Original Article

Inactivation of the paraventricular nucleus attenuates the cardiogenic sympathetic afferent reflex in the spontaneously hypertensive rat

Matthew R. Zahner[a], Mary C. Brown[a], and Michelle J. Chandley[b]

Background: Myocardial ischemia causes the release of bradykinin, which stimulates cardiac afferents, causing sympathetic excitation and chest pain. Glutamatergic activation of the paraventricular hypothalamic nucleus (PVN) in the spontaneously hypertensive rat (SHR) drives elevated basal sympathetic activity. Thus, we tested the hypothesis that inactivation of the PVN attenuates the elevated reflex response to epicardial bradykinin in the SHR and that ionotropic PVN glutamate receptors mediate the elevated reflex.

Methods: We recorded the arterial pressure and renal sympathetic nerve activity (RSNA) response to epicardial bradykinin application in anesthetized SHR and Wistar Kyoto (WKY) rats before and after PVN microinjection of $GABA_A$ agonist muscimol or ionotropic glutamate receptor antagonist kynurenic acid.

Results: Muscimol significantly decreased the arterial pressure response to bradykinin from 180.4 ± 5.8 to 119.5 ± 6.9 mmHg in the SHR and from 111.8 ± 7.0 to 84.2 ± 8.3 mmHg in the WKY and the RSNA response from 186.2 ± 7.1 to $142.7 \pm 7.3\%$ of baseline in the SHR and from 201.0 ± 11.5 to $160.2 \pm 9.3\%$ of baseline in the WKY. Kynurenic acid significantly decreased the arterial pressure response in the SHR from 164.5 ± 5.0 to 126.2 ± 7.7 mmHg and the RSNA response from $189.9 \pm 13.7$to $168.5 \pm 12.7\%$ of baseline but had no effect in the WKY.

Conclusion: These results suggest that tonic PVN activity is critical for the full manifestation of the CSAR in both the WKY and SHR. Glutamatergic PVN activity contributes to the augmented CSAR observed in the SHR.

Keywords: bradykinin, cardiogenic sympathetic afferent reflex, paraventricular nucleus, renal sympathetic nerve activity, spontaneously hypertensive rat

Abbreviations: CSAR, cardiogenic sympathetic afferent reflex; GABA, gamma amino butyric acid; PVN, paraventricular hypothalamic nucleus; RSNA, renal sympathetic nerve activity; SHR, spontaneously hypertensive rat; WKY, Wistar Kyoto

INTRODUCTION

Hypertension is associated with increased mortality and adverse cardiac outcomes after acute myocardial ischemia [1–6]. During myocardial ischemia, bradykinin and other metabolites of ischemia activate cardiac sensory fibers located on the epicardial surface of the left ventricle, which causes chest pain and sympathoexcitation [7–12]. This reflex response is referred to as the 'cardiogenic sympathetic afferent reflex' (CSAR) and is characterized by a potent sympathetically mediated increase in blood pressure in an attempt to maintain proper tissue perfusion. Paradoxically, the increased sympathetic activity also increases the heart's workload and may expand the locus of ischemic injury to the myocardium, thereby worsening the clinical outcome. Thus, conditions that chronically elevate basal sympathetic activity, such as neurogenic hypertension, may potentiate the severity of the already life-threatening myocardial ischemia [13].

The SHR strain was obtained during the 1960s by Okamoto and Aoki [14] through selective breeding of hypertensive Wistar–Kyoto (WKY) rats. As such, SHR and its normotensive WKY control have been a model of neurogenic hypertension for decades. The increase in sympathetic activity in the SHR relies on central mechanisms, particularly within the hypothalamus and brain stem medulla [15–17].

The hypothalamic paraventricular nucleus (PVN) is a key source of sympathetic activity and is essential in regulating sympathetic vasomotor activity [18–21]. Tonically active PVN neurons send direct projections to the brain stem rostroventrolateral medulla (RVLM) and other nuclei within the brain and brain stem to maintain blood pressure via sympathetic activity [22–27]. GABA and glutamate are the central nervous system's primary inhibitory and excitatory neurotransmitters [28–30]. In-vivo [17,31–35] and in-vitro [33–38] electrophysiological data show that an imbalance of GABAergic and glutaminergic activity within the PVN occurs in neurogenic hypertension observed in the SHR.

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The CSAR is also elevated in the SHR, and PVN hyperactivity plays an essential role [39,40]. We have shown that tonic PVN activity is critical for the full manifestation of the CSAR in the Sprague–Dawley rat [41]. Li and Pan [17] in 2007 show that microinjection of ionotropic glutamate receptor antagonists within the PVN of the SHR decreases the elevated sympathetic activity and normalizes basal blood pressure. However, it is unknown if the imbalance of GABA and glutamate within the PVN is involved in the elevated CSAR observed in the SHR. Therefore, we tested the hypothesis that inactivation of the PVN attenuates the elevated reflex response to epicardial bradykinin in the SHR and that ionotropic PVN glutamate receptors mediate the elevated reflex.

METHODS

A more detailed Material and Methods section is available as Supplemental Material, http://links.lww.com/HJH/C270. Additional details of the materials, data, and analysis that support the findings of this study are available from the corresponding author upon reasonable request.

Animals

Adult, male, SHR or WKY rats (Envigo, Indianapolis, Indiana, USA) 14–18 weeks old were surgically prepared following the Guide for the Care and Use of Laboratory Animals using procedures approved by the East Tennessee State University Committee on Animal Care and Use [42]. Rats were initially anesthetized using 2% isoflurane in O_2 through a nose cone to allow for the cannulation of vessels and tracheostomy. Adequate depth of anesthesia before surgical preparation was confirmed by the absence of a withdrawal response to tail pinch.

Surgical preparation

We maintained each rat's body temperature between 37 and 38 °C throughout the surgery. The left carotid artery was cannulated for the measurement of arterial pressure. Heart rate was counted by triggering from the arterial pressure pulse. The left jugular vein was cannulated for intravenous administration of drugs. The trachea was cannulated for mechanical ventilation. A lateral thoracotomy was performed to expose the heart for the epicardial application of bradykinin. A craniotomy was performed for bilateral microinjection of drugs into the right and left PVN. A left flank incision was made for renal sympathetic nerve recordings.

After the laparotomy, rats were slowly administered 1% αchloralose (100 mg/kg intravenously) and weaned from isoflurane over approximately 20 min. This approach maintained a complete surgical anesthetic state and was verified by the absence of a corneal reflex or an increase in arterial pressure or RSNA to tail pinch. After dissecting a renal sympathetic nerve and determining that rats remained completely surgically anesthetized, they were paralyzed with D-tubocurarine (0.1 mg/kg intravenously) to block any spontaneous muscle twitching that typically occurs in the retracted external oblique. This dose of D-tubocurarine lasted ~30 to 45 min and was allowed to wear off to assess the adequacy of the anesthetized state of the rat. In all cases, the rats' arterial pressure, heart rate, and RSNA remained completely unresponsive to corneal stimulation or tail pinch.

