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Short communication

Ventral tegmental injection of nicotine induces locomotor activity and L-DOPA release from nucleus accumbens

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Abstract

Effects of nicotine administered systemically or locally on locomotor activity and L-3,4-dihydroxyphenylalanine (L-DOPA) release were studied using microdialysis in the nucleus accumbens of freely moving rats. The basal L-DOPA release was Ca^{2+}-dependent and tetrodotoxin-sensitive. Systemic nicotine (1 mg/kg s.c.) increased locomotor activity and L-DOPA release preferentially in the nucleus accumbens as compared with the striatum. Injection of nicotine (30 μg) into the ventral tegmental area increased locomotor activity and L-DOPA release from the nucleus accumbens. These increases were antagonized by prior injection of mecamylamine into the ventral tegmental area. Nicotine induces locomotor activity and L-DOPA release from the nucleus accumbens via nicotinic receptors in the ventral tegmental area. The release may be relevant to behavioral actions of nicotine.

Keywords: Nicotine; L-DOPA (3,4-dihydroxyphenylalanine) release; Locomotor activity, rat; Nucleus accumbens; Ventral tegmental area; Microdialysis

1. Introduction

We have proposed that L-3,4-dihydroxyphenylalanine (L-DOPA) is a neurotransmitter in the central nervous system (CNS) (Misu and Goshima, 1993; Misu et al., 1995). Endogenous L-DOPA is released in a transmitter-like manner in vivo and in vitro from some areas of the brain (Nakamura et al., 1992a,b; Misu and Goshima, 1993; Yue et al., 1994b; Misu et al., 1995). L-DOPA produces in vivo postsynaptic responses (Yue et al., 1994b) and in vitro presynaptic responses (Misu and Goshima, 1993). These effects appear to be mediated via a specific recognition site for L-DOPA itself since these effects are stereoselective in nature and are antagonized by L-3,4-dihydroxyphenylalanine methyl ester in a competitive fashion (Goshima et al., 1991). Furthermore, these effects of L-DOPA are not mimicked by dopamine (Misu and Goshima, 1993; Misu et al., 1995). In the nucleus tractus solitarii, L-DOPA probably acts as a neurotransmitter of the primary baroreceptor afferents and is tonically involved in vasodepressor control (Yue et al., 1994b). In the striatum, L-DOPA appears to be an endogenous potentiator for presynaptic β-adrenoceptors, facilitating dopamine release (Misu and Goshima, 1993) and for postsynaptic dopamine D_2 receptors (Yue et al., 1994a; Misu et al., 1995).

Nicotine changes various types of behavioral parameters, including locomotor activity (Imperato et al., 1986; Clarke et al., 1988; Di Chiara and Imperato, 1988; Nakamura et al., 1993; Vezina et al., 1994). The behavioral action of nicotine has been attributed in part to its ability to induce neurotransmitter and/or neuromodulator release via nicotinic receptors on nerve terminals and/or cell bodies in the CNS. Nicotine induces Ca^{2+}-dependent and tetrodotoxin-sensitive L-DOPA release in rat striatum, in vivo, via tonically functioning nicotinic receptors (Nakamura et al., 1992b). However, several lines of evidence have suggested an important role of the mesolimbic dopaminergic system in the action of nicotine. In the rat, in vivo, nicotine preferentially stimulates dopamine release from the nucleus accumbens when compared with the striatum (Imperato et al., 1986). Locomotor activity induced by systemic nicotine is abolished by prior bilateral injection of 6-hydroxydopamine into the nucleus accumbens (Clarke et al., 1988). Furthermore, pretreatment with either dopamine D_1 or D_2 antagonist blocks nicotine-induced locomotor activity (O'Neill et al., 1991). Recent

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studies indicate that nicotinic receptors located in the ventral tegmental area may be of greater importance than those located in the nucleus accumbens for the stimulatory action of systemic nicotine (Reavill and Stolerman, 1990; Nisell et al., 1994). In this study, we have attempted to clarify whether systemic nicotine or nicotine locally injected into the ventral tegmental area similarly releases transmitter-like L-DOPA from the nucleus accumbens of freely moving rats and compared it with the effect on the neurotransmitter dopamine.

