Brain Research 1646 (2016) 366–376

Contents lists available at ScienceDirect

Brain Research

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

Leptin receptor-positive and leptin receptor-negative proopiomelanocortin neurons innervate an identical set of brain structures

Leandro B. Lima, Martin Metzger, Isadora C. Furigo, J. Donato Jr.*

Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, São Paulo, SP, Brazil

ARTICLE INFO

Article history:
Received 19 January 2016
Received in revised form
1 June 2016
Accepted 15 June 2016
Available online 16 June 2016

Keywords:
POMC
α-MSH
β-endorphin
Melanocortin
ROSA26 Cre reporter
Hypothalamus

ABSTRACT

Neurons that express the prohormone proopiomelanocortin (POMC) in the arcuate hypothalamic nucleus (Arc) are engaged in the regulation of energy balance and glucose homeostasis. Additionally, POMC neurons are considered key first-order cells regulated by leptin. Interestingly, in the Arc, POMC cells that express the leptin receptor (POMC/LepR+ cells) are found side by side with POMC cells not directly responsive to leptin (POMC/LepR− cells). However, it remains unknown whether these distinct populations innervate different target regions. Therefore, the objective of the present study was to compare the projections of POMC/LepR+ and POMC/LepR− neurons. Using genetically modified LepR-reporter mice to identify leptin receptor-expressing cells and immunohistochemistry to stain POMC-derived peptides (α-MSH or β-endorphin) we confirmed that approximately 80% of Arc β-endorphin-positive neurons co-expressed leptin receptors. POMC/LepR+ and POMC/LepR− axons were intermingled in all of their target regions. As revealed by confocal microscopy, we found an elevated degree of co-localization between α-MSH+ axons and the reporter protein (tdTomato) in all brain regions analyzed, with co-localization coefficients ranging from 0.889 to 0.701. Thus, these two populations of POMC neurons seem to project to the same set of brain structures, although one of the two subtypes of POMC axons was sometimes found to be more abundant than the other in distinct subregions of the same nucleus. Therefore, POMC/LepR+ and POMC/LepR− cells may target separate neuronal populations and consequently activate distinct neuronal circuits within some target nuclei. These findings contribute to unravel the neuronal circuits involved in the regulation of energy balance and glucose homeostasis.

1. Introduction

Leptin plays a pivotal role in the regulation of food intake, energy balance and glucose homeostasis. These functions are mediated predominantly by leptin receptor (LepR)-expressing neurons located in the hypothalamus and brainstem (Coppari and Bjorbaek, 2012; Morton et al., 2014; Williams and Elmquist, 2012). Although nowadays it is well recognized that a distributed network of neuronal populations contributes to mediate the multiple biological effects of leptin, including the regulation of energy balance (Dhillon et al., 2006; Leinninger et al., 2011; Myers et al., 2009), glucose homeostasis (Flak et al., 2014; Scott et al., 2011), reproduction (Donato Jr. et al., 2011), and modulation of the cardiovascular system (Simonds et al., 2014), the arcuate hypothalamic nucleus (Arc) undoubtedly is a major site of leptin action (Coppari et al., 2005). In the Arc, neurochemically defined neuronal populations express the LepR, including proopiomelanocortin (POMC) positive cells and neurons that co-express neuropeptide Y (NPY) and agouti-related peptide (AgRP) (Cheung et al., 1997; Elias et al., 1999; Mizuno and Mobbs, 1999; Schwartz et al., 1996). POMC

Abbreviations: 3V, 3rd ventricle; ac, anterior commissure; AgRP, agouti-related peptide; Aq, aqueduct; Arc, arcuate hypothalamic nucleus; CC, central canal; ChaT, choline acetyltransferase; ChAT-ir, choline acetyltransferase-immunoreactivity; D3V, dorsal 3rd ventricle; DM, dorsomedial hypothalamic nucleus; DMC, dorsomedial hypothalamic nucleus, compact part; DMD, dorsomedial hypothalamic nucleus, dorsal part; DMV, dorsomedial hypothalamic nucleus, ventral part; DRL, dorsal raphe nucleus, lateral part; IML, intermediolateral column; IMM, intermediomedial column; KPBS, potassium phosphate-buffered saline; LPB, lateral parabrachial nucleus; LepR, leptin receptor; MC4R, melanocortin 4 receptor; MD, mediodorsal thalamic nucleus; ME, median eminence; MPO, medial preoptic nucleus; MPOL, medial preoptic nucleus, lateral part; MPOM, medial preoptic nucleus, medial part; NPY, neuropeptide Y; MSH, melanocyte-stimulating hormone; Pa, paraventricular hypothalamic nucleus; PBS, phosphate-buffered saline; PLH, peduncular part of lateral hypothalamus; POMC, proopiomelanocortin; PrC, precommissural nucleus; pSTAT3, phosphorylated form of signal transducer and activator of transcription-3; pSTAT3-ir, pSTAT3 immunoreactivity; scp, superior cerebellar peduncle; Sol, solitary nucleus; STMV, bed nucleus of the stria terminalis, medial division, ventral part; Vh, ventral horn; VLPAG, ventrolateral periaqueductal gray; VMH, ventromedial hypothalamic nucleus; VMPO, ventromedial preoptic nucleus