Experimental design

After a high-quality nerve recording was obtained, an ~15 min stabilization period was given followed by a 5 min control period that was used to normalize baseline and reflex RSNA before and after microinjection treatments. We tested the CSAR by applying bradykinin (10 μg/ml; Sigma, St. Louis, Missouri, USA) to the anterior epicardial surface (~1 cm^2) of the left ventricle with a cotton applicator through the exposed window in the left chest as previously demonstrated [12,41,43,44]. The responses to epicardial bradykinin were examined at least twice, separated by ~5 min, to ensure a reproducible response. After each bradykinin application, the heart was washed using ~10 ml of room temperature physiological saline, and the arterial pressure, heart rate, and RSNA returned to baseline levels. To ensure that rats do not respond to the mechanical application of the cotton applicator touching the epicardium, we applied saline to the heart before any reflex trials, as we have in previous studies [12,41,43,44].

Arterial pressure, heart rate, and RSNA response to epicardial bradykinin application were tested before (control) and after microinjection into the PVN. Microinjections of either vehicle (saline), the broad-spectrum ionotropic glutamate receptor antagonist kynurenic acid (5.0 nmol in 50 nl, Tocris, Minneapolis, Minnesota, USA), or $GABA_A$ receptor agonist muscimol (0.5 nmol in 50 nl, Sigma) were performed in separate groups of rats such that each rat received only one treatment. The muscimol and kynurenic acid doses were based on previous studies [15,17,41,45].

The location of the microinjector pipette tip and diffusion of the injectant within the PVN was examined and confirmed histologically in all rats. All microinjections contained 5% rhodamine-labeled fluorescent microspheres (0.04 μm, Molecular Probes, Eugene, Oregon, USA). Microinjection locations were plotted on standardized sections from the Paxinos and Watson atlas [46]. Rats with micropipette misplacement outside the PVN were excluded from the analysis.

Data analysis

Values are presented as means ± SE. Baseline arterial pressure, heart rate, and RSNA were averaged during 30 s of the baseline period before epicardial bradykinin. The peak blood pressure, heart rate, and RSNA responses were measured immediately after the bradykinin application. The RSNA was normalized to the control period and expressed as a percentage change from control baseline period prior to microinjection to account for the variability in each rat. Data were analyzed using one-way ANOVA or two-way repeated-measure ANOVA and plotted with GraphPad Prism software version 9.4.1 (Boston, Massachusetts, USA). We used the Bonferroni post hoc test to compare the difference between group means when F values were significant. P less than 0.05 was considered statistically significant.

RESULTS

We conducted these studies with 72 rats, of which 38 were SHRs, and 34 were WKYs. All microinjections into the PVN were verified histologically and plotted according to Paxinos and Watson's stereotaxic atlas (Fig. 1) [46]. We dismissed 10 rats from the analysis (6 SHR and 4 WKY) because of inaccurate microinjection position. In one WKY rat, one of the bilateral muscimol microinjections penetrated the third ventricle and was, therefore, considered a misplacement. Table 1 shows the grouped mean (±SE) arterial pressure and RSNA values during baseline and the reflex response to epicardial bradykinin (10 μg/ml) in WKY and SHR before and after microinjection treatments. Bradykinin significantly increased arterial pressure, heart rate, and RSNA in all rats before and after microinjection treatment. Although the bradykinin-elicited tachycardia was statistically significant ($P < 0.05$), albeit subtle, neither vehicle, kynurenic acid nor muscimol microinjection into the PVN significantly affected the baseline nor the bradykinin response in the SHR or WKY rats (Supplemental Fig 1, http://links.lww.com/HJH/C271). As such, heart rate will not be discussed further.

Effect of vehicle and muscimol microinjections into the paraventricular nucleus on arterial pressure and renal sympathetic nerve activity responses to epicardial bradykinin

Vehicle microinjection into the PVN did not affect the baseline arterial pressure or RSNA in the SHR or the WKY (Supplemental Fig 2, http://links.lww.com/HJH/C272). The mean baseline arterial pressure during control and after vehicle microinjection into the PVN as well as the mean bradykinin-induced increase in arterial pressure was significantly greater in the SHR compared with the WKY ($P < 0.05$). The mean baseline RSNA during control and after vehicle microinjection into the PVN, as well as the mean bradykinin-induced RSNA response, did not differ between WKY and SHR ($P > 0.999$).

To determine the importance of PVN activity in the CSAR in the WKY and SHR, inactivation of the PVN was performed using bilateral muscimol (0.5 nmol/50 nl) microinjection. Figure 2a and b shows a representative response to epicardial bradykinin (10 μg/ml) before and after bilateral muscimol microinjection into the PVN in one WKY and one SHR rat. Prior to muscimol treatment, the mean baseline arterial pressure during control was higher in the SHR than in the WKY ($P < 0.05$). During control, bradykinin elicited a significant increase in arterial pressure and RSNA in both the WKY ($P < 0.05$) and the SHR ($P < 0.05$).

Muscimol inactivation of the PVN significantly reduced baseline arterial pressure and RSNA and attenuated the arterial pressure and RSNA responses to epicardial bradykinin in the WKY and SHR (Fig. 2c and d). The attenuated bradykinin-induced arterial pressure response after muscimol treatment remained greater in SHR than in the WKY ($P < 0.05$). However, the attenuated RSNA response after muscimol treatment did not differ between the SHR and the WKY ($P = 0.2291$).

Effect of microinjection of kynurenic acid into the paraventricular nucleus on arterial pressure and renal sympathetic nerve activity responses to epicardial bradykinin

To determine if PVN ionotropic glutamate receptors are involved in the elevated bradykinin-induced sympathetic

