

Exercise changes regional vascular control by commissural NTS in spontaneously hypertensive rats

Cristiana A. Ogihara,¹ Gerhardus H. M. Schoorlemmer,¹ Adriana C. Levada,² Tania C. Pithon-Curi,² Rui Curi,² Oswaldo Ubriaco Lopes,¹ Eduardo Colombari,^{1,3} and Monica A. Sato³

¹Department of Physiology, Federal University of Sao Paulo, Sao Paulo, Brazil; ²Department of Physiology, Biomedical Sciences Institute—University of Sao Paulo, Sao Paulo, Brazil; and ³Faculdade de Medicina do ABC, Santo Andre, Brazil

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Ogihara CA, Schoorlemmer GHM, Levada AC, Pithon-Curi TC, Curi R, Lopes OU, Colombari E, Sato MA. Exercise changes regional vascular control by commissural NTS in spontaneously hypertensive rats. *Am J Physiol Regul Integr Comp Physiol* 299: R291–R297, 2010. First published April 21, 2010; doi:10.1152/ajpregu.00055.2009.—Inhibition of the commissural nucleus of the solitary tract (commNTS) induces a fall in sympathetic nerve activity and blood pressure in spontaneously hypertensive rats (SHR), which suggests that this subnucleus of the NTS is a source of sympathoexcitation. Exercise training reduces sympathetic activity and arterial pressure. The purpose of the present study was to investigate whether the swimming exercise can modify the regional vascular responses evoked by inhibition of the commNTS neurons in SHR and normotensive Wistar-Kyoto (WKY) rats. Exercise consisted of swimming, 1 h/day, 5 days/wk for 6 wks, with a load of 2% of the body weight. The day after the last exercise session, the rats were anesthetized with intravenous α -chloralose, tracheostomized, and artificially ventilated. The femoral artery was cannulated for mean arterial pressure (MAP) and heart rate recordings, and Doppler flow probes were placed around the lower abdominal aorta and superior mesenteric artery. Microinjection of 50 mM GABA into the commNTS caused similar reductions in MAP in swimming and sedentary SHR (-25 ± 6 and -30 ± 5 mmHg, respectively), but hindlimb vascular conductance increased twofold in exercised vs. sedentary SHR (54 ± 8 vs. $24 \pm 5\%$). GABA into the commNTS caused smaller reductions in MAP in swimming and sedentary WKY rats (-20 ± 4 and -16 ± 2 mmHg). Hindlimb conductance increased fourfold in exercised vs. sedentary WKY rats ($75 \pm 2\%$ vs. $19 \pm 3\%$). Therefore, our data suggest that the swimming exercise induced changes in commNTS neurons, as shown by a greater enhancement of hindlimb vasodilatation in WKY vs. SHR rats in response to GABAergic inhibition of these neurons.

GABA; cardiovascular; citrate synthase

THE NUCLEUS OF THE SOLITARY tract (NTS) is the primary site in the central nervous system that receives the afferents arising from arterial baro- and chemoreceptors (10, 15). The intermediate NTS receives inputs particularly from arterial baroreceptors, but some of these afferents achieve the commNTS (10, 16). The commNTS also receives inputs from arterial chemoreceptors, and seems to be highly sensitive to carotid chemoreceptor stimulation (8, 9, 11).

The possible role of the commNTS as a source of sympathoexcitation was first shown by Nelson et al. (33), which demonstrated that nanoliter injections of L-glutamate into the

commNTS evoke elevations in heart rate and arterial pressure mediated by the sympathetic nervous system.

Electrolytic lesions or chemical inhibition of the commNTS have been shown to markedly decrease the splanchnic sympathetic nerve activity and arterial pressure in spontaneously hypertensive rats (SHR), which suggested that this subnucleus of the NTS contains neurons tonically active in SHR, responsible for the high blood pressure in these animals (40, 41).

Evidence suggests that moderate exercise reduces the diastolic and systolic pressure in hypertensive humans and animals (2, 28, 39). The heart rate seems to be reduced by low-intensity exercise training in SHR, likely due to a decrease in the sympathetic tone to the heart (17). This effect has been also attributed to causing the decrease in the cardiac output in low-intensity exercise-trained SHR and, consequently, for reducing the hypertension (46). Postexercise hypotension may persist for more than 12 h (16, 19), and it has been reported that arterial baroreceptor reflex is required for this reduction in arterial pressure (7), but all of the mechanisms underlying this effect are not fully understood.

Most of the previous studies have shown that exercise training reduces hypertension, and the peripheral mechanisms involved to produce this effect have been largely investigated in humans and animals (3, 7, 12, 13, 17, 19, 27, 30, 39, 44). On the other hand, there are few studies showing the possible central mechanisms underlying the cardiovascular changes evoked by exercise training in animals.

