Cardiovascular and Systemic Responses to Inhaled Pollutants in Rodents: Effects of Ozone and Particulate Matter

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Striking similarities have been observed in a number of extrapulmonary responses of rodents to seemingly disparate ambient pollutants. These responses are often characterized by primary decreases in important indices of cardiac and thermoregulatory function, along with secondary decreases in associated parameters. For example, when rats are exposed to typical experimental concentrations of ozone (O_3), they demonstrate robust and consistent decreases in heart rate (HR) ranging from 50 to 100 beats per minute, whereas core temperature (T_{co}) often falls 1.5–2.5°C. Other related indices, such as metabolism, minute ventilation, blood pressure, and cardiac output, appear to exhibit similar deficits. The magnitudes of the observed decreases may be modulated by changes in experimental conditions and appear to vary inversely with both ambient temperature and body mass. More recent studies in which both healthy and compromised rats were exposed to either particulate matter or its specific components yielded similar results. The agents studied included representative examples of ambient, combustion, and natural source particles, along with individual or combined exposures to their primary metallic constituents. In addition to the substantial decreases in HR and T_{co}, similar to those seen with the O_3-exposed rats, these animals also displayed numerous adverse changes in electrocardiographic waveforms and cardiac rhythm, frequently resulting in fatal outcomes. Although there is only limited experimental evidence that addresses the underlying mechanisms of these responses, there is some indication that they may be related to stimulation of pulmonary irritant receptors and that they may be at least partially mediated via the parasympathetic nervous system. *Key words*: arrhythmia, heart rate, metals, ozone, particulate matter, radiotelemetry, rat, temperature. — *Environ Health Perspect* 109(suppl 4):539–546 (2001).
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Over the past decade our laboratory has been investigating the nature and extent of specific extrapulmonary responses observed in rodents following exposure to ambient pollutants and other xenobiotic agents. These responses are characterized by primary decreases in important indices of cardiac and thermoregulatory functions, along with secondary decreases in associated parameters. The summation of these effects, termed the *hypothermic response* (*1*), has both physiological and behavioral components, appears to be most pronounced in (and possibly unique to) the rodent, and may significantly impact the interpretation of experimental data and its subsequent extrapolation to the human situation. Typically, healthy adult rats may exhibit decreases in heart rate (HR) of 50–100 beats per minute (bpm) and decreases in core temperature (T_{co}) of 1.5–2.5°C following routine exposures to moderate levels of ambient pollutants, such as ozone (O_3) or particulate matter (PM). In addition, there appear to be similar, albeit related, decreases in other functional parameters, including metabolism, minute ventilation, blood pressure, and cardiac output. Furthermore, it has been demonstrated that the magnitudes of these observed decreases may be significantly modulated by changes in experimental conditions. For example, experimental stresses related to exercise, restraint, and handling, as well as the imposition of

changes in ambient temperature (T_a), have all been shown to have profound effects on these responses. Finally, it has been proposed that a moderate hypothermic response may afford protection and improve survivability following toxic exposures, whereas a more severe response may actually potentiate the toxicity. Although the underlying mechanisms of the hypothermic response are still largely unexplored and unknown, preliminary experimental evidence suggests that these effects may be mediated, at least partly, via components of the parasympathetic nervous system.

O_3 is a ubiquitous ambient pollutant and a known pulmonary irritant (*2*). In a series of earlier studies from our laboratory (*3–6*), we

investigated both the pulmonary and systemic consequences of O_3 exposures (0.25–2.0 ppm) in rodents. With respect to nonpulmonary end points, these studies used radiotelemetry procedures to examine HR, T_{co}, and electrocardiographic changes in rats and mice over a variety of experimental conditions. These studies were among the first to report consistent, robust concentration-related decreases in the above-mentioned parameters in rodents following exposure to routine experimental levels of O_3.

More recently, the putative toxicity of PM has attracted considerable attention, and numerous epidemiological studies published over the last few years have reported a slight but consistent association between the concentration of ambient PM and the incidence of adverse health effects in man (*7–12*). Despite limited supporting experimental evidence, there is a growing consensus that higher levels of PM in the air are associated with increased morbidity and mortality, and that persons with preexisting cardiopulmonary disease are especially at risk for acute, life-threatening illnesses. To investigate these hypotheses, we recently examined the changes induced in pulmonary and nonpulmonary end points in various rodent models of cardiopulmonary disease/stress following exposure to PM or its metallic constituents (*13–19*). In all cases, in addition to the effects on standard pulmonary indices, we observed substantial decreases in both HR and T_{co}, accompanied by increases in the incidences of arrhythmias and lethalities.

Interestingly, despite the seemingly dissimilar characteristics of the ambient pollutants to which the rats in the above studies were exposed, the nonpulmonary responses (e.g., decreases in HR and T_{co}) were remarkably

similar. This singular observation raises a number of intriguing questions. For example, how can such disparate agents produce such similar effects on the cardiac and thermoregulatory systems? How are these effects transduced from the portal of entry, i.e., the lung, to the effectors of the cardiac and central nervous systems? Do they share a common pathway or mechanism of action? How can ambient pollutants that sometimes produce relatively limited, nonlethal effects on their primary target organ system (pulmonary) cause fatal responses in a secondary organ system (cardiac)? One important commonality of these pollutants may be their ability to stimulate sensory or irritant receptors in the lung. Such stimulation may initiate mechanisms that converge via a shared pathway to produce similar end responses in the intact animal. Despite a recent upsurge of interest in the above issues, few published studies address these questions. In this article we summarize the results of some of our previous studies and briefly explore plausible explanations that may link these observations.

Materials and Methods
General

In this article, we describe representative results from a number of studies conducted in our laboratory to examine the effects of ambient pollutants in healthy and cardiopulmonary-compromised rodents maintained under varied environmental conditions. The strains of rats (Charles River Breeding Laboratory, Raleigh, NC, USA) used included Fischer-344, Sprague-Dawley (SD), spontaneously hypertensive (SH), and Wistar-Kyoto (WKY), with some groups of animals subjected to further procedures to induce cardiopulmonary stress or dysfunction. Additional studies used mice (C57BL/6J, C3H/HeJ) and guinea pigs (Hartley) (Charles River Breeding Laboratories). Care and treatment of all animals were conducted in accordance with the established guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care, International.

