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Short communication

Excitatory responses of cardiovascular activities to urocortin3 administration into the PVN of the rat

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ABSTRACT

Urocortin3 (Ucn3) is an endogenous ligand for corticotropin-releasing factor receptor subtype 2 (CRF_2R). In this study, we examined its potential cardiovascular effects by microinjection of Ucn3 and anti-sauvagine 30 (ASV30), a selective antagonist of CRF_2R, into the paraventricular nucleus (PVN) of the hypothalamus. After Ucn3 (10 pmol/100 nl) was microinjected into the PVN of anesthetized rats, significant increases of systolic blood pressure, heart rate and renal sympathetic nerve activity were observed. Furthermore, all these cardiovascular and autonomic effects induced by Ucn3 could be blocked totally by administration of ASV30 into the PVN. These results are consistent with the idea that Ucn3 might be involved in the central nervous control of cardiovascular function by acting centrally to increase sympathetic outflow via the activation of CRF_2R within the PVN.

Corticotropin-releasing factor (CRF) is well documented as a mediator in stress responses. In mammals, two major receptor subtypes of CRF have been identified, namely CRF receptor type 1 (CRF_1R) and CRF receptor type 2 (CRF_2R) (Dautzenberg et al., 2001). In recent years, three new peptides, urocortin, urocortin2 and urocortin3 (Ucn3), have been cloned and recognized as members of CRF family (Vaughan et al., 1995; Hsu and Hsueh, 2001; Reyes et al., 2001; Lewis et al., 2001). Among these new peptides, Ucn3 is distributed broadly both centrally and peripherally (Vita et al., 1993; Potter et al., 1994) and is considered as an exclusive ligand for CRF_2R. In the central nervous system (CNS), highly labeled neurons of Ucn3 and Ucn3 immunoreactive positive fibers were detected in the hypothalamus (including the hypothalamic median preoptic nucleus, the rostral perifornical area, the medial preoptic area, paraventricular nucleus (PVN), and ventromedial nuclei) (Li et al., 2002). Moreover, its selective receptor, CRF_2R mRNAs are also highly expressed in the hypothalamus especially in the hypothalamic ventromedial nucleus and the PVN (Chalmers et al., 1995; Van Pett et al., 2000), suggesting an involvement of an Ucn3-CRF_2R pathway in the hypothalamus under certain conditions.

There is evidence that central Ucn3 might be involved in the regulation of cardiovascular activity, but possible sites and mechanisms of action are unclear (Chu et al., 2004; Nakamura et al., 2009). Chu et al. reported that intracerebroventricular (i.c.v) administration of stresscopin, a human homologue of Ucn3, induced an increase of systolic blood pressure (SBP) and heart rate (HR) (Chu et al., 2004). In contrast, administration of Ucn3 into the medial nucleus of solitary tractus (mNTS) elicited depressor and bradycardic responses accompanied by a decrease of efferent greater splanchnic nerve activity (Nakamura et al., 2009). The PVN of the hypothalamus is an important central area involving in the integration of cardiovascular activities (Coote, 2005), but cardiovascular effects of Ucn3 in this site have not been identified. In this study Ucn3 was microinjected into the PVN of Sprague–Dawley (SD) rats to further clarify its central actions on cardiovascular activity.

In the current study, male SD rats (250–300 g, $n = 32$) were used. All rats were housed under controlled conditions with a 12-h:12-h light–dark cycle, free access to food and water. Experiments were performed with the approval of the Institutional Animal Ethical Committee of Fudan University. After rats were anesthetized with a mixture of urethane and chloralose (700 mg/kg and 35 mg/kg, respectively, i.p.), the left femoral artery was cannulated using a catheter which was connected to a computer-based data acquisition system (SMUP-PC, Shanghai) via a pressure transducer. Electrocardial signals of the rats were also sequentially recorded via a transducer by a biological signal recording system (SMUP-PC, Shanghai). Body temperature of the rats was monitored with a rectal thermometer and maintained at around 37.8 °C throughout the duration of the experiment by using a feedback controlled heating blanket.

After that, the rats were fixed in a stereotaxic frame in a prone position (Narishige, Japan). A midline incision was made to expose the skull such that a small burr hole could be drilled to permit the

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advancement of an injection pipette into the region of the right PVN according to the coordinates of Paxinos and Watson (caudal to Bregma: 1.6–2.0 mm; lateral: 0.5–0.7 mm; ventral to dura: 7.0–7.5 mm). All reagents were microinjected manually (100 nl over about 2 min) using a microsyringe (Hemilton, USA) connected to the stainless steel pipette (outer diameter: 0.125 mm) by a plastic tube. Microinjection of aCSF (pH 7.4) into the PVN was used as a control.

To record renal sympathetic nerve activity (RSNA), the left kidney was exposed retroperitoneally. The renal nerve bundle was isolated from surrounding tissues and mounted on a bipolar electrode. The renal nerve branch and the electrode were immersed in warm liquid paraffin. Nerve activity was amplified (SMUP-E, Shanghai) and rectified. The sampling rate was 1000 Hz. Mean RSNA was obtained by integrating the rectified nerve signal (bin width, 1 s). Mean level of integrated RSNA in 2 min before drug administration was determined as the 100% activity level. To reflect the RSNA change induced by reagent administration, the ratio of integrated RSNA after reagent application to the pre-reagent was calculated.

