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Peptide therapeutics from venom: Current status and potential

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ABSTRACT

Peptides are recognized as being highly selective, potent and relatively safe as potential therapeutics. Peptides isolated from the venom of different animals satisfy most of these criteria with the possible exception of safety, but when isolated as single compounds and used at appropriate concentrations, venom-derived peptides can become useful drugs. Although the number of venom-derived peptides that have successfully progressed to the clinic is currently limited, the prospects for venom-derived peptides look very optimistic. As proteomic and transcriptomic approaches continue to identify new sequences, the potential of venom-derived peptides to find applications as therapeutics, cosmetics and insecticides grows accordingly.

1. Introduction

Animals evolved venom for both protection and predation. The diversity of animals employing this strategy for survival encompasses nearly all of the different phyla in the animal kingdom, including annelids (bearded fireworm), cnidarians (sea anemones, jellyfish and hydra), echinoderms (sea urchins and starfish), mollusks (cone snails and octopuses), arthropods (spiders, ants, centipedes, bees, wasps, scorpions, mosquitos, and ticks) and vertebrates (fish, frogs, snakes, lizards, birds and mammals). Venom is delivered to the predator or the prey via a stinger, fang, stinging cell, barb, pincer, proboscis or spine. Venom composition varies from animal to animal, but most venoms are a heterogeneous mixture of inorganic salts, low molecular weight organic molecules, peptides (2–10 kDa) and enzymes (>10 kDa).[1] This mixture provides the animal with a multipronged approach to immobilizing and/or killing the prey or predator.

Since ancient times, mankind has recognized that venomous creatures are extremely dangerous and potentially fatal to victims who are stung or bitten. As humans are quite creative in producing improved weapons, it did not take long for ancient peoples to develop a deadly tactic and dip their projectiles in venoms from different animals. As recorded in 326 B.C.E., Alexander the Great's army encountered arrows dipped in Russel's viper (*Daboia russelii*) venom in India, as evidenced by the symptoms that were recorded of his dying soldiers.[2] One of the first recorded medical uses of venom was described by Appian, the Roman historian of Greek origin, in 37 B.C.E., when Mithradates suffered a grievous sword wound to his thigh; as he was near death, his Scythian doctor administered a small amount of steppe viper venom (*Vipera ursinii*) to stop the profuse bleeding and saved his life.[3]

Venoms from snakes, toads, spiders and scorpions have been used for millennia in many traditional remedies and medicines for treating a variety of ailments such as arthritis, cancers, and gastrointestinal issues, to name just a few. Most of these traditional medicines used small doses of whole venoms to accomplish their therapeutic goals. It was not until the late 20th century that modern medicine adopted a more systematic and rigorous approach to utilising venoms as therapeutic agents. [4]

2. Harnessing the potential benefits of venom

Most drug discovery efforts begin with some form of screening of chemical compound libraries, many of which also contain a variety of natural products. The rationale is that nature has developed a veritable cornucopia of molecular scaffolds that offer nearly infinite possibilities for finding a potential lead. Coupling this with our knowledge of animals and plants that have been found to produce toxins, poisons or potential remedies can help focus our search for a potential lead molecule.

As noted above, most venoms are a complex mixture of components, including peptides, proteins and enzymes. As technology improved over the past century, it became easier and easier to analyze venoms and to separate and fully characterize the individual peptides and proteins. Classical chromatography methods were replaced with HPLC methods in the 1980's to obtain pure samples that were subsequently sequenced using Edman degradation or faster and more powerful mass-spectroscopic techniques[5] (Fig. 1). Many of the more interesting peptides are highly

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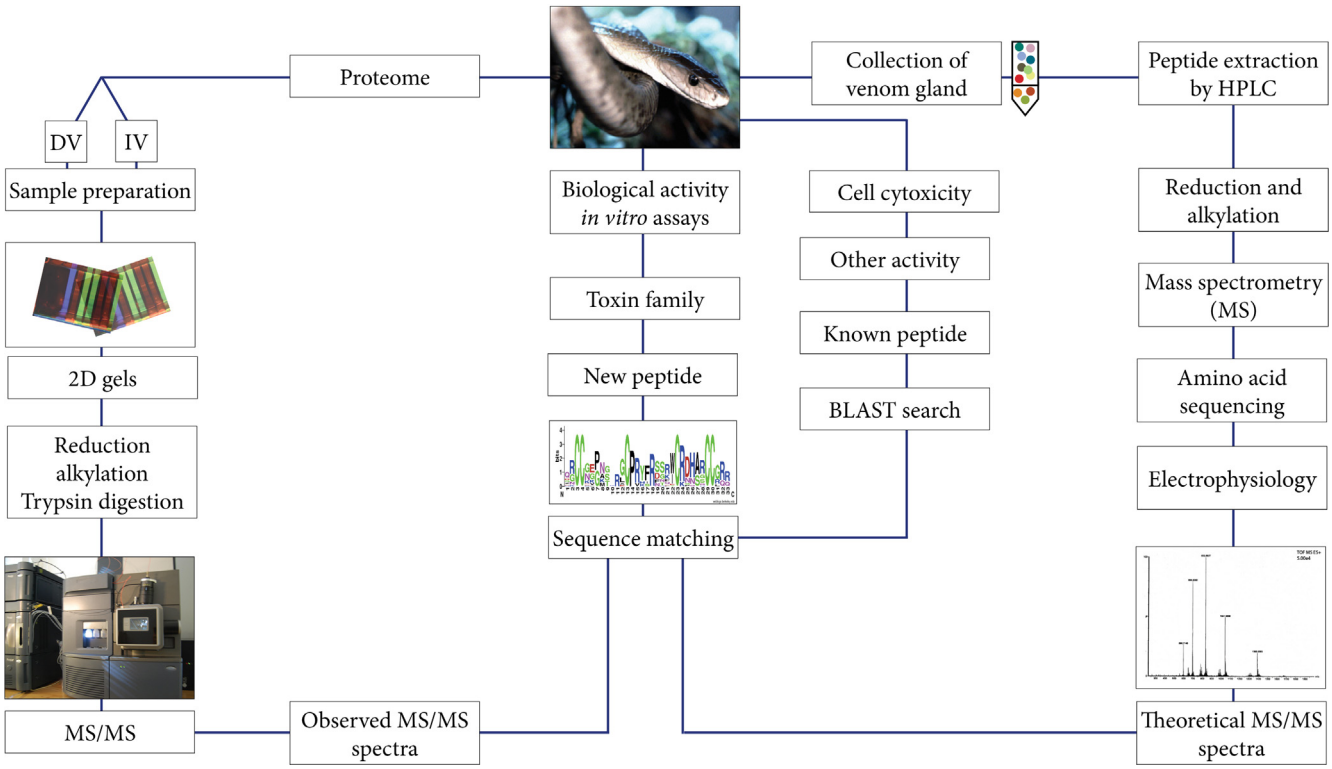

Fig. 1. A typical workflow for isolation and screening of peptides and other bioactive compounds from a venomous animal such as the black mamba (*Dendroaspis polylepis*).

structured owing to the presence of multiple disulfide bonds.[6,7] With improvements in NMR instrumentation, three-dimensional solution structures of these molecules have been obtained fairly quickly since the early 1990's, although the quality continues to improve with access to modern spectrometers and isotopic labelling.[8] All of these technological improvements made it feasible to gain insight into how each venom component acted on its potential target (Fig. 1).

Venomics, which involves the global study of venom and venom glands, targeting comprehensive characterization of the whole toxin profile of a venomous animal by means of genomics, transcriptomics, proteomics and bioinformatics studies, has significantly advanced the number of peptides available for screening and lead development.[9–11] This integrative approach has been made possible by the rapid evolution of DNA, RNA and protein sequencing techniques, as well as databases and computing algorithms. Even micro-components are detected using this approach, which would otherwise be missed during conventional peptide isolation methods (Fig. 1).

Functional activity assays have also advanced during the same period. Early assays often involved intracerebroventricular (ICV) injection of isolated fractions into mice followed by observation of their activity, as shown in Fig. 2,[5] and this remains an informative approach.[12] More recently, heterologous expression in oocytes or mammalian cells microinjected with cDNA coding for specific ion channels has become a standard tool to determine the specificity of ion channel blocking molecules.[13] Development of fluorometric or colorimetric substrate-based assays became useful for measuring inhibitors of blood-clotting enzymes.[14] Other assay innovations, such as the fluorescence imaging plate reader (FLIPR)[15] and surface plasmon resonance,[16] have significantly accelerated lead molecule identification and development.

This review covers peptide drugs and related products that have originated from animal venoms. Perhaps the most commercially successful toxins to be developed to date are the botulinum toxins A and B, although these are in fact proteins from prokaryotic sources that are beyond the scope of this review, which is focussed on peptides (of fewer than 70 amino acid residues). The initial examples are well-established drugs that have been used widely in the clinic. This is followed by a summary of several clinical candidates that ultimately failed or were abandoned during clinical development. We then describe peptides that are either in clinical development or very close to moving into the clinic. Finally, we exemplify other interesting applications of peptides in cosmetics and crop protection.

3. Approved venom-derived drugs

3.1. ACE inhibitors: captopril

In the 1970's, the blockbuster angiotensin converting enzyme (ACE) inhibitor captopril (Fig. 3) was developed based on bradykinin-potentiating peptides (BPF) isolated from the venom of *Bothrops jararaca*, a pit viper endemic to southeastern South America.[17] The discovery of bradykinin in 1949 by Rocha e Silva et al.[18] came from studying cases of patients bitten by *B. jararaca*. This discovery began our understanding of the kallikrein-kinin system and the role that ACE plays in the physiology of blood circulation, which was unraveled over nearly four decades.[19] These BPF peptides were later determined to be short Pro-rich peptides (the first to be sequenced being Pyr-Lys-Trp-Ala-Pro-OH),[20] which act by blocking the processing and generation of angiotensin-II by somatic ACE.[21] The Squibb Institute for Medical Research took on the challenge on the advice of their consultant, John Vane, to target ACE as a means of controlling blood pressure regulation. The BPF peptides helped elucidate one of the key structural requirements for inhibiting this Zn-metalloprotease. They were believed to act as competitive substrates/inhibitors with the pharmacophore sequence Phe-Ala-Pro.[22–24] However, these BPF peptides, such as

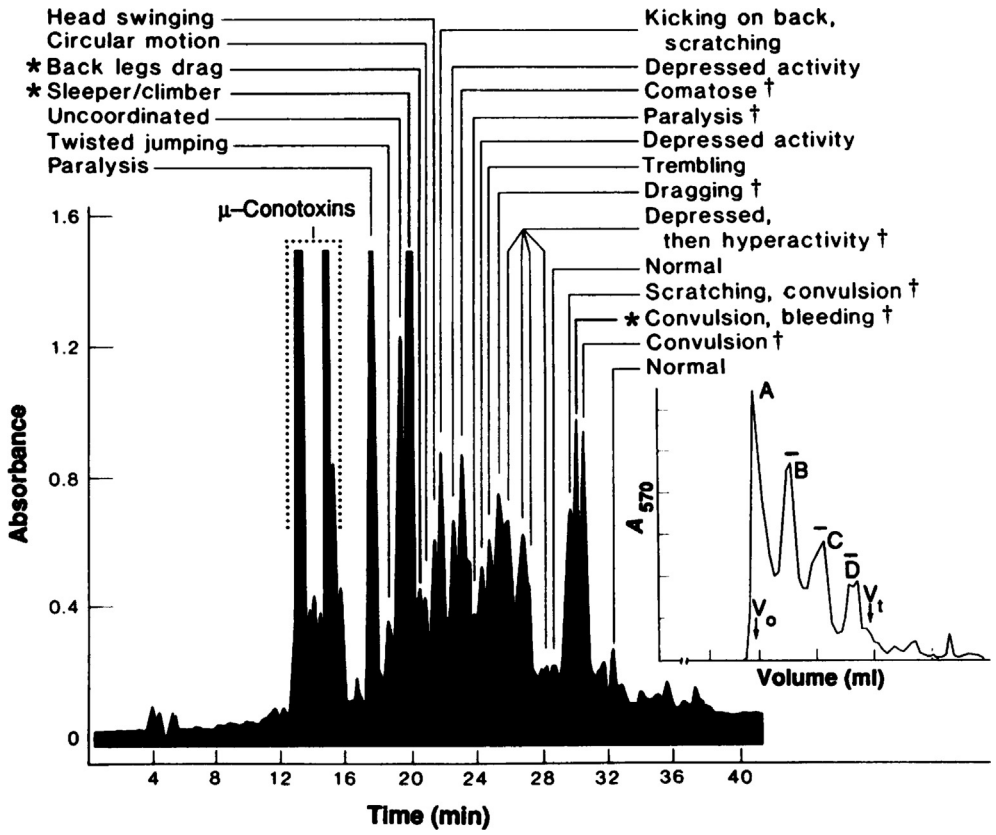

Fig. 2. RP-HPLC chromatogram of *Conus geographus* venom demonstrating the multitude of compounds and their activities following intracerebroventricular (ICV) injection into mice. Inset: initial fractionation of the venom by size-exclusion chromatography. From Olivera et al.[5] Reproduced with permission from AAAS.

