Secondhand tobacco smoke, arterial stiffness, and altered circadian blood pressure patterns are associated with lung inflammation and oxidative stress in rats

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Gentner NJ, Weber LP. Secondhand tobacco smoke, arterial stiffness, and altered circadian blood pressure patterns are associated with lung inflammation and oxidative stress in rats. Am J Physiol Heart Circ Physiol 302: H818–H825, 2012. First published December 2, 2011; doi:10.1152/ajpheart.00852.2011.—Chronic smoking and secondhand tobacco smoke exposure are major risk factors for cardiovascular disease that are known to adversely alter the structural and mechanical properties of arteries. The objective of this study was to determine the effects of subchronic secondhand tobacco smoke exposure on circadian blood pressure patterns, arterial stiffness, and possible sources of oxidative stress in conscious, unsedated radiotelemetry-implanted rats. Pulse wave change in pressure over time (dP/dt) was used an indicator of arterial stiffness and was compared with both structural (wall thickness) and functional (nitric oxide production and bioactivity and endothelin-1 levels) features of the arterial wall. In addition, histology of lung, heart, and liver was examined as well as pulmonary and hepatic detoxifying enzyme activity (cytochrome P450, specifically CYP1A1). Subchronic secondhand tobacco smoke exposure altered the circadian pattern of heart rate and blood pressure, with a loss in the normal dipping pattern of blood pressure during sleep. Secondhand tobacco smoke exposure also increased pulse wave dP/dt in the absence of any structural modifications in the arterial wall. Furthermore, although nitric oxide production and endothelin-1 levels were not altered by secondhand tobacco smoke, there was increased inactivation of nitric oxide as indicated by peroxynitrite production. Increased lung neutrophils or pulmonary CYP1A1 may be responsible for the increase in oxidative stress in rats exposed to secondhand tobacco smoke. In turn, this may be related to the observed failure of blood pressure to dip during periods of sleep and a possible increase in arterial stiffness.

nitric oxide; circadian rhythm; endothelial dysfunction; inflammation

SUBSTANTIAL EPIDEMIOLOGICAL evidence indicates that smoking is a major factor, if not the number one preventable risk factor for cardiovascular disease (21). Many of the traditional risk factors for cardiovascular disease, including smoking, adversely alter endothelial function as well as the structure and mechanical properties of the arterial wall (14). The endothelium releases nitric oxide (NO), which in turn causes vasodilation (11), but also releases powerful vasoconstrictors such as endothelin-1 (ET-1) that physiologically balance the effect of NO (40). Although much attention has been paid to alterations in NO release or activity in cardiovascular disease (11), elevated ET-1 release is also commonly reported in atherosclerosis and hypertension (40). Arterial stiffness is a powerful, independent predictor of cardiovascular risk (2) and is influenced by both structural and functional features of the arterial wall (17). Increasingly, it is being recognized that secondhand tobacco smoke [also commonly referred to as environmental tobacco smoke (ETS)] poses a similar risk for cardiovascular disease (1). However, it is not clear whether the same smoking-associated changes in endothelial function or arterial stiffness also occur with secondhand tobacco smoke exposure.

