Divergent Electrocardiographic Responses to Whole and Particle-Free Diesel Exhaust Inhalation in Spontaneously Hypertensive Rats

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Diesel exhaust (DE) is a major contributor to traffic-related fine particulate matter (PM$_{2.5}$). Although inroads have been made in understanding the mechanisms of PM-related health effects, DE’s complex mixture of PM, gases, and volatile organics makes it difficult to determine how the constituents contribute to DE’s effects. We hypothesized that exposure to particle-filtered DE (fDE; gases alone) will elicit less cardiac effects than whole DE (wDE; particles plus gases). In addition, we hypothesized that spontaneously hypertensive (SH) rats will be more sensitive to the electrocardiographic effects of DE exposure than Wistar Kyoto rats (WKY; background strain with normal blood pressure). SH and WKY rats, implanted with telemeters to monitor electrocardiogram and heart rate (HR), were exposed once for 4 h to 150 m$g/m^3$ of wDE (gases plus PM) or fDE (gases alone) DE, or gasses alone plus PM) DE, or filtered air. Exposure to fDE, but not wDE, caused immediate electrocardiographic alterations in cardiac repolarization (ST depression) and ativoventricular conduction block (PR prolongation) as well as bradycardia in SH rats. Exposure to wDE, but not fDE, caused postexposure ST depression and increased sensitivity to the pulmonary C fiber agonist capsaicin in SH rats. The only notable effect of DE exposure in WKY rats was a decrease in HR. Taken together, hypertension may predispose to the potential cardiac effects of DE and components of DE may have divergent effects with some eliciting immediate irritant effects (e.g., gases), whereas others (e.g., PM) trigger delayed effects potentially via separate mechanisms.

Key Words: diesel; exhaust; gases; SH rat; cardiac; electrophysiology.

Inhalation of fine particulate matter (PM) air pollution at concentrations frequently encountered in ambient air sheds increases cardiovascular morbidity and mortality (Brook et al., 2010), especially in individuals with preexisting cardiovascular diseases (Brook and Rajagopalan, 2009). Vehicular traffic is a dominant source of ambient PM particularly in urban environments (Zhu et al., 2002) and studies have shown that proximity to traffic sources (e.g., highway or tunnel) is a major determinant of cardiovascular health outcomes (Hock et al., 2002; Hoffmann et al., 2006; Van Hee et al., 2009). Diesel exhaust (DE), largely emanating from heavy duty diesel engines, is a significant source of fine (PM$_{2.5}$) and ultrafine PM air pollution (EPA/600/8-90/057F 2002) and is a major contributor to near roadway emissions and near road-related adverse clinical outcomes. Peters et al. (2004) found that exposure to traffic with high levels of DE was associated with onset of myocardial infarction. Similarly, a study in eight European countries attributed hospitalizations for acute coronary syndrome in older patients to exposure to DE (Le Tertre et al., 2002). In a controlled human exposure study, Mills et al. (2007) found that DE exposure accentuated exercise-induced electrocardiographic ST depression in subjects with known coronary artery disease and exercise-induced ischemic electrocardiogram (ECG) changes.

Several mechanisms of ambient PM effects have been postulated including pulmonary receptor–mediated modulation of autonomic balance, systemic inflammation/oxidative stress leading to altered vasomotor regulation, and direct actions through particles entering systemic circulation (Brook et al., 2010). DE is a chemically complex source of ambient PM, and thus, defining modes of action are challenging. In addition to PM, DE also consists of a mixture of gases including nitrogen oxides (NO$_x$), sulfur oxides (SO$_x$), carbon monoxide (CO), and volatile organics including aldehydes, benzene, and polycyclic aromatic hydrocarbons. Although DE PM has been linked to
altered cardiovascular effects (Anselme et al., 2007; Mills et al., 2011b), other studies have shown that DE gases affect health adversely. In addition to noting the effects of PM, Berger et al. (2006) found that increased risk of supraventricular tachycardia in men with coronary heart disease was associated with NO₂ and Dockery et al. (2005) found similar responses associated with exposure to NO₂ and CO gases known to originate from DE. In addition, atherosclerotic mice exposed to DE gases showed increased endothelin-1–induced vasoconstriction and altered T-wave morphology (Campen et al., 2005).

Abnormal impulse formation and conduction can lead to clinically important heart rhythm disorders and even sudden cardiac death. Despite clear associations with cardiac dysfunction, it is unclear what modifying effects DE components may have when in combination on heart rate (HR) and cardiac electrophysiology in individuals with preexisting cardiovascular disease. We have previously shown that exposure to PM (Carll et al., 2010; Farraj et al., 2009) or the irritant acrolein (Hazari et al., 2009) in rat models of hypertension or heart failure causes bradycardia, increased parasympathetic tone, ST depression, and arrhythmia. In addition, we have previously shown that air pollution exposure (acrolein) enhances sensitivity of pulmonary C fibers (Hazari et al., 2008), suggesting that air pollution exposure may modify chemoreflex responses and potentially autonomic effects. Given the heightened sensitivity to air pollution in individuals with cardiovascular disease, we hypothesized that (1) spontaneously hypertensive (SH) rats will be more sensitive to the electrocardiographic effects of a single DE exposure than similarly exposed Wistar Kyoto rats (WKY; background strain with normal blood pressure), (2) exposure to particle-filtered DE (fDE; gases alone) will elicit less cardiac effects than exposure to whole DE (wDE; particles plus gases), and (3) the pulmonary chemoreflex response to capsaicin (C fiber agonist) provocation will be potentiated 24 h after DE exposure. Physiological endpoints were monitored during and up to 1 day after exposure to two different concentrations of DE as studies have shown that exposure to traffic-related air pollution can trigger short-term effects within hours after exposure (Peters et al., 2004).

