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Nicotine activation of neuronal nitric oxide synthase and guanylyl cyclase in the medulla increases blood flow of the common carotid artery in cats

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ABSTRACT

Individual activation of nicotinic acetylcholine receptor (nAChR) or nitric oxide (NO) synthase in the dorsal facial area (DFA) increases blood flow of common carotid artery (CCA) supplying intra- and extracranial tissues. We investigated whether the activation of nAChR initiated the activation of NO synthase and guanylyl cyclase to increase CCA blood flow in anesthetized cats. Microinjections of nicotine (a non-selective nAChR agonist), or choline (a selective α7-nAChR agonist) in the DFA produced increases in CCA blood flow ipsilaterally. These increases were significantly reduced by pretreatment with NG-nitro-arginine methyl ester (L-NAME, a non-specific NO synthase inhibitor), 7-nitroindazole (7-NI, a relatively selective neuronal NO synthase inhibitor) or methylene blue (MB, a guanylyl cyclase inhibitor) but not by that with N5-(1-iminoethyl)-L-ornithine (L-NIO, a potent endothelial NO synthase inhibitor). Control microinjection with D-NAME (an isomer of L-NAME), artificial cerebrospinal fluid or DMSO (a solvent for 7-NI) did not affect resting CCA blood flow, nor did they affect nicotine- or choline-induced response. In conclusion, activation of nAChR, at least α7-nAChR, led to the activation of neuronal NO synthase and guanylyl cyclase in the DFA, which induced an increase in CCA blood flow.

Kuo et al. [15] for the first time reported that stimulation of the dorsal facial area (DFA) in the cat medulla induced an ipsilateral increase in blood flow of common carotid artery (CCA) without significant changes in other cardiovascular parameters. This effect is mediated by the parasympathetic branches of the 7th and 9th nerves [1] and is attenuated by atropine, a parasympathetic blocker [1]. The DFA thus is likely the rostral extension of the dorsal motor nucleus of the vagus nerve (DMN) [2]. Kuo and colleagues have serially reported about neurochemical actions of glutamate [1,2,9,10,15], serotonin [14,16], nicotine [8,13], and nitric oxide synthase (NOS) [7,12] in the DFA. It is established that activation in the DFA on either nicotinic acetylcholine receptor (nAChR) [8,13] or NOS [7,12] increases CCA blood flow. Yet it is not known whether activation of the nAChR in the DFA may thereby activate the NOS to induce the increase in CCA blood flow.

In the present study, we demonstrated that in the DFA, stimulation of the α7-nicotinic receptor activated neuronal NOS and guanylyl cyclase, which then led to the increase in CCA blood flow in anesthetized cats.

The experiments were carried out according to the guidelines of the China Medical University Ethical Committee for Animal Research. This study was approved by the Committees.

Cats (2.0–3.5 kg) of either sex were anesthetized with α-chloralose (40 mg/kg) and urethane (400 mg/kg) intraperitoneally, and paralyzed with atracurium with an initial dose of 0.05 mg/kg and a maintaining dose of 0.02 mg/kg (IV) every 20 min to eliminate interference in recording of blood flow. Tracheotomy was performed for artificial ventilation that maintained end expiratory CO_2 concentration at 3.5–4.5%. The rectal temperature was kept at 37.5 ± 0.5 °C by an electrical heating pad. The femoral artery and vein were cannulated with PE-90 polyethylene tubing for monitoring the systemic arterial pressure and supplying fluid, respectively. The ultrasound Doppler probes (diameter 1.5–2.0 mm), which were placed around the right and left CCA, were connected with a directional pulsed Doppler flowmeter (University of Iowa, Bioengineering, 545C-4, Iowa, USA) for monitoring CCA blood flow.

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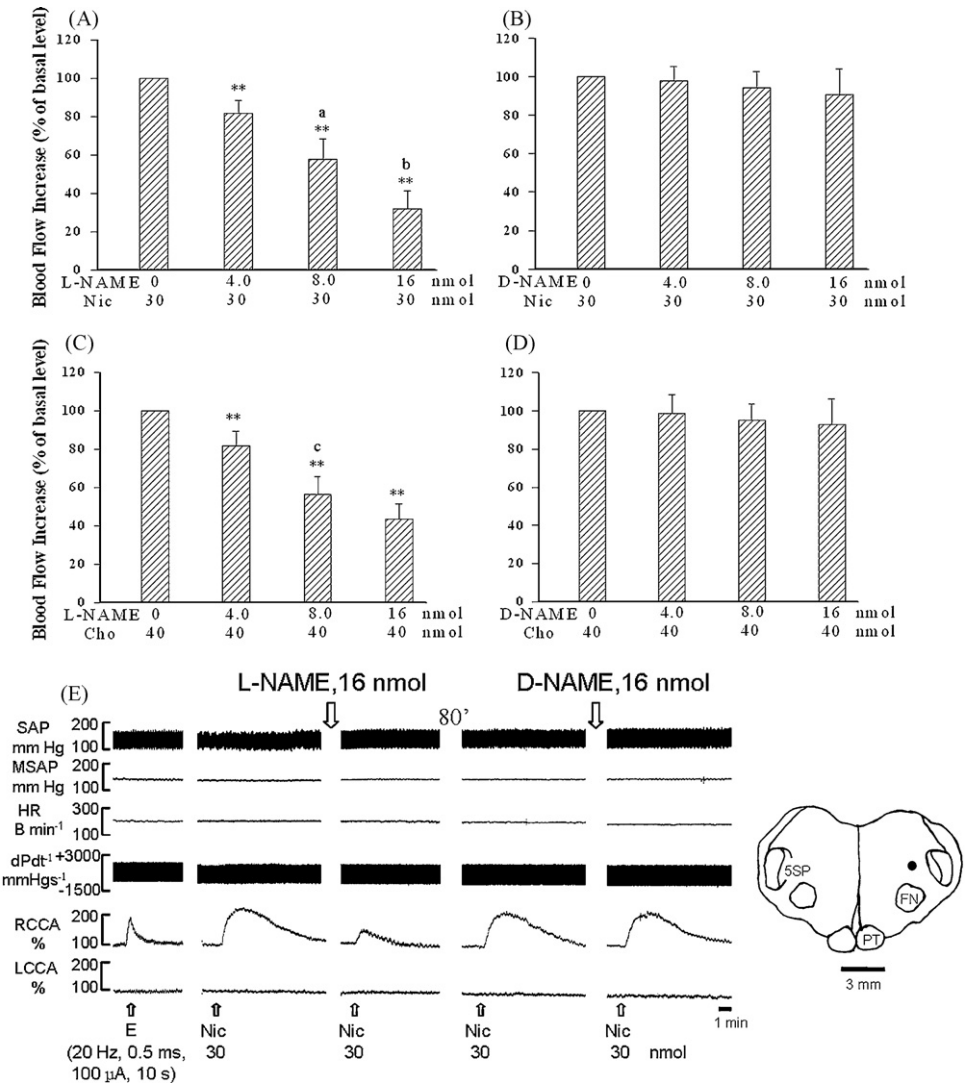

