Afferent signaling drives oxytocinergic preautonomic neurons and mediates training-induced plasticity

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Cavalleri MT, Burgi K, Cruz JC, Jordão MT, Ceroni A, Michelini LC. Afferent signaling drives oxytocinergic preautonomic neurons and mediates training-induced plasticity. Am J Physiol Regul Integr Comp Physiol 301: R958–R966, 2011. First published July 27, 2011; doi:10.1152/ajpregu.00104.2011.—We showed previously that oxytocinergic (OTergic) projections from the hypothalamic paraventricular nucleus (PVN) to the dorsal brain stem mediate training-induced heart rate (HR) adjustments and that beneficial effects of training are blocked by sinoaortic denervation (SAD; Exp Physiol 94: 630–640; 1103–1113, 2009). We sought now to determine the combined effect of training and SAD on PVN OTergic neurons in spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats. Rats underwent SAD or sham surgery and were trained (55% of maximal capacity) or kept sedentary for 3 mo. After hemodynamic measurements were taken at rest, rats were deeply anesthetized. Fresh brains were frozen and sliced to isolate the PVN; samples were processed for OT expression (real-time PCR) and fixed brains were processed for OT immunofluorescence. In sham rats, training improved treadmill performance and increased the gain of baroreflex control of HR. Training reduced resting HR (~8%) in both groups, with a fall in blood pressure (~10%) only in SHR rats. These changes were accompanied by marked increases in PVN OT mRNA expression (3.9- and 2.2-fold in WKY and SHR rats, respectively) and peptide density in PVN OTergic neurons (2.6-fold in both groups), with significant correlations between OT content and training-induced resting bradycardia. SAD abolished PVN OT mRNA expression and markedly reduced PVN OT density in WKY and SHR. Training had no effect on HR, PVN OT mRNA, or OT content following SAD. The chronic absence of inputs from baroreceptors and chemoreceptors uncovers the pivotal role of afferent signaling in driving both the plasticity and activity of PVN OTergic neurons, as well as the beneficial effects of training on cardiovascular control.

sinoaortic denervation; exercise training; hypothalamus; paraventricular nucleus; supraoptic nucleus; oxytocin; spontaneous hypertension

ACCUMULATING EXPERIMENTAL evidence from our and other laboratories has shown that aerobic training promotes several beneficial cardiovascular effects in normotensive and hypertensive individuals. Training causes remodeling of the heart with a simultaneous stroke volume increase and heart rate (HR) decrease (5, 34, 40), outward eutrophic remodeling of arterioles, capillary angiogenesis, and venule neoinformation in the exercised muscles (1–3, 10, 24). Exercise training is also accompanied by a predominance of relaxation over contractile endothelium-derived factors (15, 44). These adaptive mechanisms by improving blood flow and tissue conductance, by reducing vascular resistance, and restoring normal endothelial function favor the amelioration of impaired functions in cardiovascular disease.

Training reduces both the activity of the renin-angiotensin system and oxidative stress (13, 22, 38) and effectively induces neuronal plasticity in brain areas involved in autonomic control (29). A series of studies indicated that low-intensity aerobic training in normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rats improved afferent signaling by aortic baroreceptors (7) and increased noradrenergic drive from the brain stem to hypothalamic preautonomic neurons (18). Training also caused structural remodeling and increased the intrinsic excitability of oxytocinergic (OTergic) and vasopressinergic (VPergic) neurons within the paraventricular nucleus (PVN) of the hypothalamus that project to the brain stem (20, 29), thus activating OTergic and VPergic drive to dorsal brain stem areas (23, 26, 29). Oxytocinergic input is involved in the modulation of vagal outflow to the heart, causing resting bradycardia and smaller exercise tachycardia in trained WKY and SHR rats (6, 16, 17, 25). It was also shown that central OTergic pathways are deprived in SHR (23), but a significant, although smaller, oxytocin-induced restraint of exercise tachycardia is still observed in the trained SHR (17). In contrast, activation of VPergic projections to the nucleus tractus solitarius (NTS) blunted pressure-induced activity of aortic afferents (4), contributing to the upward resetting of baroreflex during exercise that facilitated the tachycardic response (12, 27–29).

