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Deleterious effect of salusin-β in paraventricular nucleus on sympathetic activity and blood pressure via NF-κB signaling in a rat model of obesity hypertension

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The paraventricular nucleus (PVN) has been shown to play a critical role in regulating blood pressure and sympathetic activity in obesity hypertension (OH). Salusin-β is a bioactive peptide with potential roles in mediating cardiovascular activity. The study was designed to test the hypothesis that salusin-β in the PVN can modulate sympathetic activity and blood pressure in OH. Male Sprague-Dawley rats were used to induce OH by a 12-week feeding of a high-fat diet (42% kcal as fat). Microinjection of salusin-β into the PVN increased the renal sympathetic nerve activity (RSNA), mean arterial pressure (MAP) and heart rate (HR) in a dose-dependent manner, whereas salusin-β antibody elicited significant decreases in RSNA, MAP and HR, and abolished the effects of salusin-β only in the OH rats. As expected, the OH rats had a higher norepinephrine level, which was further increased by salusin-β. Furthermore, salusin-β in the PVN accelerated the nuclear translocation of the p65 subunit of nuclear factor kappa B (NF-κB) and the degradation of IκB-α (an endogenous inhibitor of NF-κB). Pretreatment with pyrrolidine dithiocarbamate (an exogenous inhibitor of NF-κB) decreased RSNA, MAP and HR, and abolished the effects of salusin-β in the PVN in the OH rats. We concluded that salusin-β in the PVN markedly increased sympathetic outflow and blood pressure in diet-induced OH rats via NF-κB signaling.

1. Introduction

Salusins, multifunctional bioactive peptides, were initially identified from a human full-length cDNA bank by bioinformatics analyses (Shichiri et al. 2003). Two previously reported salusins, named salusin-α and salusin-β, consist of 28 and 20 amino acids, respectively. They were generated from an alternatively-spliced mRNA of torsin family 2 member A (Shichiri et al. 2003). Intravenous injection of salusin-α or salusin-β was found to produce a rapid and marked hypotension in rats (Shichiri et al. 2003). Salusin-α or salusin-β displayed different localizations and expression patterns, and were expected to exert different effects in specific cell types (Suzuki et al. 2011; Koya et al. 2012). For instance, vascular inflammation was promoted by salusin-β rather than salusin-α in apoE-deficient mice (Zhou et al. 2014). Salusin-β had more potent mitogenic effects on human vascular smooth muscle cells than salusin-α (Koya et al. 2012). Formation of human macrophage foam cells was stimulated by salusin-β but suppressed by salusin-α (Watanabe et al. 2008). Recently, accumulating evidence indicated that salusin-β may play an important role in the regulation of cardiovascular activity (Sun et al. 2012; Chen et al. 2013).

Abbreviations: BW, body weight; MAP, mean arterial pressure; NF-κB, nuclear factor kappa B; PVN, paraventricular nucleus; HFD, high-fat diet; HR, heart rate; MAP, mean blood pressure; OH, obesity hypertension; PDTC, pyrrolidine dithiocarbamate; RD, regular diet; RSNA, renal sympathetic nerve activity; SBP, systolic blood pressure.

The hypothalamic paraventricular nucleus (PVN) is an important integrative site involved in the control of blood pressure and sympathetic outflow (Badoer 2001; Grisk 2011). The PVN regulates several sympatho-excitatory reflexes, such as cardiac sympathetic afferent reflex (Zhong et al. 2008; Zhu et al. 2009), carotid body chemoreflex (Reddy et al. 2007) and adipose afferent reflex (Shi et al. 2012; Xiong et al. 2012). It is well known that PVN participates in the sympathetic modulation and regulation of blood pressure in many animal models of hypertension (Zhu et al. 2009; Nishihara et al. 2012; Sun et al. 2012; Xiong et al. 2012; Yi et al. 2012). In a previous study, salusin-β-immunopositive cells were observed in both parvocellular and magnocellular regions of the rat PVN (Takenoya et al. 2005; Suzuki et al. 2007). A recent study further showed that salusin-β in the PVN contributed to the hypertension and sympathetic activation in renovascular hypertensive rats (Chen et al. 2013). Thus, salusin-β in the PVN participates in regulating blood pressure and sympathetic activity in hypertension.

Obesity markedly increases the risk of developing hypertension (Van Gaal et al. 2006). It is well known that sympathetic activity is evidently over-excited in human and experimental obesity hypertension (OH) (Lambert et al. 2010; Xiong et al. 2012). The antihypertensive effect of adrenergic blockers was much stronger in obese hypertensives than in lean hypertensives (Wofford et al. 2001). Blocking the sympathetic nervous system caused a greater depressor response in Zucker obese *versus* lean rats (Carlson et al. 2000). In addition, autonomic

withdrawal with the ganglionic blocker caused an exaggerated blood pressure fall in obese compared with lean subjects (Shibao et al. 2007). Renal sympathetic denervation markedly prevented the blood pressure elevation in diet-induced obesity (Kassab et al. 1995). Furthermore, enhanced sympathetic activity greatly contributed to the pathogenesis and development of the OH (Lambert et al. 2010; Kalil et al. 2012). However, it is unknown whether salusin-β in the PVN is involved in regulating blood pressure and sympathetic activity in diet-induced OH rats.

