Neuroscience Letters 551 (2013) 39–42

Contents lists available at ScienceDirect

Neuroscience Letters

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

Paraventricular nucleus nesfatin-1 neurons are regulated by pituitary adenylate cyclase-activating polypeptide (PACAP)

Yuko Maejima[a], Kenju Shimomura[a], Kazuya Sakuma[a], Yifei Yang[a], Takeshi Arai[a], Masatomo Mori[b], Toshihiko Yada[a,*]

[a] Division of Integrative Physiology, Department of Physiology, Jichi Medical University School of Medicine, Shimotsuke, Tochigi 329-0498, Japan
[b] Department of Medicine and Molecular Science, Gunma University Graduate School of Medicine, Showa-machi, Maebashi 371-8511, Japan

HIGHLIGHTS

- We examined the effect of PACAP on nesfatin-1 neurons from PVN.
- Cytosolic free calcium ($[Ca^{2+}]_i$) in response to PACAP was measured.
- PACAP increased ($[Ca^{2+}]_i$) with dose-dependent manner in nesfatin-1 neurons.
- Nesfatin-1 may regulate feeding, stress and autonomic response under PACAP control.

ARTICLE INFO

Article history:
Received 2 May 2013
Received in revised form 19 June 2013
Accepted 2 July 2013

Keywords:
Nesfatin-1
Paraventricular nucleus
Cytosolic calcium
Pituitary adenylate cyclase-activating polypeptide

ABSTRACT

Nesfatin-1 is a neuropeptide localized in hypothalamic paraventricular nucleus (PVN). Previously, we have reported the mechanism of feeding suppression by nesfatin-1, and also reported the ability of nesfatin-1 in regulating stress response and the circadian feeding pattern.

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide also related to the stress response, feeding, and regulation of cardiovascular and autonomic nervous systems. The neurons with receptors for PACAP are distributed in PVN. However, there are no reports showing the direct effect of PACAP on nesfatin-1 neurons. In order to explore the direct effect of PACAP on PVN nesfatin-1 neuron, we have measured the cytosolic free calcium ($[Ca^{2+}]_i$) using fura-2 microfluorometry in single neurons isolated from PVN of adult rats, followed by immunocytochemical identification of nesfatin-1 neurons. PACAP at 10^{-15} M to 10^{-9} M increased $[Ca^{2+}]_i$ in dose dependent manner. PAC1 and VPAC2 receptor agonists also increased $[Ca^{2+}]_i$. Sixteen out of 40 neurons (40%) in PVN responded to 10^{-9} M PACAP, and 12 out of 16 neurons (75%) which responded to 10^{-9} M PACAP were found to be nesfatin-1 neurons. In this paper we show that PACAP directly activates nesfatin-1 neurons in PVN. The data suggest that nesfatin-1 controls feeding, stress response or autonomic response under PACAP regulation.

© 2013 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Nesfatin-1 is a neuropeptide, derived from nucleobindin 2 (NUCB2) [12]. Nesfatin-1 neurons are distributed in hypothalamic areas related to feeding regulation, such as paraventricular nucleus (PVN), lateral hypothalamic area (LHA), and arcuate nucleus (ARC) [12]. Nesfatin-1 is known to colocalize with other neuropeptides, such as arginine vasopressin (AVP), corticotropin releasing hormone (CRH) or oxytocin (Oxt) [5]. Our previous report demonstrated that nesfatin-1 induces anorexia *via* Oxt neurons in PVN [7]. In addition, recent reports have revealed that, aside from feeding regulation, nesfatin-1 is related to the regulation of stress responses [19], blood pressure [20] and sleeping [17].

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide and a member of vasoactive intestinal polypeptide (VIP)/secretin/glucagon family [10]. PACAP neurons are distributed in various brain regions including hypothalamus, hyppocampus, spinal cord and amygdaloid complex [9]. Among these regions, PACAP is most abundant in the nucleus of hypothalamus, such as ARC and ventromedial hypothalamic nucleus [8] which are the major components of the feeding regulation center. Similar to nesfatin-1, PACAP regulates homeostatic functions such as feeding [11], stress response [2], energy metabolism [3] and the cardiovascular systems [16].

