Salusin-β in paraventricular nucleus increases blood pressure and sympathetic outflow via vasopressin in hypertensive rats

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Aims Salusin-β is a bioactive peptide with peripheral hypotensive, mitogenic and pro-atherosclerotic effects. The present study was designed to determine the roles of salusin-β in the paraventricular nucleus (PVN) and its relationship with arginine vasopressin (AVP) in hypertension and sympathetic activation.

Methods and results Renovascular hypertension was induced by two-kidney, one-clip (2K1C) in male Sprague–Dawley rats. Acute experiments were carried out 4 weeks after 2K1C or sham-operation under urethane and alpha-chloralose anaesthesia. Renal sympathetic nerve activity (RSNA), mean arterial pressure (MAP), and heart rate (HR) were recorded. Microinjection of salusin-β into the PVN increased the RSNA, MAP, and HR in a dose-related manner, whereas antisalusin-β IgG in the PVN decreased the RSNA and MAP, and abolished the effects of salusin-β in 2K1C rats. However, either salusin-β or antisalusin-β IgG in the PVN failed to cause any significant effects in sham-operated rats. The number of salusin-β-like immunopositive neurons in the PVN was significantly increased in 2K1C rats. Salusin-β in the PVN increased the plasma AVP and norepinephrine levels in 2K1C rats, but not in sham-operated rats. Iv injection of dTyr(CH2)5(Me)AVP (an AVP V1-receptor antagonist, AAVP) decreased RSNA and MAP, and abolished the effects of salusin-β in the PVN in 2K1C rats. Microinjection of AAVP into the rostral ventrolateral medulla (RVLM), but not into the PVN, abolished the effects of salusin-β on RSNA and HR.

Conclusion Salusin-β in the PVN increases blood pressure, heart rate, and sympathetic outflow via both circulating AVP and AVP in the RVLM in hypertensive rats.

Keywords Salusin • Hypertension • Sympathetic activity • Vasopressin • Paraventricular nucleus

1. Introduction

Salusins are originally identified from full-length human cDNAs by bioinformatics analyses. They are translated from an alternatively spliced mRNA of the human torsion dystonia-related gene (TOR2A), a gene encoding a protein of the torsion dystonia family. Two related peptides salusin-α and salusin-β in human are identified, comprising 28 and 20 amino acids, respectively. Iv administration of salusin-α and salusin-β to rats causes rapid, profound hypotension and bradycardia.1 Salusin-β-like immunoreactivity was detected strongly in the hypothalamus and posterior pituitary, and less abundantly in anterior pituitary and gastrointestinal, immune, and haematopoietic systems, whereas salusin-α-like immunoreactivity was not detected in any of the rat tissues, indicating that rat salusin-β is immunologically similar to human salusin-β.2

Paraventricular nucleus (PVN) is an important integrative site in the control of cardiovascular activity and sympathetic outflow via its projections to the intermediolateral column of the spinal cord and the rostral ventrolateral medulla (RVLM).3,4 The PVN plays a role in regulating several sympa-thothalamic and gastrointestinal, immune, and haematopoietic systems, whereas salusin-α-like immunoreactivity was not detected in any of the rat tissues, indicating that rat salusin-β is immunologically similar to human salusin-β.2

Paraventricular nucleus (PVN) is an important integrative site in the control of cardiovascular activity and sympathetic outflow via its projections to the intermediolateral column of the spinal cord and the rostral ventrolateral medulla (RVLM).3,4 The PVN plays a role in regulating several sympatho-excitatory reflexes such as a cardiac sympathetic afferent reflex5,6 and a adipose afferent reflex7,8. The PVN is known to be very important in sympathetic activation and hypertension in 2K1C hypertensive rats,9 spontaneously hypertensive rats,10 and obesity hypertensive rats.9