As the central mechanisms underlying the cardiovascular adjustments elicited by exercise are not well understood, the commNTS is a site of convergence of cardiovascular afferents, and the influence of commNTS neurons on sympathetic nerve activity control has been demonstrated in SHR, our purpose was that the low-intensity exercise could modify the commNTS-regulated hemodynamic responses in SHR and Wistar-Kyoto (WKY) rats. To test this hypothesis, we evaluated the regional vascular responses evoked by GABAergic inhibition of the commNTS neurons in SHR and WKY rats sedentary or submitted to swimming exercise.

MATERIALS AND METHODS

Animals. Adult male SHR and normotensive WKY rats (250–300 g, 14 to 16 wk old) were obtained from the central animal facility of the Federal University of Sao Paulo, Sao Paulo, Brazil. Rats were housed in groups of three in plastic cages in an air-conditioned room (20–24°C) with a 12:12-h light-dark cycle and had free access to standard chow pellets and water. All procedures were performed according to the Guide for Care and Use of Laboratory Animals endorsed by The American Physiological Society and by The Brazilian College of Animal Experimentation, and were approved by the

Address for reprint requests and other correspondence: M. A. Sato, Dept. of Physiology, Faculdade de Medicina do ABC, Av. Lauro Gomes, 2000, Vila Sacadura Cabral, Santo Andre, SP, Brazil 09060-650 (e-mail: monica.akemi.sato@gmail.com).

Animal Ethics Committee of the Federal University of Sao Paulo (protocol no. 1566/06).

Drugs. Halothane (Tanohalo) was obtained from Cristalia Laboratory, Itapira, SP, Brazil; α -chloralose, GABA, and urethane were obtained from Sigma (St. Louis, MO). The vehicle used to dissolve α -chloralose was propyleneglicol (Sigma). GABA was dissolved directly in saline and urethane was dissolved in distilled water.

Exercise. Swimming pools were plastic cylinders with a diameter of 30 cm and a height of 60 cm, filled to a height of 50 cm with lukewarm water (30–34°C), one for each rat. Rats were subjected to daily swimming sessions for 6 wk, 5 days/wk, always between 10:00 AM and 12:00 PM. The duration of the sessions gradually increased during the first days (first day: 10 min, second day: 15 min, third and fourth days: 30 min, subsequent days: 1 h). After the first week, exercise intensity was increased by placement of a small extra weight (2% of the body weight) around the chest of the rat during swimming. This load was used previously by Sturek et al. (42). During the exercise period, the age-matched sedentary control group was exposed to similar noise and handling, but were maintained in the individual empty swimming pools.

Measurement of tail cuff blood pressure and heart rate in awake rats. The tail pressure of the SHR was determined at the beginning of experiments and at the end of 6 wk of swimming to ensure that all of the SHR used were actually hypertensive and also to evaluate the effects of exercise.

The tail pressure was measured in gently heated rats by using a cuff placed around the rat's tail. The cuff was inflated until blood flow was occluded and then released until the first pulse of arterial flow could be detected. The cuff was connected to a transducer (model MLT 1010; AD Instruments, Melbourne, Australia). The signal was amplified and recorded in a data acquisition system (MacLab Power Lab System 4SP).

The ECG was recorded from transcutaneous electrodes, implanted on the rat's back the day before the experiment under brief (30 s) halothane anesthesia. Recordings lasted 30 min, and started when the rat had settled down. ECG signals were amplified (model ETH-250; CB Sciences, Dover, NH) and recorded on a data acquisition system (Power Lab 4SP). Heart rate was derived from the ECG with Chart 5.5 software (AD Instruments) by using stretches of the recording during which behavioral activity was low.

Assessment of the exercise effect. At the end of 6 wk of exercise, 2 h after the last swimming session, citrate synthase activity was assessed in the soleus muscle from both legs in sedentary and swimming rats. The animals were deeply anesthetized with 3% halothane in 100% O₂ and killed. Muscles were removed, frozen in liquid nitrogen, and stored at –70°C. Citrate synthase activity was determined following the method described by Alp (1) and used in previous studies (25, 29). Soleus muscles from both legs of each animal were homogenized in an extraction buffer (50 mM Tris-HCl and 1 mM EDTA, pH 7.4). After centrifugation at 13,000 rpm, for 1 min, at 4°C, aliquots of supernatants were used for the measurement of the enzyme activity. The activity of citrate synthase was expressed as nanomoles per minute per milligram of protein. Protein content of muscle homogenate was determined as described by Bradford (5) using bovine serum albumin as a standard.