At the end of each experiment, 2% pontamine sky blue (100 nl) was microinjected into the PVN where the reagents had been applied.

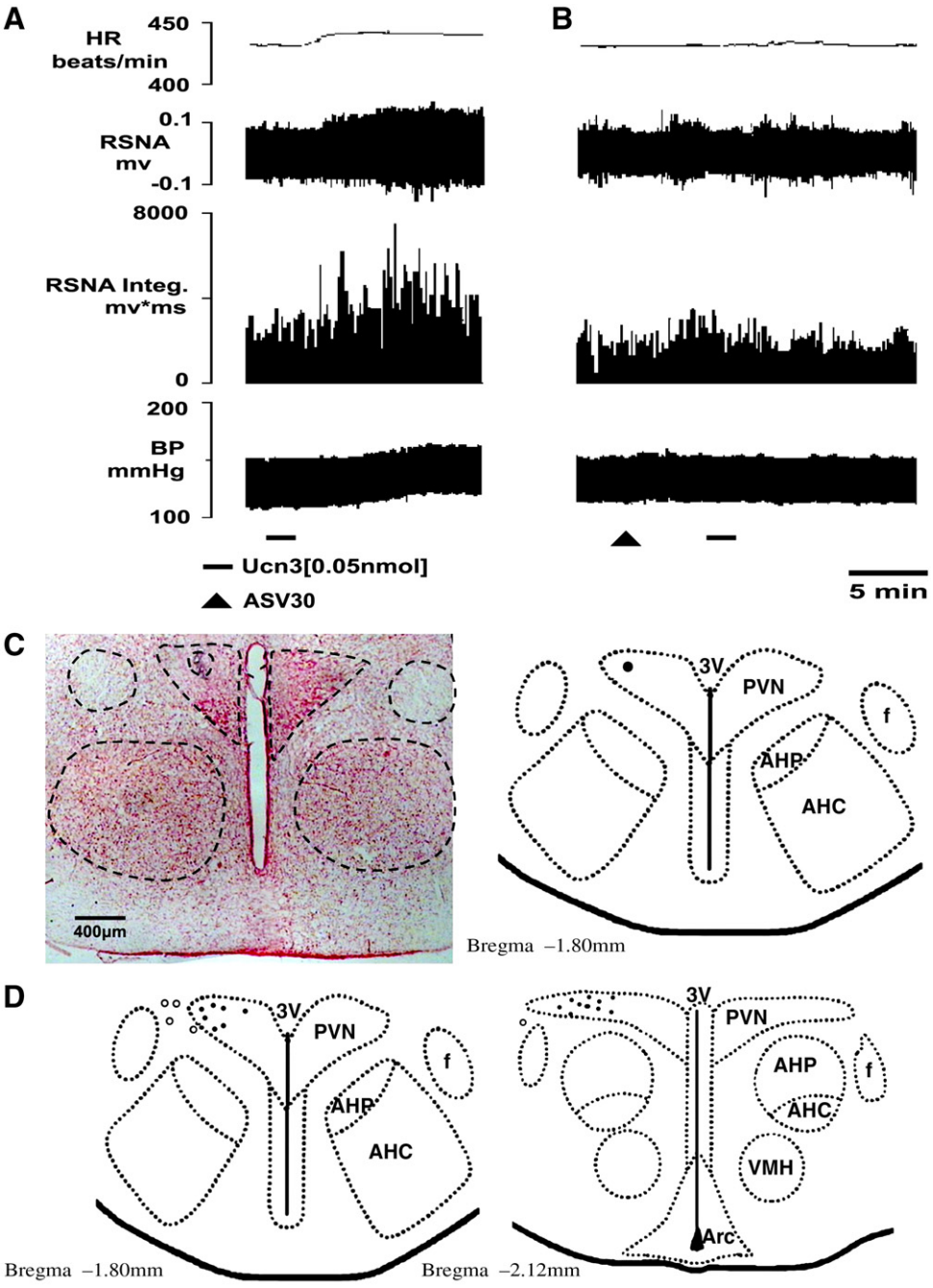

Fig. 1. Increases of SBP, HR and RSNA were observed post Ucn3 (10 pmol/100 nl) microinjection into the hypothalamic paraventricular nucleus (PVN) of Sprague Dawley rats. Traces showed systolic blood pressure (SBP, bottom), integrated and raw data of renal sympathetic nerve activity (RSNA, middle) as well as heart rate (HR, top) responses to: A, microinjection of Ucn3 (bar) into the PVN; and B, subsequent administration of Ucn3 (10 pmol/100 nl) 5 min after selective CRF_2R antagonist ASV30 (0.1 nmol/100 nl, arrowhead). Illustration showed a representative microinjection site within the PVN (C) and schema showed the distribution of microinjection sites in the hypothalamus (D). Dots represent microinjection sites within the PVN ($n=17$); circles represent microinjection sites outside the anatomical boundaries of the PVN ($n=5$). PVN, paraventricular nucleus of hypothalamus; AHP, anterior hypothalamic nucleus, posterior part; AHC, anterior hypothalamic nucleus, central part; VMH, ventralmedial nucleus of hypothalamus; Arc, arcuate nucleus of hypothalamus; f, fornix; 3 V, third ventricule.