Immunohistochemical analysis of rat tissues showed the presence of salusin-β in the PVN.11 Salusin-like immunoreactivity was detected...
in the hypothalamo-neurohypophyseal tract and immunopositive cells were distributed in both parvocellular and magnocellular part of the PVN, supraopticohypophysis, and supraoptic nucleus, and most of the salusin-like immunoreactivity was detected in vasopressin—but not in oxytocin-containing neurons in these nuclei. Salusin-β stimulates the arginine vasopressin (AVP) release from the perfused rat pituitary. However, it is unknown whether salusin-β in the PVN is involved in regulating blood pressure and sympathetic activity. The present study was designed to determine whether salusin-β in PVN contributes to the hypertension and sympathetic activation in renovascular hypertensive rats, and whether the AVP is involved in the effects of salusin-β.

2. Methods

Experiments were carried out in male Sprague–Dawley rats. The procedures were approved by the Experimental Animal Care and Use Committee of Nanjing Medical University and complied with the Guide for the Care and Use of Laboratory Animals (NIH publication, 8th edition, 2011). The rats were housed in a temperature-controlled and humidity-controlled room with a 12-h light–dark cycle with a standard chow and tap water ad libitum.

2.1 Rat model of renovascular hypertension

Renovascular hypertension was induced by the two-kidney one-clip (2K1C) method as we previously reported. Briefly, the rat weighing 160–180 g was anesthetized by pentobarbital sodium (60 mg/kg) ip. The adequacy of anesthesia was determined by the loss of a pedal withdrawal reflex. A right retroperitoneal flank incision was performed with sterile techniques. The right renal artery was exposed, and partly occluded with a U-shaped silver clip with an internal diameter of 0.20 mm on the vessel to induce renovascular hypertension. The sham-operated rats (Sham) received similar surgery except the clip was removed before the surgery. Rats were anaesthetized with urethane (800 mg/kg) and α-chloralose (40 mg/kg) ip. The adequacy of anesthesia was determined by the loss of a pedal withdrawal reflex. A midline incision in the neck was made to expose the trachea and carotid artery. The trachea was intubated and connected to a rodent ventilator (Model 683, Harvard Apparatus, Inc., Holliston, MA, USA) for mechanical ventilation. The mean arterial pressure (MAP) and heart rate (HR) were measured with a pressure transducer (MLT0380, ADInstruments, Castle Hill, Australia) via a catheter in the right carotid artery. The MAP, HR, and renal sympathetic nerve activity (RSNA) were simultaneously recorded with a PowerLab data acquisition system (B/35, ADInstruments, Castle Hill, Australia).

2.2 SBP measurement

SBP of the tail artery was measured in a conscious state with a non-invasive computerized tail-cuff system (NIHB, ADInstruments, Sydney, Australia). The rats were warmed for 10–20 min at 28°C before the measurements to allow the detection of tail artery pulsations and to achieve a steady pulse level. The SBP was obtained by averaging 10 measurements at weekly intervals.

2.3 Salusin-β and anti-salusin-β antibody

Human salusin-β was obtained from Phoenix Pharmaceuticals (CA, USA). Rabbit anti-salusin-β (human) IgG and rabbit anti-salusin-β (human) serum were obtained from Bachem (Bubendorf, Switzerland). Human salusin-β has high homology with the rat salusin-β. As shown in the instruction of the commercial antibody, the rabbit anti-salusin-β (human) IgG is a purified polyclonal antibody, and its specificity has been determined with radiimmunoassay. The cross-reaction data show that the anti-salusin-β IgG has no cross-reaction with salusin-α. Rabbit anti-salusin-β (human) IgG is supplied as a lyophillized powder, and is reconstituted by adding 0.01 M PBS (pH 7.4) to get the solution (100 ng in 50 nL), which was used for PVN microinjection. Rabbit anti-salusin-β (human) serum was diluted with 0.01 M PBS and the titre was 1:500 for immunohistochemistry.

