Postnatal Sulfur Dioxide Exposure Reversibly Alters Parasympathetic Regulation of Heart Rate

Amanda L. Woerman, David Mendelowitz

Abstract—Perinatal sulfur dioxide exposure disrupts parasympathetic regulation of cardiovascular activity. Here, we examine the relative risks of prenatal versus postnatal exposure to the air pollutant and the reversibility of the cardiovascular effects. Two groups of animals were used for this study. For prenatal exposure, pregnant Sprague–Dawley dams were exposed to 5 parts per million sulfur dioxide for 1 hour daily throughout gestation and with their pups after birth to medical-grade air through 6 days postnatal. For postnatal exposure, dams were exposed to air, and after delivery along with their pups to 5 parts per million sulfur dioxide through postnatal day 6. ECGs were recorded from pups on postnatal day 5 to examine changes in heart rate. Whole-cell patch-clamp electrophysiology was used to examine changes in neurotransmission to cardiac vagal neurons in the nucleus ambiguus on sulfur dioxide exposure. Postnatal sulfur dioxide exposure diminished glutamatergic neurotransmission to cardiac vagal neurons by 40.9% and increased heart rate, whereas prenatal exposure altered neither of these properties. When postnatal exposure concluded on postnatal day 5, excitatory neurotransmission remained decreased through day 6 and returned to basal levels by day 7. ECGs showed that heart rate remained elevated through day 6 and recovered by day 7. On activation of the parasympathetic diving reflex, the response was significantly blunted by postnatal sulfur dioxide exposure through day 7 but recovered by day 8. Postnatal, but not prenatal, exposure to sulfur dioxide can disrupt parasympathetic regulation of cardiovascular activity. Neonates can recover from these effects within 2 to 3 days of discontinued exposure. **(*Hypertension*. 2013;62:274-280.)**

Key Words: air pollution ■ electrocardiography ■ electrophysiology ■ heart rate ■ parasympathetic nervous system ■ sulfur dioxide

The health effects associated with exposure to polluted air present an increasing public health threat. In 1990, air pollution exposure was responsible for 800 000 premature deaths worldwide. By 2010, that number skyrocketed to 3.2 million, an increase of 300%, in addition to the 3.5 million individuals killed by indoor air pollutants.[1] Although air pollution exposure has mainly been associated with respiratory disease, a shift in this primary association has been spurred by several findings that have identified cardiovascular disease, and the disruption of cardiovascular homeostasis, as a major impact of air pollution exposure, particularly as a result of exposure to the pollutant sulfur dioxide (SO_2).[2–9]

Several epidemiological studies have identified changes in cardiovascular function as a result of SO_2 exposure. Specifically, tachycardia[8,9] and a decrease in heart rate (HR) variability,[3,9–11] a measure of autonomic tone, have led many to hypothesize that SO_2 acts by altering autonomic regulation of the cardiovascular system.[9,10] Our recent publication demonstrated significant changes in the reflex control of HR and changes in the brain stem that are likely to be responsible for the impaired parasympathetic activity to the heart that occurs with SO_2 exposure.[12] The increase in basal HR induced by perinatal SO_2 exposure was accompanied by a diminished diving reflex-evoked bradycardia, indicating a loss of reflexive parasympathetic activity. In vitro electrophysiology recordings showed SO_2 induces a loss of glutamatergic neurotransmission to cardiac vagal neurons (CVNs), which are responsible for tonic parasympathetic outflow to the heart.[13]

Although these findings made significant strides forward in understanding the influence of SO_2 on cardiovascular disease, several questions remain. In particular, the Environmental Protection Agency has stated that a lack of research pertaining to prenatal and neonatal exposure to SO_2 limits its ability to define safe short-term exposure standards.[14] In this study, we tested the relative risks of prenatal versus postnatal exposure to SO_2 and whether the cardiovascular effects are reversible. The findings of these experiments have provided a clearer understanding of how SO_2 disrupts the neurons that maintain cardiovascular homeostasis and the specific risks associated with perinatal exposure to the pollutant.

Methods

Experiments were performed on Sprague–Dawley rats (Hilltop Laboratory Animals Inc, Scottdale, PA) housed in The George Washington University animal care facility. All animal procedures were approved by the George Washington University Institutional Animal Care and Use Committee, and all procedures are in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Rats were maintained under standard

Received April 10, 2013; first decision May 7, 2013; revision accepted May 26, 2013.

From the Department of Pharmacology and Physiology, The George Washington University, Washington, DC.

Correspondence to David Mendelowitz, Department of Pharmacology and Physiology, The George Washington University, 2300 Eye St NW, Ross Hall 640, Washington, DC. E-mail dmendel@gwu.edu

Hypertension **is available at http://hyper.ahajournals.org** **DOI: 10.1161/HYPERTENSIONAHA.113.01552**

environmental conditions (12:12-hour light:dark cycle) with free access to food and water. A total of 146 rat pups were used in this study.

