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Research Report

Postnatal developmental profile of urocortin 1 and cocaine- and amphetamine-regulated transcript in the perioculomotor region of C57BL/6J mice

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ABSTRACT

Urocortin 1 (Ucn 1) is an endogenous corticotropin releasing factor (CRF)-related peptide. Ucn 1 is most highly expressed in the perioculomotor urocortin containing neurons (pIIIu), previously known as the non-preganglionic Edinger-Westphal nucleus (npEW). Various studies indicate that these cells are involved in stress adaptation and the regulation of ethanol (EtOH) intake. However, the developmental trajectory of these neurons remained unexamined. Expression of the cocaine- and amphetamine-regulated transcript (CART), which co-localizes with Ucn 1 in the perioculomotor area (pIII) has been examined prenatally, but not postnatally. The goal of the current study was to characterize the ontogenetic profile of Ucn 1 and CART during postnatal development in C57BL/6J (B6) mice. B6 mice were bred, and brains were collected at postnatal days (PND) 1, 4, 8, 12, 16, 24 and 45. Brightfield immunohistochemical staining for Ucn 1 and CART showed that Ucn 1-immunoreactivity (ir) was absent at PND 1, while CART-ir was already apparent in pIIIu at birth, a finding indicating that although the pIIIu neurons have already migrated to their adult position, Ucn 1 expression is triggered in them at later postnatal stages. Ucn 1-ir gradually increased with age, approaching adult levels at PND 16. This developmental profile was confirmed by double-immunofluorescence, which showed that Ucn 1 was absent in CART-positive cells of pIII at PND 4 and that Ucn 1 and CART are strongly but not completely co-localized in pIII at PND 24. Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) analysis confirmed that Ucn 1 mRNA levels are significantly lower at PND 4 and PND 12 than in adult animals. The lack of brain Ucn 1 immunoreactivity at birth and the gradual postnatal increase in Ucn 1 in pIIIu suggests that this peptide plays a greater behavioral role in adulthood than during the early postnatal development of an organism.

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Abbreviations: ANOVA, analysis of variance; BSA, bovine serum albumin; CART, cocaine- and amphetamine-regulated transcript; CRF, corticotropin releasing factor; DAB, diaminobenzidine; EtOH, ethanol; ir, immunoreactivity; NaN3, sodium azide; npEW, non-preganglionic Edinger-Westphal nucleus; pIII, perioculomotor area; pIIIu, periocolumotor urocortin-containing neurons; PBS, phosphate-buffered saline; PLSD, Protected Least Significant Difference; qRT-PCR, quantitative reverse transcriptase polymerase chain reaction; Ucn 1, urocortin 1

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1. Introduction

Urocortin 1 (Ucn 1) is a member of the corticotrophin releasing factor (CRF) family of peptides and is known to act with high affinity on both CRF1 and CRF2 receptors (Vaughan et al., 1995). In the brain Ucn 1 is most highly expressed in the perioculomotor urocortin containing neurons (pIIIu) (May et al., 2008; Ryabinin et al., 2008; Spangler et al., 2009), a brain region previously referred to as the non-preganglionic Edinger-Westphal nucleus (npEW) (Bittencourt et al., 1999; Kozicz et al., 1998; Ryabinin et al., 2005; Vaughan et al., 1995; Weitemier et al., 2005). To a lesser degree, Ucn 1 is also expressed in the lateral superior olive (Bittencourt et al., 1999; Weitemier et al., 2005). The distribution of this peptide has been examined in various animal models in both the central nervous system and the periphery (Boorse and Denver, 2006; Cunha et al., 2007; Kozicz et al., 1998; Kozicz and Arimura, 2002; Kozicz et al., 2002; Lim et al., 2006; May et al., 2008), but the developmental trajectory of Ucn 1 immunoreactivity (ir) has been limited in the literature.

Ucn 1 expression has been reported in fetal ovine pituitary and human colon (Holloway et al., 2002; Muramatsu et al., 2000). In the nervous tissue, many studies have found that exogenous application of Ucn 1 has a neuroprotective and neurotrophic role, suggesting that Ucn 1 could play a role during development (Abuirmeileh et al., 2007; Brar et al., 2000; Calle et al., 2005; Choi et al., 2006; Facci et al., 2003; Gounko et al., 2005; Swinny et al., 2004b). However, this interpretation is complicated by the fact that exogenous application of Ucn 1 does not distinguish between the endogenous actions of Ucn 1 and other CRF-like peptides. It has been previously reported that CRF and CRF receptors show brain region-specific profiles of expression, appear in the brain already prenatally, but are not found in pIIIu at any age (Avishai-Eliner et al., 1996; Baram and Lerner, 1991; Bittencourt et al., 1999; Bugnon et al., 1982; Chalmers et al., 1995; Eghbal-Ahmadi et al., 1998; Grino et al., 1989; Korosi and Baram, 2008; Potter et al., 1992). To evaluate the potential role of Ucn 1 in development, it would be beneficial to study the ontogenic profile of Ucn 1 expression. To this date only one study attempted to characterize this profile (Swinny et al., 2004a). This study performed in the rat, reported presence of Ucn 1-positive fibers in cerebellum and Ucn 1-positive cells in inferior olive at postnatal day (PND) 3, an increase of Ucn 1-positive fibers in the cerebellum at PND 8 and PND 15 and presence of Ucn 1-positive cells at PND 15. These findings suggested a substantial change in brain Ucn 1 expression during postnatal development warranting future more detailed investigations.

