A Single Exposure to Particulate or Gaseous Air Pollution Increases the Risk of Aconitine-Induced Cardiac Arrhythmia in Hypertensive Rats

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Epidemiological studies demonstrate an association between arrhythmias and air pollution. Aconitine-induced cardiac arrhythmia is widely used experimentally to examine factors that alter the risk of arrhythmogenesis. In this study, Wistar-Kyoto (WKY) and spontaneously hypertensive (SH) rats acutely exposed to synthetic residual oil fly ash (s-ROFA) particles (450 μg/m³) were “challenged” with aconitine to examine whether a single exposure could predispose to arrhythmogenesis. Separately, SH rats were exposed to varied particulate matter (PM) concentrations (0.45, 1.0, or 3.5 mg/m³ s-ROFA), or the irritant gas acrolein (3 ppm), to better assess the generalization of this challenge response. Rather than directly cause arrhythmias, we hypothesized that inhaled air pollutants sensitize the heart to subsequent dysrhythmic stimuli. Twenty-four hour postexposure, urethane-anesthetized rats were monitored for heart rate (HR), electrocardiogram, and blood pressure (BP). SH rats had higher baseline HR and BP and significantly longer PR intervals, QRS duration, QTc, and JTc than WKY rats. PM exposure caused a significant increase in the PR interval, QRS duration, and QTc in WKY rats but not in SH rats. Heart rate variability was significantly decreased in WKY rats after PM exposure but increased in SH rats. Cumulative dose of aconitine that triggered arrhythmias in air-exposed SH rats was lower than WKY rats and even lower for each strain postexposure. SH rats exposed to varied concentrations of PM or acrolein developed arrhythmia at significantly lower doses of aconitine than controls; however, there was no PM concentration–dependent response. In conclusion, a single exposure to air pollution may increase the sensitivity of cardiac electrical conduction to disruption. Moreover, there seem to be host factors (e.g., cardiovascular disease) that increase vulnerability to triggered arrhythmias regardless of the pollutant or its concentration.

Key Words: aconitine; air pollution; cardiac arrhythmia; inhalation.

Epidemiological, experimental, and clinical studies point to a significant association between arrhythmias and air pollutant exposures. For example, hospital admissions for arrhythmias increased by 49% during a 1985 smog episode in West Germany (Wichmann et al., 1989). Peters et al. (2000) showed a significant correlation between the frequency of cardioverter defibrillator discharge and the levels of certain gaseous and particulate matter (PM) air pollutants, also indicating a relationship between air pollution and cardiac arrhythmia. While the relative risk of death due to PM air pollution is higher for exacerbation of respiratory conditions, the greater proportion of the population with underlying cardiovascular disease yields substantially more deaths related to cardiovascular-associated mortality (Dockery, 2001). Hospitalization due to cardiovascular complications were found to be significantly associated with PM₁₀, CO, and NO₂ (Linn et al., 2000) between 1992 and 1995 in Los Angeles, CA, USA, and when related to air pollution appear to be quite dependent on underlying heart disease (Pope et al., 2008). In rats with severe cardiac disease, exposures of increasing doses of a model PM raised the incidence of cardiac arrhythmia as well as other cardiographic abnormalities, a response not observed in normal rats (Watkinson et al., 1998; Wellenius et al., 2002). These similar findings between the humans and rats linked by underlying cardiac disease suggest commonalities that might be exploited in studies with the rat which could shed light on the conditions or circumstances which promote these events in humans.

A novel approach has been employed to explore the tendency or sensitivity to arrhythmogenic episodes associated with air pollutant exposure. This approach takes advantage of the potential for pollutants to “sensitize” individuals to subsequent cardiac stimuli rather than examine direct exposure-related cardiac events per se. Aconitine, a cardiotoxic alkaloid derived from the plant Aconitum (wolfsbane), is widely used to produce ventricular arrhythmias in laboratory animals. Aconitine targets voltage-dependent sodium channels in various...
excitable tissues, including the myocardium, suppressing their inactivation and thereby interfering with repolarization of the cardiomyocyte membrane in preparation for the next beat. Therefore, this drug has come to be used to produce experimental arrhythmia, often as a positive control in drug-testing paradigms, which assess potential arrhythmogenic properties (e.g., doxorubicin; Dragojevic-Simic et al., 2004) and test the efficacy of antiarrhythmic drugs (Bartosova et al., 2007; Lu and De Clerck, 1993). Recently, aconitine was used to compare arrhythmia susceptibility of healthy animals and those with cardiovascular disease (Li et al., 2007). The present study takes advantage of the arrhythmogenic properties of aconitine to, for the first time, test arrhythmia sensitivity following exposure to air pollutants. Here, we sought to determine whether inhalation exposure to particulate or gaseous air pollutants increases the risk of arrhythmogenesis in a rat model of hypertension.

