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Original Article

Central administration of resistin into the paraventricular nucleus (PVN) produces significant cardiovascular responses

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Abstract

Introduction: Resistin, a complex multimeric structure which is secreted by adipose tissue and circulates in the blood, acts on the hypothalamus to increase sympathetic nerve activity, inhibit appetite and is associated with obesity, insulin resistance and cardiovascular disorders. In this study, we survey the cardiovascular effects of direct injection of resistin into specific cell group of the paraventricular nucleus (PVN) that is known as one of the centers which control the baseline of arterial pressure and heart rate.

Methods: Adult male rats were anesthetized with urethane (1.4g/kg intraperitoneally). Arterial pressure (AP) and heart rate (HR) were monitored before and after treatment. Resistin (1, 3 and 5µg/rat), norepinephrine (2.5 nM), muscimol (250ng/rat) and saline as control (vehicle solution, 1µl) were injected into the PVN parvocellular neurons.

Results: The results showed that resistin (3 and 5µg/rat) caused a significant increase in AP, HR and high QRS compared to control group and prior to its injection. Injection of norepinephrine into the PVN evoked a significant increase in AP, HR and QRS amplitude, whereas injection of muscimol significantly decreased these parameters. In the control group, saline injection into the PVN had no significant effect on these parameters.

Conclusion: It can be concluded that the PVN can be one of the important central areas for actions of resistin which had obvious effects on HR and AP. These results provide a base for future studies to explore the role of resistin in cardiovascular responses in conditions like metabolic syndrome and hypertension.

Keywords:
Resistin;
Paraventricular Nucleus;
Heart Rate;
Arterial Pressure

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Introduction

Resistin, a novel 12.5-kDa cysteine-rich polypeptide, is primarily released from adipocytes as the major source in rodents macrophages (Steppan et al., 2001) and human monocytes (Nagaev and Smith, 2001; Patel et al., 2003). However, resistin is released from other types of cells and tissues including macrophages (Savage et al., 2001), fat and differentiated 3T3L1 cells (Steppan et al., 2001), pre-adipocytes (Janke et al., 2002), trophoblastic cells (Yura et al., 2003), brown adipocytes (Viengchareun et al., 2002), white blood cells (Lu et al., 2002) and

mammary gland (Steppan et al., 2001). Resistin expression is regulated in a nutritional-, age- and gender-specific manner (Morash et al., 2004) and is influenced by other factors including tumor necrosis factor-κ (Fasshauer et al., 2001a, b), β-adrenergic agonists (Fasshauer et al., 2001a) and testosterone (Ling et al., 2001).

Metabolic syndrome refers to a set of conditions that include hypertension, insulin resistance, obesity and increased levels of blood lipids. Type II diabetes is characterized by insulin resistance in certain peripheral tissues and is associated with obesity (Steppan et al., 2001). Type II diabetes and obesity are major risk factors for the development of cardiovascular complications, especially hypertension and premature death (Frankel et al., 2009; Steppan et al., 2001). Studies in the last decade have shown that increase of resistin is involved in the development of diabetes, insulin resistance, obesity, cardiovascular disorders, hypertension and heart failure (Frankel et al., 2009; Steppan et al., 2001). Resistin is also involved in insulin signaling in 3T3-L1 adipocytes and expression of suppressor of cytokine signaling-3 either 3T3-L1 adipocytes or murine adipose tissues (Steppan et al., 2005). Moreover, some studies have shown that resistin, like leptin, exists in cerebrospinal fluid (Kos et al., 2007) and is widely expressed (mRNA and protein) in the pituitary (Morash et al., 2004) and hypothalamus nuclei including arcuate nucleus and the ventromedial nucleus (Tovar et al., 2005) which plays a central role in control of food intake and energy homeostasis.

The hypothalamus has various nuclei with different functions, such as the paraventricular nucleus (PVN) which is involved in control of food intake, energy homeostasis and cardiovascular activity. The PVN can be one of the most important brain nuclei which controls the physiological response to energetic challenges (Hill, 2012), environmental stress and cardiovascular diseases (Benarroch, 2005). The PVN contains two general populations of neurons, the magnocellular neurons project to the median eminence and the posterior pituitary, and parvocellular elements that project to the anterior pituitary, hindbrain, spinal cord and other areas of the brain (Benarroch, 2005). Recent studies showed that the PVN contains autonomic neurons which are directly associated with cardiac sympathetic nerve by sympathetic preganglionic neurons (Dampney, 1994; Ranson et al., 1998). In addition, the PVN, as a forebrain structure, contains sympathetic pre-motor neurons (Dampney, 1994).

