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Effects of Ambient Particles and Carbon Monoxide on Supraventricular Arrhythmias in a Rat Model of Myocardial Infarction

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The association between short-term increases in particulate air pollution and increased cardiovascular morbidity and mortality is well documented. Recent studies suggest an association between particulate matter with aerodynamic diameter $<2.5\ \mu$m ($PM_{2.5}$) and supraventricular arrhythmias (SVA), but the results have been inconsistent. We evaluated this hypothesis in a rat model of acute myocardial infarction (AMI). Diazepam-sedated Sprague-Dawley rats with AMI were exposed (1 h) to either filtered air ($n = 16$), concentrated ambient fine particles (CAPS; mean $= 645.7\ \mu g/m^3$; $n = 23$), carbon monoxide (CO; 35 ppm; $n = 19$), or CAPs and CO ($n = 24$). Each exposure was immediately preceded and followed by a 1-h exposure to filtered air (baseline and postexposure periods, respectively). Surface electrocardiograms were recorded and the frequency of supraventricular premature beats was quantified. Among rats in the CAPS group, the probability of observing any SVA decreased from baseline to the exposure and postexposure periods. This patterm was significantly different than that observed for the filtered air group during the exposure period ($p = .048$) only. In the subset of rats with one or more SVA during the baseline period, the change in SVA rate from baseline to exposure period was significantly lower in the CAPS ($p = .04$) and CO ($p = .007$) groups only, as compared to the filtered air group. No significant effects were observed in the group simultaneously exposed to CAPS and CO. Thus, the results of this study do not support the hypothesis that exposure to ambient air pollution increases the risk or frequency of supraventricular arrhythmias.

The association between short-term increases in particulate air pollution and increased cardiovascular morbidity and mortality is well documented. Putative biologic mechanisms include changes in autonomic nervous system function, hemodynamics, hemostatic factors, and a systemic inflammatory response (Brook et al., 2004).

Several recent epidemiologic studies have reported an association between particulate matter with aerodynamic diameter $<2.5\ \mu$m ($PM_{2.5}$) and ventricular arrhythmias (Peters et al., 2000; Rich et al., 2005; Riediker et al., 2004). A similar association has been observed in a controlled exposure study in humans (Gong et al., 2004) and in animals exposed to residual oil fly ash, a component of $PM_{2.5}$ (Campen et al., 2000; Watkinson et al., 1998; Wellenius et al., 2002).

Although not generally considered life-threatening, supraventricular arrhythmias reduce cardiac output and can initiate ventricular arrhythmias (Podrid, 2006). Relatively few epidemiologic studies have evaluated the link between $PM_{2.5}$ and supraventricular arrhythmias. While there is some evidence

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from these studies in favor of such a link (Brauer et al., 2001; Gong et al., 2004; Riediker et al., 2004), the results have been inconsistent (Devlin et al., 2003; Gong et al., 2003; Rich et al., 2006). To our knowledge, only one previous animal toxicological study has specifically addressed this question and that study found no effect (Nadziejko et al., 2004).

A complicating factor in many epidemiological studies is that $PM_{2.5}$ exists in outdoor air as a complex mixture that includes gaseous pollutants such as carbon monoxide (CO). CO is a ubiquitous gaseous pollutant produced by incomplete combustion of carbonaceous fuels and substances. Typical sources of CO include vehicle exhaust, industrial processes, home heating systems, and cigarette smoke. Numerous studies have found an association between short-term increases in ambient CO levels and increased risk of cardiovascular morbidity and mortality (Burnett et al., 1997; Hoek et al., 2001; Morris et al., 1995). In the United States, daily mean ambient levels of CO range from 0.5 to 2 ppm (Samet et al., 2000). Acute CO poisoning, which occurs at much higher CO levels, has historically been associated with the development of cardiac arrhythmias, including conduction disorders, atrial and ventricular fibrillation, and atrial and ventricular premature beats (Marius-Nunez, 1990).

