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Effects of arundic acid, an astrocytic modulator, on the cerebral and respiratory functions in severe hypoxia

Isato Fukushi[a,b], Kotaro Takeda[b,c], Shigefumi Yokota[d], Yohei Hasebe[b,e], Yutaka Sato[f], Mieczyslaw Pokorski[b,g], Jouji Horiuchi[a], Yasumasa Okada[b,*]

[a] Department of Biomedical Engineering, Graduate School of Science and Engineering, Toyo University, 2100 Kujirai, Kawagoe, Saitama 350-8585, Japan
[b] Clinical Research Center, Murayama Medical Center, 2-37-1 Gakuen, Musashimurayama, Tokyo 208-0011, Japan
[c] Fujita Memorial Nanakuri Institute, Fujita Health University, 423 Oodori-cho, Tsu, Mie 514-1296, Japan
[d] Department of Anatomy and Morphological Neuroscience, Shimane University School of Medicine, 89-1 Enya-cho, Izumo 693-8501, Japan
[e] Department of Pediatrics, School of Medicine, University of Yamanashi, 1110 Shimokawato, Chuo, Yamanashi 409-3898, Japan
[f] Institute of Socio-Arts and Sciences, The University of Tokushima, 1-1 Minamijosanjima-cho, Tokushima 770-8502, Japan
[g] Public Higher Medical Professional School in Opole, 68 Katowicka Street, Opole 45-060, Poland

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ABSTRACT

Mild hypoxia increases ventilation, but severe hypoxia depresses it. The mechanism of hypoxic ventilatory depression, in particular, the functional role of the cerebrum, is not fully understood. Recent progress in glial physiology has provided evidence that astrocytes play active roles in information processing in various brain functions. We investigated the hypothesis that astrocytic activation is necessary to maintain the cerebral function and ventilation in hypoxia, by examining the responses of EEG and ventilation to severe hypoxia before and after administration of a modulator of astrocytic function, arundic acid, in unanesthetized mice. Ventilatory parameters were measured by whole body plethysmography. When hypoxic ventilatory depression occurred, gamma frequency band of EEG was suppressed. Arundic acid further suppressed ventilation, and the EEG power was suppressed in a dose-dependent manner. Arundic acid also suppressed hypoxia-induced c-Fos expression in the hypothalamus. We conclude that severe hypoxia suppresses the cerebral function which could reduce the stimulus to the brainstem resulting in ventilatory depression. Astrocytic activation in hypoxia may counteract both cerebral and ventilatory suppression.

1. Introduction

Mild hypoxia in conscious men induces shortness of breath and increases ventilation. The brainstem respiratory neuronal network receives redundant stimulatory input on exposure to hypoxia. One comes from the sensory discharge of carotid arterial chemoreceptors which sense a reduction in oxygen content and the other comes from the sensory feeling of dyspnea perceived by the forebrain and relayed via the hypothalamic pathway. When hypoxia is too severe, ventilation is reduced, which further worsens hypoxia and eventually causes respiratory arrest and death. This phenomenon is seen in patients with severe asthma (Kikuchi et al., 1994) as well as in healthy subjects who are accidentally exposed to severe hypoxic environment.

The mechanism of severe hypoxia-induced ventilatory depression is not fully understood. Several explanations have been proposed focusing on the brainstem neuron network function: (1) alkaline shift in brain extracellular fluid due to increased cerebral blood flow which would suppress the central respiratory chemosensitivity, (2) increases in the synthesis or release of the inhibitory neurotransmitters and neuromodulators including GABA, adenosine, and endogenous opioids, and (3) metabolic impairment of respiratory neurons due to O_2 deficiency (Hayashi and Fukuda, 2000; Neubauer et al., 1990). Regarding the role of the higher brain in the hypoxic ventilatory depression, there has been no convincing study. In particular, the functional role of the cerebrum in hypoxic ventilatory depression has not been clarified.

Recent progress in glial physiology has provided evidence that astrocytes play an active role in information processing in various brain functions (Halassa and Haydon, 2010; Parpura et al., 2012). Therefore, in the present study we investigated the hypothesis that astrocytes are involved in the maintenance of the cerebral function and ventilation in a hypoxic condition. We further presumed

* Corresponding author at: Clinical Research Center, Murayama Medical Center, 2-37-1 Gakuen, Musashimurayama, Tokyo 208-0011, Japan. Fax: +81 42 564 2210.
E-mail address: yasumasaokada@1979.jukuin.keio.ac.jp (Y. Okada).

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that hypoxia, if severe enough, would suppress the higher brain function, manifested by the dimmed consciousness and suppressed hypothalamic activity, resulting in hypoxic ventilatory depression. We addressed the issue by examining the responses of EEG and ventilation to severe hypoxia before and after administration of a modulator of astrocytic function.

2. Materials and methods

2.1. Animals

All experiments were performed with the approval of the Animal Experiment Ethics Committee of the Murayama Medical Center, and were in accordance with the Guiding Principles for the Care and Use of Animals of the Physiological Society of Japan. Sixteen conscious, spontaneously breathing, adult male C57BL/6 mice were used in functional study. Two adult male mice were used in immunohistochemistry study. The mice were housed in separate cages at 23–24 °C, 50–60% relative humidity, and 12/12 h light/dark cycle, and were fed with commercial chow and water ad libitum.

