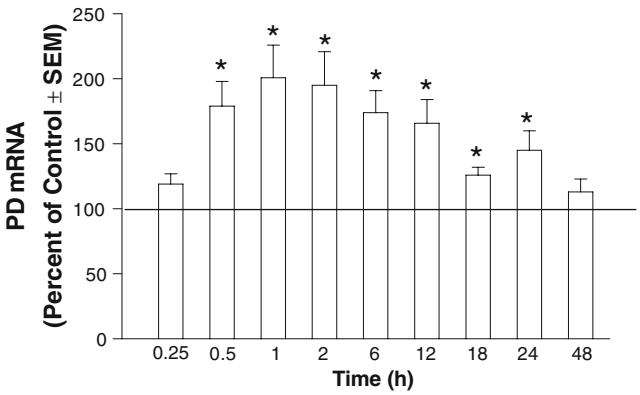


**Fig. 2** Increased PD mRNA in the striatum following acute administration of nicotine: time response. Mice were treated with nicotine, 1 mg/kg, sc, or vehicle (Control) and killed at the indicated times post-injection. PD mRNA was estimated by Northern blot as described in the "Materials and methods". $N=8–12$. $^*P<0.05$ compared with Control

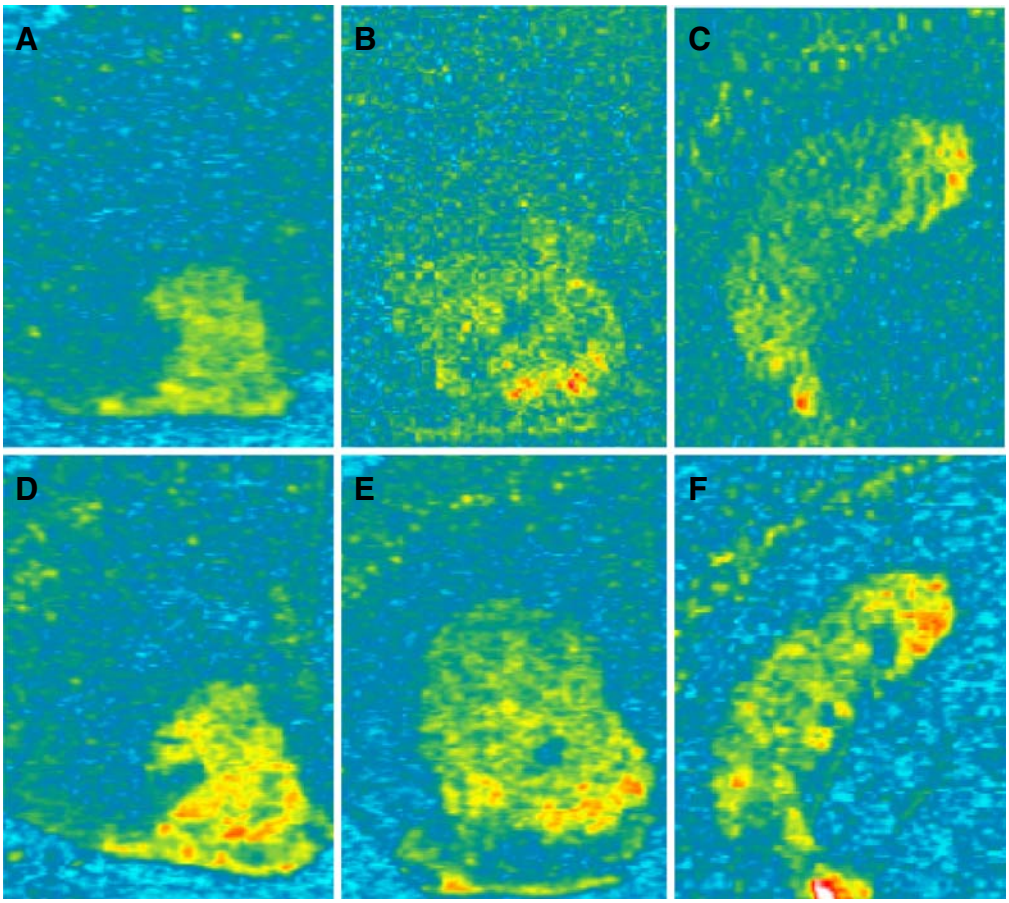


**Fig. 3** Subregional distribution of the nicotine-induced elevation of PD mRNA in the striatum. Mice were treated as described in Fig. 2, and PD in situ hybridization was performed as described in the "Materials and methods" at 3 h post-nicotine. Representative images showing the distribution of PD mRNA expression in control mice: **a** rostral pole; **b** caudate/putamen (rostral aspects) and nucleus accumbens; **c** caudate/putamen (caudal aspects); and nicotine-treated mice: **d** rostral pole; **e** caudate/putamen (rostral aspects); and nucleus accumbens; **f** caudate/putamen (caudal aspects)

Table 2; 72% and 89% over control, respectively). Significant message increase was found in the rostral pole, shell, and core of the nucleus accumbens (Fig. 3d and e and Table 2; 44%, 56%, and 54% over control, respectively).

