General and Comparative Endocrinology 158 (2008) 183–190

Contents lists available at ScienceDirect

General and Comparative Endocrinology

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

Hormonal and synaptic influences of serotonin on adult neurogenesis

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ARTICLE INFO

Article history:
Received 2 June 2008
Accepted 16 July 2008
Available online 3 August 2008

Keywords:
Sinus gland
Bromodeoxyuridine
BrdU
Neurogenic niche
Molt inhibiting hormone
Crustacean hyperglycemic hormone

ABSTRACT

New neurons are incorporated into the adult brains of a variety of organisms, from humans and higher vertebrates, to non-vertebrates such as crustaceans. In virtually all of these systems serotonergic pathways appear to provide important regulatory influences over the machinery producing the new neurons. We have developed an in vitro preparation where adult neurogenesis can be maintained under highly controlled conditions, and are using this to test the influence of hormones on the production of neurons in the crustacean (*Homarus americanus*) brain. Serotonin levels have been manipulated in this in vitro preparation, and the resulting effects on the rate of neurogenesis have been documented. In addition we have compared in vitro influences of serotonin with results acquired from in vivo exposure of whole animals to serotonin. These experiments suggest that there are multiple mechanisms and pathways by which serotonin may regulate neurogenesis in the crustacean brain: (1) serotonin is effective in regulating neurogenesis at levels as low as 10^{-10} M, suggesting that circulating serotonin may have hormonal influences on neuronal precursor cells residing in a vascular niche or the proliferation zones; (2) contrasting effects of serotonin on neurogenesis (up- vs. down-regulation) at high concentrations (10^{-4} M), dependent upon whether eyestalk tissue is present or absent, indicate that serotonin elicits the release of substances from the sinus glands that are capable of suppressing neurogenesis; (3) previously demonstrated (Beltz, B.S., Benton, J.L., Sullivan, J.M., 2001. Transient uptake of serotonin by newborn olfactory projection neurons. Proc. Natl. Acad. Sci. USA 98, 12730–12735) serotonergic fibers from the dorsal giant neuron project directly into the proliferation zone in Cluster 10, suggest synaptic or local influences on neurogenesis in the proliferation zones where the final cell divisions and neuronal differentiation occur. Serotonin therefore regulates neurogenesis by multiple pathways, and the specific mode of influence is concentration-dependent.

1. Introduction

Serotonergic pathways are important regulators of neuronal birth during embryogenesis and in adult organisms (Whitaker-Azmitia et al., 1996; Brezun and Daszuta, 1999; Benton and Beltz, 2001; Fricker et al., 2005). We are interested in how serotonergic mechanisms are involved in regulating adult neurogenesis in the crustacean brain, where serotonin (5-hydroxytryptamine; 5HT) is localized in a handful of neurons including the dorsal giant neuron (DGN), whose anatomy and physiological functions have been extensively examined (Sandeman and Sandeman, 1987; Sandeman and Sandeman, 1994; Sandeman et al., 1995b; Sandeman et al., 1995a; Benton and Beltz, 2001). The paired DGNs innervate (1) the ipsilateral primary olfactory centers of the crustacean brain, the olfactory lobes (OLs); (2) synaptic regions that process higher-order olfactory, visual and mechanosensory information, the accessory lobes (ALs) (Sandeman et al., 1995a,b; Sullivan and Beltz, 2005a); and (3) the proliferation zone in Cluster 10 where new olfactory projection neurons are born throughout life (Fig. 1A) (Beltz et al., 2001; anatomical terminology from Sandeman et al., 1992). We have proposed that the DGN regulates adult neurogenesis in the crustacean brain via the release of serotonin directly into the Cluster 10 proliferation zone, thereby promoting cell division of the immediate precursors of the olfactory projection neurons (Fig. 1B) (Beltz et al., 2001). In the current paper, we explore serotonergic mechanisms further, with experiments demonstrating that multiple serotonergic pathways are likely to be involved in regulating neurogenesis in the adult crustacean brain.