There is increasing evidence that the PVN is implicated in disorders such as congestive heart failure (CHF), hypertension and metabolic syndrome (Benarroch, 2005). Hence, the PVN may be one of the major central areas for actions of resistin, like leptin (Shih et al., 2003). On the other hand, it was reported that there is a relationship between plasma concentration of resistin and metabolic syndrome, CHF and hypertension (Bhalla et al., 2010; Takeishi et al., 2007). However, the relationship between central resistin and cardiovascular responses has not been examined to date. Thus, the aim of the present study was to survey the effect of direct injection of resistin into the PVN on arterial blood pressure (AP) and heart rate (HR) in adult male rat.

Materials and methods

General procedures

All investigations conducted in this study are in accordance with the 'Guiding Principles for the Care and Use of Research Animals' approved by Shiraz University. Adult male Sprague-Dawley rats (280-320 g, age 9-10 weeks), colony-bred in the Animal House Center, Shiraz, Iran, were housed in the animal room under controlled lighting (12 h light: 12 h darkness) and temperature (20 ± 2°C) conditions and had free access to pelleted food (formulated and made by Javaneh Khorasan Company, Iran) and tap water. To study the effect of injection of resistin into the PVN parvocellular neurons on AP and HR, rats were randomly allocated in six equal groups (n=6) as follows:

Group 1: injection of 1µl saline as vehicle (as control group); all of the procedures were exactly the same in all groups; however, instead of saline other drugs were injected into the PVN in the other group. All injections were performed in the same volume (1µl). Group 2: injection of resistin (1µg/rat, Phoenix Pharmaceuticals Inc., Karlsruhe, Germany); group 3: injection of resistin (3µg/rat); group 4: injection of resistin (5µg/rat); group 5: injection of norepinephrine (2.5nM, a α_1 receptor agonist; Sigma) and group 6: injection of muscimol (250ng/rat, a $GABA_A$ receptor agonist; Sigma). All injections were made unilaterally into the PVN parvocellular neurons.

Fig.1. Diagrammatic representation is based on the rat brain atlas of Paxinos & Watson (2005), showing the dispersion of injection sites among experiments. Filled circles (muscimol), open circles (norepinephrine), filled triangle (resistin) and open triangle (saline) indicate injection sites in the PVN.
The left panel is a photomicrograph of a rat brain coronal section showing the injection site in the PVN of one representative animal. PVN: paraventricular nucleus of hypothalamus; 3V: third ventricle.

Experimental procedures and cardiovascular response

All animals were anesthetized with urethane (1.4 g/kg intraperitoneally). The paw pinch reflex was used to assess the depth of anesthesia. Continuous cardiovascular response measurements were made directly using polyethylene catheters (PE-50) inserted into the left femoral artery. Polyethylene catheter was filled with heparinized saline, and was connected to a pressure transducer (Grass Instrument Company, USA). Heart rate and arterial pressure were continuously recorded by both a Grass polygraph (Model 7D Polygraph, Grass Instrument Co., USA) and a computer program written in this laboratory and by a digital electrocardiography (ECG) recorder (SuzukenKenz, ECG 110, Suzuken Co., Japan**)**. Body temperature was monitored by rectal temperature and maintained in the range 37-38°C. The animals were placed in prone position in a stereotaxic apparatus (Stoelting Co., USA) and a small hole was drilled through the parietal bone over the PVN. Stereotaxic position for injection of drugs into the PVN was selected from the Rat Brain Atlas of Paxinos and Watson (Paxinos and Watson, 2005). Coordination of PVN was as follows: 7.44 mm rostral to the interaural line, right+0.5-0.7mm from the medial suture and 7.8 mm deep from the bregma.

