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DOPA cyclohexyl ester, a DOPA antagonist, blocks the depressor responses elicited by microinjections of nicotine into the nucleus tractus solitarii of rats

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ABSTRACT

Nicotinic cholinergic receptors play a role in cardiovascular regulation in the lower brain stem. Herein, we present evidence that L-3,4-dihydroxyphenylalanine (DOPA), a putative neurotransmitter in the central nervous system, is involved in the depressor response to microinjection of nicotine into the nucleus tractus solitarii (NTS). Microinjection of nicotine into the medial area of the NTS led to decreases in arterial blood pressure and heart rate in anesthetized rats. Mecamylamine, a nicotinic receptor antagonist, microinjected into NTS, blocked the depressor and bradycardic responses to nicotine. Nicotine-induced depressor and bradycardic responses were blocked by DOPA cyclohexyl ester (DOPA CHE), an antagonist for DOPA. DOPA CHE did not modify the action of carbachol on excitatory postsynaptic potential in rat cortical slices. These results suggest that endogenous DOPA is involved in nicotine-induced depressor responses in the NTS of anesthetized rats.

The nucleus tractus solitarii (NTS) is the first site at which afferents mediating different cardiovascular reflexes form their primary synapse [2]. The NTS plays essential roles in baroreflex neurotransmission and central regulation of arterial blood pressure, and various neurotransmitters and/or neuromodulators have been implicated in this process [2,1,20,21,23]. There is a general consensus that glutamate receptors in the NTS are involved in mediating different cardiovascular reflexes. Nicotinic receptors, the other excitatory neurotransmitter receptors, have also been identified on subpopulations of NTS neurons in rat brain stem slices [18], dissociated NTS neurons [22], and the nicotinic receptors in the NTS have been involved in cardiovascular regulation [3,4,9,17]. Activation of nicotinic receptors has been reported to release an excitatory amino acid in the NTS [3], and some of the nicotinic effects may be attributed to active endogenous substances released in response to nicotine [16]. However, there are very few detailed studies on the role of nicotinic receptors in the NTS mediating or modulating cardiovascular function [4,9].

L-3,4-Dihydroxyphenyalanine (DOPA) is believed to be an inert amino acid that exerts its actions via its conversion to dopamine (DA) by aromatic L-amino acid decarboxylase (AADC). Contrary to this generally accepted idea, we have proposed that DOPA itself is a neurotransmitter in the central nervous system [12,13]. There is immunocytochemical evidence for the existence of neurons that may contain DOPA as an end product. The NTS is one of the brain areas where DOPA likely acts as a neurotransmitter [12,26]. Electrical aortic depressor nerve stimulation releases DOPA ipsilaterally in the NTS [26], which is accompanied by hypotension and bradycardia. The evoked release of DOPA is abolished following the blockade of Na^+ channels by tetrodotoxin (TTX) infused in the NTS. DOPA is therefore a highly probable neurotransmitter of the primary baroreceptor afferents [10,26]. Microinjection of DOPA into the medial area of the NTS leads to dose-dependent decreases in arterial blood pressure and heart rate in rats [10], and these cardiovascular responses are antagonized by prior injection of DOPA methyl ester and DOPA cyclohexyl ester (DOPA CHE), competitive antagonists for DOPA [5,7]. In rats treated by central administration of an AADC inhibitor, DOPA can induce c-Fos expression in the NTS, thereby suggesting that NTS includes target sites for DOPA itself [19]. In the present study, we have attempted to clarify whether some interaction between DOPA and nicotinic receptors to regulate cardiovascular function exists by determining whether or not DOPA CHE blocks the nicotine-induced depressor and bradycardic responses in rat.

All procedures were conducted according to the guidelines outlined in the Institutional Animal Care and Use Committee of the Yokohama City University Graduate School of Medicine. All experimental procedures were conducted with maximum effort to

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minimize the number of animals used and their suffering. Male Wistar rats (240–320 g) were anesthetized with urethane (1.2 g/kg, i.p.). The femoral artery was cannulated. Rats were paralyzed with D-tubocurarine (1 mg/kg, i.m.), artificially ventilated with a respirator, and placed in a stereotaxic apparatus with the head fixed at 45°. The dorsal surface of the lower brainstem was exposed by a limited occipital craniotomy. A glass micropipette pulled to an outside tip diameter of 50–100 μm was introduced into the NTS (0.6 mm rostral and 0.6 mm lateral to the caudal tip of the area postrema and 0.6 mm beneath the dorsal surface of the brainstem) [10]. Drugs dissolved in saline were injected unilaterally into NTS in a volume of 50 nl in 2 s.