Our understanding of serotonergic influences on neuronal development in the crustacean brain began with a series of studies in embryonic lobsters where serotonin levels were depleted using the pharmacological agent 5,7-dihydroxytryptamine (5,7-DHT). The brains of these embryos were reduced in size and structurally immature compared to the brains of embryos that developed with-

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doi:10.1016/j.ygcen.2008.07.016

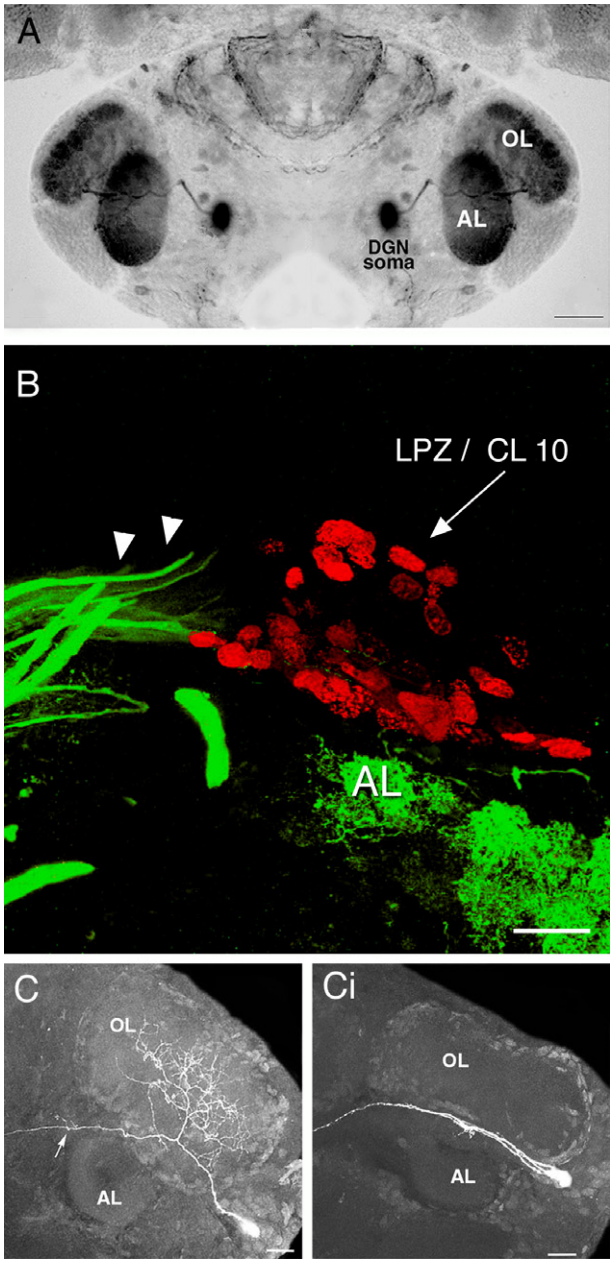

Fig. 1. (A) Serotonin immunostaining of an embryonic lobster brain at hatching. Each dorsal giant neuron (DGN) innervates the ipsilateral olfactory (OL) and accessory (AL) lobe. The intense labeling of the OL and AL is due to the massive DGN projection to these areas. (B) Following a 6-h survival time after BrdU injection, labeled nuclei (red) are found in the lateral proliferation zone (LPZ) immediately adjacent to the AL. Serotonin antibodies label a group of fibers (green; arrowheads) that terminate blindly at the proliferation zone. These fibers have been traced back to the DGN in the adult, juvenile, and larval brains (adapted from Beltz et al. (2001). (C) Stacked confocal images illustrate the morphology of normal OL (Ci) when these late embryos are chemically depleted of serotonin. The cells were filled intracellularly with Lucifer Yellow. The arrow in C points to the primary neurite that courses anteriorly to innervate target regions in the medulla terminalis (adapted from Sullivan et al. (2000). Scale bars: A = 200 μm. B, C = 20 μm.

