CLINICS

BASIC RESEARCH

Effects of the administration of a catalase inhibitor into the fourth cerebral ventricle on cardiovascular responses in spontaneously hypertensive rats exposed to sidestream cigarette smoke

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OBJECTIVE: Previous studies have demonstrated a relationship between brain oxidative stress and cardiovascular regulation. We evaluated the effects of central catalase inhibition on cardiovascular responses in spontaneously hypertensive rats exposed to sidestream cigarette smoke.

METHODS: Male Wistar Kyoto (WKY) rats and spontaneously hypertensive rats (SH) (16 weeks old) were implanted with a stainless steel guide cannula leading into the fourth cerebral ventricle (4^{th} V). The femoral artery and vein were cannulated for arterial pressure and heart rate measurement and drug infusion, respectively. The rats were exposed to sidestream cigarette smoke for 180 minutes/day, 5 days/week for 3 weeks (CO: 100-300 ppm). The baroreflex was tested using a pressor dose of phenylephrine (8 μg/kg, bolus) and a depressor dose of sodium nitroprusside (50 μg/kg, bolus). Cardiovascular responses were evaluated before and 5, 15, 30 and 60 minutes after injection of a catalase inhibitor (3-amino-1,2,4-triazole, 0.001 g/100 μL) into the 4^{th} V.

RESULTS: Vehicle administration into the 4^{th} V did not affect the cardiovascular response, whereas administration of the central catalase inhibitor increased the basal HR and attenuated the bradycardic peak ($p<0.05$) to a greater extent in WKY rats exposed to sidestream cigarette smoke than in WKY rats exposed to fresh air. However, in spontaneously hypertensive rats, the effect of the catalase inhibitor treatment was stronger in the fresh air condition ($p<0.05$).

CONCLUSION: Administration of a catalase inhibitor into the 4^{th} V combined with exposure to sidestream cigarette smoke has a stronger effect in WKY rats than in SH rats.

KEYWORDS: Oxidative Stress; Catalase; Medulla Oblongata; Tobacco; Air Pollutants.

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INTRODUCTION

The effects of cigarette smoke on the cardiovascular system underlie the adverse effects of smoking on cardiovascular (1,2) and brain health, (3-5) in addition to detrimental effects in different systems (6-8). Cigarette smoke is classified into 2 categories: the mainstream smoke usually inhaled by active smokers and the sidestream smoke emitted from a cigarette and inhaled by so-called ''passive smokers.'' Sidestream cigarette smoke (SSCS) is known to contain greater amounts of various oxidants and other harmful compounds than mainstream smoke (9). Thus, passive smokers are exposed to nearly the same chemicals in cigarette smoke as active smokers, and passive smoking has been found to increase the risk of cardiac or other related diseases in nonsmokers (10).

Increased production of reactive oxygen species (ROS) by cigarette smoke occurs as a direct effect of the radicals present in smoke (11-13). For instance, it was previously shown that cigarette exposure over 24 consecutive days

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increased mRNA levels of catalase in the heart by two fold relative to only 1 day of exposure (14). ROS, such as superoxide anions (O_2^-) and hydrogen peroxide (H_2O_2), were once thought only to be harmful byproducts of oxidative metabolism but are now recognized as critical second messengers in a wide range of cellular processes (15). ROS are produced by the incomplete reduction of oxygen to O_2^-, which is spontaneously or enzymatically dismutated to H_2O_2 by superoxide dismutase (SOD). H_2O_2 is transformed to H_2O and O_2 by the activity of catalase (16). Previous investigations have associated brain ROS with increased sympathetic activity (17,18), and systemic ROS have also been associated with an impaired baroreflex (19). Drugs injected into the fourth cerebral ventricle (4^{th} V) may easily reach structures surrounding the ventricular system such as the area postrema (20). Previous studies have indicated that ROS in the 4^{th} V influence cardiovascular responses (21). Moreover, administration of a catalase inhibitor into the 4^{th} V has also been demonstrated to influence cardiovascular responses in normotensive rats (22,23). Luchese et al. (24) indicated that acute cigarette smoke exposure increases oxidative stress in the brain by increasing the activity of reactive species and reducing the activity of superoxide dismutase and catalase. Bartoli et al. (25) suggested that increased baroreceptor reflex sensitivity may compensate for particle-induced alterations in blood pressure in dogs. In addition, our group previously demonstrated that SSCS affects the cardiovascular responses induced by central catalase inhibition in normotensive rats (26). However, to the best of our knowledge, no previous study has evaluated the effects of SSCS and central catalase inhibition in spontaneously hypertensive (SH) rats. Thus, to study the detailed mechanism of catalase inhibition, we investigated the effects of administration of a catalase inhibitor into the 4^{th} V on cardiovascular responses in SH rats exposed to SSCS.