SO_2 Exposure Protocol

Prenatal and postnatal exposure to SO_2 was carried out on the basis of modifications to the perinatal exposure model previously published.[12] Briefly, there were 2 groups of animals. In the first group, pregnant Sprague–Dawley dams were exposed to 5 ppm (13.1 mg/m^3) SO_2 for 1 hour daily throughout gestation and along with their pups on delivery to medical-grade air from birth through postnatal day 6 (P6; prenatal exposure). In the second group (postnatal exposure), pregnant Sprague–Dawley dams were exposed to air and along with their pups on delivery to 5 ppm SO_2 for 1 hour daily through P6. In experiments studying recovery from postnatal exposure, pregnant rats were exposed to medical-grade air throughout gestation for 1 hour daily. After birth, the dam and her pups were exposed to 5 ppm SO_2 for 1 hour daily through P5. Control animals underwent similar exposure during gestation but were exposed to medical-grade air. A Single Toxic Gas Monitor for SO_2 (RKI Instruments, Union City, CA) was used to monitor the concentration of SO_2 inside a sealed chamber (E-Z Systems, Palmer, PA). SO_2 and medical-grade air tanks were purchased from Roberts Oxygen (Rockville, MD).

Unrestrained ECG Recording

Three standard ECG electrodes were affixed to unanesthetized and unrestrained P5 pups, and ECG activity was amplified (CWE, Inc, Ardmore, PA), digitized, and recorded using DSI Ponemah software (St. Paul, MN). The effect of postnatal SO_2 exposure on basal and reflex control of HR was measured by eliciting the diving reflex by placing 1 to 2 drops of cold (10°C) water on the pup's nose during ECG recordings. Pups were kept on a heating pad to ensure stable body temperature at 34°C. None of the pups used for ECG recordings underwent any surgical procedures.

Labeling of CVNs

In a separate group of animals from those used for ECG recordings, pups at P1 were anesthetized via hypothermia and cooled to ≈4°C. Once HR significantly slowed and a pain reflex could not be elicited, a right thoracotomy was performed, exposing the heart, and the retrograde tracer rhodamine (XRITC, Invitrogen, 2% solution, 20–50 μL) was injected into the pericardial sac to label CVNs. After surgery, buprenorphine (0.1–0.5 mg/kg, s.c.) was immediately administered and pups were warmed to raise their body temperatures on a heating pad. Pups were monitored for 30 minutes and every 20 minutes thereafter until ambulatory, and 2 more injections of buprenorphine were given within the next 2 to 12 hours.

Brain Stem Slice Preparation

After 4 to 8 days of recovery, animals were overdosed with isoflurane and euthanized by cervical dislocation. Brain tissue was collected and placed in 4°C physiological saline buffer solution with the following composition: NaCl (140 mmol/L), KCl (5 mmol/L), $CaCl_2$ (2 mmol/L), glucose (5 mmol/L), and HEPES (10 mmol/L) and sectioned. A single slice of tissue from the medulla that included the nucleus ambiguus was obtained and submerged in a recording chamber allowing perfusion above and below the slice with room temperature artificial cerebrospinal fluid with the following composition: NaCl (125 mmol/L), KCl (3 mmol/L), $CaCl_2$ (2 mmol/L), $NaHCO_3$ (26 mmol/L), glucose (5 mmol/L), and HEPES (5 mmol/L) in equilibrium with 95% O_2-5% CO_2.

Patch-Clamp Techniques and Drug Application

CVNs were identified by the presence of the fluorescent tracer and differential interference contrast optics along with infrared illumination and video detection cameras to gain enhanced spatial resolution. Patch pipettes were filled with a solution at pH 7.3 consisting of K-gluconic acid (150 mmol/L), HEPES (10 mmol/L), EGTA (10 mmol/L), $MgCl_2$ (1 mmol/L), and $CaCl_2$ (1 mmol/L). Identified CVNs were voltage-clamped at a holding potential of −80 mV.

Glutamatergic currents were isolated in patched cells by application of gabazine (25 μmol/L), to block GABAergic neurotransmission, and strychnine (1 μmol/L), to block glycinergic neurotransmission, using a pneumatic picopump pressure delivery system (WPI, Sarasota, FL). Drugs were received from Sigma-Aldrich (St. Louis, MO).

Data Analysis

Initial ECG traces were analyzed by dividing the final 2 minutes of uninterrupted recordings from a 5-minute recording into 5-second bins, and the R wave to R wave (RR) interval for each bin was used to determine baseline HR using the Ponemah Standard ECG Analysis Software (DSI, Minneapolis, MN). Data from each 5-second bin were averaged for each animal and combined for an overall average from each experimental group. Recordings of HR or HR responses were excluded if 2 minutes of uninterrupted recordings were not available, or were considered outliers on the basis of conservative estimation of being more than 4 SD away from the mean.

In recovery studies, baseline HR was determined from the 15 seconds before diving reflex stimulation. The data were divided into 5-second bins and the average RR-interval for each bin was used to determine HR. The change in HR on evoking the diving reflex was obtained within 5 to 10 seconds after placing 1 to 2 drops of cold water on the nose. Averages for baseline HR and the change in HR were compared between control and postnatal SO_2-exposed animals.

