Published in final edited form as:

Biochem Pharmacol. 2020 November 01; 181: 114129. doi:10.1016/j.bcp.2020.114129.

Neuropeptide signalling systems – an underexplored target for venom drug discovery

Helen C. Mendel^a, Quentin Kaas^a, Markus Muttenthaler^{a,b}

^aInstitute for Molecular Bioscience, The University of Queensland, Brisbane, Australia

^bUniversity of Vienna, Faculty of Chemistry, Institute of Biological Chemistry, Vienna, Austria

Abstract

Neuropeptides are signalling molecules mainly secreted from neurons that act as neurotransmitters or peptide hormones to affect physiological processes and modulate behaviours. In humans, neuropeptides are implicated in numerous diseases and understanding their role in physiological processes and pathologies is important for therapeutic development. Teasing apart the (patho)physiology of neuropeptides remains difficult due to ligand and receptor promiscuity and the complexity of the signalling pathways. The current approach relies on a pharmacological toolbox of agonists and antagonists displaying high selectivity for independent receptor subtypes, with the caveat that only few selective ligands have been discovered or developed. Animal venoms represent an underexplored source for novel receptor subtype-selective ligands that could aid in dissecting human neuropeptide signalling systems. Multiple endogenous-like neuropeptides as well as peptides acting on neuropeptide receptors are present in venoms. In this review, we summarise current knowledge on neuropeptides and discuss venoms as a source for ligands targeting neuropeptide signalling systems.

Keywords

venom peptide drug discovery; pharmacological probes; neuropeptides; selectivity; G protein-coupled receptor (GPCR)

1 Neuropeptides – ancient signalling peptides widely distributed across the animal kingdom

Neuropeptides are the largest and most diverse class of cell-to-cell signalling molecules with significant roles in animal physiology, behaviour and homeostasis [1]. To date, over 6,000 neuropeptides have been identified belonging to more than 60 neuropeptide families found across nearly 500 different species (source: NeuroPep [2]). The widespread distribution of neuropeptides across Bilateria is due to whole genome as well as local gene duplication events [3].

Corresponding author: A/Prof. Markus Muttenthaler, Währingerstraße 38, 1090 Vienna, Tel +43 1 4277 70515, markus.muttenthaler@univie.ac.at.

Conflict of interest statement

The authors declare there is no conflict of interest.

Neuropeptide signalling systems are evolutionarily ancient and seem to have evolved in the earliest of the metazoans, predating bilaterians, as they are found in the pre-bilaterian metazoans Ctenophora (comb jellies), Cnidaria, Porifera (sponges) and Placozoa (Figure 1A) [3–5]. Porifera and Placozoa do not technically possess a nervous system, meaning that they do not possess neurons, yet studies have found neuropeptide-like sequences in both [4–8]. Neuropeptides mediate their effects mainly through G protein-coupled receptors (GPCRs) [1]. There is ample evidence for the presence of GPCRs in Porifera, Placozoa, Cnidaria and Ctenophora [9, 10], and the study of GPCRs in early pre-bilaterian metazoans has provided insights into the evolution of neuropeptide signalling systems [4, 5]. For instance, genomic evidence was found for bilaterian neuropeptide receptor orthologues of several glycoprotein hormones (follicle-stimulating hormone, luteinizing hormone, and thyroid stimulating hormone) as well as relaxin, and bursicon in Cnidaria and Placozoans [11]. In addition, several genes encoding proteins with characteristics of neuropeptides were identified in the placozoan *Trichoplax adhaerens* [12] and cnidarian *Nematostella vectensis* [13]. However, in most cases neuropeptide receptor families are considered evolutionarily ancient based on the occurrence of orthologous neuropeptide-type receptors in one or more deuterostome species and one or more protostome species [3]. Within this definition, at least 31 different neuropeptide families are of pre-bilaterian origin and considered evolutionarily ancient [3].

High conservation of evolutionarily ancient neuropeptide families is generally associated with important physiological roles. Although the function of many conserved neuropeptide families has diverged across animal lineages, emerging evidence demonstrates evolutionary conservation of related neuropeptide-receptor pairs in the regulation of similar aspects of animal physiology and behaviour across species [14]. For example, the neurohypophyseal neuropeptide family (oxytocin and vasopressin) has a conserved role in reproduction, water homeostasis and feeding [15–18], which are essential functions for survival. Other examples include insulin in regulating growth and metabolism [19], thyrotropin-releasing hormone (TRH) in growth [20], prolactin water and salt in metabolism [21], adrenocorticotrophic hormone (ACTH) in the immune system [22], and gonadotropin in reproduction [23].

Many neuropeptide families have evolved more recently, yet still regulate important physiological processes. For example, the opioid family, which is a very diverse family and therapeutically important for pain management [24]. Current evidence suggests that opioids are a vertebrate novelty as there is no evidence of opioid signalling system in invertebrates [3]. Similarly, bradykinin is only found in vertebrates and plays a crucial role in inflammation and is also involved in fluid homeostasis, pain and blood pressure [25].

2 Neuropeptide biosynthesis and signalling

Neuropeptides are produced as precursor proteins which consist of an N-terminal signal peptide, a propeptide and one or more mature neuropeptides (Figure 1C) [26]. The precursor protein requires further proteolytic processing to generate the smaller, bioactive neuropeptides. After removal of the signal peptide in the endoplasmic reticulum by signal peptidase, the peptide precursor is transported to the Golgi, where most neuropeptides are released from the precursor protein through cleavage at dibasic residues (K/R-K/R) and, in

some cases, single basic residues (K/R) by proprotein convertases 1/3 and 2 (Figure 1B, C) [26]. The neuropeptides are then transported in large dense core vesicles to the nerve terminals. Neuropeptides can undergo post-translational modifications (PTMs) including C-terminal amidation, N-terminal pyroglutamylation, acetylation, phosphorylation, sulfation, and glycosylation to generate the final mature neuropeptides [1, 26–29]. Mature neuropeptide release is mediated by prolonged increases in calcium levels at the axon terminals, leading to the fusion of the secretory vesicles to the cell membrane. In some cases, such as bradykinin and angiotensin II, further peptide processing by enzymes occurs after release to produce the mature peptide [30, 31]. Once released, peptides can either act at short range (within the synapse or local diffusion) [32] or act at a distance ('peptide hormones') [33], typically after being released into the bloodstream (Figure 1B) [14].

A neuropeptide precursor can contain one or more of the same or different mature peptides and proteolytic processing plays a key regulatory function [1, 34]. In addition, mature peptides within one neuropeptide precursor can belong to different neuropeptide families; for instance, the proopiomelanocortin (POMC) precursor contains the melanocortin neuropeptides ACTH and melanocyte-stimulating hormone (MSH) as well as the opioid peptide b-endorphin [26, 35]. Further neuropeptide diversity results from alternative splicing of neuropeptide genes, resulting in neuropeptide precursor isoforms as is the case for the calcitonin/calcitonin gene-related peptide (CGRP) family which is alternatively spliced to generate either calcitonin precursor mRNA or CGRP precursor mRNA [26, 36]. In this instance, alternative splicing is tissue-dependent and results in tissue-specific expression of calcitonin and CGRP [26, 36]. The nature of PTMs can also be tissue- and state-dependent as a result of differential expression of processing enzymes. For example, during fasting, a-MSH from POMC accumulates due to an increased rate of processing of POMC, likely as a result of altered expression of proprotein convertases [37]. In summary, neuropeptide precursors can contain more than one mature peptide, and gene splicing and precursor processing contribute to neuropeptide diversity.

A single neuropeptide may bind to a variety of receptor subtypes and thereby mediate a wide range of effects [38]. Vasopressin is a clear example for this since vasopressin activates all three vasopressin receptors (V1aR, V1bR, V2R) as well as the oxytocin receptor (OTR) with varying potencies [39, 40]. In blood vessels, for example, vasopressin acts on the V1aR to mediate pressor effects *via* smooth muscle contraction, whereas in the kidney vasopressin mediates antidiuretic effects through the V2R. In addition, different ligands binding at the same receptor subtype can activate different signalling pathways; binding of morphine or endogenous enkephalin to the μ -opiate receptor results in recruitment of distinct b-arrestin isoforms, which differentially affect desensitisation [14, 41]. Thus, neuropeptide signalling systems can be very complex as they involve several GPCR subtypes linked to multiple signalling cascades and are expressed in various tissues with different functions [42].

3 Neuropeptides in humans

So far, over 200 different neuropeptides from 47 different neuropeptide families have been identified in humans (source: NeuroPep [2]). They are distributed in both the central nervous system (CNS) and the peripheral nervous system (PNS), where they regulate numerous

physiological processes including reproduction, fluid homeostasis, cardiovascular function, energy homeostasis, pain, memory and learning, and circadian rhythm (Figure 2) [1]. In some cases, neuropeptides play essential physiological roles, for example, pituitary adenylate cyclase-activating peptide (PACAP) is necessary for nerve growth, nerve differentiation, and neuroprotection, and PACAP gene knockout in mice leads to early death after birth [43]. In addition, neuropeptides modulate behaviour including social behaviour (oxytocin and vasopressin), feeding behaviour (leptin, gastrin, ghrelin, neuropeptide Y, oxytocin, and melanin-concentrating hormone), and stress and anxiety (neuromedins, galanin, cholecystokinin, melanin-concentrating hormone, neuropeptide S, neuropeptide Y, somatostatin, tachykinins, urotensin-2, oxytocin, vasopressin). A single neuropeptide can contribute to and regulate many different physiological processes and/or behaviours. This is mostly mediated through tissue-dependent release and receptor subtype expression. For example, V1bR activation in the paraventricular nucleus of the hypothalamus initiates ACTH secretion signalling whereas V1bR activation in the colon mediates proinflammatory properties [44].

Although some neuropeptides are crucial for survival and development, many are modulatory in nature or play a minor role [41]. Subsequently, neuropeptide gene knockout mice often show little change in phenotype [41]. For example, vasoactive intestinal peptide is an immunoregulatory neuropeptide, yet vasoactive intestinal peptide knock out mice survived weaning and have normal basal immune characteristics [45]. In addition, there is evidence of functional compensation of neuropeptide signalling systems; oxytocin knockout mice are unable to release milk but have otherwise largely normal parturition and maternal behaviour, possibly due to compensation from the closely-related vasopressin signalling system [46].

Under pathological conditions neuropeptide signalling can play a more important role and become therapeutically relevant [41]. For example, the vasoconstriction of blood vessels caused by vasopressin binding to V1aR in the vascular system plays a minor role in the regulation of blood pressure in a healthy individual. However, under pathophysiological conditions such as severe extracellular fluid volume depletion, hypotension, general anaesthesia, or haemorrhage, high levels of vasopressin in the blood can significantly exacerbate vasopressor effects [44].

4 Clinical application of neuropeptides

Neuropeptides are implicated in multiple physiological processes and, consequently, numerous disease processes [47]. Disease areas where neuropeptides play a key role and where neuropeptide signalling systems are a validated therapeutic target or are actively being investigated as a therapeutic target include obesity, heart failure, epilepsy, sleep disorders, autism and depression [47–55].

Both neuropeptide analogues and small molecules targeting neuropeptide receptors have been developed for therapeutic treatments ranging from cardiovascular disorders, diabetes, pain, and labour to gastrointestinal disorders. Table 1 provides an overview of approved

drugs targeting neuropeptide signalling systems. A range of other neuropeptide targets are also under investigation [47].

There are clear examples of the clinical importance of neuropeptides including opioid analgesics, insulin treatment for type 2 diabetes mellitus, oxytocin to induce labour, and parathyroid hormone to treat osteoporosis (Table 1). However, many trials targeting neuropeptide signalling systems have been disappointing in part due to lack of efficacy, receptor dimerization, unexpected side effects, and dosage-dependent effects [41, 47, 56, 57]. One potential cause of lack of efficacy is the complexity of neuropeptide signalling, which displays temporal variations and signalling redundancy, but also a lack of knowledge on signalling mechanisms [41, 47, 58]. Consequently, it is no longer the expectation that a single neuropeptide will cure a single disease and there is much scope for targeting neuropeptide signalling systems for therapeutic treatment in a wide range of diseases [41].

Neuropeptides, and in some cases neuropeptide precursors, are also valuable biomarkers and diagnostic probes to aid disease diagnosis and prognosis (Table 1). As the various roles of neuropeptides in (patho)physiological processes are elucidated, their value as biomarkers and diagnostic markers is often assessed.

Neuropeptide signalling systems represent a great opportunity for the development of therapeutics, in particular for peptide therapeutics. An in-depth understanding of the mechanistic roles of neuropeptides in health and disease is essential to translate their therapeutic potential into drug targets, therapeutic leads, diagnostics and biomarkers. Although technical advances within next-generation sequencing and mass spectrometry have greatly facilitated neuropeptide discovery and characterisation of their signalling systems [59–62], most neuropeptide signalling systems remain poorly understood [14]. In addition to their complex signalling pathways (multiple G protein-dependent and -independent pathways), neuropeptides have numerous characteristics that make them difficult to identify using mass spectrometry and sequencing technology, especially because of the high occurrence of PTMs, and the existence of isoforms [62]. Identification based on mass is further complicated by (i) varying and typically low in vivo concentrations (up to 1,000-fold lower than classical neurotransmitters); (ii) lack of enzymatic fragments, requiring highly-sensitive proteomic detection methods; and (iii) rapid in vivo degradation [62]. Consequently, the therapeutic potential of many neuropeptide systems remain unrealised [41]. Subtype selective ligands that can selectively inhibit or stimulate neuropeptide receptors are key pharmacological tools to dissect their functions in health and disease [58]. These ligands also constitute therapeutic leads. Such pharmacological toolboxes already exist for some neuropeptide signalling systems. For instance, bradykinin acts via two receptors, B1, B2, and receptor-selective agonists and antagonists have been developed for both receptors [63, 64].

