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5-HT₂ receptors modulate excitatory neurotransmission to cardiac vagal neurons within the nucleus ambiguus evoked during and after hypoxia

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Abstract

To examine the role of 5-HT₂ receptors in the central cardiorespiratory network, and in particular the respiratory modulation of parasympathetic activity to the heart, we used an *in vitro* medullary slice that allowed simultaneous examination of rhythmic inspiratory-related activity recorded from hypoglossal rootlet and excitatory inspiratory-related neurotransmission to cardioinhibitory vagal neurons (CVNs) within the nucleus ambiguus (NA). Focal application of ketanserin, a 5-HT₂ receptor antagonist, did not significantly alter the frequency of spontaneous excitatory postsynaptic excitatory currents (EPSCs) in CVNs in control conditions. However, ketanserin diminished spontaneous excitatory neurotransmission to CVNs during hypoxia. The inhibitory action of ketanserin was on 5-HT₃ mediated EPSCs during hypoxia since these responses were blocked by the 5-HT₃ receptor antagonist ondansetron. In addition, a robust inspiratory-related excitatory neurotransmission was recruited during recovery from hypoxia. Focal application of ketanserin during this posthypoxia period evoked a significant augmentation of the frequency of inspiratory-related, but not spontaneous EPSCs in CVNs. This excitatory effect of ketanserin was prevented by application of the purinergic receptor blocker pyridoxal-phosphate-6-azophenyl-2',4'-disulfonic acid (PPADS). These results demonstrate 5-HT₂ receptors differentially modulate excitatory neurotransmission to CVNs during and after hypoxia. Activation of 5-HT₂ receptors acts to maintain excitatory neurotransmission to CVNs during hypoxia, likely via presynaptic facilitation of 5-HT₃ receptor-mediated neurotransmission to CVNs. However, activation of 5HT₂ receptors diminishes the subsequent inspiratory-related excitatory neurotransmission to CVNs that is recruited during the recovery from hypoxia likely exerting an inhibitory action on inspiratory-related purinergic signaling.

Keywords

parasympathetic; neurons; postsynaptic; serotonin; brainstem

Infants who succumb to sudden infant death syndrome (SIDS) often experience episodes of prolonged apnea and bradycardia prior to or during the fatal outcome (Kelly et al., 1986; Meny

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et al., 1994; Poets et al., 1999). Victims of SIDS typically have an increased frequency of apnea events during rapid eye movement sleep (Franco et al., 1999), altered parasympathetic and sympathetic activities, including increased heart rate, reduced heart rate variation, and prolonged QT intervals relative to controls (Kelly et al., 1986; Schechtman et al., 1992; Franco et al., 2008). In addition, infants who succumb to SIDS often possess altered autonomic responses to obstructive apnea during sleep compared with age-matched infants (Franco et al., 1999).

Although a specific cause in a majority of SIDS death is unknown, developmental abnormalities of serotonin (5-HT) function in the medullary regions involved in “protective” cardiorespiratory reflexes and responses to hypoxia have recently been closely correlated with SIDS (Kinney et al., 2001; Nattie and Kinney, 2002). These abnormalities involve multiple elements of 5-HT function including increased number of 5-HT neurons, reduction of 5-HT_{1A} receptor binding, and reduction of 5-HT transporter function (Paterson et al., 2006). In agreement with these findings the study of cerebrospinal fluids of SIDS victims showed a significant increase of the metabolites of 5-HT (Caroff et al., 1992; Cann-Moisan et al., 1999). Medullary 5-HT abnormalities may result in alteration of autonomic responses to hypoxia including deleterious bradyarrhythmias.

Heart rate at rest, as well as during challenges such as hypoxia, is dominated by the activity of the cardioinhibitory parasympathetic autonomic system (Andresen et al., 2004). Hypoxia evokes an initial increase in heart rate followed by bradycardia and ultimately, cessation of cardiac contractions (Slotkin et al., 1997; Neff et al., 2004). The cellular mechanisms for this phenomenon include transient inhibition and subsequently disinhibition of cardiac vagal neurons (CVNs) within the nucleus ambiguus (NA) during hypoxia by a biphasic increase, followed by a decrease, in the frequency of spontaneous and inspiratory-related GABAergic and glycinergic neurotransmission to CVNs (Neff et al., 2004). In addition to the changes in inhibitory neurotransmission to CVNs, hypoxia also recruits 5-HT₃ receptor-mediated excitatory neurotransmission to CVNs (Dergacheva et al., 2009).