* Corresponding author.
E-mail address: jdonato@icb.usp.br (J. Donato Jr.).

http://dx.doi.org/10.1016/j.brainres.2016.06.024
0006-8993/

is a prohormone and after protein cleavage several bioactive peptides are produced, including adrenocorticotropic hormone, β-endorphin and α-, β- or γ-melanocyte-stimulating hormone (α-/β-/γ-MSH). Leptin stimulates POMC mRNA expression and α-MSH secretion which binds to the melanocortin 4 receptor (MC4R) (Kishi et al., 2003; Schwartz et al., 1997; Seeley et al., 1997; Thornton et al., 1997; Yaswen et al., 1999). On the other hand, AgRP acts as a competitive antagonist of the MC4R (Ollmann et al., 1997). Together, POMC and NPY/AgRP cells act as first-order neurons and MC4R positive cells as second-order neurons in the neuronal circuitry known as central melanocortin system, which controls important biological effects of leptin (Balthasar et al., 2004; Kishi et al., 2003; Seeley et al., 1997; van de Wall et al., 2008). Accordingly, MC4R deficiency recapitulates key defects caused by the lack of leptin signaling (Farooqi et al., 2003; Huszar et al., 1997; Kim et al., 2000; Marsh et al., 1999; Yeo et al., 1998).

Notably, just a subset of Arc POMC cells expresses the LepR. For example, using double label in situ hybridization techniques, Cheung et al. (1997) observed that, depending on the signal to background ratio criteria utilized, 33–89% of POMC neurons co-expressed LepR mRNA. In another study, 79% of Arc POMC cells exhibited LepR mRNA expression (Baskin et al., 1999). Since LepR activation induces the phosphorylation of the signal transducer and activator of transcription-3 (pSTAT3) (Rosenblum et al., 1996), several studies have nowadays used leptin-induced pSTAT3 as a marker to visualize leptin-responsive cells in different brain regions (Donato Jr. et al., 2010; Hosoi et al., 2002; Munzberg et al., 2003). Using this approach, three independent studies observed that approximately 40–60% of Arc POMC cells exhibited leptin-induced pSTAT3-immunoreactivity (pSTAT3-ir) (Berglund et al., 2012; Huo et al., 2009; Lam et al., 2015). Thus, there is manifold evidence that in the Arc exist, side by side, two distinct populations of POMC cells, one expressing the LepR (POMC/LepR+ cells), and another that is not directly responsive to leptin (POMC/LepR− cells).

Given the importance of Arc POMC neurons for the regulation of metabolism (Balthasar et al., 2004; Berglund et al., 2012; Coppari and Bjorbaek, 2012; Huo et al., 2009; Morton et al., 2014; Williams and Elmquist, 2012), a careful characterization of the different subpopulations of POMC cells may improve our understanding about the neuronal circuits involved in the regulation of energy balance and glucose homeostasis. Therefore, the objective of the present study was to investigate and compare the axonal projections of POMC/LepR+ and POMC/LepR− neurons.

