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Research article

Microinjection of acetylcholine into cerebellar fastigial nucleus induces blood depressor response in anesthetized rats

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HIGHLIGHTS

- Microinjection of ACh into the FN induces systemic blood depressor response.
- nACh receptors in the FN are ineffective against the ACh-mediated depressor response.
- mACh receptors in the FN contribute substantially to the ACh-mediated depressor response.

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ABSTRACT

It is well known that the cerebellar fastigial nucleus (FN) is involved in cardiovascular modulation, and has direct evidence of cholinergic activity; however, whether and how acetylcholine (ACh) in the FN modulates blood pressure has not been investigated. In this study, we analyzed mean arterial pressure, maximal change in mean arterial pressure, and the reaction time of blood pressure changes after microinjection of cholinergic reagents into the FN in anesthetized rats. The results showed that ACh evoked a concentration-dependent (10, 30 and 100 mM) effect on blood pressure down-regulation. The muscarinic ACh (mACh) receptor antagonist atropine, but not the nicotinic ACh (nACh) receptor antagonist mecamylamine, blocked the ACh-mediated depressor response. The mACh receptor agonist oxotremorine M, rather than nACh receptor agonist nicotine, mimicked the ACh-mediated blood pressure decrease in a dose-dependent manner (10, 30 and 100 mM). These results indicate that cholinergic input in the cerebellar FN exerts a depressor effect on systemic blood pressure regulation, and such effects are substantially contributed by mACh rather than nACh receptors, although the precise mechanism concerning the role of mACh receptor in FN-mediated blood pressure modulation remains to be elucidated.

1. Introduction

The fastigial nucleus (FN) is phylogenetically the oldest nucleus of the deep nuclei (including FN and interpositus and dentate nuclei) in the cerebellum, and plays an important role in cerebellum-mediated somatic and visceral integration [6,23,28]. In the FN circuits, the neurons receive inhibitory synapses from the Purkinje cell axons, excitatory afferents from the climbing and mossy fibers, as well as diverse modulatory information from various other inputs, such as serotonergic, norepinephrinergic, histaminergic, dopaminergic, orexinergic and cholinergic projections [6,17,28]. Among these modulatory afferents, the role of cholinergic inputs receives much less attention, although it is known that the FN is innervated with cholinergic fibers [7,11,13] and contains several subtypes of cholinergic receptors [2,7].

Central cholinergic roles on blood pressure modulation have been widely studied. The outcomes depend on different brain sites and/or different acetylcholine (ACh) receptors. For example, microinjection of ACh into the ventrolateral periaqueductal gray areas and cuneiform nucleus, or activation of nicotinic ACh (nACh) receptor in the caudal ventrolateral medulla, causes a marked blood depressor response [1,3,19], while activation of muscarinic ACh

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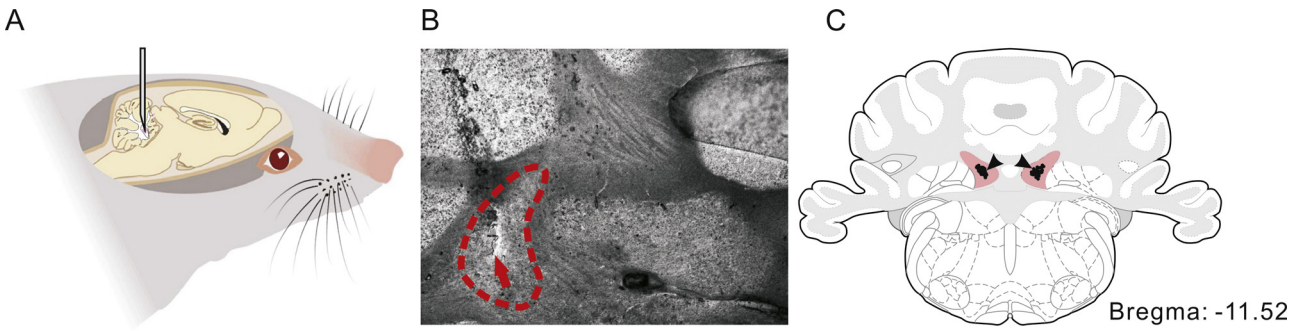

Fig. 1. A, Schematic diagram indicates the microinjection site from a sagittal view of a rat brain. B, Representative histological section (60 μm thick) shows the injection site (arrow) in cerebellar FN (surrounded by red dotted line). C, Histological reconstruction shows the microinjection sites (arrowheads) in cerebellar FN among 40 animals according to a rat brain atlas [14] (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article)..