Unless otherwise noted, the following methodologies were common to all rat studies and were only slightly modified for the studies involving mice and guinea pigs. Only male animals were used and all were approximately 60 days of age at the beginning of the study. Animals were implanted intraperitoneally with radiotransmitters (Model TA11CTA-F40; Data Sciences International, Inc., St. Paul, MN, USA) under aseptic conditions and allowed at least 1 week for recovery and restoration of circadian rhythm. Thereafter, animals were maintained in a climate-controlled exposure chamber (T_a: 22°C; relative humidity: 50%; 12 hr light:12 hr dark) and radiotelemetry methodology was used to

monitor electrocardiogram (ECG), HR, and T_{co} at either 5- or 10-min intervals throughout the experimental period. Preexposure data allowed each animal to serve as its own control, while matched group animals exposed to neutral conditions (filtered air or acidified saline) provided time-paired control data. The following ambient pollutants were studied: O_3; various types of PM, including a representative ambient particle [Ottawa dust (OTT)], combustion particle [residual oil fly ash (ROFA)], and natural source particle [Mt. St. Helen's dust (MSH)]; and the primary transition metal constituents of these PM (e.g., nickel, vanadium, and iron). Exposure scenarios involved whole-body inhalation [(IH-W), O_3, PM, and metals], nose-only inhalation [(IH-N), O_3, and PM], and intratracheal instillations [(IT), PM, and metals]. Although the protocols employed permitted examination of a number of physiological, biochemical, and morphological parameters, in this article we focus on the cardiac and systemic effects induced by exposure to these pollutants and their potential role in the observed morbidity and mortality. Experimental details specific to a particular exposure scenario or study are described in the legend to each figure.

Ozone Inhalation

O_3 studies primarily used IH-W procedures, although a few studies employed IH-N

procedures. Fischer-344 rats, mice, and guinea pigs were used in these studies, with exposure concentrations ranging from 0.25–2.0 ppm. Exposure scenarios were both continuous and intermittent, whereas exposure periods ranged from a minimum of 2 hr to a maximum of 5 days. Last, exposures were conducted at T_a's of 10, 22, and 34°C.

Particulate Matter/Metals Instillation

In the initial PM studies, particles were dissolved in acidified saline vehicle (0.3 mL; pH 2), and rats were exposed via direct instillation into the trachea. Instillation was performed under halothane anesthesia and generally required less than 1 min to complete. The strains of rats used included SD, SH, and WKY, and all studies paired healthy animals with cardiopulmonary-compromised animals. The stress/disease models employed were chosen to simulate *a*) physiological increases in cardiac work (SD rats maintained and exposed at a T_a of 10°C); *b*) acute pulmonary inflammation (SD rats exposed to 1 ppm $O_3 \times 6$ hr at 18 hr pre-IT); *c*) pulmonary vasculitis with hypertension [SD rats exposed to 60 mg/kg monocrotaline (MCT) at 12 days pre-IT]; *d*) systemic hypertension (SH rats); *e*) and old age (SH rats 15 months of age). Instillation methodology was used to examine the effects of exposure to whole particles (e.g., ROFA, OTT, MSH), as well as to individual and
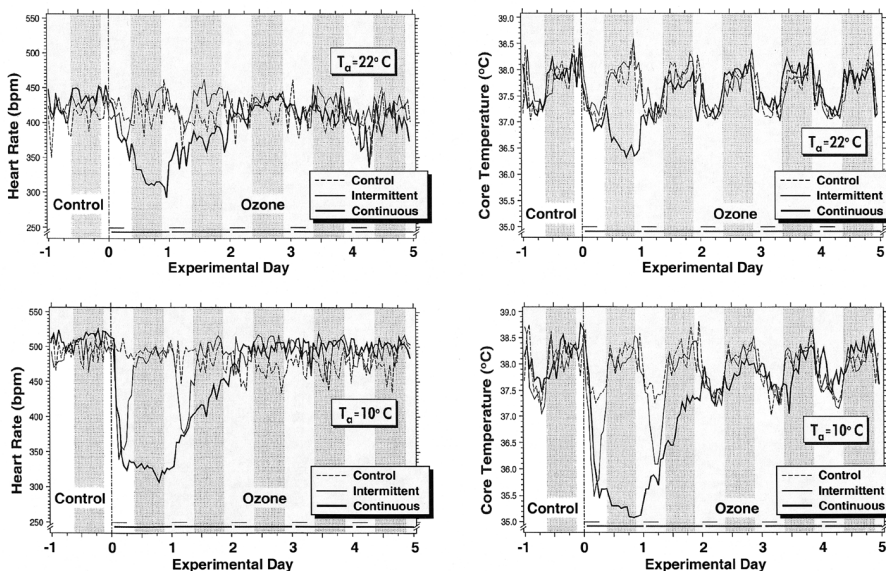

Figure 1. Changes in heart rate and core temperature in healthy rats exposed to O_3 while maintained at different ambient temperatures (T_a's). bpm, beats per minute. Effects of intermittent and nearly continuous O_3 exposure on heart rate and core temperature of awake, adult Fischer-344 rats (n = 4–6/group) maintained at an ambient temperature of either 10 or 22°C. All animals were exposed to filtered air for the first day. Control animals continued to be exposed to filtered air throughout the experimental period, whereas treated animals were exposed to O_3 (0.5 ppm) for either 6 hr/day or for 23 hr/day. O_3 exposure regimens for intermittent and continuous exposures are indicated by the length of the light and heavy horizontal lines, respectively, across the bottom of the figure. Data were obtained at 10-min intervals but for presentation purposes have been averaged over 1-hr periods across groups. Thus, each data point represents the combined mean of 24–36 individual values (6 time points/hr/rat). Light:dark cycles (0600:1800) are indicated by the light and shaded vertical bands, respectively. T_a's at which the exposures were conducted are indicated on the individual graphs. For complete details see Watkinson et al. (*4*).

combined exposures to the primary transition metal constituents (e.g., Ni, V, and Fe) of these particles.