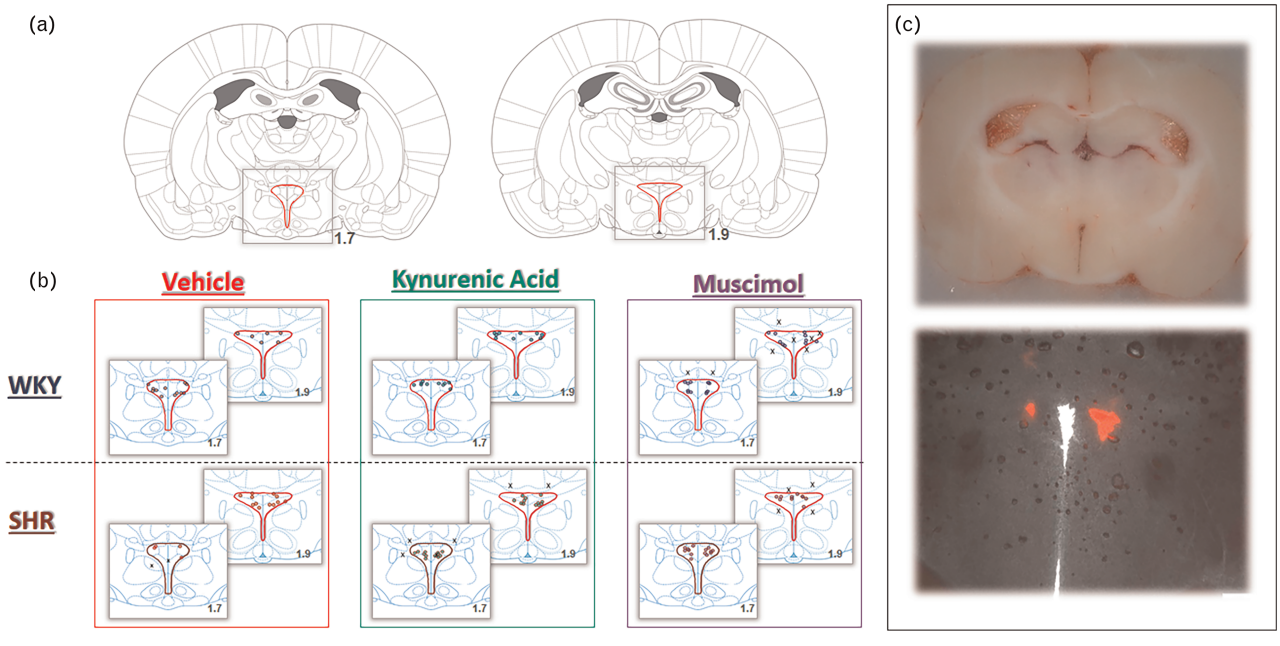

FIGURE 1 Locations of the paraventricular nucleus microinjections (50 nl). (a) Coronal atlas images at the level of the PVN 1.7 and 1.9 mm caudal from bregma as shown in the Paxinos and Watson atlas [46]. Squares show the area of the hypothalamus shown in b with paraventricular nucleus (PVN) outlined in red. (b) Grouped data showing the microinjection needle placement of vehicle (saline), kynurenic acid, or muscimol in the Wistar Kyoto (WKY) or spontaneously hypertensive rats (SHR). O indicates bilateral microinjections were within the PVN, and X indicates microinjection where one or both were outside the PVN. (c) Image of the brain section observed during cutting (top) and merged fluorescent and light microscope (4×, bottom) showing the injection area.

TABLE 1. Cardiogenic sympathetic reflex in Wistar Kyoto and spontaneously hypertensive rats

	WKY							
	Arterial pressure (mmHg)				RSNA (% baseline)			
	Control		Treatment		Control		Treatment	
	Baseline	Reflex	Baseline	Reflex	Baseline	Reflex	Baseline	Reflex
Vehicle ($n=9$)	80.9 ± 3.6	104.4 ± 3.5*	79.3 ± 4.1	103.0 ± 5.5*	100.4% ± 1.2%	193.3% ± 9.9%*	101.9% ± 1.8%	195.6% ± 10.6%*
Kynurenic acid ($n=10$)	84.8 ± 3.6	105.7 ± 4.3*	81.8 ± 4.2	102.3 ± 5.7*	100.9% ± 0.7%	196.3% ± 14.8%*	102.3% ± 4.0%	205.0% ± 15.5%*
Muscimol ($n=11$)	88.9 ± 4.8	111.8 ± 7.0*	67.3 ± 5.2$^{\#}$	84.2 ± 8.3*,**	99.9% ± 0.5%	201.0% ± 11.5%*	81.5% ± 3.6%$^{\#}$	160.2% ± 9.3%*,**
	SHR							
	Arterial pressure (mmHg)				RSNA (% baseline)			
	Control		Treatment		Control		Treatment	
	Baseline	Reflex	Baseline	Reflex	Baseline	Reflex	Baseline	Reflex
Vehicle ($n=8$)	137.1 ± 3.7$^{\diamond}$	181.4 ± 5.6$^{*,\diamond}$	134.5 ± 4.7$^{\diamond}$	181.3 ± 5.5 $^{*,\diamond}$	102.1% ± 1.7%	195.7% ± 11.6%*	100.1% ± 4.2%	189.1% ± 13.8%*
Kynurenic acid ($n=12$)	125.8 ± 2.6$^{\diamond}$	164.5 ± 5.0$^{*,\diamond}$	86.6 ± 5.4$^{\#,\diamond}$	126.2 ± 7.7$^{*,**,\diamond}$	100.9% ± 0.4%	189.9% ± 13.7%*	73.3% ± 4.9%$^{\#}$	168.5% ± 12.7%*,**
Muscimol ($n=12$)	140.6 ± 3.6$^{\diamond}$	180.4 ± 5.8$^{*,\diamond}$	89.2 ± 5.6$^{\#}$	119.5 ± 6.9$^{*,**,\diamond}$	100.2% ± 0.2%	186.2% ± 7.1%*	76.2% ± 4.6%$^{\#}$	142.7% ± 7.3%*,**

Arterial pressure (mmHg) and renal sympathetic nerve activity (RSNA % baseline) during baseline and the reflex response after epicardial bradykinin (10 μg/ml) application before (control) and after microinjection of vehicle, kynurenic acid, or muscimol. RSNA, renal sympathetic nerve activity; SHR, spontaneously hypertensive rat; WKY, Wistar Kyoto.
$^{*}P<0.05$ compared with the respective baseline.
$^{\#}P<0.05$ compared with the respective control baseline.
$^{**}P<0.05$ compared with the respective control response.
$^{\diamond}P<0.05$ compared between the WKY and SHR.

response observed in the SHR, we tested the reflex response to epicardial bradykinin before and after the microinjection of kynurenic acid (5.0 nmol/50 nl) into the PVN. Figure 3a shows a recording from one experiment in a SHR showing baseline activity and the reflex responses to epicardial bradykinin before and after bilateral microinjection of kynurenic acid into the PVN.

In the WKY, inhibition of ionotropic glutamate receptors with kynurenic acid microinjection into the PVN did not affect arterial pressure or RSNA during baseline or the reflex response to epicardial bradykinin (Fig. 3b and c). Before kynurenic acid treatment, baseline arterial pressure and the arterial pressure response to epicardial bradykinin were significantly higher in the SHR compared with the WKY ($P<0.05$). In the SHR, microinjection of kynurenic acid significantly reduced baseline arterial pressure and RSNA and attenuated the arterial pressure and RSNA responses to epicardial bradykinin ($P<0.05$). After kynurenic acid treatment, the elevated baseline arterial pressure in the SHR was not significantly different from baseline arterial pressure in the WKY ($P>0.999$). Although kynurenic acid microinjection in the SHR significantly decreased both the baseline RSNA ($P<0.05$) and the reflex response to epicardial bradykinin ($P<0.05$) compared with its pretreatment control, neither the decrease in baseline RSNA ($P=0.196$) nor the attenuated reflex response to epicardial bradykinin ($P=0.057$) reached significance when compared with WKY. Also, while the reflex arterial pressure response to epicardial bradykinin after kynurenic acid treatment was significantly reduced in the SHR compared with the pretreatment control response in the SHR ($P<0.05$), it remained significantly greater than the reflex response to bradykinin in the WKY ($P=0.0043$).

DISCUSSION

Myocardial ischemia causes the production and release of ischemic metabolites that activate cardiac sensory spinal afferents [7,10,47]. Activation of these fibers is often associated with pain and elicits a sympathetically mediated increase in arterial pressure [10–12,41,48,49]. We have previously shown that cardiac sensory afferents are expressed on the ventricular epicardium and that the abolition of these fibers abolishes the reflex response to epicardial bradykinin [12]. Although the brain stem nuclei mainly mediate the CSAR [43,44,50–52], modulation of supramedullary sites, particularly by the PVN, has also been shown to influence the CSAR [39,40,53–57].

