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Aerobic training normalizes autonomic dysfunction, HMGB1 content, microglia activation and inflammation in hypothalamic paraventricular nucleus of SHR

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1Comparative Biomedical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, Louisiana; 2Department of Physiology & Biophysics, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil; and 3Department of Physiology & Pharmacology, Fluminense Federal University, Niterói, Brazil

Submitted 14 May 2015; accepted in final form 25 July 2015

Masson GS, Nair AR, Silva Soares PP, Micheliní LC, Francis J. Aerobic training normalizes autonomic dysfunction, HMGB1 content, microglia activation and inflammation in hypothalamic paraventricular nucleus of SHR. Am J Physiol Heart Circ Physiol 309: H1115–H1122, 2015. First published August 7, 2015; doi:10.1152/ajpheart.00349.2015.—Exercise training (ExT) is recommended to treat hypertension along with pharmaceutical antihypertensive therapies. Effects of ExT in hypothalamic content of high mobility box 1 (HMGB1) and microglial activation remain unknown. We examined whether ExT would decrease autonomic and cardiovascular abnormalities in spontaneously hypertensive rats (SHR), and whether these effects were associated with decreased HMGB1 content, microglial activation, and inflammation in the hypothalamic paraventricular nucleus (PVN). Normotensive Wistar-Kyoto (WKY) rats and SHR underwent moderate-intensity ExT for 2 wk. After ExT, cardiovascular (heart rate and arterial pressure) and autonomic parameters (arterial pressure and heart rate variability, peripheral sympathetic activity, cardiac vagal activity, and baroreflex function) were measured in conscious and freely-moving rats through chronic arterial and venous catheterization. Cerebrospinal fluid, plasma, and brain were collected for molecular and immunohistochemistry analyses of the PVN. In addition to reduced heart rate variability, decreased vagal cardiac activity and increased mean arterial pressure, heart rate, arterial pressure variability, cardiac, and vasomotor sympathetic activity, SHR had higher HMGB1 protein expression, pIkB-α phosphorylation, TNF-α and IL-6 protein expression, and microglia activation in the PVN. These changes were accompanied by higher plasma and cerebrospinal fluid levels of HMGB1. The ExT + SHR group had decreased expression of HMGB1, CXCR4, SDF-1, and phosphorylation of p42/44 and pIkB-α. ExT reduced microglial activation and proinflammatory cytokines content in the PVN, and improved autonomic control as well. Data suggest that training-induced downregulation of activated HMGB1/CXCR4/microglia/proinflammatory cytokines axis in the PVN of SHR is a prompt neural adaptation to counterbalance the deleterious effects of inflammation on autonomic control.

NEW & NOTEWORTHY

Spontaneously hypertensive rats have increased HMGB1 content and CXCR4 signaling in the hypothalamic paraventricular nucleus, which contributes to microglial activation, proinflammatory cytokines production, and, finally, to autonomic dysfunction. Aerobic training decreases HMGB1 content, microglia activation and proinflammatory cytokines expression by inhibiting HMGB1-CXCR4 signaling pathway in the hypothalamic paraventricular nucleus. Aerobic training induces neuroinflammatory adaptations and autonomic benefits independent of the arterial pressure fall.

AUTONOMIC DYSFUNCTION is defined as a misbalance between sympathetic and vagal activity associated with baroreflex dysfunction and increased arterial pressure variability. This major pathological feature in hypertension is closely related to end-organ injuries, as retinopathy, glomerular sclerosis, stroke, and myocardial infarction (25, 42). Experimental studies from our group have shown that hypertensive rats had increased proinflammatory cytokines expression in autonomic control areas, such as the hypothalamic paraventricular nucleus (PVN) (2, 31). In addition, it was demonstrated that acute microinjection of proinflammatory molecules into the PVN of normotensive rats causes elevation of both arterial pressure and renal sympathetic nerve activity (38). On the other hand, chronic inhibition of proinflammatory signaling in the brain decreased arterial pressure and renal sympathetic nerve activity, and reversed the deleterious cardiac remodeling in hypertensive rats (7, 13, 18, 39). Therefore, brain inflammation emerges as a central factor that contributes to autonomic and cardiovascular abnormalities in hypertension.