Using a rat model of myocardial infarction, we previously showed that exposure to CO was associated with a 60.4% *reduction* (95% confidence interval [CI]: 80.7, 18.8; $p = .012$) in the frequency of ventricular arrhythmias, while exposure to concentrated ambient fine particles (CAPS) was associated with a 64.2% *increase* (95% CI: 17.7, 227.6%; $p = .16$) in the frequency of ventricular arrhythmias (Wellenius et al., 2004). The purpose of the current analysis was to assess whether exposure to CAPS and/or CO alters the risk of supraventricular arrhythmias in this animal model.

METHODS

Animals

Adult, male Sprague-Dawley rats weighing $\sim$ 250 g (Charles River Laboratories, Inc., Wilmington, MA) were maintained and studied in accordance with the National 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

Left-ventricular MI was induced by thermocoagulation as previously described (Wellenius et al., 2002). Briefly, under inhalation anesthesia, a left thoracotomy was performed via the third or fourth intercostal space to gain access to the left ventricular wall of the heart. Myocardial infarction was induced by briefly and repeatedly applying the tip (0.5 inch fine electrode) of a portable thermocautery unit (2200°C, Aaron Medical Industries, Inc., St. Petersburg, FL) to one or more visible branches of the left coronary artery. Visible discoloration of the affected region indicated that blood flow had been successfully interrupted. Each animal was allowed to recover for a minimum of 12 h.

Experimental Design

To investigate the cardiac effects of air pollution, 85 rats were randomized to one of four groups: (1) filtered air ($n = 17$), (2) CAPS only ($n = 23$), (3) CO only ($n = 21$), or (4) both CAPS and CO (CAPS + CO; $n = 24$). Data from one rat in the filtered air group and two rats in the CO-only group were lost due to technical problems. The CO target dose was 35 ppm, equal to the current 1-h U.S. National Ambient Air Quality Standard. All exposures were 1 h in duration (exposure period), and were immediately preceded and followed by 1 h of exposure to filtered air (preexposure and postexposure periods, respectively).

Exposure Technology and Characterization

For all experiments, animals were placed in one of four sealed Plexiglas chambers for exposure, as previously described (Wellenius et al., 2004). Briefly, within each exposure chamber, rats were sedated (diazepam, ip, 12 mg/kg) and placed in individual holders facing the air inlet. The flow rate for each chamber was maintained at 15 L/min (LPM). CO exposures were generated by the addition of a small constant flow (approximately 230 cm^3/min) of high concentration CO from a certified cylinder (2510 ppm, Matheson Tri-Gas, Inc., Montgomeryville, PA) upstream of the two CO exposure chambers (CO only and CO + CAPS chambers). CO in both chambers was measured continuously using two Langan monitors adapted for active sampling (Chang et al., 2001).

Ambient fine particles were concentrated using the Harvard ambient particle concentrator (HAPC), the characteristics of which have been described in detail previously (Godleski et al., 2000; Lawrence et al., 2004; Savage et al., 2003). Briefly, the HAPC concentrates ambient fine particulate matter with an aerodynamic diameter $\leq 2.5\,\mu$m ($PM_{2.5}$) to $\sim 30\times$ ambient levels without altering its size distribution or chemical composition. Particles with diameters $>2.5\,\mu$m are removed upstream of the HAPC, while ultrafine particles ($<0.1\,\mu$m) and ambient gases are neither enriched nor excluded. CAPS mass concentration was determined gravimetrically from 1-h integrated samples and particle number concentration was measured continuously (5-min averages) using a condensation particle counter (CPC model 3022A; TSI, Inc., Shoreview, MN).

Electrocardiographic Data Acquisition and Analysis

The day of an experiment, electrodes for obtaining electrocardiograms (ECG) were implanted subcutaneously in a standard Lead II configuration (right arm, left leg, and right leg) under light inhalation anesthesia, as previously described (Wellenius et al., 2002). ECG signals were bandpass filtered, amplified,

digitized (500 Hz/animal), and stored using a customized PC-based data acquisition system (Mathworks, Inc., Natick, MA) with a 12-bit analog-to-digital converter (National Instruments Corp., Austin, TX). 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 measures of heart rate were consistent from minute to minute.

