African Journal of Pharmacy and Pharmacology Vol. 5(21), pp. 2376-2385, 8 December, 2011
Available online at http://www.academicjournals.org/AJPP
DOI: 10.5897/AJPP11.216
ISSN 1996-0816 ©2011 Academic Journals

Full Length Research Paper

The cardiovascular inhibition functions evoked by salusin β within the caudal ventrolateral medulla are mediated by muscarinic receptors mechanism

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Accepted 14 November, 2011

Salusin α and salusin β are newly found bioactive peptides of 20 and 28 amino acid respectively, distributed widely in hematopoietic system, endocrine system and the central nervous system. Our present study is to determine the cardiovascular functions of salusin β within the caudal ventrolateral medulla (CVLM) of rats. Unilateral microinjection of the artificial cerebrospinal fluid (aCSF) into the CVLM did not affect the blood pressure (BP) and heart rate (HR) in anesthetized rats. Topical application of salusin β into the CVLM produced a dose-dependently hypotension (0.04 pmol: -4 ± 2 mmHg; 0.4 pmol: -13 ± 4 mmHg; 4 pmol: -14 ± 2 mmHg) in anesthetized rats (P < 0.05, compared with aCSF: -2 ± 1 mmHg). Microinjection of higher dose salusin β (4 pmol) produced bradycardia (-15 ± 3 bpm vs. -4 ± 5 bpm with aCSF, P<0.05) significantly. Pretreatment with α2 receptor antagonist yohimbine (YOH, 12.8 nmol, n = 9), non-selective glutamate receptor antagonist kynurenic acid (KYN, 1 nmol, n = 7) or nicotinic receptor antagonist hexamethonium (HEX, 120 pmol, n = 7) into the CVLM of rats did not alter the hypotension and bradycardia induced by intra-CVLM salusin β (4 pmol). However, pretreatment with muscarinic receptor antagonist atropine (120 pmol, n = 9) or scopolamine (5 nmol, n = 7) within the CVLM effectively decreased the cardiovascular inhibition functions of intra-CVLM salusin β. Our present study shows that hypotension and bradycardia induced by microinjection of salusin β into the CVLM is mainly mediated by muscarinic receptors.

Key words: Salusin, rat, central, blood pressure, heart rate.

INTRODUCTION

Salusin α and salusin β are novel bioactive peptides of 28 and 20 amino acid residues, respectively (Shichiri et al., 2003). They were originally predicted from a human full-length enriched cDNA library (Shichiri et al., 2003). Both salusinα and salusin β originated from preprosalusin, an alternative-splicing product of the torsion dystonia-related gene (TOR2A) (Shichiri et al., 2003). The ubiquitous expression of salusins in kinds of tissues including brain (Shichiri et al., 2003; Izumiyama et al., 2005; Takenoya et al., 2005; Suzuki et al., 2007; Nakayama et al., 2009; Suzuki et al., 2011) has been detected. However, it is not clear whether salusins exert their physiological functions in the central cardiovascular regions. Previous studies show that salusins have multiple functions in the endocrine, immune including cardiovascular system (Shichiri et al., 2003; Wang et al., 2006; Watanabe et al., 2008; Shi et al., 2010). In the central nervous system (CNS), preprosalusin is expressed abundantly in the hypothalamus and pituitary (Takenoya et al., 2005; Suzuki et al., 2007). Salusin β stimulates the release of arginin–vasopressin (AVP) from the posterior pituitary, suggesting that salusin β is a potential candidate of neuropeptide (Shichiri et al., 2003; Takenoya et al., 2005; Wang et al., 2006; Saito et al., 2008). Although salusin β expresses abundantly in the CNS, the central cardiova-

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scular effects of salusins have not been fully determined. In our present study, we hypothesized that salusins influence the activities of central cardiovascular regions.

The caudal ventrolateral medulla (CVLM) is a major vasodepressor area in the brain stem. Chemical stimulition of the CVLM produces hypotension and bradycardia due to a reduction in sympathetic nerve activity (SNA). Anatomical studies indicated that the CVLM receives numerous afferents from the nucleus tractus solitarii (NTS) areas that receive primary baroreceptor afferent fibers. Stimulation of baroreceptor afferents is generally thought to activate excitatory amino acid receptors within the NTS. In turn, the NTS sends excitatory amino acid projections to the CVLM and the NTS stimulates a γ-Aminobutyric acid (GABA) inhibitory pathway to the rostral ventrolateral medulla (RVLM) (Sapru, 1996). Because the CVLM is an important area for integrating the tonic and reflex control of the cardio-vascular activity, the present work was designed to observe the effect of exogenous salusin β on cardio-vascular activity at the CVLM level and to explore their mechanisms.

MATERIAL AND METHODS

General procedure

Experiments were performed on 74 adult male Sprague-Dawley rats (body weight, 250 to 300 g), which were provided by Lanzhou University Laboratory Animal Center. All animals received human care in compliance with the NIH Guide for the care and use of laboratory animals (National Institutes of Health Publications, No. 80 to 23, revised 1978). The methods for animal preparation, microinjection and histological procedures were similar to those described previously (Lu et al., 2007; Liu et al., 2011; Qiao et al., 2011).