We adapted a simple cardiac challenge test using the well-established arrhythmogenic properties of aconitine to (1) determine if hypertensive rats are more at risk of developing cardiac arrhythmia when compared to normotensive rats; (2) compare the impact of a single inhalation exposure to a synthetic PM that is similar in transition metal content to residual oil fly ash (ROFA), an emission source pollutant particle found in fossil fuel emissions, on arrhythmogenicity in normotensive and hypertensive rats; and (3) determine if increasing concentrations of PM or acrolein, a potent gaseous pulmonary irritant found in cigarette smoke, increase the risk of developing cardiac arrhythmia in hypertensive rats. We hypothesized that inhaled air pollutants sensitize the heart to dysrhythmic stimuli rather than directly cause arrhythmias.

MATERIALS AND METHODS

Animals

Eleven-week-old male Wistar-Kyoto (WKY) and spontaneously hypertensive (SH) rats (Charles River, Wilmington, MA) weighing 300–400 g were studied. Upon arrival, animals were housed two per cage with food and water available ad libitum in a facility approved by the Association for Assessment and Accreditation of Laboratory Animal Care. All experimental protocols were approved by and in accordance with the guidelines of the Institutional Animal Care and Use Committee of the U.S. Environmental Protection Agency, Research Triangle Park, NC.

Implantation of Radiotelemeters

Radiotelemeters were implanted in all animals as previously described (Campen et al., 2000; Watkinson et al., 1995). Briefly, animals were weighed and anesthetized with ketamine hydrochloride/xylazine hydrochloride solution (1 ml/kg, ip; Sigma-Aldrich, St Louis, MO). Using aseptic technique, each animal was implanted with a radiotelemetry transmitter (Model TA11TCATF40; Data Sciences International, Inc., St Paul, MN) in the abdominal cavity through a small incision. The electrode leads were guided through the abdominal musculature via separate stab wounds and tunneled sc across the lateral ventral thorax; the distal portions of the leads were secured in positions that approximated those of the lead II of a standard electrocardiogram (ECG).

Body heat was maintained both during and immediately following the surgery. Animals were given food and water and were housed individually. All animals were allowed 7–10 days to recover from the surgery and reestablish circadian rhythms.

Radiotelemetry Data Acquisition and Analysis

Radiotelemetry methodology (Data Sciences International, Inc.) was used to track changes in cardiovascular function by monitoring ECG and heart rate (HR). This methodology provided continuous monitoring and collection of physiologic data from individual rats to a remote receiver (DataART2.1; Data Sciences International, Inc.). ECG waveforms were continuously acquired and saved during the 5-min baseline period and aconitine challenge, which did not last longer than 40 min. HR was obtained from the ECG.

ECG Analysis

ECGAuto software (EMKA Technologies USA, Falls Church, VA) was used to visualize individual ECG signals, analyze and quantify ECG segment durations, and identify cardiac arrhythmias. Using ECGAuto, P-wave, QRS complex, and T-wave were identified for individual ECG waveforms and compiled into a library. Analysis of all experimental ECG traces was then based on established libraries. The following parameters were determined for each ECG waveform: PR interval; QRS duration, which was measured as the beginning of the R-wave (nadir of Q-wave) until the S-wave reached the isoelectric line because Q-waves were not clearly discernible in many traces; QT interval; QT corrected for HR (QTC) using Bazett’s formula; corrected JT interval (JTc = QTC – QRS); and ST interval, which was measured from the nadir of the S-wave until the end of the T-wave or when it reached the isoelectric line. The Lambeth Conventions (Walker et al., 1988) were used as guidelines for the identification of cardiac arrhythmic events in rats. Arrhythmias were identified as occurring sequentially during aconitine challenge as ventricular premature beats (VPBs), ventricular tachycardia (VT), and ventricular fibrillation (VF). Figure 1 shows examples of arrhythmias typically found in the animals in this study.

Heart rate variability (HRV) was calculated as the mean of the differences between sequential HRs for the complete set of ECG signals using ECGAuto. For each 1-min stream of ECG waveforms, mean time between successive QRS complex peaks (RR interval), mean HR, and mean HRV-analysis–generated time-domain measures were acquired. The time-domain measures included standard deviation of the time between normal-to-normal beats (SDNN) and root mean squared successive differences (RMSSD). HRV analysis was also conducted in the frequency domain using a fast Fourier transform. The spectral power obtained from this transformation represents the total harmonic variability for the frequency range being analyzed. In this study, the spectrum was divided into low-frequency (LF) and high-frequency (HF) regions. The ratio of these two frequency domains (LF/HF) was calculated as an estimate of the relative balance between sympathetic (LF) and vagal (HF) activity.

