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Cardiac Effects of Carbon Monoxide and Ambient Particles in a Rat Model of Myocardial Infarction

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Ambient air pollution is a complex mixture of particulate matter (PM) and gaseous pollutants such as carbon monoxide (CO). The effect of exposure to CO, alone or in combination with ambient PM, on arrhythmia incidence is unclear. To evaluate these effects, left-ventricular myocardial infarction was induced in Sprague-Dawley rats by thermocoagulation. Diazepam-sedated rats were exposed (1 h) to either filtered air ($n = 40$), CO (35 ppm, $n = 19$), concentrated air particles (CAPs, median concentration = 350.5 μg/m^3, $n = 53$), or CAPs and CO (CAPs median concentration = 318.2 μg/m^3, $n = 23$), 12–18 h after surgery. Each exposure was immediately preceded and followed by a 1 h exposure to filtered air (pre-exposure and post-exposure periods, respectively). The CO target dose of 35 ppm is related to the 1 h U.S. National Ambient Air Quality Standard. Surface electrocardiograms were recorded and heart rate and arrhythmia incidence were quantified. CO exposure reduced ventricular premature beat (VPB) frequency by 60.4% ($p = 0.012$) during the exposure period compared to controls. This effect was modified by both infarct type and the number of pre-exposure VPBs, and was not mediated through changes in heart rate. Overall, CAPs exposure increased VPB frequency during the exposure period, but this effect did not reach statistical significance. This effect was modified by the number of pre-exposure VPBs. Overall, neither CAPs nor CO had any effect on heart rate, but CAPs increased heart rate in specific subgroups. No significant interactions were observed between the effects of CO and CAPs. In this animal model, the responses to CO and CAPs are distinctly different.

Key Words: **arrhythmia; myocardial infarction; carbon monoxide; particulate matter; air pollution; cardiovascular; Sprague-Dawley rats.**

The association between short-term increases in particulate air pollution and increased cardiovascular morbidity and mortality is well documented. Specifically, short-term increases in levels of ambient particulate matter (PM) have been associated with triggering of cardiac arrhythmias (Peters *et al*., 2000), myocardial infarction (D'Ippoliti *et al*., 2003; Peters *et al*., 2001), decompensation of heart failure patients (Burnett *et al*., 1997; Schwartz and Morris, 1995), and the exacerbation of myocardial ischemia (Pekkanen *et al*., 2002; Wellenius *et al*., 2003).

However, PM exists in outdoor air as a complex mixture that includes gaseous pollutants such as sulfur oxides, ozone, and carbon monoxide, some of which are known to affect cardiovascular health. Ambient levels of gaseous pollutants are often highly correlated with ambient PM levels and, thus, may confound the associations between PM and specific health effects observed in epidemiologic studies. Indeed, this issue has generated much debate among epidemiologists (Chen *et al*., 1999; Lipfert and Wyzga, 1999; Sarnat *et al*., 2001; Schwartz, 2000; Schwartz and Coull, 2003). Additionally, gas-particle interactions in the induction of health effects have been observed among multiple gases typically present in ambient air. For example, increased lung injury is observed in rats exposed simultaneously to PM and ozone as compared to rats exposed to either pollutant alone (Bouthillier *et al*., 1998; Goldsmith *et al*., 2002; Jakab and Hemenway, 1994; Vincent *et al*., 1997). These observations are likely due to a combination of atmospheric gas-particle chemical reactions that increase particle toxicity (Madden *et al*., 2000), lung gas-particle interactions that increase delivery of oxidants to lower regions of the lung (Laskin *et al*., 2003), and gas-lung interactions that alter regional particle deposition patterns (reviewed by Gerrity, 1995).

Carbon monoxide (CO) is a ubiquitous gaseous pollutant produced by the incomplete combustion of carbonaceous fuels and substances. Typical sources of CO include vehicle exhaust, industrial processes, home heating systems, and cigarette smoke. In the U.S., daily mean ambient levels of CO range from 0.5 to 2 ppm (Samet *et al*., 2000). Numerous studies have found an association between short-term increases in ambient CO levels and increased risk of cardiovascular morbidity (Burnett *et al*., 1997; Morris *et al*., 1995; Schwartz, 1997; Schwartz and Morris, 1995; Yang *et al*., 1998) and mortality

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(Hoek *et al*., 2001; Mar *et al*., 2000). An interaction between CO and PM in eliciting these effects has not been examined.

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). At least one experimental study supports the notion that acute exposure to CO may trigger ventricular premature beats in humans (Sheps *et al*., 1990), but a number of other studies have found no effect.

We have previously shown that inhalation exposure to combustion-derived PM increases the incidence of ventricular arrhythmias in rats with acute myocardial infarction (MI; Wellenius *et al*., 2002). However, it is unknown whether exposure to ambient PM would elicit the same response. Additionally, ambient levels of CO may confound or modify the PM-arrhythmia association observed in epidemiologic studies, but this hypothesis has not been evaluated in a controlled setting. Accordingly, the goal of this study was to examine the cardiac effects of exposure to ambient PM and CO, individually and together, in a rat model of acute MI. The specific hypotheses to be tested were: (1) CAPs exposure will increase arrhythmia incidence in a rat model of MI; (2) exposure to CO will increase arrhythmia incidence in this model; and (3) exposure to a combination of CAPs and CO will synergistically increase arrhythmia incidence.

