

Insect background sodium channel as a new target for scorpion alpha toxin[†]

Maria Stankiewicz,¹ Françoise Grolleau,² Hervé Rochat,³ Eliahu Zlotkin,⁴ Marcel Pelhate² and Bruno Lapied^{2*}

¹Laboratory of Biophysics, Institute of General and Molecular Biology, N Copernicus University, 87–100 Torun, Poland

²Laboratoire de Neurophysiologie, UPRES EA 2647, Université d'Angers, rue Haute-de-Reculée, F-49045 Angers cedex, France

³Laboratoire de Biochimie – Ingénierie des Protéines, UMR 6560 CNRS - Université de la Méditerranée, I F R Jean Roche, Bd Pierre Dramard, F-13916, Marseille, cedex 20, France

⁴Institute of Life Sciences, Hebrew University of Jerusalem, Jerusalem 91904, Israel

Abstract: The effects of an anti-insect scorpion alpha toxin, Lqh α IT, isolated from the venom of the Israeli scorpion *Leiurus quinquestriatus hebraeus* was studied on isolated adult dorsal unpaired median (DUM) neurones isolated from the cockroach *Periplaneta americana* terminal abdominal ganglion. Using the cell-attached patch-clamp configuration, a new type of sodium channel, called background sodium channel (bNa), was recently characterized. At -50 mV, the channel activity was observed as unclustered brief single openings. For hyperpolarized steady-state holding potential (-100 mV) the patches contained large unitary current steps, appearing generally in bursts. The open probability (P_o) calculated at -50 mV was low ($0.008 (\pm 0.004)$, $n = 5$) and displayed a typical bell-shaped voltage dependence. Lqh α IT (10^{-8} M) altered the bNa activity in a time-dependent manner. At -50 mV the channel activity appeared in bursts. P_o calculated at -50 mV was about 20 times greater than P_o calculated in controls and also showed bell-shaped voltage dependence. At 10^{-7} M, Lqh α IT induced longer silent periods interrupted by bursts of increased channel activity. Whole-cell recordings revealed that 10^{-7} M Lqh α IT transformed regular beating DUM neurone pacemaker activity into a rhythmic bursting. In this paper we demonstrate, for the first time, that bNa is a new target for anti-insect scorpion toxin.

© 1999 Society of Chemical Industry

Keywords: background Na channels; scorpion alpha toxin; *Periplaneta americana*

1 INTRODUCTION

Rapid paralysis or knock-down following application of toxins or insecticides is the result of disruption of normal functioning of voltage-dependent sodium channels in insects.^{1,2} It is thought that sodium channels are the primary site of action because of their highly conserved function.

Three groups of peptide neurotoxins derived from scorpion venoms exhibit a preferential higher activity on insects than on mammals.^{3–5} Among them the scorpion alpha-toxin Lqh α IT, from *Leiurus quinquestriatus hebraeus*, like other classical alpha toxins, slows down inactivation of the voltage-dependent sodium current in the cockroach giant axon.⁶ Recently, another type of sodium channel activity has been characterized in adult neurosecretory dorsal unpaired median (DUM) neurones, isolated from the terminal abdominal ganglion (TAG) of the cockroach *Periplaneta americana* L.⁷ These channels, named

background sodium channels, show some electrophysiological characteristics different from those described for the classical voltage-dependent sodium channels expressed in the same preparation.⁸ It has been suggested that the DUM neurone background sodium channels could be involved in driving the membrane potential to threshold for action potential generation.⁹

In this paper we report an investigation with the action of Lqh α IT on the background sodium channels of cockroach DUM neurones. In particular, we were interested in its specific mode of action on these channels as a potential new target site for insecticides and its effect on beating pacemaker activity.

2 EXPERIMENTAL METHODS

Experiments were performed on DUM neurone cell bodies isolated from the midline of the TAG of the

* Correspondence to: Bruno Lapied, Laboratoire de Neurophysiologie, UPRES EA 2647, Université d'Angers, rue Haute-de-Reculée F-49045 Angers cedex, France

E-mail: Bruno.Lapied@univ.angers.fr

[†] Based on a poster presented at 'Neurotox '98' organised by DJ Beadle, IS Blagborough, MG Ford, R Greenwood, DJ Ray and PN Usherwood on behalf of the Physicochemical and Biophysical Panel of the SCI Pesticides Group and held at St Catherine's College, Oxford, on 28–31 July 1998

