Short-term secondhand smoke exposure decreases heart rate variability and increases arrhythmia susceptibility in mice

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SECONDHAND SMOKE (SHS), a major source of indoor air pollution, poses a significant threat to the cardiovascular system (4). SHS is classified as a toxic air contaminant (43), and the American Heart Association has concluded that the risk of death due to heart disease is increased by 30% in those exposed to SHS in the home. The risk is thought to be higher in those exposed to SHS in the workplace (40). Epidemiological studies have shown that 70–80% of deaths attributable to SHS are caused by heart disease, suggesting that the cardiovascular system is vulnerable to SHS (7, 26). Despite the widely recognized association of cardiovascular morbidity and mortality with SHS exposure, only nineteen states in the United States require 100% smoke-free public places (2).

Epidemiological studies indicate significant associations between exposure to SHS and cardiovascular-related morbidity and mortality, including sudden cardiac death (17, 18, 28, 39, 41). A number of potential mechanisms have been proposed, including platelet aggregation, endothelial dysfunction, accelerated atherosclerotic lesions, increased circulating inflammatory mediators, oxidative stress, and changes in autonomic function, as indexed by reduced heart rate variability (HRV) (17, 18, 35, 36).

It is likely that multiple mechanisms contribute to SHS-induced cardiovascular-related morbidity and mortality; however, there is a particularly compelling association between exposure to SHS and decreased HRV. Pope and colleagues (35) provided the first direct evidence of SHS-induced decreased HRV. With the use of ambulatory electrocardiogram (ECG) monitoring, ECG signals were recorded in an airport setting while subjects moved between smoking and nonsmoking areas, spending 2 h in each area. The data showed that exposure to SHS was associated with decrements in all measures of HRV (35). Similarly, using 24-h ECG (Holter) recording, Dietrich and colleagues (13) examined 24-h HRV in nonsmokers with or without SHS exposure. The data showed that exposure to SHS either at home or at work for more than 2 h/day is associated with a higher heart rate and lower HRV.

Reduced exercise performance, including impaired heart rate responses, has also been observed when exercise is performed in the presence of SHS (33). The recovery time to return to preexercise heart rate, an indicator of vagal regulation of heart rate, has also been found to be significantly longer when subjects exercise in a smoking environment, suggesting that SHS impairs autonomic regulation of heart rate as well as HRV (29). In addition, a growing number of human studies suggest that decreased autonomic regulation of heart rate, including HRV, is an underlying cause of adverse cardiovascular consequences associated with particulate exposure (34), and SHS and particulate exposure have similar cardiovascular end points (29, 33, 35). These data suggest that SHS-induced reduction in HRV may be a mechanism linking SHS exposure and increased cardiovascular consequences, including arrhythmia vulnerability (8, 23, 27).

The present study used telemetry in conscious mice to determine directly the effects of short-term SHS exposure (3 days, 6
h/day) on HRV, at concentrations close to those that surround a person in the act of smoking or several people smoking in a confined space. Moreover, with the use of in vivo electrophysiological study, the effects of short-term SHS exposure in cardiac excitability and arrhythmia susceptibility was determined. This study is the first to provide evidence for short-term SHS exposure on the decrease in HRV and the associated increase in arrhythmia inducibility in an animal model mimicking the effects of SHS. The developed animal model will allow future studies to test directly the effects of SHS exposure and autonomic dysfunction to further understand the cellular mechanisms.