MATERIALS AND METHODS

Animals. Adult, male Sprague-Dawley rats weighing ~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, animals were placed under inhalation anesthesia and mechanically ventilated via a 2 mm-diameter tracheal tube (Kent Scientific Corp., Torrington, CT). 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″ 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. The lungs were hyperinflated and the chest closed. Each animal was 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) filtered air, (2) concentrated ambient air particles (CAPs) only, (3) CO only, or (4) both CAPs and CO. In initial experiments, rats were randomized to receive either CAPs only or filtered air. Starting on 8 June 2001, rats were randomized to receive one of the four treatments above (Table 1). 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 (pre-exposure and post-exposure periods, respectively).

TABLE 1
Number of Animals Studied, by Exposure Group and Experiment Date

Date	Filtered air	CO only	CAPs only	CO + CAPs	Total
07/06/2000	4	0	3	0	7
10/12/2000	1	0	2	0	3
10/17/2000	1	0	2	0	3
10/18/2000	2	0	2	0	4
10/19/2000	2	0	2	0	4
10/27/2000	1	0	0	0	1
01/18/2001	2	0	2	0	4
01/19/2001	2	0	4	0	6
01/29/2001	3	0	3	0	6
01/30/2001	2	0	4	0	6
01/31/2001	2	0	4	0	6
02/02/2001	2	0	3	0	5
02/05/2001	2	0	4	0	6
06/08/2001	1	2	2	2	7
06/15/2001	1	2	2	2	7
06/22/2001	0	1	2	2	5
07/06/2001	1	0	1	1	3
08/24/2001	2	2	2	0	6
09/21/2001	2	1	2	2	7
10/12/2001	0	1	0	0	1
10/26/2001	1	1	1	1	4
11/20/2001	1	1	2	2	6
11/27/2001	2	2	2	2	8
11/30/2001	2	2	1	2	7
12/12/2002	1	2	2	1	6
12/20/2002	1	2	1	2	6
01/03/2003	0	2	1	3	6
01/22/2003	2	0	2	2	6
Total	43	21	58	24	146

Note. Abbreviations: CAPs, concentrated air particles; CO, carbon monoxide.

Exposure technology and characterization. For all experiments, animals were placed in one of four sealed plexiglass chambers for exposure. Within each exposure chamber, rats were sedated (diazepam, ip, 12 mg/kg) and comfortably placed in individual holders such that they were facing the air inlet and at approximately the same height as the inlet. The flow rate for each chamber was maintained at 15 LPM regardless of the exposure. Chamber flows were measured downstream using inline calibrated rotameters.

CO exposures were generated by 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). Flows of concentrated CO were regulated using valves and calibrated inline rotameters, and were checked and recorded routinely every 10–15 min during exposure. Levels of CO in both chambers were measured continuously during pre-exposure, exposure, and post-exposure periods using two Langan monitors adapted for active sampling (Chang *et al*., 2001).

Ambient particles were concentrated using the Harvard Ambient Particle Concentrator (HAPC). The characteristics of the HAPC and exposure chamber have been described in detail previously (Godleski *et al*., 2000; Sioutas *et al*., 1995). Briefly, the HAPC concentrates ambient fine particulate matter with an aerodynamic diameter ≤ 2.5 mm ($PM_{2.5}$) to ~30× ambient levels without altering its size distribution or chemical composition. Particles with diameters >2.5 mm are removed upstream of the HAPC, while ultrafine particles (<0.1 mm) and ambient gases are neither enriched nor excluded.

Integrated and continuous measures were used for CAPs exposure characterization, as previously described (Godleski *et al*., 2000). Briefly, CAPs particle characterization included analysis of 1-h integrated samples: gravimetric determinations for particle mass, X-ray fluorescence analysis for elemental composition (Dzubay and Stevens, 1975), and thermal and optical reflectance analysis for elemental (EC) and organic carbon (OC) (Chow *et al*., 1993). Continuous measurements (5-min averages) of particle number concentration were obtained using a condensation particle counter (CPC Model 3022A; TSI, Inc., Shoreview, MN) and continuous measurements of black carbon (BC) mass concentration were obtained using an aethalometer (Hansen *et al*., 1984). The average value for each day was calculated as the arithmetic mean of all 5-min averages during the exposure period excluding the first and last 10 min of the exposure period.

Electrocardiographic data acquisition and analysis. Pharmacological sedation with diazepam (ip, 12 mg/kg) was chosen over physical restraint or implantation of radiotelemetry devices for obtaining high quality ECG recordings in a large number of animals. 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). Physical restraint of conscious rats significantly increases plasma catecholamine levels (Kvetnansky *et al*., 1978). The use of diazepam 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. These conditions could have also been achieved with implantable radiotelemetry devices. However, given the large number of animals to be studied, the relatively high mortality associated with the MI surgery, and that each rat was to be exposed only once, we chose not to use implanted telemetry devices.

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 Halothane or Isofluorane anaesthesia, as previously described (Wellenius *et al*., 2002). Each electrode was made of a brass clip soldered to a lead wire. ECG signals were band-pass 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. Representative ECG recordings are shown in Figure 1. The most commonly observed cardiac arrhythmia was isolated ventricular premature beats (VPBs; Fig. 1A). Couplets and triplets of VPBs, atrial premature beats (Fig. 1B), and atrioventricular heart block (Fig. 1C) were also observed. The number of each type of arrhythmia observed in the hour before exposure (pre-exposure value), during the exposure hour (exposure value), and in the hour following exposure (post-exposure value) was recorded for each animal. Only the results from the analysis of heart rate and ventricular arrhythmias are presented here; results from the analysis of supraventricular arrhythmias will be presented separately.

