Sidestream Cigarette Smoke Exposure Effects on Baroreflex in Adult Rats

Vitor E. Valenti ¹, ², Luiz Carlos de Abreu ², Celso Ferreira ³, ¹
Departamento de Medicina, Disciplina de Cardiologia, UNIFESP, São Paulo, SP; Departamento de Morfologia e Fisiologia, Faculdade de Medicina do ABC ²; Departamento de Clínica Médica, Disciplina de Cardiologia, Faculdade de Medicina do ABC ³, Santo André, SP – Brazil

Abstract
Background: It has been evidenced in the literature that exposure to cigarette smoke causes hypertension in rats; however, it has not been demonstrated if the baroreflex function is impaired before the animal becomes hypertensive.

Objective: We evaluated short-term effects of sidestream cigarette smoke (SSCS) exposure on baroreflex function in Wistar normotensive rats.

Methods: Rats were exposed to SSCS during three weeks, 180 minutes, five days per week, at a concentration of monoxide carbon between 100-300 ppm. Mean arterial pressure (MAP) and heart rate (HR) were evaluated through cannulation of the femoral vein and artery.

Results: There was no significant difference between control and SSCS groups regarding basal mean arterial pressure and heart rate, sympathetic and parasympathetic components of the baroreflex function.

Conclusion: Our data suggest that three weeks of exposure to SSCS is not enough to significantly impair cardiovascular parameters and baroreflex sensitivity in normotensive Wistar rats. (Arq Bras Cardiol. 2010; [online].ahead print, PP.0-0)

Keywords: Tobacco; smoke; smoke inhalation injury; baroreflex; rats.

Introduction
Cigarette smoking plays an important role in terms of toxic trace metal distribution towards human health and environmental pollution ¹. Cigarette smoke can be classified into two categories, with one being the mainstream smoke, usually inhaled by active smokers, and the other being the sidestream cigarette smoke (SSCS) emitted from a cigarette and inhaled by so-called “passive smokers”. It is known that sidestream smoke contains a variety of oxidants and other harmful compounds much more than that contained in mainstream smoke. Passive smokers are thus exposed to almost the same chemicals in cigarette smoke as active smokers are. Therefore, passive smoking increases the risk of cardiac or other related diseases in nonsmokers ², ³.

In cardiovascular physiology, the baroreflex or baroreceptor reflex is one of the body’s homeostatic mechanisms to maintain blood pressure ⁴. Although it has been evidenced in the literature that exposure to cigarette smoke increases arterial pressure in rats ⁵, it has not demonstrated if baroreflex function is impaired before the animal becomes hypertensive. Therefore, in this study we investigated the baroreceptor reflex in rats exposed to SSCS for a short term.

Method

Animals
Wistar rats (300-400g) were kept in the Animal Care Unit of our University. Rats were housed individually in plastic cages under standard laboratory conditions. They were kept under a 12-h light/dark cycle (lights on at 07:00 h) and had free access to food and water. Animals were divided into two groups: Control (n=18), rats exposed to fresh air and; SSCS (n=15), rats exposed to SSCS. The Institution’s Animal Ethics Committee authorized housing conditions and experimental procedures. Efforts were made to minimize the number of animals used.

Sidestream cigarette smoke (SSCS) exposure
The rats were placed in the transparent chamber, with a volume of approximately 95x80x65 cm³, where four rats remained. Rats were maintained at 23±1°C and 50-60% relative humidity. Smoke carbon monoxide (CO) concentration in the chamber was maintained between 100-300 ppm⁶. Rats were placed in the clear chamber.
Cigarettes were placed inside the chamber in a small box which prevented the rats from touching the cigarettes. SSCS was produced by burning the cigarettes inside the chamber without filtering, which is the main profile of SSCS. When CO concentration reached 100 ppm we started to keep track of time (up to 180 minutes). Cigarettes were replaced by new cigarettes in order to maintain CO concentrations between 100-300 ppm. Rats were exposed to SSCS during 180 minutes, five days/week; the total duration of these experiments was three weeks and all the exposures were carried out in the morning, between 8 a.m. and 12 p.m. The cigarette used was a commercial brand with the following composition: 1.1 mg of nicotine, 14 mg of tar and 15 mg of carbon monoxide. Control animals were maintained in the same place and under the same conditions as the SSCS group, but were exposed to fresh air.

