Toxicon 56 (2010) 1293–1298

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Review

"The King is dead": Checkmating ion channels with tethered toxins

Sebastian Auer, Inés Ibañez-Tallon*

Molecular Neurobiology Group, Department of Neuroscience, Max Delbrück Center for Molecular Medicine, 13125 Berlin, Germany

ARTICLE INFO

Article history:
Received 4 August 2010
Received in revised form 24 September 2010
Accepted 28 September 2010
Available online 7 October 2010

Keywords:
Antagonist
Ion channel
Neuron
Peptide toxin
Receptor
Tethered toxin

ABSTRACT

The quickest possible checkmate in the game of chess requires two moves using a pawn and the queen. Metaphorically speaking, the pawn (a membrane tether) and the queen (a toxin) work together to checkmate an ion channel within a neuronal circuit. This strategy termed "tethered toxin" (t-toxin) is based on the use of genetically encoded peptide toxins that are anchored to the cell-membrane via a glycolipid or transmembrane tether. Because of their mode of action at the cell surface, t-toxins act only on ion channels and receptors of the cell that is expressing the t-toxin, and not on identical receptors present in neighboring cells that do not express the t-toxin. In this mini-review we discuss the design of these genetic tools and their application for cell-specific and temporal manipulation of ion channel-mediated activities *in vivo*.

1. Introduction

There are approximately 160 and 350 genes that encode for channel proteins in insects and mammals, respectively (www.membranetransport.org), illustrating the importance of ion transport for cell communication and function. Given this diversity, it is a challenge to understand how a particular class of ion channel contributes to the function of a given cell network and, more generally, how this information is processed under physiological and pathophysiological conditions. Historically, methods for manipulating neuronal circuits have included electrolytic or mechanical lesions, microinjection of tetrodotoxin or other neurotoxic agents into target areas, and the local administration of anesthetics or other pharmacologic agents. Many of these manipulations, however, are not reversible and frequently disrupt more than one neuronal circuit. Powerful genetic approaches have provided new opportunities for manipulations of ion channels *in vivo*. For example, targeted gene deletion of ion channel subunits or downregulation by siRNAs methodologies can be used to interfere with ion channel function. Yet, since the majority of receptors and ion channels are multimeric, gene deletion of one subunit might be accompanied by compensatory upregulations of closely related receptors that share common subunits or compete for the same binding partners (Akopian et al., 1999; Drago et al., 2003; Inchauspe et al., 2004). The study of ion channels has also advanced with the identification of gene promoters (i.e. Bacterial Artificial Chromosomes (BACs)) that allow cell-type specific manipulations of functionally related neuronal populations (Gong et al., 2002). These mouse models can be used to trace neuronal connections, achieve cell-specific conditional mutagenesis or drive functional changes within circuits by *in vivo* microinjections with viral vectors (Hatten and Heintz, 2005; Luo et al., 2008; Tolu et al., 2010).

Here we describe a genetic approach based on tethered toxins that extends our ability to modify singular ionic currents in specific neurons *in vivo*. Venoms of snakes, scorpions, spiders, sea anemones, and cone snails contain a complex mixture of peptide toxins as a means to capture

* Corresponding author. Tel.: +49 30 9406 3411; fax: +49 30 9406 3433.
E-mail address: ibanezi@mdc-berlin.de (I. Ibañez-Tallon).

0041-0101/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.toxicon.2010.09.016

prey or to protect themselves against predators. Adaptive evolution driven by natural selection has led to strong variations in venom composition providing a vast array of naturally occurring peptide toxins with different affinities for specific ion channel subclasses. The last decade of research on venom peptide toxins has identified an important number of specific inhibitors and modulators of ion channels and receptors. Thus, single venom peptide toxins with characteristic cysteine backbones and selective affinities for voltage-gated sodium (Na_v), calcium (Ca_v), and potassium (K_v) ion channels, and ligand-gated receptors, such as nicotinic acetylcholine receptors (nAChRs), N-methyl-D-aspartate (NMDA) and G-protein coupled receptors (GPCRs) have been identified (for reviews see: (Dutertre and Lewis, 2010; Phui Yee et al., 2004; Terlau and Olivera, 2004; Twede et al., 2009)). Their high specificity makes them ideal tools for deciphering the contribution of ionic currents to neurophysiology, but their activity cannot be restricted to a single cell-population in brain slices or in a living organism and usually requires constant administration (Fig. 1A). To bypass these limitations, we developed genetically encoded tethered toxins (t-toxins) that are bound to the cell surface by membrane tethers and act only on ion channels and receptors of the cell-population that expresses the t-toxin and not on identical receptors present in neighboring cells that do not express the t-toxin (Ibañez-Tallon et al., 2004) (Fig. 1B). In this review, we discuss the development of modular t-toxins with preserved activity and specificity and their application to studies of neurocircuitry. Examples include expression of tethered bungarotoxin constructs for simple cell-specific and genetic manipulation of different nAChR functions *in vivo*, and inducible expression of tethered conotoxins and agatoxins that target specific neuronal Na^+ and Ca^{2+} channels and can be used for reversible suppression of neuronal activity by blockade of action potentials or presynaptic vesicle fusion, respectively. The strength of this approach lies in the impressive functional diversity of the peptide neurotoxins, the simple strategy to harness their potency for use *in vivo*, and its applicability across species for the genetic dissection of ion channels and pathways that influence development and function of the CNS.