2. Materials and methods

2.1. General procedures and locomotor activity record

Male Sprague-Dawley rats weighing 250–350 g were anesthetized with sodium pentobarbital (50 mg/kg i.p.). Animals were placed in a stereotaxic apparatus (Narishige, SR-6). An intracerebral guide- and dummy-cannula (CMA/10, BAS) for microdialysis was stereotaxically implanted in the left nucleus accumbens (A 1.7, L 1.5 and V 8.0 from the bregma) or striatum (A 1.0, L 3.0 and V 4.0), and/or another cannula (Unique Medical) for local injection was implanted in the ipsilateral ventral tegmental area (P 5.1, L 0.9 and V 8.0) according to a stereotaxic atlas (Paxinos and Watson, 1986). After recovery from surgery (at least 3 days), rats were individually placed in a photo cage (30 cm in both height and diameter) on autoactivity detectors (Colombus Instruments) and habituated to their environment at least for 2 h. Locomotor activity was measured continuously between 09:00 and 18:00 h each day, with number of counts/30 min recorded.

2.2. Microdialysis and measurement of L-DOPA and dopamine

On the day before experiments, the dummy cannula was replaced by a dialysis probe (membrane length 2 mm; CMA/10). Ringer solution was perfused through these probes at a rate of 2 μl/min and perfusates were collected at 4°C every 30 min. L-DOPA and dopamine in each 59-μl sample were measured by high-performance liquid chromatography with electrochemical detection. Chromatographic conditions are described elsewhere (Yue et al., 1994b). The mean absolute value for L-DOPA and dopamine release in three samples, 3 h after the start of perfusion, is given as a percentage of control in each group. For characterization of the basal release, either Ca^{2+} was removed from the perfusate and replaced with 12.5 mM Mg^{2+} or 1 μM tetrodotoxin (Sigma) was perfused, starting 4 h after the start of perfusion and continuing for the duration of the experiments.

2.3. Drug treatment

Drugs were dissolved in phosphate-buffered saline (pH 7.4). (±)-Nicotine tartrate (Wako) solution was neutralized to pH 7.4 with NaOH. Saline vehicle and nicotine were injected systemically (1 mg/kg s.c.) or locally (30 μg) into the ventral tegmental area. For local injection, rats received a volume of 1 μl over a 1-min period. In some cases, 100 μg mecamylamine hydrochloride (RBI) was injected into the ventral tegmental area 5 min before local nicotine injection. At the end of experiments, the injection site was marked by injecting 1 μl of Evans blue dye solution for later histological identification (Yue et al., 1994b).

2.4. Data analysis

The statistical analysis of the behavioral and biochemical data was performed by analysis of variance (ANOVA) for repeated measures with treatment and time as the independent factors analyzed. The statistical significance was assessed using Dunn's multiple comparison test.