(Received 10 November 1998; revised version received 10 May 1999; accepted 30 June 1999)

nerve cord of adult male cockroaches, *P americana*, taken from our stock colonies maintained at 29°C with a photoperiod of 12h light–12h dark. Isolation of adult DUM neurone cell bodies was performed under sterile conditions using enzymatic digestion and mechanical dissociation of the median parts of the TAG from the nerve cord of the cockroach as previously described.^{9,10}

2.1 Single channel recording

Single background sodium channel activity was recorded using the cell-attached configuration of the patch-clamp technique.¹¹ Patch pipettes were pulled from borosilicate glass capillary tubes (Clark Electromedical Instruments, Reading, UK) and heat-polished to 2–5 MΩ when filled with solution (see Section 2.3). Patch pipettes were coated with Sylgard (Dow Corning Corporation, Midland, USA) to reduce stray capacitance. The liquid junction potential between bath and internal pipette solution was always corrected before the formation of a gigaohm seal (>30 GΩ). Current was recorded using an Axopatch 200A (Axon Instruments, Foster City, CA) amplifier, filtered at 2 kHz. These signals were stored on-line on the hard disk of an IBM Pentium 100 computer connected to a 125-kHz labmaster DMA data acquisition system (TL-1-125 interface, Axon Instruments). The pClamp package (version 6.03, Axon Instruments) was used for data acquisition and analysis (sampling frequency 12.5 kHz). Transitions from closed to open states were determined by a 50% threshold detection method.

2.2 Whole-cell recording

The whole cell patch-clamp technique was used to record spontaneous action potentials and membrane potential. Patch electrodes had resistances ranging from 1 to 3 MΩ when filled with the pipette solution (Section 2.3). Action potentials were displayed on a digital oscilloscope (310, Nicolet Instrument Corporation, Madison, WI, USA) and stored on a Digital Tape Recorder 1202 (Biologic, Claix, France) for subsequent off-line analysis.

2.3 Solutions

For single channel recording, the pipette solution contained (mM): sodium chloride, 200; calcium chloride, 0.5; tetraethylammonium (TEA) chloride, 10; Hepes, 10; pH was adjusted to 7.4 with sodium hydroxide. During formation of the seal, the DUM neurone cell bodies were initially superfused with an external saline containing (mM): sodium chloride, 100; TEA-Cl, 100; potassium chloride, 3.1; calcium chloride, 2; magnesium chloride, 7; cadmium chloride, 1; 4-aminopyridine, 4; Hepes, 10; pH was adjusted to 7.4 with TEAOH. Once the seal was established, a high-potassium external solution was used to lower the membrane potential to close to 0 mV. Thus the command potentials applied during cell-attached recordings were easily converted to patch

potentials. This solution contained (mM): potassium aspartate, 170; sodium chloride, 30; magnesium chloride, 4; EGTA, 5; Hepes, 20; pH was adjusted to 7.4 with potassium hydroxide. For whole-cell recording, the bathing solution contained (mM): sodium chloride, 200; potassium chloride, 3.1; calcium chloride, 5; magnesium chloride, 4; Hepes, 10; pH was adjusted to 7.4 with sodium hydroxide. The patch electrode was filled with a solution of the following composition (mM): potassium chloride, 170; sodium chloride, 15; ATP-Mg, 3; EGTA, 10; calcium chloride, 0.5; Hepes, 10; pH was adjusted to 7.4 with potassium hydroxide. All compounds were purchased from Sigma Chemicals (L'isle d'Abeau Chesnes, France). The scorpion alpha toxin LqhαIT isolated from the venom of the Israeli scorpion *L quinquestratus hebraeus*³ was dissolved in the saline at the final concentration just before use. Experiments were carried out at room temperature (21 °C). Data, when quantified, were expressed as mean (± SEM).

3 RESULTS

As illustrated in Fig 1A, DUM neurone background sodium channel activity recorded in control preparations at a steady-state holding potential of –50 mV, occurred as unclustered, brief single openings with a mean amplitude of –3.93 (±0.21) pA (*n*=5). The background channel, whose main biophysical properties have already been characterized,⁷ has a very low open probability (*P*_o, 0.008 (±0.004; *n*=5) which displays a bell-shaped voltage dependence (Fig 2A).

