Showa Univ J Med Sci **26**(3), 211～217, September 2014

Original

Early Onset of Ventilatory and Airway Response to Hypercapnia is Mediated by Medullary 5-HT_{1A} Receptors in Infant Rats

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Abstract: Medullary 5-hydroxytryptamine (5-HT) neurons are involved in ventilatory responses to hypercapnia. Underdeveloped medullary 5-HT neurons and reduced 5-HT_{1A} receptor binding activity in the dorsomedial medulla oblongata (DMM) have been found in infants with sudden infant death syndrome (SIDS). The DMM includes the solitary tract nucleus, which receives primary afferent inputs from the lung, and the hypoglossal nucleus, which affects genioglossal muscle tone. We hypothesized that hypercapnia would elicit 5-HT release in the DMM and that local 5-HT_{1A} receptors would affect ventilatory and airway responses to hypercapnia. This hypothesis was investigated in unanesthetized infant Wistar rats by microdialysis of the DMM coupled with double-chamber plethysmography. After microdialysis probe placement, the rats were acclimatized to the chamber for over 5 h, and artificial cerebrospinal fluid (aCSF) or a 5-HT_{1A} receptor antagonist, WAY-100635, was then perfused into the DMM, and extracellular fluid was collected. Respiratory flow curves were recorded while the rats inhaled five concentrations of CO_2 in O_2 for 10 min each (0% [100% O_2], 5%, 7%, 9%, and 0% again). 5-HT concentration was measured using high-performance liquid chromatography with electrochemical detection. 5-HT release in the DMM and hypercapnic ventilatory and airway responses increased dose dependently with CO_2 concentration during both aCSF and WAY-100635 perfusion, with no difference between groups. However, early-onset ventilatory and airway responses to hypercapnia were significantly delayed or reduced by WAY-100635 perfusion in the DMM. These results suggest that 5-HT release in the DMM is dependent on hypercapnia, while early ventilatory and airway responses to hypercapnia are mediated by 5-HT_{1A} receptors in the DMM. Blunted early onset of hypercapnic ventilatory and airway responses may be one cause of SIDS.

Key words: 5-HT, hypercapnia, upper airway, respiration, sudden infant death syndrome (SIDS)

Introduction

Caudal 5-hydroxytryptamine (5-HT) neurons are concentrated in the raphe nuclei in the medulla oblongata of the brainstem. These medullary 5-HT neurons receive sensory inputs

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for O_2, CO_2, and temperature, and regulate protective responses to physical stresses such as hyperthermia, hypoxia, and hypercapnia[1]. Medullary 5-HT neurons project to respiration-related neurons in the ventral medulla and affect respiratory patterns[2]. They act as sensors of CO_2 and pH[3] and enhance ventilatory responses to hypercapnia[4]. They also project to the hypoglossal and solitary tract nuclei of the dorsomedial medulla oblongata (DMM)[5,6]. The hypoglossal nucleus regulates genioglossal muscle tone, which is known to act against obstructive sleep apnea[7]. The solitary tract nucleus receives primary afferent inputs from the lungs. Therefore, it is likely that ventilatory and airway responses to hypercapnia are controlled, at least in part, by medullary 5-HT projections to the DMM.

Sudden infant death syndrome (SIDS) is one of the leading causes of infant mortality in developed countries. The syndrome occurs while the child is sleeping. The mechanism of SIDS may involve deficits in the medullary serotonergic network[8]. Postmortem brains of infants with SIDS show immature medullary 5-HT neurons and decreased binding activity to 5-HT_{1A} receptors in the DMM[9]. Binding density of 5-HT_{1A} receptors in the hypoglossal nucleus increases during infancy in humans[10]. Perfusion of 5-HT into the hypoglossal nucleus increases genioglossal muscle activity, suggesting a mechanism underlying obstructive sleep apnea[11,12]. Therefore, it is important to determine the role of 5-HT_{1A} receptors in upper airway control in the hypoglossal nucleus, and their role in respiratory control in the solitary tract nucleus.

We hypothesized that hypercapnia elicits 5-HT release and that 5-HT acting on 5-HT_{1A} receptors in the DMM affects ventilatory and airway responses to hypercapnia in infant rats. We tested this hypothesis using microdialysis with double-chamber plethysmography in unanesthetized infant rats.