Following insertion of catheter in femoral artery and stereotaxic here was a waiting period of 20-25 minutes before measuring cardiovascular variables. Arterial pressure and heart rate were then recorded for an additional 15 to 30 minutes before injections, and for a period of 45 minutes following injection of resistin, vehicle and other drugs in the PVN parvocellular neurons.

Injection of drugs

Injection of recombinant murine resistin, saline and other drugs was made by pressure into the right side in the PVN using a hand-driven 1µl syringe (KH7001; Hamilton, Reno, NV, USA) connected to a 33 gauge needle by PE-10 tubing. Injections were performed in a volume of 1µl. After injection, the needle was kept within the guide cannula for 1min. The needle was 0.5mm longer than the guide cannula.

Confirming the site of injection

At the end of the each experiment, for confirming injected site, 1µl Evan's blue dye was injected as a marker into the PVN. After 10 minutes, 5ml of 10% formalin was injected directly into the left ventricle. Then the brain was removed and fixed in a 10% formalin solution for at least 3 weeks. After this period, serial sections were prepared from the tissues. The injection sites were confirmed according to rat brain atlas of Paxinos and Watson (2005) under the light microscope (Fig. 1).

Data analysis

The average values of AP and HR were measured before and during 45 minutes after each injection. The average values of these variables for a 20-minute period prior to injection were measured as the baseline levels. The maximum changes in AP and

HR during the 45 minutes after injections into the PVN were compared to before injection by pairedt-test and or were compared to those after vehicle injection into the same region by the independent t-test. A probability value $P<0.05$ was taken to indicate a statistically significant difference. All data are presented as mean±standard error of mean (±SEM). Data were analyzed by the Statistical Package for Social Sciences (SPSS 16.0 for Windows).

Results

The mean values (±SEM) of arterial pressure, heart rate and complex QRS amplitude are presented in Fig. 3 and 4. The baseline levels of AP and HR which are averages of value over the 20-minute period before injections were 92.03±3.19 mmHg and 375.5±18.6 beats/minute, respectively, and in the control group (saline) after injection of saline into PVN were 94.22±3.02 mmHg and 381.38±16.39 beat/minute, respectively.

Injection of resistin (3 and 5µg/rat) into the PVN caused a significant pressor response (Fig. 3) on HR and AP compared to control group ($P<0.05$). The responses were very sharp and quick compared to before injection and control group. These responses began in the first moments of the resistin injection and reached a peak after 1-3 minutes (Fig. 2). The

