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Mechanism of neuronal activity and synaptic transmission in rostral ventrolateral medulla

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ABSTRACT

Rostral ventrolateral medulla (RVLM) plays an essential role in blood pressure homeostasis. This study was aimed to investigate the mechanism of neuronal activity and synaptic transmission in the RVLM. Medulla oblongata slices were carefully obtained from brainstem of rats. With continues perfusion of artificial cerebrospinal fluid (ACSF), the spontaneous firing of slices and amplitudes were assayed by conventional whole cell patch-clamp recording after addition of gamma-aminobutyric acid (GABA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) and N-methyl D-aspartate (NMDA). Furthermore, the effects of agonist or antagonist targeted Type-A GABA (GABAA) or glutamate receptors on spontaneous excitatory postsynaptic potential (sEPSP) and spontaneous inhibitory postsynaptic potential (sIPSP) of neurons in RVLM were determined. The spontaneous firing of neurons in RVLM were inhibited by GABA ($P < 0.001$) while were promoted by NMDA or AMPA ($P < 0.01$ or $P < 0.001$). In terms of sEPSP and sIPSP, the numbers of firing neurons in RVLM were both improved by GABAA receptor antagonist ($P < 0.01$ or $P < 0.001$) while were both decreased by GABAA receptor agonist or glutamate receptor antagonist ($P < 0.05$, $P < 0.01$ or $P < 0.001$). The corresponding effects of agonist and antagonist on amplitudes were the same as the effects on number of firing neurons in RVLM. The spontaneous firing, sEPSP and sIPSP of neurons in RVLM were all activated by GABAA receptor antagonist while were all suppressed by GABAA receptor agonist or glutamate receptor antagonist.

1. Introduction

Blood pressure homeostasis is a complex process, which refers to a continuum of actions of cardiovascular, neural, renal and endocrine system [1]. When the blood pressure is too low for normal function, termed hypotension, the patients suffer from dizziness, syncope, orthostatic hypotension symptoms and ischemia of brain and heart [2]. Conversely, the blood pressure which is too high for normal function, termed hypertension, leads to heart failure, coronary artery disease, stroke and peripheral arterial disease [3].

Vascular resistance and cardiac output are two vital factors for control of blood pressure at rest. During movement, it has been reported that vestibular system offers a spatially convergent input to the presympathetic central nervous system (CNS) cell group for blood pressure control [4]. Thus the baroreceptor reflex and vestibular inputs operate in concert to control the blood pressure both at rest and during movement. The signals are conveyed and finally projected from CVLM to the rostral ventrolateral medulla (RVLM) and then innervate the intermediolateral cell column in the spinal cord [5,6]. In addition, the vestibular inputs are also integrated by RVLM and are constituted the sympathetic nerve circuitry with baroreceptor. Therefore, the RVLM is import for blood pressure control.

Acted as the primary region of the brainstem, the RVLM is located dorsally in the hindbrain [7]. Two major neuronal subpopulations are reported to compose the RVLM including presympathetic neurons and expiratory neurons [8,9]. There are plenty of neurons whose somata lie in RVLM while whose axons are stretched to the spinal cord. In spinal cord, axons interact with sympathetic preganglionic neurons and then provide basal activity to ensure adequate circulation for all organs [6]. It is widely accepted that neurons in RVLM possess a tonic activity which is

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greatly affected by inputs from the CNS. Meanwhile, RVLM modulates sympathetic outflow via integrating various descending and barosensor inputs [10–12]. If the RVLM is destructed or inactivated, the arterial blood pressure is tumbled to the levels equivalent to the results of spinal transection [13]. The activation of RVLM exhibits the opposite results for arterial blood [14].

Previous investigations demonstrated that gamma-aminobutyric acid (GABA) receptor, α-amino-3-hydroxy-5-methy l-4-isoxazole propionate (AMPA) receptor and N-methyl D-aspartate (NMDA) receptor were expressed in the RVLM [15–17]. As a consequence, agonist and antagonist of these receptors are hypothesized to play roles in homeostasis of blood pressure. The present study was conducted to determine if GABA receptor agonist and antagonist, AMPA receptor antagonist and NMDA receptor antagonist indeed affect spontaneous firing, spontaneous excitatory postsynaptic potential (sEPSP) and spontaneous inhibitory postsynaptic potential (sIPSP) of neurons in RVLM.

2. Materials and methods

2.1. Animals

A total of 42 adult male Wistar rats (7–8 weeks of age, 260–280 g body weight), which were purchased from the Shanghai Experimental Animal Centre (Chinese Academy of Sciences, Shanghai, China), were randomly assigned to experimental group (33 rats) and control group (9 rats). All the experimental operations on rats in this study were in line with the guidelines of the international association for the study of pain (IASP) and were approved by the local ethical committee. All efforts were made to minimize the number and suffering of rats involved in our study.