Materials and methods

Male and female pre-weaned Wistar rats (14 ～ 22 days old, 34.4 ± 2.2 g; Saitama Experimental Animals Supply Co., Saitama, Japan) were used. The mothers had free access to food and water, and were housed under a 12 / 12 h light / dark cycle with lights on at 8:00 AM. This study was reviewed and approved by the Institutional Animal Care and Use Committee of Showa University.

The pups were anesthetized with intraperitoneal pentobarbital sodium (0.5 mg / 0.1 ml saline / 10 g body weight), fixed on a stereotaxic frame for mice with the tooth bar lowered 10 mm for pups, and the head region locally anesthetized with 0.5% bupivacaine hydrochloride hydrate injection. A microdialysis probe (CMA / 7; 1-mm membrane length; 0.24-mm diameter; 6000-Da cutoff; Carnegie Medicin, Stockholm, Sweden) was inserted into the DMM (0.5 mm lateral to the midline, 0.8 mm rostral to the obex, and 1 mm ventral to the dorsal brain surface) and fixed to the cranial bone with dental cement. The subsequent experimental procedures have been described in detail previously[13,14]. Briefly, the rats were placed in a double-chamber plethysmograph with air flow provided to each chamber at 150 ml / min. Rectal temperature was controlled at 37°C by a heating blanket during surgery and a heat lamp throughout the entire experimental period. Artificial cerebrospinal fluid (aCSF; 121.1 mM NaCl, 5 mM KCl,

24 mM $NaHCO_3$, and 1.5 mM $CaCl_2$) was adjusted to pH 7.4 with 95% O_2 and 5% CO_2. A 5-HT_{1A} receptor antagonist, WAY-100635 (Sigma-Aldrich, St. Louis, MO) was dissolved in aCSF to 10^{-5} M. The animals were allowed over 5 h to recover from anesthesia and to become acclimatized to the chamber. Extracellular fluid was then collected every 10 min. 5-HT concentrations were measured by high-performance liquid chromatography with electrochemical detection (HTEC-500; EiCOM, Kyoto, Japan).

In the aCSF group (n = 4), aCSF was perfused at 1.2 μl/min throughout the entire experimental period; in the 5-HT_{1A} antagonist group (n = 4), aCSF was perfused for 10 min then changed to 10^{-5} M WAY-100635. Both groups inhaled five concentrations of CO_2 in O_2 balanced gas (0% [100% O_2], 5%, 7%, 9%, and 0% again) for 10 min each at a flow rate of 2 l/min.

Respiratory flow curves from the head and body chambers were obtained and recorded by a PowerLab system (ADInstruments, Bella Vista, NSW, Australia). Tidal volume (V_T) was measured by a respiratory flow curve from the head chamber and calibrated using five 0.5 ml injections of air. Specific airway resistance (sR_{aw}) was calculated with a time delay between the head and body chamber flows. Ventilatory and airway variables were measured for the last 0.5 min of inhalation at each CO_2 concentration except 5%, where measurements were made every 0.5 min.

After experimentation, the animals were humanely killed and their brains were removed and fixed in formalin. Microdialysis probe placement was verified in 50-μm thick coronal sections under a light microscope. 5-HT release in the DMM and ventilatory and airway variables were expressed as mean ± SEM in both perfusion groups. Results were analyzed with a two-way repeated-measures analysis of variance using SPSS software. $P < 0.05$ was considered statistically significant.

Results

Hypercapnic gas inhalation significantly increased 5-HT release in the DMM in a dose-dependent manner, with no difference between the aCSF and WAY-100635 groups (Fig. 1, top). The microdialysis probe sites were in the solitary tract nucleus, the dorsal motor nucleus of the vagus nerve, and the hypoglossal nucleus. Their distributions were similar in both groups (Fig. 1, bottom).

Inhalation of gas at increasing CO_2 concentration significantly increased the respiratory rate (RR), V_T, minute ventilation ($\dot{V}_E$), and the change in sR_{aw} (ΔsR_{aw}) in a dose-dependent manner, with no significant difference between the aCSF and WAY-100635 groups (Fig. 2).