The effectiveness of the exercise was also determined by comparison of the body weight gain of swimming and sedentary SHR and WKY rats. The percentage of body weight gain was determined by $[(\text{final body wt} - \text{initial body wt})/\text{initial body wt}] \times 100$. The difference in the %body wt gain in SHR and WKY rats was calculated by %body wt gain in sedentary rats – %body wt gain in swimming rats.

Preparation. Two hours after the completion of the last exercise session, rats were anesthetized with 2% halothane in 100% O₂, which was used during all of the surgical procedures. Femoral artery and vein were cannulated for pulsatile arterial pressure recording and infusion of drugs. Mean arterial pressure (MAP) and heart rate were

derived from the pulsatile arterial pressure signal. The trachea was cannulated for artificial ventilation with 100% O₂. Rectal temperature was maintained between 37 and 38°C.

Miniature Doppler flow probes (Iowa Doppler Products, Iowa City, IA) were placed around the lower abdominal aorta (1.3 mm of lumen) and superior mesenteric artery (1.0 mm of lumen) through a midline laparotomy for indirect measurement of hindquarter and mesenteric blood flow, respectively. The probe wires were exteriorized through a small opening left in the sutured wound. The leads of the flow probes were connected to a Doppler flowmeter (Department of Bioengineering, The University of Iowa, Iowa City, IA) for indirect blood flow measurement. More details about the Doppler technique, including the reliability of this method for estimation of the blood velocity have been previously described by Haywood et al. (1981). Relative hindquarter and mesenteric vascular conductance were calculated as the ratio of Doppler shift and MAP. Data were presented as percentage of change from the baseline $[(\text{final conductance} - \text{initial conductance})/\text{initial conductance}] \times 100$. The pulsatile arterial pressure, MAP, heart rate, and blood flow were digitalized and recorded in a MacLab (PowerLab 8SP System).

The animals were placed in a stereotaxic apparatus and a partial craniotomy of the occipital bone was performed. The dorsal surface of the brainstem was exposed. The coordinates for drug injections into the commNTS were taken at the midline from *calamus scriptorius* (0.5 mm caudal and 0.3 mm ventral to the dorsal surface of the medulla).

α -Chloralose (60 mg/kg iv) was used for maintenance of anesthesia while physiological variables were being recorded after halothane withdrawal at the end of the surgical procedures. Animals were unresponsive to noxious toe pinch and maintained a steady level of arterial pressure. A supplementary dose of α -chloralose (20 mg/kg iv) was infused, according to the stability of MAP and heart rate. After baseline MAP, heart rate, and blood flow recording, microinjections of GABA (50 mM) into the commNTS were made with glass pipettes (20 μ m tip diameter) coupled to a pressure injection apparatus (PicoSpritzer II). The volume of injection (60 nl) was estimated from displacement of the fluid meniscus in the pipette using a calibrated reticule.

At the end of the experiments, the animals were deeply anesthetized with an overdose of 2.0 g/kg iv urethane, and Evans blue dye was injected into the brainstem site of drug injection. The animals were transcardially infused with 10% formalin solution, and the brains were removed and stored in the same solution for at least 24 h. The brainstem was sliced in a freezing microtome in sections of 40 μ m and stained with 2% neutral red solution. The sites of drug injections were confirmed by histological analysis of the brainstem slices in a light field microscope. Figure 1 shows a typical example of GABA injection site and depicts the site of dye deposition (bregma: –14.6 mm) from the atlas of Paxinos and Watson (34).

Data analysis. Changes in the cardiovascular variables measured after commNTS injections were evaluated at the peak response. Results are expressed as means \pm SE. Data were submitted to two-way ANOVA followed by the Tukey's post hoc test for comparisons among the strains at the level of MAP, heart rate, and percentage of change from baseline in hindquarter and mesenteric conductances in anesthetized rats. The same statistical test was also used for comparisons of tail pressure and heart rate derived from ECG and body weights before and after 6 wk of exercise in conscious SHR and WKY rats. The significance level was set at $P < 0.05$.

RESULTS

Effects of swimming exercise on resting values. The swimming exercise significantly reduced resting heart rate derived from ECG and tail pressure in SHR compared with the values at the beginning of the experiments, but not in WKY rats as shown in Table 1.