Recently, high-mobility group protein box-1 (HMGB1) was described as a cytokine that triggers injury-induced inflammatory response (29). Toll-like receptor 4 (TLR4) and chemokine receptor CXC receptor 4 (CXCR4) signaling are activated by HMGB1 (36, 37). In the central nervous system, HMGB1 seems to regulate microglial activation and the subsequent production of proinflammatory cytokines (15). Previous stud-
ities demonstrated that CXCR4 signaling induces microglial activation, MAPK p42/44 phosphorylation, and the expression of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) (5, 30). These findings suggest a novel mechanism that could drive inflammation-induced autonomic dysfunction in hypertension.

Exercise training (ExT) is broadly recognized as a preventive and adjuvant therapeutic tool in hypertension. Experimental and clinical data demonstrated cardiovascular, hemodynamic, and autonomic adaptations in trained individuals and attenuated end-organ injuries (4, 11, 26). Decreased tissue oxidative stress and inflammation were described as key mechanisms that contribute to these physiological benefits (31). In the PVN, we previously showed that ExT decreases the expression of proinflammatory cytokines and reduces the production of superoxide in spontaneously hypertensive rats (SHR), which attenuates hypertension and improves autonomic function (2, 31). Improvement of cardiac vagal activity and baroreflex sensitivity was recently demonstrated after short-term ExT in experimental (31) and clinical (21) studies. Therefore, we hypothesized that short-term exercise training could attenuate HMGB1 expression, inflammation, and microglial activation in the PVN of SHR, thereby improving both autonomic function and cardiovascular control in these animals.

MATERIALS AND METHODS

Ethics statement and animals. All experimental procedures in this study were approved by the Institutional Animal Care and Use Committee (IACUC) at Louisiana State University in compliance with National Institutes of Health Guide for the Care and Use of Laboratory Animals. Twelve-week-old SHR and Wistar-Kyoto rats (WKY) were housed at a controlled room temperature (22°C) with a 12:12-h dark-light cycle with free access to standard chow and water.

Experimental design. We analyzed the effects of short-term exercise training on autonomic and cardiovascular parameters, HMGB1 content, SDF-1 and CXCR4 expression, microglial activation, and proinflammatory cytokine levels in the PVN of WKY and SHR. After a 1-wk acclimation period to treadmill, a progressive maximal exercise test was conducted, as previously reported (10, 31), to allocate SHR and WKY with similar physical capacity to trained (T) and sedentary (S) protocols. SHR and WKY were submitted to moderate-intensity training (50–60% of maximal exercise capacity, 0% grade, performed 5 days/wk, 1 h/day for 2 wk) (10, 31). Rats allocated to the S protocol were handled every day and were not subjected to ExT protocol.

Analysis of cardiovascular parameters and baroreflex function. At the end of the protocols, rats were anesthetized (ketamine, 80 mg/kg, plus xylazine, 12 mg/kg, ip) for chronic implantation of catheters in the left femoral artery and vein (10, 31). Instrumented rats were treated with antibiotic and analgesic and allowed to recover for 2 days. At least 48 h after the last exercise session, resting mean arterial pressure (MAP) and heart rate (HR) were recorded beat-to-beat for 60 min (computer, 2,000-Hz sampling frequency, LabChart 2.0.1) as described previously (10, 31). Baroreflex function was determined by loading/unloading of baroreceptors (phenylephrine and sodium nitroprusside iv, 0.1–6.4 and 0.2–12.8 μg/kg, respectively; 100 μl bolus injection given in a random order); subsequent injections were not made until the recorded parameters had returned to preinjection levels. Mean arterial pressure (AP) and HR values were measured before (control) and at the peak of each response. Baroreceptor reflex control of HR, determined for each rat, was estimated by the sigmoidal logistic equation fitted to data points, as described previously (31). The equation linking HR responses to pressure changes was HR = [(P1 + P2)/[1 + e3(BP - P4)]], where P1 = HR plateau, P2 = HR range, P3 = the curvature coefficient, and P4 = BP50 (the value of blood pressure at half of the HR range). The average gain of baroreflex function (BrS) was calculated as BrS = - (P2 × P3)/4.

After the physiological measurements, animals were anesthetized (ketamine, 80 mg/kg plus xylazine 12 mg/kg, ip) for blood and cerebrospinal fluid collection. Blood samples were centrifuged for plasma separation. Plasma and cerebrospinal fluid were stored at −80°C until assayed. Rats were then euthanized, perfused with saline or 4% PFA, and brains were removed and stored for later processing.