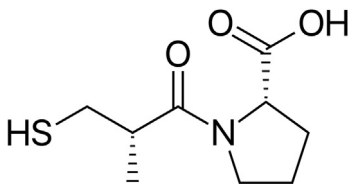

Fig. 3. Chemical structure of captopril.

SQ-20881 (a nonapeptide: Pyr-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro-OH), were not effective by oral administration and consequently were not developed directly.[25] Instead, structure-based screening of small organic molecules commenced that used the key binding requirements to inhibit related Zn-metalloproteases such as carboxypeptidase A: a proline residue coupled with a coordinating residue such as a carboxylate. After screening more than 1800 compounds, 1-((2s)-3-mercapto-2-methylpropionyl)-l-proline (captopril) (Fig. 3) was found to be more than 1000-fold more potent than D-2-methylsuccinyl-Pro-OH. This potent inhibition was facilitated by positioning the Pro residue in the substrate binding pocket of ACE coupled with a thiol moiety to coordinate with the active site Zn.[26] With the commercial success of captopril, the foundation was laid for the concept of venoms to drugs.

3.2. Antiplatelet drugs: eptifibatide and tirofiban

Unimpeded blood circulation is required for survival of all vertebrates. When an injury occurs to a blood vessel, hemostasis or the spontaneous stopping of blood loss occurs as a result of the interplay of vasoconstriction, platelet activation and aggregation, blood coagulation and fibrinolysis.[27] Following vascular injury, platelets immediately begin to adhere to the exposed sub-endothelial tissue by way of the exposed collagen, fibronectin and von Willebrand factor binding to the surface integrin receptors (α2β1, α5β1) and glycoprotein IIb-IX on the platelets. Additional activation of platelets by ADP, thrombin and thromboxane A2 released at the wound site stimulates aggregation between the platelet integrin αIIbβ3 (also known as glycoprotein GPIIB-IIIa) and fibrinogen, ultimately forming a platelet thrombus to stop bleeding.[28]

The disintegrins are a family of disulfide-rich mini-proteins isolated from viperid snake venoms. Disintegrins block platelet aggregation by binding to the αIIbβ3 receptor, which prevents fibrinogen binding.[29,30] The drugs Aggrestat® (tirofiban, Merck & Co.) and Integrilin® (eptifibatide, Cor Therapeutics, now part of Millennium Pharmaceuticals) (Fig. 4) were developed based upon echistatin from the saw-scaled viper, *Echis carinatus*, and barbourin from the southeastern pygmy rattlesnake, *Sistrusus miliarius barbouiri*. Both of these mini-proteins mimic the key Arg-Gly-Asp or Lys-Gly-Asp sequence, which is responsible for binding to the αIIbβ3 receptor. Tirofiban was designed based on the spacing of the side chains of the RGD pharmacophore in echistatin,[31–33] whereas eptifibatide is a small cyclic disulfide-constrained peptide containing the key Lys-Gly-Asp pharmacophore from barbourin.[34] It eventuates that the substitution of Lys for Arg in the key RGD pharmacophore of barbourin increases the specificity for αIIbβ3. Both of these drugs have been instrumental in reducing the risk of death and/or myocardial infarction in patients with unstable angina or non-ST segment elevation myocardial infarction.[35]

3.3. Thrombin inhibitors: lepirudin and bivalirudin

For centuries, blood-letting was used as a treatment for various medical ailments, based on an ancient practice of medicine in

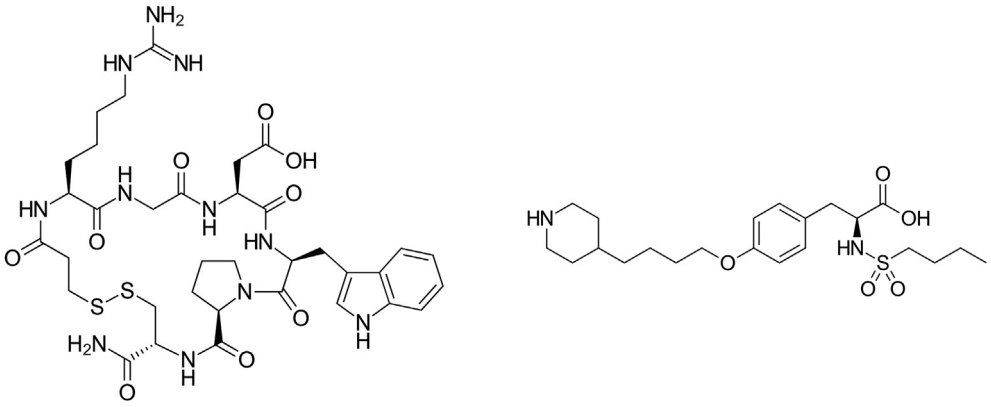

Fig. 4. Chemical structures of eptifibatide (left) and tirofiban (right).

which bodily fluids or humors needed to stay in balance. Thus, patients were treated by removing excess fluid via bleeding from a vein. One form of medical blood-letting utilizing leeches became popular in Europe (especially in France) in the nineteenth century.[36] Live leeches placed on a patient would adhere to the patient's body and begin sucking blood. The leeches would become engorged with the "excess" blood and would subsequently be removed from the patient, at which point his or her humors would be balanced.

The leech-mediated therapy led to an understanding of how this animal could continue to suck a patient's blood without it coagulating at the site of the wound.[37] With the use of improved biochemical techniques, the saliva from the medical leech was separated and a protein called hirudin was isolated in the 1950's.[38,39] Hirudin is an extremely potent inhibitor of the blood-clotting enzyme thrombin,[40] which is responsible for cleaving fibrinogen to fibrin in the final step in forming a blood clot. Hirudin binds only to the activated form of thrombin and not the zymogen prothrombin.

The primary structure of this 65-residue mini-protein with three disulfide bonds was finally determined in 1976 (Fig. 5).[41] Interestingly, the protein has a post-translational modification of a sulfated Tyr at position 63. The solution structure is characterized by an N-terminal compactly folded domain stabilized by three intramolecular disulfide bonds and an extended disordered C-terminal domain.[43] The N-terminal residues 1–3 bind at the active site region, forming a parallel β-sheet with residues 214–217 of thrombin, with the NH of Ile1 forming a hydrogen bond with the catalytic Ser195 oxygen atom. The extended conformation of the C-terminal domain makes numerous electrostatic interactions with an anionic binding exosite on thrombin.[42,44]

Hirudin itself has been developed as an injectable drug named Refludan® (lepirudin). This peptide, which is produced by recombinant methods, has two minor changes from native hirudin: Ile1 to Leu and no sulfation of Tyr63. Refludan® had been utilized in cases where heparins are contraindicated because of thrombocytopenia. Lepirudin was withdrawn from the market by Bayer in 2012,[45] reportedly as a result of a third-party production site discontinuing manufacture of the recombinant product.

Hirulog® (bivalirudin) (Fig. 6) is a 20-residue linear peptide that was designed based on hirudin. This peptide incorporates the key N-terminal residues (H-D-Phe-Pro-Arg-Pro) and C-terminal domain (Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Tyr-Leu) of hirudin connected by a tetra-Gly spacer. It also incorporates an N-terminal D-Phe to improve binding and inhibit degradation.[46] It inhibits both circulating and clot-bound thrombin, while also inhibiting thrombin-mediated platelet activation and aggregation.[47] Bivalirudin was approved in 2000 for use as an anticoagulant in patients with unstable angina undergoing percutaneous transluminal coronary angioplasty. Bivalirudin in conjunction with a glycoprotein GPIIb/IIIa inhibitor (eptifibatide or tirofiban) is also provisionally approved for use as an anticoagulant in patients undergoing percutaneous coronary intervention (PCI). Bivalirudin is indicated for patients with, or at risk of, heparin-induced thrombocytopenia undergoing PCI.

3.4. Chronic pain: ziconotide

There are more than 700 species of cone snails around the world.[48] Several human encounters with cone snails have resulted in fatalities, primarily in Australia and the South Pacific. In particular, cone snails from the genus *Conus*, such as *C. geographus, C. textile, C. tulipa, C. magus, C. marmoreus, C. aulicus, C. catus* and *C. pennaceus*, can be considered particularly dangerous.[49] Cone snails have evolved a sophisticated hunting strategy whereby they use a harpoon to lance their prey and deliver a potent venom to immobilize and/or kill the prey.[7,50] Human encounters with these snails usually occur when someone picks up a live cone snail and places it in their pocket, whereupon the cone snail defensively harpoons the person and injects its venom, sometimes with lethal consequences. The most dangerous cone snail to humans is *C. geographus*.[5]

Early work on the venom of *C. geographus* was conducted by Alan Kohn from Australia, who first observed that these snails hunted fish,[51] and followed up by Bob Endean and co-workers, who began to characterize the pharmacology of the venom.[52] Spence and co-workers fractionated *C. geographus* venom and characterized three neurotoxic components by conventional ion exchange chromatography followed by gel permeation in the 1970's.[53] Purified homogeneous peptides were finally obtained and sequenced in the early 1980's by Gray et al.[54] for the α conotoxins, which target nicotinic acetylcholine receptors (nAChR), and Sato et al.[55] and Cruz et al.[56] for μ-conotoxins, which primarily target voltage-gated sodium channels (Na_V) in muscle. In the 1980's, Olivera's group at University of Utah used HPLC to separate the venom into different fractions, then injected these fractions into mice and observed their behavior (Fig. 2).[5,57] The venom was made up of a complex mixture of disulfide-rich peptides (~8–30 residues), henceforth known as conotoxins. Bioassays of these conotoxins induced a variety of conditions such as severe tremors, shaking, paralysis, scratching and death.[5]

The ω-conotoxins, which all originate from fish-hunting cone snails, were characterized shortly after the α- and μ-conotoxins. These peptides were called "shaker" toxins owing to the persistent tremors they invoked upon intracerebral injection in mice. The ω-conotoxins are typically 24–30 residues long and contain three intramolecular disulfide bonds.[5,11] They target voltage-activated

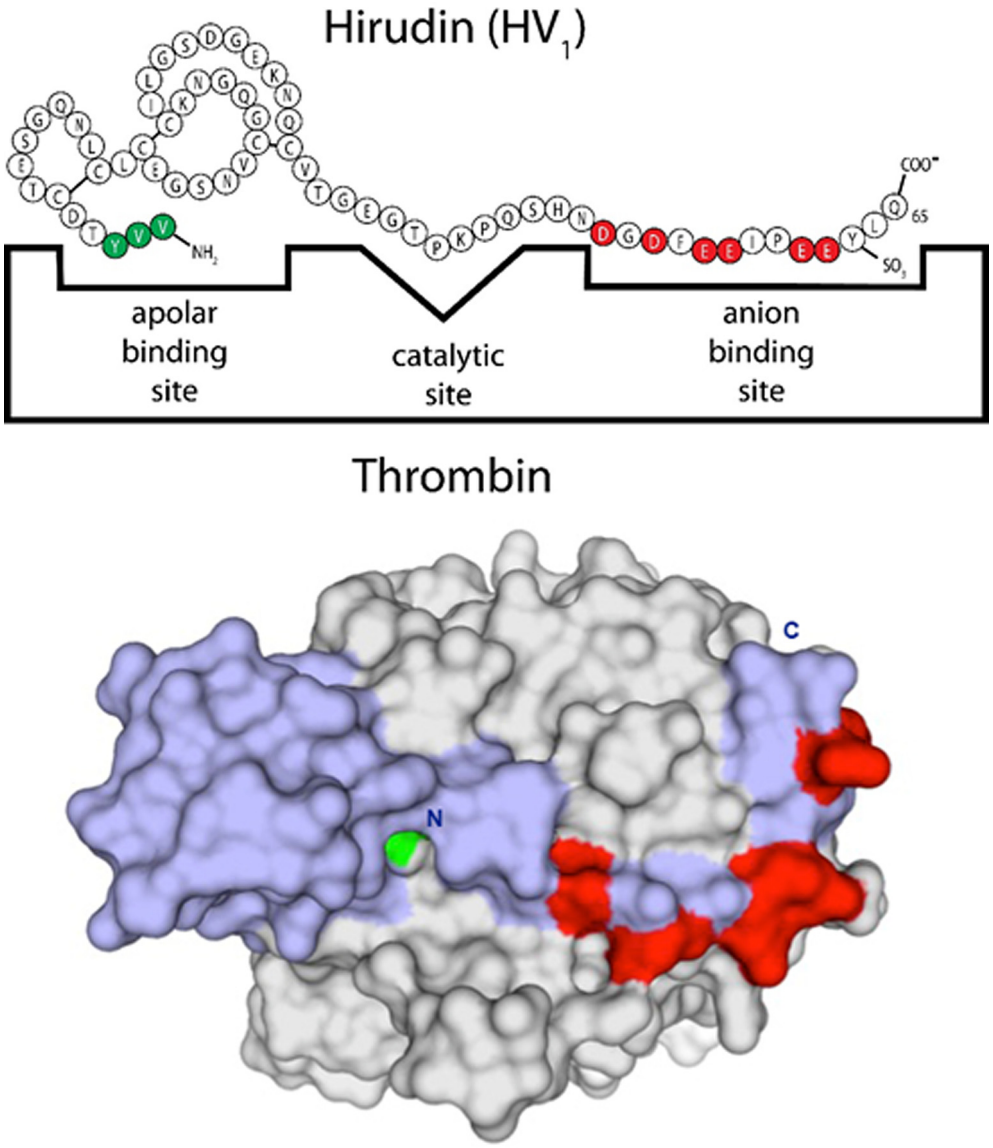

Fig. 5. Binding of hirudin to thrombin, with the key hydrophobic pocket binding residues in green and the anionic pocket binding residues in red.[41] Its 3D structure bound to thrombin (pdb id 4HTC)[42] is also shown as molecular surfaces. Hirudin is shown in lightblue, thrombin in light grey and the green and red residues are coloured as in the cartoon (although some are obscured because of their proximity to thrombin). The N- and C-termini of hirudin are labelled. Structural images in this and other figures were created in PyMOL (https://www.pymol.org/).