2.4 General procedures of acute experiment

Acute experiment was carried out at the end of the fourth week after surgery. Rats were anaesthetized with urethane (800 mg/kg) and α-chloralose (40 mg/kg) ip. The adequacy of anesthesia was determined by the loss of a pedal withdrawal reflex. A midline incision in the neck was made to expose the trachea and carotid artery. The trachea was intubated and connected to a rodent ventilator (Model 683, Harvard Apparatus, Inc., Holliston, MA, USA) for mechanical ventilation. The mean arterial pressure (MAP) and heart rate (HR) were measured with a pressure transducer (MLT0380, ADInstruments, Castle Hill, Australia) via a catheter in the right carotid artery. The MAP, HR, and renal sympathetic nerve activity (RSNA) were simultaneously recorded with a PowerLab data acquisition system (B/35, ADInstruments, Castle Hill, Australia).

2.5 RSNA recording

A retroperitoneal incision was made and the left renal sympathetic nerve was isolated. The renal nerve was cut distally to eliminate its afferent activity. The nerve was placed on a pair of silver electrodes and immersed in warm mineral oil. The signals were amplified with a four channel AC/DC differential amplifier (DP-304, Warner Instruments, Hamden, CT, USA) with a high pass filter at 10 Hz and a low pass filter at 3000 Hz. The RSNA was integrated at a time constant of 100 ms. At the end of each experiment, the background noise was determined after section of the central end of the nerve and was subtracted from the integrated values of the RSNA.

2.6 PVN and RVLM microinjection

The stereotaxic coordinates for the PVN are 1.8 mm caudal from bregma, 0.4 mm lateral to the midline, and 7.9 mm ventral to the dorsal surface. The coordinates for the RVLM are 4.7 mm posterior to lambda, 1.8 mm lateral to midline, and 8.2 mm below the dorsal surface of the cerebellum. The bilateral microinjections were completed within 1 min and the microinjection volume was 50 nL for each microinjection site. Functional location of RVLM neurons on either side was carried out at the beginning of each experiment by eliciting a transient increase in SBP (20–30 mmHg) by microinjection of glutamate (2 nmol). The histological identification was made to verify each microinjection site. Rats with microinjection sites outside the PVN or RVLM were excluded from data analysis.

2.7 Immunohistochemistry for salusin-β

Brain sections were incubated with a rabbit anti-salusin-β antibody diluted in 0.01 M PBS (1:500) at 4°C overnight. After washing in PBS, sections were further incubated with a biotinylated secondary antibody (ABC staining system kit, Santa Cruz, CA, USA) in PBS containing 1.5% goat serum for 30 min at 37°C. Finally, sections were incubated according to the manufacturer’s descriptions of the ABC kit. The salusin-positive neurons in PVN were observed using a conventional light microscopy (DP70, Olympus, Tokyo, Japan). The number of neurons with salusin-β-like immunoreactivity in the PVN was determined by averaging the numbers of four sections for each animal.

2.8 Measurement of plasma AVP, norepinephrine and angiotensin (Ang) II

Commercial ELISA kits were used for the measurement of plasma AVP (MyBiosource, San Diego, USA) and Ang II (Uscn Life Science, Inc., Houston, USA). According to the manufacturer’s descriptions, the standards or samples diluted were added and incubated in the appropriate well of specific antibody pre-coated microtitre plate. Conjugate was added and incubate for 1 h at 37°C and then washed. The reactions were stopped with stop solution and read at 450 nm using a microtitre plate reader (ELX800, BioTek, Vermont, USA). Plasma norepinephrine was measured with commercial ELISA kits (ALPCO Diagnostics, formerly known as BioVendor). Plasma angiotensin II levels were measured with a commercially available enzyme-linked immunosorbent assay (ELISA) kit (ALPCO Diagnostics, formerly known as BioVendor).
Windham, NH, USA). The procedures were similar to the AVP measurement except that the antibody was not pre-coated.  

