

An Autonomic Link Between Inhaled Diesel Exhaust and Impaired Cardiac Performance: Insight From Treadmill and Dobutamine Challenges in Heart Failure–Prone Rats

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Cardiac disease exacerbation is associated with short-term exposure to vehicular emissions. Diesel exhaust (DE) might impair cardiac performance in part through perturbing efferent sympathetic and parasympathetic autonomic nervous system (ANS) input to the heart. We hypothesized that acute changes in ANS balance mediate decreased cardiac performance upon DE inhalation. Young adult heart failure–prone rats were implanted with radiotelemeters to measure heart rate (HR), HR variability (HRV), blood pressure (BP), core body temperature, and pre-ejection period (PEP, a contractility index). Animals pretreated with sympathetic antagonist (atenolol), parasympathetic antagonist (atropine), or saline were exposed to DE (500 $\mu\text{g}/\text{m}^3$ fine particulate matter, 4h) or filtered air and then treadmill exercise challenged. At 1 day postexposure, separate rats were catheterized for left ventricular pressure (LVP), contractility, and lusitropy and assessed for autonomic influence using the sympathoagonist dobutamine and surgical vagotomy. During DE exposure, atenolol inhibited increases in HR, BP, and contractility, but not body temperature, suggesting a role for sympathetic dominance. During treadmill recovery at 4h post-DE exposure, HR and HRV indicated parasympathetic dominance in saline- and atenolol-pretreated groups that atropine inhibited. Conversely, at treadmill recovery 21h post-DE exposure, HRV and PEP indicated sympathetic dominance and subsequently diminished contractility that only atenolol inhibited. LVP at 1 day postexposure indicated that DE impaired contractility and lusitropy while abolishing parasympathetic-regulated cardiac responses to dobutamine. This is the first evidence that air pollutant inhalation both causes time-dependent oscillations between sympathetic and

parasympathetic dominance and decreases cardiac performance via aberrant sympathetic dominance.

Key Words: air pollution; autonomic; cardiac function; cardiovascular; diesel exhaust; electrocardiography; heart failure; heart rate variability; rat; stress test.

Near-road air pollution exposure is associated with adverse clinical events, especially in those with preexisting cardiac disease (Bell *et al.*, 2009; Brook, 2008; Mann *et al.*, 2002; Pope *et al.*, 2008). Multiple pollutants are implicated, including fine and ultrafine particulate matter (PM_{2.5} and UFP, diameters < 2.5 μm and < 0.1 μm , respectively), nitrogen dioxide (NO₂), carbon monoxide (CO), and sulfur dioxide (SO₂). Diesel exhaust (DE) is a major urban source of these pollutants, volatile organics, and carbonyls and may thus contribute to pollutant-induced adverse cardiac events. Leading candidate mechanisms of DE's cardiac toxicity include changes in myocardial perfusion, ion channel and exchanger function, and autonomic nervous system (ANS) regulation that can compromise the viability, electrical stability, and pump function (performance) of the heart.

Several studies indicate that DE inhalation can impair perfusion and electrical stability within the myocardium and alter autonomic modulation (Anselme *et al.*, 2007; Campen *et al.*, 2005; Carll *et al.*, 2012, 2013; Hazari *et al.*, 2011, 2012; Lamb *et al.*, 2012; Mills *et al.*, 2007). We recently showed that short-term DE inhalation increases arrhythmia susceptibility in hypertensive rats through sympathetic dominance 1 day after exposure (Hazari *et al.*, 2011). The belief that DE may also impair the heart's ability to contract and relax has been supported only by studies involving crude exposures and lacking insight on potential mechanisms (Gordon *et al.*, 2012; Huang *et al.*, 2010; Minami *et al.*, 1999; Yan *et al.*, 2008). Conversely,

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we observed in aged heart failure-prone rats that DE inhalation caused parasympathetic dominance and, 1 h after exposure, increased cardiac output (Carll *et al.*, 2013). As such, research has yet to demonstrate that DE inhalation impairs cardiac performance or that autonomic imbalance mediates such effects. Therefore, we examined the effects of acute DE inhalation on cardiac performance and autonomic imbalance in heart failure-prone rats, including the day after exposure, which we have found corresponds with sympathetic-mediated arrhythmia susceptibility. To more directly determine whether the ANS mediates impairments in cardiac performance, we incorporated pharmacologic, surgical, and physiological interventions in heart failure-prone rats, which have particular cardiac susceptibility to DE. We hypothesized that DE exposure would adversely affect cardiac performance by inducing autonomic imbalance.

Epidemiologists have used the electrocardiogram (ECG) to link air pollution exposure to changes in autonomic balance in part by identifying changes in heart rate variability (HRV). Because exertion increases cardiac workload and provokes autonomic compensatory reflexes (sympathetic activation during exercise and parasympathetic activation thereafter), ECG measurements during exercise stress tests are useful for unmasking aberrant autonomic regulation of the heart (Goldberger *et al.*, 2006) and cardiovascular risk. Increases in heart rate (HR) or HRV during recovery from treadmill exercise are predictive of cardiovascular mortality (Dewey *et al.*, 2007; Watanabe *et al.*, 2001). Additionally, exercise tests in patients with hypertrophic cardiomyopathy can provoke a transient cardiac pump dysfunction that is predictive of adverse outcomes (Pelliccia *et al.*, 2007). As we have shown, sympathetic agonists (e.g., dobutamine) may also be used to mimic exercise and unmask latent effects of DE exposure, including impaired HR recovery, increased arrhythmia, and ECG indications of ischemia (Hazari *et al.*, 2012). To determine the influence of DE on cardiac performance and assess the role of the ANS in these effects, we measured responses in blood pressure (BP), HR, ECG, pre-ejection period (PEP), and HRV to exposure and subsequent treadmill exercise in heart failure-prone rats pretreated with pharmacologic inhibitors of either sympathetic (β_1 -adrenergic) or parasympathetic (M_{1-5} -muscarinic) modulation of the heart. At 1 day after exposure, we also assessed cardiac performance in a subset of rats by left ventricular pressure (LVP) before and during two dobutamine infusions separated by surgical vagotomy to test for parasympathetic mediation of effects. Here we provide the first evidence that DE exposure may impair cardiac mechanical function through sympathetic dominance.

MATERIALS AND METHODS

Animals. Lean male spontaneously hypertensive heart failure rats (SHHF *Mccr1-Lep^r*) were obtained from Charles River Laboratories. These rats acquire cardiac hypertrophy by 2 months and dilated cardiomyopathy and overt heart failure at 18 months (Carll *et al.*, 2012). Intermediately, neurohormonal

TABLE 1
Inhalation Exposure Characterization

	Air	DE
PM _{2.5} ($\mu\text{g}/\text{m}^3$)	1.9 (0.3)	502 (2.8)
Volume median diameter of PM (nm)	184	89
O ₂ (%)	20.9 (0.0)	20.3 (0.1)
CO (ppm)	< 0.5	33.1 (2.3)
NO (ppm)	< 0.5	23.4 (1.8)
NO ₂ (ppm)	< 0.5	3.8 (0.22)
SO ₂ (ppm)	< 0.5	< 0.5
Temperature ($^{\circ}\text{F}$)	73.9 (1.3)	71.7 (0.8)
Humidity (%)	43.8 (2.6)	56.1 (1.2)

Note. Means (SEM in parentheses) from continuous (concentrations of O₂, CO, NO, and NO₂), single (PM_{2.5} mass concentration), or six (DE PM_{2.5} number) measurements per exposure day. Volume diameter was calculated from number-based mobility diameters and assumed spherical particles. Air indicates filtered air; DE, diesel exhaust; PM_{2.5}, fine particulate matter.

and sympathetic activation compensates for a loss of contractile function and promotes myocardial remodeling.