Briefly, after 3-days accommodation, the rats were anesthetized with pentobarbital sodium (40 mg/kg, intraperitoneal administration). An arterial femoral catheter was inserted into the right femoral artery to continuously record blood pressure (BP) and a venous femoral catheter was inserted into right femoral vein to administer drugs. The arterial femoral catheter connected to a pressure transducer (P-300B) and BP was sequentially measured and displayed on one channel of a recording system (RM6240, China). BP was recorded continuously and heart rate (HR) was computed from the BP waveforms. After a tracheotomy was performed in all animals, each rat was paralyzed with gallamine triethiodide (10 mg/kg initially and 4 mg/kg every 30 min, i.v.) for ventilating with oxygen-enriched room air through a tracheal cannula, connected to an artificial ventilator. Urethane was injected intravenously to maintain surgical anaesthesia. Anesthetics were supplemented when necessary. Depth of anaesthesia was monitoring the stability of BP, and BP response to noxious stimulation. The rat was placed in a stereotaxic frame (MK-8003, China). Body temperature was maintained at about 37°C with an infrared heating lamp.

Microinjection procedure

In the rats which received the CVLM microinjection, part of the occipital bone and the cerebellum were removed to expose the dorsal surface of the medulla. The vertex of which was taken as a landmark for the stereotaxic co-ordinates. Under the guidance of a stereotaxic apparatus, the multi-barreled micropipette (tip diameter 20 to 30 μm) was inserted into the CVLM. The co-ordinates for the CVLM were determined from the rat atlas of Paxinos and Watson (Paxinos and Watson, 2007) (0.3 to 0.8 mm rostral to the obex, 1.6 to 2.0 mm lateral to the midline, and 2.8 to 3.2 mm below the dorsal surface of the medulla). The micropipette was filled with L-glutamate, salusin β or antagonist [atropine, scopolamine (SCOP), hexamethonium (HEX), kynurenic acid (KYN) or yohimbine (YOH)]. Salusin β was obtained from Phoenix (USA). Other chemicals were obtained from the Sigma-Aldrich (USA). KYN was initially dissolved in 10% sodium hydroxide (NaOH), and diluted with in an artificial cerebrospinal fluid (aCSF, in mM: 133.3 NaCl, 3.4 KCl, 1.3 $CaCl_2$, 1.2 $MgCl_2$, 0.6 NaH_2PO_4, 32.0 $NaHCO_3$, and 3.4 glucose, pH 7.4) to the final concentration (pH was adjusted to 7.4 with 10% HCl). The doses of KYN, atropine, SCOP, HEX, YOH were based on previous studies (Criscione et al., 1983; Willette et al., 1984; Mosqueda-Garcia et al., 1991; Hasser and Bishop, 1993; Kubo et al., 2000; Menegaz et al., 2000; Ferreira et al., 2005; Kitchen et al., 2006; Sartor and Verberne, 2007; Mandel and Schreihofer, 2008; Bhuiyan et al., 2009). All drugs were administered into the CVLM in a volume of 100 μl, and delivered approximately 10 s by a syringe under the guidance of operating microscope. As previously described, the Chemical identification of the CVLM was based on the depressor response induced by injecting 5 nmol L-glutamate at the beginning of the experiment.

Protocol

Rats were divided into 4 groups (n = 4 to 7) to test the dose effects of salusin β on cardiovascular responses within the CVLM; salusin β (0.04, 0.4, and 4 pmol) or vehicle (aCSF, 100 nl) was unilaterally injected into the CVLM (Table 1). To investigate whether the cardiovascular effects of intra-the CVLM salusin β were mediated by glutamate receptor, α2 receptor, muscarinic receptor or nicotinic receptor, rats were divided into 5 groups, and glutamate receptor antagonist KYN (1 nmol, n = 7), α2 receptor antagonist YOH (12.8 nmol, n = 9), muscarinic receptor antagonist atropine (120 pmol, n = 9) or SCOP (5 nmol, n = 7), nicotinic receptor antagonist HEX (120 pmol, n = 7) or vehicle (aCSF, 100 nl, n = 5) was prior injected into CVLM 10 min before salusin β (4 pmol) was injected into the same site, and the responses were followed for 60 min after salusin β (Table 1).

Histological analysis

At the end of the experiment, 20 μl of 2% pontamine sky blue solution was injected to verify the sites of microinjection. Each animal was perfused transcardially with 0.9% NaCl and 10% formalin. The brainstem was removed, stored overnight in 10% phosphate-buffered formalin, and then transferred to fixative containing 30% sucrose. Frozen brain tissue was sectioned in the coronal plane (50 μm) stained with neutral red. The location for drug injections within the CVLM were reconstructed from the dye spots according to the atlases of Paxinos and Watson (Figure 1) (Paxinos and Watson, 2007).

Statistical analysis

All of the values are expressed as mean ± SE. BP is expressed as mean arterial pressure (MAP), which was calculated as diastolic + [(systolic-diastolic)/3]. The magnitudes of the changes in MAP and HR at different time after injections of salusin β were compared with

Table 1. Baseline values of MAP and HR in experimental groups.