METHODS

Animals

We used male Wistar Kyoto (WKY) rats and SH rats (16 weeks old) that were kept in the Animal Care Unit of our university. The rats were housed individually in plastic cages under standard laboratory conditions. The animals were divided into 4 groups: WKY rats exposed to fresh air (N = 7), WKY rats exposed to SSCS (N = 7), SH rats exposed to fresh air (N = 7) and SH rats exposed to SSCS (N = 7). The rats 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 the housing conditions and experimental procedures (number 0255/10). Efforts were made to minimize the number of animals used.

SSCS exposure

The rats were placed in a transparent chamber with a volume of approximately 95x80x65 cm^3, with 4 rats per chamber. The rats were maintained at 23 ± 1°C and 50-60% relative humidity. The carbon monoxide (CO) concentration of the smoke in the chamber was maintained between 100-300 ppm. 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. When the CO concentration reached 100 ppm, the 180-minute interval began. The cigarettes were replaced by new cigarettes to maintain a CO concentration between 100-300 ppm. The rats were exposed to SSCS for 180 minutes on 5 days per week, and the total duration of the experiment was 3 weeks. All of the exposures per performed in the morning, between 8 a.m. and 12 p.m. The cigarettes used were of a commercial brand with the following composition: 1.1 mg of nicotine, 14 mg of tar and 15 mg of carbon monoxide. The control animals were maintained in the same manner and same conditions as the SSCS group but exposed to fresh air (27-29).

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.). After anesthesia was applied to the scalp using 2% lidocaine, the skull was exposed, and stainless steel guide cannulas (26 G) were implanted into the 4^{th} V 1 mm above the site of injection using a stereotaxic apparatus (Stoelting, USA). The stereotactic coordinates for implantation of the cannula into the 4^{th} V were as follows: AP = −13 mm from the bregma, L = 0 mm from the medial suture and V = −6 mm from the skull. The cannulas were fixed to the skull using dental cement and 1 metal screw (30).

One day before the experiment, 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 monitoring of blood pressure and heart rate. The catheters consisted of 4-cm segments of PE-10 polyethylene tubing (Clay Adams, USA) that were heat-bound to a 13-cm segment of PE-50 polyethylene tubing. The catheters were tunneled under the skin and exteriorized at the animal's dorsum (31,32). After each surgery, the animals received a single dose of an antibiotic (ampicillin, 100 mg/kg) and a single dose of the analgesic ketorolac (0.6 mg/kg).

Recording of arterial pressure and heart rate

After surgery, the animals were kept in the individual cages used in their transport to the experiment room. The animals were allowed 60 minutes to adapt to the conditions of the experimental room, such as sound and illumination, before the recording of the blood pressure and heart rate was initiated. The experiment room was acoustically isolated and had a constant background noise produced by an air exhauster. At least another 30-minute period was allowed before the experiments were initiated. The pulsatile arterial pressure of the freely moving animals was recorded using an HP-7754A preamplifier at a sampling frequency of 1,000 Hz (Hewlett Packard, USA) and an acquisition board (MP100A, Biopac Systems Inc., USA) connected to a computer. The mean arterial pressure (MAP) and heart rate (HR) were derived from the pulsatile arterial pressure recordings and processed on-line (33).

Baroreflex evaluation

The baroreflex was activated by intravenous phenylephrine (PHE, 8 μg/kg, bolus) or sodium nitroprusside (SNP, 50 μg/kg, bolus). The baroreflex gain was calculated as the derivation of HR in the function of the MAP variation (ΔHR/ΔMAP, maximum changes in MAP and HR). The sympathetic baroreflex gain (SBG) was defined as the ΔHR/ΔMAP ratio in response to i.v. SNP, and parasympathetic

Table 1 - Baseline mean arterial pressure (MAP) and heart rate (HR), bradycardic and tachycardic peak and sympathetic (SBG) and parasympathetic baroreflex gain (PBG) in Wistar Kyoto rats exposed to fresh air and treated with ATZ administered into the 4th V. N = 7. Mean ± SEM. *$p<0.05$ for comparison with 0′.