Groups

Table 1 shows the exposure groups. SH rats (n = 6 per group) were assigned to one of the following exposure groups: (1) filtered air, (2) 450 μg/m³ (low), (3) 1 mg/m³ (medium), (4) 3.5 mg/m³ (high) synthetic residual oil fly ash (s-ROFA), or (5) 3 ppm acrolein, exposed and then challenged with aconitine 24 h later to measure sensitivity to arrhythmias associated with increasing concentrations of s-ROFA and to assess the transferability of this approach to a known gaseous irritant, acrolein. Concurrently, WKY rats (n = 6 per group) were exposed to either filtered air or 450 μg/m³ (low) s-ROFA and then challenged with aconitine 24 h later to assess the effect of strain on arrhythmogenicity following exposure.

Exposure

s-ROFA-like PM. The PM used in this study was synthetically generated by combining metal compounds in molar ratios reflecting those observed in a model historic PM ROFA (Southern Research Institute, Birmingham, AL; an emission air pollution particle that was collected as a fugitive stack emission postemission control at Florida Power & Light plant that burned #6 grade residual oil containing 1% sulfur [Hatch et al., 1985]). After a 2-day
acclimatization routine in the nose-only exposure system, the rats were exposed one time for 3 h to 450 \( \mu g/m^3 \), 1 mg/m \(^3\), or 3.5 mg/m \(^3\) of the PM \(_{2.5}\) fraction (mass median aerodynamic diameter was 2.09 \( \mu m \) and the geometric standard deviation was 3.37) of the PM or filtered air in two separate 24-port nose-only flow-by inhalation chambers (Lab Products, Seaford, DE) as previously described. Particle concentration was determined gravimetrically on Teflon filters (45-mm diameter with 1-\( \mu m \) pore size; VWR Scientific, West Chester, PA) using a Mercer cascade impactor (Intox Products, Albuquerque, NM), and real-time PM concentration was estimated with an aerosol monitor (Dust Track; TSI Inc., St Paul, MN) on the chamber exhaust. Extracts from the Teflon filters were analyzed quantitatively for 28 metals and sulfur (expressed as sulfate) on a PerkinElmer 4300DV ICP-OES elemental analyzer (PerkinsElmer, Waltham, MA). Mean (± SD) of H\(_2\)O-soluble content of metals and SO\(_4\) in microgram per gram were as follows: Fe = 1118 ± 210, Ni = 10,144 ± 947, V = 21,849 ± 2342, and SO\(_4\) = 335,077 ± 28,529. Mean (± SD) 1M HCl-soluble content of metals and SO\(_4\) in microgram per gram were as follows: Fe = 135,586 ± 7904, Ni = 12,398 ± 713, V = 89,852 ± 5432, and SO\(_4\) = 421,845 ± 22,923.

**Acrolein.** Animals were exposed to 3 ppm acrolein for 3 h, while a separate control group was simultaneously exposed to filtered air. Gas was metered from a 1000-ppm cylinder into a glass mixing chamber where the gas was mixed with dry filtered dilution air to achieve a final concentration of 3 ppm of acrolein with a total flow of 6 l/min. All animals were monitored for a 15-min baseline period prior to being exposed in a whole-body plethysmograph (Model PLY3213; Buxco Electronics, Inc., Wilmington, NC), which continuously and noninvasively monitored respiratory function in the conscious animals. The actual chamber concentration was measured using an HP5890 gas chromatograph (Hewlett Packard, Palo Alto, CA) equipped with manual injection, a flame ionization detector, and a DB-VRX capillary column (Agilent Technologies, Foster City, CA). The plethysmograph pressure was monitored using Biosystems XA software (Buxco Electronics, Inc.).

Although these concentrations are relatively high compared to ambient air, such challenges can be reached occupationally as in the case of ROFA and cigarette smoke exposures (10 ppm mainstream smoke) in the case of acrolein (Reinhardt and Ottmar, 2004; Song et al., 2004). For instance, Cavallari et al. (2008a) found that boilermaker welders were exposed to mean PM\(_{2.5}\) concentrations of 1.12 mg/m \(^3\); however, the concentrations actually ranged from 0.12 to 3.99 mg/m \(^3\). Other studies in similar cohorts have documented occupational concentrations of 0.47 mg/m \(^3\) of PM\(_{10}\) (Liu et al., 2005) and 0.44 mg/m \(^3\) (Kim et al., 2004) and 0.65 mg/m \(^3\) (Cavallari et al., 2008b) of PM\(_{2.5}\). Nevertheless, the specific aim of the study was to ascertain the feasibility of a postexposure challenge approach for assessing enhanced cardio-sensitivity to dysrhythmias.

