ELSEVIER

Neuroscience Letters 181 (1994) 107–112

NEUROSCIENCE LETTERS

# Cholinergic modulation of dopamine overflow in the rat neostriatum: a fast cyclic voltammetric study in vitro

Martina Kudernatsch, Bernd Sutor*

*Department of Physiology, University of Munich, Pettenkoferstrasse 12, D-80336 Munich, Germany*

Received 13 September 1994; Revised version received 22 September 1994; Accepted 22 September 1994

**Abstract**

Stimulus-evoked dopamine overflow in rat neostriatal slices was determined using fast cyclic voltammetry. The dopamine efflux induced by intrastriatal stimulation increased with stimulus intensity and was found to be enhanced by more than 100% upon application of the dopamine uptake inhibitor nomifensine. The acetylcholine esterase inhibitor eserine concentration-dependently and reversibly depressed stimulus-induced dopamine overflow. This effect was mediated by both, muscarinic and nicotinic cholinergic receptors: the action of eserine was mimicked by cholinergic agonists (muscarine and nicotine) and the effects of these agonists were blocked by muscarinic and nicotinic antagonists (atropine and dihydro-$\beta$-erythroidine). These experiments suggest that endogenous acetylcholine exerts an inhibitory control on stimulus-evoked (i.e. phasic) dopamine overflow in vitro by affecting striatal dopaminergic nerve terminals.

*Key words:* Neostriatum; Dopamine; Acetylcholine; Fast cyclic voltammetry; Brain slice

The function of neostriatal neurons is modulated by various neurotransmitters including dopamine and acetylcholine [5,12]. The dopaminergic control is exerted via fibers originating from nuclei in the brain stem (A8–A10, [3]), whereas the cholinergic modulation is accomplished predominantly by interneurons of the neostriatum [5,15]. Many studies indicated that the modulatory action of both transmitters is antagonistic (for review see [15]). It is now well established that dopamine inhibits stimulus-evoked acetylcholine release from cholinergic interneurons and that this action is mediated by dopamine D2-receptors [15]. There is also evidence that acetylcholine regulates dopamine release within the striatum. However, the present data do not allow clear conclusions. It has been demonstrated that the cholinergic agonist nicotine stimulates dopamine release from striatal synaptosomes [11] and from rat striatal slices [4,10]. This effect was explained by the presence of presynaptic nicotinic acetylcholine receptors on dopaminergic nerve terminals. On the other hand, in slice preparations of the cat striatum, acetylcholine facilitated dopamine efflux even in the presence of TTX [8]. The blockade by atropine of this stimulatory cholinergic action suggested the existence of presynaptic muscarinic receptors on striatal dopaminergic nerve terminals. These experiments were performed by loading the preparation with tritium-labelled markers and monitoring spontaneous release of radioactivity or measuring release of radioactivity induced by electrical stimulation, high concentrations of extracellular potassium or by excitatory amino acids. In order to investigate the influence of endogenous acetylcholine on the stimulus-induced efflux of endogenous dopamine, we used a slice preparation of the rat neostriatum to analyse the effects of the acetylcholine esterase inhibitor eserine on stimulus-evoked overflow of dopamine. Dopamine overflow was determined by fast cyclic voltammetry (FCV). This technique allows the measurement of dopamine efflux in 'real time' [1]. Since there is no evidence for dopaminergic interneurons within the rat neostriatum [3,5,15], the dopamine overflow determined by FCV in slice preparations can be directly related to the release of dopamine from synaptic terminals belonging to fibers originally arising from brain stem nuclei.

*Corresponding author. Fax: (49) (89) 5996-216.


