Altered Inflammatory Response Is Associated With an Impaired Autonomic Input to the Bone Marrow in the Spontaneously Hypertensive Rat

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Abstract—Autonomic nervous system dysfunction, exaggerated inflammation, and impaired vascular repair are all hallmarks of hypertension. Considering that bone marrow (BM) is a major source of the inflammatory cells (ICs) and endothelial progenitor cells (EPCs), we hypothesized that impaired BM–autonomic nervous system interaction contributes to dysfunctional BM activity in hypertension. In the spontaneously hypertensive rat (SHR), we observed a >30% increase in BM and blood ICs ($CD4.8^+$) and a >50% decrease in EPCs ($CD90^+.CD4.5.8^-$) when compared with the normotensive Wistar–Kyoto rat. Increased tyrosine hydroxylase (70%) and norepinephrine (160%) and decreased choline acetyl transferase (30%) and acetylcholine esterase (55%) indicated imbalanced autonomic nervous system in SHR BM. In Wistar–Kyoto rat, night time–associated elevation in sympathetic nerve activity (50%) and BM norepinephrine (41%) was associated with increased ICs (50%) and decreased EPCs (350%) although BM sympathetic denervation decreased ICs (25%) and increased EPCs (40%). In contrast, these effects were blunted in SHR, possibly because of chronic downregulation of BM adrenergic receptor α2a (by 50%–80%) and β2 (30%–45%). Application of norepinephrine resulted in increased BM IC activation/release, which was prevented by preadministration of acetylcholine. Electrophysiological recordings of femoral sympathetic nerve activity showed a more robust femoral sympathetic nerve activity in SHR when compared with Wistar–Kyoto rat, peaking earlier in the respiratory cycle, indicative of increased sympathetic tone. Finally, manganese-enhanced MRI demonstrated that presympathetic neuronal activation in SHR was associated with an accelerated retrograde transport of the green fluorescent protein–labeled pseudorabies virus from the BM. These observations demonstrate that a dysfunctional BM autonomic nervous system is associated with imbalanced EPCs and ICs in hypertension. (***Hypertension*****. 2014;63:542-550.)** ● **Online Data Supplement**

Key Word: bone marrow ■ EPCs ■ hypertension ■ inflammation ■ rats, inbred SHR ■ sympathetic drive

Autonomic dysfunction, characterized by increased sympathetic and decreased parasympathetic activity, is a hallmark of neurogenic hypertension.[1,2] Recent evidence has indicated a direct interaction of the autonomic nervous system with the immune system to regulate normal cardiovascular homeostasis. Thus, a dysfunctional neural-immune communication has been implicated in the pathogenesis of cardiovascular diseases and hypertension.[3,4] Evidence of increased sympathetic nervous system activity to immune organs in hypertension supports this contention.[5] Moreover, atherosclerotic vasculature is further compromised by mobilization of the bone marrow (BM)–derived inflammatory cells (ICs) after myocardial infarction, characterized by increased sympathetic nerve activity (SNA).[6] However, the anti-inflammatory effects of the vagus nerve (ie, parasympathetic) stimulation are demonstrated by lowered levels of the inflammatory cytokines and suppressed activation of ICs.[7,8] Therefore, the imbalance of the parasympathetic/sympathetic influence in hypertension contributes to the hypertensive phenotype by perpetuating the inflammatory response.[9] In contrast to the ICs, decreased circulating levels of endothelial progenitor cells (EPCs) and their dysfunction are demonstrated in hypertension and cardiovascular diseases,[10–13] suggesting EPCs' impaired abilities in repairing vascular damage. The EPCs, like ICs, seem to be neuroregulated, as suggested by the diurnal pattern of their release into the circulation.[13] As the EPC numbers and function are inversely correlated in patients and rat models of hypertension,[10,11,14] the overactive sympathetic drive to the BM and the imbalance in the overall parasympathetic/

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sympathetic tone in hypertension may also directly contribute to the dampened EPC numbers and function, leading to the impairment of the endothelial reparative processes and accelerating the vascular dysfunction and hypertension-associated pathophysiology. Taken together, these observations led us to hypothesize that the autonomic imbalance influences the release of the progenitor cells from the BM, thereby affecting the circulating IC and EPC levels. Spontaneously hypertensive rat (SHR), a model of neurogenic hypertension that exhibits an early onset of autonomic and endothelial dysfunctions and increased inflammatory response, has been used in this study to evaluate this hypothesis. We present direct evidence that an altered autonomic regulation of BM is associated with an imbalance in the EPC and IC levels in hypertension.

Methods

All experimental protocols are presented in the Methods section and are available in the online-only Data Supplement. All animal procedures were approved by the University of Florida Institute Animal Care and Use Committee.

Results

Circadian Regulation of BM ICs and EPCs

First, we investigated whether increased sympathetic drive affected the BM activity in the SHR by comparing the levels of EPCs and ICs in the BM at the times of lowest (ie, 11 am, day) and highest (ie, 8 pm, night) sympathetic drive. We observed a 50% increase in the low frequency of systolic blood pressure (SBP), LF:SBP, in the Wistar–Kyoto (WKY) rat and a 130% increase in the LF:SBP in the SHR at night versus day (Figure 1A). This was associated with increased BM norepinephrine (WKY, 41%; SHR, 38%) at night versus day (Figure 1B). Furthermore, the overall sympathetic drive as measured by LF:SBP was higher in the SHR versus the WKY rats at both day and night (Figure 1A and 1B). Furthermore, a 158% increase in the density of tyrosine hydroxylase (TH) immunoreactivity (Figure 4A), a 30% decrease in the density of choline acetyl transferase (Figure 4B), and a 55% decrease in the density of acetylcholine esterase (Figure 4C) immunoreactivity around the femoral BM blood vessels were observed in the SHR when compared with the WKY, suggesting impaired BM autonomic nervous system input. Increased sympathetic drive at night in the WKY rats was associated with a 50% and 33% increase in the IC levels in the BM and blood, respectively (Figure 1C and 1D, left), and a 350% decrease in the blood EPCs (Figure 1F, left). In comparison, the ICs were higher and the EPCs were lower in the BM of the SHR both at day and night (Figure 1C and 1D) and in the blood of the SHR at day (Figure 1E and 1F, left) when compared with the WKY rat. Furthermore, there was a lack of the night time–associated increase in the ICs and decrease in the EPCs in the SHR (Figure 1C and 1F, right), suggesting a dysfunctional response of the SHR BM to the circadian-related sympathetic drive changes. To investigate this further, we performed BM sympathetic denervation by dissection of the superior cervical