5 Neuropeptides in venoms – an underexplored field

Endogenous neuropeptides from animals throughout the animal kingdom represent a solid starting point for discovery. Neuropeptide receptor sequence conservation means that there are numerous occurrences of neuropeptides from one phylum activating the corresponding

receptors in another phylum. For example, vertebrate substance P is able to activate a *Drosophila* tachykinin receptor expressed in *Xenopus* oocytes [65], and mammalian neuropeptide Y peptides can activate the *Drosophila* neuropeptide F receptor, the invertebrate neuropeptide Y receptor orthologue [66]. Small sequence variations in ligands and receptors across species result in diverse pharmacological profiles including differences in selectivity, affinity and function (e.g. agonism, antagonism, and biased signalling) [67, 68]. This diversity can be exploited to benefit human health as neuropeptides identified from different organisms represent a rich source for molecular probes and therapeutic leads to dissect neuropeptide signalling in health and disease. This hypothesis has been underpinned by multiple studies, including new oxytocin and vasopressin ligands identified from insects [69, 70], salmon calcitonin used to treat hypercalcemia [71]; and pramlintide (based on rat amylin) to reduce blood glucose levels in type 1 and type 2 diabetes mellitus patients [72].

Another, and maybe even richer, source for ligands targeting neuropeptide signalling systems is animal venom. Venoms are highly complex mixtures mostly made up of proteins and peptides (~90%) that disrupt normal biochemical and physiological processes in the prey/predator for the benefit of the venomous animal [73–76]. Venom peptides have an array of targets including receptors and enzymes affecting biological functions in various locations including the CNS, PNS, blood and muscle tissue [77]. Importantly, venom components have undergone strong natural selection for in vivo stability, potency, selectivity and delivery to engage in precise molecular interactions exogenously to warrant their survival through defence and predation [73–76, 78, 79]. Many of these features are also highly desirable in drug development, thus venoms represent an advantageous drug discovery starting point compared to combinatorial or DNA/RNA display libraries [78, 80–83]. Indeed, venoms are a highly successful source for drug leads with five venom-derived peptide drugs on the market, including Exenatide (Byetta®) and Lixisenatide (Adlyxin, Lyxumia) from the Gila monster to treat type 2 diabetes [84, 85]; Eptifibatide (Integrilin®), an antiplatelet agent from the rattlesnake *Sistrurus miliarius barbourin* [86]; and Bivalirudin (Angiomax, Angiox) from the leech *Hirudo medicinalis*, which is used as an anticoagulant agent [87]. Furthermore, conotoxins from the marine predatory cone snail have transformed neuropathic pain research with one conotoxin drug approved to treat severe chronic pain (Ziconotide, Prialt®) [88]. Spider toxins have also provided outstanding therapeutic leads for stroke, pain, and epilepsy through their actions on ion channels Nav1.1 [89, 90], Nav1.7 [91, 92], and acid-sensing ion channels 1a (ASIC1a) [93], respectively. Chlorotoxin, isolated from the venom of the scorpion *Leiurus quinquestriatus* and initially characterised as a voltage-gated chloride channel ligand [94], was subsequently identified as a potent and selective matrix metalloproteinase-2 receptor ligand and is currently undergoing clinical trials to visualise glioblastomas [95]. Finally, venom peptides and their use as receptor-subtype selective pharmacological probes have revolutionised our understanding of many human receptors and links to disease [96–102]. For instance, bradykinin-potentiating peptides, originally identified in the venom of the snake *Bothrops jararaca*, led to the development of a new class of hypotensive drugs, angiotensin converting enzyme inhibitors, including captopril, for the treatment of high blood pressure and heart failure [103].

Venom proteins and peptides are a result of gene duplication and selective expression of an endogenous protein/peptide in the venom gland, a process called neofunctionalization [73,

75, 76, 104–107]. Neuropeptides are no exception, and at least 176 different neuropeptides from 16 different neuropeptide families have been found in the venom gland transcriptomes or venoms of 107 different species, including spiders, scorpions, insects, centipedes, cone snails, octopus, monotremes, snakes, lizards and fish (Table 2).

There exist several examples where venom neuropeptides have demonstrated their therapeutic potential. Exendin-4, a GLP-1 receptor agonist isolated from the venom of the Gila monster (*Heloderma suspectum*), was used to develop Exenatide as well as Lixisenatide for the treatment of type 2 diabetes mellitus [85, 108]; contulakin-G, a neurotensin homologue isolated from *Conus geographus* venom is under investigation for the treatment of acute and chronic pain [109]; natriuretic peptides found in snake venoms are used to understand human natriuretic peptide receptor signalling [110]; sarafotoxin, identified in the venom of the snake *Atractaspis engaddensis*, acts on endothelin receptors, has been used to understand endothelin receptor signalling and aided development of endothelin receptor antagonists for the treatment of cardiovascular disorders [111]; conopressin-T, a vasopressin-like peptide identified in the cone snail *Conus tulipa* venom, contributed towards the development of novel vasopressin receptor antagonists that are used for the treatment of hyponatremia [112].

Although venom neuropeptides have been utilised for their therapeutic potential, very little is known on the physiological role venom neuropeptides play in predation and defence. Venom natriuretic peptides and sarafotoxins likely contribute to prey capture by targeting the cardiovascular system and affecting vasodilation or vasoconstriction and heart contractility [110, 113, 114]. Cono-insulins reduce blood glucose levels in fish which results in sluggish behaviour facilitating capture [115]. Pain and inflammation are common responses to envenomation, often for defensive purposes [116] and for envenomation of mice by the wasp *Polistes lanio lanio* these effects were attributed to tachykinin-like peptides acting on the neurokinin-1 (NK1) receptor [117, 118]. Conorfamide-Sr2, an FMRF-amide related peptide isolated from worm-eating *Conus spurius* venom, was hypothesized to have a defensive function as it had mild paralytic effects in the limpet *Patella opea* [119]. In addition, based on the neuropeptide families they belong to, venom neuropeptides are implicated in various physiological processes including cardiovascular functions (natriuretic peptide [120], crustacean cardioactive peptide [121], RFamide [122], neurotensin [123]), metabolism (insulin [124], glucagon-like peptide-1 [GLP-1] [125], RFamide [122]), reproduction (oxytocin [16]), fluid homeostasis (natriuretic peptide [126], vasopressin [44], bradykinin [127]) and pain (tachykinin [128], bradykinin [25], enkephalin [129], RFamide [122]).

In addition to venom neuropeptides with high homology to the human neuropeptides, venoms also contain peptides with very little homology to neuropeptides that potently bind to neuropeptide receptors. Venom peptides are generally characterised by well-conserved disulfide bond frameworks with loops that are highly mutable, thereby providing an evolutionary easily amendable scaffold able to adapt to a wide range of targets [76, 130]. A good example of this is mambaquearin-1 isolated from the green mamba (*Dendroaspis angusticeps*), which binds allosterically to the vasopressin V2R (GPCR) and protects against renal cyst development, making it an interesting therapeutic lead for the treatment of polycystic kidney disease [131]. This was unexpected, since Mambaquearin-1 has a Kunitz-

fold, which is a well-characterised scaffold with three disulfide bonds, two antiparallel beta strands and a short alpha helix [131, 132] that is commonly found in venoms and known to target ion channels and proteases [110, 131, 133, 134]. Other examples of venom peptides that have no homology to neuropeptides but target neuropeptide receptors include hypotensin-I isolated from the scorpion *Tityus serrulatus* and which is a B2 bradykinin receptor agonist [135]; d-ctenitoxin-Pn1a from the spider *Phoneutria nigriventer* that targets opioid receptors [136]; t-CnVA and conorphins from cone snail venom that target the somatostatin and opioid receptors, respectively [137, 138]; and Crotalpine isolated from the venom of the snake *Crotalus durissus terrificus* that targets the κ -opioid receptor and causes analgesia in rats [139]. The overall chemical and functional diversity of venom toxins is what renders venoms such interesting sources for the discovery of novel drug leads, diagnostic probes and pharmacological tools, which could accelerate our understanding of neuropeptide signalling in humans, if explored properly.

The number of venom neuropeptides listed in Table 2 is likely to represent only a small fraction of the total number of neuropeptides recruited into animal venoms as a result of limited characterisation. The broad phylogenetic range of venomous animals and number of known venom compounds (~6,000) versus the number of known venom neuropeptides suggests there are many more venom neuropeptides to be discovered [76, 140]. One explanation for the low number of neuropeptides is that only a limited number of venoms have been characterised to date and there is a bias towards certain animal lineages at the expense of others [140]. For example, snakes and cone snails have been heavily studied in contrast to invertebrates such as centipedes, cephalopods (octopus, squid and cuttlefish) and cnidarians [140, 141]. In fact, it is hypothesised that less than 1% of the total number of venom proteins and peptides have been characterised [77].

Another reason for the low number of known neuropeptides in animal venoms may have to do with the methods employed to characterise them. The majority of neuropeptides listed in Table 2 were identified by transcriptomics. The transcriptome is the sum of all mRNA molecules in one cell or a population of cells [142]; it provides information on transcript expression level, transcriptome dynamics across different tissues or conditions, and is not dependent on an existing genomic sequence. Transcriptomics, often combined with proteomics, is the most common venom characterisation method and allows a non-biased and non-targeted approach to characterise venom glands [140, 143–145]. However, it has also revealed high intraspecific variation due to diet, geographical location, age, and gender which necessitates care when interpreting results [146–148]. The correct and efficient use of bioinformatics is essential to be able to process and integrate the large amount of data generated in a transcriptomic study [149]. Annotation of transcriptome sequences relies on sequence comparison to known, annotated proteins and peptides in databases such as UniProt and the National Center for Biotechnology Information (NCBI) database [150]. The short length of neuropeptides and high sequence diversity of neuropeptide precursors makes them difficult to identify using sequence homology methods such as the NCBI Basic Local Alignment Search Tool (BLAST) [61, 151]. Consequently, it is likely that a large number of neuropeptide homologues in venoms have not been detected in ‘omics’ data. Therefore, it seems reasonable to expect identification of a higher number of neuropeptides in animal venoms using a targeted approach. This seems to have been the case in cone snails where

neuropeptide-specific search in the venom gland transcriptome of *Conus victoriae* led to the identification of five different neuropeptides [152], in contrast to the two neuropeptides previously identified in *C. victoriae* [153].

6 Neuropeptide identification

Venom and neuropeptide research employ comparable strategies and technologies to identify peptides and face similar challenges including low peptide concentrations, PTMs, variable size, high degree of structural diversity and a large number of isoforms [60, 62]. A combined approach of mass spectrometry, sequencing technologies (i.e. transcriptomics) and bioinformatics (termed ‘Integrated Venomics’) has been very successful for both venom characterisation [77, 102, 140, 145] and neuropeptide identification [29, 59, 154]. Mass spectrometry is very sensitive, fast, allows de novo sequencing and enables identification of PTMs, although more material is required compared to transcriptomics [60, 62]. For animals with no or only a partial reference genome, transcriptomics in particular has greatly accelerated characterisation of animal venoms and identification of neuropeptides [140, 155]. Advances in sequencing technology and mass spectrometry combined with in silico prediction and bioinformatic tools has facilitated research in both areas [59, 60, 150, 156].

PTMs include the precursor protein cleavage sites that produce the mature peptides as well as residue modifications and can only be partially predicted from the nucleotide sequence [151]. Although advancements in mass spectrometry have increased selectivity and sensitivity, PTM determination of low abundance peptides remains challenging and requires sample preparation and separation methods such as liquid chromatography or capillary electrophoresis to maximise sensitivity [59, 62]. This is particularly relevant for venom characterisation of animals that produce very small amounts of venom. D-amino acids, a PTM identified in spider, mollusc and mammalian venom peptides, as well as mollusc neuroexcitatory peptides and crustacean neurohormones, are notoriously difficult to detect due to a lack of a sequence change or mass defect [157]. New mass spectrometry methods have been developed that measure the distinct molecular fragmentation patterns among peptide diastereomers with tandem mass spectrometry (MS/MS) [59].

Computational methods are necessary to filter, process, store and integrate the multifaceted information generated in mass spectrometry and sequencing studies. In addition, bioinformatic tools have been developed to facilitate neuropeptide and venom peptide identification including prohormone prediction tools (NeuroPID [158]), precursor protein cleavage site prediction tools (NeuroPred [159, 160], ProP [161], SignalP [162]), and peptide databases (neuropeptide database NeuroPep [2] and venom peptide databases [163–166]). The advancement and development of novel bioinformatic tools is critical to meet the computational demands of high-throughput mass spectrometry and sequencing technology which produce ever larger and more complex datasets [60].

Identification of a peptide, be that a neuropeptide or venom peptide, using mass spectrometry or polynucleotide sequencing technology does not provide information on the peptide target. After being identified with an ‘omics’ approach, peptides are typically synthesised and their activity characterised. Fortunately, synthesis of peptides (under 50

residues) is relatively straightforward using solid-phase peptide synthesis and the use of selective amino acid protecting groups allows directed disulfide-bond formation, which is important for characterising disulfide-rich peptides displaying potentially several disulfide bond isomers [167, 168]. In addition, recombinant expression, native chemical ligation, semisynthetic methods, and ligase/enzymatic ligations enable synthesis of longer and more complex peptides [100, 169, 170]. Subsequent testing on high-throughput assays such as fluorescent plate readers which measure Ca^{2+} , Na^{+} , K^{+} signalling (such as Fluorescence Imaging Plate Reader, FLIPR), electrophysiology, or single cell assays enables assessing of activity at various receptors [171, 172]. However, in vitro characterization of venom peptides provides limited insight into their function, and often unexpected effects are observed once administered in vivo [173].

7 Summary and future perspectives

Neuropeptide signalling systems are ubiquitous throughout the animal kingdom and regulate many fundamental physiological processes. They typically contain multiple receptor subtypes with complex signalling cascades that remain poorly understood due to a lack of receptor subtype-selective ligands. Because of their high level of conservation, endogenous neuropeptides, and in particular venom neuropeptides, represent a source for novel neuropeptide receptor ligands with varying pharmacological properties. Known venom neuropeptides are likely to be the tip of the iceberg and technological advances in mass spectrometry and sequencing technology combined with the development of novel bioinformatics tools promise to facilitate the identification of venom neuropeptides and characterisation of neuropeptide signalling systems. However, many challenges remain including a limited venom species diversity and identification of PTMs. Nevertheless, neuropeptides recruited into animal venoms offer a viable source for novel pharmacological tools and therapeutic leads to study the role of neuropeptides in health and disease.

Acknowledgements

M.M. was supported by the European Research Council under the European Union's Horizon 2020 research and innovation program (714366) and by the Australian Research Council (DE150100784, DP190101667).