There was a significant interaction between time and perfusion medium on the onset of hypercapnic responses at 5% CO_2. RR, V_T, and $\dot{V}_E$ increased gradually, reaching a peak at 1.5 ~ 2.0 min in the aCSF group, and values were significantly higher than in the WAY-100635 group until 2 min (Fig. 3). There was a main effect of perfusion medium on ΔsR_{aw}. In the aCSF group, ΔsR_{aw} increased slowly and reached a peak at 3.5 min during 5% CO_2 inhalation, and was significantly higher than the WAY-100635 group.

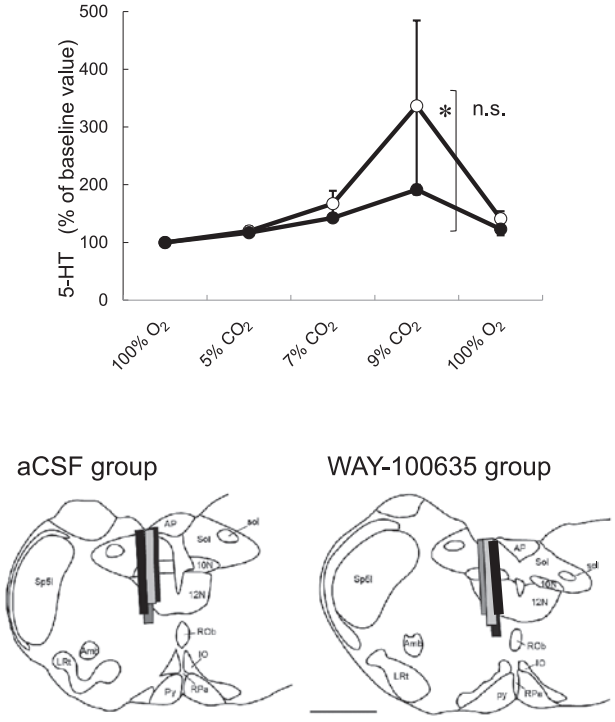

Fig. 1. Effect of CO_2 inhalation on 5-hydroxytryptamine (5-HT) release during artificial cerebrospinal fluid (aCSF) or WAY-100635 perfusion in the dorsomedial medulla oblongata.
Top: 5-HT release, expressed as the percentage of 5-HT released during the first inhalation of 100% O_2. Data are the mean ± SEM (n = 4 in the aCSF group, open circles; n = 4 in the WAY-100635 group, closed circles). *significant effect of CO_2 concentration; n.s., no significant difference between groups.
Bottom: microdialysis probe placement. AP, area postrema; 10N, dorsal motor nucleus of the vagus nerve; sol, solitary tract; Sol, solitary tract nucleus; 12N, hypoglossal nucleus; Sp5I, spinal trigeminal nucleus, interpolar part; ROb, raphe obscurus nucleus; Amb, ambiguus nucleus; LRt, lateral reticular nucleus; IO, inferior olivary nucleus; RPa, raphe pallidus nucleus; py, pyramidal tract. Scale bar = 1 mm.

Discussion

In this study we found that 1) 5-HT release in the DMM, including the solitary tract nucleus and the hypoglossal nucleus, of infant rats increased dose dependently with increasing CO_2 concentration; 2) the values of RR, V_T, $\dot{V}_E$, and ΔsR_{aw} also showed a dose-dependent increase with CO_2 concentration, which was not affected by the 5-HT_{1A} receptor antagonist; and 3) the early onset of hypercapnic ventilatory and airway responses was significantly delayed and reduced by 5-HT_{1A} blockade in the DMM.

Hypercapnia dose-dependently increased the 5-HT release in the DMM in the present study. The pH / CO_2 level is detected by medullary raphe 5-HT neurons[3], which project to the hypoglossal and solitary tract nuclei[5,6]. Therefore, the DMM is one of the projection and activation sites of medullary 5-HT neurons when they act as CO_2 / pH sensors.