A recent study by Ceroni et al. (8) showed that the chronic absence of baroreceptor inputs blocked the training-induced reduction in vasomotor sympathetic tonus, the pressure fall in SHR rats, and training-induced resting bradycardia in SHR and WKY rats, suggesting that baroreceptor afferent signaling is an important stimulus that orchestrates training-induced plasticity within central autonomic pathways. The effects of training on HR responses were attributable to activation of OTergic pathways from the hypothalamus to dorsal brain stem (6, 17, 23, 25, 27), and OT has been shown to be involved in the baroreceptor reflex control of HR (11, 16, 37, 39, 43) and heart protection (14, 19, 21). We hypothesized that sinoaortic denervation (SAD) would affect the central OTergic drive that modulates the autonomic control of the heart in normotensive and hypertensive rats. Therefore, the present study investigated the effects of SAD on PVN OTergic neurons in SHR and WKY controls subjected to exercise training or kept sedentary. The combined effect of chronic afferent removal and training on resting HR and brain OT content were analyzed by hemodynamic recordings in conscious rats, followed by measurements of OT mRNA expression and OT immunoreactivity within PVN neurons. To distinguish between the effects of SAD on OTergic autonomic circuitry and the effects on plasma OT...
release, we also analyzed OT immunoreactivity within suprachiasmatic neurons (SOns).

MATERIALS AND METHODS

Animal surgery and experimental protocols. All surgical procedures and experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the University of São Paulo, in compliance with the Ethical Principles in Animal Research of the Brazilian College of Animal Experimentation.

Male WKY and SHR rats, aged 2 mo, were housed in Plexiglas cages in the Animal Facilities of the Department of Physiology and Biophysics under controlled temperature and a 12:12-h light-dark cycle, with free access to tap water and food. After an acclimatization period, active rats were preselected for their ability to walk/run on a treadmill (KT-300, Inbramed, Porto Alegre, Brazil; 5–10 sessions from 0.3 to 0.7 km/h, 0% grade, 10 min/day). The rats were then subjected to a progressive maximal exercise test on the treadmill (beginning at 0.3 km/h and increasing by increments of 0.3 km/h every 3 min until exhaustion). Sinoaortic denervation was performed according to Ceroni et al. (8) in half of the animals in both the WKY and SHR groups. Briefly, the rats were anesthetized [80 mg/kg ip ketamine (Fort Dodge, IA) plus 12 mg/kg ip xylazine (Fort Worth, TX)] to expose the neurovascular trunk bilaterally in the neck. The common carotid artery and vagus and sympathetic nerves were dissected to allow the identification and specific sectioning of aortic depressor nerves (traveling together as sympathetic nerves or as a separate branch, with preservation of the sympathetic trunk). The third contingent of afferentafferent fibers was interrupted by resection of the superior laryngeal nerve. The carotid bifurcation was exposed for resection of the sinus and carotid body nerves. The other half of the rats in the WKY and SHR groups was subjected to sham surgery without nerve sectioning. The rats were then treated subcutaneously with ketoprofen (1% Biofen, 2 mg/kg sc; Biofarm, Jaboraciab, Brazil) and penicillin (24,000 IU/kg sc; Pentabiotico Veterinario, Fontoura Wyeth, Brazil) and allowed to recover for 1 wk. The rats were then readapted to the treadmill for 1 wk. Before beginning the experimental protocols, the Sham and SAD WKY and SHR groups were subjected to a second maximal exercise test (week 0) to identify the effects of surgery on treadmill performance and to determine the intensity of training to assign the rats equal performance to the training and sedentary groups.

Low-intensity aerobic training (T = 50–60% of maximal exercise capacity, performed 5 days/wk, 1 h/day for 3 mo) was similar to that used previously in age-matched SHR and WKY rats (6, 8, 17, 23). The maximal exercise tests were repeated for all groups at weeks 6 and 12 to adjust the training intensity and compare the efficacy of the training and sedentary protocols, respectively. The rats allocated to the sedentary protocols (S) were handled every day and subjected once per week to a short period of mild exercise (5–10 min, 0.4–0.8 km/h, 0% grade) to keep the sedentary rats in conditions that approximated those experienced by the training groups. At the end of the experimental protocols, the rats were anesthetized intraperitoneally (ketamine plus xylazine) for chronic implantation of arterial and venous catheters in the left femoral artery and vein, as previously reported (8). The rats were treated again with analgesic plus antibiotic and allowed to recover for 1 day.

