DIRECT MASS SPECTROMETRY OF BUCCAL GANGLIA AND NERVES REVEALS THE PROCESSING AND TARGETING OF PEPTIDE MESSENGERS INVOLVED IN FEEDING BEHAVIOUR OF LYMNAEA

by

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ABSTRACT

Feeding in molluscs is initiated and modulated by central neurons, especially those located in the buccal ganglia. Many of these neurons use diverse peptides as neuromessengers. Direct matrix-assisted laser desorption mass spectrometric analysis of tissues demonstrates that a large number of peptides are present in the buccal ganglia. Furthermore, the newly identified -LFRFamide peptides are shown to target to both the buccal mass and the oesophagus, whereas Lymnaea inhibitory peptide is shown to target to the oesophagus. In accordance to the mass spectrometric data, immunocytochemistry using antibodies against Lymnaea inhibitory peptide indicates that the nerves that innervate the oesophagus are indeed heavily immunoreactive, and the nerves that innervate the buccal mass are only faintly immunoreactive.

KEY WORDS: MALDI, immunocytochemistry, buccal mass, oesophagus.

INTRODUCTION

Feeding in molluscs, e.g., Lymnaea stagnalis and Aplysia californica, is an advantageous behavioural system for a multidisciplinary approach at various levels of analysis ( Benjamin & Elliott, 1989; Weiss et al., 1992). The intake of food particles consists of a sequence of events, involving rhythmic protrusion and retraction of the radula, and is controlled by several buccal muscles (Weiss et al., 1992). Food is scraped from the substrate into the cavity of the buccal mass and delivered to the oesophagus for swallowing. The distension of the gut by food particles contributes to satiety (Elliott & Benjamin, 1989). The rhythmic movement of the radula is initiated and maintained to a large extent by a set of neurons that make up the central pattern generator ( Benjamin & Elliott, 1989). The central pattern generator drives the various motor neurons that control and/or modulate the buccal muscle activities. These neurons, together with sensory neurons and interneurons that at various levels modulate the neuronal outputs (Yeoman et al., 1993), are mostly located in the buccal ganglia.
Studies on *Aplysia* (Weiss *et al.*, 1992) showed that the different buccal neurons co-express overlapping yet distinct sets of peptides such as FMRFamide, myomodulins, buccalins and small cardioactive peptides. When released, the peptides have interactive effects on the buccal muscles, thereby modulating the degree and activity of scraping. Similarly, the second phase of feeding, *i.e.*, swallowing, may also be modulated by multiple peptide messengers released by buccal neurons. For example, the *Lymnaea* tetradecapeptide that in the *in vitro* condition excites the contraction of the oesophagus (Li *et al.*, 1993) might influence the rate of passage of food particles to the stomach in the *in vivo* situation.

In order to fully understand the molecular basis underlying feeding behaviour, the identity and localization of messengers involved, including hitherto unidentified peptides, needs to be determined. Here, we explore a novel method, direct matrix-assisted laser desorption ionization mass spectrometry, to gain insight into the peptide diversity of the various components of the feeding network, and the differential transport of these peptides to the peripheral target tissues. We focus on two classes of peptides, of which the precursor structures have been recently elucidated, namely the -LFRFamide peptides (R.M. Hoek, unpubl. res.) and the *Lymnaea* inhibitory peptides (A.B. Smit, unpubl. res.). In addition, we performed immunocytochemical studies to substantiate the mass spectrometry data.

**MATERIALS AND METHODS**

**Mass spectrometry**

Adult laboratory bred *L. stagnalis* were used. Buccal ganglia and the connecting nerves were separately dissected and immediately transferred to the 1 μl matrix solution (2,5-dihydroxybenzoic acid, 10g/L) previously deposited on a metal target (cf. Jiménez *et al.*, 1994; Li *et al.*, 1994a,b,c). The tissues were ruptured in the matrix solution and then dried by a gentle stream of cold air. The target was inserted into the mass spectrometer immediately afterwards. Mass spectrometry was performed on a Finnigan Mat Vision 2000 laser desorption time of flight mass spectrometer.

**Whole mount immunocytochemistry**

Whole mount immunocytochemical studies were performed as described previously (Hoek *et al.*, 1992) using antiserum against synthetic *Lymnaea* inhibitory peptide.