Power spectral analysis. Five-minute time series of systolic arterial pressure (SAP) and pulse interval (PI) were analyzed on the frequency domain. The fast Fourier transformation was used to obtain power spectral density. Spectral power for low-frequency (LF: 0.20–0.75 Hz) and high-frequency (HF: 0.75–4.0 Hz) bands was calculated by means of power spectrum density integration within each frequency bandwidth, using a customized routine (MATLAB 6.0, Mathworks), as described previously (10, 31).

Western blotting. PVN was isolated from frozen brain sections using a Stoeling brain punch (Stoelting) and, then, the tissue was homogenized with RIPA lysis buffer, as previously described (2, 7, 18, 39). The protein concentration was measured using a bicinchoninic acid (BCA) protein assay kit (Pierce). Equal amounts of protein (5 μg) were size-separated in 10% SDS-PAGE and blotted into a PVDF membrane (Millipore). Primary antibodies used were anti-phospho-ERK1/2 (1:1,000, Cell Signaling Technology), anti-total-ERK1/2 (1:2,000, Santa Cruz Biotechnology), anti-HMGB1 (1:1,000, Abcam), anti-SDF-1 (1:2,000, Abcam), anti-CXCR4 (1:500, Abcam), anti-TNFα (1:500, Cell Signaling Technology), anti-IL-6 (1:200, Santa Cruz Biotechnology), anti-phospho-IkBα (1:100, Cell Signaling Technology), and anti-actin (1:1,000, Santa Cruz Biotechnology). HRP-conjugated secondary antibody (1:2,000, Sigma) was applied and blots developed using an ECL kit (Millipore). Data analyses were performed using ImageJ software (NIH).

Measurement of plasma and cerebrospinal fluid HMGB1. Plasma and cerebrospinal fluid levels of HMGB1 were quantified using a commercially available rat HMGB1 ELISA kit (IBL, Hamburg, Germany) according to manufacturer’s instructions and as described previously (14).

Immunofluorescence. Immunofluorescence staining in the PVN was performed as previously reported (2, 13, 31). Briefly, sequential hypothalamic coronal sections (30 μm, −1.80 to −2.12 caudal to the bregma, 3 rats/subgroup) were cut with a cryostat (Leica CM 1850; Nussloch, Germany) and collected in tissue culture wells with 0.1 M PB. Free-floating sections were pretreated with 1% H2O2 for 30 min, washed with 0.1 M PB for 30 min, and incubated with 2% normal donkey serum for 30 min. For immunofluorescence reactions, the sections were incubated with primary antibody (rabbit polyclonal anti-Iba1, 1:800 dilution, Wako, Japan; rabbit polyclonal anti-HMGB1, 1:500 dilution, Abcam) for 48 h at 4°C. The sections were washed with 0.1 M PB for 30 min and incubated for 60 min at room temperature with secondary antibody (donkey anti-goat labeled with Alexa 594, 1:1,000 Jackson ImmunoResearch Laboratories). Four to six slices were placed in each slide and mounted with a coverslip and Vectashield. Negative controls omitted the primary or the secondary antibodies. The sections were examined to localize PVN (Leica DMLB, Wetzlar, Germany). Imaging analysis was performed with Image ProPlus software (Media Cybernetics, Silver Spring, MD).

Statistical analysis. The results are expressed as means ± SE. All analyses were conducted with two-way ANOVA, with Tukey’s post hoc test. All the groups were analyzed simultaneously. Correlation analyses were performed using Pearson statistics. Differences were considered significant at P < 0.05.

