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Research Report

Retrograde study of CART- or NPY-neuronal projection from the hypothalamic arcuate nucleus to the dorsal raphe and/or the locus coeruleus in the rat

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ABSTRACT

The present study was designed to reveal cocaine- and amphetamine-regulated transcript (CART)- or neuropeptide Y (NPY)-immunoreactive neuronal projections from the hypothalamic arcuate nucleus (Arc) to the dorsal raphe (DR) and/or the locus coeruleus (LC) in the rat. Our results demonstrated that CART or NPY axon terminals formed close appositions to the neuronal profiles in the DR and the LC. Thus, arcuate sections were immunostained for the CART or NPY after the injections of green RetroBeads™ into the DR and red tracer into the LC (or vice versa). First, retrogradely-labeled CART cells were mainly observed in the lateral Arc without colchicine. Of the total population of arcuate CART neurons, DR- and LC-projecting cells were 5.7%±0.9% and 6.6%±0.7%, respectively. In addition, a subset (3.3%±0.7%) of CART neurons provided divergent axon collaterals to the DR and the LC. Second, retrogradely-labeled NPY cells were observed in lateral or ventral borders of the medial Arc only after colchicine injection. Of the entire NPY cell population, DR- and LC-projecting neurons were 1.5%±0.3% and 1.3%±0.3%, respectively. Only a scanty proportion (0.1%±0.0%) sent axon collaterals to the DR and the LC. These observations suggested that arcuate CART or NPY system might have a potential influence on the brainstem monoaminergic nuclei, modulating their roles in feeding, nociception, emotional behaviors, arousal, and stress responses. Furthermore, a portion of arcuate CART neurons (along with only a few NPY cells) sending divergent axon collaterals to the DR/LC might have a simultaneous (and possibly more efficient) way to exert their specific influences on the monoaminergic nuclei.

1. Introduction

The arcuate nucleus (Arc) in the mediobasal hypothalamus consists of several important populations of neurons including neuroendocrine and centrally-projecting ones. Neuroendocrine neurons with nerve endings in the median eminence release dopamine or growth hormone-releasing factor into the hypophysial portal blood. Centrally-projecting neurons consist of two major cell populations that influence food intake antagonistically (Sergeyev et al., 2001). One group of neurons in the medial Arc

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contains orexigenic neuropeptide Y (NPY), agouti-related protein (AGRP), and GABA, projecting strongly to the lateral hypothalamus (LH) as well as the paraventricular hypothalamic nucleus (PVN). The other group of cells in the lateral Arc contains anorexigenic, cocaine- and amphetamine-regulated transcript (CART), pro-opiomelanocortin (POMC), and glutamate, projecting to the hypothalamus, brainstem, and the spinal cord (Khorooshi and Klingenspor, 2005; Vicentic and Jones, 2007). The arcuate CART neurons are strongly involved in energy balance in that they are activated by circulating leptin/insulin and directly inhibited by orexigenic NPY neurons (Kristensen et al., 1998; van den Pol, 2003; Meister, 2007).

The feeding-control action of hypothalamic neuropeptides can be modulated by monoamines (Rowland et al., 2000; Saper et al., 2002). First, serotonin produces a hypophagic effect through 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2C} receptors in specific hypothalamic regions (Leibowitz et al., 1988; Parada et al., 1992; Leibowitz and Alexander, 1998; Nonogaki et al., 1998). Second, histamine plays a modulatory role in food intake; appetite is suppressed through activation of the H_1 receptor or inhibition of the H_3 receptor in the ventromedial hypothalamic nucleus (VMN) or the PVN (Kalra et al., 1999; Morimoto et al., 2001; Haas and Panula, 2003). Third, dopamine exerts a site-specific influence on feeding behavior; it depresses melanin-concentrating hormone (MCH)-containing neuronal activity in the LH, but reinforces feeding through the nucleus accumbens (Meguid et al., 2000; Conductier et al., 2011). Finally, chemical/electrolytic lesion of the locus coeruleus (LC) causes overeating and obesity, whereas the injection of exogenous norepinephrine into the hypothalamus elicits eating behavior (Currie et al., 1994; Luque and Rey, 1999; Wellman, 2000). Norepinephrine is also known to modulate hypothalamic, arcuate neurons via α_1 and β adrenergic receptors (Kang et al., 2000).