2. Modular structure of t-toxins: origin, development and optimization

The tethered toxin concept was triggered by the discovery of naturally occurring cell-membrane bound prototoxins of the lynx1 family (Miwa et al., 1999). *Lynx1*, an evolutionary precursor to snake venom toxins, shares structural characteristics with the three-finger α- and κ-bungarotoxins, which bind tightly to nicotinic acetylcholine receptors (nAChRs) and inhibit their activation. *Lynx1* is a member of the Ly-6/neurotoxin gene family, a group of small proteins, of which some members, including lynx1, are attached to the cell surface by a glycosylphosphatidylinositol (GPI) anchor. Furthermore, they share a unique structure containing 8–10 conserved cysteine residues with a characteristic spacing pattern (Holford et al., 2009). Functional analyses indicate that lynx1, and the closely related molecule lynx2, are not ligands or neurotransmitters, but directly assemble with nAChRs at the cell-membrane and have the capacity to modulate their function in the presence of acetylcholine or nicotine (Ibañez-Tallon et al., 2002; Miwa et al., 2006, 1999; Tekinay et al., 2009). Lynx1 consists of a secretory signal sequence, followed by the sequences encoding the 79 amino acid mature protein and a glycosylphosphatidylinositol (GPI) anchor signal. The first recombinant membrane-bound toxins were designed by replacing lynx1 with the sequences encoding for bungarotoxins or alpha-conotoxins, followed by a short linker and sequences controlling the addition of the lynx1 GPI

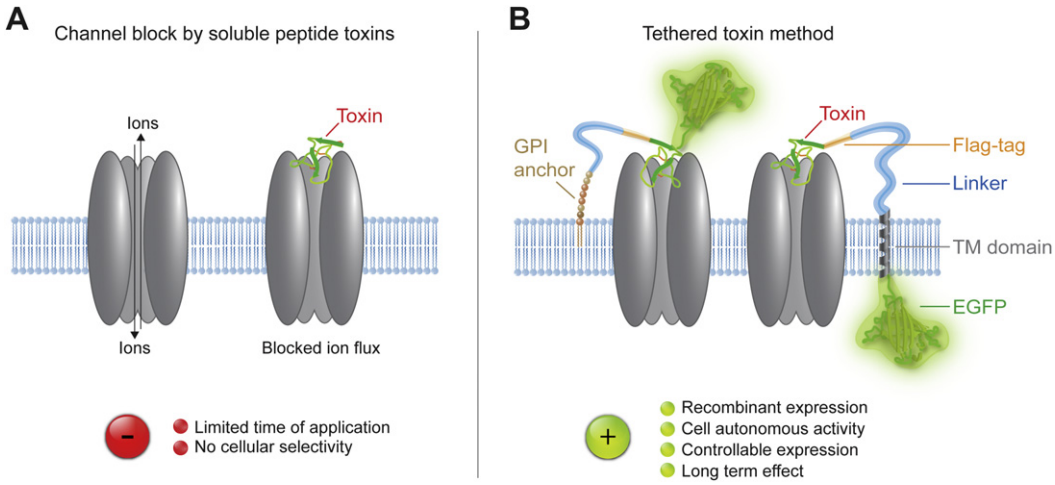

Fig. 1. Illustration of ion channel inhibition by soluble peptide toxins or genetic expression of recombinant tethered toxins (A) Soluble peptide toxins cause instantaneous block of target ion channels, but their use *in vivo* is limited by the accessibility of the brain structure to be targeted, the necessity of constant re-administration and the lack of cellular selectivity within a circuit or cell network (B) Genetically encoded toxins tethered to the membrane via a glycosylphosphatidylinositol (GPI) anchor or a transmembrane (TM) domain allow cell-autonomous block of ion channels and the integration of domains (i.e. Flag or other epitopes, EGFP or other markers) to monitor their long-term expression in targeted cells.

