Symposium Report

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 gven 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.* 2008; Gruber *et al.* 2010).

In humans, oxytocin and vasopressin are structurally very similar; they differ by only two amino acids (in positions 3 and 8; see Table 1). Both nonapeptides contain an N-terminal cyclic 6-residue ring structure stabilized by an intramolecular disulfide bond and a flexible C-terminal 3-residue tail. They mediate their distinct function by signalling through four G-protein-coupled receptors (OTR, $V_{1a}R$, $V_{1b}R$ and V_2R; Gimpl & Fahrenholz, 2001; Gruber *et al.* 2010), which share ∼80% sequence homology. The structural similarity of oxytocin and vasopressin together with the high sequence conservation of their receptors, in particular of the extracellular binding domains, results in significant cross-reactivity. This 'selectivity dilemma' constitutes a major burden for the development of receptor-specific ligands (agonists and antagonists), in particular for the human receptors (Chini & Manning, 2007; Gruber *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)
	Non-mammalian vertebrates	Vasotocin	CYIQNCPRG*	Acher *et al.* (1960)
Arthropoda	Insects	Inotocin	CLITNCPRG*	Proux *et al.* (1987); Stafflinger *et al.* (2008); Gruber & Muttenthaler (2012)
		Inotocin	CLIVNCPRG*	Gruber & Muttenthaler (2012)
	Arachnids	Arachnotocin	CFITNCPPG*†	Present study
		Arachnotocin	CFITNCPIG*‡	Present study
	Myriapods	Myriatocin	CYITNCPPG*§	Present study
	Crustaceans/brachiopods	Oxytocin-/vasopressin-like	CFITNCPPG*	Stafflinger *et al.* (2008)
Annelida	Earthworms	Annetocin	CFVRNCPTG*	Oumi *et al.* (1994)
	Leeches	Lys-conopressin-G	CFIRNCPKG*	Salzet *et al.* (1993)
Mollusca	Cephalopods	Cephalotocin	CYFRNCPIG*	Reich (1992)
		Octopressin	CFWTSCPIG*	Takuwa-Kuroda *et al.* (2003)
	Gastropods	Lys-conopressin-G	CFIRNCPKG*	Cruz *et al.* (1987); Martínez-Padrón *et al.* (1992); McMaster *et al.* (1992); van Kesteren *et al.* (1992)
Nematoda	*Caenorhabditis elegans*	Nematocin	CFLNSCPYRRY*	Beets *et al.* (2012); Garrison *et al.* (2012)

*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: CAEY01002026.1); ‡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 *Tetranychus 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)
	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)
Mollusca	*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.* (1995*a*,*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	Martínez-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,

oxytocin- and vasopressin-like signalling seems to be involved in neurotransmission, metabolism and osmoregulation in molluscs and annelids, and in osmoregulation and possibly neurotransmission in insects, such as the migratory locust, the black field cricket and the red flour beetle. In nematode worms (*Caenorhabditis elegans*), this neuropeptide system facilitates gustatory associative learning and co-ordinates reproductive behaviour. Moreover, oxytocin and vasopressin are involved in the reproduction of leeches, earthworms and snails (Table 2).

It therefore seems that, across evolutionary lineages, the oxytocin and vasopressin neuropeptide signalling system shows conserved functions in physiology, including water homeostasis, reproductive behaviour, learning and memory. However, except for the studies on *C. elegans* (Beets *et al.* 2012; Garrison *et al.* 2012) and *Tribolium castaneum* (Aikins *et al.* 2008), most studies are based on the following techniques: (i) transcript analysis and immunolocalization of the receptors; (ii) *in vitro* cell culture assays; or (iii) observations of behavioural changes upon administration of artificial peptide. Neither functional genomics data (such as gene knock-down) nor detailed biochemical analysis have yet been performed, but they are required in order to obtain understanding of the *in vivo* physiological and biological roles of this important neuropeptide system in invertebrates. Thus, the discovery of novel oxytocin- and vasopressin-like signalling systems of species with completed genome projects will allow the detailed pharmacological characterization of oxytocin and vasopressin neuropeptides, as well as functional genomics analysis to study the effect on the physiology and behaviour of these animals. In turn, this information will advance drug discovery efforts for human receptors, as described in the next section.

Application of natural oxytocin- and vasopressin-like peptides for human ligand design

Endogenous analogues of oxytocin and vasopressin 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 vasopressin 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 in only two other endogenous vasopressin analogues, cephalotocin (*Octopus vulgaris*) and annetocin (*Eisenia foetida*). Conopressin-S was 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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References

Acher R & Chauvet J (1953). The structure of bovine vasopressin. *Biochim Biophys Acta* **12**, 487–488.