Particulate Matter/Metals Inhalation

These studies simulated portions of the above IT studies using both IH-W and IH-N exposure protocols. Similar rat strains (e.g., SD, SH, and WKY) and compromised models (pulmonary inflammation, pulmonary hypertension, systemic hypertension) were employed, with exposures to ROFA and individual or combined transition metals. Exposures were conducted over several days (generally 6 hr/day × 4 days), and the concentrations of PM or metals used were calculated to deliver a cumulative dose similar to that given as a bolus in the previous IT studies.
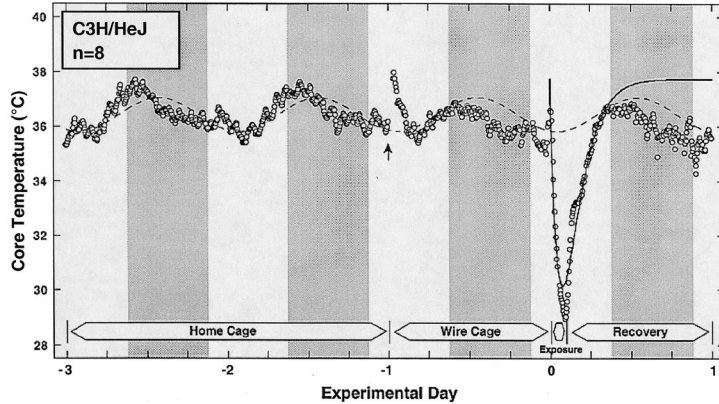

Figure 2. Changes in core temperature in healthy mice exposed to O_3 while maintained at normal ambient temperature. Effects of acute exposure to O_3 on core temperature of awake, adult C3H/HeJ mice (n = 8) maintained at an ambient temperature of 22°C. Animals were exposed to either filtered air or O_3 (2.0 ppm × 2 hr) while subjected to different housing conditions throughout the 5-day experimental period. Time and duration of experimental conditions are indicated on the figure. Data were obtained at 5-min intervals and were averaged across the group. Light:dark cycles (0600:1800) are indicated by the light and shaded vertical bands, respectively. Note the abrupt increase (arrow) in core temperature due to handling as the mice were changed from home to wire cages. Core temperature data over the entire home cage period have been modeled using a cosine model function (dashed line). The core temperature response during the exposure and recovery periods has been fit using a one-compartment model function (solid line). For complete details see Watkinson et al. (*5*).
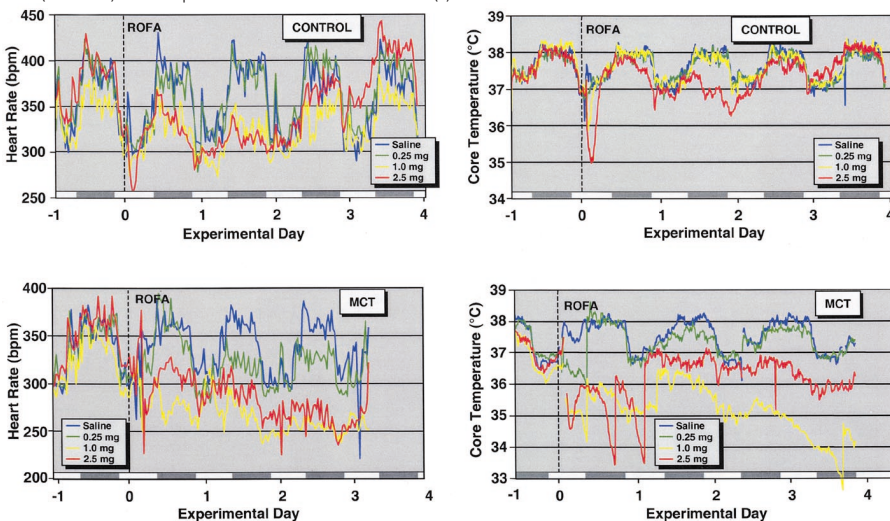

Figure 3. Changes in heart rate and core temperature in healthy and cardiopulmonary-compromised rats exposed to instilled residual oil fly ash (ROFA). Effects of intratracheal instillation of ROFA particulate matter on heart rate and core temperature of awake, adult Sprague-Dawley rats (n = 4/group) maintained at an ambient temperature of 22°C. Studies compared the effects of up to four doses of ROFA particles (0, 0.25, 1.0, or 2.5 mg/kg) in healthy rats with those of rats preexposed to monocrotaline (MCT; 60 mg/kg, intraperitoneal) to induce pulmonary inflammation and pulmonary hypertension. The healthy rats were allowed 5 days to acclimate to the experimental conditions, instilled intratracheally with ROFA particles (dashed vertical line), and monitored for 96 hr postinstillation. The MCT-treated rats were allowed 12 days after MCT administration for the development of pulmonary inflammation and pulmonary hypertension before ROFA instillation was performed. Data were obtained at 10-min intervals but have been averaged over 30-min periods across groups. Intermittent open and shaded boxes above the abscissa indicate daytime and nighttime, respectively. Note: The abrupt negative spikes in the data of the MCT-treated animals are due to lethalities (n = 2). For complete details see Watkinson et al. (*14*) and Campen et al. (*19*).