**Aconitine Challenge**

We have previously demonstrated that the number of arrhythmias are dramatically increased in SH rats in the 24-h period following a single ROFA exposure (Farraj et al., 2009). Similarly, Dockery et al. (2005) have suggested, in the reverse situation, that assessment of pollutant concentrations 24 h prior to the arrhythmia would provide a good estimate of a subject’s exposure. Therefore, 24 h after particle or gaseous irritant exposure, animals were

**TABLE 1**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Exposure (n = 6)</th>
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<tr>
<td>WKY (air)</td>
<td>Filtered air</td>
</tr>
<tr>
<td>WKY (s-ROFA)</td>
<td>450 ( \mu g/m^3 ) s-ROFA</td>
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<tr>
<td>SH rat (air)</td>
<td>Filtered air</td>
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<tr>
<td>SH rat (s-ROFA)</td>
<td>450 ( \mu g/m^3 ) s-ROFA</td>
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<tr>
<td>SH rat (s-ROFA medium)</td>
<td>1.0 mg/m(^3) s-ROFA</td>
</tr>
<tr>
<td>SH rat (s-ROFA high)</td>
<td>3.5 mg/m(^3) s-ROFA</td>
</tr>
<tr>
<td>SH rat (acrolein)</td>
<td>3 ppm acrolein</td>
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**FIG. 1.** Typical ECG traces showing normal sinus rhythm, VPB, VT, and VF.
anesthetized with urethane (1.5 g/kg, ip) for the aconitine challenge; supplemental doses of the anesthetic were administered iv when necessary to abolish pain reflex. Animal body temperature was maintained at ~36°C with a heating pad. The left jugular vein and right carotid artery were cannulated with P.E. 50 polyethylene tubing (Becton-Dickinson, Sparks, MD) for the administration of aconitine and measurement of blood pressure (BP), respectively. Ten microgram per milliliter aconitine was continuously infused at a speed of 0.2 ml/min, while ECG was continuously monitored and timed. Sensitivity to arrhythmia was measured as the threshold dose of aconitine required to produce VPBs, VT, and VF and was calculated using the following formula:

Threshold dose (µg/kg) for arrhythmia = 10 µg/ml × 0.2ml/min × time required for inducing arrhythmia(min)/body weight(kg).

Statistics

The statistical analyses for all the data in this study were performed using SAS version 9.1.3 software (SAS Institute Inc., Cary, NC). PROC MIXED and PROC GLIMMIX procedures were used to analyze all ECG- and HRV-generated data. Tests of normality were performed for all continuous variables, and parametric methods of analysis were used. A linear mixed model with restricted maximum-likelihood estimation analysis (SAS) and least squares means post hoc test were used to determine statistical differences for all data. All aconitine dose-response data were analyzed using an ANOVA. A p value of < 0.05 was considered as statistically significant. A significant interaction resulted in pairwise comparisons performed as a subtest of the ANOVA model adjusting the significance level for multiple comparisons using Tukey’s post hoc test. Reported values represent means ± SE.

RESULTS

Postexposure Comparison of WKY and SH Rat Strains

There were some basic differences among WKY and SH rats; they were largely consistent with what were expected to be observed for these two strains.

Body Weight and Heart Rate

There were no significant differences in the preexposure body weights of any group, although SH rats were slightly heavier. There was also no significant change in body weight within a group between preexposure and postexposure (data not shown). However, 24 h after exposure, WKY rats exposed to s-ROFA had significantly lower body weights than air-exposed WKY rats (Fig. 2A). There was no change in SH body weights due to exposure to s-ROFA. However, 1 day following exposure, urethane-anesthetized SH rats exposed to air only had higher HR when compared to WKY rats. SH rats exposed to s-ROFA had a significant decrease in HR relative to the air controls (Fig. 2B).

Blood Pressure

There was no significant difference in the systolic and diastolic BPs, as well as mean arterial pressure (MAP), of SH rats when compared to WKY rats. However, a single exposure to s-ROFA caused a small decrease in both systolic and diastolic BP in both strains (Figs. 3A and 3B). Exposure to s-ROFA caused a significant decrease in MAP in WKY rats but had only a minor, not significant, decrease in the SH rats (Fig. 3C).

Electrocardiogram

SH rats had significantly longer PR intervals, QRS duration, QTc, and JTc when compared to WKY rats. Exposure to s-ROFA caused a significant increase in PR interval, QRS duration, and QTc in WKY rats but had no effect on SH rats. There was no difference in ST interval duration between any of the groups (Fig. 4).