MATERIALS AND METHODS

Animals. Twelve-week-old male SH (n = 65) and WKY normotensive (n = 15) rats (Charles River, Raleigh, NC) were housed in plastic cages (one per cage), maintained on a 12-h light/dark cycle at approximately 22°C and 50% relative humidity in our Association for Assessment and Accreditation of Laboratory Animal Care-approved facility, and held for a minimum of 1 week before implantation. The Institutional Animal Care and Use Committee of the U.S. Environmental Protection Agency approved all protocols. Food (Prolab RMH 3000; PMI Nutrition International, St Louis, MO) and water were provided ad libitum, and all rats were randomized by weight.

Radiotelemetry implantation. Animals (SH rats; n = 7 per group; 35 total and WKY rats; n = 5 per group; 15 total) were anesthetized with a ketamine/xylazine solution (80 mg/ml ketamine HCL and 12 mg/ml xylazine HCL; 1 ml/kg ip; Sigma Chemical Co., St Louis, MO) and were implanted with radiotelemetry as previously described (Watkinson et al., 1995). Briefly, an aseptic surgical technique was used to implant a radiotelemetry transmitter (Model TA11CTA-F40; Data Science International, Inc., St Paul, MN) in the abdominal cavity. Electrode leads were guided through the abdominal musculature through stab wounds. Leads were tunneled sc and secured in a lead II configuration. Body heat was maintained during and after surgery using a heating pad. Animals recovered for 2 weeks after surgery before inhalation studies.

DE generation and exposure. Rats were assigned to exposure groups and acclimated to exposure chambers for 1 h once/day beginning 2 days before exposure. On the exposure day, rats were allowed to acclimate to the chambers for 1 h and then baseline data were recorded for the next hour. SH rats were then exposed once to filtered air, 150 μg/m³ IDE, or wDE or 500 μg/m³ IDE or wDE for 4 h in whole-body exposure chambers. We hypothesized that the highest concentration of IDE and wDE would cause the most severe effects. Because of this, WKY rats were exposed to only 500 μg/m³ IDE or wDE for 4 h in whole-body exposure chambers. wDE for exposure experiments was generated using a 4.8 kW (6.4 hp) direct injection single-cylinder 0.320 l displacement Yannmar L70 V diesel generator operated at a constant 3600 rpm. Resistance heating elements provided a constant 3 kW load. Low-sulfur diesel fuel (32 ppm) purchased from a local distributor was available from a large storage tank. Engine lubrication oil (Shell Rotella, 15W-40) was changed before each set of exposure tests. From the engine, the exhaust was mixed with particulate (high-efficiency particulate air [HEPA]) filtered room air. wDE concentrations were based on the fine PM (PM₂.₅; Mass Median Aerodynamic Diameter < 2.5 microns) fractions of the diluted exhaust. Approximately 85 l/min of the exhaust was directed to a cone diluter and mixed with approximately 595 l/min (7:1 dilution) of HEPA-filtered room air. The diluted exhaust then traveled approximately 12 m through 7.1 cm diameter stainless steel tubing to a Hazleton 1000 (984 l) exposure chamber housed in an isolated animal exposure room. Target wDE concentration of the diluted exhaust was 500 μg of PM/m³ (high) and 150 μg of PM/m³ (low), which was routed to filtered and unfiltered exposure chambers. Multiple human and rodent studies have performed DE studies at concentrations similar to and/or greater than the present study (Harkema et al., 2009; Mills et al., 2007). The filtered chamber was operated at the same pressure, temperature, flow rate, and gas concentrations as the whole particle chamber. The only difference was that the filtered chamber pulled its exposure gas through a Solberg (Itaska, IL) filter housing (model number is CSL-851-200HC) containing a HEPA canister filter. The housing has an inlet to the outside of the pleated canister filter and discharges through the core. The housing was equipped with 2 in. national pipe thread inlet and exit ports. The HEPA canister filter (part number HE-851) had a height of 8.75 in. and an outer diameter of 5.75 in. This filter features a 99.97% removal efficiency standard to 0.3 micron and a temperature range from −15°F to 220°F. Although the filtered chamber had nearly no PM present, it still contained all the diluted combustion gases as the unfiltered chamber. The chamber concentrations were controlled by periodic adjustments of dilution air based on continuous mass concentrations determined by tapered element oscillating microbalance (TEOM; Rupprecht and Patashnick Co., series 1400, Albany, NY) instruments. These instruments include a heated (50°C) chamber that could theoretically vaporize low-temperature volatiles. Control animals were placed in a third chamber supplied with the same HEPA-filtered room air. The chambers were operated at the same flow rate (424 l/min) resulting in approximately 25 air exchanges/hour. Integrated 4-h filter samples (14.1 l/min) were collected daily from each chamber and analyzed gravimetrically to determine particle concentrations. Continuous emission monitors were used to measure chamber concentrations of PM by TEOM, oxygen (O₂, Beckman Corp., model 755, La Habra, CA), carbon monoxide (CO, Thermo Electron Corp., model 48, Franklin, MA), nitrogen oxides (NO and NO₂, Teledyne Technology Co., model 200A, San Diego, CA), and sulfur dioxide (SO₂, model 43c; Thermo Electron Corp.). Samples were extracted through fixed stainless steel probes in the exposure chambers. Gas samples were passed through a particulate filter prior to the individual gas analyzers. Dilution of air
was adjusted periodically to maintain target PM concentrations as measured by the TEOM. Particle size distributions were characterized during each exposure using an engine exhaust particle sizer (model 3090: TSI Inc., St Paul, MN). Chamber temperatures, relative humidity, and noise were also monitored and maintained within acceptable ranges.

