Cardiovascular responses induced by Catalase Inhibitior into the Fourth Cerebral Ventricle is changed in Wistar rats exposed to sidestream cigarette smoke

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Abstract:

Objectives: This experimental study aimed to evaluate the effects of central catalase inhibition on cardiovascular responses in rats exposed to sidestream cigarette smoke (SSCS) for 3 weeks.

Methodology: A total of 20 males Wistar rats (320-370g) were implanted with a stainless steel guide cannula into the fourth cerebral ventricle (4^{th}V). Femoral artery and vein were cannulated for mean arterial pressure (MAP) and heart rate (HR) measurement and drug infusion, respectively. Rats were exposed to SSCS for three weeks, 180 minutes per day, 5 days/week [carbon monoxide (CO): 100-300 ppm]. Baroreflex was tested with one pressor dose of phenylephrine (PHE, 8 µg/kg, bolus) and one depressor dose of sodium nitroprusside (SNP, 50 µg/kg, bolus). Cardiovascular responses were evaluated before and 15 minutes after 3-amino-1, 2, 4-triazole (ATZ, catalase inhibitor, 0.001g/100µL) injection into the 4th V.

Results: Vehicle treatment into the 4th V did not change cardiovascular responses. Central catalase inhibition increased tachycardic peak, attenuated bradycardic peak and reduced HR range at 15 minutes, increased MAP at 5, 15 and 30 min and increased HR at 5 and 15 min. In rats exposed to SSCS, central ATZ increased basal MAP after 5 min and increased HR at 5, 15 and 30 minutes, respectively, and attenuated bradycardic peak at 15 minutes.

Conclusion: This study suggests that brain oxidative stress caused by SSCS influences autonomic regulation of the cardiovascular system.

Keywords: Baroreflex; Oxidative Stress; Catalase inhibition; Medulla Oblongata; sidestream cigarette smoking.

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Introduction:

The increased production of reactive oxidative stress (ROS) induced by cigarette is caused by the effects of the radicals in smoke.[1-3] Some ROS components, including hydrogen peroxide (H_2O_2) and superoxide anions (O_2^-) are considered as dangerous second messengers in a range of cellular mechanisms.[4] ROS are resulted from incomplete reduction of oxygen to O_2^-, which is usually or enzymatically dismutated to H_2O_2 by superoxide dismutase (SOD). Catalase is responsible for transforming H_2O and O_2 from H_2O_2.[5]

Previous investigations suggested that brain ROS is associated to increased sympathetic activity[6, 7] and systemic ROS is also related to impaired baroreflex.[8] The activity of the autonomic nervous system is regulated by a brainstem circuitry that is composed by the nucleus ambiguous, nucleus of the solitary tract (NTS), caudal (CVLM) and rostral ventrolateral medulla (RVLM). In this context, drugs injection into the fourth cerebral ventricle (4th V) present a preference for the parasympathetic system activity on the heart.[6]

Cigarette smoke and exposure to ambient tobacco smoke is considered as a relevant factor for increased mortality and morbidity.[9-11] Regarding cigarette smoke, it is divided in mainstream and sidestream cigarette smoke (SSCS). The mainstream cigarette smoke is the smoke in the air that was inspired by the smokers. The SSCS is emitted from a cigarette that was not inhaled by the active smoker. The SSCS is composed by several oxidants and different injurious components, its concentrations are higher than that observed in the mainstream smoke.[12-15]

Cigarette smoke exposure for 15 minutes increases oxidative stress in the brain of mice.[16] Moreover, upregulation of the substance P in the NTS area was suggested to contribute to the increased NTS excitability and enhanced reflex responses to lung C-fiber stimulation in pigs exposed to SSCS[16] and the increased baroreceptor reflex sensitivity may compensate for particle-induced alterations in blood pressure in dogs.[17] In addition, our group has already demonstrated that SSCS affects cardiovascular responses induced by central catalase inhibition in normotensive rats.[18] In this study cardiovascular reflex was tested five times in 60 minutes. It is possible that the volume of injection influenced cardiovascular responses.

