Regulatory Peptides 166 (2011) 112–120

Contents lists available at ScienceDirect

Regulatory Peptides

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

Activation of the melanocortin-4 receptor causes enhanced excitation in presympathetic paraventricular neurons in obese Zucker rats

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ARTICLE INFO

Article history:
Received 8 July 2010
Received in revised form 26 August 2010
Accepted 4 October 2010
Available online 16 October 2010

Keywords:
Obese zucker rats
Melanocortin 4 receptors
Hypothalamus
Sympathetic nervous system

ABSTRACT

Sympathetic nerve activity is increased in obesity-related hypertension. However, the central mechanisms involved in the increased sympathetic outflow remain unclear. The hypothalamic melanocortin system is important for regulating energy balance and sympathetic outflow. To understand the mechanisms by which the melanocortin systems regulates sympathetic outflow, we investigated the role of melanocortin 4 receptors (MC4R) in regulating presympathetic paraventricular nucleus (PVN) neurons. We performed whole-cell patch-clamp recordings on retrogradely labeled PVN neurons projecting to the rostral ventrolateral medulla in brain slices from obese zucker rats (OZRs) and lean zucker rats (LZRs). The MC4R agonists melanotan II (MTII) and α-melanocyte-stimulating hormone (α-MSH) increased the firing activity and depolarized the labeled PVN neurons from both LZRs and OZRs in a concentration-dependent manner. MTII produced significant greater increase in the firing activity in OZRs than in LZRs. Blocking MC4R with the specific antagonist SHU9119 had no effect on the basal firing rate but abolished the MTII-induced increase in the firing rate in both OZRs and LZRs. Furthermore, intracellular dialysis of guanosine 5′-*O*-(2-thodiphosphate), but not bath application of kynurenic acid and bicuculline, eliminated the MTII-induced increase in firing activity. In addition, MTII had no effect on the frequency and amplitude of glutamatergic excitatory postsynaptic currents and GABAergic inhibitory postsynaptic currents in labeled PVN neurons. Collectively, our findings suggest that MC4R contributes to the elevated excitability of PVN presympathetic neurons, which may be involved in obesity-related hypertension.

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1. Introduction

Obesity is a major health problem and confers an increased risk of cardiovascular diseases, type II diabetes, and end-stage renal disease [1,2]. Excessive weight gain, especially with visceral obesity, is associated with increased sympathetic nerve activity, which contributes to high blood pressure in obese humans as well as in animal models of obesity [1,3,4]. Obese Zucker rats (OZRs), which have nonfunctional leptin receptors owing to gene mutation, possess metabolic abnormalities such as hyperphagia, insulin resistance, hyperinsulinemia, and hyperlipidemia [5–7]. Furthermore, OZRs have cardiovascular complications such as elevated sympathetic outflow and arterial blood pressure [4,8,9]. For example, the arterial blood pressure in free-moving conscious OZRs is significantly higher than that in age-matched lean Zucker rats (LZRs) [10–12]. Furthermore, the resting sympathetic nerve activity is higher in OZRs than in LZRs [9]. Autonomic ganglionic blockade with hexamethonium decreases the mean arterial blood pressure more in OZRs than in LZRs [4,12], suggesting that the heightened sympathetic nerve activity contributes to the elevated blood pressure in OZRs. However, the mechanisms underlying the increased sympathetic activation and hypertension associated with obesity remain unclear.