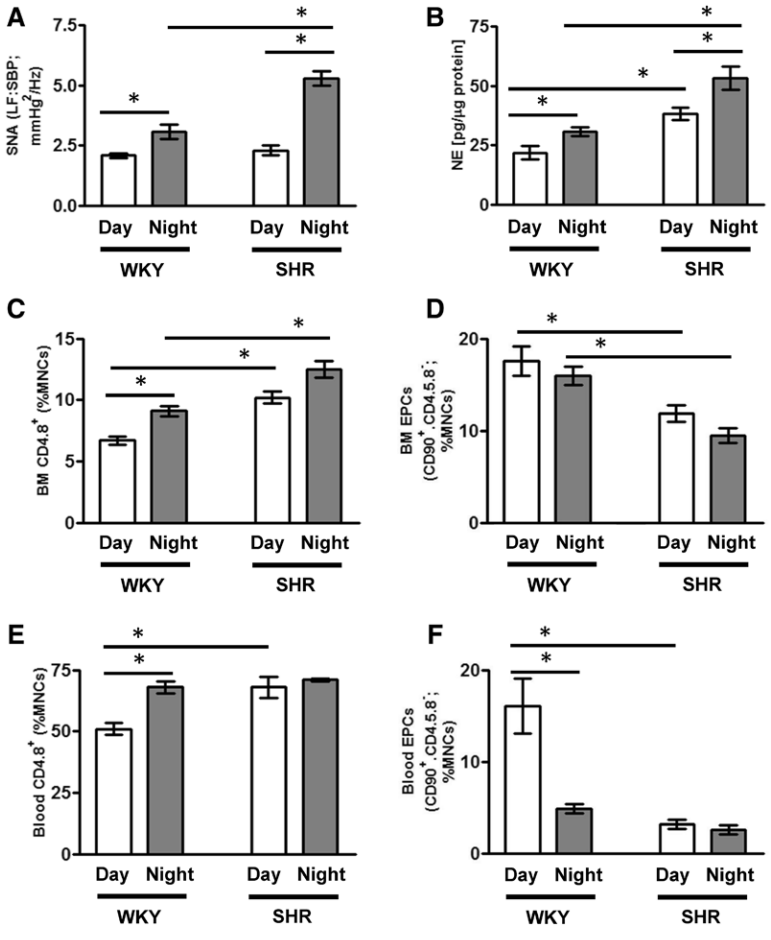

Figure 1. Effects of elevated sympathetic drive at night vs day on endothelial progenitor cell (EPC) and inflammatory cell (IC) levels in bone marrow (BM) and blood in spontaneously hypertensive rat (SHR) and Wistar–Kyoto (WKY) rat. **A**, Spectral analysis of systolic blood pressure (SBP) reveals elevation in the overall sympathetic drive (LF:SBP) at night vs day in both the SHR and WKY ($P<0.05$ vs day) and higher overall sympathetic drive at night in the SHR vs WKY ($P<0.05$ vs WKY night), n=4 per strain. **B**, Significantly higher norepinephrine (NE) protein levels were observed in the BM cell supernatant at night vs day in both the SHR and WKY ($P<0.05$ vs day; n=12 per strain) and higher NE protein levels at both day and night in the SHR vs WKY ($P<0.05$ vs WKY; n=12 per strain). **C**–**F**, Significantly higher ICs in the BM (**C**) and blood (**E**), and lower EPCs in the blood (**F**) were observed at night in the WKY but not in the SHR ($P<0.05$ vs day; n=6 time of the day). Significantly lower ICs and higher EPCs were observed at day in the BM (**C** and **D**) and blood (**E** and **F**) in the WKY vs SHR ($P<0.05$ vs SHR day), n=6 per strain.

ganglion in the WKY and SHR as previously described.[15] Forty-eight hours after the superior cervical ganglion, we observed a 50% and 30% decrease in the BM norepinephrine in the WKY and SHR, respectively (Figure 2A) when compared with the naïve controls. This was associated with a 25% decrease in the BM IC levels and a 40% increase in the BM EPC levels in the WKY (Figure 2B, left), similar to the decrease in ICs and increase in EPCs observed from night to day in the WKY (Figure 1C and 1D). However, BM ICs showed a trend toward an increase but produced no significant change after superior cervical ganglion, whereas we observed ≈18% increase in the BM EPC levels in the SHR (Figure 2B, right), suggesting attenuated responsiveness of the BM HSPCs to the BM sympathetic changes in the SHR when compared with the WKY. Quantitative polymerase chain reaction showed a significant decrease in both the α2a (by ≈50 at day and ≈80% at night)- and β2-adrenergic receptors (by ≈45% at day and ≈30% at night) in BM mononuclear cells of SHR when compared with the WKY. This suggested a possible mechanism for loss of circadian regulation of BM cells in the SHR (Figure 3).