DE exposure and generation. Animals were exposed to either whole DE (target 500 μg PM_{2.5}/m³) or filtered air (Air) under conditions previously described (Table 1) (Carll *et al.*, 2012, 2013). DE exposures were at ultrafine PM and NO₂ concentrations comparable to observations in United States and European traffic tunnels and roadways (Anselme *et al.*, 2007; Svartengren *et al.*, 2000; Zhu *et al.*, 2007). DE originated from a single-cylinder 0.320 l displacement Yanmar L70V diesel generator operated at 3600rpm on low sulfur diesel fuel (16 ppm) at a 3 kW load and was diluted with high efficiency particulate air (HEPA)-filtered room air and delivered to exposure chambers as previously described (Carll *et al.*, 2012). Control (“Air”) animals received HEPA-filtered room air in a second exposure chamber.

Study 1—Treadmill Exercise Stress Test and Pharmacologic Autonomic Inhibition

Radiotelemeter implantation. Rats were implanted with radiotelemeters transmitting ECG, aortic BP, and core body temperature ($n = 24$, 8 weeks old, telemeter model TL11M2-C50-PXT, Data Sciences International) at Charles River as described previously (Carll *et al.*, 2010) and shipped after a 10-day recovery to our AAALAC International-approved animal facility at the EPA. Additional SHHF rats ($n = 15$, 11–12 weeks old) were implanted in-house with radiotelemeters measuring ECG, HR, and core body temperature (model TA11CTA-F40) (Lamb *et al.*, 2012). The rats were housed individually in Plexiglas cages with pine-shave bedding in an animal holding room ($22 \pm 1^{\circ}\text{C}$, $50 \pm 5\%$ relative humidity, 12-h light:dark cycle, 0600:1800 h) and provided standard Purina rat chow (5001; Brentwood, MO) and water *ad libitum*. All studies conformed to the guidelines of the U.S. EPA Institutional Animal Care and Use Committee (IACUC). After ≥ 10 days of surgical recovery, rats were transferred to a satellite facility and maintained as previously detailed (Carll *et al.*, 2013).

Drugs and exercise challenge. Animals were assigned to one of six treatment groups (Air-Saline, Air-Atropine, Air-Atenolol, DE-Saline, DE-Atropine, and DE-Atenolol) maintaining equivalent mean body weights and ages between groups. Rats were trained for treadmill challenge on 2 days before baseline treadmill challenges with telemetry. Each challenge involved an initial 4-min run (Run A), a 20-min resting period, and a 5-min run (Run B). See Supplementary figure S1.

The rats were placed in exposure chambers for a 2-h acclimation and returned 2 days later for a 5-h exposure to filtered air (“sham”) (Fig. 1). At

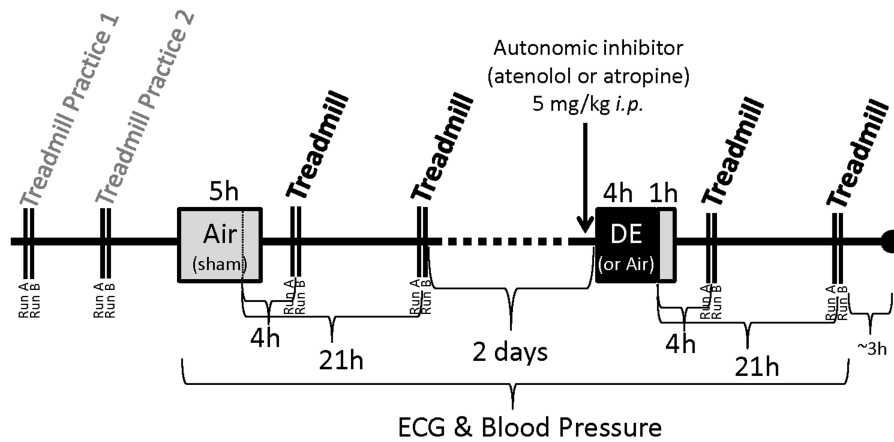


FIG. 1. Study 1 regimen. Effects of DE inhalation on cardiovascular physiology during exposure and after treadmill exercise. ECG and BP were measured by radiotelemetry. Animals were pretreated with an autonomic inhibitor or saline control 30 min before whole-body exposure to clean air or DE at a target PM concentration of $500 \mu\text{g}/\text{m}^3$ and challenged 4 and 21 h later by repeat treadmill exercise (Run A and Run B) separated by a 20 min resting period. Sham exposure to filtered air for both groups occurred 3 days before actual exposure.

approximately 3 and 20 h after end of sham, animals received treadmill challenges. Inhalation exposures began 3 days after sham. At 1 h before exposure, atropine and atenolol were each dissolved into saline, twice sonicated and vortexed for 2 min, and maintained at 38°C . Rats (12–15 weeks old) were weighed and injected ip with saline vehicle (0.9% NaCl, Sigma), atropine or atenolol (5 mg/kg each, Sigma) at 2.5 ml/kg, placed in exposure chambers immediately thereafter, and allowed 30 min to equilibrate before whole-body exposure for 4 h to either whole DE or filtered air, followed by a 1 h washout period in which only filtered air was circulated through exposure chambers. Animals were returned to home cages and treadmill challenged again at 4 and 21 h after cessation of DE exposures (3 and 20 h post washout).

Radiotelemetry and ECG. Radiotelemetry was used to continuously monitor ECG, BP, core body temperature, and activity in conscious unrestrained rats from 3 days before exposure until euthanasia 24 h postexposure. Arterial BP (mean, systolic, diastolic, and pulse), HR, and aortic PEP were automatically calculated by software (DataART 3.01; DSI) from pressure and ECG waveforms sampled at 1000 Hz for 2 of every 10 min within home cages, 2 of every 12 min within exposure chambers, and continuously during treadmill challenges. The PEP (sometimes termed QA interval) provides an inverse index of contractility measured by the delay between onset of left ventricular (LV) depolarization and ejection, indicated by initializations of the R wave and an increase in aortic pressure, respectively (Cambridge and Whiting, 1986). We previously found that drug-induced cardiomyopathy increased PEP in proportion to decreases in aortic dP/dt_{max} , another index of contractility not examined in this study (Carll *et al.*, 2010). From treadmill HR data, we also calculated HR recovery time (time required from peak exercise for HR to return to within 25 BPM of 3-min prerun mean), HR increase (change at peak exercise from 3-min prerun mean), and HR decrease (change from peak exercise at 3-min recovery).

