Prenatal nicotine exposure enhances the trigeminocardiac reflex via serotonin receptor facilitation in brainstem pathways

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Gorini C, Jameson H, Woerman AL, Perry DC, Mendelowitz D. Prenatal nicotine exposure enhances the trigeminocardiac reflex via serotonin receptor facilitation in brainstem pathways. *J Appl Physiol* 115: 415–421, 2013. First published June 13, 2013; doi:10.1152/japplphysiol.00552.2013.—In this study we used a rat model for prenatal nicotine exposure to test whether clinically relevant concentrations of brain nicotine and cotinine are passed from dams exposed to nicotine to her pups, whether this changes the trigeminocardiac reflex (TCR), and whether serotonergic function in the TCR brainstem circuitry is altered. Pregnant Sprague-Dawley dams were exposed to 6 mg·kg^{-1}·day^{-1} of nicotine via osmotic minipumps for the duration of pregnancy. Following birth dams and pups were killed, blood was collected, and brain nicotine and cotinine levels were measured. A separate group of prenatal nicotine-exposed pups was used for electrophysiological recordings. A horizontal brainstem slice was obtained by carefully preserving the trigeminal nerve with fluorescent identification of cardiac vagal neurons (CVNs) in the nucleus ambiguus. Stimulation of the trigeminal nerve evoked excitatory postsynaptic current in CVNs. Our data demonstrate that prenatal nicotine exposure significantly exaggerates both the TCR-evoked changes in heart rate in conscious unrestrained pups, and the excitatory neurotransmission to CVNs upon trigeminal afferent nerve stimulation within this brainstem reflex circuit. Application of the 5-HT$_{1A}$ receptor antagonist WAY 100635 (100 μM) and 5-HT$_{2A/C}$ receptor antagonist ketanserin (10 μM) significantly decreased neurotransmission, indicating an increased facilitation of 5-HT function in prenatal nicotine-exposed animals. Prenatal nicotine exposure enhances activation of 5-HT receptors and exaggerates the trigeminocardiac reflex.

prenatal nicotine; serotonin; parasympathetic; heart rate; SIDS

THE TRIGEMINOCARDIAC REFLEX (TCR) is one of the most powerful autonomic reflexes (21). Activation of sensory trigeminal afferents by mechanical or electrical stimulation elicits a polysynaptic glutamatergic neurotransmission to cardiac vagal neurons (CVNs) in the nucleus ambiguus (NA) that causes pronounced bradycardia, significant decrease in blood pressure, and apnea (17, 18, 21, 43–45). In adults this reflex can be triggered by tooth extractions or by orofacial, maxillofacial, or craniofacial surgery (2, 45, 46). A subset of this reflex, the diving response, can be initiated by fluid or airborne irritant stimulation of nasotrigeminal afferent fibers located in the nasal mucosa (11, 16, 61). The diving reflex is most prominent in infants with heart rate decreases up to 51% upon a single facial submersion (16). Although normally cardioprotective, an exaggerated TCR and resulting bradycardia can be detrimental. Sudden infant death syndrome (SIDS) is the leading cause of death in infants aged 1 mo to 1 yr (20) and is associated with bradycardia, centrally mediated apnea (13, 27, 31, 41), and

an exaggeration of parasympathetic activity to the heart (9, 19, 27, 47). Additionally, it has been reported that an exacerbated or abnormal response to trigeminal sensory nerve stimulation may be associated with the onset of SIDS and that exaggerated activation of the TCR may be involved in the heightened parasympathetic activity to the heart in infants that succumb to SIDS (24, 25, 28).

One of the highest risk factors for SIDS is maternal cigarette smoking and accompanying prenatal exposure to nicotine (10, 52). Despite this well-known risk factor, smoking during pregnancy has only slightly declined since 2003 (38). Some likely targets of prenatal nicotine exposure are most likely serotonergic and cholinergic function in the brainstem of the developing fetus as prenatal nicotine exposure alters serotonin transporter function, 5-HT receptor density, and cholinergic receptor density (6, 10, 32, 53, 54). Additionally, fetal exposure to nicotine perturbs cardiac and brainstem monoamine pathways, downregulating some subtypes of 5-HT receptors and facilitating the risk for SIDS (54). Prenatal nicotine exposure has been shown to alter both 5-HT$_{1A}$ and 5-HT$_2$ receptors in addition to increasing 5-HT transporter activity in systems that are critical components of brainstem cardiorespiratory function, particularly the TCR (53).