Spontaneous excitatory postsynaptic currents (EPSCs) were recorded for ≥5 minutes. The frequency of EPSCs was determined by dividing the number of excitatory events by the duration of the recording. Data were obtained and averaged under each condition. MiniAnalysis (Synaptosoft version 4.3.1) was used to analyze all experimental traces. The threshold for glutamatergic events was set to 5× the root mean square of noise.

All data are represented by mean±SEM. A 2-tailed unpaired Student *t* test was used to determine statistical significance. An *F* test was used to determine whether equal or unequal variance was used for the *t* test, and statistical significance was determined with a P value <0.05.

Results

Postnatal, but Not Prenatal, Exposure to SO_2 Disrupts Neurotransmission to Cardiac Vagal Neurons

Previously published in vitro studies showed that perinatal SO_2 exposure disrupts excitatory glutamatergic, but not inhibitory, neurotransmission to CVNs.[12] To determine whether prenatal-only exposure, postnatal-only exposure, or both were responsible for this change in activity, glutamatergic EPSCs were isolated in an 800-μm-thick slice of the brain stem from P5 or P6 pups exposed to SO_2 prenatally or postnatally only (Figure 1A) and were compared with recordings previously published from air-exposed (4.3±0.8 Hz; n=5) and perinatal-exposed animals (2.1±0.3 Hz; n=11).[12] The frequency of EPSCs in CVNs from pups exposed to SO_2 during the prenatal time period only was not statistically different from the frequency of EPSCs in CVNs obtained from air-exposed animals (previously published; 3.5±0.4 Hz; n=13; P>0.05).[12] However, pups exposed to SO_2 during the postnatal time period only showed a significant decrease in glutamatergic EPSC frequency recorded from CVNs (2.6±0.2 Hz; n=11; P<0.05; Figure 1B). These data indicate that postnatal exposure to SO_2 alone is sufficient to disrupt central regulation of parasympathetic activity.

Postnatal Exposure to SO_2 Increases Basal HR

To test whether the in vitro electrophysiology data demonstrating impaired brain stem parasympathetic cardiac activity

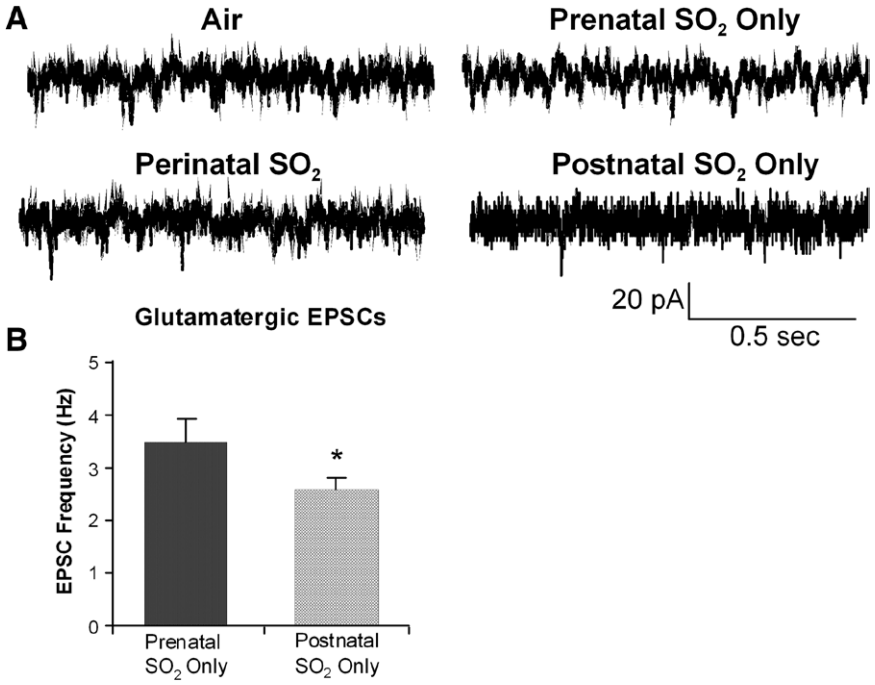

Figure 1. Glutamatergic excitatory postsynaptic currents (EPSCs) were isolated and recorded from cardiac vagal neurons (CVNs) in pups exposed to SO_2 during the prenatal and postnatal time points only, and were compared with EPSCs isolated from control air-exposed animals and perinatally SO_2-exposed pups, as previously published (**A**). Initial experiments showed perinatal SO_2 exposure significantly decreased glutamatergic neurotransmission to CVNs (2.1±0.3 Hz; n=11) compared with air-exposed animals (4.3±0.8 Hz; n=5). Prenatal-only exposure did not statistically alter EPSC frequency (3.5±0.4 Hz; n=13), whereas postnatal-only exposure did significantly decrease excitatory neurotransmission to CVNs (2.6±0.2 Hz; n=11; **P*<0.05; **B**).

were predictive of an increase in basal HR, in vivo ECGs were recorded from P5 air-exposed animals and pups exposed to SO_2 prenatally, postnatally, and perinatally (Figure 2). HR was determined using the final 2 minutes of a 5-minute recording from each animal. Consistent with the predictions based on the in vitro experiments, perinatal SO_2 exposure produced a significant increase in HR (388±3 bpm; n=8) compared with control air-exposed animals (356±7 bpm; n=8; *P*<0.05). Prenatal exposure to SO_2 only, however, did not statistically alter basal HR (348±5 bpm; n=11; *P*>0.05) compared with those from control animals, but postnatal exposure significantly increased HR (378±5 bpm; n=9). These data are consistent with, and expand on, the in vitro data, indicating that postnatal exposure to SO_2 alone is sufficient to disrupt parasympathetic regulation of cardiovascular function.