It is a paradox that while smoking increases arterial stiffness, epidemiological studies have generally shown that the blood pressure of smokers is lower than that of nonsmokers (e.g., 16). A previous study (12) from this laboratory on the acute effects of secondhand tobacco smoke in rodents supports this observation, while another study (43) from this laboratory showed that acute secondhand tobacco smoke effects increased blood pressure in pigs. It has long been known that circadian rhythms are a characteristic feature of blood pressure regulation and hypertension (36). Ambulatory blood pressure monitoring is increasingly being used in humans to evaluate the effects of lifestyle (e.g., smoking) on blood pressure. It provides greater accuracy than casual measurements and allows for the analysis of circadian rhythms in blood pressure (32). A previous study (31) reported that stress and mainstream cigarette smoke in rats altered the daily rhythm characteristics of heart rate, body temperature, and locomotor activity. However, whether secondhand tobacco smoke has a similar effect on blood pressure patterns is not known. Polycyclic aromatic hydrocarbons (PAHs), agonists at the aryl hydrocarbon receptor (AhR), are found in the tar particulate phase of cigarette smoke (19, 33). This receptor is an orphan receptor that is known to be activated by dioxins, PAHs, and many other environmental contaminants but has an unknown function in normal physiology (28). It is, however, part of the Per-ARNT-Sim superfamily of proteins, many of which are involved in circadian clock signaling. Recent findings have led to the hypothesis that the AhR plays a role in circadian rhythms (48). Two recent studies have reported that subchronic exposure to AhR agonists, either dietary 2,3,7,8-tetrachlorodibenzo-p-dioxin (23) or intranasal administration of the PAH benzo-a-pyrene (13) caused significant increases in blood pressure. A striking finding of the latter study conducted in this laboratory (13) was that intranasal benzo-a-pyrene provoked a failure of blood pressure to dip during periods of sleep inactivity in rats. The altered circadian blood pressure pattern elicited by intranasal benzo-a-pyrene was associated with pulmonary inflammation and increased oxidative-stress-mediated inactivation of NO. Thus a major goal of this study was to examine whether subchronic secondhand tobacco smoke exposure would cause a similar alteration in the circadian rhythm of blood pressure and then to explore
Tobacco smoke alters circadian blood pressure pattern

If this is related to pulmonary oxidative stress, inflammation, and AhR activation.

We hypothesize that similar to smoking, exposure to a high, but environmentally relevant, level of secondhand tobacco smoke will increase ET-1 and that oxidative stress arising from lung inflammation and/or AhR activation will reduce NO bioactivity, leading to increased arterial stiffness and blood pressure. We (12) have previously demonstrated that pulse wave change in pressure over time (dP/dt) collected from blood pressure telemetry-implanted rats can be used as an indicator of acute, active changes in arterial stiffness in rats. Therefore, the objective of this study was to determine whether daily 1-h secondhand tobacco smoke exposure for 28 days would induce changes in arterial stiffness and circadian blood pressure patterns in rats. A second objective was to examine whether any changes were related to oxidative stress, inflammation, and/or cytochrome P450 1A1 activity (CYP1A1; an indicator of AhR activation) in the lung and liver. In addition, structural (wall thickness) and functional (NO production and bioavailability and ET-1) features of the arterial wall were examined.

Materials and methods

Animals and surgery. All protocols were approved by the Animal Research Ethics Board at the University of Saskatchewan in accordance with the Canadian Council on Animal Care guidelines. Male Sprague-Dawley rats (195–225 g) were housed individually under standard conditions (12:12-h light-dark) with food and water available ad libitum except during exposures. A subsample of rats from each treatment group (n = 4 rats/group) was surgically implanted with blood pressure radiotelemetry devices as described previously (12).

Briefly, a PA-C10 radiotelemetry blood pressure transmitter (Data Sciences International, St. Paul, MN) was implanted into the femoral artery and advanced toward the iliac artery with the transmitter body placed subcutaneously in the left flank. Rats were allowed to recover from surgery for ≥14 days before experiments.

Secondhand tobacco smoke exposures. Secondhand tobacco smoke (mainstream plus sidestream smoke) was generated with a single cigarette manual smoking machine from CH Technologies at a rate of 3 puffs/min (57 ml/puff, 2-s duration). The secondhand tobacco smoke was mixed with indoor air and pumped into a 89.5-l inhalation chamber. Pumps controlling inflow and outflow were both set at 6 l/min. Animals were entered into the exposure study in a staggered fashion (n = 2 rats per day for each treatment). During exposures, rats were restrained in individual wire mesh tubes within the chamber and exposed together (n = 8) to secondhand tobacco smoke from three regular-sized cigarettes (Canadian Classics, Rothmans, and Benson & Hedges) during a 1-h exposure period every day for 28 days. Sham-exposed rats (n = 8) were restrained and placed in an identical clean exposure chamber under the same conditions except clean, unfiltered room air was pumped into the chambers for 1 h every day for 28 days. Exposures were conducted between 8:00–10:00 AM every day (corresponding to the beginning of the “lights on” or sleep period every day) except on days when weekly 24-h reads were performed when exposures were performed after 10:00 AM. A 1-mo exposure period was chosen based on a previous report (49) indicating that a similar exposure period was sufficient to induce myocardial remodeling in rats.