anchor (Ibañez-Tallon et al., 2004). This design directs the toxin peptide to the secretory pathway, where the signal sequence is cleaved and the GPI targeting sequence is substituted by a covalent bond to the GPI, anchoring the peptide to the extracellular side of the plasma membrane of the cell in which it is expressed (Fig. 1B). While this design was optimal to achieve inactivation of nicotine-evoked currents by tethered bungarotoxins and α-conotoxins, other receptor–toxin combinations required the inclusion of a longer flexible linker preceding the GPI signal (Ibañez-Tallon et al., 2004). This was the case for a number of conotoxins which were only functional upon insertion of a flexible linker of $(asn–gly)_n$, which joins the mature toxin molecule to the GPI sequence and provides rotational freedom for the t-toxin to bind within the vestibule of voltage-gated channels. However, experiments varying the length of the linker region of t-GID conotoxin indicated that when the linker exceeds a certain length, the inactivation of nAChRs is incomplete (Holford et al., 2009). Thus tethered toxins with different linker length can be used to study ion channel–toxin interfaces. The t-toxin design has been further optimized by the integration of fluorescent markers and other membrane tethers (i.e. transmembrane domain of the PDGF receptor (Auer et al., 2010) and (Fig. 1B)). These modules have increased the ability to monitor the expression levels and subcellular localization of the recombinant molecules upon genetic delivery into neurons, which are important prerequisites for their use in neurocircuitry. More details on the modular scaffold of t-toxins encompassing linkers, epitopes, fluorescent markers and membrane tethers can be found in Holford et al., 2009. So far, approximately 40 different t-toxins derived from the venom of predatory animals have been cloned, and their activity has been characterized on voltage and ligand-gated ion channels (Holford et al., 2009). Studies *in vivo* have been possible using different genetic approaches to drive their cell-autonomous action. These include transgenesis in zebrafish (Ibañez-Tallon et al., 2004), Drosophila (Wu et al., 2008) and mouse (Stürzebecher et al., 2010), as well as recombinant viral systems (Auer et al., 2010; Hruska et al., 2009). In particular, the possibility to encode t-toxins in viral vectors has further allowed the development of temporal-inducible and Cre recombinase-dependent approaches for regulated and cell-specific expression in the mouse that we will discuss later (Auer et al., 2010). Altogether the fact that this approach combines the extremely high affinity of peptide toxins for blockade of specific ionic currents, the ability to restrict their action by membrane tethering and enhance the working concentration of the toxin close to its point of activity in the plasma membrane, and the flexibility to implement this system with cell-specific and temporal regulated genetic methodologies, makes the t-toxin approach an extremely useful tool for investigating ion channels *in vivo*.

3. Cell-autonomous inhibition of nicotinic acetylcholine receptors with t-toxins

In mammals, 16 subunits of nAChR (α1–7, α9–10, β1–4, γ, δ, ε) assemble in hetero- and homopentameric receptors with distinct structural and pharmacological properties (Le Novere and Changeux, 1995; Leonard and Bertrand, 2001). The muscle nicotinic receptor mediates synaptic transmission at the neuromuscular junction, while the neuronal forms of the receptor can be found both post-synaptically and presynaptically in the central and peripheral nervous system. Early studies, in 1970, took advantage of the irreversible binding of α-bungarotoxin to the cholinergic receptor at the motor endplate to characterize and purify it (Changeux et al., 1970). Since then a great number of other snake venom toxins and α-conotoxins with specific affinities for different nAChR combinations have been identified (Lewis, 2009; Terlau and Olivera, 2004; Tsetlin and Hucho, 2004). Given that the vast majority of peptide toxins bind to nAChR combinations and not to single subunits, with few exceptions, toxins have been used as a means to localize and functionally identify the pentamer combinations present in different neuronal and non-neuronal populations. For instance, α-bungarotoxin, but not κ-bungarotoxin, binds to the muscle α1β1γδ and α7 pentamers, and not to other neuronal nAChR. Other examples include α-conotoxins such as PnIB, which blocks homopentameric α7 nAChRs (Quiram et al., 2000), and MII, which inhibits heteromeric nAChRs made of combinations of α3 or α6 subunits with the β2 subunit (Salminen et al., 2005). We demonstrated that these bungarotoxins and conotoxins retained their selective antagonistic properties against specific nAChR combinations when tethered to the cell-membrane (Ibañez-Tallon et al., 2004). To further test their efficacy *in vivo*, we generated transgenic zebrafish in which expression of either tethered alpha (t-α-Btx) or kappa bungarotoxin (t-κ-Btx) was driven by a cell-type specific promoter to muscle fibers (Table 1). These experiments demonstrated targeted cell-autonomous elimination of nAChR function in muscle fibers expressing t-α-Btx but not t-κ-Btx, consistent with the specific activity of the two toxins. Functionally, the t-α-Btx mediated inhibition of nAChR currents resulted in silencing of neuromuscular neurotransmission without perturbing synapse formation (Ibañez-Tallon et al., 2004). In similar studies, we used t-α-Btx to inhibit α7 receptors *in vivo* in chick ciliary ganglia with retroviral vectors (Table 1). By blocking the intracellular calcium influx mediated by ganglionic α7 receptors with t-α-Btx, these studies demonstrated the critical role of this receptor type in the early programmed cell-death that occurs during development of the autonomic nervous system (Hruska et al., 2007). These findings illustrate also some of the possible uses of t-toxins to dissect the contribution of nAChR combinations to cell network functions in the living organism.