List of abbreviations

ACTH	Adrenocorticotrophic hormone
BLAST	Basic Local Alignment Search Tool
CGRP	Calcitonin/calcitonin gene-related peptide
CNS	Central nervous system
CRH	Corticotropin-releasing hormone
ER	Endoplasmic reticulum
FLIPR	Fluorescence Imaging Plate Reader
GLP-1	Glucagon-like peptide-1

GnRH	Gonadotropin-releasing hormone
GPCR	G protein-coupled receptors
MCH	Melanin-concentrating hormone
MS/MS	Tandem mass spectrometry
MSH	Melanocyte-stimulating hormone
NCBI	National Center for Biotechnology Information
NPY	Neuropeptide Y
OTR	Oxytocin receptor
PACAP	Pituitary adenylate cyclase-activating peptide
PNS	Peripheral nervous system
POMC	Proopiomelanocortin
PTM	Post-translational modifications
TRH	Thyrotropin-releasing hormone

References

- [1]. Hokfelt T, Broberger C, Xu ZQ, Sergeev V, Ubink R, Diez M. Neuropeptides--an overview. *Neuropharmacology*. 2000; 39(8):1337–1356. [PubMed: 10818251]
- [2]. Wang Y, Wang M, Yin S, Jang R, Wang J, Xue Z, et al. NeuroPep: a comprehensive resource of neuropeptides. *Database (Oxford)*. 2015; 2015
- [3]. Elphick MR, Mirabeau O, Larhammar D. Evolution of neuropeptide signalling systems. *J Exp Biol*. 2018; 221(3):jeb151092. [PubMed: 29440283]
- [4]. Jekely G. Global view of the evolution and diversity of metazoan neuropeptide signaling. *Proc Natl Acad Sci U S A*. 2013; 110(21):8702–8707. [PubMed: 23637342]
- [5]. Mirabeau O, Joly JS. Molecular evolution of peptidergic signaling systems in bilaterians. *Proc Natl Acad Sci U S A*. 2013; 110(22):E2028–2037. [PubMed: 23671109]
- [6]. Krishnan A, Dnyansagar R, Almen MS, Williams MJ, Fredriksson R, Manoj N, et al. The GPCR repertoire in the demosponge *Amphimedon queenslandica*: insights into the GPCR system at the early divergence of animals. *BMC Evol Biol*. 2014; 14:270. [PubMed: 25528161]
- [7]. Grimmelikhuijzen CJ, Hauser F. Mini-review: the evolution of neuropeptide signaling. *Regul. Pept*. 2012; 177:S6–S9. [PubMed: 22726357]
- [8]. Jekely G, Paps J, Nielsen C. The phylogenetic position of ctenophores and the origin(s) of nervous systems. *Evodevo*. 2015; 6(1)
- [9]. Hauser F, Cazzamali G, Williamson M, Blenau W, Grimmelikhuijzen CJ. A review of neurohormone GPCRs present in the fruitfly *Drosophila melanogaster* and the honey bee *Apis mellifera*. *Prog Neurobiol*. 2006; 80(1):1–19. [PubMed: 17070981]
- [10]. Civelli O, Saito Y, Wang Z, Nothacker HP, Reinscheid RK. Orphan GPCRs and their ligands. *Pharmacol Ther*. 2006; 110(3):525–532. [PubMed: 16289308]
- [11]. Roch GJ, Sherwood NM. Glycoprotein Hormones and Their Receptors Emerged at the Origin of Metazoans. *Genome Biol Evol*. 2014; 6(6):1466–1479. [PubMed: 24904013]
- [12]. Nikitin M. Bioinformatic prediction of *Trichoplax adhaerens* regulatory peptides. *Gen Comp Endocrinol*. 2015; 212:145–155. [PubMed: 24747483]

- [13]. Anctil M. Chemical transmission in the sea anemone *Nematostella vectensis*: A genomic perspective. *Comp Biochem Physiol., Part D: Genomics Proteomics*. 2009; 4(4):268–289. [PubMed: 20403752]
- [14]. Jekely G, Melzer S, Beets I, Kadow ICG, Koene J, Haddad S, et al. The long and the short of it - a perspective on peptidergic regulation of circuits and behaviour. *J Exp Biol*. 2018; 221(3)
- [15]. Beets I, Temmerman L, Janssen T, Schoofs L. Ancient neuromodulation by vasopressin/oxytocin-related peptides. *Worm*. 2013; 2(2):e24246. [PubMed: 24058873]
- [16]. Garrison JL, Macosko EZ, Bernstein S, Pokala N, Albrecht DR, Bargmann CI. Oxytocin/vasopressin-related peptides have an ancient role in reproductive behavior. *Science*. 2012; 338(6106):540–543. [PubMed: 23112335]
- [17]. Lockard MA, Ebert MS, Bargmann CI. Oxytocin mediated behavior in invertebrates: An evolutionary perspective. *Dev Neurobiol*. 2017; 77(2):128–142. [PubMed: 27804275]
- [18]. Odekunle EA, Semmens DC, Martynyuk N, Tinoco AB, Garewal AK, Patel RR, et al. Ancient role of vasopressin/oxytocin-type neuropeptides as regulators of feeding revealed in an echinoderm. *BMC Biol*. 2019; 17(1)
- [19]. De Meyts P. Insulin and its receptor: structure, function and evolution. *Bioessays*. 2004; 26(12):1351–1362. [PubMed: 15551269]
- [20]. Van Sinay E, Mirabeau O, Depuydt G, Van Hiel MB, Peymen K, Watteyne J, et al. Evolutionarily conserved TRH neuropeptide pathway regulates growth in *Caenorhabditis elegans*. *Proc Natl Acad Sci U S A*. 2017; 114(20):e4065–e4074. [PubMed: 28461507]
- [21]. Sakamoto, T. Subchapter 15B - Prolactin *Handbook of Hormones: Comparative Endocrinology for Basic and Clinical Research*. Takei, Y, Ando, H, Tsutsui, K, editors. Academic Press; San Diego; 2016. 112–113.
- [22]. Malagoli D, Ottaviani E. ACTH in invertebrates: a molecule for all seasons. *Isj-Invertebrate Survival Journal*. 2016; 13(1):28–33.
- [23]. Tsai PS. Gonadotropin-releasing hormone in invertebrates: Structure, function, and evolution. *Gen Comp Endocrinol*. 2006; 148(1):48–53. [PubMed: 16256989]
- [24]. Takahashi, A. Chapter 7 - Opioid Peptide Family *Handbook of Hormones: Comparative Endocrinology for Basic and Clinical Research*. Takei, Y, Ando, H, Tsutsui, K, editors. Academic Press; San Diego; 2016. 53–54.
- [25]. Maurer M, Bader M, Bas M, Bossi F, Cicardi M, Cugno M, et al. New topics in bradykinin research. *Allergy*. 2011; 66(11):1397–1406. [PubMed: 21859431]
- [26]. Fricker LD. *Neuropeptides and Other Bioactive Peptides: From Discovery to Function*. Morgan and Claypool Life Sciences. 2012
- [27]. Lee JE. Neuropeptidomics: Mass Spectrometry-Based Identification and Quantitation of Neuropeptides. *Genome Inform*. 2016; 14(1):12–19.
- [28]. Schilling S, Wasternack C, Demuth HU. Glutaminy cyclases from animals and plants: a case of functionally convergent protein evolution. *Biol Chem*. 2008; 389(8):983–991. [PubMed: 18979624]
- [29]. Hook V, Lietz CB, Podvin S, Cajka T, Fiehn O. Diversity of Neuropeptide Cell-Cell Signaling Molecules Generated by Proteolytic Processing Revealed by Neuropeptidomics Mass Spectrometry. *J Am Soc Mass Spectrom*. 2018; 29(5):807–816. [PubMed: 29667161]
- [30]. Paul M, Mehr AP, Kreutz R. Physiology of local renin-angiotensin systems. *Physiol Rev*. 2006; 86(3):747–803. [PubMed: 16816138]
- [31]. Campbell, DJ. Chapter 188 - Bradykinin Peptides *Handbook of Biologically Active Peptides (Second Edition)*. Kastin, AJ, editor. Academic Press; Boston; 2013. 1386–1393.
- [32]. van den Pol AN. Neuropeptide transmission in brain circuits. *Neuron*. 2012; 76(1):98–115. [PubMed: 23040809]
- [33]. Burbach JP. What are neuropeptides? *Methods Mol. Biol*. 2011; 789:1–36.
- [34]. Hallberg M. Neuropeptides: metabolism to bioactive fragments and the pharmacology of their receptors. *Med Res Rev*. 2015; 35(3):464–519. [PubMed: 24894913]

- [35]. Takahashi, A. Chapter 16 - Proopiomelanocortin Family Handbook of Hormones: Comparative Endocrinology for Basic and Clinical Research. Takei, Y, Ando, H, Tsutsui, K, editors. Academic Press; San Diego: 2016. 116–117.
- [36]. Suzuki, N. Chapter 27 - Calcitonin/Calcitonin Gene-Related Peptide Family Handbook of Hormones: Comparative Endocrinology for Basic and Clinical Research. Takei, Y, Ando, H, Tsutsui, K, editors. Academic Press; San Diego: 2016. 230–231.
- [37]. Tung YCL, Piper SJ, Yeung D, O’Rahilly S, Coll AP. A Comparative Study of the Central Effects of Specific Proopiomelanocortin (POMC)-Derived Melanocortin Peptides on Food Intake and Body Weight in Pomc Null Mice. *Endocrinology*. 2006; 147(12):5940–5947. [PubMed: 16959830]
- [38]. Holmes CL, Landry DW, Granton JT. Science review: Vasopressin and the cardiovascular system part 1--receptor physiology. *Crit Care*. 2003; 7(6):427–434. [PubMed: 14624682]
- [39]. Barberis C, Mouillac B, Durroux T. Structural bases of vasopressin/oxytocin receptor function. *J Endocrinol*. 1998; 156(2):223–229. [PubMed: 9518866]
- [40]. Chini, B, Manning, M, Guillon, G. Affinity and efficacy of selective agonists and antagonists for vasopressin and oxytocin receptors: an “easy guide” to receptor pharmacology, *Prog Brain Res*. Elsevier; 2008. 513–517.
- [41]. Strand FL. Neuropeptides: general characteristics and neuropharmaceutical potential in treating CNS disorders. *Prog Drug Res*. 2003; 61:1–37. [PubMed: 14674607]
- [42]. Alexander SPH, Davenport AP, Kelly E, Marrion N, Peters JA, Benson HE, et al. The Concise Guide to Pharmacology 2015/16: G protein-coupled receptors. *Br J Pharmacol*. 2015; 172(24):5744–5869. [PubMed: 26650439]
- [43]. Takei, Y. Chapter 18 - Secretin (Pituitary Adenylate Cyclase-Activating Polypeptide) Family Handbook of Hormones: Comparative Endocrinology for Basic and Clinical Research. Takei, Y, Ando, H, Tsutsui, K, editors. Academic Press; San Diego: 2016. 140–141.
- [44]. Koshimizu TA, Nakamura K, Egashira N, Hiroshima M, Nonoguchi H, Tanoue A. Vasopressin V1a and V1b receptors: from molecules to physiological systems. *Physiol Rev*. 2012; 92(4):1813–1864. [PubMed: 23073632]
- [45]. Abad C, Tan Y-V. Immunomodulatory Roles of PACAP and VIP: Lessons from Knockout Mice. *J. Mol Neurosci*. 2018; 66(1):102–113. [PubMed: 30105629]
- [46]. Winslow JT, Insel TR. The social deficits of the oxytocin knockout mouse. *Neuropeptides*. 2002; 36(2-3):221–229. [PubMed: 12359512]
- [47]. Hoyer D, Bartfai T. Neuropeptides and neuropeptide receptors: drug targets, and peptide and non-peptide ligands: a tribute to Prof. Dieter Seebach *Chem Biodivers*. 2012; 9(11):2367–2387.
- [48]. van der Klaauw AA. Neuropeptides in Obesity and Metabolic Disease. *Clin Chem*. 2018; 64(1):173–182. [PubMed: 29097517]
- [49]. Holmes A, Heilig M, Rupniak NMJ, Steckler T, Griebel G. Neuropeptide systems as novel therapeutic targets for depression and anxiety disorders. *Trends Pharmacol Sci*. 2003; 24(11):580–588. [PubMed: 14607081]
- [50]. Menon N, Prabhavalkar KS, Bhatt LK. Neuropeptides: A promising target for treating seizures. *Neuropeptides*. 2017; 65:63–70. [PubMed: 28559061]
- [51]. Widiapradja A, Chunduri P, Levick SP. The role of neuropeptides in adverse myocardial remodeling and heart failure. *Cell Mol Life Sci*. 2017; 74(11):2019–2038. [PubMed: 28097372]
- [52]. Nixon JP, Mavanji V, Butterick TA, Billington CJ, Kotz CM, Teske JA. Sleep disorders, obesity, and aging: The role of orexin. *Ageing Res Rev*. 2015; 20:63–73. [PubMed: 25462194]
- [53]. Guastella AJ, Gray KM, Rinehart NJ, Alvares GA, Tonge BJ, Hickie IB, et al. The effects of a course of intranasal oxytocin on social behaviors in youth diagnosed with autism spectrum disorders: a randomized controlled trial. *J Child Psychol Psychiatry*. 2015; 56(4):444–452. [PubMed: 25087908]
- [54]. Menegueti BT, Cardoso MH, Ribeiro CFA, Felicio MR, Pinto IB, Santos NC, et al. Neuropeptide receptors as potential pharmacological targets for obesity. *Pharmacol Ther*. 2019; 196:59–78. [PubMed: 30439454]