RESULTS

Short-term aerobic training promoted cardiovascular and autonomic benefits in SHR. We evaluated the cardiovascular and autonomic effects of training in SHR and WKY. SHR-S
presented a higher resting mean AP and HR compared with WKY-S. Although 2 wk of aerobic training did not significantly attenuate mean AP, trained SHR (SHR-T), compared with sedentary SHR (SHR-S), exhibited a lower HR, which was not different from that of WKY-S (Table 1). SHR-S also showed a lower PI variability and HF component, as well as a higher LF component, compared with WKY-S. Two weeks of aerobic training promptly normalized all these autonomic ab-

normalities, as SHR-T exhibited values similar to the sedentary WKY (WKY-S), which were significantly different from those of the SHR-S. In addition, SHR-S showed higher SAP variability and LF component, compared with WKY-S. Even though 2 wk of aerobic training did not significantly change SAP variability in the SHR, SHR-T exhibited a lower LF component of SAP variability compared with SHR-S. Aerobic training did not alter any of these cardiovascular and autonomic parameters in the WKY group (Table 1). In addition to SAP and PI variability, SHR-S vs. WKY-S also had decreased BrS (2.16 ± 0.20 vs. 4.26 ± 0.51 beats·min⁻¹·mmHg, \( P < 0.01, F = 9.71, \text{Fig. 1A} \)) and an upward displacement of the lower plateau (301 ± 4 vs. 250 ± 9 beats/min, \( P < 0.05, F = 12.02, \text{Fig. 1B} \)). Two weeks of aerobic training was able to normalize both BrS (4.34 ± 0.29 beats·min⁻¹·mmHg, \( P < 0.01, F = 9.71, \text{Fig. 1A} \)) and lower plateau (263 ± 6 beats/min, \( P < 0.01, F = 12.02, \text{Fig. 1B} \)), whose values were similar to those in the WKY group. Sigmoidal fitting of HR \( \times \) MAP, shifted to the right in the SHR-S according to the higher pressure exhibited, confirmed the depressed baroreflex sensitivity and the reduced operational HR range. In contrast, ExT did not modify MAP, but corrected the baroreceptor reflex control of HR, which was similar to that of WKY groups (Fig. 1C). Further on autonomic function, at the end of the study, trained and sedentary SHR and trained and sedentary WKY exhibited 320 ± 5, 328 ± 5, 335 ± 5, and 344 ± 4 g of body weight, respectively.

**Short-term aerobic training decreased HMGB1 content in the PVN, cerebrospinal fluid, and plasma in the SHR.** We analyzed in both groups of rats the effects of short-term aerobic training on HMGB1 content in the PVN, cerebrospinal fluid, and plasma in the SHR. We analyzed in both groups of rats the effects of short-term aerobic training on HMGB1 content in the PVN, cerebrospinal fluid, and plasma in the SHR. We analyzed in both groups of rats the effects of short-term aerobic training on HMGB1 content in the PVN, cerebrospinal fluid, and plasma in the SHR. We analyzed in both groups of rats the effects of short-term aerobic training on HMGB1 content in the PVN, cerebrospinal fluid, and plasma in the SHR.
and plasma. Our qualitative immunofluorescence analysis revealed that HMGB1 is highly expressed in SHR-S (Fig. 2A). This effect was confirmed by immunoblotting data showing higher HMGB1 protein expression in the PVN of the SHR-S compared with WKY-S (1.2 ± 0.3 vs. 0.7 ± 0.1 AU, *P < 0.01, F = 11.59, Fig. 2B). Two weeks of aerobic training normalized HMGB1 content in the PVN (from 1.2 ± 0.3 to 0.6 ± 0.1 AU, *P < 0.01, F = 11.59, Fig. 2B). Two weeks of aerobic training normalized HMGB1 immunoreactivity (Fig. 2, B and C). PVN HMGB1 expression was not altered by training in the WKY group.

In addition SHR-S showed elevated HMGB1 concentration in the cerebrospinal fluid (26 ± 3 vs. 13 ± 1 ng/ml, *P < 0.01, F = 15.20) and plasma (27 ± 7 vs. 9 ± 2 ng/ml, *P < 0.01, F = 5.04) compared with WKY-S, but normalized content after exercise training (13 ± 1 and 7 ± 1 ng/ml for cerebrospinal fluid and plasma, respectively, *P < 0.01 and F > 5.04 for SHR-T vs. SHR-S). As observed in the PVN, training did not change HMGB1 levels in the cerebrospinal fluid and plasma in the WKY group.