The developmental expression profile of cocaine- and amphetamine-regulated transcript (CART), a peptide that strongly but not completely co-localizes with Ucn 1 in the perioculomotor area (pIII) (Kozicz, 2003; Lazar et al., 2004; Lima et al., 2008; Xu et al., 2009), has been examined prenatally (Brischoux et al., 2002). It was reported that CART is expressed during early embryonic development, and the finding that these cells migrate through the ventral tegmentum area and settle in the pIII, has led to the argument that CART may be one of the earliest neuropeptides with a neuromodulatory role that is expressed in the brain (Brischoux et al., 2002; Risold et al., 2006). Although, this prenatal developmental profile was reported, the postnatal developmental expression profile of CART has only been examined in other brain regions (Abraham et al., 2007).

Various studies indicate that cells in the pIIIu are sensitive to stress (Cunha et al., 2007; Gaszner et al., 2004; Kozicz, 2007), ethanol (EtOH) administration and self-administration (Bachtell et al., 1999; Chang et al., 1995; Ryabinin et al., 1997; Sharpe et al., 2005; Topple et al., 1998), and administration of other drugs of abuse (Bachtell et al., 2002a; Spangler et al., 2009). A greater number of Ucn 1-positive cells in pIIIu can be found in many ethanol-preferring strains of mice and rats, including C57BL/6J (B6) mice, versus alcohol avoiding strains (Bachtell et al., 2002b; Bachtell et al., 2003; Fonareva et al., 2009; Ryabinin and Weitemier, 2006; Turek et al., 2005) and both Ucn 1 and CART play an important role in the actions of alcohol and other addictive drugs in adult animals (Bachtell et al., 2004; Dandekar et al., 2008; Jaworski et al., 2008; Ryabinin et al., 2008; Salinas et al., 2006; Turek and Ryabinin, 2005; Weitemier and Ryabinin, 2005). However, since the postnatal development of these peptides has not been extensively studied, it remains unknown whether these peptides may play a role in responses to stress and addictive drugs during infant and juvenile stages of ontogeny.

The goal of the current study is to characterize the postnatal developmental expression profile of Ucn 1 with a focus on pIIIu and compare it to CART with the use of immunohistochemistry and quantitative reverse transcription polymerase chain reaction (qRT-PCR).

2. Results

Brightfield immunohistochemistry revealed that Ucn 1-positive cells in the B6 mice were completely absent at PND 1, and only one of three mice showed Ucn 1-ir in the pIIIu at PND 4. Furthermore, no additional Ucn 1-ir was observed in the slices stained laterally from the midline at PND 1 or PND 4. At PND 8, Ucn 1-ir was present in pIIIu of all mice examined and had increased 3.2 fold from levels at PND 4. Another 2.4 fold increase was observed at PND 12, with mature cell counts leveling off at PND 16, as similar values were observed in the late juvenile/early adolescent mice at PND 24. Representative sagittal sections of the pIIIu and Ucn 1-positive cells can be seen in Figs. 1 and 2.

CART-positive cells were strongly labeled in pIII starting at PND 1. The number of detected CART-labeled cells doubled during the first postnatal week and then slightly declined. At PND 24, CART-ir was 1.4 fold of PND 1 counts (Figs. 1 and 3).

Double fluorescence immunohistochemistry of Ucn 1 and CART performed in additional mice on coronal sections at PND 4 and PND 24 showed positive CART labeling at PND 4, while Ucn 1 was completely absent from the pIIIu. At PND 24, fluorescent labeling of both Ucn 1 and CART was present. Double labeling confirmed that Ucn 1 and CART are largely co-localized in pIII, but a small number of cells showed either only Ucn 1 or only CART staining (Fig. 4).

Quantitative real-time polymerase chain reaction (qRT-PCR) performed on dissections of pIIIu from PND 4, PND 12 and PND 45 confirmed the increase in Ucn 1 expression during postnatal development (Fig. 5). Specifically, the relative level