Abolishment of Serotonergic Neurotransmission to Cardiac Vagal Neurons During and After Hypoxia and Hypercapnia With Prenatal Nicotine Exposure


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INTRODUCTION

Infants that die from Sudden Infant Death Syndrome (SIDS) often succumb to death following respiratory challenges such as hypoxia and hypercapnia (H/H) that occur during sleep. These challenges result from failure to autoresuscitate resulting in complete lack of arousal from sleep, a necessary mechanism for survival (Poets et al. 1999; Sridhar et al. 2003). While the causes of these failures have not been elucidated, several mechanisms have been suggested, and cardiovascular irregularities have been observed in active and sleeping babies that subsequently died of SIDS. These irregularities are parasympathetic in nature and include decreased heart rate, increased vagal tone, and prolonged QT intervals (Kelly et al. 1986; Schwartz et al. 1998). In infants that succumb to SIDS, a centrally mediated slowing of the heart that precedes or accompanies apnea often occurs prior to and during the fatal outcome (Meny et al. 1994; Schechtman et al. 1992).

One of the predisposing factors to SIDS is exposure to maternal smoking. Children born to mothers who smoke have a higher incidence of SIDS than those who do not smoke. In rats, exposure to prenatal nicotine (PNN) impairs the ability of rat pups to autoresuscitate following repeated bouts of hypoxia (Fewell and Smith 1998; Slotkin et al. 2005) and has been hypothesized as a likely link to SIDS (Hunt and Brouillette 1987). While the implications of maternal nicotine are known, the altered physiological mechanisms are still unclear. Previous studies showed that the inability to autoresuscitate probably results from faulty cardiorespiratory interactions rather than failed ventilatory chemosensory responses (Bamford et al. 1996). PNN also alters the expression and function of various receptor families and has been implicated in numerous disease pathologies (Huang et al. 2004; Kamendi et al. 2006; Slotkin et al. 2006).

The most ubiquitous cardiorespiratory interaction is respiratory sinus arrhythmia (RSA). During control conditions, cardiac vagal neurons (CVNs) receive inspiratory-related inhibitory GABAergic and glycinegic neurotransmission (Neff et al. 2003), but CVNs do not receive any respiratory-related excitatory neurotransmission. During hypoxia and/or hypercapnia, inhibitory synaptic inputs initially increase then decrease while excitatory pathways remain unchanged (Neff et al. 2003). After an acute episode of H/H, however, an inspiratory-related excitatory neurotransmission is recruited to CVNs that includes serotonergic and purinergic synaptic pathways (Kamendi et al. 2008) resulting in increased vagal tone. Exposure to PNN alters these excitatory synaptic inputs by recruiting inspiratory-related excitatory neurotransmission to CVNs earlier, including during the H/H episode as well as in recovery. The increased excitatory neurotransmission to CVNs in PNN animals during H/H is mediated by nicotinic receptors, which have only been identified as non-β2- and non-α7-containing nicotinic receptors, and the role of serotonergic pathways have not yet been investigated in PNN animals (Huang et al. 2007). Changes in the serotonergic input to CVNs with H/H in PNN animals is particularly intriguing as recent work has suggested diminished serotonergic activity is strongly correlated with autonomic dysfunction and SIDS (Aud-
METHODOLOGY

