Review

Analysis of the melanotrope cell neuroendocrine interface in two amphibian species, *Rana ridibunda* and *Xenopus laevis*: A celebration of 35 years of collaborative research

Bruce G. Jenks [a,*], Ludovic Galas [b], Miyuki Kuribara [a], Laurence Desrues [c], Adhanet H. Kidane [a], Hubert Vaudry [c], Wim J.J.M. Scheenen [a], Eric W. Roubos [a], Marie-Christine Tonon [c]

[a] Dept. of Cellular Animal Physiology, Donders Institute for Brain, Cognition and Behaviour, Radboud University Nijmegen, Heyendaalseweg 135, 6525 AJ Nijmegen, The Netherlands
[b] Regional Platform of Research in Cellular Imaging of Haute-Normandie (PRIMACEN), European Institute for Peptide Research (IFRMP 23), University of Rouen, 76821 Mont-Saint-Aignan, France
[c] Inserm U982, Laboratory of Neuronal and Neuroendocrine Differentiation and Communication, European Institute for Peptide Research (IFRMP 23), University of Rouen, 76821 Mont-Saint-Aignan, France

ABSTRACT

This review gives an overview of the functioning of the hypothalamo–hypophyseal neuroendocrine interface in the pituitary neurointermediate lobe, as it relates to melanotrope cell function in two amphibian species, *Rana ridibunda* and *Xenopus laevis*. It primarily but not exclusively concerns the work of two collaborating laboratories, the Laboratory for Molecular and Cellular Neuroendocrinology (University of Rouen, France) and the Department of Cellular Animal Physiology (Radboud University Nijmegen, The Netherlands). In the course of this review it will become apparent that *Rana* and *Xenopus* have, for the most part, developed the same or similar strategies to regulate the release of α-melanophore-stimulating hormone (α-MSH). The review concludes by highlighting the molecular and cellular mechanisms utilized by thyrotropin-releasing hormone (TRH) to activate *Rana* melanotrope cells and the function of autocrine brain-derived neurotrophic factor (BDNF) in the regulation of *Xenopus* melanotrope cell function.

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1. Introduction

Most amphibians have the remarkable ability to adjust skin color in relation to the color of their environment, a process referred to as background adaptation. This often involves a redistribution of the black pigment melanin in skin melanophores. Early research shows that "background adaptation" is a neuroendocrine reflex involving the regulated release of α-melanophore-stimulating hormone (α-MSH) from melanotrope cells in the pituitary pars intermedia. In animals on a dark (or black) background there is release of this peptide hormone, and a subsequent dispersion of melanin pigment in skin melanophores and thus skin darkening; in animals on a light (or white) background, release of α-MSH is inhibited and thus there is a blanching of the skin (Bagnara and Hadley, 1973; Waring, 1963). In recent years considerable effort has been made to discover the molecular and cellular mechanisms involved in the neuroendocrine integration at the level of the intermediate lobe melanotrope cell. Two amphibian models have been particularly prominent in these endeavors, namely the European green frog, *Rana ridibunda* and the South African clawed toad, *Xenopus laevis*. It is apparent that these two amphibian species have developed similar, and in some respects even identical, strategies to regulate the biosynthesis and secretion of α-MSH. This review gives an overview of the neuroendocrine strategies utilized by *Rana* and *Xenopus* to regulate melanotrope cell function. This is followed by a more in depth analysis of two features of the regulatory systems involved, namely the actions of the neuropeptide thyrotropin-releasing hormone (TRH) in the regulation of melanotrope cells of *R. ridibunda* and the role of autocrine brain-derived neurotrophic factor (BDNF) in the regulation of melanotrope cell function in *X. laevis*.