- [55]. Yamasue, H, Domes, G. Oxytocin and Autism Spectrum Disorders Behavioral Pharmacology of Neuropeptides: Oxytocin. Hurlemann, R, Grinevich, V, editors. Springer International Publishing; Cham: 2018. 449–465.
- [56]. Leighton GE, Rodriguez RE, Hill RG, Hughes J. kappa-Opioid agonists produce antinociception after i.v and i.c.v. but not intrathecal administration in the rat. *Br J Pharmacol*. 1988; 93(3):553–560. [PubMed: 2836010]
- [57]. Jordan BA, Devi LA. G-protein-coupled receptor heterodimerization modulates receptor function. *Nature*. 1999; 399(6737):697–700. [PubMed: 10385123]
- [58]. Hokfelt T, Bartfai T, Bloom F. Neuropeptides: opportunities for drug discovery. *Lancet Neurol*. 2003; 2(8):463–472. [PubMed: 12878434]
- [59]. Romanova EV, Sweedler JV. Peptidomics for the discovery and characterization of neuropeptides and hormones. *Trends Pharmacol Sci*. 2015; 36(9):579–586. [PubMed: 26143240]
- [60]. Buchberger A, Yu Q, Li L. Advances in Mass Spectrometric Tools for Probing Neuropeptides. *Annu Rev Anal Chem*. 2015; 8:485–509.
- [61]. Clynen E, Liu F, Husson SJ, Landuyt B, Hayakawa E, Baggerman G, et al. Bioinformatic approaches to the identification of novel neuropeptide precursors. *Methods Mol Biol*. 2010; 615:357–374. [PubMed: 20013220]
- [62]. DeLaney K, Buchberger AR, Atkinson L, Grunder S, Mousley A, Li L. New techniques, applications and perspectives in neuropeptide research. *J Exp Biol*. 2018; 221(3)
- [63]. Coulson J, Couture R, Faussner A FG Jr, Leeb-Lundberg F, Marceau F, et al. Bradykinin receptors (version 2019.4) in the IUPHAR/BPS Guide to Pharmacology Database. *IUPHAR/BPS Guide to Pharmacology CITE*. 2019; 2019(4)
- [64]. Marceau F, Bachelard H, Bouthillier J, Fortin JP, Morissette G, Bawolak MT, et al. Bradykinin receptors: Agonists, antagonists, expression, signaling, and adaptation to sustained stimulation. *Int Immunopharmacol*. 2020; 82:106305. [PubMed: 32106060]
- [65]. Li XJ, Wolfgang W, Wu YN, North RA, Forte M. Cloning, heterologous expression and developmental regulation of a *Drosophila* receptor for tachykinin-like peptides. *EMBO J*. 1991; 10(11):3221–3229. [PubMed: 1717263]
- [66]. Li XJ, Wu YN, North RA, Forte M. Cloning, Functional Expression, and Developmental Regulation of a Neuropeptide-Y Receptor from *Drosophila-Melanogaster*. *J Biol Chem*. 1992; 267(1):9–12. [PubMed: 1370455]
- [67]. Muratspahic E, Freissmuth M, Gruber CW. Nature-Derived Peptides: A Growing Niche for GPCR Ligand Discovery. *Trends Pharmacol Sci*. 2019; 40(5):309–326. [PubMed: 30955896]
- [68]. Seyedabadi M, Ghahremani MH, Albert PR. Biased signaling of G protein coupled receptors (GPCRs): Molecular determinants of GPCR/transducer selectivity and therapeutic potential. *Pharmacol Ther*. 2019; 200:148–178. [PubMed: 31075355]
- [69]. Di Giglio MG, Muttenthaler M, Harpsoe K, Liutkeviciute Z, Keov P, Eder T, et al. Development of a human vasopressin V1a-receptor antagonist from an evolutionary-related insect neuropeptide. *Sci Rep*. 2017; 7:41002. [PubMed: 28145450]
- [70]. Duerrauer L, Muratspahi E, Gatringer J, Keov P, Mendel HC, Pflieger KDG, et al. I8-arachnotocin—an arthropod-derived G protein-biased ligand of the human vasopressin V2 receptor. *Sci Rep*. 2019; 9(1):19295. [PubMed: 31848378]
- [71]. Hirsch P. The calcitonins: Physiology and pharmacology. *Bone and Mineral*. 1990; 11(3):363–365.
- [72]. Ogoshi, M. Subchapter 27F - Amylin Handbook of Hormones: Comparative Endocrinology for Basic and Clinical Research. Takei, Y, Ando, H, Tsutsui, K, editors. Academic Press; San Diego: 2016. 245–246.
- [73]. Casewell NR, Wuster W, Vonk FJ, Harrison RA, Fry BG. Complex cocktails: the evolutionary novelty of venoms. *Trends Ecol. Evol*. 2013; 28(4):219–229. [PubMed: 23219381]
- [74]. Arbuckle, K. Evolutionary Context of Venom in Animals Malhotra, A, editor. Toxinology, Springer Netherlands; Dordrecht: 2017. 3–31.
- [75]. Fry BG, Casewell NR, Wuster W, Vidal N, Young B, Jackson TN. The structural and functional diversification of the Toxicofera reptile venom system. *Toxicon*. 2012; 60(4):434–448. [PubMed: 22446061]

- [76]. Fry BG, Roelants K, Champagne DE, Scheib H, Tyndall JD, King GF, et al. The toxicogenomic multiverse: convergent recruitment of proteins into animal venoms. *Annu Rev Genom Hum Genet.* 2009; 10:483–511.
- [77]. Ducancel F, Durban J, Verdenaud M. Transcriptomics and venomomics: implications for medicinal chemistry. *Future Med Chem.* 2014; 6(15):1629–1643. [PubMed: 25406004]
- [78]. King GF. Venoms as a platform for human drugs: translating toxins into therapeutics. *Expert Opin Biol Th.* 2011; 11(11):1469–1484.
- [79]. Prashanth JR, Dutertre S, Lewis RJ. Revising the Role of Defense and Predation in Cone Snail Venom Evolution. *Toxinology.* 2017:105–123.
- [80]. Koh CY, Kini RM. From snake venom toxins to therapeutics--cardiovascular examples. *Toxicon.* 2012; 59(4):497–506. [PubMed: 21447352]
- [81]. Lewis RJ, Garcia ML. Therapeutic potential of venom peptides. *Nat Rev Drug Discov.* 2003; 2(10):790–802. [PubMed: 14526382]
- [82]. Undheim EA, Jenner RA, King GF. Centipede venoms as a source of drug leads. *Expert Opin Drug Discov.* 2016; 11(12):1139–1149. [PubMed: 27611363]
- [83]. Vetter I, Lewis RJ. Therapeutic potential of cone snail venom peptides (conopeptides). *Curr Top Med Chem.* 2012; 12(14):1546–1552. [PubMed: 22827523]
- [84]. Parkes DG, Mace KF, Trautmann ME. Discovery and development of exenatide: the first antidiabetic agent to leverage the multiple benefits of the incretin hormone, GLP-1. *Expert Opin Drug Discov.* 2013; 8(2):219–244. [PubMed: 23231438]
- [85]. Anderson SL, Trujillo JM. Lixisenatide in type 2 diabetes: latest evidence and clinical usefulness. *Ther Adv Chronic Dis.* 2016; 7(1):4–17. [PubMed: 26770666]
- [86]. Scarborough RM, Naughton MA, Teng W, Rose JW, Phillips DR, Nannizzi L, et al. Design of potent and specific integrin antagonists. Peptide antagonists with high specificity for glycoprotein IIb-IIIa. *J Biol Chem.* 1993; 268(2):1066–1073. [PubMed: 8419315]
- [87]. Serruys PW, Vranckx P, Allikmets K. Clinical development of bivalirudin (Angiox (R)): rationale for thrombin-specific anticoagulation in percutaneous coronary intervention and acute coronary syndromes. *Int J Clin Pract.* 2006; 60(3):344–350. [PubMed: 16494651]
- [88]. Miljanich GP. Ziconotide: neuronal calcium channel blocker for treating severe chronic pain. *Curr Med Chem.* 2004; 11(23):3029–3040. [PubMed: 15578997]
- [89]. Osteen JD, Herzig V, Gilchrist J, Emrick JJ, Zhang CC, Wang XD, et al. Selective spider toxins reveal a role for the Na(v)1.1 channel in mechanical pain. *Nature.* 2016; 534(7608):494–499. [PubMed: 27281198]
- [90]. Richards KL, Milligan CJ, Richardson RJ, Jancovski N, Grunnet M, Jacobson LH, et al. Selective Na(V)1.1 activation rescues Dravet syndrome mice from seizures and premature death. *Proc Natl Acad Sci U S A.* 2018; 115(34):E8077–E8085. [PubMed: 30076230]
- [91]. Deuis JR, Dekan Z, Wingerd JS, Smith JJ, Munasinghe NR, Bhola RF, et al. Pharmacological characterisation of the highly Na(V)1.7 selective spider venom peptide Pn3a. *Sci Rep.* 2017; 7(1)
- [92]. Mueller A, Starobova H, Morgan M, Dekan Z, Cheneval O, Schroeder CI, et al. Antiallodynic effects of the selective Na(V)1.7 inhibitor Pn3a in a mouse model of acute postsurgical pain: evidence for analgesic synergy with opioids and baclofen. *Pain.* 2019; 160(8):1766–1780. [PubMed: 31335646]
- [93]. Chassagnon IR, McCarthy CA, Chin YKY, Pineda SS, Keramidas A, Mobli M, et al. Potent neuroprotection after stroke afforded by a double-knot spider-venom peptide that inhibits acid-sensing ion channel 1a. *Proc Natl Acad Sci U S A.* 2017; 114(14):3750–3755. [PubMed: 28320941]
- [94]. DeBin JA, Maggio JE, Strichartz GR. Purification and characterization of chlorotoxin, a chloride channel ligand from the venom of the scorpion. *Am J Physiol.* 1993; 264(2 Pt 1):C361–369. [PubMed: 8383429]
- [95]. Cohen G, Burks SR, Frank JA. ChlorotoxinA Multimodal Imaging Platform for Targeting Glioma Tumors. *Toxins.* 2018; 10(12):496.
- [96]. Dutertre S, Lewis RJ. Use of venom peptides to probe ion channel structure and function. *J Biol Chem.* 2010; 285(18):13315–13320. [PubMed: 20189991]

- [97]. Lewis RJ, Dutertre S, Vetter I, Christie MJ. Conus venom peptide pharmacology. *Pharmacol Rev.* 2012; 64(2):259–298. [PubMed: 22407615]
- [98]. Saez NJ, Herzig V. Versatile spider venom peptides and their medical and agricultural applications. *Toxicon.* 2019; 158:109–126. [PubMed: 30543821]
- [99]. Verdes A, Anand P, Gorson J, Jannetti S, Kelly P, Leffler A, et al. From Mollusks to Medicine: A Venomics Approach for the Discovery and Characterization of Therapeutics from Terebridae Peptide Toxins. *Toxins (Basel).* 2016; 8(4):117. [PubMed: 27104567]
- [100]. Akondi KB, Muttenthaler M, Dutertre S, Kaas Q, Craik DJ, Lewis RJ, et al. Discovery, synthesis, and structure-activity relationships of conotoxins. *Chem Rev.* 2014; 114(11):5815–5847. [PubMed: 24720541]
- [101]. King GF, Hardy MC. Spider-Venom Peptides: Structure, Pharmacology, and Potential for Control of Insect Pests. *Annual Review of Entomology.* 2013; 58:475–496.
- [102]. Jin AH, Muttenthaler M, Dutertre S, Himaya SWA, Kaas Q, Craik DJ, et al. Conotoxins: Chemistry and Biology. *Chem Rev.* 2019; 119(21):11510–11549. [PubMed: 31633928]
- [103]. Sciani JM, Pimenta DC. The modular nature of bradykinin-potentiating peptides isolated from snake venoms. *J Venom Anim Tox incl Trop Dis.* 2017; 23:45.
- [104]. Fry BG. From genome to “venome”: Molecular origin and evolution of the snake venom proteome inferred from phylogenetic analysis of toxin sequences and related body proteins. *Genome Res.* 2005; 15(3):403–420. [PubMed: 15741511]
- [105]. Olivera BM. EE Just lecture, 1996 - Conus venom peptides, receptor and ion channel targets, and drug design: 50 million years of neuropharmacology. *Mol Biol Cell.* 1997; 8(11):2101–2109. [PubMed: 9362055]
- [106]. Hargreaves AD, Swain MT, Hegarty MJ, Logan DW, Mulley JF. Restriction and recruitment-gene duplication and the origin and evolution of snake venom toxins. *Genome Biol Evol.* 2014; 6(8):2088–2095. [PubMed: 25079342]
- [107]. Starcevic A, Moura-da-Silva AM, Cullum J, Hranueli D, Long PF. Combinations of long peptide sequence blocks can be used to describe toxin diversification in venomous animals. *Toxicon.* 2015; 95:84–92. [PubMed: 25595734]
- [108]. Furman BL. The development of Byetta (exenatide) from the venom of the Gila monster as an anti-diabetic agent. *Toxicon.* 2012; 59(4):464–471. [PubMed: 21194543]
- [109]. Craig AG, Norberg T, Griffin D, Hoeger C, Akhtar M, Schmidt K, et al. Contulakin-G, an O-glycosylated invertebrate neurotensin. *J Biol Chem.* 1999; 274(20):13752–13759. [PubMed: 10318778]
- [110]. Munawar A, Ali SA, Akrem A, Betzel C. Snake Venom Peptides: Tools of Biodiscovery. *Toxins.* 2018; 10(11):474.
- [111]. Mourier G, Hajj M, Cordier F, Zorba A, Gao X, Coskun T, et al. Pharmacological and structural characterization of long-sarafotoxins, a new family of endothelin-like peptides: Role of the C-terminus extension. *Biochimie.* 2012; 94(2):461–470. [PubMed: 21889567]
- [112]. Dutertre S, Croker D, Daly NL, Andersson A, Muttenthaler M, Lumsden NG, et al. Conopressin-T from *Conus tulipa* reveals an antagonist switch in vasopressin-like peptides. *J Biol Chem.* 2008; 283(11):7100–7108. [PubMed: 18174156]
- [113]. Ducancel F. Endothelin-like peptides. *Cell Mol Life Sci.* 2005; 62(23):2828–2839. [PubMed: 16261262]
- [114]. Harvey, AL. Chapter 62 - Snake Peptides. *Handbook of Biologically Active Peptides (Second Edition)*. Kastin, AJ, editor. Academic Press; Boston: 2013. 451–460.
- [115]. Safavi-Hemami H, Gajewiak J, Karanth S, Robinson SD, Ueberheide B, Douglass AD, et al. Specialized insulin is used for chemical warfare by fish-hunting cone snails. *Proc Natl Acad Sci U S A.* 2015; 112(6):1743–1748. [PubMed: 25605914]
- [116]. Jami S, Erickson A, Brierley SM, Vetter I. Pain-Causing Venom Peptides: Insights into Sensory Neuron Pharmacology. *Toxins.* 2017; 10(1):15.
- [117]. Pimenta AM, Rates B, Bloch C Jr, Gomes PC, Santoro MM, de Lima ME, et al. Electrospray ionization quadrupole time-of-flight and matrix-assisted laser desorption/ionization tandem time-of-flight mass spectrometric analyses to solve micro-heterogeneity in post-translationally