out serotonin intervention (Benton et al., 1997). These abnormalities were reflected in reduced volumes of the OLs, ALs and olfactory globular tract neuropils (Benton et al., 1997). Subsequent studies in embryonic and juvenile lobsters demonstrated that this growth retardation following chronic 5,7-DHT treatment was associated with reduced neurogenesis among the olfactory interneurons (Beltz et al., 2001; Benton and Beltz, 2001), as well as a failure of these interneurons to branch and grow into the OLs and ALs (Fig. 1C, Ci) (Sullivan et al., 2000). Serotonin therefore influences both the proliferation and differentiation of neurons in the crustacean brain.

Most of the neurons in the brains of adult decapods are born during embryonic development and are the progeny of large precursor cells, known as neuroblasts (for review see Harzsch, 2003). Neuroblasts arise during early embryonic development and divide asymmetrically, generating specific lineages of neurons before degenerating during late embryonic or early postembryonic development. Proliferation in most regions of the decapod brain ceases, therefore, in the period around hatching. The exception to this, however, is in the central olfactory pathway where mitotic activity resumes after hatching among a group of cells with glial properties that reside in a vascular niche in crayfish and clawed lobsters (Sullivan et al., 2007a,b; however, see also Schmidt (2007) regarding persistent neuroblasts in adult spiny lobsters). The daughters of the precursors migrate along glial processes towards clusters 9 and 10, niche the soma clusters containing the olfactory interneurons (Fig. 2). Proliferation within these cell clusters occurs in restricted regions, known as proliferation zones. Pulse-chase BrdU experiments show that newborn cells are translocated away from the proliferation zones over time (Harzsch et al., 1999), become dispersed among the other cells in the clusters (Beltz et al., 2001), differentiate into neurons (Sullivan and Beltz, 2005b), are wired into the appropriate circuitry (Sullivan and Beltz, 2005b), and survive for at least a year (Beltz et al., 2001; Schmidt, 2001; Sullivan and Beltz, 2005b; Sullivan et al., 2007b). We are using this pathway as a model for examining mechanisms underlying neurogenesis as well as the regulation of these processes. The niche and migratory streams are readily accessible on the ventral surface of the brain and continue to be viable when brains are placed in organ culture, providing a convenient and reliable assay for examining mechanisms underlying neurogenesis.

Our previous 5,7-DHT depletion studies in lobsters suggested that *increased* levels of serotonin should *stimulate* the production of new neurons, however, this has not been tested directly by increasing serotonin levels and observing the resulting effect on adult neurogenesis. Therefore, the present study asks how increased serotonin levels influence the rate of neurogenesis in both the isolated brain preparation and in whole animals exposed to this amine. Experiments addressing this initial question led to a second area of interest relating to the possible influences of sinus gland hormones on adult neurogenesis. An abstract describing some of these studies has been published (Benton and Beltz, 2007), and the primary findings in lobsters presented here also have been confirmed in the crayfish, *Cherax destructor* (Rogan, 2004).

2. Methods and materials

2.1. Animals

Juvenile lobsters (1–2 cm total body length; stages 5–8) were obtained from the Lobster Rearing Facility at the New England Aquarium (Boston, MA), where they were reared in filtered seawater at 16–18 °C on a 12:12 light cycle. At Wellesley College, lobsters were maintained in the same temperature and lighting conditions, but in recirculating, filtered artificial seawater (SW). Animals from these stocks were manipulated in one of three ways (in vivo, in vitro and eyestalk-ablated in vivo preparations), and then were treated with serotonin or not (the control condition). Adult neurogenesis in these animals was defined as the time when only the adult mechanisms generating new neurons (i.e., the niche and streams) were present.