Hemodynamic measurements and baroreflex testing. Arterial pressure (AP) was measured on the next day in conscious freely moving rats. The arterial catheter was connected to the recording system (Gould StatMam P23XL transducer connected to a carrier amplifier and 5900 Gould Recorder; Valley View, OH). A variable period of time (15–30 min) was allowed for the stabilization of cardiovascular parameters before beginning the simultaneous measurement of AP and HR [determined from pulse interval; Biotach Gould (Valley View, OH) for 30–40 min (resting values)]. The baroreceptor reflex control of HR [loading/unloading of baroreceptors with phenylephrine and sodium nitroprusside, intravenously, as described previously (16)] was then tested to determine baroreflex sensitivity and confirm the SAD procedure.

Tissue sampling. After the functional measurements, the rats were deeply anesthetized with 60 mg/kg ip pentobarbital sodium, leading to respiratory arrest. The rats assigned to the PCR experiments were immediately subjected to transcardiac perfusion with 0.01 M PBS, pH 7.4 [Daigger pump, Vernon Hills, IL; 20–30 ml/min, ~5 min, with the perfusion pressure maintained in the same range as the mean AP (MAP) recorded in the conscious animal] and decapitated to remove the brain, which was quickly transferred to a dry-ice box. A slice (800–1,000 μm) was taken at the hypothalamic level and immediately frozen for bilateral PVN punches that contained the medial and caudal parts of the nucleus plus surrounding structures. The samples (~1.5 mg each) were collected in 0.5 ml TRIzol reagent and stored at ~80°C. After respiratory arrest and transcardiac perfusion with 0.01 M PBS (~5 min), the rats allocated to immunofluorescence processing received an infusion of 4% paraformaldehyde in 0.1 M PBS (pH 7.2, 400–500 ml, 10 ml/min). The rats were decapitated for brain removal. The brain was postfixed in 4% paraformaldehyde for 4 h at 4°C and cryoprotected in 0.1 M PBS that contained 30% sucrose at 4°C for 3–4 days. The brain was blocked and stored at ~80°C until processing.

Real-time PCR studies. mRNA expression was estimated by real-time PCR in 7–9 samples per group obtained from the SHAMWKY, SHAMWKY, SHAMSHR, SHAMSHR, SADWKY, SADSHR, SADSHR, and SADSHR groups. Total RNA was extracted using TRIzol reagent, according to the manufacturer’s instructions. Total RNA was dissolved in 10 μl of DEPC water and stored at ~80°C. DNase I was used to digest DNA and obtain pure RNA prior to the RT reaction. The integrity of the RNA was verified by agarose gel electrophoresis. Total RNA (2 μg/reaction) was used for first-strand cDNA synthesis using SuperScript II, according to the manufacturer’s protocol. RNaseOUT was also added to protect the RNA during this process. Three pooled RNA aliquots were routinely sham reverse-transcribed (i.e., reverse transcriptase was omitted) to ensure the absence of DNA contaminants. cDNA was stored at ~20°C until processing. The samples were subjected to real-time PCR amplification using Platinum SYBR QPCR Supermix-UDG and specific oligonucleotides for OT (sense primer, TTAGGCGGATATCGGGCAAG; antisense primer, CTCGGGAGAAGGCAGACTCAG). The real-time PCR reactions were performed and analyzed using the Corbett Research System (Corbett Life Sciences, Sydney, Australia). The PCR conditions were the following: 99°C for 2 min, followed by 45 cycles at 95°C for 15 s, 60°C for 60 s, and 72°C for 20 s. The specificity of the SYBR Green assay was confirmed by melting point analysis. Hypoxianan gluaine phosphoribosyltransferase (HPRT), continually expressed in all cells, was used as the reporter gene. In a pilot experiment, HPRT was not altered by hypertension, exercise training, or SAD. The mRNA expression data were calculated by cycle threshold (Ct) values using the ΔΔCt method (33), and the results are expressed as fold increases. All reagents and oligonucleotides were purchased from Invitrogen (San Diego, CA).

Immunohistochemical studies. Sequential hypothalamic coronal sections (25 μm, −1.80 to −2.12 caudal to bregma (32)) were cut with a cryostat (Leica CM 1850; Nussloch, Germany) and were collected in tissue culture wells with 0.01 M PBS. Free-floating sections were incubated with 0.01% Triton X-100 and 10% normal horse serum for 1 h. For the immunohistochemical reaction, the sections were incubated overnight with primary antibody (polyclonal guinea pig anti-OT, 1:200,000 dilution; Bachem, Bubendorf, Switzerland), followed by a 2-h incubation with secondary antibody (donkey-anti-guinea pig Cy3-labeled, 1:400 dilution; Jackson ImmunoResearch Laboratories, West Grove, PA), diluted in PBS containing 0.01% Triton X-100. Four to eight slices were placed in each slide and mounted with a coverslip and Vectashield. Control experiments were performed by omitting the primary or secondary antibody.
marked increases in attained velocity were already observed in SAD, however, did not interfere with the training or sedentary

0.02 km/h for SHR and WKY rats, respectively) with modest, Changes on treadmill performance induced by sedentary and training protocols in WKY and SHR groups submitted Table 1.

