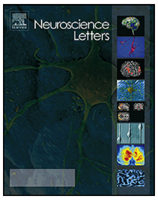
Neuroscience Letters 559 (2014) 127–131

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet



Role of rostral medulla in serotonin-induced changes of respiratory rhythm in newborn rat brainstem–spinal cord preparations

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HIGHLIGHTS

- We recorded respiratory-like nerve activity in Pons–medulla–spinal cord in vitro.
- Rostral ventrolateral medulla (RVLM) is sensitive to 5-HT application.
- Application of 10 μM 5-HT increased the activity before but not after transection.
- 5-HT suppressed the rhythm after transection at the level of the facial nucleus.
- Without the pons, 5-HT suppresses respiratory rhythm through a distinct RVLM region.

ARTICLE INFO

Article history:
Received 26 September 2013
Received in revised form
26 November 2013
Accepted 2 December 2013

Keywords:
5-HT
In vitro
Medulla
Neonatal rat
Pons
Respiratory rhythm

ABSTRACT

Abnormalities of the serotonin (5-hydroxytryptamine, 5-HT) system may induce respiratory disorders. We examined which regions in the rostral medulla are important for the effect of 5-HT on the frequency of respiratory-like nerve (fR-like) activity by transecting the preparations at different levels near the facial nucleus (nVII) in newborn rat brainstem–spinal cord preparations. The fR-like activity at the fourth cervical ventral root (C4) of the Pons–medulla–spinal cord preparations in 2–3-day-old rats ($n = 25$) was monitored at 26 °C, and the change in fR-like activity in response to application of 10 μM 5-HT before and after transection was compared among three groups, in which nVII was retained (group A, $n = 10$), partially retained (group B, $n = 7$), or eliminated (group C, $n = 8$) by the transection. Before transection, the resting fR-like activity (set to 100%) and stimulant effect of 5-HT (+101–143%) were similar among the groups. After transection, resting fR-like activity increased in all groups, but the facilitatory effects of 5-HT on the fR-like activity were abolished in groups A and C (fR-like activity of −4% and +7%, respectively). In group B, 5-HT became inhibitory (fR-like activity of −28%). In conclusion, a distinct part of the rostral medulla in the absence of pontine influences may mediate the inhibitory effects of 5-HT on the respiratory rhythm.

1. Introduction

The pre-Bötzinger complex in the ventrolateral medulla is the kernel of respiratory rhythm genesis [1]. Breathing is controlled through large hierarchical and complex neural networks [2–5]. Neurons synthesizing serotonin (5-HT) are found in the brainstem raphe nuclei, which form a column that extends from the caudal medulla to the midbrain along the midline and project to virtually all areas of the brain, including the pontine and ventral respiratory columns [6]. The 5-HT system has been suggested to impact the development and control of respiration and hence survival [6–9]. The 5-HT from the raphe supports eupneic ventilation, CO_2 chemoreception, and thermoregulation [10,11]; indeed, abnormalities of the 5-HT system may induce respiratory disorders, including sudden infant death syndrome (SIDS) [6,8,9] and sleep apnea [12]. These observations suggest the prevalence of anatomically and functionally tight relationships between the respiratory network and 5-HT systems in the brainstem.

The effects of 5-HT on respiratory rhythm generation have been investigated in brainstem–spinal cord preparations from newborn animals. For example, in Pons–medulla–spinal cord (PMS) preparations, 5-HT application affected the frequency of inspiratory discharges recorded from a phrenic nerve root in newborn rat [13,14]. These effects were suppressed after a transection performed at the level of the ponto–medullary junction [14]. In an experiment using newborn rat medulla–spinal cord (MS) preparations, 5-HT application caused excitatory or inhibitory effects depending on the resting frequency of inspiratory discharges before the 5-HT application [15]. Therefore, the effects of 5-HT on respiratory rhythm generation vary depending on the preparation or can

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http://dx.doi.org/10.1016/j.neulet.2013.12.002

be altered according to the level of transection [14], with distinct effects caused by the activation (or inactivation) of various 5-HT receptor groups and subtypes [6].