4. Silencing neurotransmitter release by inhibition of voltage-gated calcium channels with t-toxins

At presynaptic nerve terminals, two voltage-gated calcium channels $Ca_v2.1$ and $Ca_v2.2$ play an essential and joint role in the conversion of electro-chemical signals by coupling the arriving presynaptic action potential to neurotransmitter release. The membrane depolarization caused by the action potential triggers the opening of

Table 1
Overview of peptide toxins used in tethered toxin studies.

Toxin name	Species	Length [aa]	Tethered toxin construct	Activity	Reference
Agatoxin IVA	*Agenelopsis aperta*	48	Venus-flag-linker-AgaIVA-GPI anchor	Inhibition of $Ca_v2.1$ in neuronal culture and in mice *in vivo*	Auer et al., Nat Meth, 2010
Bungarotoxin	*Bungarus multicinctus*	74	αBgtx-linker-GPI anchor	Inhibition of α1β1γδ and α7nAChR in *Xenopus laevis* oocytes, zebrafish and chick ciliary ganglia	Ibañez-Tallon et al., Neuron, 2004
δ-ACTX-Hv1a	*Hadronyche versuta*	42	δ-ACTX-Hv1a-GPI anchor	Inhibition of Na_v channel inactivation in *Drosophila melanogaster*	Hruska et al., J.Neurosci. 2007 Wu et al., PLoS Biol, 2008
GID	*Conus geographus*	19	GID-flag-linker-TM domain-EGFP	Inhibition of α7 and partial block of α3β4 nAChR's in *Xenopus laevis* oocytes	Holford et al., Front Mol Neurosci, 2009
MVIIA	*Conus magus*	25	MVIIA-flag-linker-TM domain-EGFP	Inhibition of $Ca_v2.2$ in neuronal culture and in mice *in vivo*	Auer et al., Nat Meth, 2010
MrVIA	*Conus marmoreus*	31	MrVIA-flag-linker-GPI anchor	Partial inhibition of Nav1.8 in DRG culture and in mice *in vivo*	Stürzebecher et al., J Physiol, 2010

voltage-gated $Ca_v2.1$ and $Ca_v2.2$ channels. The resulting Ca^{2+} influx into the presynapse then enables the multimeric vesicle fusion machinery to fuse neurotransmitter (NT) filled vesicles with the synaptic membrane, thereby releasing NT to the synaptic cleft (Catterall and Few, 2008) (Fig. 2). As the NT release is proportional to the third or fourth power of Ca^{2+} influx, a 2-fold change in presynaptic Ca^{2+} influx results in an 8–16-fold change in NT exocytosis (Zucker and Regehr, 2002). Thus, regulation of presynaptic calcium channels is an efficient way to control synaptic transmission. Besides determining distinct neuronal connections and their physiological functions, controlling the activity of these channels could enable detailed studies of contributions of individual channels to neuronal circuits. With these aims in view, we generated recombinant t-toxins able to block $Ca_v2.1$ and $Ca_v2.2$ channels by integration of the ω-agatoxins AgaIIIA and AgaIVA, as well as ω-conotoxins MVIIA and MVIIC. We found that these t-toxins were well expressed in cultured mammalian cells, primary cultures of hippocampal neurons and in neurons of mice injected with lentivirus encoding t-toxins. The capability to block one or both channels was then confirmed by electrophysiological recordings of HEK293-$Ca_v2.2$ cells and rat hippocampal neurons *in vitro,* and by behavioral analysis of the lentivirus injected mice (Auer et al., 2010). Overall, we found that both AgaIVA and MVIIA incorporating t-toxins were as effective as the soluble toxins in fully blocking their respective target channels in a cell. Moreover, mice stereotactically injected with lentiviruses encoding these two t-toxins displayed a robust rotational phenotype, caused by an imbalance in motor coordination resulting from the inhibition of the dopaminergic nigro-striatal pathway by the action of both t-toxins. This first proof of function of the validity of using virally encoded t-toxins *in vivo* in the mouse demonstrates the general applicability of the t-toxin strategy as a straightforward method that can be used to block $Ca_v2.1$ and $Ca_v2.2$ calcium currents, resulting in cell-specific and cell-autonomous silencing of neurotransmission. These data also suggests that both t-toxins could be broadly applied for long-term inhibition of $Ca_v2.1$ and