**Table 2** Nicotine increases PD mRNA in caudate/putamen and nucleus accumbens

| Brain region | PD mRNA (percent of Control±SEM) |
|---|---|
| Caudate/putamen | |
| Rostral | 198±38* |
| Rostral/lateral | 172±39* |
| Rostral/medial | 189±27* |
| Caudal | 191±11* |
| Nucleus accumbens | |
| Rostral pole | 144±9* |
| Core | 156±7* |
| Shell | 154±8* |

Mice were treated with nicotine 1 mg/kg, sc, or vehicle (Control) and killed 3 h later. PD mRNA was estimated by in situ hybridization as described in the "Materials and methods".
*$P<0.05$ compared with Control. $N$=4–6.

## Pharmacology of the nicotine-induced increase of Dyn in the striatum

Since nicotine induced a protracted rise of Dyn content in the striatum for the pharmacological characterization of the response, an early (1 h) and a late (18 h) time were chosen. Pretreatment with the centrally acting nAChR antagonist mecamylamine prevented the nicotine-induced early and late increase of Dyn in the striatum (Table 3), confirming the specificity of the response. To investigate whether dopamine was responsible for the nicotine-induced increase of Dyn in the striatum and identify the dopamine receptor type involved, animals were pretreated with D1- and D2-like antagonists. Administration of the D1-like receptor antagonist SCH 23390 prior to nicotine prevented the early and late rise of the opioid (Table 4), and a similar effect was seen with sulpiride, a D2-like receptor antagonist, and haloperidol, a mixed D2/D1 receptor antagonist (Table 4).

The observation that both nicotine and dopamine receptor antagonists prevent the early and late nicotine-

**Table 3** Specificity of nicotine-induced increase of Dyn content in the striatum

| Treatment | Dyn (pmol/mg prot±SEM) | |
|---|---|---|
| | 1 h | 18 h |
| Vehicle | 0.21±0.01 | 0.21±0.02 |
| Nicotine | 0.33±0.02* | 0.34±0.01* |
| Mecamylamine | 0.19±0.01 | 0.22±0.03 |
| Mecamylanine+nicotine | 0.18±0.01** | 0.21±0.02** |

Mice were treated with nicotine, 1 mg/kg, sc, or vehicle (Control) and killed 1 or 18 h later. For the combined treatment, mecamylamine, 3 mg/kg, ip, or saline was administered 30 min prior to an injection of nicotine, and animals were killed 1 or 18 h after nicotine. Mice were killed 1.5 or 18.5 h after a single dose of mecamylamine, 3 mg/kg, ip, or vehicle. Dyn was estimated by RIA as described in the "Materials and methods". $N$=11–15.
*$P$<0.05 compared with Control; **$P$<0.05 compared with nicotine.

induced increase of Dyn in the striatum is suggestive of a common mechanism underlying the biphasic and protracted response of the peptide to nicotine and points to a dopamine-dependent process. Glutamate facilitates the nicotine-induced release of dopamine in the striatum via ionotropic NMDA, and perhaps AMPA, receptors (Toth et al. 1992; Sziraki et al. 1998, 2002; Schilström et al. 1998; Wonnacott et al. 2000; Fu et al. 2000), and, therefore, a role in the dopamine-mediated responses, such as the enhanced Dyn synthesis seen in our studies, appears to be reasonable. This hypothesis was tested in a series of experiments where the effect of NMDA and AMPA antagonists on the nicotine-induced increase of Dyn was investigated. Because the Dyn content in the striatum was elevated 18 h after

