Regulatory Peptides 186 (2013) 7–11

Contents lists available at ScienceDirect

Regulatory Peptides

journal homepage: www.elsevier.com/locate/regpep

Intracerebroventricular injection of stresscopin-related peptide enhances cardiovascular function in conscious rats

Ri Jin [a,b,1], Mei-Zi Li [a,1], Yan-Hua Bing [a,c], Ri-Long Piao [a], Ying-Jun Li [a], Qing-Hua Jin [a,c], De-Lai Qiu [a,c,*], Hiroshi Kannan [d], Chun-Ping Chu [a,**]

[a] Cellular Function Research Center, Yanbian University, 977 GongYuan Road, Yanji City, Jilin Province 133002, China
[b] Department of Osteology, Affiliated Hospital of Yanbian University, Yanji City, Jilin Province, China
[c] Department of Physiology and Pathophysiology, College of Medicine, Yanbian University, 977 GongYuan Road, Yanji City, Jilin Province 133002, China
[d] Department of Nutrition, Faculty of Home Economics, Kyushu Women's University, 1-1, Jiyugaoka, Yahatanishi-ku, Kitakyushu 807-8586, Japan

ARTICLE INFO

Article history:
Received 6 July 2012
Received in revised form 29 June 2013
Accepted 5 July 2013
Available online 11 July 2013

Keywords:
Neuropeptide
Mean arterial blood pressure
Heart rate
Epinephrine
Norepinephrine

ABSTRACT

Stresscopin-related peptide (SRP), which is a member of the corticotropin-releasing factor (CRF) family, is a high-affinity ligand for the type 2 corticotropin-releasing factor receptor (CRF-R2) and is involved in stress-coping responses. Central treatment with SRP suppresses food intake, delays gastric emptying and decreases heat-induced edema, but the effects of central administration of SRP on the cardiovascular system are unclear. Here we examined the effects of intracerebroventricular (i.c.v.) administration of SRP on cardiovascular function, and compared the cardiovascular effects of SRP and stresscopin (SCP). Our results showed that i.c.v. administration of SRP (0.5 nmol) increased mean arterial blood pressure (MABP) and heart rate (HR), but failed to increase plasma norepinephrine and epinephrine levels. Compared with an equivalent dose of SCP, the area under the curve (AUC) values for the changes in MABP and HR were significantly smaller with SRP, indicating that the cardiovascular effects of SRP were weaker than those mediated by SCP. Pre-treatment with a selective CRF-R2 antagonist, antisauvagine-30 (4 nmol, i.c.v.) abolished the SRP and SCP induced changes in MABP and HR. These results indicate that central administration of SRP induces a weaker enhancement of cardiovascular function through CRF-R2 than that induced by SCP and that these effects are mediated without increasing plasma norepinephrine and epinephrine levels.

1. Introduction

Stresscopin-related peptide (SRP), a human homolog of urocortin II (Ucn II), is a member of the mammalian corticotrophin-releasing factor (CRF) family of peptides [1,2]. The CRF family includes CRF, urocortin (Ucn I), urocortin II (Ucn II) and urocortin III (Ucn III) in mammals, and the human orthologs of Ucn II and Ucn III are SRP and stresscopin (SCP) respectively. SRP is more closely related to Ucn III than to Ucn I, which is associated with high biological activity [3–5].

CRF is the main mediator of stress responses. Two G-protein-coupled receptors, termed CRF-receptor 1 (CRF-R1) and CRF-receptor 2 (CRF-R2), have been identified as CRF receptors [6]. CRF binds with high affinity to CRF-R1, but with lower affinity to CRF-R2 [7,8]. However, human SRP is a specific ligand for CRF-R2 [1]. In vitro binding studies showed that SRP binds CRF-R2 with high affinity but with lowest affinity for CRF-R1 [1,3]. CRF-R2 is expressed mainly in the paraventricular nucleus (PVN) and hypothalamic ventromedial nucleus, medial amygdaloid nucleus and lateral septal nucleus of the brain [9,10]. SRP is expressed in discrete regions of the central nervous system (CNS), including in the parvocellular and magnocellular divisions of the PVN, the supraoptic nucleus (SON) and arcuate nuclei of the hypothalamus and locus coeruleus [2,11]. This suggests that the action of SRP on the CNS might affect cardiovascular regulation via CRF-R2.