The PVN is one of five major autonomic premotor cell groups [25,58] and is an important site in controlling sympathetic outflow [26,59,60]. Inhibition of the tonic sympathetic activity from PVN in Sprague–Dawley as well as SHR and WKY rats by microinjection of GABA or $GABA_A$ receptor agonist, muscimol, suppresses sympathetic activity and lowers blood pressure [15,41,61]. Reduced GABAergic neurotransmission and increased glutamatergic activity within the PVN play a key factor in hypertension observed in the SHR [17,31,32,36]. Blockade of glutamatergic neurotransmission within the PVN lowers lumbar sympathetic nerve activity and normalizes the high blood pressure in the SHR [17]. Although we have previously shown that activation of the PVN is required for the full manifestation of the CSAR in the normotensive Sprague–Dawley rat and that inhibition of PVN glutamate receptors is not involved [41], glutamate receptors are involved in the high blood pressure observed in the SHR [17,33,36,62–64]. However, the role of increased PVN glutamatergic activity in the SHR on the reflex response to activation of cardiac sensory spinal afferents is not well known. In this study, we show that the reflex response in arterial pressure to epicardial bradykinin is greater in the SHR than the WKY and that inactivation of the PVN, using microinjection of the long-lasting $GABA_A$ agonist, muscimol, decreased basal arterial pressure, and RSNA and significantly diminished the reflex response to epicardial bradykinin in both the SHR and the WKY. We

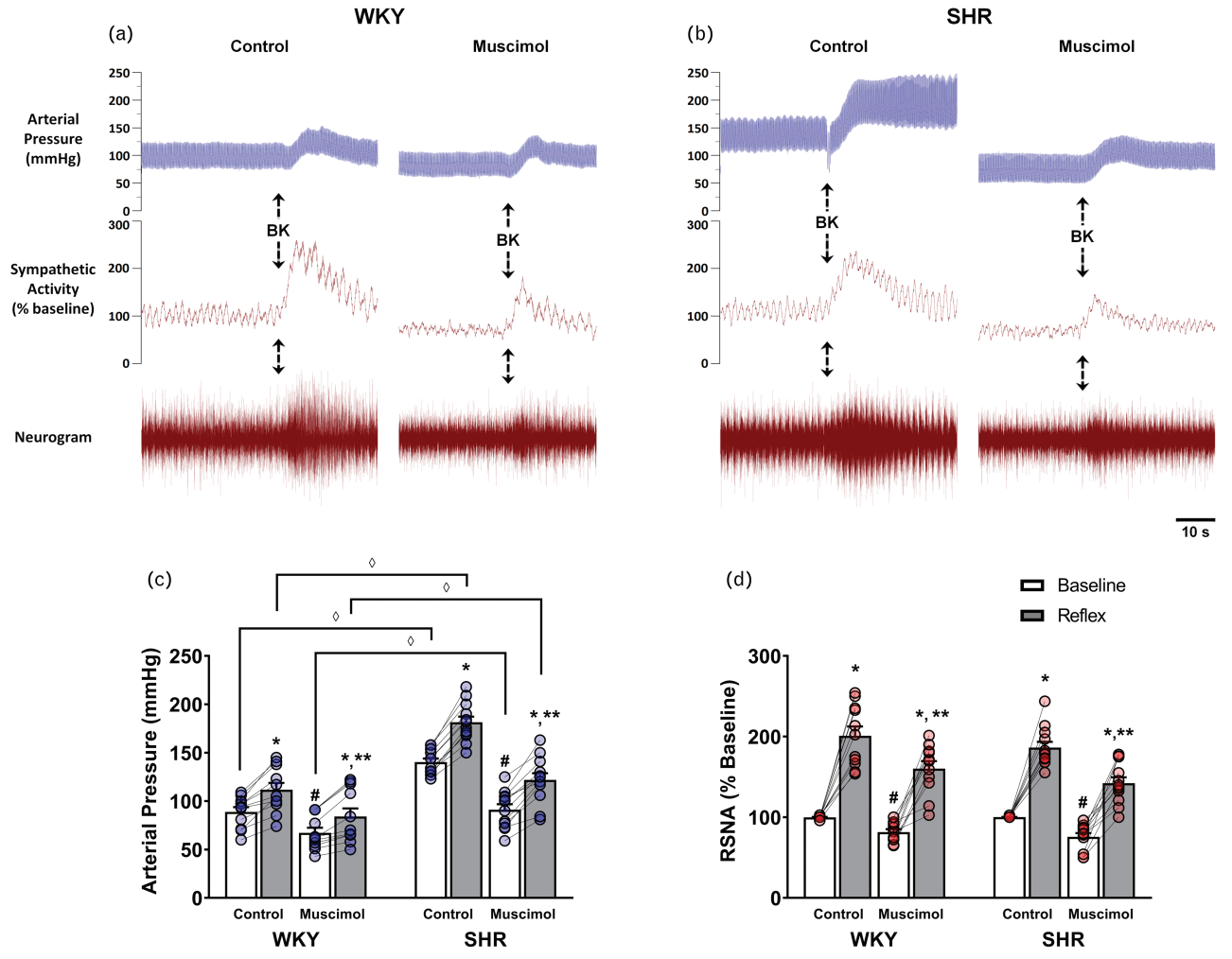

FIGURE 2 Muscimol-induced inactivation of the paraventricular nucleus attenuates the epicardial bradykinin-elicited sympathoexcitatory reflex in spontaneously hypertensive and Wistar Kyoto rats. Original tracings from one WKY (a) and one SHR (b) rat showing the arterial pressure, renal sympathetic nerve activity averaged, and the raw neurogram response to epicardial application of bradykinin (BK, 10 μg/ml) before (control) and after 50 nl microinjection of muscimol (0.5 nmol) bilaterally into the PVN. Arrow indicates epicardial bradykinin (BK) application. Grouped data from WKY ($n = 10$) and SHR ($n = 12$) showing the arterial pressure (c) and RSNA (d) during baseline and the responses to epicardial bradykinin (10 μg/ml) before and after bilateral microinjection of muscimol into the PVN. The RSNA responses are expressed as a percentage of the control baseline before epicardial bradykinin or microinjections. Data are represented as means ± SE. $^{*}P < 0.05$ compared with the respective baseline. $^{\#}P < 0.05$ compared with the respective control baseline. $^{**}P < 0.05$ compared with the respective control response. $^{\diamond}P < 0.05$ compared between the WKY and SHR. Two-way repeated–measures ANOVA with baseline/reflex and treatment (control/muscimol and BK reflex) as factors, and one-way ANOVA between groups (WKY and SHR) with Bonferroni posttest. PVN, paraventricular nucleus; RSNA, renal sympathetic nerve activity; SHR, spontaneously hypertensive rat; WKY, Wistar Kyoto.

also show that, unlike in the Sprague–Dawley, the elevated magnitude of the reflex is mediated in part by the PVN glutamatergic activation. These experiments suggest that activation of PVN glutamate receptors is necessary for the enhanced CSAR observed in the SHR.

We have previously shown that muscimol inactivation of the PVN attenuates the CSAR in the Sprague–Dawley [41], and other labs have confirmed this [53,56]. Although inhibition of ionotropic glutamate receptors in the Sprague–Dawley is not involved, this study shows that glutamate receptors are involved in the augmented reflex response observed in the SHR. Microinjection of the ionotropic glutamate receptor antagonist kynurenic acid into the PVN reduced RSNA and normalized arterial pressure in SHR and significantly reduced the reflex response to epicardial bradykinin in the SHR, not the WKY. These findings suggest that similar to the Sprague–Dawley rat, tonic PVN activity is critical for the full manifestation of the CSAR in both the SHR and the WKY, and the elevated CSAR observed in the SHR is likely mediated by increased glutamatergic PVN activity. The role of PVN glutamate receptors in the CSAR is important because it appears they play a critical role in maintaining the elevated tonic sympathetic activity observed in the SHR, as previously described but also contribute to the augmented CSAR. Therefore, this study provides new information that the glutamatergic activity within the PVN contributes to the augmented sympathetic activity elicited by stimulating cardiac afferents in the SHR.