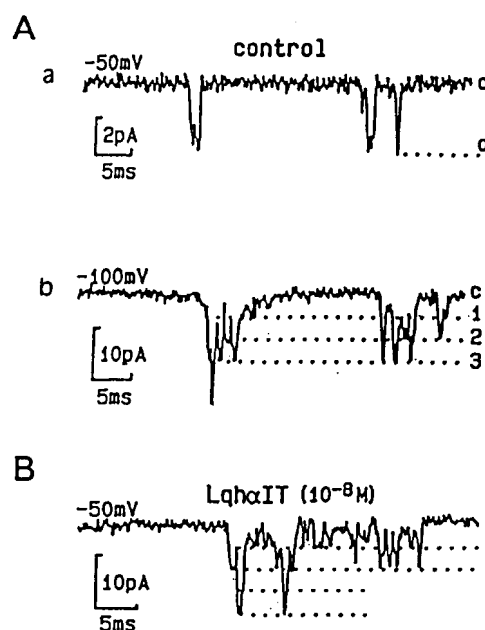


Figure 1. A(a, b): Background sodium channel currents (recorded under the cell-attached patch-clamp configuration) of adult DUM cell body recorded at two steady-state holding potentials indicated above each trace. The sample frequency is 12.5 kHz. C: closed state, O: 1, 2, 3: open current levels. B: Example trace of inward sodium current recorded at a holding potential of –50 mV, after 6 min of toxin application with a patch pipette containing 10⁻⁸ M LqhαIT.

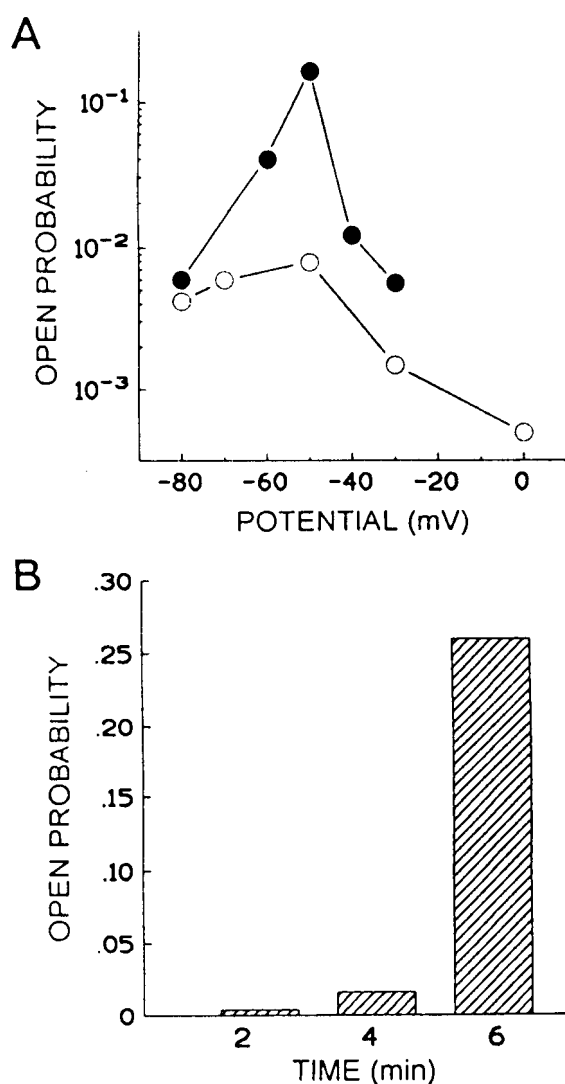


Figure 2. A: Graph illustrating the voltage dependence of the open probability (P_o) of background sodium channel calculated in control conditions (\circ , $n=5$) and after 10^{-8} M Lqh α IT treatment (\bullet , $n=4$), plotted as a function of the steady-state holding potentials. B: Histogram obtained from a typical experiment illustrating the time-dependent effect of 10^{-8} M Lqh α IT on the P_o calculated at a steady-state holding potential of -50 mV, 2, 4 and 6 min after toxin application.

P_o is maximum at -50 mV (corresponding to the resting membrane potential of DUM neurones)⁹ and decreases for lower and higher steady-state holding potentials. Steady-state hyperpolarization to -100 mV, for instance, transforms channel activity into large unitary current steps, due to superimposed channel activity (Fig 1Ab). In this case, the channel activity appears in bursts with rapid flickering between different open and closed states.