Heart Rate Variability

SH rats had shorter RR interval than WKY rats as might be expected with higher HRs. When adjusted for HR, air-exposed SH rats had lower baseline overall and short-term HRV when compared to air-exposed WKY rats. Lf, Hf, and Lf/Hf ratio was lower in air-exposed SH rats when compared to air-exposed WKY rats.

s-ROFA-exposed WKY rats exhibited lower mean RR interval, significantly decreased overall and short-term HRV, and significantly decreased power in the low and high-frequency range when compared to air-exposed WKY rats. In contrast, s-ROFA-exposed SH rats had higher RR interval, significantly increased overall and short-term HRV, and increased power in the low and high-frequency range when compared to air-exposed SH rats. Exposure to s-ROFA caused an increase in Lf/Hf in WKY rats and decrease in SH rats (Fig. 5).

Aconitine Challenge

During aconitine infusion, the first arrhythmia to be manifested was the VPB, a form of irregular heartbeat in which the lower chambers of the heart contract prematurely. These anomalies, which are the most common form of arrhythmia, are often perceived and described as skipped beats or palpitations. Continued infusion of aconitine then elicited three or more successive VPBs or VT, which is fast abnormal heart rhythm that is potentially life threatening. VTs are
dangerous, because if persistent, they can lead to VF or fatal, uncoordinated, discordant contraction of the ventricles (Fig. 1). Significant drops in BP occurred as VT became sustained and transitioned to VF, which occurred at lower cumulative doses of aconitine in SH rats when compared to WKY rats.

Figure 6 shows the dose of constantly infused aconitine that elicited arrhythmia in WKY and SH rats. The cumulative dose of aconitine necessary to trigger VPB, VT, and VF in SH rats was significantly lower than WKY rats. Exposure to s-ROFA further decreased the dose of aconitine that elicited arrhythmia when compared to the respective air-exposed animals for each strain (Fig. 6).

Figure 7 shows that exposure to varying concentrations of s-ROFA or acrolein increases the risk of developing cardiac arrhythmias in hypertensive rats. The cumulative dose of aconitine necessary to trigger VPB, VT, and VF in SH rats exposed to 0.45, 1.0, or 3.5 mg/m³ s-ROFA was significantly lower than air-exposed controls. Interestingly, there was no effect of increasing s-ROFA concentration on the dose of aconitine that elicited arrhythmia. Exposure to acrolein also significantly decreased the dose of aconitine that triggered arrhythmia when compared to air-exposed controls; the doses were comparable to those observed in the s-ROFA-exposed rats (Fig. 7).

DISCUSSION

Routinely, toxicological assessments of air pollutants focus on the direct impacts of one or more pollutants on the targeted tissue of organ systems. With air pollution, notably PM, there...
are extensive reports of cardiac impacts, including arrhythmias, which require hospitalization or potentially lead to other morbidities or death. The findings presented here suggest that perhaps the impact of pollutant exposure is less direct in eliciting effects but more in the prestaging of a response to other cardiostimulatory challenges, i.e., a “sensitization.” A single inhalation exposure to toxic air pollutants (either PM or gaseous irritant), as used in this study, appeared to increase the “sensitivity” of the cardiac electrical conduction system, much as the lungs become hyperresponsive to nonspecific stimuli (e.g., methacholine, saline, dryness) following exposure to respiratory toxicants (Bateson and Schwartz, 2008). It is also apparent that part of this sensitivity results from underlying cardiovascular disease. The stress to the body from exposure to PM and acrolein suggests that they may not only enhance sensitivity but also impart a temporal longevity beyond the initial period of insult, 24 h in this case. Although air pollution is often seen as a factor that sets off adverse cardiac events, here we demonstrate that a single exposure increases the vulnerability of the heart to developing arrhythmia by a subsequent trigger. This cardiac “hypersensitivity” may be a more serious risk than direct toxic injury because it is insidious and may occur at substantially lower doses.

The SH rat is widely used as a model for the study of hypertension and progressive cardiovascular disease. Intrinsic differences in the BP, myocardial substrate, or autonomic nervous system function of the WKY and SH rat strains likely contribute significantly to arrhythmogenic vulnerability. Although measured under anesthesia in this study, baseline HR and BP were still higher in SH rats when compared to WKY

**FIG. 5.** Group data of HRV in WKY and SH rats exposed to s-ROFA. One day after exposure, s-ROFA-exposed WKY rats had lower RR interval (A), significantly decreased SDNN (B) and RMSSD (C), and significantly decreased $L_f$ (D) and $H_f$ (E) when compared to air-exposed WKY rats (black bars). s-ROFA-exposed SH rats had higher RR interval (A), significantly increased SDNN (B) and RMSSD (C), and increased $L_f$ (D) and $H_f$ (E) when compared to air-exposed SH rats (gray bars). Exposure to s-ROFA caused an increase in $L_f/H_f$ in WKY rats and decrease in SH rats (F). * $p < 0.05$, $n = 6$.