Experimental group	n	Blood pressure (mmHg)	Heart rate (bpm)
aCSF	4	108±5	483±16
Salusin β (4 pmol)	6	87±1	448±6
Salsuin β (0.4 pmol)	7	92±5	442±9
Salusin β (0.04 pmol)	7	108±3	484±8
aCSF + Salusin β (4 pmol)	5	87 ± 8	479 ± 14
artopine+ Salusin β (4 pmol)	9	82±17	432 ± 29
Yohimbine + Salusin β (4 pmol)	9	84 ± 7	436 ± 18
Scopolamine + Salusin β (4 pmol)	7	100+4	434±38
Hexamethonium + Salusin β (4 pmol)	7	83±8	471 ± 24
KYN + Salusin β (4 pmol)	7	93 ± 6	473 ± 15

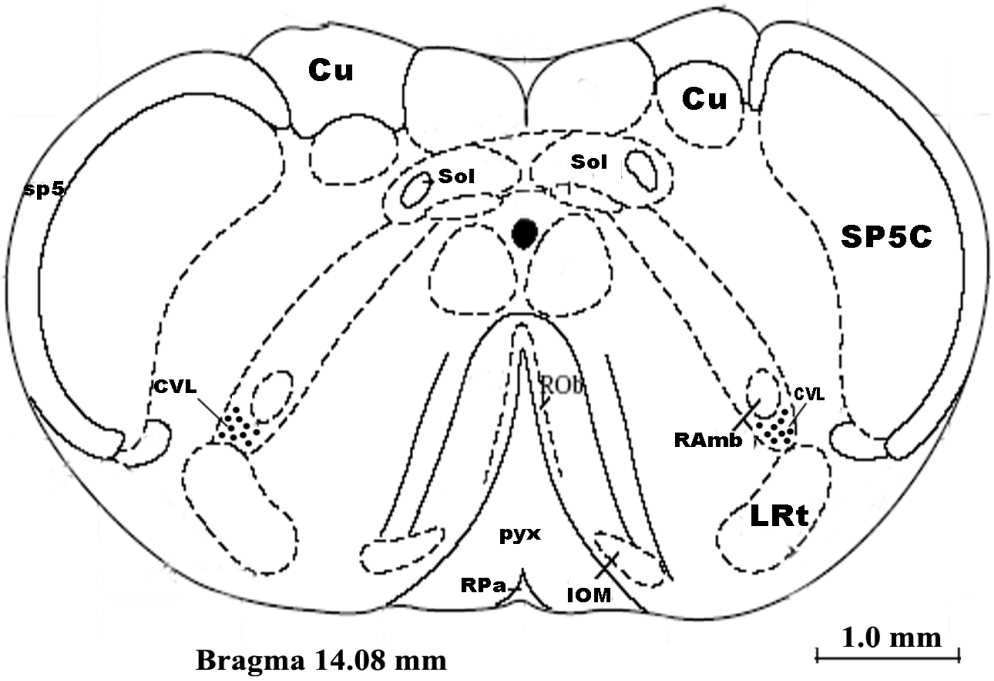

Figure 1. The injection sites (·) in the caudal ventrolateral medulla (CVLM) mapped on a standard section through medulla 0.3 to 0.8 mm rostral to the obex. Cu, Cuneate nucleus; sol, solitary tract; sp5, spinal trigeminal tract; SP5C, spinal trigeminal nucleus, caudal part; CVL, caudoventrolateral reticular nucleus; Rob, raphe obscurus nucleus; RAmb, retroambiguus nucleus; pyx, pyramidal decussation; RPa, raphe pallidus nucleus; LRt, lateral reticular nucleus; IOM, inferior olive, medial nucleus.

those of vehicle by analysis of Student's t-test for unpaired observations. A one- or two-way repeated-measure ANOVA followed with the Newman-Keuls test for post hoc analysis was used when multiple comparisons were made. A P value of <0.05 was regarded as statistically significant.

RESULTS

The cardiovascular responses of microinjection of salusin β (0.04 to -4 pmol) into the CVLM in anesthetized rats. Figure 2a presented the representative original tracings of BP and HR response to microinjection of salusin β (0.04, 0.4, or 4 pmol) or aCSF (100 nl) into the CVLM in rats. Intra-CVLM injection of aCSF did not alter the basal MAP (108 ± 5 vs. 107±5 mmHg, P > 0.05) or HR (483 ± 16 vs. 479 ± 15 bpm, P > 0.05) in rats. However, topically application of salusin β produced a dose-dependent hypotension (0.04 pmol: -4 ± 2 mmHg; 0.4 pmol: -13 ± 2

