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Review

SALMFamide *salmagundi*: The biology of a neuropeptide family in echinoderms

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ABSTRACT

The SALMFamides are a family of neuropeptides that occur in species belonging to the phylum Echinodermata. The prototypes for this neuropeptide family (S1 and S2) were discovered in starfish but subsequently SALMFamides were identified in other echinoderms. There are two types of SALMFamides: L-type, which have the C-terminal motif S*x*L*x*Famide, and F-type, which have the C-terminal motif S*x*F*x*Famide. They are derived from two types of precursor proteins: an L-type SALMFamide precursor, which comprises only L-type or L-type-like SALMFamides and an F-type SALMFamide precursor, which contains several F-type or F-type-like SALMFamides and, typically, one or more L-type SALMFamides. Thus, SALMFamides occur as heterogeneous mixtures of neuropeptides – a SALMFamide *salmagundi*. SALMFamides are produced by distinct populations of neurons in echinoderm larval and adult nervous systems and are present in the innervation of neuromuscular organs. Both L-type and F-type SALMFamides cause muscle relaxation in echinoderms and, for example, in starfish this effect of SALMFamides may mediate neural control of cardiac stomach eversion in species that feed extra-orally (e.g., *Asterias rubens*). The SALMFamide S1 also causes inhibition of neural release of a relaxin-like gonadotropin in the starfish *Asterina pectinifera*. An important issue that remains to be resolved are the relationships of SALMFamides with neuropeptides that have been identified in other phyla. However, it has been noted that the C-terminal S*x*L*x*Famide motif of L-type SALMFamides is a feature of some members of a bilaterian neuropeptide family that includes gonadotropin-inhibitory hormone (GnIH) in vertebrates and SIFamide-type neuropeptides in protostomes. Similarly, the C-terminal F*x*Famide motif of F-type SALMFamides is a feature of vertebrate QRFP (26RFa)-type neuropeptides. These sequence similarities may provide a basis for molecular identification of receptors that mediate effects of SALMFamides. Furthermore, analysis of the actions of the heterogeneous mixtures of SALMFamides that occur in echinoderms may provide new insights into the physiological significance of the general phenomenon of precursor proteins that give rise to neuropeptide "cocktails".

1. Introduction

Twenty-five years ago a paper reporting "FMRFamide-like immunoreactivity in the nervous system of the starfish *Asterias rubens*" was published in *Biological Bulletin* (Elphick et al., 1989). When the paper was submitted for peer review, the feedback from reviewers was supportive but the tone leaned towards "yet another paper reporting FMRFamide-like immunoreactivity in an invertebrate!" This was not unreasonable because by 1989, twelve years after FMRFamide was identified as a cardioexcitatory neuropeptide in molluscs (Price and Greenberg, 1977), there was already a long list of species and phyla in which the presence of FMRFamide-like immunoreactivity had been reported (Price and Greenberg, 1989). In fact, a paper reporting the *absence* of FMRFamide-like immunoreactivity in starfish would have been more surprising! What made the paper of interest was that it was the first to reveal the anatomical distribution of any neuropeptide(s) in the nervous system of an echinoderm. Furthermore, it laid the foundations for discovery of the first neuropeptides to be identified in echinoderms, SALMFamide neuropeptides, which are the focus of this review article.

The review is divided into five main sections corresponding to the five classes of extant echinoderms. The Asteroidea (starfish) lead the review because it was in species belonging to this class (*A. rubens* and *Asterias forbesi*) that SALMFamide neuropeptides (S1 and S2) were first identified (Elphick et al., 1991a). The Holothuroidea follow because soon after the discovery of S1 and S2, two SALMFamide neuropeptides were identified in the sea cucumber *Holothuria glaberrima* (Díaz-Miranda et al., 1992), providing the first evidence

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that SALMFamides may occur throughout the phylum Echinodermata. Then come the Echinoidea, which through analysis of genome/transcriptome data from the sea urchin *Strongylocentrotus purpuratus* provided the first insights into the diversity of SALMFamides that occur in an echinoderm species (Elphick and Thorndyke, 2005; Rowe and Elphick, 2010). Lastly the Ophiuroidea and Crinoidea, the two echinoderm classes for which least is currently known but which have the potential to provide fascinating insights into the evolution and physiological roles of SALMFamide neuropeptides.

Before proceeding, perhaps an explanation for the title of this review is necessary. The word *salmagundi* is thought to originate from the French word *salmigondis*, which translates as "an assortment" or "a collection containing a variety of things". In English the word salmagundi has become associated with a 17th century salad dish comprising a rich variety of ingredients including meats, seafood, nuts, fruit, vegetables etc. However, like its French counterpart, salmagundi also has the more general meaning of a "heterogeneous mixture". As described in more detail below, genome sequence data and/or transcriptome sequence data have revealed that there are indeed heterogeneous mixtures of SALMFamide neuropeptides in echinoderms. Thus, there are both L-type SALMFamides and F-type SALMFamides; L-type SALMFamides are derived from L-type SALMFamide precursors and F-type SALMFamides are derived from F-type SALMFamide precursors but in some cases F-type SALMFamide precursors also give rise to L-type SALMFamides. Furthermore, there are SALMFamides that are not strictly L-type but are L-type-like and there are SALMFamides that are not strictly F-type but are F-type-like (Elphick et al., 2013). This is the SALMFamide *salmagundi*; a lexiconic marriage just waiting to happen!

