Respiratory Sinus Arrhythmia
Endogenous Activation of Nicotinic Receptors Mediates Respiratory Modulation of Brainstem Cardioinhibitory Parasympathetic Neurons

Robert A. Neff, Jijiang Wang, Sunit Baxi, Cory Evans, David Mendelowitz

Abstract—The heart rate increases during inspiration and decreases during expiration. This respiratory sinus arrhythmia (RSA) occurs by modulation of premotor cardioinhibitory parasympathetic neuron (CPN) activity. However, RSA has not been fully characterized in rats, and despite the critical role of CPNs in the generation of RSA, little is known about the mechanisms that mediate this cardiorespiratory interaction. This study demonstrates that RSA in conscious rats is similar to that in other species. The mechanism of RSA was then examined in vitro. Rhythmic inspiratory-related activity was recorded from the hypoglossal rootlet of 700- to 800-μm medullary sections. CPNs were identified by retrograde fluorescent labeling, and neurotransmission to CPNs was examined using patch-clamp electrophysiological techniques. During inspiratory bursts, the frequency of both spontaneous γ-aminobutyric acidergic (GABAergic) and spontaneous glycinergic synaptic events in CPNs was significantly increased. Focal application of the nicotinic antagonist dihydro-β-erythroidine in an αβ2-selective concentration (3 μmol/L) abolished the respiratory-evoked increase in GABAergic frequency. In contrast, the increase in glycinergic frequency during inspiration was not altered by nicotinic antagonists. Prenatal nicotine exposure exaggerated the increase in GABAergic frequency during inspiration and enhanced GABAergic synaptic amplitude both between and during inspiratory events. Glycinergic synaptic frequency and amplitude were unchanged by prenatal nicotine exposure. This study establishes a neurochemical link between neurons essential for respiration and CPNs, reveals a functional role for endogenous acetylcholine release and the activation of nicotinic receptors in the generation of RSA, and demonstrates that this cardiorespiratory interaction is exaggerated in rats prenatally exposed to nicotine. (Circ Res. 2003;93:565-572.)

Key Words: nucleus ambiguus ■ vagal activity ■ respiratory sinus arrhythmia ■ prenatal nicotine ■ sudden infant death syndrome

The heart rate increases during inspiration and decreases during the post-inspiration/expiration period. This respiratory-related change in heart rate, respiratory sinus arrhythmia (RSA), helps to match pulmonary blood flow to lung inflation and to maintain an appropriate diffusion gradient for oxygen in the lungs.1–3 RSA has been observed in neonatal4 and adult5,6 humans, baboons,7 dogs,8 rabbits,8 and seals9 but has not been well characterized in rats.

Heart rate is controlled by the activity of premotor cardioinhibitory parasympathetic neurons (CPNs) in the brainstem, and RSA is mediated in part by central respiratory modulation of CPN activity. CPNs are primarily located in the nucleus ambiguus (NA), in proximity to neurons thought to be essential for respiratory rhythmogenesis.2,3,10–13 CPNs in the NA are intrinsically silent and therefore rely on synaptic inputs to dictate their activity.14

Although the pathways and transmitters responsible for respiratory modulation of CPNs are unknown, γ-aminobutyric acid (GABA), glycine, and acetylcholine (ACh) are all neurotransmitters that have been implicated in the central generation of RSA. CPNs are inhibited during inspiration, and this inhibition has been reversed by the intracellular injection of Cl−.15 This suggests that GABA- and/or glycine-mediated chloride channels may be involved in respiratory modulation of CPNs. Paradoxically, however, one author of the same study described in a later review that the inhibition of CPNs during inspiration could not be inhibited by the GABA,A antagonist bicuculline or the glycine antagonist strychnine.2

ACh has been shown to inhibit CPN activity,15 and recent work has shown that endogenous ACh activates presynaptic nicotinic acetylcholine receptors (nAChRs), which enhance both GABAergic and glycinergic inputs to CPNs.16 The possible involvement of nicotinic receptors in mediating RSA is interesting because prenatal nicotine augments parasympathetic and reduces sympathetic control of the heart rate17 and is among the highest risk factors for sudden infant death syndrome (SIDS).18,19 Infants that succumb to SIDS often

