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5HT1A receptors inhibit glutamate inputs to cardiac vagal neurons post-hypoxia/hypercapnia

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ABSTRACT

Synaptic inputs to cardiac vagal neurons (CVNs) regulate parasympathetic activity to the heart. Previous work has shown insults such as hypoxia and hypercapnia (H/H) alter CVN activity by activating post-synaptic serotonergic, purinergic, and glutamatergic receptors in CVNs. This study examines the role of serotonergic 5HT1A receptors in modulating these excitatory neurotransmissions to CVNs during control conditions, H/H and recovery from H/H. Excitatory post-synaptic currents (EPSCs) were recorded from identified CVNs *in vitro* before, during and post H/H. The 5HT1A receptor antagonist WAY 100635 had no effect on EPSCs in CVNs before, and during H/H. However, during recovery from H/H inspiratory-related excitatory serotonergic and purinergic pathways were recruited to excite CVNs. However, when these serotonergic and purinergic pathways are blocked, the 5HT1A receptor antagonist WAY 100635 restores an excitatory glutamatergic neurotransmission to CVNs. This study indicates endogenous activation of serotonergic 5HT1A receptors diminishes glutamatergic neurotransmission to CVNs following H/H, likely via a presynaptic site of action.

1. Introduction

SIDS remains the leading cause of infant mortality yet, despite many years of research, the underlying basis of SIDS remains unknown. SIDS is postulated to result from failed autoresuscitation and inadequate or inappropriate cardiorespiratory network function and responses to challenges such as hypoxia and/or hypercapnia. Multiple postmortem reports of SIDS victims indicate that hypoxic episodes precipitate respiratory distress which is accompanied and/or preceded by bradycardia before the fatal event (Meny et al., 1994). Genetic and receptor binding studies in postmortem tissues of infants that succumbed to SIDS indicate gross serotonergic and cholinergic abnormalities (Kinney et al., 2001; Nattie and Kinney, 2002; Weese-Mayer et al., 2003; Paterson et al., 2006a; Slotkin et al., 2006a,b; Rand et al., 2007).

Among cardiorespiratory network interactions originating in the brainstem the most ubiquitous is respiratory sinus arrhythmia (RSA). Mechanisms of RSA have been extensively characterized at the level of cardiac vagal neurons (CVNs) located within the nucleus ambiguus (NA) (Neff et al., 2003; Huang et al., 2005). During inspiration CVNs receive tonic non-respiratory related excitatory glutamatergic neurotransmission (Huang et al., 2005), little if any serotonergic or purinergic input, and inspiratory evoked inhibitory GABAergic and glycinergic synaptic inputs (Neff et al., 2003). Glutamatergic neurotransmission to CVNs is diminished while serotonergic and purinergic neurotransmission via post-synaptic 5HT3 and P2X receptor activation, respectively, sustains excitatory neurotransmission to CVNs during recovery from hypoxia/hypercapnia (H/H) (Kamendi et al., 2008). However, the mechanisms responsible for the loss of glutamatergic neurotransmission to CVNs post H/H remain unknown. Given the evidence SIDS is associated with abnormal 5HT receptor function, this study tested whether endogenous activation of serotonergic 5HT1A receptors modulates excitatory neurotransmission to CVNs during normal respiration, H/H or recovery from H/H.

2. Materials and methods

All animal procedures were performed in compliance with the institutional guidelines at George Washington University and are in accordance with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association and the National Institutes of Health publication *Guide for the Care and*

Abbreviations: AP-5, d-2-amino-5-phosphonovalerate; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; CVNS, cardiac vagal neurons; EPSC, excitatory post-synaptic current; H/H, hypoxia/hypercapnia; NA, nucleus ambiguus; PPADS, pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonic acid; RSA, respiratory sinus arrhythmia.

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Use of Laboratory Animals. All efforts were made to minimize the number of animals used and their suffering.

