Expression of AMPA Glutamate Receptors on RVLM-projecting nTS Neurons in Response to Chronic Intermittent Hypoxia

Jessica R. Howard, Eileen M. Hasser, David D. Kline
Dept. of Biomedical Sciences and Dalton Cardiovascular Res. Ctr., Univ. of Missouri, Columbia, MO 65211

Abstract

Obliterative sleep apnea (OSA) is a common disorder affecting 10% of the adult population. Individuals with OSA experience periods of arterial hypoxemia during sleep. Chronic intermittent hypoxia (CIH) in rats is a model for OSA. Humans with OSA and rats with CIH both show an increase in sympathetic nerve activity and hypertension. CIH has been attributed to an augmented carotid body chemoreflex. Chemosensor afferents form a synapse on second-order neurones in the nucleus of the solitary tract (NTS) before projecting to other areas of the brain. Projections from the NTS to the ventrolateral medulla (RVLM) have been shown to be responsible for the increases in sympathetic nerve activity with CIH. The primary excitatory neurotransmitter released by chemosensor afferents is glutamate, which binds to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptors within the NTS. Our lab has shown that synaptic glutamatergic plasticity within the NTS in response to CIH is associated with the physiological effects observed with CIH. Based on our previous results and the observed increase in sympathetic nerve activity in response to CIH, we hypothesized that AMPA glutamate receptors in the NTS are up-regulated after CIH. To test this hypothesis, rats will be exposed to CIH for 10 days, perfused, and tissue sections of the NTS will be generated for immunohistochemistry of AMPA receptor subunits in normoxically labeled RVLM-projecting cells. Western blot analysis of CIH issue will be performed to confirm expression levels after exposure to CIH.

Introduction

The carotid body chemoreflex serves as a physiological mechanism to increase respiration, blood pressure, and sympathetic nerve system activity when hypoxic chemoreflex saturation decreases past 75%. Decreases in arterial O2 (hypoxic) are sensed by arterial chemoreceptors, which increaseafferent chemoreceptor impulses to the brainstem nucleus of the solitary tract (NTS). These chemosensory afferents form a synapse on second-order neurones in the NTS. Afferent information is processed and integrated in the NTS before being sent to other cardiorespiratory nuclei.

Patients with obstructive sleep apnea (OSA) exhibit alterations in respiration, elevated sympathetic nerve activity, and sustained hypertension. These alterations are due to an increased sensitivity of the chemoreflex. Animal models of OSA using chronic intermittent hypoxia (CIH) have shown similar physiologic effects. Patients with OSA and rats exposed to CIH experience cyclic periods of arterial hypoxemia during their nocturnal hours. The cyclic periods of hypoxia activate the chemoreflex and culminate in persistently increased chemoreceptor afferent signaling to the NTS. The primary excitatory neurotransmitter released by chemoreceptor afferents is glutamate, which binds to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptors. AMPA glutamate receptors are ionotropic, heterodimeric receptors consisting of at least one GluR1 subunit, with a combination of GluR2, GluR3 and GluR4 subunits.

Methods

Abdominopereineal resection mice (TU), Sprague-Dawley rats and Fischer 344 rats were housed in a climatic chamber at 22°C, with a 12:12 h light-dark cycle. Food and water were available ad lib. For CIH, rats were placed in a whole body plethysmograph (Buxco) and exposed to 12% O2 for 10 h/day for 14 days. Exposure to hypoxia by CIH for 14 days induced an increase in glutamate receptor subunits and proteins in the NTS. The increase in receptor subunits and proteins corresponded with an increase in chemoreflex activity.

Immunohistochemistry: Rats were deeply anesthetized and perfused transcardially with 4% paraformaldehyde. The brainstem was collected and postfixed in 4% paraformaldehyde before being cryoprotected and frozen. Coronal sections were cut at 10 µm, mounted on gelatin-coated slides, and stored at -20°C. The sections were then immunostained with antibodies specific to GluR2 (2 µg/mL, Chemicon) in 1% normal donkey serum, 0.1% PBS, and 0.2% Triton-X 100 in PBS for 2 h at room temperature. Immunoreactive tissue was visualized using an FITC-conjugated antirabbit IgG (1:100, Molecular Probes). Sections were counterstained with DAPI (1:1000, Molecular Probes). Sections were imaged with an Axioplan 2 Imaging microscope (Zeiss) with a 20× objective, and images were acquired using ImageJ software.

Results

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Conclusions

Studies in Progress

Immunohistochemistry (IHC) has been performed on GluR1 subunits and data is being analyzed. Preliminary data shows high GluR1 subunits within the region of the nTS and AP border. GluR2 subunit IHC is being analyzed to determine if rostral-caudal location within the nTS affects number of subunits and/or degree to which they are expressed on RVLM projecting neurons. Immunohistochemistry will be performed on rostral and caudal sections of nTS.

Summary

There is an increase in cells expressing the GluR2 subunit that project to the RVLM in response to CIH.

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