Endogenously Released DOPA Is Probably Relevant to Nicotine-Induced Increases in Locomotor Activities of Rats

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ABSTRACT—The relevance of endogenously released DOPA to the (±)-nicotine-induced increase in locomotor activities was explored in rats. This increase was dose (0.1–1.0 mg/kg, s.c.)-dependent, stereoselective and mecamylamine (1.0 mg/kg, s.c.)-sensitive, but was not antagonized by L-dopa methyl ester (200 μg, i.v.t.), a competitive L-dopa antagonist. Then, by microdialysis, low doses of α-methyl-p-tyrosine (α-MPT, i.p.) at 1–10 mg/kg, were tested to find a dose that selectively inhibits the basal DOPA release in the striata: the release was consistently inhibited by 3 mg/kg without any tendency to inhibit the basal dopamine release. Pretreatment with this dose did inhibit the nicotine-induced increases in locomotor activities. This suggests that endogenously released DOPA is in part relevant to the nicotine-induced behavior in rats.

Keywords: DOPA (endogenous neuroactive substance), Nicotine (increase in locomotor activity), α-Methyl-p-tyrosine

1-L-3,4-Dihydroxyphenylalanine (L-dopa) is believed to exert its actions via conversion to dopamine (DA) by L-aromatic amino acid decarboxylase (AADC) (1). In contrast to this, we have proposed that DOPA itself is a neurotransmitter and/or -modulator in the central nervous system. Depolarizing stimuli released endogenous transmitter-like DOPA from rat striatal slices (2, 3). A transmitter-like basal DOPA release was seen under physiological conditions in the striata of conscious rats (4). On the other hand, in vitro rat brain slices (5–7), exogenous nanomolar L-dopa itself stereoselectively facilitated the evoked release of DA and noradrenaline (NA) via propranolol-sensitive presynaptic β-adrenoceptors even under complete inhibition of AADC. However, this facilitation was antagonized competitively by L-dopa methyl ester, whereas it was noncompetitively antagonized by propranolol (7). Then, picomolar L-dopa itself stereoselectively potentiated the iso-propranolol-induced facilitation of the NA release (8). This potentiation was selectively antagonized by L-dopa methyl ester, whereas propranolol antagonized both the facilitation by isopropranolol alone and its potentiation by L-dopa. Thus, there exists a recognition site for DOPA itself, which differs from presynaptic β-adrenoceptors. Furthermore, L-dopa methyl ester antagonized in vivo postsynaptic depressor responses of rats to L-dopa microinjected into the nucleus tractus solitarii (9).

Nicotine releases various neurotransmitters or -modulators in the central and peripheral nervous systems. Peripheral application of nicotine increases locomotor activities of rats (10), and this increase seems to be linked to the DA release (11). On the other hand, local perfusion of nicotine into the rat striatum released transmitter-like DOPA via tonically functioning nicotinic acetylcholine receptors (12). Thus, we have attempted to clarify whether i.v.t.-application of L-dopa methyl ester antagonizes nicotine-induced increases in locomotor activities and then whether endogenously released DOPA is relevant to this behavior in rats.

Male Sprague-Dawley rats (300–400 g) were given food pellets and water ad libitum and kept on a regular day and night schedule (lights on at 5:00 hr and off at 19:00 hr). Rats were individually placed in a cage (25 cm in height and 30 cm in diameter) on an autoactivity detector (AUTOMEX, Colombus Instruments, Columbus, OH, USA) and habituated to their environment for 2 to 3 hr. Then, the counts of locomotor activities/10 min were continuously recorded and 0.1–3.0 mg/kg (±)-nicotine tartrate (Wako, Osaka) or (+)-nicotine (free base, Smoking Research Foundation, Japan) was s.c.-injected. Pretreatment with 1 mg/kg mecamylamine (Sigma, St.
Louis, MO, USA), s.c. or 3 mg/kg α-methyl-p-tyrosine (α-MPT, Nacalai, Kyoto), i.p. was initiated 20 or 90 min before nicotine injection. Saline was used as the control. The volume injected s.c. or i.p. was 1 or 5 ml/kg. At 7 days before the experiments, under anesthesia with 50 mg/kg pentobarbital Na, i.p., some rats received stereotaxic implantation (A = −1.0, L 1.5 and V 4.0 mm from the bregma) of a stainless-steel guide cannula (length of 10 mm, external diameter of 0.7 mm) into the left lateral ventricle. L-Dopa methyl ester (Research Biochemicals, Natick, MA, USA; 200 µg) was i.v.t.-injected in 10 µl over a 10-min period with a microinfusion pump (A-99, Razel, Stamford, CT, USA) immediately before nicotine injection. Each drug was dissolved in saline.

Under anesthesia, a guide- and dummy-cannula (CMA12, Carnegie Medicin, Loughborough, England) was stereotaxically implanted into the striatum (A 1.0, L 3.0 and V 4.0 mm from the bregma) according to a stereotaxic atlas (4). The dummy cannula was replaced by a probe on the day before the experiments. The length and external diameter of the dialysis membrane was 3 and 0.5 mm. Ringer solution was perfused through the probe at the rate of 2 µl/min. The composition of the medium without any kind of buffer was as follows: 147 mM Na⁺, 2.3 mM Ca²⁺, 4 mM K⁺ and 155.6 mM Cl⁻. Dialysates were successively collected every 20 min from 3 to 7 hr after the start of perfusion. α-MPT at 1–10 mg/kg or saline was injected i.p. 4 hr after the start of perfusion. DOPA and DA in the perfusates were measured by a high performance liquid chromatography (HPLC) (114 M, Beckman, San Ramon, CA, USA) with an electrochemical detector (VMD-101A, Yanaco, Kyoto). A 40-µl sample of perfusate was directly injected into the HPLC column without any treatment. Chromatographic data are described elsewhere (4). The limit of sensitivity, defined as a signal-to-noise ratio <2, was 5 fmol for DOPA and DA.