2.2. Recording of EEG

To monitor the functional status of the forebrain, the electroencephalogram (EEG) was recorded. Surgical procedures to implant EEG electrodes on the skull were carried out under isoflurane anesthesia, followed by intraperitoneally injected pentobarbital. The skull was exposed and 3 miniature screws were inserted; two were over the frontal lobes at 2.5 mm laterally and at 2.5 mm posterior to the bregma as recording electrodes, and one was along the midline at 4.5 mm anterior to the bregma as a ground electrode. The implanted electrodes, together with an additional screw for mounting the head, were fixed to the skull with dental resin and adhesive. The mice were allowed to recover from surgery for at least 1 week until EEG recordings began. EEG signal was amplified (JB-101J and AB-651J, Nihon Kohden, Tokyo, Japan) and filtered in 0.08–100 Hz frequency range. To calculate the power of gamma band (55–95 Hz), which is a hallmark of the waking state (Bosman et al., 2014; Ishibashi et al., 2015), the EEG time signal was transferred to frequency domain via fast Fourier transform.

2.3. Recording of ventilation

The hypoxic ventilatory response was measured in a whole body rodent plethysmograph (PLY 310, EMMS, Bordon, UK) consisting of the recording (volume 530 mL) and reference chambers, as previously described (Oyamada et al., 2008; Pokorski et al., 2014). Briefly, the chambers were placed inside a transparent acrylic box (size 20 × 20 × 20 cm). Each mouse was placed in the pre-calibrated recording chamber. Chamber temperature was maintained constant at 25 °C throughout the experiment. The air in the recording chamber was suctioned with a constant flow generator. To calculate the respiratory flow, the pressure difference between the recording and reference chambers was measured with a differential pressure transducer (TPF100, EMMS), connected to an amplifier (AIU060, Information & Display Systems, Bordon, UK), and was bandpass filtered at 0.1–20 Hz. The signal was integrated to obtain tidal volume (V_T [μL/weight (gram)]) for each respiratory cycle, which was then averaged throughout the period of interest. Respiratory rate (RR [breath/min]) was counted. Minute ventilation ($\dot{V}_E$ [mL/g/min]) was calculated as $V_T \times RR$.

The O_2 concentration in the chamber was monitored with an O_2 analyzer incorporating a polarographic sensor (Respina IH 26, San-ei, Tokyo, Japan), and was adjusted by controlling the mixing of N_2 and air flows blown into the acrylic box. The pressure and EEG signals, together with O_2 concentration data were simultaneously digitized at 400 Hz sampling with an A/D converter (PowerLab4/26, ADInstruments, Colorado Springs, CO., USA) and stored in a PC with LabChart7 software (ADInstruments).

2.4. Experimental protocol for whole body plethysmography

The hypoxic ventilatory depression was analyzed before and after administration of arundic acid, a modulator of astrocytic function (Asano et al., 2005a,b; Mori et al., 2004; Tateishi et al., 2002; Wajima et al., 2013; Yamamura et al., 2013; Yanagisawa et al., 2015). Firstly, dimethyl sulfoxide (DMSO), a solvent for arundic acid, was injected as a vehicle, and the mouse was put into the chamber to acclimatize in room air for 40–60 min, after which the measurements of ventilation and EEG began. The gas mixture was then switched to 6% O_2 (N_2 balanced) and ventilatory variables were continuously recorded until the ventilatory fall-off started. Hypoxic loading was discontinued if the ventilatory fall-off did not occur in 20 min. Data in mice that did not show the fall-off were excluded from analysis. Ventilation was then recorded for further 10–20 s, followed by a switch back to room air. The hypoxic responses were tested after injection of DMSO and two doses of arundic acid with 60 min intervals. The injections were made in the following sequence: (1) vehicle—0.47 mL/kg DMSO, (2) arundic acid—100 mg/kg, (3) arundic acid—200 mg/kg,—cumulative dose of 300 mg/kg. Arundic acid was dissolved in a mixture of DMSO and saline, and the proportion of arundic acid, DMSO and saline was 1:4:5 v/v. A total dose of DMSO did not exceed 2.0 g/kg. Although DMSO alone could affect brain function when the dose is high (Hülsmann et al., 1999; Jacob and de la Torre, 2009), the total dose in the present study does not affect respiratory function (Takeda et al., in press). All injections were intraperitoneal.

2.5. Immunohistochemistry of c-Fos

We compared c-Fos expression without and with administration of arundic acid (300 mg/kg) in mice that were exposed to severe hypoxia. In a control mouse, DMSO (0.47 mL/kg) was injected as a vehicle. After the mice were acclimated in a whole body plethysmography chamber breathing room air for 60 min, they were exposed to 7% O_2 hypoxia (N_2 balanced) for 40 min followed by another 60 min of room air breathing. Because 6% O_2 hypoxia is too severe when exposed for 40 min, we chose 7% O_2 in immunohistochemistry (Miyake et al., 2007). Then the mice were deeply anesthetized with diethyl ether and transcardially perfused with saline, followed by 10% formalin. Brains were removed, postfixed overnight, and immersed in 20% sucrose, and the hypothalamus were cut into frontal sections at 25 μm thickness on a frozen microtome. Sections were Nissl stained for the identification of the dorsomedial hypothalamic nucleus (DMH) an important region in respiratory control (Dampney et al., 2008; Horiuchi et al., 2009), and were processed for detection of hypoxia-induced c-Fos. The technique of c-Fos immunohistochemistry was as previously described (Yokota et al., 2015), with the difference that we presently used goat anti-c-Fos antibody, sc-52-G, (diluted 1:500, Santa Cruz Biotechnology, Dallas, TX, USA) as primary antibody and biotinylated donkey anti-goat IgG (diluted 1:500, Jackson ImmunoResearch Laboratories, West Grove, PA, USA) as secondary antibody.

2.6. Data analysis

The periods during which the mouse moved (e.g., sniffing, grooming, and licking) were excluded from the calculations. The mean values of V_T, RR, $\dot{V}_E$ and EEG power of the gamma band were respectively submitted to a two factor within-subject ANOVA; with