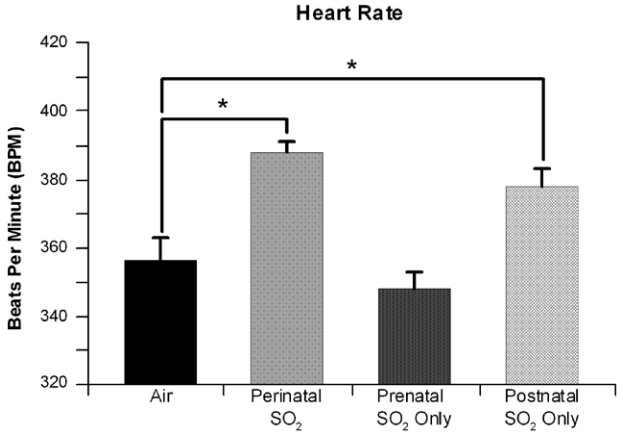

Figure 2. The R wave to R wave (RR)-intervals from the final 2 minutes of a 5-minute ECG were used to determine baseline heart rate (HR) in P5 pups exposed to medical-grade air or 5 ppm SO_2 during prenatal, postnatal, or perinatal development. Perinatal exposure to SO_2 significantly increased basal HR (388±3 bpm; n=8) compared with air-exposed animals (356±7 bpm; n=8). Prenatal-only exposure did not statistically change baseline HR (348±5 bpm; n=11), whereas postnatal-only exposure significantly increased basal HR (378±5 bpm; n=9; **P*<0.05).

The SO_2-Mediated Decrease in Glutamatergic Neurotransmission to CVNs Returns to Control Levels After Exposure Has Discontinued

After determining that postnatal exposure to SO_2 alone was enough to elicit physiological changes in cardiovascular regulation and parasympathetic activity to the heart, we tested whether these effects were reversible, and if so with what time course. To examine this, we used the postnatal SO_2-only exposure protocol previously established and exposed pups to medical-grade air for 1 hour daily throughout gestation and to 5 ppm SO_2 for 1 hour from P0 to P5. After P5, the pups were no longer exposed to either gas. Glutamatergic EPSCs were isolated and recorded from CVNs in P5 to P9 pups (Figure 3A), however, whereas an 800-μm-thick slice of the brain stem was previously used, a 400-μm-thick slice was used for all recovery experiments to avoid the likelihood of hypoxia in tissue from more susceptible older animals.

The average EPSC frequency for each day was compared with age-matched control animals exposed to medical-grade air from the first day of gestation through P5. At P5, EPSC frequency to CVNs was significantly blunted in pups postnatally