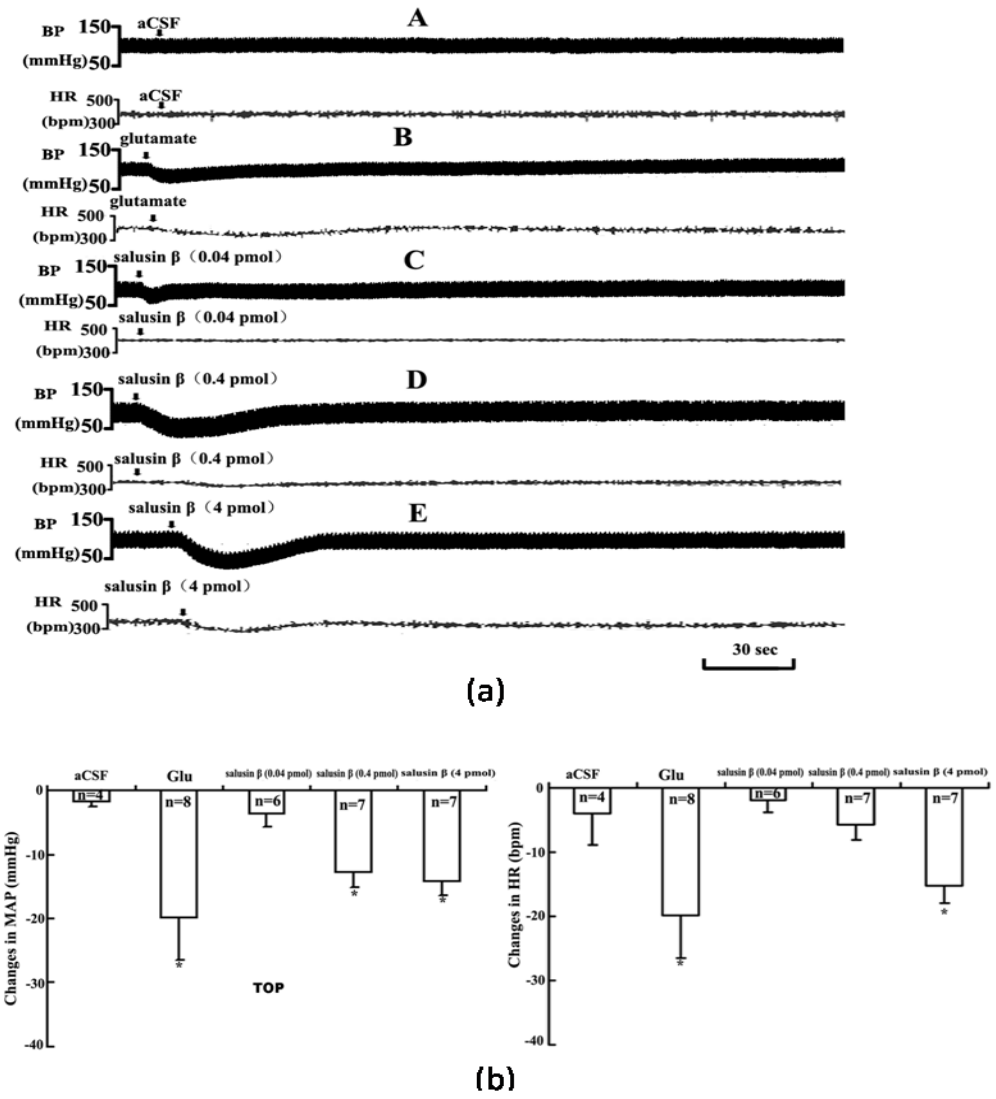

Figure 2. (a) Representative traces showing the responses of microinjection of salusin β (0.04, 0.4, 4 pmol, n = 6 - 7), glutamate (5 nmol, n = 8) or artificial cerebrospinal fluid (aCSF, 100 nl, n = 4) into the caudal ventrolateral medulla (CVLM) on the blood pressure response (BP) and heart rate (HR) of rats. The microinjection site was verified functionally by the depressor response to L-glutamate (5 nmol). (b) The magnitude of changes (mean ± SE) in mean arterial pressure [MAP (a)] and heart [HR (b)] by microinjection of salusin β (0.04, 0.4, 4 pmol, n = 6 - 7), glutamate (5 nmol, n = 8) or artificial cerebrospinal fluid (aCSF, 100 nl, n = 4) into the caudal ventrolateral medulla (CVLM). *P <0.05 vs. aCSF.

mmHg; 4 pmol: -14 ± 2 mmHg) in anesthetized rats (P < 0.05, compared with microinjection of aCSF: -2 ± 1 mmHg). Besides, although microinjection of lower dose salusin β (0.04 and 0.4 pmol) into CVLM did not significantly influence HR (0.04 pmol: -2 ± 2 bpm; 0.4 pmol: -6 ± 2 bpm; P > 0.05, compared with microinjection of aCSF: -4 ± 5 bpm) of rats, higher dose salusin β (4 pmol) produced significantly bradycardia (-15 ± 3 bpm with microinjection of 4 pmol salusin β vs. -4 ± 5 bpm with aCSF, P < 0.05). The hypotension and bradycardia occurred 5 s after topical application of salusin β, reached the nadir after 30 s, and returned to baseline after about 3 min. The cardiovascular responses of microinjection of aCSF or salusin β (0.04, 0.4, or 4 pmol) are summarized in Figure 2b.