All animal procedures were performed with the approval of the Animal Care and Use Committee of The George Washington University 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.” Animal procedures for PNN exposure, fluorescent labeling, and slice preparation in adult female rats are identical to those previously published (Neff et al. 2003). Briefly, adult female rats were anesthetized with ketamine-xylazine (87/13 mg/kg ip; Phoenix Pharmaceuticals, St. Joseph, MO) on the third day of gestation and implanted with Alzet osmotic minipumps (Durect, Cupertino, CA) that delivered 6 mg·kg$^{-1}$·d$^{-1}$ of nicotine, a level approximately equivalent to those that occur in moderate to heavy smokers, for 28 days (Slotkin et al. 1997). After birth, 3- to 5-day-old rat pups were anesthetized and cooled to $-4^\circ$C to slow the heart rate. A right thoracotomy was performed, and the retrograde fluorescent tracer X-rhodamine-5-(and-6)-isothiocyanate (Molecular Probes, Eugene, OR) was injected into the fat pads at the base of the heart. Buprenorphine was administered for postoperative analgesia, and the animals were observed continuously for 30 min after surgery and thereafter every 20 min until recovery. After 24–48 h of recovery, animals were anesthetized with isoflurane and killed by cervical dislocation, and the brain tissue was placed in a 4°C physiologic saline solution. A single thick (770–870 μm) section that included CVNs, the hypoglossal nerve rootlet, the pre-Bötzinger complex, and the rostral portion of the hypoglossal nucleus was cut and submerged in a recording chamber that allowed perfusion (4 ml/min) above and below the slice with room temperature artificial cerebrospinal fluid (ACSF). Procedures for recording from these cells in the whole cell configuration with spontaneous respiratory network activity are similar to those previously published (Neff et al. 2003). Spontaneous inspiratory-related activity was recorded by monitoring motor-neuron population activity from hypoglossal nerve rootlets using a suction electrode. Identified CVNs were voltage-clamped at a holding potential of $-80$ mV. As previously described, rhythmic inspiratory-related and spontaneous excitatory postsynaptic currents (EPSCs) in a single CVN were recorded simultaneously for 4 min (control period) in ACSF equilibrated with 95% O$_2$-5% CO$_2$. Slices were then perfused with H/H ACSF (equilibrated with 85% N$_2$-6% O$_2$-9% CO$_2$) for an acute period of 10 min and then returned to the original perfusate for $\leq$60 min during which different drug regimens (see focal drug application in the following text) were applied to isolate the excitatory inputs recruited to CVNs during and in recovery from the H/H period.

All the drugs used in these experiments were applied using a pneumatic picopump pressure delivery system and were continuously applied until the end of the experiment or drug-free recovery period (WPI, Sarasota, FL). Drugs were ejected from a patch pipette positioned within 30 μm from the patched CVN. The maximum range of drug application has been previously determined to be 100–120 μm downstream from the drug pipette and considerably less behind the drug pipette (Wang et al. 2001). Ondansetron (100 μM), pyridoxalphosphate-6-azophenyl-2′, 4′-disulphonic acid (PPADS, 100 μM), 6-cyano-7-nitroquinoline-2,3-dione (CNQX, 50 μM), d(-)-2-amino-5-phosphono pentanoic acid (AP5, 50 μM), α-conotoxin AuIB (α-CTX AuIB, 100 μM), and α-CTX MII (20 nM) were used to characterize the role of 5HT3, P$_{2X}$, AMPA/kainate, N-methyl-d-aspartate (NMDA), α3β4 and α3α and α6 nicotinic acetylcholine receptors (nAChRs), respectively.

Data analysis

Synaptic events were detected using MiniAnalysis version 5.6.12 (Synaptosoft, Decatur, GA) using a threshold of five times RMS noise level for each experiment. The frequencies of EPSCs that occurred in CVNs were grouped into 1-s bins and cross-correlated with the onset of inspiratory-related hypoglossal activity. Data were analyzed from all

**FIG. 1.** N-methyl-d-aspartate (NMDA) and non-NMDA receptors mediate excitatory neurotransmission to cardiac vagal neurons (CVNs) of rats treated prenatally with nicotine (PNN) during control conditions. Inspiratory-related bursting activity was recorded from the hypoglossal rootlet (XII) and electronically integrated (top). Fluorescently identified CVNs of PNN animals were patch clamped in the whole cell configuration, and excitatory neurotransmission was isolated by focal application of GABAergic (gabazine; 25 μM) and glycinerenic (strychnine; 1 μM) receptor antagonists in this and all subsequent figures. Ondansetron (100 μM), 100 μM pyridoxalphosphate-6-azophenyl-2′, 4′-disulphonic acid (PPADS), and 25 μM 6-cyano-7-nitroquinoloxalene-2,3-dione (CNQX)/1 μM d(-)-2-amino-5-phosphono pentanoic acid (AP5) were applied sequentially to CVNs with a 5-min period between applications. A typical experiment is shown, left, including an expanded section of each excitatory postsynaptic current (EPSC) trace to better visualize individual events before and during the inspiratory-related activity in this and all subsequent figures. A bar on top of the expanded trace indicates inspiratory-related activity in this and all subsequent figures.
bursts during the last 2 min of the control period, during minutes 8–10 of the 10-min H/H period and from minutes 6–8 during each of the 8-min drug regimen application periods. In addition, at the end of the experiment, the last 2 min recorded during the drug-free period were analyzed. These periods were chosen for analysis because during these periods, H/H-evoked changes and drug application had reached a steady state. Statistical comparisons were performed within a condition using a one-way ANOVA with repeated measures to examine the differences between the spontaneous and inspiratory-related EPSCs in response to various time-dependent or drug application periods. Repeated-measures two-way ANOVA comparisons were made between the averaged 5 s of spontaneous EPSC frequency recorded before the inspiratory burst and the averaged inspiratory-related EPSC frequency. The PNN data were compared with that of controls previously published (Kamendi et al. 2008) to compare differences in the mechanisms of CVN activation between unexposed and animals exposed to PNN. Some of the summary data from experiments in unexposed animals published previously but conducted concurrently with these PNN exposed animals (Kamendi et al. 2008) are shown in Figs. 2 and 6 to permit a direct comparison in the results from prenatal nicotine exposed and unexposed animals. A Bonferroni post hoc analysis was used following every significant one-way or two-way RM-ANOVA and significant difference was set at $P < 0.05$.