Acher R, Chauvet J, Lenci MT, Morel F & Maetz J (1960). Presence of a vasotocin in the neurohypophysis of the frog (*Rana esculenta* L.). *Biochim Biophys Acta* **42**, 379–380.

Aikins MJ, Schooley DA, Begum K, Detheux M, Beeman RW & Park Y (2008). Vasopressin-like peptide and its receptor function in an indirect diuretic signaling pathway in the red flour beetle. *Insect Biochem Mol Biol* **38**, 740–748.

Bardou I, Leprince J, Chichery R, Vaudry H & Agin V (2010). Vasopressin/oxytocin-related peptides influence long-term memory of a passive avoidance task in the cuttlefish, *Sepia officinalis*. *Neurobiol Learn Mem* **93**, 240–247.

Beets I, Janssen T, Meelkop E, Temmerman L, Suetens N, Rademakers S, Jansen G & Schoofs L (2012). Vasopressin/oxytocin-related signaling regulates gustatory associative learning in *C. elegans*. *Science* **338**, 543–545.

Busnelli M, Bulgheroni E, Manning M, Kleinau G & Chini B (2013). Selective and potent agonists and antagonists for investigating the role of mouse oxytocin receptors. *J Pharmacol Exp Ther* **346**, 318–327.

Busnelli M, Sauliere A, Manning M, Bouvier M, Gales C & Chini B (2012). Functional selective oxytocin-derived agonists discriminate between individual G protein family subtypes. *J Biol Chem* **287**, 3617–3629.

Chini B & Manning M (2007). Agonist selectivity in the oxytocin/vasopressin receptor family: new insights and challenges. *Biochem Soc Trans* **35**, 737–741.

Cottet M, Albizu L, Perkovska S, Jean-Alphonse F, Rahmeh R, Orcel H, Méjean C, Granier S, Mendre C, Mouillac B & Durroux T (2010). Past, present and future of vasopressin and oxytocin receptor oligomers, prototypical GPCR models to study dimerization processes. *Curr Opin Pharmacol* **10**, 59–66.

Cruz LJ, de Santos V, Zafaralla GC, Ramilo CA, Zeikus R, Gray WR & Olivera BM (1987). Invertebrate vasopressin/oxytocin homologs. Characterization of peptides from *Conus geographus* and *Conus straitus* venoms. *J Biol Chem* **262**, 15821–15824.

Dircksen H, Neupert S, Predel R, Verleyen P, Huybrechts J, Strauss J, Hauser F, Stafflinger E, Schneider M, Pauwels K, Schoofs L & Grimmelikhuijzen CJ (2011). Genomics, transcriptomics, and peptidomics of *Daphnia pulex* neuropeptides and protein hormones. *J Proteome Res* **10**, 4478–4504.

Donaldson ZR & Young LJ (2008). Oxytocin, vasopressin, and the neurogenetics of sociality. *Science* **322**, 900–904.

Dutertre S, Croker D, Daly NL, Andersson A, Muttenthaler M, Lumsden NG, Craik DJ, Alewood PF, Guillon G & Lewis RJ (2008). Conopressin-T from *Conus tulipa* reveals an antagonist switch in vasopressin-like peptides. *J Biol Chem* **283**, 7100–7108.

Du Vigneaud V, Ressler C & Trippett S (1953). The sequence of amino acids in oxytocin, with a proposal for the structure of oxytocin. *J Biol Chem* **205**, 949–957.

El Filali Z, Van Minnen J, Liu WK, Smit AB & Li KW (2006). Peptidomics analysis of neuropeptides involved in copulatory behavior of the mollusk *Lymnaea stagnalis*. *J Proteome Res* **5**, 1611–1617.

Fujino Y, Nagahama T, Oumi T, Ukena K, Morishita F, Furukawa Y, Matsushima O, Ando M, Takahama H, Satake H, Minakata H & Nomoto K (1999). Possible functions of oxytocin/vasopressin-superfamily peptides in annelids with special reference to reproduction and osmoregulation. *J Exp Zool* **284**, 401–406.

Garrison JL, Macosko EZ, Bernstein S, Pokala N, Albrecht DR & Bargmann CI (2012). Oxytocin/vasopressin-related peptides have an ancient role in reproductive behavior. *Science* **338**, 540–543.

Gimpl G & Fahrenholz F (2001). The oxytocin receptor system: structure, function, and regulation. *Physiol Rev* **81**, 629–683.

Gruber CW, Koehbach J & Muttenthaler M (2012). Exploring bioactive peptides from natural sources for oxytocin and vasopressin drug discovery. *Future Med Chem* **4**, 1791–1798.

Gruber CW & Muttenthaler M (2012). Discovery of defense- and neuropeptides in social ants by genome-mining. *PLoS One* **7**, e32559.

Gruber CW, Muttenthaler M & Freissmuth M (2010). Ligand-based peptide design and combinatorial peptide libraries to target G protein-coupled receptors. *Curr Pharm Des* **16**, 3071–3088.