Previous work has shown that trigeminally evoked synaptic responses to CVNs in the brainstem from which parasympathetic activity to the heart originates, are differently modulated by endogenously active 5-HT$_{1A}$ and 5-HT$_{2A/C}$ receptors (17). In this study we expanded from this foundation and tested whether prenatal nicotine exposure alters the TCR in unanesthetized, unrestrained rat pups and TCR reflex brainstem circuitry, and in particular the endogenous serotonergic modulation of excitatory neurotransmission to CVNs in the NA upon trigeminal afferent activation.

METHODS

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.*

Adult female Sprague-Dawley rats (Hilltop, Scottdale, PA) 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}·day^{-1} of nicotine free base for 28 days. This dosing regimen produces nicotine blood levels approximately equivalent to those that occur in moderate to heavy smokers for 28 days (51). After birth, 2- to 5-day-old male and female pups (P2-P5; Hilltop) were anesthetized and cooled to 4°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. After 24–48 h of recovery, animals were anesthetized with isoflurane and

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killed by cervical dislocation, and the brain tissue was placed in a 4°C physiologic saline solution [containing (in mM) 140 NaCl, 5 KCl, 2 $CaCl_2$, 5 glucose, and 10 HEPES] bubbled with 100% O_2 (pH 7.4).

The medulla was removed with care to preserve the trigeminal cranial nerve rootlet and was mounted on a cutting block and placed into a vibrating blade microtome (Leica, Nussloch, Germany). A modified horizontal slice (800–900 μm) was obtained to allow stimulation of the trigeminal nerve rootlet and recording of synaptic responses in CVNs. The tissue was submerged in a recording chamber that allowed perfusion (4 ml/min) above and below the slice with room temperature artificial cerebrospinal fluid [containing (in mM) 125 NaCl, 3 KCl, 2 $CaCl_2$, 26 $NaHCO_3$, 5 glucose, and 5 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid] equilibrated with 95% O_2 5% CO_2 (pH 7.4).

CVNs in the NA were identified by the presence of the retrograde fluorescent tracer as described previously (26). Briefly, slices were viewed with infrared illumination and differential interference optics (Zeiss, Oberkochen, Germany) and under fluorescent illumination with an infrared-sensitive cooled charged-coupled device camera (Photometrics, Tucson, AZ). Neurons that contained the fluorescent tracer were identified by superimposing the fluorescent and infrared images on a video monitor (Sony, Tokyo, Japan). Patch pipettes (2.5–3.5 MΩ) were visually guided to the surface of individual CVNs using differential interference optics and infrared illumination (Zeiss). CVNs were voltage-clamped at a holding potential of −80 mV. The patch pipettes were filled with a solution that consisted of (in mM) 135 K-gluconic acid, 10 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, 10 ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid, 1 $CaCl_2$, and 1 $MgCl_2$, at a pH of 7.3.

Afferent fibers in the trigeminal nerve were stimulated with a bipolar concentric stimulating electrode (W.P.I., Sarasota, FL) using a flexible stimulus isolator (A.M.P.I., Jerusalem, Israel). Stimulus intensity was increased in each experiment, ranging from 0.1 to 1 mA, until a consistent synaptic pathway was evoked in each CVN during control conditions. The stimulation parameters were then maintained at that intensity and duration throughout the experiment. A series of 10 consecutive stimulations in each neuron was averaged, and this mean value from each neuron in the population was then averaged to create a summary of results for each condition. Data presented in scatterplots represent the last 6 consecutive stimulations before drug application followed by 10 consecutive stimulations after 5 min of drug bath application. Excitatory events were measured using pClamp 8 software (Molecular Devices, Sunnyvale, CA). Results are presented as means ± SEM and statistically compared with a paired Student's t-test (for significance of difference, $P < 0.05$). One slice per animal was obtained, with only one cell per slice utilized for experiments.

The 5-HT_{1A} antagonist WAY 100635 maleate salt (WAY, 100 μM) and the 5-$HT_{2A/C}$ receptor antagonist ketanserin tartrate salt (ketanserin, 10 μM) were applied by inclusion in the perfusate. All drugs were purchased from Sigma Aldrich (St. Louis, MO).