ECG waveforms were analyzed during exposure and treadmill for HRV, morphology, and arrhythmia with computer software (ECGauto 2.8.1.26; EMKA Technologies, Falls Church, VA) as previously detailed (Carll *et al.*, 2012). HRV analysis generated HR and time-domain measures, including mean time between adjacent QRS-complex peaks (RR interval), standard deviation of the RR interval (SDNN), square root of the mean of squared differences of adjacent RR intervals (RMSSD), and percent of adjacent normal RR intervals differing by ≥ 15 ms (pNN15). SDNN represents overall HRV, whereas pNN15 and RMSSD represent parasympathetic influence over HR (Rowan *et al.*, 2007). HRV analysis also provided frequency-domain parameters, including low frequency (LF: 0.200–0.750 Hz, representing a combination of sympathetic and parasympathetic tone) and high frequency (HF: 0.75–3.50

Hz, indicating parasympathetic tone), and the ratio of these two (LF/HF, indicating sympathovagal balance) analyzed with a Hanning window for segment lengths of 512 samples with 50% overlapping (Rowan *et al.*, 2007). Arrhythmia identification and exclusion from HRV and ECG morphology analyses were performed blind to treatment and according to previously described criteria (Carll *et al.*, 2012, 2013).

We limited midexposure HRV analysis to 1–2 h after initialization of DE because this period corresponded with the most significant alterations in HR and BP in the DE-Saline group relative to the Air-Saline group. Because significant effects of DE on HRV and HR during treadmill occurred only at recovery from Run B at 4 h postexposure and recovery from Run A at 21 h postexposure, we only report these values. Because significant changes in PEP and systolic BP were limited to recovery from Run B for 21 h postexposure, we only report these parameters during this period at 4 and 21 h postexposure.

Tissue collection and analysis. At 24 h after the 4-h exposure, rats were deeply anesthetized with sodium pentobarbital/phenytoin solution, ip. Tissue samples of blood, lung lavage fluid, heart, and lungs were collected, processed, and analyzed as previously described (Carll *et al.*, 2012). To examine for indications of cardiopulmonary inflammation, injury, oxidative stress, and risk, assays of multiple biochemical markers were performed identical to our previous description (Carll *et al.*, 2013).

Study 2—LV Pressure, Dobutamine Stress Test, and Vagotomy

SHHF rats ($n = 10$, 13.5 weeks old) were exposed by whole-body inhalation to DE or Air and, at 20–24 h after exposure, anesthetized with urethane (1.5 mg/kg ip, Sigma) and then prepared for LVP measurement by right carotid arterial catheterization with a 2 French transducer (SPR-320, Millar Instruments). The LV probe was connected via a Pressure Control Unit (Model 2000, Millar Instruments) to a receiver (Powerlab 4/30, ADInstruments) and a computer acquiring data at 1000 Hz. The left jugular vein was cannulated for cardiac stress test by sympathomimetic infusion (dobutamine). The transducer was advanced into the left ventricle for a 4-min baseline, and freshly diluted dobutamine hydrochloride (dissolved in 0.9% NaCl saline at 640 $\mu\text{g}/\text{ml}$) was infused for 2 min at a dose of 320 $\mu\text{g}/\text{kg}/\text{min}$ iv (infusion A). For regimen, see Figure 6. Rats were observed for 12 min after infusion cessation, which pilot studies revealed as adequate time for recovery to resting HR and dP/dt_{max} . Animals then received bilateral vagotomy by suture occlusion followed by a stabilization period (3 min), another 2-min infusion at the same dose (infusion B), a postinfusion observation period (2.5 min), and subsequent euthanasia by exsanguination. Software (LabChart Pro 7.3.2, ADInstruments) generated HR,

pressure at end diastole and end systole (EDP and ESP), and the maximum and minimum pressure slopes (dP/dt_{max} and dP/dt_{min} , respectively) per beat, indicative of contractility and relaxation rate (lusitropy), respectively. HR peaked within the first 110 s of infusion at equal raw values for both groups and began decelerating in control rats thereafter. Because the differences in HR deceleration (difference from peak) and dP/dt_{min} (difference from baseline) were most pronounced at the last 10 s of dobutamine infusion, we analyzed and report values from this time point.

Statistics. Analyses were performed using Prism 4.03 (GraphPad Software, San Diego, CA). Repeated measures two-way ANOVA with Bonferroni *post hoc* test was performed on LV pressure data. To control for effects of containment in exposure chambers and enable comparisons between groups in effects of treatment, treadmill data at 4 and 21 h after sham exposure ("Baseline") were subtracted from treadmill data at 4 and 21 h after actual exposure and compared between all groups at corresponding treadmill periods ("Prerun," "Run," or "Recovery") by two-way ANOVA with Bonferroni *post hoc* test and (to control for autonomic effects of pharmacologic inhibitors) by repeat measures two-way ANOVA between the DE- and Air-exposed groups administered the same drug. Additionally, ANOVA with Tukey test was used on nonphysiological endpoints. For all analyses, $p < 0.05$ was considered statistically significant.

RESULTS

Study 1. Effects During Exposure and Treadmill Recovery

Exposure. Relative to the corresponding Air groups, DE significantly increased HR in saline- and atropine-treated rats at hour 1 of exposure (+ 50 and 78 BPM, respectively) while increasing mean arterial pressure (MAP, + 25 mmHg) and shortening PEP (−3.3 ms) only in atropine-treated rats at this time (all $p < 0.05$, data not shown). At hour 2 of exposure, DE continued to increase HR for both saline- (+ 85 BPM) and atropine-treated groups (+ 51 BPM) from their corresponding Air groups, while DE increased MAP (+ 33 mmHg) and shortened PEP (−2.8 ms) only for the saline-treated group (Fig. 2; all $p < 0.05$), indicating an increase in contractility. DE significantly increased core temperature at hour 1 for the saline-, atropine-, and atenolol-pretreated groups (+ 0.9°C, + 1.3°C, + 1.2°C, respectively, data not shown) and hour 2 (+ 2.1°C, + 1.8°C, and + 0.8°C, Fig. 2) relative to Air controls, respectively

