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Research report

Evidence that urocortin I acts as a neurohormone to stimulate αMSH release in the toad *Xenopus laevis*

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Abstract

We have raised the hypothesis that in the South African clawed toad *Xenopus laevis*, urocortin 1 (UCN1), a member of the corticotropin-releasing factor (CRF) peptide family, functions not only within the brain as a neurotransmitter/neuromodulator but also as a neurohormone, promoting the release of α-melanophore-stimulating hormone (αMSH) from the neuroendocrine melanotrope cells in the intermediate lobe of the pituitary gland. This hypothesis has been investigated by (1) assessing the distribution of UCN1 and CRF by light immunocytochemistry, (2) determining the subcellular presence of UCN1 in the neural lobe of the pituitary gland by immuno-electron microscopy applying high-pressure freezing and cryosubstitution, and (3) testing the effect of UCN1 on MSH release from toad melanotrope cells using in vitro superfusion.

In the *X. laevis* brain, the main site of UCN1-positive somata was found to be the Edinger–Westphal nucleus. UCN1 immunoreactivity (ir) also occurs in the nucleus posteroventralis tegmenti, central gray, nucleus reticularis medius, nucleus motorius nervi facialis, and nucleus motorius nervi vagi. UCN1 occurs together with CRF in the nucleus motorius nervi trigemini, and in the magnocellular nucleus, which send a UCN1- and CRF-containing fiber tract to the median eminence. Strong UCN1-ir and CRF-ir were found in the external zone of the median eminence. From the internal zone of the median eminence, UCN1-ir fibers, but few CRF-ir fibers, were found to project to the pituitary neural lobe, where they form numerous neurohemal axon terminals. Ultrastructurally, two types of terminal containing UCN1-ir secretory granules were distinguished: type A contains large, moderately electron-dense, round secretory granules and type B is filled with smaller, strongly electron-dense, ellipsoid secretory granules. In vitro superfusion studies showed that UCN1 stimulated the release of αMSH from melanotrope cells in a dose-dependent manner.

Our results support the hypothesis that in *X. laevis*, UCN1 released from neurohemal axon terminals in the pituitary neural lobe functions as a stimulatory neurohormone for αMSH release from melanotrope cells of the pituitary intermediate lobe.

Theme: Endocrine and autonomic regulation
Topic: Neuroendocrine regulation: other

Keywords: Neural pituitary lobe; Corticotropin-releasing factor; Melanotrope cells; Magnocellular nucleus; High-pressure freezing; Cryosubstitution; Immuno-electron microscopy

1. Introduction

Urocortin I (UCN1) is a 40 amino acid peptide first isolated from rat brain by molecular cloning in 1995 [44]. It is a member of the corticotropin-releasing factor (CRF) peptide family and exhibits 45% amino acid sequence

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similarity to rat CRF, 63% sequence similarity to carp urotensin I and 35% sequence similarity to sauvagine [44]. The main sites of UCN1 production in the mammalian brain are the Edinger–Westphal nucleus, from which UCN1 is transported to the spinal cord, the lateral septum [2,33], and the lateral superior olive where UCN1 is proposed to target accessory optic, precerebellar and auditory structures, and the spinal intermediate gray. In addition, moderate levels of UCN1-immunoreactivity (ir) occur in the cerebellum, hippocampus, neocortex, olfactory system, basal ganglia, amygdala, and the supraoptic, ventromedial, and paraventricular nuclei of the hypothalamus [2,17,22]. In amphibians, UCN1-ir was reported in the brain of the frog *Rana esculenta*, where it mainly occurs in the Edinger–Westphal nucleus, anterior preoptic area, ventromedial thalamic nucleus, nucleus of the posterior tuberculum, and the nucleus of the medial longitudinal fasciculus [18].