Nuclear factor kappa B (NF-κB) is an inducible transcription factor involved in the regulation of gene expression for immune response, inflammation and cellular growth control (Koya et al. 2012; Zhou et al. 2014). Particularly, many investigators indicated that NF-κB played an important role in mediating the biofunction of salusin-β (Koya et al. 2012; Zhou et al. 2014). A previous study revealed that salusin-β accelerated inflammatory responses in vascular endothelial cells *via* NF-κB signaling in LDL receptor-deficient mice (Koya et al. 2012). Moreover, salusin-β also promoted vascular inflammation *via* NF-κB pathway in ApoE-deficient mice (Zhou et al. 2014). These findings provide the functional basis supporting the hypothesis that salusin-β may regulate the cardiovascular activity through NF-κB signaling.

To clarify the effect of salusin-β in PVN on sympathetic activity and blood pressure in diet-induced OH, we have examined the effect of centrally microinjection of salusin-β and salusin-β antibody (anti-salusin-β IgG) on renal sympathetic nerve activity (RSNA), mean arterial pressure (MAP) and heart rate (HR). In addition, the effect of pyrrolidine dithiocarbamate (PDTC, an inhibitor of NF-κB) on these responses has been examined to establish whether they are mediated by NF-κB signaling. In this study, the activity of NF-κB signaling was evaluated by determining the nuclear translocation of the p65 subunit and phosphorylation of IκB-α.

2. Investigations and results

2.1. General data

After 12 weeks of high-fat diet (HFD) feeding, the final body weight (BW) and BW gain of OH rats were higher than those of Control (Ctrl) rats. In addition, there were increased systolic blood pressure (SBP), baseline MAP and HR in OH rats compared with Ctrl rats (Table 1).

Table 1: Anatomic data, blood pressure and HR

Variables	Ctrl	OH
Initial BW (g)	317 ± 3	315 ± 6
Final BW (g)	463 ± 6	637 ± 11[a]
BW gain (g)	165 ± 7	312 ± 13[a]
Final BW range (g)	362−541	558-784
SBP (mm Hg)	126.3 ± 3.2	182 ± 4.5[a]
Baseline MAP (mm Hg)	93.2 ± 1.9	126.5 ± 2.7[a]
Baseline HR (bpm)	338 ± 7	372 ± 10[a]

Values are mean ± SE. MAP and HR were determined under anesthesia. [a] $P < 0.05$ *vs.* Ctrl

2.2. Effect of salusin-β microinjection

Significant dose-dependent increases in RSNA, MAP and HR were observed in the salusin-β (1-100 pmol) treated OH rats, but not in Ctrl rats. The dose of salusin-β was found to be positively correlated with RSNA ($r = 0.557$, $P < 0.01$), MAP ($r = 0.354$, $P < 0.01$) and HR ($r = 0.613$, $P < 0.01$) only in OH rats (Fig. 1).

2.3. Effect of anti-salusin-β IgG microinjection

Microinjection of anti-salusin-β IgG into the PVN elicited significant decreases in RSNA, MAP and HR in OH rats rather than in Ctrl rats. Effects of salusin-β (100 pmol) on RSNA, MAP and HR were abolished by the pretreatment with anti-salusin-β IgG in the PVN of OH rats (Fig. 2).

2.4. Effect of salusin-β microinjection on plasma norepinephrine

The OH rats had a higher norepinephrine level than Ctrl rats. PVN microinjection of salusin-β (100 pmol) further increased the plasma norepinephrine level only in OH rats, which was abolished by the pretreatment with anti-salusin-β IgG (Fig. 3).

2.5. Effect of salusin-β microinjection on NF-κB signaling

In OH rats, microinjection of salusin-β (100 pmol) into the PVN promoted the nuclear translocation of the p65 subunit of NF-κB, which was blocked by the pretreatment with anti-salusin-β IgG. Moreover, IκB-α was an endogenous inhibitor of NF-κB and degraded in a phosphorylation form induced by NF-κB activation. Microinjection of salusin-β caused a higher level of phospho-IκB-α in OH rats than in Ctrl rats, while pretreatment with anti-salusin-β IgG abolished the IκB-α degradation induced by salusin-β in the PVN of OH rats (Fig. 4).

