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Review

The role of brain-derived neurotrophic factor in the regulation of cell growth and gene expression in melanotrope cells of *Xenopus laevis*

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ABSTRACT

Brain-derived neurotrophic factor (BDNF) is, despite its name, also found outside the central nervous system (CNS), but the functional significance of this observation is largely unknown. This review concerns the expression of BDNF in the pituitary gland. While the presence of the neurotrophin in the mammalian pituitary gland is well documented its functional significance remains obscure. Studies on the pars intermedia of the pituitary of the amphibian *Xenopus laevis* have shown that BDNF is produced by the neuroendocrine melanotrope cells, its expression is physiologically regulated, and the melanotrope cells themselves express receptors for the neurotrophin. The neurotrophin has been shown to act as an autocrine factor on the melanotrope to promote cell growth and regulate gene expression. In doing so BDNF supports the physiological function of the cell to produce and release α-melanophore-stimulating hormone for the purpose of adjusting the animal's skin color to that of its background.

1. Introduction and overview of BDNF in the vertebrate pituitary gland

Brain-derived neurotrophic factor (BDNF) is a 14 KDa protein belonging to the neurotrophin family of growth factors. It supports neuron survival and stimulates the growth and differentiation of neurons [8,13]. BDNF also plays an essential role in the regulation of long-term potentiation, where there is a strengthening of synapses between neurons in support of memory processes [42,52,64]. Despite its name, BDNF is not only found in the brain but also in many peripheral organs [32,65,80] including the rat pituitary gland. Here, BDNF and/or its mRNA is found in both the anterior [14,23,25,36,37,58,68] and intermediate lobe [14,26,36,37,53,58] as well as in nerve terminals of the pars nervosa [14]. The above studies on the rat do not report the cell-type expressing BDNF in the anterior lobe, with the exception of Höpker et al. [25] who showed colocalization of BDNF-immunoreactivity with thyroid-stimulating hormone, and Rage et al. [58], reporting colocalization of the neurotrophin in the anterior pituitary lobe with S-100 protein, a marker in the pituitary for folliculo-stellate cells. The presence of the BDNF-specific tropomyosin-receptor-kinase B (TrkB) receptor in the rat pituitary gland [23,36,37,58] suggests that the neurotrophin could have a local autocrine/paracrine function. Immobilization stress increases anterior lobe *BDNF* mRNA [23,68], suggesting involvement of BDNF in the regulation of the hypothalamo–pituitary–adrenal axis. This idea is supported by the observation that adrenalectomy decreases the amount of *BDNF* mRNA in the anterior pituitary [37].

In the rat pars intermedia BDNF-immunoreactivity is found throughout the lobe, reflecting BDNF's presence in melanotrope cells [58]. *In vitro* addition of BDNF to intermediate lobe fragments from young (but not middle-aged or old) rats weakly stimulates secretion of α-melanocyte-stimulating hormone (α-MSH), an observation favoring the idea of an autocrine/paracrine function for the neurotrophin [58]. Höpker et al. [26] noted a depletion of immunoreactive BDNF from incubated intermediate lobe cells, which could be prevented by adding the dopamine receptor agonist apomorphine during the dissociation and cultivation of the cells. This result indicates that melanotrope cells sequester BDNF in the regulated secretory pathway, because secretory activity of rat melanotropes is known to be under inhibitory dopaminergic control [4].

From the above it is evident that BDNF and its receptor are present in the rat pituitary gland, but the functional significance of these observations is elusive. To our knowledge, the only other

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vertebrate species where pituitary BDNF has been studied is the amphibian, *Xenopus laevis*. Here, BDNF-immunoreactivity is found in melanotropes throughout the intermediate lobe [40] and in neurohemal nerve terminals ending in the pars nervosa [5,73]. Because the function of the *Xenopus* melanotrope cell is well established (see below) this is an ideal cell-type to investigate the functional significance of endocrine BDNF. This review introduces the *Xenopus* melanotrope cell as a model to study neuroendocrine integration, and then gives an overview of our studies on the role of BDNF in this integrative process.

2. The *Xenopus* melanotrope cell as a model for neuroendocrine integration

Amphibian intermediate lobe melanotrope cells are responsible for regulating skin color in a process known as "background adaptation". They produce and release α-MSH when an animal is on a black background and, through the action of circulating α-MSH on dermal melanophores, the black pigment melanin in the melanophores disperses and consequently the skin darkens. When the animal is placed on a white background release of the hormone is inhibited, the melanin pigment becomes punctuate in a perinuclear position, and the skin blanches [2,77]. The neuroendocrine reflex regulating this background adaptation process involves the eyes, with the optic information concerning color of background being processed by various brain centers that regulate the secretory activity of the melanotrope cell via neuronal projections to the pituitary gland (for reviews see: [41,63,71]). This regulation involves the action of a number of inhibitory and stimulatory neurotransmitters and neuropeptides acting directly on the endocrine cell. Details of this regulation have been extensively studied in two amphibian species, *Rana ridibunda* and *Xenopus laevis*; these studies reveal that the two species have developed similar strategies for the regulation of the secretory activity of the melanotrope cell (for reviews see: [21,31,50,62,63,74]). For the *Xenopus* melanotrope, many of the regulatory transmitters, acting through their specific receptors, converge on the adenylyl cyclase system to regulate the production of cyclic-AMP (see Fig. 1). This second messenger activates protein kinase A (PKA), which in turn regulates membrane ion channel complexes to stimulate the influx of Ca^{2+} through voltage-operated Ca^{2+} channels (VOCC). This Ca^{2+} signal induces exocytosis of α-MSH and, through the process of Ca^{2+}-induced Ca^{2+} release, it mobilizes Ca^{2+} from intracellular Ca^{2+} stores, thereby creating a self-propagating Ca^{2+} wave that travels through the cytoplasm and enters the nucleus. Activation of the melanotrope cell not only stimulates release of α-MSH but also increases the transcription and translation of proopiomelanocortin (POMC), the precursor protein of α-MSH. The intracellular signaling cascades regulating *Xenopus* melanotrope cell function have been extensively reviewed [30,29]. While cyclic-AMP and Ca^{2+} are the two major intracellular second messengers generated by the signal transduction machinery of *Xenopus* melanotropes, surprisingly, the promoter of *POMC* lacks cyclic-AMP responsive elements and Ca^{2+} responsive elements [16,28]. *POMC* expression in these cells is regulated, at least in part, through indirect mechanisms involving the immediate early genes *c-Fos* [35] and *Nur77* [45].