Offline, ECG signals were viewed and analyzed using customized software scripts in Matlab (Mathworks, Inc.). Arrhythmia grade and frequency were manually determined by an investigator blinded to the exposure status of each rat. The number of each type of arrhythmia observed in the hour before exposure (baseline value), during the exposure hour (exposure value), and in the hour following exposure (postexposure value) was recorded for each animal. ECG data were unusable for 10 of 246 (4.1%) possible observation periods. Results from the analysis of supraventricular ectopic beats (SVEB; including premature atrial complexes and premature junctional complexes) are presented here; results from the analysis of heart rate and ventricular arrhythmias have been previously published (Wellenius et al., 2004).

Statistical Analysis

First, we tested the hypothesis that exposure to CAPS and CO increases the risk of observing one or more SVEB (Hypothesis 1). We used repeated-measures logistic regression (Diggle et al., 2002) to model the odds of a given rat having one or more SVEB during a given time period. This model, fitted using generalized estimating equations (GEE), included indicator variables for time (exposure and postexposure periods), group (CAPS, CO, and CAPS + CO), and two-way interactions between these variables. An exchangeable covariance structure was assumed and inferences were based on empirical (robust) standard errors. To explore the dose-response relationship we also considered models where CAPS mass concentration or CAPS number concentrations were treated as linear continuous variables.

Second, we tested the hypothesis that among rats with one or more SVEB during the pre-exposure period, exposure to CAPS or CO increases SVEB frequency either during or following exposure (Hypothesis 2). We used repeated-measures Poisson regression fit by GEE to model the SVEB frequency in each period. This model included indicator variables for time and group, as well as 2-way interactions between these variables. We allowed for Poisson overdispersion in the data, assumed an exchangeable covariance structure, and based inferences on empirical (robust) standard errors.

The above approach is analogous to methods developed for zero-inflated count data (Agresti & Min, 2005) which are appropriate when there are many more subjects with no arrhythmias than would be expected under a Poisson model (Lambert, 1992). Statistical analyses were performed using PROC GENMOD in SAS version 9.1 (SAS Institute, Cary, NC). Statistical significance for all models was based on a two-sided $\alpha = 0.05$.

RESULTS

CAPS mass concentration over the 13 exposure days ranged from 78.0 to 2202.5 $\mu g/m^3$ (mean: 645.7; standard deviation (SD): 760.3). Particle number concentration ranged from 13,900 to 93,500 particles/cm^3 (mean: 38,500; SD: 23,300). The mean CO concentrations in the CO and CAPS+CO groups were 37.9 and 38.0 ppm, respectively.

Hypothesis 1: Exposure to CAPS and CO Increases the Risk of Observing One or More SVEB

In a first analysis we used repeated-measures logistic regression to determine whether exposure to CAPS or CO increased the probability of observing any SVEB. Figure 1A shows the mean probability of observing any SVEB during each time period for each group. Differences among the groups during the preexposure period were not statistically significant.

Among rats in the filtered air group, the probability of observing any SVEB increased over time, but this effect was not statistically significant. Among rats in the CAPS group, the probability of observing any SVEB decreased from baseline to the exposure and postexposure periods (Figure 1A). Compared to the filtered air group, this difference was statistically significant during the exposure period ($p = .048$), but not during the postexposure period ($p = .10$). The change in probability of observing any SVEB was not significantly different in the CO or CAPS + CO groups during either the exposure or postexposure periods, as compared to the filtered air group. We found no association between the probability of observing any SVEB and either CAPS mass concentration or CAPS number concentration in any time period.

Hypothesis 2: Among Rats With SVEB at Baseline, Exposure to CAPS or CO Increases the Number of SVEB Observed Either During or Following Exposure

In the subset of rats with one or more SVEB at baseline ($n = 30$), we used repeated-measures Poisson regression to determine whether exposure to CAPS or CO altered SVEB frequency. Figure 1B shows the mean number of SVEB/hour during each time period for each group. During the preexposure period, SVEB frequency was significantly greater in the CO group as compared to the filtered air group ($p = .03$). No other baseline differences reached statistical significance.