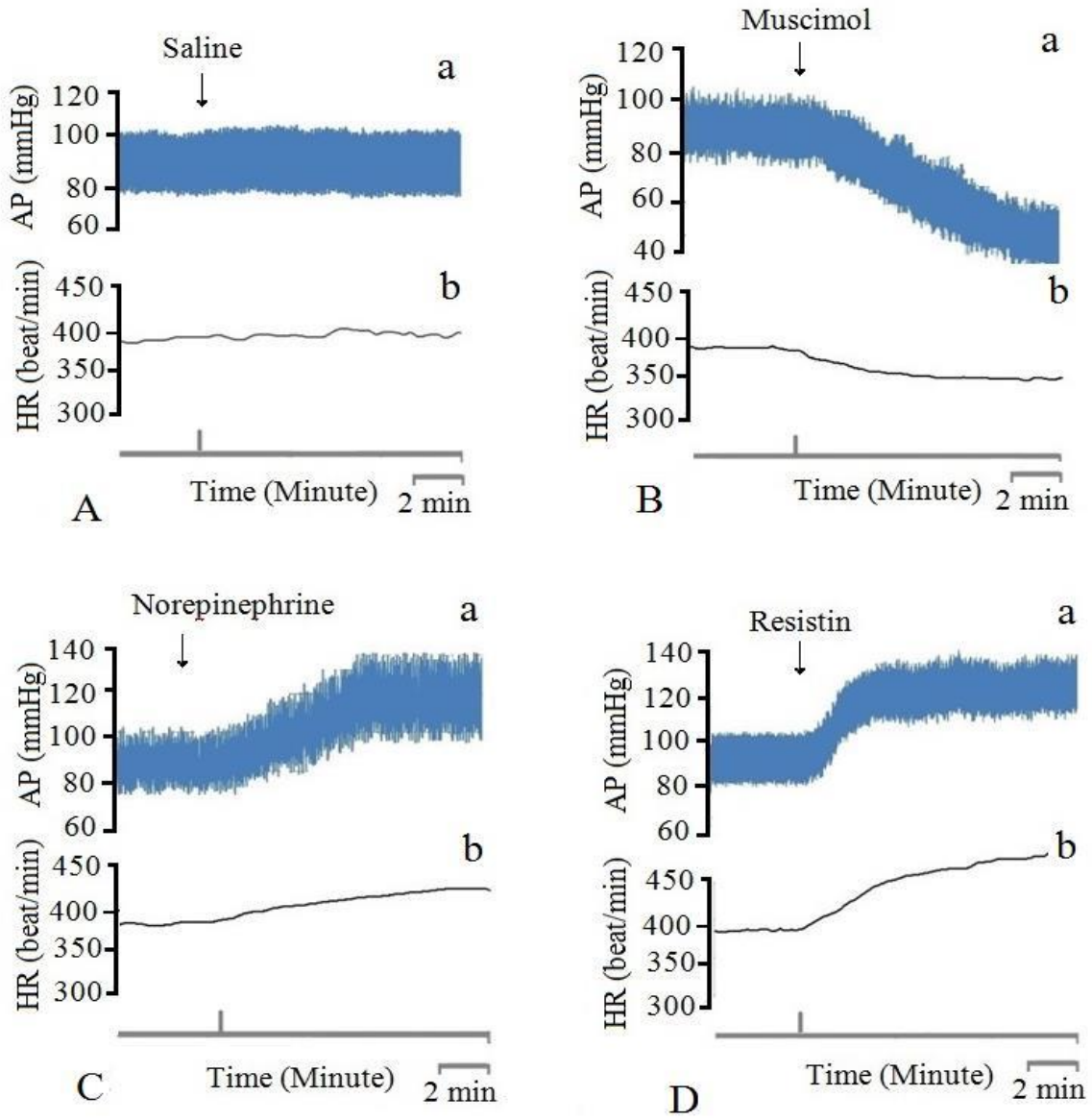

Fig.2. Typical recording of (a) arterial blood pressure (AP) and (b) heart rate (HR) in response to different injections in PVN. The vertical lines indicate the injection time.
Panel A-D demonstrate injection of different drugs including: Saline, Muscimol, Norepinephrine and Resistin.