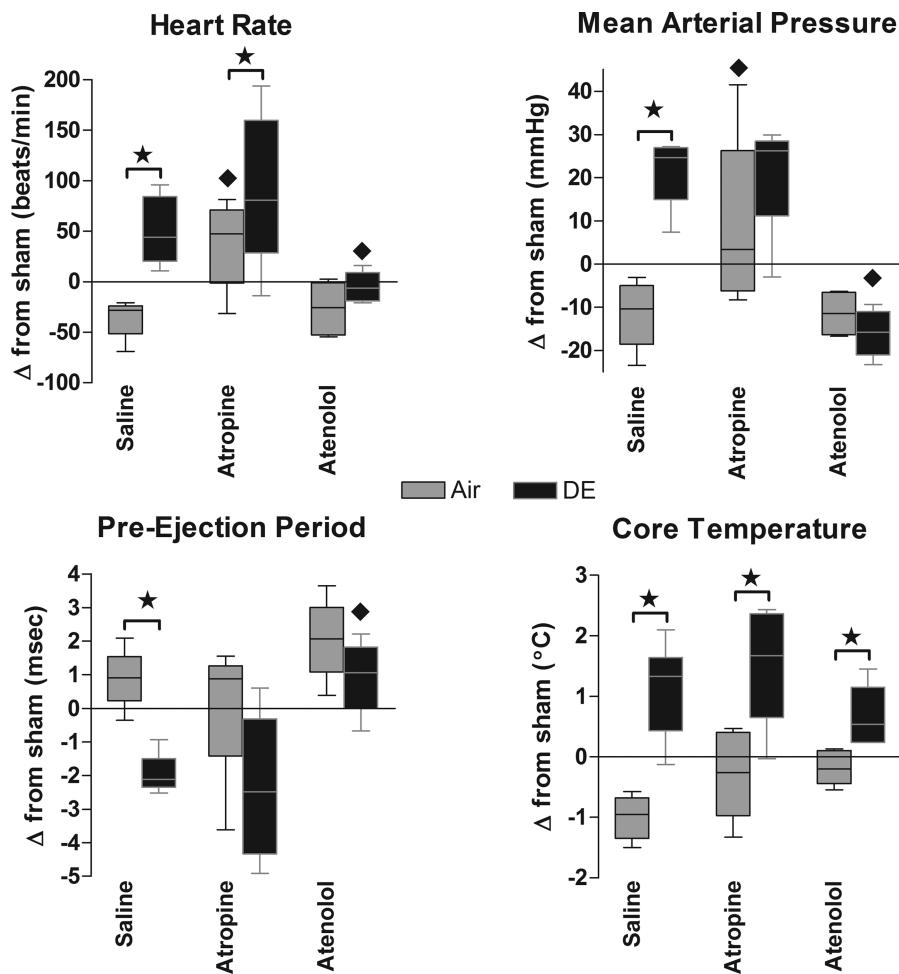


FIG. 2. Box and whisker plots of physiological changes from sham exposure. DE increased HR, BP, cardiac contractility (shortened PEP), and body temperature during exposure hour 2 relative to Air. Atenolol blocked all effects except on temperature. Dash indicates median, box ends mark 75th and 25th percentile, respectively, and whiskers indicate maximum and minimum values. Stars indicate differences between Air and DE groups, and diamonds indicate differences from Saline-pretreated group of corresponding inhalation exposure ($p < 0.05$). HR and core temperature: $N = 5-9$ per group. MAP and PEP: $N = 4$ per group.

(all $p < 0.05$). Mid-exposure HRV analysis was restricted to hour 2 of exposure, during which DE did not affect HRV relative to Air regardless of pretreatment. During exposure hour 3 and also hour 5 (washout) in exposure chambers when only clean filtered air was delivered, DE exposure had no significant effect on the aforementioned parameters regardless of pretreatment. Sympathetic inhibition with atenolol prevented DE from affecting HR, MAP, and PEP at all time points of exposure. The hypertensive effects of DE appeared to reverse to hypotensive effects by hour 4 of exposure for rats pretreated with saline (-13 mmHg ; $p > 0.05$) and significantly reversed for rats pretreated with atropine (-25 mmHg ; $p < 0.05$) relative to their corresponding Air group.

With Air exposure, drug pretreatments also had noteworthy effects relative to saline. Atropine significantly increased HR and MAP at exposure hour 2 (Fig. 2) as well as over the entire exposure relative to Air-saline (48 ± 3 vs. -11 ± 3 BPM and 18 ± 6 vs. -3 ± 2 mmHg) driven by increases at hours 1–3 in HR ($+53$ – 74 BPM) and hours 2–3 in MAP ($+20$ – 22 mmHg; all $p < 0.05$). At hour 2 of exposure, Atropine also significantly decreased RMSSD relative to saline regardless of DE or Air

exposure (Supplementary fig. S2), likely obscuring effects of DE on HRV. Atenolol did not significantly affect these measures during any hour of exposure.

Treadmill stress test. Prior to treatment, treadmill exercise increased HR to approximately 500 BPM in all groups. At 4 h postexposure, HR and HRV responses differed significantly between the DE and Air groups only during the recovery from the second consecutive run (Run B) of the exercise challenge (Fig. 3). Specifically, DE exposure in saline-pretreated rats increased SDNN and RMSSD by 4.4 and 1.2 ms, respectively, relative to the Air-Saline group (both $p < 0.05$). Meanwhile, DE at 4 h postexposure in atenolol-pretreated rats decreased HR (Fig. 3, -67 BPM vs. Air-Atenolol) but did not significantly affect HRV. DE had no similar effects on the atropine group at 4 h postexposure ($p > 0.05$).

At 21 h postexposure (Fig. 4), the DE-Saline group exceeded its air-exposed counterpart in change in HR and LF/HF by 32 BPM and 0.99 units, respectively, while it fell significantly below the Air-Saline group in RMSSD (-1.0 ms; all $p < 0.05$), collectively suggesting sympathetic dominance. Importantly,