Pretreatment with a glutamate receptor antagonist KYN or a α2 adrenoreceptor antagonist yohimbine on the BP responses of intra-the CVLM salusin β

Figure 3a presented the representative trace of pretreatment with a nonselective glutamate receptor antagonist KYN (1 nmol, n = 7) or a α2 adrenoreceptor

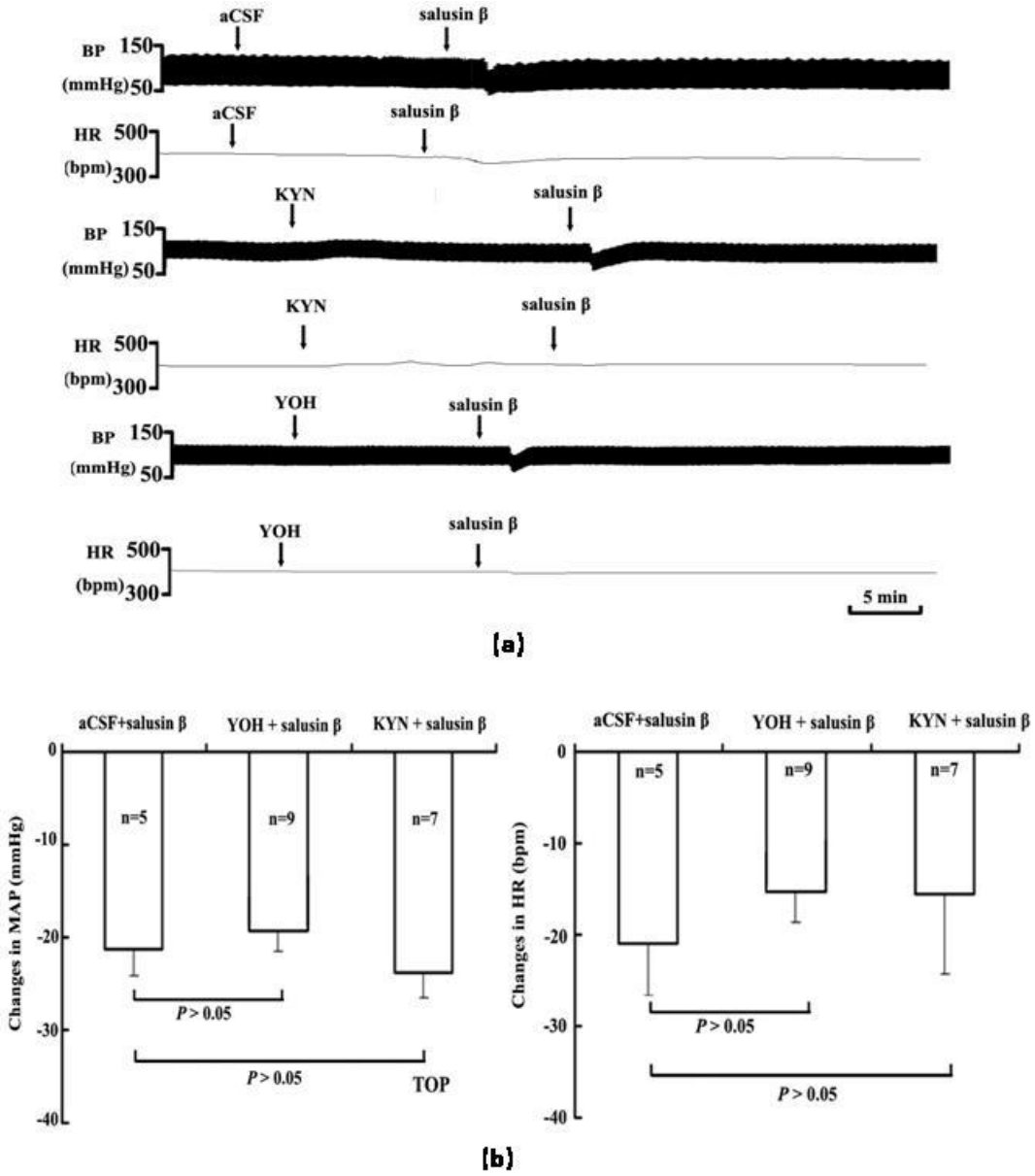

Figure 3. (a) Representative traces showing the blood pressure (BP) and heart rate (HR) responses to microinjection of salusin β (4 pmol) into the caudal ventrolateral medulla (CVLM) 10 min after pretreatment with aCSF (100 nl), glutamate receptor antagonist kynurenic acid (KYN, 100 pmol) or α2-adrenoceptor antagonist yohimibine (YOH, 12.8 nmol). (b) The magnitude of changes (mean ± SE) in mean arterial pressure [MAP (a)] and heart [HR (b)] induced by microinjections of salusin β (4 pmol) into the caudal ventrolateral medulla (CVLM) following pretreatment with vehicle (aCSF, 100 nl, n = 4), α2-receptor antagonist yohimibine (YOH, 12.8 nmol, n = 9) or glutamate receptor antagonist kynurenic acid (KYN, 100 pmol, n = 7).P <0.05 vs. pretreatment with aCSF (100 nl).

antagonist YOH (12.8 nmol, n = 9) on the BP and HR responses of intra-CVLM salusin β (4 pmol). In 5 rats, prior microinjection of aCSF did not alter the basal MAP (87 ± 8 vs. 88 ± 9 mmHg, P > 0.05) and HR (479 ± 14 vs. 469 ± 18 bpm, P > 0.05) of rat. At the same time, pre-treatment with aCSF did not influence the MAP (from 96 ± 5 to 95 ± 2 mmHg, P > 0.05) and HR (from 481 ± 13 to 475 ± 11, P>0.05) responses of intra-CVLM salusin β. Microinjection of KYN significantly increased basal MAP (from 93 ± 6 to 102 ± 4 mmHg, P < 0.05), but did not significantly alter basal HR (473 ± 15 vs. 493 ± 10 bpm, P > 0.05) of rats. At the same time, pretreatment with KYN did not affect the MAP (-21 ± 3 mmHg pretreatment with aCSF vs: -24 ± 3 mmHg pretreatment with KYN, P > 0.05)