Therefore, this study investigated the effects of catalase inhibition into the 4th V on cardiovascular responses in rats exposed to SSCS for three weeks, with a lower volume of infusion.

Methods

Animals

A total old 20 male Wistar rats (320-370g) which 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. The Institution's Animal Ethics Committee authorized housing conditions and experimental procedures (number 0255/10). Efforts were made to minimize the number of animals used.

Sidestream Cigarette Smoke (SSCS) Exposure

The rats were placed in a transparent chamber, with a volume of approximately 95x80x65 cm^3, where four rats remained. Rats were maintained at $23\pm1°C$ and 50-60% relative humidity. Smoke carbon monoxide (CO) concentration in the chamber was maintained between 100-300 ppm. Rats were placed in the clear chamber. Cigarettes were placed inside the chamber in a small box which avoided the rats to touch 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 count (until 180 minutes). Cigarettes were replaced by new cigarettes in order to maintain CO concentration 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 at 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 maintained at the same place and same conditions as the SSCS group but exposed to fresh air.[19-21]

Surgical Preparation

Five days before the experiment (one day after the last SSCS exposure), the rats were anesthetized with ketamine (50 mg/kg i.p.) and xylazine (50 mg/kg i.m.). This period was necessary to allow a good recovery from the

surgical procedures. After scalp anesthesia with 2% lidocaine, the skull was exposed and a stainless steel guide cannulas (26G) were implanted into the 4th V 1 mm above site injection, using a stereotaxic apparatus (Stoelting, USA). Stereotaxic coordinates for cannula implantation into the 4th V were: AP=−13 mm from the bregma; L=0 mm from the medial suture, V=−6 mm from the skull. Cannulas were fixed to the skull with dental cement and one metal screw.[22, 23]

One day before the experiments, 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. The period of one day was necessary in order to allow a good recovery from the surgical procedures according to previous studies from our group.[24-26] Catheters were made of 4 cm segments of PE-10 polyethylene (Clay Adams, USA) heat bound to a 13 cm segment of PE-50. Catheters were tunneled under the skin and exteriorized at the animal's dorsum.[24-26]

Arterial pressure and heart rate recording

After surgery, the animals were kept in individual cages used in the transport to the experimental room. Animals were allowed 60 minutes to adapt to the conditions of the experimental room such as sound and illumination before starting blood pressure and heart rate recording. The experimental room was acoustically isolated and had constant background noise produced by an air exhauster. At least another 30 minutes period was allowed before beginning experiments. Pulsatile arterial pressure 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 pulsatile arterial pressure recordings and processed on-line.[27]

Baroreflex evaluation

Baroreflex was activated by intravenous phenylephrine (PHE, 8 μg/kg, bolus) or sodium nitroprusside (SNP, 50 μg/kg, bolus). Injections were made at the following sequence: PHE i.v. injection before central injection of the catalase inhibitor, approximately 60 seconds later SNP was i.v. injected. After 15 minutes the central administration of the catalase inhibitor, PHE i.v. injection was performed and approximately

60 seconds later SNP was i.v. was injected. Each i.v. injection spent 1-3 seconds. Baroreflex gain was calculated as the derivation of HR in function of the MAP variation (ΔHR/ΔMAP, maximum changes in MAP and HR). Sympathetic baroreflex gain (SBG) was considered as the ΔHR/ΔMAP ratio in response to i.v. SNP and parasympathetic baroreflex gain (PBG) was considered as the ΔHR/ΔMAP ratio in response to i.v. PHE. We also analyzed bradycardiac and tachycardiac peak and HR range (the difference between bradycardiac and tachycardiac peak).[28]

Injections into the 4th V

Injections into the 4th V were made with 10 μl Hamilton syringes connected by polyethylene tubing (PE-10) to an injector needle. The injector, when completely inserted, protruded 2mm beyond the tip of the guide cannula. Injections into the 4th V were 1.0 μl for about 5–10 s.[29]

Experimental Procedure

Baroreflex and cardiovascular responses were evaluated before (control) and 15 minutes after 3-Amino-1,2,4-triazole (ATZ, catalase inhibitor, 0.001g/100μL) or vehicle (0.9% NaCl) injection into the 4th V of conscious rats.[23]

Statistical Analysis

The results were reported as means ± standard error of means (S.E.M.). In order to compare all variables (basal MAP and HR, bradycardic and tachycardic peak, HR range, SBG, PBG, PHE-induced increase and SNP-induced decrease in MAP, bradycardic and tachycardic reflex) analyses of variance (one way ANOVA) for repeated measures followed by the Tukey post test were applied. We compared variables between before (control), 5, 15, 30 and 60 minutes after ATZ injection into the 4th V in the same rat. Differences were considered significant when the probability of a Type I error was less than 5% ($p < 0.05$).