2.9 Experimental design  
2.9.1 Experiment 1  
To determine the different doses of salusin-β on the RSNA, MAP, and HR, either Sham or 2K1C rats were randomly subjected to the PVN microinjection of saline, 0.1, 1, 10, or 100 pmol of salusin-β (n = 6 for each group). The intervals between injections were at least 40 min for a complete recovery. To exclude the possibility that the effect of salusin-β was caused by diffusion to other brain area, the effects of microinjection of high dose of salusin-β into the anterior hypothalamic area, which is adjacent to the PVN, were determined (n = 3).  

2.9.2 Experiment 2  
To determine the effects of endogenous salusins, anti-salusin-β IgG was used to immunoneutralize salusins in the PVN. Either Sham or 2K1C rats were randomly divided into four groups (n = 6 for each group), which were subjected to the PVN microinjection of control IgG (100 ng), anti-salusin-β IgG (100 ng), salusin-β (100 pmol) pre-treated with control IgG, or salusin-β pre-treated with anti-salusin-β IgG. Salusin-β was administered 5 min after the pre-treatment.  

2.9.3 Experiment 3  
Salusin-β-like immunoreactivity in the PVN was investigated in Sham and 2K1C rats (four sections for each rat and three rats for each group).  

2.9.4 Experiment 4  
To determine the effects of salusin-β in the PVN on the sympathetic activation, AVP release, and Ang II production, either Sham or 2K1C rats were successively and randomly subjected to the PVN microinjection of saline or salusin-β (100 pmol). The intervals between injections were at least 40 min for a complete recovery (n = 6 for each group). The blood samples were obtained 10 min after the microinjection for noradrenergine, AVP, and Ang II measurements.  

2.9.5 Experiment 5  
To determine whether peripheral AVP V1 receptors mediate the effects of salusin-β in the PVN, 2K1C rats were randomly divided into four groups (n = 6 for each group), which were subjected to the iv injection of saline (20 µL/min) or d[Tyr(CH2)5(Me)AVP (AAVP, an AVP V1-receptor antagonist, 4.0 nmol/min), or iv infusion of saline or losartan plus the PVN microinjection of salusin-β (100 pmol). Salusin-β was administered 5 min after the iv infusion.  

2.9.6 Experiment 6  
To determine whether AVP V1 receptors in the PVN mediate the effects of salusin-β in the PVN, 2K1C rats were randomly divided into four groups (n = 6 for each group), which were subjected to the PVN microinjection of saline or AAVP (40 pmol), or the PVN microinjection of salusin-β (100 pmol) pre-treated with PVN microinjection of saline or AAVP. Salusin-β was administered 5 min after the pre-treatment.  

2.9.7 Experiment 7  
To determine whether AVP V1 receptors in the RVLM mediate the effects of salusin-β in the PVN, 2K1C rats were randomly divided into four groups (n = 6 for each group), which were subjected to the RVLM microinjection of saline or AAVP (40 pmol), or the PVN microinjection of salusin-β (100 pmol) pre-treated with RVLM microinjection of saline or AAVP. Salusin-β was administered 5 min after the pre-treatment.  

2.9.8 Experiment 8  
To determine whether AT1 receptors mediate the effects of salusin-β in the PVN, 2K1C rats were randomly divided into four groups (n = 6 for each group), which were subjected to the iv injection of saline (20 µL/min) or losartan (AT1 receptor antagonist, 150 nmol/min), or iv infusion of saline or losartan plus the PVN microinjection of salusin-β (100 pmol). Salusin-β was administered 5 min after the iv infusion.  

2.10 Statistics  
Comparisons between two groups were made by Student’s t-test. ANOVA followed by the post hoc Bonferroni test was used when multiple comparisons were made. Regression analysis was made to determine the correlation between the doses and responses. All data were expressed as mean ± SE. A value of P < 0.05 was considered statistically significant.  

3. Results  
3.1 General data  
At the end of the fourth week, SBP and MAP in 2K1C rats were significantly higher than those in Sham rats, but no significant difference in body weight or HR was found between Sham and 2K1C rats (Table 1).  