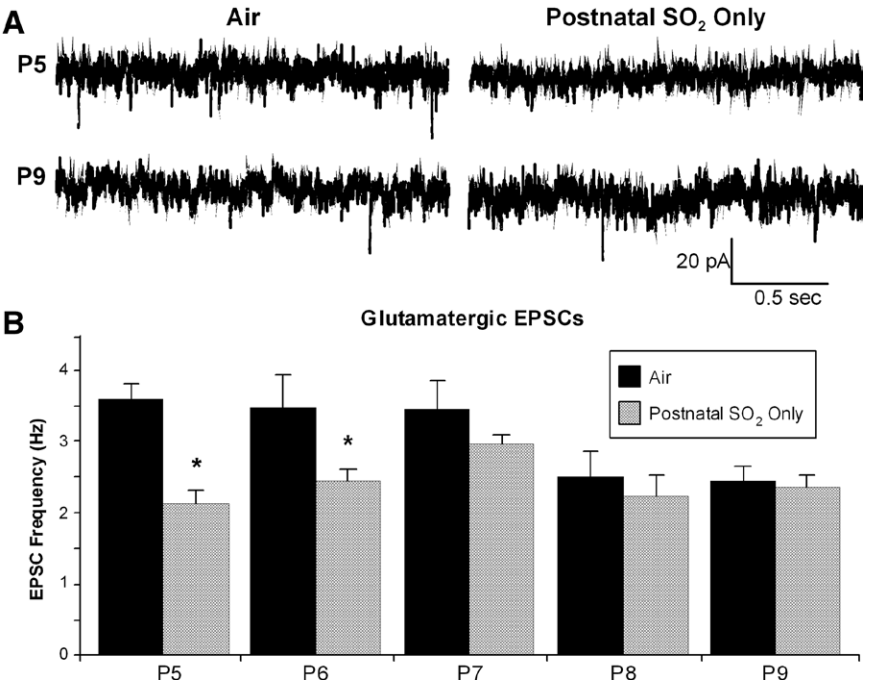

Figure 3. Excitatory postsynaptic currents (EPSCs) were isolated and recorded from cardiac vagal neurons (CVNs) in a 400-µm-thick slice of the brain stem from pups exposed to medical-grade air 1 hour daily throughout gestation and to 5 ppm SO_2 1 hour daily through P5. Average frequencies were compared with control pups exposed to medical-grade air throughout perinatal development. All exposures were discontinued on P5 to determine reversibility of the effects of exposure. Sample traces (**A**) from P5 pups show a significant decrease in neurotransmission to CVNs in postnatally exposed animals, but by P9, neurotransmission was recovered to control frequencies. EPSC frequency (**B**) was significantly decreased in pups exposed to SO_2 during postnatal development at P5 (control, 3.6±0.2 Hz; n=8 and postnatal SO_2, 2.1±0.2 Hz; n=8; **P*<0.05) and P6 (control, 3.5±0.5 Hz; n=8 and postnatal SO_2, 2.4±0.2 Hz; n=12). The decrease was no longer significant by P7 (control, 3.4±0.4 Hz; n=7 and postnatal SO_2, 2.9±0.1 Hz; n=10) and P8 (2.5±0.3 Hz; n=7 and postnatal SO_2, 2.2±0.3 Hz; n=10). Pups fully recovered by P9 (2.3±0.2 Hz; n=8) to control frequencies (2.4±0.2 Hz; n=7).

exposed to SO_2 (2.1±0.2 Hz; n=8) compared with control pups (3.6±0.2 Hz; n=8; *P*<0.05; Figure 3B). On P6, the first day after exposure, neurotransmission remained significantly decreased in pups exposed to SO_2 postnatally (2.4±0.2 Hz; n=12) compared with control animals (3.5±0.5 Hz; n=8), but by P7, the decrease seen in the postnatally exposed pups (2.9±0.1 Hz; n=10) was no longer statistically different than activity recorded from control pups (3.4±0.4 Hz; n=7; *P*>0.05). This recovery persisted through P8 and P9. The trending developmental decrease in EPSC frequency recorded from both air- and SO_2-exposed animals is likely a result of animal growth during the 5-day period, resulting in fewer active connections remaining in the 400-µm-thick slice preparation.

The SO_2-Mediated Increase in Basal HR and Blunting of the Diving Reflex Both Return to Control Levels After Exposure Has Discontinued

After determining that glutamatergic neurotransmission to CVNs was able to recover to baseline levels after cessation of postnatal SO_2 exposure, ECG recordings were used to measure basal and diving reflex–mediated changes in HR to examine whether the changes in the in vitro measures of cardiovascular function predict recovery of cardiovascular function in vivo. Postnatal SO_2 exposure significantly elevated baseline HR in P5 pups (387±5 bpm; n=17; *P*<0.05) compared with air-exposed animals (368±5 bpm; n=16; Figure 4A). HR remained significantly elevated at P6 (381±5 bpm) compared with air-exposed pups (358±6 bpm), but by P7 the increase in HR in postnatally exposed pups was no longer significant (control, 372±6 bpm; postnatal SO_2, 383±6; *P*>0.05) and this recovery persisted through P8 and P9.

In addition to baseline HR, the change in HR that was elicited by the diving reflex was also examined. The diving reflex is a powerful autonomic reflex[15] that results in bradycardia elicited by placing 1 to 2 drops of cold (10°C) water on the pup's nose. In pups that were exposed to SO_2 postnatally only, there was a significant blunting of the diving reflex at P5 (21±6 bpm; n=17; *P*<0.05) compared with air-exposed animals (43±8 bpm; n=15; Figure 4B). This significant loss of reflex activity was still present in P6 (control, 37±5 bpm; n=13 and postnatal SO_2, 18±7 bpm; n=17) and P7 pups (control, 69±6 bpm; n=15 and postnatal SO_2, 25±4 bpm; n=11). But by P8, the reflex evoked in postnatally exposed pups (73±11 bpm; n=15) was no longer significantly different from air-exposed pups (96±12 bpm; n=15; *P*>0.05), and by P9 the change in HR between the 2 groups was insignificant (control, 97±11 bpm; n=13 and postnatal SO_2, 86±13 bpm; n=13). The increase in HR response to the diving reflex in both groups of animals is likely a result of continued parasympathetic nervous system maturation during postnatal development, manifesting as a stronger reflex response.

Discussion

To protect vulnerable groups within the population from exposure to toxins, research must identify the most susceptible periods

