Angewandte International Edition Chemie

Natural Products

DOI: 10.1002/anie.201200340

A Heteromeric Snake Toxin and the Molecular Details of Pain Perception

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ion channels · pain · natural products · snake toxins

Compounds from organisms of all kingdoms of life have been the primary source of human medications, initially as active ingredients in traditional remedies. Despite the emergence of high-throughput screening, in silico drug design, and combinatorial chemistry, natural products have continued to serve as a most fertile pool for the discovery of novel compounds of therapeutic interest, which are applied directly or as templates for synthetic modification developed as drug leads. Despite this dominant role in drug discovery, research in natural products has declined significantly in recent years in particular in the pharmaceutical industry.

Historically, small-molecule natural products from plants and microbes provided the majority of leads for the development of drugs. However, in recent years advances in biotechnology and protein chemistry have accelerated the use of peptide and protein natural products as human drugs. So far, most biopharmaceuticals are recombinant antibodies and peptide hormones, but drugs from venom peptides or proteins are emerging. Notable examples include the 25 amino acid ω-conotoxin MVIIA (ziconotide, Prialt) from the *Conus magus* sea snail, which blocks voltage-gated Ca^{2+} channels and is used for the treatment of neuropathic pain, and the 39 amino acid exendin-4 (exenatide, Byetta) isolated from the gila monster (a venomous lizard), which targets the glucagon-like peptide 1 (GLP-1) receptor and is used in the treatment of type 2 diabetes.

In addition to their role in drug discovery, natural products play a prominent role as pharmacological tools in biological research. It is in this area that the Julius group at the University of California in San Francisco has excelled in the application of natural products as tools to understand, in particular, sensory signal transduction in the nervous system. For example, they used capsaicin, the small molecule responsible for the burning sensation associated with chili peppers, to reveal the molecular and cellular basis of heat sensation by showing that capsaicin activates an hitherto unidentified ion channel, the transient receptor potential V1 (TRPV1) receptor, also known as the capsaicin receptor.[2a] TRPV1 is a member of a superfamily of sensory receptors for various stimuli, including the TRPM8 receptor, which senses cold. This receptor was also discovered by the Julius laboratory using menthol, the ingredient in peppermint leaves that elicits a cooling sensation.[2b] More recently, the Julius group has identified and applied peptide toxins, vanillotoxins, and a bivalent peptide toxin from tarantula spiders that target the TRPV1 receptor.[3]

In their most recent work, the Julius group again demonstrated the tremendous potential of combining the isolation and application of novel natural products with state-of-the-art biomolecular technologies to establish previously unknown aspects of pain signaling.[4] This time they embarked on identifying the active components in the venom of the Texas coral snake that produce the uniquely excruciating pain sensation caused by bites of this snake. Using an array of biochemical, biophysical, and genetic experiments, they first identified the pain-producing venom component, a heteromeric protein, then established its molecular target, which proves to be a subgroup of acid-sensing ion channels (ASICs) found on sensory neurons. Finally they applied the toxin to establish a previously unknown role of this sensory receptor in pain perception.[4]

Interestingly, they found that only a combination of two fractions from the crude venom, each containing a single protein compound, can elicit an increase in intracellular Ca^{2+} concentration, which is a tell-tale sign of activation of the intracellular pain-signaling cascades in sensory neurons. Thermodynamic analysis of these two protein components, MitTx-α and MitTx-β, show that they readily noncovalently assemble to form a heterodimeric complex, MitTx. Thus, only the protein complex, but not the individual MitTx-α and MitTx-β components, activates sensatory signaling in these neurons. The amino acid sequences of MitTx-α and MitTx-β show that these are similar to well-known proteins (Figure 1). MitTx-α is a 6 kDa Kunitz-type protein, which usually are protease inhibitors, and MitTx-β is similar to phospholipase-A2 (PLA_2) enzymes, which are found in a wide variety of snake venoms. This heteromeric complex resembles another group of snake toxins, β-bungarotoxins, which consist of a functional PLA_2 domain and a Kunitz-type domain that form a covalently linked heterodimer connected by an inter-domain disulfide bridge (Figure 1).[5]

The Julius group focused on establishing the mechanism by which the MitTx complex triggers an intracellular response in the sensory neurons. After excluding that that the enzymatic activity of PLA_2 plays a role, they employed