Hypoxia elicits dramatic alterations in 5-HT function within the brainstem. Hypoxia induces Fos-like immunoreactivity in 5-HT neurons in the nucleus raphe pallidus, the nucleus raphe magnus, and along the ventral medullary surface (Erickson and Millhorn, 1994). In the medullary region of ventral respiratory group (VRG), which is located in the close proximity to CVNs in the NA, 5-HT levels significantly increase during acute hypoxia and remains elevated for 8-32 min following reoxygenation (Richter et al., 1999).

Although 5-HT may activate many types of 5-HT receptors, 5-HT₂ receptors are likely essential in the cardiorespiratory responses to hypoxia. Activation of 5-HT₂ receptors is required to sustain hypoxic gasping and to restore respiratory activity during posthypoxia (Tryba et al., 2006; St-John and Leiter, 2008). 5-HT₂ receptors are also critical for long-term facilitation in respiratory activity followed by intermittent hypoxia, as the 5-HT₂ receptor antagonist ketanserin either attenuates or completely abolishes this hypoxia-evoked long-term facilitation (Fuller et al., 2001; McGuire et al., 2004; Tryba et al., 2006).

Although 5-HT_{2A} receptors participate in a facilitatory modulation of reflex-evoked bradycardia within the brainstem (N'Diaye et al., 2001; Sevoz-Couche et al., 2006) and the 5-HT contacts on surrounding neurons in the NA are among the most dense in the brainstem (Takeuchi et al., 1983), it is not yet known if central 5-HT₂ receptors modulate hypoxia-evoked excitatory signaling in CVNs. In this study we examined the endogenous role of 5-HT₂ receptors in modulating spontaneous and inspiratory-related excitatory neurotransmission to CVNs before, during, and in recovery from hypoxia.

EXPERIMENTAL PROCEDURES

To identify cardiac vagal neurons *in vitro* a two-stage procedure was utilized. In an initial surgery, Sprague–Dawley rats (postnatal days 2–6; Hilltop, Scottsdale, PA, USA) were anesthetized with hypothermia and received a right thoracotomy. The heart was exposed, and 0.05 ml of 1–5% rhodamine (XRITC, Molecular Probes) was injected into the pericardial sac to retrogradely label CVNs. The location and identification of these neurons, particularly in juxtaposition to other cholinergic neurons in the NA, was previously described (Bouairi et al., 2006). On the day of experiment (2–4 days later), the animals were anesthetized with isoflurane and killed by rapid cervical dislocation. The brain was submerged in cold (4 °C) buffer composed of 140 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 5 mM glucose, and 10 mM HEPES and continually gassed with 100% O₂. Using a dissection microscope the cerebellum was removed and the hindbrain was isolated. A single slice of the medulla (800- μ m thickness) that included CVNs, the rostral hypoglossal nucleus and rootlets and the pre-Botzinger complex was obtained and submerged in a recording chamber, which allowed perfusion (5–10 ml/min) of Artificial CerebroSpinal Fluid (ACSF) at room temperature containing 125 mM NaCl, 3 mM KCl, 2 mM CaCl₂, 26 mM NaHCO₃, 5 mM glucose, and 5 mM HEPES equilibrated with carbogen (95% O₂ and 5% CO₂, pH 7.4). 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 Use of Laboratory Animals*. All efforts were made to minimize the number of animals used and any possible discomfort.

The thick medullary slice preparation generates rhythmic fictive inspiratory-related motor discharge in hypoglossal cranial nerves. Spontaneous inspiratory-related activity was recorded by monitoring motoneuron population activity from hypoglossal nerve rootlets using a suction electrode. Hypoglossal rootlet activity was amplified 50,000 times, filtered (10–300 HZ bandpass; CWE, Ardmore, PA, USA) and electronically integrated (τ =50 ms; CWE).