**Short-term aerobic training reduced CXCR4 signaling in the PVN.** Hypertension and exercise training also altered the signaling pathway activated by the HMGB1 (SDF1-CXCR4-p42/p44-NF-κB) in the PVN. Compared with WKY-S, SHR-S exhibited higher SDF-1 and CXCR4 protein expression (2.7- and 2.0-fold increase, respectively) as shown in Fig. 3. A and B, and C and D, respectively. Although MAPK p42/p44 was not changed by hypertension (1.3 ± 0.1 vs. 1.1 ± 0.1 AU, *P > 0.05, Fig. 3, E and F), SHR-S showed a higher IκB-α phosphorylation (a 3.8-fold increase, *P < 0.01, F = 12.85, Fig. 3, G and H). Interestingly, 2 wk of aerobic training was able to markedly decrease MAPK p42/p44 (from 1.3 ± 0.1 to 0.5 ± 0.1 AU, *P < 0.01, F = 11.32, Fig. 3F) and to normalize SDF1, CXCR4, and p-IκBα (data on Fig. 3). Again no changes were observed in WKY rats that underwent aerobic training.

**Short-term aerobic training decreased proinflammatory cytokines expression and microglia activation in the PVN.** Our immunofluorescence data demonstrated that SHR-S, compared with WKY-S, exhibited an intense Iba1 immunoreactivity in the PVN (Fig. 4A). It was confirmed by Western blotting analyses, since Iba1 protein expression in the PVN was higher in SHR-S (1.8 ± 0.2 vs. 0.7 ± 0.1 AU in the WKY-S, *P < 0.01, F = 19.02, Fig. 4, B and C). Two weeks of aerobic training was able to decrease Iba1 immunoreactivity (Fig. 4A) and to normalize protein expression (Fig. 4B) without changing its expression in WKY rats. In agreement with microglia activation analyses, SHR-S showed higher IL-6 and TNF-α (5.3- and 1.8-fold increases vs. WKY-S, *P < 0.01, F > 12.95, Fig. 4, D and E, and F and G, respectively). Similar to that observed in upstream signaling pathways, 2 wk of aerobic training were enough to completely normalize IL-6 and TNF-α expression within the PVN of the SHR-T (Fig. 4, E and G, *P < 0.01, F > 12.95), without changing proinflammatory cytokines expression in the WKY group.

**Microglial activation and HMGB1 expression were correlated with autonomic parameters.** In the present study, we used Iba1 protein expression as an activated microglial cells marker in the hypothalamic paraventricular nucleus, which

Fig. 2. Effects of short-term aerobic training (ExT) on HMGB1 expression in the paraventricular nucleus (PVN). As demonstrated on fluorescent photomicrographs, sedentary SHR had intense HMGB1 reactivity in the PVN (A). Sedentary SHR exhibited increased HMGB1 protein expression in the PVN compared with sedentary WKY. In SHR, short-term aerobic training was able to normalize HMGB1 immunoreactivity (A) and protein expression (B and C) in the PVN. HMGB1, high mobility group box-1. n = 5 per group, *P < 0.01, #P < 0.05.
was negatively correlated with PI variability (\( P = 0.0053; R^2 = 0.30; f = 9.58 \)), baroreflex sensitivity (\( P = 0.001; R^2 = 0.48; f = 21.09 \)), and resting HR (\( P < 0.0001; R^2 = 0.73; f = 61.81 \)). In addition, Iba1 protein expression was positively correlated with systolic arterial pressure variability (\( P = 0.02; R^2 = 0.21; f = 6.21 \)). Besides microglia activation, we also identified negative correlation between HMGB1 protein expression in the hypothalamic paraventricular nucleus and PI variability (\( P = 0.02; R^2 = 0.26; f = 6.64 \)), baroreflex sensitivity (\( P = 0.04; R^2 = 0.20; f = 4.65 \)), and resting HR (\( P < 0.0031; R^2 = 0.39; f = 11.68 \)). These data strongly suggest a cause-effect relation between neuroinflammatory adaptations and autonomic alterations evoked by hypertension and aerobic exercise training.

**DISCUSSION**

In the present study, we show that short-term aerobic training decreases HMGB1, SDF1, and CXCR4 expression, inhibits microglia activation, and attenuates proinflammatory cytokine expression in the PVN of SHR. These effects, at least in part, contribute to the improvement of autonomic function, even in persistence of hypertension.