**Surgical procedures**

On the third day after the last SSCS exposure, the rats were anesthetized with ketamine (50 mg/kg i.p.) and xylazine (50 mg/kg i.m.) and a catheter was inserted into the abdominal aorta through the femoral artery for blood pressure and heart rate recording. Catheters were made of 4 cm segments of PE-10 polyethylene (Clay Adams, USA) heat bound to a 13 cm segment of PE-50. The catheters were tunneled under the skin and exteriorized at the animal’s dorsum.

**Arterial pressure and heart rate recording in awaken rats**

After surgery, the animals were kept in individual cages used in the transport to the experimental room. Animals were allowed 20 min to adapt to the conditions of the experimental room such as sound and illumination before the recording of blood pressure and heart rate was started. The experimental room was acoustically isolated and had constant background noise produced by an air exhauster. At least another 15-min period was allowed before the beginning of the experiments. Pulsatile arterial pressure (PAP) of freely moving animals was recorded using an HP-7754A preamplifier (Hewlett Packard, USA) and an acquisition board (MP100A, Biopac Systems Inc, USA) connected to a computer. Mean arterial pressure (MAP) and heart rate (HR) values were derived from the PAP recordings and processed on-line.

**Baroreflex test**

The baroreflex was tested with a pressor dose of 0.1mL phenylephrine (PHE-bolus-8μg/kg IV; Sigma Chemical) and depressor doses of 0.1mL sodium nitroprusside (SNP-bolus-50μg/kg IV; RBI). The baroreflex gain was calculated as the derivation of Hn as a function of the MAP variation (ΔHR/ΔMAP). We also analyzed bradycardic and tachycardic peaks and HR range (the difference between bradycardic and tachycardic peak).

**Statistical Analysis**

Values are reported as means ± standard error of means. HR, MAP, ΔHR, ΔMAP and ΔHR/ΔMAP were compared between rats exposed to SSCS and ambient air. After the distributions were evaluated through the Kolmogorov normality test, the unpaired Student’s T test was used to verify differences between normal distributions (MAP, HR, PHE-induced hypertension, bradycardic reflex, SNP-induced decrease in MAP, tachycardic reflex, bradycardic peak tachycardic peak, HR range and baroreflex gain tested with SNP), and the Mann-Whitney test was used to assess differences between non-parametric distributions (baroreflex gain tested with PHE). In order to compare body weight between before and after SSCS exposure, we applied paired Student’s T test. Differences were considered significant when the probability of a Type I error was less than 5% (p < 0.05).

**Results**

We compared body weights between before and after SSCS exposure and we observed that it significantly decreased in the SSCS group (352.5±14.7g vs. 338.5±13.6g; p=0.0003), while there was no difference in the control group (before : 365.7±17.5g vs. after : 366.8±18.3g; p=0.7374).

As shown in Table 1, we observed no significant difference between SSCS and control groups regarding baseline MAP and HR, bradycardic and tachycardic peak, HR range and baroreflex gain. Furthermore, the bradycardic reflex response to changes in MAP increase was not attenuated in rats exposed to SSCS (C : -82.7±5.2 vs. SSCS : -88±6.9; p=0.392) and PHE-induced increase in MAP was not significantly different between the groups (C : 48.6±2.3 vs. SSCS : 46.3±1.8; p=0.395). Figure 1 presents representative recordings obtained during baroreflex testing with PHE in conscious rats, showing no expressive difference between control and SSCS groups. The reflex bradycardia in response to PHE-induced increase in arterial pressure was similar in both groups.

