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Electrocardiographic Changes during Exposure to Residual Oil Fly Ash (ROFA) Particles in a Rat Model of Myocardial Infarction

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Epidemiological studies have reported a positive association of short-term increases in ambient particulate matter (PM) with daily mortality and hospital admissions for cardiovascular disease. Although patients with cardiopulmonary disease appear to be most at risk, particulate-related cardiac effects following myocardial infarction (MI) have not been examined. To improve understanding of mechanisms, we developed and tested a model for investigating the effects of inhaled PM on arrhythmias and heart rate variability (HRV), a measure of autonomic nervous system activity, in rats with acute MI. Left-ventricular MI was induced in 31 Sprague-Dawley rats by thermocoagulation of the left coronary artery; 32 additional rats served as sham-operated controls. Diazepam-sedated rats were exposed (1 h) to residual oil fly ash (ROFA), carbon black, or room air at 12–18 h after surgery. Each exposure was immediately preceded and followed by a 1-h exposure to room air (baseline and recovery periods, respectively). Lead-II electrocardiograms were recorded. In the MI group, 41% of rats exhibited one or more premature ventricular complexes (PVCs) during the baseline period. Exposure to ROFA, but not to carbon black or room air, increased arrhythmia frequency in animals with preexisting PVCs. Furthermore, MI rats exposed to ROFA, but not to carbon black or room air, decreased HRV. There was no difference in arrhythmia frequency or HRV among sham-operated animals. These results underscore the usefulness of this model for elucidating the physiologic mechanisms of pollution-induced cardiovascular arrhythmias and contribute to defining the specific constituents of ambient particles responsible for arrhythmias.

Key Words: **myocardial infarction; particulate matter; arrhythmia; heart rate variability; residual oil fly ash; air pollution; electrocardiogram; Sprague-Dawley rats.**

A growing number of epidemiological studies have shown a positive association between daily mortality and short-term increases in particulate air pollution (reviewed by Pope, 2000). Pope (2000) estimates that 69% of the excess deaths attributable to respirable particulate matter (PM) are due to cardiovascular disease. Elevations in ambient pollution have also been associated with increased hospitalizations for cardiovascular disease (Ballester *et al.*, 2001; Burnett *et al.*, 1995, 1999; Künzli *et al.*, 2000; Moolgavkar, 2000; Poloniecki *et al.*, 1997; Schwartz, 1997, 1999; Schwartz and Morris, 1995; Zanobetti *et al.*, 2000), increased incidence of cardiac arrhythmias (Peters *et al.*, 2000; Santos *et al.*, 2001), and increased risk of myocardial infarction (Peters *et al.*, 2001).

Epidemiological studies indicate that the elderly and those with pre-existing cardiopulmonary disease may be most susceptible to the effects of PM exposure. Accordingly, several animal models that mimic susceptible segments of the population are currently being used to elucidate physiologic mechanisms. Experimental studies using concentrated ambient particles or residual oil fly ash (ROFA) have shown effects of PM exposure on cardiovascular function in normal dogs (Godleski *et al.*, 2000; Nearing *et al.*, 1996), hypertensive rats (Kodavanti *et al.*, 2000), a rat model of pulmonary hypertension (Campen *et al.*, 2000; Killingsworth *et al.* 1997; Watkinson *et al.*, 1998), and in a canine model of acute myocardial ischemia (Godleski *et al.*, 2000; Wellenius *et al.*, 2000).

An important gap in the literature has been the lack of an animal model to study the effects of air particulates following myocardial infarction. This is an important deficiency because of the evolving clinical evidence that patients with ischemic heart disease may be at elevated risk of life-threatening arrhythmias. To address this problem, we adapted a rat model of myocardial infarction (Johns and Olson, 1954), in order to explore the possibility that rats with a recent myocardial infarction would be more responsive to the effects of PM exposure with inhaled ROFA than normal animals, and that the cardiovascular effects would be evident in observable changes in electrocardiographic measures.

MATERIALS AND METHODS

Animals. Adult, male Sprague-Dawley rats weighing ~250g (Taconic, Germantown, NY) were maintained and studied in accordance with the Na-

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TABLE 1
Number of Animals in Each Treatment Group

	ROFA	Carbon black	Room air	Total
MI	10	11	10	31
Sham-operated	11	9	12	32
Total	21	20	22	63

tional Institutes of Health guidelines for the care and use of animals in research. Animals were housed (12-h light/dark cycle) in plastic cages with pine-chip bedding (Northeastern Products Corp., Warrensburg, NY) and received food (LabDiet, PMI Nutrition International, Inc., Brentwood, MO) and water *ad libitum*. All protocols were approved by the Harvard Medical Area Standing Committee on Animals.