To identify CVNs *in vitro*, a two-stage procedure was used. In an initial surgery, Sprague-Dawley rats (postnatal days 2–6; Hilltop, Scottdale, PA) were anesthetized with hypothermia and received a right thoracotomy. Rhodamine (0.05 mL, 1–5%) (XRITC, Life Technologies Corporation, Carlsbad, CA) was injected into the pericardial sac to retrogradely label CVNs. The location and identification of these neurons in the NA, was previously described (Bouairi et al., 2006). Specificity of the cardiac vagal labeling was confirmed by the absence of any labeled neurons in the brainstem when rhodamine is injected either outside the pericardial sac or within the pericardial sac after the cardiac branch of the vagus nerve was sectioned ($n = 4$). Recent work demonstrated that this method identifies CVNs localized in the external formation of the NA (Bouairi et al., 2006). In other control experiments ($n = 10$), i.v. injection of up to 10 mg of rhodamine failed to label any neurons in the medulla except for rare labeling of neurons in the area postrema, an area with a deficient blood–brain barrier.

1–4 Days later, the animals were anesthetized with isoflurane and killed by rapid cervical dislocation. Electrophysiological experiments were conducted at postnatal age P3–P8. The brain was submerged in cold (4 °C) buffer: 140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 5 mM glucose, and 10 mM HEPES and continually gassed with 100% O_2. A single slice of the medulla (800 μm thickness) that included CVNs, the rostral hypoglossal nucleus and rootlets, and the preBotzinger complex was obtained and submerged in a recording chamber, which allowed perfusion (5–10 mL/min) of ACF at room temperature (24–25 °C) containing 125 mM NaCl, 3 mM KCl, 2 mM $CaCl_2$, 26 mM $NaHCO_3$, 5 mM glucose, and 5 mM HEPES equilibrated with carbogen (95% O_2 and 5% CO_2, pH 7.4). The thick medullary slice preparation generates rhythmic inspiratory-related motor discharge in hypoglossal cranial nerves. Spontaneous inspiratory-related activity was recorded by monitoring motor neuron population activity from hypoglossal nerve rootlets using a suction electrode. Hypoglossal rootlet activity was amplified 50,000 times and filtered (10–300 Hz bandpass; CWE, Ardmore, PA).

Individual CVNs in the NA were identified by the presence of the fluorescent tracer using a Zeiss Axioskop upright microscope (Carl Zeiss Inc., Thornwood, NY) and a 40× water immersion objective. These identified CVNs were then imaged with differential interference contrast optics, infrared illumination, and infrared-sensitive video detection cameras to gain better spatial resolution. Patch pipettes (2.5–3.5 μm) were filled with a solution consisting of 135 mM K-gluconic acid, 10 mM HEPES, 10 mM EGTA, 1 mM CaCl2, and 1 mM MgCl2, pH 7.35 and guided to the surface of individual CVNs. Voltage clamp whole-cell recordings were made at a holding potential of −80 mV with an Axopatch 200B and pClamp 8 software (Axon Instruments, Union City, CA).

All drugs used in these experiments were applied using a pneumatic picopump pressure system (WPI, Sarasota, FL). All drugs were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO). Drugs were focally released using a picrospritzer and pressure ejected from a patch pipette positioned within 30 μm of the patched CVN. The maximum range of drug application was determined previously to be 100–120 μm downstream from the drug pipette and was considerably less behind the drug pipette (Wang et al., 2002). Excitatory postsynaptic currents (EPSCs) were isolated by continuous focal application of strychnine (1 μM) and gabazine (25 μM) to block glycine and GABAergic receptors, respectively. Other drugs used included ondansetron (100 μM) to block 5HT3 receptors, pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS, 100 μM) to block purinergic receptors; and finally D-2-amino-5-phosphonovalerate (AP-5, 50 μM) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 50 μM) were used to block NMDA and AMPA/kainite glutamatergic neurotransmission, respectively.