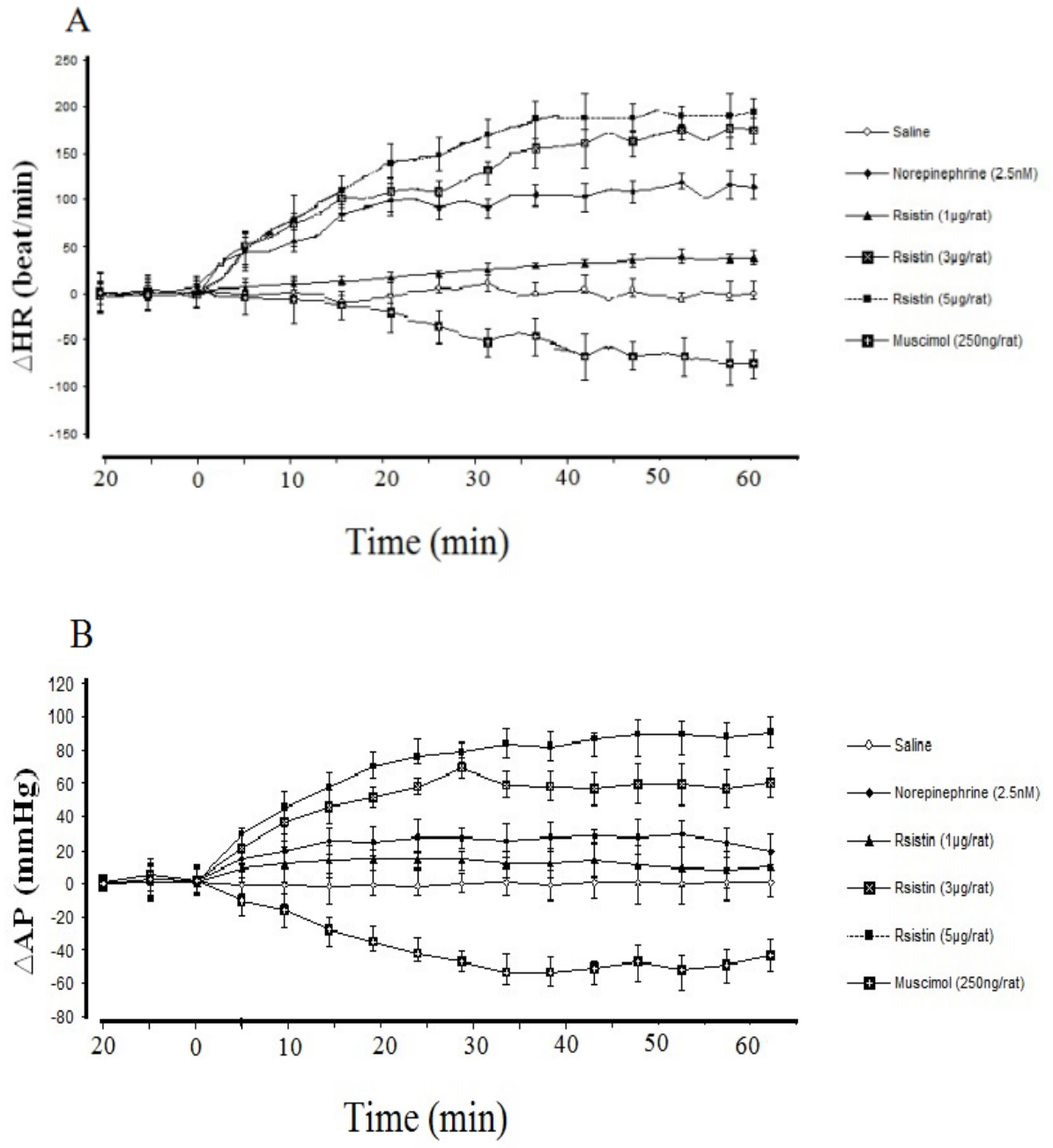

Fig.3. Time course for resistin, muscimol, norepinephrine and saline injection in PVN on changes in (A) heart rate (HR) and (B) arterial pressure (AP) in anesthetized rats. In each case, the time zero represents the point at which injections were made. Significant difference was observed among groups from 5min after injection of drugs and later. All values are represented as mean±SEM.

AP in the first moments after resistin (3μg/rat) injection began to rise and reached to its maximum (152±5 mmHg) and remained unchanged for over 45 minutes. This value was also significantly greater than before resistin injection (91.96±4.045 mmHg, $P<0.05$) and compared to the control group (92.2±3.19 mmHg, $P<0.05$). Injection of resistin (3μg/rat) into the PVN also significantly increased HR from 376.36±21.69 to 463.586±19.34 and compared to the control group (381.38±16.39 beat/minute, $P<0.05$). Injection of resistin (5 μg/rat) into the PVN significantly raises AP from 90.54±4.78 to 168.84±4.68 and HR from 368.36±19.54 to 496.652±21.51 ($P<0.05$, Fig 3) and also increased QRS amplitude compared with before injection and compared to the control group ($P<0.05$, Fig. 4). However, injection of low dose of resistin (1 μg/rat) into PVN (Fig. 3) does not increase AP, HR and QRS amplitude significantly ($P>0.05$).