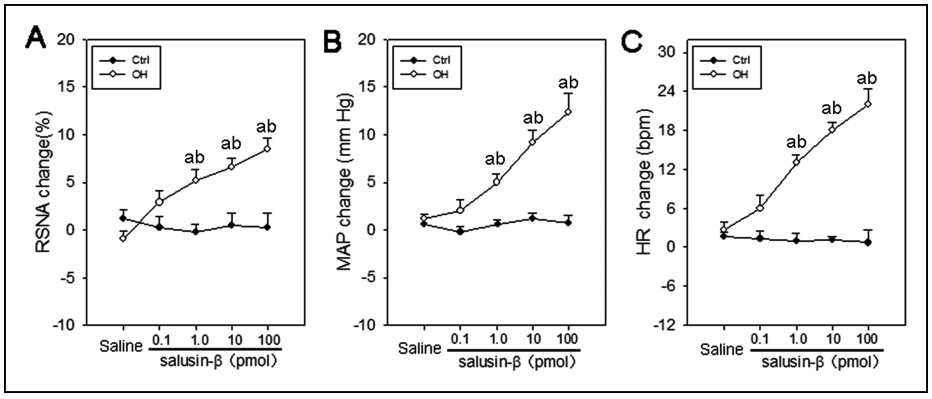

Fig. 1: Changes in RSNA (A), MAP (B) and HR (C) after PVN microinjection of salusin-β in the Ctrl and OH rats. Either Ctrl or OH rats randomly received PVN microinjections of saline and different doses of salusin-β (0.1, 1.0, 10 and 100 pmol). The 40-min interval between injections was chosen to ensure a complete recovery. Values are mean ± SE; $n = 8$ for each group. [a] $P < 0.05$ *vs.* saline; [b] $P < 0.05$ *vs.* Ctrl.

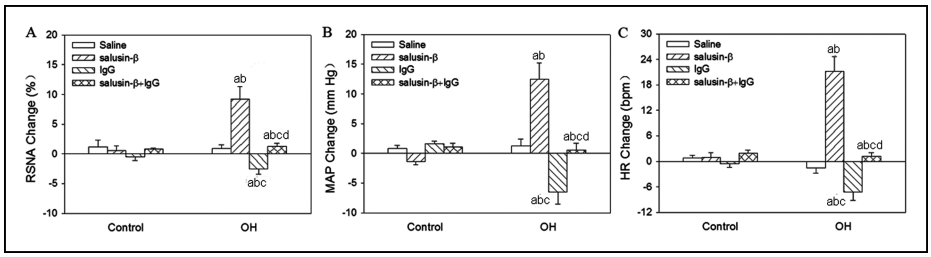

Fig. 2: Changes in RSNA (A), MAP (B) and HR (C) after PVN microinjection of anti-salusin-β IgG (IgG, 100 ng) in the Ctrl and OH rats. In the salusin-β + IgG group, salusin-β (100 pmol) was administered 5 min after the pretreatment of IgG. Values are mean ± SE; $n = 6$ for each group. [a] $P < 0.05$ *vs.* Ctrl; [b] $P < 0.05$ *vs.* saline; [c] $P < 0.05$ *vs.* salusin-β; [d] $P < 0.05$ *vs.* IgG.

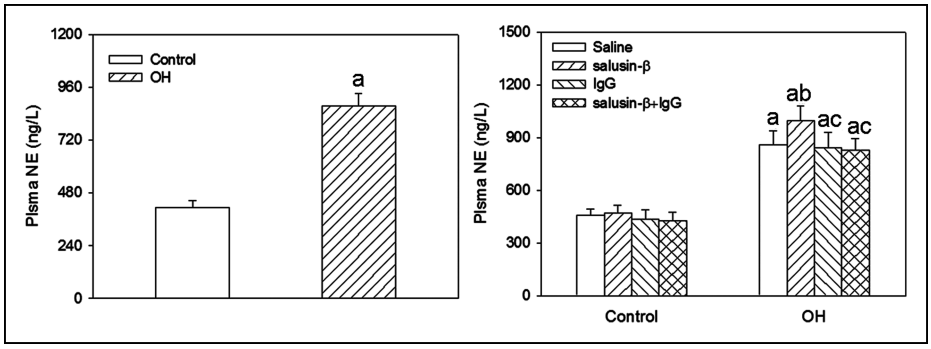

Fig. 3: Baseline level of plasma norepinephrine (A) or changes in plasma norepinephrine in response to salusin-β (100 pmol) alone or in combination with anti-salusin-β IgG (B) in the Ctrl and OH rats. Baseline plasma norepinephrine was measured before any PVN microinjection. Other measurements of plasma norepinephrine were carried out 10 min after the corresponding PVN microinjection. In the salusin-β + IgG group, salusin-β was administered 5 min after the pretreatment of IgG. Values are mean ± SE; $n = 6$ for each group. [a] $P < 0.05$ *vs.* Ctrl; [b] $P < 0.05$ *vs.* saline; [c] $P < 0.05$ *vs.* salusin-β.