RESULTS
Mechanisms of CVN excitation during control conditions are not altered by PNN

Previous studies have shown that under control conditions, CVNs exclusively receive spontaneous glutamatergic EPSCs that are blocked by AP5 and CNQX, NMDA and AMPA/kainate receptor blockers, respectively (Evans et al. 2005; Neff et al. 1998). We tested the hypothesis that following PNN exposure, CVNs receive additional signaling during control conditions. Similar to unexposed animals, (Kamendi et al. 2008), PNN exposed rats do not receive any inspiratory-related EPSC events [spontaneous $4.7 \pm 0.1$ Hz, inspiratory period $4.6 \pm 0.2$ Hz, $n = 8$, (not significant (NS), see Fig. 1]. The frequency and amplitude of EPSCs was not altered by ondansetron (100 $\mu$M) or PPADS (100 $\mu$M) see Fig. 1. However, AP5 and CNQX, abolished all EPSCs, ($4.7 \pm 0.1$ to $1.0 \pm 0.6 n = 8$, $P < 0.05$), and this abolishment of EPSCs with AP5 and CNQX was reversible (Fig. 1).

PNN alters inspiratory-related inputs to CVNs during H/H and during recovery

In animals exposed to PNN, there was a significant recruitment of an inspiratory related excitatory pathway to CVNs during H/H, see Fig. 2, top. This is in contrast to the absence of any inspiratory related excitatory neurotransmission to CVNs in unexposed animals during H/H reported previously (Kamendi et al. 2008) and illustrated in Fig. 2, bottom. The significant increase in PNN animals in the frequency of inspiratory-related EPSCs persisted for a long period during recovery from H/H, see Fig. 2, top. In PNN animals, EPSC frequency increased from $5.1 \pm 0.4$ to $9.9 \pm 0.2$ Hz ($n = 8$, $P < 0.05$) during inspiratory activity in the H/H period. In

FIG. 2. Hypoxia/hypercapnia (H/H) recruits a long-lasting inspiratory-related neurotransmission to CVNs of rats exposed to PNN. Changing the perfusate from artificial cerebrospinal fluid (ACSF) equilibrated with 95% $O_2$-5% $CO_2$ to ACSF equilibrated with 9% $CO_2$-6% $O_2$, 85% $N_2$, induced an increase in the inspiratory-related EPSC frequency of CVNs during H/H that also persisted during recovery from H/H and lasted for ≥60 min. A typical experiment is shown at the top, and the average data from 8 neurons are shown in the histograms (bottom). In this and all subsequent figures, *, statistically significant difference of $P < 0.0001$ comparing inspiratory-related EPSC frequency to spontaneous EPSC frequencies 1–5 s prior to inspiratory activity using 1-way ANOVA with repeated measures, #, statistically significant difference of $P < 0.0001$ comparing the inspiratory related EPSC frequency between that condition and initial control conditions; +, significant ($P < 0.001$) difference, using 2-way ANOVA with repeated measures, comparing the spontaneous EPSC frequency (average of 5 s before inspiratory activity) between control and that condition in this and all subsequent figures. In this figure, and Fig. 6, the unfilled histograms, bottom, illustrate the summary data from experiments conducted in unexposed animals previously published (Kamendi et al. 2008) to permit direct comparisons between PNN exposed (top) and unexposed animals (bottom).