*SSDI* 0304-3940(94)00737-3

The experiments were performed on corticostriatal slices prepared from adult male Wistar rats. The preparation was done according to the method described by Kawaguchi et al. [7]. In a recording chamber, the slices were kept submerged in artificial cerebrospinal fluid (ACSF) perfused through the chamber at a flow rate of 4–5 ml/min. The ACSF consisted of (in mM): NaCl 125, KCl 3, $NaH_2PO_4$ 1.25, $CaCl_2$ 2.5, $MgCl_2$ 1.5, $NaHCO_3$ 25 and D-glucose 10. The ACSF was saturated with a mixture of 95% $O_2$ and 5% $CO_2$. At a recording temperature of 31–32°C the pH of the solution was 7.4. Dopamine overflow in the caudate/putamen was detected using carbon-fiber electrodes. Single carbon-fibers (7 μm diameter) were introduced into glass pipettes so that the fiber protruded the tip of the pipette by 20–40 μm. These electrodes were connected via a headstage to a potentiostatic amplifier [1]. A waveform consisting of 1.5 cycles of a triangular ramp scanning between −1.0 and +1.4 V was applied to the carbon-fiber. The slope of the ramp was 480 V/s and the scan frequency was 4 or 8 Hz. In the absence of dopamine, the voltage transient produced a typical background current signal [1]. In the presence of dopamine, a shift occurred in the current waveform when the voltage transient passed the dopamine oxidation potential of +0.6 V [1]. A second shift was seen when the ramp crossed the dopamine reduction potential. The current signal amplitude sampled at a potential of +0.6 V in the absence of dopamine was subtracted from that registered in the presence of the compound. The amplitude difference was fed into a sample-and-hold buffer and the output of this buffer was monitored on a Y-t chart recorder and stored on a computer. Following each experiment the carbon-fiber electrodes were calibrated in standard dopamine solutions. The measurement of calibration curves in the absence and presence of eserine showed that the compound did not interfere with the oxidation of dopamine at the carbon-fiber electrode.

Throughout the paper, we use the term 'dopamine overflow' instead of 'dopamine release' to point out that we have determined the stimulus-induced change in