Results
Effect of vehicle injection into the 4th V

Based on Table (1), injection of vehicle (0.9% NaCl) into the 4th V did not affect basal MAP and HR, tachycardic and bradycardic peak, HR range, SBG and PBG in Wistar rats exposed to fresh air.

Table 1. Baseline level of mean arterial pressure (MAP) and heart rate (HR), bradycardic and tachycardic peak and sympathetic (SBG) and parasympathetic baroreflex gain (PBG) in Wistar rats (n=6) exposed to filtered air treated with vehicle into the 4th V.

Variable	Control	5 minutes	15 minutes	30 minutes	60 minutes
MAP (mmHg)	102.11±5.23	101.28±6.29	106.58±2.22	101.49±5.22	106.47±3.29
HR (bpm)	361.21±12.13	351.84±12.12	357.46±6.19	369.83±11.37	370.09±10.05
Bradycardic Peak (bpm)	221.45±22.43	228.01±12.67	240.27±4.34	232.56±20.95	239.37±5.22
Tachycardic Peak (bpm)	472.34±11.01	489.63±19.22	481.35±13.57	471.25±10.31	464.94±23.51
HR range (bpm)	248.81±14.91	248.33±10.03	243.34±12.25	259.21±11.03	255.92±12.05
PBG (bpm x mmHg^{-1})	-1.84±0.12	-2.43±0.46	-2.52±0.51	-2.17±0.11	-1.93±0.21
SBG (bpm x mmHg^{-1})	-3.41±0.31	-3.54±0.22	-2.92±0.38	-3.14±0.43	-2.42±0.15

Data expressed as means±SEM.

Effect of ATZ injection into the 4th V

In rats exposed to fresh air, injections of ATZ into the 4th V increased basal MAP at 5, 15, 30 minutes and increased of basal HR at 5 and 15 minutes (p<0.05) compared to control levels,. Furthermore, during baroreflex activation, at 15 minutes, it attenuated bradycardic peak, decreased HR range and increased tachycardic peak, however, it did not affect PBG (p=0.3217) and SBG (p=0.1983) compared to control levels (Table 2).

Table 2. Baseline level of mean arterial pressure (MAP) and heart rate (HR), bradycardic and tachycardic peak and sympathetic (SBG) and parasympathetic baroreflex gain (PBG) in Wistar rats (n=7) exposed to filtered air treated with ATZ into the 4th ventricle.

Variables	Control	5 minutes	15 minutes	30 minutes	60 minutes
MAP (mmHg)	113.12±1.61	**123.53±1.14***	**121.91±1.11***	**124.25±17***	118.75±0.28
HR (bpm)	354.42±9.21	**433.75±12.67***	**437.25±14.87***	386.37±17.82	347.75±11.52
Bradycardic Peak (bpm)	184.07±2.91	-	**294.87±10.05***	-	-
Tachycardic Peak (bpm)	487.55±3.75	-	**519.12±6.61***	-	-
HR range (bpm)	303.51±4.73	-	**225.12±8.37***	-	-
PBG (bpm x mmHg^{-1})	-2.58±0.13	-	-2.81±0.16	-	-
SBG (bpm x mmHg^{-1})	-3.15±0.25	-	-2.76±0.21	-	-

Data expressed as means±SEM.
Significance versus control group

In rats group exposed to SSCS we observed increased basal MAP at 5 minutes and increased basal HR at 5, 15 and 30 minutes after ATZ administration inhibition into the 4th V (p<0.05) compare to control levels. Moreover, during baroreflex activation, bradycardic peak was attenuated at 15 minutes after catalase inhibition into the 4th V (p<0.05). On the other hand, there were no changes with respect to tachycardic peak (p=0.22), HR range (p=0.399), PBG (p=0.62) and SBG (p=0.07) at 15 minutes after ATZ treatment in Wistar rats exposed to SSCS (Table 3).

