Physiology of invertebrate oxytocin and vasopressin neuropeptides

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New findings

- What is the topic of this review?
  This article describes the discovery and function of invertebrate oxytocin and vasopressin neuropeptides.
- What advances does it highlight?
  The novel discovery of oxytocin-like peptides in arthropods is described. An up-to-date overview is given of the functional role (physiology and behaviour) of oxytocin and vasopressin signalling. The application of natural peptides for drug development is discussed.

Neuropeptides and regulatory peptide hormones control many developmental, physiological and behavioural processes in animals, including humans. The nonapeptides oxytocin and arginine vasopressin are produced and released by the pituitary gland and have actions on many organs and tissues. Receptive cells possess particular receptors to which the peptides bind as ligands, leading to activation of G-protein-coupled receptors, hence cellular responses. In humans and other mammalian species, oxytocin and vasopressin mediate a range of peripheral and central physiological functions that are important for osmoregulation, reproduction, complex social behaviours, memory and learning. The origin of the oxytocin/vasopressin signalling system is thought to date back more than 600 million years. All vertebrate oxytocin- and vasopressin-like peptides have presumably evolved from the ancestral nonapeptide vasotocin by gene duplication and today are present in vertebrates, including mammals, birds, reptiles, amphibians and fish. Oxytocin- and vasopressin-like peptides have been identified in several invertebrate species, including molluscs, annelids, nematodes and arthropods. Members of this peptide family share high sequence similarity, and it is possible that they are functionally related across the entire animal kingdom. However, it is evident that not all animals express oxytocin/vasopressin neuropeptides and that there is little information available about the biology and physiology of this signalling system of invertebrates and, in particular, of insects, which represent more than half of all known living organisms. This report describes the discovery of novel oxytocin- and vasopressin-like peptides in arthropods and summarizes the status quo of the functional relevance of this neuropeptide signalling system in invertebrates, which will have beneficial implications for the design of selective and potent ligands to human oxytocin and vasopressin receptors.

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Introduction

Oxytocin and arginine vasopressin are regulatory neuropeptides that are involved in many peripheral and central functions in mammals. For oxytocin, these functions include uterine smooth muscle contraction during parturition, ejaculation, milk ejection from the mammary glands and complex social behaviour, while for vasopressin they include regulation of peripheral fluid balance and blood pressure, as well as central implications in memory, learning and stress-related disorders. Owing to this physiological importance, ligands of oxytocin and vasopressin receptors have potential therapeutic applications for novel treatment approaches to mental disorders characterized by social dysfunction, such as autism, social anxiety disorder, borderline personality disorder and schizophrenia (Meyer-Lindenberg et al., 2011), childbirth-related conditions, such as premature labour and postpartum haemorrhage (Gruber & O’Brien, 2011), osmoregulatory dysfunction, such as diabetes insipidus, as well as cardiovascular disorders, such as congestive heart failure (Treschan & Peters, 2006; Manning et al., 2012; Manning et al., 2012). In addition, it is known that oxytocin and vasopressin receptors signal via multiple G-protein coupling modes (Busnelli et al., 2012, 2013), and they can form functional homo- and hetero-oligomers (Cottet et al., 2010), which further complicates the quest for selective and ‘biased’ ligands. Over recent decades, at least 1000 oxytocin and vasopressin peptide ligands have been synthesized and characterized for therapeutic applications (Manning et al., 2012; Busnelli et al., 2013), but there is still a great demand for selective ligands that activate or block only a specific cellular pathway. Peptide sequences identified from natural sources should provide an evolutionary advantage over random chemical synthetic approaches and may yield novel lead compounds for therapeutic applications (Gruber et al., 2012; Gruber & Muttenthaler, 2012).

Table 1. Oxytocin- and vasopressin-like peptide sequences across the animal kingdom