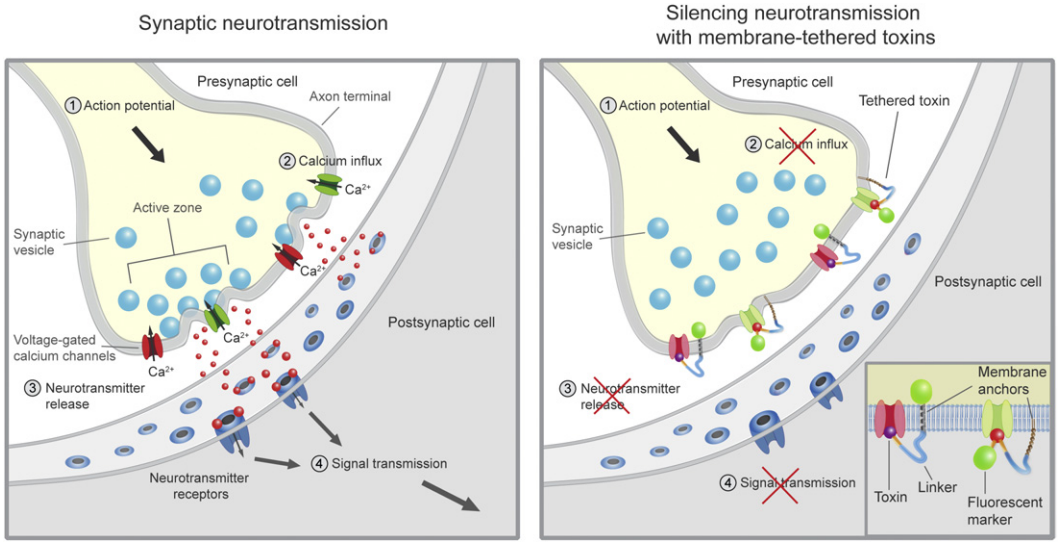

Fig. 2. Schematic representation of tethered-toxin mediated silencing of neurotransmission by inhibition of voltage-gated calcium channels $Ca_v2.1$ and $Ca_v2.2$ at the presynapse. The block of calcium influx upon binding and inactivation of the calcium channels by the t-toxins prevents the vesicle fusion machinery from releasing neurotransmitter to the synaptic cleft, thereby interrupting neurotransmission.

$Ca_v2.2$ channels individually or simultaneously to allow the characterization of the channel contribution to physiological functions and circuit analyses in a wide variety of species.

5. Selective inactivation or inhibition of inactivation of sodium voltage-gated currents by t-toxins

Given the key role of voltage-gated sodium channels (VGSC, Na_v) in the initiation and propagation of electrical signals in excitable tissues, it is not surprising that many venom peptides specifically target several VGSC isoforms. Venom toxins act at six or more distinct receptor sites on the channel protein. Although a few polypeptide toxins physically block the pore and prevent sodium conductance, the majority of toxins alter channel gating by voltage-sensor trapping through binding to extracellular receptor sites (Catterall et al., 2007). For example, MrVIA and MrVIB μO-conotoxins seem to bind near the pore of specific sodium channel types expressed in the heart ($Na_v1.5$), muscle ($Na_v1.4$) and peripheral nociceptive neurons ($Na_v1.8$) and partially block ionic influx (Leipold et al., 2007), while δ-atracotoxins or β-scorpion toxins act by inhibiting inactivation of activated channels. In this manner, the venom induces spontaneous repetitive firing or tetanus-like burst of action potentials followed by plateau potentials, block of neuronal transmission and paralysis of the prey. The tethered toxin strategy has been used for *in vivo* studies using sodium toxins with these two opposed types of activities (Table 1). One study has employed δ-atracotoxin Hv1a with the GAL4-UAS transgenic system in Drosophila to alter the rhythmicity of circadian neurons (Wu et al., 2008), while another study applied mouse BAC transgenesis to target nociceptive neurons and interfere with pain perception (Auer et al., 2010; Stürzebecher et al., 2010). Preferential inactivation of $Na_v1.8$ channels in nociceptors by the t-MrVIA toxin, without compensation by $Na_v1.7$ as it occurs in $Na_v1.8$ knockout mice, has shown the essential role of $Na_v1.8$ channels in cold perception (Stürzebecher et al., 2010). This is interesting, since this channel isoform has been the subject of research over many years as a target for the treatment of pain, and its relatively depolarized activation voltage dependence may allow it to continue to function when nociceptive neurons are depolarized, for example in the cold (Baker and Wen, 2010). Thus these studies using t-toxins taken together with previous studies on cold-sensitive neurons (Carr et al., 2002; Zimmermann et al., 2007) indicate that $Na_v1.8$ is the likely channel to encode for cold perception. As research on venom peptide toxins and synthetic peptide ligands progresses, it would be interesting to identify and test a similar antagonist of $Na_v1.6$ channels expressed in central neurons for neurocircuitry studies.