Results

Ozone Inhalation

Exposure of rodents (via both IH-W and IH-N procedures) to experimental levels of O_3 (0.25–2.0 ppm) resulted in concentration-related decreases in HR and T_{co}, in conjunction with increases in important biochemical indices of pulmonary damage (*3–6,20,21*). The magnitudes of these responses were clearly affected by changes in experimental conditions associated with increases in physiological or emotional stress, e.g., cold stress, exercise, handling, and restraint. For example, rats maintained at the "standard" room temperature of 22°C and exposed either continuously (23 hr/day × 5 days) or intermittently (6 hr/day × 5 days) to 0.5 ppm O_3 (*4*) exhibited moderate decreases (Figure 1) in HR (75–100 bpm) and T_{co} (1.5–2.0°C). Similar rats maintained at a T_a of 10°C showed decreases in HR (150–200 bpm) and T_{co} (2.5–4.0°C) significantly greater than those of the rats exposed at normal room temperature. Results of bronchoalveolar lavage were similarly exacerbated (*6*). Rats were only slightly affected by exposure to O_3 concentrations of 0.25 ppm when kept under normal housing conditions, whereas the combination of 1.0 ppm O_3 with a T_a of 10°C produced substantial lethality [3 of 4 rats; (*22*)]. When rats were exposed to 0.5 ppm O_3 in conjunction with moderate treadmill exercise (*20,21*), the expected O_3-induced decreases in the above-described functional parameters were offset by exercise-related increases in these parameters. However, when carbon dioxide was employed to promote increases in ventilation, and thus serve as an "exercise surrogate," the O_3-induced systemic effects were significantly potentiated. This hypothermic response has been shown to be much greater in mice (*5*), where exposure to 2.0 ppm O_3 produced decreases in HR of 200–250 bpm and decreases in T_{co} of 9–10°C (Figure 2). Interestingly, guinea pigs do not appear to demonstrate a hypothermic response following exposure to up to 2.0 ppm O_3 (*23*). Despite consistent, significant decreases in the above-described parameters, there was essentially no lethality in any of the studies conducted at standard room temperature.

Particulate Matter/Metals Instillation

Healthy rats exposed via IT procedures to ROFA and other PM demonstrated significant biphasic, dose-related responses consisting of both acute (0–6 hr post-IT) and delayed (12–72 hr post-IT) bradycardia and hypothermia, accompanied by a high incidence of cardiac arrhythmias and substantial pulmonary inflammation (*13,14,17–19*). Healthy SD rats instilled with the highest dose (2.5 mg) of ROFA (*19*) demonstrated an initial 50-bpm decrease in HR, in conjunction with a 2.0°C decrease in T_{co} (Figure 3). These

parameters returned to control levels within 6 hr but showed further sporadic decreases over the next 3 days. Changes were most apparent during nocturnal cycles and reached their nadir (approx. 100 bpm; approx. 1.5°C) during the second night after exposure. On the fourth day post-IT, there was a slight increase in HR and T_{co} (approx. 60 bpm; approx. 0.6°C) in these animals. Healthy SD rats exposed to medium and low doses of ROFA (1.0 mg and 0.25 mg, respectively) exhibited similar response patterns but of reduced magnitude and duration. In contrast, cardiopulmonary-compromised SD rats (e.g., rats subjected to cold-stress, O_3-induced pulmonary inflammation, or MCT-induced pulmonary hypertension) demonstrated exaggerated bradycardic and hypothermic

responses to IT ROFA. Acute decreases in HR that ranged from 75–150 bpm were observed in all groups, along with T_{co} decreases in excess of 3.0°C. Delayed decreases were both potentiated and prolonged. These effects were most pronounced in the cold-stressed animals and were more variable in the O_3-pretreated and MCT-pretreated rats (Figure 3). In general, there was an increased frequency of arrhythmic events on the first day post-IT ROFA for the first two compromised animal groups, which decreased over the next 3 days, whereas the MCT-treated animals exhibited a sustained rate of arrhythmias over the entire experimental period (14). Further studies monitored the ECGs of healthy SD rats during the administration of various doses of acetylcholine (0.05–0.25 mg). These studies

demonstrated that intravenous infusions of acetylcholine were able to reproduce essentially similar arrhythmic events as those produced in the previous studies by IT ROFA (Figure 4). Lethality (6 of 12) was seen only in the MCT-treated group animals exposed to ROFA. Analyses of the ECGs of the animals that died implicated two potential cardiovascular-related scenarios for the observed lethalities. Thus, in half of the deaths it appeared there may have been a (relatively) slow failure of the myocardium due to the presence of pulmonary edema, with consequent hypoxemia and myocardial ischemia, whereas in the remaining animals there seemed to be an abrupt failure of the heart due to a fatal arrhythmia, possibly consequent to a parasympathetic-mediated conduction deficit.

Additional studies conducted using protocols similar to those described above compared IT exposures to ROFA, OTT, and MSH in aged (15 months) SH rats (17,18). The dose of OTT instilled (2.5 mg) was selected to be equal in mass to that of the highest ROFA dose in the previous studies, and the dose of ROFA (0.5 mg) was chosen to match the metal content of the OTT. These PM produced extrapulmonary responses (Figure 5) that were generally similar to each other, although the responses of the OTT animals appeared to be slightly more severe than those of the ROFA animals. In both cases there were pronounced, acute, biphasic bradycardic and hypothermic responses. In contrast, exposure to MSH (2.5 mg), a generally inert natural source particle, produced no discernable effects. In the OTT-exposed SH rats, the magnitudes of the initial decreases in HR and T_{co} were approximately 35 bpm and approximately 2.5°C, respectively, with a duration of less than 2 hr. There was a brief, incomplete recovery in these parameters, followed by further drops of approximately 45 bpm and approximately 0.6°C that required nearly 4 hr to return to control levels. Thus, the overall acute bradycardic and hypothermic responses in these rats reached maximums of approximately 80 bpm and approximately 3.0°C while spanning almost 9 hr. After near-complete recovery to normal levels, HR failed to demonstrate the expected nighttime rise and remained 20–30 bpm below control levels throughout the first dark period. These responses in OTT-exposed SH rats were also accompanied by a significant increase in the incidence of cardiac arrhythmias. In the ROFA-exposed SH rats, both components of the acute HR and T_{co} decreases were generally briefer and of reduced magnitude. There was an essentially complete recovery of these parameters to control levels between the induced acute episodes in the ROFA-exposed SH animals, followed by a prolonged delayed bradycardia and
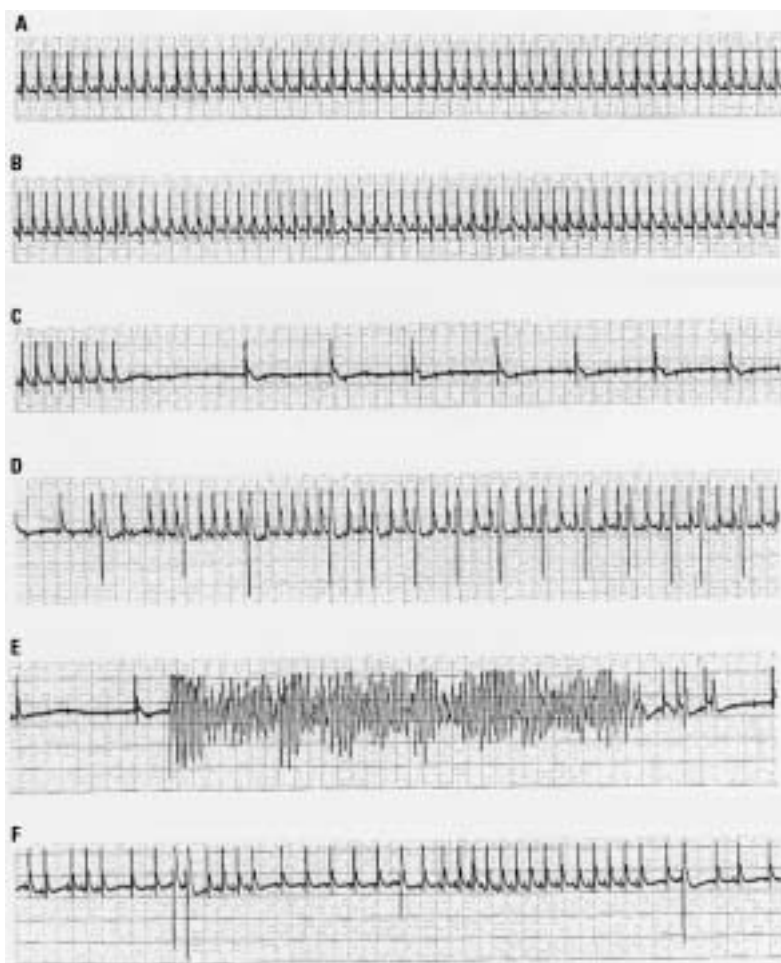