**Radiotelemetry data acquisition.** Radiotelemetry methodology (Data Sciences International, Inc.) allowed constant monitoring of electrocardiographic data in unrestrained unanesthetized rats from implantation until sacrifice. Electrocardiographic data were monitored by remote receivers (DataART3.01; Data Sciences International, Inc.) positioned under the home cages within the animal facility and under the exposure cages within the exposure chambers. The exposure cages were modified with plastic siding to limit signal noise from metal interference and positioned away from other animals to prevent signal cross talk. In home cages, 60-s segments of ECG waveforms were acquired and saved at 15-min intervals from surgical recovery through sacrifice not including the exposure period. Preexposure baseline data were collected from home cages as well as a 30-min baseline in exposure cages after a 1-h acclimation period. During the 4-h exposure, 60-s segments were acquired and saved at 5-min intervals. After exposure, rats were monitored in home cages until sacrifice, approximately 18 h after the end of exposure. HR was automatically obtained from the ECG waveforms with data acquisition software (DataART3.01; Data Sciences International, Inc.).

**ECG, arrhythmia identification and heart rate variability analysis.** ECG Auto software (EMKA Technologies, Falls Church, VA) was used for automated analyses of ECG wave amplitudes and segment durations and areas as well as for the visual identification and enumeration of cardiac arrhythmias. Several parameters were determined for each ECG waveform: PR interval; R amplitude and interval; QRS duration, amplitude, and area; ST interval, amplitude, and area; and T-wave amplitude and area; QT interval; HR-corrected QT interval (QTc). To account for potential effects of normal circadian rhythm, ECG parameters were quantified over four 6-h periods for time-matched comparisons between preexposure and postexposure periods while the rats were unrestrained in their home cages. The times analyzed included 12 A.M.–6 A.M., 6 A.M.–12 P.M., 12 P.M.–6 P.M., and 6 P.M.–12 A.M.. ECG parameters during exposure were analyzed in terms of baseline (1-h recordings while in the exposure chambers immediately before the beginning of exposure) and hours 1–4 (constituting the entire exposure period between 7:00 A.M. and 11:00 A.M.) For magnitude change, exposure values were subtracted from the baseline values. Magnitude changes were assessed to allow for comparisons between strains and exposures and are presented where appropriate. Most of the data are presented as baseline versus exposure information and are separated by hour in some cases.

Cardiac arrhythmic events were identified in part by using the Lambeth Conventions (Walker et al., 1988) as a guideline for the identification of arrhythmias in rats. Arrhythmias were identified as atrial premature beats, ventricular premature beats, sinoatrial blocks atrioventricular blocks, or ventricular tachycardia. Arrhythmias were quantified and totaled over an 18-h period prior to exposure (this corresponded to the same times assessed after exposure), during the 4-h exposure period, or during the 18-h period beginning immediately after exposure. Total arrhythmia counts during exposure were quantified (total of 48 two-min segments during 4-h exposure period). To arrive at counts per hour, the total amount of time sampled in minutes (960) was divided by the number of minutes per hour (60).

For the analysis of heart rate variability (HRV), thorough visual inspection was conducted to identify and exclude arrhythmias, artifacts, and 1-min ECG waveforms lacking distinguishable R waves for more than 30 s. The analysis of HRV generated HR and time-domain measures, including mean time between adjacent QRS complex peaks (the RR interval), a SD of the RR interval (SDNN), SDNN normalized for the effects of HR [SDNN/RR interval × 100], and the square root of the mean of squared differences of adjacent RR intervals (RMSSD). The SDNN represents overall HRV, whereas RMSSD represents parasympathetic influence over HR. The analysis of HRV also calculated frequency domain parameters, particularly low frequency (LF) and high frequency (HF), and the ratio of these two frequency domains (LF/HF). LF is generally believed to represent a combination of sympathetic and parasympathetic tones, whereas HF indicates cardiac vagal (parasympathetic) tone and LF/HF serves as an index of sympathovagal balance.