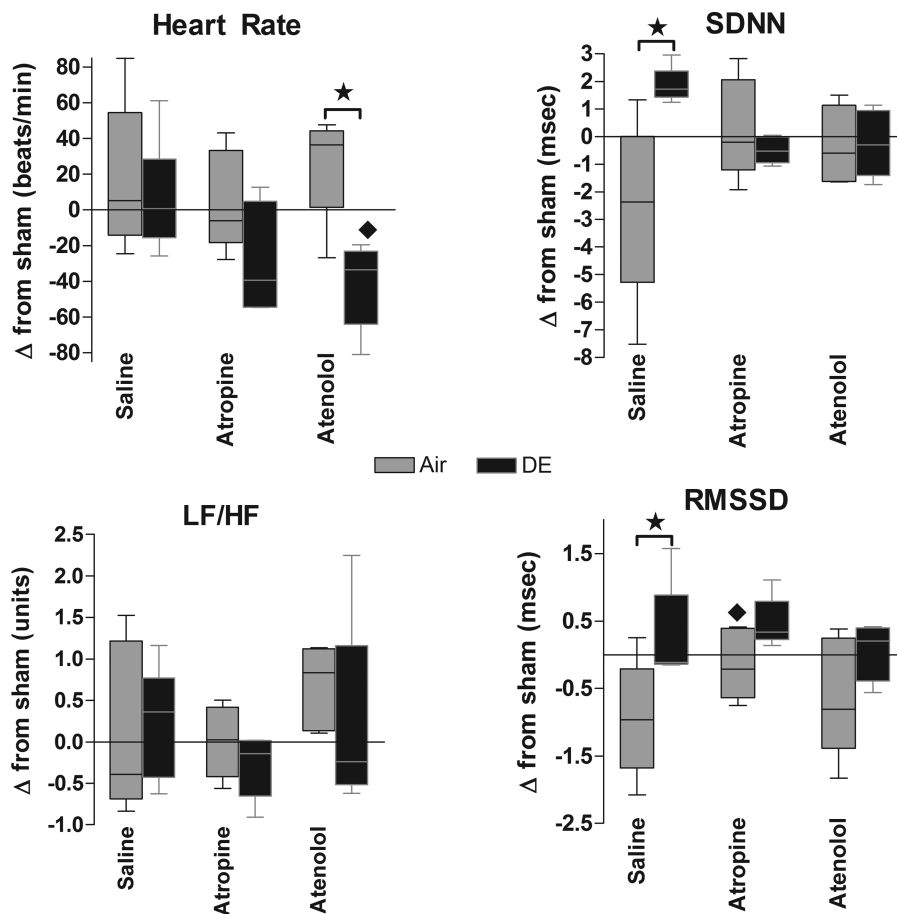


FIG. 3. HR and HRV during recovery from the second of two treadmill runs at 4 h postexposure. DE increased SDNN and RMSSD in saline-pretreated rats and decreased HR in atenolol-pretreated rats. Atropine inhibited these effects of DE. $N = 5$ – 9 per group. Stars indicate differences between Air and DE groups, and diamonds indicate differences from Saline-pretreated group of corresponding inhalation exposure ($p < 0.05$).

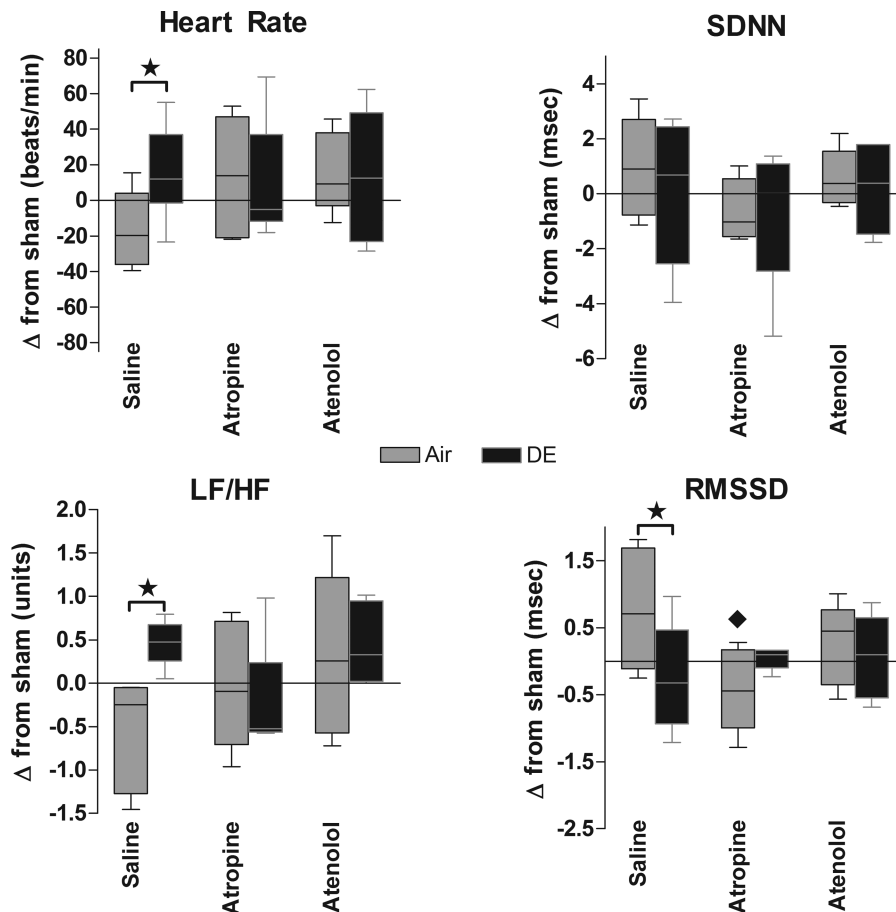


FIG. 4. HR and HRV during recovery from the initial of two treadmill runs at 21 h postexposure. DE increased HR and LF/HF (a measure of sympathetic/parasympathetic influence), and it decreased RMSSD. Treatment with either atropine or atenolol inhibited all of these effects. $N = 5-9$ per group. Stars indicate differences between Air and DE groups, and diamonds indicate differences from Saline-pretreated group of corresponding inhalation exposure ($p < 0.05$).

all of these effects occurred at recovery from the initial treadmill run (Run A). Conversely, DE prolonged PEP relative to Air at recovery from the second consecutive run (Run B) in saline- and atropine-treated rats, suggesting decreased contractility (Fig. 5; +2.2 and 2.1 ms, respectively, $p < 0.05$). Concomitantly, DE decreased systolic BP in atropine-pretreated rats relative to their air-exposed counterparts and trended toward a similar effect in saline-pretreated rats ($p = 0.12$). In contrast, atenolol pretreatment inhibited the aforementioned changes in HRV, BP, and PEP seen 24 h after DE exposure.

There were no significant effects of DE or Air exposure on arrhythmia frequency, HR increase, HR decrease, HR recovery time, or ECG morphology during treadmill exercise challenges (data not shown). There were no clear effects of DE or autonomic inhibitors on biochemical measures of oxidative stress, inflammation, or cardiopulmonary injury (data not shown).

Study 2. Effects on LV Pressure and Autonomic Modulation

Predobutamine infusion. At 1 day after inhalation exposure, DE decreased contractility (dP/dt_{max} : Air, 6489 ± 399 vs.

DE, 5425 ± 259 mmHg/s; $p = 0.056$), slowed LV relaxation (dP/dt_{min} : Air, -8294 ± 409 vs. DE, -6145 ± 460 mmHg/s; $p < 0.05$), and increased LV filling pressure (EDP: Air, 0.42 ± 1.6 vs. DE, 4.4 ± 0.9 mmHg; $p < 0.05$, Fig. 6B). DE did not affect HR, arterial pressure, or ESP before infusion (all $p > 0.05$).

Infusion A. Dobutamine increased HR by approximately 150 BPM for both groups during the first 110 s of infusion relative to preinfusion, with no significant raw differences between peak HRs (Air vs. DE: 464.9 ± 6.4 vs. 480.0 ± 6.3 BPM; $p > 0.05$). Dobutamine also increased dP/dt_{max} and dP/dt_{min} with no difference between the peak values of DE and Air groups; however, during the final 10 s of infusion, the Air group had a greater increase in dP/dt_{min} from preinfusion and a greater deceleration from peak HR than the DE group (Figs. 6C and D) ($p < 0.05$ for all). At the “Recovery” period 12 min after infusion stopped, both groups decreased in HR and dP/dt_{max} to values comparable to preinfusion, whereas only the Air group maintained an elevated dP/dt_{min} relative to its preinfusion value (Supplementary fig. S3, $p < 0.05$), which was now equivalent to the DE group.