Figure 4. Acetylcholine-induced electrocardiographic waveform anomalies. Effects of intravenous infusion of acetylcholine (0.05–0.25 mg) on electrocardiographic waveforms of an anesthetized, healthy adult Sprague-Dawley rat. Individual waveform strips illustrate frequently observed arrhythmias at various time points (≤ 2 min) following infusion. Chart speed for these records was 25 mm/sec and each strip represents approximately 10 sec sampling time. (*A*) Normal waveform prior to infusion. (*B*) Occasional premature junctional contractions. (*C*) Second-degree atrioventricular (Mobitz type II) block. (*D*) Second-degree atrioventricular (Mobitz type II) block followed by frequent premature ventricular contractions. (*E*) Second-degree atrioventricular (Mobitz type II) block bounding episode of severe tachyarrhythmia. (*F*) Multiple interspersed arrhythmias including prolonged R–R intervals, premature ventricular contractions, skipped beats, and escape beats. For complete details see Watkinson et al. (*14*) and Campen (*25*).

hypothermia from 36 to 72 hr postexposure. Effects on arrhythmogenesis were also less severe in the ROFA-exposed SH animals. No significant cardiac or thermoregulatory effects were seen with exposure to MSH. There was no lethality among any of these rats.

More recent studies investigated both individual and combined IT exposures of healthy and MCT-treated SD rats to the primary transition metal constituents of ROFA, e.g., Ni, V, and Fe (*24,25*). The V-treated healthy animals exhibited a rapid drop in HR (90 bpm) and T_{co} (2.5°C), followed by a quick return (approx. 6 hr) to control levels (Figure 6). In contrast, the HR of the Ni-treated healthy animals remained stable until approximately 24 hr postexposure, when it began to drop precipitously (100 bpm), ultimately stabilizing at approximately 30–70 bpm below control levels for the next 36 hr. Likewise, there was a delayed decrease in T_{co} beginning approximately 24 hr postexposure that remained approximately 1.4°C below control for the duration of the exposure

period. In both of the above groups, there were concomitant effects on arrhythmogenesis, with V-induced arrhythmias predominating on the first day post-IT and Ni-induced effects beginning on the second day post-IT and continuing throughout the experiment. The HR, T_{co}, and arrhythmia frequency of rats exposed to Fe did not differ from those of control rats. Lethalities were observed only in the Ni-treated group animals (2 of 4) in this initial individual metals study. The MCT-treated animals exhibited similar responses to those of the healthy animals, with the responses of the V-treated animals somewhat attenuated and those of the Ni-treated animals slightly potentiated compared to those of the healthy animals. Analyses of interactions among these metals appear to confirm and extend these results. Groups of MCT-treated SD rats receiving Ni and V showed the greatest toxicity, with an apparent greater-than-additive effect associated with their combined exposure. Interestingly, although exposure to Fe alone caused little or no response, exposure to combinations of metals that included Fe induced a smaller response than similar exposures that did not include Fe. Thus, exposures to Ni and V caused decreases in HR and T_{co} of 100 bpm and 3.0°C, respectively. However, when rats were exposed to the same concentrations of Ni and V with Fe added, HR and T_{co} decreased only 50 bpm and 2.5°C. Lethalities (14 of 42)

among the rats exposed to metal combinations in this series of studies also primarily involved animals treated with Ni and/or V and occurred over the entire experimental period. Again, the data suggest that Fe may be slightly protective, as the mortality rate in Ni + V–treated rats (67%) was higher than that of Ni + V + Fe–treated rats (50%). When HR patterns of animals that subsequently died were examined, it was found that HR increased dramatically in the hours before death, in contrast to the previously observed toxicant-induced decreases in these animals (Figure 7). Thus, an abrupt increase in HR was able to serve as a biomarker for lethality and permitted the subsequent capture of ECG waveforms at the time of death for multiple animals. In general, these ECGs supported the lethality scenarios previously described. Further analysis of the ECGs of some of the animals that died indicated the presence of T-wave alternans, often cited (*26*) as a potential precursor to fatal arrhythmias in man (Figure 8).