PACAP is known to act *via* binding to G-protein coupled receptors known as PAC1, VPAC1 and VPAC2 [1]. It has been reported that these three types of receptors are distributed in PVN [4] and nerve

* Corresponding author. Tel.: +81 0285 58 7319.
E-mail address: tyada@jichi.ac.jp (T. Yada).

0304-3940/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neulet.2013.07.006

fibers containing PACAP densely innervate to the PVN, particularly to CRH neurons [6]. These reports strongly suggest that PACAP has influence on PVN neurons.

Therefore, we hypothesized that physiological role of PACAP, such as regulation of feeding, stress response and blood pressure are mediated by nesfatin-1 in PVN. In order to elucidate the regulatory mechanism of nesfatin-1 neurons by PACAP, we examined the direct effect of PACAP on PVN nesfatin-1 neurons by measuring cytosolic calcium ($[Ca^{2+}]_i$) using fura-2 microfluorometry.

2. Materials and methods

2.1. Animals

Male Wistar rats aged 6 weeks were used in this study. Animals were maintained on 12-h light/dark cycle and given conventional food and water *ad libitum*. Experimental procedures and care of animals were carried out according to the Jichi Medical School Institute of Animal Care and Committee.

2.2. Preparation of single neurons from PVN

We used 1 mM glucose containing Krebs-Ringer bicarbonate buffer solution (KRB) composed of (in mM) 129 NaCl, 5.0 $NaHCO_3$, 4.7 KCl, 1.2 KH_2PO_4, 2.0 $CaCl_2$, 1.2 $MgSO_4$, and 10.0 HEPES at pH 7.4. The PVN was isolated from the rat brain, and then single neurons were prepared according to the previous report [18]. Briefly, the whole PVN was incubated with 20 units/ml papain (Sigma Chemical, St. Louis, MO), 1 mM cysteine, 0.015 mg/ml deoxyribonuclease, and 0.75 mg/ml BSA for 15 min at 36 °C in the shaking water bath, followed by gentle mechanical trituration. The cell suspension was centrifuged at 700 rpm for 5 min. The obtained single neurons were distributed onto coverslips and incubated in the humidified chamber at 30 °C for 30 min to 6 h until experiment.

2.3. Measurements of $[Ca^{2+}]_i$ in single PVN neurons

$[Ca^{2+}]_i$ was measured by ratiometric fura-2 microfluorometry combined with digital imaging, as reported previously[7]. Briefly, single neurons on coverslips were incubated with 2 mM fura-2/AM (Dojin chemical, Kumamoto, Japan) for 1 h at room temperature, mounted in chamber and superfused with HKRB at 1 ml/min at 30 °C. Fluorescence images due to excitation at 340 and 380 nm were captured and the ratio (F340/F380) images were produced by Argus-50 system (Hamamatsu Photonics, Hamamatsu, Japan). PACAP-38 was purchased from Peptide Institute (Osaka Japan). PAC1 receptor agonist, Maxadilan was provided from Dr. A. Miyata at Kagosihma University. VPAC2 receptor agonist, Ro 25-1553 was provided from Dr. P. Robberecht at Universite Libre de Bruxelles Belgium.

3. Criteria for responses

When changes in ratio (F340/380) took place within 5 min after administration of reagents and their amplitudes were more than 0.4 ratio unit, they were considered as the responses. To check viability of single cells, responses to 30 mM KCl were examined at the end of whole $[Ca^{2+}]_i$ measurements procedure.

3.1. Immunocytochemistry and identification of nesfatin-1 neurons

Immunocytochemistry of nesfatin-1 neurons were carried out as reported previously [7]. After $[Ca^{2+}]_i$ measurements, the cells were fixed with 4% paraformaldehyde for overnight. They were incubated with a rabbit antiserum against NUCB2/nesfatin-1 (1:1000, generous gift from Prof. Mori) for overnight at 4 °C, followed by incubation with biotinylated antibodies raised against rabbit IgG for 1 h and then with avidin-biotin complex for 1 h.