2.6. PVN microinjection of PDTC on salusin-β related effect

The PVN microinjection of PDTC alone decreased RSNA, MAP and HR in OH rats but not in Ctrl rats. The effects of salusin-β (100 pmol) on RSNA, MAP and HR were abolished by pretreatment with PDTC in the PVN of OH rats (Fig. 5).

3. Discussion

The primary findings in the present study were that the exogenous salusin-β in the PVN caused dose-related increases in RSNA, MAP and HR in OH rats but not in Ctrl rats, and we observed a clear positive correlation between the doses of salusin-β and changes of RSNA, MAP or HR. Microinjection of the antibody targeting the salusin-β into the PVN blocked the enhancement of RSNA, MAP and HR, strongly indicating a deleterious role of salusin-β in the sympathetic activation and high blood pressure in OH. Moreover, we observed a significantly higher level of plasma norepinephrine in response to salusin-β in the OH rats. The plasma norepinephrine elevation induced by salusin-β was normalized by the pretreatment with the antibody against salusin-β. The data presented here indicates that salusin-β in the PVN may contribute to the sympathetic activation and hypertension in the OH rats. Our present results demonstrate a critical role of the NF-κB signaling for enhancement effect of salusin-β on sympathetic activity and blood pressure in OH.

It is well established that PVN plays an important role in the regulation of sympathetic nerve activity (Reddy et al. 2007; Zhong et al. 2008; Shi et al. 2012; Xiong et al. 2012). Indeed, a previous study reported that PVN participated in sympathetic modulation and blood pressure regulation in the OH rats (Xiong et al. 2012). The sympathetic activity was enhanced in OH (Lambert et al.

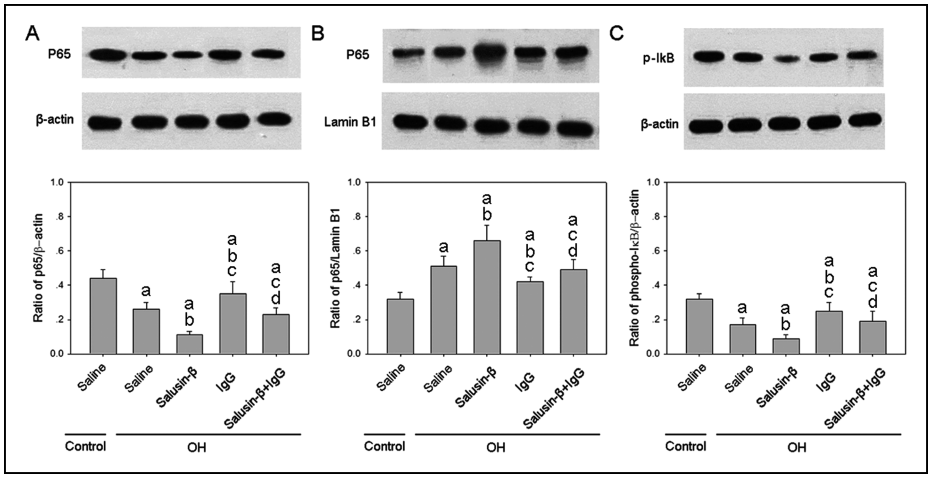

Fig. 4: Levels of p65 subunit of NF-κB in cytoplasm (A) or nucleus (B) and phospho-IκB in whole cell (C) after PVN microinjection of salusin-β (100 pmol) alone or in combination with anti-salusin-β IgG (IgG, 100 ng) in the Ctrl and OH rats. In the salusin-β + IgG group, salusin-β was administered 5 min after the pretreatment of IgG. Values are mean ± SE; $n = 6$ for each group. [a] $P < 0.05$ *vs.* Ctrl; [b] $P < 0.05$ *vs.* saline; [c] $P < 0.05$ *vs.* salusin-β; [d] $P < 0.05$ *vs.* IgG.

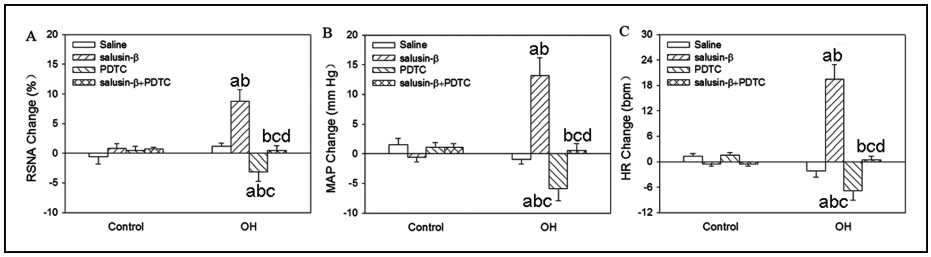

Fig. 5: Changes in RSNA (A), MAP (B) and HR (C) after PVN microinjection of salusin-β (100 pmol) alone or in combination with PDTC (3 nmol) in the Ctrl and OH rats. In the salusin-β + PDTC group, salusin-β was administered 5 min after the pretreatment of PDTC. Values are mean ± SE; $n = 6$ for each group. [a] $P < 0.05$ *vs.* Ctrl; [b] $P < 0.05$ *vs.* saline; [c] $P < 0.05$ *vs.* salusin-β; [d] $P < 0.05$ *vs.* PDTC.