In such in vitro preparations, specific actions of 5-HT on neurons located in the rostral ventrolateral medulla (RVLM) have been detected in PMS preparations [14]. Specifically, 5-HT application may disturb the correlation between pre-inspiratory and inspiratory neuronal discharges in RVLM of newborn rat MS preparations [15]. Pre-inspiratory and inspiratory neuronal activities in RVLM have been observed particularly in the region ventrolateral to the facial nucleus (nVII) [16] and in the functional group of neurons that correspond to the retrotrapezoid nucleus/parafacial respiratory group (RTN/pFRG). More recently, the importance of RTN/pFRG in central respiratory rhythm generation and chemoreception has become widely recognized [4,5,17]. Therefore, special attention should be accorded to the RTN/pFRG region in the RVLM as a potential regulator of respiratory rhythm.

In this study, we transected newborn rat PMS preparations at different levels relative to nVII, which we used as a landmark for the RTN/pFRG region [4,5,18]. We applied 5-HT to the preparations before and after the transection, and examined whether varying the level of transection changed the effects of 5-HT application on the frequency of respiratory-like nerve (f_R-like) activities.

2. Materials and methods

The experimental protocol was reviewed and approved by the Animal Research Committee of Nippon Dental University, School of Life Dentistry at Tokyo. The animals were treated in accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences (The Physiological Society of Japan) and the American Physiological Society's Guiding Principles for Research Involving Animals and Human Beings. In addition, efforts were made to minimize suffering and the number of animals used.

Newborn rats (Wistar, 2–3 days old, $n = 45$) were deeply anesthetized with ether. The brainstem was rostrally decerebrated at the intercollicular level, the cerebellum was removed, and the spinal cord was transected at the C7–C8 level to create the PMS preparations. As will be described below, the pons was further eliminated from the PMS preparation [19] by pontomedullary transection to create the MS preparation. Each preparation was superfused at a rate of 2.5 ml min^{-1} in a 3-ml recording chamber with artificial cerebrospinal fluid (aCSF; pH, 7.4) and equilibrated with oxygenated gas (95% O_2 plus 5% CO_2). The aCSF had the following composition (mM) [19,20]: KCl, 3.0; NaCl, 128; $MgSO_4$, 1.0; $NaHCO_3$, 24; NaH_2PO_4, 0.5; $CaCl_2$, 1.5; and D-glucose, 30. The chamber temperature was continuously monitored throughout the experiments and controlled to 25.6 ± 0.9 °C.

As described previously [19], each preparation was placed with the ventral surface upward in the chamber. To monitor respiratory-like activity and obtain its frequency (f_R-like, min^{-1}), the dissected cervical C4 ventral root was recorded with glass suction electrode connected to amplifiers (DAM-50, World Precision Instruments, Inc., FL). The nerve signal was amplified and band-pass filtered (0.3–3 kHz). Data were recorded on paper (Omniace 8100, NEC, Tokyo, Japan) and stored on a computer using a software interface (PowerLab®, ADInstruments Japan, Tokyo, Japan) at a sampling frequency of 2 kHz on each recording signal for subsequent data analysis.