Individual CVNs in the NA were identified by the presence of the fluorescent tracer. 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 M Ω) were filled with a solution consisting of 135 mM K-gluconic acid, 10 mM HEPES, 10 mM ethylene glucol-bis(β -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (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, USA).

Drugs were focally released using a picrospritzer and pressure ejected from a patch pipette positioned within 30 μ m of the patched CVN. The maximum range of drug application was determined previously to be 100–120 μ m downstream from the drug pipette and was considerably less behind the drug pipette (Wang et al., 2002). Continual focal drug applications were performed using a pneumatic picopump pressure system (WPI, Sarasota, FL, USA). Excitatory neurotransmission was isolated by continuous application throughout the experiments of strychnine (1 μ M) and gabazine (25 μ M) to block glycine and GABAergic receptors, respectively. Other antagonists used in this study included the 5-HT₂ receptor antagonist ketanserin (0.1, 1, and 10 μ M), the 5-HT₃ receptor antagonist ondansetron (100 μ M), and the purinergic receptor antagonist pyridoxal-phosphate-6-azophenyl-2',4'-disulfonic acid (PPADS, 100 μ M). Ketanserin was purchased from Tocris (Ellisville, MO), all other drugs were purchased from Sigma-Aldrich (St. Louis, MO).

Rhythmic inspiratory-related activity and EPSCs of CVNs were recorded simultaneously for 10 min in control ACSF. Slices were then exposed for 10-min to hypoxia by changing control ACSF to ACSF equilibrated with 5% CO₂, 20% O₂, and 75% N₂, and then slices were reoxygenated during 10 min by returning the perfusate to initial control ACSF (posthypoxia). Because of the absence of blood perfusion in the in-vitro brain slice preparation and inherent susceptibility of the preparation to tissue hypoxia (Torgerson et al., 1997) the thick medullary slice preparation used in this study was bathed in control ACSF equilibrated with 95% O₂, and 5% CO₂. Under these control conditions oxygen pressure levels in the slice tissue were found to be 583 Torr at the upper surface of the tissue and 157 Torr in the core of the slice (Neff et al., 2004). Although these values are higher than brain oxygen pressure found in in-vivo studies (Huang et al., 1995; Song et al., 1997), we have labeled these as normoxia given their widespread use as control conditions for in-vitro electrophysiological experiments. When the solution is equilibrated with 20% O₂ the O₂ tension in the tissue decreases to 142 Torr at the surface and to 0 Torr in the core of the slice and this has been used previously as a model of hypoxia (Neff et al., 2004). Only one experiment was conducted per preparation. In total 53 animals were used in this study.

Synaptic events were detected using MiniAnalysis (version 5.6.12; Synaptosoft, Decatur, GA, USA). Threshold was set at root-mean-square noise multiplied by 5. 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 during the last 2 min of the control period, during the last 2 min of hypoxia, and during the last 2 min of 10-min posthypoxia period. 10 s of EPSC events were analyzed for each inspiratory burst, 5 s prior and 5 s after the onset of inspiratory burst. All data from bursts in the last 2 min of each period were then condensed into a single time series of values for each slice. Values from multiple slices were then analyzed for time-dependent and condition-dependent differences. To examine changes with ketanserin corresponding periods were compared between ketanserin and control (no drug). This protocol for data analysis was also used to analyze EPSC amplitude and decay times. To evaluate any possible time-dependent changes during hypoxia in spontaneous neurotransmission independent of respiratory bursting 2-min averages of EPSC frequency in periods of 0-2 min, 4-6 min, and 8-10 min of the hypoxia period were analyzed.

Results are presented as means $\pm$ SEM and were statistically compared using Student's t-test to examine spontaneous EPSCs before and after drug application within a condition. One-way ANOVA with repeated measures and Dunnett's post test was utilized to examine the differences between spontaneous and respiratory related EPSCs within a condition. To determine the concentration-related differences in various time-dependent periods two-ways ANOVA test, following by Bonferroni post test was utilized. Significant differences for all data were set at $P < 0.05$.