Exposure conditions were assessed in preliminary experiments by monitoring the total particulate concentrations in the chambers for sham and secondhand tobacco smoke exposures without rats using a SKC constant airflow pump (Universal 224-PCXR-8; Eighty Four, PA) fitted with preweighed mixed cellulose ester filters (0.8 µm, SKC; Eighty Four). The SKC pump sampled air at 2 l/min via a separate air sampling port than those used for air inflow/outflow. The regular outflow pump was adjusted to 4 l/min so that total outflow remained balanced with total inflow at 6 l/min. Air was sampled for 1 h for sham conditions or for a shorter time (20 min) for secondhand tobacco smoke exposure conditions to prevent cellulosic filters from becoming saturated with the higher particulate conditions. For both exposure conditions, data were calculated and expressed as total particulates that would have been generated during a 1-h continuous exposure. Carbon monoxide (CO) levels produced during exposures were also measured by placing a T40 Rattler CO monitor (Industrial Scientific, Oakdale, CA) within the chamber without rats during preliminary experiments. Separate preliminary experiments to assess O2–CO2 air levels were conducted with eight rats in the exposure chamber using a Criticare Poet IQ Multiparameter gas monitor (Criticare Systems). CO2 levels remained <1% and mean O2 levels were 21% (n = 3 determinations) during the sham exposures. The measured particulate concentrations (Table 1) during secondhand tobacco smoke exposures were found to be within the maximum range reported in smoking homes in the United States (5) but lower than those reported for tobacco smoke levels in automobiles with the windows closed (20). Therefore, the secondhand tobacco smoke exposure conditions in the current study are on the high end but still relevant to what humans may encounter in the real world.

Plasma nitrate/nitrite, ET-1, cotinine, nitrotyrosine, and ethoxyresorufin-o-deethylase. Blood samples were collected at 30 min after sham and secondhand tobacco smoke exposures by immersing the tail in warm tap water for 2 min and then inserting a butterfly catheter into the tail vein and allowing gravity to facilitate bleeding into a tube from the catheter. Plasma nitrate/nitrite (NOx) levels were measured using a commercially available enzyme-based kit (nitric oxide quantitation kit; Active Motif North America, Carlsbad, CA). Plasma ET-1 (R&D Systems, Minneapolis, MN), cotinine (Bio-Quant, San Diego, CA), and nitrotyrosine (Cell Sciences) levels were quantitated using commercially available ELISA. Rat liver and lung homogenates were analyzed for ethoxyresorufin-o-deethylase (EROD) activity (a CYP1A1-specific marker enzyme activity), as previously described (44). A positive control sample (liver microsomes prepared from rainbow trout injected 2 min with 10 mg/kg benzo-a-pyrene over 72 h) was analyzed in every sample series to ensure assay performance.

Endothelium-dependent vasorelaxation measurements. All chemicals were purchased from Sigma-Aldrich measurements. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) unless specified otherwise. On day 29 of the experiment, rats (n = 8 per group) were exposed to sham or secondhand tobacco smoke after surgery and then were housed individually under standard conditions (12:12-h light-dark) with food and water available ad libitum except during exposures. A subsample of rats from each group was used for plasma cotinine quantitation (n = 8 rats per group), and data are means ± SE. Temperature and carbon monoxide (CO) were measured every 30 s for a total of 1 h (sham) or 20 min (secondhand tobacco smoke) in preliminary experiments in the absence of rats and are expressed as mean CO for a 1-h exposure. Particulates were sampled continuously for 1 h (sham) or 20 min (secondhand tobacco smoke) and are expressed as total particulate matter for a 1-h exposure period. Rats were then exposed daily for 1 h to sham (unlit cigarette) or secondhand tobacco smoke (3 cigarettes) for 28 days. Ethoxyresorufin-o-deethylase (EROD) activity was analyzed in liver, and lung microsomes were prepared from rats (n = 8 per group) after 28 days of secondhand tobacco smoke or sham exposure. *P < 0.01 and †P < 0.0001 vs. sham-exposed group one-way ANOVA.