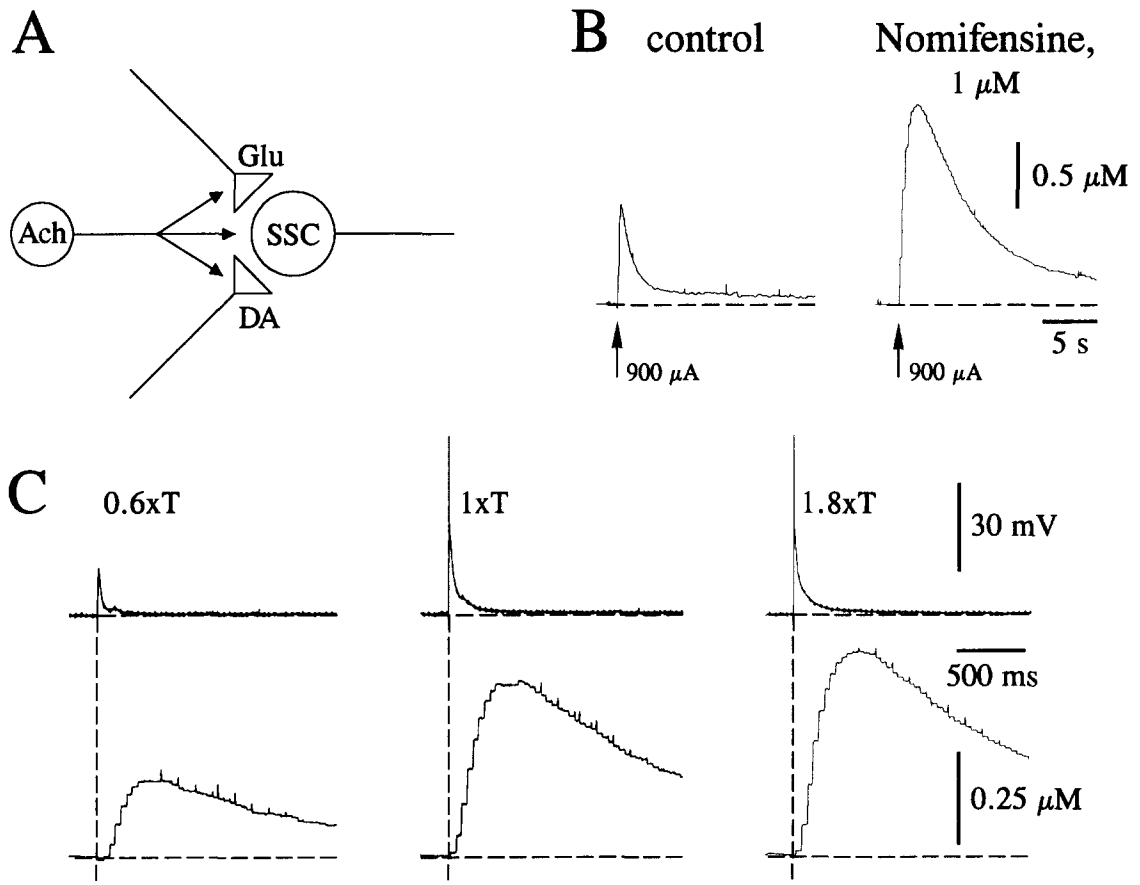


Fig. 1. A: simplified scheme of intrastriatal connections relevant for the experiments described. Abbreviations are: SSC, spiny stellate cell; Ach, cholinergic interneuron; Glu, glutamatergic afferents; DA, dopaminergic afferents. B: stimulus-induced dopamine overflow was enhanced by nomifensine (1 μM). The records were taken from the same slice in the absence (left) and the presence of the dopamine uptake inhibitor (right), respectively. Stimulation is indicated by arrows. C: synaptic potentials of a single striatal neuron (upper traces, RMP: −84 mV) recorded intracellularly and simultaneously to the extracellular dopamine overflow (lower traces). Both responses were evoked by the same stimulus (time point of stimulation is indicated by vertical dashed lines). The traces represent the average of 4 subsequent measurements. $1 \times T$ corresponds to the stimulus intensity necessary to induce a synaptically generated action potential ($1 \times T = 520$ μA at a duration of 50 μs). Recordings were taken in the absence of nomifensine.