Sham groups during S and T (n = 14) (n = 13) (n = 14) (n = 15) Week 0 0.74 ± 0.04 0.76 ± 0.04 1.38 ± 0.06 * 1.38 ± 0.04 * Week 6 0.71 ± 0.03 1.34 ± 0.05 †# 1.19 ± 0.05 * 1.77 ± 0.06 **# Week 12 0.69 ± 0.03 1.39 ± 0.03 †# 1.23 ± 0.05 * 1.97 ± 0.05 **# Gain −0.05 ± 0.03 +0.64 ± 0.04 † −0.15 ± 0.08 +0.59 ± 0.05 † SAD groups during S and T (n = 14) (n = 13) (n = 14) (n = 15) Week 0 0.65 ± 0.03 0.72 ± 0.04 1.21 ± 0.04 * 1.31 ± 0.07 * Week 6 0.68 ± 0.03 1.24 ± 0.04 †# 0.89 ± 0.03 †# 1.80 ± 0.06 **†# Week 12 0.64 ± 0.03 1.34 ± 0.04 †# 0.86 ± 0.03 †# 1.95 ± 0.05 **†# Gain −0.01 ± 0.04 +0.62 ± 0.05 † −0.35 ± 0.05 +0.64 ± 0.06 †

Values, measured during maximal exercise tests on treadmill, are expressed as means ± SE. “S” denotes sedentary, while “T” denotes training, WKY, and WKYt, Wistar-Kyoto, sedentary and training, respectively. SHRs and SHRt, spontaneously hypertensive rats, sedentary and training, respectively. SAD, sinoaortic denervation. Significant differences (P < 0.05) are * vs. WKY; † vs. S; # vs. week 0.

The histological sections were carefully examined (Leica DMLB, Wetzlar, Germany) to localize the regions of interest (ROIs). An EBQ 100 mc-L system (Leistungslelektronik, Jena, Germany) was used to excite the Cy3 fluorochrome at 543 nm. The images from the medial and posterior PVN and SON from all experimental groups were digitized with identical acquisition settings and analyzed. Immunoreactive signals were acquired and quantified as previously described (18). Imaging analysis was performed with Image ProPlus software (Media Cybernetics, Silver Spring, MD). An automated tracing procedure that incorporated a threshold paradigm was applied to the OT immunoreactivity observations. Background intensity was calculated from random adjacent areas in the neuropil. The threshold was set to pass intensities 1.5-fold above background immunofluorescence. The ROIs of predetermined sizes were drawn within PVN subnuclei (posterior, post; ventromedial, vm; dorsal cap, dc; magnocellular, mg) and SON, and the density of the OT threshold signal within each ROI (expressed as a percentage of threshold area; i.e., area occupied by threshold signal/total ROI area × 100) was calculated to obtain the mean value for each area per rat. Average density values were then obtained for each experimental condition in each group (18).

Statistical analysis. The results are expressed as means ± SE. Treadmill performance in the Sham and SAD groups for both strains during the sedentary and training protocols was analyzed by three-way ANOVA with repeated measurements (time). The comparisons of the hemodynamic parameter, baroreceptor reflex control of HR, and OT content data between strains (SHR and WKY), conditions (sedentary and trained), and the presence or absence of surgery (sham and SAD) were performed with three-way ANOVA, followed by Fisher’s post hoc test. Correlation analyses were performed using Pearson statistics. Differences were considered significant at P < 0.05.