To investigate the mechanism of increased mobilization of the BM ICs at high sympathetic drive, we used in vivo real-time imaging of the tibial BM (Figure 5) to determine whether increase in the local norepinephrine in the BM would influence mobilization of inflammatory BM cells by studying the mobilization of green fluorescent protein (GFP)–labeled ICs in response to administration of 6 μg/kg norepinephrine, in the absence and in the presence of 80 mg/kg acetylcholine. A significant increase in the movement of ICs in response to norepinephrine was observed (Figure 5; Movies in the online-only Data Supplement). This movement was significantly attenuated by preadministration of acetylcholine (Figure 5).

Loss of EPC Function in the SHR

The overall decrease in the EPC numbers in the SHR when compared with the WKY was accompanied with the loss

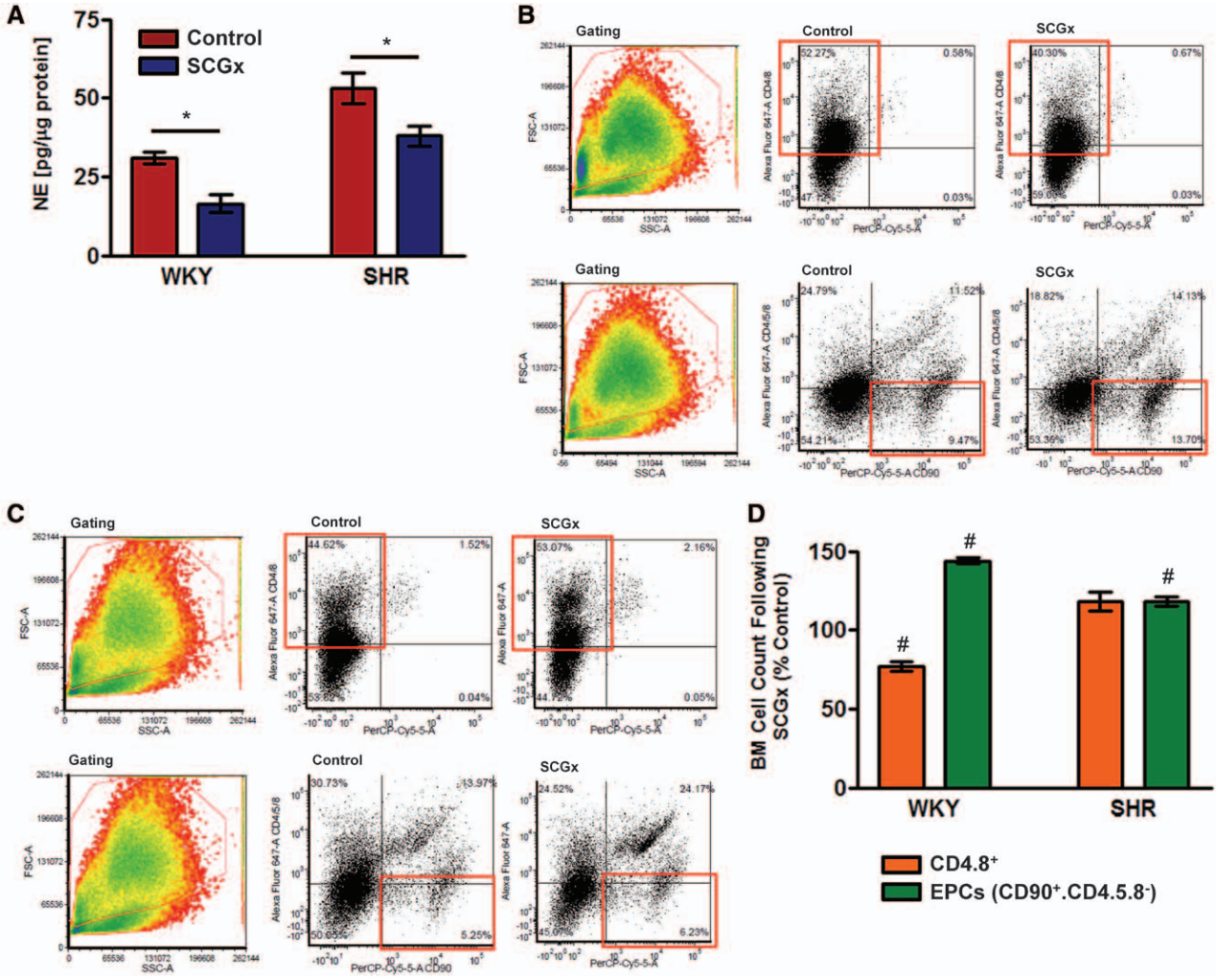

Figure 2. Effects of superior cervical ganglion denervation (SCGx) on the bone marrow (BM) endothelial progenitor cell (EPC) and inflammatory cell (IC) levels in spontaneously hypertensive rat (SHR) and Wistar–Kyoto (WKY) rats. **A**, A significant reduction in norepinephrine (NE) protein levels was observed in the BM cell supernatant in both the SHR and the WKY after SCGx ($P<0.05$; n=3). **B** and **C**, Examples of raw FACS (fluorescence activated cell sorting-area) data for WKY (**B**) and SHR (**C**), showing the changes in $CD4.8^+$ (**top**, highlighted in red box) and $CD90^+.CD4.5.8^-$ BM cells (**bottom**, highlighted in red box) in control and SCGx rats. **D**, A significant decrease in the ICs in the WKY and an increase in EPCs in both WKY and SHR were observed in the BM after SCGx ($P<0.05$ vs naïve control; n=3). FSC-A indicates forward scattered light; and SSC-Area, side scattered light.