To assess the effects of 5-HT application on respiratory-like (f_R-like) activity, serotonin creatinine sulfate complex (Sigma–Aldrich Co., St. Louis, MO) was first dissolved in aCSF at concentrations of 1, 5, 10, 30, and 50 μM 5-HT. Each concentration of 5-HT was applied for 5–7 min to a preparation by superfusion, followed by inflow of normal aCSF for 10–15 min. For normal aCSF superfusion and 5-HT application, we placed three pipes over the chamber: a main pipe to provide continuous inflow of normal aCSF during the experiment, and two small pipes (PE50, Becton Dickinson and Co., Sparks, MD, USA) to superfuse a vehicle or 5-HT solution (Fig. 1A). Before and after consecutive applications of vehicle and 5-HT, the small flow pipes were entirely closed and normal aCSF was applied through the main pipe at a flow rate of 2.5 ml min^{-1}. During consecutive applications of vehicle and 5-HT through the small flow pipes (1.5 ml min^{-1}), the flow rate of normal aCSF through the main pipe was reduced from 2.5 to 1.0 ml min^{-1}. The change in f_R-like activity during 5-HT application was expressed as the ratio (%) of the activity obtained from 2 to 5 min after drug application to the resting

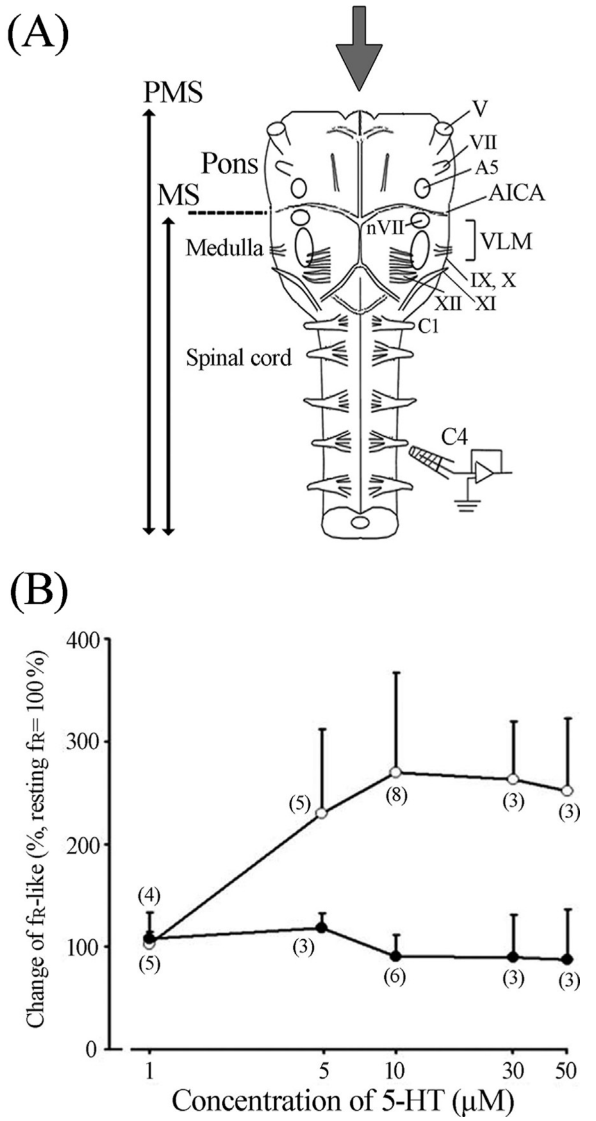

Fig. 1. (A) Pons–medulla–spinal cord (PMS) and medulla–spinal cord (MS) preparations, drawn with the ventral surface facing upward. Dashed line indicates the approximate boundary between the pons and medulla. The gray arrow at the top indicates the direction of inflow of the normal aCSF and 5-HT solutions. *Abbreviations*: AICA: anterior inferior cerebellar artery; A5: area of A5 noradrenergic neurons; C1 and C4: 1st and 4th cervical spinal ventral roots; nVII: facial nucleus; VLM: ventrolateral medulla. (B) Dose–response curves of the effect of 5-HT on the f_R-like activity in the PMS preparations (open circles) and MS preparations (filled circles) measured at C4. The response of the f_R-like activity to 5-HT application is expressed as the change from the resting f_R-like activity (=100%), which was obtained during application of the vehicle just before each 5-HT application. In total, we used 15 preparations (PMS, $n = 8$; MS, $n = 7$); the number of preparations used for each data point are indicated in parentheses.