Among rats in the filtered air group, the SVEB frequency increased over time. This difference was statistically significant during both the exposure ($p = .003$) and postexposure ($p = .03$) periods. Compared to the filtered air group, the change from the preexposure to exposure period in SVEB frequency was significantly lower in the CAPS ($p = .04$) and CO ($p = .007$) groups and marginally lower in the CAPS + CO group ($p = .06$). The change in SVEB frequency from

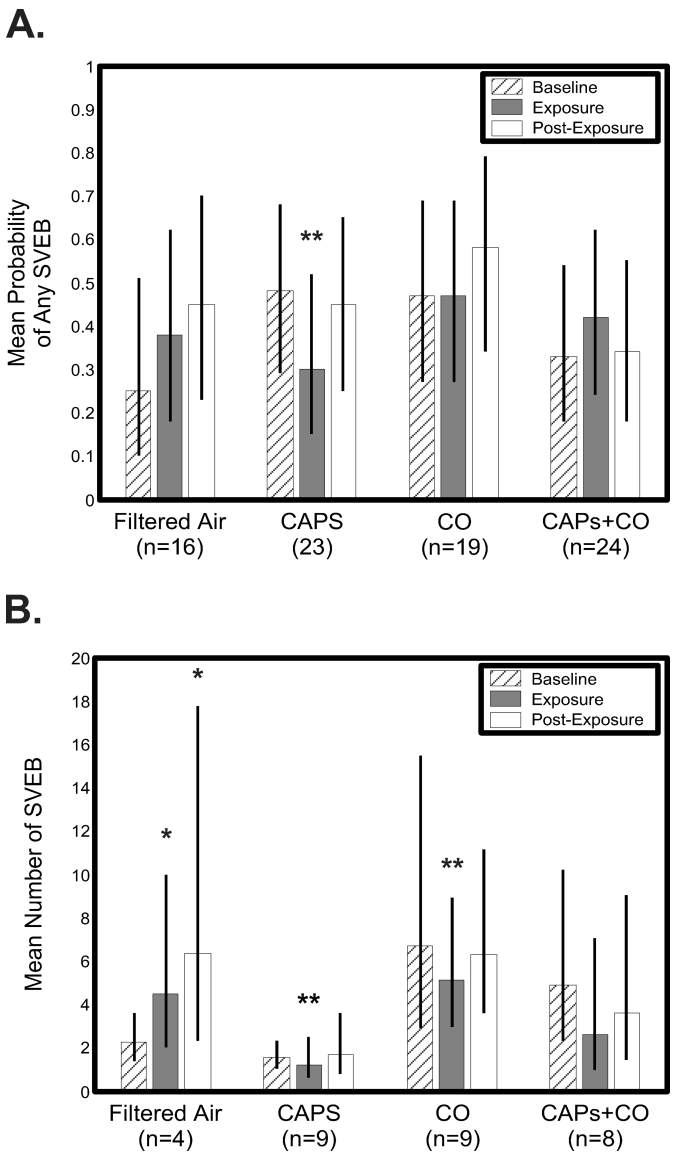

FIG. 1. (A) Predicted mean probability (95% confidence intervals) of observing one or more SVEB by time period and treatment group. (B) Predicted mean number of SVEB per hour by time period and treatment group. Asterisk indicates statistically significant ($p < .05$) change from baseline; double asterisk, statistically significant ($p < .05$) change from baseline compared to change from baseline in filtered air group.

the preexposure to the postexposure period was not significantly different in any group as compared to the filtered air group. We found no association between SVEB frequency and CAPS mass concentration in any time period. However, we found that a 1000 particles/cm^3 increase in CAPS number concentration was associated with a 3.3% decrease in SVEB frequency during the exposure period (95% confidence interval: -5.3, -1.2%; $p = .0024$).

DISCUSSION

Watkinson et al. (1998) first noted the development of premature atrial beats in rats following intratracheal instillation of residual oil fly ash. To our knowledge, only one previous toxicological study has evaluated the effects of ambient particulate matter on the risk of SVEB (Nadziejko et al., 2004). In that study, the authors exposed aged Fischer 344 rats to filtered air, CAPS, ultrafine carbon particles, or sulfur dioxide for 4 h and found no effect of any exposure on the frequency of premature supraventricular beats. Similarly, in the current study, we found no evidence in support of the hypothesis that exposure to either CAPS or CO increases the risk of SVEB. On the contrary, we found that CAPS exposure was associated with a lower risk of SVEB and that both CAPS and CO exposure were associated with a lower rate of SVEB in the subset of animals with supraventricular arrhythmias at baseline.