Table 3. Baseline level of mean arterial pressure (MAP) and heart rate (HR), bradycardic and tachycardic peak and sympathetic (SBG) and parasympathetic baroreflex gain (PBG) in Wistar rats (n=7) exposed to SSCS treated with ATZ into the 4th ventricle. *

Variable	Control	5 minutes	15 minutes	30 minutes	60 minutes
MAP (mmHg)	107.12$\pm$3.63	**118.37$\pm$3.16 ***	115.25$\pm$2.78	106.75$\pm$1.78	104.52$\pm$2.64
HR (bpm)	312.25$\pm$11.36	**403.12$\pm$9.36***	**379.37$\pm$9.13***	**362.25$\pm$21.61***	314.12$\pm$1.17
Bradycardic Peak (bpm)	222.51$\pm$9.51	-	**257.58$\pm$1.63***	-	-
Tachycardic Peak (bpm)	483.12$\pm$8.83	-	500.37$\pm$6.71	-	-
HR range (bpm)	285.82$\pm$27.98	-	279.12$\pm$29.08	-	-
PBG (bpm x mmHg^{-1})	-1.64$\pm$0.19	-	-1.72$\pm$0.18	-	-
SBG (bpm x mmHg^{-1})	-2.85$\pm$0.14	-	-3.92$\pm$0.46	-	-

Data expressed as means$\pm$SEM.
Significance versus control group

Discussion:

This study was undertaken to investigate the cardiovascular responses induced by central catalase inhibition into the 4th V in animals exposed to SSCS. As a main finding, it was observed that endogenous H_2O_2 increase induced by catalase inhibition influenced the cardiovascular responses in a lower intensity in rats exposed to SSCS. According to our findings, we suggest that SSCS exposure decreased the cardiovascular responses caused by catalase inhibition into the 4th V. Furthermore, the absence of alterations in the vehicle group supports this assumption.

A recent study published by our group demonstrated that central catalase inhibition increased basal HR and attenuated the bradycardic peak with more intensity in Wistar rats exposed to SSCS in different periods. [18] That investigation used an increased volume of injection, since baroreflex was tested five times. In this case the baroceptor reflex was tested twice, the volume of injection was two times lower than our previous study. [18] Conversely, we observed that blood pressure was significantly increased by ATZ into the 4th V. We hypothesize that the volume of i.v. injection influence the cardiovascular responses induced by central catalase inhibition.

Regarding brain oxidative stress and cardiovascular responses, according to our results, acute endogenous H_2O_2 increase into the 4th V caused by catalase inhibition affected the cardiovascular responses in a lower intensity in animals exposed to SSCS. We suggest that cigarette exposure can adapt the animals to such situation, since they presented smaller responses to increased oxidative stress. The nose anatomy is well suitable for the transport of exogenous agents into the brain, since the olfactory nerve is susceptible to such infiltration. Those cells dispersed around the rostral part of the nasal cavity. Distinct from other receptor cells, those cells are first-order neurons that end axons to the brain without synapse intervention.[30] In view of the above considerations, we suggest that the olfactory vector hypothesis as a hypothesis to start to elucidate our results.[31] Nevertheless, it is not possible to confirm the area affected by SSCS exposure.

It is clear in the literature that anesthesia is a factor that influences cardiovascular reflexes. We tested baroreflex in conscious rats, since cardiorespiratory reflex activity is attenuated under anesthesia [32] decreasing the HR range, which results in an analysis of a restricted portion of the cardiovascular response. Thus, our research provides trustful data.