2.2. Medulla oblongata slice preparation

Rats were deeply anesthetized with an intraperitoneal injection of sodium pentobarbital (80 mg/kg Sigma–Aldrich, St. Louis, Mo, USA) and were quickly decapitated while anesthetization. The medulla oblongata was carefully but rapidly isolated and quickly immersed into artificial cerebrospinal fluid (ACSF) which was pre-cold and bubbled with a carbogen mixture (95% O_2-5% CO_2). The ACSF was consisted of (in mM) 126 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, 1.3 $MgCl_2$, 26 $NaHCO_3$ and 20 glucose, buffered to pH 7.4. Utilizing a vibrating microtome VT 1200S (Leica, Wetzlar, Germany), medulla oblongata slices (300 μm) containing RVLM were successfully obtained under a temperature of 0 °C. After incubation in ACSF for 30 min at 35 °C, the slices were kept in ACSF at room temperature until the subsequent experiments.

2.3. The conventional whole cell patch-clamp recording

The isolated slice was placed in a submerged recording chamber and then was fixed by nylon strings attaching to a platinum frame. Continuously perfused with warm oxygenated ACSF (30 °C), the slice was viewed under an upright microscope (Nikon FN1, Nikon, Mississauga, Canada). Patch electrode for recording sEPSPs was filled with a solution containing (in mM) 140 K-gluconate, 10 EGTA, 10 HEPES, 2 $MgCl_2$, 3 KCl, and 2 Na_2ATP, buffered to pH 7.3–7.4 (adjusted with KOH). Patch electrode for recording sIPSPs was filled with a solution containing (in mM) 125 Cs-methanesulfonate, 10 CsCl, 2 $MgCl_2$, 5 NaCl, 10 HEPES, 1 EGTA, 5 Mg-ATP and 0.3 Na_3GTP, buffered to pH 7.3–7.4 (adjusted with CsOH). Seals were formed by patch pipettes according to the standard methods [18]. Using patch pipettes of 4–8 MΩ, slices were voltage-clamped at −70 mV for sEPSP examination and 0 mV for sIPSP examination, respectively. Electrophysiological signals were recorded by Muticlamp 700B amplifier (Axon Instruments, Oceanside, CA, USA) digitized at 10 kHz, low-pass filtered at 1 kHz, and the recording data were analyzed by Clampex 9.2 software (Axon Instrument).

2.4. Slices treatment

For examination of spontaneous firing, AMPA (250 μM), NMDA (250 μM) or GABA (250 μM) were respectively ejected under computer control through micropipettes with a microinjector and a picospritzer. The slice was perfused constantly with ACSF and the number of firing neurons was recorded as well as the amplitude. Slice with no treatment acted as control.

For sEPSP and sIPSP examination, Type-A GABA (GABAA) receptor agonist Muscimol (5 μM), antagonist SR95531 hydrobromide (SR95531, 10 μM), AMPA receptor antagonist 6-cyano-2,3-dihy droxy-7-nitroquinoxaline (CNQX, 10 μM) or NMDA receptor antagonist D-2-amino-5-phosphonopentanoate (D-AP5, 50 μM) (all from Tocris Bioscience, Ellisville, MO, USA) was respectively dissolved in ACSF and applied as bath buffer in certain experiments.

2.5. Statistical analysis

All experiments were repeated three times. The results were presented as the mean ± standard deviation (SD). Statistical analysis was performed using Graphpad Prism 5 software (GraphPad, San Diego, CA, USA). The *P*-values were calculated using the two-way analysis of variance (ANOVA). Difference between groups was considered significant at $P < 0.05$.

3. Results

3.1. The effects of AMPA, NMDA and GABA on spontaneous firing of neurons in RVLM

To investigate the effects of excitatory AMPA, NMDA, and inhibitory GABA on spontaneous firing of neurons in RVLM, number of firing neurons (NFN) and the corresponding amplitude were recorded. Average NFN was presented in Fig. 1A, AMPA and NMDA significantly enhanced NFN in RVLM in comparison with control ($P < 0.01$ or $P < 0.001$) at the same time point. In contrast, GABA markedly decreased NFN in RVLM in comparison with control ($P < 0.001$). The examples of original 1.0 s traces were shown in Fig. 1B, AMPA and NMDA significantly increased amplitudes while GABA remarkably decreased amplitudes. Thus, we concluded that excitatory AMPA, NMDA, and inhibitory GABA prominently affected spontaneous firing of neurons in RVLM.

3.2. The effects of GABAA receptor agonist, antagonist, AMPA and NMDA receptor antagonist on sEPSPs of neurons in RVLM

To assess the effects of various agonist or antagonist on glutamatergic synaptic transmission in RVLM slices, NFN and the corresponding amplitude were recorded bathing with Muscimol, SR95531, CNQX or D-AP5. Fig. 2A and C showed that Muscimol, CNQX and D-AP5 respectively decreased NFN in comparison with control ($P < 0.01$ or $P < 0.001$), while SR95531 notably increased NFN in comparison with control ($P < 0.01$ or $P < 0.001$). The examples of original 1.0 s traces were shown in Fig. 2B and D, the amplitudes were significantly reduced by Muscimol, CNQX and D-AP5 when compared with control while were markedly increased by SR95531. Therefore, we proposed that GABAA receptor agonist, antagonist, AMPA and NMDA receptor antagonist could affect sEPSPs.