Only one of the 182 rats studied died during the course of the exposure protocol. Data from 74.6% (135 of 181) of the surviving rats was available for analysis. The remaining 46 animals were excluded because of poor ECG signal quality ($n = 37$) or very high number of VPBs during the pre-exposure period ($n = 10$). To exclude a small number of animals with an uncharacteristically high number of VPBs, we excluded from further analysis rats with 50 or more VPBs during any time period.

To assess heart rate, normal sinus beats were automatically labeled by customized software and subsequently verified by an investigator. We assessed heart rate, calculated as the reciprocal of the 5-min mean normal beat-to-beat interval, at 0, 55, and 115 min after the start of the exposure period. If the ECG at any time point could not be automatically labeled by the software or was otherwise of insufficient quality, no value was reported for that time point for that rat. Heart rate analysis was carried out on the 135 rats included in the arrhythmia analysis. Of the 405 possible data points (135 rats × 3 time points), 29 points (7.2%) were excluded because of failure to meet the above quality criteria.

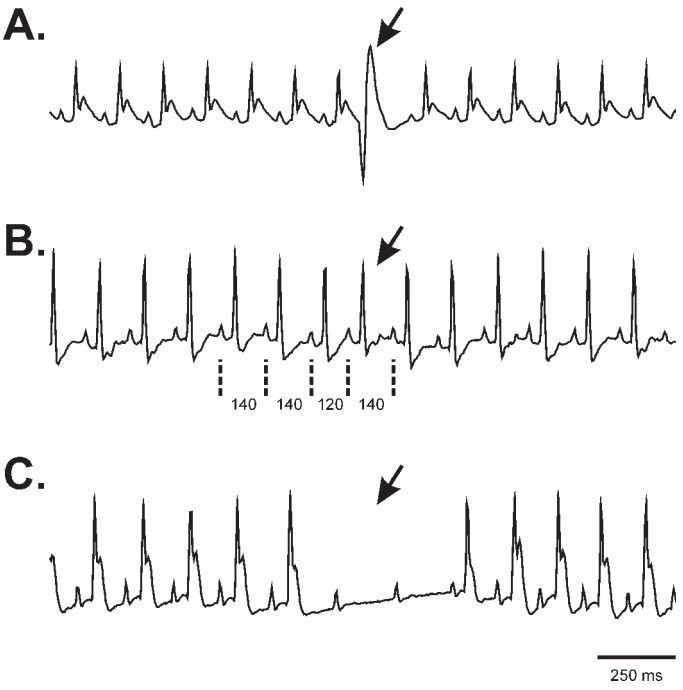

FIG. 1. Representative ECG tracings illustrating various cardiac arrhythmias. (A) Isolated ventricular premature beats (VPBs, arrow) are premature beats characterized by a large amplitude, wide, and often inverted QRS complex. (B) Atrial premature beats (arrow) usually have normal QRS complex morphology and are clearly associated with a premature P-wave. In this example, the P-P interval in the surrounding normal beats (denoted by the dashed lines) was ~140 ms, but only 120 ms prior to the atrial premature beat. (C) The sudden appearance of one or more nonconducted normal sinus P wave without progressive prolongation of PR intervals is indicative of second degree (Mobitz type II) atrioventricular heart block. Tracings A and C exhibit pronounced elevation of the ST-segment, as would be expected in animals with severe infarcts.

Histopathology. Histopathology was carried out in all animals 14–21 days after infarction specifically to confirm that the surgical procedure had successfully induced an MI. Additionally, we wished to distinguish transmural from nontransmural infarcts. At autopsy, the operative site and thoracic cavity was observed for evidence of inflammation or infection. The heart was removed and placed in 10% buffered formalin (Fisher Scientific International, Inc., Pittsburgh, PA). After fixation, the heart was dissected with serial 2–3 mm cross sections from the apex to base. These cross sections of the ventricles were processed routinely for paraffin histology, sectioned at 4 μm thickness, stained with hematoxylin and eosin, and examined by light microscopy to assess the extent of myocardial injury resulting from the infarction. By two weeks post-MI, necrotic fibers have been phagocytosed and replaced by fibrosis and vascular granulation tissue. The presence of infarcted tissue as well as the extent of injury resulting from the infarct can be readily appreciated by light microscopy. Histopathology was assessed in 134 (99.3%) of the 135 rats included in the arrhythmia analysis.

Statistical analysis. We chose *a priori* to assess the effect of exposure on ventricular arrhythmia frequency by comparing the number of VPBs during

either the exposure or post-exposure periods with the number of VPBs during the pre-exposure period in each animal. We modeled VPB frequency with a repeated-measures Poisson regression using Generalized Estimating Equations (Diggle *et al*., 2002). Inferences were based on empirical (robust) standard errors, which adjust for Poisson overdispersion in the data. Models included indicator variables for time (exposure and post-exposure periods), CAPs exposure, CO exposure, and 2- and 3-way interactions between these variables. Stratified analyses were carried out to examine effect modification by infarct type (transmural vs. subepicardial) and by the number of VPBs during the pre-exposure period (low [≤ 4 VPBs] vs. high [>4 VPBs]). The cutoff for classifying pre-exposure VPBs is related to the median non-zero VPB frequency observed during the pre-exposure period across all exposure groups.