**Necropsy, blood collection, and bronchoalveolar lavage.** Rats were deeply anesthetized with an ip injection of Euthasol (200 mg/kg Na pentobarbital and 25 mg/kg phenytoin; Virbac Animal Health, Fort Worth, TX). The 35 SH rats and 15 WKY rats implanted with radiotelemeters were sacrificed 18 h after exposure. The remaining 30 SH rats were sacrificed 1 h after exposure. Blood samples were collected from the abdominal aorta with a syringe. The trachea was cannulated, and the lungs were lavaged with a total volume of 35 ml/kg of Ca2+, Mg2+, and phenol red-free Dulbecco’s PBS (SAFC Biosciences, Lenexa, MD), divided into two equal aliquots, Cytospins and cell differentials from lavaged cell samples, assays for total protein (Coomassie Plus Protein Reagent; Pierce, Rockford, IL), albumin (Diasorin, Inc., Stillwater, MN), lactate dehydrogenase (Thermo DNA, Louisville, CO), and N-acetyl-b-d-glucosaminidase (Roche Diagnostics, Mannheim, Germany) in lavage supernatants, and serum C-reactive protein and creatine kinase (kit from Diasorin, Inc.; standard from Kamiya Biomedical Co., Seattle, WA) were analyzed as previously described (Ghio et al., 2002).

**Capsaicin challenge.** A separate cohort of 15 male age-matched SH rats (not implanted with telemeters) were used for this challenge test. Iv capsaicin challenge was performed as described by Hazari et al. (2008). Capsaicin (Sigma-Aldrich, St Louis, MO) was prepared as a stock solution (500 µg/ml) in a vehicle of 10% Tween 80, 10% ethyl alcohol, and 80% saline. On the day of the experiment, solutions with incremental capsaicin concentrations were made by diluting the stock solution in saline based on individual animal body weights. Briefly, 24 h after exposure, animals were anesthetized with urethane (1.5 g/kg, ip), supplemental doses of the anesthetic were administered intravenously as necessary to abolish pain reflex. Body temperature was maintained at ~36°C with a heating pad. The left jugular vein was cannulated with polyethylene 50 tubing for the administration of capsaicin. The cannula was exteriorized through an airtight port in the whole-body plethysmograph (Buxco Electronics, Inc, Wilmington, NC). The flow signal was integrated to give V̇ and f was computed from the amount of time it took for one breath. A flow threshold of approximately 60% of the peak inspiratory flow was programmed using Biosystems XA software (Buxco Electronics, Inc, Wilmington, NC) into the analyzing computer. To be registered, the flow signal had to drop below this threshold, indicating an adequate inspiration. T was calculated as the time between the start of the breath, or where the flow last crossed zero flow, and the start of expiratory flow, or the point where the flow signal rose to zero. T was calculated as the difference between the length of the breath and the inspiratory time. A breath was “rejected” when the expired volume differed by more than two-thirds of the inspired volume. The parameters were measured and recorded on a breath-by-breath basis and averaged over 10-s intervals. For each dose, 0.1 ml of capsaicin was first injected into the catheter and then flushed into the animal with 0.2 ml of physiological saline. The volume of the catheter was approximately 0.2 ml. Administration of capsaicin in this manner initiated the pulmonary chemoreflex apneic response (rapid decrease in f and increase in T), at least 15 min elapsed between doses to allow complete recovery. Data from 10 breaths immediately preceding the capsaicin injection were pooled to represent the baseline values. Animals were euthanized with an overdose of Na pentobarbital after the experiment.

**Real-time PCR.** Total RNA was isolated from the left ventricle of the heart, using the TRIzol (Invitrogen, Carlsbad, CA) method of RNA isolation. RNA concentration and integrity were confirmed using the NanoDrop ND-1000 spectrophotometer (ThermoScientific, Wilmington, DE) and the Agilent 2100 Bioanalyzer (ThermoScientific), respectively. RNA was converted to complementary DNA using the TaqMan reverse transcription reagents (Applied Biosystems, Foster City, CA). Real-time PCR was performed using the TaqMan Universal PCR Master Mix and TaqMan gene expression assays: Hmox1 (Rn01536933_m1), IL-6 (Rn01410330_m1), and Gapdh housekeeping gene (Rn01426261_g1) (Applied Biosystems). Reaction plates (96 wells) were...
TABLE 1

Summary of Concentrations and Characteristics of the DE Particles and Gases Within the Animal Exposure Chambersa