Particulate Matter/Metals Inhalation

Both pulmonary hypertensive (MCT-treated SD) and systemically hypertensive (SH) rats exposed to ROFA via IH-N procedures demonstrated cardiac and thermoregulatory responses (data not shown) that were similar to those described above but of much diminished magnitude (*16–18*). There were
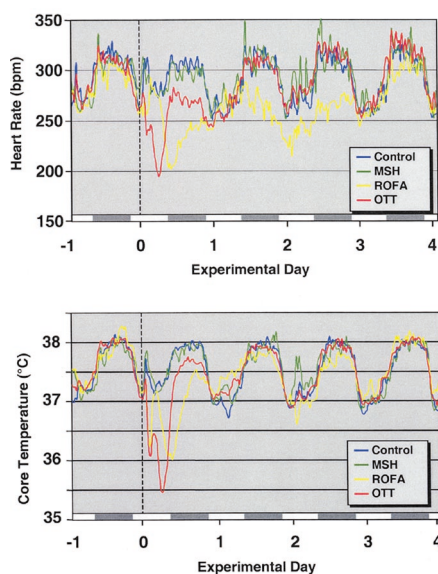
Figure 5. Changes in heart rate and core temperature in aged spontaneously hypertensive rats exposed to instilled Mt. St. Helens volcanic ash (MSH), residual oil fly ash (ROFA), and Ottawa dust (OTT). Effects of intratracheal instillation of representative examples of natural source (MSH), combustion (ROFA), and ambient (OTT) particulate matter on heart rate and core temperature of awake, aged spontaneously hypertensive rats (*n* = 6/group; 15 months) maintained at an ambient temperature of 22°C. Rats were allowed 5 days to acclimate to the experimental conditions, instilled intratracheally (dashed vertical line) with either acidified saline vehicle (0.3 mL; pH 2) or OTT (2.5 mg), ROFA (0.5 mg), or MSH (2.5 mg) particles dissolved in acidified saline vehicle, and monitored for 96 hr postinstillation. Data were obtained at 10-min intervals but have been averaged over 30-min periods across groups. Intermittent open and shaded boxes above the abscissa indicate daytime and nighttime, respectively. For complete details see Watkinson et al. (*17,18*).
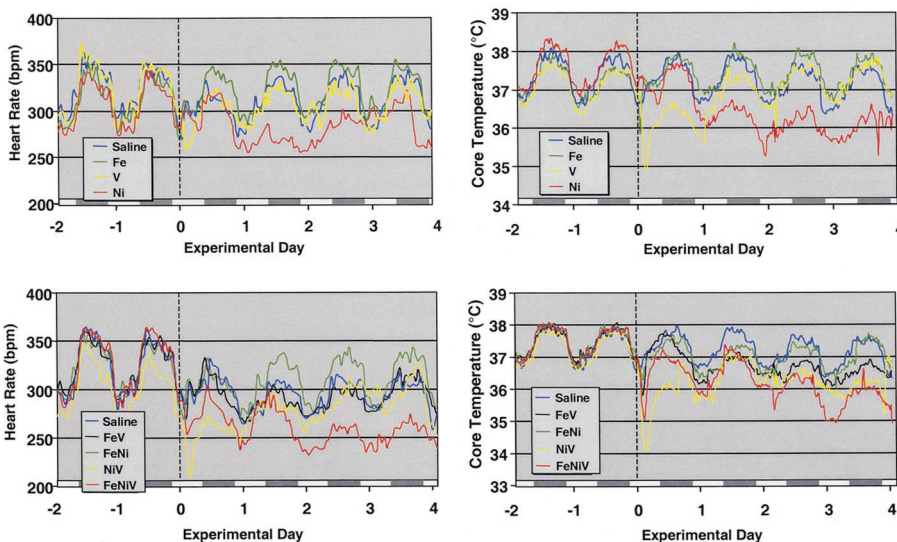
Figure 6. Changes in heart rate and core temperature in cardiopulmonary-compromised rats exposed to instilled metallic constituents of residual oil fly ash. Effects of intratracheal instillation of primary transition metal constituents of residual oil fly ash particulate matter on heart rate and core temperature of awake, adult Sprague-Dawley rats (*n* = 10/group) maintained at an ambient temperature of 22°C. Studies compared the effects of individual and combined doses of nickel (263 µg $NiSO_2$), vanadium (245 µg VSO_4), and iron [105 µg $Fe_2(SO_4)_3$] in rats preexposed to monocrotaline (60 mg/kg, intraperitoneal). Rats were allowed 12 days after monocrotaline administration for the development of pulmonary inflammation and pulmonary hypertension. Rats were allowed 5 days to acclimate to the experimental conditions, instilled intratracheally with metals (dashed vertical line), and monitored for 96 hr postinstillation. Data were obtained at 10-min intervals but have been averaged over 30-min periods across groups. Intermittent open and shaded boxes above the abscissa indicate daytime and nighttime, respectively. For complete details see Campen et al. (*24*) and Campen (*25*).

decreases in HR and T_{co} in the MCT-treated SD rats exposed to ROFA of approximately 30–40 bpm and approximately 0.8°C, respectively. However, these responses were only slightly greater than those of the control animals during confinement in the nose-only exposure tubes. Interestingly, in the MCT-treated group animals, HR a) was considerably

Figure 7. Lethal heart rate patterns in rats exposed to instilled metallic constituents of residual oil fly ash. Examples of heart rate patterns in awake, adult Sprague-Dawley rats that subsequently died following exposure via intratracheal instillation to various individual or combined doses of primary transition metal constituents of residual oil fly ash particulate matter. Doses of each metal were as follows: nickel (263 μg $NiSO_2$), vanadium (245 μg VSO_4), and iron [105 μg $Fe_2(SO_4)_3$]. Animals were maintained at an ambient temperature of 22°C throughout the experimental procedure. The rats were allowed 5 days to acclimate to the experimental conditions, instilled intratracheally with metals (dashed vertical line), and monitored for 96 hr postinstillation. Intermittent open and shaded boxes above the abscissa indicate daytime and nighttime, respectively. Note: Despite the bradycardia commonly observed following exposure to these metals, heart rate increased markedly in the hours preceding death. For complete details see Campen et al. (24) and Campen (25).