2. Asteroidea

2.1. FMRFamide-like immunoreactivity in the nervous system of the starfish A. rubens

In order that patterns of neuropeptide expression in starfish and other echinoderms can be described, it is necessary to first briefly outline the architecture of the nervous systems in these animals. The organisation of the nervous system in adult starfish reflects its pentaradial body plan; there are five radial nerve cords that extend along the midline of each arm linked by a circumoral nerve ring in the central disk. The radial nerve cords control the activity of rows of tube feet that enable locomotor activity. The radial nerve cords and the circumoral nerve ring comprise two parts, the ectoneural and the hyponeural, which are separated by a basement membrane. The ectoneural division comprises sensory, inter- and motor neurons, and is continuous with an extensive basiepithelial nerve plexus underlying the body wall surface. The hyponeural division is considered to be purely motor. In visceral organs such as the cardiac stomach and the associated digestive glands (pyloric caecae), bipolar neuronal somata are located in the mucosal epithelium and have processes that form a basiepithelial nerve plexus. Neurons are also located within the coelomic epithelium of the gut and their processes innervate an underlying muscle layer, which is separated from the basiepithelial plexus by a basement membrane (Cobb, 1987, 1989; García-Arrarás et al., 2001; Heinzeller and Welsch, 2001; Pentreath and Cobb, 1972).

Immunocytochemical studies using antibodies to the molluscan neuropeptide FMRFamide revealed immunoreactivity in the radial nerve cords and circumoral nerve ring of the starfish *A. rubens* (Elphick et al., 1989). The immunostaining was localised in cell bodies and axonal fibres in both the ectoneural and hyponeural parts of the nerve cords and nerve ring. Furthermore, immunoreactive fibres were also evident in the basiepithelial nerve plexus of the tube feet, indicating a potential role for the immunoreactive peptides in control of tube foot activity. These findings were of interest because they provided the first insight into the neuroanatomical organisation of peptidergic signalling systems in the nervous system of an echinoderm. Furthermore, although by the time this study was published FMRFamide-like immunoreactive peptides had been identified in vertebrates and a variety of protostomian invertebrates, FMRFamide-like peptides had not been identified in any deuterostomian invertebrate species. A pattern was beginning to emerge, with peptides sharing the motif F*x*RFamide (where *x* is variable) with FMRFamide only being found in protostomian invertebrates. Accordingly, it was proposed that there is a family of orthologous FMRFamide-related peptides (FaRPs) in protostomians, with other FMRFamide-like peptides that have a C-terminal RFamide motif being more widely distributed phylogenetically (e.g., in cnidarians and vertebrates) (Price and Greenberg, 1989). It was against this backdrop that it was of particular interest from an evolutionary perspective to determine the molecular identity of the peptides responsible for the FMRFamide-like immunoreactivity detected in the starfish *A. rubens*.

2.2. Discovery of the starfish SALMFamide neuropeptides S1 and S2

The detection of FMRFamide-like immunoreactivity (ir) in the nervous system of *A. rubens*, as discussed above, provided a basis for efforts to purify and identify the peptide(s) responsible for this immunoreactivity. Initially a radioimmunoassay (RIA) employing antibodies to FMRFamide was used to screen extracts of nerves from *A. rubens* and *A. forbesi* that had been fractionated using high-performance liquid chromatography (HPLC). However, subsequently it was found that an antiserum (Q2) to a leucine-containing FMRFamide-like peptide (pQDPFLRFamide) detected more immunoreactivity in starfish nerve extracts and therefore Q2 was used to monitor purification of immunoreactive peaks (Elphick et al., 1991a). Four peaks (B–E) were purified to homogeneity and sequenced. Peak E was identified as the amidated octapeptide GFNSALMFamide, peak C was identified as the oxidised form of the peak E peptide and peak B was identified as a C-terminal fragment (SALMFamide) of the peak E peptide. Peak D was identified as the amidated dodecapeptide SGPYSFNSGLTFamide, which shares sequence similarity (underlined) with the peak E peptide (GFNSALMFamide). Interestingly, the presence of the LxFamide motif in both peptides provided an explanation for why antibodies to pQDPFLRFamide detected more immunoreactivity in starfish nerve extracts than antibodies to FMRFamide. However, the two starfish peptides differ from FMRFamide-like peptides identified in invertebrates and vertebrates because they do not have an arginine residue in the penultimate position from the C-terminal amide. Thus, the starfish peptides are not strictly "RFamide-type" neuropeptides and therefore they were designated as founding members of a new family of neuropeptides – the SALMFamides. The octapeptide GFNSALMFamide was designated as SALMFamide-1 (or S1) and the dodecapeptide SGPYSFNSGLTFamide was designated as SALMFamide-2 (or S2) (Elphick et al., 1991a,b). S1 and S2 were the first neuropeptides to be identified in a species belonging to the phylum Echinodermata and therefore it was of interest to investigate the physiological roles of these neuropeptides in starfish. To facilitate investigation of the physiological roles of S1 and S2, antibodies to these two peptides were generated and characterised using RIA methods (Elphick et al., 1995).

2.3. Localisation of SALMFamide neuropeptides in starfish larvae

The development of antibodies to S1 and S2 enabled the first investigations of the expression of native neuropeptides in