Neuropeptides 56 (2016) 115–123

Contents lists available at ScienceDirect

Neuropeptides

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

Fasted/fed states regulate postsynaptic hub protein DYNLL2 and glutamatergic transmission in oxytocin neurons in the hypothalamic paraventricular nucleus

Shigetomo Suyama [a,1], Misato Kodaira-Hirano [a,1], Zesemdorj Otgon-Uul [a], Yoichi Ueta [b], Masanori Nakata [a], Toshihiko Yada [a,c,*]

[a] Division of Integrative Physiology, Department of Physiology, Jichi Medical University School of Medicine, 3311-1 Yakushiji, Shimotsuke, Tochigi 320-0498, Japan
[b] Department of Physiology, School of Medicine, University of Occupational and Environmental Health, Kitakyushu 807-8555, Japan
[c] Division of Adaptation Development, Department of Developmental Physiology, National Institute for Physiological Sciences, Okazaki, Aichi 444-8585, Japan

ARTICLE INFO

Article history:
Received 16 July 2015
Received in revised form 24 August 2015
Accepted 25 August 2015
Available online 28 August 2015

Keywords:
Oxytocin
Paraventricular nucleus
Fasting
Feeding
Synaptic plasticity
NMDA receptor
Slice patch clamp
DYNLL2
Proteomics

ABSTRACT

The neurons in the hypothalamus regulate food intake and energy metabolism on reception of systemic energy states. Accumulating evidences have indicated that synaptic transmission on the hypothalamic neurons is modulated by the metabolic condition related to fasted/fed states, and that this modulation of synaptic plasticity plays a role in regulation of feeding. It has been shown that oxytocin (Oxt) neurons in the paraventricular nucleus (PVN) of the hypothalamus sense and integrate various peripheral and central signals and thereby induce satiety. However, whether metabolic conditions regulate the synaptic transmission on Oxt neurons in PVN remains unclear. The present study examined whether the fasted/fed states regulate synaptic transmission on Oxt neurons in PVN. The miniature excitatory postsynaptic currents (mEPSCs) onto Oxt neurons in PVN were increased under ad lib fed condition compared to 24 h fasted condition. Furthermore, the NMDA receptor-mediated EPSC on Oxt neurons was increased under fed, compared to fasted, condition. In Oxt neurons, dynein light chain 2 (DYNLL2), a protein suggested to be implicated in the NMDA receptor trafficking to the postsynaptic site, was increased under fed, compared to fasted, condition. The present results suggest that feeding increases excitatory synaptic input on PVN Oxt neurons via mechanisms involving DYNLL2 upregulation and NMDA receptor-mediated synaptic reorganization.

1. Introduction

The hypothalamic arcuate nucleus (ARC) is equipped with the orexigenic neurons expressing neuropeptide Y (NPY) and agouti-related peptide (AgRP), popularly named NPY/AgRP neurons, and the anorexigenic neurons expressing proopiomelanocortin (POMC), being considered as the first order neurons of the feeding center (Schwartz et al., 2000). They sense and integrate peripheral metabolic signals, and project to the second order neurons in the hypothalamic paraventricular nucleus (PVN), an area recognized as the integrative center of feeding and energy balance (Schwartz et al., 2000).

Recent studies have revealed that the metabolic states and related nutrient and hormonal signals regulate the efficacy of synaptic transmission, defined as synaptic plasticity, of NPY/AgRP and POMC neurons for fine regulation of energy homeostasis. The positive energy state and satiety signals such as high-fat diet and leptin increase excitatory synaptic inputs and decrease inhibitory synaptic inputs onto POMC neurons in ARC, while exerting opposite effects on NPY/AgRP neurons in ARC (Benani et al., 2012; Horvath et al., 2010; Pinto et al., 2004). Conversely, the negative energy state and hunger signals such as starvation and ghrelin increase excitatory inputs on NPY/AgRP neurons (Yang et al., 2011) and inhibitory inputs on POMC neurons (Pinto et al., 2004). The N-methyl-D-aspartic acid type glutamate receptor (NMDAR) is involved in regulation of synaptic plasticity, but not synaptic transmission. In the mice lacking NMDAR specifically in AgRP neurons, fasting fails to up-regulate spine formation, excitatory postsynaptic current (EPSC) and firing rate on AgRP neurons, and fasting incompletely promotes food intake and body weight (Liu et al., 2012). By contrast, the mice lacking NMDAR specifically in POMC neurons show normal synaptic morphology and transmission (Liu et al., 2012). These results show that 1) modulation of synaptic plasticity regulates feeding and energy metabolism, and that 2) the molecular mechanism underlying the synaptic plasticity operates in particular neurons, but not all neurons, in the hypothalamus.