Surgical protocol. Two groups of animals were studied: animals with myocardial infarction (MI) and sham-operated controls. Animals were placed under halothane anesthesia and mechanically ventilated via a 2-mm-diameter tracheal tube (Kent Scientific Corp., Torrington, CT). A left thoracotomy was performed via the fifth intercostal space to gain access to the left ventricular wall of the heart. For those in the MI group, myocardial infarction was induced by briefly and repeatedly applying the fine tip electrode of a portable high-temperature (2200°C)-thermocautery unit (Aaron Medical Industries, Inc., St. Petersburg, FL) to one or more branches of the left coronary artery. Visible discoloration of the affected region indicated that blood flow had been successfully interrupted. This is a modified version of a previously described technique (Adler *et al.*, 1976), which involved the use of a fine-tipped soldering iron to induce myocardial injury. In sham-operated controls, the cautery was similarly applied, but was not activated. The lungs were hyperinflated and the chest closed. Each animal was given a single dose of Buprenorphine HCL (IP, 0.24 mg/kg) and allowed to recover for a minimum of 12 h.

Experimental design. To investigate the cardiac effects of air pollution, rats were exposed to either (1) room air, (2) a fine-particle aerosol produced by resuspending residual oil fly ash (ROFA) obtained from a Boston area power plant, or (3) a fine-particle aerosol produced by resuspending carbon black. ROFA particles were chosen as the test substance to simulate outdoor air particles because (1) ROFA is a highly toxic emission source component of ambient air pollution, (2) the chemical components of ROFA and urban air particles are similar (Fisher *et al.*, 1983; Spengler and Thurston, 1983), and (3) ROFA samples obtained from local Boston oil-fired electrical power plants have been well characterized in terms of both their chemical composition and biologic responses in humans (Hauser *et al.*, 1995). Carbon black (Sigma, St. Louis, MO) was used as a control for exposure to inert particles.

All exposures were 1 h in duration (exposure period) and were immediately preceded and followed by 1 h of exposure to room air (baseline and recovery periods, respectively). Given the short exposure duration, we chose to use high particle concentrations ($\sim$3 mg/m^3) relative to typical ambient levels. However, these concentrations are similar to those observed in occupational settings (Hauser *et al.*, 1995). Each rat was assigned to one of 6 possible treatment groups (Table 1): (1) MI rat exposed to ROFA, (2) MI rat exposed to carbon black, (3) MI rat exposed to room air, (4) sham-operated rat exposed to ROFA, 5) sham-operated rat exposed to carbon black, and (6) sham-operated rat exposed to room air.

Particle exposure system. The methods for the generation and delivery of aerosolized ROFA and carbon black particles have been previously described (Killingsworth *et al.*, 1997). Briefly, a Wright Dust Feed Aerosol Generator (Model MK-II; L. Adams, Ltd., London, UK) resuspended particles into a stream of air (10.6 l/min). Of that stream, 4.0 l/min particles were passed through a Harvard Marple Impactor to eliminate particles larger than 2.5 μm, diluted with 20 l/min of filtered air in a mixing chamber, and subsequently delivered to the exposure chamber. At the entrance to the exposure chamber, 2 l/min was used for continuous measures of airborne concentration and particle size or passed through a 0.2 μm filter (Millipore, Bedford, MA) to recollect particles for gravimetric analysis. A sealed Plexiglass exposure chamber measuring 38.0 $\times$ 29.1 $\times$ 20.7 cm (length $\times$ width $\times$ height) was used for all experiments. The flow of air was along the length of the chamber. Rats were positioned on an adjustable shelf so that they were at the same height as the airflow. No more than 5 animals (median: 3, range: 1–5) were placed in the exposure chamber at any time.

Particle size and airborne concentration were measured for each exposure. Particle concentration was determined gravimetrically from the mass change on the Millipore filters and by continuous monitoring with a real-time aerosol monitor (Model RAS-1; MIE, Inc., Bedford, MA). The average particle concentration determined gravimetrically for ROFA ($n = 6$) and carbon black ($n = 4$) exposures was 3.42 $\pm$ 0.91 and 2.96 $\pm$ 1.13 mg/m^3 (mean $\pm$ SEM, NS), respectively. Particle size distributions were determined continuously with an aerosizer (Model MACCII; API, Inc., Amherst, MA) and found to be 1.81 $\pm$ 1.54 and 0.95 $\pm$ 1.42 μm (MMAD $\pm$ σg, $p < 0.05$) for ROFA and carbon black exposures, respectively. Scanning electron microscopy with energy dispersive x-ray spectroscopy has revealed that ROFA samples from the same source were rich in sulfur, silicon, iron, calcium, aluminum, nickel, and vanadium (Killingsworth *et al.*, 1997).

Electrocardiographic data acquisition and analysis. The day of an experiment, electrodes for obtaining electrocardiograms (ECG) were implanted subcutaneously in a Lead II configuration (right arm, left leg, and right leg) under light Halothane anesthesia. Each electrode consisted of a brass clip soldered to a lead wire. ECG signals were band-pass filtered, amplified, digitized (500 Hz/animal), and stored using a PC-based data acquisition system (Streamer, Kiethley Instruments, Inc., Cleveland, OH) with a 12-bit analog-to-digital converter (DAS-20; Kiethley).

It has been shown that physical restraint of conscious rats significantly increases plasma catecholamine levels (Kvetnansky *et al.*, 1978; Livezey *et al.*, 1985; Popper *et al.*, 1977). In order to obtain stable ECG recordings in unrestrained animals, rats were lightly sedated with a single dose of diazepam (ip, 12 mg/kg) 15–20 min before the beginning of the experiment. ECG recordings from diazepam-sedated animals were of high quality and our cardiac measures were consistent from minute to minute.