H-fPRPGGGGNGDFEEIPEEYL-OH

Fig. 6. The amino acid sequence of bivalirudin. f = D-phenylalanine.

calcium (Ca_V) channels. The first to be isolated was ω-conotoxin GVIA from *C. geographus*.[58,59] Subsequently, ω-conotoxins have been isolated from many other species, including *C. magus* and *C. catus* (Fig. 7).[60,61] Selectivity differences among these peptides led to ω-conotoxins MVIIA and CVID being developed as N-type Ca channel blockers.

In a quest for alternatives to opioid-based pain management that avoid the highly addictive nature of the opiates, the Ca_V channel was identified as a potential target owing to its involvement in neurotransmitter release. There are six pharmacologically distinct Ca_V channel subtypes, of which the N-type ($Ca_V2.2$), T-type (Ca_V3) and P/Q-type ($Ca_V2.1$) are considered optimal targets for the treatment of pain. The localization of the N-type Ca_V channels in the dorsal horn region of the spine helps convey nociceptive signals from the peripheral nervous system to the central nervous system. Inhibition of the nociceptive pain signal in animals has been demonstrated in clinically relevant pain relief models.[67] Thus, at Neurex (now part of Elan Pharmaceuticals), ω-conotoxin MVIIA (SNX-111) was tested as a therapeutic agent that acted by blocking the N-type Ca_V found in the spine. Development of this as a drug required specialized intrathecal infusion using a subdermal pump. This peptide progressed through clinical trials and was renamed ziconotide (Prialt®), which was approved for the treatment of intractable pain in 2004.[68] Dose limiting side-effects observed in patients restrict this drug to cases of intractable pain. A second, even more selective ω-conotoxin, CVID, was also being developed by Xenome as AM-336 or leconotide. This peptide failed in clinical trials as a result of side-effects encountered in the patient pool upon intrathecal administration.[69]

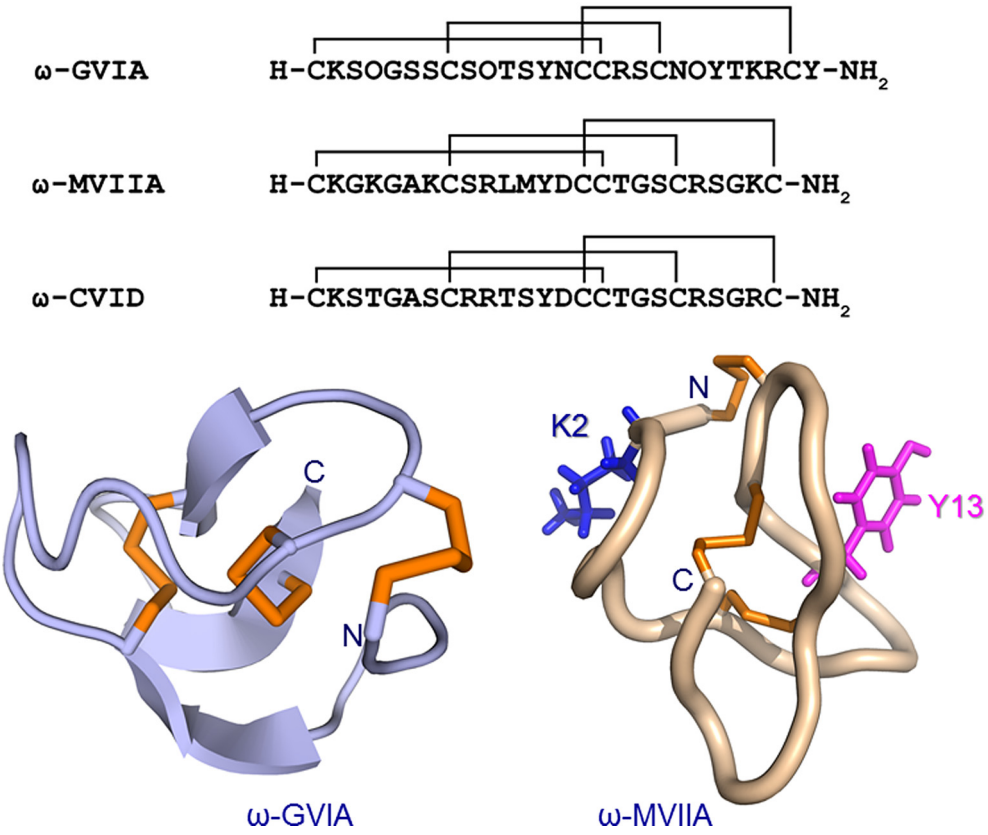

Fig. 7. Amino acid sequences and 3D structures of ω-conotoxin GVIA (pdb id 2CCO),[62] ω-conotoxin-MVIIA (pdb id 1OMG)[63] and ω-conotoxin CVID, with disulfide bonds indicated. Backbones are shown in lightblue (ω-GVIA) and wheat (ω-MVIIA) and disulfides in orange. The view of ω- GVIA highlights the ICK structure created by the peptide backbone and the three disulfide bridges.[64,65] The view of ω-MVIIA highlights Lys2 and Tyr13, which have been shown to have a role in Ca_V channel binding.[66] β-sheets have not been flattened in the graphics program. The structures are shown approximately to scale but have not been aligned.

3.5. Type 2 diabetes: exenatide

Type 2 diabetes is a major global public health crisis, with the World Health Organization estimating that more than 300 million people worldwide suffer with this disease.[70,71] Obesity and associated insulin resistance are key contributors to its rising prevalence. Also associated with the disease are impaired or reduced insulin secretion and lower B-cell levels. Complications from diabetes manifest themselves as increased mortality rates from conditions such as coronary heart disease, stroke and peripheral vascular disease.[72]

Drugs to treat type 2 diabetes include biguanides (metformin) and sulphonylureas (glibenclamide, tolbutamide), which have been used for many years. Only recently have new drugs emerged such as meglitinide analogs (repaglinide, nateglinide), which stimulate insulin secretion in a similar manner to the sulfonylureas.[73] In addition, the thiazolidinediones (rosiglitazone, pioglitazone) are agonists of the nuclear PPAR-γ receptor, which cause a greater increase in insulin sensitivity. Also emerging are incretin pathway agonists such as glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide, also known as gastric inhibitory peptide (GIP), shown in Fig. 8. GIP is a 42-residue peptide derived from a 153-residue precursor and GLP-1 is a 29-residue peptide corresponding to residues 7–36 from proglucagon. These two peptide hormones are secreted by intestinal L-cells and K-cells, respectively, in response to orally administered glucose; they act on their G-protein-coupled receptors (GPCR) to cause an increase in intracellular cAMP, which amplifies glucose-induced insulin secretion.[74] These peptides have very short plasma half-lives (~2 min) owing to their rapid degradation by dipeptidyl peptidase IV (DPPIV), which cleaves the N-terminal His-Ala dipeptide and inactivates these peptides. Inhibitors of DPPIV (sitagliptin, saxagliptin and vildagliptin) prevent the hydrolysis of this dipeptide from GIP and GLP-1, resulting in lower blood glucose and glucagon levels, which causes insulin secretion and slows or decreases gastric emptying.[75,76]

The Gila monster (*Heloderma suspectum*), one of the very few venomous lizards in the world, originates from New Mexico and Arizona in the USA. Reports of deaths from the late 1800's in cases of envenomation by Gila monsters were as overblown as most of

Exendin-3 H-SDGTFTSDLSKQMEEEAVRLFIEWLKNGGPSGAPPPS-NH_2

Exendin-4 H-GEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS-NH_2

GLP (7-36) H-HAEGTFTSDVSSYLEGQAAKEFIAWLVKGR-NH_2

GIP (Human) H-YAEGTFISDYSIAMDKIHQQDFVNWLLAQKGKKNDWKHNITQ-OH

Fig. 8. The amino acid sequences of exendin-3,[77] exendin-4,[78] GLP-1 (7–36)[79] and gastric inhibitory polypeptide (human).[80]

the Wild West gunfights of that same period. The venom from these animals is produced by submandibular glands in the lower jaw and oozes onto grooved teeth and into the victim by chewing motion rather than through a hollow sharp fang such as in snakes.[81] Isolation of the venom and subsequent separation using conventional and HPLC-based methods led to the identification of numerous enzymes (hyaluronidases, phospholipases, phosphodiesterases, and proteases).[81] The most lethal component, gilatoxin, is a serine protease similar to other snake-derived thrombin-like proteases.[82] One of the most interesting groups of products isolated from the venom are the exendins.[77,78] These peptides all have high sequence homology to GLP-1 (Fig. 8). In exendin-4, there is a substitution of Gly for Ala at position 2, which enhances protease resistance, especially to DPP-IV;[83,84] as a result, exendin-4 has a serum half-life *in vivo* of 2.4 h versus 2 min for GLP-1. Exendin-4 was developed by Amylin Pharmaceuticals as the drug Byetta® (exenatide) and was approved for treatment of type 2 diabetes in 2005.[72] Improvements in stability and delivery systems have resulted in two FDA-approved Byetta-derived products: Bydureon® (exenatide extended release) (Astra Zeneca), a once-a-week administration, and Adlyxin® or Lyxumia®, once-a-day administration (lixisenatide) (Sanofi).[85] Currently, another slow-release exenatide product (subdermal mini-osmotic pump for 90 days), ITCA-650, is being developed by Intarcia Pharmaceuticals. Intarcia submitted a new drug application (NDA) with the FDA in November 2016, which was accepted in February 2017 (http://www.intarcia.com/pipeline-technology/itca-650.html).

In 2008, some side-effects led to an FDA-mandated warning that Byetta may increase the possibility of pancreatitis. Also, in 2010 Bydureon® received a Black Box warning for a possible increase in risk for thyroid cancer similar to that of a related GLP-1 type drug Victoza® (liraglutide).

3.6. Prokaryotic toxins for gastrointestinal conditions: linaclotide and plecanatide

Certain strains of bacteria such as enterotoxigenic *Escherichia coli* secrete short disulfide-rich peptides that are highly stable to thermal denaturation.[86] These peptides typically contain 14–18 residues and three intramolecular disulfide bonds (Fig. 9). These "heat-stable" enterotoxins are the leading cause of acute infant's and traveler's diarrhea.[87] These peptides are a close mimic of uroguanylin, the naturally-occurring hormone in animals (Fig. 9), which is a 16-residue peptide containing two intramolecular disulfide bonds.[88] Both of these peptides are highly specific agonists of the GPCR 2C in the large intestine, activation of which leads to an increase in intracellular cGMP that in turn results in chloride, bicarbonate and water release into the lumen of the large intestine by way of the cystic fibrosis transmembrane conductance regulator CFTR ion channel. When not carefully controlled, this results in diarrhea.[89]

Two drugs have been developed that target the 2C receptor in the lumen of the large intestine. The first to market was an analog of heat-stable enterotoxin named Linzess® (linaclotide) in 2012 (Fig. 9), which was developed by Ironwood Labs and Forrest Labs and was one of the first peptides developed with an oral application.[92,93] Owing to the highly stable compact structure, the peptide successfully survives passage through the gut with only a single residue being clipped from the C-terminus. As the site of action is GPCRs in the lumen of the large intestine, the drug requires no adsorption into the blood stream and oral administration is possible.[90,94]

The second drug to market (in 2017) was Trulance® (plecanatide) from Synergy Pharmaceuticals. This drug is a uroguanylin analog with Asp3 replaced by Glu (Fig. 9).[95,96] This substitution helps to slow the thermodynamically-controlled interconversion of two topoisomers, which occurs naturally with this peptide (Fig. 9). The topoisomers result from a conformational change in the peptide at acidic pH, the A topoisomer being active and the B isomer inactive.[91] As a result, plecanatide is also marketed to the same constipation market as it has fewer cases of severe diarrhea owing to the spontaneous inactivation to the B topoisomer, which is accelerated at physiological temperature and thereby reduces the major side-effect of linaclotide, which is diarrhea.[97]

4. Peptide toxins discontinued in clinical trials

4.1. α-Conotoxin Vc1.1: pain

nAChRs are members of the Cys-loop ligand-gated ion channels, which participate in rapid synaptic transmission and mediate a range of neurophysiological functions.[98,99] They have been implicated in many nervous system diseases and disorders, including Parkinson's disease, Alzheimer's disease, schizophrenia, neuropathic pain, memory loss, and stress mediation. As such, nAChRs have emerged as important potential targets for pharmaceutical development.[100] nAChR subtypes are homo- or hetero-pentamers of α1−10, β1−4, γ, δ, or ε subunits that are expressed in various regions of the nervous system.[101] The α9α10 nAChR subtype is a hetero-pentamer comprising two α9 and three α10 subunits ($(\alpha9)_2(\alpha10)_3$).[102] The α9α10 nAChR involvement in analgesia suggests that it might also be expressed in the brain and/or in the peripheral nervous system and is a potential target for the treatment of pain.[103]

α-Conotoxins are a large conotoxin family that specifically targets nAChR subtypes.[7,11,50] Most α-conotoxins have a similar three-dimensional structure, comprising a small helical segment stabilized by two disulfide bonds.[104,105] The loops between the cystine residues and the number of residues in these loops are used to subclassify α-conotoxins.[106] Vc1.1 is a 16-residue α-conotoxin (Fig. 10), originally isolated from the venom of *Conus victoriae*, that has potent analgesic activity and shows potential as a novel drug lead for the treatment of neuropathic pain.[107–109] Vc1.1 contains four residues in the first loop and seven in the second, and thus belongs to the 4/7 loop family.