**FIG. 6.** Increased risk of arrhythmia in hypertensive rats is exacerbated by exposure to air pollution. Constant infusion of aconitine (2 μg/min) triggers VPB, followed by VT, VF, and progression to cardiac arrest (CA) in air-exposed WKY (black bars) and SH rats (dark gray bars). The cumulative dose of aconitine necessary to trigger VPB, VT, VF, and CA in SH rats was lower than WKY rats and lower following exposure to s-ROFA for each strain of rat (light gray bars). *Significantly different from WKY (Air); §Significantly different from WKY (s-ROFA); $p < 0.05$, $n = 6$. 
rats, regardless of exposure group. Anesthesia has depressive effects on centrally mediated physiological function, e.g., one study showed that sodium pentobarbital anesthesia lowered the dose of aconitine at which ventricular arrhythmias occurred, possibly due to decreased arterial baroreceptor function (Shu et al., 2004). The authors demonstrated that conscious rats with sinoaortic denervation, or depressed baroreflex sensitivity (BRS), responded to a lower threshold dose of aconitine than control rats. However, under pentobarbital anesthesia, control rats and sinoaortic denervated rats had comparable responses, suggesting depression of BRS by pentobarbital increased sensitivity to aconitine. In the current study, the effects of anesthesia were assumed to be minimal since urethane, which is considered to have minor effects on the cardiovascular system and its reflexes (Koblin, 2001; Maggi and Meli, 1986), was used instead of pentobarbital. Thus, it may be likely that SH rats are more sensitive to the arrhythmogenic effects of aconitine than WKY rats because of inherent depressed BRS, which is known to be impaired in hypertensive states (Gerritsen et al., 2001; Mortara et al., 1997; Stryker-Boudier et al., 1982).

It is important to note that at 11 weeks of age, despite some elevation in BP, the SH rats probably had not fully developed the hypertensive phenotype; older animals may have been a better representation of this group. Regardless, the finding that SH rats had greater baseline arrhythmia susceptibility when compared to the normal WKY rats was in agreement with the results of Li et al., who showed that 20-week-old Lyon Hypertensive (LH) rats were more prone to ventricular arrhythmias when compared to normotensive Lyon rats. However, Li et al. (2007) found that BP level was not an independent determinant of arrhythmia sensitivity to aconitine in LH rats, which when considered with the results from the younger less hypertensive SH rats in our study indicates that BP by itself probably does not affect the dose at which aconitine induces arrhythmia. Instead, it may be a combination of hemodynamic abnormalities, cardiac electrical disturbance, and other factors that contribute to the heightened sensitivity of hypertensive rats.

Although we were cautious about making any definitive conclusions about ECG in our urethane-anesthetized rats and the role anesthesia plays in arrhythmogenesis, these findings were consistent with our previous results showing that there were baseline differences in the ECG of conscious WKY and SH rats and the results of others who used anesthetized rats (Hazari et al., 2009; Kelishomi et al., 2008; Xin et al., 2007). SH rats had longer PR intervals than WKY rats. PR interval prolongation might be due to slowing of intra-atrial propagation or more likely slowing of conduction in the atrioventricular (AV) node since no change in P-wave duration was detected. This baseline difference between the normotensive and hypertensive rat strains may contribute to the sensitivity of the animals to cardiotoxic insults, particularly electrical dysfunction, and therefore arrhythmogenesis. However, since changes in PR intervals are associated with supraventricular factors (i.e., nonconducted P-waves and AV block in humans) (Scheidt, 1986), it is unclear whether they represent a predisposition to the types of ventricular arrhythmia observed during aconitine challenge. Nonetheless, given the PM exposure-related increase, PR interval may be a sensitive indicator of developing cardiotoxicity or cardiac stress in the normotensive strain, particularly since naive SH rats have comparatively elevated baseline PR intervals, which ostensibly are unaffected by exposure.