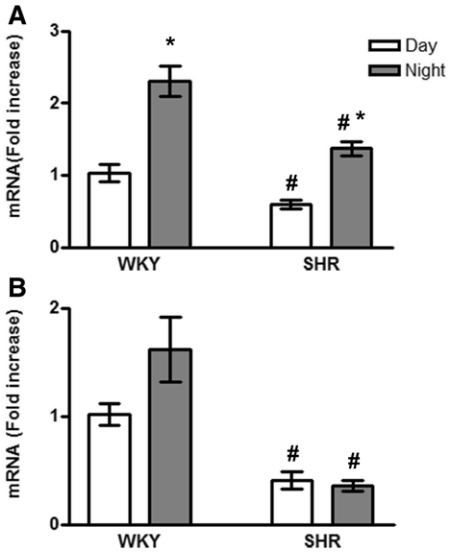

Figure 3. Chronic downregulation of adrenergic receptors β2 and α2a in the bone marrow (BM) of spontaneously hypertensive rat (SHR). **A** and **B**, Quantitative real-time-polymerase chain reaction in the whole BM mononuclear cells shows significantly lower relative expression levels of adrenergic receptors β2 (**A**) and α2a (**B**) at both day (white bars) and night time (gray bars). *P<0.05 vs day; #P<0.05 vs Wistar–Kyoto (WKY) rats; n=6 per strain.

of cell function: the angiogenic ability of the BM-derived cells was also reduced in the SHR when compared with the age-matched WKY, as evidenced by a 65% decrease in the formed tube length, a 50% reduction in tube width formation ex vivo (Figure S1A–S1C in the online-only Data Supplement), and a 35% decrease in the SHR BM EPC's ability to proliferate in response to SDF (Figure S1D).

SNA to the BM Is Altered in the SHR

Next, we characterized the activity of the sympathetic nerve innervating the femoral bone. Respiratory cycle triggered averages of simultaneously recorded phrenic and femoral sympathetic nerve activities (fSNA) were made in the decerebrate artificially perfused rat (Figure 6A) as established previously.[16] This in situ decerebrate artificially perfused rat revealed a classic phrenic nerve activity pattern (Figure 6B, second and third panels) and a robust fSNA (Figure 6B, fourth and fifth panels). The influence of respiration on fSNA was enhanced by 9% CO_2 (Figure 6D, third panel, red arrows) and was completely blocked by hexamethonium (Figure 6D, fourth panel). Next, we compared the respiratory modulation of fSNA between the hypertensive and normotensive rat. The peak firing of fSNA in the SHR occurred earlier in the phrenic nerve activity cycle (ie, at the end of the inspiration [I] phase; Figure 6E, third panel, red arrow) and was ≈25% more robust when compared with the normotensive control, the peak of which occurred in the postinspiration (P-I) phase (Figure 6E, second panel, red arrow), typical of respiratory-sympathetic patterning.[17,18] These responses were consistent and repeatable over several preparations (n=4 per strain).

Retrograde Viral Tracing Reveals Dysfunctional Autonomic-BM Communication in the SHR

Finally, MRI and GFP-pseudorabies virus (PRV) retrograde labeling experiments were performed to investigate increased autonomic-BM communication in hypertension further. Mn2+-enhanced MRI, a technique commonly used to visualize elevated cellular activity in vivo, showed a 20% to 25% higher neuronal activity in the hypothalamic paraventricular nucleus (PVN) of the SHR when compared with the WKY rats (Figure 7). In addition,

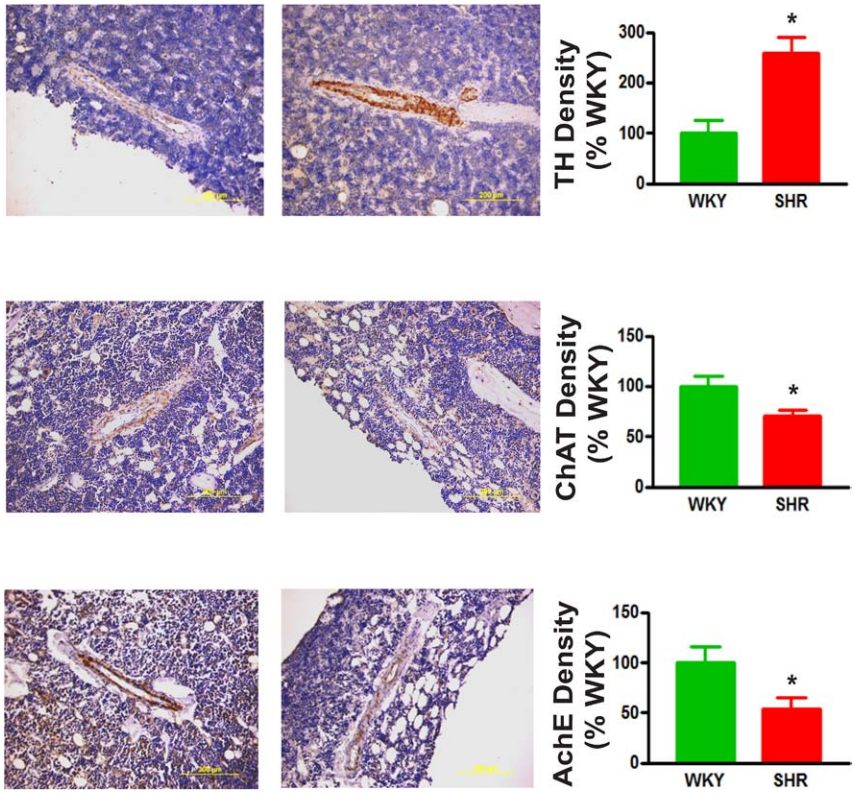

Figure 4. Elevated tyrosine hydroxylase (TH) and decreased choline acetyltransferase (ChAT) and acetylecholinesterase (AchE) immunostaining in the bone marrow (BM) of the spontaneously hypertensive rat (SHR). Immunohistochemistry reveals higher TH density and lower ChAT and AchE densities around the blood vessels (brown staining) in the BM of SHR when compared to Wistar–Kyoto (WKY) rats. The quantification was a result of extensive image analysis using Image J to analyze 40 to 50 images per strain (P<0.05 vs WKY; n=6 per strain).