Variable	0′	5′	15′	30′	60′
MAP (mmHg)	112 ± 13	116 ± 15	124 ± 16	127 ± 14	113 ± 14
HR (bpm)	315 ± 29	308 ± 24	318 ± 22	**370 ± 22***	323 ± 25
Bradycardic Peak (bpm)	230 ± 28	260 ± 20	263 ± 29	**318 ± 22***	252 ± 21
Tachycardic Peak (bpm)	417 ± 25	419 ± 23	454 ± 28	469 ± 31	425 ± 36
HR range (bpm)	187 ± 19	166 ± 11	191 ± 16	151 ± 18	173 ± 17
PBG (bpm x mmHg^{-1})	-1.73 ± 0.24	-1.54 ± 0.03	-1.56 ± 0.14	-1.9 ± 0.6	-1.35 ± 0.04
SBG (bpm x mmHg^{-1})	-2.25 ± 0.23	-2.24 ± 0.43	-1.93 ± 0.16	-2.64 ± 0.45	-2.5 ± 0.26

baroreflex gain (PBG) was defined as the ΔHR/ΔMAP ratio in response to i.v. PHE. We also analyzed the bradycardic and tachycardic peak and HR range (i.e., the difference between the bradycardic and tachycardic peaks) (34).

Injections into the 4th V

Injections into the 4th V were performed using 10-μl Hamilton syringes connected by polyethylene tubing (PE-10) to an injector needle. The injector, when completely inserted, protruded 2 mm beyond the tip of the guide cannula. The injections into the 4th V consisted of a volume of 1.0 μl for approximately 5-10 s (35).

Protocol

Baroreflex and cardiovascular responses were evaluated before (control) and 5, 15, 30 and 60 minutes after injection of the catalase inhibitor (3-amino-1,2,4-triazole, ATZ, 0.001 g/100 μL) or vehicle (0.9% NaCl) into the 4th V of conscious rats (23).

Histology

At the end of the experiments, the animals were anesthetized using urethane (1.25 g/kg, i.p.), and 200 ml of 1% Evan's blue dye was injected in the 4th V as a marker of the injection site. The chest was surgically opened, the descending aorta occluded, the right atrium severed and the brain perfused with 10% formalin through the left ventricle. The brains were post-fixed for 24 h at 4℃, and 40-μm sections were cut in a cryostat (model CM 1900, Leica, Germany). The brain sections were stained with 1% neutral red. The actual placement of the injection needle was verified using serial sections (36).

Statistical analysis

The results were reported as the mean ± standard error of the mean (SEM). Analyses of variance (ANOVA) for repeated measures followed by the Tukey post-test were applied 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 and bradycardic and tachycardic reflex). We compared the variables at baseline (control) with the 5-, 15-, 30- and 60-minute time points after injection of ATZ into the 4th V in the same rat. We applied Student's t-test to compare the cardiovascular responses between WKY groups and SH groups exposed to SSCS. 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

Injection of vehicle (0.9% NaCl) into the 4th V did not affect the basal MAP and HR, tachycardic and bradycardic peak, HR range, SBG and PBG in WKY or SH rats exposed to fresh air.

Effect of ATZ injection into the 4th V

Injection of ATZ into the 4th V did not affect the basal MAP; however, the basal HR was increased at 30 minutes ($p<0.05$) after ATZ administration in WKY rats exposed to fresh air. Furthermore, the bradycardic peak was also attenuated at 30 minutes. In contrast, we did not observe significant changes in the bradycardic and tachycardic peak, HR range, PBG and SBG after central catalase inhibition in WKY rats exposed to fresh air (Table 1).

As shown in Table 4, injection of ATZ into the 4th V produced a strong response in SH rats exposed to fresh air. The basal HR increased was at 60 minutes and the bradycardic peak was attenuated at 30 minutes after ATZ administration. PBG was increased at 60 minutes after ATZ treatment, whereas SBG was decreased at 30 and 60 minutes (Table 2).