In a separate group of dams and pups, nicotine and cotinine levels in rat brain and serum were measured. Levels were measured by liquid chromatography tandem mass spectrometry (LC-MS/MS) in the laboratory of Dr. John James at Virginia Commonwealth University. Trunk blood was collected following decapitation of pups at P2 and from dams immediately following birthing. Serum was prepared in heparinized tubes, which were then frozen at −70°C until shipment. Brains were removed, rinsed in saline, dried, and weighed. Extraction of brain tissue was performed as previously described (15). Tissue was homogenized in three volumes of ice-cold 1.15% KCl; after 30 min centrifugation at 3,000 g at 4°C, the supernatant was treated with 1 ml of 2% $ZnSO_4$ for 1 h at 34°C to precipitate proteins. This mixture was then centrifuged for 1 h at 30,000 g at 4°C, after which the supernatant was decanted and volume recorded, and then frozen at −70°C until shipment.

Table 1. *Brain levels of nicotine and cotinine in pregnant dams and offspring**

	Brain Nicotine Levels, μM			Brain Cotinine Levels, μM		
	Mean	SEM	n	Mean	SEM	n
Dams	0.69	0.07	4	0.77	0.11	4
P2, all	1.53	0.20	12	0.69	0.14	12
P2, males	1.40	0.21	7	0.76	0.22	7
P2, females	1.70	0.38	5	0.59	0.08	5

*Nicotine was delivered via osmotic pump, 6 mg/kg per day.

Three standard electrocardiogram (ECG) electrodes were affixed to unanesthetized and unrestrained P6 pups in the lead II configuration, and ECG activity was amplified (CWE, Ardmore, PA), digitized, and recorded using DSI Ponemah software (St. Paul, MN). The effect of fetal nicotine exposure on the TCR control of heart rate was examined by placing 1–2 drops of cold (10°C) water on the pup's nose. Pups were kept on a heating pad and rectal body temperature was recorded and maintained at a stable temperature of 34°C. None of the pups used for ECG recordings underwent any surgical procedures. To determine baseline heart rate, the 15 sec prior to nasal afferent stimulation was divided into 5-sec bins and the average R-R interval for each bin was used to determine heart rate using the Ponemah Standard ECG Analysis Software (DSI). The change in heart rate upon evoking the TCR was obtained within 5–10 sec after placing 1–2 drops of cold water on the nose.

For nicotine and cotinine analysis, samples were thawed at room temperature and 50 μl was removed and spiked with appropriate deuterated internal standard. After addition of 90:10 methyl-t-butyl ether:tetrahydrofuran and vortexing, the organic layer was evaporated to dryness under a nitrogen stream. Samples were then reconstituted with 1% formic acid in acetonitrile and a 10-μl sample was injected into the LC-MS/MS for analysis (4). The LC-MS/MS method employed electrospray ionization–positive multiple reaction monitoring (MRM) mode. Nicotine, cotinine, and their respective deuterated internal standards were monitored using the following MRM transitions: nicotine 163→130, nicotine-d4 167→134, cotinine 176→80, and cotinine-d3 179→101. Chromatographic separation was achieved using a Polaris Si-A column (50 × 3.0 mm, 5 μm; Agilent Technologies, Palo Alto, CA). Chromatographic separation used hydrophilic interaction liquid chromatography. A gradient initially of 100% 1:1 acetonitrile:methanol with 0.05% formic acid slowly changing to 90% over 3 min and 10% 10 mM ammonium formate with 0.05% formic acid was used. The linear range used for nicotine was 2–75 ng/ml, and for cotinine 1–3,000 ng/ml, with a $1/x^2$ weighted regression model.