QTc was also prolonged in control SH rats when compared to control WKY rats. Prolonged QRS complex duration accounted for the relatively increased QTcs in SH rats since there was no difference in ST interval between the strains. Lengthening of QRS complex duration is thought to facilitate ventricular arrhythmogenesis (Kashani and Barold, 2005; Madias, 2008; Scheidt, 1986). Prolonged QTc, which is classically used to measure repolarization time, is also well recognized as a risk factor of arrhythmogenesis and adverse cardiac events in healthy individuals (De Bruyne et al., 1999) and is predictive of mortality in patients with advanced heart failure (Vrtovec et al., 2003). However, it has been suggested in humans that because the QT interval includes ventricular depolarization, its prognostic value is limited when QRS complex contributes to QT prolongation. Alternatively, JTc (QTc – QRS) may be a more appropriate and independent measure of ventricular repolarization (Crow et al., 2003; Das, 1990; Spodick, 1992). Our findings demonstrate that SH rats had longer action potential durations at baseline than WKY rats, which suggests that the presence of QRS complex lengthening may point to a predisposition to ventricular arrhythmia. SH rats also had prolonged time of repolarization, as demonstrated by the increased JTc, which may have increased the likelihood of arrhythmic events (Fossa, 2008). It may also have enhanced the heterogeneity of repolarization, which is considered a significant prerequisite for ventricular
arrhythmia in humans and animals (Akar and Rosenbaum, 2003; Kuo et al., 1983). Moreover, the occurrence of arrhythmia, which is more frequent in naive SH rats when compared to WKY rats (Hazari et al., 2009), could induce further electrical remodeling of cardiac tissues leading to alterations in activation sequence (Rosen, 2001).

Exposure to PM also increased QTc in WKY rats reflecting in part the increase in QRS complex duration as was seen in the SH strain. It is uncertain whether these changes represent the mechanism underlying the increased sensitivity of the PM-exposed WKY rats to aconitine when compared to the air-exposed controls since the response was not statistically significant, although it is not entirely unlikely that increases in the same ECG parameters that were prolonged in SH rats at baseline indicate a tendency to develop electrical dysfunction and cardiotoxicity in the background normotensive strain. There was no significant effect of PM on the ECG parameters of SH rats, suggesting the exposure caused arrhythmia sensitivity through mechanisms that may not include an effect on ECG or that the strain already had changes, which did not become further altered. This is in agreement with a study in conscious SH rats, which showed that a single inhalation exposure to ROFA had no effect on ECG parameters (Farraj et al., 2009). Instead, it may be a combination of hypertension, underlying cardiac electrical dysfunction, autonomic imbalance, and/or other hemodynamic mechanisms that contribute to the heightened sensitivity of the SH strain to aconitine postexposure. Oxidative stress, which has been shown to mediate PM-related effects on cardiovascular function and ECG (Brook et al., 2004; Gurgueira et al., 2002), and which is known to be elevated in hypertensive rats (Bartha et al., 2009), may also play a role in the strain’s response to aconitine. Subchronic exposure and ECG monitoring studies are necessary to clarify this issue and further determine the persistence of arrhythmia sensitivity in these strains of rat.

Decreased HRV has been associated with cardiovascular disorders such as congestive heart failure, hypertension, arrhythmia, and sudden cardiac death (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). In the current study, changes in HRV cannot be definitively assessed as a biomarker of effect because ECG, and therefore HRV, was not measured prenose-only restraint but rather was compared across the air and pollutant cohorts. Nevertheless, prior to aconitine challenge, air-exposed control SH rats had decreased HRV when compared to air-exposed WKY rats, particularly in the RMSSD (short-term component). Since SDNN only reflects long-term global components, the value of its decrease in the context of this study is probably limited. Nonetheless, these data and lower Hf values in air-exposed SH rats indicate a diminished parasympathetic component regulating HR (European Society of Cardiology, 1997; Rowan et al., 2007). Decreases in HRV, which reflect imbalance in the parasympathetic and sympathetic components of cardiovascular control, are associated with increased cardiac risk especially in those with preexisting disease or cardiac events (Devlin et al., 2003; Pope et al., 2004; Tsuji et al., 1996). In particular, reduced parasympathetic tone has been found to play an important role in ventricular arrhythmogenesis (Kjellgren and Gomes, 1993; Zareba et al., 2001); this propensity may partially explain the observed sensitivity in the SH strain.

Exposure to PM caused a decrease in HRV in WKY rats when compared to air-exposed controls. Similar decreases in HRV have been observed in a cardiac-infarcted Sprague-Dawley rat model exposed to ROFA that also exhibited an increased incidence of arrhythmia (Wellenius et al., 2002). As was suggested previously, oxidative stress due to exposure may mediate the decreased HRV and when combined with electrical changes lead to heightened arrhythmia sensitivity. In contrast, SH rats had an increase in HRV following PM exposure. Similar increases in HRV, particularly the parasympathetic component, have also been reported in young human subjects exposed to ultrafine particles (UFP) (Zareba et al., 2009) and concentrated ambient particles (CAPs) (Gong et al., 2003). The mechanisms governing this increase in HRV postexposure are not yet fully understood. In our experiments, the 24-h postexposure delay in ECG measurement, use of anesthesia, age, and degree of hypertension may have impacted the response. Studies of HRV and air pollution seem to indicate that older people, particularly those with underlying cardiovascular disease, experience decreases in HRV upon exposure. It is plausible that the HRV may have decreased if the SH rats used in this study were older and had further developed their hypertensive phenotype.