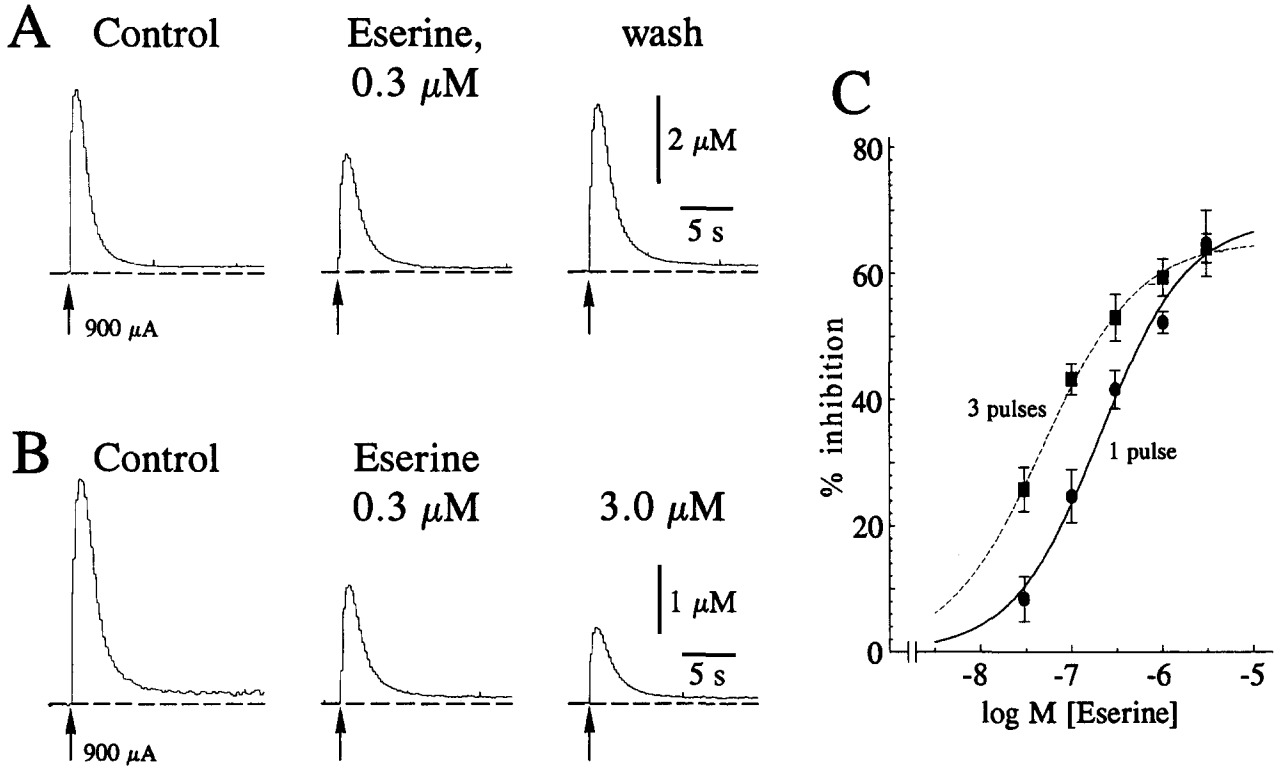


Fig. 2. A: dopamine overflow was evoked by single pulse stimulation at an intensity of 900 μA. Application of eserine (0.3 μM) depressed dopamine overflow by 34%. This effect was reversible upon washout of the compound. B: specimen records of dopamine overflow in the absence and presence of increasing concentrations of eserine. Dopamine efflux was induced by single pulses at maximum intensity. C: concentration-response relation for the effect of eserine on stimulus-evoked dopamine overflow. The percent inhibition was plotted as a function of the logarithm of the eserine concentration. Each curve represents data obtained from at least 3 different experiments. The data points were fitted to a sigmoid function. Note the increased efficiency of eserine when stimulation was performed using three pulses instead of one pulse. Time points of stimulation are indicated by arrows.

dopamine concentration in the extracellular space probably in the close vicinity of synapses. The concentrations given do not correspond to those present in the synaptic cleft. Dopamine overflow was induced by intrastriatal electrical stimulation using a concentric bipolar stainless steel electrode. The stimulation electrode was positioned at a distance of 100–200 μm from the carbon-fiber electrode. The stimulus duration was set to 50 or 100 μs and the interval between two stimulations ranged between 3 and 7 min. Intracellular recordings were made using techniques as previously described [16]. Cells were recorded in an area corresponding to a circle around the carbon-fiber electrode with a diameter of 100–200 μm. In order to reduce the artefacts introduced by the FCV voltage ramp, a second extracellular electrode was positioned in close vicinity to the intracellular recording electrode. The signal recorded with the extracellular electrode was then subtracted from that registered with the intracellular electrode. Recording electrodes were filled with either 3 M KCl (intracellular, 60–80 MΩ) or with 1 M NaCl (extracellular, 10–15 MΩ). Drugs were applied by addition to the bathing solution. All experiments with cholinergic agonists and antagonists were performed in the presence of nomifensine (1 μM). Data are expressed as the mean ± standard error of mean (S.E.M.).

The stimulus-induced oxidation signals were identified to be due to the oxidation of dopamine by the following criteria: (1) under the given experimental conditions, the oxidation potential of dopamine is +0.6 V [1]; (2) the oxidation signal observed in the tissue following electrical stimulation was identical to that seen upon application of dopamine to the bathing solution in the absence of a slice; (3) the stimulus-evoked oxidation signal was enhanced by addition of the selective dopamine uptake inhibitor nomifensine [14] to the bathing solution; (4) the oxidation signal was absent in slices obtained from rats with a lesion of the median forebrain bundle (data not shown).