6. Further applications and developments

In addition to the broad range of possible applications of tethered toxins in basic research, this strategy also represents a potential new avenue for the development of genetic therapies for chronic diseases caused by malfunction of ion channels and peptide ligand receptors. Several human disorders that affect nervous system functions have been traced to mutations in genes encoding ion channels or regulatory proteins (George, 2005). These disorders, referred to as channelopathies, could potentially be targeted by the t-toxin strategy when the disorder is caused by ion channel hyperactivity. For instance, ω-conotoxin MVIIA (commercialized as Prialt) is already used to treat severe chronic pain in humans (Miljanich, 2004; Staats et al., 2004; Zamponi et al., 2009). However, its use requires the implantation of intrathecal microinfusion pumps, which allow constant administration of the soluble drug to minimize the still substantial side effects due to block of CNS channels. Genetic targeting of t-MVIIA to nociceptive neurons in transgenic mice has shown that these mice are protected from inflammatory and neuropathic pain (Auer et al., 2010), suggesting that this strategy could be a viable alternative therapy to avoid uncontrolled diffusion of the injected toxin and the necessity for repetitive treatments. Further examples of hyperactive disorders include gain-of-function mutations in $Ca_v2.1$ calcium channels, linked to familial hemiplegic migraine type 1 (FHM-1) (Ophoff et al., 1996; Tottene et al., 2009), and mutations in neuronal nAChRs associated to autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) (Klaassen et al., 2006; Steinlein et al., 1995). Here, one potential application would be to use t-toxins in corresponding mouse mutant models to dissect the circuitry of the diseases in a cell-specific manner.

7. Concluding remarks

The physiological properties of all cells depend upon the activities of ion channels and receptors expressed at the cell surface. Since the panoply of peptide toxins targeting these molecules is only now being explored, the tethered toxin strategy offers the potential for experimental studies that will greatly expand our knowledge of molecules, cells and circuits *in vivo*. In particular, the combination of t-toxins, transgenic and viral methodologies for cell-specific gene expression, and inducible and reversible strategies provides a new genetic-based method to map functional neuronal circuits and synaptic changes *in vivo*. The ability to successfully activate or inactivate specific currents within neural circuits in response to the inducible expression of cell and ion channel-selective tethered toxins will be a powerful technology for understanding the function of the targeted neurons in the awake behaving animal, and therefore will be a critical first step in the effort to create integrated functional connectivity maps of the mammalian nervous system.

Acknowledgements

This work was supported by Helmholtz Association (31-002) and Sonderforschungsbereich (SFB 665).

Conflict of interest

The authors declare that there are no conflicts of interest.

References

Akopian, A.N., Souslova, V., England, S., Okuse, K., Ogata, N., Ure, J., Smith, A., Kerr, B.J., McMahon, S.B., Boyce, S., Hill, R., Stanfa, L.C., Dickenson, A.H., Wood, J.N., 1999. The tetrodotoxin-resistant sodium channel SNS has a specialized function in pain pathways. Nat. Neurosci. 2, 541–548.

Auer, S., Sturzebecher, A.S., Juttner, R., Santos-Torres, J., Hanack, C., Frahm, S., Liehl, B., Ibanez-Tallon, I., 2010. Silencing neurotransmission with membrane-tethered toxins. Nat. Methods 7, 229–236.

Baker, M.D., Wen, H., 2010. Tethered-toxin debut gets cold reception. J. Physiol. 588, 1663.

Carr, R.W., Pianova, S., Brock, J.A., 2002. The effects of polarizing current on nerve terminal impulses recorded from polymodal and cold receptors in the guinea-pig cornea. J. Gen. Physiol. 120, 395–405.

Catterall, W.A., Cestele, S., Yarov-Yarovoy, V., Yu, F.H., Konoki, K., Scheuer, T., 2007. Voltage-gated ion channels and gating modifier toxins. Toxicon 49, 124–141.

Catterall, W.A., Few, A.P., 2008. Calcium channel regulation and presynaptic plasticity. Neuron 59, 882–901.

Changeux, J.P., Kasai, M., Lee, C.Y., 1970. Use of a snake venom toxin to characterize the cholinergic receptor protein. Proc. Natl. Acad. Sci. U S A 67, 1241–1247.

Drago, J., McColl, C.D., Horne, M.K., Finkelstein, D.I., Ross, S.A., 2003. Neuronal nicotinic receptors: insights gained from gene knockout and knockin mutant mice. Cell. Mol. Life Sci. 60, 1267–1280.