The number of rats studied was based upon power calculations from our previous study (Wellenius *et al*., 2002) which showed that 1 h exposure to combustion-derived particles increased the number of ventricular premature beats more than six-fold compared to control animals. We estimated needing five rats in each of the CAPs groups to detect a significant effect of this size with $\alpha = 0.05$ and 90% power. We also performed a post-hoc power analysis to estimate the minimum detectable increase in VPB frequency associated with CAPs exposure given the number of animals included in this study. These calculations were performed in S-PLUS (Insightful Corp., Seattle, WA) using a simulation-based approach which accounted for both correlation among observations taken on the same rat as well as general Poisson overdispersion. These extra sources of variability were introduced into the data-generating Poisson model by adding both rat-specific and observation-specific Gaussian random effects with means 0 and SDs of 0.2 (within-rat correlation) and 0.7 (overdispersion). These values were selected to yield overdispersion in the simulated data matching that present in the observed results. All power calculations were based on two-sided tests at the $\alpha = 0.05$ significance level.

We chose *a priori* to assess the effect of exposure on heart rate by comparing the heart rate at the end of the exposure period or at the end of the post-exposure period with that at the end of the pre-exposure period in each animal. We modeled heart rate with a repeated-measures linear regression model that included indicator variables for time (exposure and post-exposure periods), CAPs exposure, CO exposure, and 2- and 3-way interactions between these variables. Statistical analyses were performed using PROC GENMOD in SAS version 8 (SAS Institute, Cary, NC). Statistical significance for all models was based on $\alpha = 0.05$.

RESULTS

Effect of CO on the Frequency of Ventricular Arrhythmias

Isolated VPBs were the most commonly observed cardiac arrhythmia. Of the 135 rats, 102 (75.6%) exhibited one or more VPBs. The distribution of VPB frequency during the pre-exposure, exposure, and post-exposure periods in each exposure group is shown in Figure 2. In a repeated-measures Poisson regression model treating CO and CAPs exposure as dichotomous variables, there was no significant interaction between CO and CAPs on VPB frequency during either the exposure or post-exposure periods. Therefore, a term representing the interaction of CO and CAPs was not included in subsequent models.

In a regression model assuming no interaction between CAPs and CO and treating CAPs as a dichotomous variable, CO exposure reduced VPB frequency by 60.4% (95% CI: −80.7, −18.8; $p = 0.012$) during the exposure period and by 19.5% (−64.3, 81.5; $p = 0.60$) during the post-exposure period, as compared to the filtered air group (Fig. 3). Note that the effect of CO was statistically significant only during the exposure period. The effect of CO on VPB frequency during the exposure and post-exposure periods was qualitatively similar even when more sensitive metrics of CAPs exposures such as CAPs mass concentration, number concentration, or the concentration of individual elements were used (data not shown).

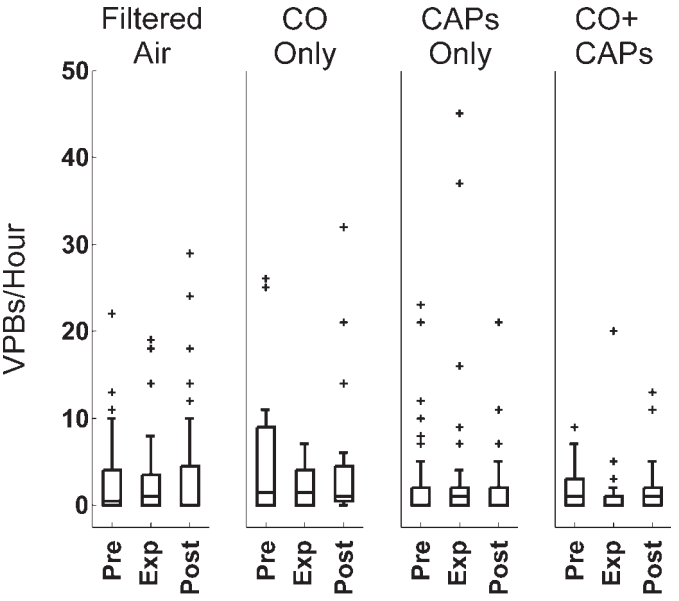

FIG. 2. Box and whisker plots summarizing the number of VPBs/h observed during the pre-exposure (Pre), exposure (Exp), and post-exposure (Post) periods in animals exposed to filtered air, carbon monoxide (CO) only, concentrated air particles (CAPs) only, or both CO and CAPs. The box has lines at the lower quartile, median, and upper quartile values. The whiskers are lines extending from each end of the box to show the extent of the rest of the data (up to 1.5 times the interquartile range). (+) indicate data points beyond the ends of the whiskers.

Histologic evaluation revealed that of the 134 animals included in the above analysis for which histopatholic data were available, 44.0% had a transmural infarct, 35.8% had a subepicardial infarct, and the remaining 20.1% had just a cautery lesion and no infarct (Fig. 4, Table 2). To evaluate whether the effects of CO on VPB frequency were modified by infarct type, we fit the regression model to the data stratified by infarct type (Fig. 3). In animals with a transmural infarct, CO exposure significantly reduced VPB frequency during the exposure period (−77.8% [95% CI: −88.5, −57.0]; $p < 0.001$), as compared to control animals. A reduction in VPB frequency was also observed during the post-exposure period (−56.8% [−83.7, 14.6]; $p = 0.091$), but this effect was not robust. In contrast, in animals with a subepicardial infarct, CO exposure had a small nonsignificant effect on VPB frequency during both time periods.