It is still unclear whether the metabolic state-dependent synaptic reorganization takes place in the neurons in the hypothalamic nuclei other than ARC. Oxytocin (Oxt), synthesized in the PVN and supraoptic

* Corresponding author at: Division of Integrative Physiology, Department of Physiology, Jichi Medical University School of Medicine, 3311-1 Yakushiji, Shimotsuke, Tochigi 329-0498, Japan.
E-mail address: tyada@jichi.ac.jp (T. Yada).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.npep.2015.08.008
0143-4179/

nucleus (SON) of the hypothalamus and released from the pituitary, promotes uterine contraction and milk ejection, as its classical functions. Recent studies have demonstrated that Oxt neurons in the PVN project to the POMC neurons in the nucleus tractus solitarius (NTS) and ARC (Maejima et al., 2009, 2011) to regulate feeding. Oxt neurons in the PVN receive inhibitory and excitatory inputs from NPY/AgRP and POMC neurons of ARC, respectively. A study of optogenetic approach using channelrhodopsin2 demonstrated that the food intake stimulated by photo-activation of AgRP neurons was canceled by concurrent photo-activation of Oxt neurons in the PVN (Atasoy et al., 2012). Furthermore, Oxt neurons in the PVN largely mediate anorectic ability of nesfatin-1, a potent anorexigenic neuropeptide (Maejima et al., 2009). These results indicate an essential role of PVN Oxt neurons in regulation of feeding. However, little is known about metabolic state-dependent synaptic plasticity on Oxt neurons.

To examine whether the fed/fasted states influence synaptic plasticity on PVN Oxt neurons, we conducted the electrophysiology on the PVN slices prepared from Oxt-monomeric red fluorescent protein1 (mRFP) rats (Ohkubo et al., 2014), and examined the effect of fed vs. fasted conditions on excitatory synaptic reorganization on PVN Oxt neurons by measuring the currents mediated by NMDAR and by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid type glutamate receptor (AMPAR). Furthermore, to gain insight on the molecular mechanism underlying the fed/fast-dependent changes in the synaptic plasticity, we took the proteomics approach to explore a novel protein implicated in synaptic plasticity, with particular attention on dynein light chain 2 (DYNLL2). DYNLL2 is a component of motor protein complex that works as a cargo in retrograde transport (Naisbitt et al., 2000) and is associated with transport of NMDAR toward postsynaptic density (Moutin et al., 2012, 2014). Two dimensional gel electrophoresis, an effective method to exhaustively analyze protein expression, was combined with mass spectrometry to identify changed proteins (Agrawal et al., 2005; Celis and Gromov, 1999; Hirano et al., 2006). We found fed state-associated enhancement of the NMDAR- and AMPAR-mediated currents and DYNLL2 expression in the PVN Oxt neurons.

2. Materials and methods

2.1. Animals and experimental protocol

Male Wistar rat (Nihon SLC, Hamamatsu, Japan) and Oxt-mRFP transgenic Rat aged at 6–7 weeks were used in these experiments. Animals were housed individually on a 12 h light/dark cycle (19:30 lights off) and given conventional food (CE-2; Clea, Osaka, Japan) and tap water ad libitum for at least 1 week before the experiments. Experimental procedures and care of animals were carried out according to Jichi Medical University Institute of Animal Care and Use Committee.