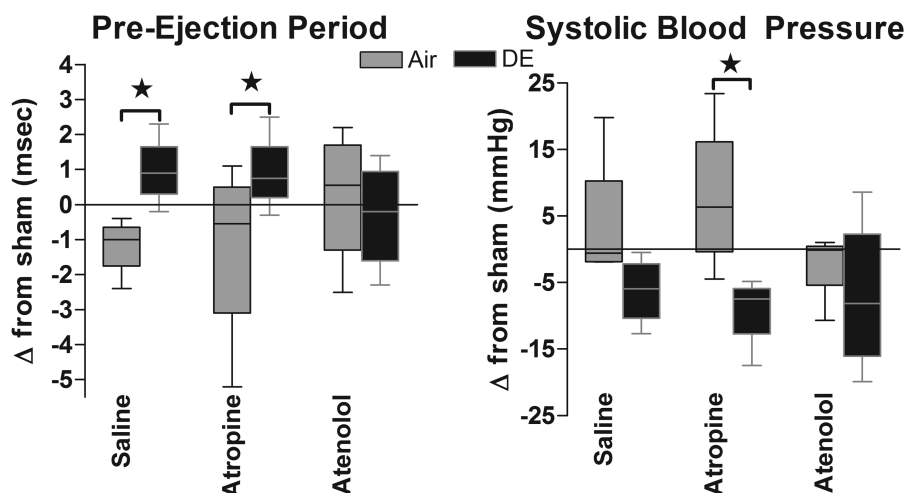


FIG. 5. PEP (inverse index of contractility) and systolic BP during recovery from the second of two treadmill runs at 21 h postexposure. DE significantly decreased contractility (prolonged PEP) in both saline- and atropine-pretreated groups, and it also decreased systolic BP in atropine-pretreated rats. $N = 4$ per group. Stars indicate differences between Air and DE groups, and diamonds indicate differences from Saline-pretreated group of corresponding inhalation exposure ($p < 0.05$).

Vagus nerve ablation and infusion B. Vagotomy increased HR and dP/dt_{max} for both groups relative to their own values at preinfusion and recovery ($p < 0.05$; [Supplementary fig. S3](#)), indicating successful inhibition of parasympathetic input. In addition, vagotomy did not alter the DE group's lusitropy, whereas it restored the Air group to preinfusion dP/dt_{min} . Importantly, vagotomy significantly decreased the Air group's responses to dobutamine in both dP/dt_{min} and HR deceleration ([Figs. 6C and D](#), relative to infusion A; $p < 0.05$), making them comparable to those of DE-exposed animals, which were unchanged from prevagotomy. There were no significant differences between groups in HR or dP/dt_{max} during or after infusion B nor in BP after infusion.

DISCUSSION

We demonstrate in hypertensive heart failure-prone rats that a single 4-h exposure to DE by inhalation impairs cardiac performance, in part, through sympathetic dominance. One day after exposure, DE altered intracardiac pressures consistent with diminished systolic and diastolic mechanical function, while DE also decreased cardiac contractility during exercise recovery and depressed vagal-mediated chronotropic and lusitropic reflexes to adrenergic stimulation ([Table 2](#)). Additionally, DE increased LV filling pressure (EDP) and impaired LV relaxation (dP/dt_{min}). These effects might offer insight into epidemiological findings that short-term air pollution exposure increases heart failure-related hospitalizations and deaths ([Bell et al., 2009](#); [Mann et al., 2002](#); [Pope et al., 2008](#)) and complement our observation that DE causes LV dilation in aged SHHF rats ([Carll et al., 2013](#)).

Our findings suggest that DE inhalation causes sympathetic dominance, which subsequently diminishes cardiac mechanical function. Importantly, only atenolol inhibited sympathetic

cardiovascular effects during DE exposure and prevented decreased contractility 1 day later. Interestingly, at 1 day after exposure, sympathetic dominance upon recovery from the initial treadmill run appeared to obscure any effects of DE on contractility, which only emerged after a repeat treadmill run, when autonomic imbalance dissipated. Similarly, a repeated exercise in humans with hypertrophic cardiomyopathy has been shown to unmask latent contractility deficits that were more predictive of long-term health decline than systolic decrements during an initial exercise ([Pelliccia et al., 2007](#)). Thus, our observations may bear particular clinical relevance.

The autonomic effects of DE in saline-treated rats involved oscillations from sympathetic dominance during exposure to parasympathetic dominance 4 h after exposure and back to sympathetic dominance 1 day after exposure. Inhalation of concentrated ambient particles in dogs has been shown to similarly alter HRV in a bipolar manner ([Godleski et al., 2000](#)). These oscillations in autonomic imbalance offer some resolution to conflicting reports of only parasympathetic or sympathetic dominance upon air pollutant exposure. Direct assessments of receptor and ion channel expression and functionality may offer more precise insight into the etiology of these effects. Nevertheless, the data reveal that short-term DE exposure causes time-dependent oscillations in autonomic balance, among which early sympathetic dominance may initiate the bulk of systolic dysfunction. Ultimately, this study complements clinical observations ([de Hartog et al., 2009](#); [Pekkanen et al., 2002](#)) by demonstrating that β_1 -adrenergic blockade might prevent air pollutant-induced cardiac dysfunction.

Notably, sympathetic dominance 1 day after exposure potentially compensated for impaired inotropy, as it dissipated upon repeat exercise concomitant with decreased contractility. Yet, atropine inhibited this sympathetic dominance but did not prevent DE from decreasing contractility after the