To explore if rats with a greater number of VPBs during the pre-exposure period were more susceptible to the effects of CO, we fit the regression model to the data stratified by the number

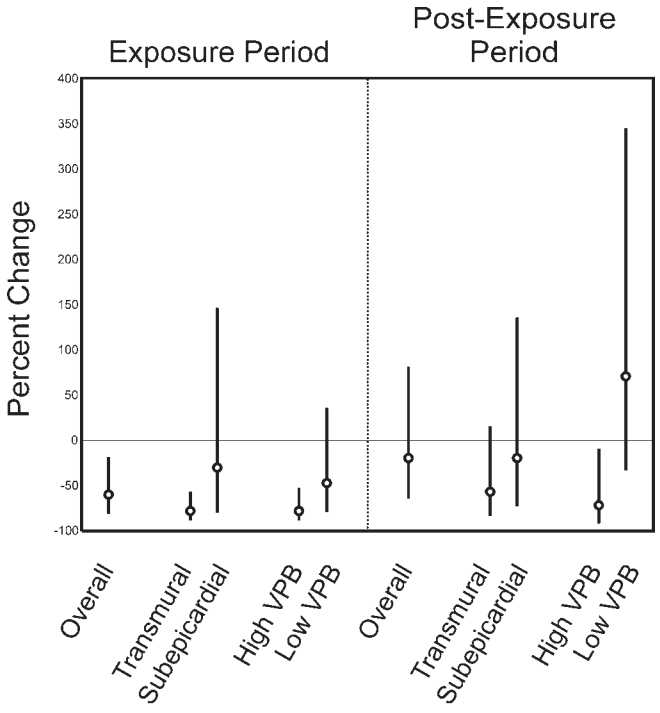

FIG. 3. Percent change (and 95% confidence interval) in the frequency of premature ventricular beats due to CO exposure, during the exposure (left) and post-exposure (right) periods. Overall results and results stratified by infarct type (transmural vs. subepicardial) and by the frequency of premature ventricular beats during the pre-exposure period (high vs. low) are shown. Note that 0% change denotes no effect.

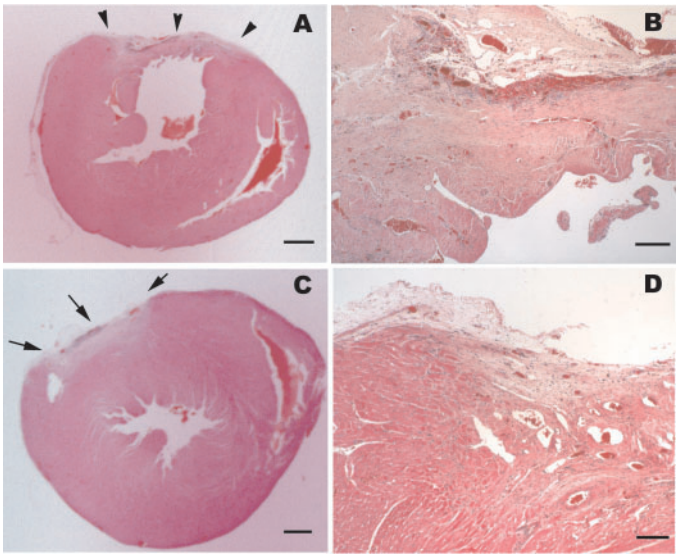

FIG. 4. H&E stained heart sections established the presence of MI in the model. (A) The typical size of a transmural infarction in this model at 14 days. In this very low magnification photomicrograph (Bar = 1000 μm), the anterior wall of the left ventricle has significant transmural tissue loss and replacement with fibrous tissue (area highlighted by arrow heads). (B) Higher magnification of the transmural infarct seen in A (Bar = 200 μm) showing the fibrosis through the entire thickness of the wall. (C) The typical size of a subepicardial infarction in this model at 14 days. In this very low magnification photomicrograph (Bar = 1000 μm), the anterior wall has a subepicardial area of fibrosis (highlighted by the arrows), but there is considerable myocardium in the subendocardial area uninvolved by the infarction. (D) Higher magnification of the subepicardial infarct seen in C (Bar = 200 μm) showing the localized fibrosis.

of VPBs during the pre-exposure period (low: $\leqslant 4$ VPBs vs. high: >4 VPBs; Fig. 3). In animals with a high number of pre-exposure VPBs, CO exposure significantly reduced VPB frequency during both the exposure (−77.5% [−89.3, −52.9]; $p < 0.001$) and post-exposure (−72.25% [−91.5, −9.3]; $p = 0.034$) periods, as compared to control animals. In contrast, among animals with few VPBs during the pre-exposure period, CO exposure decreased VPB frequency only slightly during the exposure period and increased VPB frequency during the post-exposure period. Note however, that neither of these effects was statistically significant.

Effect of CAPs on the Frequency of Ventricular Arrhythmias

In a regression model treating CAPs as a dichotomous variable and assuming no interaction between CAPs and CO (Fig. 5), CAPs exposure increased VPB frequency during the exposure period (64.2%, [−17.7, 227.6%]; $p = 0.16$) and reduced VPB frequency during the post-exposure period (−35.0% [−65.8, 23.7]; $p = 0.19$). Note that neither of these changes was statistically significant. Additionally controlling for either pre-exposure heart rate or change in heart rate did not materially alter the results.

Daily CAPs mass concentration varied widely over the 28 exposure days, ranging from 60.30 to 2202.50 μg/m^3. Particle composition also exhibited substantial daily variability (Table 3). The CAPs mass concentration was similar among rats exposed to CAPs only (median: 350.5 μg/m^3) and those exposed to CAPs and CO (median: 318.2 μg/m^3). Neither CAPs mass concentration, CAPs number concentration, nor the mass concentration of any single element was a significant predictor of VPB frequency during either the exposure or post-exposure periods (data not shown). We performed a post-hoc power analysis to demonstrate that there was sufficient power in the study to detect a meaningful increase in the VPB frequency resulting from CAPs exposure. The estimated minimum

TABLE 2
Number of Animals Included in the Analysis, by Exposure Group and Infarct Type

Infarct type	Filtered air	CO only	CAPs only	CO + CAPs	Total
Transmural	19	11	17	12	59
Subepicardial	13	7	18	10	48
Other	8	1	17	1	27
Total	40	19	52	23	134

Note. Abbreviations as in Table 1.