Dutertre, S., Lewis, R.J., 2010. Use of venom peptides to probe ion channel structure and function. J. Biol. Chem. 285, 13315–13320.

George, A.L., 2005. Inherited disorders of voltage-gated sodium channels. J. Clin. Invest. 115, 1990–1999.

Gong, S., Yang, X.W., Li, C., Heintz, N., 2002. Highly efficient modification of bacterial artificial chromosomes (BACs) using novel shuttle vectors containing the R6Kgamma origin of replication. Genome Res. 12, 1992–1998.

Hatten, M.E., Heintz, N., 2005. Large-scale genomic approaches to brain development and circuitry. Annu. Rev. Neurosci. 28, 89–108.

Holford, M., Auer, S., Laqua, M., Ibañez-Tallon, I., 2009. Manipulating neuronal circuits with endogenous and recombinant cell-surface tethered modulators. Front. Mol. Neurosci. 2, 21.

Hruska, M., Ibañez-Tallon, I., Nishi, R., 2007. Cell-autonomous inhibition of alpha 7-containing nicotinic acetylcholine receptors prevents death of parasympathetic neurons during development. J. Neurosci. 27, 11501–11509.

Hruska, M., Keefe, J., Wert, D., Tekinay, A.B., Hulce, J., Ibanez-Tallon, I., Nishi, R., 2009. Prostate stem cell antigen is an endogenous lynx1-like prototoxin that antagonizes alpha7 containing nicotinic receptors and prevents programmed cell death of parasympathetic neurons. J. Neurosci..

Ibañez-Tallon, I., Miwa, J.M., Wang, H.L., Adams, N.C., Crabtree, G.W., Sine, S.M., Heintz, N., 2002. Novel modulation of neuronal nicotinic acetylcholine receptors by association with the endogenous prototoxin lynx1. Neuron 33, 893–903.

Ibañez-Tallon, I., Wen, H., Miwa, J.M., Xing, J., Tekinay, A.B., Ono, F., Brehm, P., Heintz, N., 2004. Tethering naturally occurring peptide toxins for cell-autonomous modulation of ion channels and receptors in vivo. Neuron 43, 305–311.

Inchauspe, C.G., Martini, F.J., Forsythe, I.D., Uchitel, O.D., 2004. Functional Compensation of P/Q by N-type channels blocks short-term plasticity at the calyx of held presynaptic terminal. J. Neurosci. 24, 10379–10383.

Klaassen, A., Glykys, J., Maguire, J., Labarca, C., Mody, I., Boulter, J., 2006. Seizures and enhanced cortical GABAergic inhibition in two mouse models of human autosomal dominant nocturnal frontal lobe epilepsy. Proc. Natl. Acad. Sci. U S A 103, 19152–19157.

Le Novere, N., Changeux, J.P., 1995. Molecular evolution of the nicotinic acetylcholine receptor: an example of multigene family in excitable cells. J. Mol. Evol. 40, 155–172.

Leipold, E., DeBie, H., Zorn, S., Borges, A., Olivera, B.M., Terlau, H., Heinemann, S.H., 2007. muO conotoxins inhibit NaV channels by interfering with their voltage sensors in domain-2. Channels (Austin) 1, 253–262.

Leonard, S., Bertrand, D., 2001. Neuronal nicotinic receptors: from structure to function. Nicotine Tob. Res. 3, 203–223.

Lewis, R.J., 2009. Conotoxins: molecular and therapeutic targets. Prog. Mol. Subcell. Biol. 46, 45–65.

Luo, L., Callaway, E.M., Svoboda, K., 2008. Genetic dissection of neural circuits. Neuron 57, 634–660.

Miljanich, G.P., 2004. Ziconotide: neuronal calcium channel blocker for treating severe chronic pain. Curr. Med. Chem. 11, 3029–3040.

Miwa, J., Stevens, T., King, S., Caldarone, B., Ibaneztallon, I., Xiao, C., Fitzsimonds, R., Pavlides, C., Lester, H., Picciotto, M., 2006. The prototoxin lynx1 acts on nicotinic acetylcholine receptors to balance neuronal activity and survival in vivo. Neuron 51, 587–600.

Miwa, J.M., Ibanez-Tallon, I., Crabtree, G.W., Sánchez, R., Sali, A., Role, L.W., Heintz, N., 1999. lynx1, an endogenous toxin-like modulator of nicotinic acetylcholine receptors in the mammalian CNS. Neuron 23, 105–114.

Ophoff, R.A., Terwindt, G.M., Vergouwe, M.N., van Eijk, R., Oefner, P.J., Hoffman, S.M., Lamerdin, J.E., Mohrenweiser, H.W., Bulman, D.E., Ferrari, M., Haan, J., Lindhout, D., van Ommen, G.J., Hofker, M.H., Ferrari, M.D., Frants, R.R., 1996. Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca2+ channel gene CACNL1A4. Cellule 87, 543–552.

