A NOVEL METHOD TO STUDY DIVERSITY AND FUNCTIONS OF PEPTIDES IN NEURONAL NETWORKS: PEPTIDES OF THE NETWORK UNDERLYING MALE COPULATION BEHAVIOUR IN THE MOLLUSC LYMNAEA STAGNALIS

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ABSTRACT

Direct matrix-assisted laser desorption ionization mass spectrometry of neurons and nerves was used to study the diversity, localization and transport of neuropeptides that are produced in the neuronal network that underlies male copulation behaviour in the mollusc Lymnaea stagnalis. These studies reveal a large peptide diversity and distinct peptide profiles in different parts of the network, suggesting a complex regulation of copulation behaviour. Two peptides, e.g., conopressin and APGWamide, which are co-localized in neurons of the network, are involved in the control of vas deferens activities. Conopressin, which is structurally related to vasopressin, induces muscular contractions of the vas deferens, whereas APGWamide inhibits the conopressin-induced contractions. Together, these peptides may be involved in the modulation of peristaltic movements of the vas deferens.

KEY WORDS: direct peptide profiling by mass spectrometry, peptidergic neurons, male sexual behaviour, vas deferens, co-transmission of antagonistic peptides, APGWamide, conopressin, mollusc, Lymnaea stagnalis.

INTRODUCTION

In Lymnaea, male mating behaviour is a flexible behaviour consisting of a series of events that are a prelude to copulation (VAN DUIVENBODEN, 1984). During copulation, the penis complex is everted and intromission takes place. These activities are accurately tuned in to the production and ejaculation of semen by the prostate gland and vas deferens. At the same time, other reproductive activities (e.g., egg laying) are suppressed.

Male copulation is controlled by a neuronal network consisting of several clusters of central neurons that are interconnected and in addition send an axon into the penis nerve (VAN DUIVENBODEN, 1984; SMIT et al., 1992), the sole nerve that innervates the penis complex, its associated muscles, the vas deferens and the prostate gland. Most neurons of this network are peptidergic and initial experiments using

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well-established peptide chemical and cDNA cloning methods have resulted in the structural characterization of some of the peptides. We have now applied mass spectrometry directly to nervous tissue and show that many more peptides are present in the copulation network. Current studies focus on the molecular characterization and functional aspects of these peptides. In the present paper, we will restrict our discussions to conopressin and APGWamide.

PEPTIDE DIVERSITY IN THE NEURONAL NETWORK UNDERLYING MALE COPULATION AS REVEALED BY MATRIX-ASSISTED LASER DESORPTION IONIZATION MASS SPECTROMETRY (MALDI-MS)

Cobalt chloride backfilling experiments of the penis nerve (Van Duivenboden, 1984; Smit et al., 1992) have revealed central neurons in the anterior and ventral lobes of the right cerebral ganglion that most likely are involved in the regulation of copulation when Lymnaea acts as a male. Using conventional peptide chemical and cDNA cloning methods, a number of peptides have been identified in these neuronal clusters as well as in the male reproductive organs, i.e., APGWamide (Smit et al., 1992), conopressin (Van Kesteren et al., 1995), neuropeptide tyrosine (NPY; C.P. Tensen, unpublished data), FMRFamide and related peptides (Linacre et al., 1990), various myomodulins (Li et al., 1994; F.A. Van Golen, unpublished data), Lymnaea inhibitory peptide (L-IP; K.W. Li, unpublished data) and pedal peptide (R.M. Hoek, unpublished data). Immunocytochemistry indicated that most of these peptides are probably produced by neurons in the anterior and ventral lobes of the right cerebral ganglion. In order to unequivocally establish the site of synthesis and the direction of axonal transport of these peptides, and also to identify novel peptides involved in the control of male copulation behaviour, we decided to use MALDI-MS. As to applications in biological research, major advantages of MALDI-MS over other mass spectrometry methods are: 1) its sensitivity, 2) its accuracy, 3) the simplicity of its information content, and 4) its high tolerance for sample impurities. For these reasons, MALDI-MS is especially suited for the identification of multiple peptides in tissue homogenates. Recently, we demonstrated that MALDI-MS can indeed be utilized for direct and semi-quantitative measurements of peptides and proteins in tissue (Li et al., 1994) and even in single cells (Jiménez et al., 1994).

Application of MALDI-MS to clusters of neurons located in the anterior and ventral lobes of the right cerebral ganglion as well as to small samples of the penis nerve revealed the presence of molecules with apparent molecular masses corresponding to the above mentioned peptides (fig. 1). However, in particular the mass spectrum of