Sequence analysis of Somatolactin from *Cichlasoma dimerus* and its relation with MCH and GnRH systems

by
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**ABSTRACT.** - In this study we detected a clear morphological close association between MCH fibers and SL cells in the Pars Intermedia. According to this finding, pituitaries incubated with MCH showed an increase of SL release in a dose-dependent manner. GnRH was used as control stimuli and showed dose dependence as well. These results propose that MCH and GnRH play a role in the cdSL release. On the other hand, cdSL is highly related to Perciform fish and contains a N-Glycosylation site at position 121 and includes the seven characteristic cysteine residues of the mature peptide.

Key words. - somatolactin - GnRH - MCH - pituitary.

**Introduction**

Somatolactin (SL) is proposed as a multifunctional hormone. In *Cichlasoma dimerus*, we found clear evidence of a possible involvement of SL together with α melanocyte stimulating hormone (MSH) and melanin concentrating hormone (MCH) in background colour adaptation (Cánepa et al., 2006). However, there is scarce information on the regulation of SL release. This study is focused on the relation between SL, MCH and GnRH. In addition we report the characterization of partial *C. dimerus* SL (cdSL) cDNA.

**Methods**

Coronal sections (7 mm) of brain at pituitary level were processed for double immunohistochemistry (IHC) with anti-salmon MCH (1:1000 dilution) and anti-*Sparus aurata* (sa) SL (1:1000 dilution). The MCH fibers were developed with anti-rabbit rodamine-conjugated secondary antibody. SL cells were developed with anti-rabbit Fluorescein-conjugated secondary antibody and then mounted and observed with a confocal microscope.

Pituitary glands were removed from *C. dimerus* and cultured during 2 days in 96 wells plate with L15. On day 1, medium was removed and fresh medium was added with or without GnRH or MCH (0.1 to 10 µM). After a 24 h-incubation period, medium was collected and frozen for further analysis by western blot.

Degenerate primers were used to characterize, by PCR and further sequence analysis, the internal region of cdSL cDNA from pituitary gland.

**Results and discussion**

We found a clear morphological association between MCH fibers and SL cells suggesting a possible interaction. Besides, we found GnRH fibers in association with SL cells as observed in trout and pejerrey (Parhar and Iwata 1994, Stefano et al., 1999).

The analysis by immunoblots showed that, after a 24 h-incubation period with MCH or GnRH, SL release by male’s pituitaries increases significantly in a dose-dependent manner (p < 0.05) (Fig. 1A, 1B).

In addition, we characterized a partial cdSL cDNA that contains 879-bp (Accession number: EF192603) and shares 59-86% nucleotide sequence identity with other teleost SLs. Besides cdSL cDNA encodes a partial signal peptide and the mature protein of 207 aa. The cdSL has a N-Glycosylation site at position 121 and includes the seven characteristic cysteine residues of the mature peptide. This fact validates the use of heterologous SL antisera in *C. dimerus*.

**Conclusions**

Results of double-labelled IHC and *in vitro* cultures of pituitary glands propose that MCH and GnRH could play a role in the cdSL release. On the other hand the characterization of cdSL indicates that it is highly related to Perciform fish. The cdSL characterization will allow us to develop the necessary tools for future physiological studies on SL function.

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![Figure 1.](image_url) - Action of MCH and GnRH on SL released from males and females pituitaries. (ROD relative optical density).

**Figure 1.** - Action of MCH and GnRH on SL released from males and females pituitaries. (ROD relative optical density).

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