Among the groups exposed to SSCS, we observed stronger effects for catalase inhibition in WKY rats. Central ATZ

Table 2 - Baseline mean arterial pressure (MAP) and heart rate (HR), bradycardic and tachycardic peak and sympathetic (SBG) and parasympathetic baroreflex gain (PBG) in SH rats exposed to fresh air and treated with ATZ administered into the 4th V. N = 7. Mean ± SEM. *$p<0.05$ for comparison with 0′.

Variable	0′	5′	15′	30′	60′
MAP (mmHg)	176 ± 16	192 ± 14	180 ± 11	175 ± 15	175 ± 16
HR (bpm)	322 ± 22	**407 ± 22***	**340 ± 21***	**343 ± 27***	**341 ± 29***
Bradycardic Peak (bpm)	273 ± 19	**352 ± 19***	**305 ± 21***	**308 ± 28***	309 ± 28
Tachycardic Peak (bpm)	442 ± 37	463 ± 31	445 ± 31	455 ± 34	445 ± 32
HR range (bpm)	154 ± 17	111 ± 12	142 ± 11	147 ± 11	148 ± 12
PBG (bpm x mmHg^{-1})	-0.35 ± 0.03	**-1.2 ± 0.14***	**-0.8 ± 0.05***	**-0.67 ± 0.13***	**-0.7 ± 0.12***
SBG (bpm x mmHg^{-1})	-2 ± 0.18	-1.96 ± 0.01	-2.16 ± 0.08	**-1.39 ± 0.13***	**-1.36 ± 0.1***

Table 3 - Baseline mean arterial pressure (MAP) and heart rate (HR), bradycardic and tachycardic peak and sympathetic (SBG) and parasympathetic baroreflex gain (PBG) in Wistar Kyoto rats exposed to SSCS and treated with ATZ administered into the 4th V. N = 7. Mean ± SEM. *$p<0.05$ for comparison with 0′.

Variable	0′	5′	15′	30′	60′
MAP (mmHg)	106 ± 13	118 ± 14	111 ± 15	107 ± 12	107 ± 13
HR (bpm)	344 ± 16	**412 ± 19***	**440 ± 13***	**429 ± 17***	397 ± 13
Bradycardic Peak (bpm)	279 ± 14	323 ± 19	**364 ± 26***	**380 ± 18***	318 ± 10
Tachycardic Peak (bpm)	489 ± 11	506 ± 18	525 ± 19	527 ± 14	502 ± 12
HR range (bpm)	203 ± 12	183 ± 13	161 ± 22	169 ± 25	183 ± 11
PBG (bpm x mmHg^{-1})	-1.2 ± 0.34	-1.19 ± 0.11	-1.08 ± 0.22	-0.85 ± 0.13	-1.11 ± 0.19
SBG (bpm x mmHg^{-1})	-1.48 ± 0.2	-1.26 ± 0.14	-1.39 ± 0.26	-1.13 ± 0.1	-1.84 ± 0.21

administration increased the basal HR at 5, 15 and 30 minutes and reduced the bradycardic peak at 15 and 30 minutes (Table 3).

In the SH rats exposed to SSCS, we observed that ATZ treatment increased the basal HR during the first 30 minutes, reduced the bradycardic peak at 5 and 15 minutes and reduced the tachycardic peak at 60 minutes (Table 4).

Comparison of cardiovascular responses between WKY rats and SH rats exposed to SSCS and treated with ATZ

In Table 5, we present a comparison of the cardiovascular responses induced by central ATZ administration between the WKY and SH rats exposed to SSCS at each time point. It should be noted that almost all variables were different between the groups before ATZ injection. However, the differences were not significant after the injection of ATZ.

■ DISCUSSION

This study was undertaken to evaluate the effects of central catalase inhibitor administration on cardiovascular responses in WKY and SH rats exposed to SSCS. We observed that administration of a catalase inhibitor into the 4th V produced a strong effect on cardiovascular responses in WKY rats exposed to SSCS but not in SH rats exposed to SSCS. The lack of any change in the vehicle-treated group is consistent with these findings.