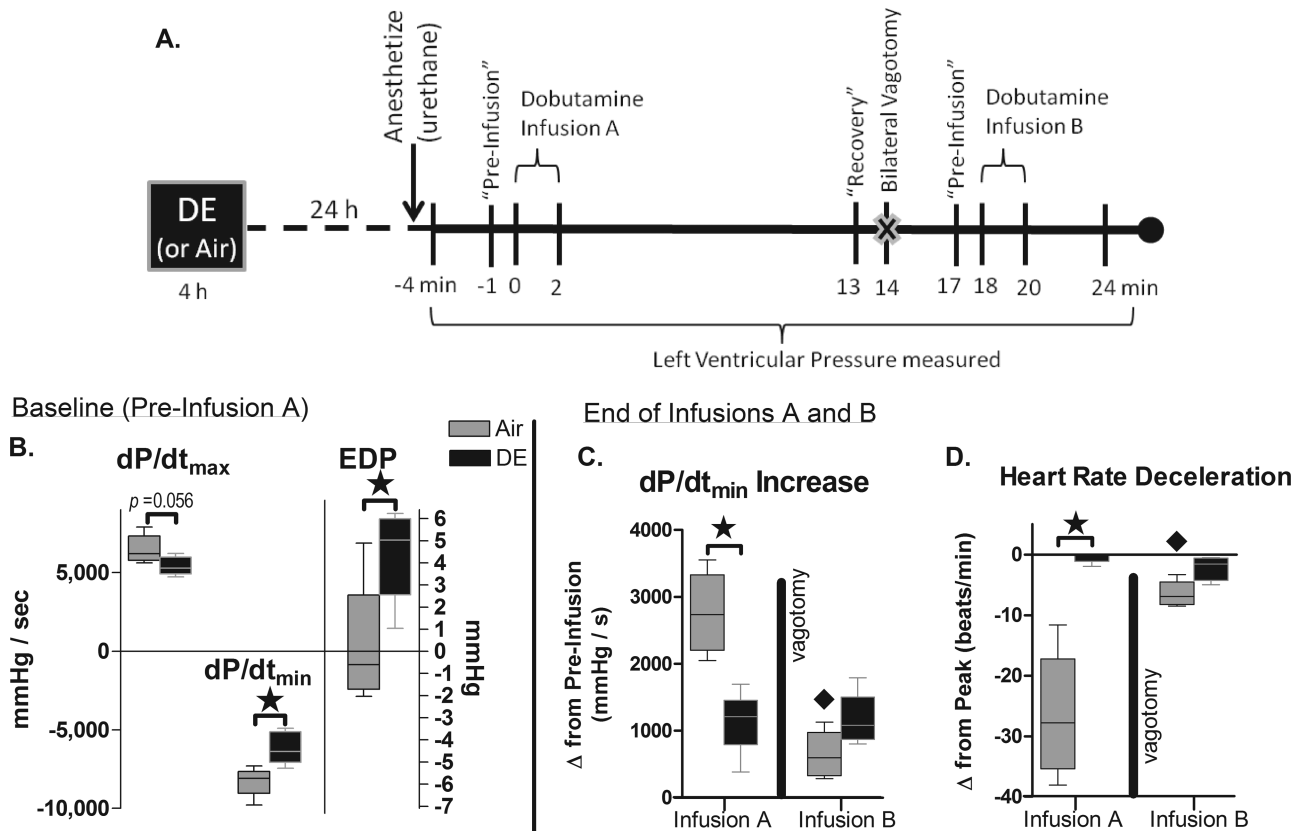


FIG. 6. Study 2 regimen (A). DE impaired baseline LV systolic function (decreased dP/dt_{max}), diastolic relaxation (increased dP/dt_{min}), and chamber filling (B). DE blunted parasympathetic-mediated responses to sympathetic activation (C and D). Box and whisker plots of (B) LV contractility and lusitropy before dobutamine infusion (dP/dt_{max} and dP/dt_{min} , respectively), (C) change in dP/dt_{min} from preinfusion to final 10 s of infusion, and (D) change from peak HR during infusion to final 10 s of infusion. Stars indicate differences between Air and DE groups, and diamonds indicate differences from a group's own values at infusion A ($p < 0.05$). $N = 5$ per group.

subsequent run. Thus, this delayed sympathetic dominance may have resulted from mechanisms independent of contractile dysfunction, such as muscarinic receptor desensitization secondary to parasympathetic hyperactivation (Maloteaux and Hermans, 1994). Accordingly, atropine could have preempted sympathetic dominance on the day after exposure through parasympathetic inhibition on the day of exposure. Yet, the benefits of this late sympathoinhibition are unclear because only sympathetic dominance during DE exposure appeared to mediate a subsequent decrease in contractility. Intracardiac pressure measurements in atenolol-pretreated animals might provide a more clear demonstration of when and if DE-induced sympathetic dominance mediates decreased cardiac performance.

We observed several effects of DE that indicate a central role for sympathetic mediation and deserve comparison to our previous findings (Fig. 7). We have found that aged SHHF rats have parasympathetic dominance during whole DE exposure and bradyarrhythmias immediately thereafter (Carll et al., 2013), contrasting with the sympathetic dominance and steady heart rhythms that we observed in young adult SHHFs here and previously (Carll et al., 2012). Bred for its genetic

predisposition to heart failure, this strain replicates important human cardiovascular risk factors and cardiac deficits, progressing from compensated hypertrophic cardiomyopathy at 2 months to dilated cardiomyopathy and decompensated heart failure at 18 months of age primarily as a consequence of hypertension (Carll et al., 2011, 2012). Heart failure progression might increase sensitivity to inhaled irritants or hypertension, thereby provoking more pronounced trigeminocardiac (Gorini et al., 2010), baroreceptor, or cardiac mechanoreceptor reflexes that increase parasympathetic tone and bradyarrhythmia (Carll et al., 2013). Nevertheless, our data from young adult SHHF rats indicate sympathetic dominance during or shortly after exposure, parasympathetic dominance at about 4 h postexposure, and—in both Spontaneously Hypertensive and SHHF rats—sympathetic dominance at 1 day postexposure (Fig. 7).

The current study revealed unique chronotropic, hypertensive, hyperthermic, and inotropic effects during DE exposure. These findings may have emerged because each animal was normalized by its own time-matched 4-h sham exposure instead of the 1-h preexposure baseline we have traditionally used, thereby controlling for the confounding reductions in

TABLE 2

Summary of DE Effects on Cardiovascular Physiology for Both Studies Relative to Air Groups of the Corresponding Drug Treatment

	Time	Challenge	DE effects (vs. Air)			Interpretations
			Saline	Atropine	Atenolol	
Treadmill study	Exposure hour 1	—	↑HR	↑HR, BP, CTY	—	DE caused <i>sympathetic</i> dominance, inhibited by atenolol
	Exposure hour 2	—	↑HR, BP, CTY	↑HR	—	
	Exposure hour 4	—	—	↓BP	—	DE caused <i>parasympathetic</i> dominance, inhibited by atropine
	4 h post-DE	After repeat run	↑SDNN, RMSSD	—	↓HR	
	1 day post-DE	After initial run	↑HR, LF/HF, ↓RMSSD	—	—	
		After repeat run	↓CTY	↓CTY, BP	—	DE impaired LV contraction, inhibited by atenolol
LVP study	Immediately before dobutamine (1 day post-DE)			↓LTY, CTY*, ↑EDP		DE impaired LV contraction, relaxation, and chamber filling
	First 2-min infusion of dobutamine (110–120 s)		Diminished HR deceleration and lusitropic response during final 10 s of dobutamine infusion			DE diminished parasympathetic inhibitory reflexes to sympathetic activation

Note. DE caused oscillations from sympathetic dominance (midexposure) to parasympathetic dominance (4h postexposure) to sympathetic dominance (day after exposure) in saline-treated rats. In the LVP study, contractility (CTY), lusitropy (LTY), and end-diastolic pressure (EDP) were all altered on the day after exposure, indicating impaired LV contraction and relaxation. Only statistically significant effects ($p < 0.05$) are reported, with exception to * $p = 0.056$. No significant effects were seen at exposure hour 3 or the exposure washout period (hour 5), during which filtered air was delivered to all groups.

activity that tend to occur over the course of our chamber exposures (Carll *et al.*, 2012). We saw a hyperthermic response in the current study that was consistent with sympathetic dominance and likely persisted during exposure despite β_1 -blockade because thermogenesis is mediated by sympathetic stimulation of the β_3 -receptor. Finally, by performing treadmill challenges and analyzing HRV during exercise recovery, we found new evidence that DE caused sympathetic dominance and decreased contractility on the day after exposure.