Fig. 1. Effects of pretreatment with non-specific NOS antagonist L-NAME (A, $n = 7$; C, $n = 5$) and its D-form isomer D-NAME (B, $n = 4$; D, $n = 4$) on nicotine- and choline-induced increases in the CCA blood flow. All chemicals were microinjected into the DFA. Original tracings are shown in E. Data are expressed as mean ± S.E.M. and analyzed by ANOVA and Tukey's test. For A, $^{**}P < 0.01$ vs. L-NAME 0 nmol; $^{a}P < 0.01$ vs. L-NAME 4.0 nmol; $^{b}P < 0.01$ vs. L-NAME 8.0 nmol. For C, $^{**}P < 0.01$ vs. L-NAME 0 nmol; $^{c}P < 0.01$ vs. L-NAME 4.0 nmol. The dot on the drawing medullary section indicates injected locus. Abbreviations for this and following figures: B min^{-1}, beats per min; dPdt^{-1}, cardiac contractile force; Cho, choline; E, electrical stimulation; FN, facial nucleus; HR, heart rate; MSAP, mean systemic arterial pressure; Nic, nicotine; PT, pyramidal tract; RCCA or LCCA, right or left common carotid arterial blood flow; SAP, systemic arterial pressure; 5ST, spinal trigeminal nucleus.

Systemic arterial pressure, heart rate, cardiac contractile force, and CCA blood flows were routinely recorded on a Gould Recorder RS3800 (Cleveland, OH, USA) [7,8,10–13].

The head of the cat was immobilized in a David-Kopf stereotaxic instrument. The stereotaxic coordinates of the center of DFA were about 6.0 mm rostral to the obex, 3.5 mm lateral to the midline, and 3.5 mm ventral to the floor of the fourth cerebral ventricle [2,7,8,11–13]. Aiming at this point, a four-barrel microinjection tube [11,13] was placed at an angle of 34° from the vertical axis of the stereotaxic instrument. The DFA was confirmed by an increase of CCA blood flow in response to an electrical stimulation (20 Hz, 0.5 ms, 100 μA, 10 s) through the tubing. Misplaced injection of glutamate does not cause any response in CCA blood flow [2]. Each barrel was used for microinjecting 200 nl nicotine (a non-selective nAChR agonist), choline (an α7-nAChR agonist), L-NAME (a non-selective NOS inhibitor), D-NAME (an isomer of L-NAME), methylene blue (a guanylyl cyclase inhibitor), 7-NI (a neuronal NOS inhibitor), or L-NIO (an endothelial NOS inhibitor). All of these drugs were purchased from Sigma–Aldrich Inc. St. Louis, USA. Except 7-NI which was dissolved in DMSO, all drugs were dissolved in artificial cerebrospinal fluid (aCSF) containing the following chemicals in mM: NaCl 119, KCl 2.5, $MgCl_2$ 4, $CaCl_2$ 4, $NaHCO_3$ 26.2, NaH_2PO_4 1, and glucose 11. These solutions were gassed with 95% O_2 and 5% CO_2 at pH 7.4. They were microinjected at a rate of 1.2 μl/min into the DFA over 10 s with a microinjection pump (CMA/100, Carnegie Medicin, North Chelmsford, MA, USA).

At the end of the whole experiment, the animals were sacrificed by intravenous injection of saturated KCl. The brains were removed and frozen-sectioned by a microtome (2800 Frigocut) at 40 μm thickness. Only brain sections in which the injection point had been correctly positioned into the DFA were considered for data analysis.

Changes in systemic arterial pressure, heart rate, cardiac contractile force (dP/dt), and CCA blood flows in response to microinjections of chemicals were calculated as (response value − control value)/(control value) × 100% and then normalized with the control. The normalized data (means ± S.E.M.) were