Table 1. Second hand tobacco smoke exposure conditions, plasma cotinine concentrations, and liver and lung ethoxyresorufin-o-deethylase activity

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Secondhand Smoke</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature, °C</td>
<td>25</td>
<td>26</td>
</tr>
<tr>
<td>CO, mean parts per million</td>
<td>358</td>
<td>360</td>
</tr>
<tr>
<td>Particulates, µg/m³</td>
<td>30</td>
<td>360</td>
</tr>
<tr>
<td>Plasma cotinine, ng/ml</td>
<td>2.1 ± 0.4</td>
<td>51.6 ± 4.8†</td>
</tr>
<tr>
<td>Liver EROD, fmol·min⁻¹·mg⁻¹</td>
<td>0.46 ± 0.03</td>
<td>0.46 ± 0.04</td>
</tr>
<tr>
<td>Lung EROD, fmol·min⁻¹·mg⁻¹</td>
<td>0.06 ± 0.02</td>
<td>0.22 ± 0.04*</td>
</tr>
</tbody>
</table>

Blood samples collected after exposure on day 28 were used for plasma cotinine quantitation (n = 8 rats per group), and data are means ± SE. Temperature and carbon monoxide (CO) were measured every 30 s for a total of 1 h (sham) or 20 min (secondhand tobacco smoke) in preliminary experiments in the absence of rats and are expressed as mean CO for a 1-h exposure. Particulates were sampled continuously for 1 h (sham) or 20 min (secondhand tobacco smoke) and are expressed as total particulate matter for a 1-h exposure period. Rats were then exposed daily for 1 h to sham (unlit cigarette) or secondhand tobacco smoke (3 cigarettes) for 28 days. Ethoxyresorufin-o-deethylase (EROD) activity was analyzed in liver, and lung microsomes were prepared from rats (n = 8 per group) after 28 days of secondhand tobacco smoke or sham exposure. *P < 0.01 and †P < 0.0001 vs. sham-exposed group one-way ANOVA.
treatment group) were anaesthetized with isoflurane and euthanized with pentobarbital sodium (0.44 ml/kg iv). Ring segments were rapidly excised from the aorta for tissue bath studies of endothelial-dependent relaxation, as previously described (45). Phenylephrine (10^{-7} M) was then added to each bath to preconstrict the aortic rings. At the plateau in force, carbachol was added in cumulative concentrations from 10^{-8} M to 10^{-3} M. Vasorelaxation was expressed as a percentage of the plateau force to phenylephrine.

**Histological analysis.** Whole heart, a portion of the lung (lower left lobe), thoracic aorta immediately after the arch, and the abdominal aorta with iliac branches were dissected from rats euthanized at day 29 of treatment (n = 8 per treatment group) and transferred into neutral buffered formalin. After 24 h, samples were transferred to 70% ethanol and stored until histological analysis could be performed. Samples were paraffin-embedded and cross-sectioned (5 μm, serial sections) and then stained with hematoxylin and eosin. Size analyses (wall thickness and luminal diameter) and histopathology were performed in heart and all arteries collected. However, none of these analyses showed any significant effect of secondhand tobacco smoke exposure (data not shown). For the lung, cross sections were scanned for four zones of high neutrophils, where the number of neutrophils was counted at ×400 magnification. A single mean value per animal was used for all statistical comparisons.