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experience a sustained bradycardia, which is preceded or accompanied by a life-threatening apnea.\textsuperscript{19,20} These life-threatening events in SIDS victims are thought to be caused by exaggerated central cardiorespiratory interactions.\textsuperscript{17,19}

The aim of the present study was to test whether rats have an RSA pattern similar to that of other species and to elucidate the cellular mechanisms responsible for the respiratory modulation of CPNs. Specifically, we tested the hypothesis that heart rate increases during inspiration in rats. In addition, we tested whether CPNs receive increased GABAergic and glycineric synaptic inputs during inspiratory-related activity. Furthermore, we examined whether the respiratory modulation of CPNs is dependent on endogenous activation of nicotinic receptors. Because our work demonstrates that endogenous activation of nicotinic receptors is responsible for RSA, we tested whether prenatal nicotine exposure alters these cardiorespiratory interactions.

### Materials and Methods

#### Plethysmographic/Blood Pressure Recordings

Adult female Sprague-Dawley rats were anesthetized with a combination of ketamine (40 mg/kg) and xylazine (5 mg/kg IP, Phoenix Pharmaceuticals). The femoral artery was exposed and catheterized with Micro-Renthane tubing (Braintree Scientific) that had been soaked overnight in heparinized bacteriostatic saline. The animals were placed in a Covance infusion harness (Instech Labs, Inc) and allowed to recover for 24 to 48 hours. After recovery, the unanesthetized, freely moving rats were placed in a whole-body plethysmograph chamber, which allowed simultaneous measurement of blood pressure, heart rate, and respiratory airflow using Biosystem XA software (Buxco Electronics, Inc). Only measurements recorded during periods in which the animals were awake and sedentary were analyzed.

#### Fluorescent Labeling of CPNs and Medullary Slice Preparations

Neonatal Sprague-Dawley rats (P1-P5, Hilltop, Scottdale, Pa) were initially exposed to isoflurane (Abbott Laboratories) until anesthetized and cooled to \(\sim 4^\circ C\). A right thoracotomy was performed, and the retrograde fluorescent tracer X-rhodamine-5- (and -6)-isothiocyanate (Molecular Probes) was injected into the fat pads at the base of the heart. After 24 hours of recovery, each animal was anesthetized with isoflurane and decapitated, and the head was placed in a 4°C physiological saline solution (mmol/L: NaCl 140, KCl 5, CaCl\(_2\) 2, glucose 5, and HEPES 10) bubbled with 100% \(O_2\), pH 7.4. 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. The medulla was removed with care to preserve the hypoglossal cranial nerve rootlet. The medulla was mounted on a cutting block and placed into a vibrating blade microtome (Leica). Serial transverse sections were sliced in a rostrocaudal progression until the inferior olives and the NA could be visualized on the rostral surface of the tissue. A single thick (700 to 800 \(\mu m\)) section that included CPNs, the hypoglossal nerve rootlet, the pre-Botzinger complex, and the rostral portion of the hypoglossal nucleus was cut, transferred to a recording chamber and perfused (4 \(mL/min\)) with room temperature artificial cerebrospinal fluid (mmol/L: NaCl 125, KCl 3, CaCl\(_2\) 2, NaHCO\(_3\) 26, glucose 5, and HEPES 5) equilibrated with 95% \(O_2/5%\) \(CO_2\), pH 7.4.

#### Recording Respiratory Network Activity

The thick medullary slice preparation contains the pre-Botzinger complex, local circuits for motor output generation, and respiratory hypoglossal motor neurons, which generate inspiratory-phase motor discharge in hypoglossal cranial nerves.\textsuperscript{15} As seen in other similar medullary respiratory slice preparations, the frequency of respiratory-related hypoglossal discharge is significantly lower than that in in vivo preparations; this is likely due to the reduced temperature of the preparation and the absence of sensory input to the medulla.\textsuperscript{21} Spontaneous respiratory-related activity was recorded by monitoring the motor neuron population activity from hypoglossal nerve rootlets using a suction electrode. Hypoglossal rootlet activity was amplified (50 000 times), filtered (10- to 300-Hz bandpass, CWE Inc), and adjacent-averaged (50-ms windows). Respiratory activity was also electronically integrated (\(\tau=50\ ms\), CWE Inc) during experiments examining glycine-related synaptic inputs to CPNs.