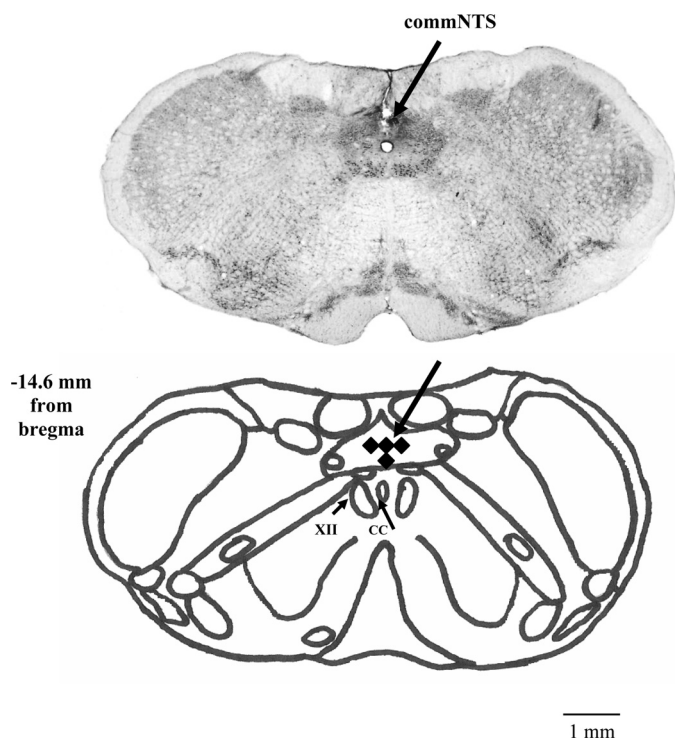


Fig. 1. *Top*: photomicrograph of one slice of the medulla showing the site of microinjection of GABA into the commissural nucleus of the solitary tract (commNTS) marked with 2% Evans blue in one rat of the group. *Bottom*: schematic representation depicting the localization of the site of microinjection of GABA approximately at -14.60 mm from bregma. XII, hypoglossal nucleus; CC, central canal.

After the period of 6 wk of swimming, the activity of citrate synthase in the soleus muscle was slightly higher in SHR (21%) and WKY (9%) rats compared with sedentary rats; however, these increases were not statistically significant in either strain. Citrate synthase activity was higher in WKY rats compared with SHR either in the swimming or in the sedentary group (Table 2).

The SHR and WKY rats submitted to exercise during 6 wk showed lower body weight gain than sedentary rats (Table 3).

Cardiovascular changes evoked by commNTS inhibition in exercised and sedentary rats. MAP was similar in swimming and sedentary α -chloralose-anesthetized SHR (155 ± 5 in exercised vs. 165 ± 5 mmHg in sedentary SHR). Heart rate was identical in both groups (322 ± 11 in exercised and 322 ± 12 beats/min in sedentary SHR).

Table 1. Tail-cuff arterial pressure (mmHg) and heart rate (beats/min) in awake spontaneously hypertensive rat (SHR) and Wistar-Kyoto (WKY) rats maintained sedentary or submitted to 6 wk of exercise

Strain	Tail Pressure		Heart Rate	
	Initial	After 6 wk	Initial	After 6 wk
SHR-sedentary	215 ± 2	220 ± 2	355 ± 2	337 ± 3
SHR-exercised	221 ± 3	$204 \pm 1^*$	380 ± 2	$323 \pm 2^*$
WKY-sedentary	119 ± 1	116 ± 2	335 ± 1	335 ± 1
WKY-exercised	122 ± 4	120 ± 1	309 ± 5	311 ± 5

Values are means \pm SE, $n = 6$ rats/group. *Different from initial.

Table 2. Citrate synthase activity ($\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg}$ of protein $^{-1}$) in sedentary or exercised SHR and WKY

Strain	Sedentary	Exercised
SHR	91 ± 7 ($n = 7$)	110 ± 8 ($n = 9$)
WKY	206 ± 37 ($n = 7$)*	225 ± 28 ($n = 7$)*

Values are means \pm SE, $n =$ number of rats/group. *Different from SHR.

Microinjection of GABA into the commNTS significantly reduced MAP either in exercised or sedentary SHR (Figs. 2 and 4). The injections did not significantly alter heart rate (1 ± 2 beats/min in swimming, 6 ± 2 beats/min in sedentary SHR, both groups $n = 6$). Swimming (-25 ± 6 mmHg) and sedentary SHR (-30 ± 5 mmHg) showed similar decrease in blood pressure after GABA into the commNTS, but a greater increase in the aortic conductance ($54 \pm 8\%$) was observed in exercised-SHR compared with sedentary SHR ($24 \pm 5\%$). Microinjections of GABA produced no significant effect on mesenteric vascular conductance ($-7 \pm 3\%$ in swimming SHR and $-2 \pm 3\%$ in sedentary SHR) (Figs. 3 and 4).

MAP and heart rate showed similar values in α -chloralose anesthetized WKY rats submitted to exercise (116 ± 5 mmHg and 281 ± 14 beats/min) and sedentary WKY rats (108 ± 2 mmHg and 332 ± 18 beats/min).