**Arterial stiffness, blood pressure, and statistical analyses.** Over the course of the 28-day exposure period, blood pressure and heart rate data were collected weekly for 20 min before daily exposures began (~22 h after the previous day’s exposure). Manual rather than continuous reads were performed daily to conserve battery life of the telemetry implant since implants were turned off between reads. Also, to obtain the circadian pattern of blood pressure and heart rate, data were collected once a week for a period of 24 consecutive hours (10 min of automatic sampling per rat per hour) starting within 2 h of the end of the daily exposure and ending before the exposure periods the next day. Arterial wave pulse dP/dt has previously been validated by our laboratory (12) to reflect active changes in arterial stiffness using radiotelemetry in rats. With arterial stiffening (whether through active or structural changes), pulse wave velocity throughout the arterial tree increases and the reflected wave tends to increase systolic pressure. Thus the amplitude of the incident wave has been shown to increase in humans, along with the slope of the rise in arterial dP/dt (30). Therefore, values for dP/dt were extracted from arterial pressure waveforms and averaged over 10-min segments every hour using DSI Dataquest ART analysis software (St. Paul, MN). To minimize effects of variation among individuals in baseline blood pressure values and determine treatment effects, preexposure (baseline or day 0) blood pressure, heart rate, and dP/dt were subtracted from postexposure values from the same individual. The percent dip in blood pressure was calculated as the blood pressure, heart rate, or dP/dt value during the dark (mean value for 12-h period) minus the value during the light period (mean value for 12-h period) and multiplied by 100. The mean values during each portion of the light cycle were chosen to represent periods when the rats were expected to be awake/active vs. sleeping/inactive, respectively. All data are expressed as means ± SE. Differences among groups were detected using one-way (single time point data), two-way (data with factors for week of exposure, and treatment), or three-way (data with factors for time of day, week of exposure and treatment) ANOVA followed by Tukey’s posteriori tests as appropriate. However, all organ weights and tissue sizes were analyzed by analysis of covariance with body weight as a covariate.

**RESULTS**

Secondhand tobacco smoke exposure had higher total suspended particulates and carbon monoxide levels compared with sham exposure (Table 1). Plasma cotinine levels of tobacco smoke-exposed rats were also significantly higher compared with sham-exposed rats at 30 min after exposure. EROD activity was analyzed in liver and lung microsomes as an enzymatic marker for CYP1A1 induction and biomarker of exposure to the PAH component of secondhand tobacco smoke. Lung microsomes of tobacco smoke-exposed rats showed higher EROD activity than those of sham-exposed rats while liver EROD activity was not significantly different between treatment groups (Table 1). There were no significant differences in body, lung, or liver weight of rats exposed to secondhand tobacco smoke compared with sham after 28 days of exposure (data not shown).

To obtain the circadian pattern of blood pressure, data were collected once a week for a period of 24 consecutive hours and are expressed as a change from baseline (values obtained in the same individual rat before entering experiment subtracted from value obtained during experiment). Secondhand tobacco smoke altered the circadian pattern of systolic pressure, diastolic pressure and heart rate (Fig. 1; P < 0.05 for treatment factor in separate three-way ANOVAs for each end point) as well as pulse pressure (data not shown). Systolic pressure, diastolic pressures, and pulse wave dP/dt were higher in tobacco smoke-exposed compared with sham-exposed rats (Fig. 1, A, B, and D) with this difference becoming significantly greater over time (P < 0.05 for week factor in three-way ANOVAs). In contrast, heart rate in tobacco smoke-exposed rats was significantly lower at several time points during the period where lights were out compared with sham-exposed rats (Fig. 1C). Paradoxically, heart rate tended to be higher in most time points during periods where lights were on in the same tobacco smoke-exposed compared with sham-exposed rats (Fig. 1C). Blood pressure and dP/dt of sham-exposed rats were slightly lower at week 4 than they were at baseline (a negative change from baseline; Table 2) while in the tobacco smoke group, blood pressure, and dP/dt had increased from the baseline values (a positive change from baseline; Table 2 and Fig. 1).