3.2 Effects of different doses of salusin-β  
Microinjection of salusin-β into the PVN increased RSNA, MAP, and HR in a dose-related manner in 2K1C rats, but not in Sham rats. There was a significant linear correlation between the dose of salusin-β and RSNA, MAP, or HR change (Figure 1). PVN microinjection of salusin-β (100 pmol) caused immediate increases in the RSNA, MAP, and HR in the 2K1C rat, lasting for 20–30 min (see Supplementary material online, Figure S1). Microinjection of salusin-β into the anterior hypothalamic area, which is adjacent to the PVN, had no significant effects on RSNA, MAP, or HR.  

3.3 Effects of anti-salusin-β IgG  
Microinjection of anti-salusin-β IgG into the PVN decreased the baseline RSNA and MAP, and almost abolished the effects of salusin-β in the PVN on RSNA, MAP, and HR in 2K1C rats. No significant effects were found in Sham rats (Figure 2).  

3.4 Salusin-β-like immunoreactivity in PVN  
Compared with Sham rats, the number of salusin-β-like immunopositive neurons in the PVN was significantly increased in 2K1C rats (Figure 3).  

<table>
<thead>
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<th>Variables</th>
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<tbody>
<tr>
<td>n</td>
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</tr>
<tr>
<td>Body weight, g</td>
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<td>338 ± 3</td>
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<td>SBP, mm Hg</td>
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<td>191 ± 3*</td>
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<td>Baseline MAP, mmHg</td>
<td>89 ± 2</td>
<td>136 ± 2*</td>
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<tr>
<td>Baseline HR, b.p.m.</td>
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<td>361 ± 4</td>
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SBP was measured in conscious state with a non-invasive computerized tail-cuff system. Baseline MAP and HR were measured under anaesthesia with a pressure transducer through a catheter placed in the right carotid artery. Values are mean ± SE. *P < 0.05 compared with Sham.
3.5 Plasma norepinephrine, AVP and Ang II levels

The plasma norepinephrine, AVP and Ang II levels were higher in 2K1C rats than Sham rats. Microinjection of salusin-β into the PVN further increased the plasma norepinephrine and AVP levels in 2K1C rats, but not in Sham rats. However, salusin-β had no significant effect on plasma Ang II level in both Sham and 2K1C rats (Figure 4).

3.6 Effects of iv injection of AAVP or losartan

Iv injection of AVP V1-receptor antagonist AAVP decreased the baseline RSNA and MAP, and abolished the effects of salusin-β in the PVN. However, iv injection of AAVP had no significant effects on baseline HR in 2K1C rats. Iv injection of AT1 receptor antagonist losartan in 2K1C rats decreased the baseline RSNA, MAP, and HR, but failed to attenuate the effects of salusin-β in the PVN (Figure 5).

3.7 Effects of PVN microinjection of AAVP

Microinjection of AAVP into the PVN had no significant effects on the baseline RSNA, MAP, or HR as well as the effects of salusin-β in the PVN (see Supplementary material online, Figure S2).

3.8 Effects of RVLM microinjection of AAVP

Microinjection of AAVP into the RVLM had no significant effects on the baseline RSNA, MAP, or HR, but abolished the effects of salusin-β on RSNA and HR in the PVN (Figure 6).

4. Discussion

PVN is important in the control of cardiovascular activity and sympathetic outflow. Salusin-β and hypertension
immunopositive neurons in the PVN was increased in the PVN in 2K1C rats compared with Sham rats. Furthermore, salusin-β in the PVN increased plasma norepinephrine in 2K1C rats but not in Sham rats. These results suggest that increased salusin-β in the PVN of 2K1C rats may contribute to the sympathetic activation and hypertension. We excluded the possibility that the effects of salusin-β were caused by diffusion into peripheral circulation, because iv salusin-β administration to intact anaesthetized rats caused hypertension and bradycardia, which was abolished by pre-treatment with atropine, a muscarinic receptor antagonist. Microinjection of salusin-β into the anterior hypothalamic area, which is adjacent to the PVN had no significant effects on RSNA, MAP, or HR.

