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Research report

Maturation of the hypothalamic arcuate agouti-related protein system during postnatal development in the mouse

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Abstract

The hypothalamic arcuate nucleus (Arc) and its neurons expressing agouti-related protein (AgRP) are key components of the forebrain circuitry involved in long-term regulation of energy homeostasis, including conveying leptin signaling to other hypothalamic and extrahypothalamic regions. In the present work, we investigated the postnatal development (P0, P5, P10, P15, and P21) of this system (AgRP transcript and peptide) in the mouse brain using in situ hybridization and immunohistochemistry. At all stages, AgRP mRNA expression was detected exclusively in the Arc. At P0, AgRP mRNA levels were low, and only a few AgRP-immunoreactive fibers were present reaching, rostrally, the bed nucleus of the stria terminalis and, caudally, the dorsal raphe nucleus. During the following period (P5–P21), the levels of AgRP mRNA gradually increased in the Arc along with a parallel increase in the AgRP fiber density in the hypothalamic regions responsible for control of appetite, including the paraventricular nucleus, as well as in extrahypothalamic regions, including locus coeruleus. These data provide evidence that, in the mouse, the maturation of the AgRP Arc system occurs mainly during the first three postnatal weeks. Together with the existing data on the physiology of appetite and body weight, our data suggest that the first three postnatal weeks in the mouse represents a critical period for the formation of brain mechanisms underlying appetite control via peripheral hormones.

Theme: Development and regeneration
Topic: 40.013 Peptides: anatomy and physiology

Keywords: Central nervous system; Development; Food intake; Neuroendocrinology; Neuropeptides

1. Introduction

The hypothalamic arcuate nucleus (Arc) is a key component in the neuropeptidergic circuitry of the forebrain involved in long-term regulation of appetite and body weight [12,21,28,42]. Here leptin, insulin, ghrelin, and other peripheral hormones act on arcuate neurons expressing the corresponding receptors [15,27,32] and regulate expression of two coexisting messenger molecules, orexigenic agouti-related protein (AgRP) and neuropeptide Y (NPY) and of the anorexigenic α-melanocyte stimulating hormone (α-MSH) and some other neuropeptides [13]. These neurons in turn send projections to other hypothalamic areas important for the regulation of energy balance, e.g., the hypothalamic paraventricular nucleus (PVN) and the lateral hypothalamic area (LHA) [19]. The mapping of this circuitry [3,8] was facilitated by the fact that AgRP was found to be exclusively expressed by the arcuate NPY neurons [26,34], whereas NPY, as known since long, is present in multiple systems in the brain (and other tissues).

The critical involvement of the arcuate AgRP system in appetite control makes it important to study its ontogenesis. In fact, development of the arcuate AgRP/NPY system has been thoroughly studied in the rat, showing that it is not mature until the third postnatal week [24,25]. In contrast the normal development of the AgRP system in the mouse

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has not yet been studied, in spite of the fact that mice are now extensively used for studies of central mechanisms of appetite and related disorders, such as obesity and anorexia [9,29,30]. Therefore, in the present work using in situ hybridization and immunohistochemistry, we investigated the early postnatal development of AgRP expression in the mouse brain at P0, P5, P10, P15, and P21.

2. Materials and methods

2.1. Animals

Experiments involving animals followed the guidelines approved by the local ethical committee (Stockholms norra djurförsöksetiska nämnd). All procedures were designed to minimize suffering of experimental animals. Pregnant C57B16 mice (B&K Universal, Sollentuna, Sweden) were housed in ventilated cages at 25 °C in an animal room with a 12-h light–dark cycle (lights on 7:00 a.m.). Food and water were available ad libitum. Mice were checked daily for the presence of pups; the day of delivery was considered P0. Litter size was adjusted to 8 pups on P2. Pups were sacrificed on postnatal days P0, P5, P10, P15, and P21. From each litter, 3 mice were used for immunohistochemistry and 5 for in situ hybridization.

2.2. In situ hybridization

Antisense oligoprobes complementary to nucleotides 1–48 of AgRP mRNA [34] were synthesized by CyberGene AB (Huddinge, Sweden). The oligonucleotides were labeled at the 3′ end using terminal deoxynucleotidyltransferase (Amersham, Buckinghamshire, UK) with [^{33}P]dATP (NEN, Boston, MA, USA) to a specific activity of 1–4 × 10^6 cpm/ng oligonucleotide. The oligoprobes were purified through QIA quick spin columns (Qiagen/GmbH, Hilden, Germany).