Intravenous injections of SNP produced a vasodepressor response, which was not statistically reduced in rats exposed to SSCS compared with control rats (C : 34.7±2.1 vs. SSCS : -39.4±1.7; p=0.189). Moreover, tachycardic reflex in response to SNP-induced decrease in MAP was not significantly impaired in SSCS group (C : 104.4±7.4 vs. SSCS : 110±4.9; p=0.557). Figure 2 shows the representative

### Table 1 - Baseline levels of mean arterial pressure (MAP) and heart rate (HR), bradycardic and tachycardic peaks, HR range and baroreflex gain (BG) in rats exposed to SSCS (n=15) and to ambient air (n=18)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>SSCS</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mmHg)</td>
<td>108.3±2.43</td>
<td>108.07±2.49</td>
<td>0.8282</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>328.56±5.38</td>
<td>319.67±7.89</td>
<td>0.591</td>
</tr>
<tr>
<td>Bradycardic Peak (bpm)</td>
<td>229.39±12.44</td>
<td>226.48±7.4</td>
<td>0.8317</td>
</tr>
<tr>
<td>Tachycardic Peak (bpm)</td>
<td>464.72±10.87</td>
<td>490.13±6.89</td>
<td>0.0689</td>
</tr>
<tr>
<td>HR range (bpm)</td>
<td>240.6±12.89</td>
<td>259±6.4</td>
<td>0.2484</td>
</tr>
<tr>
<td>BG (bpm x mmHg⁻¹) PHE</td>
<td>-1.71±0.09</td>
<td>-1.92±0.16</td>
<td>0.2617</td>
</tr>
<tr>
<td>BG (bpm x mmHg⁻¹) NaNP</td>
<td>-3.23±0.28</td>
<td>-2.86±0.15</td>
<td>0.2765</td>
</tr>
</tbody>
</table>
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Sidestream cigarette smoke and baroreflex recordings obtained during baroreflex testing with SNP in conscious rats and no significant difference was observed between the control and SSCS groups. The reflex tachycardia in response to SNP-induced decrease in arterial pressure was similar in both groups.

**Discussion**

In view of the toxic effects of cigarette components and other pollutant agents\(^1\) on cardiovascular system previously presented in the literature\(^11, 14\), we aimed to evaluate the baroreflex function in rats exposed to SSCS during a short term. We report that only three weeks of exposure, five days per week at a concentration of 100-300 ppm of CO was not

![Figure 1](image1.png)

**Figure 1** - Recordings from one rat from control group (n=18) and other rat from SSCS group (n=15) illustrating reflex bradycardia (top) in response to blood pressure increases. Infusions were given in bolus. MAP: mean arterial pressure; PAP: pulsatile arterial pressure; HR: heart rate; PHE: phenylephrine.

![Figure 2](image2.png)

**Figure 2** - Recordings from one rat from control group (n=18) and other rat from SSCS group (n=15) illustrating reflex tachycardia (top) in response to blood pressure decreases. Infusions were given in bolus. MAP: mean arterial pressure; PAP: pulsatile arterial pressure; HR: heart rate; SNP: sodium nitroprusside.

Although our results did not indicate an effect of SSCS on baroreflex function, previous researches investigated the effects of cigarette components on autonomic function. Xiao et al\(^21\) demonstrated that intrauterine nicotine administration affects bradycardic response to angiotensin. Shinozaki et al\(^22\) evidenced that cigarette smoking in smokers stimulates sympathetic nerve activity by both a direct peripheral effect and a centrally mediated effect. Apart from nicotine, cigarette smoke contains thousands of other chemical substances, including carbon monoxide, hydrogen cyanide, nitrogen oxides, aldehydes, N-nitrosamines and polyaromatic hydrocarbons. SSCS has a higher concentration of toxic substances compared to mainstream smoke due to a lower temperature of combustion, as well as lack of filtering\(^17\). For instance, there is five times more...
enough to significantly damage any baroreflex component in conscious Wistar rats. We only verified differences regarding body weight before and after SSCS exposure.