RESULTS

There is controversy in the literature concerning brain nicotine levels in both dams and pups when dams are administered prenatal nicotine during gestation (14, 30, 36, 37). To address this issue we measured brain levels of nicotine and cotinine in pregnant dams and pups when exposed to gestational/prenatal nicotine (6 mg·kg^{-1}·day^{-1} via osmotic minipumps) and blood levels of nicotine in dams at the end of gestation. Blood levels of nicotine in dams were 0.36 ± 0.07 μM. Data in Table 1 show that in dams there was a mean nicotine brain level of 0.69 ± 0.07 μM and a mean cotinine level of 0.77 ± 0.11 μM ($n = 4$). Brain nicotine levels in P2 male and female pups were 1.5 ± 0.2 μM, and average brain cotinine levels for P2 male and female pups was 0.69 ± 0.14 μM ($n = 12$). These results demonstrate that this dosage regimen leads to clinically relevant brain levels of nicotine in the nursing offspring of treated rat dams.
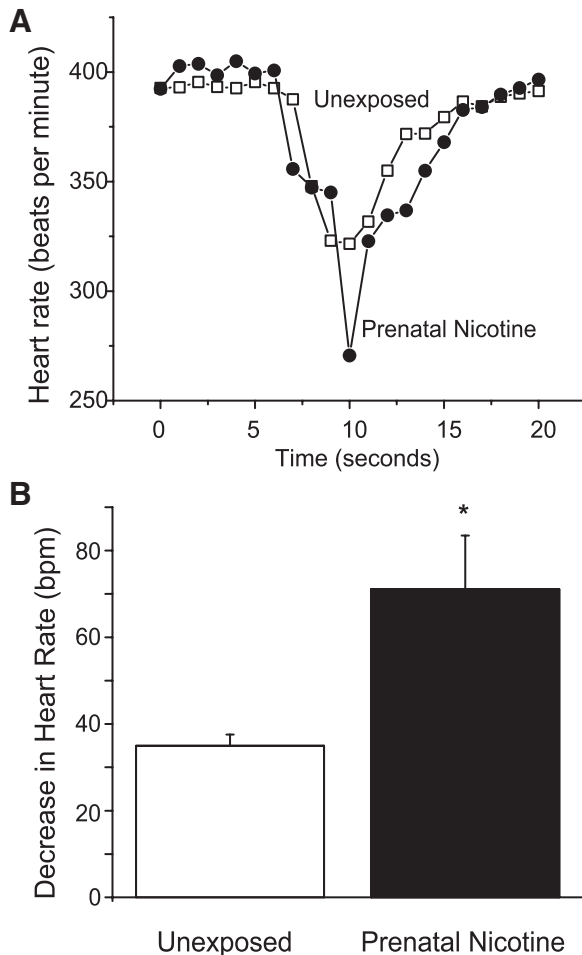
Fig. 1. Activation of the trigeminocardiac reflex (TCR) by applying 1–2 drops of 10°C water to the nose of P6 pups exposed to fetal nicotine evoked a significant decrease in heart rate compared with unexposed animals. *Top*: Time course of the TCR activated at 6 s from one unexposed and one prenatal nicotine-exposed animal. *Bottom*: Average decrease in heart rate responses of 71 ± 12.4 beats per minute (BPM) ($n = 8$; $P < 0.05$) in prenatal nicotine-exposed pups was significantly greater than the responses in unexposed animals (35 ± 2.6 BPM; $n = 10$).

To determine the effect of prenatal nicotine on the TCR reflex, ECGs were recorded in both unexposed pups and pups exposed to fetal nicotine prior to and during application of cold water (10°C) to pups' noses. As shown in Fig. 1 *top*, application of cold water (10°C) to a pup's nose evoked a rapid (within 1–2 s) transient decrease in heart rate in both unexposed and prenatal nicotine-exposed animals. This TCR response was significantly greater in animals exposed to prenatal nicotine than in unexposed pups (Fig. 1 *bottom*). Average heart rate decreased by 71 ± 12.4 beats per minute (BPM) ($n = 8$) in prenatal nicotine-exposed animals, compared with the decrease in heart rate of 35 ± 2.6 BPM in unexposed animals ($n = 10$).

To determine the electrophysiological basis for the prenatal nicotine exposure elicited exaggeration in the TCR we examined changes in the brainstem reflex circuitry responsible for the TCR by determining the synaptic responses in CVNs upon electrical stimulation of the trigeminal nerve rootlet. Animals exposed to prenatal nicotine had an exaggerated excitatory response in CVNs; the trigeminally evoked glutamatergic neurotransmission in prenatal nicotine-exposed animals was in-

creased by 64.2 ± 7.2% pA compared with unexposed animals ($n = 20$; $P < 0.05$), with mean peak amplitude increasing from 37.2 ± 3.1 pA in unexposed animals to 61.1 ± 11.1 pA in prenatal nicotine-exposed animals, as shown with a representative example of these responses in Fig. 2*A*, with the summary data from 40 cells (20 unexposed, 20 prenatal nicotine-exposed) shown in Fig. 2*B*.