The 24-h circadian pattern shows that secondhand tobacco smoke increased systolic and diastolic pressure to a greater extent during the period of light, a time of inactivity/sleep in rats (Fig. 1, A and B). More specifically, there was a significant reduction in the percent dip of systolic and diastolic pressures from the dark/active period to the light/inactive period in the tobacco smoke-exposed rats (Table 2), while the percent dip for heart rate and dP/dt was not significantly affected by secondhand tobacco smoke exposure (Table 2). When the data were averaged for the entire 24-h period, the change in systolic and diastolic pressures from baseline were also significantly increased in tobacco smoke-exposed rat compared with sham-exposed rats (Table 2). In the tobacco smoke-exposed group, the 24-h average blood pressure and heart rate at week 4 of the exposure were 124 ± 1 mmHg (systolic blood pressure), 87 ± 2 mmHg (diastolic blood pressure), and 309 ± 5 beats/min (heart rate). At 4 wk of sham exposure, blood pressure and heart rate were 122 ± 1 mmHg (systolic), 84 ± 2 mmHg (diastolic), and 324 ± 10 beats/min. It is important to note that while the data for systolic pressure, diastolic pressure, and heart rate in the current experiment all exhibited significant circadian fluctuations (P < 0.05 for time of day factor in three-way ANOVAs; Fig. 1), dP/dt did not (P = 0.656 for time of day factor; Fig. 1D). However, secondhand tobacco smoke exposure did cause a significant increase in dP/dt that...
Results were analyzed by three-way ANOVA with treatment, week, and time of day as factors. After three-way ANOVA, there were no significant interactions between the factors in three-way ANOVA analyses. Significant difference in contractile response to 10^{-7} M phenylephrine in aorta isolated from rats after 4 wk of secondhand tobacco smoke exposure (0.20 ± 0.04 g tension/mg tissue; n = 7) compared with sham exposure (0.22 ± 0.03 g tension/mg tissue; n = 8). Furthermore, endothelium-dependent vasodilation to acetylcholine in the same isolated aorta was not significantly different between tobacco smoke- and sham-exposed rats (Fig. 4).

Histological analysis of lung tissue showed an increased number of neutrophils in the lungs of rats exposed to secondhand tobacco smoke after 28 days (Fig. 5). In quantitative analyses, the number of lung neutrophils was significantly increased in tobacco smoke-exposed rats (5.4 ± 0.7 neutrophils/view; n = 8) compared with sham-exposed rats (3.0 ± 0.2 neutrophils/view; n = 8 rats). No other differences in any other blood cell type (e.g., no change in macrophage numbers) or pathological changes were noted in the lungs (Fig. 5), liver, or arteries. Secondhand tobacco smoke also had no significant effect on heart weight, heart morphology, or left ventricular wall thickness (data not shown).

Table 2. Percent dip and average blood pressure, heart rate, and dP/dt of the arterial pressure pulse 4 wk after secondhand tobacco smoke exposure

<table>
<thead>
<tr>
<th>Treatment</th>
<th>%Systolic Pressure</th>
<th>%Diastolic Pressure</th>
<th>%Heart Rate</th>
<th>%dP/dt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>6.5 ± 1.0</td>
<td>8.3 ± 2.1</td>
<td>17.1 ± 0.7</td>
<td>7.9 ± 2.2</td>
</tr>
<tr>
<td>Secondhand tobacco smoke</td>
<td>−0.3 ± 1.6*</td>
<td>−0.4 ± 1.5*</td>
<td>13.2 ± 1.9</td>
<td>6.6 ± 3.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>24-h Average at Week 4</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>−2 ± 2</td>
<td>−3 ± 2</td>
<td>−75 ± 2</td>
<td>−457 ± 15</td>
</tr>
<tr>
<td>Secondhand tobacco smoke</td>
<td>8 ± 2*</td>
<td>4 ± 1*</td>
<td>−71 ± 11</td>
<td>171 ± 216</td>
</tr>
</tbody>
</table>

Data are means ± SE. Blood pressure telemetry-implanted rats were exposed daily for 1 h to sham (unlit cigarette; n = 4) or secondhand tobacco smoke (3 cigarettes; n = 4) for 28 days. Blood pressure data collected during a 24-h period were divided into light and dark periods and expressed as a percent dip (from dark/active to light/inactive period) or were averaged for the entire 24 h and expressed as a change from individual baseline values. dP/dt, change in pressure over time. *P < 0.05 vs. sham-exposed group in one-way ANOVA.
DISCUSSION

The major finding of the current study is that secondhand tobacco smoke exposure alters the circadian pattern of blood pressure and heart rate, significantly reducing the dipping pattern of blood pressure during sleep. The increase in pulse wave dP/dt observed with secondhand tobacco smoke occurred early in the exposure period (by the end of week 1) and did not show a circadian pattern similar to blood pressure and heart rate. Furthermore, the increase in pulse wave dP/dt occurred in the absence of any structural alterations of the arterial wall, which may instead be related to the observed reduction in NO bioactivity or other changes in functional regulation of arterial contractility.