The changes in RR, V_T, $\dot{V}_E$, and ΔsR_{aw} also increased dose dependently with CO_2 concentration, but these changes were not dependent on 5-HT_{1A} receptor activity in the DMM. Medullary raphe 5-HT neurons enhance ventilatory responses to hypercapnia owing to an increase in V_T in rats[4]. In 5-HT_{1A} receptor knockout mice, ventilation is lower than in wild-type mice in early postnatal development, but ventilatory responses to hypercapnia are not affected[15]. It is likely that the effects of 5-HT release in the DMM of our infant rats are mediated by 5-HT receptors other than 5-HT_{1A}.

In the present study, however, the early onset of ventilatory and airway responses to hypercapnia was significantly delayed and reduced by a 5-HT_{1A} antagonist in the DMM of infant rats. Upper airway muscle activity is increased by increasing upper airway negative pressure[16].

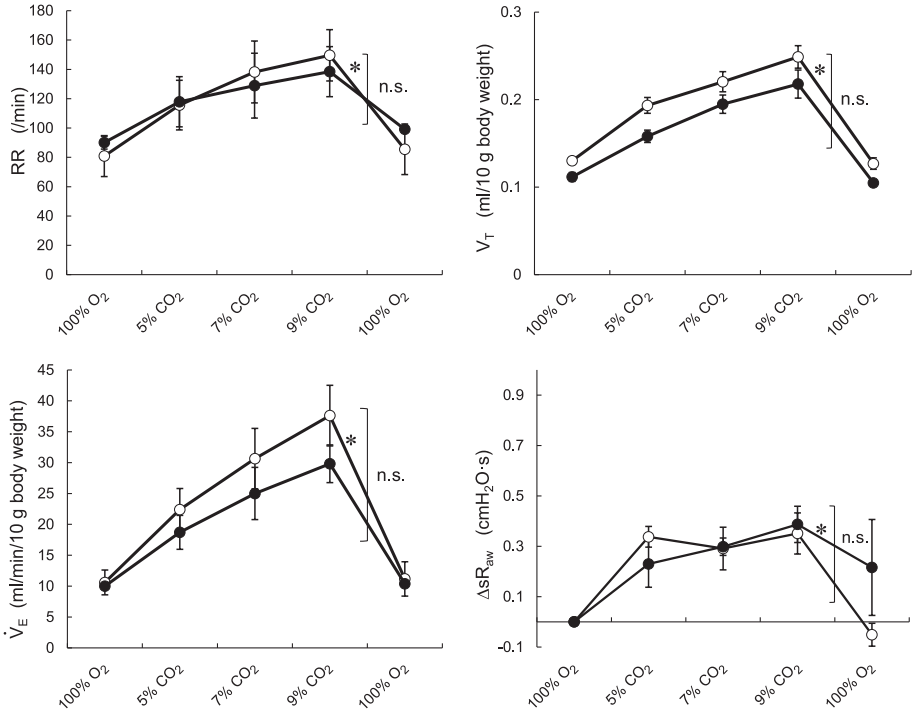

Fig. 2. Effect of CO_2 inhalation on ventilatory and airway variables during artificial cerebrospinal fluid (aCSF) or WAY-100635 perfusion in the dorsomedial medulla oblongata. Data are the mean ± SEM (n = 4 in the aCSF group, open circles; n = 4 in the WAY-100635 group, closed circles). RR, respiratory rate; V_T, tidal volume; $\dot{V}_E$, minute ventilation; ΔsR_{aw}, change in specific airway resistance from first inhalation of 100% O_2. *significant effect of CO_2 concentration; n.s., no significant difference between perfusion groups.

Additionally, negative pressure of the upper airway decreases the RR depending on the magnitude of the pressure change[17]. These findings are contrary to our results which suggest that the hypercapnic ventilatory and airway responses are not reflex responses to hypercapnic ventilatory augmentation.

5-HT release in the DMM, including the hypoglossal nucleus, is related to tone in the postural and respiratory muscles[18] and hypoxia / hypercapnia[13, 14, 19]. In infant rats, hypoglossal motoneuronal inputs from the raphe pallidus are reduced by a decrease in glutamate release via presynaptic 5-HT$_{1A}$ receptors[20], and medullary 5-HT neurons are CO_2 / pH sensors[3]. We therefore suggest the following pathway: 1) medullary raphe 5-HT neurons sense the CO_2 / pH level, leading to a co-release of 5-HT and glutamate in the hypoglossal nucleus and decreased glutamate release via presynaptic 5-HT$_{1A}$ receptors; and 2) the lowered glutamate release suppresses excitability of the hypoglossal motoneurons, hypoglossal nerves, and genioglossal muscle tone, leading to airway narrowing.