#### Patch-Clamp Techniques

CPNs in the NA were identified by the presence of the fluorescent tracer.\textsuperscript{22} Patch pipettes (2.5 to 3.5 \(M\Omega\)) were visually guided to the surface of individual CPNs using differential interference optics and infrared illumination (Zeiss). Patch pipettes contained (mmol/L) KCl 150, MgCl\(_2\) 4, EGTA 2, Na-ATP 2, and HEPES 10, pH 7.4. This pipette solution causes the \(Cl^-\) current induced by the activation of GABA or glycine receptors to be recorded as an inward current (calculated reversal potential of \(Cl^-\) 4 \(mV\)). Voltage-clamp recordings were made with an Axopatch 200B and pClamp 8 software (Axon Instruments). All synaptic activity in CPNs was recorded at \(-80\ mV\). Only preparations in which synaptic activity increased in CPNs during inspiration (in 95 of 117 [81\%] of the preparations) were used for further experimentation and analysis. Only one cell was recorded per nucleus for an experiment. In 12 slices, an additional cell was recorded from the same slice in the contralateral NA.

#### Focal Drug Application

Focal drug application was performed using a pneumatic Picopump pressure delivery system (WPI). Drugs were ejected from a patch pipette positioned within 30 \(\mu m\) from the patched CPN. The maximum range of drug application has been previously determined to 100 to 120 \(\mu m\) downstream from the drug pipette and considerably less behind the drug pipette.\textsuperscript{23} GABAergic neurotransmission was isolated by focal application of \(\alpha2\)-amino-5-phosphonovalerate (AP-5, 50 \(\mu mol/L\)), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 50\(\mu mol/L\)), and strychnine (1 \(mmol/L\)) to block \(N\)-methyl-D-aspartate (NMDA), non-NMDA, and glycine receptors, respectively. Nicotinic receptors were blocked with dihydro-\(\beta\)-erythroidine (DH\(\beta\)E, 100 \(\mu mol/L\)) or curare (10 \(\mu mol/L\)). The role of different receptor subtypes was tested by applying DH\(\beta\)E at concentration selective for the \(\alpha\beta\) nicotinic receptor (3 \(\mu mol/L\)), and \(\alpha\) nicotinic receptor subtypes were tested with the \(\alpha\) nicotinic receptor antagonist \(\alpha\)-bungarotoxin (\(\alpha\)BTX, 100 \(\mu mol/L\)). All drugs were obtained from Sigma.

#### Prenatal Nicotine Exposure

Prenatal nicotine exposure was achieved by ketamine (40 mg/kg)/xylazine (5 mg/kg IP, Phoenix Pharmaceuticals) on the third day of gestation and implanted with Alzet osmotic minipumps (Durect) containing (–)-nicotine (56.1 mg/mL bacteriostatic saline, Sigma). Osmotic minipumps were chosen to avoid the high plasma nicotine concentrations and subsequent episodic fetal hypoxia/ischemia that can be produced by nicotine injections.\textsuperscript{25} Pumps delivered 2.1 mg nicotine per day, a level approximately equivalent to levels that occur in moderate to heavy smokers, for 28 days.\textsuperscript{17}

#### Data Analysis

### Plethysmographic/Blood Pressure Experiments

Heart rate intervals were measured during inspiratory and subsequent expiratory periods in 6 animals using Acqknowledge (version 3.7.3,
Biopac Systems). Heart rate was recorded from at least 20 respiratory cycles while the animal was awake and sedentary. Data are presented as mean ± SEM. Statistical comparisons were made using paired Student t tests. A value of $P < 0.05$ indicates significant differences.