Fig. 1A depicts a simplified scheme which represents the presumed fiber connections relevant for our experiments. Intrastriatal stimulation in the slice preparation excited dopaminergic afferents which terminate predominantly on spiny stellate cells, the principal neurons in the neostriatum [5]. In the absence of the dopamine uptake inhibitor nomifensine, stimulation using single pulses elicited detectable dopamine overflow in all slices tested

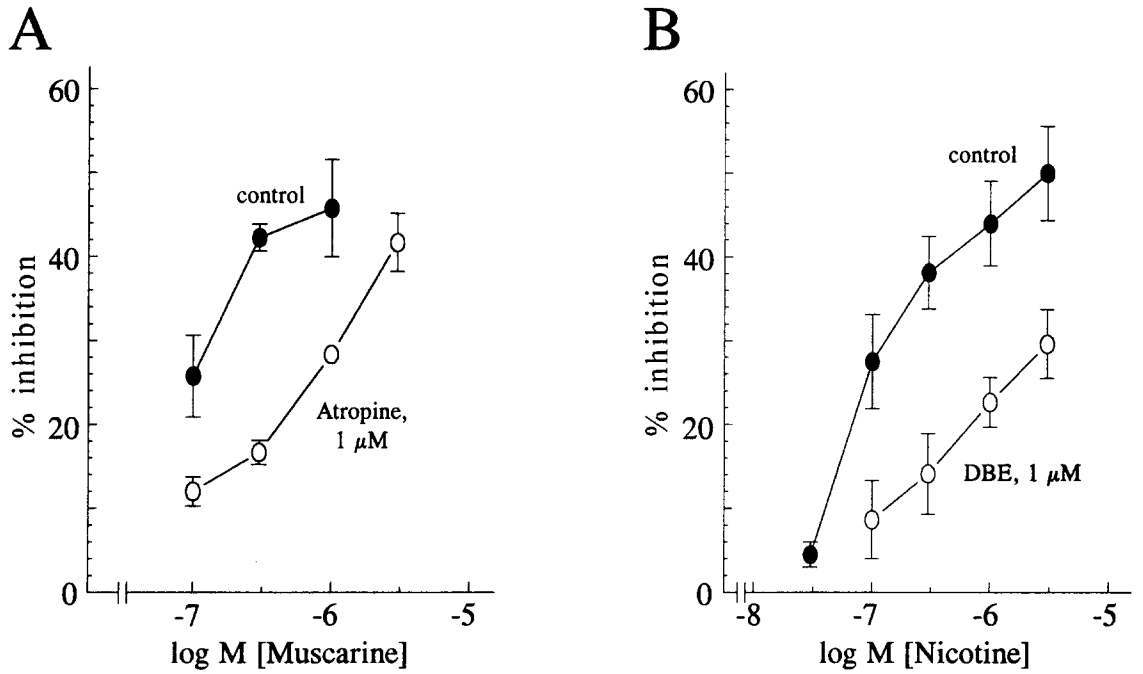


Fig. 3. A: concentration-response relation for the action of muscarine on stimulus-evoked dopamine overflow in the absence (filled circles, $n = 4$) and presence (open circles, $n = 3$) of 1 $\mu$M atropine. B: concentration-response relation for the action of nicotine on stimulus-evoked dopamine overflow in the absence (filled circles, $n = 4$) and presence (open circles, $n = 3$) of 1 $\mu$M dihydro-$\beta$-erythroidine (DBE). In both diagrams the percent inhibition was plotted as a function of the logarithm of the agonist concentration.