Microinjection of GABA into the commNTS elicited similar decreases in MAP in swimming (-20 ± 4 mmHg, $n = 6$) and sedentary WKY rats (-16 ± 2 mmHg, $n = 6$) (Figs. 2 and 4). Nevertheless, the decrease in MAP in sedentary WKY animals elicited by GABA into the commNTS was significantly smaller compared with sedentary SHR. Swimming SHR and WKY rats showed similar decreases in MAP evoked by GABA into the commNTS. The inhibition of the commNTS produced a greater increase in the aortic conductance ($75 \pm 2\%$) in swimming compared with sedentary WKY rats ($19 \pm 3\%$). No significant changes were produced in the mesenteric conductance by GABA injections into commNTS either in swimming ($-7 \pm 4\%$) or sedentary WKY rats ($-11 \pm 5\%$) (Figs. 3 and 4). No significant responses in heart rate were induced by GABA injections in exercised (-2 ± 2) and sedentary WKY rats (1 ± 2 beats/min).

The change in aortic conductance evoked by GABA into the commNTS increased to a greater extent in WKY rats submitted to exercise (~ 4 -fold) than in exercised SHR (~ 2 -fold) compared with the respective sedentary rats (Figs. 3 and 4).

Table 3. Body weights (g) and %body weight gain in SHR and WKY maintained sedentary or submitted to 6 wk of exercise

Strain	Initial Body Weight	Body Weight After 6 wk	%Body Weight Gain
SHR-sedentary	293.3 ± 3.2	350.0 ± 5.5	19.3
SHR-exercised	318.3 ± 9.2	343.0 ± 8.5	7.8*
WKY-sedentary	300.6 ± 9.8	363.8 ± 9.9	21.1
WKY-exercised	281.5 ± 1.9	309.0 ± 3.0	9.8*

Values are means \pm SE, $n = 6$. *Different from sedentary rats.

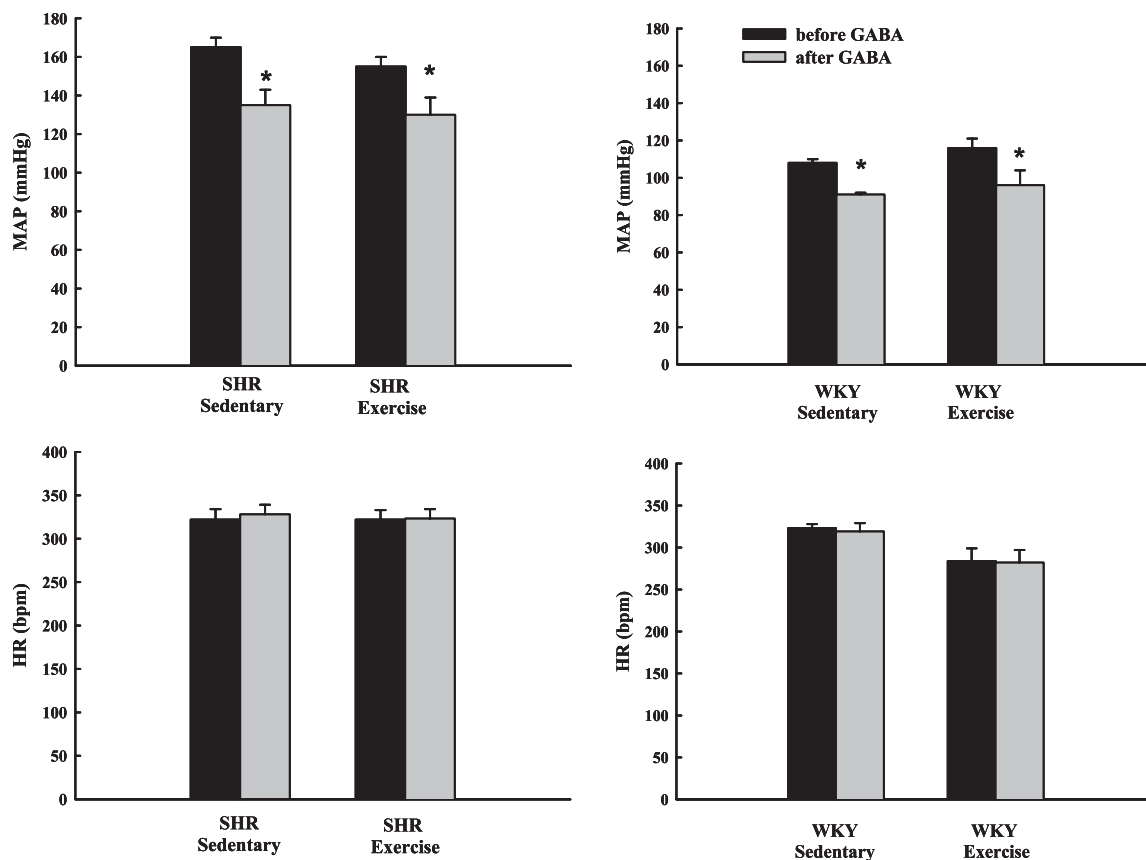


Fig. 2. Mean arterial pressure (MAP, mmHg) and heart rate (HR, beats/min) before and after microinjection of 50 mM (60 nl) GABA into the commNTS in sedentary and swimming exercise SHR (*left*) and WKY rats (*right*). *Different from before GABA.