2010; Xiong et al. 2012), consistent with our finding that the OH rats had a higher level of plasma norepinephrine. Salusin-β, a multifunctional bioactive peptide, was expected to exert an important role in the regulation of cardiovascular activity (Shichiri et al. 2003; Sun et al. 2012; Chen et al. 2013). A recent study showed that the hypertensive rats had more salusin-β-like immunopositive neurons in the PVN than normotensive rats (Chen et al. 2013). Therefore, we proposed that salusin-β in the PVN could facilitate the development of excessive sympathetic outflow and hypertension in OH.

The dose and time period of salusin-β for microinjection are based on a previous report (Chen et al. 2013). As expected, we observed a dose-related enhancement of RSNA, MAP and HR in response to salusin-β in OH rats, and salusin-β could result in a further release of plasma norepinephrine. The current findings are consistent with the results of a previously published study of another hypertensive rat model, suggesting that the salusin-β in the PVN led to sympathetic activation and high blood pressure. The initial 18 amino acids of human salusin-β had high homology with the N-terminal sequence of rat salusin-β (Suzuki et al. 2007). The affinity-purified antibody recognized rat salusin in the hypothalamo-pituitary system, and the previous studies showed that anti-salusin antibodies could inhibit the effects of endogenous salusins (Takenoya et al. 2005; Suzuki et al. 2007). Therefore, anti-salusin-β IgG was applied to verify the effects of endogenous salusin-β in the PVN of OH rats in the current study. Expectedly, anti-salusin-β IgG almost abolished the effects of salusin-β in the PVN, implying an adequate dose of anti-salusin-β IgG for blocking the endogenous salusin-β. Moreover, it was observed that immunoneutralizing endogenous salusin-β with its antibody reduced the RSNA, MAP, and HR in the OH rats, but not in the Ctrl rats. These findings highlighted that the endogenous salusin-β in the PVN contributed to hypertension and sympathetic activation in disease state but not in normal state. It is particularly worth noting that blocking endogenous salusin-β can mitigate its potentially deleterious effects on sympathetic activation and high blood pressure in OH, which may be beneficial to reduce the risk of developing OH-related cardiovascular complications. However, the absence of a salusin-β receptor antagonist limitsthe future use of salusin-β in clinical practice.

NF-κB signaling is involved in the regulation of several physiological responses (Wang et al. 2013; Hong et al. 2014). Recent studies revealed that NF-κB signaling was proven to play an important role in mediating the biofunction of salusin-β (Koya et al. 2012; Zhou et al. 2014). To elucidate the possible mechanism underlying the pernicious effect of salusin-β in the PVN, we consequently evaluated the activity of NF-κB signaling by determining the nuclear translocation of the p65 subunit and phosphorylation of IκB-α (Chi et al. 2012). The results showed that microinjection of salusin-β into the PVN could profoundly accelerate nuclear translocation of the p65 subunit and promote IκB-α degradation, hinting that salusin-β in the PVN could activeate NF-κB signaling. Similarly, the antibody against salusin-β totally abolished its effect on the activation of NF-κB signaling, strongly indicating the pivotal role of NF-κB signaling in mediating the biofunction of salusin-β. When the PVN was pre-treated with PDTC, the subsequent application of salusin-β was unable to evoke an elevation of RSNA, MAP and HR, further confirming a necessary role of NF-κB signaling in salusin-β's biofunction on regulating sympathetic activity and blood pressure in diet-induced OH. Interesting, Chen et al. (2013) demonstrated that salusin-β in PVN increased blood pressure and sympathetic outflow *via* vasopressin in hypertensive rats. We failed to observe the change of vasopressin after the application of salusin-β and its antibody (data not shown), which differed from the previous experiment (Chen et al. 2013). Different animal models of hypertension were used in the current (diet-induced OH) and previous experiments (two-kidney, one-clip hypertensive) which may explain the divergent results across studies. The above observations highlighted an important role of obesity in the development of hypertension.

In conclusion, we have demonstrated that salusin-β in the PVN markedly increased sympathetic outflow and blood pressure in diet-induced OH rats. Blocking the effect of salusin-β with its antibody is efficient in alleviating the sympathetic overdrive and attenuating the rise in blood pressure in OH. Taken together, our results provide evidence that salusin-β in the PVN increases blood pressure and sympathetic outflow *via* NF-κB signaling in diet-induced OH rats.