Blood pressure in mammals is known to exhibit a circadian rhythm where blood pressure dips during the night and rises in the morning hours (27), a cycle that is inverted in nocturnal animals (25). In preliminary studies, we have observed an average 5 and 8% dip in systolic and diastolic pressures, respectively, in untreated, healthy Sprague-Dawley rats using radiotelemetry. In humans, nondipping (<10% decrease in nocturnal blood pressure) and reverse-dipping (increased nocturnal blood pressure) blood pressure patterns are associated with increased cardiovascular mortality (32). Studies in humans have reported that heart rate (9) and daytime blood pressure (4, 29) are increased in smokers, while nocturnal blood pressure dipping has been reported to be similar between smokers and nonsmokers (29). However, whether secondhand tobacco smoke causes similar effects on blood pressure patterns has not been previously reported. In humans, elevated heart rate and blood pressure during the day have been attributed to the acute effects of nicotine from continuous smoking, with withdrawal occurring at night. In previous studies from this laboratory, acute secondhand tobacco smoke exposure was found to increase pig blood pressure (43) but had no acute effect on blood pressure in rats (12). Instead, subchronic tobacco smoke-exposed rats in the current study exhibited a
nondipping or reverse dipping blood pressure during sleep. Rats have previously been reported to exhibit increased locomotor activity after mainstream smoke exposure (31), but the current study found no significant change in activity with secondhand tobacco smoke exposure (data not shown). Thus the failure of blood pressure to dip in the tobacco smoke-exposed rats was not due to an acute hyperactivity response to nicotine and does not appear to be attributable to acute effects of nicotine.

The circadian clock influences both central and peripheral mechanisms of blood pressure regulation (36). NO has been implicated in the control of circadian variation in blood pressure in rats (47) and increased production of reactive oxygen species can disrupt timing of the circadian clock (18). In hypertensive individuals, a reversed or nondipping nocturnal blood pressure pattern is associated with increased arterial stiffness (24). In the current study, secondhand tobacco smoke significantly increased pulse wave dP/dt, which may indicate arterial stiffness increased. However, this conclusion requires corroboration with other tests of arterial stiffness in future experiments. Since there was no significant circadian pattern to dP/dt, the influence of secondhand tobacco smoke on circadian blood pressure patterns and arterial stiffness may occur via different mechanisms.

Endothelium-derived NO and ET-1 function as mutual antagonists in the determination of vascular tone, and several lines of evidence indicate that they play direct roles in the regulation of arterial stiffness (10, 12, 26). A reduction in NO bioavailability is a key feature of endothelial dysfunction, and chronic smoking in humans is associated with impaired endothelium-dependent vasodilation in arteries exhibiting increased stiffness (34). In the current study, although plasma ET-1 and nitrate/nitrite levels were not significantly altered in tobacco smoke-exposed rats, there was an increase in peroxynitrite formation indicating reduced NO bioavailability. This increased oxidative stress-mediated inactivation of NO after 28-day secondhand tobacco smoke exposure agrees well with a recent study (39) reporting increased oxidative stress in mice exposed for 16 and 32 wk to mainstream cigarette smoke. With increased oxidative stress and NO inactivation, one would expect to observe endothelial dysfunction. However, this was not evident in the current study in aorta isolated from rats exposed to secondhand tobacco smoke for 28 days compared with sham-exposed rats. Instead endothelium-dependent relaxation in the current was intact in isolated aorta from tobacco smoke-exposed rats. Again, these findings agree well with the recent mouse study (39) that found that isolated aorta did not exhibit impaired endothelium-dependent dilation to acetylcholine at 16-wk exposure but did by 32 wk of tobacco smoke exposure.