**Table 4** Dopamine receptor antagonists reverse the nicotine-induced Dyn increase in the striatum

| Treatment | Dyn (pmol/mg prot±SEM) | |
|---|---|---|
| | 1 h | 18 h |
| Vehicle | 0.21±0.01 | 0.22±0.01 |
| Nicotine | 0.38±0.02* | 0.29±0.02* |
| SCH 23390 | 0.24±0.02 | 0.20±0.02 |
| Sulpiride | 0.25±0.03 | 0.25±0.02 |
| Haloperidol | 0.21±0.01 | 0.25±0.02 |
| SCH 23390+nicotine | 0.23±0.02** | 0.17±0.03** |
| Sulpiride+nicotine | 0.26±0.02** | 0.21±0.03** |
| Haloperidol+nicotine | 0.22±0.02** | 0.18±0.02** |

Mice were treated with nicotine, 1 mg/kg, sc, or vehicle (Control) and killed 1 or 18 h later. Mice were killed 1.5 or 18.5 h after a single dose of SCH 23390, 1 mg/kg, ip, sulpiride, 50 mg/kg, ip, haloperidol, 1 mg/kg, ip, or vehicle. For the combined treatments, SCH 23390, sulpiride, haloperidol, or drug vehicle was administered 30 min prior to an injection with nicotine and animals killed 1 or 18 h after nicotine. Dyn was estimated by RIA as described in the "Materials and methods". $N$=8–15.
*$P$<0.05 compared with Control; **$P$<0.05 compared with nicotine.

DMSO, the vehicle was used for dissolving the glutamatergic antagonist drugs (saline 0.20±0.02; DMSO 0.39±0.06 Dyn pmol/mg prot), and results only from the 60-min time point are presented where the DMSO had no effect. The NMDA antagonist MK-801 when given alone had no effect on the Dyn content, but prevented the Dyn response when administered prior to nicotine (Table 5). Pretreatment with the AMPA antagonist NBQX showed a trend to reduce the increase of Dyn content after nicotine, but the overall response was variable and small, about 15%, and did not reach statistical significance (Table 5).

## Discussion

These studies demonstrate that the administration of a single dose of nicotine increases the expression of PD mRNA and PD content in the striatum for a prolonged time. The temporal pattern of PD and Dyn response to nicotine points to an association between precursor and opioid change and implies enhanced Dyn synthesis and release. Indeed, the rise of PD mRNA occurred early, 30 min after nicotine, which preceded that of the Dyn content, and the mRNA levels remained elevated for over 24 h. The content of Dyn increased soon after the rise of PD mRNA, at 1 h after nicotine, but the response displayed a biphasic pattern suggesting post-translational processing of the peptide product. The decline of Dyn content, observed between 2 and 6 h, after the initial increase could be construed to reflect accelerated peptide release and subsequent breakdown resulting in lower, but still above control, tissue levels. Increased Dyn levels in the presence of increased precursor mRNA, as seen from 12 to 24 h post-nicotine, could indicate an imbalanced synthesis and release resulting in intracellular peptide storage. Despite our efforts, we were

**Table 5** Involvement of NMDA receptors in the nicotine-induced increase of Dyn in the striatum

| Treatment | Dyn (pmol/mg prot±SEM) |
|---|---|
| Vehicle | 0.21±0.01 |
| Nicotine | 0.32±0.02* |
| MK-801 | 0.22±0.01 |
| NBQX | 0.20±0.03 |
| MK-801+nicotine | 0.21±0.02** |
| NBQX+nicotine | 0.27±0.03 |

Mice were treated with nicotine, 1 mg/kg, sc, or vehicle (Control) and killed 1 h later. Mice were killed 1.5 h after a single dose of MK-801, 0.5 mg/kg, ip, NBQX, 5 mg/kg, ip, or vehicle. For the combined treatments, MK-801, NBQX, or vehicle was administered 30 min prior to an injection of nicotine, and animals were killed 1 h after nicotine. Dyn was estimated by RIA as described in the "Materials and methods". $N$=6–12.
*$P$<0.05 compared with Control; **$P$<0.05 compared with Nicotine.

not able to determine the effect on nicotine on Dyn release in vitro and ex vivo because the released Dyn is low and below the detection sensitivity of our assay. Notwithstanding, our observations are congruent with reports that acute administration of psychostimulant drugs enhances the PD mRNA expression as well as the synthesis and release of Dyn in the striatum for a protracted time (Hanson et al. 1988; Trujillo et al. 1990; Hurd and Herkenham 1992, Smith and McGinty 1994; Wang and McGinty 1995; Wang et al. 1995; Adams et al. 2000, 2003). Nicotine enhances Dyn biosynthesis in both the caudate/putamen and nucleus accumbens, and it appears that like the psychostimulants, it has a larger effect on caudate/putamen than on nucleus accumbens (Wang and McGinty 1995; Wang et al. 1995).