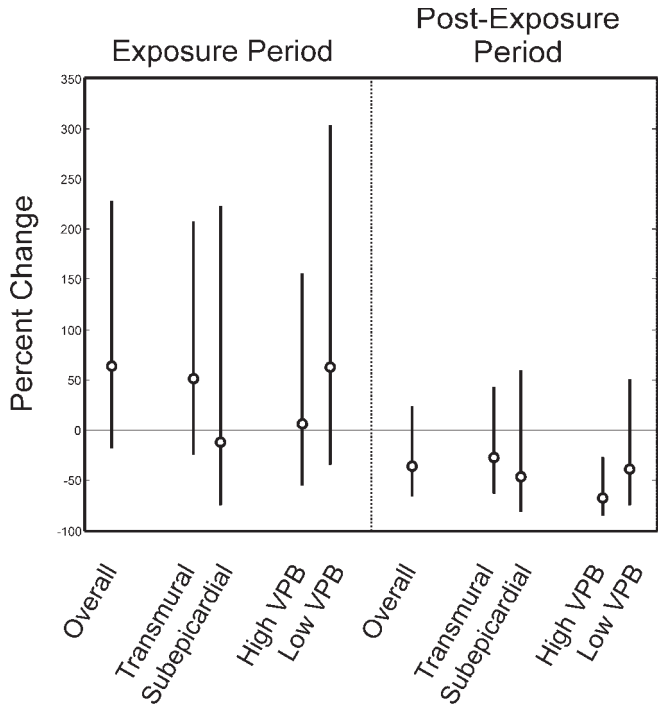

FIG. 5. Percent change (and 95% confidence interval) in the frequency of premature ventricular beats due to CAPs exposure, during the exposure (left) and post-exposure (right) periods. Overall results and results stratified by infarct type (transmural vs. subepicardial) and by the frequency of premature ventricular beats during the pre-exposure period (high vs. low) are shown. Note that 0% change denotes no effect.

TABLE 3
Summary of CAPs Characteristics for 26 Exposure Days

Exposure parameter[a]	N	Mean	SD	Median	Minimum	Maximum
Mass concentration	25	523.11	581.60	348.40	60.30	2202.50
Number concentration	17	35.45	21.60	30.98	7.78	93.48
BC	24	12.03	12.33	6.94	1.38	51.49
EC	22	28.87	27.71	17.97	BD	101.94
OC	22	100.48	75.50	74.55	17.97	319.17
S	19	60.42	86.88	28.32	5.32	299.51
Al	19	3.19	3.11	2.75	BD	14.08
Si	19	11.14	8.48	9.23	2.99	39.77
Na	19	12.06	15.51	7.96	BD	62.21
Cl	19	13.22	19.55	1.53	BD	72.32
V	19	0.18	0.15	0.11	0.03	0.47
Ni	19	0.15	0.19	0.08	BD	0.63
K	19	3.08	1.73	2.80	1.10	7.48
Ca	19	6.22	4.40	5.28	2.03	17.03
Ti	19	0.53	0.33	0.51	0.19	1.37
Cr	19	0.03	0.03	0.03	BD	0.10
Mn	19	0.34	0.21	0.35	0.07	0.64
Fe	19	10.37	5.39	10.12	3.66	24.18
Cu	19	0.20	0.09	0.16	0.09	0.41
Zn	19	1.52	1.28	1.00	0.18	4.48
As	19	0.03	0.03	0.03	BD	0.09
Se	19	0.04	0.05	0.02	BD	0.22
Br	19	0.12	0.09	0.09	0.02	0.32
Cd	19	0.02	0.03	0.01	BD	0.11
Ba	19	0.89	0.35	0.87	0.36	1.73
Pb	19	0.19	0.15	0.15	0.01	0.65

Note. Abbreviations: N, number of days for which data was available; SD, standard deviation; BC, black carbon; EC, elemental carbon; OC, organic carbon; BD, minimum value was less than limit of detection; other abbreviations as in Table 1.

[a]All measures are reported as $\mu g/m^3$ except particle number concentration, which is reported as 1000 particles/cm^3.

detectable increase in VPB frequency with $\alpha = 0.05$ and 80% power was a 110% increase.

We carried out a stratified analysis to evaluate whether the effects of CAPs on VPB frequency were modified by infarct type (Fig. 5). In rats with transmural infarcts, CAPs exposure increased VPB frequency by an average of 52.3% (95% CI: −24.8, 208.4; $p = 0.24$) during the exposure period, but this effect was highly variable and not statistically significant. CAPs exposure also had no significant effect on VPB frequency during the post-exposure period regardless of infarct type.

We carried out a stratified analysis to evaluate whether rats with a greater number of VPBs during the pre-exposure period were more susceptible to the effects of CAPs (Fig. 5). Regardless of the number of pre-exposure VPBs, CAPs exposure had no significant effect on VPB frequency during the exposure period. However, during the post-exposure period, CAPs exposure significantly decreased VPB frequency (−67.1% [−85.2, −27.0]; $p < 0.001$) in rats with a high number of pre-exposure VPBs.