MATERIALS AND METHODS

All protocols were approved by the Institutional Animal Care and Use Committee in compliance with the Animal Welfare Act and Public Health Service Policy on Humane Care and Use of Laboratory Animals. SHS exposure. Sidestream smoke, a surrogate for SHS, was generated by a modified ADL/II (Little Cambridge, MA) system using 1R4F cigarettes from the University of Kentucky Tobacco and Health Research Institute (Lexington, KY). Two to five cigarettes at a time were smoked under Federal Trade Commission conditions in a staggered fashion at a rate of 1 puff/min (35 ml/puff, 2-s duration). The smoke was diluted with high-efficiency particulate air filters to the appropriate concentration in a mixing chamber and then passed into a stainless steel and glass Hinnertype exposure chamber that was 0.44 m$^3$ in size. Nicotine was sampled daily for 15 min during each 6-h exposure period. Likewise, the total suspended particle (TSP) concentration was sampled with the piezobalance technique for 30 min during each 6-h daily exposure period. During exposures, the mice were housed in their home cage with wire lids, rodent chow, and water ad libitum. The mice were randomly assigned to either a filtered air (FA) exposure, low-concentration SHS exposure, rodent chow, and water ad libitum. The mice were randomly assigned to either a FA exposure ($n$ = 18), low-concentration SHS exposure ($n$ = 18), or high-concentration SHS exposure ($n$ = 17) group. The exposure and ECG recording protocol is shown in Fig. 1A. All mice were exposed to either FA or SHS for 3 days (6 h/day). Continuous ECG signals were recorded in freely moving mice in their home cage at the following time periods: 1) the night after the first day of exposure (6:00 PM to 6:00 AM), 2) the night after the second day of exposure (6:00 PM to 6:00 AM), and 3) the time period encompassing 24 h after the third and last day of exposure (6:00 PM to 6:00 PM). The mice remained in their home cage throughout the exposure and recording period. Figure 1B shows an example trace of the ECG signals recorded with the telemetry system from one conscious mouse.

In vivo electrophysiological protocols. In separate mice, the arrhythmia susceptibility and cardiac conduction properties were tested after 3 days of SHS exposure (Fig. 1A). Since the low concentration of SHS produced no effect on HRV, only the FA control ($n$ = 15) and high concentration of SHS ($n$ = 12) were included in this part of the studies. In vivo electrophysiological studies were performed as previously described in a blinded fashion (6, 46). Briefly, the animals were anesthetized with pentobarbital sodium (40 mg/kg ip). A 1.7-Fr octopolar catheter with an interelectrode spacing of 0.5 mm (CIBer mouse EP, NuMed, Hopkinton, NY) was inserted via the internal jugular vein into the right atrium and ventricle. Surface electrocardiograms and intracardiac electrograms were recorded using Recording System (CardioLab, Prucka, GE Medical System). Pacing protocols were performed via the catheter. Standard pacing protocols were used to determine the electrophysiological parameters, including sinus node recovery; atrial, atrioventricular (AV) nodal, and ventricular refractory periods; and AV nodal conduction properties. To induce atrial and ventricular tachycardia and fibrillation, programmed extra-stimulation techniques and burst pacing were used. Programmed right atrial and right ventricular double and triple extra-stimulation techniques were performed at 100-ms drive cycle length (CL), down to a minimum coupling interval of 10 ms. Right atrial and right ventricular burst pacing were performed as eight 50-ms and four 30-ms CL train episodes repeated several times, up to a maximum 1-min time limit of total stimulation. For heart conduction properties, standard pacing protocols were used to determine the electrophysiological parameters, including sinus node recovery time; atrial, AV nodal, and ventricular refractory periods; and AV nodal conduction properties. Each animal underwent an identical pacing and programmed stimulation protocol.