Norepinephrine injection into the PVN significantly

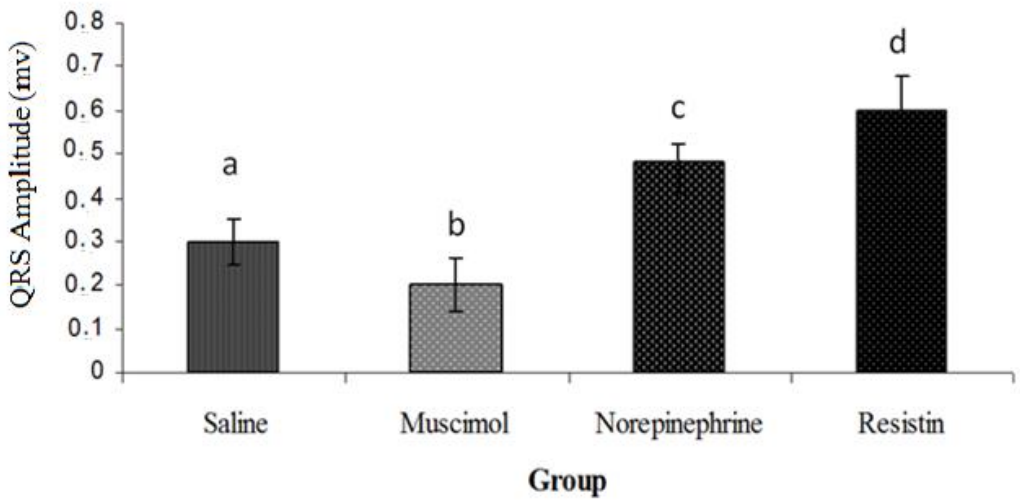

Fig.4. Mean ±SEM changes in QRS amplitude in different groups (n=6). Small letters shows the statistical difference among groups.

increased AP, HR and QRS amplitude compared with before injection and compared to the control group (P<0.05, Fig. 3 and 4). While, injection of muscimol into the PVN significantly reduced these responses compared with before injection and compared to the control group (P<0.05, Fig. 3 and 4).

Discussion

In the present study we showed that the paraventricular nucleus was one of the major central areas for actions of resistin, and resistin caused pressor responses and tachycardia. PVN plays a key role in central controlling activity of the cardiovascular system, so that both chemical and electrical stimulations of it generate cardiovascular responses in various species. Stimulation of the PVN by L-glutamate increases renal sympathetic nerve activity, plasma concentrations of adrenaline and nor-adrenaline, blood pressure and heart rate in conscious rats (Busnardo et al., 2009; Coote et al., 1998), whereas in anaesthetized rats a depressor response and bradycardia (Kannan et al., 1988) are generated. These responses are mediated by a local nitric oxide-guanylatecyclase mechanism associated with NMDA glutamate receptors (Busnardo et al., 2010; Busnardo et al., 2009). Activation of NMDA glutamate receptors will result in production of nitric oxide, which potentiates the inhibitory effects of GABA via $GABA_A$ receptors in PVN (Benarroch, 2005).

Injection of muscimol, a selective $GABA_A$ receptor agonist, into the PVN was reported to decrease renal sympathetic activity, blood pressure and heart rate (Allen, 2002), which was consistent with our results. Some studies also showed that injection of bicuculline, a selective $GABA_A$ receptor antagonist, into the PVN caused an increase in glutamate release and produced a reverse effect on these cardiovascular parameters (Chen et al., 2003). These results suggest that the PVN GABAergic neurotransmission has a tonic inhibition in glutamate action on sympathetic activity and cardiovascular responses (Chen et al., 2003; Li et al., 2006). The PVN receives noradrenergic nerve endings from medullary A1 and A2 noradrenergic cells and the A6 locus coeruleus cells (Rinaman, 2011; Samuels and Szabadi, 2008). The α_1 and α_2-adrenoceptors were detected in the PVN (Papay et al., 2004; Stone et al., 2007) and the activation of α_1-adrenoceptor in the PVN is associated with the autonomic response to stress (Stone et al., 2007) and increases sympathetic outflow (Chen et al., 2006). These responses occur through increasing the excitability of PVN pre-sympathetic neurons primarily via enhancement of glutamatergic tone and attenuation of GABAergic input (Chen et al., 2006). In addition, in an electrophysiological study, Daftary et al. suggested that “the noradrenergic regulation of PVN parvocellular neurons takes place either via a direct β-adrenoreceptor-mediated inhibition or via α_1-receptor-mediated activation of intra-hypothalamic glutamatergic circuits” (Daftary et al., 2000). The α_2-adrenoceptor has also been detected on inhibitory

GABAergic neurons synapsing with these spinally-projecting PVN neurons, these projections via the release of norepinephrine from the locus coeruleus (Li et al., 2006; Samuels and Szabadi, 2008) influence functions of the autonomic nervous system relating to cardiovascular regulation, for example, suppression of the baroreceptor reflex (Hwang et al., 1998; Samuels and Szabadi, 2008). Therefore, it can be concluded that cardiovascular effects of norepinephrine in PVN are mediated by synergy between noradrenergic and glutamatergic synaptic and inhibition of GABAergic inputs which play an important role in controlling PVN activity and sympathetic outflow.