Figure 8. Lethal changes in electrocardiographic waveform of pulmonary hypertensive rat exposed to instilled metallic constituents of residual oil fly ash. Effects of intratracheal instillation of nickel (263 μg $NiSO_2$) and vanadium (245 μg VSO_4) on electrocardiogram of awake, adult pulmonary hypertensive Sprague-Dawley rat just prior to death. Death occurred approximately 77 hr postinstillation.

Strip 1: slightly accelerated rhythm; normal morphology; P-, R-, and T waves all present. *Strip 2:* slowed rate, but still somewhat elevated; presence of occasional depolarizations (a) which may be ectopic in origin. *Strip 3:* increased frequency of ectopic depolarizations; periods of noise are noted (b). *Strip 4:* abrupt rhythm change (50%↓) following ectopic depolarization (c); P-wave present but alternating rhythm predominates. *Strip 5:* couplet rhythm (d) lost; dramatically slowed rate; P-wave absent; rate may reflect intrinsic junctional or ventricular rhythm. *Strip 6:* P-waves, ectopic depolarizations absent (e); death. NOTE: Magnification of Strip 1 shows presence of T-wave alternans, indicated by alternating amplitudes of T waves (arrows), an electrocardiographic anomaly often associated with subsequent fatal arrhythmias. For complete details see Campen et al. (24) and Campen (25).

lower (approx. 50 bpm) in the preexposure period than that of the saline-treated rats, b) exhibited substantial stress-induced increases (approx. 100 bpm) when the rats were placed within the nose-only exposure tubes, and c) showed postexposure decreases (25–80 bpm) that were sustained for the duration of the study. The HR and T_{co} of the SH rats exposed to ROFA were decreased approximately 40–60 bpm and approximately 1.0–1.5°C, respectively, when compared to their air-exposed SH and WKY controls, and were also slightly lower than their ROFA-exposed WKY controls. Both MCT-treated SD and SH rats demonstrated somewhat lower preexposure HRs and T_{co}'s, along with substantial stress-induced increases in these parameters when placed in the nose-only exposure tubes, than their paired controls. ROFA-induced arrhythmogenesis was also diminished in these rats compared to that seen in the previous studies. The ECGs of SH rats showed evidence of myocardial ischemia (e.g., depressed ST-segment area), which was exacerbated with exposure to ROFA (16). There were no lethalities in any of the groups following inhalation of ROFA.

A series of recent studies exposed healthy rats to individual and combined transition metals (Ni and V) via IH-W procedures (27,28). Groups of rats were exposed to a range of concentrations for the individual (four concentrations each) and combined (two concentrations) metals to bracket the exposure doses of previous studies. Specifically, the concentrations of metals used were calculated such that at the highest concentration used, the estimated burden to be deposited in the lung over the 4-day exposure period would match that of the high dose (2.5 mg) of the previous IT studies, given as a bolus. The lowest concentrations of V (0.3, 0.6, 0.9 mg/m³) caused no observable effects on HR, T_{co}, or arrythmic frequency. Exposure to the highest concentration of V (1.7 mg/m³) produced only minimal decreases in HR (20 bpm) and T_{co} (0.2°C) and no changes in arrhythmia frequency. Similarly, the lowest concentrations of Ni (0.36, 0.49 mg/m³) alone caused no discernable effects on HR, T_{co}, or arrhythmia frequency. However, inhalation of 1.3 mg/m³ of Ni caused significant decreases in HR (75 bpm) and T_{co} (2.0°C), beginning on the third exposure day and lasting for approximately 72 hr. The highest concentration of Ni (2.1 mg/m³) induced severe bradycardic (100 bpm) and hypothermic (3.3°C) responses that began on the second exposure day and lasted for the entire 96-hr monitoring period. Effects on arrhythmia frequency for the two highest concentrations of Ni were commensurate with these responses. The final series of studies exposed healthy rats to combinations of Ni and V at concentrations that individually
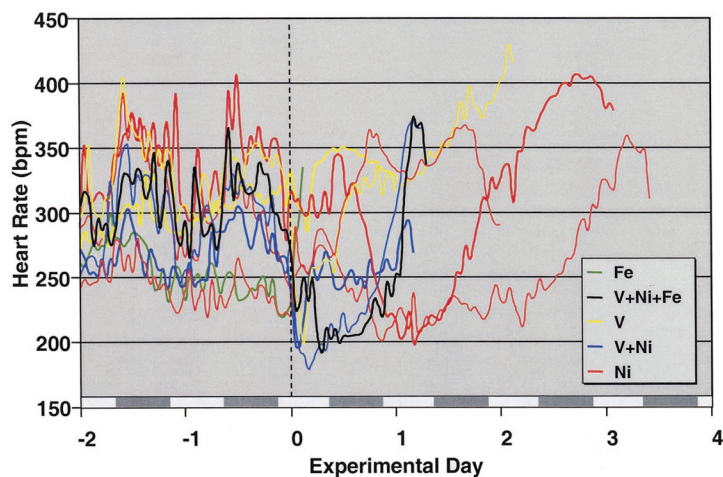
produced either no effects or low effects. Thus, rats exposed to Ni + V (0.5 mg/m³ each) demonstrated decreases in HR (50 bpm) and T_{co} (1.0°C) that began on the third exposure day and persisted for approximately 30 hr (Figure 9). Arrhythmogenesis was markedly increased on the second and third days of exposure. Exposure to the highest concentrations of Ni + V (1.3 mg/m³ each) produced severe delayed bradycardia and hypothermia, with decreases of 160 bpm and 4.0°C, respectively. In addition, there was a 4-fold increase in arrhythmia frequency in these animals along with a mortality rate of 50%.