When administered peripherally, SRP causes hypotension, tachycardia, and vasodilatation and reduces plasma catecholamine levels in rats or mice [12,13]. Intracerebroventricular (i.c.v.) injection of SRP was shown to attenuate night-time feeding but failed to increase gross motor activity in conscious rats [2,14]. It has been suggested that peptides that affect food intake influence cardiovascular responses and sympathetic nervous system activity [15–17]. The PVN, which is an important site of endocrine and autonomic function, is involved in autonomic control of cardiovascular activity and stress responses. The PVN magnocellular neurons integrate incoming information and secrete vasopressin and oxytocin into the posterior pituitary, whereas the PVN parvocellular neurons comprise neuroendocrine neurons and pre-autonomic neurons [18]. The PVN pre-autonomic neurons project directly towards the sympathetic preganglionic neurons in the spinal

* Correspondence to: D.-L. Qiu, Cellular Function Research Center, Yanbian University, 977 GongYuan Road, Yanji City, Jilin Province 133002, China.
** Corresponding author. Tel.: +86 433 2435050; fax: +86 433 2435051.
E-mail addresses: dlqiu@ybu.edu.cn, dlqiu_neuron@hotmail.com (D.-L. Qiu), cpchu@ybu.edu.cn, chpchu@hotmail.com (C.-P. Chu).
[1] These authors contributed equally to this work.

0167-0115/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.regpep.2013.07.001

cord and control sympathetic outflow [19]. We have previously reported that i.c.v. administration of SCP, another specific ligand of CRF-R2, elicits an increase in blood pressure (BP), heart rate (HR) and plasma catecholamine levels in conscious rats, suggesting that SRP might affect cardiovascular function through activation of CRF-R2 [10,17]. In addition, SRP appeared to have a higher potency (>10 fold) as a CRF-R2 than SCP [1], but the anti-apoptotic and protective effects mediated by SCP in cardiomyocytes were more potent than those mediated by SRP in rats [20]. Moreover, SRP caused significant hypotension and tachycardia in the periphery [12,13], but i.c.v. administration of SCP has been shown to affect the cardiovascular system in conscious rats [17].

Taken together, SRP may affect cardiovascular function through activation of central CRF-R2, but the effects and possible mechanisms of central administration of SRP on the cardiovascular system are currently unclear. Therefore, we here examined the effects of i.c.v. administration of SRP on mean arterial blood pressure (MABP), HR and plasma norepinephrine and epinephrine levels and compared with SCP in conscious, freely moving rats.

2. Materials and methods

2.1. Animal preparation and data collection

The experimental procedures used in this study were approved by the Animal Care and Use Committee of Jilin University and were in accordance with the animal welfare guidelines of the National Institutes of Health. The anesthesia and surgical procedures used in this study have been described previously [17]. In brief, male Sprague–Dawley rats (350–380 g) were implanted with a lateral cerebroventricular cannula under anesthesia [50 mg/kg, intraperitoneal (i.p.) injection of pentobarbital sodium]. A guide cannula was positioned 2.5 mm from the cortex surface, 1 mm above the left lateral cerebroventricle, 0.8 mm posterior and 1.5 mm lateral to the bregma. SP-31 tubing heat-coupled to SP-50 tubing was then inserted into the abdominal aorta for measurement of MABP, and a PE-50 catheter was inserted into the inferior vena cava for intravenous administration of drugs. The arterial catheter, filled with heparinized (10 U/ml) saline solution, was connected to a PT-100 blood pressure transducer (Chengdu Technology & Market Co., Ltd., China) and data were recorded via a BL-420 biological signal processing system. HR was determined according to the BP wave. BP and HR were monitored simultaneously and recorded on a Data Acquisition and Analysis System (BL-420S, Chengdu TME Technology Co., Ltd., China). Arterial and venous catheters were tunneled under the skin to exteriorize at the nape of the neck. I.c.v. administration of 10 pmol angiotensin II induced acute elevation (>20 mm Hg) of MABP and persistent (at least 10 min) water-drinking were considered to be indicators of both cannula patency and correct placement in the ventricular system.

2.2. Measurement of plasma catecholamine levels

Another group of weight- and age-matched rats were anesthetized with pentobarbital sodium (50 mg/kg i.p.) to measure plasma catecholamine levels. Arterial and venous catheters were inserted into the abdominal aorta and inferior vena cava, respectively. Arterial blood (1 mL) was withdrawn through the cannula from the abdominal aorta. To avoid the confounding effects of acute hemorrhage, the same amount of physiological saline solution was infused into the intravenous catheter at the same rate at which the blood was withdrawn. These blood samples were immediately heparinized and centrifuged at 3000 rpm, followed by reconstitution in 0.3 mL of 0.5 M acetic acid solution to obtain the final samples. Catecholamine concentration was measured by high-performance liquid chromatography (HPLC) and electrochemical detection using an Eicompak CA-5ODS column (Eicom, Kyoto, Japan).