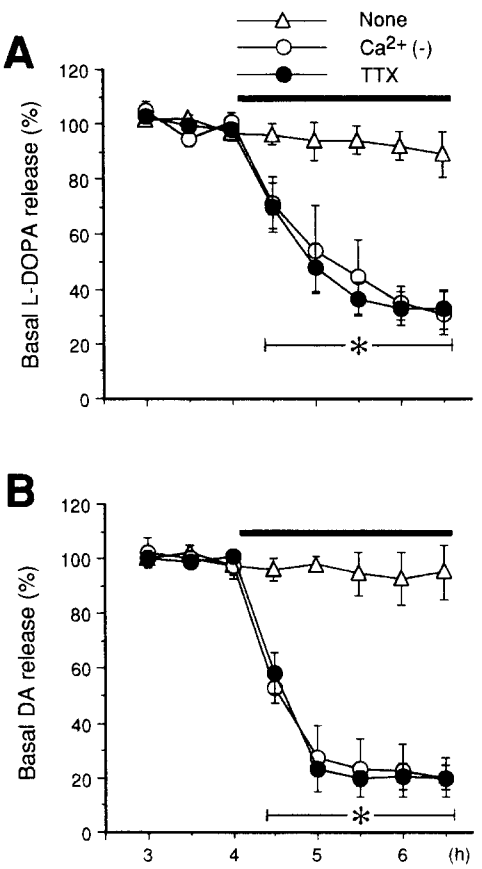

Fig. 1. Time course of Ca^{2+} deprivation- and tetrodotoxin (TTX)-induced decreases in basal L-DOPA and dopamine (DA) release during microdialysis of the left nucleus accumbens in freely moving rats. Ringer solution was perfused at a rate of 2 μl/min and perfusates were collected every 30 min. Ca^{2+}-free or TTX perfusion (1 μM) was done at the time indicated by horizontal bars, 4 h after the start of perfusion. Ordinates show basal L-DOPA (A) and DA (B) release as percentage of control, the mean of three of the initial stable absolute values, 3–4 h after the start of perfusion. Each value represents the mean ± S.E.M. * $P < 0.05$ vs. none (Dunn's multiple comparison test). Control (fmol) for L-DOPA and DA was 78 ± 25 and 49 ± 7 for none ($n = 3$), 39 ± 6 and 92 ± 22 for Ca^{2+}-free ($n = 3$) and 48 ± 20 and 80 ± 12 for TTX ($n = 3$), respectively.

3. Results

3.1. Basal release of L-DOPA and dopamine

During microdialysis of the left nucleus accumbens of freely moving rats, basal L-DOPA and dopamine release was consistently detectable and became stable 3 h after the start of perfusion. The respective mean values (fmol) for L-DOPA and dopamine were 68 ± 9 and 73 ± 8 in the nucleus accumbens ($n = 31$). Ca^{2+} removal reduced L-DOPA and dopamine release, compared to control, in the nucleus accumbens (Fig. 1). Tetrodotoxin (1 μM) perfusion reduced the release of L-DOPA and dopamine. These results for basal L-DOPA release in the nucleus accumbens were similar to those in the striatum (Nakamura et al., 1992a). The striatal mean values (fmol) for L-DOPA and dopamine were 66 ± 15 and 93 ± 23 ($n = 6$) in the present experiments.

3.2. Effects of systemic injection of nicotine

Nicotine (1 mg/kg s.c.) caused behavioral changes, such as rearing and sniffing, and increased the total loco-