- modified peptides from *Phoneutria nigriventer* (Aranea, Ctenidae) venom. *Rapid Commun. Mass Spectrom.* 2005; 19(1):31–37. [PubMed: 15573368]
- [118]. Yshii LM, Souza GH, Camargo EA, Eberlin MN, Ribela MT, Muscara MN, et al. Characterization of the mechanisms underlying the inflammatory response to *Polistes lanio lanio* (paper wasp) venom in mouse dorsal skin. *Toxicon.* 2009; 53(1):42–52. [PubMed: 18977380]
- [119]. Aguilar MB, Luna-Ramirez KS, Echeverria D, Falcon A, Olivera BM, Heimer de la Coteria EP, et al. Conorfamide-Sr2, a gamma-carboxyglutamate-containing FMRFamide-related peptide from the venom of *Conus spurius* with activity in mice and mollusks. *Peptides.* 2008; 29(2):186–195. [PubMed: 18201803]
- [120]. Volpe M, Rubattu S, Burnett J Jr. Natriuretic peptides in cardiovascular diseases: current use and perspectives. *Eur Heart J.* 2014; 35(7):419–425.
- [121]. Moller C, Melaun C, Castillo C, Diaz ME, Renzelman CM, Estrada O, et al. Functional hypervariability and gene diversity of cardioactive neuropeptides (vol 285, pg 40673, 2010). *J Biol Chem.* 2012; 287(5):3609–3609.
- [122]. Tsutsui, K, Ubuka, T. Chapter 1 - RFamide Peptide Family Handbook of Hormones. Takei, Y, Ando, H, Tsutsui, K, editors. Academic Press; San Diego: 2016. 5–e1-2.
- [123]. Osadchii OE. Emerging role of neurotensin in regulation of the cardiovascular system. *Eur J Pharmacol.* 2015; 762:184–192. [PubMed: 26004530]
- [124]. DeFronzo RA. Insulin resistance, lipotoxicity, type 2 diabetes and atherosclerosis: the missing links. The Claude Bernard Lecture 2009. *Diabetologia.* 2010; 53(7):1270–1287.
- [125]. Hayes MR, De Jonghe BC, Kanoski SE. Role of the glucagon-like-peptide-1 receptor in the control of energy balance. *Physiol Behav.* 2010; 100(5):503–510. [PubMed: 20226203]
- [126]. Potter LR, Yoder AR, Flora DR, Antos LK, Dickey DM. Natriuretic peptides: their structures, receptors, physiologic functions and therapeutic applications. *Handb Exp Pharmacol.* 2009; 191:341–366.
- [127]. Marceau F, Regoli D. Bradykinin receptor ligands: therapeutic perspectives. *Nat Rev Drug Discov.* 2004; 3(10):845–852. [PubMed: 15459675]
- [128]. Zieglansberger W. Substance P and pain chronicity. *Cell Tissue Res.* 2019; 375(1):227–241. [PubMed: 30284083]
- [129]. Corder G, Castro DC, Bruchas MR, Scherrer G. Endogenous and Exogenous Opioids in Pain. *Annu Rev Neurosci.* 2018; 41:453–473. [PubMed: 29852083]
- [130]. Gruber CW, Muttenthaler M, Freissmuth M. Ligand-based peptide design and combinatorial peptide libraries to target G protein-coupled receptors. *Curr Pharm Des.* 2010; 16(28):3071–3088. [PubMed: 20687879]
- [131]. Ciolek J, Reinfrank H, Quinton L, Viengchareun S, Stura EA, Vera L, et al. Green mamba peptide targets type-2 vasopressin receptor against polycystic kidney disease. *Proc Natl Acad Sci U S A.* 2017; 114(27):7154–7159. [PubMed: 28630289]
- [132]. Zweckstetter M, Czisch M, Mayer U, Chu ML, Zinth W, Timpl R, et al. Structure and multiple conformations of the Kunitz-type domain from human type VI collagen alpha 3(VI) chain in solution. *Structure.* 1996; 4(2):195–209. [PubMed: 8805527]
- [133]. Zupunski V, Kordis D. Strong and widespread action of site-specific positive selection in the snake venom Kunitz/BPTI protein family. *Sci Rep.* 2016; 6:37054. [PubMed: 27841308]
- [134]. Fry BG, Koludarov I, Jackson TNW, Holford M, Terrat Y, Casewelle NR, et al. Seeing the Woods for the Trees: Understanding Venom Evolution as a Guide for Biodiscovery. *Rsc Drug Discov.* 2015; 42:1–36.
- [135]. Verano-Braga T, Figueiredo-Rezende F, Melo MN, Lautner RQ, Gomes ERM, Mata-Machado LT, et al. Structure-function studies of *Tityus serrulatus* Hypotensin-I (TsHpt-I): A new agonist of B-2 kinin receptor. *Toxicon.* 2010; 56(7):1162–1171. [PubMed: 20417225]
- [136]. Emerich BL, Ferreira RCM, Cordeiro MN, Borges MH, Pimenta AMC, Figueiredo SG, et al. δ -Ctenitoxin-Pn1a, a Peptide from *Phoneutria nigriventer* Spider Venom, Shows Antinociceptive Effect Involving Opioid and Cannabinoid Systems, in Rats. *Toxins.* 2016; 8(4):106. [PubMed: 27077886]
- [137]. Brust A, Croker DE, Colless B, Ragnarsson L, Andersson A, Jain K, et al. Conopeptide-Derived kappa-Opioid Agonists (Conorphins): Potent, Selective, and Metabolic Stable Dynorphin A

- Mimetics with Antinociceptive Properties. *J Med Chem.* 2016; 59(6):2381–2395. [PubMed: 26859603]
- [138]. Petrel C, Hocking HG, Reynaud M, Upert G, Favreau P, Biass D, et al. Identification, structural and pharmacological characterization of tau-CnVA, a conopeptide that selectively interacts with somatostatin sst(3) receptor. *Biochem Pharmacol.* 2013; 85(11):1663–1671. [PubMed: 23567999]
- [139]. Gutierrez VP, Konno K, Chacur M, Sampaio SC, Picolo G, Brigatte P, et al. Crotalphine induces potent antinociception in neuropathic pain by acting at peripheral opioid receptors. *Eur J Pharmacol.* 2008; 594(1-3):84–92. [PubMed: 18703042]
- [140]. Oldrati V, Arrell M, Violette A, Perret F, Sprungli X, Wolfender JL, et al. Advances in venomomics. *Mol Biosyst.* 2016; 12(12):3530–3543. [PubMed: 27787525]
- [141]. Von Reumont BM. Studying Smaller and Neglected Organisms in Modern Evolutionary Venomomics Implementing RNASeq (Transcriptomics)—A Critical Guide. *Toxins.* 2018; 10(7):292.
- [142]. Wang Z, Gerstein M, Snyder M. RNA-Seq: a revolutionary tool for transcriptomics. *Nat Rev Genet.* 2009; 10(1):57–63. [PubMed: 19015660]
- [143]. Calvete JJ. Venomomics: integrative venom proteomics and beyond. *Biochem J.* 2017; 474:611–634. [PubMed: 28219972]
- [144]. Chen YP, Chen F. Identifying targets for drug discovery using bioinformatics. *Expert Opin Ther Tar.* 2008; 12(4):383–389.
- [145]. Escoubas P, King GF. Venomomics as a drug discovery platform. *Expert Rev Proteomics.* 2009; 6(3):221–224. [PubMed: 19489692]
- [146]. Augusto-de-Oliveira C, Stuginski DR, Kitano ES, Andrade-Silva D, Liberato T, Fukushima I, et al. Dynamic Rearrangement in Snake Venom Gland Proteome: Insights into Bothrops jararaca Intraspecific Venom Variation. *J Proteome Res.* 2016; 15(10):3752–3762. [PubMed: 27575776]
- [147]. Dutertre S, Jin AH, Vetter I, Hamilton B, Sunagar K, Laverne V, et al. Evolution of separate predation- and defence-evoked venoms in carnivorous cone snails. *Nat Commun.* 2014; 5:3521. [PubMed: 24662800]
- [148]. Gao B, Peng C, Zhu Y, Sun Y, Zhao T, Huang Y, et al. High Throughput Identification of Novel Conotoxins from the Vermivorous Oak Cone Snail (*Conus quercinus*) by Transcriptome Sequencing. *Int J Mol Sci.* 2018; 19(12)
- [149]. Prashanth JR, Lewis RJ. An efficient transcriptome analysis pipeline to accelerate venom peptide discovery and characterisation. *Toxicon.* 2015; 107(B):282–289. [PubMed: 26376071]
- [150]. Kaas Q, Craik DJ. Bioinformatics-Aided Venomomics. *Toxins (Basel).* 2015; 7(6):2159–2187. [PubMed: 26110505]
- [151]. Caers J, Verlinden H, Zels S, Vandersmissen HP, Vuerinckx K, Schoofs L. More than two decades of research on insect neuropeptide GPCRs: an overview. *Front Endocrinol.* 2012; 3:151.
- [152]. Robinson SD, Li Q, Bandyopadhyay PK, Gajewiak J, Yandell M, Papenfuss AT, et al. Hormone-like peptides in the venoms of marine cone snails. *Gen Comp Endocrinol.* 2017; 244:11–18. [PubMed: 26301480]
- [153]. Robinson SD, Safavi-Hemami H, McIntosh LD, Purcell AW, Norton RS, Papenfuss AT. Diversity of conotoxin gene superfamilies in the venomous snail, *Conus victoriae*. *PLoS One.* 2014; 9(2):e87648. [PubMed: 24505301]
- [154]. Southey BR, Romanova EV, Rodriguez-Zas SL, Sweedler JV. Bioinformatics for Prohormone and Neuropeptide Discovery. *Methods Mol Biol.* 2018; 1719:71–96. [PubMed: 29476505]
- [155]. Christie AE. Prediction of the peptidomes of *Tigriopus californicus* and *Lepeophtheirus salmonis* (Copepoda, Crustacea). *Gen Comp Endocrinol.* 2014; 201:87–106. [PubMed: 24613138]
- [156]. Boerjan B, Cardoen D, Verdonck R, Caers J, Schoofs L. Insect omics research coming of age. *Can J Zool.* 2012; 90(4):440–455.
- [157]. Torres AM, Tsampazi C, Geraghty DP, Bansal PS, Alewood PF, Kuchel PW. D-amino acid residue in a defensin-like peptide from platypus venom: effect on structure and chromatographic properties. *Biochem J.* 2005; 391:215–220. [PubMed: 16033333]
- [158]. Karsenty S, Rappoport N, Ofer D, Zair A, Linial M. NeuroPID: a classifier of neuropeptide precursors. *Nucleic Acids Res.* 2014; 42(Web Server issue):W182–186. [PubMed: 24792159]

- [159]. Southey BR, Amare A, Zimmerman TA, Rodriguez-Zas SL, Sweedler JV. NeuroPred: a tool to predict cleavage sites in neuropeptide precursors and provide the masses of the resulting peptides. *Nucleic Acids Res.* 2006; 34(Web Server issue):W267–272. [PubMed: 16845008]
- [160]. Southey BR, Sweedler JV, Rodriguez-Zas SL. A python analytical pipeline to identify prohormone precursors and predict prohormone cleavage sites. *Front Neuroinform.* 2008; 2:7. [PubMed: 19169350]
- [161]. Duckert P, Brunak S, Blom N. Prediction of proprotein convertase cleavage sites. *Protein Eng Des Sel.* 2004; 17(1):107–112. [PubMed: 14985543]
- [162]. Petersen TN, Brunak S, von Heijne G, Nielsen H. SignalP 4.0: discriminating signal peptides from transmembrane regions. *Nat Methods.* 2011; 8(10):785–786. [PubMed: 21959131]
- [163]. Lavergne V, Dutertre S, Jin AH, Lewis RJ, Taft RJ, Alewood PF. Systematic interrogation of the *Conus marmoreus* venom duct transcriptome with ConoSorter reveals 158 novel conotoxins and 13 new gene superfamilies. *BMC Genomics.* 2013; 14:708. [PubMed: 24131469]
- [164]. Kaas Q, Yu R, Jin AH, Dutertre S, Craik DJ. ConoServer: updated content, knowledge, and discovery tools in the conopeptide database. *Nucleic Acids Res.* 2012; 40(Database issue):D325–330. [PubMed: 22058133]
- [165]. Pineda SS, Chaumeil PA, Kunert A, Kaas Q, Thang MWC, Le L, et al. ArachnoServer 3.0: an online resource for automated discovery, analysis and annotation of spider toxins. *Bioinformatics.* 2018; 34(6):1074–1076. [PubMed: 29069336]
- [166]. Roly ZY, Hakim MA, Zahan AS, Hossain MM, Reza MA. ISOB: A Database of Indigenous Snake Species of Bangladesh with respective known venom composition. *Bioinformation.* 2015; 11(2):107–114. [PubMed: 25848172]
- [167]. Behrendt R, White P, Offer J. Advances in Fmoc solid-phase peptide synthesis. *JPept Sci.* 2016; 22(1):4–27. [PubMed: 26785684]
- [168]. Postma TM, Albericio F. Disulfide Formation Strategies in Peptide Synthesis. *Eur J Org Chem.* 2014; 2014(17):3519–3530.
- [169]. Heeke, GV, Stout, JS, Wagner, FW. *Synthesis of Recombinant Peptides* Peptide Analysis Protocols. Dunn, BM, Pennington, MW, editors. Humana Press; Totowa, NJ: 1994. 245–260.
- [170]. Thompson RE, Muir TW. Chemoenzymatic Semisynthesis of Proteins. *Chem Rev.* 2020; 120(6):3051–3126. [PubMed: 31774265]
- [171]. Muttenthaler M, Andersson A, Vetter I, Menon R, Busnelli M, Ragnarsson L, et al. Subtle modifications to oxytocin produce ligands that retain potency and improved selectivity across species. *Sci Signal.* 2017; 10(508)
- [172]. Vetter, I, Carter, D, Bassett, J, Deuis, JR, Tay, B, Jami, S. , et al. High-Throughput Fluorescence Assays for Ion Channels and GPCRs Calcium Signaling. Islam, MS, editor. Springer International Publishing; Cham: 2020. 27–72.
- [173]. Harvey AL. Toxins and drug discovery. *Toxicon.* 2014; 92:193–200. [PubMed: 25448391]
- [174]. Azria, M. The calcitonins: physiology and pharmacology Karger; 1989.
- [175]. Boss C, Bolli MH, Gatfield J. From bosentan (Tracleer®) to macitentan (Opsumit®): The medicinal chemistry perspective. *Bioorg Med Chem Lett.* 2016; 26(15):3381–3394. [PubMed: 27321813]
- [176]. Rogier van der Velde, A, Meijers, WC, de Boer, RA. Chapter 3.7.1 - Cardiovascular Biomarkers: Translational Aspects of Hypertension, Atherosclerosis, and Heart Failure in Drug Development Principles of Translational Science in Medicine (Second Edition). Wehling, M, editor. Academic Press; Boston: 2015. 167–183.
- [177]. Gut P, Czarnywojtek A, Fischbach J, Baczyk M, Ziemnicka K, Wrotkowska E, et al. Chromogranin A - unspecific neuroendocrine marker. Clinical utility and potential diagnostic pitfalls. *Arch. Med. Sci.* 2016; 12(1):1–9.
- [178]. Sekiguchi, T. Subchapter 20A - Gastrin Handbook of Hormones: Comparative Endocrinology for Basic and Clinical Research. Takei, Y, Ando, H, Tsutsui, K, editors. Academic Press; San Diego: 2016. 174–176.
- [179]. Andoh, T. Subchapter 19A - Insulin Handbook of Hormones: Comparative Endocrinology for Basic and Clinical Research. Takei, Y, Ando, H, Tsutsui, K, editors. Academic Press; San Diego: 2016. 157–160.