4. Data analysis

Statistical analysis was carried out using one-way ANOVA, followed by Bonferroni's multiple range test. $P < 0.05$ was considered significant.

5. Result

To examine whether PACAP acts directly on the PVN nesfatin-1 neurons, we used an *in vitro* method that allows one to monitor cytosolic free calcium ($[Ca^{2+}]_i$) in isolated single neurons. After finishing $[Ca^{2+}]_i$ measurements, these neurons were stained immunocytochemically with a specific antiserum for nesfatin-1.

The administration of PACAP to single PVN neuron increased $[Ca^{2+}]_i$ (Fig. 1A, left panel) which was subsequently shown to be containing nesfatin-1(Fig. 1A, right panel). Twelve out of 16 (75%) of PACAP (10^{-9} M)-responsive neurons were identified as nesfatin-1 neurons by immunocytochemistry (Fig. 1B).

The amplitude of $[Ca^{2+}]_i$ (Fig. 1C) and the percentage of PACAP responsive neurons (Fig. 1D) increased followed by ascending concentration of PACAP (10^{-15} to 10^{-9}M). PAC1 agonist maxadilan (10^{-9} M) and VPAC2 agonist Ro25-1553 (10^{-9}M) increased $[Ca^{2+}]_i$ in nesfatin-1 positive neuron (Fig. 1E). Fifteen of 33 (45%) PVN neurons responded to 10^{-9} M maxadilan, 20 of 33 (61%) to 10^{-9} M Ro25-1553, and 26 of 33 (79%) to 10^{-9} M PACAP (Fig. 1F). Eighteen of 26 (69%) PACAP responsive neurons also responded to Ro25-1553, 13 of 26 (50%) PACAP responsive neurons responded to Maxadilan, and 9 of 26 (35%) PACAP responsive neurons responded to both agonists. In addition, 17 of 18 (94%) nesfatin-1 neurons responded to Ro25-1553 and/or, maxadilan and/or, PACAP.

6. Discussion

Nesfatin-1 is the N-terminal fragment of nucleobindin-2 (NUCB2) that has been identified as novel anorectic peptides [12]. Recently, new physiological roles of nesfatin-1 have been reported. In the past, we have found that nesfatin-1 has ability to determine the feeding pattern of light/dark phase [14] and also found that nesfatin-1 can regulate stress response by activating CRH neurons in PVN and increasing adrenocorticotropic hormone and glucocorticoid hormone levels in the plasma [19]. Other reports include nesfatin-1's effect on blood pressure [20] and even on sleeping regulation [17].

PACAP is a neuropeptide first isolated from ovine hypothalamic extracts with ability to stimulate cAMP production in pituitary gland [10]. Similar to multiple roles reported for nesfatin-1, PACAP is known to regulate multiple homeostatic functions such as feeding [11], stress response [2], energy metabolism [3] and the cardiovascular functions [16]. Therefore, we hypothesized that physiological role of PACAP may be mediated by nesfatin-1 in PVN.

Consistent to our hypothesis, we have found that the administration of PACAP to single nesfatin-1 neuron can increase $[Ca^{2+}]_i$. The data strongly suggests that nesfatin-1 regulates feeding, stress response or autonomic response under PACAP regulation. In other words, PACAP is one of the key factors to determine the activity of nesfatin-1 neurons.

Three types of receptors have been reported for PACAP: VPAC1, VPAC2 and PAC1. The activation of these three receptors all mediates the increases of cAMP level. In this study, we examined the effects of selective VPAC2 receptor agonist, Ro 25-1553, and

Download English Version:

https://daneshyari.com/en/article/6282737

Download Persian Version:

https://daneshyari.com/article/6282737

Daneshyari.com