Offline, digitized ECG signals were displayed and normal beats were labeled using software scripts in Matlab (Mathworks, Inc., Natick, MA). Arrhythmias were viewed, graded, and enumerated manually by one investigator and subsequently verified by a second. Investigators were blinded as to the exposure status of each rat until after the analysis was completed. Representative ECG recordings are shown in Figure 1. The most commonly observed cardiac arrhythmia was isolated premature ventricular complexes (PVCs; Fig. 1, bottom panel). PVC couplets and triplets, and atrioventricular block were also occasionally observed. The total number of PVCs in the hour before exposure (baseline value) and during the exposure hour (exposure value) was determined, and the change in PVC frequency was calculated for each animal. The data from 97% (61 of 63) rats that completed the experimental protocol were included in the PVC analysis. The 2 remaining animals were excluded because of poor ECG signal quality.

To assess heart rate and heart rate variability changes, only verified normal sinus beats were used. Heart rate, calculated as the reciprocal of the mean beat-to-beat interval, and the standard deviation of beat-to-beat intervals (SDNN), were calculated at 0, 30, 60, 90, and 110 min after the start of the exposure. The exposure period ended after 60 min and was followed by exposure to room air. For each time point, a 1-min interval was selected that was free of ventricular arrhythmias, atrioventricular conduction block, and motion or other signal artifacts, and that began within 1 min of the specified time point. If these conditions could not be met, no value was reported for that time point for that particular rat. The data from 92% (58 of 63) rats that completed the experimental protocol were included in this analysis. Of the possible 290 data points (58 rats $\times$ 5 time points), 21 points (7%) were excluded because of failure to meet the data inclusion criteria. The majority (12 of 21) of the excluded data points corresponded to the 110 min time point and

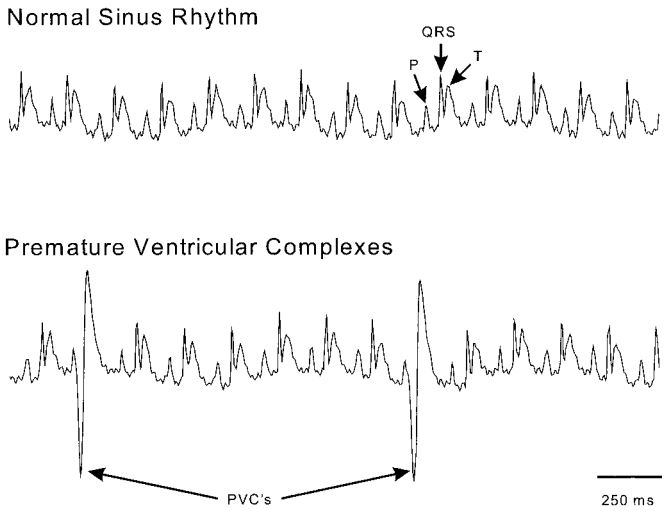

FIG. 1. Representative ECG tracings showing normal sinus rhythm and isolated premature ventricular complexes (PVCs).

were generally due to motion artifacts as the diazepam sedation decreased. Change in heart rate and change in SDNN were calculated for each animal as the end-exposure value (t = 60 min) minus the start-exposure value (t = 0 min).

Histopathology. Histopathologic confirmation of MI was carried out in selected animals at various times after infarction. Rats were sacrificed at 1, 5, and 9 to 14 days after infarction. At autopsy, the operative site and thoracic cavity were observed for evidence of inflammation or infection. The heart was removed and dissected with serial 2–3 mm cross sections from the apex to base. These cross sections of the ventricles were then placed in 10% buffered formalin (Fisher Scientific, Pittsburgh, PA), processed routinely for paraffin histology, sectioned at 4 μm thickness, stained with hematoxylin and eosin, and examined by light microscopy to assess the presence and extent of infarction.

Statistical analysis. We chose, *a priori*, to assess the effect of exposure on arrhythmia frequency by comparing the number of PVCs during the baseline and exposure periods in each animal. Specifically, 2-by-2 tables (ROFA by increase in PVC frequency) were constructed and Fisher's exact test (2-tail) was used to establish statistical significance. We chose, *a priori*, to assess the effect of exposure on heart rate and SDNN by calculating the end-exposure change in value. Change in heart rate, due to exposure, was assessed with a general linear model that included terms for MI, ROFA exposure, carbon black exposure, and the interaction of each exposure with MI. Change in SDNN due to exposure was examined with a general linear model that additionally included terms to control for the baseline SDNN value of each rat. Effects were considered statistically significant when $p < 0.05$.

RESULTS

Mortality and Confirmation of Infarction

Surgical mortality using the thermocautery technique was less than 20%, and this technique produces the histological changes typical of MI (Fig. 2). Exposure to ROFA was not associated with immediate mortality in either the sham-operated or MI animals. None of the 63 animals studied died during the course of the experiment. A specific cause of death was not determined for the 2 animals that died during the night following exposure, both of which were in the MI group and had been exposed to ROFA.