- [180]. Katsuura, G, Inui, A. Subchapter 25A - Pancreatic Polypeptide Handbook of Hormones: Comparative Endocrinology for Basic and Clinical Research. Takei, Y, Ando, H, Tsutsui, K, editors. Academic Press; San Diego: 2016. 213–214.
- [181]. Hausfater P. Biomarkers and infection in the emergency unit. Med Mal Infect. 2014; 44(4):139–145. [PubMed: 24556451]
- [182]. Sridharan P, Chamberlain RS. The Efficacy of Procalcitonin as a Biomarker in the Management of Sepsis: Slaying Dragons or Tilting at Windmills? Surgical Infections. 2013; 14(6):489–511. [PubMed: 24274059]
- [183]. Ando, H. Chapter 4 - Thyrotropin-Releasing Hormone Handbook of Hormones: Comparative Endocrinology for Basic and Clinical Research. Takei, Y, Ando, H, Tsutsui, K, editors. Academic Press; San Diego: 2016. 34–35.
- [184]. Wishart DS, Feunang YD, Guo AC, Lo EJ, Marcu A, Grant JR, et al. DrugBank 5.0: a major update to the DrugBank database for 2018. Nucleic Acids Res. 2018; 46(D1):D1074–D1082. [PubMed: 29126136]

Figure 1. Overview of animal evolution, neuropeptide biosynthesis, release and signalling.

(A) Simplified phylogenetic tree illustrating selected animal phyla where neuropeptides are found, including non-bilateria phyla. The deuterostome and protostome phyla diverged ~600 million years ago (mya). *, phyla with no evidence of a nervous system. Adapted from [7]. (B) Neuropeptide precursors are synthesised in the soma of the neuron. The precursor neuropeptide is produced in the endoplasmic reticulum (ER) from mRNA that is translated on the cytosolic ER membrane. After folding of the protein and addition of N-linked carbohydrate (if the appropriate sites are present in the precursor), the neuropeptide is transported to the Golgi via small vesicles that bud from the ER and fuse with the Golgi. The precursor protein is packaged into secretory vesicles, where they are further processed. Upon stimuli, the vesicle fuses with the cell membrane and releases the mature neuropeptides. Mature neuropeptides can be released from nerve endings, dendrites or the cell soma. Neuropeptides can either act at short range on the secreting neuron or through diffusion on neighbouring cells (1), or reach long range target receptors through secretion into the blood stream (2) or through non-synaptic dispersion (3). Once released, the neuropeptides are

subject to processing by extracellular peptidases. This generally results in degradation and inactivation of the neuropeptides, except for some cases (e.g., bradykinin and angiotensin II) where this extracellular processing is required to produce the active mature neuropeptide.

(C) Schematic of a model neuropeptide precursor made up of a signal peptide (red), propeptide (green) and mature peptides (blue and yellow). Precursors are cleaved by endopeptidases and carboxypeptidases at dibasic cleavage sites to produce the neuropeptides, which can further mature through additional post-translational modifications once in the Golgi. Image was created with Biorender.com.

Figure 2. Selected neuropeptides and the physiological processes they are involved in. MCH, melanin-concentrating hormone; CRH, corticotropin-releasing hormone; NPY, neuropeptide Y; GnRH, gonadotropin-releasing hormone. This figure has been designed using resources from Freepik.com.

Table 1
Approved drugs targeting neuropeptide receptors and neuropeptides used as biomarkers or diagnostic tools.^a

DRUGS		
Receptor	Receptor subtypes	Drug and indication
Angiotensin (AT) receptor	AT ₁ and AT ₂ receptors	<i>Small molecules:</i> Valsartan, Losartan, Irbesartan, Olmesartan, Eprosartan, Telmisartan , AT ₁ antagonists for hypertension; Candesartan , AT ₁ antagonist for chronic heart failure.
Bradykinin receptors	B ₁ , B ₂ receptors	<i>Peptide:</i> Icatibant , selective B ₂ receptor antagonist, for hereditary angioedema.
Calcitonin (CT) receptor	CT receptor	<i>Peptides:</i> Synthetic salmon calcitonin or synthetic human calcitonin for the treatment of hypercalcemia (postmenopausal osteoporosis, Paget's disease of bone, Sudeck's atrophy, and malignancy-associated hypercalcemia)[174].
Endothelin (ET) receptors	ET _A and ET _B receptors	<i>Small molecules:</i> Bosentan and Macitentan , dual receptor antagonists; Ambrisentan , a selective ET _A receptor antagonist; All used for the treatment of pulmonary hypertension [175].
Glucagon receptors	Glucagon receptor, GLP-1 receptor ^b , GLP-2 receptor	<i>Peptides:</i> Recombinant glucagon , for severe hypoglycaemia; Exenatide, liraglutide, albiglutide, lixisenatide, dulaglutide, semaglutide , GLP-1 receptor agonists, used for diabetes treatment; Teduglutide , human GLP-2 analogue, used for short bowel syndrome, malabsorption.
Gonadotropin-releasing hormone (GnRH) receptor	GnRH receptor	<i>Peptides:</i> Nafarelin , GnRH receptor agonist used for treatment of central precocious puberty and endometriosis; Cetrorelix , GnRH receptor antagonist used to inhibit premature luteinizing hormone surges in controlled ovarian stimulation; Elagolix , GnRH receptor antagonist, used for management of moderate to severe pain associated with endometriosis; Degarelix , GnRH receptor antagonist, used for management of advanced prostate cancer; Leuprolide , GnRH agonist, used to treat prostate cancer, endometriosis, uterine fibroids and premature puberty.
Growth hormone receptor	Growth hormone receptor	<i>Peptide / protein:</i> Somatotropin , recombinant human growth hormone, used for growth hormone deficiency, Turner syndrome, Noonan syndrome, Prader-Willi syndrome, short stature homeobox-containing gene deficiency, chronic renal insufficiency, idiopathic short stature and children small for gestational age; Pegvisomant , growth hormone receptor antagonist, used for acromegaly.
Growth hormone releasing hormone (GHRH) receptor	GHRH receptor	<i>Peptides:</i> Sermorelin , GHRH ₁₋₂₉ peptide fragment, for treatment of growth failure in children; Tesamorelin , GHRH ₁₋₄₄ peptide fragment, for lipodystrophy in HIV patients.
Insulin receptor	Insulin receptor, IGF-1 ^c receptor	<i>Peptides:</i> Human insulin , used for glycaemic control in patients with diabetes mellitus; Mecasermin , recombinant human IGF-1, approved for growth failure in children with primary insulin-like growth factor-1 deficiency or growth hormone gene deletion.
Leptin receptor	Leptin receptor	<i>Peptide:</i> Metreleptin , a leptin analogue used for diabetes and/or hypertriglyceridemia.
Melanocortin (MC) receptors	MC ₁₋₅ receptors	<i>Peptide:</i>

DRUGS		
Receptor	Receptor subtypes	Drug and indication
Natriuretic peptide receptors (NPR)	NPR-A, -B, and -C receptors	Bremelanotide , MC ₁ , MC ₃₋₅ agonist, used for premenopausal women with hypoactive sexual desire disorder. <i>Small molecules:</i> Nitroprusside, Nitroglycerin, Isosorbide dinitrate, Erythrityl tetranitrate, and Amyl nitrite , act as nitric oxide source, vasodilators used for chest pain and high blood pressure. <i>Peptide:</i> Nesiritide , recombinant human BNP ^d for congestive heart failure.
Opioid receptors	μ-, δ-, κ-opioid receptors	<i>Small molecules:</i> Butorphanol, Tramadol, Sufentanil, Alfentanil, Hydrocodone, Fentanyl, Dextropropoxyphene, Morphine, Codeine, Hydromorphone, Meperidine , μ-, δ-, and/or κ-opioid receptor agonists for pain management, anaesthetic, analgesia; Naltrexone, Nalbuphine, Buprenorphine, Dezocine, Pentazocine, Naloxone, Levallorphan , μ-, δ-, and/or κ-opioid receptor antagonists for pain and/or addiction management, treatment of narcotic depression.
Orexin (OX) receptors	OX ₁ , OX ₂ receptors	<i>Small molecule:</i> Suvorexant , OX ₁ R and OX ₂ R antagonist used for insomnia.
Parathyroid hormone (PTH) type 1 receptor	PTH type 1 receptor	<i>Peptides:</i> Teriparatide , recombinant human PTH used in treatment of osteoporosis; Abaloparatide , PTH-related protein analogue, used for post-menopausal women with osteoporosis.
Somatostatin (SST) receptors	SST ₁₋₅ receptors	<i>Peptides:</i> Octreotide, lanreotide , SST ₂ / SST ₅ receptor agonists used for acromegaly and to reduce cancer chemotherapy side effects; Pasireotide , pan agonist used for Cushing's Disease;
Tachykinin receptors	NK ₁ , NK ₂ , NK ₃ , Neuromedin-K receptors	<i>Small molecules:</i> Fosaprepitant, Aprepitant, Netupitant, Rolapitant , NK ₁ antagonist used for chemotherapy-induced emesis.
Oxytocin (OT) and vasopressin receptors	OT, V _{1a} , V _{1b} , V ₂ receptors	<i>Peptides:</i> Atosiban , OTR antagonist used as labour suppressant and IVF treatment; Pitocin and Carbetocin , OTR agonists used for labour induction, post-partum bleeding, and haemorrhage; Desmopressin , V ₂ R agonist for diabetes insipidus and nocturnal enuresis; Terlipressin , V ₂ R agonist used to stop bleeding in the oesophagus. <i>Small molecules:</i> Conivaptan , V _{1a} R and V ₂ R antagonist used for hyponatremia; Tolvaptan , V ₂ R antagonist used for hyponatremia.

BIOMARKERS OR DIAGNOSTIC TOOLS

Neuropeptide	Receptor	Compound and application
ATCH	MC ₂ receptor	Diagnostic agent used to screen for adrenocortical insufficiency.
BNP	n.a. ^e	Diagnostic marker for heart failure [176].
Chromogranin A	n.a.	An indicator for pancreas and prostate cancer in carcinoid syndrome [177].
Corticotropin-releasing hormone (CRH)	CRH receptor 1	Corticoirelin , synthetic human CRH, is used to test the HPA axis and ATCH secretion.
Gastrin	n.a.	Diagnostic marker for gastrin-secreting tumours, atrophic gastritis, gastric ulcers, and pernicious anaemia [178].
Ghrelin	Ghrelin receptor	Ghrelin mimetic Macimorelin is used to diagnose adult GH deficiency.

DRUGS		
Receptor	Receptor subtypes	Drug and indication
GnRH	GnRH receptor	Gonadorelin , synthetic GnRH, is used to evaluate anterior pituitary performance.
Insulin	Insulin receptor	Biomarker for diabetes (complementary); used for identification of insulinomas; insulin-induced hypoglycaemia test used to diagnose suspected ACTH or growth hormone deficiency [179].
Pancreatic polypeptide	n.a.	Diagnostic test of vagal nerve function [180].
Procalcitonin	n.a. n.a.	A biomarker of infection and sepsis [181, 182]. Diagnostic marker in sex hormone check-up; epilepsy type diagnosis [21].
Prolactin		
Thyrotropin-releasing hormone (TRH)	TRH receptor	Protirelin , a TRH analogue, is used to test anterior pituitary gland function to aid thyroid disorder diagnosis [183].
Somatostatin	SST ₁₋₅ receptors	Various radiolabelled somatostatin analogues used for cancer treatment (e.g. Edotreotide gallium Ga-68 and Lutetium Lu 177 dotatate).

^a Information from DrugBank database (www.drugbank.ca) [184] where an 'approved' drug has been approved in at least one jurisdiction, anywhere;

^b GLP, Glucagon-like peptide;

^c IGF, insulin-like growth factor;

^d BNP, B-type natriuretic peptide;

^e n.a. Not applicable.

Table 2
Neuropeptides in animal venoms (proteomic evidence and venom organ transcriptome evidence).