RESULTS

Treadmill performance: effects of SAD and training. At the beginning of the experiments, the SHR rats exhibited better performance on the treadmill compared with the WKY group (1.40 ± 0.03 vs. 0.83 ± 0.02 km/h, respectively). Sinoaortic denervation induced a small but significant decrease in treadmill performance in both groups (−0.18 ± 0.04 and −0.21 ± 0.02 km/h for SHR and WKY rats, respectively) with modest, nonsignificant changes in the Sham groups (−0.02 ± 0.03 and −0.06 ± 0.02 km/h for SHR and WKY rats, respectively). SAD, however, did not interfere with the training or sedentary protocols, which began 2 wk later. As shown in Table 1, marked increases in attained velocity were already observed in all trained groups at week 6, with a further small increase from week 6 to week 12 of training. At the end of the protocols, the trained groups attained similar velocities (SHR, 1.96 ± 0.05 km/h; WKY, 1.37 ± 0.03 km/h), with a similar performance gain in the WKY and SHR groups subjected to sham and SAD surgery (Table 1). The sedentary groups showed no significant changes or a small decrease (SHR rats subjected to SAD; Table 1) in treadmill performance.

Effects of SAD and training on hemodynamic parameters. The average basal values of MAP and HR are depicted on Fig. 1. As expected, sedentary SHR rats exhibited higher MAP and HR [180 ± 2 mmHg and 359 ± 6 beats per min (bpm)] than WKY controls. In the Sham groups, training caused significant resting bradycardia (from 311 ± 5 to 285 ± 5 bpm and 359 ± 6 to 332 ± 11 bpm in WKY and SHR rats, respectively; P < 0.05, corresponding to a 7–8% reduction), accompanied by a pressure decrease only in the SAD group (from 180 ± 2 to 162 ±
5 mmHg; \( P < 0.05 \), corresponding to a 10% decrease). In contrast, SAD completely abolished these beneficial effects of training, which were replaced by resting tachycardia (from 320 ± 6 to 343 ± 7 bpm in WKY rats and from 347 ± 8 to 375 ± 7 bpm in SHR rats; \( P < 0.05 \); Fig. 1) and a further pressure increase in the trained SHR rats (from 183 ± 2 to 195 ± 4 mmHg; \( P < 0.05 \)). WKY rats subjected to SAD exhibited higher MAP (140 ± 2 vs. 131 ± 2 mmHg in Sham controls; \( P < 0.05 \)). No training-induced pressure change was observed in SAD and Sham normotensive controls.

Training also improved the baroreceptor reflex control of HR in the Sham groups, reflected by the calculation of the reflex bradycardia and reflex tachycardia indices (Table 2). Bradycardic and tachycardic responses to loading and unloading of baroreceptors were absent after SAD, confirming the efficacy of sinoaortic afferent removal (Table 2).

### Effects of SAD and training on PVN OTergic neurons.

Oxytocin mRNA content in the PVN was greatly affected by SAD and training \( (F_{1,41} = 34.96, P < 0.001 \) and \( F_{1,41} = 11.77, P = 0.001 \), respectively) with a significant interaction between both \( (F_{1,41} = 11.41, P = 0.002) \). No effect of strain was observed \( (F_{1,41} = 1.98, P = 0.167) \). In the Sham groups, training was accompanied by a marked increase in OT expression \( [1.32 ± 0.51 to 5.16 ± 1.26 arbitrary units (AU) and from 1.42 ± 0.32 to 3.05 ± 0.61 AU in trained WKY and SHR rats, respectively, corresponding to 3.9- and 2.2-fold increases; \( P < 0.001 \) and \( P = 0.042 \), respectively; Fig. 2A]. Oxytocin mRNA content in the PVN was significantly correlated with resting HR in intact WKY and SHR rats. Negative relationships showed that the training-induced increase in OT mRNA content was accompanied by a training-induced decrease in HR (Fig. 2B). Surprisingly, SAD blocked OT mRNA expression within the PVN and abolished its correlation with HR values (Fig. 2, A and B). After SAD, training did not change OT mRNA expression.