α-Conotoxin Vc1.1 was taken into clinical trials as ACV1 by Melbourne-based Metabolic Pharmaceuticals Ltd. for the treatment of neuropathic pain. ACV1 was safe and well tolerated at all administered doses in the first human study (Phase 1), completed in November 2005. ACV1 was tested in several well-established animal pain models and showed efficacy in relieving the characteristic pain symptoms of neuropathy, allodynia and hyperalgesia. However, in 2007 ACV1 failed in Phase 2a trails owing to a lack of efficacy in humans. Apparently, the human α9α10 nAChR did not have the same sensitivity to ACV1 as its counterpart in the rat models used in the preclinical stage of development.[111,112]

More recently, backbone cyclization of Vc1.1 improved its stability, further enhancing its potential as a drug.[113] Cyclized Vc1.1 is active when administered orally to reduce mechanical allodynia in animal models of neuropathic pain.[113] Two potential modes of action of Vc1.1 have been identified: (1) inhibiting the $Ca_V2.2$ channel by activating the $GABA_B$ receptor,[105,114] and (2) inhibiting the α9α10 nAChR subtype.[103] Both of these receptors could be involved in the observed analgesia, but further studies are needed to fully understand the analgesic activity of Vc1.1 at the molecular level.[105] As discussed below (Section 6.2), targeting the α9α10 nAChR for treatment of pain with α-conotoxin RgIA is still an area of active clinical development at Kineta Inc.[115]

4.2. χ-Conotoxin-MrIA: pain

As described above, pain therapy is truly in need of non-addictive alternatives to opioid-based drugs. Since neuronal pathways

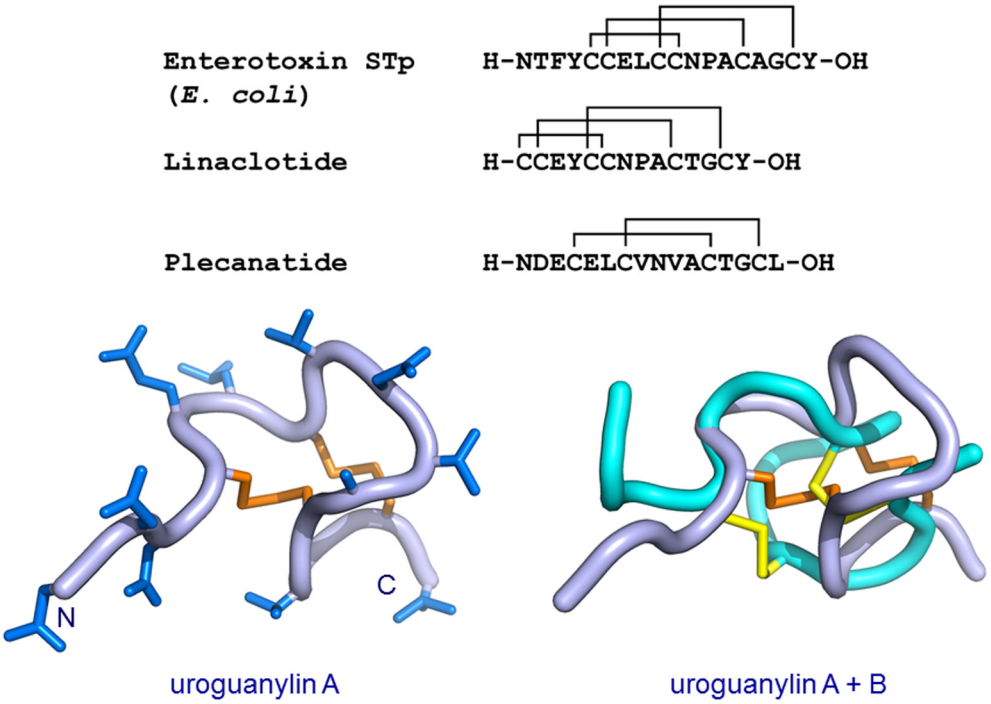

Fig. 9. The amino acid sequences of enterotoxin STp (*E. coli*), linaclotide and plecanatide, with disulfide bonds indicated. The solution structure of linaclotide was reported by Busby et al.[90] but there is no pdb deposition. The structure of uroguanylin A (pdb id 1UYA) is shown on the left. Superposition of the topoisomers of uroguanylin A (lightblue backbone, orange disulfides) and B (pdb id 1UYB)[91] (cyan backbone, yellow disulfides), aligned over the backbone heavy atoms, is shown on the right.

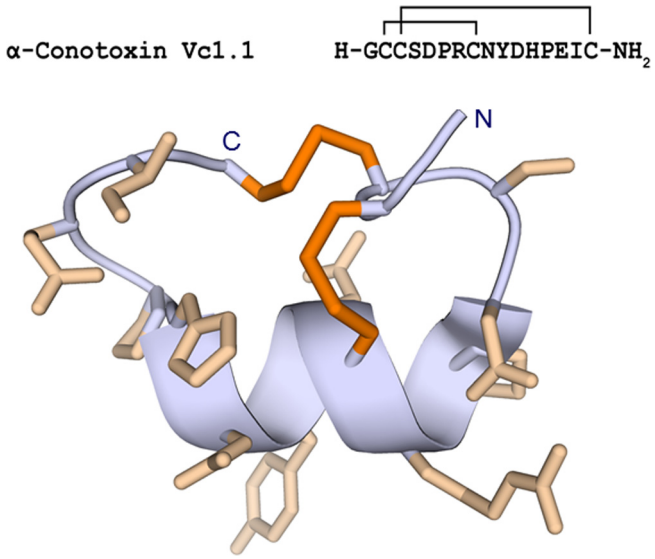

Fig. 10. Amino acid sequence of α-conotoxin Vc1.1, with disulfide bonds indicated, and its 3D structure (pdb id 2H8S).[110] Backbone bluewhite, disulfides orange, other side chains wheat.

controlled by ion channels propagate pain signals to the brain, selective blockers or modulators of such channels are promising candidates for development. Fortuitously, fish-, worm- and mollusk-hunting cone snails produce venoms with hundreds of small peptides that block nearly all of the major ion channels (Na^+, K^+ and Ca^{2+}) as well as specific receptors such as nAChR and noradrenaline transporters.

In 2000, McIntosh et al.[116] identified a novel hydroxy-Pro-containing 13-residue conotoxin from *C. marmoreus* with a 1–4/2–3 disulfide pattern (also known as the ribbon form), quite different from the 1–3/2–4 disulfide pattern found in the α-conotoxins that block nACh receptors (Fig. 11). This peptide was designated χ-MrIA or Mr10a. The target of χ-MrIA is the noradrenaline (norepinephrine (NE)) transporter (NET), where it acts as a non-competitive or allosteric modulator.[117,118] Noradrenaline, which accumulates in chemical synapses, is removed by the action of NET. As noradrenaline magnifies the intensity of descending pain inhibition, inhibitors of spinal NET, such as χ-MrIA, are potential clinical leads in the management of pain. A stabilized analog with Pyr at the N-terminus was developed as Xen2174 by the Australian company Xenome.[119] Xen2174 shows good selectivity relative to other monoamine neurotransmitter transports such as serotonin and dopamine.[118,119] Unfortunately, despite showing early encouraging results in Phase 1 trials, Xen2174 was found to have dose-limiting toxicity issues in humans and was discontinued (Groeneveld, 2013; cited in[4])

4.3. Contulakin-G: pain

Another pain pathway involves the 13-residue neuropeptide neurotensin (NT). NT (Fig. 12) is found throughout the central nervous system (hypothalamus and amygdala) and functions as a neurotransmitter in dopaminergic neurons as well as in enteroendocrine cells of the small intestine.[121,122] The NT receptor is a GPCR. The pleiotropic actions of NT are apparent from its involvement in Parkinson's disease, nociception, cancer blood pressure and other conditions[123,124] Modifications of NT have led to a variety of interesting compounds, including anti-nociceptives[125] and some anti-convulsants[126] among others.

Predatory cone snails have evolved venom components with highly homologous sequences to NT. Contulakin-G, isolated from *C. geographus*, is a 16-residue peptide with an N-terminal Pyr, as in NT, as well a Thr-O-linked disaccharide at position 10 (Fig. 12)[127] This peptide has been shown to be a NT receptor agonist for all three subtypes with fM potency.[126,127] PK-PD studies showed the peptide to be remarkably stable in serum with a

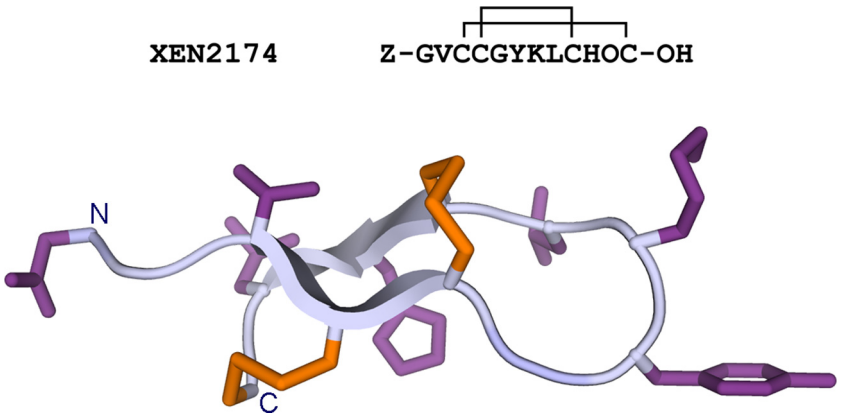

Fig. 11. Amino acid sequence of Xen2174, with disulfide bonds indicated, and its solution structure (pdb id 2EW4).[120] Z = pyroglutamic acid. Backbone bluewhite, disulfides orange, other sidechains violetpurple. β-sheets have not been flattened in the graphics program.

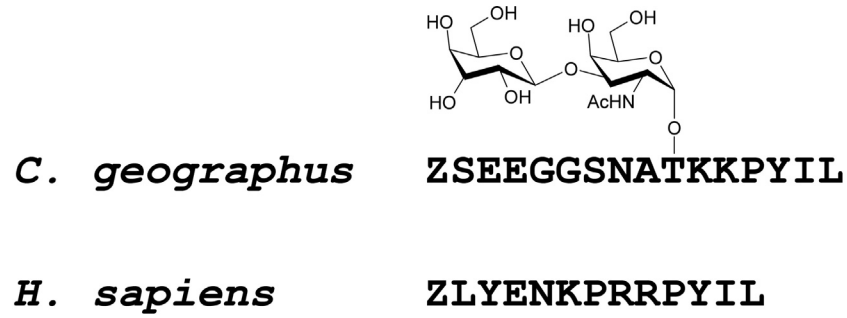

Fig. 12. Amino acid sequences of *C. geographus* contulakin-G and human neurotensin. Z = pyroglutamate. The Thr residue in contulakin-G contains the O-linked disaccharide β-D-Galp-(1 → 3)-α-D-GalpNAc-(1→).

half-life of several hours, which is unusual for a short linear peptide.[128] Contulakin-G was taken into clinical trials by Cognetix in Salt Lake City as CGX-1160 for patients with neuropathic pain from spinal cord injury or post-operative pain,[129] and received orphan drug status in 2005. However, it was discontinued by Cognetix, at which point Cognetix was liquidated. More recently, CGX-1160 was tested in a Phase 1a trial in humans and was found to be generally well tolerated when administered intrathecally at doses up to 1 mg/h. Peak analgesia occurred after the peak intrathecal concentration. It was noted in this study that the effect of the drug was best achieved when the compound was able to circulate in the cerebral spinal fluid following IT administration. [130] However, the FDA put a hold on further studies with CGX-1160 in man because of safety concerns arising from animal toxicity studies.