Neuropeptide family	Neuropeptide	Species	Taxonomic group	UniProt/ Genbank/ PMID ID
Bradykinin	Megascoliakinin	<i>Megascolia flavifrons</i>	Insect	P12797
Bradykinin	Thr6-Bradykinin	<i>Polybia occidentalis</i>	Insect	P0DM70
CCAP ^a	ConoCAP-a	<i>Conus viliepinii</i>	Mollusc	E3PQQ8
CCAP	ConoCAP-Gm	<i>Conus gloriamus</i>	Mollusc	28531118
Elevenin	Elevenin-Vc1	<i>Conus victoriae</i>	Mollusc	A0A0F7YZQ7
Endothelin	Bibrotoxin	<i>Atractaspis bibroni</i>	Lepidosauromorpha (Lamprophiidae)	P80163
Endothelin	Sarafotoxin-A, Serisoform, S6A	<i>Atractaspis engaddensis</i>	Lepidosauromorpha (Lamprophiidae)	P13209
Endothelin	Sarafotoxin-A, Thrisoform	<i>Atractaspis engaddensis</i>	Lepidosauromorpha (Lamprophiidae)	P13208
Endothelin	Sarafotoxin-B, S6B	<i>Atractaspis engaddensis</i>	Lepidosauromorpha (Lamprophiidae)	P13208
Endothelin	Sarafotoxin-C, S6C	<i>Atractaspis engaddensis</i>	Lepidosauromorpha (Lamprophiidae)	P13208
Endothelin	Sarafotoxin-E, S6E	<i>Atractaspis engaddensis</i>	Lepidosauromorpha (Lamprophiidae)	P13208
Endothelin	Sarafotoxin-i1	<i>Atractaspis irregularis</i>	Lepidosauromorpha (Lamprophiidae)	P0DJK0
Endothelin	Sarafotoxin-i2	<i>Atractaspis irregularis</i>	Lepidosauromorpha (Lamprophiidae)	P0DJK1
Endothelin	Sarafotoxin-m (SRTX-m)	<i>Atractaspis microlepidota microlepidota</i>	Lepidosauromorpha (Lamprophiidae)	Q6RY99
Endothelin	Sarafotoxin-m1 (SRTX-m1)	<i>Atractaspis microlepidota microlepidota</i>	Lepidosauromorpha (Lamprophiidae)	Q6RY100
Endothelin	Sarafotoxin-m2 (SRTX-m2)	<i>Atractaspis microlepidota microlepidota</i>	Lepidosauromorpha (Lamprophiidae)	Q6RY101
Endothelin	Sarafotoxin-m3 (SRTX-m3)	<i>Atractaspis microlepidota microlepidota</i>	Lepidosauromorpha (Lamprophiidae)	Q6RY102
Endothelin	Sarafotoxin-m4 (SRTX-m4)	<i>Atractaspis microlepidota microlepidota</i>	Lepidosauromorpha (Lamprophiidae)	Q6RY103
Enkephalin	BmK-YA	<i>Buthus martensii</i>	Arachnid (scorpion)	22792309
Enkephalin	BmK-YA	<i>Mesobuthus martensii</i>	Arachnid (scorpion)	Q9Y0X6
Enkephalin	His4-BmK-YA	<i>Buthus martensii</i>	Arachnid (scorpion)	22792309
Enkephalin	Met-enkephalin	<i>Meiactanthus atrodorsalis</i>	Actinopterygii	P0DP56
Enkephalin	Met-enkephalin-His-Asp	<i>Meiactanthus atrodorsalis</i>	Actinopterygii	P0DP56
Enkephalin	Met-enkephalin-Se	<i>Meiactanthus atrodorsalis</i>	Actinopterygii	P0DP56

Neuropeptide family	Neuropeptide	Species	Taxonomic group	UniProt/ Genbank/ PMID ID
Enkephalin	Synenkephalin	<i>Meiacanthus atrodorsalis</i>	Actinopterygii	P0DP56
FARP ^b	Conorfamide Tx1.3	<i>Conus textile</i>	Mollusc	P0DL71
FARP	Conorfamide-As1	<i>Conus cancellatus</i>	Mollusc	P0DQH7
FARP	Conorfamide-As2	<i>Conus cancellatus</i>	Mollusc	P0DQH8
FARP	Conorfamide-Gm	<i>Conus gloriatus</i>	Mollusc	28531118
FARP	Conorfamide-Sr1	<i>Conus spurius</i>	Mollusc	P58805
FARP	Conorfamide-Sr2	<i>Conus spurius</i>	Mollusc	P85871
FARP	Conorfamide-Sr3	<i>Conus spurius</i>	Mollusc	P0DM28
FARP	Conorfamide-Tx1	<i>Conus textile</i>	Mollusc	P0DM26
FARP	Conorfamide-Tx2	<i>Conus textile</i>	Mollusc	P0DM27
FARP	Conorfamide-Vc1	<i>Conus victoriae</i>	Mollusc	P0DOZ7
GLP-1 ^c	Exendin-4	<i>Heloderma suspectum</i>	Lepidosauromorpha (lizard; Helodermatidae)	8396143
GLP-1	GLP-1 like	<i>Ornithorhynchus anatinus</i>	Monotreme	A0A1L3MY50
Insulin	Cono-insulin P11	<i>Conus planorbis</i>	Mollusc	KX034591
Insulin	Cono-insulin Tx1	<i>Conus textile</i>	Mollusc	A0A0B5A7N8
Insulin	Cono-insulin Tx2	<i>Conus textile</i>	Mollusc	KX034587
Insulin	Cono-insulin Bn1	<i>Conus bandanus</i>	Mollusc	KX034581.1
Insulin	Cono-insulin Eb1	<i>Conus eburneus</i>	Mollusc	KX034589
Insulin	Cono-insulin Eb2	<i>Conus eburneus</i>	Mollusc	KX034590
Insulin	Cono-insulin F1	<i>Conus floridulus</i>	Mollusc	A0A0B5AC98
Insulin	Cono-insulin F2	<i>Conus floridulus</i>	Mollusc	A0A0B5ADT9
Insulin	Cono-insulin F2b	<i>Conus floridulus</i>	Mollusc	A0A0B5A7N1
Insulin	Cono-insulin F2c	<i>Conus floridulus</i>	Mollusc	A0A0B5A7N5
Insulin	Cono-insulin G1	<i>Conus geographus</i>	Mollusc	A0A0B5AC95
Insulin	Cono-insulin G121	<i>Conus geographus</i>	Mollusc	BAO65654.1
Insulin	Cono-insulin G1b	<i>Conus geographus</i>	Mollusc	A0A0B5A8Q2
Insulin	Cono-insulin G1c	<i>Conus geographus</i>	Mollusc	A0A0B5A7P2
Insulin	Cono-insulin G2	<i>Conus geographus</i>	Mollusc	A0A0B5ABD9
Insulin	Cono-insulin G2b	<i>Conus geographus</i>	Mollusc	A0A0B5ADT3
Insulin	Cono-insulin G3	<i>Conus geographus</i>	Mollusc	A0A0B5A8P4
Insulin	Cono-insulin G3b	<i>Conus geographus</i>	Mollusc	A0A0B5AC86
Insulin	Cono-insulin Im1	<i>Conus imperialis</i>	Mollusc	A0A0B5A7M7
Insulin	Cono-insulin Im2	<i>Conus imperialis</i>	Mollusc	A0A0B5A8P8
Insulin	Cono-insulin K1	<i>Conus kinoshitai</i>	Mollusc	AZS18883.1
Insulin	Cono-insulin K2	<i>Conus kinoshitai</i>	Mollusc	AZS18884.1
Insulin	Cono-insulin M1	<i>Conus marmoreus</i>	Mollusc	A0A0B5A8Q6
Insulin	Cono-insulin M2	<i>Conus marmoreus</i>	Mollusc	KX034588
Insulin	Cono-insulin Me1	<i>Conus memiae</i>	Mollusc	A0A0B5ADV0
Insulin	Cono-insulin Pu1	<i>Conus pulicarius</i>	Mollusc	KX034592
Insulin	Cono-insulin Q1	<i>Conus quercinus</i>	Mollusc	A0A0B5ABE6
Insulin	Cono-insulin Q1b	<i>Conus quercinus</i>	Mollusc	A0A0B5ABE4

Neuropeptide family	Neuropeptide	Species	Taxonomic group	UniProt/ Genbank/ PMID ID
Insulin	Cono-insulin T1	<i>Conus tulipa</i>	Mollusc	A0A0B5ADU4
Insulin	Cono-insulin T2	<i>Conus tulipa</i>	Mollusc	A0A0B5AC90
Insulin	Cono-insulin T2	<i>Conus tulipa</i>	Mollusc	MH879035.1
Insulin	Cono-insulin T3	<i>Conus tulipa</i>	Mollusc	A0A0B5ABD5
Insulin	Cono-insulin Tr1	<i>Conus tribblei</i>	Mollusc	KX034595
Insulin	Cono-insulin Ts1	<i>Conus tessulatus</i>	Mollusc	KX034593
Insulin	Cono-insulin Ts2	<i>Conus tessulatus</i>	Mollusc	KX034594
Insulin	Cono-insulin Va1	<i>Conus varius</i>	Mollusc	KX034596
Insulin	Cono-insulin Va2	<i>Conus varius</i>	Mollusc	KX034597
Insulin	Cono-insulin Vc1	<i>Conus victoriae</i>	Mollusc	A0A0F7YYV0
Insulin	Cono-insulin Vi1	<i>Conus virgo</i>	Mollusc	AOF40168.1
Insulin	Insulin	<i>Conus lividus</i>	Mollusc	ATG85034.1
Insulin	Insulin partial	<i>Conus araneosus</i>	Mollusc	AQM52451.1
Insulin	Insulin-like protein	<i>Conus amadis</i>	Mollusc	AKZ17800.1
Insulin	Insulin-like protein partial	<i>Conus buxus lorisii</i>	Mollusc	AMB57283.1
Insulin	Insulin-like protein partial	<i>Conus frigidus</i>	Mollusc	ARU12135.1
Insulin	Insulin-like protein partial	<i>Conus litteratus</i>	Mollusc	ARS01446.1
Insulin	Insulin-related protein	<i>Conus ermineus</i>	Mollusc	AXL95338.1
Insulin	Insulin-related protein	<i>Conus ermineus</i>	Mollusc	AXL95359.1
Insulin	Insulin-related protein	<i>Conus ermineus</i>	Mollusc	AXL95374.1
Insulin	Insulin-related protein	<i>Conus ermineus</i>	Mollusc	AXL95395.1
Insulin	Insulin-related protein	<i>Conus ermineus</i>	Mollusc	AXL95461.1
Insulin	Insulin-related protein	<i>Conus ermineus</i>	Mollusc	AXL95708.1
Insulin	Tu304	<i>Conus tulipa</i>	Mollusc	30669642
Insulin	Tu478	<i>Conus tulipa</i>	Mollusc	30669642
Insulin	Tu479	<i>Conus tulipa</i>	Mollusc	30669642
ITP/CHH ^d	α -latrotoxin associated LMWP ^e	<i>Latrodectus geometricus</i>	Arachnid (spider)	V9QF69
ITP/CHH	α -latrotoxin associated LMWP	<i>Latrodectus hesperus</i>	Arachnid (spider)	V9QFH5
ITP/CHH	α -latrotoxin associated LMWP	<i>Latrodectus tredecimguttatus</i>	Arachnid (spider)	P49125
ITP/CHH	α -latrotoxin associated LMWP 2	<i>Latrodectus geometricus</i>	Arachnid (spider)	V9QFG7
ITP/CHH	α -latrotoxin associated LMWP 2	<i>Latrodectus hesperus</i>	Arachnid (spider)	V9QEI7
ITP/CHH	α -latrotoxin associated LMWP 2	<i>Latrodectus tredecimguttatus</i>	Arachnid (spider)	Q4U4N3
ITP/CHH	α -latrotoxin associated LMWP 2	<i>Steatoda grossa</i>	Arachnid (spider)	V9QFH8
ITP/CHH	α -latrotoxin associated LMWP SGV150-311	<i>Steatoda grossa</i>	Arachnid (spider)	V9QFG9
ITP/CHH	α -latrotoxin associated LMWP SGV242-280	<i>Steatoda grossa</i>	Arachnid (spider)	V9QER4
ITP/CHH	BLTX280	<i>Nephila pilipes</i>	Arachnid (spider)	A0A076KUK2
ITP/CHH	k-scoloptoxin(03)-Ssd1a	<i>Scolopendra subspinipes dehaani</i>	Myriapod	A0A0R4I951

Neuropeptide family	Neuropeptide	Species	Taxonomic group	UniProt/ Genbank/ PMID ID
ITP/CHH	k-scoloptoxin(03)-Ssm1a	<i>Scolopendra mutilans</i>	Myriapod	I6RU32
ITP/CHH	k-scoloptoxin(03)-Ssm1d	<i>Scolopendra mutilans</i>	Myriapod	I6RU46
ITP/CHH	k-scoloptoxin(03)-Ssm1e	<i>Scolopendra mutilans</i>	Myriapod	I6RA73
ITP/CHH	μ-scoloptoxin(03)-Ssm2a	<i>Scolopendra mutilans</i>	Myriapod	P0DL36
ITP/CHH	U-scoloptoxin(03)-Sa1b	<i>Phoneutria nigriventer</i>	Myriapod	P0DPW8
ITP/CHH	U-scoloptoxin(03)-Ssd1b	<i>Polistes lanio</i>	Insect	P0DPV3
ITP/CHH	U1-agatoxin-Ta1a	<i>Scolopendra mutilans</i>	Myriapod	O46166
ITP/CHH	U1-agatoxin-Ta1b	<i>Scolopendra subspinipes dehaani</i>	Myriapod	O46167
ITP/CHH	U1-agatoxin-Ta1c	<i>Scolopendra alternans</i>	Myriapod	O46168
ITP/CHH	Venom protein 10	<i>Microctonus hyperodae</i>	Insect	A9YME6
Myoactive tetradeca-peptide	Conomap-Vt	<i>Conus vitulinus</i>	Mollusc	P0C260
Natriuretic peptide	CNP ^f	<i>Philodryas olfersii</i>	Lepidosauromorpha (colubrid)	Q09GK2
Natriuretic peptide	CNP	<i>Bothrops insularis</i>	Lepidosauromorpha (viper)	P68515
Natriuretic peptide	CNP	<i>Cerastes cerastes</i>	Lepidosauromorpha (viper)	A8YPR9
Natriuretic peptide	CNP	<i>Crotalus atrox</i>	Lepidosauromorpha (viper)	P0CV87
Natriuretic peptide	CNP	<i>Crotalus durissus collilineatus</i>	Lepidosauromorpha (viper)	Q2PE51
Natriuretic peptide	CNP	<i>Crotalus durissus terrificus</i>	Lepidosauromorpha (viper)	Q90Y12
Natriuretic peptide	CNP	<i>Echis ocellatus</i>	Lepidosauromorpha (viper)	A8YPR6
Natriuretic peptide	CNP	<i>Gloydius blomhoffii</i>	Lepidosauromorpha (viper)	P01021
Natriuretic peptide	CNP	<i>Lachesis muta muta</i>	Lepidosauromorpha (viper)	Q27J49
Natriuretic peptide	CNP	<i>Sistrurus catenatus edwardsii</i>	Lepidosauromorpha (viper)	B0VXV8
Natriuretic peptide	CNP	<i>Trimeresurus gramineus</i>	Lepidosauromorpha (viper)	P0C7P6
Natriuretic peptide	CNP 2	<i>Takifugu rubripes</i>	Actinopterygii	Q805D5
Natriuretic peptide	CNP 39	<i>Ornithorhynchus anatinus</i>	Monotreme	P84715
Natriuretic peptide	Tf-CNP	<i>Probothrops flavoviridis</i>	Lepidosauromorpha (viper)	P0C7P5
Natriuretic peptide	Natriuretic peptide	<i>Rhabdophis tigrinus tigrinus</i>	Lepidosauromorpha (colubrid)	D1MZV3
Natriuretic peptide	Natriuretic peptide	<i>Heloderma horridum</i>	Lepidosauromorpha (lizard; Helodermatidae)	E8ZCG5
Natriuretic peptide	Natriuretic peptide	<i>Heloderma suspectum cinctum</i>	Lepidosauromorpha (lizard; Helodermatidae)	C6EVG7
Natriuretic peptide	Natriuretic peptide	<i>Micrurus altirostris</i>	Lepidosauromorpha (elapid)	F5CPE8
Natriuretic peptide	AsNP-a (Fragment)	<i>Austrelaps superbus</i>	Lepidosauromorpha (elapid)	A8S6B3