UCN1 may have a diversity of functions. It clearly has anorectic activity, inhibiting food intake via the ventromedial hypothalamic nucleus and the hypothalamic paraventricular nucleus [23,48]. A role for UCN1 in stress adaptation is suggested by the fact that it may be released from the rat Edinger–Westphal nucleus under conditions of chronic stress [16] and is down-regulated in the Edinger–Westphal nucleus in mice over-expressing CRF [19]. While UCN1 in the brain probably acts on central neurons in both synaptic and non-synaptic ("volume transmission") ways, there is circumstantial evidence that UCN1 can also control peripheral targets. In the rat, UCN1 increases heart rate, probably via its action on CRF type 2 receptors (CRF_2) [4,26] and may induce a drop in blood pressure via mesenteric vasodilatation [26]. Moreover, the stimulatory action of UCN1 on ACTH release from the distal lobe of the rat pituitary gland suggests that UCN1 can act as a neurohormone [1]. However, the sites of synthesis and release of corticotrope-stimulating UCN1 are unknown.

In the present study, we analyzed the distribution and possible neurohormonal actions of UCN1 in the South African clawed toad, *Xenopus laevis.* This species was chosen because it is a well-established model for studying the functioning and regulation of the intermediate lobe of the pituitary gland [14,15,27–30,37,38,45] but the roles of CRF and UCN in these processes are unclear. The neurohormonal and neural control of the release of α-melanophore-stimulating hormone (αMSH) from the melanotrope cells of the *Xenopus* intermediate pituitary lobe has been extensively studied. A rise in circulating αMSH causes darkening of the animal's skin, a process known to occur in response to environmental challenges like placing the animal on a dark background or lowering the ambient water temperature [27–30,37,38]. Various inhibitory and stimulatory factors are proposed to control the release of αMSH from the melanotrope cells, including CRF [14,15,45]. Because both the *X. laevis* magnocellular nucleus and the neural lobe of the pituitary gland stained positively with an antiserum raised against mammalian CRF [45], it has been proposed that CRF is released from neurohemal axon terminals in the neural lobe and diffuses towards the pars intermedia to activate the melanotropes [14]. However, CRF-immunopositivity was also seen in the median eminence [45], leaving open the possibility that it acts on the melanotropes via the classical median eminence–pituitary portal system.

The homology of xCRF with both rat and human CRF is 93% [34] and the homologies of xUCN1 with rat and human UCN1 are 70% and 65%, respectively [8,53]. Recently, Dautzenberg and colleagues [5–7] isolated cDNAs for two *X. laevis* CRF receptors, $xCRF_1$ and $xCRF_2$, which share a high degree of sequence similarity with their mammalian counterparts. We recently demonstrated by RT-PCR the expression of both $xCRF_1$ and $xCRF_2$ mRNAs in the *X. laevis* neurointermediate lobe (B.G. Jenks, unpublished results). The $xCRF_1$ binds xCRF with higher affinity than *X. laevis* UCN1, but the $xCRF_2$ binds both peptides with similar high affinity (G.C. Boorse and R.J. Denver, unpublished results). Based on these results, we have raised the hypothesis that in *X. laevis* both CRF and UCN1 function as neurohormones controlling the pituitary melanotrope cells.

We have tested this hypothesis by analyzing the distribution of UCN1-ir in comparison to that of CRF-ir in the brain and pituitary gland of *X. laevis*. We paid special attention to potential sites where these peptides could be released to act on the melanotrope cells, viz., the median eminence of the hypothalamus and, in particular, the neural lobe of the pituitary gland. Affinity-purified antibodies were used that had been generated against synthetic xCRF to detect xCRF-ir. To detect UCN1-ir in *X. laevis*, we used an antiserum to rat UCN1, and verified the specificity of the immunoreaction by preabsorption with synthetic *X. laevis* peptides. We also determined the subcellular distribution of UCN1 in the pituitary neural lobe by immuno-electron microscopy, applying our recently developed high-pressure freezing (HPF) and cryosubstitution method [49]. Finally, the effect of UCN1 on αMSH release from *X. laevis* melanotrope cells was tested in an in vitro superfusion study.

2. Methods

2.1. Animals

Forty adult (6 months) specimens of *X. laevis*, with a body weight of 28–32 g, were raised under standard laboratory conditions and fed weekly on ground beef heart and Trouvit trout pellets (Trouw, Putten, The Netherlands). They were kept under constant illumination at a water temperature of 22 ± 1 °C, and adapted to a gray background for 3 weeks. All experiments were carried out under the guidelines of the Dutch law concerning animal welfare.