RESULTS

Central cardiorespiratory responses to hypoxia

In agreement with previous published data (Evans et al., 2005; Griffioen et al., 2007), there was no significant alteration (Fig. 1, $n = 10$; $P > 0.05$) in the frequency of EPSCs in CVNs during inspiratory bursts under either control or hypoxic conditions. Similarly, the amplitude and decay time constant of EPSCs were not altered by inspiratory bursts ($P > 0.05$). There were no significant changes in the spontaneous neurotransmission to CVNs throughout the hypoxia period (control, 2.2 ± 0.4 Hz; 0-2 minutes of hypoxia, 2.3 ± 0.3 Hz; 4-6 minutes of hypoxia, 1.7 ± 0.5 Hz; 8-10 minutes of hypoxia, 1.4 ± 0.4 Hz; $n = 10$, $P > 0.05$). However, on recovery from hypoxia there was a significant increase in the frequency of excitatory neurotransmission to CVNs simultaneous with inspiratory bursts (see Fig. 1, $n = 8$). This increase in the EPSC frequency was often accompanied by a small, but significant, increase in the EPSCs amplitude.

For example, during first second of inspiratory burst the frequency of EPSCs was increased from 1.9 ± 0.7 Hz to 5.6 ± 1.4 Hz ($n = 8$, $P < 0.001$) while the amplitude of EPSCs was increased from 13.8 ± 1.6 Hz to 16.3 ± 1.8 Hz ($n = 8$, $P < 0.01$). The decay time constant of EPSCs was not altered by inspiratory bursts ($P > 0.05$) during posthypoxia.

Ketanserin did not alter excitatory neurotransmission to CVNs during normoxic conditions

Continuous focal application of ketanserin, a 5-HT₂ receptor antagonist, did not significantly alter the frequency of spontaneous EPSCs in CVNs during normoxia in concentrations of either 0.1 μ M ($n = 7$), 1 μ M ($n = 8$), or 10 μ M ($n = 8$), see Fig. 2, $P > 0.05$. Similarly, the amplitude and decay time constant of spontaneous EPSCs during normoxia were not altered by ketanserin in concentrations of either 0.1 μ M ($n = 7$), 1 μ M ($n = 8$), or 10 μ M ($n = 8$), $P > 0.05$.

Ketanserin dose-dependently diminished excitatory neurotransmission to CVNs during hypoxia

While ketanserin, at a concentration of 0.1 μ M, did not significantly alter the frequency of EPSCs in CVNs during hypoxia (Fig. 3, A, $n = 7$, $p > 0.05$), at concentrations of both 1 μ M ($n = 8$) and 10 μ M ($n = 8$) ketanserin evoked a significant decrease in the frequency of spontaneous EPSCs in CVNs during hypoxic conditions (see Fig. 3, A, $P < 0.05$). The decrease in the EPSC frequency evoked by focal application of ketanserin (10 μ M) persisted in the presence of NMDA and non-NMDA glutamatergic receptor antagonists AP-5 (50 μ M) and CNQX (50 μ M), respectively: from 0.9 ± 0.1 Hz to 0.5 ± 0.1 Hz, $n = 8$, $p < 0.01$, Fig. 3, B. However, in the presence of the 5-HT₃ receptor antagonist ondansetron (100 μ M) ketanserin (10 μ M) had no effect on the EPSC frequency (0.8 ± 0.1 Hz versus 0.8 ± 0.1 Hz, $n = 6$). The amplitude and decay time constant of spontaneous EPSCs during hypoxia were not altered by ketanserin in concentrations of either 0.1 μ M ($n = 7$), 1 μ M ($n = 8$), or 10 μ M ($n = 8$), $P > 0.05$.

Ketanserin facilitated excitatory inspiratory-related, but not spontaneous neurotransmission to CVNs during posthypoxia

Continuous focal application of ketanserin at a concentration of 0.1 μ M did not significantly change the frequency of either spontaneous or inspiratory-related EPSCs in CVNs posthypoxia (Fig. 4, A, $n = 6$, $P > 0.05$). However, ketanserin at concentrations of both 1 μ M ($n = 6$) and 10 μ M ($n = 6$) evoked a significant increase in the frequency of inspiratory-related but not spontaneous EPSCs during recovery from hypoxia (see Fig. 4, A). The amplitude and decay time constant of both spontaneous and inspiratory-related EPSCs during posthypoxia were not altered by ketanserin in concentrations of either 0.1 μ M ($n = 6$), 1 μ M ($n = 6$), or 10 μ M ($n = 6$), $P > 0.05$. Continuous focal co-application of the purinergic receptor blocker PPADS (100 μ M) and ketanserin (10 μ M) abolished inspiratory-related increase in the EPSC frequency in CVNs (Fig. 4, B, $n = 6$, $p > 0.05$).