4.4. Conantokin-G: pain, epilepsy

One of the more unusual families of peptides isolated from cone snail venom consists of linear peptides with a high proportion of the post-translationally modified amino acid γ-carboxy-glutatamic acid (Gla). The first peptide characterised was conantokin-G from *C. geographus*,[131] which has 5 Gla residues in its 17-residue sequence (Fig. 13). This peptide was named the "sleeper" peptide as it induces a sleep-like state in mice under two weeks old, but causes a hyperactive state in older mice when injected ICV. The peptide is highly helical in the presence of Ca^{2+} and all of its Ca^{2+}-binding Gla residues cluster on the same face of the helix.[132] Its site of action was determined to be the NMDA receptor,[133] with which two residues in particular at the N-terminus of the peptide, Gly and Glu, engage. Further studies with conantokin-G using cloned subtypes of the NMDA receptor showed that it was selective for the NR2B subtype.[134] Cognetix advanced conantokin-G into clinical development as CGX-1007 for treatment of intractable epilepsy via intrathecal administration, pain and as a potential neuroprotectant in ischemic stroke.[135] CGX-1007 progressed through Phase 1 trials but was discontinued at Phase 2.

4.5. Cenderitide: cardiovascular diseases

Atrial natriuretic peptide (ANP) was discovered in the early 1980s.[136] It was observed that rat atrial extracts contained a factor that increased salt and urine output in the kidneys. Subsequently, the substance was purified from the heart by several groups and named ANF (atrial natriuretic factor) or ANP.[137] ANP is a 28-residue peptide containing a 17-residue disulfide-constrained ring (C7-C23) in the middle of the molecule that is characteristic of all the related natriuretic peptides (Fig. 14). ANP is closely related to brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP), which share a similar structure.

ANP is a potent vasodilator hormone secreted by heart muscle cells (atrial myocytes) in response to elevated blood volume. It is involved in the homeostatic control of body water, sodium, potassium and fat, reducing the water, sodium, and adipose loads on the circulatory system, and thereby reducing blood pressure. With regard to its effect on sodium in the kidney, ANP has exactly the opposite function to aldosterone, secreted by the zona glomerulosa; specifically, aldosterone stimulates sodium retention and ANP stimulates sodium loss.[138]

Conantokin-G H-GEγγLQγNQγLIRγKSN-NH_2

Fig. 13. Amino acid sequence of conantokin-G. γ = gamma-carboxy-L-glutamic acid.

Fig. 14. Amino acid sequence of C-type natriuretic peptide (CNP) and a chimeric natriuretic peptide, CD-NP (cenderitide), made up of CNP (in red) and the C-terminus of *Dendroaspis* natriuretic peptide (DNP) in green.

As snakes and lizards produce venoms that contain a plethora of components to incapacitate or immobilize prey or predators, it is no surprise that they contain natriuretic peptides, which cause hypotensive effects that contribute significantly to a rapid loss of consciousness in envenomated animals.[139,140] One of the first reptilian natriuretic peptides discovered was from venom glands of the eastern green mamba, *Dendroaspis angusticeps* (DNP).[141] DNP is a potent natriuretic and diuretic peptide which is similar to ANP and BNP and caused an increase in urinary and plasma cGMP.[141,142] In canine models, DNP decreases blood pressure, left ventricular end diastolic pressure, stroke volume and left ventricular afterload; the latter appears to be due to a reduction in preload rather than arterial vasodilatation.[143] DNP was also found to have greater stability to neutral endopeptidease 24.11, which is the primary inactivator of the natriuretic peptides, compared to ANP, BNP or CNP, as a consequence of the elongation of both the N- and C-terminus.[144] This enhanced stability and high potency observed with DNP has stimulated screening of other reptilian venoms for novel natriuretic peptides.[139]

Cenderitide (CD-NP) (Fig. 14), a chimeric natriuretic peptide created by fusing the 22-residue structure of CNP with the 15-residue carboxyl terminus of DNP, was recently assessed in clinical trials for heart failure This novel peptide was engineered to uniquely co-activate the two particulate guanylyl cyclase (pGC) receptors (pGC-A and pGC-B) so as to take advantage of distinct receptor-mediated actions through cGMP.[144] In recent studies, cenderitide activated pGC-A, but less effectively than ANP, and was closely equivalent to CNP in activating pGC-B. *In vivo* CD-NP, like ANP, BNP, and DNP, but unlike CNP, possesses renal-enhancing actions through pGC-A/cGMP activation. Specifically, cenderitide is a 200-fold greater activator of pGC-A than CNP and 5-fold less potent activator of GC-B.[145] Cenderitide had only 50% potency in activating pGC-A and 40-fold greater GC-B-activation when compared with ANP. In contrast, CNP has potent anti-fibrotic properties through pGC-B activation and cGMP generation without renal-enhancing actions. In 2015, a Phase 2 dose-ranging study was completed in 14 patients with stable, chronic heart failure through subcutaneous infusion. This was an open-label trial that assessed the safety, tolerability and pharmacodynamic response to increasing dose levels of cenderitide. The drug was well-tolerated and there were no significant adverse events. Capricor, located in Beverly Hills, California, recently completed an additional study to further assess the safety, tolerability, pharmacokinetic profile and pharmacodynamic response to increasing dose levels of cenderitide in patients with stable heart failure with moderate renal impairment. Following this additional study, clinical development by Capricor was terminated in early 2017 (http://irdirect.net/prviewer/release/id/2342502).

5. Venom-derived peptides currently in clinical development

5.1. Chlorotoxin: tumor imaging

Perhaps one of the most dreaded diseases in human history is cancer. The first written record of cancer occurs in an ancient Egypt papyrus document from around 1600 BCE.[146–148] Hippocrates around 400 BCE was the first to use the Greek word for crab, karkinos, to describe the appearance of the veins forming the feet of the tumor, appearing like those of a crab.[149] Celsus in ~50 AD translated karkinos into Latin and thus was born the word cancer for the disease as it is currently known.[146,147] The ideas of Galen (131–201 CE) in the second century AD regarding the uses of purgatives were the basis for the primary treatment for cancer for more than 1000 years.[146,147] Sometimes these purgatives involved the use of venoms from snakes and scorpions according to local traditions.[146–148] It would take two millennia for research to ultimately catch up with why certain venoms may have had beneficial properties.

While screening venom of the death stalker scorpion, *Leiurus quinquestriatus hebraeus*, a toxin was isolated that had the property of blocking chloride channels.[150] This peptide, named chlorotoxin, contains 36 residues and four disulfide bonds (Fig. 15).[151] It is one of the dominant peptides in the venom in terms of concentration and was the first high-affinity chloride channel-blocking peptide.

One of the unique properties of chlorotoxin is that it also binds with high affinity to glioma cells[153] via matrix metalloprotease MMP-2 subtypes that are upregulated on the surfaces of glioma and other cancer cells but are not normally present.[154] As a result, chlorotoxin has been undergoing development as both a potential therapeutic delivery peptide for radiochemical treatment of malignant cells as well as an *in vivo* diagnostic tool for cancers. The biotech company Transmolecular (Birmingham, Alabama) took an I^{131}-labeled chlorotoxin (TM-601) into clinical trials for recurrent high grade glioma (https://clinicaltrials.gov/ct2/show/NCT00040573) in 2003 and for malignant melanoma in 2008 (https://clinicaltrials.gov/ct2/show/NCT00733798). Results of the Phase 1 and 2 trials for glioma showed that intracavitary administration was well tolerated, with no dose-limiting toxicities observed. I^{131}-TM-601 bound to the tumor periphery and demonstrated long-term retention at the tumor with minimal uptake in any other organ system. Unbound peptide was eliminated from the body within 24 to 48 h. Only minor adverse events were reported during the 22 days of administration. At day 180, four patients had radiographically stable disease, and one had a partial response. Two of these patients further improved and were without evidence of disease for more than 30 months.[155]

Blaze Biotech (Seattle) coupled a cyanine dye (Cy5.5) to chlorotoxin (BLZ-100; Tumor Paint®; tozuleristide) and used this analog as an imaging agent for cancer cells. Cy5.5 is a fluorescent dye that emits photons in the near infrared spectrum, enabling visualization in the operating room using infrared glasses. This fluorescent peptide improves surgeons' ability to remove all of the cancerous cells without injuring the surrounding healthy tissue. Studies in mouse models have shown that tozuleristide can visualize tumors with as few as 2000 cancer cells, making it 500 times more sensitive than MRI methods.[156] Treated animals exhibited no neurologic or behavioral deficits, and postmortem studies revealed no evidence of neuropathy.[157] Results of the preclinical trials demonstrate both the safety and superior imaging properties of this chlorotoxin

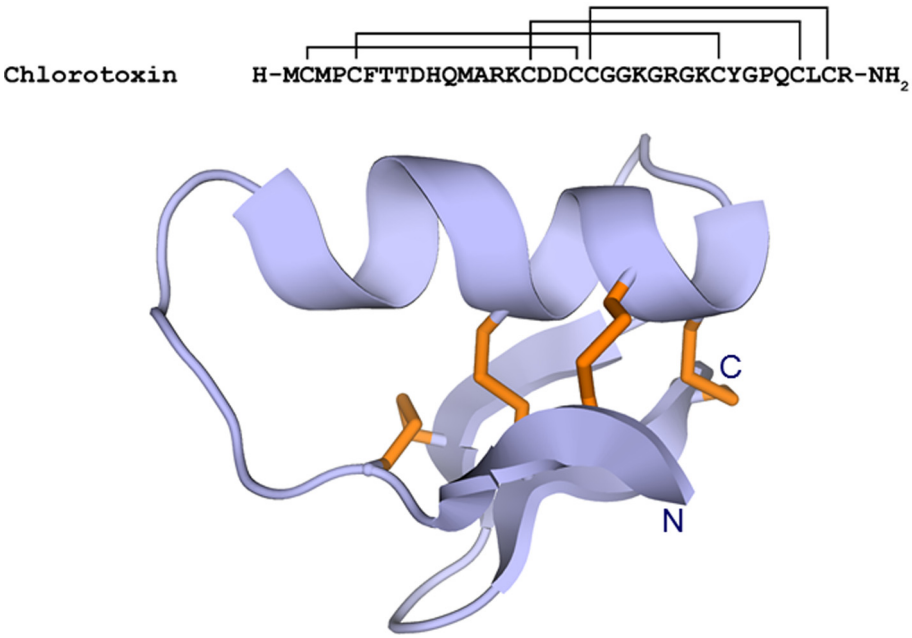

Fig. 15. Amino acid sequence indicating disulfide pairings and 3D structure of chlorotoxin (pdb id 1CHL).[152] Backbone is shown in lightblue and disulfides in orange. β-sheets have not been flattened in the graphics program.

analog.[158] Tozuleristide is currently ongoing Phase 1 clinical studies in pediatric brain cancer and breast cancer, and has completed Phase 1 testing in skin cancer and adult brain cancer.

5.2. ShK: autoimmune diseases

Voltage-gated K^+ (K_V) channels were found in T lymphocytes in 1984.[159,160] $K_V1.3$ is directly involved in the activation of a sub-set of T cells known as effector memory T (T_{EM}) cells as it sets the membrane potential during activation by allowing K^+ efflux to counterbalance the influx of Ca^{2+} through CRAC channels. As the $K_V1.3$-mediated efflux is required for activation of these T_{EM} cells, blocking these channels prevents their activation. T_{EM} cells are key mediators of autoimmune diseases and are therefore an attractive target for drug development.[161]

Charybdotoxin, from venom of the death stalker scorpion *Leiurus quinquestriatus hebraeus* (Fig. 16), was first discovered as an inhibitor of Ca^{2+}-activated K^+ channels[162] but is also active against $K_V1.3$.[163] Additional screening of a number of other scorpion species led to the discovery of margatoxin in the Central American bark scorpion, *Centruroides margaritatus*, which was more than 20-fold more potent than charybdotoxin against $K_V1.3$ (K_i 50 pM) and did not affect Ca^{2+}-activated K^+ channels.[164] Each of these toxins adopted a fold consisting of a short α-helix and a three-stranded antiparallel β-sheet,[163,165] and has a key Lys that inserts into the pore of the $K_V1.3$ channel to achieve blockade.[166,167] Margatoxin (Fig. 16) was taken into preclinical development by Merck and Co and shown to be effective in a mini-pig model of delayed-type hypersensitivity (DTH).[168] Merck subsequently moved into developing a small molecule drug, correolide, which was also effective in DTH models[169] before ultimately abandoning $K_V1.3$ as a target.