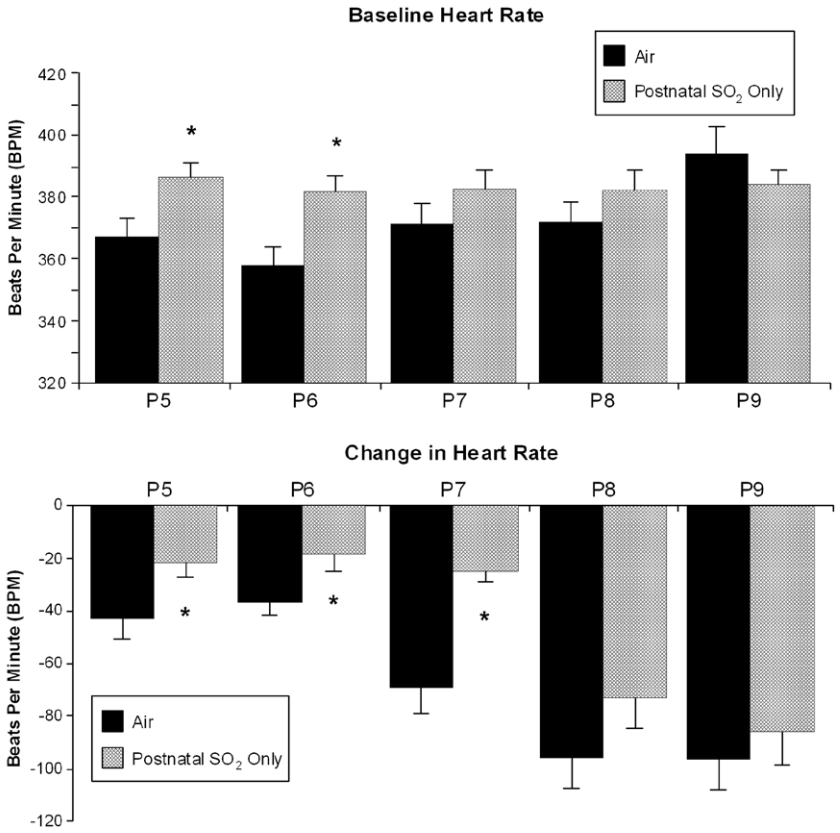

Figure 4. The R wave to R wave (RR)-intervals isolated from 15 seconds of an ECG were used to determine basal heart rate (HR) in air-exposed animals and pups exposed to 5 ppm SO_2 postnatally for 1 hour daily through P5 (**A**). Basal HR was measured daily from P5 to P9. Postnatal SO_2 exposure significantly elevated baseline HR in P5 (control, 368±5 bpm; n=16 and postnatal SO_2, 387±5 bpm; n=17; **P*<0.05) and P6 pups (control, 358±6 bpm and postnatal SO_2, 381±5 bpm). By P7, postnatally exposed pups began to recover and the increase in HR was no longer significant (control, 372±6 bpm and postnatal SO_2, 383±6 bpm). As the pups continued to recover, HR was statistically unchanged at P8 (control, 372±7 bpm and postnatal SO_2, 382±7 bpm) and P9 (control, 394±10 bpm and postnatal SO_2, 384±5 bpm). RR-intervals recorded during the 5 to 10 seconds after the diving reflex was elicited by placing 1 to 2 drops of 10°C water on the nose were compared with the 15 seconds prior, and the average decrease in HR between pups exposed to 5 ppm SO_2 for 1 hour daily postnatally through P5 was compared with air-exposed pups (**B**). At P5, postnatal SO_2 exposure significantly blunted the decrease in HR elicited by the diving reflex (–21±6 bpm; n=17) compared with air-exposed pups (–43±8 bpm; n=15). This loss of reflexive parasympathetic activity was still present in P6 (control, –37±5 bpm; n=13 and postnatal SO_2, –18±7 bpm; n=17) and P7 pups (control, –69±6 bpm; n=15 and postnatal SO_2, –25±4 bpm; n=11). However, as the pups recovered from SO_2 exposure, parasympathetic reflex activity began to return, and the decrease seen in postnatally exposed pups was no longer significant at P8 (–73±11 bpm; n=15) compared with air-exposed animals (–96±12 bpm; n 15). At P9, reflexive activity in postnatally exposed animals (–86±13 bpm; n=13) continued to approach baseline levels (–97±11 bpm; n=13).

of exposure and whether the impacts of exposure are reversible. As such, there are 2 main findings of this study: (1) postnatal, but not prenatal, exposure to SO_2 is sufficient to disrupt excitatory synaptic inputs to CVNs, reflexive control of HR, and induce tachycardia. (2) Recovery from these deleterious effects of exposure to SO_2 occurs within 2 to 3 days after exposure has ended, indicating reversibility and fast recovery from SO_2 exposure.

Having previously identified the physiological changes to brain stem parasympathetic activity induced by perinatal SO_2 exposure,[12] this study focused on the relative risks of prenatal versus postnatal exposure to the air pollutant. To address this question, the perinatal exposure model was altered to examine the specific outcomes of prenatal-only versus postnatal-only exposure to SO_2. Isolation of excitatory neurotransmission to CVNs showed that the frequency of EPSCs in CVNs recorded from pups exposed to SO_2 prenatally was not different from those obtained from control animals. However, postnatal exposure to SO_2 alone produced a significant decrease in EPSCs in CVNs by 40.9%, which is very similar to the previously published 51.2% decrease in EPSC frequency in CVNs after combined perinatal exposure. To test whether these physiological changes correlated with changes in cardiovascular function, basal HR was recorded from P5 pups. These in vivo studies showed that HR measured after prenatal-only exposure was not statistically different from control pups, whereas both perinatal- and postnatal-only exposure to SO_2 elicited very similar and significantly elevated HRs.