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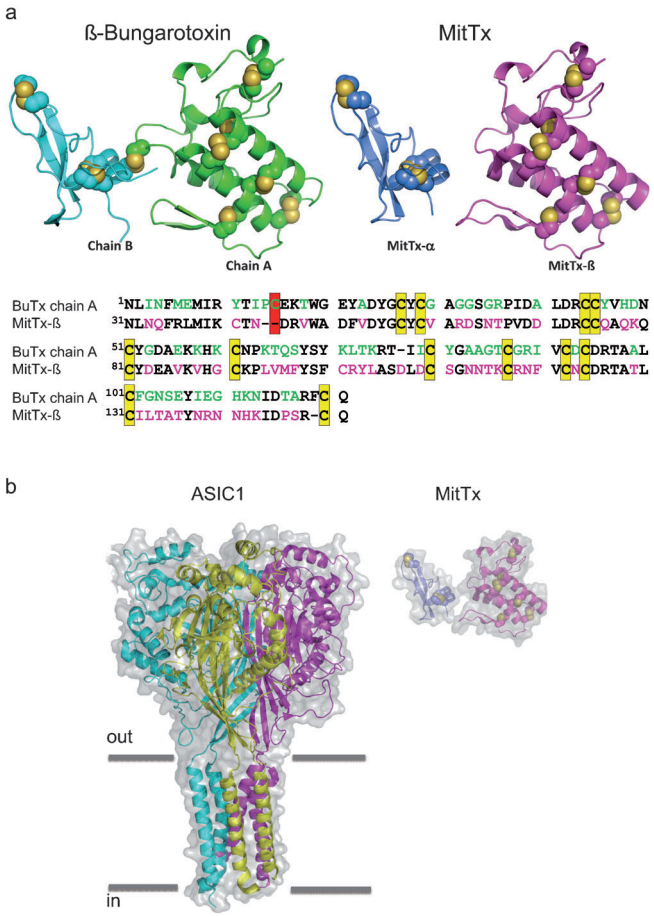

Figure 1. a) Model of the MitTx structure (right) based on β-bungarotoxin (PDB code 1BUN) and alignment of the amino acid sequence of MitTx-β with that of chain A of β-bungarotoxin. Cys residues involved in intramolecular disulfide bonds are highlighted as yellow spheres. The Cys residues forming an intradomain disulfide bond in BuTX, but absent in MitTx are highlighted in red in the alignment. b) X-ray crystal structure of ASIC1 and the model of MitTx.

electrophysiology to monitor ion-channel activity in the sensory neurons upon MitTx application, and they found that the triggering mechanism most likely involves initial activation of cation channels. They therefore considered a class of ionotropic sensory receptors known as acid-sensitive ion channels (ASICs), which are known to be involved in pain sensation. They found that MitTx, but not the individual components, is a potent and selective activator of a range of ASIC subtypes. ASICs are ligand-gated ion channels that are activated by changes in pH and expressed on sensory neurons of the peripheral nervous system (PNS) as well as in central neurons of the brain. Structurally, they are composed of three protein subunits, and the subunits assemble as either homomers or heteromers. ASICs contain an extracellular proton-sensing domain that couples protonation of the sensor to the opening of a membrane-embedded ion channel, which is primarily permeable to Na^+ ions. Lowering of the physiological pH (approximately pH 7.4) is often associated with tissue damage or inflammation, and ASICs are used in the PNS by sensory neurons as pH-sensing pain receptors to report tissue damage. In humans and rodents, four genes encoding ASIC subunits (ASIC1–ASIC4) are found, and ASIC1 and ASIC2 can further be expressed as different splice variants (ASIC1a, ASIC1b and ASIC2a, ASIC2b). When MitTx was tested at subtypes of ASICs expressed in *Xenopus* oocytes, it was found that MitTx acts as an apparent agonist at all the tested ASIC subtypes. Most importantly, however, MitTx showed highly differential potency, displaying low nanomolar potency at the ASIC1a and ASIC1b subtypes with more than 100-fold lower potency at the ASIC2 and ASIC3 subtypes. MitTx did not show any effect at a range of other sensory receptors that typically are present in sensory neurons such as TPRV1, and MitTx is therefore a highly selective agonist of ASIC1 receptors.