To determine the possible interaction of DOPA CHE with nicotinic receptors, we performed slice patch analysis using rat cortical slices. Briefly, rats (2–3 weeks old) were anesthetized by inhalation of isoflurane, and decapitated. Brains were quickly transferred into ice-cold dissection buffer (25.0 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, 2.5 mM KCl, 0.5 mM $CaCl_2$, 7.0 mM $MgCl_2$, 25.0 mM glucose, 110.0 mM choline chloride, 11.6 mM ascorbic acid, 3.1 mM pyruvic acid) gassed with 5%CO_2/95%O_2. Coronal brain slices were cut (300 μm, Leica vibratome) in dissection buffer and transferred to physiological solution (22–25 °C, 118 mM NaCl, 2.5 mM KCl, 26.2 mM $NaHCO_3$, 1 mM NaH_2PO_4, 11 mM glucose, 1.3 mM $MgCl_2$, 2.5 mM $CaCl_2$, pH 7.4), gassed with 5%CO_2/95%O_2. Patch recording pipettes (4–7 MΩ) were filled with intracellular solution (115 mM cesium methanesulfonate, 20 mM CsCl, 10 mM HEPES, 2.5 mM $MgCl_2$, 4 mM Na_2ATP, 0.4 mM Na_3GTP, 10 mM sodium phosphocreatine, 0.6 mM EGTA at pH 7.25). A whole cell voltage-clamp technique was used to record postsynaptic currents in layer 2/3 pyramidal neurons (150–500 μm from pial surface) of rat barrel cortex with Axopatch –1D amplifier (Axon Instruments), digitized at 10 kHz with an A/D converter (Digidata 1322, Axon Instruments), filtered at 1–2 kHz, and saved on a personal computer using pClamp 10.0 software (Axon Instruments). The slice was placed in a recording chamber and fixed with a grid of parallel nylon threads supported by a U-shaped stainless steel weight. The physiological solution saturated with 5%CO_2/95%O_2 was perfused into the chamber at 3.0 ml/min at 22–25 °C. All drugs (carbachol 50 μM, DOPA CHE 100 μM) were dissolved in the physiological solution and applied to the bath by manually switching the perfusates. After 3 min of baseline data were obtained, the responses of the frequency, number of excitatory postsynaptic current (EPSC) per min, and the amplitude (mean amplitude of EPSC) of spontaneous EPSC were recorded.

The paired Student's *t*-test for comparisons of cardiovascular parameters before and after treatment was applied to compare group differences. Experimental data on the amplitude and frequency were analyzed with one-way ANOVA, followed by Fisher's protected least significant difference post hoc. The threshold for statistical significance was 0.05.

In control rats, the arterial pressure was 78 ± 6 mmHg and heart rate 400 ± 11 beats/min (n = 5). Unilateral microinjections of L-glutamate (monosodium salt, 30 ng, Nacalai Tesque) into the medial area of NTS produced a fall in blood pressure and heart rate. When delivered at the depressor sites identified with L-glutamate, nicotine (300 pmol) elicited decreases in blood pressure and heart rate by 27 ± 5 mmHg and 51 ± 11 beats/min (n = 6), respectively. Mecamylamine (5 nmol) alone produced no effect on these parameters. Mecamylamine, microinjected 10 min previously, markedly inhibited the depressor and bradycardic responses to nicotine (300 pmol): this antagonist elicited a reduction in the decrease induced by nicotine in blood pressure and heart rate (−6 ± 2 mmHg, −10 ± 5 beats/min, n = 6, P < 0.05 vs. nicotine alone, n = 6), respectively. This result is consistent with previous findings [4,9]. Under these experimental conditions, we