For in situ hybridization, mice were sacrificed by decapitation, the brains dissected (brains from P0 and P5 mice were dissected with the skull), immersed in ice-cold phosphate-buffered saline, immediately thereafter snap-frozen with CO_2, cut from the entire rostro-caudal extent of the brain (14 μm thick coronal sections) using a cryostat (Microm, Heidelberg, Germany), and thaw-mounted onto ‘Probe On’ slides (Fisher Scientific, Pittsburgh, PA, USA). Brain sections were hybridized as described previously [16,40]. In brief, sections were air-dried and incubated in a hybridization cocktail (50% formamide, 4 × SSC, 1 × Denhardt’s solution [1% sarcosyl, 0.02 M phosphate buffer, 10% dextran sulphate], 500 μg/ml heat-denatured salmon sperm DNA, 1 × 10^7 cpm/ml of the labeled probe) in a humidified chamber for 16 h at 42 °C. After hybridization, sections were rinsed in 1 × SSC at 55 °C for 4 × 15 min and 30 min at room temperature then air-dried and dipped into NTB2 nuclear emulsion (Kodak, Rochester, NY, USA) diluted 1:1 with water. After exposure for 2 weeks (slides from P0 mice were exposed for 2 month), the slides were developed in Kodak D19, fixed in Kodak 3000 and mounted in glycerol. Sections were analyzed using a microscope equipped with a dark-field condenser (Nikon Microphot-MX) and a digital camera DXM1200 (Nikon). Illustrations were produced using the ×20 objective (Fig. 1).

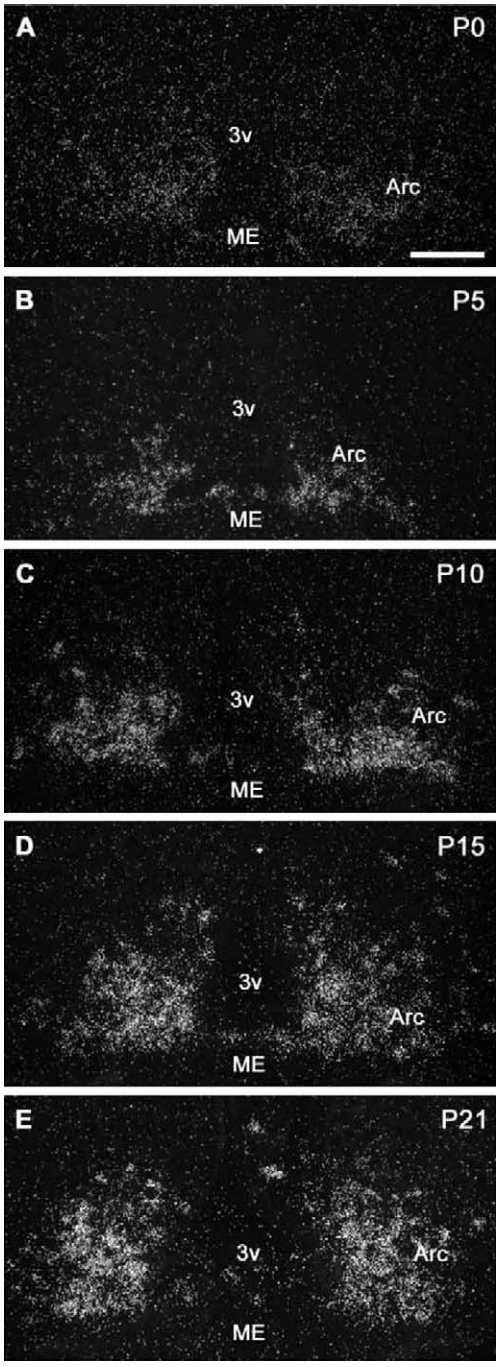

Fig. 1. AgRP mRNA expression in the mouse Arc at different postnatal developmental stages. (A) P0; (B) P5; (C) P10; (D) P15; and (E) P21. ME, median eminence, 3v, 3rd ventricle, scale bar = 100 μm.