It is known that the initial 18 amino acids of human salusin-β have high homology with the estimated N-terminal sequence of rat salusin. The affinity-purified antibody recognizes rat salusin in the hypothalamo-pituitary system, and the specificity of the salusin-β staining is assessed by pre-absorption of the antibody with the full-length human salusin-β, which completely abolishes salusin-β staining. Anti-salusin antibody was used to investigate the effects of endogenous salusins due to unavailable salusin receptor antagonist. In the present study, anti-salusin-β IgG almost abolished the effects of salusin-β in the PVN, implying that the dose of anti-salusin-β IgG was high enough to block the effects of endogenous salusin-β. It was found that immunoneutralizing endogenous salusin-β in the PVN with anti-salusin-β IgG reduced the RSNA, MAP, and HR in 2K1C rats, but not in Sham rats. The results suggest that endogenous salusin-β in the PVN contributes to hypertension and sympathetic activation in 2K1C rats, but is not involved in regulating blood pressure and sympathetic activity in the normal state.

AVP is synthesized predominantly in the magnocellular neurons of the PVN and supraoptic nuclei (SON) of the hypothalamus, and then transported to the neurohypophysis from which it is released into the systemic circulation. AVP causes powerful constriction in a variety of vascular regions, and the AVP V1 receptors mediate the vasoconstrictor action of the peptide. Salusin-β stimulates the AVP release from the perfused rat pituitary. Salusin-β-like immunopositive cells were found in the parvocellular and magnocellular part of the PVN, and most of them located in the AVP-containing neurons in rats. Furthermore, many salusin-positive nerve fibres and their terminals were identified in the internal layer of the median eminence and posterior pituitary.

In the present study, salusin-β-like immunoreactivity was greatly increased in both parvocellular and magnocellular part of the PVN in 2K1C rats. The number of neurons with salusin-β-like immunoreactivity in 2K1C rats was more than three times of that in Sham rats. The immunohistological findings support a dual role of salusin-β in the PVN in regulating both AVP release and sympathetic nerve activity, which contributes to hypertension and sympathetic activation in 2K1C rats.
The plasma AVP level was higher in 2K1C rats, which is similar to the previous report. Microinjection of salusin-β into the PVN further increased the plasma AVP level in 2K1C rats, but not in Sham rats. Iv administration of AVP V1 receptor antagonist AAVP not only reduced baseline MAP and RSNA, but also abolished the effects of salusin-β on the RSNA, MAP, and HR in 2K1C rats. These results suggest that circulating AVP contribute to both hypertension and sympathetic activation in 2K1C rats, and the increased circulating AVP mediates the pressor and sympatho-excitatory responses to salusin-β in the PVN via V1 receptors in 2K1C rats. It is well known that renin–angiotensin system contributes to hypertension, which is confirmed by the present findings. However, PVN microinjection of salusin-β had no a significant effect on the plasma Ang II level. Iv injection of losartan, which can cross blood–brain barrier in rats, failed to attenuate the effects of salusin-β. These results suggest that AT1 receptors do not mediate the effects of salusin-β.

Excessive sympathetic nerve activity plays a pathogenic role in triggering and sustaining the hypertensive state and contributes to the pathogenesis of hypertension and progression of organ damage. It

**Figure 5** Effects of iv injection of V1 receptor antagonist AAVP (upper panel) or AT1 receptor antagonist losartan (lower panel) on the RSNA, MAP, and HR, as well as the effects of pre-treatment with AAVP or losartan on the RSNA, MAP, and HR responses to salusin-β in the PVN in 2K1C rats. n = 6 for each group. Values are mean ± SE. *P < 0.05 vs. Saline; †P < 0.05 vs. Saline + Salusin-β.

