Acute hypoxia (AH) activates nNOS and astrocyte associated nNOS containing cells in the paraventricular nucleus of the hypothalamus (PVN)

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Introduction

Patients with chronic obstructive sleep apnea suffer from hypertension (Bradley 2005), which can last into waking hours (Narkiewicz and Somers 2003). Hypoxia experienced during apneic episodes excites chemoreceptor afferent nerves and causes increased activation of effenter sympathetic nerves and hypertension (Dempsey et al. 2010). The paraventricular nucleus (PVN) of the hypothalamus integrates cardiorespiratory signals including those from the arterial chemoreflex (King et al. 2012) and is activated during acute hypoxia (Berguin et al. 2000). However, previous studies show that during acute hypoxia, hypotension is experienced rather than hypertension (King et al. 2012). Consistent with this observation, pre-sympathetic, spinally projecting cells within the PVN do not exhibit increased activation during acute hypoxia. Rather, increased activation (as indicated by Fos-IR) was observed in cells containing neuronal nitric oxide synthase (nNOS); these cells were neither pre-sympathetic spinally projecting nor vasopressinergic (Coldren et al. 2013). It is possible the activated nNOS containing cells are producing nitric oxide, which has a known inhibitory action on pre-sympathetic (Rossi et al. 2015) and magnocellular neurons (Stenn and Zhang 2005) within the PVN. Astrocytes and CRH-releasing neurons are known to contain nNOS and could be activated under hypoxic conditions. Moreover, along with the pre-sympathetic terminal and post-sympathetic neuron, astrocytes are an important component of the tripartite synapse and play an important role in modulation of synaptic transmission.

Hypothesis

Therefore, we hypothesize that activated nNOS containing cells (as indicated by Fos-IR) and activated pre-sympathetic spinally projecting cells will show increased association with astrocytes (as indicated by GFAP) under acute hypoxia compared to normoxic conditions.

Methods

Prior retrograde labeling of pre-sympathetic neurons in the PVN: Microinjection of 6% PFA into the PVN was performed bilaterally into the rostral and caudal portion of the PVN. Animals and treatments: Male Sprague-Dawley rats were exposed to acute hypoxia (AH, 10% oxygen for 3 hours) or normoxia (N, 21% oxygen). Immunohistochemistry (IHC): IHC was performed on free-floating sections (1 in 6 series). Primary antibodies: 24 hr incubation (overnight). Secondary antibodies: 2 hr incubation. Analysis: Images were obtained for two rostral-caudal levels of the PVN.

Central Chemoreflex Pathways

Figure 1. ARTERIAL CHEMOREFLEX PATHWAYS. Chemoreceptor afferents project to the nucleus tractus solitarii (nTS). The nTS projects to the PVN directly or to the central ventrolateral medulla (CVLM) which projects to the PVN. Pre-sympathetic spinally projecting efferent neurons project to the intermediolateral cell column (IML) directly or also to the central ventrolateral medulla which projects to the IML.

Modulation in the PVN

Figure 2. NITRIC OXIDE (NO) MODULATION OF PRE-SYMPATHETIC NEURONS IN THE PVN. Upon excitation by glutamate, nNOS within nNOS containing cells is activated and potentially releases NO, which has a stimulatory effect or release of the inhibitory neurotransmitter GABA at the nerve terminal within the PVN.

Immunohistochemistry

Figure 3. PVN SECTIONS FROM AH RATS (Fos-IR-Red, nNOS-IR-Green, spinally projecting neuron-Blue). Figure 4. ASTROCYTE ASSOCIATED CELLS (A) Level A (Bregma ~1.8) (B) Left: co-labeled Fos-IR and nNOS-IR cell. Right: co-labeled cell associated with an astrocyte. Figure 5. AN INCREASED NUMBER OF ACTIVATED nNOS CELLS (A) Fos-IR singly projecting cells were small and tended (P<0.05) to be greater in level B. AH was without effect. (B) All activated nNOS cells level A of the PVN. Figure 6. ACTIVATED nNOS CELLS WERE ASSOCIATED WITH ASTROCYTES. Few GFAP-spinally projecting cells were located primarily in level A of the PVN and were predominantly spinally projecting cells in level B.

Results

Table 1. EFFECTS OF ACUTE HYPOXIA AND NORMOXIA ON CELLS WITHIN THE PVN. Mean cell counts and SEM for all cells. * indicates a significant difference (P<0.05) from the normoxic condition at the same level.

Summary and Conclusion

As seen in previous studies, a larger proportion of nNOS containing cells was seen in level A and a larger proportion of spinally projecting cells was seen in level B. Rats exposed to acute hypoxia show an overall increased cellular activation (Fos-IR) and activated 14% of nNOS containing cells in the PVN (primarily level A).

Current data indicate that acute hypoxia activated 17% nNOS containing cells associated with astrocytes in level A. Although a substantial number of spinally projecting cells were associated with astrocytes, very few of these cells were activated by AH (6%). The majority (62%) of cells activated (Fos-IR) by acute hypoxia are phenotypically unknown.

Nitric oxide is a highly diffusible inhibitor of sympathetic outflow from the PVN, and increased activation of nNOS could serve as an early compensatory response to prevent hypertension found in apnic patients. Considering the role of astrocytes in modulating synaptic transmission, it is possible that astrocytes closely associated with activated cells contribute to nNOS production in the PVN during acute hypoxic insult.