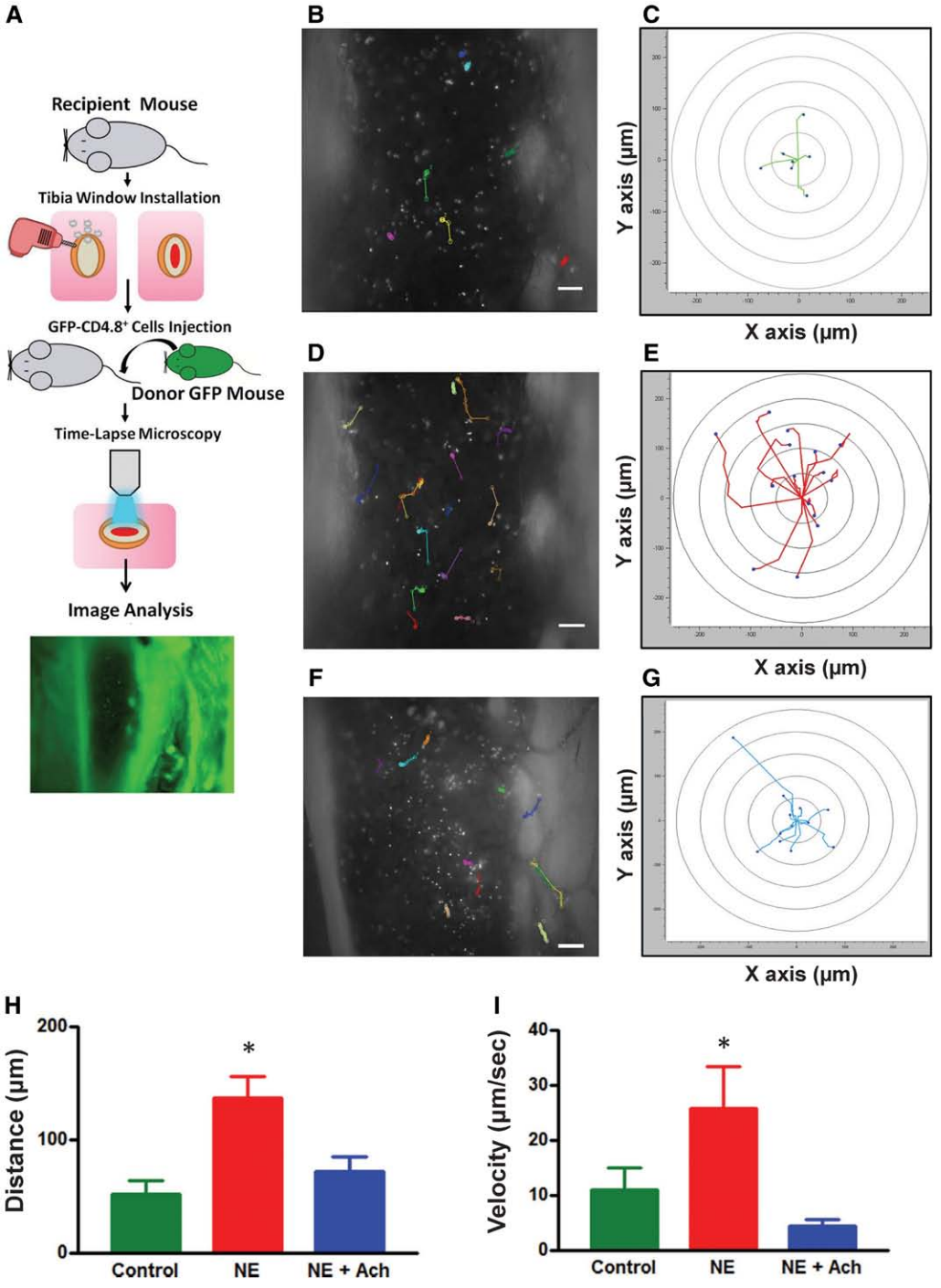

Figure 5. Direct effect of norepinephrine (NE) on activation and migration of bone marrow (BM) inflammatory cells (ICs). **A**, Green fluorescent protein (GFP)–labeled CD4.8⁺ T cells were injected into a recipient mouse with an exposed tibial BM and imaged in vivo under the fluorescence microscope. **B** and **C**, GFP-labeled ICs' movement was tracked in vivo before the NE injection. **Left**, A still image of the BM niche with GFP-labeled cells showing as bright gray and their trajectory labeled by colored lines. **Right**, The summation of each of the cells' trajectories plotted as the distance and velocity travelled. **D** and **E**, Representative movement of each individual GFP-labeled IC was plotted after the NE injection. **F** and **G**, Representative movement of each individual GFP-labeled IC was plotted after the NE injection in the presence of preadministered acetylcholine (Ach). **H** and **I**, NE significantly increased the distance (**H**) and the velocity of travel (**I**) of GFP-labeled BM ICs, which was attenuated by preadministration of Ach ($P<0.05$ vs control; n=7–15).

the rate of the PVN retrograde labeling by the GFP-PRV injected in the BM was significantly faster in the SHR when compared with the WKY rats (Figure 7B). For example, 7 days after GFP-PRV administration, the PVN neurons from the SHR showed robust GFP fluorescence, whereas little fluorescence was seen in the PVN of the WKY rats (Figure 7B). A similar increase in the labeling of neurons in other SHR autonomic brain regions (such as the NTS [nucleus of the solitary tract], RVLM [rostral ventrolateral medulla], SFO [subfornical organ]) was observed (Figures S2 and S3). These responses were consistent and repeatable over several preparations (n=3 per strain for MRI; n=3 per strain per time point for GFP-PRV retrograde labeling).