Data acquisition and analysis. All values are means ± SE unless otherwise indicated. Differences were considered significant at $P < 0.05$. The ECG signals were recorded at 5 kHz with Dataquest A.R.T. (Data Sciences International) program. The raw data were converted to binary format with the MiniAnalysis program (Synaptosoft, Deca-
tur, GA) and analyzed with the Nevorkard SA-HRV (Intelectual Services, Ljubljana, Slovenia) program. The accuracy of the R-wave detection was visually confirmed. All arrhythmic events, ectopics, and missing beats were excluded from data analysis. Only normal-to-normal R-R intervals were used for HRV analysis in the time domain. Five-minute CLs are commonly used for human HRV analysis, whereas most mouse studies used 2-min CLs. In a preliminary analysis in three mice, the standard deviation of all normal-to-normal R-R interval averages (SDANNs) obtained from 2-min CLs were similarly lower than that of 5-min CLs in control (4.9%) and exposed (4.4%) mice. Therefore, the 2-min CL was used because 2-min CL is most frequently used in mice. The standard HRV parameters determined are listed and defined in Table 1. Since the duration of the recording used for HRV analysis could affect the outcome of the HRV, the recordings taken over the 24-h period after the third day of exposure were divided into 12-h sections for HRV analysis to match the time length of recording periods 1 and 2. Three mice in each group only had recordings for the first 24 h after the third day of exposure; a two-way mixed-model ANOVA was used for analyzing the difference between the FA- and SHS-exposed mice with exposure (FA, low SHS, and high SHS) as one factor and time (recording periods) as the other factor and followed by Fisher least significant differences test when appropriate. The raw numbers of HRV were used for statistical analysis. Even though the statistical analysis was performed with the raw numbers, to demonstrate the SHS-induced changes, all exposure data in the figures are expressed as percent changes from the averages of the FA-exposed control group.

For in vivo electrophysiological studies, the programmed extrastimulation techniques and the stimulation duration of atrial and ventricular burst pacing were the same in all mice for the comparison of the inducibility in each mouse. The sinus node function was evaluated at the same pacing CL, and the sinus node recovery time (SNRT) and corrected SNRT were measured (6, 25, 45). Refractory periods at the same pacing CL were measured for the AV node and the right ventricle (6, 25, 45). Inducibility for atrial and ventricular arrhythmias was examined, as well as the presence of heart block or sinus arrest. Sustained atrial or ventricular arrhythmias were defined as atrial arrhythmias lasting longer than 30 s. Reproducibility was defined as greater than one episode of induced atrial or ventricular tachycardia.

Fisher exact test was used for noncontinuous variables, and Student’s t-test was used for continuous variables. For atrial and ventricular arrhythmias and heart block, a Fisher exact test was used for comparisons between the FA control and SHS-exposed mice. For recovery time and refractory periods, a Student’s t-test was used for a comparison between the FA control and SHS-exposed mice.

RESULTS

Table 2 shows the 12-h baseline day- and nighttime heart rate and HRV recorded after 3 days of FA exposure. The mice displayed a typical circadian rhythm having lower heart rate and higher HRV during the daytime when the vagal regulation is expected to be higher.

Short-term exposure to SHS reduced measures of HRV. Exposure to SHS for 6 h/day for 3 days reduced measures of HRV in C57BL/6 mice. Figure 2 shows an example of a tachogram from a FA-exposed control mouse (Fig. 2A) and from a high SHS-exposed mouse (Fig. 2B) recorded during the night after the first day of exposure. The SHS-exposed mouse showed a reduced HRV as indicated by having less frequent and lower magnitude “fluctuations” in the R-R intervals.

The group data show that short-term exposures to the low concentration of SHS had no significant effect on R-R interval or measures of HRV during the nonsmoking periods (Fig. 3). Exposure to the higher concentration of SHS resulted in a physiologically small but statistically significant increase in R-R interval (Fig. 4A, \( P < 0.05 \)) compared with FA-exposed controls. Exposure to the higher concentration of SHS also significantly decreased measures of overall HRV: SDNN (Fig. 4B, \( P < 0.05 \)), SDNNIDX, and short-term HRV (rMSSD, Fig. 4F, \( P < 0.05 \)). The HRV component longer than 2 min in CL (SDANN, Fig. 4D, \( P < 0.05 \)), SDANNIDX, and short-term HRV (rMSSD, Fig. 4F, \( P < 0.05 \), high SHS vs. FA and low SHS) were also significantly decreased. There was no significant difference in the HRV component due to the 2-min CL (SDNNIDX, Fig. 4E). The SHS-induced