Oxidative stress might underlie the observed effects, as it can cause sympathetic dominance with PM exposure (Rhoden *et al.*, 2005) and decrease cardiac performance (Dhalla *et al.*, 2000). Air pollutant exposure has been repeatedly linked with oxidative stress (cardiac and systemic) and increased

sympathetic influence (Brook, 2008). Alternatively, increased circulating NO has been observed for up to 2h after DE inhalation (Knuckles *et al.*, 2011) and can increase HR by directly stimulating the sinoatrial node (Chowdhary *et al.*, 2002) while possibly leaving HRV unaffected. This latter mechanism may explain why the typical inverse relationship between HRV and HR was absent *during* this and our prior inhalation exposures (when animals ranged from ambulatory to asleep). Yet, the restoration of this relationship while animals remained still during treadmill recovery suggests that HRV and HR may uncouple with spontaneous activity, further highlighting the importance of physiologic controls in HRV assessments (Hautala *et al.*, 2010). Finally, although we controlled for PM_{2.5} concentrations between studies, the NO₂ and CO levels were, for reasons

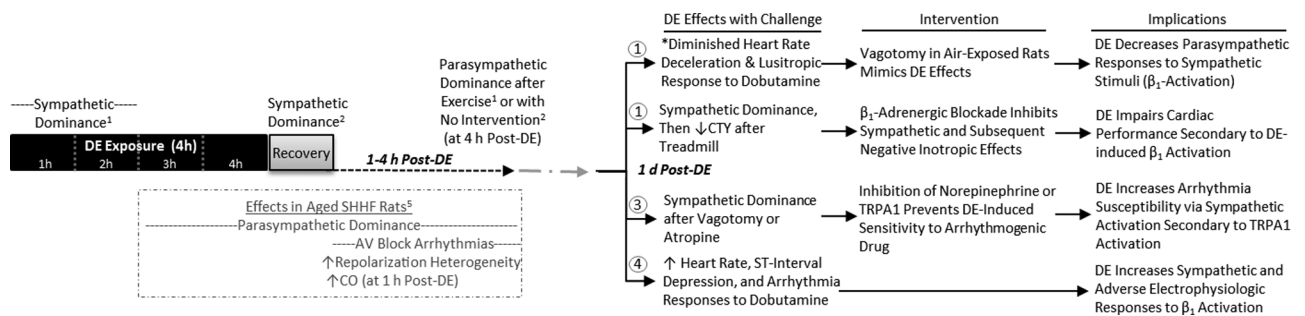


FIG. 7. Our recent investigations show that DE inhalation and cardiac dysfunction are linked to autonomic imbalance in heart failure-prone SHHF rats and in Spontaneously Hypertensive (SH) rats. *, before challenge DE diminished LV contractility (CTY) and lusitropy and increased LV filling pressure. Results are shown for exposure to whole DE only. CO, cardiac output. Circled or superscript numbers represent the following investigations: ¹present studies with young adult SHHF rats; ²Carll *et al.*, 2012, young adult SHHF rats; ³Hazari *et al.*, 2011, SH rats; ⁴Hazari *et al.*, 2012, SH rats; ⁵Carll *et al.*, 2013, aged SHHF rats.

unclear, several-fold higher in this study than previously. Therefore, the discrepancies between our current and past findings (Carll *et al.*, 2012, 2013; Hazari *et al.*, 2012; Lamb *et al.*, 2012) may stem from differences in study design, including anesthesia, concentrations of gaseous components, animal susceptibility, differences in baselines (i.e., a full sham exposure vs. a brief baseline), and the divergent effects of exercise relative to sympathomimetic challenge or spontaneous activity. Our studies collectively indicate that both parasympathetic and sympathetic dominance can occur with DE exposure in a time- and susceptibility-dependent manner.

The responses of DE- and air-exposed rats to dobutamine infusion and vagotomy indicate that DE impaired parasympathetic modulation of the heart. Importantly, DE exposure and vagotomy each individually inhibited lusitropic and chronotropic responses to dobutamine, indicating that DE, at 24h postexposure, elicits similar effects on heart function as parasympathectomy. In concordance with our recent findings (Hazari *et al.*, 2012), air-exposed rats had inhibitory chronotropic reflexes to dobutamine that DE exposure abolished. Recapitulation of this effect by vagotomy in air-exposed rats appears to confirm that this effect was parasympathetic in origin. Thus, the responses to both dobutamine and vagotomy demonstrate that DE may degrade normal parasympathetic modulation, thereby enabling increased sympathetic influence and inevitably impeding cardiovascular function.

DE exposure caused autonomic imbalance that may relate to the triggering of pulmonary irritant receptors, including the transient receptor potential ankyrin 1 (TRPA1) channel, which activate sensory nerves (C-fibers) (Hazari *et al.*, 2011), thereby causing acute autonomic cardiovascular reflexes (Widdicombe & Lee, 2001). We have previously shown that both (1) the inhibition of TRPA1 channels prior to DE exposure and (2) the administration of a sympathetic antagonist 1 day after DE exposure prevent DE-enhanced sensitivity to aconitine, a proarrhythmic drug (Hazari *et al.*, 2011). Others have found that a 3-day PM_{2.5} inhalation exposure in mice decreases cardiac vagal neuron excitability and HRV, indicating that air pollutant exposure can compromise the parasympathetic counterbalance to sympathoexcitation through induced neuroplasticity (Pham *et al.*, 2009). Similar effects have recently been found in SO₂-exposed rats (Woerman and Mendelowitz, 2013). Importantly, although the parasympathetic branch has minimal effects on vascular tone relative to sympathetic input (Friberg *et al.*, 1988), it suppresses sympathetic influence over the cardiovascular system through a number of mechanisms, including presynaptic inhibition of sympathetic neurons, inhibition of catecholamine release, and decreased firing rates of the sinoatrial and atrioventricular pacemaker nodes (Katz, 2006). Thus, it remains unclear whether our observations derive from diminished parasympathetic or enhanced sympathetic neural regulation of the heart. Nevertheless, decreased vagal tone and increased sympathetic influence over the heart have been found to correspond with heart failure exacerbation and predict

arrhythmia and sudden cardiac death in humans (La Rovere *et al.*, 1994; Nolan *et al.*, 1998). Similarly, increased HR and HRV during exercise recovery are predictive of cardiovascular mortality (Dewey *et al.*, 2007; Watanabe *et al.*, 2001). Therefore, regardless of the exact neural etiology, our findings that sympathetic dominance preceded impaired cardiac function may illuminate epidemiological observations of increased cardiovascular morbidity and mortality with acute air pollution exposure. Moreover, these observations support the growing hypothesis that indications of autonomic imbalance may serve as biomarkers of air pollutant cardiotoxicity.

CONCLUSION

We incorporated treadmill exercise with autonomic-inhibiting drugs and, separately, a sympathoagonist with parasympathetic ablation to reveal that air pollution caused cardiac performance decrements that may be partly mediated by the ANS. We found that a commonly prescribed inhibitor of sympathetic neural regulation of the heart (a β_1 -adrenergic receptor blocker) can prevent DE from impairing a measure of contractility. We also revealed that DE impaired cardiac performance concomitant with diminished parasympathetic modulation. Although the relevance of this to long-term exposures remains unclear, our findings offer important insight into the mechanistic basis and prevention of air pollution's adverse effects on the heart.

SUPPLEMENTARY DATA

Supplementary data are available online at <http://toxsci.oxfordjournals.org/>.

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