DISCUSSION

The present study showed that the inhibition of commNTS neurons evoked similar decrease in MAP in exercised and sedentary SHR. However, sedentary SHR had a significantly greater decrease in MAP evoked by GABA into the commNTS than sedentary WKY rats. Swimming and sedentary WKY rats showed equivalent decreases in MAP evoked by inhibition of the commNTS. Nevertheless, the regional vascular changes were different. In exercised SHR and WKY rats, we observed a greater increase in aortic conductance compared with sedentary rats, suggesting an enhanced vasodilatation in the hindlimb in exercised animals evoked by inhibition of the commNTS. This vasodilatation observed in the hindlimb was much greater in swimming WKY rats than in SHR, likely due to the impaired vasorelaxation in SHR as described in earlier studies (2, 4, 18, 20, 25). In contrast, the mesenteric bed showed no significant changes in conductance after inhibition of the commNTS comparing exercised and sedentary SHR and WKY rats. Considering previous findings that the commNTS may contribute to hypertension via increase in sympathetic nerve activity (41), we expected that the mesenteric conductance would increase and consequently produce vasodilatation instead of no change upon inhibition of the commNTS. This apparent discrepancy could lie in the fact that inhibition of the commNTS would produce the withdrawal of splanchnic nerve tone and also, simultaneously, inhibition of the adrenal nerve, a branch of the splanchnic nerve. Thus, no epinephrine would be released to produce vasodilatation through adrenergic mecha-

nisms. If the resting β -adrenergic vasodilatory tone was greater than the resting vasoconstrictor tone in the mesenteric vascular bed, it is likely that inhibition of the commNTS would result in no response of the vasculature.

Although the measurement of blood flow in other vascular beds was not accomplished in our study, we cannot exclude the possibility that inhibition of the commNTS could induce changes in the conductance in other blood vessels differentially comparing sedentary and exercised rats. Indeed, our findings suggest that the enhanced vasodilatory responses in the hindlimb by inhibition of the commNTS after swimming exercise are likely due to alterations in the regulation of these neurons involved in cardiovascular function.

The NTS plays a pivotal role in the central network, integrating the baroreceptor inputs and other visceral and somatic afferents. Within the intermediate NTS, GABAergic neurons play an important role in baroreceptor signal processing. Most of the intermediate NTS neurons receive tonic GABA inputs from either interneurons in the NTS or projections from other brain regions (31). There is considerable evidence suggesting that increased GABAergic inhibition in the intermediate NTS contributes to the development of hypertension (6, 31, 45, 47). On the other hand, the inhibition of the commNTS reduces splanchnic sympathetic nerve activity and, consequently, produces a marked fall in blood pressure in SHR, suggesting this subnucleus of the NTS is tonically active in SHR (41). Thereby, according to the previous reports, the intermediate and commissural NTS have different neurons that control the