Table 1. Time domain measures of HRV and definitions

<table>
<thead>
<tr>
<th>Measures of variability</th>
<th>Definitions</th>
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<tr>
<td>Overall HRV</td>
<td>SD of all normal-to-normal R-R intervals</td>
</tr>
<tr>
<td>SDNN</td>
<td>Coefficient of variance, 100(SDNN/mean R-R)</td>
</tr>
<tr>
<td>CV%</td>
<td>SD of all 2-min R-R interval averages</td>
</tr>
<tr>
<td>HRV due to CL &gt; 2 min</td>
<td>Averages of SD of all 2-min R-R intervals</td>
</tr>
<tr>
<td>SDANN</td>
<td>Short-term HRV</td>
</tr>
<tr>
<td>HRV due to 2-min cycles</td>
<td>rMSSD, Root mean square of successive difference</td>
</tr>
</tbody>
</table>

Values are means ± SE. HRV, heart rate variability; CL, cycle length.
reduction on SDANN and rMSSD were greater when the baseline R-R interval was taken into account (data not shown).

The overall HRV (SDNN, Fig. 4B, left) and the HRV due to CL longer than 2 min (SDANN, Fig. 4D) show the greatest decrease on the night after the first day of exposure (10–19% decrease). The decrease in these HRV measures persisted to a smaller extent (6–10% decline) after the second and third day of exposure and 24 h after the third day of exposure (recording periods 2, 3, and 4, respectively). In contrast, short-term HRV (rMSSD, Fig. 4F) gradually decreased over the 3 days of exposure and reached a maximum reduction (16–18%) after the cessation of the 3-day exposure (recording periods 3 and 4). The data suggest that the SHS-induced decrease in HRV can extend to the nonsmoking period.

Short-term exposure to SHS increased arrhythmia inducibility in mice. Short-term SHS exposure increased the arrhythmia inducibility, including atrial fibrillation and ventricular tachycardia/fibrillation. Figure 5 shows examples of sustained ventricular tachycardia/fibrillation (Fig. 5A), atrial fibrillation (Fig. 5B), and intermittent AV block (Fig. 5C) from SHS-
HRV. Our data also suggest that SHS effects on short-term (SDANN) rather than the short-term components (rMSSD) of HRV are associated with measures of long-term components of HRV, whereas both cardiac sympathetic and parasympathetic modulation have a longer-term and overall effect on HRV. In mice, the blockade of cardiac parasympathetic modulation was associated with a decrease in long-term HRV parameters and only had a minimal effect on the short-term HRV. The data suggest that, in mice, although sympathetic modulation had greater influence on the baseline heart rate, the long-term HRV is mostly under cardiac parasympathetic regulation and the long-term HRV is under dual regulation as in humans.

In humans, exposure to SHS for 2 h in an airport smoking area significantly decreased long-term measures of HRV (35). Similarly, SHS exposure for longer than 2 h/day significantly decreased the long-term HRV as well as HR analyzed over a 24-h period (13). The data suggest that SHS exposure may change the autonomic balance toward sympathetic modulation. Similar to these studies in humans (13, 35), the initial drop in HRV in mice, after 1 day of exposure, was predominately associated with a decrease in long-term components (SDANN) rather than the short-term components (rMSSD) of HRV. The data suggest that the activation of the cardiovascular sympathetic nervous system during exposure period and immediately after exposure might contribute to increase the risk factor for arrhythmias in both humans and mice. In addition, with repeated exposure for 3 days, the accumulative effect of SHS on short-term HRV suggested that a reduced cardiac parasympathetic modulation may play a more important role with repeated exposures.