Our results showed that cardiovascular responses are produced by injection of resistin into PVN, it has already been reported that this nucleus has a role in regulation of cardiovascular system (Benarroch, 2005; Guyenet, 2006), hence the observed effect could to due to presence or expression of resistin receptors in this nucleus, which need to be investigated. Resistin-sensitive neurons within the PVN might modulate cardiovascular responses both via direct projections to sympathetic preganglionic neurons in the spinal intermediolateral cell column (IML) and via collateral projections to neurons of the rostral ventrolateral medulla that send excitatory inputs to the IML (Benarroch, 2005; Guyenet, 2006). In our study, we indicated that resistin in a dose dependent manner increases the cardiovascular parameters and the time course of cardiovascular responses to resistin injection into the PVN was very fast, so that in the first moments after injection of resistin the blood pressure began to rise and reached a peak after 1-2 minutes, somewhat similar to injection of norepinephrine. The intracellular mechanisms that are activated by resistin in the PVN neurons are not completely understood, although Rodriguez-Pacheco et al. showed that the stimulatory action of resistin take places through a Gs protein-dependent mechanism, adenylatecyclase/cAMP/protein kinas A pathway, the phosphatidylinositol 3-kinase/Akt pathway, protein kinas C and extracellular Ca^{2+} entry through L-type Ca^{2+} channels in pituitary somatotrope cells (Rodriguez-Pacheco et al., 2009). Thus, the fast cardiovascular responses to action of resistin presumably reflect the time course of activation of these or other components of the intracellular signaling pathway. On the other hand, resistin regulates dopamine, norepinephrine and serotonin release in the hypothalamus (Brunetti et al., 2004), which could partially mediate their cardiovascular effects. Resistin may act on its receptors in the PVN nerve endings causing entry of extracellular Ca^{2+} through L-type Ca^{2+} channels and release of norepinephrine, which in itself increases blood pressure and heart rate. The proposed mechanism is consistent with the results of the norepinephrine and resistin groups. According to our results, cardiovascular response to resistin injection was significantly greater and longer than norepinephrine injection. This may be due to stimulating effect of resistin on other excitatory neural systems or inhibition of GABAergic inputs. Moreover, since the injection site of resistin was PVN parvocellular neurons, it may spread out to other PVN are as, especially the magnocellular, and causes release of vasopressin. Recent studies have shown that central administration of resistin in lateral ventricle regulates hypothalamic and peripheral lipid metabolism in a nutritional-dependent fashion (Vazquez et al., 2008) and promotes short term satiety in rats (Tovar et al., 2005). Although these studies suggested that resistin can activate neurons in the different regions of the hypothalamus that regulate sympathetic nerves affecting energy homeostasis and food intake, no measurements were made of cardiovascular variables. Kosari et al. reported that central administration of resistin (7µg/rat) into lateral ventricle (LV) enhances sympathetic nerve activity to the hind limb but attenuates the activity to brown adipose tissue. They also showed that injection of resistin into LV increased distribution of the protein Fos, a marker of increased neuronal activity, in the hypothalamic nuclei, however, their reported indicated that administration of resistin into LV had no effect on cardiovascular parameters (Kosari et al., 2011), which was inconsistent with our results. This could be due to difference in the site and doses of injected resistin.

Conclusion

Based on above mentioned reports and our findings, it can be assumed that brain-derived resistin as a new neuropeptide in addition to regulating food intake and energy homeostasis could control cardiovascular

responses in PVN as a central site for regulating cardiovascular activity. Understanding the central action of resistin provides a unique model for unraveling the interactions among the brain, resistin, and peripheral organs, and could potentially shed new light on the pathogenesis of metabolic disorders associated with hypertension, cardiovascular disorder and obesity.

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Conflict of interest

The authors report no conflict of interest.

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