Effect of CO and CAPs on Heart Rate

In a repeated-measures linear regression model treating CAPs as a dichotomous variable, there was no significant interaction between CO and CAPs on heart rate during any time period. Therefore, a term representing the interaction of CO and CAPs was not included in subsequent models. In a regression model treating CAPs as a dichotomous variable and assuming no interaction between CAPs and CO, neither exposure to CO nor exposure to CAPs had a significant effect on heart rate. Moreover, neither CAPs mass concentration nor CAPs number concentration was a significant predictor of heart rate during either the exposure or post-exposure periods. However, a statistically significant increase in heart rate was associated with the mass concentration of sulfur (6.4 beats/min per 100 $\mu g/m^3$ [0.21, 12.5]; $p = 0.043$).

We carried out a stratified analysis to evaluate whether the effects of CO or CAPs on heart rate were modified by pre-exposure heart rate. CO had no effect on heart rate during either time period regardless of pre-exposure heart rate. In rats with a low pre-exposure heart rate, CAPs exposure increased heart rate by 15.7 beats/min (95% CI: 2.0, 29.4; $p = 0.025$) during the exposure period and by 12.1 beats/min (95% CI: −4.7, 28.8; $p = 0.16$) during the post-exposure period. The effect on heart rate during the exposure period was related to CAPs mass concentration

(14.1 beats/min per 100 μg/m^3 [1.2, 27.1]; $p = 0.032$), but not CAPs number concentration. CAPs had no effect on heart rate in those animals with a high pre-exposure heart rate (data not shown).

To parallel the stratified analysis of the VPB data above, we evaluated whether the effects of CO or CAPs on heart rate were modified by infarct type. Neither exposure to CO nor CAPs had a significant effect on heart rate regardless of infarct type. We also evaluated whether the effects of CO or CAPs on heart rate were modified by the number of VPBs during the pre-exposure period. Exposure to CO had no effect on heart rate during either time period regardless of the number of VPBs during the pre-exposure period. In rats with low pre-exposure VPBs, exposure to CAPs had no significant effect on heart rate during either time period. However, in rats with a high number of pre-exposure VPBs, exposure to CAPs significantly increased heart rate during both the exposure (20.5 beats/min [0.9, 40.1]; $p = 0.040$) and post-exposure (21.5 beats/min [1.6, 41.3]; $p = 0.034$) periods. This effect was related to CAPs mass concentration during the exposure (1.6 beats/min per 100 μg/m^3 [0.4, 2.8]; $p < 0.001$) and post-exposure (0.9 beats/min per 100 μg/m^3 [0.0, 1.9]; $p = 0.068$) periods. In contrast, no relationship was found with CAPs number concentration.

DISCUSSION

The purpose of this study was to assess the effects of exposure to CO and CAPs, separately and together, on arrhythmia frequency and heart rate in a rat model of myocardial infarction. The results of this study are qualitatively summarized in Tables 4 and 5. Several important findings emerge from this investigation. First, 1 h exposure to CO reduced the frequency of ventricular arrhythmias during the exposure hour, and to a lesser degree during the subsequent hour. This effect was modified by both infarct type and the frequency of VPBs during the pre-exposure period. Second, overall, 1 h exposure to CAPs tended to increase VPB frequency during the exposure period, but this effect was not statistically significant. This effect was modified by the frequency of VPBs during the pre-exposure period. Third, overall, neither CO nor CAPs had any effect on heart rate during either the exposure hour or in the subsequent hour. However, exposure to CAPs significantly increased heart rate during the exposure hour in animals with a low pre-exposure heart rate and in the post-exposure hour in animals with a high number of pre-exposure VPBs. Fourth, no significant interactions were observed between the effects of CO and CAPs on either arrhythmia frequency or heart rate.

TABLE 4
Summary of Results for the Effect of CO on VPB Frequency and Heart Rate

		Infarct type		Pre-exposure VPBs		Pre-exposure heart rate	
	Overall	Transmural	Subepicardial	High	Low	High	Low
Exposure period							
VPB	↓↓	↓↓	↓	↓↓	↓	na	na
Heart rate	—	—	—	—	—	—	—
Post-exposure period							
VPB	—	↓	—	↓	↑	na	na
Heart rate	—	—	—	—	—	—	—

Note. CO and CAPs were treated as binary variables and no interaction between the effects of CO and CAPs was assumed. Symbols: ↓, tended to decrease; ↓↓, significantly decreased; —, no change; ↑, tended to increase; ↑↑, significantly increased; na, not applicable.

TABLE 5
Summary of Results for the Effect of CAPs on VPB Frequency and Heart Rate

		Infarct type		Pre-exposure VPBs		Pre-exposure heart rate	
	Overall	Transmural	Subepicardial	High	Low	High	Low
Exposure period							
VPB	↑	↑	—	—	↑	na	na
HR	—	—	—	↑↑	—	—	↑↑
Post-exposure period							
VPB	↓	↓	↓	↓↓	↓	na	na
HR	—	—	—	↑↑	—	—	↑

Note. CO and CAPs were treated as binary variables and no interaction between the effects of CO and CAPs was assumed. Symbols as in Table 4.