Several factors should be taken into consideration when comparing the current study to the previous human studies. First, there is the species difference in heart rate regulation between humans and mice. Second, heart rate and HRV re-

Table 3. Electrophysiological changes and arrhythmia inducibility in SHS-exposed mice

<table>
<thead>
<tr>
<th>Control</th>
<th>SHS Exposure</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>15</td>
</tr>
<tr>
<td>VT/VF (%)</td>
<td>0/15 (0)</td>
</tr>
<tr>
<td>AV block (%)</td>
<td>0/15 (0)</td>
</tr>
<tr>
<td>SNRT (at CL 80 ms), ms</td>
<td>145±35</td>
</tr>
<tr>
<td>AVNERP, ms</td>
<td>50±12</td>
</tr>
<tr>
<td>VERP, ms</td>
<td>73±8</td>
</tr>
<tr>
<td>VERP, ms</td>
<td>47±8</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of mice. SHS, secondhand smoke; VT/VF, ventricular tachycardia or fibrillation; SNRT, sinus node recovery time; AVNERP, atrioventricular (AV) node refractory period; VERP, right ventricular refractory period. *P < 0.05 (Fisher exact test); †P < 0.05 (Student’s t-test).
sponses to SHS exposure in the present study were not recorded during the exposure period and no direct comparison can be made. Finally, the particle levels, nicotine concentrations, and carbon monoxide levels were higher in the present study. In this regard, we wished to study the effects of SHS within a range of concentrations that could be generated under a wide variety of conditions. The high dose of SHS is a concentration that can be easily achieved in poorly ventilated, small enclosed spaces (such as automobiles) (24). Although the “high” concentration of SHS would not be typically encountered for prolonged periods of time, this provides a range to begin to elucidate whether a dose-response effect might be seen for HRV following short-term exposure to SHS. Although testing the exposure effects with a lower concentration over a longer period of time is also important, the present study provides the first evidence for the potential mechanisms mediating the SHS-induced cardiovascular effects. It is conceivable that a more extended exposure, such as when working in a smoky bar, might result in a more prolonged effect with a lower exposure concentration. This is particularly important when considering lifetime exposures; it is estimated that 63% of nonsmokers are exposed to SHS for more than 1 h/wk, 35% of nonsmokers are exposed to SHS for more than 10 h/wk, and 16% of nonsmokers are exposed for at least 40 h/wk (40).

Studies have shown that a decreased HRV is associated with deleterious health outcomes and an increased risk for cardiovascular-related sudden death, particularly in individuals with cardiovascular disease (5, 10), diabetes (37), Parkinson’s disease (20), and depression (1). More importantly, the risks are not just limited to susceptible groups. Reduced HRV may predict mortality even in the absence of overt disease (12). Although it is well recognized that a reduced HRV is a marker for a reduced autonomic function, the mechanism by which the reduced autonomic function contributes to cardiovascular-related mortality is not well understood. The present study suggests that the increase in arrhythmias inducibility associated with a reduced autonomic function in SHS-exposed mice may account for the findings in the epidemiologic data linking SHS to cardiac arrhythmias and sudden cardiac death. Indeed, previous published studies have documented that control mice do not show evidence of inducible atrial fibrillation without pharmacological provocation (0% incidence of atrial fibrillation), and wild-type mice have only a 2% incidence of ventricular tachycardia or fibrillation (6, 25, 45). Recently, Smith and colleagues (38) presented the most direct evidence showing the link between autonomic modulation and ventricular repolarization that is independent of heart rate. In humans, autonomic blockade exagerrated drug-induced prolongation of the Q-T interval, a known substrate for polymorphic ventricular tachycardia. Although the exact cellular mechanisms underlying the effect are unclear, the study demonstrated the link between a reduced autonomic function and the susceptibility to arrhythmia.