Arrhythmia Frequency

The most commonly observed arrhythmia in all groups of animals was isolated premature ventricular complexes (PVCs). Animals with MI exhibited PVCs more often (41% or 12 of 29) than sham-operated animals (16% or 5 of 32) during the baseline period ($p < 0.05$, Fisher's Exact test).

In the MI group, an increase in PVC frequency was observed when animals were exposed to ROFA (40% or 4 of 10) but not to carbon black ($n = 9$) or room air ($n = 10$); see Table 2. Statistical analysis indicates that exposure to ROFA, but not carbon black, was associated with an increase in arrhythmia frequency in a significant ($p < 0.01$) number of animals. In the sham-operated group, neither ROFA nor carbon black exposure had a significant effect on arrhythmia frequency.

A large portion, 71% (12 of 17), of animals with PVCs during the baseline period in either the MI or sham-operated groups also displayed PVCs during the exposure period. In contrast, only 7% (3 of 44) of animals with no PVCs during the baseline period exhibited PVCs during the exposure period ($p < 0.0001$).

To ensure that the analyses were not biased by the large number of animals that did not exhibit preexisting arrhythmias (72% or 44 of 61), further statistical analysis was conducted on those animals that exhibited PVCs during the baseline period (Table 2, Fig. 3). In this subset of animals ($n = 17$), the results of the analyses were qualitatively identical: within the MI group, exposure to ROFA, but not to carbon black or room air, increased PVC frequency ($p < 0.05$). Within the MI group (Fig. 3, solid lines), 80% of animals (4 of 5) with preexisting arrhythmias and subsequent exposure to ROFA exhibited a greater number of PVCs during the exposure period than during the baseline period. However, none of the animals with PVCs during the baseline period and subsequent exposure to either carbon black ($n = 3$) or room air ($n = 4$) exhibited increased PVCs. In the sham-operated group (Fig. 3, dashed lines), only 1 of 5 animals with baseline PVCs exhibited an increase during the exposure period, and this animal was in the ROFA-exposed group.

Thus, exposure to ROFA, but not carbon black, leads to arrhythmias in rats with myocardial infarction. Furthermore, the presence of baseline arrhythmias, which occurred in 28% of animals in our study, is necessary in order to observe the arrythmogenic effects of ROFA exposure.

Heart Rate Variability

To assess whether changes in autonomic tone were associated with exposure to ROFA or carbon black, we evaluated heart rate variability (HRV) at timed intervals for each of the 6 experimental groups. The standard deviation of beat-to-beat intervals (SDNN, a measure of HRV) of animals in the MI