At about the same time as these discoveries of K_V-blocking peptides from scorpion venoms, a novel peptide was isolated and the sequenced from the Caribbean sun anemone, *Stichodactyla helianthus*.[174] This 35-residue peptide, ShK, was shown to be a potent competitive inhibitor of α-dendrotoxin binding to rat brain synaptosomes and blocked K^+ current in dorsal root ganglion cells. The primary structure showed no homology to the K_V-blocking toxins from scorpions (Fig. 16). The disulfide bonding pattern[175] and solution structure[170] were also very different from the scorpion αβ fold. However, the two key pharmacophore residues in ShK, Lys22 and Tyr23, are spatially conserved in an arrangement common to K_V-channel blocking peptides from widely different species.[173] ShK has a very high affinity ($K_i \sim 10$ pM) for $K_V1.3$ channels but also displays high pM affinity for $K_V1.1$, $K_V1.4$ and $K_V1.6$, which are present in brain and cardiac tissues.[176,177] Thus, selectivity improvements were necessary to transform this peptide into a viable $K_V1.3$-targeted therapeutic.[178]

Following an extensive structure-activity relationship (SAR) program,[177,179,180] ShK-186 (Fig. 16) was developed, which had a 100-fold improvement in selectivity for $K_V1.3$ over $K_V1.1$, $K_V1.4$ and $K_V1.6$.[181] Models of human autoimmune diseases such as multiple sclerosis and rheumatoid arthritis have been shown to be ameliorated by ShK and its analogs,[182,183] and preclinical testing of ShK-186 produced favorable results in both rats and monkeys.[184] Intriguingly, ShK-186 was found to have a long half-life at the site of injection, resulting in sustained high pM levels in plasma and minimizing the need for improving its pharmacokinetic properties.[184] An IND was filed by Kineta and approved by the FDA in 2012. ShK-186 has been allocated the generic name dalazatide, and completed Phase 1a and 1b trials in 2016. The results of the Phase 1b trial for psoriasis were reported recently, and showed that dalazatide was well tolerated, without serious adverse events, and reduced psoriatic skin lesions.[185] It is positioned to begin Phase 2a trails at the time of writing. Dalazatide is being advanced as a treatment for multiple autoimmune diseases, including inclusion body myositis, lupus, ANCA vasculitis, multiple sclerosis, psoriasis, psoriatic arthritis, rheumatoid arthritis, type 1 diabetes, inflammatory bowel diseases, and asthma.[163,186]

Both Janssen and Amgen embarked on Kv1.3 programs utilizing $K_V1.3$-selective peptide toxins. Amgen utilized an optimized ShK peptide conjugated to a high molecular weight polyethyleneglycol (PEG) to increase plasma half-life and showed cytokine reduction in cynomolgus monkeys.[187] Janssen evaluated a $K_V1.3$-selective scorpion toxin OsK1 fused to an antibody Fc domain or human serum albumin to extend plasma half-life.[188]

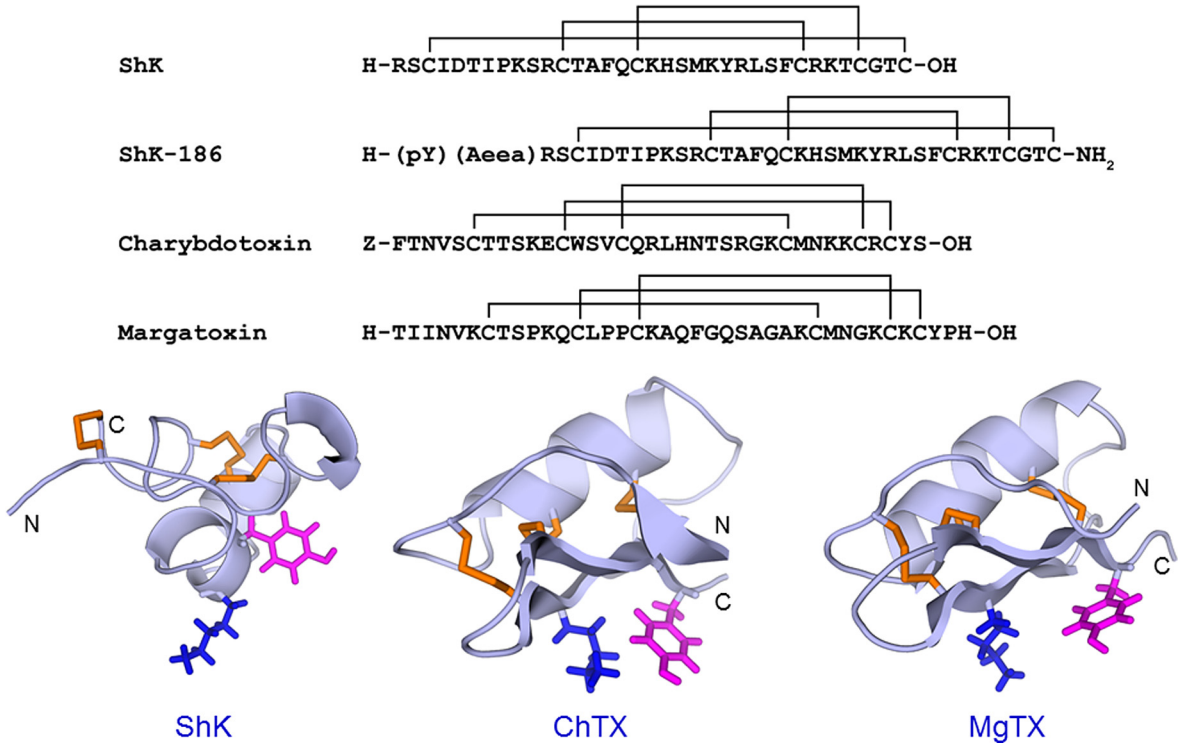

Fig. 16. Amino acid sequences of ShK, ShK-186, charybdotoxin and margatoxin, with the three disulfide bonds indicated. Aeea = 2 aminoethoxy-ethoxyacetic acid, Z = pyroglutamic acid and pY = phosphor-Tyr. Structures of ShK (pdb id 1ROO),[170] margatoxin (pdb id 1MTX)[171] and charybdotoxin (pdb id 2CRD)[172] are shown, with backbones in lightblue and disulfides in orange. β-sheets have not been flattened in the graphics program. The side chains of the Lys and Tyr that constitute the functional dyad[173] in each peptide are shown in blue and magenta, respectively. Note that this dyad is displayed on a helical scaffold in ShK but a β-sheet in charybdotoxin and margatoxin.

5.3. Shrew peptide SOR-C13: cancer

The transient receptor potential vanilloid calcium channel subtype six (TRPV6) is found predominantly in non-excitable tissues, mainly the intestinal tract, where it is responsible for capturing calcium at the apical membrane of enterocytes to begin the process of transcellular shuttling into the body. While the greatest levels of TRPV6 are in the gut, it has been variably reported in kidney, pancreas, prostate, salivary gland, placenta, and breast. TRPV6 is constitutively active and about 100-fold more selective for the apical entry of calcium over monovalent cations. Early studies of TRPV6 (named CaT1 or EaCa1 at that time) showed that the channel was significantly over-expressed in several cancers compared to corresponding normal tissues.[189] As such, TRPV6 was implicated in tumor development and progression.[190] TRPV6 is over-expressed in carcinomas of ovary and other cancer such as breast, colon, prostate and thyroid,[191] and TRPV6 mRNA is elevated in various tumor cell lines, including those of colon, human leukemia and prostate, making it an attractive potential diagnostic and therapeutic target.[192,193] In prostate cancer, TRPV6 mRNA levels are positively correlated to tumor progression and aggressiveness as indicated by Gleason score, pathological stage and extra-prostatic metastases.[192] Indeed, TRPV6-positive prostate tumors have a poor prognosis owing to their propensity to invade surrounding tissues.[194]

The northern Short-tailed shrew (*Blarina brevicauda*), found in eastern North America, is one of the very few mammals that possess venom. The venom is secreted in the animal's saliva from the submaxillary and sublingual glands and is used to subdue insect prey. One toxic component is a 253-residue serine protease with kallikrein-like activity named blarina toxin.[195] In addition, the venom contains a 54-residue paralytic peptide containing three disulfide bonds named soricidin (Fig. 17).[196] This peptide blocks Ca^{2+} uptake via TRPV6. C-terminal truncations of soricidin (SOR-C27 and SOR-C13), while not being paralytic, have been shown to block Ca^{2+} uptake by ovarian cancer cells via inhibition of TRPV6.[197] Indeed, SOR-C13 inhibits TRPV6 with an IC_{50} of 14 nM,[198] and was effective in inhibition of tumors in xenograft models of ovarian and breast cancer. Studies with fluorescently-tagged SOR-C13 showed rapid uptake by ovarian xenograft tumors with signals becoming visible in 20–30 min, maximizing at about 1 h and remaining for at least 72 h.[198]

An open-label, dose escalation Phase 1 study of SOR-C13 in 23 patients with advanced tumors of epithelial origin was conducted recently. Primary objectives were to assess safety, tolerability and pharmacokinetics,[199] and secondary goals were to assess pharmacodynamics and efficacy. No drug-related serious adverse events occurred. Some minor Grade 2 and Grade 3 dose-related toxicities were observed such as hypocalcemia and atrial fibrillation in about one quarter of the patients. One Grade 3 treatment-emergent adverse event, urticaria, was definitely related to SOR-C13. The maximum tolerated dose was not established. Stable disease suggested antitumor activity.[199]

6. Venom-derived peptides currently in preclinical development

6.1. $Na_V1.7$ blockers: pain

The 1.7 subtype of the voltage-gated sodium channels (Na_V) has emerged as one of the hottest drug targets for channel blockers or modulators over the past decade. Mutations that lead to a loss of function in *SCN9A*, the gene encoding $Na_V1.7$, cause congenital insensitivity to pain with anosmia as the only other sensory deficit, whereas mutations resulting in a gain-of-function are involved in

Soricidin* H-DCSQDCAACSILARPAELNTETCILECEGKLSSNDTEGGLCKEFLHPSKVDLPR-OH

SOR-C27 H- EGKLSSNDTEGGLCKEFLHPSKVDLPR-OH

SOR-C13 H-KEFLHPSKVDLPR-OH

*** Disulfide bonded**

Fig. 17. Amino acid sequences of soricidin, SOR-C27 and SOR-C13. The disulfide bonds for soricidin have not been reported but are known to be present from mass spectral data.

episodes of extreme spain (erythromelalgia and paroxysmal extreme pain disorder), often triggered by non-noxious stimuli.[200,201] Thus, selective inhibitors of $Na_V1.7$ are potential leads for future analgesic development. Unfortunately, non-specific blockade of other Na_V subtypes (1.1, 1.2, 1.4, 1.5 and 1.6)[202] has the potential to lead to substantial side-effects, including seizures, arrhythmias and impaired motor function.

Small disulfide-rich peptides that inhibit $Na_V1.7$ have been isolated from the venom of several species of tarantulas. The first of these to be reported was protoxin-II from the Peruvian green velvet tarantula, *Thrixopelma pruriens*,[203] which is a 30-residue peptide containing three disulfide bonds (Fig. 18). This adopts an ICK motif structure, where one disulfide bond threads a loop formed by the other two disulfide bonds and the peptide backbone to form a knot-like structure.[64,65] ProTx-II inhibits both tetrodotoxin-sensitive and tetrodotoxin-resistant voltage-gated sodium channels. ProTx-II inhibits activation by shifting the voltage-dependence of channel activation to more positive potentials. ProTx-II blocks $Na_V1.7$ with an IC_{50} value of around 300 pM, and $Na_V1.2$, 1.5 and 1.6 with IC_{50} values of 41, 79 and 26 nM, respectively.[203]

One of the best-studied venom peptide inhibitors of $Na_V1.7$ is the 35-residue huwentoxin IV, isolated from the venom of the Chinese bird-eating spider *Selenocosmia huwena* (Fig. 18).[205] This 35-residue peptide, which also adopts an ICK fold, completely inhibits Nav1.7 with an IC_{50} of ~26 nM.[208] Detailed characterization of the toxin-channel interaction revealed that the peptide binds to one of the four voltage sensor domains (VSD) of the channel,[208,209] which is in contrast to small molecule drugs such as local anesthetics that bind to the central pore region.[210] The channel pore is more highly conserved among the different Na_V1 subtypes compared to the VSD, making the VSD an attractive target for development of selective therapeutics, and small molecules that target the VSD are being developed.[211,212]

Another novel peptide, μ-theraphotoxin-Pn3a, isolated from venom of the tarantula *Pamphobeteus nigricolor,* potently inhibits $Na_V1.7$ (IC_{50} 0.9 nM) with at least 40–1000-fold selectivity over all other Nav subtypes. Despite on-target activity in small-diameter dorsal root ganglia, spinal slices, and in a mouse model of pain induced by $Na_V1.7$ activation, μ–TRTX-Pn3a alone displayed no analgesic activity in formalin-, carrageenan- or FCA-induced pain in rodents when administered systemically. However, when administered with sub-therapeutic doses of opioids or the enkephalinase inhibitor, thiorphan, μ–TRTX-Pn3a produced profound analgesia. These results suggest that in these inflammatory models, acute administration of peripherally restricted $Na_V1.7$ inhibitors may produce analgesia only when administered in combination with an opioid.[213]

Na_V channels contain four homologous but non-identical VSDs that control the gating of the channel. Each VSD consists of four transmembrane helical segments (S1-S4) connected via intra and extra-cellular loops. VSDs of domains II and IV (VSDII and VSDIV) have been shown to be excellent potential targets as they control channel opening and inactivation, respectively. HwTx-IV specifically binds to acidic residues in the S1-S2 and S3-S4 loops of VSDII; thus, rat $Na_V1.2$ and 1.3 and human $Na_V1.7$, which all possess E818 in S3-S4, are sensitive to HvTX-IV, whereas $Na_V1.4$ and 1.5, missing an acidic residue in this same position, are insensitive to HwTx-IV.[208,209] The pharmacophore of HwTX-IV was identified through extensive SAR studies, which showed that residues Trp30 and Lys32 are required for $Na_V1.7$ activity, whereas substitutions at the N- and C-terminal regions of the peptide were found to improve affinity for $Na_V1.7$ without improvement against $Na_V1.5$.[214,215] This led to Medimmune's development of a triple mutant of HwTx-IV (E1G, E4G, Y33W) which is one of the most potent blockers of $Na_V1.7$ reported to date (IC_{50} ~ 0.5 nM).[215] A recent report on the structure of this tri-substituted analog revealed that the fold is the same as that of the parent HwTX-IV peptide and that Na_V isoforms 1.1, 1.2, 1.3, 1.6 and 1.7 are sensitive to this analog whilst isoforms 1.4, 1.5 and 1.8 are not.[216]

In another program at Pfizer, potent and selective blockers of $Na_V1.7$ with improved therapeutic properties were generated from ceratotoxin-1 (CcoTx1) (Fig. 18),[217] an inhibitor of neuronal sodium channels isolated from venom of the tarantula *Ceratogyrus cornuatus*. A combination of directed evolution, saturation mutagenesis, structure-activity relationship, and chemical modification studies was pursued to create potent and selective peptide inhibitors of $Na_V1.7$.[207] Several of these peptides are highly potent (IC_{50} 2.5 nM against Nav1.7) and selective (selectivity improvements of 80-fold and 20-fold over the closely-related $Na_V1.2$ and 1.6 channels, respectively, and IC_{50} on skeletal ($Na_V1.4$) and cardiac ($Na_V1.5$) channels >3000 nM).