The melanocortin system plays a pivotal role in feeding and energy homeostasis. The five identified melanocortin receptor subtypes (MC1-MC5) belong to a superfamily of G-protein-coupled seven-transmembrane receptors, and MC3-MC5 are expressed in the brain [13,14]. The melanocortin 4 receptor (MC4R) is widely distributed in the central nervous system (CNS), including the hypothalamus, and is critically involved in the regulation of energy balance and autonomic function [13,15]. Previous studies have shown that the hypothalamic melanocortin system plays an important role in the regulation of sympathetic activity and blood pressure [1,2,16]. For example, intracerebralventricular (ICV)

Abbreviations: PVN, paraventricular nucleus; RVLM, rostral ventrolateral medulla; GABA, γ-aminobutyric acid; aCSF, artificial cerebral spinal fluid; sIPSCs, spontaneous inhibitory postsynaptic currents; sEPSCs, spontaneous excitatory postsynaptic currents; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; GDP-β-S, guanosine 5′-O-(2-thiodiphosphate); MC4R, melanocortin 4 receptors (MC4R); OZRs, obese zucker rats; LZRs, lean zucker rats; MTII, melanotan II; α-MSH, α-melanocyte-stimulating hormone; POMC, pro-opiomelanocortin; AGRP, agouti-related protein.

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0167-0115/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.regpep.2010.10.001

infusion of an MC3/4R-specific antagonist prevented the increase in blood pressure in hyperleptinemic rats [17]. In addition, MC4R-deficient mice have metabolic dysfunction including hyperphagia, hyperinsulinemia, and hyperglycemia [18], but do not have hypertension [19], indicating that MC4R is the key melanocortin receptor involved in the regulation of appetite and metabolic and cardiovascular function. MC4R signaling is modulated by both an endogenous agonist, α-melanocyte stimulation hormone (α-MSH), which is a peptide cleaved from pro-opiomelanocortin (POMC), and an endogenous antagonist, agouti-related protein (AGRP) [20]. Leptin, an adipocyte-derived hormone, acts on POMC and AGRP neurons in the arcuate nucleus of the hypothalamus, resulting in increased α-MSH and decreased AGRP formation [21,22]. It has been shown that there are more binding sites for MC4R in the hypothalamus of OZRs than in LZRs [23]. Therefore, activation of MC4R in the hypothalamus may contribute to the increased sympathetic activity in OZRs.

The hypothalamic paraventricular nucleus (PVN) is an important source of excitatory drive for sympathetic vasomotor tone and is critically involved in regulating neuroendocrine, cardiovascular, and other physiological functions [24–27]. The presympathetic neurons in the PVN project to the vasomotor neurons in the rostral ventrolateral medulla (RVLM) [25,28,29] and preganglionic sympathetic neurons in the intermediolateral cell column (IML) in the spinal cord [30,31]. To understand the mechanisms by which the melanocortin system regulates sympathetic outflow obesity, we determined the effect of activation of MC4R on the excitability of PVN neurons projecting to the RVLM in OZRs.

2. Materials and methods

2.1. Retrograde labeling of RVLM-projecting PVN neurons

Experiments were carried out using male OZRs and age-matched LZRs (13–15 weeks old, Harlan Laboratories, Inc., Indianapolis, IN, USA). The surgical procedures and experimental protocols were approved by the Animal Care and Use Committee at The University of Texas M. D. Anderson Cancer Center and conformed to the National Institutes of Health guidelines on the ethical use of animals. Briefly, rats were anesthetized with 2% to 3% isoflurane in O_2, and the head was placed in a stereotaxic apparatus. A burr hole (about 4 mm in diameter) was drilled in the occipital skull bilaterally according to the following coordinates (Bregma): −11.8 to −13.0 mm to Bregma, 1.8 to 2.2 mm to midline, and 7.8 to 8.1 mm to the surface of the dura. A rhodamine-labeled fluorescent microsphere suspension (FluoSpheres, 0.04 μm, Molecular Probes, Eugene, OR, USA) was injected bilaterally into the region of the RVLM. The microinjection of FluoSpheres was done by using a microinjector (Nanojector II, Drummond Scientific Company, Broomall, PA, USA) and was monitored by a surgical microscope. After injection, the rats were allowed to recover for 3 to 5 days to allow the FluoSpheres to be transported to the PVN [32]. After injection, rats were treated prophylactically with an antibiotic (enrofloxacin 5 mg/kg, subcutaneously daily for 3 days) and an analgesic (buprenorphine 0.2 to 0.5 mg/kg, subcutaneously every 12 h for 2 days).