Neuropeptide family	Neuropeptide	Species	Taxonomic group	UniProt/ Genbank/ PMID ID
Natriuretic peptide	BF131	<i>Bungarus flaviceps flaviceps</i>	Lepidosauromorpha (elapid)	D5J9S0
Natriuretic peptide	BM026	<i>Bungarus multicinctus</i>	Lepidosauromorpha (elapid)	P0DMD5
Natriuretic peptide	CnNP-a (Fragment)	<i>Cryptophis nigrescens</i>	Lepidosauromorpha (elapid)	Q1ZYW1
Natriuretic peptide	CnNP-b (Fragment)	<i>Cryptophis nigrescens</i>	Lepidosauromorpha (elapid)	Q1ZYW0
Natriuretic peptide	Coa_NP1	<i>Crotalus oreganus abyssus</i>	Lepidosauromorpha (viper)	B3EWY3
Natriuretic peptide	Coa_NP2	<i>Crotalus oreganus abyssus</i>	Lepidosauromorpha (viper)	B3EWY2
Natriuretic peptide	DNP	<i>Dendroaspis angusticeps</i>	Lepidosauromorpha (elapid)	P28374
Natriuretic peptide	DNP-2	<i>Dendroaspis angusticeps</i>	Lepidosauromorpha (elapid)	Q8QGP7
Natriuretic peptide	GNP1	<i>Varanus varius</i>	Lepidosauromorpha (lizard; Anguinae)	Q2XXL8
Natriuretic peptide	HsNP-a (Fragment)	<i>Hoplocephalus stephensii</i>	Lepidosauromorpha (elapid)	Q3SAE6
Natriuretic peptide	HsNP-b (Fragment)	<i>Hoplocephalus stephensii</i>	Lepidosauromorpha (elapid)	Q3SAE5
Natriuretic peptide	Mc-NP	<i>Micrurus corallinus</i>	Lepidosauromorpha (elapid)	P79799
Natriuretic peptide	Mf-NP	<i>Micrurus fulvius</i>	Lepidosauromorpha (elapid)	B8K1V9
Natriuretic peptide	Na-NP	<i>Naja atra</i>	Lepidosauromorpha (elapid)	D9IX97
Natriuretic peptide	NP2	<i>Crotalus durissus cascavella</i>	Lepidosauromorpha (viper)	P0DKY6
Natriuretic peptide	NsNP-a (Fragment)	<i>Notechis scutatus scutatus</i>	Lepidosauromorpha (elapid)	Q3SAE8
Natriuretic peptide	NsNP-b (Fragment)	<i>Notechis scutatus scutatus</i>	Lepidosauromorpha (elapid)	Q3SAE7
Natriuretic peptide	Oh-NP	<i>Ophiophagus hannah</i>	Lepidosauromorpha (elapid)	D9IX98
Natriuretic peptide	OmNP-d (Fragment)	<i>Oxyuranus microlepidotus</i>	Lepidosauromorpha (elapid)	Q3SAF8
Natriuretic peptide	OmNP-e (Fragment)	<i>Oxyuranus microlepidotus</i>	Lepidosauromorpha (elapid)	Q3SAF7
Natriuretic peptide	OsNP-d (Fragment)	<i>Oxyuranus scutellatus scutellatus</i>	Lepidosauromorpha (elapid)	Q3SAX8
Natriuretic peptide	PaNP-a (Fragment)	<i>Pseudechis australis</i>	Lepidosauromorpha (elapid)	Q3SAF5
Natriuretic peptide	PaNP-b (Fragment)	<i>Pseudechis australis</i>	Lepidosauromorpha (elapid)	Q3SAF4
Natriuretic peptide	PaNP-c (Fragment)	<i>Pseudechis australis</i>	Lepidosauromorpha (elapid)	Q3SAF3
Natriuretic peptide	PaNP-d (Fragment)	<i>Pseudechis australis</i>	Lepidosauromorpha (elapid)	Q3SAF2
Natriuretic peptide	PNP	<i>Pseudocerastes persicus</i>	Lepidosauromorpha (viper)	P82972

Neuropeptide family	Neuropeptide	Species	Taxonomic group	UniProt/ Genbank/ PMID ID
Natriuretic peptide	PpNP-a (Fragment)	<i>Pseudechis porphyriacus</i>	Lepidosauromorpha (elapid)	Q3SAF1
Natriuretic peptide	PpNP-b (Fragment)	<i>Pseudechis porphyriacus</i>	Lepidosauromorpha (elapid)	Q3SAF0
Natriuretic peptide	PtNP-a (Fragment)	<i>Pseudonaja textilis</i>	Lepidosauromorpha (elapid)	Q3SAF6
Natriuretic peptide	TcNP-a (Fragment)	<i>Tropidechis carinatus</i>	Lepidosauromorpha (elapid)	Q3SAE9
Natriuretic peptide	TNP-a	<i>Oxyuranus microlepidotus</i>	Lepidosauromorpha (elapid)	P83224
Natriuretic peptide	TNP-a	<i>Oxyuranus scutellatus canni</i>	Lepidosauromorpha (elapid)	P83226
Natriuretic peptide	TNP-a	<i>Oxyuranus scutellatus scutellatus</i>	Lepidosauromorpha (elapid)	P83225
Natriuretic peptide	TNP-b	<i>Oxyuranus microlepidotus</i>	Lepidosauromorpha (elapid)	P83227
Natriuretic peptide	TNP-b	<i>Oxyuranus scutellatus canni</i>	Lepidosauromorpha (elapid)	P83229
Natriuretic peptide	TNP-b	<i>Oxyuranus scutellatus scutellatus</i>	Lepidosauromorpha (elapid)	P83228
Natriuretic peptide	TNP-c	<i>Oxyuranus scutellatus canni</i>	Lepidosauromorpha (elapid)	P83231
Natriuretic peptide	TsNP	<i>Tityus serrulatus</i>	Arachnid	P0DMD6
Natriuretic peptide	natriuretic/ helokinstatin-Cwar1	<i>Celestus warreni</i>	Lepidosauromorpha (lizard; Anguidae)	E2E4J4
Natriuretic peptide	natriuretic/ helokinstatin-Ginf1	<i>Gerrhonotus infernalis</i>	Lepidosauromorpha (lizard; Anguidae)	E2E4J3
Natriuretic peptide	Peptide TNP-c	<i>Oxyuranus microlepidotus</i>	Lepidosauromorpha (elapid)	P83230
Neuropeptide Y	Cono-NPY	<i>Conus praecellens</i>	Mollusc	28922871
Neuropeptide Y	Cono-NPY-Gm	<i>Conus gloriamus</i>	Mollusc	28531118
Neuropeptide Y	Neuropeptide Y	<i>Meiacanthus atrodorsalis</i>	Actinopterygii	P0DP55
Neuropeptide Y	Neuropeptide Y1	<i>Conus betulinus</i>	Mollusc	P0CJ22
Neuropeptide Y	Neuropeptide Y2	<i>Conus betulinus</i>	Mollusc	P0CJ23
Neurotensin	Contulakin G	<i>Conus geographus</i>	Mollusc	Q9XYR5
Neuro-hypophyseal	Conopressin Cn	<i>Conus consors</i>	Mollusc	22079299
Neuro-hypophyseal	Conopressin G	<i>Conus andremenezi</i>	Mollusc	ATF27388.1 ; ATF27387.1
Neuro-hypophyseal	Conopressin G	<i>Conus araneosus</i>	Mollusc	ATJ04126.1
Neuro-hypophyseal	Conopressin G	<i>Conus buxeus loroisii</i>	Mollusc	ATJ04131.1
Neuro-hypophyseal	Conopressin G	<i>Conus ebraeus</i>	Mollusc	ATJ04127.1
Neuro-hypophyseal	Conopressin G	<i>Conus ermineus</i>	Mollusc	AXL95508.1
Neuro-hypophyseal	Conopressin G	<i>Conus geographus</i>	Mollusc	P05486
Neuro-hypophyseal	Conopressin G	<i>Conus gloriamus</i>	Mollusc	28531118
Neuro-hypophyseal	Conopressin G	<i>Conus imperialis</i>	Mollusc	7940591
Neuro-hypophyseal	Conopressin G	<i>Conus lividus</i>	Mollusc	ATJ04129.1
Neuro-hypophyseal	Conopressin G	<i>Conus monile</i>	Mollusc	QDE14046.1
Neuro-hypophyseal	Conopressin G	<i>Conus praecellens</i>	Mollusc	ATF27585.1

Neuropeptide family	Neuropeptide	Species	Taxonomic group	UniProt/ Genbank/ PMID ID
Neuro-hypophyseal	Conopressin S	<i>Conus striatus</i>	Mollusc	P05487
Neuro-hypophyseal	Conopressin T	<i>Conus tulipa</i>	Mollusc	P0DL76
Neuro-hypophyseal	Conopressin Tx	<i>Conus textile</i>	Mollusc	P86255.1
Neuro-hypophyseal	Conopressin Tx1	<i>Conus textile</i>	Mollusc	P02716
Neuro-hypophyseal	Conopressin Tx2	<i>Conus ermineus</i>	Mollusc	A0A346CJ19
Neuro-hypophyseal	Conopressin Tx2	<i>Conus lenavati</i>	Mollusc	A0A0K8TTS0
Neuro-hypophyseal	Conopressin Tx2	<i>Conus lividus</i>	Mollusc	A0A291NVW3
Neuro-hypophyseal	Conopressin Tx2	<i>Conus miles</i>	Mollusc	A0A291NVU9
Neuro-hypophyseal	Conopressin Tx2	<i>Conus monile</i>	Mollusc	A0A4Y5X186
Neuro-hypophyseal	Conopressin Tx2	<i>Conus textile</i>	Mollusc	Unpublished
Neuro-hypophyseal	Conopressin Tx2	<i>Conus tribblei</i>	Mollusc	A0A0C9RYL1
Neuro-hypophyseal	Conopressin Vil	<i>Conus villepini</i>	Mollusc	P85141
Prohormone-4	PH4	<i>Conus andremenezi</i>	Mollusc	28922871
Prohormone-4	PH4	<i>Conus praecellens</i>	Mollusc	28922871
Prohormone-4	PH4-Gm1	<i>Conus gloriamus</i>	Mollusc	28531118
Prohormone-4	PH4-Gm2	<i>Conus gloriamus</i>	Mollusc	28531118
Prohormone-4	PH4-Vc1	<i>Conus victoriae</i>	Mollusc	A0A0F7YYX3
Prohormone-4	Prohormone-4 like	<i>Octopus vulgaris</i>	Mollusc	<u>XP 029651323.1</u>
Tachykinin	Eledoisin	<i>Eledone cirrhosa</i>	Mollusc	P62933
Tachykinin	Eledoisin	<i>Eledone moschata</i>	Mollusc	P62934
Tachykinin	Oct-TK-I	<i>Octopus vulgaris</i>	Mollusc	Q8I6S3
Tachykinin	Oct-TK-II	<i>Octopus vulgaris</i>	Mollusc	Q8I6S2
Tachykinin	PhM1	<i>Phoneutria nigriventer</i>	Arachnid (spider)	15573368
Tachykinin	Sialokinin I	<i>Aedes aegypti</i>	Insect	P42634
Tachykinin	Sialokinin II Tachykinin-like	<i>Aedes aegypti</i>	Insect	P42634
Tachykinin	peptide-I (PIITkP-I) Tachykinin-like	<i>Polistes lanio</i>	Insect	P85879
Tachykinin	peptide-II (PIITkP-II)	<i>Polistes lanio</i>	Insect	P85880
Tachykinin	Tachykinin-like peptide-IX (PnTkP-IX)	<i>Phoneutria nigriventer</i>	Arachnid (spider)	P86306
Tachykinin	Tachykinin-like peptide-VI (PnTkP-VI)	<i>Phoneutria nigriventer</i>	Arachnid (spider)	P86303
Tachykinin	Tachykinin-like peptide-X (PnTkP-X)	<i>Phoneutria nigriventer</i>	Arachnid (spider)	P86307
Tachykinin	Tachykinin-like peptide-XI (PnTkP-XI)	<i>Phoneutria nigriventer</i>	Arachnid (spider)	P86308
Tachykinin	Tachykinin-like peptide-XIII	<i>Phoneutria nigriventer</i>	Arachnid (spider)	P86310

^aCCAP, Crustacean cardioactive peptide;

^bFARP, FMRFamide related peptide family;

^cGLP-1, Glucagon-like peptide 1;

^dITP/CHH, Ion transport peptide/crustacean hyperglycaemic hormone;

^eLMWP, low molecular weight protein;

f CNP, C-type natriuretic peptide; Genbank accession number are underlined, in bold.