2. Overview of neuroendocrine strategies in *Rana* and *Xenopus*

Early biosynthetic studies demonstrated that the production of α-MSH in amphibians begins with the synthesis of a precursor protein, proopiomelanocortin, POMC (Loh and Gainer, 1977; Martens et al., 1982a,b; van Strien et al., 1995a, 1996; Vaudry et al., 1984). Pulse-chase radiolabeling studies, used to follow processing of POMC, revealed that in both *Rana* and *Xenopus* the acetylation reaction to form the mature N-acetylated MSH is associated with the secretory process (Jenks et al., 1985; Martens et al., 1981; van Strien et al., 1995b; Vaudry et al., 1983), a phenomenon that seems unique to amphibians. This N-terminal acetylation gives rise to a 10-fold increase in the bioactivity of the peptide (Rudman

* Corresponding author. Fax: +31 24 365 2714.
  E-mail address: b.jenks@science.ru.nl (B.G. Jenks).

et al., 1983). In *Xenopus*, the terminal products of processing of the C-terminal "endorphin region" of POMC is the octapeptide N-acetyl-β-endorphin(1–8) (van Strien et al., 1993). The N-terminal acetylation of endorphins eliminates their ability to bind opiate receptors (Deakin et al., 1980). It is thus apparent that N-terminal acetylation reactions are important in setting the nature of the biological signal emanating from the melanotrope cell, namely a strong melanotropic signal. Both *Rana* and *Xenopus* produce γ-MSH, and larger N-terminal fragments of the precursor protein, such as pro-γ-MSH (Vaudry et al., 1984; van Strien et al., 1995a), but the biological significance of these peptides in amphibians has not been established.

Two approaches have been applied, in both *Rana* and *Xenopus* studies, to gain a basic outline of the regulatory mechanisms functioning in the melanotrope neuroendocrine interface. The first approach, immunohistochemistry, has been used to establish the presence of potential regulatory factors (secretagogues), either classical neurotransmitters or neuropeptides, in the vicinity of the melanotrope cells. The second approach, an analysis of the release of α-MSH from the superfused (perifused) intermediate lobe or from isolated melanotrope cells, has been applied to determine the action of these factors on the release of α-MSH. Specific receptor agonists and antagonists are then applied to the lobe or cells in superfusion experiments to establish the receptor mechanisms involved (see Fig. 1). These approaches have revealed that, in both species, there are three potent inhibitory factors regulating α-MSH release, namely dopamine (Adjeroud et al., 1986a; Tonon et al., 1983; Verburg-van Kemenade et al., 1986b), neuropeptide Y (NPY) (Danger et al., 1986; Verburg-van Kemenade et al., 1987b), and GABA (Adjeroud et al., 1986b, 1987; Verburg-van Kemenade et al., 1986a, 1987b). Ultrastructural studies established that these factors are colocalized in nerve terminals (varicosities) making synaptic contact with the melanotrope cells, with dopamine and NPY co-existing within electron-dense vesicles and GABA stored in electron-lucent vesicles (De Rijk et al., 1992; Tonon et al., 1992). In both species GABA inhibits secretion through both $GABA_A$ receptors and $GABA_B$ receptors (Adjeroud et al., 1986b, 1987; Buzzi et al., 1997; Shibuya et al., 1997; Verburg-van Kemenade et al., 1987c) while dopamine inhibits secretion through dopamine D_2 receptors (Adjeroud et al., 1986b, 1987; Buzzi et al., 1997; Shibuya et al., 1997; Verburg-van Kemenade et al., 1987c). In *Rana*, some nerve terminals only contained NPY (Tonon et al., 1992) and in this species NPY inhibits secretion through either Y_1 or Y_5 receptors (Galas et al., 2002) whereas in *Xenopus* only Y_1 receptors have been reported (Scheenen et al., 1995; Verburg-van Kemenade et al., 1987b; Zhang et al., 2006). Endogenous endozepines, probably of astroglial origin, inhibit the function of $GABA_A$ receptors in *Rana* (Tonon et al., 1989). It is not known if this modifying mechanism also functions in *Xenopus*. Tracing studies conducted in *Xenopus*, show that the inhibitory terminals synapsing on the melanotropes belong to neurons with cell bodies in the hypothalamic suprachiasmatic nucleus (Tuinhof et al., 1994; Ubink et al., 1998) and for this reason these neurons have been designated suprachiasmatic melanotrope-inhibiting neurons or "SMINs" (Ubink et al., 1998). The SMINs show high expression of NPY in *Xenopus* adapted to a white background compared to a black background (Tuinhof et al., 1993), and their synaptic varicosities proliferate and are larger in white background-adapted animals (Berghs and Roubos, 1996). This synaptic plasticity fits well with the notion that these neurons are actively inhibiting the melanotrope cells in animals on white background.