The results were analyzed by the two-tailed Student's t-test.

(±)-Nicotine at 0.1–1.0 mg/kg dose-dependently increased locomotor activities 10 and 20 min after its s.c.-injection, and a ceiling phenomenon was seen at 3 mg/kg (Fig. 1A). Stereotyped behavior such as rearing and sniffing was seen at 0.4–1.0 mg/kg. This increase was stereoselective and mecamylamine-sensitive, since 0.4 mg/kg (±)-nicotine produced no increase and 1 mg/kg mecamylamine completely antagonized the (±)-nicotine (0.4 mg/kg)-induced increase (Fig. 1B). These are consistent with previous findings (10) and are also consistent with the characteristics of the release of endogenous DOPA and DA evoked by nicotine locally perfused in the striata of conscious rats (12). This increase in locomotor activities has been suggested to be linked to the central dopaminergic function (11). However, we have proposed that endogenously released DOPA is relevant to this action of nicotine, since DOPA is released in a transmitter-like manner in vitro (2, 3) and in vivo (4, 12) striata, whereas exogenously applied L-dopa itself facilitates the DA and NA release via presynaptic β-adrenoceptors in brain slices (5–7). This facilitation is antagonized by L-dopa methyl ester, a competitive L-dopa antagonist (7).

At first, we tried to clarify whether this ester, i.v.t.-infused, antagonizes the nicotine-induced increases in locomotor activities. L-Dopa methyl ester at 200 µg alone produced no effect at least for 20 min after infusion.
However, this pretreatment produced no antagonism but even caused apparent facilitation of the action of 0.4 mg/kg (±)-nicotine: the counts for the 1st and 2nd 10-min periods were 102.3 ± 20.6 and 23.3 ± 7.3 with nicotine alone (n=6) and 133.8±23.3 and 79.6±18.1 (n=6, P<0.05, compared to nicotine alone) with the ester in combination with nicotine, respectively. This may be because this ester is readily hydrolyzed to DOPA (1, 13). This antagonist is competitively effective against L-dopa when the ester is continuously supplied by superfusion in brain slices (7, 8) and is effective within a few min when one dose is microinjected into the nucleus tractus solitarii (9). A more stable competitive antagonist has to be found to obtain clear evidence.

Next, we attempted to find a selective dose of α-MPT, a tyrosine hydroxylase inhibitor, that inhibits the basal release of endogenous DOPA without inhibiting that of DA in the striata of conscious rats. The striatum is the most important target region for l-dopa. The basal DOPA release in dialysates was consistently detectable and its identification is described elsewhere (4). The basal DOPA and DA release became constant in 4 successive samples 3 hr after the start of perfusion. The mean absolute value was 59.3±6.4 fmol for DOPA and 63.3±5.4 fmol for DA (n=16). The basal release of both DOPA and DA from the striata was markedly reduced by 200 mg/kg α-MPT (4). However, far lower doses of 3 and 10 mg/kg gradually and dose-dependently inhibited the basal DOPA release, and this inhibition reached a plateau 80 min after i.p.-injection (Fig. 2); the plateau level was approximately 60 to 50% of the control. On the other hand, these doses produced no inhibition of the basal DA release. We selected 3 mg/kg as the selective dose, since 10 mg/kg tended to inhibit the basal DA release.

Pretreatment with this dose of α-MPT 90 min prior to nicotine injection did inhibit the (±)-nicotine (0.4 mg/kg)-induced increases in locomotor activities by approximately 40% to 60% of those by nicotine alone 10 and 20 min after the injection (Fig. 1B). This is a new finding to support the idea that endogenously released DOPA is at least partially relevant to the nicotine-induced behavior in rats. α-MPT alone at 3 mg/kg produced no effect.

Our present thinking is that DOPA is an endogenous neuromodulator in the striatum. DOPA is an endogenous potentiator for presynaptic β-adrenergic receptors to facilitate the DA release (7, 8). Furthermore, DOPA is probably a potentiator for postsynaptic D2-receptors (14), as is exogenous L-dopa for presynaptic β-adrenoceptors (8), since under inhibition of central AADC, a systemic noneffective dose of l-dopa (30 mg/kg, i.p.) potentiated increases in locomotor activities of rats induced by quinpirole (0.1 and 1 mg/kg, s.c.), a selective D2-agonist, before the elevation of the simultaneously monitored basal DA release, an indicator of conversion from DOPA, was seen during striatal microdialysis.

There exist DOPA-immunoreactive neurons and nerve fibers, which display no DA immunoreactivity, in various central regions of rats and cats (15). These findings support our proposal that DOPA is an endogenous neurotransmitter and/or -modulator in the central nervous system.

In conclusion, endogenously released DOPA may be partially relevant to nicotine-induced increases in locomotor activities of rats and to the central actions of nicotine.
Acknowledgments

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