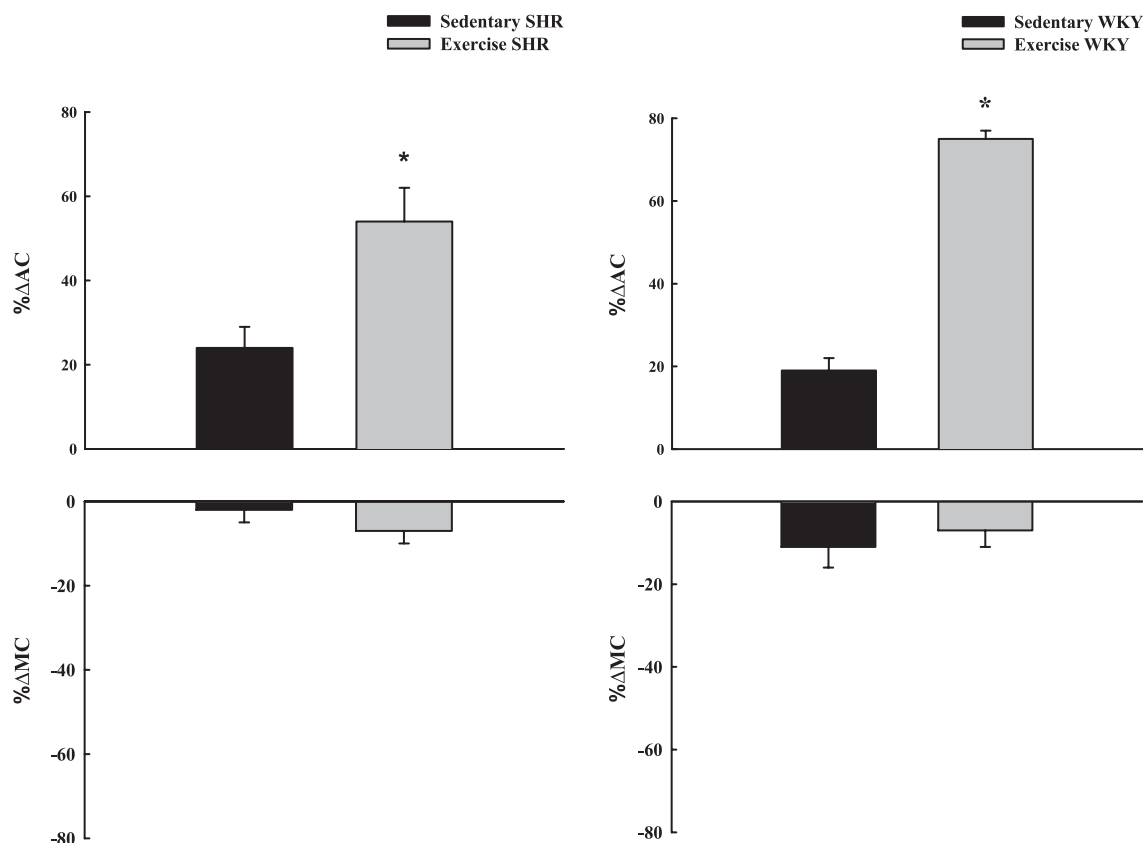


Fig. 3. Percentage changes ($\% \Delta$) in aortic (AC) and mesenteric (MC) conductance induced by microinjection of 50 mM (60 nl) GABA into the commNTS in sedentary and swimming exercise SHR (left) and WKY rats (right). *Different from sedentary rats.

sympathoexcitation. Nevertheless, cardiovascular responses evoked by L-glutamate in the intermediate NTS neurons are dependent on the integrity of commNTS, suggesting that fibers of the intermediate NTS pass through the commNTS or maybe interneurons of the intermediate NTS project to the commNTS (11).

Muscle afferents release substance P in the NTS and activate GABAergic neurons (37, 38). They seem to inhibit baroreflex neurons contributing to the resetting of baroreceptor reflex mechanism toward high blood pressure levels during exercise (38). In contrast, our data suggest the GABAergic neurons in the commNTS seem to be most important for depressor responses, and moreover, they appear essential for the enhanced vasodilation in the hindlimb in rats submitted to exercise training. Our findings also suggest this feature of the commNTS neurons on hemodynamic regulation in trained rats is likely more active due to the signalization from intermediate NTS neurons receiving muscle afferents.

Similar to previous studies that performed low-intensity exercise training for 18 wk in a treadmill or 8 wk of swimming exercise with 2% extra body weight load attached to the tail (42, 44), the approach of exercise in the current study reduced the resting heart rate and tail pressure in exercised SHR. Evidence suggested that the exercise training can reduce tonic GABA-mediated inhibition of intermediate NTS neurons involved in the control of heart rate that could explain the decrease in the resting values (32). Reduction of the resting tail pressure after swimming exercise compared with initial preex-

ercise values could be attributed to blunted baroreflex-mediated sympathoexcitation (12, 13, 27, 28, 32).

The swimming exercise approach used in the current study induced only a small increase of the citrate synthase activity, but this period of exercise was enough to produce changes on neural control of circulation. Exercise training for 8 wk or more increases the activity of citrate synthase (29, 30, 32). Indeed, our exercise protocol was not enough to produce the same metabolic muscular changes observed in trained animals. It is well known that endurance training causes an increase in the activity of oxidative enzymes (14, 22, 23). One of these enzymes is the citrate synthase, which is localized on the inner mitochondrial membrane and promotes the condensation of acetyl-CoA and oxalacetate, generating citrate in the Krebs cycle (36, 48). The increase in citrate synthase activity after an acute exercise bout indicates that there is a higher metabolic demand toward the oxidative pathway (36). Our study showed that citrate synthase activity in WKY rats at the end of 6 wk of exercise was higher than in SHR. However, the mechanisms underlying the difference in citrate synthase activity in WKY rats and SHR still requires further investigation.

Despite the small change in citrate synthase activity in exercised rats, the significantly lower percentage change in body weight gain in exercised compared with sedentary rats suggests that the swimming exercise approach used in the present study can induce other physiological or metabolic changes as reported in previous studies (43).