Based on our data, injection of ATZ into the 4th V did not affect the sympathetic and parasympathetic baroreflex gain in WKY rats. A previous study published by our group (22) reported that central ATZ administration into the 4th V did not affect the same components of the baroreflex in Wistar rats. We also reported that central catalase inhibition increased the parasympathetic baroreflex gain and reduced the sympathetic baroreflex gain in SH rats. The present study provides additional information because a different response was observed in the SH group. We therefore consider that central catalase inhibition affects the parasympathetic and sympathetic baroreflex gain to a greater extent in SH rats than in WKY rats. This hypothesis is supported by our previous study that investigated the cardiopulmonary reflex responses to catalase inhibitor administration into the 4th V in SH and WKY rats (37).

In the present study, administration of a catalase inhibitor into the 4th V strongly attenuated the bradycardic peak in WKY rats exposed to SSCS, whereas this response was not enhanced in SH rats exposed to SSCS. The parasympathetic activity of the baroreflex response is represented by the bradycardic peak, whereas the sympathetic activity during the baroreflex is represented by the tachycardic peak, and the difference between both peaks corresponds to the HR range (38). Our findings indicate that SSCS exposure has a stronger effect on parasympathetic activity in WKY rats than in SH rats. A recent study demonstrated that vagal modulation of the heart is blunted in heavy smokers, particularly during parasympathetic modulation (39). Considering that SH rats present increased levels of ROS in the brain relative to normotensive rats (40,41), we hypothesize that 3 weeks of exposure to SSCS was not sufficient to increase ROS production in the 4th V and affect cardiovascular responses.

We observed that acute administration of the catalase inhibitor into the 4th V had a strong effect on the parasympathetic regulation of the cardiovascular system in WKY rats exposed to SSCS because it strongly increased the basal HR. Conversely, in SH rats, the central catalase inhibitor treatment strongly affected cardiovascular responses in the group exposed to fresh air. In particular, administration of the catalase inhibitor into the 4th V increased the parasympathetic baroreflex gain and reduced the sympathetic baroreflex gain in the group exposed to fresh air, whereas it did not affect these components in SH rats exposed to SSCS. As mentioned above, we believe that 3 weeks of exposure to SSCS is insufficient to induce changes in ROS in the 4th V of the SH rats, possibly because the SH

Table 4 - Baseline mean arterial pressure (MAP) and heart rate (HR), bradycardic and tachycardic peak and sympathetic (SBG) and parasympathetic baroreflex gain (PBG) in SH rats exposed to SSCS and treated with ATZ administration into the 4th V. N = 7. Mean ± SEM. *$p<0.05$ for comparison with 0′.

Variable	0′	5′	15′	30′	60′
MAP (mmHg)	168 ± 13	175 ± 15	176 ± 13	173 ± 12	170 ± 11
HR (bpm)	317 ± 21	**429 ± 22***	**398 ± 24***	**380 ± 24***	344 ± 28
Bradycardic Peak (bpm)	213 ± 17	**307 ± 32***	**298 ± 14***	270 ± 17	26 ± 18
Tachycardic Peak (bpm)	504 ± 28	522 ± 28	519 ± 27	519 ± 29	**475 ± 25***
HR range (bpm)	272 ± 11	216 ± 29	222 ± 11	249 ± 15	213 ± 11
PBG (bpm x mmHg^{-1})	-1.9 ± 0.23	-2.87 ± 0.5	-2.77 ± 0.9	-2.4 ± 0.52	-1.65 ± 0.19
SBG (bpm x mmHg^{-1})	-3.16 ± 0.3	-2.46 ± 0.45	-3.03 ± 0.59	-2.73 ± 0.31	-3.31 ± 0.43

Table 5 - *p*-value for the intergroup comparison between the WKY and SH groups exposed to SSCS at each time point.