To elucidate the effect of prenatal nicotine exposure on the important serotonergic modulation of trigeminally evoked neurotransmission to CVNs, the 5-HT$_{1A}$ antagonist WAY 100635 (100 μM) was administered. WAY 100635 inhibited the evoked excitatory postsynaptic current (eEPSC) amplitude by 39.3 ± 6.0% (from −46.0 ± 9.0 pA to −27.9 ± 7.2 pA; $n = 9$; $P < 0.05$; see Fig. 3). Typical traces are illustrated in Fig. 3*A*, with a plot illustrating the time course of the changes in maximum eEPSC amplitude from nine experiments in prenatal nicotine-exposed animals shown in Fig. 3*B*. As shown in Fig. 3*C*, the blunting of the excitatory evoked neurotransmission by WAY 100635 was exaggerated in prenatal nicotine-exposed animals [summary data from unexposed animals are shown for comparison and were previously published (17) showing an inhibition of 21.6 ± 9.2%]. This inhibition of eEPSC responses by application of WAY 100635 was significantly greater (17.7 ± 8.9%; $n = 9$; $P < 0.05$) in animals exposed to prenatal nicotine compared with responses in unexposed age-matched animals.

Application of ketanserin (10 μM), a 5-HT$_{2A/C}$ antagonist, in prenatally exposed animals also significantly inhibited the excitatory glutamatergic neurotransmission to CVNs upon trigeminal afferent stimulation, as shown in Fig. 4, by an average of 14.7 ± 1.2% ($n = 8$; $P < 0.05$) (from −34.5 ± 10.7 pA to −29.4 ± 9.6 pA). A typical trace is shown in Fig. 4*A*, and time course showing the effect on eEPSC amplitude from eight cells upon application of ketanserin is shown in Fig. 4*B*. Unlike in unexposed animals in which ketanserin increased eEPSC responses by 105.4 ± 5.1% [data from unexposed animals previ-
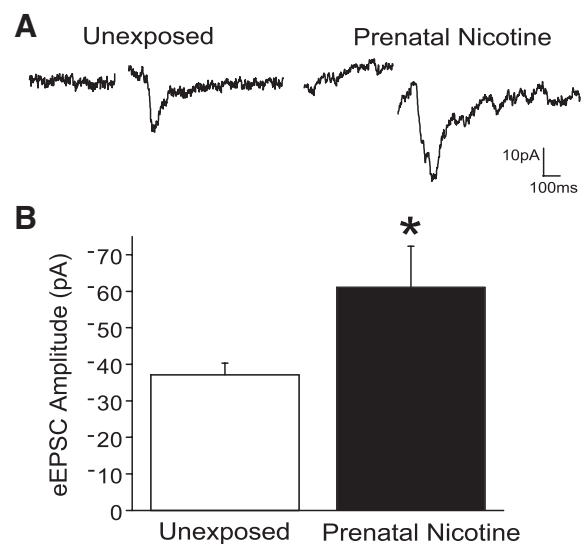

Fig. 2. Excitatory synaptic events in cardiac vagal neurons (CVNs) were evoked upon stimulation of the trigeminal nerve rootlet. *A*: traces from a typical experiment. *B*: summary data from 20 unexposed neurons and 20 prenatal nicotine-exposed neurons. Prenatal nicotine exposure significantly increased the trigeminal afferent–evoked excitatory postsynaptic current (eEPSC) in CVNs by 64.2 ± 7.2% ($n = 20$; $P < 0.05$).