4. Experimental

4.1. Animals and induction of OH

Male Sprague-Dawley rats weighing 300-320 g were used to induce OH by a 12-week feeding of a HFD (42% kcal as fat). The weight- and sex-matched rats fed with a regular diet (RD; 12% kcal as fat) were randomly selected as Ctrl. The satisfactory experimental model for OH must follow two criteria: (i) the greater BW gain than the heaviest RD-fed rat; (ii) the SBP≥150 mm Hg measured by SBP of tail artery in conscious state (Smith and Bishop 1986). All animals were housed in a light-, temperature- and humidity-controlled environment with a constant 12-h light/dark cycle, and they were maintained on *ad libitum* food and water. The experimental procedures were approved by the Experimental Animal Care and Use Committee of Jilin Medical College and complied with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996). Acute experiments were performed under anesthesia induced with a single ip injection of urethane (800 mg/kg) and α-chloralose (40 mg/kg). After an adequate depth of anesthesia was attained, supplementary doses of anesthetic were administered during experiments.

4.2. Measurement of SBP

SBP of tail artery were measured in conscious rats at 2-week intervals. The SBP was detected by use of a non-invasive computerized tail-cuff system (NIBP, ADInstruments, Sydney, Australia) in conjunction with a PowerLab system. Before the measurement, rats were warmed for 10-20 min at a temperature of 28 °C to detect the steady arterial pulsations of the tail artery. A set of 10 measurements of SBP was averaged for each animal.

4.3. *Preparation of salusin-β and anti-salusin-β IgG*

In consideration of a high degree of homology between the rat and human salusin-β, human salusin-β (Phoenix Pharmaceuticals, Inc., Belmont, CA, USA) and rabbit anti-salusin-β IgG (Bachem, Bubendorf, Switzerland) were widely used to identify the biofunction of salusin-β in rats (Yu et al. 2004; Izumiyama et al. 2005; Takenoya et al. 2005; Suzuki et al. 2007). The immunoaffinity-purified polyclonal anti-human antibody against salusin-β does not cross-react with salusin-α, so it is able to distinguish the effects of salusin-β from salusin-α. Therefore, the anti-salusin-β IgG was reconstituted and prepared for *in-vivo* use according to the manufacturer's instructions at a final concentration of 2 g/L for PVN microinjection.

4.4. *PVN microinjection*

The skull was exposed and, after drilling appropriate holes, injection cannulae were implanted in the PVN (coordinates relative to the bregma: anterio-posterior, -1.8 mm; lateral, ± 0.4 mm; dorso-ventral, -7.9 mm) according to the atlas of Paxinos & Watson. Bilateral microinjections were made in a volume of 100 nl over 30 s with two glass micropipettes (about 50 μm tip diameter). At the end of the experiments, 50 nl 2% Evans blue was injected into the same location for histological analysis.

4.5. *Recording of RSNA, MAP and HR*

RSNA was recorded as previously reported to evaluate the dynamic changes of sympathetic outflow in response to salusin-β and other stimuli (Zhu et al. 2009; Xiong et al. 2012). After the exposure of the trachea and carotid artery, a catheter with pressure transducer (MLT0380, ADInstruments, Australia) was implanted in right carotid artery to record MAP and HR. The left renal sympathetic nerve was isolated from the surrounding tissue through a left flank incision. The renal nerve was cut distally to eliminate its afferent activity, hooked onto a pair of silver electrodes and subsequently immersed in warm mineral oil. The signals from electrodes were thereby amplified with a four-channel differential amplifier, rectified and integrated (100 ms time constant) with a 10-Hz high-pass filter and a 3000-Hz low-pass filter. At the end of the experiments, the baseline noise was determined after centrally section of the nerve and then subtracted from the integrated values of the RSNA.

4.6. *Measurement of plasma norepinephrine*

Bleed samples (2 ml) were collected from the right common carotid artery into tubes containing potassium-ethylene diamine tetraacetic acid. Tubes were centrifuged at 13,000 g for 5 min at room temperature and the supernatant was collected for detection. According to the manufacturer's instructions, a commercial ELISA kit was used to measure the plasma norepinephrine level. Briefly, 96-well microtiter plates were coated with corresponding purified antibodies against norepinephrine. Supernatant was mixed with diluent buffer, incubated at 37 °C for 30 minutes and washed. After incubation with horseradish peroxidase-conjugated IgG, the reactions were stopped and the absorbance was determined at 450 nm by using an ELISA reader (ELx800; Bio-TekInstruments, Winooski, VT, USA).