2.3. Experimental protocol

All experiments were performed on conscious, freely moving rats at 3 to 5 days after surgery. Chow and water were not available during the recording time. First, the cardiovascular effects of SRP were investigated in four groups of rats. Following stabilization of MABP and HR, vehicle (physiological saline solution), or SRP (0.05 or 0.5 nmol; Peptide Institute, Japan), or SCP (0.5 nmol; Peptide Institute, Japan) was injected i.c.v. into conscious rats through an infusion cannula (30-gauge stainless steel tubing) connected to a 100-μL microsyringe by an automatic injector (LMS, Tokyo, Japan) at a rate of 5 μL/min for 1 min, and MABP and HR were recorded. Second, plasma catecholamine concentrations were measured in another four groups of rats. Each rat received one injection of vehicle or SRP (0.05 nmol or 0.5 nmol) or 0.5 nmol SCP. Blood samples were obtained 30 min before and 10 min after i.c.v. administration of the vehicle or drugs. Third, to identify the role of CRF2 receptors in the effects of SRP and SCP (0.5 nmol), a pre-treatment study with antisauvagine-30 (ASV30, Tocris Bioscience) was performed. In six groups, rats received two i.c.v. injections: the first injection (saline or 4 nmol ASV30) was followed 15 min [21] later by the second injection (saline or 0.5 nmol SRP or 0.5 nmol SCP). After each experiment, Pontamine sky blue (1 μL) was injected to verify correct placement of the i.c.v. cannula tip. The species synthesizing mouse Ucn II (Sigma) was also tested.

2.4. Statistical analysis

To provide a description of both the duration and magnitude of the cardiovascular and sympathetic responses, the area under the curve (AUC) was calculated [22]. All data are expressed as mean ± SEM. Statistical analyses of data were performed using ANOVA for repeated measurements followed by the Bonferroni multiple comparisons test. Maximum changes from control values and the AUC were analyzed using Student's t-tests. $P < 0.05$ was considered statistically significant.

3. Results

The effects of i.c.v. administration of SRP (0.05 and 0.5 nmol) and SCP (0.5 nmol) on MABP and HR are illustrated in Fig. 1. I.c.v. administration of 0.05 nmol SRP did not induce a significant change in MABP (94.8 ± 1.3 to 96.9 ± 2.9 mm Hg; $n = 6$; $P > 0.05$) or HR (302.4 ± 7.3 to 305.5 ± 15.1 bpm; $n = 6$; $P > 0.05$). However, i.c.v. administration of 0.5 nmol SRP induced a transient increase in MABP and HR; the mean values of MABP and HR were increased from 94.3 ± 1.1 mm Hg to 104.7 ± 2.1 mm Hg ($n = 6$; $P < 0.05$), and from 302.1 ± 8.4 bpm to 359.9 ± 10.2 bpm ($n = 6$; $P < 0.05$), respectively. Similar to our previous study, SCP also induced an increase in MABP and HR [17]. The CNS administered SRP induced increase in MABP and HR lasted for 20 ± 2.1 min ($n = 6$), which was significantly shorter than that induced by CNS administration of 0.5 nmol SCP (30 ± 2.3 min; $n = 6$; $P < 0.05$; data not shown). These results indicated that SRP induced a transient change in cardiovascular function, and that the SRP induced change in MABP and HR was significantly more rapid than that induced by SCP.

Furthermore, we calculated the maximal changes and AUC of MABP and HR after i.c.v. administration of SRP and SCP. I.c.v. administration of SRP and SCP induced significant increases in the maximal changes in MABP and HR. The mean values of the maximal changes in MABP for the control, SRP and SCP were 0.6 ± 0.5 mm Hg (control; $n = 6$), 9.2 ± 1.3 mm Hg (SRP; $n = 6$; $P < 0.05$ vs. control) and 8.4 ± 1.9 mm Hg (SCP; $n = 6$; $P < 0.05$ vs. control; $P > 0.05$ vs. SRP), respectively (Fig. 2A). The mean values of the maximal changes in HR for the control, SRP and SCP were 2.5 ± 3.4 bpm (control; $n = 6$), 55.5 ± 10.7 bpm (SRP; $n = 6$; $P < 0.05$ vs. control) and 47.8 ± 14.6 bpm (SCP; $n = 6$; $P < 0.05$ vs. control; $P > 0.05$ vs. SRP), respectively (Fig. 2A). The AUC values were calculated for the 30 min