A

WAY 100635 (100 μM)

10 pA | 250 ms

B

Percent Maximum eEPSC Amplitude

WAY 100635 (100 μM)

Consecutive Stimulations

C eEPCS amplitude

Prenatal Nicotine   Unexposed

WAY 100635 (100 μM)

Percent Change in eEPSC amplitude with WAY 100635
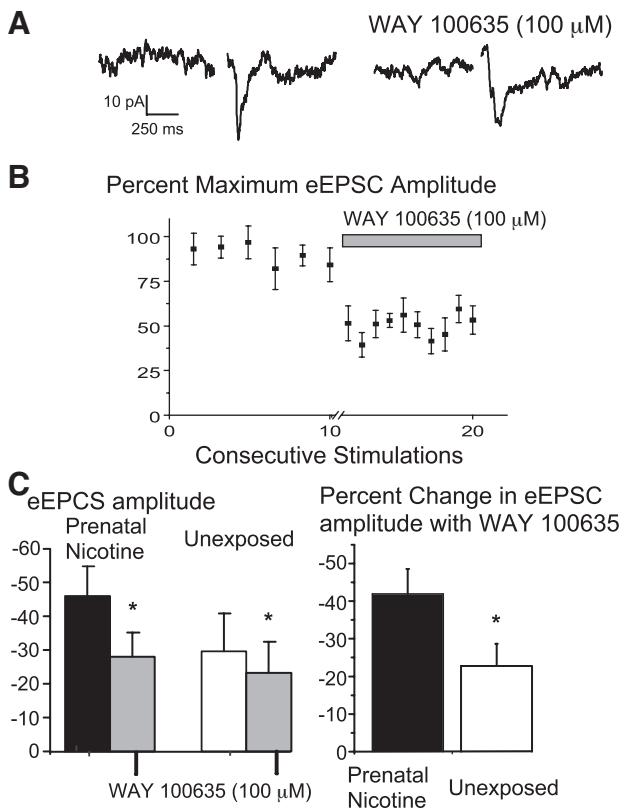

Prenatal Nicotine   Unexposed

Fig. 3. Application of the 5-HT$_{1A}$ receptor antagonist, WAY 100635 (100 μm) significantly reduced the glutamatergic TCR elicited eEPSC by an average of 39.3 ± 6.0%, from −46.0 ± 9.0 pA to −27.9 ± 7.2 pA ($n = 9$; $P < 0.05$) in prenatal nicotine-exposed animals. *A*: traces from a typical experiment. *B*: time course of the inhibition shown in a plot of maximum eEPSC amplitude averaged (with SEM) from nine prenatal nicotine-exposed animals. *C*: summary data from nine cells from prenatal nicotine-exposed animals compared with unexposed animals [borrowed with permission from (17)]. Black bars show pups exposed to prenatal nicotine, gray bars show the effect of WAY 100635, white bars show data from animals that did not undergo exposure to fetal nicotine.

ously published (17)], ketanserin evoked a significant decrease in eEPSC amplitude in prenatal nicotine-exposed animals (Fig. 4*C*).

DISCUSSION

This study has four major conclusions: *1*) pups of dams exposed to nicotine possess brain concentrations of nicotine and cotinine (1.5 μM and 0.7 μM, respectively) (42, 59) similar to values reported in heavy to moderate smokers; *2*) the TCR is exaggerated in animals exposed to prenatal nicotine; *3*) prenatal nicotine exposure significantly facilitates glutamatergic neurotransmission to CVNs upon stimulation of trigeminal afferents compared with unexposed animals; and *4*) endogenous serotonergic modulation of the TCR is enhanced by prenatal exposure to nicotine because both the 5-HT$_{1A}$ and 5-HT$_{2A/C}$ antagonists WAY and ketanserin, respectively, diminish the reflex-evoked EPSCs in CVNs from prenatal nicotine exposed animals.

Prenatal nicotine exposure increases risks for preterm delivery, spontaneous abortion, low birth weight, sleep disorders, impaired cardiac function, and SIDS (10, 12, 40, 49, 52, 60). Victims who succumb to SIDS exhibit abnormalities in glutamatergic and serotonergic function in areas of the brainstem responsible for brainstem cardiorespiratory control (10).

This study has shown that prenatal nicotine exposure significantly facilitates the TCR-evoked changes in heart rate in vivo. Exaggeration of the TCR in infants may be one component responsible for SIDS, and amplification of this reflex by fetal nicotine exposure likely plays a significant role in the disease. The prenatal nicotine exposure paradigm used in this study produced blood levels of nicotine in dams (0.36 ± 0.07 μM) that are highly clinically relevant in that previous work indicates an average plasma level in heavy to moderate smokers between 0.12 and 0.37 μM (22, 42). Our results also indicate higher concentrations of brain nicotine than cotinine in P2 pups. Previous work has shown nicotine binds to brain tissue with higher affinity compared with cotinine, and the receptor binding capacity increases due to a higher number of nicotinic ACh receptors in the brain of smokers compared with nonsmokers (3, 7, 39).