Phui Yee, J.S., Nanling, G., Afifiyan, F., Donghui, M., Siew Lay, P., Armugam, A., Jeyaseelan, K., 2004. Snake postsynaptic neurotoxins: gene structure, phylogeny and applications in research and therapy. Biochimie 86, 137–149.

Quiram, P.A., McIntosh, J.M., Sine, S.M., 2000. Pairwise interactions between neuronal alpha(7) acetylcholine receptors and alpha-conotoxin PnIB. J. Biol. Chem. 275, 4889–4896.

Salminen, O., Whiteaker, P., Grady, S.R., Collins, A.C., McIntosh, J.M., Marks, M.J., 2005. The subunit composition and pharmacology of alpha-Conotoxin MII-binding nicotinic acetylcholine receptors studied by a novel membrane-binding assay. Neuropharmacology 48, 696–705.

Staats, P.S., Yearwood, T., Charapata, S.G., Presley, R.W., Wallace, M.S., Byas-Smith, M., Fisher, R., Bryce, D.A., Mangieri, E.A., Luther, R.R., Mayo, M., McGuire, D., Ellis, D., 2004. Intrathecal ziconotide in the treatment of refractory pain in patients with cancer or AIDS: a randomized controlled trial. J. Am. Med. Assoc. 291, 63–70.

Steinlein, O.K., Mulley, J.C., Propping, P., Wallace, R.H., Phillips, H.A., Sutherland, G.R., Scheffer, I.E., Berkovic, S.F., 1995. A missense mutation in the neuronal nicotinic acetylcholine receptor alpha 4 subunit is associated with autosomal dominant nocturnal frontal lobe epilepsy. Nat. Genet. 11, 201–203.

Stürzebecher, A.S., Hu, J., Smith, E.S., Frahm, S., Santos-Torres, J., Kampfrath, B., Auer, S., Lewin, G.R., Ibanez-Tallon, I., 2010. An in vivo tethered toxin approach for the cell-autonomous inactivation of voltage-gated sodium channel currents in nociceptors. J. Physiol. 588, 1695–1707.

Tekinay, A.B., Nong, Y., Miwa, J.M., Lieberam, I., Ibanez-Tallon, I., Greengard, P., Heintz, N., 2009. A role for LYNX2 in anxiety-related behavior. Proc. Natl. Acad. Sci. 106, 4477–4482.

Terlau, H., Olivera, B.M., 2004. Conus Venoms: a rich source of novel ion channel-targeted peptides. Physiol. Rev. 84, 41–68.

Tolu, S., Avale, M.E., Nakatani, H., Pons, S., Parnaudeau, S., Tronche, F., Vogt, A., Monyer, H., Vogel, R., de Chaumont, F., Olivo-Marin, J.C., Changeux, J.P., Maskos, U., 2010. A versatile system for the neuronal subtype specific expression of lentiviral vectors. FASEB J. 24, 723–730.

Tottene, A., Conti, R., Fabbro, A., Vecchia, D., Shapovalova, M., Santello, M., van den Maagdenberg, A.M.J.M., Ferrari, M.D., Pietrobon, D., 2009. Enhanced excitatory transmission at cortical synapses as the basis for facilitated spreading depression in Ca(v)2.1 knockin migraine mice. Neuron 61, 762–773.

Tsetlin, V.I., Hucho, F., 2004. Snake and snail toxins acting on nicotinic acetylcholine receptors: fundamental aspects and medical applications. FEBS. Lett. 557, 9–13.

Twede, V.D., Miljanich, G., Olivera, B.M., Bulaj, G., 2009. Neuroprotective and cardioprotective conopeptides: an emerging class of drug leads. Curr. Opin. Drug Discov. Devel. 12, 231–239.

Wu, Y., Cao, G., Pavlicek, B., Luo, X., Nitabach, M.N., 2008. Phase coupling of a circadian neuropeptide with rest/activity rhythms detected using a membrane-tethered spider toxin. PLoS Biol. 6, e273.

Zamponi, G.W., Lewis, R.J., Todorovic, S.M., Arneric, S.P., Snutch, T.P., 2009. Role of voltage-gated calcium channels in ascending pain pathways. Brain Res. Rev. 60, 84–89.

Zimmermann, K., Leffler, A., Babes, A., Cendan, C.M., Carr, R.W., Kobayashi, J., Nau, C., Wood, J.N., Reeh, P.W., 2007. Sensory neuron sodium channel Nav1.8 is essential for pain at low temperatures. Nature 447, 855–858.

Zucker, R.S., Regehr, W.G., 2002. Short-term synaptic plasticity. Annu. Rev. Physiol. 64, 355–405.