DISCUSSION

There are 4 major findings in this study. 1) Under control conditions during either normoxia or hypoxia there is no alteration of excitatory neurotransmission to CVNs during inspiratory bursts. However, in recovery from hypoxia inspiratory bursts elicit an increase in the EPSC frequency which is often accompanied by a small but significant increase in EPSC amplitude. This increase in EPSC amplitude could be due to a postsynaptic change in receptor function and/or a result of summation occurring with the increased frequency of EPSCs that occurs during the inspiratory bursts. 2) Under control conditions there is no endogenous 5-HT₂ receptor modulation of excitatory signaling to CVNs. 3) During hypoxia, however, the 5-HT₂ antagonist ketanserin dose-dependently inhibits excitatory neurotransmission to CVNs. This modulation of excitatory neurotransmission persists in the presence of glutamatergic blockers but is abolished by the 5-HT₃ receptor blocker ondansetron indicating that 5-HT₂ receptors

endogenously facilitate 5-HT₃ mediated neurotransmission during hypoxia. 4) In recovery from hypoxia ketanserin dose-dependently facilitates inspiratory-related but not spontaneous EPSCs in CVNs and this facilitation of inspiratory-related EPSCs is blocked by the purinergic antagonist PPADS. Therefore 5-HT₂ receptors endogenously inhibit inspiratory-related purinergic neurotransmission recruited to CVNs posthypoxia.

5-HT₂ receptors are considered to be G protein coupled metabotropic receptors (Hu et al., 2004; Ase et al., 2005) and are known to exert both excitatory and inhibitory actions on central neurotransmission and receptors (Muramatsu et al., 1988; Blank et al., 1996; Arvanov et al., 1999; Yan, 2002; Hu et al., 2004; Kommalage and Hoglund, 2005). Within the NA, premotor neurons receive a high number of axosomatic 5-HT contacts, and the 5-HT contacts surrounding neurons in the NA are among the most dense in the brainstem (Takeuchi et al., 1983). 5-HT fibers also specifically surround CVNs, which have been described as “ensheathed in 5-HT immunoreactive axonal boutons” (Izzo et al., 1993). Under control conditions 5-HT_{1A/7} (Wang et al., 2007) and 5-HT_{2B} receptors (Dergacheva et al., 2007) have been shown to inhibit GABAergic inhibitory inputs to CVNs while 5-HT_{2B} receptors facilitated purinergic receptor mediated excitatory neurotransmission to CVNs (Dergacheva et al., 2008). In this study ketanserin inhibits spontaneous EPSCs during hypoxia and facilitates inspiratory-related EPSCs posthypoxia. As the pK value of ketanserin on 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors is 8.9, 5.4, 7.0, respectively (Baxter et al., 1995), ketanserin at concentrations of 1 μ M and 10 μ M used in this study acts likely on 5-HT_{2A} or 5-HT_{2A/2C} receptors. Other studies report ketanserin also displays moderate affinity (pK_i = 7.17) for 5-HT_{1D} receptors (Zgombick et al., 1995). However while 5-HT_{2A} receptors have been localized within the NA (Fay and Kubin, 2000) 5-HT_{1D} receptors are confined to discrete areas in the medulla associated with the trigeminal sensory system (Longmore et al., 1997). Since ketanserin was applied focally to the patched CVNs in the NA in this study, and has higher affinity for 5-HT_{2A} receptors in comparison with 5-HT_{1D} receptors, ketanserin likely acts on 5-HT_{2A} receptors although the role of 5-HT_{1D} receptors can not be completely excluded.