**Figure 6** Effects of RVLM microinjection of saline or V1-receptor antagonist AAVP (40 pmol) on the RSNA, MAP, and HR, as well as the effects of pre-treatment with saline or AAVP on the RSNA, MAP, and HR responses to salusin-β in the PVN in 2K1C rats. n = 6 for each group. Values are mean ± SE. *P < 0.05 vs. Saline; †P < 0.05 vs. Saline + Salusin-β.
is known that the sympathetic tone originates in the central nervous system, which is potentiated by a positive feedback reflex such as a cardiac sympathetic afferent reflex and inhibited by a negative feedback reflex such as baroreflex. A very interesting question is why iv injection of AAVP attenuated sympathetic activity and abolished the sympa-tho-excitatory response to salusin-β in the PVN. Several studies have indicated that AVP or some structurally similar peptides can be transported into the brain through blood–brain barrier carrier-mediated transport. The neurohypophyseal hormones including AVP do cross the blood–brain barrier in amounts obviously sufficient to induce central actions. We speculate that the effects of circulating AVP and iv injection of AAVP on sympathetic outflow and salusin-β-induced RSNA change may be caused by their action on certain brain regions. PVN microinjection of AAVP had no a significant effect on the RSNA, MAP, HR or effects of salusin-β on sympatho-excitatory response and attenuated MAP response to salusin-β. The abolished HR response and attenuated MAP response to salusin-β in the PVN after AAVP treatment may be caused by the blockade of RSNA response to salusin-β. The results suggest that the effects of salusin-β in the PVN on the RSNA and HR are mediated by AVP in the RVLM, and the effect of salusin-β on MAP is mediated by both circulating AVP and AVP in RVLM in hypertensive rats.

RVLM plays an important role in maintaining baseline sympathetic vasomotor tone and arterial blood pressure. It is well known that projection from the PVN to the RVLM is involved in regulating sympathetic outflow and blood pressure. AVP-immunoreactive fibres were found in the RVLM. Cholera toxin β subunit (CT-β) can be used to label neurons retrogradely. After microinjection of CT-β into the RVLM, 14.6% of CT-β-labelled PVN neurons were double-labelled with AVP. Microinjection of AVP into the RVLM increased blood pressure, while blockade of V1 receptors attenuated the PVN-evoked increases in RVLM unit discharge. These results suggest an AVP pathway from PVN to RVLM is involved in regulating sympathetic activity and blood pressure. We speculate that the AVP pathway from PVN to RVLM is responsible for the sympa-tho-excitatory effect of salusin-β in the PVN. The fact that either iv administration of AAVP or microinjection of AAVP into the RVLM abolishes the RSNA response to salusin-β in the PVN suggests that the circulating AAVP must be getting access to the V1 receptors in the RVLM. It is noted that iv injection of AAVP inhibited the baseline RSNA, but RVLM administration of AAVP failed to cause similar inhibition, suggesting that the RVLM is not responsible for the baseline RSNA inhibitory response to iv injection of AAVP. It is noteworthy that salusin-β used in the present study was human salusin-β which has high homology but a little difference with rat salusin-β in an amino acid sequence. We cannot exclude a possibility that the actions of salusin-β in the present study are the pharmacological roles rather than physiological roles of this bioactive peptide. Furthermore, the present study was carried out in the rats with anesthesia. Anaesthesia may influence or modify the haemodynamic responses to salusin-β.

5. Perspectives
The present results show that salusin-β in the PVN increased blood pressure, HR, and sympathetic outflow in renovascular hypertensive rats, but not in Sham rats. The effects of salusin-β in the PVN were mediated by AVP and its V1 receptors. Peripheral V1 receptors partially contribute to the salusin-β-induced pressor response. V1 receptors in the RVLM contribute to the salusin-β-induced sympathetic activation and its secondary tachycardia and pressor responses. Endogenous salusin-β in the PVN contributes to the hypertension and sympathetic activation via both circulating AVP and AVP in RVLM in renovascular hypertensive rats. Intervention of salusin-β in the PVN may be a target counteracting sympathetic activation and hypertension.

Supplementary material
Supplementary material is available at Cardiovascular Research online.

Conflict of interest: none declared.

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References