Rhythmic inspiratory-related activity and EPSCs of CVNs were recorded simultaneously for 10 min in control ACSF, equilibrated with 95% O_2, and 5% CO_2 (normoxia). Slices were then exposed for 10-min to hypoxia/hypercapnia by changing control ACSF to ACSF equilibrated with 9% CO_2, 6% O_2, and 85% N_2, and then slices were reoxygenated by returning the perfusate to initial control ACSF equilibrated with 95% O_2, and 5% CO_2 (posthypoxia). Only one experiment was conducted per preparation.

Synaptic events were detected using MiniAnalysis (version 5.6.12; Synaptosoft, Decatur, GA). Threshold was set at root-mean-square noise multiplied by five. The frequency of EPSCs that occurred in CVNs was grouped in 1-s bins and cross-correlated with onset of inspiratory-related hypoglossal activity. Data were analyzed from all bursts during the last 2 min of the condition. Results were presented as means ± SEM. One-way ANOVA with repeated measures and bonferroni posttest were used to examine the differences between spontaneous and inspiratory related EPSCs within a condition. To examine time-dependent differences in response to various drug application periods the results were analyzed by two-ways ANOVA test with repeated measures, followed by Bonferroni posttest. Significant differences for all data were set at $p < 0.05$.

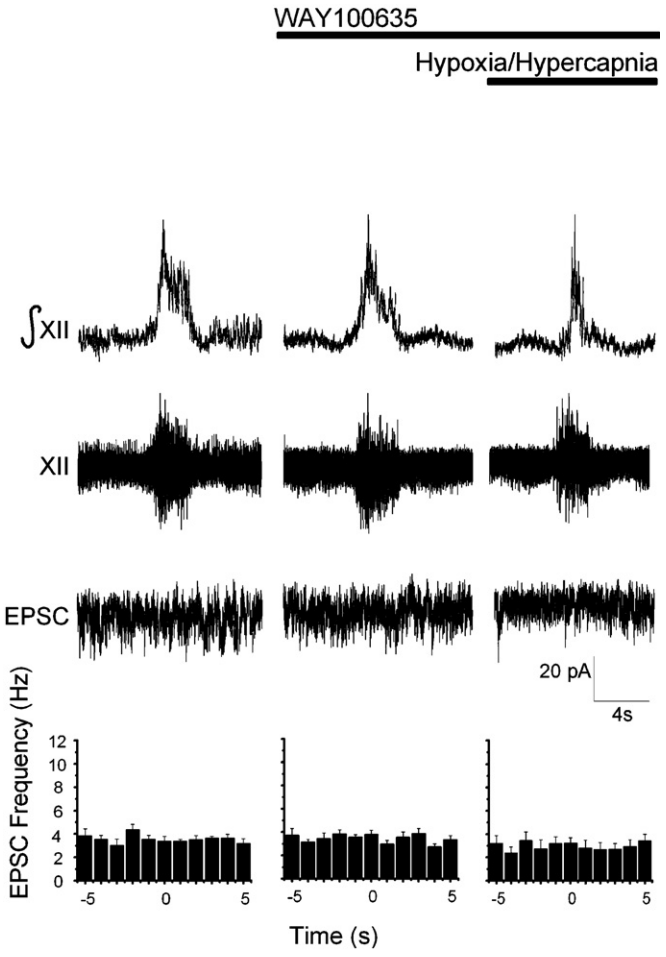

Fig. 1. Inspiratory-related bursting activity was recorded from the hypoglossal rootlet as shown in the middle traces, and integrated hypoglossal activity is shown in the top traces in this figure and Fig. 2. Fluorescently identified CVNs were patch clamped in the whole cell configuration and frequency histograms of glutamatergic EPSCs are illustrated in the bottom traces. The 5HT1A receptor antagonist WAY100635 had no effect on glutamatergic EPSCs in CVNs during the control or H/H periods.