Variable	0′	5′	15′	30′	60′
MAP (mmHg)	**<0.000001**	**<0.000001**	**<0.000001**	**<0.000001**	**<0.000001**
HR (bpm)	**0.049**	0.29	0.067	**0.02**	**0.07**
Bradycardic Peak (bpm)	**0.005**	0.38	0.15	**0.04**	0.1
Tachycardic Peak (bpm)	0.16	0.46	0.44	0.43	0.34
HR range (bpm)	**0.0005**	0.27	0.09	**0.049**	0.18
PBG (bpm x $mmHg^{-1}$)	**0.04**	0.14	0.33	0.43	0.48
SBG (bpm x $mmHg^{-1}$)	**0.0001**	0.21	0.34	0.43	0.48

rats present higher basal levels of ROS in the brain (40,41). Thus, a stronger stimulus may be necessary to induce changes in ROS in the brain of SH rats.

A recent study published by our group demonstrated that central catalase inhibition increased the basal HR and attenuated the bradycardic peak in Wistar rats exposed to SSCS (26). In our current investigation, WKY rats exposed to SSCS presented similar responses, which thus supports the results of our previous study (26). In this case, we aimed to determine whether SH rats exposed to SSCS would present increased responses. However, SH rats exposed to fresh air presented greater responses than SH rats exposed to SSCS.

In this context, we also found in this study that before injection of the catalase inhibitor into the 4th V, a difference existed between the WKY and SH groups exposed to SSCS regarding basal MAP and HR, bradycardic peak, HR range and sympathetic and parasympathetic baroreflex gain. This finding is in agreement with our previous studies (34). However, after central administration of the catalase inhibitor, these variables were no longer significantly different between the groups. Therefore administration of the catalase inhibitor into the 4th V combined with exposure to SSCS likely had a greater acute effect in WKY rats than SH rats.

Based on our findings, we suggest that SSCS exposure affects the oxidative stress balance in the 4th V in WKY rats. The morphology of the nose can be affected by substances able to achieve passage into the brain, and some substances may enter the brain through cranial nerves (42). We believe that the olfactory vector hypothesis for neurological diseases may explain our data (43). Prior studies have indicated that substances injected into the 4th V preferably reach the parasympathetic system. Areas such as the dorsal motor nucleus of the vagus and nucleus ambiguus receive glutamatergic projections from the nucleus of the solitary tract (20).

We used SSCS exposure in this investigation because it has been suggested that SSCS is more toxic than mainstream cigarette smoke (44). Indeed, the Philip Morris Company secretly performed in vivo toxicological tests of the effects of sidestream smoke (44) and observed that condensates and sidestream particulate matter present more mutagenic effects than the mainstream particulate matter. Moreover, SSCS contains approximately 200-fold more ammonia than mainstream smoke. SSCS presents a higher density of toxic substances than mainstream smoke because of its lower temperature of combustion and absence of filtering (45). For example, SSCS has five-fold more acrolein than mainstream smoke (46). On inhalation, fresh sidestream smoke is two- to six-fold more toxic per gram than mainstream smoke, depending on the end point (47). Taken together, our results and the findings of these other studies are relevant for directing and implementing public health policies.

In our research, the baroreflex was investigated in conscious, unanesthetized rats because anesthesia affects cardiovascular responses (48). We believe that our investigation provides reliable information regarding the effects of the administration of the catalase inhibitor ATZ into the 4th V on cardiovascular responses in conscious rats. Our results are useful because recently, the cardiovascular reflex has been studied in different rat models and strains (49-51) with the aim of preventing the development of cardiovascular disorders in humans (52-54), as reduced cardiovascular reflex responses are indicative of cardiovascular disease (55-57). However, some points must be considered. We did not quantify concentrations of H_2O_2 or other ROS in the 4th V during the procedures. Such data would reinforce our results by demonstrating that the ROS levels are changed in the 4th V. A previous study (58) reported a technique for continuous on-line measurement of cerebral H_2O_2.

In conclusion, our findings indicate that administration of a catalase inhibitor into the 4th V has strong effects on WKY rats but not SH rats exposed to SSCS.

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■ AUTHOR CONTRIBUTIONS

Valenti VE, Abreu LC, Fonseca FL, Adami F and Sato MA designed the study and performed the experiments. Vanderlei LC, Ferreira LL, Rodrigues LM and Ferreira C drafted the manuscript and performed the English grammar and spelling review. Valenti VE, Vanderlei LC, Ferreira LL, Rodrigues LM, Abreu LC, Fonseca FL and Ferreira C developed the experimental design, interpreted the text and drafted the manuscript. All authors read and approved the final version submitted for publication.

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