4.7. *Western blotting*

The proteins of PVN were extracted with lysis buffer (1 mM PMSF and 1 mM Na_3VO_4) as described previously (Yang et al. 2007). The supernatants containing the cytoplasmic or nuclear proteins were collected and the protein level was measured by Bradford assay. Equal amounts of proteins were mixed with loading buffer, separated by a 10% SDS-PAGE and transferred to nitrocellulose membrane (Pall, Pensacola, FL, USA) by semi-dry blotting. After overnight blocking, the membranes with cytoplasmic proteins were probed with primary antibodies against phospho-IκB-α (1:100, Santa Cruz Biotechnology, CA, USA) and p65 subunit of NF-κB (1:300, Santa Cruz Biotechnology, CA, USA), respectively. Moreover, the membranes with nuclear proteins were probed with the primary antibody against p65 subunit of NF-κB (1:300). The secondary antibody was biotinylated goat anti-rabbit IgG (1:5000). Lamin B1 and β-actin were chosen as internal references for nuclear or cytoplasmic proteins, respectively. The washed membranes were visualized using an enhanced chemiluminescence procedure (Roche Applied Science, Indianapolis, IN) with BioMax films. The optical density value of each band was measured using a computer equipped with the Image-Pro® Plus software. Results were expressed as the ratio of the optical density value of the interested band to reference band.

4.8. *Statistical analysis*

The SPSS 18.0 for Windows was employed in the data analysis. Excel (version 2007, Microsoft) was also adopted for preliminary data analysis. All data were expressed as mean ± SE of individual values. Comparisons between two observations in the same rat were assessed by Student paired *t* test. ANOVA followed by *post hoc* Bonferroni test was applied when multiple comparisons between different groups were made. A value of $P < 0.05$ was considered statistically significant.

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References

Badoer E (2001) Hypothalamic paraventricular nucleus and cardiovascular regulation. Clin Exp Pharmacol Physiol 28: 95–99.

Carlson SH, Shelton J, White CR, Wyss JM (2000) Elevated sympathetic activity contributes to hypertension and salt sensitivity in diabetic obese Zucker rats. Hypertension 35: 403–408.

Chen WW, Sun HJ, Zhang F, Zhou YB, Xiong XQ, Wang JJ, Zhu GQ (2013) Salusin-β in paraventricular nucleus increases blood pressure and sympathetic outflow via vasopressin in hypertensive rats. Cardiovasc Res 98: 344–351.

Chi F, Bo T, Wu CH, Jong A, Huang SH (2012) Vimentin and PSF act in concert to regulate IbeA + E. coli K1 induced activation and nuclear translocation of NF-κB in human brain endothelial cells. PLoS One 7: e35862.

Fan ZD, Zhang L, Shi Z, Gan XB, Gao XY, Zhu GQ (2012) Artificial microRNA interference targeting AT(1a) receptors in paraventricular nucleus attenuates hypertension in rats. Gene Ther 19: 810–817.

Grisk O (2011) Antihypertensive effects of ACE2 in the paraventricular nucleus: a consequence of reduced neuroinflammation? Cardiovasc Res 92: 365–366.

Hong D, Bai YP, Shi RZ, Tan GS, Hu CP, Zhang GG (2014) Inhibitory effect of reinioside C on vascular smooth muscle cells proliferation induced by angiotensin II via inhibiting NADPH oxidase-ROS-ERK1/2-NF-κB-AP-1 pathway. Pharmazie 69: 698–703.

Izumiyama H, Tanaka H, Egi K, Sunamori M, Hirata Y, Shichiri M (2005) Synthetic salusins as cardiac depressors in rat. Hypertension 45: 419–425.

Kalil GZ, Haynes WG (2012) Sympathetic nervous system in obesity-related hypertension: mechanisms and clinical implications. Hypertens Res 35: 4–16.

Kassab S, Kato T, Wilkins FC, Chen R, Hall JE, Granger JP (1995) Renal denervation attenuates the sodium retention and hypertension associated with obesity. Hypertension 25: 893–897.

Koya T, Miyazaki T, Watanabe T, Shichiri M, Atsumi T, Kim-Kaneyama JR, Miyazaki A (2012) Salusin-β accelerates inflammatory responses in vascular endothelial cells via NF-κB signaling in LDL receptor-deficient mice *in vivo* and HUVECs *in vitro*. Am J Physiol Heart Circ Physiol 303: 96–105.

Lambert E, Sari CI, Dawood T, Nguyen J, McGrane M, Eikelis N, Chopra R, Wong C, Chatzivlastou K, Head G, Straznicky N, Esler M, Schlaich M, Lambert G (2010) Sympathetic nervous system activity is associated with obesity-induced subclinical organ damage in young adults. Hypertension 56: 351–358.

Lambert GW, Straznicky NE, Lambert EA, Dixon JB, Schlaich MP (2010) Sympathetic nervous activation in obesity and the metabolic syndrome–causes, consequences and therapeutic implications. Pharmacol Ther 126: 159–172.

Nishihara M, Hirooka Y, Kishi T, Sunagawa K (2012) Different role of oxidative stress in paraventricular nucleus and rostral ventrolateral medulla in cardiovascular regulation in awake spontaneously hypertensive rats. J Hypertens 30: 1758–1765.