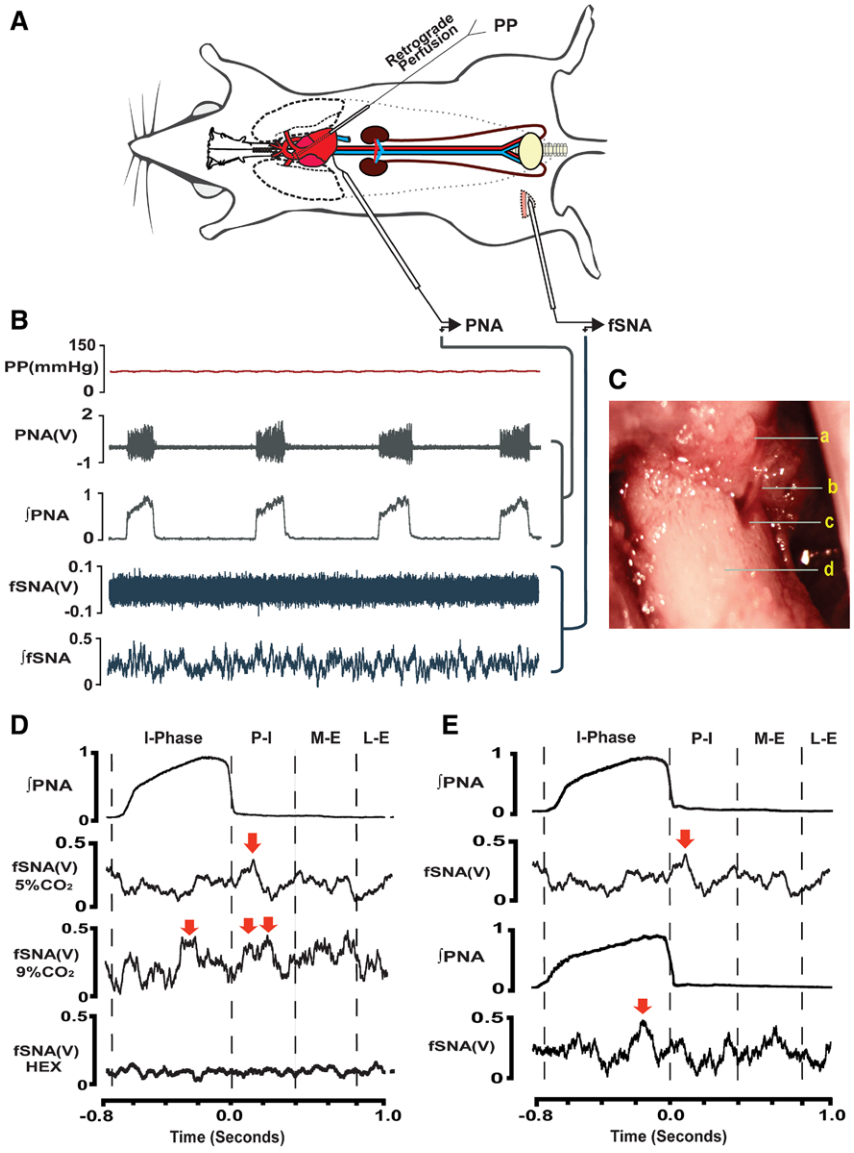

Figure 6. Elevated sympathetic drive in the bone marrow (BM) of the spontaneously hypertensive rat (SHR). **A**, Schematic of the in situ decerebrated artificially perfused rat preparation. **B**, An example of the raw and integrated tracings of phrenic nerve activity (PNA) and femoral sympathetic nerve activity (fSNA) at constant perfusion pressure (PP). **C**, Photograph of the femoral nerve bundle (b) innervating the femur (d) via the bone nutrient foramen (c). **D**, Phrenic-triggered fSNA in baseline conditions (5% CO_2) peaks immediately after the PNA peak (ie, in the P-I phase, characteristic of the SNA [panel 2, red arrow]). Nine percentage CO_2 activates the fSNA (panel 3, red arrow) and administration of HEX (300 μmol/L) abolishes the fSNA pattern (panel 4). **E**, **Top** 2 panels represent PNA and fSNA from a control normotensive rat, and the **bottom** 2 panels are from the SHR. Phrenic-triggered fSNA is more robust and occurs earlier in the PNA cycle in the SHR (panel 4, red arrow) when compared with the control rat (panel 2, red arrow). HEX indicates hexamethonium; I, inspiration, L-E, late expiration; M-E, midexpiration; and P-I, postinspiration.

Discussion

Our study is novel in a number of ways: (1) we are the first to establish the electrophysiological recordings of the sympathetic nerve innervating the femoral BM (ie, the fSNA; Figure 6). The electric properties of fSNA in the SHR are similar to those of the thoracic SNA in the SHR,[19] in that its peak activity occurs earlier in the phrenic cycle and it is more robust when compared with the normotensive control (Figure 6); (2) norepinephrine and TH levels in the BM of the SHR are increased. In addition, night time norepinephrine levels were significantly higher than daytime in the BM of both the WKY and SHR, when the overall sympathetic drive was higher in these animals; (3) IC levels increased and EPC levels decreased in the SHR when compared with the WKY rats and correlated with the increased sympathetic drive; (4) direct application of norepinephrine into the BM activated the BM ICs; (5) regular circadian regulation of the BM ICs and EPCs, which is present in the WKY, is significantly compromised in the SHR; (6) α2a- and β2-adrenergic receptor levels are significantly decreased in the SHR BM, suggesting a possible mechanism behind the loss of circadian regulation of ICs/EPCs in the SHR BM; (7) a decreased BM parasympathetic tone, as demonstrated by decreased BM choline acetyl transferase and acetylcholine esterase in the SHR, may contribute to the inflammatory activation of the BM in the SHR. Taken together, we suggest that hypertension in the SHR is associated with a persistent increase in the sympathetic drive to the periphery, including the BM. This is associated with an increase in the BM norepinephrine and TH levels, resulting in impaired BM cell activity, reflected in the increased ICs and decreased EPCs in the SHR. Chronically high fSNA eventually leads to downregulation of adrenergic receptors in the BM, leading to the loss of circadian regulation of the BM cells in the SHR. This loss of circadian regulation, coupled with persistent increase in ICs and decrease in EPCs, compromises the ability of vasculature to repair the damage induced by hypertension, which is accentuated by the residual EPCs that become dysfunctional in the SHR.