Gruber CW & O'Brien M (2011). Uterotonic plants and their bioactive constituents. *Planta Med* **77**, 207–220.

Hauser F, Neupert S, Williamson M, Predel R, Tanaka Y & Grimmelikhuijzen CJ (2010). Genomics and peptidomics of neuropeptides and protein hormones present in the parasitic wasp *Nasonia vitripennis*. *J Proteome Res* **9**, 5296–5310.

Kanda A, Satake H, Kawada T & Minakata H (2005). Novel evolutionary lineages of the invertebrate oxytocin/vasopressin superfamily peptides and their receptors in the common octopus (*Octopus vulgaris*). *Biochem J* **387**, 85–91.

Kanda A, Takuwa-Kuroda K, Iwakoshi-Ukena E, Furukawa Y, Matsushima O & Minakata H (2003). Cloning of Octopus cephalotocin receptor, a member of the oxytocin/vasopressin superfamily. *J Endocrinol* **179**, 281–291.

Kawada T, Sekiguchi T, Itoh Y, Ogasawara M & Satake H (2008). Characterization of a novel vasopressin/oxytocin superfamily peptide and its receptor from an ascidian, *Ciona intestinalis*. *Peptides* **29**, 1672–1678.

Koehbach J, Stockner T, Bergmayr C, Muttenthaler M & Gruber CW (2013). Insights into the molecular evolution of oxytocin receptor ligand binding. *Biochem Soc Trans* **41**, 197–204.

Levoye A, Mouillac B, Riviere G, Vieau D, Salzet M & Breton C (2005). Cloning, expression and pharmacological characterization of a vasopressin-related receptor in an annelid, the leech *Theromyzon tessulatum*. *J Endocrinol* **184**, 277–289.

Li B, Predel R, Neupert S, Hauser F, Tanaka Y, Cazzamali G, Williamson M, Arakane Y, Verleyen P, Schoofs L, Schachtner J, Grimmelikhuijzen CJ & Park Y (2008). Genomics, transcriptomics, and peptidomics of neuropeptides and protein hormones in the red flour beetle *Tribolium castaneum*. *Genome Res* **18**, 113–122.

McMaster D, Kobayashi Y & Lederis K (1992). A vasotocin-like peptide in *Aplysia kurodai* ganglia: HPLC and RIA evidence for its identity with Lys-conopressin G. *Peptides* **13**, 413–421.

Manning M, Misicka A, Olma A, Bankowski K, Stoev S, Chini B, Durroux T, Mouillac B, Corbani M & Guillon G (2012). Oxytocin and vasopressin agonists and antagonists as research tools and potential therapeutics. *J Neuroendocrinol* **24**, 609–628.

Manning M, Stoev S, Chini B, Durroux T, Mouillac B & Guillon G (2008). Peptide and non-peptide agonists and antagonists for the vasopressin and oxytocin V_{1a}, V_{1b}, V_2 and OT receptors: research tools and potential therapeutic agents. *Prog Brain Res* **170**, 473–512.

Martínez-Padrón M, Gray WR & Lukowiak K (1992). Conopressin G, a molluscan vasopressin-like peptide, alters gill behaviors in Aplysia. *Can J Physiol Pharmacol* **70**, 259–267.

Meyer-Lindenberg A, Domes G, Kirsch P & Heinrichs M (2011). Oxytocin and vasopressin in the human brain: social neuropeptides for translational medicine. *Nat Rev Neurosci* **12**, 524–538.

Murphy D, Si-Hoe SL, Brenner S & Venkatesh B (1998). Something fishy in the rat brain: molecular genetics of the hypothalamo-neurohypophysial system. *Bioessays* **20**, 741–749.

Musiol IM, Jirikowski GF & Pohlhammer K (1990). Immunohistochemical characterization of a widely spread Arg8-vasopressin-like neuroendocrine system in the cricket *Teleogryllus commodus* Walker (Orthoptera, Insecta). *Acta Histochem Suppl* **40**, 137–142.

Nielsen DB, Dykert J, Rivier JE & McIntosh JM (1994). Isolation of Lys-conopressin-G from the venom of the worm-hunting snail, *Conus imperialis*. *Toxicon* **32**, 845–848.

Oumi T, Ukena K, Matsushima O, Ikeda T, Fujita T, Minakata H & Nomoto K (1994). Annetocin: an oxytocin-related peptide isolated from the earthworm, *Eisenia foetida*. *Biochem Biophys Res Commun* **198**, 393–399.

Oumi T, Ukena K, Matsushima O, Ikeda T, Fujita T, Minakata H & Nomoto K (1996). Annetocin, an annelid oxytocin-related peptide, induces egg-laying behavior in the earthworm, *Eisenia foetida*. *J Exp Zool* **276**, 151–156.