3.1 Effect of Lqh α IT on background sodium channel

Figure 1B illustrates the effect of Lqh α IT on DUM neurone background sodium current recorded at -50 mV. Lqh α IT (10^{-8} M) applied on the external side of the membrane patch, induced a time-depen-

dent effect (Fig 2B). During the first two minutes, Lqh α IT did not modify the behaviour of the background sodium channel. After four minutes, channel openings occurred more frequently (Fig 2B) and after five to six minutes of 10^{-8} M toxin application, the background sodium channel activity occurred in irregular bursts, which were absent at -50 mV in control preparations. This bursting activity was separated by short silent periods during which isolated brief single events were detected (Fig 1B). Within a burst, the channel activity fluctuated between the closed state and up to five open current levels. The effect of Lqh α IT on background sodium channel has been examined further to determine whether the toxin altered the P_o or the voltage dependence of P_o . As illustrated in Fig 2A, 10^{-8} M Lqh α IT increased the channel open probability (P_o). At -50 mV, for example, P_o was about 20 times greater (from 0.008 to 0.16) than the P_o determined in the control. It is interesting to note that the bell-shaped voltage dependence of P_o was not altered during Lqh α IT treatment and declined for lower and higher steady-state holding potential. The maximum P_o remained at -50 mV (mean value of $P_o=0.17 (\pm 0.06)$, $n=4$). It should be noted that 10^{-8} M Lqh α IT did not modify the distribution of channel open time, ($0.22 (\pm 0.02)$ ms; $n=4$) calculated at -50 mV, compared with the value found in control conditions.⁷

Higher concentration of Lqh α IT (10^{-7} M) dramatically altered the background channel activity. The bursts of increased channel activity were more condensed and separated by longer (>3 s) silent periods than with a Lqh α IT concentration 10 times lower (Fig 3A compared to Fig 1B). This was confirmed by the scatter plot of current amplitude *versus* time (Fig 3B) indicating a heterogeneous distribution for the duration of the recording. In parallel, the increased P_o was not homogeneous and constant during the recording but appeared in bursts of high P_o (Fig 3C). This demonstrates the close relationship between bursts of increased channel activity and the P_o measured at -50 mV.

3.2 Effect of Lqh α IT on beating pacemaker activity

We have also studied the physiological consequences of the Lqh α IT-modified background sodium channels on the beating pacemaker activity of DUM neurones. Under control conditions, TAG DUM neurones are known to fire repetitive spontaneous action potentials at regular intervals.⁹ Figure 4A illustrates typical spontaneous action potentials (6–10 Hz in frequency) recorded from whole cells. Bath application of 10^{-7} M Lqh α IT induced a slight depolarization (about 5 mV) and transformed the regular firing into an activity pattern consisting of bursts (containing about six spikes), separated by long hyperpolarization of the membrane (>5 s), resulting in the disappearance of spontaneous electrical activity (Fig 4B). Hyperpolarization observed between the bursts of action potentials seemed to be correlated with the silent periods