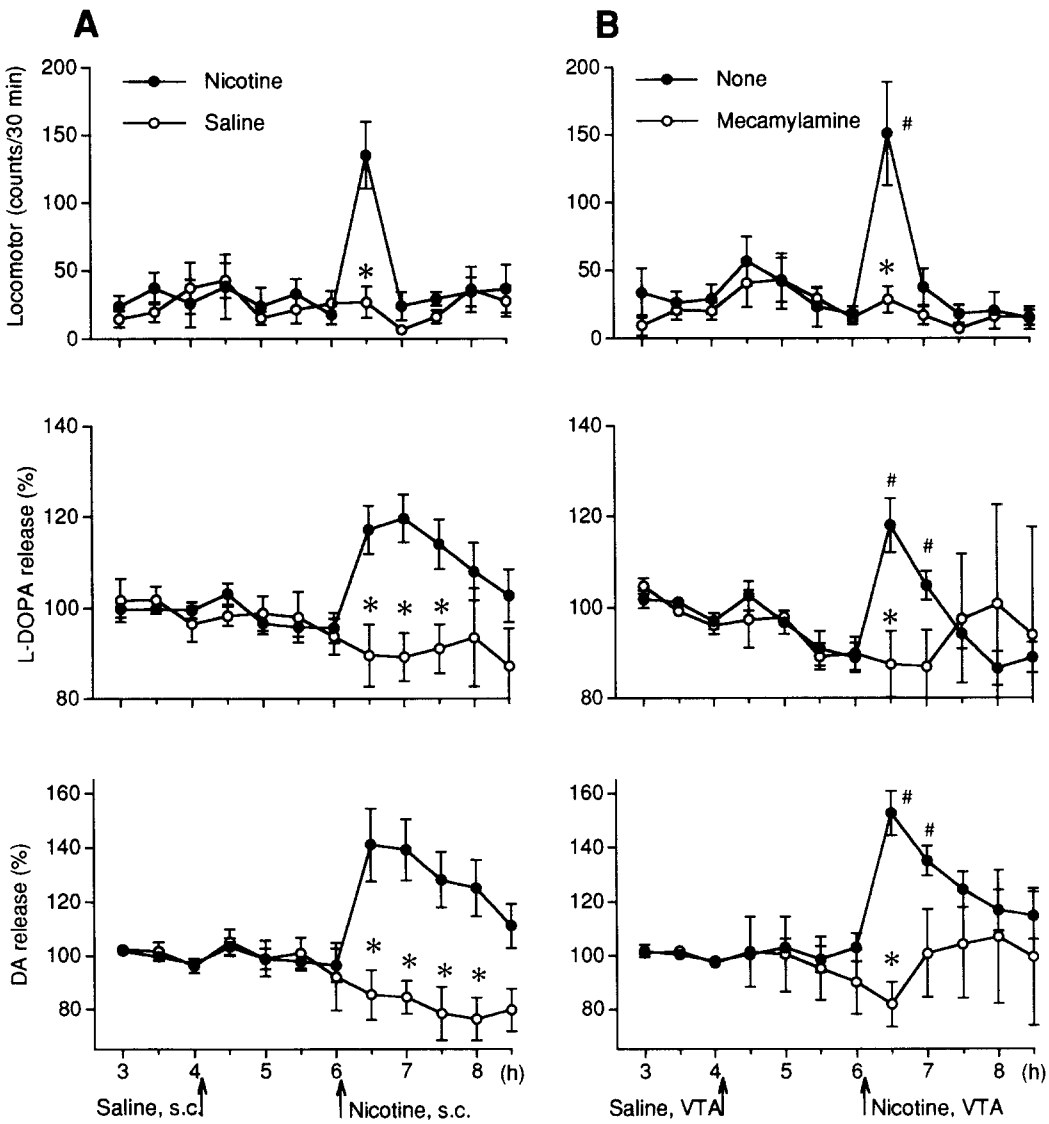

Fig. 2. Effects of systemic nicotine (A) or nicotine injected into the ventral tegmental area (VTA) (B) on locomotor activity and the release of L-DOPA and dopamine (DA) during microdialysis of the nucleus accumbens in freely moving rats. Ringer solution was perfused at a rate of 2 μl/min and perfusates were collected every 30 min. Ordinates show the counts of locomotor activity and L-DOPA and DA release as a percentage of control, the mean of the three initial stable absolute values, 3–4 h after the start of perfusion. A: nicotine 1 mg/kg s.c. injection. For control of systemic injection, saline vehicle was injected twice at times indicated by arrows, 4 and 6 h after the start of perfusion. For systemic nicotine injection, saline vehicle and nicotine were injected at 4 and 6 h after the start of perfusion, respectively. B: nicotine 30 μg locally injected into the VTA. Saline vehicle and nicotine were injected at times indicated by arrows, 4 and 6 h after the start of perfusion, respectively. In some rats, mecamylamine (100 μg) was injected into the VTA 5 min before the injection of nicotine. Each value represents the mean ± S.E.M. * $P < 0.05$ vs. the value with nicotine injection. # $P < 0.05$ vs. none (Dunn's multiple comparison test). Control (fmol) for L-DOPA and DA was 76 ± 25 and 75 ± 14 in A ($n = 8$), and was 106 ± 13 and 62 ± 9 for none ($n = 7$) and 37 ± 5 and 83 ± 28 for mecamylamine ($n = 7$) in B, respectively.

motor activity for 30 min, within 30 min of administration, the count returning to the basal levels 60 min after the injection (Fig. 2). Nicotine released L-DOPA and dopamine in the nucleus accumbens. The peak for L-DOPA and dopamine release was seen 60 and 30 min, respectively, after nicotine injection. L-DOPA and dopamine release peaked at about the same time as locomotor activity, but remained elevated for the rest of the recording period. We confirmed previous findings that peripherally applied nicotine-induced dopamine release was greater in the nucleus accumbens than in the striatum (Clarke et al., 1988; Imperato et al., 1986). This was also the case for L-DOPA. The % peak increases in nicotine-induced release of L-DOPA and dopamine were 24 ± 3 and $45 \pm 5\%$ in the nucleus accumbens ($n = 8$) and were 7 ± 4 and $27 \pm 3\%$ in the striatum ($n = 6$) ($P < 0.05$). Saline (s.c.) produced no effect.

3.3. Effects of local injection of nicotine into the ventral tegmental area

Nicotine (30 μg) injected into the left ventral tegmental area increased locomotor activity over a time course roughly similar to that of s.c. nicotine (Fig. 2). Again, the injection evoked increases in the release of L-DOPA and dopamine from the nucleus accumbens. While systemic nicotine produced a somewhat sustained increase in the release of L-DOPA and dopamine, intra VTA nicotine produced the largest response 30 min after administration, whereafter levels appeared to return to the basal control levels (Fig. 2B). Locomotor activity peaked within 30 min of administration and returned to baseline. L-DOPA and dopamine release peaked at about the same time, but remained elevated for another 30-min period. The injection of mecamylamine (100 μg) into the ventral tegmental area completely blocked nicotine-induced locomotor activity and release of L-DOPA and dopamine. Saline injected into the ventral tegmental area produced no effect.