Although we chose to record sympathetic activity from the renal sympathetic nerve, others have recorded from the lumbar sympathetic nerve [15,17]. Regional differences exist in activating sympathetic nerves that innervate the adrenal, kidney, or lumbar vascular bed. However, all are highly barosensitive, which is indicative of cardiovascular sensitivity. We have recorded from lumbar, splanchnic, and

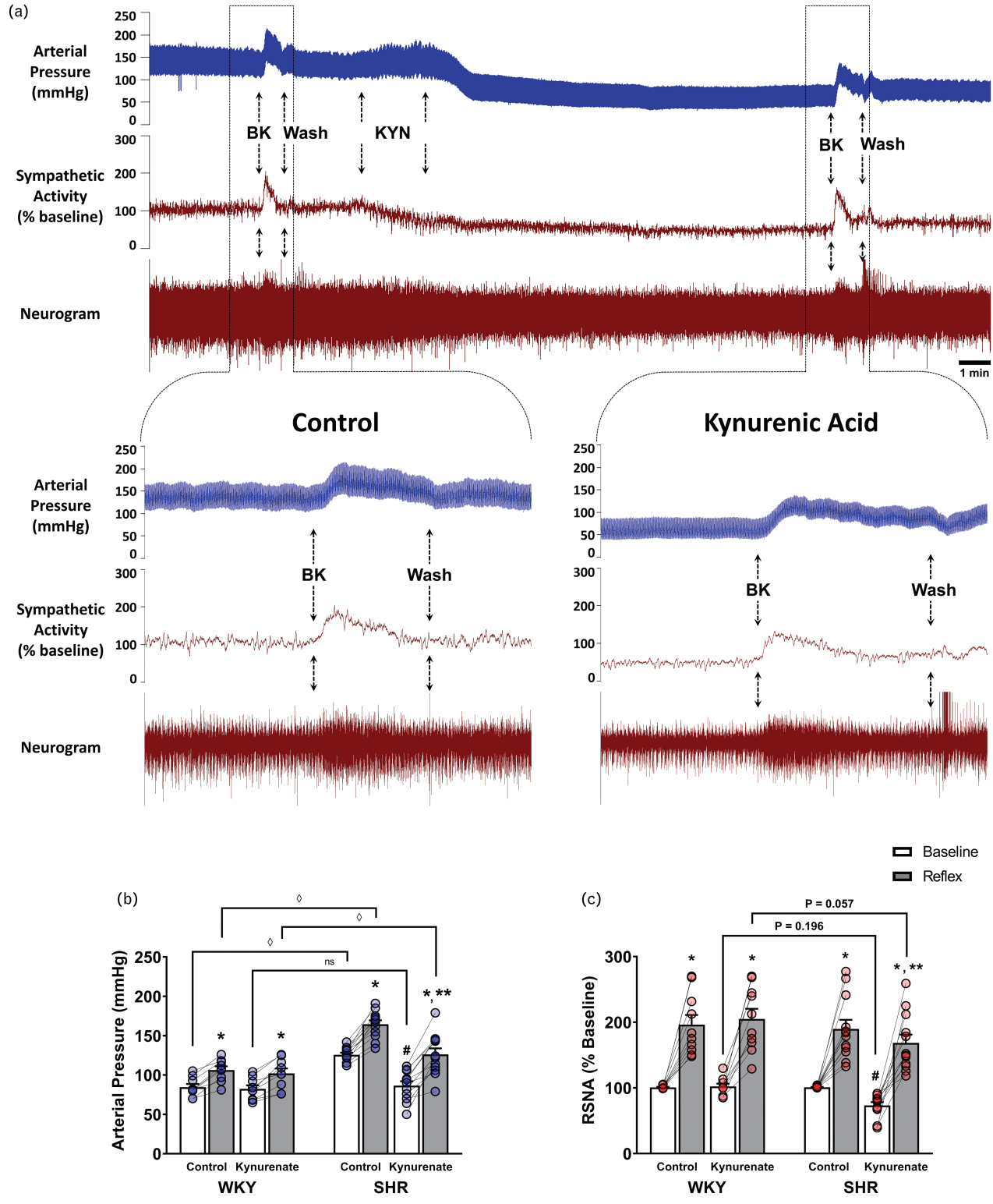

FIGURE 3 Kynurenic acid blockade of paraventricular nucleus ionotropic glutamate receptors attenuates the epicardial bradykinin-elicited sympathoexcitatory reflex in spontaneously hypertensive rats but not Wistar Kyoto rats. (a) Original tracings from one experiment in an SHR showing arterial pressure and renal sympathetic nerve activity averaged, and the raw neurogram during baseline and after epicardial application of bradykinin (BK, 10 μg/ml) before (control) and after 50 nl microinjection of kynurenic acid bilaterally into the PVN. Dashed boxes show a 2 min section of the control or kynurenic acid treatment (below). Grouped data from WKY ($n = 11$) and SHR ($n = 12$) showing the arterial pressure (b) and RSNA (c) during baseline and the responses to epicardial bradykinin (10 μg/ml) before and after bilateral microinjection of kynurenic acid (5.0 nmol) into the PVN. The RSNA responses are expressed as a percentage of the control baseline before epicardial bradykinin or microinjections. Data are represented as means ± SE. $^{*}P < 0.05$ compared with the respective baseline. $^{\#}P < 0.05$ compared with the respective control baseline. $^{**}P < 0.05$ compared with the respective control response. $^{\diamond}P < 0.05$ compared between the WKY and SHR. Two-way repeated-measures ANOVA with baseline/reflex and treatment (control/kynurenic acid and BK reflex) as factors, and one-way ANOVA between groups (WKY and SHR) with Bonferroni posttest. ANOVA, analysis of variance; PVN, paraventricular nucleus; RSNA, renal sympathetic nerve activity; SE, standard error; SHR, spontaneously hypertensive rat; WKY, Wistar Kyoto.

renal sympathetic nerves, and our experience found the renal sympathetic nerve to provide the cleanest recordings.

Sympathetic activity, measured with a chronically implanted recording device, is increased in both the conscious and anesthetized SHR compared with the WKY [65]. However, to perform our CSAR experiments, we needed to perform a thoracotomy. We, therefore, had to use acute sympathetic nerve recordings rather than chronic ones. A well known shortcoming of acute sympathetic nerve recordings is that they cannot reliably compare baseline activity from one group to another because the nerve signal must be normalized to each rat's baseline activity. Although we can see the effect of a reflex or microinjection treatment, a comparison between baseline groups cannot be accurately made. Although we observed that bradykinin application elicited a greater increase in arterial pressure in the SHR, the normalized baseline RSNA was not different between SHR and WKY. That the blood pressure response was significantly greater in the SHR compared with the WKY and that inactivation of PVN attenuated the response demonstrates the PVN is involved in the elevated arterial pressure response to epicardial bradykinin. This may be because of increased sympathetic activity in the SHR derived from PVN. We did observe a significant decrease in RSNA in the SHR after kynurenic acid treatment suggesting that inhibition of PVN ionotropic glutamate activity plays an important role in the CSAR in SHR but not the WKY. One may argue that the decrease in the magnitude of the response to epicardial bradykinin may be simply because of a reduction in baseline arterial pressure and RSNA and not reflex responsiveness. We do not believe that this is the case because we have observed that decreases in arterial pressure that are not neurogenic in nature (e.g. baroreceptor unloading) typically augment the reflex responsiveness.