According to these findings, postexposure aconitine-induced arrhythmia sensitivity seemed to be independent of the type of air pollutant, particle, or gaseous. Interestingly, increasing concentrations of the same PM did not dose-dependently affect the amount of aconitine necessary to elicit arrhythmia. Thus, the air pollutants used, gaseous or particulate, may have been sufficiently potent to trigger a common cascade of events that led to arrhythmogenesis, keeping in mind the vulnerable nature of the subjects. This result is both confounding and intriguing since it suggests that it may not be the specific components of the pollutant, but rather the generalized insult that causes toxicity, perhaps again when laid upon the vulnerable host. It has been postulated that the broad mechanisms governing responses to air pollution include neural reflexes, which alter autonomic function and control, inflammation, particularly in the upper airways of obligate nose-breathing animals, such as rats, and chemical effects on ion channel function in the heart, whether from the pollutant molecule or innate mediators (Zareba et al., 2001). Increased production of reactive oxygen species (ROS) has also been proposed as a common contributor to air pollution–related cardiotoxicity. UFP, fine, and coarse particles, diverse bulk sources of urban ambient particles, and inhalation of CAPs and diesel exhaust have been found to
cause cardiovascular effects via ROS-mediated mechanisms (see review by Simkhovich et al., 2008). ROFA-induced lung oxidative stress has been demonstrated by electron spin resonance (Kadiiska et al., 1997). In fact, in a comparison of WKY and SH rats, the latter were found to have more oxidative burden and decreased antioxidant capacity following ROFA exposure (Kodavanti et al., 2000). Although many of these studies use intratracheal instillation as the mode of exposure, a study by Costa et al. (2006) showed that either inhalation or instillation of ROFA causes similar levels of pulmonary injury and inflammation, which is another plausible cause of compromised cardiopulmonary function. This ROFA-induced pulmonary injury, which is characterized by increased bronchoalveolar lavage protein and albumin levels and inflammatory cell infiltration, has been found to be particularly robust in the SH rat (Kodavanti et al., 2000). These animal studies clearly used higher ROFA concentrations than the ones used in this study; however, human studies with comparable ROFA concentrations to ours have indicated that within 24 h of exposure, subjects experience decrements in lung function and inflammation (Hauser et al., 1995; Lees, 1980).

Similarly, gaseous pollutants promote oxidative stress and toxicity (Chuang et al., 2009; Liu et al., 2009). Acrolein has been found to cause injury and inflammation in the olfactory and respiratory epithelium of rats (Dorman et al., 2008) and increased ROS (Roy et al., 2009), and it has resulted in pulmonary edema and lung hemorrhage in rats at exposure levels between 2 and 5 ppm (see review by Faroon et al., 2008). Lastly, studies have even shown that both PM and gaseous pollutants stimulate airway sensory fibers, which alter both pulmonary and cardiovascular function by modulating airway reflexes and autonomic control (Ghelfi et al., 2008; Hazari et al., 2008; Symanowicz et al., 2004). To clarify these issues, future studies should examine lower concentrations of both gaseous and particulate pollutants and variable timing postexposure to determine if there is a threshold of response.

Here, we demonstrated that there may be a general vulnerability resulting from exposure to foreign substances in the airways, particularly since this response was observed with both pollutant particles and an irritant gas. Using a novel approach, we were able to demonstrate that although the SH rats had no overt symptoms of disease, aconitine challenge following a single exposure to an air pollutant revealed undetected cardiac sensitivity. Although studies showed that ROFA particles could increase the incidence and duration of arrhythmias in rats with severe underlying cardiopulmonary pathology (Watkinson et al., 1998; Wellenius et al., 2002), we have demonstrated that there are general factors that render a host vulnerable to triggered arrhythmias regardless of the pollutant or its concentration or in the absence of overt direct adverse health effects. More importantly, the evidence suggests that it is the host with underlying cardiovascular disease that is at greatest risk following exposure to a potentially toxic particulate or gaseous air pollutant. This response after the inhalation of pollutants likely occurs due to numerous factors; however, oxidative stress and inflammation, which persist postexposure, may be critical in the risk of arrhythmogenesis. In fact, it has been shown that the biological effects of ROFA exposure occur within 24–48 h (Ghio et al., 2002), rendering the host temporarily susceptible to triggered adverse events. More importantly, it is the impact of a single exposure that, although not considered significant, may be potentially life threatening in some individuals due to this postexposure period of sensitivity. Thus, the application of the challenge concept to studying pollutant impacts opens a new avenue for assessing imparted health risks to exposure and an alternate mode of action of pollutants on the host.

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AIR POLLUTION INCREASES RISK OF TRIGGERED CARDIAC ARRHYTHMIA 541


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