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Units</th>
<th>Filtered air control</th>
<th>150 µg/m³ fDE</th>
<th>500 µg/m³ fDE</th>
<th>150 µg/m³ wDE</th>
<th>500 µg/m³ wDE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle mass concentration (filter)</td>
<td>µg/m³</td>
<td>32.5 ± 8.5</td>
<td>15 ± 2</td>
<td>21 ± 5</td>
<td>168 ± 32</td>
<td>425 ± 31</td>
</tr>
<tr>
<td>Oxygen (O₂)</td>
<td>%</td>
<td>21 ± 0</td>
<td>20.7 ± 0</td>
<td>20.6 ± 0</td>
<td>20.8 ± 0</td>
<td>20.6 ± 0</td>
</tr>
<tr>
<td>Carbon monoxide (CO)</td>
<td>ppm</td>
<td>&lt; 1</td>
<td>6 ± 0</td>
<td>18 ± 0</td>
<td>6 ± 0</td>
<td>19 ± 0</td>
</tr>
<tr>
<td>Nitrogen oxide (NO)</td>
<td>ppm</td>
<td>&lt; 0.5</td>
<td>4 ± 0</td>
<td>13 ± 0</td>
<td>5 ± 0</td>
<td>13 ± 0</td>
</tr>
<tr>
<td>Nitrogen dioxide (NO₂)</td>
<td>ppm</td>
<td>&lt; 0.5</td>
<td>&lt; 0.5</td>
<td>&lt; 0.5</td>
<td>&lt; 0.5</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>Sulfur dioxide (SO₂)</td>
<td>ppm</td>
<td>&lt; 0.5</td>
<td>&lt; 0.5</td>
<td>&lt; 0.5</td>
<td>&lt; 0.5</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>Particle number concentration</td>
<td>#/cm³</td>
<td>1441 ± 400</td>
<td>1428 ± 182</td>
<td>459,333 ± 53,446</td>
<td>2.1 × 10⁶ ± 696 × 10³</td>
<td></td>
</tr>
<tr>
<td>Number median particle diameter</td>
<td>nm</td>
<td>26 ± 6</td>
<td>N/A</td>
<td>25 ± 6</td>
<td>60 ± 0</td>
<td>69 ± 9</td>
</tr>
<tr>
<td>Volume median particle diameter</td>
<td>nm</td>
<td>141 ± 34</td>
<td>N/A</td>
<td>133 ± 30</td>
<td>98 ± 30</td>
<td>113 ± 21</td>
</tr>
</tbody>
</table>

aTEOM, O₂, CO, NO, NO₂, and SO₂ represent mean values from continuous measurements taken over the exposure periods ± SE. TEOM was not taken at control or filtered chambers because the PM was not adjustable.

bFilter data represent mean values from one measurement from all chambers per day taken over the exposure periods ± SE.

cParticle number count and size were determined using a TSI Engine Exhaust Particle Sizer Model 3090. Instrument was operated during another study utilizing the same conditions. No data were available to the 150 µg/m³ fDE because the instrument was on loan during this study. Based upon a number of studies, particle size and count are almost equivalent to the value for 500 µg/m³ fDE.

RESULTS

Exposure Characterization

Table 1 lists average particle and gas exposure concentrations, particle size, and number. Chamber temperature and humidity were 72.45 ± 0.69°F and 43.58 ± 1.27%, respectively, for all chambers.

Heart Rate

HRs decreased in SH rats during exposure to both 150 µg/m³ and 500 µg/m³ fDE when compared with HRs before exposure (Figs. 1A–D). By the second hour of exposure, SH rats exposed to 150 µg/m³ fDE had a 16% decrease in HR, from 361 ± 8 beats/min to 311 ± 9 beats/min (p < 0.05; Fig. 1B). For SH rats exposed to 500 µg/m³ fDE, HRs decreased by 11.4% by the second hour of exposure, from 348 ± 6 beats/min to 312 ± 4 beats/min (p < 0.05; Fig. 1B). For WKY rats, exposure to 500 µg/m³ wDE caused a 14.9% decrease in HR by hour 4 from 353 ± 8 beats/min to 308 ± 4 beats/min (p < 0.05; Fig. 2D). No changes in HR were measured in SH or WKY rats exposed to filtered air.

ECG and Arrhythmia

Exposure of SH rats to 500 µg/m³ fDE increased PR interval during hour 2 of exposure, an effect that persisted throughout hours 3 and 4 of exposure (Fig. 3). There were no significant changes in PR interval in SH rats exposed to filtered air, 150 µg/m³ fDE or wDE, 500 µg/m³ fDE, or WKY rats under any exposure condition.

Only SH rats exposed to 150 µg/m³ fDE exposed had a significant increase in negative amplitude during exposure (% change, p value) (Fig. 4A). Although still reduced after exposure (a decrease of 160%), this difference was not statistically significant (Fig. 4B). There were no changes in the amplitude of the ST segment in any of the other exposure groups. No changes in ST segment amplitude were seen in WKY rats.

SH rats exposed to 150 µg/m³ fDE had a significant increase in negative ST area during exposure (Table 2), whereas only exposure to 500 µg/m³ wDE caused significant increases in negative ST area 18 h after exposure (data not shown). Negative ST area is an electrocardiographic parameter that measures the area under the curve between the S wave and the peak of the T wave and can be used to infer changes in repolarization. During exposure, 150 µg/m³ fDE caused negative ST area to increase from −0.78 ± 0.02 mVs to
−0.94 ± 0.01 mVs, a 17% increase \((p < 0.05)\). When 18 h of postexposure data are averaged, 500 \(\mu\)g/m\(^3\) wDE caused negative ST area to increase from \(-1.23 ± 0.01\) mVs to \(-1.32 ± 0.01\) mVs, a 6.8% increase \((p < 0.05)\).