To confirm the effects of training and SAD on central OTergic control, we analyzed OT immunofluorescence within the PVN. Fig. 3 shows dense OTergic innervations in the posterior PVN in Sham rats. Training was accompanied by an increased density of OTergic neurons in both the WKY and SHR groups. In contrast, OTergic neuron density was markedly depressed after SAD and not affected by training (Fig. 4). Similar to the mRNA data, the density of OTergic neurons within the PVN was not altered by hypertension \( (F_{1,16} = 0.617, P = 0.054 \) for the strain factor) but was significantly increased by training and markedly decreased by SAD \( (F_{1,16} = 68.89, P < 0.001; F_{1,16} = 230.97, P < 0.001 \) for condition and surgery factors, respectively), with interaction between factors \( (F_{1,16} = 69.76, P < 0.001) \). The quantitative data from specific PVN subnucleus (Fig. 5) showed that the OTergic neurons were largely concentrated within the ventromedial subnucleus in WKY rats \( (62 ± 3% \) vs. 20–30% in the other subnuclei; \( P < 0.05 \)). Hypertension reduced OT density within this area \( (47 ± 6\%) \), increased OT density in the magnocellular subnucleus \( (31 ± 3% \) to 46 ± 8\%, and did not change OT density in the posterior and dorsal cap subnuclei \( (29 ± 1\% \) and 21 ± 2% respectively). The data from the Sham groups in Fig. 5 also confirmed a robust training-induced increase in OT density in posterior nuclei \( (+2.9\text{-fold for both WKY and SHR}) \), dorsal cap nuclei \( (+3.3\text{- and } +2.9\text{-fold for WKY and SHR, respectively}) \), ventromedial nuclei \( (+1.4\text{- and } +1.9\text{-fold for WKY and SHR, respectively}) \), and magnocellular nuclei \( (+3.0\text{- and } +1.7\text{-fold for WKY and SHR, respectively}) \). Sinoaortic denervation decreased OT density in all PVN subnuclei.
in WKY and SHR rats (average reduction of 66% to 77%; \( P < 0.05 \) for SAD vs. Sham in all comparisons; Fig. 5). As shown in Table 3, in the posterior, ventromedial, dorsal cap, and magnocellular subnuclei, significant negative correlations were found between local OT density and resting HR in Sham sedentary/trained WKY and SHR rats. In contrast, no significant correlations were observed after SAD (see values in Table 3).

Notably, SAD had no effect on the density of OTergic neurons in the SON (Fig. 6). Within this nucleus, the relative OT density was similar for sedentary Sham WKY and SHR rats (53 ± 2% and 54 ± 2%, respectively) and unchanged by SAD (56 ± 1% and 54 ± 1%, respectively).

DISCUSSION

The present study confirmed the importance of afferent signaling for driving the beneficial effects of exercise training on resting bradycardia and pressure fall (8). Additionally, the present study revealed several new observations: 1) training caused a marked increase in OT mRNA expression in the PVN in the hypothalamus, accompanied by augmented PVN OT density in the normotensive and hypertensive groups, 2) increased OT mRNA expression and increased OT content in trained individuals were significantly correlated with reduced HR recorded in conscious rats at rest, 3) SAD blocked PVN OT mRNA expression, causing a marked decrease in the density of OT-positive neurons within the PVN in both groups, 4) removal of afferent signaling impaired training-induced resting bradycardia, blocking the negative correlation between hypothalamic OT content and HR values in both normotensive and hypertensive rats, and 5) this effect was specific for PVN OTergic neurons because SAD did not interfere with the OT immunoreactivity observed in SON neurons. Altogether, these findings strongly suggest that afferent signaling conveyed by...
baroreceptors and chemoreceptors is crucial for driving the PVN OTerergic neurons involved in HR control.

Accumulating experimental evidence in humans and animal models has demonstrated the efficacy of low-intensity aerobic training in reducing blood pressure in hypertension by decreasing HR and sympathetic activity and increasing vagal outflow to the heart in normotensive and hypertensive individuals (8, 9, 17, 27, 29, 34). Accordingly, hemodynamic recordings in the Sham groups in the present study confirmed both a decrease in pressure in trained SHR rats and training-induced resting bradycardia in both WKY and SHR rats. Previous data from our and other laboratories indicated an important role of OT within the dorsal brain stem and the involvement of PVN OTerergic projections in the modulation of HR control. Oxytocin acted in the NTS/dorsal motor nucleus of the vagus (DMV) complex to facilitate vagal outflow and reflex bradycardia during baroreceptor loading in normotensive rats (11, 16, 39). Oxytocin receptor blockade in the NTS reduced reflex bradycardia (16) and increased the tachycardic response to an acute bout of exercise (6, 25, 27).