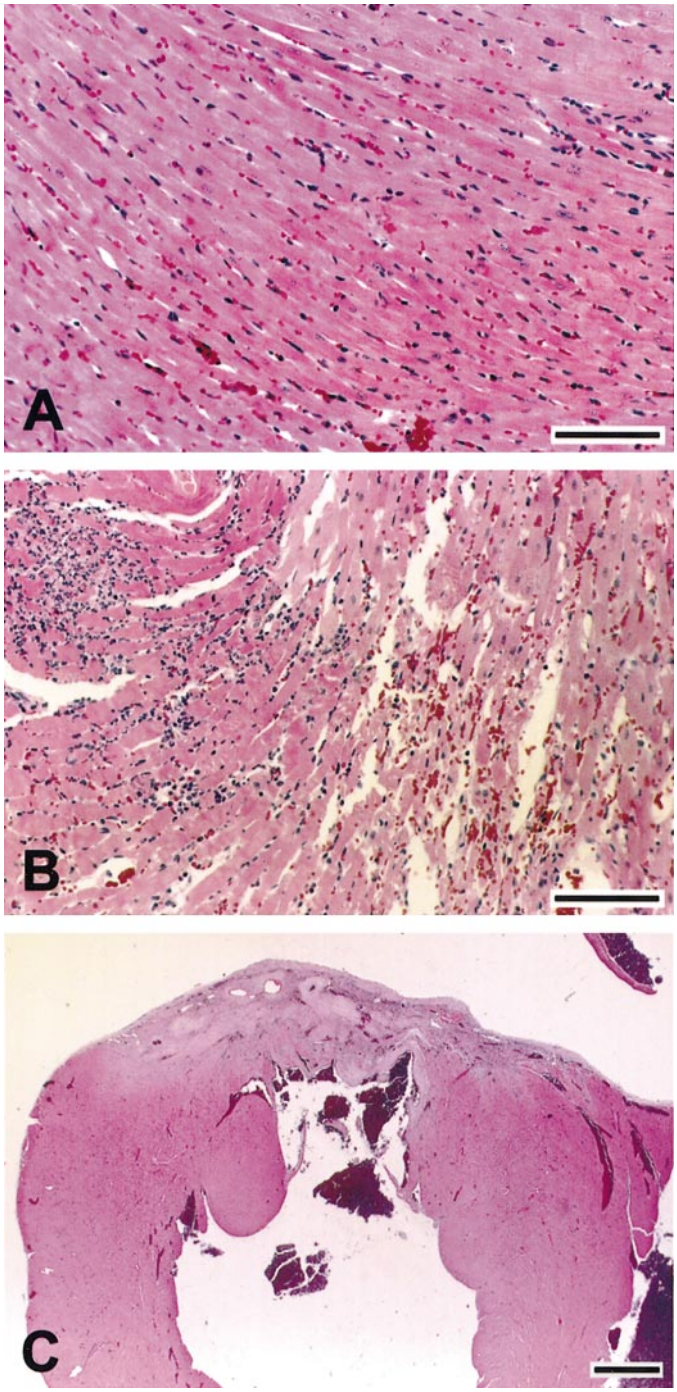

FIG. 2. H&E-stained heart sections established the presence of MI in the model. (A) Histologic findings of normal myocardium: The myocytes are normal, there is no inflammatory cell infiltrate, and red blood cells are visible in the capillaries of the myocardium. Bar =100 μm. (B) An animal with an acute infarct at 24 h: An area of infarcted tissue with early necrosis of myocytes and infiltration of neutrophils is seen on the left side of the picture. This transitions to a hyperemic, but viable, myocardium on the right. Bar = 100 μm. (C) The typical size of a transmural infarction in this model at 14 days: In this very low magnification photomicrograph (Bar = 1,000 μm), the lateral, anterior, and septal walls of the left ventricle and a small portion of right ventricle (right edge of picture) are illustrated. There is considerable loss of myocardium in the area of infarction of the anterior wall (top of picture). The area of infarction is made up of fibrous and vascular tissue with some chronic inflammatory cell infiltrate. This area is about one-half the thickness of the adjacent myocardium.

TABLE 2
Proportion of Animals Exhibiting an Increase in PVC Frequency during Exposure

	ROFA	Carbon black	Room air	Total
All animals				
MI	4/10	0/9	0/10	4/29
Sham-operated	2/11	1/9	1/12	4/32
With baseline PVCs				
MI	4/5	0/3	0/4	4/12
Sham-operated	1/3	0/0	0/2	1/5

group (Fig. 4, upper panel), decreased during exposure to ROFA (3.30 $\pm$ 0.46 vs. 2.74 $\pm$ 0.35 ms $\pm$ SEM, start vs. end of exposure), suggesting decreased protective parasympathetic nerve activity. However, the SDNN increased in animals exposed to room air (3.13 $\pm$ 0.48 vs. 4.22 $\pm$ 0.56 ms). Exposure to carbon black elicited an initial decrease in SDNN, but by the end of the exposure period, the change in SDNN was comparable to that elicited by exposure to room air (2.75 $\pm$ 0.28 vs. 3.37$\pm$0.38 ms, start vs. end of exposure). The SDNN of animals in the sham-operated group (Fig. 4, lower panel) tended to increase with time; however, this change was similar in all sham-operated animals, independent of exposure.

The change in SDNN during the exposure period (end-exposure value – starting value) was well predicted by a general linear model (Table 3) used to determine which treatments influenced the change in SDNN (model $r = 0.59$, $p < 0.005$). When compared to room air, the effect of ROFA exposure in the MI group ("ROFA*MI" term) was significantly different from that in the sham-operated controls. In contrast, when compared to room air, the effect of carbon black exposure ("Carbon*MI" term) was not significantly different between the 2 groups of animals. Exposure to particulates did not alter SDNN in the absence of an MI, as neither the "Carbon" nor the "ROFA" terms were significant in this model.

Thus, in rats with a prior MI, exposure to ROFA, but not carbon black, provoked a significant decrease in SDNN. In the absence of an MI, exposure to neither carbon black nor ROFA elicited any significant change in SDNN.

Heart Rate

Since changes in heart rate can profoundly affect measures of heart rate variability, we examined whether significant heart rate changes occurred during the exposure period. Prior to exposure (Time = 0), mean heart rate was not significantly different among any of the treatment groups, nor did mean

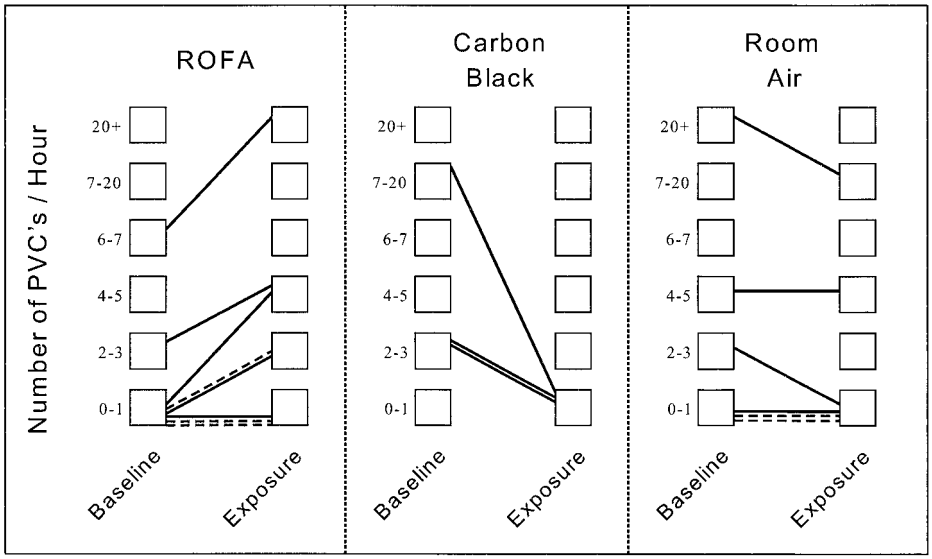

FIG. 3. Number of PVCs observed during the baseline and exposure periods. Only those animals that exhibited PVCs during the baseline period are shown. Each line represents an individual animal in either the MI (solid lines) or sham-operated (dashed lines) groups.