Previous studies have shown that upon stimulation of the trigeminal nerve an excitatory glutamatergic neurotransmission to CVNs is evoked, which can be blocked by *N*-methyl-D-aspartate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid/kainate glutamatergic receptor antagonists (17). One functional consequence of prenatal nicotine exposure is an exaggerated glutamatergic neurotransmission to CVNs upon
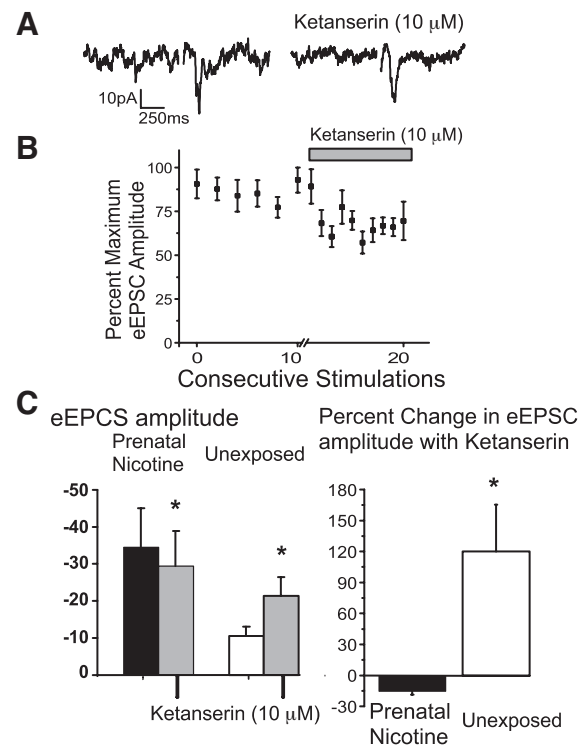
A   Ketanserin (10 μM)

10pA | 250ms

B

Ketanserin (10 μM)

Percent Maximum eEPSC Amplitude

Consecutive Stimulations

C eEPCS amplitude

Prenatal Nicotine   Unexposed

Ketanserin (10 μM)

Percent Change in eEPSC amplitude with Ketanserin

Prenatal Nicotine   Unexposed

Fig. 4. Ketanserin (10 μM), a 5-HT$_{2A/C}$ receptor antagonist, significantly inhibited excitatory glutamatergic neurotransmission to CVNs upon trigeminal afferent stimulation. *A*: traces from a typical experiment. *B*: percent maximum eEPSC amplitude averaged from eight cells (± SEM) for each stimulation time point. *C*: summary data from prenatal nicotine-exposed animals (black bars) show that TCR eEPSC amplitude decreased from −34.5 ± 10.6 pA to −29.4 ± 9.6 pA ($n = 8$; $P < 0.05$). In unexposed animals, *right* [borrowed with permission from (17)], ketanserin significantly facilitated (105.4 ± 5.1%; $P < 0.05$) the synaptic excitatory response in CVNs elicited by trigeminal afferent stimulation ($n = 8$; $P < 0.05$), whereas in prenatal nicotine-exposed animals ketanserin significantly decreased eEPSC amplitude. Gray bars indicate effect of ketanserin application, white bars indicate data from animals with no prenatal nicotine exposure.

trigeminal afferent stimulation compared with unexposed animals. In other systems fetal exposure to nicotine has been shown to increase glutamatergic neurotransmitter cycling in glutamate neurons leading to increases in glutamate release (48). Additionally, prenatal nicotine exposure has been shown to have widespread effects on brainstem serotonergic systems including changes in 5-HT receptor density, 5-HT transporter systems, and 5-HT receptor function (5, 29, 35). The experiments in this study indicate changes in 5-HT receptor function caused by prenatal exposure to nicotine produced a facilitation of evoked excitatory glutamatergic neurotransmission to CVNs. Previous work in unexposed animals has shown 5-HT_{1A} receptor activation facilitates excitatory glutamatergic neurotransmission to CVNs, whereas $5\text{-HT}_{2A/C}$ receptor activation significantly inhibits this glutamatergic transmission (17, 50). In contrast to unexposed animals, both endogenous 5-HT_{1A} and $5\text{-HT}_{2A/C}$ receptor activation increased eEPSCs in CVNs in prenatal nicotine-exposed animals. This indicates prenatal nicotine exposure likely exaggerates the TCR by increased 5-HT facilitation. Specifically, $5\text{-HT}_{2A/C}$ receptor activity augments glutamatergic neurotransmission to CVNs from prenatal nicotine-exposed animals, whereas $5\text{-HT}_{2A/C}$ receptor activity is inhibitory in unexposed animals. Although the results in this study did not address the source of the 5-HT neurons that modulate the TCR it is likely that these 5-HT neurons originate in the dorsal raphe nucleus (23, 58).