Having established the ASIC1 subtype as a potential in vivo molecular target for MitTX, the Julius group used transgenic mice, in which individual ASIC genes had been removed, to validate that the effects of MitTx in vivo indeed are mediated by ASIC1 receptors. Indeed, animals lacking expression of ASIC1 did not display MitTX pain-related behavior, whereas ASIC3-lacking animals were no different than wild-type animals. Furthermore, the tell-tale increase in the intracellular concentration of Ca^{2+} upon MitTx application was far less in the sensory neurons from mice lacking ASIC1 than those from ASIC3-lacking or wild-type mice. Thus, MitTX is a potent and selective tool to probe the role of ASIC1 receptors in pain sensation. This makes it possible to establish some immediate new insights by showing that populations of ASIC1-expressing sensory neurons play a previously unappreciated role in pain sensation, which is important because the focus on the role of ASICs in pain has so far primarily centered on the ASIC3 subtype.

Acute and chronic pain conditions, such as neuropathic pain, represent a large unmet therapeutic area. Clearly, MitTx does not have immediate potential as a drug candidate as it induces pain; however, MitTx is an important pharmacological tool in studies of pain and the role of ASICs in particular that likely will be of great benefit for the development of drugs targeting ASICs.[4] Since both MitTx-α and MitTx-β belong to protein classes with established structural scaffolds, and the heteromeric complex MitTx resembles β-bungarotoxins, it seems reasonable to predict the structure of MitTx by molecular modeling (Figure 1). It is notable that MitTX is a noncovalent complex, whereas in β-bungarotoxins the two domains are covalently linked by a disulfide bridge. However, the cysteine in the B chain of β-bungarotoxins that is responsible forming the interdomain disulfide bridge to the A chain is not present in MitTx-β (Figure 1). In turn, fine-tuning the molecular properties of MitTX might be possible by cross-linking the two moieties, and in general, protein medicinal chemistry studies of MitTx to improve potency and selectivity further would be of great interest.

Numerous questions also need to be addressed regarding the molecular mechanism of MitTx modulation of ASICs including details of how MitTx binds to the channel and how this affects the function. Julius and co-workers do not elaborate on the mechanism by which MitTx evokes agonist-like responses. Here, identification of the binding site for MitTx and its binding mode on ASIC is a pertinent next step. Furthermore, an intriguing question is whether MitTx in fact

acts as a positive modulator; for example, does it shift the proton sensititvy of the receptor so that this can be activated at physiological pH? If so, how does MitTx binding change the properties of the proton sensor? In this context, the recent X-ray structure of a chicken ASIC1 receptor from the lab of Eric Gouaux is a valuable platform to guide future experiments (Figure 1b).[6] The precise understanding of the molecular principles of MitTx modulation of ASICs will provide a structural and mechanistic basis for the rational design of molecules that can antagonize ASICs. The therapeutic potential of targeting ASICs is already established and ASICs are being pursued as drug targets for the treatment of pain.[1] Specifically, two venom proteins, psalmotoxin 1 (PcTX1) and APETx2, which are antagonists of ASIC1a and ASIC3, respectively, are in preclinical studies for the treatment of pain.

The identification of MitTx provides a new and valuable pharmacological tool in studies of the role of ASICs in pain, which inarguably will have important implications for future research in unraveling the molecular pathways of pain.

Received: January 13, 2012
Published online: March 15, 2012

[1] G. F. King, *Expert Opin. Biol. Ther.* **2011**, *11*, 1469–1484.
[2] a) M. J. Caterina, M. A. Schumacher, M. Tominaga, T. A. Rosen, J. D. Levine, D. Julius, *Nature* **1997**, *389*, 816–824; b) D. D. McKemy, W. M. Neuhausser, D. Julius, *Nature* **2002**, *416*, 52–59.
[3] a) J. Siemens, S. Zhou, R. Piskorowski, T. Nakai, E. A. Lumpkin, A. I. Basbaum, D. King, D. Julius, *Nature* **2006**, *444*, 208–212; b) C. J. Bohlen, A. Priel, S. Zhou, D. King, J. Siemens, D. Julius, *Cell* **2010**, *141*, 834–845.
[4] C. J. Bohlen, A. T. Chesler, R. Sharif-Naeini, K. F. Medzihradszky, S. Zhou, D. King, E. E. Sánchez, A. L. Burlingame, A. I. Basbaum, D. Julius, *Nature* **2011**, *479*, 410–414.
[5] R. Doley, R. M. Kini, *Cell. Mol. Life Sci.* **2009**, *66*, 2851–2871.
[6] a) J. Jasti, H. Furukawa, E. B. Gonzales, E. Gouaux, *Nature* **2007**, *449*, 316–323; b) E. B. Gonzales, T. Kawate, E. Gouaux, *Nature* **2009**, *460*, 599–604.