These data are important for 3 key reasons. Postnatal SO_2 exposure alone is sufficient to induce tachycardia and disrupt both tonic and reflexive parasympathetic regulation of HR. This loss of vagal activity and diminished reflex, in combination with tachycardia, increases the susceptibility of developing sudden cardiac death[11,16] in what is recognized to be a high-risk portion of the population. However, it should be noted that it is unclear whether pups exposed to SO_2 only during the prenatal period are altered by exposure but recover before in vivo and in vitro experiments at P5, or whether prenatal exposure does not have any effect on cardiovascular activity. In either case, this work shows that any particular effects on cardiovascular function that may arise during prenatal SO_2 exposure are not long-lasting unlike several adverse birth outcomes after prenatal air pollution exposure, including intrauterine growth restriction, very low birth weight, congenital heart defects,[17–20] and Sudden Infant Death Syndrome (SIDS).[21,22]

With these findings in mind, we wanted to determine how much time is required for recovery after postnatal exposure to SO_2. P5 pups exposed to SO_2 postnatally exhibited a significant decrease in EPSC frequency which persisted through P6, but by P7, EPSC frequency was no longer significantly different from control animals. These in vitro findings were highly predictive of in vivo changes of HR from separate P5 to P9 pups. Basal HR was significantly elevated in pups exposed to SO_2 postnatally at P5 and P6, compared with air-exposed animals, but by P7, this increase was no longer significant. In addition,

parasympathetic reflex regulation of HR, assessed by activating the diving reflex, recovered with a similar time course. Similar to the results found with perinatal exposure to SO_2,[12] postnatal SO_2 exposure significantly blunted the diving reflex in P5 pups compared with air-exposed animals. After cessation of postnatal SO_2 exposure at P5, this parasympathetic-mediated reflex was significantly diminished in P6 and P7 animals. However, by P8 the reflex was no longer significantly different from control pups. Together with the basal HR data and the in vitro data, these findings suggest that the SO_2-induced cellular changes in parasympathetic activity and reflex regulation of HR recover within 2 to 3 days after exposure.

These findings are especially important from a policy perspective. It is clearly not feasible to regulate immediate individual exposure levels during weather patterns, such as temperature inversions or after a significant volcanic eruption, but the results in this study indicate that removing individuals from SO_2 exposure for 2 to 3 days is sufficient to reverse the adverse loss of parasympathetic function and the development of tachycardia that would occur in neonates after such high-impact events. In addition, outside of the United States in rural areas of developing and undeveloped countries, coal and other biomass-fueled stoves are still highly prevalent. These stoves are used for both cooking and heating the home, and the use of low-grade coal or the incomplete combustion of biomasses escalates postnatal exposure to SO_2. The results of this study indicate exposure for as few as 5 days produces an additional 2 to 3 days of heightened risk in infants, reinforcing the need for policy changes and field projects that replace these fuel sources with cleaner options, such as solar energy or kerosene, in an effort to reduce the increasing number of individuals killed by indoor air pollution exposure each year.[1,23,24]

Perspectives

As an understudied but high-risk portion of the population,[14] the identification of poor cardiovascular health in neonates holds particular importance in determining limits for exposure standards and health policies. This work has taken significant steps forward in understanding the mechanisms behind the cardiovascular effects associated with perinatal SO_2 exposure. These findings should play an important role in crafting policies that are better designed to protect neonates from postnatal exposure to SO_2, but they can also be carried forward to examine other age groups and high-risk models, such as elderly, asthmatic, and hypertensive individuals, to determine whether or not our current policies are sufficient to also protect these members of the population.

Acknowledgments

A.L. Woerman is a predoctoral student in the Molecular Medicine Program of The Institute for Biomedical Sciences at The George Washington University. This work is from a dissertation to be presented to the above program in partial fulfillment for the Ph.D. degree.

Sources of Funding

This work was supported by the National Institutes of Health (HL49965, HL59895, and HL72006 to D. Mendelowitz) and the George Washington Institute for Sustainability Research, Education, and Policy Research Award (to A.L. Woerman).

Disclosures

None.