| Phylum                  | Animal group                  | Peptide               | Sequence                  | Reference                                                                 |
|-------------------------|-------------------------------|-----------------------|---------------------------|---------------------------------------------------------------------------|
| Vertebrata              | Mammals/humans                | Oxytocin              | CYIQNCPLG                 | Du Vigneaud et al. (1953); Tuppy (1953); Gruber & Muttenthaler (2012)    |
|                         |                               | [Arg8]-Vasopressin     | CYFQNCPRG*                | Acher & Chauvet (1953); Gruber & Muttenthaler (2012)                      |
| Arthropoda              | Non-mammalian vertebrates     | Vasotocin             | CYIQNCPRG*                | Acher et al. (1960)                                                       |
|                         | Insects                       | Inotocin              | CLTNCPRG*                 | Proux et al. (1987); Stafflinger et al. (2008); Gruber & Muttenthaler (2012) |
|                         | Arachnids                     | Inotocin              | CLVNCPRG*                 | Gruber & Muttenthaler (2012)                                              |
|                         | Myriapods                     | Arachnotocin          | CFITNCPPG†                | Present study                                                             |
|                         |                               | Arachnotocin          | CFITNCPIG‡                | Present study                                                             |
|                         |                               | Myriatocin            | CYITNCPPG*§               | Present study                                                             |
| Annelida                | Crustaceans/brachiopods       | Oxytocin-vasopressin-like | CFITNCPPG*               | Stafflinger et al. (2008)                                                 |
|                         | Earthworms                    | Annetocin             | CFVRNCPTG                 | Oumi et al. (1994)                                                        |
|                         | Leeches                       | Lys-conopressin-G     | CFIRNCPKG*                | Salzet et al. (1993)                                                      |
| Mollusca                | Cephalopods                   | Cephalotocin          | CFIRNCPIG*                | Reich (1992)                                                              |
|                         |                               | Octopressin           | CFWTSCPIG*                | Takuwa-Kuroda et al. (2003)                                               |
|                         |                               | Lys-conopressin-G     | CFIRNCPKG*                | Cruz et al. (1987); Martínez-Padrón et al. (1992); McMaster et al. (1992); van Kesteren et al. (1992); Beets et al. (2012); Garrison et al. (2012) |
| Nematoda                | Caenorhabditis elegans        | Nematocin             | CFLNSCPYRRY*              |                                                                                   |

*C-terminal amidation. Sequences were discovered by genome mining according to Gruber & Muttenthaler (2012) from the following species: †the red spider mite Tetranychus urticae (GenBank: CAEY10002026.1); ‡the predatory mite Metaseiulus occidentalis (GenBank: AFFJ01003937.1); and ‡the centipede Strigamia maritima (GenBank: AFFK01014417.1).
Discovery of novel oxytocin- and vasopressin-like peptides from invertebrates

The origin of the oxytocin and vasopressin signalling system is thought to date back at least 600 million years, and all vertebrate oxytocin- and vasopressin-like peptides are considered to have evolved from the ancestral nonapeptide vasotocin by gene duplication. These peptides are present today in many different species, including non-mammalian vertebrates, fish, mammals and humans (Donaldson & Young, 2008; Gruber et al. 2012; Koehbach et al. 2013). Importantly, oxytocin and vasopressin-like peptides were previously also identified in several classes of invertebrate animals, such as molluscs, annelids, nematodes and insects (Table 1).

When comparing the sequences of those peptides from different organisms, it is obvious that certain positions are highly variable, whereas others are highly conserved. For example, positions 2 and 3 (hydrophobic or aromatic residues), positions 4 and 5 (polar or charged residues), as well as position 7 (proline) and position 9 (glycine) are conserved, whereas position 8 is highly variable (Table 1; Gruber et al. 2012). Interestingly, the similarity in sequence has been confirmed by molecular genetics analysis. Brenner and colleagues introduced an isotocin (oxytocin-like) gene from pufferfish (Fugu rubripes) into rats, which was able to be expressed functionally in rat neurons (Venkatesh et al. 1997; Murphy et al. 1998). This kind of genetic conservation is remarkable, considering that pufferfish and rat lineages separated ~400 million years ago (Murphy et al. 1998). However, subtle differences in the amino acid sequence of the peptide ligands may have significant effects on binding of the ligand to its receptor ($K_d$) and its potency ($EC_{50}$). Using an in silico approach, these interspecies differences of the individual native ligands were recently correlated to receptor sequence variations and vice versa, bearing in mind that molecular understanding of recognition, binding and activation of oxytocin and vasopressin receptors by their native ligands could assist the design and development of novel selective ligands (Koehbach et al. 2013). The screening and discovery of naturally occurring neuropeptides will therefore be important to guaranteeing the success of future drug-development programmes.