Electrophysiology

Synaptic events were detected using MiniAnalysis (version 5.6.12, Synaptosoft). The frequency and amplitude of inhibitory postsynaptic currents (IPSCs) that occurred in CPNs during inspiratory-related hypoglossal activity were averaged to determine inspiratory values. Control values were determined by averaging IPSCs that occurred in a 5- to 10-second window ending 1 second before inspiratory burst onset and beginning at least 3 seconds after the end of any previous inspiratory activity. All data are presented as mean ± SEM. Statistical comparisons were made using paired or unpaired Student t tests, as appropriate. A value of $P < 0.05$ indicates significant differences.

**Results**

**Sprague-Dawley Rats Exhibit RSA Pattern Similar to That of Other Species**

Respiratory airflow (Figure 1, top) and blood pressure (Figure 1, bottom) were simultaneously recorded in 6 conscious, freely moving rats. During inspiration, the average heartbeat interval decreased significantly from 171 ± 4 ms during expiration to 167 ± 4 ms during inspiration ($P < 0.05$). This change in heart period corresponds to an average inspiratory-related increase in heart rate of 6.9 ± 1.9 bpm ($P < 0.05$).

**CPNs Are Inhibited During Inspiration by Endogenous Nicotinic ACh Receptor–Mediated Increases in GABAergic Activity**

To determine the cellular basis of RSA, the synaptic activity of CPNs was measured in vitro. GABAergic neurotransmission was isolated by focal application of the glutamatergic and glycinergic antagonists AP-5, CNQX, and strychnine in 22 cells (from 17 preparations). The focal application of these antagonists did not significantly alter the frequency ($P > 0.05$) or duration ($P > 0.05$) of the respiratory activity.

During inspiration, the frequency of GABAergic synaptic inputs to CPNs was significantly increased (basal 5.2 ± 0.7 Hz, inspiration 10.5 ± 1.3 Hz, $P < 0.01$, $n = 22$ cells; Figure 2a). All IPSCs under these recording conditions were blocked by nicotine. Nicotinic receptors mediate GABAergic inhibition of premotor CPNs during inspiration. Inspiratory-related bursting activity was recorded from the hypoglossal rootlet (XII), rectified, and adjacent-averaged (XII with bar above). Fluorescently identified CPNs were patch-clamped in the whole-cell configuration, and GABAergic IPSCs were isolated by focal application of the NMDA, non-NMDA, and glycine receptor antagonists AP-5 (50 μmol/L), CNQX (50 μmol/L), and strychnine (1 mmol/L), respectively. a, During inspiratory activity, the frequency of GABAergic IPSCs in CPNs was significantly increased ($P < 0.05$). b, GABA antagonist gabazine blocked all IPSCs. c, Nicotinic receptor antagonist curare (10 μmol/L) significantly inhibited ($P < 0.05$) the inspiratory-related increase in GABAergic synaptic frequency in CPNs. Representative traces in panels a, b, and c are from 3 CPNs.
by focal application of the GABA<sub>A</sub> antagonist gabazine (Figure 2b). Focal application of the nicotinic antagonist curare significantly reduced the inspiratory-related increase in GABAergic synaptic frequency (control basal 3.5±0.6 Hz, control inspiration 7.6±1.3 Hz, curare basal 2.6±0.4 Hz, curare inspiration 4.4±0.6 Hz, P<0.05, n=12 cells from 10 preparations; Figure 2c) but did not significantly affect the basal frequency of IPSCs between bursts (P>0.05, n=12 cells). GABAergic synaptic amplitude was not significantly altered by inspiratory activity or by the application of curare (P>0.05, n=12 cells).