There were no significant effects in any of the measured arrhythmias during or after exposure in any of the exposure groups in either strain.

Heart Rate Variability

There were no significant changes were in SDNN or RMSSD during exposure in SH (Table 3) or WKY rats (data not shown). All SH rats including those exposed to air had significant decreases in LF/HF during exposure, likely an effect due to chamber stress (Table 3).

Capsaicin Challenge

Capsaicin (1, 2, 4, 8 \(\mu\)g/kg, iv) caused a dose-dependent increase in Te in air exposed SH rats (Fig. 5). A single exposure to 500 \(\mu\)g/m\(^3\) wDE significantly potentiated the chemoreflex apneic response to capsaicin in SH rats (this assay was not performed in WKY rats), a greater than 60% increase in the apneic period at the two highest doses.

Heart Weight

SH rats had, on average, higher average heart weight than WKY rats (means, SEMs, and \(p\) values). There were no significant effects of exposure on heart weight in either strain (data not shown).

Oxidative Stress and Pulmonary Inflammation

There were no significant effects of exposure on the measured systemic and pulmonary markers of oxidative stress and inflammation in either strain at either 1 or 24 h after exposure (data not shown).

Real-Time PCR

There were no significant gene expression changes in heme oxygenase 1 or interleukin 6 in the left ventricles of SH and WKY rats both 1 h and 24 h after exposure.

DISCUSSION

The current study demonstrates that exposure to fDE and wDE affects electrocardiographic parameters in SH rats, but
not WKY rats, corroborating studies that demonstrate enhanced sensitivity to the effects of air pollution in susceptible clinical subgroups having underlying cardiovascular disease including hypertension (Brook and Rajagopalan, 2009). Only exposure to the gaseous components of DE (fDE) caused HR slowing, PR prolongation, and ST segment depression during exposure in SH rats. Conversely, exposure to the high concentration of wDE (both particles and gases) caused ST depression after exposure in SH rats and was associated with elevated postexposure sensitivity to the pulmonary C fiber agonist capsaicin, suggesting that the gaseous components of DE may activate separate pathways than the gas/particle mixture of wDE.

Exposure to fDE, but not wDE, in SH rats caused electrocardiographic alterations during exposure indicative of changes in the early phase of repolarization (ST depression) and atrioventricular conduction slowing (PR prolongation). In contrast, exposure to wDE caused ST depression only after exposure. The exact reason for this disparity is unclear and is discussed below. Nonetheless, multiple studies have reported ST segment depression with both particulate and gaseous air pollution exposure. In a cohort of adults with stable coronary heart disease, PM levels 2 days before clinic visits were positively associated with increased risk of ST segment depression during an exercise test (Pekkanen et al., 2002) and were attributed to the gaseous pollutants NO₂ and CO (Pekkanen et al., 2002). Likewise, ST segment depression has also been reported after exercise in elderly patients exposed to black carbon (Gold et al., 2005). Although the mechanism behind ST depression is unclear, previous studies have shown that combustion-related gases in DE such as volatile organics/aldehydes and alkanes cause coronary vasoconstriction (Campen et al., 2005). In addition, air pollution exposure causes variability in T-wave morphology and repolarization and may lead to myocardial vulnerability and the potential for adverse myocardial events (Henneberger et al., 2005). Although the ST segment changes are suggestive of ischemia, measurements of biological indicators of ischemia were not carried out in this study and will be needed to confirm ischemia in future studies. Moreover, this is the first study to report DE-induced PR prolongation. PR prolongation is fairly common irrespective of the presence of disease and is usually associated with increased vagal tone (Sapire et al., 1979). These findings are similar to our previous findings with inhaled PM (Farraj et al., 2009, 2002).

FIG. 2. wDE causes decreases in HR during exposure in WKY rats. Panels (A–D) relate to HR responses measured 1, 2, 3, and 4 h after exposure, respectively. Baseline HR is compared with the average HR value during each hour of exposure. Means and SEs are reported. Significant differences (p < 0.05) are denoted with a *.
and suggest that air pollution exposure secondarily impacts intramyocardial conduction. Although cardiac responses were the focus of this study, there were also respiratory observations, some of which may have linkage to the cardiac responses and may explain the divergence in cardiac responses with wDE and DE gases alone. Previous reports from our laboratory have demonstrated that stimulation of irritant receptors in the airways with acrolein or DE can initiate cardiac responses. Capsaicin is an agonist for the transient receptor potential channel (TRP) V1 receptor found on pulmonary C fibers; it causes reflex bronchospasm and is useful as a challenge regimen to determine enhanced