Reddy MK, Schultz HD, Zheng H, Patel KP (2007) Altered nitric oxide mechanism within the paraventricular nucleus contributes to the augmented carotid body chemoreflex in heart failure. Am J Physiol Heart Circ Physiol 292: 149–157.

Shi Z, Chen WW, Xiong XQ, Han Y, Zhou YB, Zhang F, Gao XY, Zhu GQ (2012) Sympathetic activation by chemical stimulation of white adipose tissues in rats. J Appl Physiol 112: 1008–1014.

Shibao C, Gamboa A, Diedrich A, Ertl AC, Chen KY, Byrne DW, Farley G, Paranjape SY, Davis SN, Biaggioni I (2007) Autonomic contribution to blood pressure and metabolism in obesity. Hypertension 49: 27–33.

Shichiri M, Ishimaru S, Ota T, Nishikawa T, Isogai T, Hirata Y (2003) Salusins: newly identified bioactive peptides with hemodynamic and mitogenic activities. Nat Med 9: 1166–1172.

Smith SH, Bishop SP (1986) Selection criteria for drug-treated animals in two kidney, one clip renal hypertension. Hypertension 8: 700–705.

Sun HJ, Li P, Chen WW, Xiong XQ, Han Y (2012) Angiotensin II and angiotensin-(1–7) in paraventricular nucleus modulate cardiac sympathetic afferent reflex in renovascular hypertensive rats. PLoS One 7: e52557.

Suzuki N, Shichiri M, Akashi T, Sato K, Sakurada M, Hirono Y, Yoshimoto T, Koyama T, Hirata Y (2007) Systemic distribution of salusin expression in the rat. Hypertens Res 30: 1255–1262.

Suzuki N, Shichiri M, Tateno T, Sato K, Hirata Y (2011) Distinct systemic distribution of salusin-α and salusin-β in the rat. Peptides 32: 805–810.

Takenoya F, Hori T, Kageyama H, Funahashi H, Takeuchi M, Kitamura Y, Shichiri M, Shioda, S (2005) Coexistence of salusin and vasopressin in the rat hypothalamo-hypophyseal system. Neurosci Lett 385: 110–113.

Van Gaal LF, Mertens IL, De Block CE (2006) Mechanisms linking obesity with cardiovascular disease. Nature 444: 875–880.

Wang HH, Wang XB, Li YC, Liao AJ, Fu BB, Pan HY, Liu ZG, Yang W (2013) The proteasome inhibitor bortezomib reverses P-glycoprotein-mediated leukemia multi-drug resistance through the NF-κB pathway. Pharmazie 68: 689–694.

Watanabe T, Nishio K, Kanome T, Matsuyama TA, Koba S, Sakai T (2008) Impact of salusin-α and -β on human macrophage foam cell formation and coronary atherosclerosis. Circulation 117: 643–648.

Wofford MR, Anderson DC JR, Brown CA, Jones DW, Miller ME, Hall JE (2001) Antihypertensive effect of alpha- and beta-adrenergic blockade in obese and lean hypertensive subjects. Am J Hypertens 14: 694–698.

Xiong XQ, Chen WW, Han Y, Zhou YB, Zhang F, Gao XY, ZHU GQ (2012) Enhanced adipose afferent reflex contributes to sympathetic activation in diet-induced obesity hypertension. Hypertension 60: 1280–1286.

Yang H, Shi G, Dou QP (2007) The tumor proteasome is a primary target for the natural anticancer compound Withaferin A isolated from "Indian winter cherry". Mol Pharmacol 71: 426–437.

Yi SS, Kim HJ, Do SG, Lee YB, Ahn HJ, Hwang IK (2012). Arginine vasopressin (AVP) expressional changes in the hypothalamic paraventricular and supraoptic nuclei of stroke-prone spontaneously hypertensive rats. Anat Cell Biol 45: 114–120.

Yu F, Zhao J, Yang J, Gen B, Wang S, Feng X (2004) Salusins promote cardiomyocyte growth but does not affect cardiac function in rats. Regul Pept 122: 191–197.

Zhong MK, Duan YC, Chen AD, Xu B, Gao XY, De W, ZHU GQ (2008) Paraventricular nucleus is involved in the central pathway of cardiac sympathetic afferent reflex in rats. Exp Physiol 93: 746–753.

Zhou CH, Liu L, Liu L, Zhang MX, Guo H, Pan J, Yin XX, Ma TF, Wu YQ (2014) Salusin-β not salusin-α promotes vascular inflammation in ApoE-deficient mice via the I-κBα/NF-κB pathway. PLoS One 9: e91468.

Zhu GQ, Xu Y, Zhou LM, Li YH, Fan LM, Wang W (2009) Enhanced cardiac sympathetic afferent reflex involved in sympathetic overactivity in renovascular hypertensive rats. Exp Physiol 94: 785–794.