2. Results

2.1. Similar distribution of α-MSH and β-endorphin immunoreactive axonal terminals and cells bodies in the mouse brain

Since POMC is a prohormone and is readily cleaved by post-translational mechanisms, the derived peptides can be used as reliable markers of POMC-positive cells. Therefore, in order to visualize cell bodies and axonal terminals of POMC cells, we performed immunohistochemistry to stain two major bioactive neuropeptides produced by POMC cleavage (α-MSH and β-endorphin). Initially, we performed a co-localization experiment to compare the brain distribution of α-MSH and β-endorphin immunoreactive axonal terminals and cell bodies (Fig. 1). We observed a complete overlap in the distribution pattern of α-MSH and β-endorphin immunoreactive axonal terminals in the mouse brain (Fig. 1(A)–(C)). In the Arc, virtually all β-endorphin-positive cell bodies co-expressed α-MSH-immunoreactivity (Fig. 1(D)–(F)). No other brain region exhibited α-MSH or β-endorphin cell bodies. Notably, β-endorphin immunostaining produced a superior cell body labeling (Fig. 1(E)) compared to α-MSH (Fig. 1(D)). Thus, in the following experiments, we used β-endorphin staining to identify POMC-positive cell bodies. In all, these findings indicate that α-MSH or β-endorphin immunohistochemistry essentially produces a highly similar staining pattern, and that both markers are equally suitable to label POMC-positive cell bodies and axons.

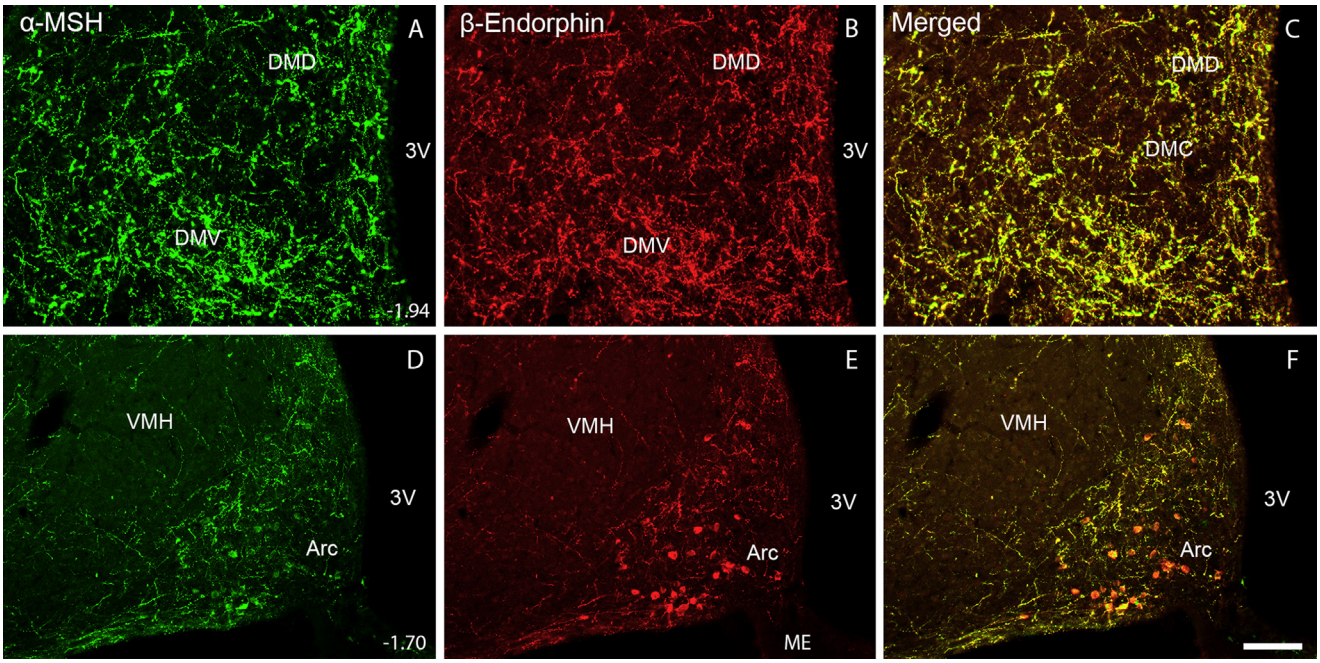

Fig. 1. Epifluorescence photomicrographs showing the co-localization between α-MSH (green) and β-endorphin (red) axonal terminals and cell bodies. (A)–(C) co-localization in the dorsomedial hypothalamic nucleus (DM). (D)–(F) co-localization in the arcuate hypothalamic nucleus (Arc). Scale bar = 50 μm. Abbreviations: 3V, 3rd ventricle; DMC, DM, compact part; DMD, DM, dorsal part; DMV, DM, ventral part; ME, median eminence; VMH, ventromedial hypothalamic nucleus.