heart rate change appreciably during the course of exposure (Fig. 5). For individual animals, the change in heart rate during the exposure period was not predicted by a general linear model (Table 4, model $r = 0.23$, $p > 0.05$). Finally, no correlation was found among measures of change in heart rate, change in SDNN, and change in arrhythmia frequency in either the MI or sham-operated groups. Thus, none of the treatments had a significant effect on heart rate, and the observed changes in arrhythmia frequency and heart rate variability associated with exposure are not attributable to changes in heart rate.

DISCUSSION

The main goal of the present study was to develop a small-animal model with a prior myocardial infarction in which the potential arrhythmogenic effects of inhaled ROFA particles could be explored. The overall intent was not only to gain insights into mechanisms, but also to make available a heuristic model that could be further applied to advance understanding of physiologic mechanisms and help to identify specific deleterious constituents in inhaled mixtures. Heart rate variability

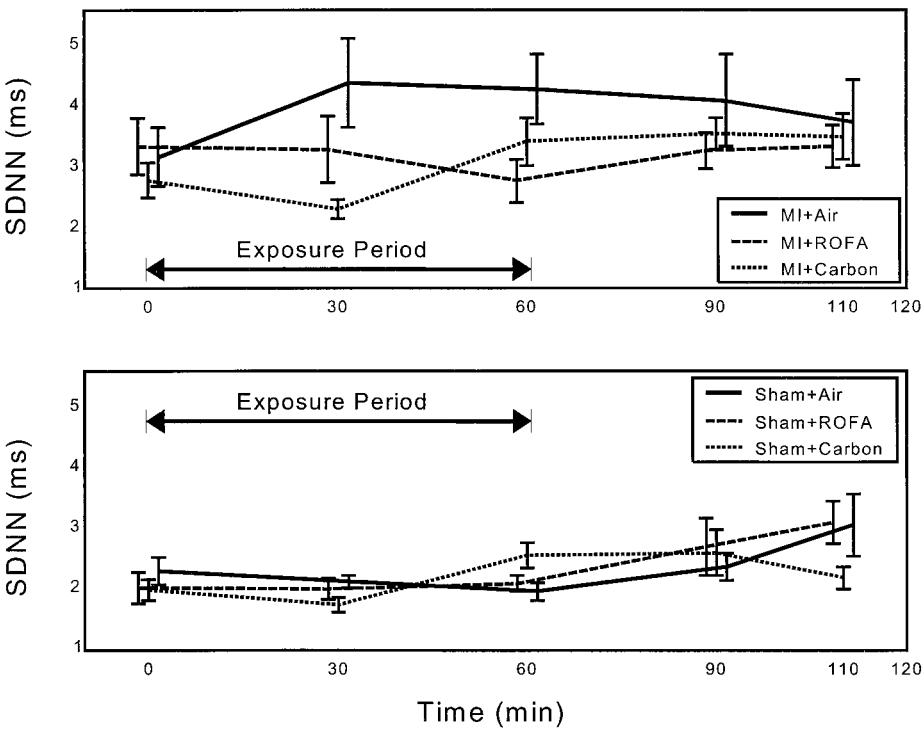

FIG. 4. SDNN as a function of time for the MI group (upper panel) and the sham-operated group (lower panel). Points represent mean ± SEM and are offset along the *x*-axis for clarity.

TABLE 3
General Linear Model Applied to Change in SDNN

Term	B	B SE	*p*
Constant	–0.136	0.369	
MI	1.464	0.507	0.006
ROFA	0.412	0.513	0.427
Carbon	0.792	0.513	0.130
ROFA*MI	–1.691	0.730	0.025
Carbon*MI	–1.230	0.721	0.095
SDNNhigh	–1.119	0.400	0.008
SDNNmed	0.251	0.380	0.512

Note. Model $R^2 = 0.35$; $p < 0.005$. B, regression coefficient; B SE, standard error of B; MI, myocardial infarction; ROFA, residual oil fly ash exposure; Carbon, carbon black exposure; ROFA*MI, interaction of ROFA and MI terms; Carbon*MI, interaction of carbon and MI terms; SDNN, standard deviation of beat-to-beat intervals; SDNNhigh, baseline SDNN in top tertial; SDNNmed, baseline SDNN in middle tertial.

was employed as a measure of autonomic nervous system balance.

Our results indicate that inhaled ROFA particles are arrhythmogenic in infarcted animals, but not in sham-operated controls, and that this effect may be mediated through reduced heart rate variability. Exposure to similar concentrations of carbon black, an inert particle, did not evoke either the increase in arrhythmia frequency or the reduction in heart rate variability that was observed following ROFA exposure in animals with MI. These results suggest that inhalation of combustion-derived PM exacerbates cardiac vulnerability following acute MI.