Our data demonstrated that in the group exposed to SSCS, body weight was reduced after the period of exposure. The lack of any change in the control group is consistent with this assumption. Conversely, Paiva et al\textsuperscript{16} did not find any significant effect of cigarette exposure on body weight. We believe that this difference between the two studies is explained by methodological factors. In our investigation, we exposed animals to SSCS 180 minutes per day in the morning; the smoke was not filtered and presented a higher density of lethal components compared to mainstream smoke\textsuperscript{17}, while Paiva et al\textsuperscript{16} exposed animals to filtered cigarette smoke 30 minutes per day, twice in the afternoon.

We report no difference in tachycardic and bradycardic peaks and HR range between rats exposed to SSCS and the age-matched control group. The tachycardic peak is associated to the maximal sympathetic response to the reduction in blood pressure; the bradycardic peak is an index of the highest parasympathetic response to the increase in arterial pressure; the HR range index represents the difference between the upper and lower HR peak and the derivation of HR as a function of MAP variation indicates baroreflex gain\textsuperscript{15}. Overton et al\textsuperscript{19} suggested that chronic food restriction reduces the development of hypertension and sympathetic support of MAP in spontaneously hypertensive rats. Considering that in our work rats exposed to SSCS reduced their body weight, it is possible that this fact contributed to the absence of sympathetic hyperactivity. However, we did not measure food intake in those animals.

There was no significant change in basal MAP and HR in those rats exposed to SSCS. A recent study suggested that acute inhalation exposure to concentrated air particulate raises blood pressure in chronically instrumented dogs\textsuperscript{20}. Several reasons may explain the difference between this investigation and our findings. First, while they exposed dogs to fine particles (diameters between 0.15 and 2.5\(\mu m\)) we exposed rats to SSCS, which contains thousands of known toxic chemical components and particles with a diameter > than 2.5\(\mu m\). Second, blood pressure was evaluated through telemetry and we cannulated the femoral artery in order to obtain more accurate measurements of arterial pressure. Third, they exposed canine models for more than 50 days and in our study rats were exposed during three weeks. Fourth, in our research rats were exposed for 180 minutes per day, and Bartoli et al\textsuperscript{20} exposed animals to concentrated air particle 5 hours per day. Even though we consider all those differences regarding the methodological aspects, we believe that three weeks of exposure to SSCS was not enough to cause the same effects observed in the study by Bartoli et al\textsuperscript{20}.

Acrolein in SSCS compared to mainstream smoke\textsuperscript{21}. Acrolein is an unsaturated aldehyde that has recently been implicated in smoke-related endothelial injury\textsuperscript{21} and could play a role in the attenuation of the acetylcholine-induced-relaxations observed with SSCS. Taken together, these data contradicts our study. We believe that the period of exposure of our study (three weeks) is not sufficient to affect baroreflex in Wistar rats.

In this investigation, baroreflex function was evaluated in conscious rats, since baroreflex activity is blunted under anesthesia\textsuperscript{25,26} reducing the range of HR, which is the outcome in an analysis of a restricted portion of the baroreflex response. Therefore, we believe that our investigation provides reliable information regarding the effects of a component of cigarette smoke on baroreflex function in Wistar rats. It would be also interesting to evaluate other cardiovascular reflex (i.e. cardiopulmonary reflex and chemoreflex).

These data present relevant information, as the baroreceptor reflex is currently being studied in different models and strains of rats aiming to prevent hypertension development in human\textsuperscript{27}, due the fact that reduced baroreflex function is indicative of cardiovascular disease\textsuperscript{27}. We recognize the limitations of our analysis in that we were unable to provide a full baroreceptor reflex function curve. However, the baroreflex components values obtained here are of physiological relevance, because they fall around the operating point of this reflex in an unrestrained conscious rat\textsuperscript{28}.

In conclusion, SSCS exposure during three consecutive weeks does not affect baroreflex function components in conscious Wistar rats.

**Authors’ contributions**

All authors participated in the design of the study and writing the manuscript as well as approving the final manuscript.

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**Potential Conflict of Interest**

No potential conflict of interest relevant to this article was reported.

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**Study Association**

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References