Pagani I, Liolios K, Jansson J, Chen IM, Smirnova T, Nosrat B, Markowitz VM & Kyrpides NC (2012). The Genomes OnLine Database (GOLD) v.4: status of genomic and metagenomic projects and their associated metadata. *Nucleic Acids Res* **40**, D571–D579.

Proux JP & Herault JP (1988). Cyclic AMP: a second messenger of the newly characterized AVP-like insect diuretic hormone, the migratory locust diuretic hormone. *Neuropeptides* **12**, 7–12.

Proux JP, Miller CA, Li JP, Carney RL, Girardie A, Delaage M & Schooley DA (1987). Identification of an arginine vasopressin-like diuretic hormone from *Locusta migratoria*. *Biochem Biophys Res Commun* **149**, 180–186.

Reich G (1992). A new peptide of the oxytocin/vasopressin family isolated from nerves of the cephalopod *Octopus vulgaris*. *Neurosci Lett* **134**, 191–194.

Salzet M, Bulet P, Van Dorsselaer A & Malecha J (1993). Isolation, structural characterization and biological function of a lysine-conopressin in the central nervous system of the pharyngobdellid leech *Erpobdella octoculata*. *Eur J Biochem* **217**, 897–903.

Stafflinger E, Hansen KK, Hauser F, Schneider M, Cazzamali G, Williamson M & Grimmelikhuijzen CJ (2008). Cloning and identification of an oxytocin/vasopressin-like receptor and its ligand from insects. *Proc Natl Acad Sci U S A* **105**, 3262–3267.

Takuwa-Kuroda K, Iwakoshi-Ukena E, Kanda A & Minakata H (2003). Octopus, which owns the most advanced brain in invertebrates, has two members of vasopressin/oxytocin superfamily as in vertebrates. *Regul Pept* **115**, 139–149.

Treschan TA & Peters J (2006). The vasopressin system: physiology and clinical strategies. *Anesthesiology* **105**, 599–612.

Tuppy H (1953). The amino-acid sequence in oxytocin. *Biochim Biophys Acta* **11**, 449–450.

Ukena K, Iwakoshi-Ukena E & Hikosaka A (2008). Unique form and osmoregulatory function of a neurohypophysial hormone in a urochordate. *Endocrinology* **149**, 5254–5261.

Ukena K, Oumi T, Matsushima O, Ikeda T, Fujita T, Minakata H & Nomoto K (1995). Effects of annetocin, an oxytocin-related peptide isolated from the earthworm *Eisenia foetida*, and some putative neurotransmitters on gut motility of the earthworm. *J Exp Zool* **272**, 184–193.

van Kesteren RE, Smit AB, De Lange RP, Kits KS, Van Golen FA, Van Der Schors RC, De With ND, Burke JF & Geraerts WP (1995*a*). Structural and functional evolution of the vasopressin/oxytocin superfamily: vasopressin-related conopressin is the only member present in Lymnaea, and is involved in the control of sexual behavior. *J Neurosci* **15**, 5989–5998.

van Kesteren RE, Smit AB, de With ND, van Minnen J, Dirks RW, van der Schors RC & Joosse J (1992). A vasopressin-related peptide in the mollusc *Lymnaea stagnalis*: peptide structure, prohormone organization, evolutionary and functional aspects of Lymnaea conopressin. *Prog Brain Res* **92**, 47–57.

van Kesteren RE, Tensen CP, Smit AB, van Minnen J, van Soest PF, Kits KS, Meyerhof W, Richter D, van Heerikhuizen H, Vreugdenhil E & Geraerts WPM (1995*b*). A novel G protein-coupled receptor mediating both vasopressin- and oxytocin-like functions of Lys-conopressin in *Lymnaea stagnalis*. *Neuron* **15**, 897–908.

van Soest PF & Kits KS (1997). Vasopressin/oxytocin-related conopressin induces two separate pacemaker currents in an identified central neuron of *Lymnaea stagnalis*. *J Neurophysiol* **78**, 1384–1393.

van Soest PF & Kits KS (1998). Conopressin affects excitability, firing, and action potential shape through stimulation of transient and persistent inward currents in mulluscan neurons. *J Neurophysiol* **79**, 1619–1632.

van Soest PF, Lodder JC & Kits KS (2000). Activation of protein kinase C by oxytocin-related conopressin underlies pacemaker current in Lymnaea central neurons. *J Neurophysiol* **84**, 2541–2551.

Venkatesh B, Si-Hoe SL, Murphy D & Brenner S (1997). Transgenic rats reveal functional conservation of regulatory controls between the Fugu isotocin and rat oxytocin genes. *Proce Natl Acad Sci U S A* **94**, 12462–12466.

Additional information

Competing interests

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