In both *Rana* and *Xenopus* multiple stimulatory mechanisms act on melanotrope cells (see Fig. 1). These mechanisms involve release of regulatory factors either from nerve terminals in close proximity to the melanotrope cells or from remote terminals, in the neurohemal pars nervosa. In the latter case the secretagogues

must presumably diffuse (or be carried by the blood) to the pars intermedia to effectuate their stimulatory actions on the melanotrope cells (e.g., van Wijk et al., 2010). The melanotropes of both species are stimulated by noradrenaline (Lamacz et al., 1995; Roubos et al., 2002); in *Rana* serotonin acts presynaptically to stimulate these noradrenergic nerve terminals (Lamacz et al., 1989b), whereas in *Xenopus* serotonin acts directly on the melanotrope cells (Ubink et al., 1999). *Xenopus* in particular has a large number of stimulatory peptides of neural lobe origin and many of the neurons involved are traced to the hypothalamic magnocellular nucleus (Roubos et al., 2010; van Wijk et al., 2010). In *Rana*, both TRH- and CRH-producing neurons penetrate into the pars intermedia (Lamacz et al., 1989a; Tonon et al., 1985) but only TRH stimulates α-MSH secretion (Tonon et al., 1980). For *Xenopus*, all members of the CRH-family of peptides (CRH, sauvagine and urocortin) are potent stimulatory secretagogues (Verburg-van Kemenade et al., 1987a; Calle et al., 2005). The significance of having so many stimulatory mechanisms converging on the melanotrope cells is unclear. Possibly, different information concerning environmental (background color, temperature, humidity) or internal (physiological) conditions is conveyed to the pars intermedia via these mechanisms. Low temperature has been shown to inhibit SMINs and to activate the hypothalamic magnocellular TRH-system of *Xenopus*, resulting in enhanced α-MSH release (Tonosaki et al., 2004). Osmotic stress (saline immersion) gives strong activation of magnocellular neurons and is accompanied by activation of intermediate lobe melanotropes (Ubink et al., 1997). Evidence for differential control of magnocellular neurons comes from studies in which *Xenopus* were fasted for three weeks, leading to decreased amounts of, e.g., urocortin and an increase in the amount of CRH (Calle et al., 2006a). Morphophysiological research is required to establish the relationships between external regulatory stimuli, specific magnocellular-neuron activations, and the response of the melanotrope cells to the diverse stimulatory inputs.

The operation of several stimulatory "autocrine" mechanisms has been shown for *Xenopus* (Fig. 1). One of these concerns the Ca^{2+} sensing receptor, a G protein-coupled receptor with extracellular Ca^{2+} as the natural ligand. Because secretory granules have high Ca^{2+} content, it has been suggested that Ca^{2+} released during exocytosis, would be the Ca^{2+} source to activate this receptor (van den Hurk et al., 2003). *Xenopus* melanotropes produce the neurotrophin BDNF (Kramer et al., 2002), which is co-packaged in secretory granules with α-MSH (Wang et al., 2004). These cells also express receptors for BDNF, including the TrkB receptor (Kidane et al., 2007), and the neurotrophin has been shown to stimulate the biosynthesis of POMC (Kramer et al., 2002). The above, to be covered in more detail later in this review, indicates that BDNF acts as an autocrine factor in the regulation of *Xenopus* melanotrope cell function. In addition, acetylcholine is produced by the *Xenopus* melanotrope and this neurotransmitter stimulates secretion through a muscarinic M_1 receptor mechanism (van Strien et al., 1996). Secretory activity of the melanotrope cell of *Rana* is also simulated by acetylcholine, also through the M_1 receptor, but in this case the acetylcholine is released by nerve terminals in the vicinity of the melanotropes (Lamacz et al., 1989c). In addition, *Rana* melanotropes are regulated by two factors, inhibitory adenosine (Chartrel et al., 1992) and stimulatory neurotensin (Desrues et al., 1998), which likely reach their targets via the general circulation.

The melanotrope cells of both *Rana* and *Xenopus* have been used extensively to determine intracellular mechanisms regulating the neuroendocrine secretory process. In *Rana*, TRH and neurotensin exert their stimulatory effects on α-MSH release by activating phospholipase C (PLC) and the formation of inositol trisphosphate (IP_3) and diacyl glycerol (Belmeguenai et al., 2003; Desrues et al., 1990), while dopamine acting through D_2 receptors, NPY acting