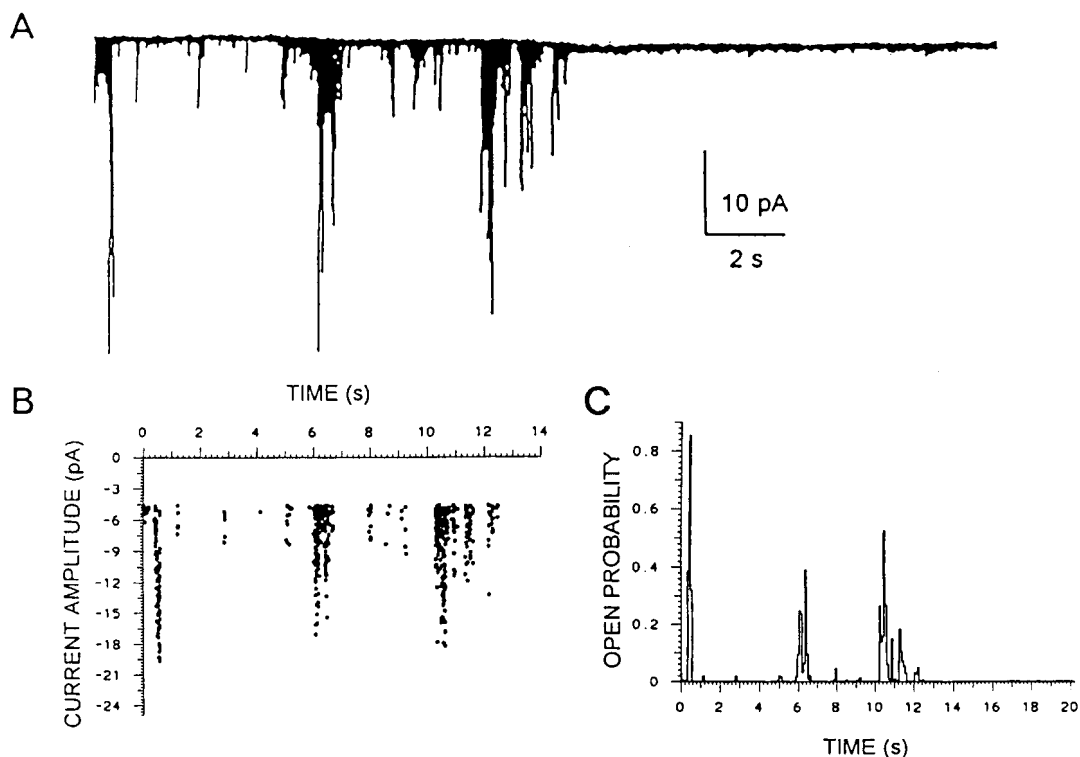


Figure 3. Effect of 10^{-7} M Lqh α IT on DUM neurone background sodium channel activity. A: Selected current trace recorded at a steady-state holding potential of -50 mV. B and C are the corresponding scatter plots of background current amplitude versus time and the channel open probability (P_o) plotted against time, respectively. Vertical bars in the last case represent the channel P_o .

separating two bursts of increased background channel activity (Fig 3A). Lqh α IT used at this concentration did not affect the different phases of action potentials as was observed, for instance, with scorpion alpha

toxin in cockroach axonal membrane.⁶ This indicated that the classical voltage-dependent sodium current was not affected by low concentration of scorpion alpha toxin. However, application of 10^{-6} M Lqh α IT was able to slow down inactivation of the voltage-dependent sodium current in TAG DUM neurones.⁶

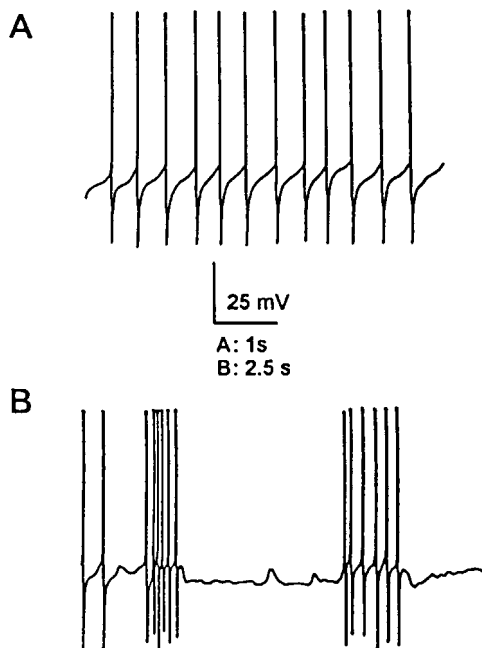


Figure 4. Spontaneous electrical activity recorded with the patch-clamp technique (whole-cell configuration) in an isolated adult DUM neurone cell body. A: Beating pacemaker activity recorded under control conditions. B: Spontaneous bursting activity recorded after bath application of 10^{-7} M Lqh α IT.

4 DISCUSSION

It is well established that scorpion and sea anemone alpha toxins specifically modify voltage-dependent sodium channel gating by interacting with the inactivation mechanism. The alpha toxins are known to slow down the global sodium current inactivation on vertebrate as well as invertebrate neuronal voltage-dependent sodium channels.¹²⁻¹⁴ At a single channel level, they prolong the mean open time, induce repetitive openings without affecting the channel ionic selectivity^{15,16} and recently it has been shown that, upon a depolarization, sea anemone toxin II-modified sodium channels could rapidly enter into a high-conductance state and more slowly into a low-conductance state.¹⁷