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recovery, the inspiratory-related EPSC frequency increased from 4.9 ± 0.4 to 9.6 ± 0.2 Hz (n = 8, P < 0.05) and persisted for ≥1 h after termination of the H/H period (9.6 ± 0.2 Hz at 6–8 min and 10.5 ± 0.5 Hz at 46–48 min; see Fig. 2, top). In contrast, in unexposed animals the inspiratory related excitatory neurotransmission to CVNs did not occur during H/H but was only recruited post H/H (see Fig. 2, bottom).

α3β4 nAChRs are recruited to modulate glutamatergic excitation of CVNs during an acute episode of H/H

To identify the neurotransmitter pathways and the receptors activated with inspiratory activity during H/H in PNN exposed animals, different receptor antagonists were applied. CNQX and AP5 nearly abolished both spontaneous-related, 3.9 ± 0.4 to 1.2 ± 0.3 Hz (n = 10, P < 0.05), and inspiratory: 3.0 ± 0.3 to 1.2 ± 0.2 Hz (n = 10, P < 0.05)-related EPSCs to CVNs during H/H (see Fig. 3). Previous studies had shown that the increase in inspiratory-related EPSCs was blocked by 100 μM dihydro β-erythroidine (DHβE) (Huang et al. 2007), suggesting that presynaptic nicotinic receptors mediate the increase in glutamatergic neurotransmission to CVNs. Therefore this study advanced this framework and tested whether α3β4 and/or α3/α6 nAChRs are involved in the recruitment of excitatory glutamatergic pathway to CVNs during H/H in PNN-exposed animals. Only α-CTX AuIB, the selective α3β4 antagonist (Luo et al. 1998) had any effect, and it nearly abolished the inspiratory evoked EPSCs to CVNs during H/H see Fig. 4. α-CTX AuIB continued to have an effect in the first few (0–4) minutes of the recovery period; however, the involvement of α3β4 nAChRs diminished rapidly in the recovery period, and no longer decreased glutamatergic neurotransmission within 4 min of the recovery period (see Fig. 4). The inhibition by CNQX, AP5, and α-CTX AuIB were reversible. α-CTX MII had no effect on the increase on EPSC frequency to CVNs before, during, or post-H/H (see Fig. 5).

NMDA and AMPA/kainate receptor antagonists diminish the excitation of CVNs during recovery from H/H

To identify the synaptic neurotransmission to CVNs post-H/H, CNQX, AP5, PPADS, and ondansetron were applied to examine the role of AMPA/kainate, NMDA, P2X, and 5HT3 receptors, respectively. Application of CNQX and AP5 prevented the recruitment of excitatory neurotransmission in PNN-exposed animals during and post-H/H, Figs. 3 and 6, top. CNQX and AP5 diminished both the inspiratory (10.8 ± 0.5 to 0.8 ± 0.2 Hz n = 8, P < 0.05) and spontaneous (5.0 ± 0.4 to 0.9 ± 0.2 Hz n = 8, P < 0.05) EPSCs to CVNs recruited post-H/H (see Figs. 3 and 6, top). The inhibition by CNQX and AP5 were reversible. This inhibition by AMPA/kainate and NMDA antagonists, CNQX and AP5, respectively, in PNN-exposed animals is in contrast to the results from unexposed animals (Kamendi et al. 2008) in which during recovery from H/H excitatory neurotransmission to CVNs is not mediated by glutamate but rather is mediated mainly by serotonergic, and to a lesser extent by purinergic pathways and receptors as illustrated in Fig. 6B.

Because both purinergic and 5-HT receptors can mediate excitation of CVNs in unexposed animals following an acute exposure to H/H (Kamendi et al. 2008), this study tested whether rat pups exposed to PNN also received serotonergic
and purinergic inputs that mediate spontaneous and inspiratory-related excitation of CVNs post-H/H. Ondansetron had no effect on the increase in inspiratory-related excitation in PNN exposed animals (9.5 ± 0.4 vs. 9.4 ± 0.4 Hz n = 8 NS) nor any change in the spontaneous activity; (4.5 ± 0.4 vs. 4.7 ± 0.4 Hz n = 8 NS) of EPSCs in CVNs post-H/H, see Fig. 7. PPADS significantly inhibited the inspiratory-related increase in EPSCs (from 9.5 ± 0.4 to 4.1 ± 0.4 Hz n = 8 P < 0.05) but had no significant effect on the spontaneous EPSC frequency (see Fig. 7). CNQX and AP5 blocked nearly all remaining synaptic inputs to CVNs, (inspiratory; from 9.5 ± 0.4 to 1.5 ± 0.3 Hz n = 8 NS, spontaneous activity; from 4.5 ± 0.4 to 1.6 ± 0.4 Hz n = 8). The inhibition by PPADS, CNQX, and AP5 were reversible (see Figs. 6 and 7). Therefore in contrast