Interestingly, exercise-induced activation of OTergic projections from the PVN to the NTS/DMV complex was only observed in trained rats (6, 25, 27). These observations were then extended to hypertensive individuals (17, 23). In both WKY and SHR rats, training did not change the expression of OT receptors within the NTS but augmented the density of OTerergic projections to this area (17, 23, 27, 29), thus facilitating the slowing of HR during exercise. In addition to the effects of training on PVN OTerergic neurons that project to the dorsal brain stem, the present data showed that training increased both PVN OT mRNA expression and OT density in the WKY and SHR groups. These changes significantly correlated with training-induced reductions in resting HR. A long cascade of events elicits these effects, triggered by an increase in pressure during repetitive exercise: 1) increased afferent signaling to the NTS (7), 2) increased noradrenergic drive from NTS to PVN preautonomic neurons (18), 3) structural remodeling of trained PVN preautonomic neurons that exhibit stronger dendritic branching and a larger surface area compared with sedentary controls (29), 4) increased OT mRNA expression in the PVN and substantial augmentation of peptide density within parvocellular PVN OTerergic neurons (present data in trained intact controls), 5) increased intrinsic excitability, specifically in PVN-NTS-projecting neurons, with diminished input-output function in PVN magnocellular neurons that project to the neurohypophysis (20), 6) augmented OTerergic projections from the PVN to the NTS/DMV complex in trained individuals (23, 26), 7) facilitated vagal outflow to the heart during OTerergic stimulation (16, 27), and 8) resting bradycardia in both trained WKY and SHR rats, which correlated with peptide content within OTergic preautonomic neurons (present study).

The cellular mechanisms through which OT acts in the NTS/DMV complex are presently poorly understood. Oxytocinergic terminals are evenly distributed throughout the NTS (23, 35), and OT-immunoreactive axons are closely apposed, suggesting synaptic contacts, with second-order NTS neurons (23, 26). Peters et al. (35) showed that OT released from OTergic preautonomic neurons that project to the neurohypophysis (20), 6) augmented OTerergic projections from the PVN to the NTS/DMV complex in trained individuals (23, 26), 7) facilitated vagal outflow to the heart during OTerergic stimulation (16, 27), and 8) resting bradycardia in both trained WKY and SHR rats, which correlated with peptide content within OTergic preautonomic neurons (present study).

Notably, training also increased OT density within the magnocellular PVN subnucleus that contains neurosecretory neu-

### Table 3. Regression equations correlating OT immunoreactivity changes with heart rate changes induced by training in different PVN subnuclei of WKY and SHR submitted to SAD or sham surgery

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<th>Sham</th>
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<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
<td>WKY</td>
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<tr>
<td>PVN post</td>
<td>$Y = -0.82x + 305^*$</td>
<td>$Y = -0.57x + 258^*$</td>
<td>$Y = 0.03x - 2$</td>
<td>$Y = 0.03x - 4$</td>
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<tr>
<td></td>
<td>($r = -0.98$)</td>
<td>($r = -0.94$)</td>
<td>($r = 0.44$)</td>
<td>($r = 0.02$)</td>
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<tr>
<td>PVN vm</td>
<td>$Y = -0.34x + 177^*$</td>
<td>$Y = -0.48x + 237^*$</td>
<td>$Y = 0.02x - 3$</td>
<td>$Y = 0.01x + 8$</td>
</tr>
<tr>
<td></td>
<td>($r = -0.88$)</td>
<td>($r = -0.96$)</td>
<td>($r = 0.37$)</td>
<td>($r = 0.56$)</td>
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<tr>
<td>PVN dc</td>
<td>$Y = -0.68x + 251^*$</td>
<td>$Y = -0.40x + 179^*$</td>
<td>$Y = 0.06x - 9$</td>
<td>$Y = 0.07x + 39$</td>
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<tr>
<td></td>
<td>($r = -0.92$)</td>
<td>($r = -0.92$)</td>
<td>($r = 0.56$)</td>
<td>($r = -0.63$)</td>
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<tr>
<td>PVN mg</td>
<td>$Y = -0.88x + 327^*$</td>
<td>$Y = -0.31x + 171^*$</td>
<td>$Y = -0.31x + 171^*$</td>
<td>$Y = -0.56x + 8$</td>
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<td>($r = -0.96$)</td>
<td>($r = -0.96$)</td>
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PVN post, posterior PVN; PVN vm, ventromedial subnucleus of medial PVN; PVN dc, dorsal cap subnucleus of medial PVN; PVN mg, magnocellular subnucleus of medial PVN. *Significant correlation ($P < 0.05$).
rons that project to the posterior pituitary. Radioimmunoassay measurements of OT in plasma and discrete brain areas obtained from trained and sedentary WKY rats revealed that training was accompanied by an increase in PVN OT content, but no change was observed in either SON or plasma levels (6). The determinants of these effects are currently unknown. The reduced intrinsic excitability exhibited by PVN magnocellular neurons after training (20) could contribute to reduced release and increased OT content within magnocellular neurons.