Evidence from the same mouse study suggested that oxidative stress originated from activated mononuclear and multinuclear leukocytes, producing oxidative stress and endothelial impairment at 32-wk exposure (39). There is increasing evidence that inflammatory reactions are involved in the pathophysiology of hypertension (37) as well as abnormalities in vascular function, including endothelial dysfunction and arterial stiffness in humans (15). Cigarette smoke exposure results in the attraction and activation of macrophages and neutrophils in the lung (38, 42) and circulation (46), agreeing well with the observed increase in pulmonary neutrophils in the current study. Not only can these cells generate reactive oxygen species themselves (35), but they also release proinflammatory cytokines such as TNF-α (3, 6) and interleukin-6 that can travel via the blood to induce vascular oxidative stress (8) and endothelial dysfunction in the systemic circulation (22). Thus there is good evidence to suggest that tobacco smoke-mediated increases in inflammation play a role in endothelial dysfunction and arterial stiffness. In addition, another study (41) suggested that a nondipping pattern of blood pressure was associated with higher C-reactive protein levels, suggesting that inflammation

![Fig. 4](http://ajpheart.physiology.org/)

Fig. 4. Ex vivo endothelium-dependent vasorelaxation in isolated aortic rings precontracted with 10^{-5} M phenylephrine from rats exposed daily to sham (unlit cigarette; n = 8 rats; ○) or secondhand tobacco smoke (also called ETS; 3 cigarettes; n = 8; ) for 28 days. No significant differences were found between treatment groups.

![Fig. 5](http://ajpheart.physiology.org/)

Fig. 5. Representative photomicrographs showing increased neutrophils (arrows) in the lungs of rats exposed daily for 1-h sham (A; unlit cigarettes) compared with secondhand tobacco smoke (B; 3 cigarettes) after 28 days. Sections were stained with hematoxylin and eosin and neutrophils counted at ×600 magnification.
may also play a role in the altered circadian blood pressure patterns.

Alternatively, superoxide anion production may instead originate from a number of other sources in cigarette smoke. While the gas-phase free radicals in cigarette smoke are only stable for a short time, tar-phase radicals can generate additional reactive oxygen species and are much more stable, having the potential to leave the pulmonary circulation and reach the peripheral vasculature (38). Tar phase PAHs in cigarette smoke cause AhR stimulation, which in turn increases expression of genes such as CYP1A1 (7, 13, 33). PAHs are also metabolically activated by CYP1A1 to form quinone structures that generate superoxide anions (28). In the current study that examined effects of secondhand tobacco smoke and not direct smoking, the majority of cytochrome P450 substrates were likely metabolized in the lung since CYP1A1 activity was increased in lung but not liver tissue. Further support for a role of CYP1A1 comes from two studies with AhR agonists. First, blood pressure increased in rats after 7 days of intranasal exposure to benzo-a-pyrene (13). Second, oral exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin in wild-type, but not CYP1A1 knockout, mice increased blood pressure after 15 days (23). Taken together, these results suggest that CYP1A1 activation by PAHs within cigarette smoke could have contributed to increasing blood pressure in the current study. It should be noted that if AhR agonists found within tobacco and other sources of smoke are verified in future studies to cause the changes in blood pressure, then implications for adverse cardiovascular effects extend much beyond tobacco smoke to air pollution and a variety of common environmental contaminants.

In summary, subchronic secondhand tobacco smoke exposure in rats results in a loss or reversal in the normal dipping pattern of blood pressure during sleep and appears to increase arterial stiffness in the absence of any structural modifications to the arterial wall. Although smoking has been implicated as a cause of failure of blood pressure to dip during sleep, this is the first study to our knowledge that establishes a causal link between secondhand tobacco smoke exposure and altered circadian blood pressure patterns. These findings were associated with increased neutrophil infiltration, increased CYP1A1 activity in the lung, and a reduction in systemic NO bioactivity, which may be causally related to arterial stiffness and overall increases in blood pressure even though endothelial dysfunction was not observed in the current study. However, which of these processes, if any, is responsible for the failure of blood pressure to dip is unclear and requires further investigation.

GRANTS
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AUTHOR CONTRIBUTIONS
Author contributions: N.J.G. performed experiments; N.J.G. analyzed data; N.J.G. and L.P.W. interpreted results of experiments; N.J.G. prepared figures; N.J.G. drafted manuscript; N.J.G. and L.P.W. approved final version of manuscript; L.P.W. conception and design of research; L.P.W. edited and revised manuscript.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES
translocation of IL-6 from the lung to the systemic circulation. Am J Respir Cell Mol Biol 44: 197–204, 2011.