Relevance to Previous Work

Previous investigators have shown that cardiopulmonary-compromised rats exhibit greater changes in cardiac electrical stability upon exposure to ROFA compared to normal animals. For example, using a rat model of pulmonary hypertension, Watkinson *et al.* (1998) and Campen *et al.* (2000) showed that compromised animals develop serious arrhythmias more readily than normal controls, following intratracheal installation of ROFA. Kodavanti *et al.* (2000) reported exaggerated ST-segment depression associated with ROFA exposure in hypertensive rats, but not in normotensive controls. Similarly, the results of the current study demonstrate that rats with myocardial infarction exhibit changes in cardiac electrical stability during ROFA exposure, whereas sham-operated controls do not.

Cardiac effects of particulate exposure have also been reported in cardiopulmonary-compromised human populations such as the elderly and those with a history of serious arrhythmia. For example, increases in levels of ambient particles have been associated with increases in arrhythmia frequency in patients with implanted cardioverter-defibrillators (Peters *et al.*, 2000) and decreases in heart rate variability in elderly subjects (Gold *et al.*, 2000; Liao *et al.*, 1999; Pope *et al.*, 1999). Thus, the rat model of MI facilitates the study of outcomes

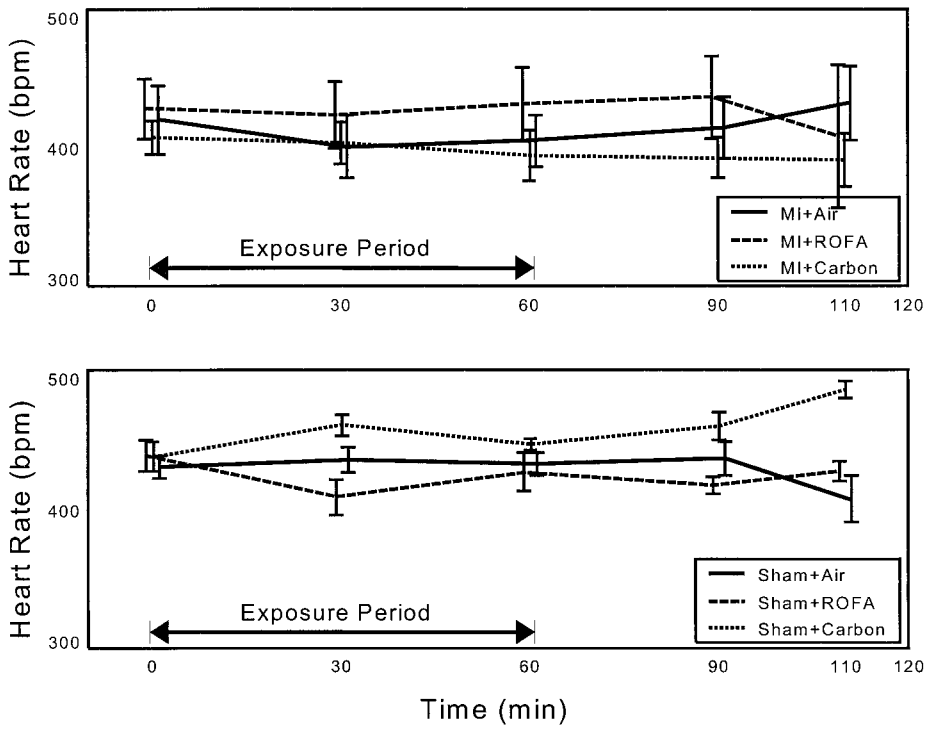

FIG. 5. Heart rate as a function of time for the MI group (upper panel) and the sham-operated group (lower panel). Points represent mean ± SEM and are offset along the *x*-axis for clarity.

TABLE 4
General Linear Model Applied to Change in Heart Rate

Term	B	B SE	*p*
Constant	1.517	9.213	
MI	–6.357	14.567	0.665
ROFA	–5.078	15.179	0.740
Carbon	7.672	15.179	0.616
ROFA*MI	13.217	21.700	0.546
Carbon*MI	–15.885	21.220	0.459

Note. Model $R^2 = 0.05$; $p > 0.05$.B, regression coefficient; B SE, standard error of B; MI, myocardial infarction; ROFA, residual oil fly ash exposure; Carbon, carbon black exposure; ROFA*MI, interaction of ROFA and MI terms; Carbon*MI, interaction of carbon and MI terms; SDNN, standard deviation of beat-to-beat intervals; SDNNhigh, baseline SDNN in top tertial; SDNNmed, baseline SDNN in middle tertial.

associated with particulate-related increases in morbidity, as identified epidemiologically in susceptible populations.

Mechanisms of Arrhythmia Provocation

In the current study, rats with MI exposed to carbon black or room air exhibited fewer arrhythmias during the exposure period than during the baseline period, while heart rate variability increased throughout the exposure period. This observation is likely due to decreasing anxiety levels with time and the onset of sedation, rather than to exposure. This possibility is supported by the finding that the simple transfer of animals from their home cage to a laboratory setup can cause short-term increases in levels of circulating catecholamines (Kvetnansky *et al.*, 1978). In contrast, rats with MI and the same baseline scenario when exposed to ROFA increased arrhythmia frequency and decreased heart rate variability during exposure. The changes in heart rate variability suggest that autonomic nervous system activation may potentiate arrhythmias.