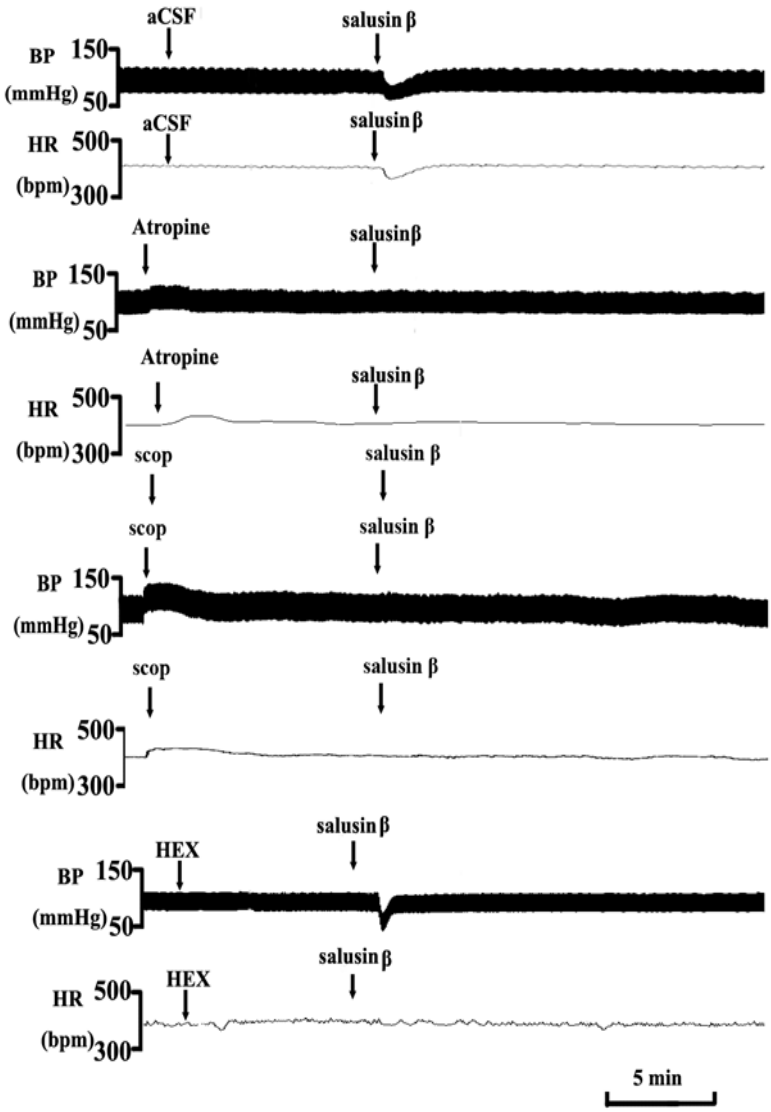

Figure 4. Representative traces showing the blood pressure (BP) and heart rate (HR) responses to microinjection of salusin β (4 pmol) into the caudal ventrolateral medulla (CVLM) 10 min after pretreatment with aCSF (100 nl, n = 4), nicotinic receptor antagonist hexamethonium (HEX, 120 pmol, n = 7), muscarinic receptor antagonist atropine (120 pmol, n = 9) or scopolamine (SCOP, 5 nmol).

and HR (pretreatment with aCSF: -21 ± 6 bpm vs. pretreatment with KYN: -15 ± 9 bpm, P > 0.05) responses of salusin β within the CVLM. Microinjection of YOH did not influence basal MAP (84 ± 7 vs. 86 ± 6 mmHg, P > 0.05) and HR (436 ± 18 vs. 422 ± 13 bpm, P > 0.05) of rats. Pretreatment with YOH also did not influence MAP (pretreatment with aCSF: -21 ± 3 mmHg vs. pretreatment with YOH: -18 ± 2 mmHg, P > 0.05) and HR (pretreatment with aCSF: -21 ± 6 bpm vs. pretreatment with YOH: -16 ± 3 bpm, P > 0.05) responses of salusin β within the CVLM. The results of pretreatment with KYN or YOH on the MAP and HR responses of intra-CVLM salusin β are summarized in Figure 3b.

Pretreatment with a muscarinic receptor antagonist atropine, scopolamine or a nicotinic receptor antagonist HEX on the cardiovascular functions of intra-CVLM salusin β

Figure 4 presented the representative original tracings of pretreatment with atropine (120 pmol, n = 9), SCOP (5 nmol, n = 7) or HEX (120 pmol, n = 7) on the BP and HR responses of intra-CVLM salusin β (4 pmol).