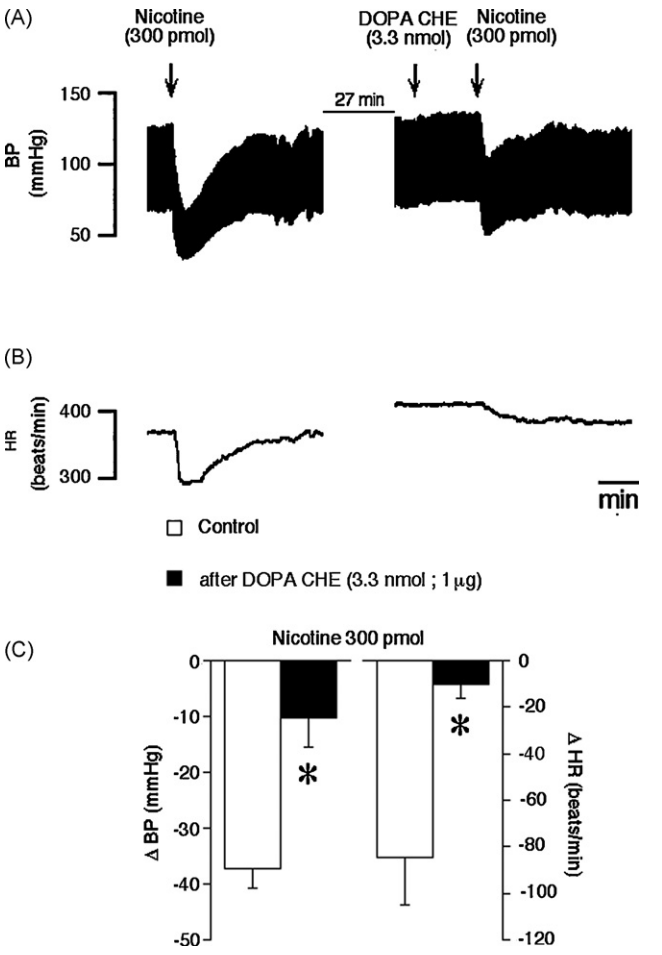

Fig. 1. Nicotine-induced depressor (A) and bradycardic (B) responses are sensitive to DOPA CHE. (C) Effect columns for depressor (blood pressure (BP), mmHg) and bradycardic (heart rate (HR), beats/min) responses to nicotine and the effects of DOPA CHE on the depressor and bradycardic responses to microinjection of nicotine. Male Wistar rats (240–320 g) were anesthetized, the femoral artery was cannulated and they were artificially ventilated with a respirator. Drugs dissolved in saline were injected unilaterally into the NTS in a volume of 50 nl in 2 s. DOPA CHE was injected into the NTS just prior to the microinjection of nicotine into the same area. (C) *P < 0.05, compared to vehicle control. Data are presented as the mean ± S.E.M. from six respective experiments.

tested the effect of DOPA CHE on the nicotine-induced depressor and bradycardic response. DOPA CHE (3.3 nmol), microinjected 1 min previously, inhibited depressor and bradycardic responses to nicotine (300 pmol) (Fig. 1). We have previously shown that DOPA agonists and DOPA antagonists, including DOPA CHE, do not inhibit specific binding of [^{3}H]-ligands of (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine (MK-801), kainite, 5,7-dichloro-kynurenic acid and DL-(*E*)-2-amino-4-propyl-5-phospho-3-pentenoic acid. DOPA ME and DOPA CHE inhibit the specific binding of [^{3}H]-MK-801 with respective IC_{50} values of 1 and 0.68 mM. Therefore DOPA CHE does not interact with ionotropic glutamate receptors [14]. However, it remains possible that DOPA CHE suppresses the action of nicotine by interacting with nicotinic cholinergic receptors. Using a whole cell voltage-clamp technique, we confirmed that DOPA CHE did not affect the action of carbachol, a non-selective cholinergic agonist, on excitatory postsynaptic potential in rat cortical slices (Fig. 2). This clearly indicates that DOPA CHE did not interact with nicotinic receptors, and further supports the idea that DOPA CHE-sensitive sites for DOPA are involved in the nicotine-induced depressor action. Glutamate in the NTS has also been implicated in the central cardiovascular regula-