Amgen identified and characterized GpTx-1, a known antagonist of TTX-sensitive sodium channels,[218] from venom of the tarantula *Grammostola porter*.[206] GpTx-1 was first reported as a Ca_V channel blocker after isolation from venom of the closely-related Chilean tarantula, *Grammostola rosea,* and named GTx1-15.[219] It was later identified in the venom of the Chilean copper tarantula, *Paraphysa scrofa* (*Phrixotrichus auratus*).[220] On the basis of its potency and desirable Nav subtype selectivity profile, GpTx-1 was developed as a lead to target Nav1.7. Murray et al.[206] described a significant peptide medicinal chemistry approach to probe GpTx-1 structure-activity relationships and engineered analogs with high potency and selectivity for $Na_V1.7$. The analog [Ala5, Phe6,Leu26,Arg28]GpTx-1 was found to be exceptionally potent and selective, with an IC_{50} of 1.6 nM against $Na_V1.7$, >1000-fold selectivity against $Na_V1.4$, and >6000-fold selectivity against $Na_V1.5$. Synthesis and folding of this analog proceeded smoothly, indicating that it would be amenable to scaled-up chemical production.

Janssen recently published on their program of optimizing ProTX-II via directed evolution.[221] Using ProTX-II as a scaffold, the engineered peptide JNJ63955918, with improved $Na_V1.7$ selectivity and *in vivo* tolerability, was developed. This analog has an N-terminal H-Gly-Pro addition, as well as the substitutions W7Q and W30L. JNJ63955918 induces a pharmacological insensitivity to pain that fully recapitulates the $Na_V1.7$-null phenotype.[221]

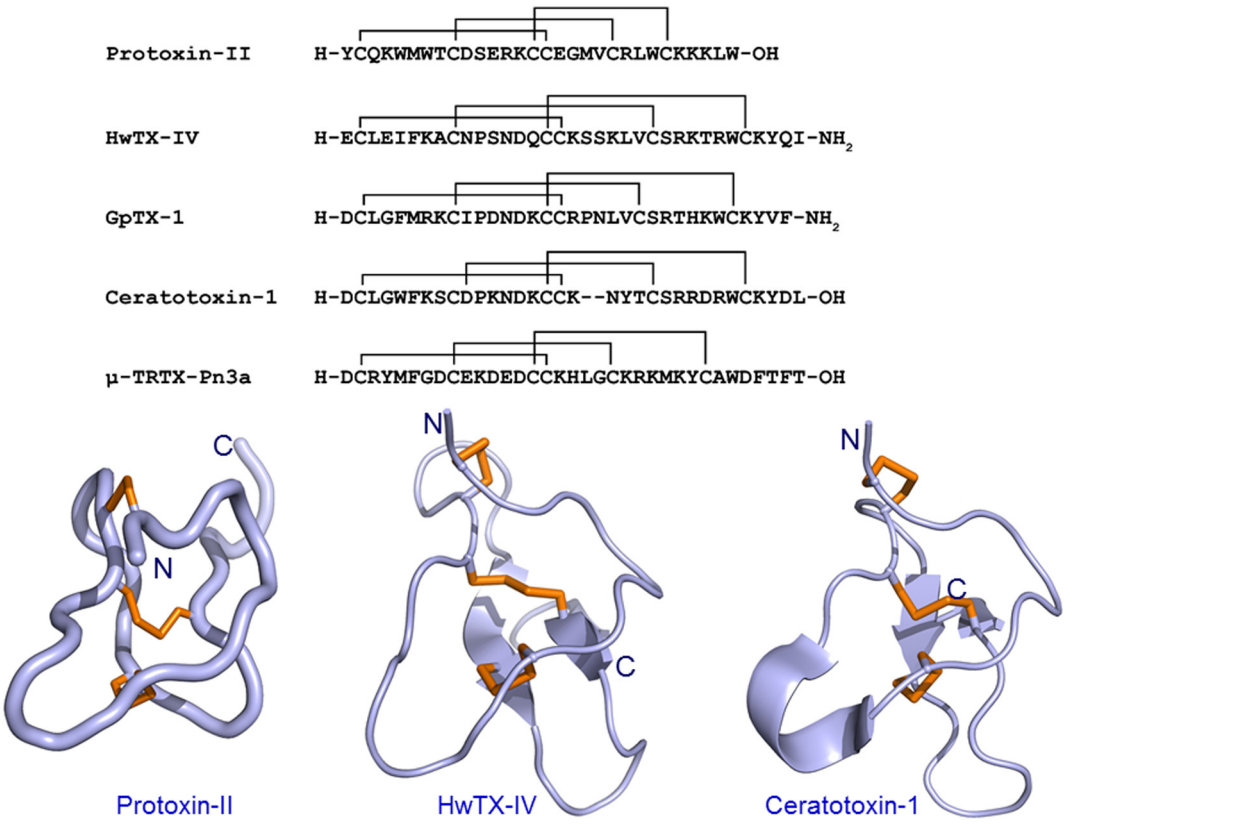

Fig. 18. Amino acid sequences and structures of spider-derived $Na_V1.7$ blockers protoxin-II (pdb id 2N9T),[204] huwentoxin-IV (pdb id 1MB6),[205], GpTx-1 (solution structure shown in Murray et al.,[206] but no pdb assigned), ceratotoxin (pdb id 5EMP of complex with $Na_V1.7$)[207] and μ–TRTX-Pn3a, with three disulfide bonds indicated in the classic ICK pattern.

Peptide engineering programs at several institutions and companies as described above have optimized the potencies and selectivities of various venom-derived leads as inhibitors of $Na_V1.7$. The larger footprint of these tarantula-derived peptides[207] compared to small molecule drugs,[212] is thought to provide an enhanced opportunity for development of more selective ligands. Peptides are therefore considered the optimal compromise between small molecules and larger proteins such as antibodies, with their superior selectivity and reasonable production costs.[222] Interestingly, many of these efforts utilize recombinant methods to generate their peptides, such that the potential lead would be classified as a biologic.[207,216,221]

6.2. α-conotoxin RgIA: pain

Originally isolated from *Conus regius*, α-conotoxin RgIA is a 13-residue two intramolecular disulfide peptide (Fig. 19).[223] As described above, α-conotoxin antagonists of α9α10 nAChRs have been proposed as potential analgesics for the treatment of neuropathic pain.[103,224] However, α-conotoxin Vc1.1 (Section 4.1) proved to be at least two orders of magnitude less potent on human than rodent nAChRs, limiting its translational application.[112] Furthermore, an alternative proposal that Vc1.1 achieves its therapeutic effects by acting as an agonist of $GABA_B$ receptors has caused uncertainty as to whether α9α10 nAChR blockade is the therapeutically relevant mechanism.[114] To address these issues, SAR studies of Rg1A were undertaken by Kineta Inc. in collaboration with researchers at the University of Utah, Salt Lake City, leading to the development of RgIA4 (KCP-400), a peptide that exhibits high potency for both human and rodent α9α10 nAChRs, and was at least 1000-fold more selective for α9α10 nAChRs over

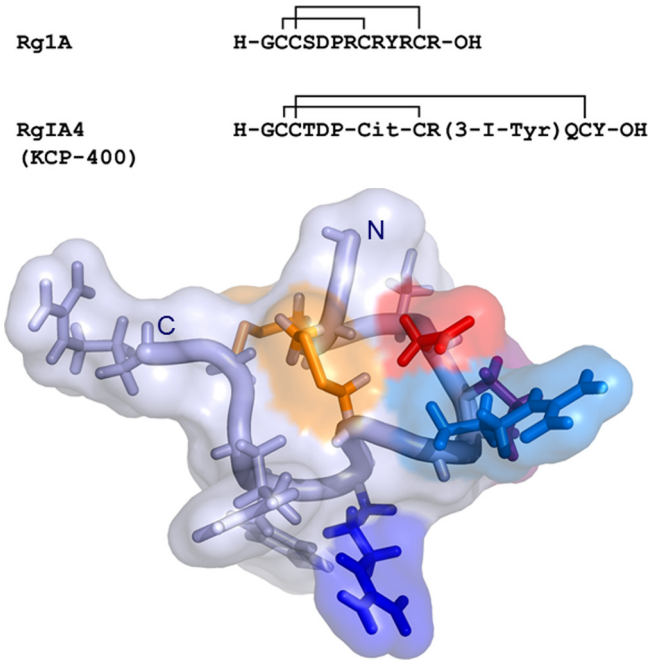

Fig. 19. Amino acid sequence of RgIA and RgIA4 (KCP-400), with the two disulfide bonds indicated. Cit = citrulline. The structure of Rg1A (pdb id 2JUT)[225] is shown with the backbone in lightblue and disulfides in orange. The side chains of Asp5, Pro6 and Arg7, which are thought to interact with the (+) face of the α9α10 receptor,[225] are coloured red, purple and marine, respectively, and that of Arg9, which may interact with the complementary face of the receptor, is shown in darkblue.

all other molecular targets tested, including opioid and $GABA_B$ receptors.[115] A daily subcutaneous dose of RgIA4 prevented chemotherapy-induced neuropathic pain in rats. In wild-type mice, oxaliplatin treatment produced cold allodynia that could be prevented by RgIA4. Additionally, in α9 knock-out mice, chemotherapy-induced development of cold allodynia was attenuated and the milder, temporary cold allodynia was not relieved by RgIA4. These results clearly establish blockade of α9-containing nAChRs as the basis for the efficacy of RgIA4, and that α9-containing nAChRs are a valid target for prevention of chronic cancer chemotherapy-induced neuropathic pain.[115]

6.3. HsTX1[R14A]: autoimmune diseases

HsTX1, which was originally discovered from the venom of the scorpion *Heterometrus spinnifer*,[226] is a 34-residue peptide with an unusual fourth disulfide bond (Fig. 20). It is a potent blocker of $K_V1.3$ channels[226] and relatively selective versus $K_V1.1$.[227] As described above, $K_V1.3$ blockers have emerged as excellent leads for treating autoimmune disorders.[186] Recently, an analog of HsTX1 was designed *in silico* with even greater selectivity for $K_V1.3$ over $K_V1.1$.[228] Complexes of the peptide with $K_V1.3$ and $K_V1.1$ were created using docking and molecular dynamics simulations, then umbrella sampling calculations were performed to construct the potential of mean force of the ligand and calculate the binding free energy for the most stable configuration. This approach predicted that substitution of Arg14 with Ala or other small hydrophobic residues would yield a 2 kcal/mol gain in the Kv1.3/Kv1.1 selectivity free energy relative to the wild-type peptide. Functional assays confirmed the predicted selectivity gain for HsTX1[R14A] and HsTX1[R14Abu], with an affinity for Kv1.3 in the low pM range and a selectivity of more than 2000-fold for Kv1.3 over Kv1.1.[228] Remarkably, the synthetic yield for this four-disulfide variant was one of the most efficient ever observed.