The Dyn response to nicotine was dose dependent, and relatively high doses, ≥0.5 mg/kg of nicotine free base, were required to increase Dyn in the striatum at 1 h. Interestingly, high doses of cocaine and methamphetamine are also needed to augment PD expression in the striatum (Hurd and Herkenham 1992; Smith and McGinty 1994; Whang et al. 1995; Adams et al. 2000), suggesting the involvement of common mechanism(s). The drug dose employed might, in part, explain the findings of Le Foll et al. (2003), who, after a single dose of 0.5 mg/kg nicotine bitartrate to rats, reported no change in the levels of PD mRNA in the caudate/putamen and core of nucleus accumbens 2 h post-injection. The decrease of PD mRNA in the shell of nucleus accumbens that the authors observed is in divergence with our findings as well the current understanding regarding PD regulation and needs further investigation.

Augmented availability of synaptic Dyn, as our data suggest, could be regarded as counteracting the facilatory action of nicotine on neurotransmitter release, particularly dopamine and glutamate, in the striatum and other limbic structures (Di Chiarra and Imperato 1988a; Hjelmstad and Fields 2003), and dampening their biochemical and behavioral consequences. The Dyn-κ opioid receptor system is known to mediate aversive behaviors (Mucha and Hertz 1985; Pfeifer et al. 1986; Bals-Kubic et al. 1993), and enhanced Dyn synthesis and release, as seen in our studies, could be, in part, responsible for the reported aversive effects of nicotine. Nicotine causes aversion in experimental animals (Fudala et al. 1985; Risinger and Oaks 1995; Risinger and Brown 1996; Gommans et al. 2000; Shoaib et al. 2002; Pescatore et al. 2005; Le Foll and Goldberg 2005) and humans exposed to nicotine for the first-time report negative subjective effects (Foulds at al. 1997; Heishman and Henningfield 2000). Notably, aversion in humans (Lundahl et al. 2000) and experimental animals (Fudala et al. 1985; Risinger and Oaks 1995; Risinger and Brown 1996; Gommans et al. 2000; Shoaib et al. 2002; Pescatore et al. 2005; Le Foll and Goldberg 2005) has been observed with relatively high doses of nicotine, in concurrence with our observation that high doses of nicotine enhance Dyn synthesis and release in the striatum.

In the striatum, nAChRs are predominantly found on neuronal afferents, and a corollary of their presynaptic locale is that the nicotine action on Dyn might be indirect via release of intermediate neurotransmitters. Our findings that both dopamine and glutamate receptor antagonists blocked the Dyn response to nicotine support such a mechanism. Nicotine stimulates dopamine release in the striatum directly via α4 and β2 subunit-containing (α4β2*) nAChRs present on midbrain dopaminergic neurons (Gotti et al. 2006) and indirectly via α7 nAChRs, expressed on glutamatergic terminals (Wonnacott et al. 2005), through glutamate release and subsequent modulation of dopamine secretion by ionotropic glutamate receptors (Toth et al. 1992; Sziraki et al. 1998, 2002; Schilström et al. 1998; Wonnacott et al. 2000; Fu et al. 2000). Direct and indirect dopamine agonists augment the expression of PD mRNA, and the levels of PD derived peptides predominantly via dopamine D1 receptors (reviewed by Trujillo et al. 1993; Angulo and McEwen 1994), although D2 receptors contribute as well, and a D1/D2 synergism has been proposed (Wang and McGinty 1996a; McGinty 2007 and discussion therein). Our finding that the nicotine-induced increase of Dyn was reversed by D1- and D2-like antagonists implies that a similar mechanism operates after nicotine and suggests that dopamine is a common link for the regulation of Dyn by stimulant drugs. The site of dopamine antagonists' action is most likely post-synaptic, targeting dopamine receptors involved with the regulation of Dyn in the medium spiny neurons of the striatum. However, reports that D1- and D2-like antagonists administered systemically or into the ventral tegmental area prevent the nicotine-induced dopamine release in the striatum and nucleus accumbens (Toth et al. 1992; Sziraki et al. 1998, 2002) provide an alternative site and mode of action for the dopamine antagonism of the nicotine effect on Dyn. Ostensibly, inhibition of dopamine release by SCH 23390, sulpiride, or haloperidol could count, in part, for the antagonists' ability to reverse the rise of striatal Dyn by nicotine and constitute a within-the-system regulatory mechanism.