We show here that the typical diurnal increases in the sympathetic vasomotor drive at night are accompanied by similar

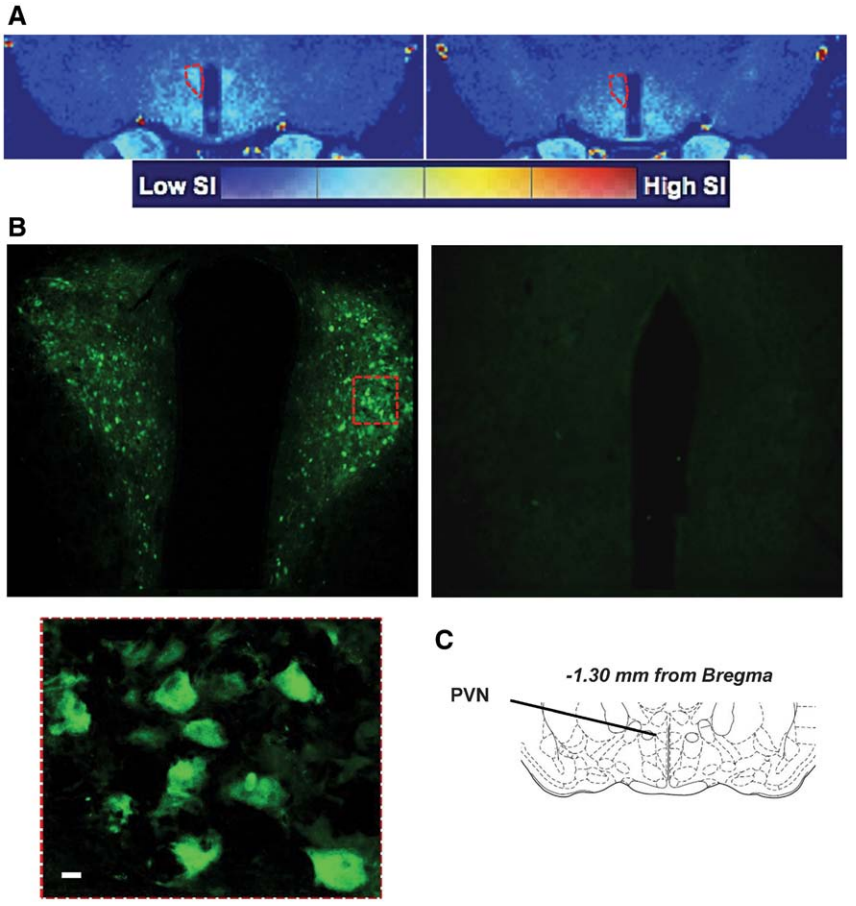

Figure 7. Higher pseudorabies virus (PRV)-green fluorescent protein (GFP) retrograde labeling from the femoral bone marrow to the paraventricular nucleus (PVN) is associated with higher neuronal activity in the PVN of the spontaneously hypertensive rat (SHR) when compared to Wistar–Kyoto (WKY) rats. **A**, MEMRI (manganese-enhanced magnetic resonance imaging) reveals significantly higher neuronal signal intensity in the PVN of the SHR (**left**, red dashed labeled area) when compared with the WKY (**right**, red dashed labeled area). **B**, GFP staining reveals robust retrograde labeling in the PVN of the SHR (**left**) with very little GFP stain present in the WKY (**right**) at day 7 after the BM PRV injection. The higher magnification image demonstrates neuronal labeling (red dashed box; scale bar, 10 μm). **C**, Paxinos–Watson stereotaxic coordinates of the PVN.

changes in the BM norepinephrine; however, both the overall sympathetic vasomotor drive, as indicated by LF:SBP, and the BM norepinephrine are significantly higher in the SHR, indicating an increased sympathetic drive to the BM of the SHR. This is corroborated by elevated BM TH protein levels in the SHR (Figure 4), as well as our electrophysiological recordings showing activity changes in the BM fSNA in the SHR, which are similar to the changes observed in the thoracic SNA of the SHR and are indicative of elevated SNA.[19] Other evidence supports this contention. For example, GFP-PRV retrograde labeling of the neurons in the PVN and other cardioregulatory brain regions from the BM is accelerated and more robust in the SHR when compared with the WKY. This is not because of the genetic diversity between the 2 rat strains but is rather associated with hypertension because accelerated labeling of the PVN neurons by BM administration of GFP-PRV is also observed in chronic angiotensin II–infused rat model of hypertension (not shown) and is in agreement with the heightened neuronal activity in the PVN of the SHR, as demonstrated by elevated manganese-enhanced MRI signals in the SHR (Figure 7).