The administration of this peptide via the buccal mucosa[232] and lung[233] has been investigated, with both routes proving to be effective in delivering plasma levels of the peptide well above those required for effective therapy. Moreover, an N-terminally PEGylated version of HsTX1[R14A] was effective in a model of inflammatory arthritis, with a single subcutaneous dose of PEG-HsTX1[R14A] reducing inflammation in pristane-induced arthritis for a longer period of time than the non-PEGylated HsTX1[R14A].[231] PEG-HsTX1[R14A] has the additional advantages of reduced non-specific adsorption to inert surfaces and enhanced circulating half-life.[231]

In order to assess the biodistribution of this peptide, it was conjugated with the chelator NOTA and radiolabelled with ^{64}Cu. [^{64}Cu]Cu-NOTA-HsTX1[R14A] was synthesized in high radiochemical purity and yield.[230] The biodistribution and positron emission tomography studies after intravenous and subcutaneous injections showed similar patterns and kinetics. The peptide was rapidly distributed, showed low accumulation in most of the organs and tissues, and demonstrated high molecular stability *in vitro* and *in vivo*. The most prominent accumulation occurred in the epiphyseal plates of trabecular bones. The high stability and bioavailability, low normal-tissue uptake and accumulation in regions of up-regulated K_V channels both *in vitro* and *in vivo* demonstrate that HsTX1[R14A] represents a valuable lead for conditions treatable by blockade of this channel. The pharmacokinetics shows that both intravenous and subcutaneous applications are viable routes for the delivery of this potent peptide.[230]

As is inevitably the case in reviews of this type, other examples of venom-derived peptides that are currently in development may have been missed owing to a lack of either published results or company press releases. In future instalments of this venoms-to-drugs compilation, these examples will be included.

7. Cosmetic applications of venom-derived peptides

Peptide applications in cosmetic products have been widespread over the past two decades. With no FDA regulation, substantiated claims are typically scarce. Some of these peptides (SNAP-8 and SNAP-25, N-terminal peptides derived from SNAP25) mimic SNAP25, which is one of the four components that forms the SNARE complex.[234] SNARE complexes form on the surface of vesicles containing ACh to bind to the neuromuscular synapse and

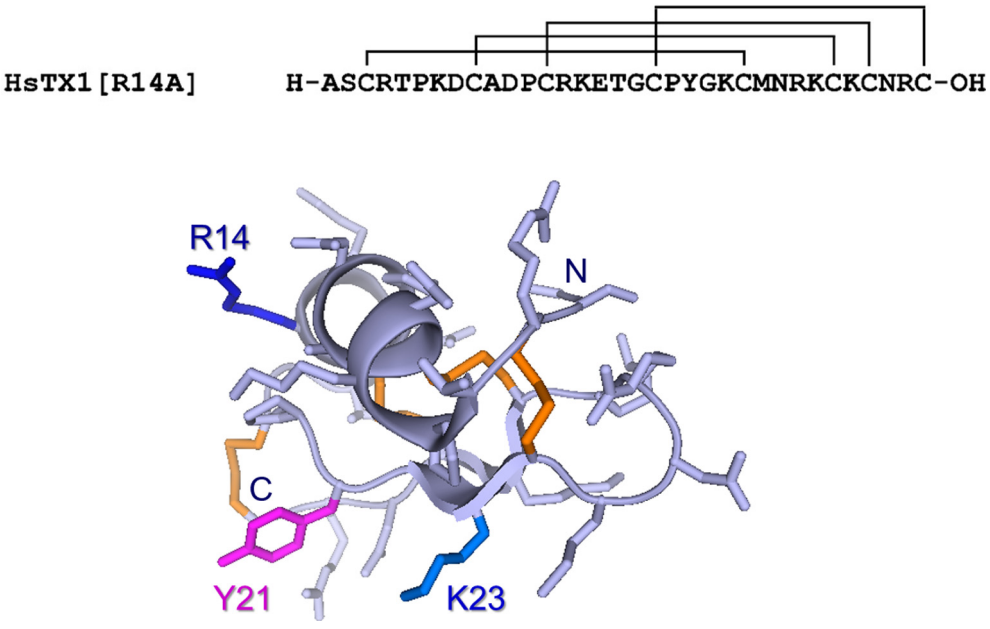

Fig. 20. Amino acid sequence of HsTX1[R14A] with four disulfide bonds indicated. The structure of HsTX1 (pdb id 1QUZ)[229] is shown with the backbone in lightblue, the disulfides in orange, and the β-sheets not flattened. The side chains of Lys23 and Tyr 21 are shown in marine and magenta, respectively, and that of Arg14 in darkblue. Note that the N-terminus is oriented away from the $K_V1.3$ channel binding surface, which facilitates conjugation of this position with tags[230] or PEG[231] without loss of selectivity.

release their neurotransmitter cargo into the synapse allowing the electrical impulse from the nerve to the muscle to be propagated. These peptides are reported to penetrate through the epidermal and dermal layers. This allows the peptides to act as competitive substrates to prevent SNAP25 from forming and preventing neuromuscular signal propagation, thereby eliminating wrinkles caused by over-stimulated neurons.[235]

7.1. Waglerin-1

Waglerin-1 is a 22-residue peptide toxin isolated from the Southeast Asian Temple Viper or Wagler's pit viper snake *Tropidolaemus wagleri* (Fig. 21).[236] Two similar peptides, Waglerin-2 and -3, were isolated from the same snake species. Waglerin-1 selectively blocks the nAChRε subunit with an IC_{50} of 50 nM and is a potent blocker of muscle nAChR. Waglerin-1 is also a modulator of $GABA_A$ receptors, with potentiating and suppressing effects. Its LD_{50} value is 0.33 mg/kg.[237] A tri-peptide mimetic peptide, SYNAKE^{Tm} (H-β-Ala-Pro-Dab-NHBzl x 2 AcOH), has been designed from waglerin-1 by Pentapharm Ltd.[238] It is reported to act as a nAChR-blocking peptide with a similar mechanism to botulinum toxin-A (BoTox^{Tm}), which cleaves SNAP-25.[239]

7.2. μ-CnIIIC

More recently, a neuromuscular blocking μ-conotoxin (μ-CnCIIIC, Fig. 22) was isolated and characterized from *C. consors*.[240] This peptide, which blocks the $Na_V1.4$ channel found in skeletal muscle, has been developed as a non-prescription alternative to botulinum toxin by Atheris Labs and marketed as Activen (XEP-018). When applied as a 1% w/v topical cream, this product is claimed to reduce fine-line wrinkles by 80% for 12–18 h after application (http://www.activen.ch/?page=products).

8. Agricultural applications of venom-derived peptides

As the human population grows at a rate of nearly 80 million per year, the population is expected to surpass 9 billion around 2050.[243] The global agricultural system will be pushed to its limit and beyond in order feed this population. The population explosion from 1960 (2.4 billion) to 2000 (6.4 billion) required a doubling of grain and a tripling of livestock production.[244] Grain and plant production to meet this demand was only possible following the invention and development of the Haber-Bosch process, immediately prior to and during World War I, which increased the production of ammonium from nitrogen gas needed for fertilizers.[245] The impact of synthetic chemical fertilizers allowed for the population explosion from 1920 to the present. The only practical way to achieve the future increases in production necessary to meet the increasing demand for food will be improvements in crop yields via genetically modified plants with increased crop yields as well as reduction in pest-mediated losses via insecticides or genetically-enhanced resistance.[244]

Insect pest are responsible for most of the reductions in crop yields around the world apart from those caused by extreme weather conditions. Controlling these pests (~1000 insect species) and the damage they cause to world crop production (estimated 10–14% reduction)[246] has been a major effort of several agricultural

Fig. 21. The amino acid sequence of waglerin-1, with the disulfide bond indicated.

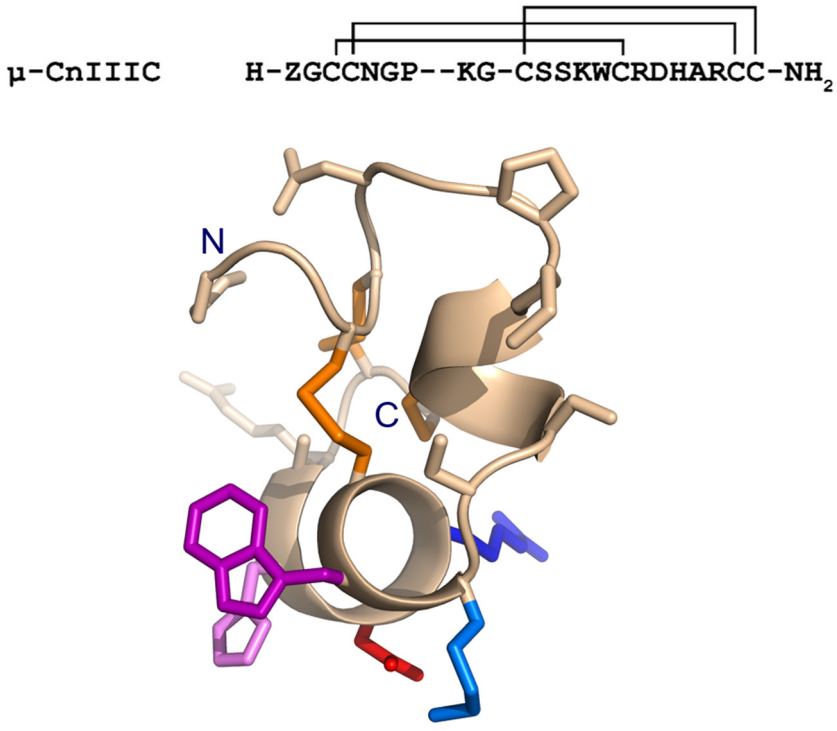

Fig. 22. Amino acid sequence of μ-CnIIIC with three disulfide bonds indicated. Z = pyroglutamic acid. The solution structure (pdb id 2YEN)[240] is shown with the backbone in wheat, disulfides in orange and the side chains of residues found to be important for activity in the related μ-conotoxin μ-KIIIA[241,242] coloured as follows: Lys13 marine, Trp14 purple, Arg16 blue, Asp17 red, His18 violet.

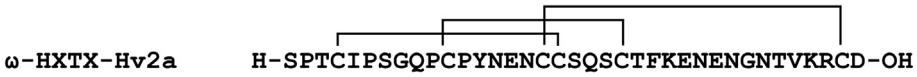

Fig. 23. Amino acid sequence of ω-HXTX-Hv2a.

chemical companies over the past century.[247] Moreover, controlling insect-borne and other arthropod-transmitted pathogens is a serious world-wide challenge for both humans and livestock, which are susceptible to diseases carried by these pests, which are primarily insects.[244]

Chemical insecticides have been the dominant control method used to reduce crop damage. Most chemical insecticides target a very small subset of ion channel targets.[248] Unfortunately, the common use of these insecticides has resulted in the development of resistance to these chemicals much like the overuse of antibiotics for treatment of bacterial infections.[249] Despite the increasing use of biological control methods, chemical insecticides remain the dominant method for controlling insect pests in both the agricultural and public health arenas. There is growing interest in the potential of insecticidal peptides derived from the venom of insect predators such as scorpions[250] and spiders[251] as an alternative to these synthetic chemicals.

Starting in the mid-1990's, genetically modified (GM) crops revolutionized global crop production. Insect-resistant GM crops, mainly corn and cotton, carrying an insecticidal protein (known as δ-endotoxin or *Bt*) from the bacterium *Bacillus thuringiensis,* dramatically reduced insecticide use and improved crop yields.[252] Overuse of GM plants constitutively expressing *Bt* will ultimately result in resistance, which has already been observed in some cases.[253] The development of GM plants containing other insecticidal peptides such as spider toxins would help mitigate this problem.

The most advanced of these insecticidal peptides for agricultural uses comes from the Australian Blue Mountains funnel web spider, *Hadronyche versuta.*[254] A published example of an insecticidal peptide toxin from this spider is ω-HXTX-Hv2a, which is a 37-residue disulfide-rich peptide (Fig. 23) and another member of the ICK-motif class of toxins. ω-HXTX-Hv2a is a potent blocker of insect Ca_V channels, with a nearly 10,000-fold selectivity for insect over vertebrate Ca_V channels. The company Vestaron, originally named Venomix, has developed a related peptide that inhibits Ca_V and K_{Ca} channels in insects. Their first product, Spear-T^{Tm} (GS-ω/κ-Hxtx-Hv1a), was launched in early 2017.[255] This product is made via a fermentation process in the yeast *Kluyveromyces lactis* to produce the correctly-folded peptide at a competitive cost to other commercial pesticides. The peptide can be applied via a spray method and kills with high selectivity upon contact. Fortuitously, this peptide is not toxic to beneficial insect species such as bees.[256] Moreover, the peptide is highly stable to thermal denaturation and organic solvents, as well as to biological digestive processes, because of its highly stable ICK fold,[257] which allows for field applications in summer conditions with no loss of activity.

Another application of this lead peptide has been in the development of GM plants, which possess a vector engineered into the plant to express potent insecticidal peptides, much like *Bt*, that would be constitutively expressed in the plant tissue.[258,259] These GM crops could be grown with vectors producing both *Bt* and the Ca_V channel-blocking peptide to enhance their effectiveness and potentially reduce the development of resistance in a principle known as pyramid stacking.[260,261]

9. Conclusions

This review summarizes the current status of many of the venom-derived peptide products that are currently on the market, under development or discontinued. As new peptides of venom origin are identified and characterized, this field will continue to grow. Moreover, as the efficiency and cost of both commercial synthesis and recombinant expression of peptides continue to improve, it is likely that more of these complex peptides will be developed further. We have attempted to summarize the current state of the field, but there are undoubtedly other developments underway that are not covered here and that promise to further advance the applications of venom-derived peptides.

Acknowledgements

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