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MULTITRANSMITTER REGULATION OF MELANOTROPE CELLS OF *XENOPUS LAEVIS*

by

C.A.F.M. BERGHS, R. TUINHOF, F.J.C. VAN STRIEN, W.J.J.M. SCHEENEN, J.R. LIESTE, C.H. DOTMAN, R. UBINK, B.G. JENKS and E.W. ROUBOS

(*Department of Cellular Animal Physiology, Nijmegen Institute for Neurosciences, Faculty of Science, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands*)

ABSTRACT

The amphibian *Xenopus laevis* is able to adapt to a dark background by releasing melanophore-stimulating hormone (α-MSH) from the melanotrope cells in the pars intermedia of the pituitary gland. This mechanism is being used as a model to study fundamentals of neuroendocrine information processing. Recent data obtained from multidisciplinary studies in our group on the functioning and hypothalamic regulation of *Xenopus* melanotropes are reviewed.

KEY WORDS: pars intermedia, suprachiasmatic nucleus, neuropeptides, *Xenopus laevis*.

MELANOTROPE CELLS

Placing the clawed toad *Xenopus laevis* on a black background stimulates the melanotrope cells in the intermediate lobe of the pituitary gland to release pro-opiomelanocortin (POMC)-derived peptides, including α-MSH and α,N-acetyl-β-endorphin$_{1\text{-}8}$ (JENKS *et al.*, 1993; VAN STRIEN *et al.*, 1993). The melanotrope cells contain dark, grey and light secretory vesicles. Only dark vesicles are formed by the Golgi apparatus (ROUBOS & BERGHS, 1993). Immunoelectron microscopy showed that desacetyl-α-MSH and β-endorphin coexist in all three vesicle types. Immunoreactivities to these peptides are lowest in dark and highest in light vesicles. It is proposed that intragranular processing of POMC to desacetyl-α-MSH and α,N-acetyl-β-endorphin is concomitant with an increase in vesicle size and a decrease in vesicle electron-density. Black background-induced activation of melanotrope cells is reflected by an increase in immunoreactivity of the secretory vesicles to each antiserum, indicating stimulation of intravesicular formation of peptides from POMC. In addition, cell activation evokes an increase in the percentage of the vesicle population that reacts with anti-α,N-acetyl-β-endorphin, probably by stimulating acetylation of β-endorphin (ROUBOS & BERGHS, 1993). Apparently, this acetylation is a regulated event that occurs within the vesicles, independently from the acetylation of desacetyl-α-MSH, which takes place near the plasmalemma at the time of vesicle exocytosis.

NEURAL CONTROL OF MELANOTROPE CELLS

The melanotrope cells are controlled by various neural factors. The stimulatory factors corticotropin-releasing hormone and thyrotropin-releasing hormone, originating in the hypothalamic magnocellular nucleus (TUINHOF *et al.*, 1994a), occur in axon terminals in the neural pituitary lobe, from where they are thought to act upon melanotrope cells after diffusion to the pars intermedia (JENKS *et al.*, 1993). Noradrenalin is present in a fibre network in the pars intermedia as well as in neurons in the locus coeruleus (GONZÁLEZ & SMEETS, 1993). The locus coeruleus and the hypothalamic suprachiasmatic nucleus (SC) become labelled upon application of retrograde tracers (DiI, DiA) into the pars intermedia (TUINHOF *et al.*, 1994a). The inhibitory factors dopamine (DA), γ-aminobutyric acid and neuropeptide Y (NPY) coexist in synaptic contacts on the melanotropes (DE RIJK *et al.*, 1992). There would appear to be a plasticity of the axonal network in the pars intermedia related to the state of adaptation of the animal to background light conditions; upon changing *Xenopus* from a dark to a white background, the size of the synaptic contacts as well as the number and length of active zones in these contacts increases (C.A.F.M. BERGHS & E.W. ROUBOS, unpublished data). *In situ* hybridisation revealed that SC neurons produce preproNPY-mRNA in animals adapted to a white background but hardly or not at all in black-adapted toads (TUINHOF *et al.*, 1993). In an immunocytochemical double labelling study using confocal laserscanning microscopy, some SC neurons were shown to contain both NPY and DA (TUINHOF *et al.*, 1994b). Furthermore, filling of the optic nerves with horseradish peroxidase indicated the existence of a direct connection between the retina and NPY- and DA-producing SC neurons (TUINHOF *et al.*, 1994a). These findings underline the importance of the SC in light-mediated control of background adaptation by *X. laevis*. Using *in vitro* superfusion, patch clamp, video-imaging and confocal laserscanning methods it has been shown that the hypothalamic factors tested control the release of α-MSH via cAMP and calcium ions. In this control N-type calcium channels and calcium oscillations play an important role (SCHEENEN *et al.*, 1994a,b; J.R. LIESTE, unpublished results). Moreover, hypothalamic factors control the expression of POMC mRNA and the biosynthesis of POMC (C.H. DOTMAN, unpublished data). Studies on the relationship between biosynthetic and secretory activity of melanotrope cells of *Xenopus* are in progress.

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