The finding that NMDA, and to some extent AMPA, glutamate ionotropic receptor antagonists reversed the nicotine-induced increase of Dyn points to a role for glutamate. Similar observations have been made after acute administration of amphetamine and methamphetamine, and the relationship between glutamatergic system and psychostimulant-stimulated synthesis of Dyn is well documented (Singh et al. 1991; Wang and McGinty 1996b and discussion therein). Collectively, the psychostimulant studies have demonstrated that NMDA and AMPA antagonists prevent the psychostimulant-induced enhancement of PD genomic expression in both ventral and dorsal striatum, and

an inhibition of dopamine release has been speculated. Systemic, intra-tegmental or local application of kynurenic acid as well as NMDA antagonists (MK-801, AP-5, CGS19755, CGP3951) prevents the effect of nicotine on dopamine overflow in caudate/putamen and nucleus accumbens in vivo (Toth et al. 1992; Sziraki et al. 1998, 2002; Schilström et al. 1998; Fu et al. 2000), and kynurenic acid partially reduces the nicotine-induced release of dopamine in striatal slices in situ (Wonnacott et al. 2000). It is possible, therefore, that the MK-801 antagonism of the nicotine-induced Dyn increase in our studies is mediated through an inhibitory action on dopamine release (Toth et al. 1992; Sziraki et al. 1998, 2002; Schilström et al. 1998; Fu et al. 2000). Unlike MK-801, the selective AMPA antagonist NBQX had a small non-significant effect on the nicotine-induced rise of Dyn in the striatum, which is in agreement with reports that AMPA receptors are not major contributors in the glutamate modulation of nicotine-stimulated dopamine release. Neither systemic nor intra-tegmental or intra-accumbal administration of AMPA antagonists (CNQX, GYKI-52466, NBQX) blocks the nicotine-induced dopamine overflow in nucleus accumbens in vivo (Sziraki et al. 1998, 2002; Schilström et al. 1998; Fu et al. 2000; Kosowski et al. 2002 and discussion therein), and blockade of AMPA receptors with DNQX inhibits about 20% of the dopamine release elicited by a high concentration of nicotine in striatal slices (Wonnacott et al. 2000). Because the quinoxalinedione-derived AMPA antagonists DNQX and NBQX also possess affinity for the Gly/NMDA site (Catarzi et al. 2007), it is possible that the observed effect on dopamine release in striatal slices and Dyn content in our studies is mediated via NMDA receptors. Based on the literature, we surmise that the excitatory amino acid could influence Dyn synthesis in the striatum indirectly by modulating nicotine-stimulated dopamine release and subsequent dopamine-mediated opioid peptide synthesis. Despite the presence of glutamate ionotropic receptors on medium spiny neurons, a direct effect of glutamate on dynorphinergic neurons is unlikely as mecamylamine, a selective antagonist for β-subunit-containing nAChRs (Gotti et al. 2006), totally reversed the nicotine-induced Dyn increase.

In summary, we provide evidence that a single dose of nicotine induces a protracted enhancement of Dyn synthesis and release in the striatum that involves both the ventral and dorsal striatum, anatomical substrates for drugs of abuse (Fasano and Brambilla 2002). Based on our pharmacological studies and the literature, we propose that following acute nicotine administration, the regulation of striatal Dyn is primarily under dopaminergic control, and other neurotransmitters, such as glutamate, contribute by fine-tuning dopamine release. Inhibition of the nicotine-stimulated dopamine release and/or blockade of dopamine receptors on striatal medium spiny neurons could prevent the enhancement of dynorphinergic function and its behavioral consequences. Interestingly, mice lacking the β2 nAChR subunit show suppressed dopamine release after nicotine with concomitantly reduced reward and aversion (Picciotto et al. 1998; Shoaib et al. 2002), and dopamine and NMDA receptor blockade prevents the aversive effects of nicotine (Laviolette and van der Kooy 2003a and 2003b). According to our studies, all these manipulations are expected to attenuate the nicotine-induced increase of Dyn synthesis and release in the striatum. The finding that Dyn is also increased in hippocampus and hypothalamus, areas associated with cognitive and stress responses, and considered to be part of the circuitry involved in drug dependence, indicates that nicotine alters Dyn along the broader limbic neuraxis. Heightened synthesis and release of Dyn after acute nicotine might be relevant to the reported dysphoria and negative mood by first-time nicotine users (Foulds et al. 1997; Heishman and Henningfield 2000), behaviors that are associated with stimulation of the Dyn-κ opioid receptor system in the brain (Mucha and Hertz 1985; Pfeifer et al. 1986; Bals-Kubik et al. 1993).

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