Increased proinflammatory cytokines expression in autonomic control areas, as the PVN, has been identified as a major central molecular mechanism to decrease baroreflex function, to reduce HR variability, and to increase pressure variability and renal sympathetic nerve activity, thus contributing to the development/maintenance of hypertension (7, 13, 18, 38, 39). It was also shown that hypertensive rats exhibit elevated proinflammatory cytokines TNF-\( \alpha \) and IL-6 expression in the PVN (2, 31). In a recent manuscript (31), we also showed that training-induced autonomic and molecular adaptations occurred in a relatively short interval of time. In addition we showed that baroreflex sensitivity and cardiac vagal activity improvement, and molecular adaptations in the PVN preceded arterial pressure fall induced by exercise training (31). Data from the present study confirmed these previous findings and demonstrated that 2 wk of aerobic training was able to atten-
activated vasomotor sympathetic activity, even in the persistence of hypertension.

It should be noted that, in addition to the autonomic-immune drive, increased arterial pressure in the SHR is maintained by hypertrophy of peripheral arteries/arterioles (1). Differently from the ANG II-induced hypertension model, which is mostly related to neurogenic drive, SHR exhibits both early autonomic dysfunction (34) and vascular remodeling (3, 8). These findings explain the conflicting data: normalization of arterial pressure in ANG II-induced hypertensive animals, arterial pressure elevation correlated with both reduced HMGB1 availability and reversal of microglia activation in the PVN. An original observation described in the present study was that only 2 wk of aerobic training was sufficient to normalize hypertension-activated microglia within the PVN. Previous studies in aged mice demonstrated that access to running wheels decreased microglia positive cells in the hippocampus (23, 24). In addition, 4 wk of exercise training in a mice model of Parkinson’s disease suppressed microglia activation in the substantia nigra pars compacta and striatum (40). Therefore, our data suggest that exercise training is able to inhibit microglia activation, which explains, at least in part, the autonomic adaptations in the trained SHR. It is important to note that these effects occurred even in the persistence of hypertension.

Interestingly, reversion of proinflammatory profile in the PVN and improvement of both baroreceptors reflex and autonomic function occurred simultaneously with the downregulation of the signaling pathway activated by HMGB1 and CXCR4 interaction in the PVN, which activates microglia, suggesting a possible cause-effect relation. It should be noted that improvement of the autonomic control of the circulation correlated with both reduced HMGB1 availability and reversal of microglia activation. These data strongly suggest a cause-

Fig. 4. Effects of short-term aerobic training on microglia activation and proinflammatory cytokines expression in the PVN. As demonstrated on fluorescent photomicrographs, SHR-S had intense microglia activation in the PVN, since a higher number of Iba1 positive cells as observed, as well as a lower number of cellular process and larger cellular nucleus on Iba1 positive cells (A). Compared with WKY-S, Iba1 protein expression in the PVN (B, representative blotting; C, densitometric quantification) was increased on SHR-S, but reduced by short-term aerobic training. In addition to microglia activation, SHR-S exhibited higher IL-6 (D, representative blots; E, densitometry quantification) and TNF-α (F, representative blots; G, densitometry quantification) protein expression in the PVN compared with WKY-S. Short-term aerobic training normalized IL-6 (D, representative blots; E, densitometry quantification) and TNF-α (F, representative blots; G, densitometry quantification) protein expression in the PVN on SHR. Iba1, ionized calcium-binding adapter molecule 1.

HMGB1 PVN INFLAMMATION, AUTONOMIC DYSFUNCTION, AND EXERCISE TRAINING
effect relationship between neuroinflammatory adaptations and autonomic alterations evoked by hypertension and exercise training. In addition, the similarity between HMGB1 and Iba1 changes within the PVN and functional effects indicated that HMGB1 availability could be one important causative factor for microglia activation.

In addition to classical proinflammatory cytokines, HMGB1 was recently identified as a cytokine-like peptide that induces proinflammatory cytokines expression through NF-kB activation in many cellular types (20, 22). According to its redox status, HMGB1 binds to TLR4 and CXCR4 (36, 37), which ultimately produce tissue inflammation and oxidative stress. In the central nervous system, previous studies identified that HMGB1 inhibition attenuated microglia activation (15) and tissue inflammation in ischemia and reperfusion models (17, 19, 28). A recent study (14) from our group showed that SHR exhibited increased HMGB1 levels in the plasma and PVN and that TLR4 blockade in the PVN attenuated inflammation and hypertension. Another original observation of this study is that short-term aerobic training was able to normalize HMGB1 expression in the PVN, as well as in the cerebrospinal fluid and plasma. In postinfarction patients, Giallauria et al. (16) demonstrated that 6-mo exercise-based cardiac rehabilitation program decreased plasma HMGB1 levels, which were significantly associated with the improvement in peak oxygen consumption and heart rate recovery, an important autonomic functional marker. So, it is reasonable to speculate that systemic and tissue HMGB1 level normalization might contribute to autonomic adaptations induced by exercise training in hypertension and that HMGB1 may act as a link between the immune and autonomic systems within the brain.