3. The *Xenopus* melanotrope cell expresses *BDNF*

In situ hybridization showed the presence of *BDNF* mRNA in the *Xenopus* melanotropes and immunohistochemistry extended this finding to the protein level [40]. Western blot analysis revealed the presence of both the precursor protein (proBDNF) and mature BDNF. Analysis of the subcellular distribution of BDNF, using a combination of high-pressure freezing, cryosubstitution and immunoelectron microscopy, demonstrated it to be sequestered within secretory granules of *Xenopus* melanotrope cells [76]. These same studies, using triple immunogold-labeling, established BDNF coexistence with POMC and α-MSH within these granules. This intragranular location and coexistence implies that BDNF follows the same regulated secretory pathway as POMC and its end-products, including α-MSH. Presumably, BDNF is released together with α-MSH from the actively secreting melanotropes of black-adapted animals. BDNF is an extremely potent protein (inducing biological responses at the sub-nanomolar level e.g. [18,56] and we have found that the amount released from the melanotrope cell remains below the detection limit of our BDNF assays (Jenks et al., unpublished).

4. Expression of *BDNF* in *Xenopus* melanotrope cells is physiologically regulated

Quantitative reverse-transcriptase polymerase chain reaction (Q-RT-PCR) revealed a 25-fold increase in *BDNF* mRNA in melanotropes of black compared to white background-adapted *Xenopus* [40]. In these studies the forward and reverse primers for the PCR were within the BDNF coding sequence, and therefore total *BDNF* mRNA was measured. The BDNF gene, however, possesses multiple promoters, each capable of producing a specific transcript [1,24,57]. To determine if there is promoter-specific expression of *BDNF* in *Xenopus*, we first characterized the structure of the *Xenopus* gene [35]. For this purpose *BDNF* transcripts of the *X. laevis* brain were sequenced and then mapped to the *Xenopus tropicalis* genome to determine exon–intron structure. This showed that *Xenopus BDNF* contains seven exons, giving rise to seven exon-specific transcripts, with each transcript containing the protein encoding exon VII (Fig. 2A). Q-RT-PCR analysis revealed transcript-specific expression in melanotropes during background adaptation [35]. The most highly upregulated transcript was transcript IV which displayed 130-fold increase in expression in animals on black background; in contrast transcript VII (a 5′ extension of exon VII), showed no difference between white- and black-adapted animals (Fig. 2B). Clearly the expression pattern of *BDNF* transcripts is highly promoter-specific. Analysis of the nucleotide sequence in the promoter region upstream of *Xenopus* exon IV revealed two potential Ca^{2+} responsive elements (CaRE1 and CaRE2) and a potential cyclic-AMP responsive element (CRE). This promoter region shows high sequence homology with that of rat and human BDNF [35]. In rat cortical neurons these elements are involved in the regulation of expression of *BDNF* transcript IV [10,69,70]. The high expression of transcript IV displayed by melanotropes of animals on black background fits well with the importance of Ca^{2+} and cyclic-AMP signaling in such cells. The *Xenopus* promoter region also has a potential binding site for a repressor transcription factor, namely a down-stream responsive element (DRE) that partially overlaps with the CRE element [35]. In mammals DRE functions as a binding site for the Ca^{2+} binding protein DRE antagonist modulator (DREAM), which in its Ca^{2+}-bound form, lifts itself from the DRE site to promote gene expression [7]. DREAM is involved in Ca^{2+}-dependent regulation of the expression of mammalian BDNF [51,60] and the same is likely true for *BDNF* expression in the *Xenopus* melanotrope. In the latter, Ca^{2+} waves generated at the membrane and entering the nucleus [38,67] might not only act on CaRE1 and CaRE2 but also on DREAM to induce *BDNF* expression.

Many of the *BDNF* transcripts of *Xenopus* possess long upstream untranslated regions (uUTRs), a phenomenon associated with inefficient mRNA translation [72]. Lengthy uUTRs are also found in *BDNF* transcripts of zebrafish [24], human [57] and rat and mouse [1]. Moreover, all *BDNF* transcripts have AUG start codons upstream to the translation initiation codon for pre–proBDNF. In