2.2. Preparation of hypothalamic slices

Brain slices containing the PVN were prepared from the FluoSphere-injected rats, as described previously [33,34]. Briefly, the rats were anesthetized with 2% isoflurane and decapitated, and the brain was quickly removed and placed in ice-cold artificial cerebral spinal fluid (aCSF, saturated with a mixture of 95% O_2 and 5% CO_2). The aCSF solution contained (in mM) 124.0 NaCl, 3.0 KCl, 1.3 $MgSO_4$, 2.4 $CaCl_2$, 1.4 NaH_2PO_4, 10.0 glucose, and 26.0 $NaHCO_3$. A tissue block containing the PVN was glued onto the stage of a vibrating microtome (Technical Products International, St. Louis, MO, USA). Coronal slices (300-μm thick) were cut as described previously [33,34]. The slices were then transferred to an incubation chamber containing aCSF continuously gassed with a mixture of 95% O_2 and 5% CO_2 at 34 °C for at least 1 h before the electrophysiological experiments were performed.

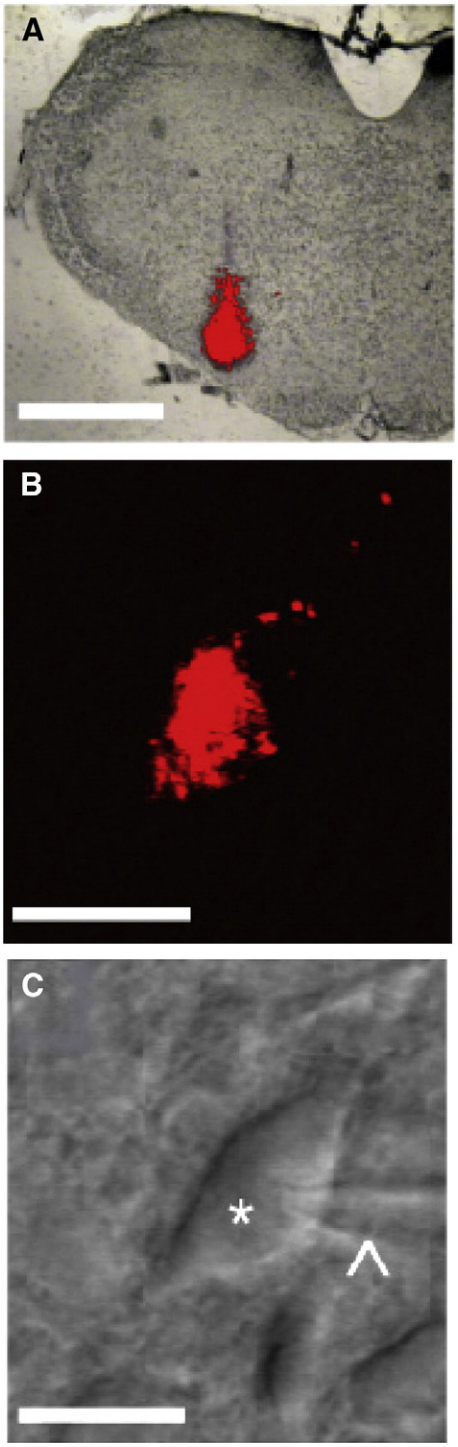

Fig. 1. Identification of retrogradely labeled RVLM-projecting PVN neurons. (A) A photomicrograph showing the FluoSphere injection site (red) in the RVLM from one rat. (B) A FluoSphere-labeled PVN neuron in brain slice viewed with fluorescence illumination. (C) A photomicrograph of the same neurons (*) shown in B with a recording electrode (∧). Scale bars in A = 1 mm and in B and C = 50 μm.