We chose to broadly focus on ionotropic glutamate receptors because it is been shown that inhibition of these channels normalized the elevated sympathetic activity and arterial pressure in the SHR [17]. Consistent with this, kynurenic acid reduced arterial pressure and RSNA in this study. Li and Pan in 2007 differentiated the ionotropic glutamate receptor subtypes within the PVN involved in the sympathetically mediated hypertension in SHRs. They showed that while α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate and N-methyl-d-aspartate (NMDA) receptors contributed to the hypertension, the greatest inhibition of sympathetic activity occurred after kynurenic acid-induced glutamatergic blockade in the PVN. In this study, we also show that kynurenic acid decreased arterial pressure and RSNA in the SHR but not the WKY. However, one may argue that the decrease in the reflex response to epicardial bradykinin may be simply because of a reduction in baseline arterial pressure and RSNA and not reflex responsiveness. We do not believe that this is the case because we have observed that decreases in arterial pressure that are not neurogenic in nature (e.g. baroreceptor unloading) augment the cardiogenic reflex.

In our study, while kynurenic acid significantly reduced the elevated reflex response to epicardial bradykinin, we cannot exclude the possibility that other PVN neurotransmitters may mediate the elevated reflex response observed in the SHR. It is well known that sympathetic activity is increased in the SHR. Increased sympathetic reflex activity [66–69], including elevated CSAR, has also been shown in the SHR [39,40,70]. Angiotensin mechanisms within the PVN at least partially mediate the augmented CSAR [71,72], and microinjection of angiotensin II into the PVN augments the sympathoexcitatory response to epicardial bradykinin in normotensive rats [57]. However, it is unlikely that angiotensin II plays a critical role in the normotensive rat in mediating the CSAR because inhibition of angiotensin 1 receptor antagonist losartan alone into the PVN does not affect the sympathoexcitatory response to epicardial bradykinin [57]. In the 2K1C renovascular hypertensive rat, pretreatment with losartan normalized the reflex response to epicardial capsaicin, demonstrating an important role for PVN angiotensin II in hypertension. In the SHR, overexpression of SOD1 in the PVN attenuates the elevated CSAR indicating that increased reactive oxygen species also contribute [40]. Increased pro-inflammatory cytokines have also increased sympathetic activity and contributed to the elevated CSAR observed in the SHR [39].

An alternative reason, from the PVN-elicited increase in sympathetic activity in the SHR, for increased hemodynamic response to epicardial bradykinin may involve increased sensitivity to stimulation of cardiac afferents. This has yet to be tested and was beyond the scope of our current study. However, a study by Shanks *et al.* [73] in 2019 demonstrated using an intrathecal infusion of the highly potent resiniferatoxin, which abolished the $TRPV_1$-expressing afferent fibers from the heart necessary for bradykinin-induced sympathetic reflex, decreased blood pressure in the SHR. This suggests that increased cardiac afferent activity drives the elevated sympathetic activity observed in the SHR. If this is the case, the increased visceral stimulation must involve glutamatergic PVN activity because inhibiting ionotropic glutamatergic activity within the PVN normalized the elevated blood pressure in the SHR.

Although the activity of presympathetic PVN neurons serves as an essential source for excitatory tonic input to the RVLM, it is also possible that supramedullary input is not critical to maintain the CSAR. In the cat, decerebration does not affect the reflex response to epicardial bradykinin [74]. This does not exclude the possibility that the supraspinal sites are also involved in this reflex response, particularly the elevated reflex observed in the SHR. The development of hypertension in the SHR is suggested to involve increased activity of glutamatergic PVN neurons [17]. The increased sympathetic reflex activity observed in the CSAR is likely because of elevated glutamatergic activity. In the absence of glutamatergic activity after kynurenic acid, the inactivation of the PVN, or muscimol inactivation, the remaining fraction of the CSAR is likely derived from brain stem nuclei.

Perspectives and significance

Neurogenic hypertension is a serious condition and may augment sympathetic reflex responses observed during myocardial ischemia. A more comprehensive understanding of the central mechanisms involving sympathetic reflex activity may provide the rationale for better therapies to minimize the adverse responses following myocardial ischemia. Collectively, these data suggest that PVN activity is