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FIG. 4. Activation of α3β4 nicotinic acetylcholine receptors (nAChRs) mediates inspiratory-related EPSC neurotransmission to CVNs during H/H. α-CTX AuIB (100 μM) was applied before, during and after H/H to characterize the role of α3β4 nAChRs in the excitatory neurotransmission to CVNs. Top: a typical experiment; bottom: the average data from 10 cells are shown in the histograms. α-CTX AuIB (100 μM) significantly inhibited both spontaneous and inspiratory-related excitatory neurotransmission to CVNs during, but not before or after, H/H. The inhibition by α-CTX AuIB, 100 μM was reversible.

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FIG. 5. α3* and/or α6* nAChRs do not mediate inspiratory-related EPSC neurotransmission to CVNs during H/H. α-CTX MII, an α3* and/or α6* nAChR antagonist, was applied before, during, and after H/H to characterize the role of α3* and/or α6* nAChRs. Top: a typical experiment; bottom: the average data from 10 cells are shown in the histograms. α-CTX MII did alter spontaneous or inspiratory-related excitatory neurotransmission to CVNs before, during, or after H/H.
to unexposed animals in which serotonergic but not glutamatergic pathways are recruited to CVNs post H/H, as shown in Fig. 6B, glutamatergic but not serotonergic pathways are recruited post H/H in PNN-exposed animals as shown in Fig. 7. However, similar to unexposed animals, in PNN-exposed animals purinergic pathways and activation of P2X receptors mediate a small but significant inspiratory related excitatory neurotransmission to CVNs post-H/H (Figs. 6 and 7).

DISCUSSION

This study sought to determine whether PNN exposure alters the pathways and mechanisms that are involved in exciting CVNs before, during, and after H/H. The major findings from this study are that in PNN-exposed animals 1) under control conditions, CVNs receive spontaneous EPSC activity that is solely glutamatergic and not purinergic or serotonergic; 2) in contrast to the lack of excitatory input in unexposed animals during H/H, PNN animals have a long-lasting inspiratory-related excitation to CVNs recruited during H/H that persists in recovery from H/H; 3) during H/H, the excitatory inputs to CVN are glutamatergic and the increase in glutamatergic EPSCs are mediated by α3β4-containing nAChRs; and 4) in contrast during recovery, the inspiratory-related increase in excitation is mediated by both glutamatergic and purinergic pathways in PNN animals, but, unlike unexposed animals, does not involve serotonergic pathways. This framework is illustrated in Fig. 8.
The results from this study show that during control conditions, similar to unexposed animals, rats treated with PNN possess glutamatergic excitatory synaptic events that are not correlated with any phase of the respiratory cycle (Kamendi et al. 2008). NMDA and AMPA/kainate receptor antagonists abolished the EPSCs in CVNs. However, as shown previously and in contrast to unexposed animals, an acute 10-min exposure to H/H recruited inspiratory-evoked EPSCs in PNN-exposed animals. Previous work showed that this increase was mediated by non-α7-containing nAChRs, with a small inhibition evoked on blocking α7-containing nAChRs (Huang et al. 2007). This study determined the neurotransmitters involved in mediating this excitation and additionally which nAChRs were responsible for recruiting and maintaining this glutamatergic neurotransmission to CVNs during H/H.