We also speculate on what signaling is driving the exercise-induced changes in the brain. It was shown that the neural signaling to the PVN is carried by several peripheral afferents as baroreceptors, chemoreceptors, cardiopulmonary receptors, and ergoreceptors (33). In accordance, previous studies have shown that 1) training increased the gain of aortic nerve activity and augmented both excitability and density of PVN oxytocinergic preautonomic neurons projecting to brain stem areas (6, 9); and 2) sinoaortic denervation and specific removal of the peripheral chemoreceptors reduced the expression of PVN oxytocinergic neurons and abrogated training-induced improvement of cardiovascular control (9, 10, 12). Therefore, baroreceptors and chemoreceptors seem to be important mechanisms driving information to the PVN.

As HMGB1 interacts with SDF1, it forms a heterodimer, and binds to CXCR4 in microglial cells (30); thus we also analyzed the effects of exercise training in the expression of SDF1 and CXCR4, as well as on its downstream signaling, within the PVN. It was recently demonstrated that acute intracerebroventricular administration of SDF-1 increased the phosphorylation of MAPK, along with arterial pressure, heart rate, and renal sympathetic nerve activity (41). In infarcted rats, Wei et al. (41) found increased SDF-1 protein expression in autonomic control areas, PVN, and subfornical organ, whereas chronic intracerebroventricular administration of SDF-1 short-hairpin RNA lentiviral particles decreased plasma norepinephrine and vasopressin levels. Here, we demonstrate, for the first time, normalized CXCR4 and SDF1 protein expression and decreased MAPK p42/p44 and IκB-α phosphorylation in the PVN of short-term trained SHR. Taken together, our data suggest that short-term exercise training decreases HMGB1 content, attenuates microglia activation, and reduces the expression of proinflammatory cytokines by inhibiting the HMGB1-CXCR4 signaling pathway in the PVN. Therefore, the downregulation of this signaling pathway contributes, at least in part, to the autonomic benefits observed in SHR. Indeed, experimental and clinical studies have extensively documented the health benefits of exercise training in hypertensive subjects. In untreated hypertensive subjects, Laterza et al. (26) observed normalized baroreflex function and peripheral sympathetic nerve activity after exercise training, which are closely related to end-organ injuries (27) and cardiovascular mortality (35) in hypertension.

In conclusion, we demonstrated that training induced down-regulation of the markedly stimulated HMGB1-SDF1-CXCR4-p42/p44-NF-κB pathway that regulates microglial activation and, consequently, the production of proinflammatory cytokines in the PVN of hypertensive rats. These molecular effects are a prompt neural adaptation to reduce inflammation in this major autonomic area. The temporal coincidence between the reversion of PVN proinflammatory profile and the improvement of cardiovascular control (normalized baroreflex function, reduced sympathetic and increased cardiac vagal activity with increased heart rate variability) reinforces the crucial role of exercise training to counterbalance inflammatory deleterious effects even in the presence of hypertension.

Perspectives and significance. The association between hemodynamic recordings and techniques to evaluate autonomic control of circulation and to investigate molecular mechanisms offers a new approach for a better understanding of the physiopathology of several diseases and possible therapeutic tools. By using an approach like that, we were able to show that increased HMGB1 content, CXCR4 signaling, microglial activation, and pro-inflammatory profile in the PVN are normalized by aerobic training, thus contributing to correcting some of the autonomic dysfunction associated with hypertension. Procedures like these might be a tool to understand integrative brain mechanisms in several diseases and to develop appropriate therapeutic targets.

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

AUTHOR CONTRIBUTIONS

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23. Kohman RA, DeYoung JK, Bhattacharya TK, Peterson LN, Rhodes JS. Wheel running attenuates microglial proliferation and increases expres-