necessary for the full manifestation of the reflex sympathetic response to epicardial bradykinin. PVN glutamatergic activity is critical for the increased arterial pressure observed in the SHR. Here we show that the augmented reflex response to epicardial bradykinin is mediated by PVN activity and that PVN inactivation normalized the reflex. We also show that, unlike Sprague–Dawley, inhibition of ionotropic glutamate receptors within the PVN attenuates the magnitude of the reflex response in the SHR but not the WKY.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Carrick D, Haig C, Maznyczka AM, Carberry J, Mangion K, Ahmed N, *et al*. Hypertension, microvascular pathology, and prognosis after an acute myocardial infarction. *Hypertension* 2018; 72:720–730.
2. Haider AW, Chen L, Larson MG, Evans JC, Chen MH, Levy D. Antecedent hypertension confers increased risk for adverse outcomes after initial myocardial infarction. *Hypertension* 1997; 30:1020–1024.
3. Herlitz J, Karlson BW, Richter A, Wiklund O, Jablonskiene D, Hjalmarson A. Prognosis in hypertensives with acute myocardial infarction. *J Hypertens* 1992; 10:1265–1271.
4. Kannel WB, Sorlie P, Castelli WP, McGee D. Blood pressure and survival after myocardial infarction: the Framingham study. *Am J Cardiol* 1980; 45:326–330.
5. Rabkin SW, Mathewson FA, Tate RB. Prognosis after acute myocardial infarction: relation to blood pressure values before infarction in a prospective cardiovascular study. *Am J Cardiol* 1977; 40:604–610.
6. Wong ND, Cupples LA, Ostfeld AM, Levy D, Kannel WB. Risk factors for long-term coronary prognosis after initial myocardial infarction: the Framingham Study. *Am J Epidemiol* 1989; 130:469–480.
7. Baker DG, Coleridge HM, Coleridge JC, Nerdrum T. Search for a cardiac nociceptor: stimulation by bradykinin of sympathetic afferent nerve endings in the heart of the cat. *J Physiol* 1980; 306:519–536.
8. Barber MJ, Mueller TM, Davies BG, Zipes DP. Phenol topically applied to canine left ventricular epicardium interrupts sympathetic but not vagal afferents. *Circ Res* 1984; 55:532–544.
9. Lombardi F, Patton CP, Della Bella PD, Pagani M, Malliani A. Cardiovascular and sympathetic responses reflexly elicited through the excitation with bradykinin of sympathetic and vagal cardiac sensory endings in the cat. *Cardiovasc Res* 1982; 16:57–65.
10. Tjen ALSC, Pan HL, Longhurst JC. Endogenous bradykinin activates ischaemically sensitive cardiac visceral afferents through kinin B2 receptors in cats. *J Physiol* 1998; 510 (Pt 2):633–641.
11. Veelken R, Glabasnia A, Stetter A, Hilgers KF, Mann JF, Schmieder RE. Epicardial bradykinin B2 receptors elicit a sympathoexcitatory reflex in rats. *Hypertension* 1996; 28:615–621.
12. Zahner MR, Li DP, Chen SR, Pan HL. Cardiac vanilloid receptor 1-expressing afferent nerves and their role in the cardiogenic sympathetic reflex in rats. *J Physiol* 2003; 551 (Pt 2):515–523.
13. Adamson PB, Vanoli E. Early autonomic and repolarization abnormalities contribute to lethal arrhythmias in chronic ischemic heart failure: characteristics of a novel heart failure model in dogs with postmyocardial infarction left ventricular dysfunction. *J Am Coll Cardiol* 2001; 37:1741–1748.
14. Okamoto K, Aoki K. Development of a strain of spontaneously hypertensive rats. *Jpn Circ J* 1963; 27:282–293.
15. Allen AM. Inhibition of the hypothalamic paraventricular nucleus in spontaneously hypertensive rats dramatically reduces sympathetic vasomotor tone. *Hypertension* 2002; 39:275–280.
16. Ito S, Komatsu K, Tsukamoto K, Sved AF. Excitatory amino acids in the rostral ventrolateral medulla support blood pressure in spontaneously hypertensive rats. *Hypertension* 2000; 35 (1 pt 2):413–417.
17. Li DP, Pan HL. Glutamatergic inputs in the hypothalamic paraventricular nucleus maintain sympathetic vasomotor tone in hypertension. *Hypertension* 2007; 49:916–925.
18. Badoer E. Hypothalamic paraventricular nucleus and cardiovascular regulation. *Clin Exp Pharmacol Physiol* 2001; 28:95–99.
19. Dampney RA, Coleman MJ, Fontes MA, Hirooka Y, Horiuchi J, Li YW, *et al*. Central mechanisms underlying short- and long-term regulation of the cardiovascular system. *Clin Exp Pharmacol Physiol* 2002; 29:261–268.
20. Porter JP, Brody MJ. Neural projections from paraventricular nucleus that subserve vasomotor functions. *Am J Physiol* 1985; 248:R271–R281.
21. Yang Z, Coote JH. Influence of the hypothalamic paraventricular nucleus on cardiovascular neurones in the rostral ventrolateral medulla of the rat. *J Physiol* 1998; 513 (Pt 2):521–530.
22. Pyner S, Coote JH. Identification of branching paraventricular neurons of the hypothalamus that project to the rostroventrolateral medulla and spinal cord. *Neuroscience* 2000; 100:549–556.
23. Saper CB, Loewy AD, Swanson LW, Cowan WM. Direct hypothalamo-autonomic connections. *Brain research* 1976; 117:305–312.
24. Shafton AD, Ryan A, Badoer E. Neurons in the hypothalamic paraventricular nucleus send collaterals to the spinal cord and to the rostral ventrolateral medulla in the rat. *Brain Res* 1998; 801:239–243.
25. Strack AM, Sawyer WB, Hughes JH, Platt KB, Loewy AD. A general pattern of CNS innervation of the sympathetic outflow demonstrated by transneuronal pseudorabies viral infections. *Brain Res* 1989; 491:156–162.
26. Swanson LW, Kuypers HG. The paraventricular nucleus of the hypothalamus: cytoarchitectonic subdivisions and organization of projections to the pituitary, dorsal vagal complex, and spinal cord as demonstrated by retrograde fluorescence double-labeling methods. *J Comp Neurol* 1980; 194:555–570.
27. Koba S, Hanai E, Kumada N, Kataoka N, Nakamura K, Watanabe T. Sympathoexcitation by hypothalamic paraventricular nucleus neurons projecting to the rostral ventrolateral medulla. *J Physiol* 2018; 596:4581–4595.
28. Bowery NG, Smart TG. GABA and glycine as neurotransmitters: a brief history. *Br J Pharmacol* 2006; 147 (Suppl 1):S109–S119.
29. Meldrum BS. Glutamate as a neurotransmitter in the brain: review of physiology and pathology. *J Nutr* 2000; 130 (4s Suppl):1007S–1015S.
30. Watkins JC. l-glutamate as a central neurotransmitter: looking back. *Biochem Soc Trans* 2000; 28:297–309.
31. Kunkler PE, Hwang BH. Lower GABAA receptor binding in the amygdala and hypothalamus of spontaneously hypertensive rats. *Brain Res Bull* 1995; 36:57–61.
32. Li D-P, Pan H-L. Role of γ-aminobutyric acid $(GABA)_A$ and $GABA_B$ receptors in paraventricular nucleus in control of sympathetic vasomotor tone in hypertension. *J Pharmacol Exp Ther* 2007; 320:615–626.
33. Li DP, Byan HS, Pan HL. Switch to glutamate receptor 2-lacking AMPA receptors increases neuronal excitability in hypothalamus and sympathetic drive in hypertension. *J Neurosci* 2012; 32:372–380.
34. Qiao X, Zhou JJ, Li DP, Pan HL. Src kinases regulate glutamatergic input to hypothalamic presympathetic neurons and sympathetic outflow in hypertension. *Hypertension* 2017; 69:154–162.
35. Ye ZY, Li DP, Li L, Pan HL. Protein kinase CK2 increases glutamatergic input in the hypothalamus and sympathetic vasomotor tone in hypertension. *J Neurosci* 2011; 31:8271–8279.
36. Li D-P, Yang Q, Pan H-M, Pan H-L. Pre and postsynaptic plasticity underlying augmented glutamatergic inputs to hypothalamic presympathetic neurons in spontaneously hypertensive rats. *J Physiol* 2008; 586:1637–1647.
37. Zhou JJ, Shao JY, Chen SR, Chen H, Pan HL. alpha2delta-1 protein promotes synaptic expression of Ca(2+) permeable-AMPA receptors by inhibiting GluA1/GluA2 heteromeric assembly in the hypothalamus in hypertension. *J Neurochem* 2022; 161:40–52.
38. Zhou JJ, Pachuau J, Li DP, Chen SR, Pan HL. Group III metabotropic glutamate receptors regulate hypothalamic presympathetic neurons through opposing presynaptic and postsynaptic actions in hypertension. *Neuropharmacology* 2020; 174:108159.
39. Shi Z, Jiang SJ, Wang GH, Xu AL, Guo L. Pro-inflammatory cytokines in paraventricular nucleus mediate the cardiac sympathetic afferent reflex in hypertension. *Auton Neurosci* 2014; 186:54–61.