In the present study, the data obtained from the WKY and SHR groups subjected to SAD (lack of training-induced decrease in pressure and HR) confirmed the importance of baroreceptor signaling for driving the beneficial effects of training on cardiovascular control (8, 30). An original observation that arose from this study was that SAD was accompanied by an absence of OT mRNA expression in the PVN and a marked reduction in OT density within autonomic and neuroendocrine PVN subnuclei, suggesting reduced activity. Importantly, SAD did not change the robust OT immunoreactivity within magnocellular SON neurons in sedentary WKY and SHR rats, indicating that this nucleus could provide elevated plasma OT levels upon specific stimulation. Indeed, a previous study demonstrated that SAD did not change OT and VP mRNA expression within the SON and did not change the plasma levels of these hormones in rats maintained under tap water drinking (36), a condition similar to that of the present study. Sinoaortic denervation also did not prevent neurohypophysial secretion but maintained its ability to cause modest or strong OT and VP release after isotonic or osmotic and hypovolemic stimuli, respectively (31, 36, 41, 42). The differential effects of SAD on OT content within PVN neurons (i.e., a marked reduction) and SON neurons (i.e., no change) indicated the specificity of baroreceptors and chemoreceptors in driving the PVN OTergic neurons that modulate autonomic control and not the regulation of fluid balance by the SON OTergic pathway. Furthermore, the strict dependence of PVN OT levels on the integrity of baroreceptor and chemoreceptor inputs strongly suggests that their targets in the PVN are associated with autonomic functions. In this regard, we showed previously that a large number of PVN OTergic neurons projects to the solitary-vagal complex (18, 20) and that OT receptor blockade within the solitary-vagal complex does alter the parasympathetic control of the heart (6, 16, 17). The depression of the PVN OTergic system associated with complete blockade of training-induced effects on HR after SAD supports our hypothesis that training exerts its beneficial effects on the

Fig. 6. A: photomicrographs comparing OTergic staining in the supraoptic nucleus (SON) of the hypothalamus in sedentary WKY and SHR rats subjected to sham or SAD surgery. B: relative OT density within the SON in sedentary WKY and SHR groups subjected to sham or SAD surgery. The values represent measurements made in 4–6 slices per rat, with 3 or 4 rats per group.
cardiovascular system by facilitating hypothalamic OTergic modulation. Our data also indicate the pivotal role of baroreceptors and chemoreceptors in driving central autonomic pathways. The relative participation of baroreceptors and chemoreceptors in driving OTergic control of HR must be evaluated in future experiments with selective carotid body removal.

Similar effects of training on PVN OT content and resting bradycardia in intact WKY and SHR rats indicate the ability of training to improve the activity of the hypothalamic preautonomic neurons that control vagal outflow in both normotensive and hypertensive individuals. In a previous study, we found similar training-induced resting bradycardia in the WKY and SHR groups but larger exercise-induced tachycardia during an acute bout of exercise in WKY rats after OT receptor blockade within the NTS (17). Oxytocin receptor density in the NTS was significantly reduced by hypertension and not affected by training (17, 23). Therefore, the smaller training-induced reduction in HR exhibited by hypertensive rats during stimulated conditions was mainly attributable to reduced OT receptor density in the target area because training-induced plasticity in PVN OTergic preautonomic neurons was nearly similar in WKY and SHR rats.

In summary, the chronic absence of baroreceptor and chemoreceptor inputs reveals the pivotal role of afferent signaling in driving the plasticity and functionality of OTergic preautonomic neurons. The beneficial effects of training on HR control in normotensive and hypertensive individuals are also triggered by afferent signaling and mediated by PVN OTergic preautonomic neurons that undergo marked augmentation in their relative density.

Perspectives and Significance

The association between hemodynamic recordings and techniques that quantify the expression and content of brain peptidergic, catecholaminergic, and cholinergic neurons offers a new approach to simultaneously study the plasticity and functionality of central pathways that integrate vegetative, neuroendocrine, and behavioral function. This approach, associated with exercise training, enabled us to uncover the important role of afferent signaling (conveyed by arterial receptors) in facilitating the OTergic control of the heart, thus determining resting bradycardia in normotensive and hypertensive individuals. The addition of SAD to these techniques allowed us to uncover the decisive role of arterial baroreceptors and chemoreceptors in driving autonomic and neuroendocrine responses. This is the first demonstration that afferent signaling is crucial for maintaining the normal activity of preautonomic PVN OTergic neurons. Procedures such as these may become more frequent in studies designed to understand integrative brain mechanisms.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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