References

1. Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H, Amann M, Anderson HR, Andrews KG, Aryee M, Atkinson C, Bacchus LJ, Bahalim AN, Balakrishnan K, Balmes J, et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the global burden of disease study 2010. *Lancet*. 2013;380:2224–2260.
2. Sang N, Yun Y, Li H, Hou L, Han M, Li G. SO2 inhalation contributes to the development and progression of ischemic stroke in the brain. *Toxicol Sci*. 2010;114:226–236.
3. de Paula Santos U, Braga ALF, Giorgi DMA, Pereira LAA, Grupi CJ, Lin CA, Bussacos MA, Zanetta DMT, do Nascimento Saldiva PH, Filho MT. Effects of air pollution on blood pressure and heart rate variability: a panel study of vehicular traffic controllers in the city of sao paulo, brazil. *Eur Heart J.*. 2005;26:193–200.
4. Guo Y, Tong S, Li S, Barnett AG, Yu W, Zhang Y, Pan X. Gaseous air pollution and emergency hospital visits for hypertension in Beijing, China: a time-stratified case-crossover study. *Environ Health*. 2010;9:57.
5. Bell ML, Davis DL. Reassessment of the lethal London fog of 1952: novel indicators of acute and chronic consequences of acute exposure to air pollution. *Environ Health Perspect*. 2001;109(suppl 3):389–394.
6. López-Villarrubia E, Ballester F, Iñiguez C, Peral N. Air pollution and mortality in the Canary Islands: a time-series analysis. *Environ Health*. 2010;9:8.
7. Naddafi K, Hassanvand MS, Yunesian M, Momeniha F, Nabizadeh R, Faridi S, Gholampour A. Health impact assessment of air pollution in megacity of Tehran, Iran. *Iranian J Environ Health Sci Eng*. 2012;9:28.
8. Peters A, von Klot S, Heier M, Trentinaglia I, Hörmann A, Wichmann HE, Löwel H; Cooperative Health Research in the Region of Augsburg Study Group. Exposure to traffic and the onset of myocardial infarction. *N Engl J Med*. 2004;351:1721–1730.
9. Routledge HC, Manney S, Harrison RM, Ayres JG, Townend JN. Effect of inhaled sulphur dioxide and carbon particles on heart rate variability and markers of inflammation and coagulation in human subjects. *Heart*. 2006;92:220–227.
10. Min KB, Min JY, Cho SI, Paek D. The relationship between air pollutants and heart-rate variability among community residents in Korea. *Inhal Toxicol*. 2008;20:435–444.
11. Liao D, Duan Y, Whitsel EA, Zheng ZJ, Heiss G, Chinchilli VM, Lin HM. Association of higher levels of ambient criteria pollutants with impaired cardiac autonomic control: a population-based study. *Am J Epidemiol*. 2004;159:768–777.
12. Woerman AL, Mendelowitz D. Perinatal sulfur dioxide exposure alters brainstem parasympathetic control of heart rate. *Cardiovasc Res*. 2013.
13. Dergacheva O, Griffioen KJ, Neff RA, Mendelowitz D. Respiratory modulation of premotor cardiac vagal neurons in the brainstem. *Respir Physiol Neurobiol*. 2010;174:102–110.
14. Environmental Protection Agency. Primary national ambient air quality standard for sulfur dioxide. *Federal Register*. 2009;74:64810–64881.
15. Schaller B. Trigeminocardiac reflex. A clinical phenomenon or a new physiological entity? *J Neurol*. 2004;251:658–665.
16. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart Rate Variability: standards of measurement, physiological interpretation and clinical use. Task force of the European society of cardiology and the North American society of pacing and electrophysiology. *Circulation*. 1996;1043–1065.
17. Bobak M, Leon DA. Pregnancy outcomes and outdoor air pollution: An ecological study in districts of the Czech Republic 1986–8. *Occup Environ Med*. 1999;56:539–543.
18. Gilboa SM, Mendola P, Olshan AF, Langlois PF, Savitz DA, Loomis D, Herring AH. Relation between ambient air quality and selected birth defects, seven county study, Texas, 1997–2000. *Am J Epidemiol*. 2005;162:238–252.

19. Liu S, Knewski D, Shi Y, Chen Y. Association between gaseous ambient air pollutants and adverse pregnancy outcomes in Vancouver, British Columbia. *Environ Health Perspect*. 2003;111:1773–1778.
20. Darrow LA, Klein M, Strickland MJ, Mulholland JA, Tolbert PE. Ambient air pollution and birth weight in full-term infants in Atlanta, 1994-2004. *Environ Health Perspect*. 2011;119:731–737.
21. Hoppenbrouwers T, Calub M, Arakawa K, Hodgman JE. Seasonal relationship of sudden infant death syndrome and environmental pollutants. *Am J Epidemiol*. 1981;113:623–635.
22. Dales R, Burnett RT, Smith-Doiron M, Stieb DM, Brook JR. Air pollution and sudden infant death syndrome. *Pediatrics*. 2004;113:e628–e631.
23. Kim KH, Jahan SA, Kabir E. A review of diseases associated with household air pollution due to the use of biomass fuels. *J Hazard Mater*. 2011;192:425–431.
24. Zhang JJ, Smith KR. Household air pollution from coal and biomass fuels in China: measurements, health impacts, and interventions. *Environ Health Perspect*. 2007;115:848–855.

Novelty and Significance

What Is New?

- Postnatal, but not prenatal, exposure to SO_2 elicits tachycardia and blunts glutamatergic neurotransmission to cardiac vagal neurons in the brain stem.
- Within 2 to 3 days of exposure, tonic and reflexive parasympathetic activity and basal heart rate return to control levels.

What Is Relevant?

- Withdrawal of brain stem parasympathetic regulation of cardiovascular activity increases the risk of developing cardiovascular disease, including hypertension and myocardial infarction.
- Because parasympathetic activity is significantly blunted in neonatal animals at physiologically relevant SO_2 concentrations, exposure standards should be designed to protect this high-risk portion of the population.

Summary

Postnatal, but not prenatal, exposure to 5 ppm SO_2 disrupted glutamatergic neurotransmission to cardiac vagal neurons, blunting brain stem parasympathetic activity and inducing tachycardia. Within 2 to 3 days of postnatal exposure to SO_2, tonic and reflexive parasympathetic activity returned to control levels, indicating that these air pollutant-induced alterations are reversible.