Epidemiological studies suggest that short-term elevations in ambient particle levels may increase the risk of supraventricular arrhythmias, but the results have been inconsistent. Riediker et al. (2004) followed 10 healthy North Carolina Highway Patrol troopers and found a positive association between $PM_{2.5}$ and the number of SVEB. Brauer et al. (2001) followed 16 elderly patients with chronic obstructive pulmonary disease (COPD) and reported a similar association. On the other hand, Rich et al. (2006) followed patients with implanted cardioverter–defibrillators (ICDs) and found no significant association between $PM_{2.5}$ and the risk of paroxysmal atrial fibrillation. Controlled exposure studies in humans have also yielded mixed results. Two studies in healthy volunteers did not find a statistically significant association between CAPS and SVEB (Devlin et al., 2003; Gong et al., 2003). However, a third study found a statistically significant positive association between CAPS and SVEB in healthy volunteers, but a statistically significant negative association in subjects with COPD (Gong et al., 2004).

Short-term inhalation exposure to low levels of CO corresponding to 2–4% carboxyhemoglobin levels (COHb) have been found to exacerbate myocardial ischemia in patients with documented coronary artery disease (Allred et al., 1989, 1991; Kleinman et al., 1989). Although still controversial, several studies suggest that there is little or no effect of CO on the incidence of ventricular arrhythmias (Dahms et al., 1993; Kizakevich et al., 2000). Furthermore, even exposure to CO resulting in COHb values as high as 20% does not appear to affect ventricular electrical properties in dogs (Foster, 1981; Verrier et al., 1990). We are not aware of any reports on the effect of CO on atrial electrical properties. One previous study in 16 healthy men noted no apparent difference in the frequency of premature atrial beats with COHb concentrations up to 19%, but very few premature beats were observed (Kizakevich et al., 2000). The results from the current study suggest that 1 h of exposure to 35 ppm CO does not increase the risk or frequency of supraventricular arrhythmias in rats. Although we did not measure COHb in the

current study, based on existing literature we estimate that rats in the CO and CAPS + CO groups may have achieved COHb as high as 5% (Brunssen et al., 2003).

SVEB frequency may be strongly influenced by changes in heart rate. In the FA group, SVEB frequency increased from the preexposure to postexposure periods and heart rate increased over time in a parallel fashion (data not shown). However, we have previously shown in these same animals that the pattern of change in heart rate over time is not significantly affected by exposure to either CAPS or CO (Wellenius et al., 2004). Therefore, it is unlikely that the results of the current study are due to exposure-related changes in heart rate.

This study has several important limitations. First, less than half of the rats in our study exhibited any SVEB. Aside from posing a methodological challenge in the analysis, this also impacted on statistical power and hence the precision of our estimates. Second, supraventricular arrhythmias are a heterogeneous group that includes atrial premature beats, junctional premature beats, supraventricular tachycardias, atrial fibrillation, and atrial flutter, only some of which were observed and quantified in the current study. Moreover, isolated supraventricular premature beats are prevalent even in the absence of cardiovascular disease and are not generally clinically important. Third, the exposure and postexposure periods were each limited to 1 h. Therefore, it is unknown whether longer exposures would elicit a different physiologic response. Moreover, it is possible that physiologic responses to CAPS and CO lagged exposure by more than 1 h. Fourth, since only mature, male, Sprague-Dawley rats were studied, it is unknown if the effects of CAPS and CO vary by gender, age, or species.

Rats were pharmacologically sedated during all experiments. This approach allowed us to carry out these experiments under conditions of minimal stress for the animals, thereby minimizing stress-induced arrhythmias unrelated to the exposures of interest. Diazepam, a benzodiazepine, was chosen as the sedative because it provides adequate sedation with only minor cardiovascular effects (Rall, 1990). Although diazepam may be vagolytic in humans and large animals, the expected effect is limited at the doses employed in this study (reviewed by Wellenius et al., 2002).

In summary, we found no evidence to support the hypothesis that short-term exposure to ambient air particles or CO increases the risk or frequency of supraventricular arrhythmias. Given the paucity of published studies and inconsistent results, further experiments in large-animal models or humans are needed to more firmly establish the effects of ambient air pollution on supraventricular arrhythmias.

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