40. Yuan N, Zhang F, Zhang L-L, Gao J, Zhou Y-B, Han Y, *et al*. SOD1 gene transfer into paraventricular nucleus attenuates hypertension and sympathetic activity in spontaneously hypertensive rats. *Pflügers Arch* 2013; 465:261–270.
41. Zahner MR, Pan HL. Role of paraventricular nucleus in the cardiogenic sympathetic reflex in rats. *Am J Physiol Regul Integr Comp Physiol* 2005; 288:R420–R426.
42. Council NR. *Guide for the care and use of laboratory animals*, 8th ed. Washington, DC: The National Academies Press; 2011.
43. Li DP, Averill DB, Pan HL. Differential roles for glutamate receptor subtypes within commissural NTS in cardiac-sympathetic reflex. *Am J Physiol Regul Integr Comp Physiol* 2001; 281:R935–R943.
44. Li DP, Pan HL. Responses of neurons in rostral ventrolateral medulla to activation of cardiac receptors in rats. *Am J Physiol Heart Circ Physiol* 2000; 279:H2549–H2557.
45. Chen QH, Haywood JR, Toney GM. Sympathoexcitation by PVN-injected bicuculline requires activation of excitatory amino acid receptors. *Hypertension* 2003; 42:725–731.
46. Paxinos G, Watson C. *The rat brain in stereotaxic coordinates*.. London: 123Library: Academic Press; 2007.
47. Pan HL, Chen SR, Scicli GM, Carretero OA. Cardiac interstitial bradykinin release during ischemia is enhanced by ischemic preconditioning. *Am J Physiol Heart Circ Physiol* 2000; 279:H116–H121.
48. White JC. Cardiac pain: anatomic pathways and physiologic mechanisms. *Circulation* 1957; 16:644–655.
49. Blair RW, Weber RN, Foreman RD. Responses of thoracic spinothalamic neurons to intracardiac injection of bradykinin in the monkey. *Circ Res* 1982; 51:83–94.
50. Guo ZL, Lai HC, Longhurst JC. Medullary pathways involved in cardiac sympathoexcitatory reflexes in the cat. *Brain Res* 2002; 925:55–66.
51. Wang WZ, Gao L, Pan YX, Zucker IH, Wang W. Differential effects of cardiac sympathetic afferent stimulation on neurons in the nucleus tractus solitarius. *Neurosci Lett* 2006; 409:146–150.
52. Weaver LC, Meckler RL, Fry HK, Donoghue S. Widespread neural excitation initiated from cardiac spinal afferent nerves. *Am J Physiol* 1983; 245:R241–250.
53. Xu B, Zheng H, Patel KP. Relative contributions of the thalamus and the paraventricular nucleus of the hypothalamus to the cardiac sympathetic afferent reflex. *Am J Physiol Regul Integr Comp Physiol* 2013; 305: R50–R59.
54. Zahner MR, Li DP, Pan HL. Benzodiazepine inhibits hypothalamic presympathetic neurons by potentiation of GABAergic synaptic input. *Neuropharmacology* 2007; 52:467–475.
55. Zhang L, Xiong X-Q, Fan Z-D, Gan X-B, Gao X-Y, Zhu G-Q. Involvement of enhanced cardiac sympathetic afferent reflex in sympathetic activation in early stage of diabetes. *J Appl Physiol* 2012; 113:47–55.
56. Zhong M-K, Duan Y-C, Chen A-D, Xu B, Gao X-Y, De W, Zhu GQ. Paraventricular nucleus is involved in the central pathway of cardiac sympathetic afferent reflex in rats. *Exp Physiol* 2008; 93:746–753.
57. Zhu GQ, Patel KP, Zucker IH, Wang W. Microinjection of ANG II into paraventricular nucleus enhances cardiac sympathetic afferent reflex in rats. *Am J Physiol Heart Circ Physiol* 2002; 282:H2039–H2045.
58. Dampney RA. Functional organization of central pathways regulating the cardiovascular system. *Physiol Rev* 1994; 74:323–364.
59. Swanson LW, Sawchenko PE. Paraventricular nucleus: a site for the integration of neuroendocrine and autonomic mechanisms. *Neuroendocrinology* 1980; 31:410–417.
60. Swanson LW, Sawchenko PE. Hypothalamic integration: organization of the paraventricular and supraoptic nuclei. *Ann Rev Neurosci* 1983; 6:269–324.
61. Zhang K, Li YF, Patel KP. Reduced endogenous GABA-mediated inhibition in the PVN on renal nerve discharge in rats with heart failure. *Am J Physiol Regul Integr Comp Physiol* 2002; 282:R1006–R1015.
62. Li DP, Pan HL. Glutamatergic regulation of hypothalamic presympathetic neurons in hypertension. *Curr Hypertens Rep* 2017; 19:78.
63. Li DP, Yang Q, Pan HM, Pan HL. Plasticity of pre and postsynaptic GABAB receptor function in the paraventricular nucleus in spontaneously hypertensive rats. *Am J Physiol Heart Circ Physiol* 2008; 295: H807–815.
64. Ma H, Chen SR, Chen H, Zhou JJ, Li DP, Pan HL. alpha2delta-1 couples to NMDA receptors in the hypothalamus to sustain sympathetic vasomotor activity in hypertension. *J Physiol* 2018; 596:4269–4283.
65. Judy WV, Watanabe AM, Henry DP, Besch HR Jr, Murphy WR, Hockel GM. Sympathetic nerve activity: role in regulation of blood pressure in the spontaenously hypertensive rat. *Circ Res* 1976; 38 (6 Suppl 2):21–29.
66. Li A, Roy SH, Nattie EE. An augmented CO2 chemoreflex and overactive orexin system are linked with hypertension in young and adult spontaneously hypertensive rats. *J Physiol* 2016; 594:4967–4980.
67. Matsukawa K, Iwamoto GA, Mitchell JH, Mizuno M, Kim HK, Williamson JW, *et al*. Exaggerated renal sympathetic nerve and pressor responses during spontaneously-occurring motor activity in hypertensive rats. *Am J Physiol Regul Integr Comp Physiol* 2023; 324:R497–R512.
68. Mizuno M, Murphy MN, Mitchell JH, Smith SA. Skeletal muscle reflex-mediated changes in sympathetic nerve activity are abnormal in spontaneously hypertensive rats. *Am J Physiol Heart Circ Physiol* 2011; 300:H968–H977.
69. Smith SA, Williams MA, Leal AK, Mitchell JH, Garry MG. Exercise pressor reflex function is altered in spontaneously hypertensive rats. *J Physiol* 2006; 577:1009–1020.
70. Zhu GQ, Xu Y, Zhou LM, Li YH, Fan LM, Wang W, *et al*. Enhanced cardiac sympathetic afferent reflex involved in sympathetic overactivity in renovascular hypertensive rats. *Exp Physiol* 2009; 94:785–794.
71. Chen AD, Zhang SJ, Yuan N, Xu Y, De W, Gao XY, Zhu GQ. Angiotensin AT1 receptors in paraventricular nucleus contribute to sympathetic activation and enhanced cardiac sympathetic afferent reflex in renovascular hypertensive rats. *Exp Physiol* 2011; 96:94–103.
72. Fan ZD, Zhang L, Shi Z, Gan XB, Gao XY, Zhu GQ. Artificial microRNA interference targeting AT(1a) receptors in paraventricular nucleus attenuates hypertension in rats. *Gene Ther* 2012; 19:810–817.
73. Shanks J, De Morais SDB, Gao L, Zucker IH, Wang H-J. TRPV1 (transient receptor potential vanilloid 1) cardiac spinal afferents contribute to hypertension in spontaneous hypertensive rat. *Hypertension* 2019; 74:910–920.
74. Weaver LC, Verghese P, Bruce JC, Fehlings MG, Krenz NR, Marsh DR. Autonomic dysreflexia and primary afferent sprouting after clip-compression injury of the rat spinal cord. *J Neurotrauma* 2001; 18:1107–1119.