During recent studies on various hypothalamic feeding-related neuropeptides, we observed that CART neurons in the Arc were unique in that none of them co-contained MCH (or NPY) and that their immunostaining intensity was higher than any other CART cell groups in the hypothalamus (Hong et al., 2011; Yoon and Lee, 2013a, b). Anterograde labeling study reported that arcuate fibers establish putative contacts with serotonergic neurons in the ventrolateral periaqueductal gray (vlPAG), DR and nucleus raphe magnus (NRM) and with noradrenergic neurons in the PAG, medullary central gray, and LC (Sim and Joseph, 1991). The neurochemical identity of arcuate neurons as the origin of the descending projections, however, has not been determined. This is the first study to determine whether anorexigenic CART cells or orexigenic NPY neurons in the Arc provide efferent projections to the DR and/or the LC in the rat. It is a sequel to our studies on hypothalamic, arousal/feeding-related neuropeptidergic projections to brainstem monoaminergic or cholinergic nuclei (Lee et al., 2005c; Hong et al., 2011; Yoon and Lee, 2013a, b). Based on our observation that certain hypothalamic neurons provide axon collaterals to the DR and the LC (Lee et al., 2005a, b; Yoon and Lee, 2013b), we also examined whether CART- or NPY-immunoreactive (ir) arcuate neurons provide divergent axon collaterals to the DR and the LC.

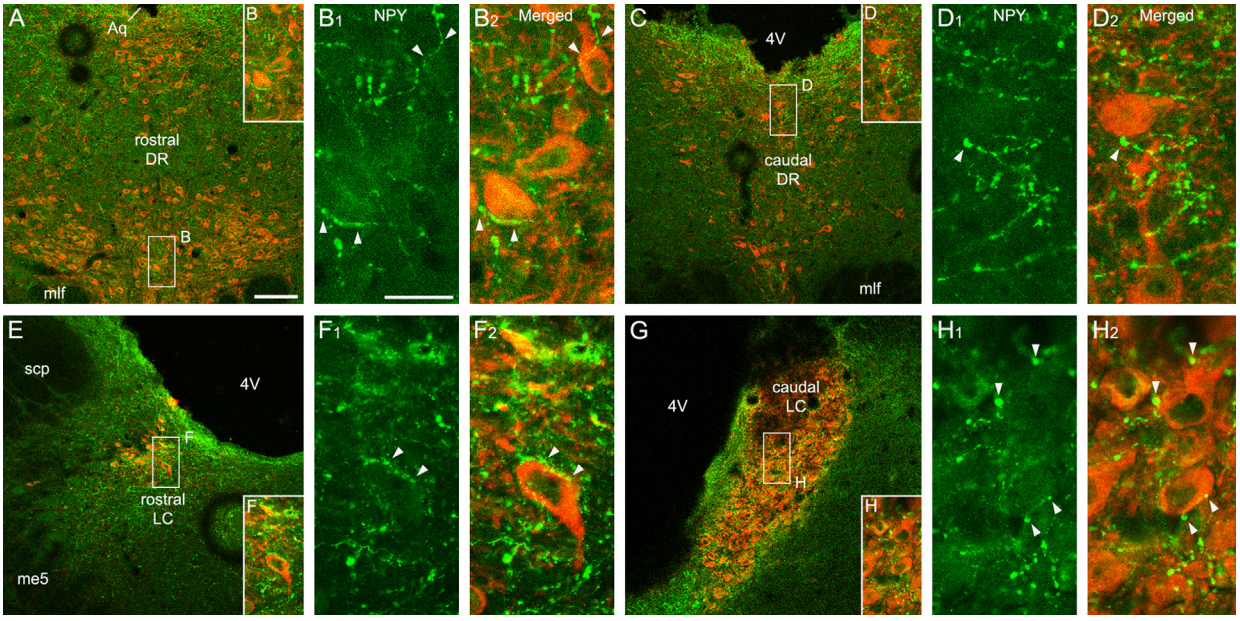

Fig. 1 – Experiments were performed to examine whether NPY-ir axon terminals formed close appositions to the neuronal profiles in the DR or LC. Without involving any tracer injections, DR and LC sections were immunostained for tryptophan hydroxylase (TPH)/NPY (A–D) and dopamine β-hydroxylase (DBH)/NPY (E–H), respectively. In the DR, NPY-ir (green-labeled) varicosities formed close appositions to TPH-ir (red-labeled) somata (B_2 and D_2). Likewise, in the LC NPY-ir (green-labeled) boutons made contact with DBH-ir (red-labeled) somata (F_2 and H_2). 4V—fourth ventricle; Aq—cerebral aqueduct; me5—mesencephalic trigeminal tract; mlf—medial longitudinal fasciculus; and scp—superior cerebellar peduncle. Scale bars = 100 μm (A), and 25 μm (B_1).