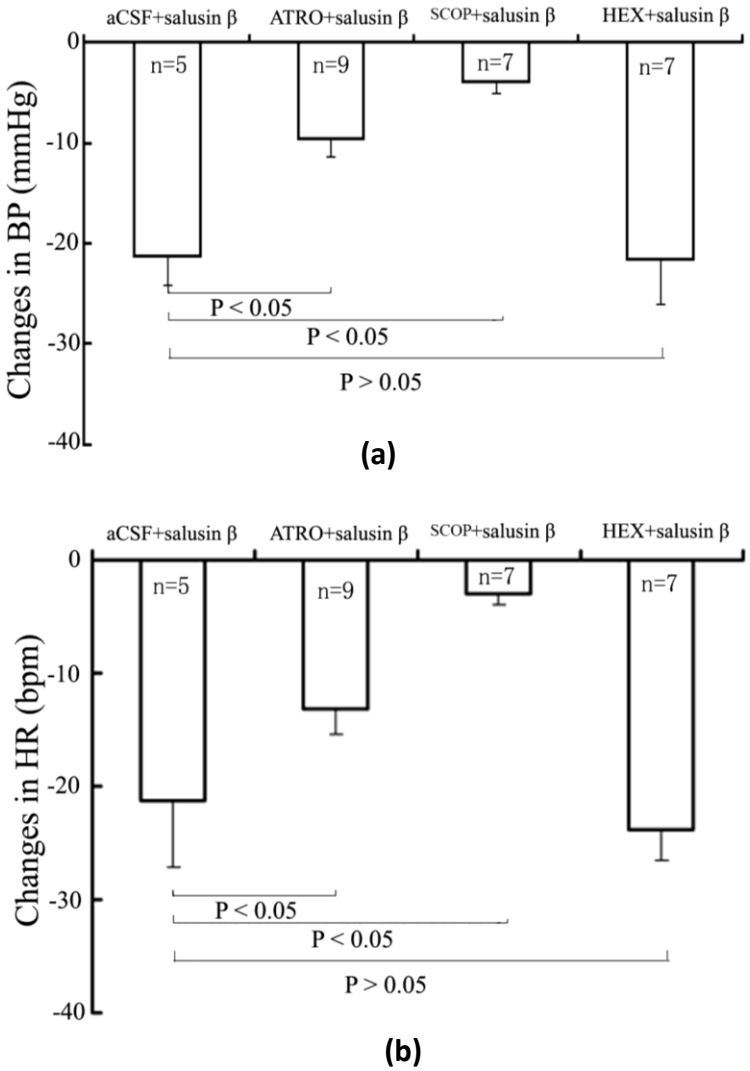

Figure 5. The magnitude of changes (mean ± SE) in MAP (A) and HR (B) induced by microinjections of salusin β (4 pmol) into caudal ventrolateral medulla (CVLM) following pretreatment with vehicle (aCSF, 100 nl, n = 4), muscarinic receptor antagonist atropine (120 pmol, n = 9), scopolamine (SCOP, 5 nmol) or nicotinic receptor antagonist hexamethonium (HEX, 120 pmol, n = 7). P < 0.05 vs. pretreatment with aCSF (100 nl).

Microinjection of atropine increase basal MAP (from 82 ± 17 to 93 ± 13 mmHg, P < 0.05) significantly, but did not significantly alter basal HR (432 ± 29 vs. 457 ± 27 bpm, P > 0.05) of rats. However, pretreatment with atropine significantly decreased hypotension (-21 ± 3 mmHg pretreatment with aCSF vs. -9 ± 2 mmHg pretreatment with atropine, P < 0.05) and bradycardia (pretreatment with aCSF: -21 ± 6 bpm vs. pretreatment with atropine: -13 ± 2 bpm, P < 0.05) of intra-CVLM salusin β. Similarly, microinjection of SCOP into the CVLM not only increase BP (from 98 ±11 to 110 ± 10 mmHg, P<0.05) and HR (from 426 ±45 to 468 ± 38 bpm, P < 0.05) of rats significantly but also effectively decreased the BP (-21 ± 3 mmHg pretreatment with aCSF vs. -4 ± 1 mmHg pretreatment with SCOP, P< 0.05) and HR (pretreatment with aCSF: -21 ± 6 bpm vs. pretreatment with SCOP: -3 ± 1 bpm, P <0.05) responses induced by intra-CVLM salusin β (4 pmol). Microinjection of HEX did not alter basal MAP (from 83 ± 8 to 85 ± 9 mmHg, P > 0.05) and HR (471 ± 24 vs. 460 ± 28 bpm, P > 0.05) of rats. Pretreatment with HEX did not alter the hypotension (-21 ± 3 mmHg pretreatment with aCSF vs.-22 ± 5 mmHg, P > 0.05) and bradycardia (-21 ± 6 bpm pretreatment with aCSF vs.-24 ± 3 bpm, P > 0.05) of intra-CVLM salusin β. Figure 5 summarized the effects of pretreatment with muscarinic receptor antagonist atropine, SCOP or nicotinic receptor antagonist HEX on the cardiovascular responses of intra-CVLM salusin β.

DISCUSSION

In our present study, our most important findings are: 1. intra-CVLM application of salusin β produces a dose-dependant hypotension and bradycardia in anesthetized rats. 2. The hypotension and bradycardia induced by intra-CVLM salusin β is mainly mediated by muscarinic receptors.