($n = 70$). The stimulus-induced oxidation signals reached their maxima within 300–500 ms (Fig. 1C, lower traces) and declined to baseline levels within 4–7 s (Fig. 1B, left trace). The amplitudes of the signals increased with stimulus strength up to a maximum (Fig. 1C, lower traces) which was reached at intensities around 800–900 $\mu$A. Further enhancement of the stimulus strength did not produce any further increase in the signal amplitude. Upon application of nomifensine (1 $\mu$M), the amplitudes of the oxidation signals evoked at maximum intensity were found to be enhanced by a factor of 2 (Fig. 1B). In addition, the duration of the signals increased resulting in an increase in the area under the signal by a factor of 5. Similar results were obtained upon application of the selective dopamine uptake inhibitor GBR 12909 (10 $\mu$M, data not shown).

As revealed by intracellular recordings from single neurons simultaneously to extracellular determination of dopamine overflow, intrastriatal stimulation activated, in addition to dopaminergic fibers, glutamatergic afferents terminating on spiny stellate cells (see Fig. 1A, [7]). The stimulus intensities necessary to induce subthreshold synaptic potentials were identical to those capable of evoking detectable dopamine overflow. Fig. 1C shows the synaptic responses of a striatal cell recorded intracellularly and simultaneously to the extracellular dopamine overflow determined in the close proximity of the neuron. Synaptic potentials and dopamine overflow were evoked by the same stimulus. At threshold intensity $1 \times T$, the stimulus induced a short-latency synaptic potential giving rise to an action potential. At the same time, dopamine overflow was observed reaching its maximum at about 400 ms post-stimulus. Lowering the stimulus intensity to $0.6 \times T$ led to the elicitation of a subthreshold synaptic response and a still clearly detectable dopamine overflow. The minimum stimulus strengths necessary to evoke synaptic responses and dopamine overflow, respectively, were almost identical. Both, synaptic responses as well as dopamine overflow were abolished by the application of TTX (0.6 $\mu$M) or by removal of $Ca^{2+}$ ions from the ACSF, indicating that the dopamine overflow determined under the given experimental conditions was due to release of dopamine from synaptic terminals.

As shown in Fig. 2A, the application of the acetylcholine esterase inhibitor eserine led to a reversible reduction in dopamine overflow suggesting that intrastriatal stimulation activated cholinergic interneurons (see Fig. 1A). The effects of eserine were tested in 24 slices and in 22 of these preparations, eserine inhibited dopamine overflow. In two slices, no effect of eserine was observed. The depressing action of eserine on dopamine overflow was concentration-dependent. The cumulative application of increasing concentrations of eserine resulted in an increasing reduction of the signal amplitudes (Fig. 2B). Fig. 2C depicts concentration response curves obtained from the summated results of different experiments. The diagram reflects two characteristics of the

action of eserine: (1) the maximum effect achieved was a 65% inhibition of dopamine overflow at an eserine concentration of 3 $\mu$M. Further increase in the eserine concentration (10 $\mu$M) did not produce a larger depression of dopamine overflow. (2) The efficiency of eserine was depending on the number of stimulus pulses used to elicit dopamine overflow. Stimulation with one pulse resulted in an apparent $EC_{50}$ of 200 nM as determined from a sigmoid function fitted to the data points. When dopamine overflow was evoked by 3 pulses (10 Hz), the apparent $EC_{50}$ was 65 nM. This enhancement in efficiency of eserine most probably reflects an increase in acetylcholine release with increasing numbers of stimulus pulses.

Application of the non-selective cholinergic agonist carbachol had similar effects to that of eserine. At a concentration of 10 $\mu$M, carbachol depressed stimulus-induced dopamine overflow by 39.5% ($n = 2$) and at a concentration of 30 $\mu$M, the reduction was found to be 41.5% of control ($n = 2$).