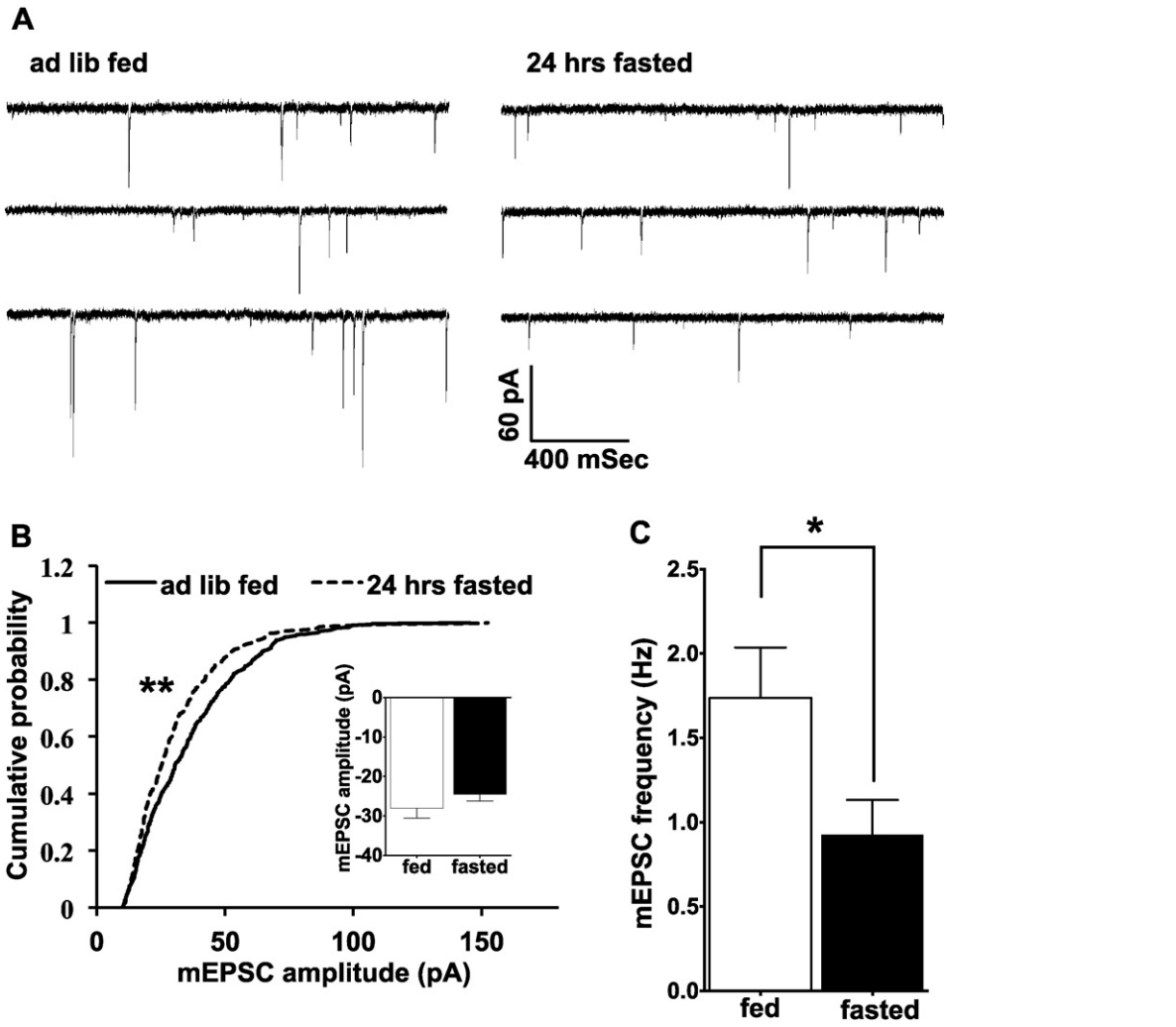

Fig. 1. Fed state enhances mEPSCs in PVN Oxt neurons. A, Representative traces of mEPSC from PVN Oxt neurons in acute hypothalamic slices from ad lib fed and 24 h fasted rats. B, Cumulative probability distribution of mEPSC amplitudes in ad lib fed and 24 h fasted groups. $^{**}p < 0.01$ (Kolmogorov–Smirnov test), n = 10–11. Bar graph indicates the averaged median amplitude of mEPSCs from ad lib fed and 24 h fasted groups. C, Frequency of mEPSCs in ad lib fed and 24 h fasted groups. $^{*}p < 0.05$ (unpaired t-test).