Although short-term exposure to low levels of CO is known to exacerbate myocardial ischemia in patients with documented coronary artery disease (Allred *et al*., 1989, 1991; Kleinman *et al*., 1989), the effect on the incidence of ventricular arrhythmias remains controversial. In one series of studies, CO exposure increased the number of premature ventricular beats among exercising subjects with coronary artery disease and baseline arrhythmias (Sheps *et al*., 1990), but not in those without baseline arrhythmias (Hinderliter *et al*., 1989). However, other studies in humans (Dahms *et al*., 1993; Kizakevich *et al*., 2000) and conscious dogs (Vanoli *et al*., 1989) have failed to observe a change in the incidence of ventricular arrhythmias associated with CO exposure. Exposure to even high concentrations of CO did not appear to affect ventricular electrical properties such as ventricular conduction velocity (Foster, 1981) and ventricular effective refractory period (Verrier *et al*., 1990) in experimental dogs during occlusion-induced myocardial ischemia. The lack of change in these parameters suggests that exposure to low to moderate concentrations of CO is unlikely to be arrhythmogenic, despite the conflicting results cited above.

In the current study, contrary to our initial hypothesis, we found that CO exposure decreased the frequency of ventricular arrhythmias. Given that the occurrence of ventricular arrhythmias is sensitive to changes in heart rate, one possible explanation for this finding is that CO exposure induced changes in heart rate. This is unlikely, however, as we did not observe CO-related heart rate changes either overall or in any of the subgroups. The lack of heart rate changes following CO exposure is in agreement with those of previous studies (Hausberg and Somers, 1997; Kleinman *et al*., 1989; Tarkiainen *et al*., 2003; Verrier *et al*., 1990). An alternative explanation is that the very low levels of CO employed in this study acted as an endothelium-independent vasodilator (Lin and McGrath, 1988) and therefore alleviated ongoing myocardial ischemia. Indeed, low concentrations of inhaled CO have been shown to inhibit hypoxic pulmonary vasoconstriction in rats (Tamayo *et al*., 1997) and sheep (Nachov *et al*., 2001) and may decrease systemic arterial resistance (Hausberg and Somers, 1997). However, two observations in this model argue against this hypothesis. First, the rat has little or no collateral coronary blood flow (Maxwell *et al*., 1987), making it difficult for vasodilation to alleviate ongoing ischemia. Second, in the rat heart it is anticipated that 12 h after coronary artery occlusion, it is likely that very little viable myocardial tissue remains in the underperfused area (Hearse *et al*., 1988). Thus, the underperfused region is expected to have little or no viable tissue where ischemia could be alleviated by changes in collateral flow. Nonetheless, if the ectopic foci of the observed arrhythmias were located at the junction of viable and nonviable tissue, improving flow to the viable tissue at that site might have some benefit.

That we did not observe a significant CAPs-related increase in the frequency of ventricular arrhythmias was also unexpected for three reasons. First, epidemiological studies have linked ambient PM levels with the risk of hospitalization for arrhythmias (Burnett *et al*., 1999; Poloniecki *et al*., 1997) and the risk of arrhythmic events in patients with implantable cardioverter-defibrillators (ICD; Peters *et al*., 2000). Second, exposure to combustion-derived PM has been shown to increase total arrhythmia incidence in a rat model of pulmonary hypertension (Campen *et al*., 2000; Watkinson *et al*., 1998) and to increase the incidence of ventricular premature beats in the rat model of MI employed in the current study (Wellenius *et al*., 2002). This contrast highlights the difficulty of extrapolating results from studies employing surrogate particles to the effects of real-world ambient particles. Third, our study was adequately powered to detect a doubling in the frequency of ventricular arrhythmias associated with CAPs exposure. This is a modest increase in comparison to the more than six-fold increase in ventricular arrhythmia frequency observed in our previous study (Wellenius *et al*., 2002). However, we cannot rule out the possibility of a more modest increase in the frequency of ventricular arrhythmias associated with CAPs exposure in this model.

Although overall there was not a significant increase in arrhythmia frequency with CAPs exposure, there was a significant CAPs-related decrease during the post-exposure period in rats with a high number of pre-exposure VPBs. In this subgroup we also observed a concomitant CAPs-induced increase in heart rate. In humans, increased heart rate may lead to a decrease in the frequency of ventricular arrhythmias through overdrive suppression of ventricular ectopic foci. The CAPs-related decrease in VPBs we observed may have been mediated by a similar mechanism. Interestingly, the change in heart rate was associated with CAPs mass concentration, in agreement with studies in humans (Pope *et al*., 1999a,b). The change in heart rate was not associated with CAPs number concentration.

The present study has several potential limitations which may restrict the implications of these findings. First, the duration of exposure was limited to 1 h because of the short time which rats could be sedated with a single dose of diazepam. Thus, it is not known whether a longer exposure would produce similar results. Second, the number of animals in each group is unbalanced because initially animals were only randomized to either CAPs or filtered air exposure. While this might have affected the power of our study to detect an effect of CO exposure, the imbalance is not expected to affect the validity of the results. Third, to reduce biologic variability, only mature, male, Sprague-Dawley rats were studied. Thus, it is unknown how the effect of ambient particles and CO varies by gender, age, or species. Fourth, there are important differences between the rat and human heart, including differences in the degree of collateral blood flow, ventricular mass, and electrical properties (Janse *et al*., 1998). As such, results from experiments conducted in a large animal model would likely be more directly comparable to epidemiologic findings.

Ambient air pollution represents a complex mixture of PM and gaseous pollutants including CO. It is unclear whether the cardiovascular effects of PM observed in epidemiologic studies may be confounded or modified by CO exposure. The findings of the current study do not support the notion that CO exposure

increases the incidence of cardiac arrhythmias. Additionally, we found no evidence that the cardiovascular effects of PM are modified by simultaneous exposure to CO. Further experiments are needed to clarify the impact of ambient PM on cardiac arrhythmias and to elucidate the mechanism of this effect.

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