**Determination of the nAChR Subtype Mediating Inspiratory-Related Increase in GABAergic Input to CPNs**

To determine the specific nicotinic ACh receptor (nAChR) subtype mediating the respiratory-related increase in GABAergic frequency, subtype-selective nicotinic antagonists were used. Focal application of αβ<sub>2</sub> receptor subtype-specific nicotinic antagonists had no significant effect on the frequency of GABAergic synaptic inputs to CPNs between inspiratory bursts (control 4.9±1.2 Hz, αBTX 5.5±1.2 Hz, P>0.05, n=4 cells) and did not alter the inspiratory-related increase in GABAergic synaptic frequency (control basal 5.0±1.2 Hz, control inspiration 15.2±2.7 Hz, αBTX basal 5.7±1.1 Hz, αBTX inspiration 11.4±2.4, P>0.05, n=4 cells from 4 preparations).

However, focal application of the nicotinic antagonist DHβE, in a concentration selective for the αβ<sub>2</sub> receptor subtype (3 μmol/L), abolished the increase in GABAergic frequency during inspiration (control basal 8.5±1.7 Hz, control inspiration 13.9±2.7 Hz, DHβE control 6.4±1.2 Hz, DHβE inspiration 8.2±1.8 Hz, P<0.05, n=6 cells; Figures 3a, 3b, and 3e) but did not affect GABAergic synaptic frequency between bursts (control 8.5±1.7 Hz, DHβE 6.4±1.2 Hz, P>0.05, n=6 cells from 3 preparations). GABAergic synaptic amplitude was not significantly altered by αBTX (control 49.7±8.2 pA, αBTX 40.5±8.9 pA, P>0.05, n=4 cells) or DHβE (control 57.2±11.4 pA, DHβE 52.9±9.2 pA, P>0.05, n=6 cells). Focal application of gabazine reversibly blocked all GABAergic synaptic events (Figures 3c through 3e).

**Glycinergic Respiratory Inputs to CPNs**

In an additional 13 cells (from 10 preparations), glycinergic activity was isolated by focal application of the glutamatergic and GABAergic antagonists AP-5, CNQX, and gabazine. The application of these antagonists did not significantly alter the frequency (P>0.05) or duration (P>0.05) of the respiratory activity. During inspiration, glycinergic synaptic frequency was also significantly increased (basal 11.0±2.2 Hz, inspiration 19.0±3.5 Hz, P<0.01, n=13 cells; Figure 4a). Focal application of the nicotinic antagonist DHβE in a high concentration that blocks all nicotinic receptors (100 μmol/L, Figures 4b, 4c, and 4f) did not significantly alter the inspiratory-related increase in glycinergic frequency (control basal 13.4±3.8 Hz, control inspiration 22.3±6.3 Hz, DHβE 13.4±4.0 to 22.0±6.1 Hz, P>0.05, n=7 cells from 5 preparations). DHβE did not significantly alter the frequency of glycinergic synaptic events between inspiratory events (Figure 4f, n=7 cells, P>0.05). All IPSCs under these recording conditions were reversibly blocked by focal application of strychnine (Figures 4d through 4f). Glycinergic amplitude was not altered by respiratory activity (basal 52.0±5.1 pA, inspiration 59.6±9.1 pA, P>0.05, n=13 cells) or by application of DHβE (control 52.0±5.1 pA, DHβE 44.9±7.7 pA, P>0.05, n=7 cells). Application of 100 μmol/L DHβE did not significantly alter the frequency (P>0.05) or duration (P>0.05) of the respiratory activity.

**Effect of Prenatal Nicotine Exposure on GABAergic and Glycinergic Synaptic Inputs to CPNs**

**GABA**

The frequency of GABAergic synaptic events increased 460±90% in animals prenatally exposed to nicotine (n=18 cells from 17 preparations), a significant exaggeration of the
260±40% increase observed in unexposed animals (n=22 cells, P<0.01; Figures 5a through 5c and 5e). Prenatal nicotine did not significantly alter the frequency of GABAergic synaptic events between inspiratory bursts (unexposed 5.2±0.7 Hz [n=22 cells], prenatal nicotine 7.7±0.7 [n=18 cells], P>0.05). Focal application of DHβE at a concentration (3 µmol/L) selective for αβ nicotinic receptors significantly inhibited the inspiratory-related increase in GABAergic activity in animals prenatally exposed to nicotine (control basal 6.2±1.2 Hz, control inspiration 14.8±2.2 Hz, DHβE basal 2.8±0.8 Hz, DHβE inspiration 4.6±1.3 Hz, P<0.05, n=6 cells from 5 preparations). In addition, DHβE significantly reduced basal GABAergic synaptic frequency (P>0.05, n=6 cells).