In the WKY, the night time–associated increase in the BM sympathetic drive was accompanied by elevated BM and blood $CD4.8^+$ levels and decreased EPC levels, suggesting circadian regulation of the BM cells (Figure 1). This concept is not new because both animal and human studies have shown that the release of BM cells follows a regular circadian pattern, and that in rodents; the immune cells (ie, the surveillance cells) are released at night, whereas the repair cells (including the EPCs) are released during the day.[20] This circadian regulation of BM activity seems to be dependent on the sympathetic innervation, interruption of which results in pathological situations, as evidenced in diabetes mellitus.[13] Our observations in the SHR are consistent with this because the circadian regulation of the BM cells seems to be impaired in the SHR (Figure 1). Thus, in the SHR, the IC levels remain chronically high, whereas the EPC levels remain low. Therefore, one can postulate that the presence of the functioning circadian rhythmic regulation of the highs and lows in ICs and EPCs at night and day, associated with diurnal sympathetic changes, is the reason that the WKY rat does not develop hypertension. In contrast, loss of circadian regulation in the SHR results in persistently higher ICs and lower EPCs throughout, which, combined with the reduction in the EPC function in the SHR (Figure S1) may contribute to increased inflammation and compromised repair of the vascular damage, thereby perpetuating the hypertension-related cardiovascular pathophysiology in the SHR. The loss of circadian regulation of the BM cell activity in the SHR is also reflected in the lack of BM cell response after the BM denervation. As the BM norepinephrine levels are reduced after the BM sympathetic denervation in both rat strains, this results in decreased ICs and increased EPCs but only in the WKY and not in the SHR (Figure 2D). This may be because of the remaining high norepinephrine levels in the BM of SHR when compared with

the WKY rats (Figure 2A). However, the lack of the BM cell response after BM denervation in the SHR, as well as the loss of circadian regulation of the BM cell activity discussed above, is more likely because of chronic downregulation of specific adrenergic receptors in the BM of the SHR, perhaps occurring in compensation of the chronically high sympathetic drive.[21] Alternatively, it is pertinent to point out that the BM denervation was a relatively short-term experiment (<48 hours), which may not be sufficient time to correct the chronic adrenergic receptor dysfunction in the BM of SHR.

BM adrenergic receptors are crucial in the BM cell responses.[20] In line with this, we observed that norepinephrine applied locally to the BM activated the BM ICs, confirming a direct effect of norepinephrine on the BM cells. Interestingly, this effect of norepinephrine was blocked by preapplication of acetylcholine in the BM, suggesting that the parasympathetic influence in the BM may counteract/dampen the effects of the SNA. As phrenic nerve activity is generally reduced in the SHR,[22,23] and, as it appears, in the BM too, as demonstrated by reduced acetylcholine esterase and choline acetyl transferase protein levels in the BM of SHR when compared with the WKY rats, it may be that this also contributes to dysfunctional BM cell activity in the SHR. This is consistent with previous data showing that stimulation of the vagus nerve ameliorates experimental inflammatory diseases.[7,24] However, further experiments, perhaps with the use of genetically modified animals where chimeric mice are generated by adoptive BM transfer from the nicotinic AchR knockout mice, are needed to elaborate the involvement of parasympathetic influence in the BM in hypertension. We also recognize a limitation of this study in that it does not distinguish the effects of the BM norepinephrine, delivered directly via the increased BM fSNA, from the peripheral norepinephrine delivered via the BM blood vessels. Availability of adrenergic receptor knockout mice may be useful in chimeric experiments to address this issue in the near future.

In summary, we propose that there is a loss of circadian regulation of the BM cells because of dysfunctional sympathetic/adrenergic mechanisms in the BM, resulting in chronically high ICs and low EPCs in the SHR. This study raises key questions: do changes in the BM sympathetic drive precede the development of high BP or are they involved in the establishment of the hypertensive pathophysiology? Although both animal and human studies implicate prehypertensive elevation in the sympathetic drive,[3,25] further experiments to measure changes in fSNA before development of high BP are warranted. Nonetheless, the present study demonstrates a dysfunctional BM activity in hypertension, which is associated with changes in fSNA.

Perspectives

In this study, we present the first direct evidence for an impaired circadian regulation of the BM cell activity in the SHR. We present the hypothesis that an impaired sympathetic input to the BM promotes imbalance in the BM ICs and EPCs, which may result in an increased inflammatory-dependent vascular injury, and compromises the vascular repair in hypertension. This hypothesis is supported by the following: (1) enhanced functional neural connections between the presympathetic brain regions and the BM in hypertension; (2) norepinephrine directly activates the BM ICs, which is attenuated by preapplication of acetylcholine; (3) elevated TH and norepinephrine in the BM of SHR; (4) circadian control of the BM activity was impaired in the SHR, which exhibited increased ICs and decreased EPCs when compared with the WKY because of chronic downregulation of adrenergic receptor levels in the SHR BM. Thus, targeting the sympathoadrenergic mechanisms, the BM presents a novel strategy for consideration in neurogenic hypertension.

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Disclosures

None.

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Novelty and Significance

What Is New?

- This article presents direct evidence of altered sympathetic drive to the BM, which is associated with the dysfunctional BM-derived endothelial progenitor cells and inflammatory cells in the rat model of neurogenic hypertension.
- Retrograde labeling using green fluorescent protein–labeled pseudorabies virus shows increased neuronal communication between the brain presympathetic nuclei and the BM in the spontaneously hypertensive rat.
- Increased sympathetic drive to the BM is associated with increased BM tyrosine hydroxylase and norepinephrine. Decreased choline acetyl transferase and acetylcholine esterase suggests impaired parasympathetic influence to the BM. Furthermore, local delivery of norepinephrine to the BM increases the mobilization of the inflammatory cells in vivo which can be antagonized by similar localized delivery of acetylcholine.

What Is Relevant?

- Increased inflammation and reduced vascular repair are hallmarks of hypertension and cardiovascular diseases.
- Increased sympathetic drive contributes to inflammation by mobilizing the inflammatory cells from the spleen in hypertension and from the bone marrow (BM) in myocardial infarction.
- The anti-inflammatory effects of the vagus nerve stimulation are demonstrated by lowered levels of the inflammatory cytokines and suppressed activation of inflammatory cells in inflammatory diseases.

Summary

The study is the first direct evidence of elevated sympathetic drive to the BM in hypertension, which is associated with decreased BM-derived endothelial progenitor cell counts and function and increased BM-derived inflammatory cell counts and mobilization. We suggest that repairing the balance in the EPCs and inflammatory cells presents a novel antihypertensive target in drug-resistant hypertension.