In addition to the increase in arrhythmia inducibility, the present study showed that the incidence of AV block was increased from near 0% in control mice to 33% after SHS exposure. Because of the nature of the experiment, although desirable, the 24-h HRV and the cardiac electrophysiology were not performed in the same animals. Additional studies are required to further delineate the underlying mechanisms for the observed AV block. For example, the use of autonomic blockade on the SHS-induced changes in cardiac electrophysiology will help to reveal the contribution of each autonomic limb in the modulation of cardiac electrophysiology after exposure.

Short-term SHS exposure alters the cardiac electrical properties of the intact mouse (Table 3). The SHS exposure results in the prolongation of the recovery of the sinus node function, which is one mechanism speculated to cause atrial fibrillation and sick sinus syndrome in humans. Additionally, SHS exposure shortened the refractory period over the AV node, which could accelerate the conduction to the ventricular and promote the development of ventricular arrhythmias. Finally, SHS shortened the ventricular refractory period, which may result in the increase in ventricular susceptibility to the development of ventricular arrhythmias as the electrical wavelets have more time to synergize and regenerate.

With more than 4,000 components in SHS, the exposure-induced cardiovascular consequences are unlikely to be caused by a single SHS component. Suspect components affecting cardiovascular health include carbon monoxide, nicotine, and particulate matter. The main concern with increased carbon monoxide levels resides in the carboxyhemoglobin levels. Although not measured in this study, the carboxyhemoglobin levels were below 1% immediately following exposure to the low concentration of SHS (unpublished observation). Based on the prediction models, it is estimated that the carboxyhemoglobin levels increased gradually over the 6 h of exposure and reached a maximal of 7.6–10% at the end of 6 h of exposure (19, 42). At these carboxyhemoglobin levels, the available studies on the effect of increased carboxyhemoglobin on the cardiovascular regulation are mixed; some showed no change in the occurrence of arrhythmias (9, 15, 44) and no change in heart rate and blood pressure (14, 22, 44), whereas others showed a decrease in ventricular fibrillation threshold (3, 11). Importantly, the carboxyhemoglobin levels after high-concentration SHS exposure are expected to decrease down to near control levels within an hour after the exposure (42). Therefore, the carboxyhemoglobin level during the HRV recording periods is not expected to be elevated. In addition, Pope et al. (35) found that the individuals consistently showed a lower HRV in the smoking areas of an airport even though the carbon monoxide levels were extremely low. Taken together, carbon monoxide does not seem to play a major role in SHS-induced reduction of HRV, arrhythmias, and heart block.

In terms of the contribution from nicotine, in the present study, the SHS environment is generated in a glass and stainless steel chamber of small capacity. Because of the limited surface found in such a chamber, nicotine can rapidly saturate this surface and lead to the off-gassing of nicotine from the surfaces into the air, resulting in a higher nicotine concentration. This is a limitation of the exposure system but is consistent with the properties of SHS and a small enclosed space in human exposure. This would not be considered as active smoking since the gases and particulates emitted arise predominantly from the smoke being emitted off the end of the cigarette between puffs, and the particle concentrations are well below any that is designed to simulate active smoking (21, 31, 32). Importantly, Lucini and colleagues (30) showed that nicotine patches produce a much smaller reduction in HRV compared with smoking, suggesting that nicotine is not the only component causing the reduction in HRV.

Finally, suspended particles from a burning cigarette may play an important role in SHS-induced reduced HRV (35).
Particulate matter exposure has the same characteristic health consequences as SHS exposure, such as cardiovascular mortality and morbidity (34). More importantly, a number of studies show that exposure to particulate matter, particularly in the fine and ultrafine range, is associated with a decreased HRV (34).

In summary, the present study shows that SHS exposure results in a reduced HRV and increased arrhythmia susceptibility, suggesting that the mouse is a suitable research model for future studies investigating the biochemical, cellular, and physiological mechanisms of SHS-induced cardiovascular morbidity and mortality. Further electrophysiological investigations will help elucidate any neuroplastic changes that may occur in the regions responsible for autonomic cardiovascular control, whereas biochemical experiments may help explain the neuroplasticity.

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