Fetal exposure to nicotine has been reported to cause 5-HT_{1A} receptor overexpression in the brainstem of Rhesus monkeys compared with unexposed animals (50), and SIDS victims have a higher number of 5-HT neurons in the medulla compared with a control population (34, 35). The increased number of 5-HT neurons has been localized to the midline raphe, lateral extraraphe, and at the ventral surface of the medulla (34). Additionally, transgenic 5-HT_{1A} receptor-overexpressing mice display abnormal and prolonged periods of bradycardia, hypothermia, diminished chemosensory activation, apnea, and respiratory distress (1, 8). An increase in 5-HT_{1A} receptor expression is one likely explanation for exaggerated eEPSC amplitudes in prenatal nicotine-exposed animals compared with unexposed animals in this study.

Interestingly, we have shown blocking $5\text{-HT}_{2A/C}$ receptors in prenatal nicotine-exposed animals produces an opposite modulatory effect on glutamatergic neurotransmission to CVNs upon trigeminal afferent stimulation compared with unexposed animals. In prenatal nicotine-exposed animals $5\text{-HT}_{2A/C}$ receptor activation facilitates eEPSCs, whereas in unexposed animals $5\text{-HT}_{2A/C}$ receptor activation depresses this neurotransmission. Data regarding fetal nicotine exposure and brainstem-specific 5-HT_2 receptor interaction are limited. One possible explanation for the exaggeration of glutamatergic neurotransmission in animals exposed to prenatal nicotine, however, is a combination of prenatal nicotine-induced increased extracellular glutamate and decreased inhibitory 5-HT_2 receptor function in the dorsal raphe nucleus (56, 57). The resulting effect is in increased glutamatergic neurotransmission to CVNs upon $5\text{-HT}_{2A/C}$ receptor activation.

Data from this study indicate that nicotine administered to pregnant dams leads to concentrations of both nicotine and its major metabolite, cotinine, in rat brain and serum that are similar to the values obtained in smokers. One potential explanation for the higher nicotine levels in the brains of pups compared with dams is a lower rate of nicotine metabolism in

the pups. Unfortunately, data concerning nicotine metabolism in pups is limited and difficult to calculate (33).

We have also shown that prenatal exposure to nicotine exaggerates the TCR-evoked changes in heart rate and the glutamatergic neurotransmission to CVNs upon trigeminal afferent stimulation. Additionally, 5-HT_{1A} and $5\text{-HT}_{2A/C}$ receptor activation increases the reflex-evoked eEPSCs in CVNs, and this facilitation by blocking $5\text{-HT}_{2A/C}$ receptors is opposite to the inhibitory effect of blocking these receptors in unexposed animals. In conclusion, fetal exposure to nicotine and heightened activation of both 5-HT_{1A} and $5\text{-HT}_{2A/C}$ receptors involved in the TCR may be detrimental to infant survival and is a likely mechanism that increases the risk of bradyarrhythmias that occurs in SIDS victims.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: C.G., H.J., A.L.W., D.C.P., and D.M. conception and design of research; C.G., H.J., and A.L.W. performed experiments; C.G., A.L.W., D.C.P., and D.M. analyzed data; C.G., A.L.W., D.C.P., and D.M. interpreted results of experiments; C.G., A.L.W., and D.M. prepared figures; C.G., H.J., and D.M. drafted manuscript; C.G., H.J., A.L.W., D.C.P., and D.M. edited and revised manuscript; C.G., H.J., A.L.W., D.C.P., and D.M. approved final version of manuscript.

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