4. Discussion

The present experiments showed that the basal release of L-DOPA was markedly inhibited by Ca^{2+} removal or tetrodotoxin addition, thereby indicating that L-DOPA is released via a spontaneously occurring impulse-dependent neuronal mechanism. Systemically applied nicotine-induced locomotor activity was associated with preferential increases in L-DOPA and dopamine release from the nucleus accumbens in vivo, compared to those in the striatum. These effects were mimicked by local injection of nicotine into the ventral tegmental area, and the effects of the injection were almost completely blocked by mecamylamine applied locally into the ventral tegmental area. These findings provide the first evidence that nicotine induces release of transmitter-like L-DOPA in the nucleus accumbens via nicotinic acetylcholine receptors located in the ventral tegmental area. This L-DOPA release had characteristics similar to those of transmitter dopamine release. This is in accordance with the findings that of the brain areas studied, infusion of nicotine into the ventral tegmental area only increases locomotor activity (Reavill and Stolerman, 1990), and that systemic nicotine-induced locomotor activity and dopamine release from the nucleus accumbens are antagonized by local infusion of mecamylamine into the ventral tegmental area but not into the nucleus accumbens (Nisell et al., 1994). Our finding that nicotine preferentially stimulates L-DOPA release from the nucleus accumbens is consistent with the idea that the mesolimbic system is of primary importance in the behavioral actions of nicotine (Imperato et al., 1986; Clarke et al., 1988; Di Chiara and Imperato, 1988). In addition to nicotine, other drugs self-administered by humans or animals, such as cocaine and amphetamine, preferentially release dopamine from the nucleus accumbens (Di Chiara and Imperato, 1988). It will be interesting to know whether or not these drugs induce the release of L-DOPA, as well as dopamine, from the nucleus accumbens.

Endogenously released L-DOPA as well as dopamine could be involved indirectly and/or directly in the nicotine-induced locomotor activity of rats. This view is supported by our previous findings that nicotine-induced locomotor activity is partially but significantly reduced by systemic pretreatment with a selective dose (3 mg/kg) of α-methyl-*p*-tyrosine, a tyrosine hydroxylase inhibitor, which decreases L-DOPA release without modifying dopamine release from in vivo striatum (Nakamura et al., 1993).

We have previously provided evidence that L-DOPA potentiates the activity of postsynaptic dopamine D_2 receptors (Misu et al., 1995). An exogenously applied non-effective dose of L-DOPA (i.p.) itself stereoselectively potentiates the locomotor activity induced by quinpirole, a selective dopamine D_2 receptor agonist, under inhibition of central aromatic L-amino acid decarboxylase (AADC) in freely moving rats (Misu et al., 1995). Furthermore, the levels of locomotor activity induced by quinpirole appear to depend on those of endogenously released L-DOPA monitored in the striatum: the action of quinpirole is augmented, when, by inhibition of AADC, the level of extracellular L-DOPA is increased without modification of dopamine release, and is diminished, when, by a selective dose of α-methyl-*p*-tyrosine, the level is decreased without modification of dopamine release (Yue et al., 1994a). It is thus likely that nicotine-induced L-DOPA release and dopamine synergistically activated postsynaptic dopamine receptors to facilitate the locomotor activity. Consistent with this, several dopamine antagonists block the locomotor activity induced by systemic nicotine (O'Neill et al., 1991). In addition, L-DOPA may act presynaptically to augment the release of dopamine by potentiating facilitatory presynaptic β-adrenoceptors (Misu and Goshima,

1993). It is also possible that a postsynaptic action of L-DOPA may have relevance to the nicotinic actions, since L-DOPA probably acts as an excitatory neurotransmitter of the primary baroreceptor afferents in depressor sites of the rat nucleus tractus solitarii (Yue et al., 1994b). Interestingly, it has recently been suggested that nicotine might produce some of its locomotor effects via non-dopaminergic systems in the brain (Vezina et al., 1994).

In conclusion, nicotine induces the release of transmitter-like L-DOPA from the nucleus accumbens terminal area of the mesolimbic system via nicotinic acetylcholine receptors located in the ventral tegmental area, in a manner similar to that for neurotransmitter dopamine. This release may be partially relevant to nicotine-induced locomotor activity in the rat.

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