Prenatal nicotine also significantly increased GABAergic synaptic amplitude relative to unexposed animals both between (unexposed 43.7±4.5 pA [n=22 cells], prenatal nicotine 61.0±5.5 pA [n=18 cells], P<0.05) and during (unexposed 45.3±5.3 pA [n=22 cells], prenatal nicotine 66.1±6.2 pA [n=18 cells], P<0.05) inspiratory bursts (Figures 5d and 5e). All GABAergic synaptic events were reversibly blocked by focal application of gabazine.

**Glycine**

The inspiratory-related increase in glycine-mediated inhibition of CPNs during inspiration was significantly altered by prenatal nicotine exposure between (unexposed 52.0±5.0 pA [n=13 cells], prenatal nicotine 43.3±5.5 pA [n=11 cells], P>0.05) or during inspiratory bursts (unexposed 59.6±9.1 pA [n=13 cells], prenatal nicotine 45.2±7.9 pA [n=11 cells], P<0.05). All glycine-mediated, inhibitory postsynaptic currents were reversibly blocked by focal application of strychnine.

**Discussion**

There are four major findings from the present study: (1) Heart rate increases during inspiration and decreases during expiration in conscious, unrestrained rats. (2) CPNs in the brainstem are inhibited during inspiration by an increase in both GABAergic and glycine-mediated synaptic inputs. (3) The respiratory-related increase in GABAergic activity, but not glycine-mediated, is mediated by the endogenous activation of αβ nicotinic ACh receptors. (4) Prenatal nicotine exposure significantly exaggerates the GABA-mediated, but not glycine-mediated, inhibition of CPNs during inspiration.

**RSA Pattern in Rats**

It is well established in many species (including neonatal and adult humans, baboons, dogs, seals, and rabbits) that the heart rate increases during inspiration and decreases during expiration. This RSA improves the efficiency of pulmonary gas exchange by better matching ventilation and pulmonary blood flow.1,2,9 This inspiratory-related tachycardia is predominantly mediated by a reduction in cardiac vagal activity due to the decreased activity of CPNs in the NA.2,3,8,15,27–30 However, data describing cardiorespiratory interactions in the rat are contradictory. A recent in vitro study has paradoxically shown that in contrast to all other studied species, the activity of CPNs is enhanced during inspiration in rats.31 However, there are several factors that make the results from that study difficult to interpret. These include a desynchronization of central respiratory activity.
from lung inflation and using anesthetics, which in general reduce or eliminate parasympathetic cardiac activity. In contrast, a recent study using the rat working heart brainstem preparation\(^1\) (a preparation without anesthetics) demonstrates that heart rate increases during inspiration, consistent with studies using other species.\(^4–9,26\) The results from the present study unequivocally demonstrate that similar to the RSA that occurs in other species, heart rate increases during inspiration in conscious, freely moving rats.

**Inhibitory Respiratory-Related Synaptic Inputs to CPNs**

Previous in vivo work has shown that CPNs are inhibited during inspiration via a chloride-mediated current.\(^15\) The present study demonstrates that CPNs receive an increased frequency of both GABAergic and glycinergic IPSCs during inspiration. This respiratory-dependent inhibition of CPN activity provides a cellular mechanism for the tachycardia that occurs during the inspiratory phase of the respiratory cycle.

**Involvement of Nicotinic Receptors in RSA**

The GABA-mediated inhibition of CPNs during inspiration was significantly inhibited by curare, indicating that the increase in GABAergic frequency is mediated by the activation of nicotinic receptors. Further investigation revealed that this increase in GABAergic frequency was unaffected by \(\alpha\)-BTX, demonstrating that it was not mediated by the activation of the \(\alpha_4\) nAChR subtype. However, an \(\alpha_2\)-selective concentration of DH\(\beta\)E abolished the GABAergic inhibition of CPNs during inspiration, demonstrating that activation of \(\alpha_4\beta_2\) nAChRs by endogenous ACh is essential for the GABAergic component of this cardiorespiratory interaction.