Hypoxia elicits a bradycardia in both neonatal and adult rats (Bao et al., 1997; Fewell et al., 2007; Cummings et al., 2009; Ishii et al., 2009). In adult animals the decrease in heart rate during hypoxia is mediated by increased activity of the cardioinhibitory parasympathetic autonomic system (Bao et al., 1997). Similarly, in fetal sheep acute hypoxia produces a marked bradycardia and this fall in heart rate can be abolished by either vagotomy or muscarinic receptor blockage (Boddy et al., 1974; Giussani et al., 1993). However, in a study conducted on rats during early postnatal maturation (Fewell et al., 2007), bilateral vagotomy did not alter hypoxia-elicited bradycardia although bradycardia was attenuated by blocking adenosine A₁ receptors. These results conflict with other work that indicates parasympathetic control of the rat heart is functional as early as day 21 of gestation (Marvin et al., 1980). Parasympathetic control of heart remains stable throughout the early postnatal period of the rat (Quigley et al., 1996). In addition, in-vitro studies carried out on medullary slices obtained from 2-6 day old rat pups suggest hypoxia can elicit robust changes in activity of parasympathetic CVNs (Neff et al., 2004; Dergacheva et al., 2009). In summary, it is likely the parasympathetic cardioinhibitory system is involved in mediation of hypoxia-elicited bradycardia in animals of different ages, including neonates, although other factors such as adenosine-mediated effects during hypoxia can not be excluded.

Acute hypoxia elicits a biphasic change in inhibitory neurotransmission to CVNs (Neff et al., 2004). In addition, hypoxia recruits excitatory 5-HT₃ mediated signaling to CVNs (Dergacheva et al., 2009). Measurement of neurotransmitter release within the VRG, an area located close to CVNs, in response to hypoxia indicates the concentration of 5-HT in the extracellular fluids increases significantly during hypoxia and declines slowly in the posthypoxia period (Richter et al., 1999). Taken together these studies indicate under control conditions 5-HT release in

the ventral brainstem is comparatively low and 5-HT neurotransmission is not involved in unchallenged cardiorespiratory brainstem function. In agreement with this hypothesis, glutamatergic, but not 5-HT₃ receptors, mediate excitation of CVNs under control conditions, (Kamendi et al., 2008; Dergacheva et al., 2009). However, during hypoxia 5-HT signaling, acting via postsynaptic 5-HT₃ receptors, is recruited to maintain excitation of CVNs while glutamatergic neurotransmission is also facilitated to CVNs during hypoxia (Dergacheva et al., 2009). The results from this study extend this framework and suggest that during hypoxia 5-HT₂ receptors are also recruited and act to maintain excitation of CVNs during hypoxia likely via facilitation of 5-HT₃ receptor activation.

Similarly, recent data also suggest purinergic receptors do not mediate endogenous excitation of CVNs under either normal conditions or during hypoxia (Griffioen et al., 2007). However purinergic receptors are involved in inspiratory-related excitation of CVNs during recovery from hypoxia (Griffioen et al., 2007). In agreement with these results, within the VRG adenosine 5'-triphosphate is released at low levels during hypoxia and adenosine 5'-triphosphate levels peak and remain elevated after termination of hypoxia (Richter et al., 1999). This study has shown there is endogenous activation of 5-HT₂ receptors during recovery from hypoxia that likely exerts an inhibitory influence on the purinergic inspiratory-related neurotransmission to CVNs. The inhibitory influence of 5-HT₂ receptors on excitatory purinergic neurotransmission to CVNs may prevent excessive excitation CVNs and an exaggerated bradycardia posthypoxia.

Implications for SIDS

SIDS is the leading cause of postneonatal infant mortality and 5-HT abnormalities are strongly implicated in SIDS (Kinney et al., 2001). Despite the clinical importance of understanding the neurochemical mechanisms by which 5-HT system influences central cardiorespiratory responses to hypoxia, these mechanisms remain incomplete. In this study we show 5-HT₂ receptors play a critical role in control of CVNs during and posthypoxia. Endogenous activation of 5-HT₂ receptors facilitates excitatory 5-HT₃ mediated neurotransmission to CVNs during hypoxia and inhibits excitatory purinergic inspiratory-related signaling to CVNs on recovery from hypoxia. Unfortunately, it is not known which 5-HT functions are altered in SIDS victims. Increased number of 5-HT neurons along with an increase of the metabolites of 5-HT in cerebrospinal fluids of SIDS victims (Caroff et al., 1992; Cann-Moisan et al., 1999; Paterson et al., 2006) suggest exaggerated 5-HT function occurs in SIDS. Hyperactivity within 5-HT pathways may lead to supranormal stimulation of 5-HT₂ receptors during hypoxia which may result in enhanced activation of CVNs and extreme bradycardia during hypoxia in SIDS victims.

