Cell Tissue Res (1993) 271:375–380

Cell & Tissue Research

Regulatory peptides in gut endocrine cells and nerves in the starfish *Marthasterias glacialis*

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Received: 14 April 1992 / Accepted: 20 August 1992

Abstract. The endocrine cells of the starfish digestive tract are spindle-shaped, contacting both the lumen and the basiepithelial plexus. Silver impregnation labels the basiepithelial and subcoelomic plexuses as well as these cells. Twenty antisera have been tested using the avidin-biotin method, in order to identify the regulatory substances involved in this system. Endocrine cells and nerves immunoreactive to GFNSALMFamide- (S1), FMRFamide-, peptide tyrosine-tyrosine- (PYY), pancreatic polypeptide- (PP), melanocyte stimulating hormone- (αMSH) and peptidylglycine alpha-amidating monooxygenase- (PAM) specific antisera have been found in the epithelium. The antibodies against S1, a peptide isolated from the nervous system of a starfish, and αMSH, stain both the basiepithelial plexus and the subcoelomic plexus, but the others react only with nerves in the basiepithelial plexus. Absorption controls show that antibodies for S1 and FMRFamide totally cross-react recognizing the same molecule, possibly S1. The other antibodies do not show cross-reactivity to any of the rest, and thus we conclude that these regulatory peptides are present in starfish. This is the first report of the presence of FMRFamide, PYY, αMSH and PAM in the Echinodermata. Under the electron microscope the endocrine cells exhibit secretory granules, microtubules and mitochondria. Direct contact with the subcoelomic plexus can be observed.

Key words: Immunocytochemistry – Gut – Innervation – Regulatory peptides – Endocrine cells – *Marthasterias glacialis* (Echinodermata)

Introduction

The innervation of the alimentary tract of the starfish, *Marthasterias glacialis*, consists of two nervous plexuses, separated by a connective layer: a basiepithelial plexus derived from the ectoneural system, and a subcoelomic plexus, related to the entoneural system, which innervates the muscle cells (Cobb and Raymond 1979). Although some authors have described the ultrastructure of the nervous system in different echinoderms (Bachmann and Goldschmid 1978; Cobb 1969, 1978; Doyle 1967; Fontaine 1962; Welsch et al. 1989), a systematic study of the involved peptides does not exist.

In a previous study, we described, for the first time, a diffuse endocrine system in the epithelium of the pyloric caeca of *M. glacialis*. This consists of endocrine cells connected to the basiepithelial plexus (Martínez et al. 1989), which are positive to pancreatic polypeptide (PP), glucagon and somatostatin. A positive reaction to PP is also given by the basiepithelial plexus. Some immunoreactive products have also been reported in crinoids (Welsch et al. 1989a, b), and CCK-positive cells have been demonstrated in an holothurian (García-Arrarás et al. 1991). However, a study on calcitonin and CGRP distribution in echinoderms (Sasayama et al. 1991) has failed to show any immunoreactivity in starfish. Recently, an octapeptide, the GFNSALMFamide (S1), has been sequenced (Elphick et al. 1990, 1991a, 1991b) and immunocytochemically located (Moore et al. 1990) in the central nervous system of the starfish *Asterias rubens*.

The present work has been undertaken to: (1) complete our former study using new antibodies raised against regulatory substances, in order to identify new starfish-specific peptides; and (2) try and immunocytochemically detect the enzymes that are involved in the peptide-amidating process.

Materials and methods

Twelve adult specimens of the starfish *Marthasterias glacialis* were collected from the Cantabrian sea (Spain). After anaesthesia with magnesium chloride, a careful dissection of the digestive tract was performed. Pieces of cardiac and pyloric stomachs, pyloric caeca, intestine and rectal caeca were processed.

The fragments to be embedded in paraffin were fixed in isotonic GPA (glutaraldehyde, picric and acetic acids) as described in Martínez et al. (1989). Tissues were sectioned at a thickness of 3 μm,

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