(mACh) receptor in the rostral ventrolateral medulla significantly increases blood pressure [10]. Our previous findings demonstrated that microinjection of ACh or activation of mACh receptor in the cerebellar cortex markedly down-regulated blood pressure [27,29]. In the present study, we focused on whether and how ACh in the FN participates in blood pressure modulation, and the underlying receptor mechanism.

2. Materials and methods

2.1. Animals

Forty young adult male Sprague–Dawley rats (aged 2 months, weighing 230–260 g) were obtained from Shanghai Sippr BK Laboratory Animals Ltd. (Shanghai, China). The rats were individually housed in a temperature-controlled (23 ± 1 °C) environment with a 12/12-h light/dark cycle with food and water *ad libitum*. All animal experiments were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23, revised in 1996). The minimal possible number of animals was used, and all efforts were accordingly made to minimize their suffering.

2.2. Surgical procedure

The rat brain surgical procedure was as described previously [24,27,29]. The rats were anesthetized with 800 mg/kg urethane (intraperitoneal injection; Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) and then processed for cervical surgery with tracheal intubation. The carotid artery pressure was measured via a catheter (BL-2020; Taimeng Sci-Tec Co. Ltd., Chongqing, China), which was linked to a signal collecting and processing apparatus (BL-420F; Taimeng Sci-Tech Co. Ltd.) through a blood pressure transducer (PT-100; Taimeng Sci-Tech Co. Ltd.). After craniotomy, a Hamilton syringe needle (0.5 mm outer diameter, 0.3 mm internal diameter) was lowered into the cerebellum under stereotaxic guidance (51503 New Standard Stereotaxic; Stoelting, Wood Dale, IL, USA) using a computer-controlled stepper motor (IVM-1000; Scientifica, Uckfield, Sussex, UK), which extended to the bilateral FN (x: −11.5; y: ±1.1; z: −5.8 to −6.0) according to a rat brain atlas [14] (Fig. 1A–C). Repeated injections were administered at intervals of at least 30 min, ensuring the blood pressure recovered to the basal level and remained stable for >10 min to avoid interference between administrations.

2.3. Drug microinjection and blood pressure measurements

ACh chloride (ACh; 10, 30 or 100 mM; Sinopharm Chemical Reagent Co. Ltd.), nonselective nACh receptor agonist nicotine ditartrate (10, 30 or 100 mM; Tocris Cookson Ltd., Bristol, UK), 100 mM ACh mixed with the nonselective nACh receptor antagonist mecamylamine hydrochloride (Meca; 1, 3 or 10 mM; Tocris Cookson Ltd.), nonselective mACh receptor agonist oxotremorine M (OXO; 10, 30 or 100 mM; Sigma, St. Louis, MO, USA), 100 mM ACh mixed with nonselective mACh receptor antagonist atropine sulfate (1, 3 or 10 mM; Sinopharm Chemical Reagent Co. Ltd.), and normal saline (0.9% NaCl) was separately microinjected (0.3 μl/5 s) into the FN. The drug effects on blood pressure regulation were considered substance-specific provided they were reversible and reproducible. The mean arterial pressure (MAP), maximal change in MAP (MCMAP), and reaction time (RT; duration required for BP to return to basal level) were calculated and analyzed.

2.4. Statistical analysis

All the data are presented as the mean ± standard error. Student's t-test and one-way analysis of variance followed by a Fisher's least significant difference post hoc test were conducted for statistical analyses. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Microinjection of ACh into FN down-regulates blood pressure in rats

Before microinjection, basal MAP ranged from 82.7 to 122.8 mmHg, with an average of 104.4 ± 7.4 mmHg, in line with our previous and other studies [18,27,29]. MAP was maintained at 103.2 ± 8.5 mmHg after microinjection of saline into the FN, which showed no significant difference from before injection (Fig. 2A1, A2), but there was a marked decrease after ACh treatment (Fig. 2B1–B3).

ACh at 10, 30 and 100 mM showed a concentration-dependent effect on MAP ($F_{3,76} = 52.173$, $P < 0.001$), which declined by 9.1%, 21.0% and 29.5%, respectively (Fig. 3A); MCMAP ($F_{2,57} = 151.508$, $P < 0.001$), which was 12.4 ± 2.9, 25.5 ± 3.6 and 35.2 ± 4.8 mmHg, respectively (Fig. 3B); and RT ($F_{2,57} = 46.987$, $P < 0.001$), which was 94.2 ± 11.4, 139.4 ± 16.8 and 173.1 ± 20.8 s, respectively (Fig. 3C). These results indicate that microinjection of ACh into the cerebellar FN induces a significant depressor response in systemic blood pressure.

3.2. Role of nACh receptor in FN in ACh-mediated depressor response

As the cholinergic effects occur by either or both of the two ACh receptor superfamilies (nACh and mACh receptors), we examined the role of nACh receptor in FN in ACh-mediated blood