The facilitation of GABAergic inputs to CPNs by activation of nicotinic receptors is consistent with previous work, which has shown that spontaneous GABAergic synaptic inputs to CPNs are enhanced by the activation of \(\alpha_4\beta_2\) receptors located in the presynaptic terminals of GABAergic neurons.\(^16,33\) The present study also indicates that the nicotinic receptors responsible for the increased GABAergic activity during inspiration are in proximity to the CPNs, inasmuch as the focal application of the nicotinic antagonist DH\(\beta\)E abolished this increase.

In contrast, greater (100 \(\mu\)mol/L) concentrations of DH\(\beta\)E did not significantly alter the respiratory-related increase in glycinergic synaptic frequency in CPNs. Interestingly, previous studies have shown that spontaneous glycinergic inputs to CPNs are also enhanced by the activation of \(\alpha_4\beta_2\) nicotinic receptors in glycinergic presynaptic terminals.\(^16\) This suggests that although glycinergic inputs to CPNs possess presynaptic nicotinic receptors, the respiratory-evoked increase in glycinergic synaptic input to CPNs is not mediated by the activation of nicotinic receptors. Alternatively, there may be nicotinic modulation of glycinergic activity that occurs at a location distant from the CPNs and out of the range of the focal application of drugs in the present study.

**Prenatal Nicotine Exposure and Respiratory-Related Synaptic Inputs to CPNs**

In animals prenatally exposed to nicotine, the inspiratory-related increase in GABAergic synaptic frequency was nearly...
twice that of unexposed animals, whereas the inspiratory-dependent increase in glycine-ergic frequency was not significantly different in control and nicotine-exposed animals. This is consistent with the results that nicotinic antagonists abolished the inspiratory-related increase in GABAergic frequency to CPNs but did not alter the respiratory modulation of glycine-ergic IPSCs to CPNs. In addition to enhancing the increase in frequency of GABAergic synaptic inputs to CPNs during inspiration, prenatal nicotine exposure also caused a significant increase in the amplitude of both spontaneous and inspiratory-evoked GABAergic synaptic inputs to CPNs.

Other studies have shown that the α2β2 nAChR subtype is significantly upregulated in rat brains chronically exposed to nicotine34,35 and that α2β2 receptors chronically exposed to nicotine exhibit enhanced responses to ACh and are less sensitive to desensitization.36 A greater number of, enhanced responses to, and reduced desensitization of α2β2 nicotinic receptors may be responsible for the prenatal nicotine-induced exaggeration of the α2β2-mediated increase in GABAergic synaptic frequency in CPNs during inspiration.

The α2β2 receptor antagonist DHβE (3 μmol/L) significantly inhibited the inspiratory-related increase in GABAergic frequency in animals prenatally exposed to nicotine, indicating that activation of α2β2 nicotinic receptors by endogenous ACh also mediates this cardiorespiratory interaction in animals prenatally exposed to nicotine. In addition, α2β2 block significantly reduced the frequency of spontaneous, non–inspiratory-related GABAergic synaptic inputs to CPNs in animals prenatally exposed to nicotine but not in unexposed animals. This suggests that prenatal nicotine augments endogenous cholinergic control of GABAergic inputs to CPNs in animals prenatally exposed to nicotine.

These alterations in cardiorespiratory control with prenatal nicotine exposure may be clinically important. Maternal cigarette smoking is highly correlated with SIDS, and it has been suggested that SIDS is caused by an alteration of brainstem sites responsible for cardiorespiratory control.17,19,37,38 Infants that subsequently succumb to SIDS have brainstem sites responsible for cardiorespiratory control. These data show a neurochemical link from the American Heart Association, Mid-Atlantic affiliate.

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