An increase in respiratory load enhances intercostal motor activity, which some believe compensates for respiratory load[21]. 5-HT in the hypoglossal nucleus affects hypoglossal nerve and genioglossal muscle activities[11, 12, 22, 23]. Therefore, in the current study, the early onset of ventilatory responses to hypercapnia may be a type of respiratory load compensation due to

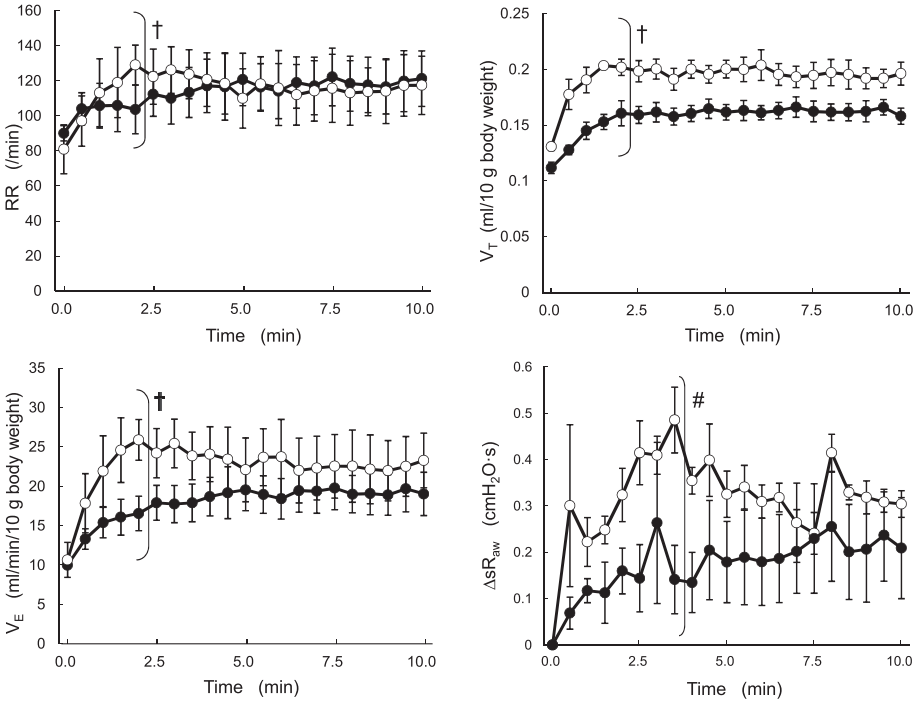

Fig. 3. Effect of 5% CO_2 inhalation on ventilatory and airway variables during artificial cerebrospinal fluid (aCSF) or WAY-100635 perfusion in the dorsomedial medulla oblongata. Data are the mean ± SEM (n = 4 in the aCSF group, open circles; n = 4 in the WAY-100635 group, closed circles). RR, respiratory rate; V_T, tidal volume; $\dot{V}_E$, minute ventilation; ΔsR_{aw}, change in specific airway resistance from first inhalation of 100% O_2. † significant interaction between time and perfusion medium. #significant effect of perfusion medium.

airway narrowing elicited by 5-HT_{1A} receptors in the hypoglossal nucleus.

In summary, the present findings suggest that in infant rats, 1) 5-HT release is present in the DMM and dependent on the degree of hypercapnia; and 2) early onset of hypercapnic ventilatory augmentation and hypercapnic airway narrowing is mediated by 5-HT_{1A} receptors in the DMM. Although future research is needed to validate these claims, a delayed and reduced onset of ventilatory and airway responses to hypercapnia may be one of the causes of SIDS.

Conflict of interest

There is no conflict of interest to disclose concerning this study.

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[Received June 24, 2014 : Accepted July 3, 2014]