Glossary

5-HT, serotonin; ACSF, Artificial CerebroSpinal Fluid; AP-5, d-2-amino-5-phosphonovalerate; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; CVNs, cardiac vagal neurons; EPSCs, excitatory postsynaptic currents; NA, nucleus ambiguus; PPADS, pyridoxal-phosphate-6-azophenyl-2',4'-disulfonic acid; SIDS, sudden infant death syndrome; VRG, ventral respiratory group.

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Figure 1.

Central cardiorespiratory responses to hypoxia. Inspiratory-related bursting activity was recorded from hypoglossal rootlet (XII) simultaneously with synaptic events of fluorescently identified CVNs within the NA. Changing the perfusate from ACSF, equilibrated with 95% O₂, and 5% CO₂ to ACSF equilibrated with 5% CO₂, 20% O₂, and 75% N₂, did not alter excitatory neurotransmission to CVNs. However, on recovery from hypoxia the frequency of EPSCs was significantly increased and correlated with the onset of inspiratory burst activity as shown in a typical experiment (top) and in the summary data (bottom). * and *** denotes $P < 0.05$ and $P < 0.001$, respectively, comparing the EPSC frequency 5 s before the burst onset to all other time series values using one-way ANOVA with repeated measures.

Figure 2.

Ketanserin did not alter excitatory neurotransmission to CVNs during normoxic conditions. Under normoxic conditions, there were no excitatory inspiratory-related inputs to CVNs. Continuous focal application of ketanserin, a 5-HT₂ receptor antagonist, did not significantly alter the frequency of spontaneous EPSCs in CVNs in concentrations of either 0.1 μM , 1 μM , or 10 μM . A typical experiment is shown at the top while the summary data are illustrated on the bottom.

Figure 3.

Ketanserin dose-dependently diminished excitatory neurotransmission to CVNs during hypoxia. Under hypoxic conditions, there were no excitatory inspiratory-related inputs to CVNs. Continuous focal application of ketanserin, at a concentration of 0.1, did not significantly alter the frequency of EPSCs in CVNs. However, at concentrations of both 1 μ M and 10 μ M ketanserin evoked a significant decrease in the frequency of spontaneous EPSCs in CVNs. A typical experiment is shown in A (top), while in the summary data are illustrated in A (bottom). In this figure and figure 4 ■ denotes a significant ($p < 0.005$) difference between EPSC frequency compared with corresponded control values using two-ways ANOVA. In the presence of glutamatergic blockers AP-5 and CNQX ketanserin persisted to decrease the

spontaneous EPSC frequency as shown in a typical experiment (B, left top) and in the summary data (B, left bottom). However, ketanserin did not alter the EPSC frequency in the presence of 5-HT₃ receptor blocker ondansetron, as shown in a typical experiment (B, right top) and in the summary data (B, right bottom). ++ denotes a significant difference ($P < 0.01$, Student's t-test) between spontaneous EPSC frequency prior and after ketanserin applications during hypoxia.

Figure 4.

Ketanserin facilitated inspiratory-related, but not spontaneous EPSCs in CVNs posthypoxia. On recovery from hypoxia CVNs receive inspiratory-related modulation of EPSCs. Continuous focal application of ketanserin at a concentration of 0.1 μM did not significantly change the frequency of either spontaneous or inspiratory-related EPSCs in CVNs. However, ketanserin at concentrations of both 1 μM and 10 μM evoked a significant increase in the frequency of inspiratory-related but not spontaneous EPSCs. A typical experiment is shown in A (top), while the summary data are illustrated in A (bottom). As shown in a typical experiment (B, top) and in the summary data (B, bottom), continuous focal co-application of the purinergic receptor blocker PPADS and ketanserin abolished inspiratory-related increase in the EPSC frequency in CVNs ($P > 0.05$). *, **, and *** denotes $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively, comparing the EPSC frequency 5 s before the burst onset to all other time series values using one-way ANOVA with repeated measures.