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Timing</th>
<th>QRS (ms)</th>
<th>QTc (ms)</th>
<th>Negative ST area (mV × s)</th>
<th>T-wave area (mV × s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>Baseline</td>
<td>36.04 ± 0.69</td>
<td>91.45 ± 0.61</td>
<td>−0.88 ± 0.04</td>
<td>1.59 ± 0.10</td>
</tr>
<tr>
<td>Air</td>
<td>Exposure</td>
<td>34.69 ± 0.32</td>
<td>90.77 ± 0.22</td>
<td>−0.86 ± 0.01</td>
<td>1.83 ± 0.04</td>
</tr>
<tr>
<td>fDE 150 µg/m³</td>
<td>Baseline</td>
<td>30.10 ± 0.96</td>
<td>93.8 ± 0.57</td>
<td>−0.78 ± 0.02</td>
<td>3.37 ± 0.14</td>
</tr>
<tr>
<td>fDE 150 µg/m³</td>
<td>Exposure</td>
<td>32.71 ± 0.28</td>
<td>93.16 ± 0.16</td>
<td>−0.94 ± 0.01*</td>
<td>2.04 ± 0.04*</td>
</tr>
<tr>
<td>fDE 500 µg/m³</td>
<td>Baseline</td>
<td>30.27 ± 0.60</td>
<td>90.96 ± 0.41</td>
<td>−1.03 ± 0.04</td>
<td>1.84 ± 0.07</td>
</tr>
<tr>
<td>fDE 500 µg/m³</td>
<td>Exposure</td>
<td>29.79 ± 0.16</td>
<td>87.74 ± 0.15*</td>
<td>−1.05 ± 0.01</td>
<td>1.89 ± 0.03</td>
</tr>
<tr>
<td>wDE 150 µg/m³</td>
<td>Baseline</td>
<td>38.49 ± 1.15</td>
<td>88.20 ± 0.57</td>
<td>−0.74 ± 0.04</td>
<td>1.94 ± 0.10</td>
</tr>
<tr>
<td>wDE 150 µg/m³</td>
<td>Exposure</td>
<td>40.66 ± 0.40</td>
<td>90.75 ± 0.19*</td>
<td>−0.82 ± 0.01</td>
<td>1.87 ± 0.03</td>
</tr>
<tr>
<td>wDE 500 µg/m³</td>
<td>Baseline</td>
<td>40.96 ± 0.18</td>
<td>94.00 ± 0.57</td>
<td>−1.12 ± 0.04</td>
<td>1.66 ± 0.07</td>
</tr>
<tr>
<td>wDE 500 µg/m³</td>
<td>Exposure</td>
<td>41.38 ± 0.36</td>
<td>93.23 ± 0.19</td>
<td>−1.10 ± 0.02</td>
<td>1.44 ± 0.03</td>
</tr>
</tbody>
</table>

Note. * Denotes a significant change from baseline (p < 0.05). Values represent means ± SE.

FIG. 3. SH rats exposed to fDE have increased PR interval during exposure. Panels (A–D) relate to PR responses measured 1, 2, 3, and 4 h after exposure, respectively. Baseline PR interval is compared with the average PR interval value during each hour of exposure. Means and SEs are reported. Significant differences (p < 0.05) are denoted with a *.
sensitivity to pulmonary irritation and injury (Hazari et al., 2008). In this study, only SH rats exposed to high wDE had significant increases in $T_e$, a measure of apnea, after iv injection of capsaicin 24 h after exposure, suggesting enhanced sensitivity to capsaicin-induced respiratory dysfunction. The discrepancy in effects with wDE and fDE suggests the activation of separate mechanisms. Traditionally, air pollution toxicity is believed to occur through three major pathways: (1) irritant or sensory receptor activation leading to changes in autonomic balance, (2) activation of systemic inflammatory pathways that impact vascular function, and (3) direct effects of translocated PM (Brook et al., 2010). Although there was no evidence of inflammatory changes in the heart, lung, and circulation, the immediacy of effects with fDE exposure (HR slowing, PR prolongation, and ST depression during exposure) suggests the activation of irritant pathways, whereas the delayed effects with wDE exposure suggest nonirritant pathways that are potentially mediated by oxidative or inflammatory pathways. There were no significant HRV changes after exposure to DE. Interestingly, Mills et al. (2011a) had similar finding showing that exposure to 300 $\mu$g/m$^3$ DE did not affect HRV in human volunteers. Although there was no evidence of altered HRV with fDE during exposure, high fDE did cause bradycardia and accompanying PR prolongation that are associated with increased vagal tone. Conversely, the delayed response with the wDE (i.e., ST segment depression after exposure) was accompanied by exaggerated sensitivity to capsaicin, suggesting potential mediation by neurogenic/neuroimmune mechanisms. Further work is needed to decipher the mechanisms that mediate these responses.

The divergence in responsiveness is likely due to the distinct composition of fDE and wDE. Several studies have shown that several components within DE, including particles, particle/organic compound mixtures, and organic compounds found in the gas phase, are capable of eliciting adverse cardiovascular effects (Ris, 2007). For example, atherosclerotic mice exposed to gaseous components of DE had increased coronary vasoconstrictive responses (Campen et al., 2005).