The postgenomic era greatly facilitates the search for natural oxytocin- and vasopressin-like peptide sequences, owing to the steadily increasing number of ongoing genome-sequencing projects, as well as advanced bioinformatics tools. For example, the number of completed genome-sequencing projects stands at 6577, with another 20,522 currently being in progress (information as of 10 July 2013; www.genomesonline.org; Pagani et al. 2012). As proof of concept, we have recently established a simple genome-mining workflow to analyse endogenous neuropeptides from insects, in particular from several ant species. We discovered inotocin (insect oxytocin-/vasopressin-like) sequences and their putative receptors in the genomes of a South American leaf-cutter ant (Atta cephalotes), the Florida carpenter ant (Camponotus floridanus) and the Jerdon’s jumping ant (Harpegnathos saltator; Gruber & Muttenthaler, 2012). Two newly identified ant inotocin peptide sequences display high similarity to vasotocin. These novel sequences show amino acid variations in position 2 and position 4 (Table 1), and structure–activity studies are underway to determine whether these modifications provide any novel selectivity leads for the human receptors.

In all the analysed ant genomes, the short mature peptides (nine amino acids long) are first translated within a longer precursor protein, which shares molecular features with precursors of other insect inotocin proteins, snail conopressin and even human oxytocin and vasopressin precursors. Besides the sequence of the mature nonapeptides, all precursors contain conserved protein domains for cellular secretion, enzymatic processing and physiological transport, and are characterized by identical intron sites and similar lengths (Gruber & Muttenthaler, 2012). The mature peptides have the same length and position of Cys residues, but the molecular sequence is slightly different between species. Also, the receptor sequences in ants share high similarity to those of other insects, such as the beetle Tribolium castaneum (Stafflinger et al. 2008; Gruber & Muttenthaler, 2012), suggesting that it is possible that not only the genetic structure but also the function of these receptors and their nonapeptide ligands may be conserved across species.

This work has now been extended by characterizing oxytocin- and vasopressin-like precursors and receptor sequences from the genomes of several arthropod species, such as the red spider mite Tetramychus urticae, the predatory mite Metaseiulus occidentalis and the centipede Strigamia maritima (C. W. Gruber, unpublished data). These novel nonapeptide sequences are presented in Table 1.

In addition to genome-mining approaches, there are several reports about using state-of-the-art peptidomics technology for identification of novel neuropeptides from invertebrates. For example, oxytocin- and vasopressin-like peptides were identified from the beetle Tribolium castaneum (Li et al. 2008), the parasitic wasp Nasonia vitripennis (Hauser et al. 2010), the water flea Daphnia pulex (Dircksen et al. 2011) and the great pond snail Lymnaea stagnalis (El Filali et al. 2006), confirming the initial discovery of these peptides in genomic or transcript sequences.

Following this description of recent efforts in the discovery of oxytocin- and vasopressin-like peptides, I would like to summarize the available information about the physiology and behavioural role of the oxytocin and vasopressin signalling system, focusing on invertebrate animals, which are by far the biggest group of living animals.
Table 2. Overview of invertebrate oxytocin and vasopressin physiology and behaviour