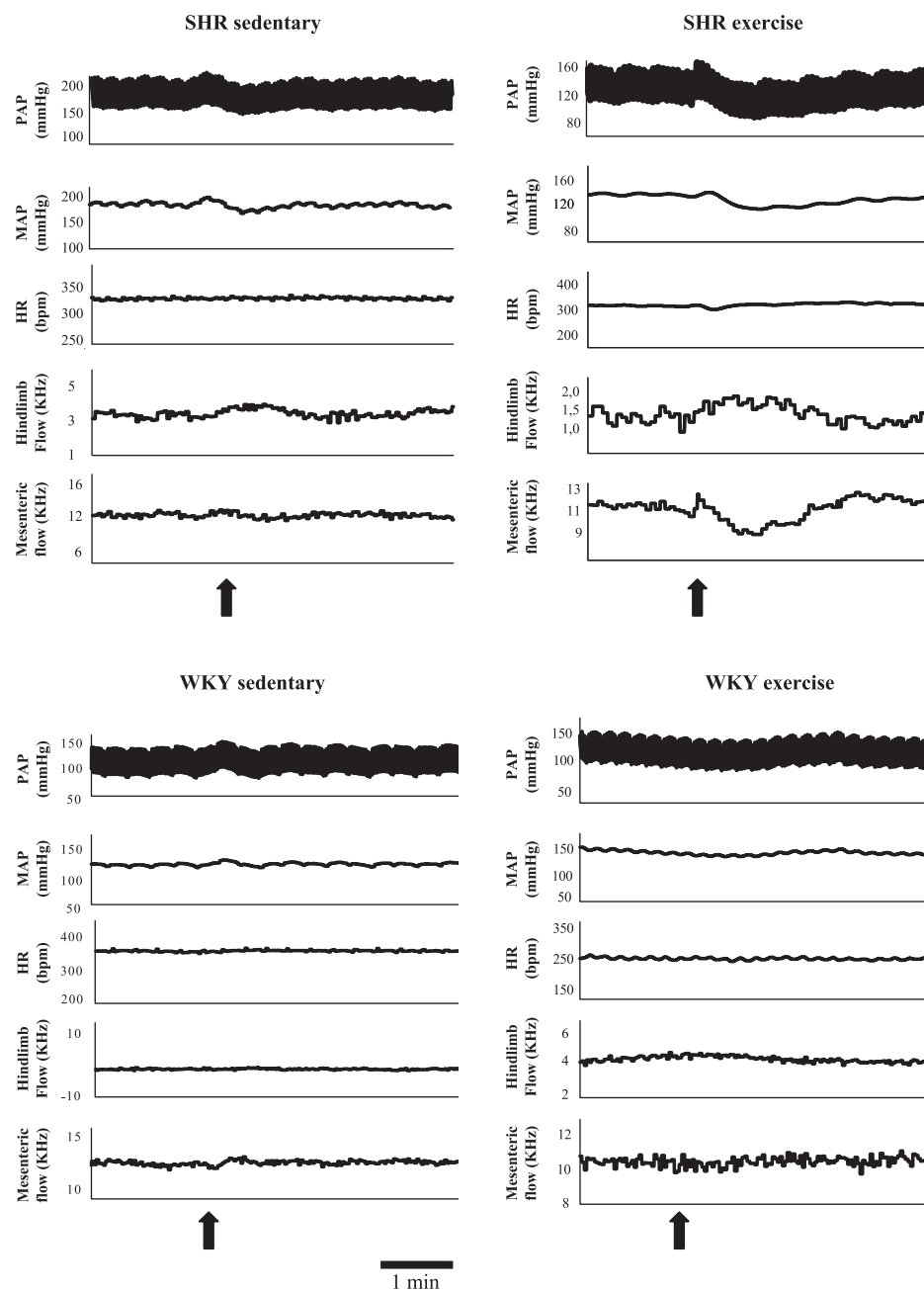


Fig. 4. Tracings showing the effects of GABA microinjections (arrows) into the commNTS on pulsatile arterial pressure (PAP, mmHg), MAP, HR, hindlimb flow (KHz), and mesenteric flow (KHz) in sedentary and swimming exercise SHR and WKY rats.

In conclusion, our findings suggest the low-intensity exercise can induce changes on commNTS neurons, and the GABAergic inhibition of this subnucleus of the NTS is important for the enhanced hindlimb vasodilatation either in exercised SHR or WKY rats. This effect seems to be greater in WKY than in SHR rats.

Perspectives and Significance

The marked decrease in splanchnic sympathetic nerve activity showed in previous studies (41) was not reflected by respective changes in mesenteric vascular bed in swimming SHR in the present study. As the aortic vasodilation evoked by inhibition of the commNTS was enhanced to a greater extent in WKY rats than SHR submitted to swimming exercise, it would be interesting to investigate whether the lumbar sympathetic

nerve activity is also inhibited to a great extent in trained WKY than SHR rats.

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DISCLOSURES

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

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