A previous study evaluated the effects of SSCS on parasympathetic and sympathetic responses induced by decrease and increase in arterial blood pressure in Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR).[25] It was reported that SSCS during three weeks affected the sympathetic and parasympathetic responses in WKY whereas it influenced the sympathetic responses in SHR. The exposure protocol used in this study indicated that SSCS attenuated tachycardic peak, bradycardic reflex and heart rate range in WKY rats, while it affected the heart rate range and tachycardic peak in SHR. These

results suggest a hypothesis that a period of SSCS lower than 30 days is sufficient to induce changes in the sympathetic nervous activity on the heart while there is no change on baseline arterial blood pressure. This method is based on intravenous phenylephrine that enhances arterial pressure and activates the parasympathetic nervous system, leading to the bradycardic reflex and then the bradycardic baroreflex gain is calculated. The intravenous sodium nitroprusside decreases arterial blood pressure and activates the sympathetic nervous system, leading to the tachycardic reflex. [21, 24, 28] The difference between the results from this study and the other reported in Wistar rats regarding autonomic cardiac responses to SSCS is possible due to the difference between WKY and Wistar rats, since WKY rats come from normotensive strains of SHR. [27]

The novelty of this study is the protocol of the analysis of cardiovascular responses induced y catalase inhibition into the 4th cerebral ventricle. In the study published previously by group[19] we analyzed baroreflex before, 5, 15, 30 and 60 minutes after central ATZ injection, while in this study we investigated baroreflex function two times, before and 15 minutes after the central ATZ injection. We measured baroreflex gain only at 15 minutes after central catalase inhibition in order to verify if a reduced infusion volume present influence, since we have measured baroreflex previously with a higher infusion volume.[20] Therefore, a reduced volume of i.v. saline with drug was injected. Moreover, we found different responses in basal mean arterial pressure, heart rate and bradycardic peak induced by central ATZ compared to the study previously published. [19]

A relevant matter to be raised that was not previously mentioned in this text is the role of endothelial function in the cardiac autonomic regulation. Different mechanisms of modulations are indicated as hormonal and autonomic regulation. The range of regulatory processes implies in interactions. [33-35] For instance, nitric oxide is released by the endothelium and by the heart. It is indicated that the triggering type is involved in the role of this substance in the cardiovascular system, influencing the heart and the endothelium. [35] Moreover, its production is affected by the expression of calcium/calmodulin-dependent kinase IV that modulates arterial pressure. [36] A previous study [37] failed to report an effect of cigarette smoke exposure on the forearm vascular responses induced by vasodilators in non-smokers volunteers. Nevertheless, other study [38] observed that tobacco SSCS extract damaged endothelium-dependent relaxation in the isolated aorta of rats. They also reported that this mechanism was related to increased oxidative stress and to tobacco and the increased superoxide production was not accompanied with changes in acetylcholine-induced relaxation. Gairola and colleagues [39] investigated the effects of SSCS exposure on atherosclerotic lesions in apoE−/− mice. They reported an elevation of atherosclerotic injury progress induced by SSCS in this strain, suggesting that a genetic component is also involved. Therefore, it is suggested that the interaction between autonomic and endocrine systems may be involved in the induced-SSCS cardiovascular injuries.

There are some points in our study that are worth to be raised. It was not measured the concentration of H_2O_2 or other ROS inside the 4th V before and after the injection of ATZ. It would significantly strengthen the impact of our results to show that H_2O_2 or some other ROS is actually altered in the 4th V with this duration and level of treatment. We did not measure this component in the 4th V due to the lack of such equipment in our laboratory. On the other hand, our method is well accepted in the literature, since there is significant association between antioxidant injections and antioxidant activity into the brainstem. [40] The last exposure to cigarette smoke occurred 5 days before the experimental tests due to our surgery protocols. Oxidative stress may occur rapidly and reactive oxygen species dissipate quickly. In order to address to what extent the surgery may contribute to the creation of ROS and other harmful metabolites, another indirect indication would be to determine the number of apoptotic cells in this region of the brain (e.g. Caspase-3 staining) and the amount of fibrotic tissue (e.g. Masson's trichrome staining). Future protocols could investigate this mechanism through those procedures.

Conclusion:

The inhibition of catalase into the 4th V presented weaker effects on cardiovascular responses in rats exposed to SSCS. We propose that catalase is an enzyme involved in the central oxidative stress related to cardiovascular disorders induced by the cigarette.

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