In order to determine the type of acetylcholine receptors involved in the depressing action of eserine on dopamine overflow, we investigated the effects of the selective acetylcholine receptor agonists muscarine and nicotine, respectively. As shown by Fig. 3A (filled circles), muscarine depressed dopamine overflow in a concentration-dependent manner. The effects of muscarine were significantly reduced, when the agonist was applied in the presence of the muscarinic antagonist atropine (1 $\mu$M, open circles). The action of the selective cholinergic agonist nicotine is shown in Fig. 3B. This agonist concentration-dependently depressed dopamine overflow (filled circles). In the presence of the selective nicotinic antagonist dihydro-$\beta$-erythroidine (DBE, 1 $\mu$M, open circles), the efficacy of nicotine was significantly depressed.

These experiments demonstrate that in a slice preparation of the rat neostriatum intrastriatal stimulation with single pulses simultaneously releases several neurotransmitters including dopamine, acetylcholine and glutamate. Dopamine overflow determined by FCV depended on the presence of $Ca^{2+}$ ions and sodium-dependent action potentials. The dopamine efflux increased with stimulus intensity and the stimulus strengths necessary to evoke detectable overflow were identical to those necessary to elicit synaptic responses in single neurons. These observations indicate that the dopamine overflow was due to the release of the compound from synaptic terminals belonging to fibers originally arising from brain stem nuclei. The stimulation parameters used to induce dopamine overflow were found to be in a 'normal' range (under the given experimental conditions), since they were identical to those triggering the release of glutamate which is known to mediate sub- and suprathreshold excitatory synaptic responses in neostriatal neurons [6,7]. The stimulus-induced release of acetylcholine, predominantly from intrinsic cholinergic interneurons, was inferred from the effects of the selective acetylcholine esterase inhibitor eserine. As indicated by the increasing efficiency of eserine on dopamine overflow following increases in number of stimulus pulses, the release of acetylcholine seems to depend on stimulus strength. The depressant effect of eserine on stimulus-evoked dopamine overflow suggests that endogenous acetylcholine inhibits the release of dopamine by acting on dopaminergic nerve terminals (see Fig. 1A). The present data show that both, muscarinic as well as nicotinic receptors are involved in this action. In the rat striatum, muscarinic M1-and non-M1-receptors have been shown to be significantly downregulated following lesions of the median forebrain bundle suggesting their location on dopaminergic terminals [2]. The activation of these presynaptic receptors obviously inhibits dopamine release similar to the muscarinic receptor-mediated depression of acetylcholine release [9,17] and glutamate release [13,16] (see Fig. 1A). There is evidence for the existence of presynaptic nicotinic receptors on striatal dopaminergic nerve terminals [4,8,10,11]. However, in contrast to the data presented, direct activation of these receptors by nicotine or acetylcholine leads to an increase in dopamine release [4,8,10,11]. The reason for this discrepancy is not clear. In the slice preparation, nicotine might stimulate cholinergic interneurons, thereby enhancing acetylcholine release which in turn depresses dopamine release via muscarinic receptors. Another possibility is a strong nicotine-induced depolarization of synaptic terminals which would reduce transmitter release. Further experiments are necessary to exclude a possible indirect inhibitory effect of nicotine on dopamine overflow in the neostriatal slice.

The authors thank G. ten Bruggencate, R. Morgenstern and C.D. Earl for helpful discussions and for reading the manuscript. Some of the results in this paper are part of a doctoral thesis of M. Kudernatsch which is to be submitted to the Medical Faculty of the Ludwig-Maximilians-Universität of Munich. This work was supported by the BMFT (01 ZZ 9101/3 in cooperation with the Institute of Pharmacology and Toxicology, Humboldt-University, Berlin).

[1] Bull, D.R., Palij, P., Sheehan, M.J., Millar, J., Stamford, J.A., Kruk, Z.L. and Humphrey, P.P., Application of fast cyclic voltammetry to measurement of electrically evoked dopamine overflow from brain slices in vitro, J. Neurosci. Methods, 32 (1990) 37–44.