Previous work has shown acetylcholine, acting via nicotinic receptors, endogenously enhances inspiratory-related GABAergic neurotransmission to CVNs via β2-containing nAChRs (Neff et al. 2003). PNN augments the inspiratory-related GABAergic neurotransmission and in addition also facilitates the spontaneous GABAergic neurotransmission to CVNs (Neff et al. 2004). This study extends the role of endogenous activation of nAChRs in PNN and demonstrates PNN recruits endogenous cholinergic activity to CVNs during H/H that facilitates glutamatergic neurotransmission to CVNs, but this excitatory neurotransmission to CVNs in PNN animals is absent in unexposed animals. The origin of this cholinergic innervation is still undetermined. However, a study by (Metz 1966), showed that during exposure to hypercapnia and H/H, respiratory-responsive regions of the reticular formation released acetylcholine. The respiratory-responsive regions may involve the cholinergic cell groups involved in spontaneous arousal located in the pontine nucleus or the chemosensitive arcuate nucleus located in the ventral medullary surface (VMS). In addition, polysynaptic cholinergic connections exist between the arcuate nucleus and unidentified neurons within the nucleus ambiguus that potentially could be involved in the
cholinergic modulation during H/H (Lewis 1998). The H/H-induced increase in inspiratory-related cholinergic-mediated glutamatergic excitation is probably a major contributor to the more immediate and pronounced bradycardia that occurs in PNN animals in contrast to the biphasic heart rate responses seen in unexposed animals in response to H/H. Excitatory neurotransmission to CVNs is not altered during H/H in control animals. However, in PNN animals (bottom), during H/H, CVNs have a precipitous decrease in inhibitory GABAergic and glycinergic neurotransmission that is accompanied by an inspiratory-related α2β4-containing nAChR-mediated glutamatergic neurotransmission. The precipitous decrease in inhibitory, and recruitment of excitatory neurotransmission to CVNs during H/H is likely responsible for the exaggerated bradycardia that occurs in PNN animals. In control animals, during the recovery from H/H inspiratory-related excitatory neurotransmission is recruited and is primarily mediated by both serotonergic and purinergic neurotransmission activating 5-HT3 and P2x receptors, respectively (top right). In contrast PNN animals do not recruit a serotonergic neurotransmission to CVNs during recovery from H/H, but instead CVNs in PNN animals continue to receive glutamatergic neurotransmission which is now modulated by P2X receptors during inspiratory activity (bottom right).

These results also demonstrate exposure to nicotine in utero impairs 5HT3 physiological functions that are normally evoked post-H/H in unexposed animals. While the present study cannot address the mechanisms responsible for the loss of 5HT3 function, we speculate that it can occur from compensatory desensitization or down regulation of 5HT3 receptors in PNN animals due to over stimulation by prior excess 5HT (Paterson et al. 2006). Alternatively, PNN exposure could lead to direct

![Diagram](image-url)
alteration of the 5HT3 receptor rendering it inactive or preventing its translocation to the membrane. PNN has long-lasting effects on the expression of 5HT1A and 5HT2 receptors in rats (Slotkin et al. 2007). It is interesting to note that PNN treatment causes similar etiology to that presented by SIDS victims as faulty serotonergic mechanisms are implicated (Kinney et al. 2001; Paterson et al. 2006) including genetic polymorphisms of the serotonin transporter (Weese-Mayer et al. 2003) and mutations of the Fifth Ewing Variant gene (Rand et al. 2007) in infants that have died of SIDS. Furthermore, there are higher numbers of 5HT neurons, decreased expression of 5HT1A, and increased expression of 5HT2A receptors in SIDS victims as compared with age-matched controls (Kinney et al. 2003; Ozawa and Takashima 2002; Panagryha et al. 2000; Paterson et al. 2006; Say et al. 2007).

In conclusion, this study reports that PNN exposure alters the brain stem cardiorespiratory responses to H/H. In control animals, inhibitory mechanisms likely mediate the biphasic changes in heart rate observed during H/H, while excitatory 5HT and purinergic pathways to CVNs likely mediate the resulting bradycardia in recovery from H/H (see Fig. 8) (Ka-mendi et al. 2008; Neff et al. 2003, 2004). These data reveal new mechanisms for heart rate control in PNN animals. During H/H, there is an increase in inspiratory-related α3β4-containing nicotinic receptor-dependent glutamatergic excitatory neurotransmission to CVNs that likely results in the conversion of the biphasic changes in heart rate seen in control animals to only sustained bradycardia in PNN animals. In addition, these data reveal PNN decreases serotonergic function to CVNs post-H/H and enhances glutamatergic excitatory neurotransmission to CVNs. Taken together, these data suggest the failed mechanisms of autoresuscitation that occur in PNN animals are due to decreased 5HT function resulting in altered cardiorespiratory interactions and that this loss of 5HT function may impede the efforts to autoresuscitate in PNN animals challenged with H/H (Fewell et al. 2001; Slotkin et al. 1997) or SIDS victims (Meny et al. 1994).

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