![FIG. 4. SH rats exposed to fDE had increased negative ST amplitude during and after exposure. Magnitude of ST segment shift compared with baseline (A) during exposure and (B) 18 h after exposure to low and high dose wDE and fDE. SH rats exposed to 150 $\mu$g/m$^3$ fDE had significant ST depression during exposure. Significant differences ($p < 0.05$) are denoted with a *.

| TABLE 3 | Four Hour Average of HRV Parameters in SH Rats During Exposure |
|---|---|---|---|---|---|
| Exposure | Timing | SDNN | RMSSD | LF | HF | LF/HF |
| Air | Baseline | 6.14 ± 0.38 | 3.76 ± 0.48 | 1.45 ± 0.52 | 0.74 ± 0.91 | 2.26 ± 0.37 |
| Exposure | 8.74 ± 0.75* | 3.97 ± 0.47 | 1.15 ± 0.26 | 0.90 ± 0.14 | 1.28 ± 0.14* |
| fDE 150 $\mu$g/m$^3$ | Baseline | 8.50 ± 0.38 | 4.00 ± 0.98 | 2.10 ± 0.46 | 0.60 ± 0.065 | 2.73 ± 0.47 |
| Exposure | 8.50 ± 1.00 | 4.20 ± 0.96 | 1.00 ± 0.29* | 1.15 ± 0.25* | 1.5 ± 0.32* |
| fDE 500 $\mu$g/m$^3$ | Baseline | 7.70 ± 0.77 | 3.49 ± 0.72 | 0.84 ± 0.18 | 0.50 ± 0.09 | 2.54 ± 0.58 |
| Exposure | 7.93 ± 0.56 | 3.19 ± 0.24 | 0.73 ± 0.12 | 0.84 ± 0.10 | 1.07 ± 0.16* |
| wDE 150 $\mu$g/m$^3$ | Baseline | 8.38 ± 0.69 | 4.78 ± 0.62 | 1.26 ± 0.27 | 0.76 ± 0.11 | 1.72 ± 0.13 |
| Exposure | 8.09 ± 0.65 | 3.89 ± 0.46 | 0.89 ± 0.16 | 1.08 ± 0.24 | 1.14 ± 0.14 |
| wDE 500 $\mu$g/m$^3$ | Baseline | 7.82 ± 0.64 | 4.34 ± 0.84 | 2.35 ± 0.83 | 0.95 ± 0.28 | 2.33 ± 0.28 |
| Exposure | 7.75 ± 0.57 | 3.64 ± 0.42 | 0.87 ± 0.20* | 1.01 ± 0.24 | 1.10 ± 0.12* |

Note. * Denotes a significant change from baseline ($p < 0.05$). Values represent means ± SE.
Epidemiological studies have also shown that gaseous components of ambient air pollution drive some of the adverse cardiovascular effects of air pollution. For example, pollutant models excluding the effects of PM$_{2.5}$ found increased resting diastolic blood pressure due to ozone concentrations in patients with preexisting cardiovascular disease (Zanobetti et al., 2004). In addition, gaseous and particulate components of DE interact, and these interactions may have driven some of the divergent responses seen in this study. The carbonaceous core of diesel particles creates a high-surface area vehicle suitable for absorption of a number of organic compounds including volatile organic compounds and polyaromatic hydrocarbons that can then be transported more deeply into the lung (Sosnowski et al., 2011). In addition, Kamm et al. (1999) found that the surface properties of diesel soot were significantly altered after interaction with ozone. Thus, the composition of the exhaust affects particle and chemical characteristics, which in turn might affect both the nature of the cardiopulmonary responses and their time to onset.

Although DE exposure decreased HR in both strains of rats, only the hypertensive rat had significant electrocardiographic changes during and after exposure to DE. The mechanisms accounting for the elevated sensitivity of the SH rat are uncertain but may relate to the structural, biochemical, and physiological characteristics of the cardiovascular system attendant to prolonged hypertension. Previous studies have shown that SH rats have, on average, 40 mmHg higher mean arterial pressure than background control rats with normal blood pressure at the stage of life used in the present study (El-Mas and Abdel-Rahman, 2005) as well as greater arterial wall thickness (Mulvany and Halpern, 1977). Over time hypertension leads to structural and biological remodeling of the left ventricle characterized by hypertrophy, fibrosis, and changes in membrane channels, cellular energetic, and ion regulation that combine to heighten myocardial sensitivity (Bernardo et al., 2010). Such remodeling has been demonstrated in SH rats (Goltz et al., 2007) and may account for the differences in responsiveness to DE among the SH and WKY rat. The potential effects of low-DE concentration on electrocardiographic response in WKY rats are unknown and should be determined in future studies.

Collectively, the present findings demonstrate for the first time that WDE and particulate-free DE from the same diesel engine source trigger divergent electrocardiographic responses. As illustrated with DE in this study, the specific composition of ambient pollutants at any one location likely exerts unique physicochemical characteristics that influence both the quality and the magnitude of toxicity. The mixing of particles and gases likely results in the generation of multiple byproducts not found in particles or gases alone. Thus, as evident from the data, traditional dose-response relationships may not readily apply. DE exhaust exposure also caused little to no effect in healthy rats, stressing the necessity of modeling susceptible human populations. Taken together, these findings highlight the need for additional studies that focus on the effects of multipollutant exposures to assess more accurately the comparative cardiopulmonary toxicity of different air sheds.

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