The finding that exposure to ROFA, but not to carbon black, elicits these changes suggests that our results are not due to nonspecific particle effects, but rather that the composition of the particles is important. ROFA particles are rich in transition metals, many of which are soluble and have the ability to reach the heart (Dreher *et al.*, 1997). Transition metals are also an important component of ambient air particles (Spengler and Thurston, 1983), and recent evidence suggests that ambient particles resulting from fuel oil combustion may be associated with daily mortality (Laden *et al.*, 2000). There is also evidence suggestive of an association between mobile emission sources and daily mortality from ischemic heart disease, although this association was not statistically significant (Laden *et al.*, 2000). In light of the uncertainty that exists, this model provides a valuable tool for determining the toxic components of ambient particulate matter responsible for the observed cardiovascular morbidity and mortality.

Work with monocrotaline-treated rats suggests that the cardiovascular effects of ROFA exposure may result, in part, from myocardial ischemia. Monocrotaline treatment of rats has been shown to induce lesions in the endothelium of the pulmonary vasculature, resulting in pulmonary inflammation and hypertension, and in right ventricular hypertrophy (e.g., Meyrick *et al.*, 1980; Reindel *et al.*, 1990). Upon subsequent exposure to ROFA, some rats exhibit progressive hypoxic failure of the myocardium, as evidenced by severe bradycardia and ST-segment depression (Campen *et al.*, 2000; Watkinson *et al.*, 1998), and by a reduction in right ventricular systolic pressure (Killingsworth *et al.*, 1997). In the current study, heart rate was not significantly affected by exposure in either the MI or sham-operated animals, nor were ST-segment changes evident over the course of the exposure period. These findings suggest that the acute changes in arrhythmia frequency that we observed were not due to progressive myocardial ischemia.

Model Considerations

Pharmacological sedation was chosen over physical restraint or implantation of telemetry devices for obtaining high quality ECG recordings. Diazepam, a benzodiazepine, was chosen as a sedative because it provides adequate sedation with minimal cardiovascular and autonomic nervous system effects. The use of diazepam allowed us to carry out these experiments under conditions of minimum stress for the animals, thereby minimizing stress-induced arrhythmias (Corbalan *et al.*, 1974) unrelated to particulate exposure.

However, studies indicate that diazepam reduces vagal tone and suppresses vagally mediated reflexes in humans and large animals (Adinoff *et al.*, 1992; Hockman and Livingston, 1971; Keim and Sigg, 1973; Marty *et al.*, 1986; Sakamoto *et al.*, 1990; Sigg *et al.*, 1971; Taneyama *et al.*, 1993). It is unlikely that the dose used in our experiments significantly reduced vagal tone, as our average baseline heart rates in both the sham-operated and MI animals were similar to those reported in the literature for conscious rats (e.g., Krüger *et al.*, 2000; Opitz *et al.*, 1995). Diazepam also acts directly on the myocardium, leading to a reduction in contractility (Bianco *et al.*, 1971; Chai and Wang, 1966; Daniell, 1975; Hernández 1991; Jones *et al.*, 1979; Nakae *et al.*, 1997), a possible reduction in arrhythmia (Muir *et al.*, 1975; Ruiz *et al.*, 1989; Van Loon 1968), and a reduction in myocardial oxygen consumption (Daniell, 1975; Rossetti *et al.*, 1994). Since these effects are largely cardioprotective, it is likely that the use of diazepam masked, rather than potentiated, the cardiac effects of particulate exposure.

The arrhythmogenic effects of particulate exposure were most evident in those animals with arrhythmias prior to exposure. However, only 41% of the MI animals in the current study exhibited any baseline arrhythmias. Our surgical technique has evolved and improved since the completion of this study. We now induce MI by cauterizing the left coronary artery near its origin as it passes between the pulmonary artery

and the left atrial appendage. This approach results in more consistent and more severe infarcts than were previously possible. As well, the proportion of infarcted animals exhibiting baseline arrhythmias is now ~60%. Thus, this model is useful for studying the effect of inhaled particles on cardiac electrical stability in the presence of myocardial infarction, especially when multiple cardiovascular endpoints are considered.

Conclusions and Clinical Implications

Based on the results of the current study, we anticipate that increased levels of ambient particles are associated with increased disturbances in cardiac rhythm and cardiac electrical stability in patients with a recent MI. The limited clinical data available supports this possibility. For example, in an emerging study, Speizer and colleagues (unpublished observations) found a greater effect of ambient pollution on heart rate variability in patients within 30 days of an MI, as compared to the same patients 3 months later.

Air pollution may not only exacerbate acute MI, but may also be a risk factor in the precipitation of MI. For example, Gustavsson *et al.* (2001) found that occupational exposure to motor exhaust and combustion products increased the risk of first-time MI in men and women, while Peters *et al.* (2001) reported an association between increased levels of ambient particulate matter and a transient increased risk for the onset of acute MI. Given the potential impact of air pollution in conditions with high risk of cardiac mortality, this model is particularly valuable for elucidating mechanism and defining the specific constituents of ambient particulate matter that precipitate and exacerbate life-threatening cardiac conditions.

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