| Phylum       | Species (common name)                      | Functional role of oxytocin-/vasopressin-like peptide signalling                                                                 | References                      |
|--------------|--------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------|---------------------------------|
| Chordata     | *Ciona intestinalis* (vase tunicate, sea squirt) | Expression in neurons: genetic/transcript analysis of peptide indicated exclusive expression in neurons of the brain, pharmacologically active in recombinant system; 13-mer peptide (lacking C-terminal amidation) | Kawada et al. (2008)            |
|              | *Styela plicata* (sea squirt)               | Osmoregulation: contractile activity measured on siphons; localization of peptides in cerebral ganglion; 14-mer peptide    | Ukena et al. (2008)             |
| Arthropoda*  | *Tribolium castaneum* (red flour beetle)     | Regulation of water homeostasis: diuretic activity *in vivo*; pharmacologically active *in vitro*; receptor and precursor mainly expressed in CNS; indirect action on Malpighian tubules | Aikins et al. (2008); Stafflinger et al. (2008) |
|              | *Teleogryllus commodus* (black field cricket) | Expression in nerve tissue: axonal tracts extend backwards through the ventral nerve cord to the terminal ganglion (immunofluorescence); numerous beaded axons with specific immunostaining were detected within the lateral nerves | Musiol et al. (1990)            |
|              | *Locusta migratoria* (migratory locust)     | Diuretic hormone (arginine vasopressin-like) function: administration of peptide enhanced the excretion of urine from the Malpighian tubules; signalling via the second messenger cyclic AMP | Proux et al. (1987); Proux & Herault (1988) |
| Annelida     | *Theromyzon tessulatum* (duck leech)        | Possible reproductive function: vasopressin-like pharmacological profile; mRNA expression in the genital tract, the ovary and the CNS | Levoye et al. (2005)            |
|              | *Whitmania pigra* (leech)                   | Role in reproduction and osmoregulation: mediation of egg-laying-like behaviour; reduction of body weight in the animals (due to water loss) | Oumi et al. (1996); Fujino et al. (1999) |
| Mollusca     | *Eisenia foetida* (earthworm)               | Role in reproduction and gut motility: triggering of stereotyped egg-laying behaviour; stimulation of spontaneous contractions of the gut | Ukena et al. (1995); Oumi et al. (1996); Fujino et al. (1999) |
|              | *Erpobdella octoculata* (dog leech)         | Diuretic effects: mass loss after administration of peptide due to water excretion                                            | Salzet et al. (1993)            |
|              | *Sepia officinalis* (common cuttlefish)     | Role in memory processing: enhanced long-term memory formation after *in vivo* administration of peptide                       | Bardou et al. (2010)            |
|              | *Octopus vulgaris* (common octopus)         | Receptor expression in nervous and reproductive tissues; receptor localization in the nervous system and peripheral tissues, the pancreas, the oviduct and the ovary; possibly involved in neurotransmission, reproduction and metabolism (no functional evidence); two distinct peptides and receptors | Kanda et al. (2003, 2005)        |
|              | *Lymnaea stagnalis* (great pond snail)      | Role in reproduction and metabolism: control of male copulatory behaviour; autotransmitter-like functions; oxytocin-like reproductive functions; vasopressin-like metabolic functions; two distinct peptides and receptors | van Kesteren et al. (1995a,b); van Soest & Kits (1997, 1998); van Soest et al. (2000) |
|              | *Aplysia californica* (California sea hare) | Role in neurophysiology and behaviour: *in vitro* modulation of gill behaviours, possibly associated with the food-aroused state | Martinez-Padrón et al. (1992)    |
| Nematoda     | *Caenorhabditis elegans* (roundworm)        | Role in learning and reproduction: modulation of gustatory associative learning (salt chemotaxis) and sensory processing in neural circuits; co-ordination of reproductive behaviour; two distinct receptors, 11-mer peptide | Beets et al. (2012); Garrison et al. (2012) |

*Functional data within the phylum Arthropoda are available only for insect species.

Physiology and behavioural biology of the oxytocin and vasopressin signalling system in invertebrates

The function of oxytocin-like peptides has been studied across several invertebrate model organisms, such as annelids, nematodes, molluscs and chordates. Within the arthropods, this signalling system was studied for insects of the orders Orthoptera and Coleoptera (Table 2). Similar to the well-characterized mammalian systems,
Invertebrate oxytocin and vasopressin neuropeptides

Although the endogenous oxytocin and vasopressin neuropeptides have been reported in several groups and lineages of the animal kingdom (Table 1; Donaldson & Young, 2008; Gruber et al., 2012), but, at least for invertebrates, information about their physiology and function is very sparse (Table 2). Surprisingly, several variants of neuropeptides were also found in the venom of predatory cone snails (Cruz et al., 1987). The original discovery of two of these conopressin analogues was later characterized by the observation of grooming and scratching behaviour upon intracerebral injection into mice (Nielsen et al., 1994). Although the sequences of conopressins are similar to that of human vasopressin, they have an additional positive charge in position 4, which is so far found only in two other endogenous vasopressin analogues, cephalotocin (Octopus vulgaris) and annetocin (Eisenia fetida). Conopressin-G was first isolated from Conus striatus, whereas conopressin-G was first isolated from Conus geographus venom, but later also found to be present in the venom of Conus imperialis, as well as in tissue extracts of the non-venomous snails Lymnea stagnalis and Aplysia californica, and the leech Erpobdella octoculata (references cited in Tables 1 and 2). It is not yet clear what evolutionary advantage is conferred by the presence of these peptides in the venom of the cone snail. Nevertheless, the discovery and characterization of conopressin-T and comparison with the human neuropeptides vasopressin and oxytocin led to the identification of an interesting agonist–antagonist switch, which is currently being investigated with regard to the design of a novel antagonist for the human receptors (Dutertre et al., 2008).

This is one of several examples of the use of natural peptides in drug-design applications (Gruber et al., 2010, 2012). In conclusion, it appears that the discovery and functional characterization of oxytocin- and vasopressin-like neuropeptides from natural sources shows promise as an effective strategy for applications in drug-discovery efforts and selective ligand development to target human oxytocin and vasopressin receptors (Dutertre et al., 2008; Gruber et al., 2010, 2012; Koehbach et al., 2013).

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