Salusins are not only considered as a novel bioactive peptide involving in hypotension, mitogenic activities and intracellular signaling pathways etc, but also characterized as a novel candidate of neuropeptide because (1) salusin β stimulates the secretion of AVP from rat neurohypophysis *in vitro* (Shichiri et al., 2003; Saito et al., 2008); (2) salusin β coexists with AVP in the hypothalamo-neurohypophyseal system of the rat under normal condition (Takenoya et al., 2005). Although salusin β has various physiological functions, the receptors of salusin β have not been identified. Previous study showed that human salusin β is a surrogate ligand of the mouse MrgA1 (mas-related G protein- coupled receptors), however it could not activate human MrgA1 (Wang et al., 2006). Because the exact receptor and post-receptor signaling pathways are not clear, it is difficult to elucidate the mechanisms of cardiovascular roles of salusin β.

The CVLM is the important site of baro- and chemoreceptors in medullary reticular formation (Kubo and Kihara 1990; Lawrence and Jarrott, 1996). Various transmitters or active peptides alter the sensitivity of baro- or chemoreflex in the CVLM level (Chai et al., 1995; Chen and Chai, 1999; Yang et al., 1999). ACh, glutamate and norepinephrine are widely distributed in the CVLM and play important roles in central autonomic regulation, including control of arterial pressure (Pedrino et al., 2006; Wu et al., 2009). The activation of glutamate receptors or muscarinic receptors into the CVLM elicits hypotension and bradycardia responses (Gieroba and Blessing, 1992; Chen et al., 2001), which are very similar to those induced by stimulation of arterial baroreceptors and could be effectively inhibited by pretreatment with a muscarinic receptor antagonist, atropine or glutamate antagonist KYN. The widely distribution of glutamate receptors in CNS (Ambalavanar et al., 1998) provides the potential hypothesis that the cardiovascular functions of intra-CVLM salusin β may be mediated by glutamate receptors. However, in our present study, we indicated that non-selective glutamate receptor antagonist KYN, which has been reported to effectively abolish the cardiovascular functions of glutamate within the CVLM or RVLM (Bergamaschi et al. 1999; Natarajan and Morrison 2000), could not decrease the BP responses of intra-CVLM salusin β. It suggests that glutamate receptor might not mediate the cardiovascular effects of salusin β within the CVLM. We also speculated that α2-adrenoreceptor was probably involved in the cardiovascular effects of intra-CVLM salusin β. Single-unit recordings from the NTS have identified groups of chemoreceptor afferent-sensitive neurons that are sensitive to local application of catecholamine receptor agonists and antagonists (Reis et al., 1984). Because stimulation of α2-adrenoreceptor in the CVLM produces a gradual hypotension and bradycardia, similar to responses of intra-CVLM salusin β, we hypothesized that the cardiovascular functions of intra-CVLM salusin β are probably directly or indirectly mediated by α2-adrenoreceptor. However, prior application of selective α2-adrenoreceptor antagonist yohimbine produced no significant influences on the cardiovascular functions of intra-CVLM salusin β. Moreover, the cardiovascular responses peak of α2-adrenoreceptor activation in the NTS is about 10 to 20 min (Fior et al., 1995; Hayward et al., 2002), much longer than that of salusin β within the CVLM (about 30 s). Both of them suggest that the cardiovascular responses of intra-CVLM salusin β are not mediated by α2-adrenoreceptor.

ACh has been widely accepted as a neurotransmitter in the CNS. Electrophysiological, behavioral, and biochemical studies demonstrate the wide distribution of Ach and muscarinic receptors in the caudal medulla (including the CVLM) (Jhamandas et al., 1970; Criscione et al., 1983). Microinjection of ACh into the CVLM or NTS elicits hypotension and bradycardia, mainly mediated by muscarinic receptors but not by nicotinic receptors (Reis et al., 1981; Tsukamoto et al., 1994; Yang et al., 1999), which is very similar to those of microinjection of salusin β. Additionally, it has been reported that the hypotension and bradycardia of bolus intravenous injection of salusin β in rats are probably mediated by muscarinic receptors (Izumiyama et al., 2005). Base on these observations, we conjectured that muscarinic receptors might mediate the cardiovascular functions of intra-CVLM salusin β. To test this hypothesis, muscarinic receptor antagonist atropine, SCOP and nicotinic receptor antagonist HEX was microinjected into the CVLM to observe whether the cardiovascular functions of intra-CVLM salusin β were mediated by muscarinic or nicotinic receptor. Our study indicated that the cardiovascular functions of intra-CVLM salusin β were significantly decreased not only by prior application of atropine, but also by prior application of SCOP, the other muscarinic receptor antagonist SCOP. However, nicotine receptor antagonist HEX produced no significantly influences on the cardiovascular functions of salusin β. It suggests that the depressor and bradycardia of intra-CVLM salusin β is directly or indirectly mediated by muscarinic receptors but not by nicotinic receptors. In the CNS, the salusin β is expressed in the neuroendocrine system and posterior pituitary including brain stem (Nakayama et al., 2009; Suzuki et al., 2011). Our present study provided a probably basis that salusin β may be involved in the pathogenesis of vasodepressor syncope by CVLM mechanism.

Based on the aforementioned observations, we proposed that: Salusin β directly or indirectly stimulates

muscarinic neurons within the CVLM, and then produces hypotension and bradycardia probably by suppressing the activities of presympathetic neurons in the RVLM.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (No. 30700266). We thank Prof. Ya-Na BAI for her professional statistical assistance.

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