Discussion and Conclusions

The results of the preceding studies present an extensive body of largely observational work conducted to characterize the hypothermic response in rodents and to document the dramatic effects of ambient pollutants on extrapulmonary and systemic functional parameters. Despite the variety of agents tested and the differing modes of exposure, there are some striking similarities in the resultant extrapulmonary response outcomes. Thus, the hallmarks of this multifaceted response involve primary decreases in HR and T_{co}, along with secondary decreases in related parameters such as metabolism, cardiac output, blood pressure, and ventilation. In the studies summarized above, these effects were often accompanied by increases in the rates of occurrence of serious arrhythmic events and lethalities, particularly in cardiopulmonary-compromised animals. These effects are clearly potentiated by stress-inducing changes in experimental conditions and have been postulated to convey a survival advantage for the rodent. Unfortunately, despite the robust and ubiquitous nature of the extrapulmonary

effects observed following exposures to O_3 and PM, to date very little is known concerning the underlying mechanisms of these responses, and the proposed involvement of potential pathways remains largely speculative.

There have been several plausible theories advanced to explain the transducing mechanisms that may link the initial exposure to an inhaled pollutant with the resultant hypothermic response, as well as with other potential adverse outcomes; however, credible supportive experimental evidence is generally lacking. For example, it is reasonable to speculate that there may be a direct effect of an agent (or its metabolite) on the myocardium. Indeed, the lung is well suited for the uptake and distribution of inhaled substances into the bloodstream. The large surface area of the pulmonary tree, the intimate nature of the capillary–alveolar intersection, and the ready absorption of both lipid- and water-soluble constituents all serve to contribute to the rapid uptake of inhaled agents. Further uptake may result from the leaching or dissolution of PM-borne constituents in the lung lining fluid, with subsequent transport via diffusion or phagocytic action across the thin air–blood barrier. Once in the bloodstream, the extensive branching pattern of the coronary vasculature ensures efficient distribution and effective exposure of the myocardial musculature to bloodborne toxicants. Both *in vivo* and *in vitro* studies have demonstrated the bioavailability and persistence of water-soluble metals known to directly induce cardiac muscle and cell dysfunctions (*29–31*). A recent report by Killingsworth et al. (*32*) described the induction of cardiac MIP-2 as an indicator of direct lung inflammation resulting from inhalation of a metal-rich ROFA. Alternatively, lung injury that results

in inflammation of pulmonary tissues can induce a spectrum of cytokines and associated mediators (second messengers) that may diffuse into the circulation and likewise alter cardiac tissue or function (*13,15,33,34*).

A second and perhaps more likely theory proposed to explain these phenomena involves reflex mediation via both local and central neural pathways. These pathways are composed of reflex arcs that carry afferent information from sensory stimulation, either locally through both myelinated and unmyelinated networks and/or more centrally through higher levels of the nervous system, to efferent autonomic neurons that may synapse directly in the myocardium. Stimulation of these reflex arcs, well known in rodents for many years (*35*) and only recently appreciated in humans, may have a significant impact on cardiac performance. With respect to the above studies, as O_3 is a known irritant and PM is often acidic, it is plausible to speculate that the initial step in the stimulus–response reflex arc may involve activation of pulmonary irritant receptors leading to irritant receptor-mediated stimulation of parasympathetic pathways. Efferent responses to such stimulation may involve direct effects on pacemaker activity, cardiac muscle contractility, and coronary vascular tone, leading to both chronotrophic and iontrophic deficits and an ensuing cascade of related deleterious events. Unfortunately, at this time there is only limited experimental evidence to support the above scenario. For a more thorough review of the complexities of these reflex arcs, see Yeates (*36*).

Despite our meager knowledge regarding the causative factors responsible for the above effects, elucidation of the underlying mechanism(s) of these responses is important for a number of reasons. First, as the hypothermic response appears to be so prominent in rodents, and the rodent is used in the overwhelming majority of toxicologic studies, it is imperative to consider the impact of these dramatic changes in cardiac and thermoregulatory functional parameters when interpreting the results of rodent studies and when subsequently extrapolating those results to humans (who seem to have only limited capacity for this response under routine circumstances). Second, if it can be demonstrated that this is indeed an irritant receptor-mediated response, then the rodent may prove to be an ideal model for the study of such responses. Finally, if the intricacies of these stimulus–response patterns can be unraveled in the rodent, it may help to explain the often subtle effects of ambient pollutants reported in epidemiologic studies of cardiopulmonary-compromised populations. Clearly, much more work must be done to resolve these issues.
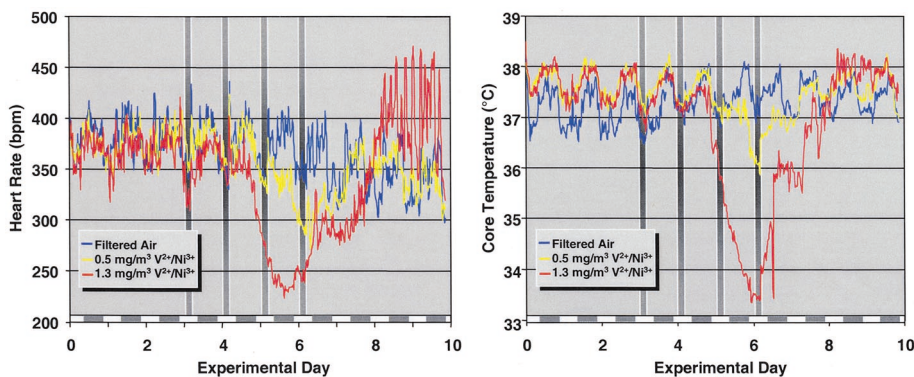

Figure 9. Changes in heart rate and core temperature in healthy rats exposed via inhalation to metallic constituents of residual oil fly ash. Effects of inhalation exposure to primary transition metal constituents of residual oil fly ash particulate matter on heart rate and core temperature of awake, adult Sprague-Dawley rats (*n* = 4/group) maintained at an ambient temperature of 22°C. Studies compared the effects of combined exposures to nickel and vanadium (0.5 mg/m³ or 1.3 mg/m³ NiSO₂ + VSO₄) in healthy rats. The rats were allowed 3 days to acclimate to the experimental conditions, exposed via inhalation (6 hr/day × 4 days; vertical bars), and monitored for 96 hr postexposure. Data were obtained at 10-min intervals but have been averaged over 30-min periods across groups. Intermittent open and shaded boxes above the abscissa indicate daytime and nighttime, respectively. For complete details, see Campen (*25*) and Watkinson et al. (*27*).

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