[2] Dawson, T.M., Dawson, V.L., Gage, F.H., Fisher, L.J., Hunt, A.A. and Wamsley, J.K., Downregulation of muscarinic receptors in the rat caudate-putamen after lesioning of the ipsilateral dopamine pathway with 6-hydroxydopamine (6-OHDA): normalization by fetal mesencephalic transplants, Brain Res., 540 (1991) 145–152.

[3] Gerfen, C.R., Herkenham, M. and Thibault, J., The neostriatal mosaic. II. Patch-and matrix-directed mesostriatal dopaminergic and non-dopaminergic systems, J. Neurosci., 7 (1987) 3915–3934.

[4] Giorguieff-Chesselet, M.F., Kemel, M.L., Wandscheer, D. and Glowinski, J., Regulation of dopamine release by presynaptic nicotinic receptors in rat striatal slices: effect of nicotine at low concentration, Life Sci., 25 (1979) 1257–1262.

[5] Graybiel, A.M. and Ragsdale Jr., C.W., Biochemical anatomy of the striatum. In P.C. Emson (Ed.), Chemical neuroanatomy, Raven Press, New York, 1983, pp. 427–504.

[6] Jiang, Z.-G. and North, R.A., Membrane properties and synaptic responses of rat striatal neurones in vitro, J. Physiol., 443 (1991) 533–553.

[7] Kawaguchi, Y., Wilson, C.J. and Emson, P.C., Intracellular recording of identified neostriatal patch and matrix spiny cells in a slice preparation preserving cortical inputs, J. Neurophysiol., 62 (1989) 1052–1068.

[8] Kemel, M.-L., Desban, M., Glowinski, J. and Gauchy, C., Distinct presynaptic control of dopamine release in striosomal and matrix areas of the cat caudate nucleus, Proc. Natl. Acad. Sci. USA, 86 (1989) 9006–9010

[9] Misgeld, U., Weiler, M.H. and Cheong, D.K., Atropine enhances nicotinic cholinergic EPSPs in rat neostriatal slices, Brain Res., 253 (1982) 317–320.

[10] Misu, Y., Goshima, Y., Nakamura, S. and Kubo, T., Nicotine releases stereoselectively and $Ca^{2+}$-dependently endogenous 3,4-dihydroxyphenylalanine from rat striatal slices, Brain Res., 520 (1990) 334–337.

[11] Rapier, C., Lunt, G.G. and Wonnacott, S., Stereoselective nicotine-induced release of dopamine from striatal synaptosomes: concentration dependence and repetitive stimulation, J. Neurochem., 50 (1988) 1123–1130.

[12] Semba, K., Fibiger, H.C. and Vincent, S.R., Neurotransmitters in the mammalian striatum: neuronal circuits and heterogeneity, Can. J. Neurol. Sci., 14 (1987) 386–394.

[13] Sheridan, R.D. and Sutor, B., Presynaptic $M_1$ muscarinic cholinoceptors mediate inhibition of excitatory synaptic transmission in the hippocampus in vitro, Neurosci. Lett., 108 (1990) 273–278.

[14] Stamford, J.A., Kruk, Z.L., Palij, P. and Millar, J., Diffusion and uptake of dopamine in rat caudate and nucleus accumbens compared using fast cyclic voltammetry, Brain Res., 448 (1988) 381–385.

[15] Stoof, J.C., Drukarch, B., de Boer, P., Westerink, B.H.C. and Groenewegen, H.J., Regulation of the activity of striatal cholinergic neurons by dopamine, Neuroscience, 47 (1992) 755–770.

[16] Sutor, B. and Hablitz, J.J., Cholinergic modulation of epileptiform activity in the developing rat neocortex, Dev. Brain Res., 46 (1989) 155–160.

[17] Weiler, M.H., Muscarinic modulation of endogenous acetylcholine release in rat neostriatal slices, J. Pharmacol. Exp. Ther., 250 (1989) 617–623.