In cockroach TAG DUM neurones, two types of sodium current have been described, the voltage-dependent sodium current responsible for the depolarizing phase of action potential⁸ and the background sodium current essential to maintain the beating pacemaker activity of these neurones.^{7,9} In this paper, we demonstrate for the first time the existence of a new type of target for the well-known

scorpion alpha toxin Lqh α IT. Very low concentrations of Lqh α IT preferentially affected the background sodium channels without any effect on the voltage-dependent sodium channels. It was necessary to use a concentration of Lqh α IT 100 times higher to observe an effect on the voltage-dependent sodium current on this preparation. The effect of Lqh α IT is unusual. This scorpion alpha toxin modified homogenous distribution of the channel activity which characterizes the behaviour of the background channels.⁷ Lqh α IT causes the development of a bursting channel activity in the range of normal resting membrane potential (ie -50 mV) by increasing the number of open current levels separated by several closures to different intermediate levels and/or to the zero current level. In contrast, at -50 mV, bursting activity was always absent in the control. However, at very negative steady-state holding potentials (eg -100 mV), different current levels were observed, such as those seen under scorpion alpha toxin treatment. This similarity leads us to suggest the existence of a complex mechanism of background sodium channels that involves simultaneous openings of several conducting units forming the channel, as has already been proposed for other background channels,^{18,19} most of these units being 'inactivated' (ie, not functional) at -50 mV. Hyperpolarization or Lqh α IT treatment at -50 mV could enhance the opening of most of these units, inducing the bursting phenomenon.

Although the classical voltage-dependent sodium channels play a fundamental role in the generation and the conduction of electrical activity, we report in this study that background sodium channels are not only involved in driving the membrane potential to threshold for action potential generation⁹ but can play an additional physiological role in determining the firing pattern of pacemaker neurosecretory cells. Lqh α IT is capable of transforming the regular firing pattern of TAG DUM neurones into a bursting electrical activity. This fundamental alteration of the somatic intrinsic electrical activity has already been observed in a variety of invertebrate, as well as vertebrate, neurones, and different mechanisms of such alteration have been proposed.^{20–22} In our study, the transition from beating activity to rhythmic bursting seems to be due to an alteration of the background sodium channel behaviour. Correlation between the long silent periods separating two bursts of action potentials during which the neurone is often hyperpolarized and the silent periods observed between two bursts of increased channel activity is obvious. It is clear that Lqh α IT is capable of inducing the switch between these two intrinsic electrical properties, probably being conditional upon changes in background channel behaviour (ie transition between homogenous distribution of single events into bursting activity of the background channels). Transformation from beating activity to rhythmic bursting in a given DUM neurone could strongly influence the neurosecretory function (eg release of octopamine, known to

be involved in the modulation of different vital functions in insects).²³ Although the background sodium channels have not yet been characterized in many neuronal preparations, they may exist in other types of insect neurosecretory cells. In this case, their functional alteration may profoundly affect poisoned insects. Consequently, this high scorpion alpha toxin sensitivity makes the background sodium channels suitable candidates as primary targets for other toxins or insecticides. From our results emerges an interesting question: can insect selective toxins be used as biological control agents? The pyrethrins are well-known examples of defensive plant compounds that are toxic to insects. In the last three decades, pyrethroids have been successfully developed for controlling insect pests,¹ but the rapidly developing resistance to conventional insecticides makes it necessary to discover new targets for insecticides. Scorpion toxins and pyrethroids bind to insect voltage-dependent sodium channels at different binding sites.²⁴ Some cases of resistance to pyrethroids have been demonstrated to be due to point mutations in a locus encoding a voltage-gated sodium channel.²⁵ Because of the results reported here, it would be interesting to perform further comparative studies between native and mutant voltage-gated, as well as background, sodium channel interactions with pyrethroids and scorpion toxins. This may contribute to overcoming pyrethroid resistance. In parallel the development of new insect control methods is necessary. As an example, the baculovirus, which is an insect pathogen, has been used as an expression vector of anti-insect toxins. It was demonstrated that baculovirus pathogenicity could be enhanced by 30–40% by the expression of such toxins.^{26,27} Furthermore, a recent study reported that baculoviruses expressing depressant and excitatory scorpion anti-insect selective toxins possessed high insecticidal activity.²⁸

REFERENCES

- 1 Narahashi T, Nerve membrane Na⁺ channels as targets of insecticides. *TIPS* 13:236–241 (1992).
- 2 Zlotkin E, Moskowicz H, Herrmann R, Pelhate M and Gordon D, Insect sodium channel as the target for insect-selective neurotoxins from scorpion venom, in *Molecular Action of Insecticides on Ion Channels*, ed by Marshall Clark J, *ACS Symp Series* 591, pp 56–85 (1995).
- 3 Zlotkin E, Miranda F and Rochat H, Chemistry and pharmacology of *Buthinae* scorpion venoms, in *Arthropod venoms*, ed by Bettini S, Springer Verlag, pp 317–369 (1978).
- 4 Lester D, Lazarovici P, Pelhate M and Zlotkin E, Purification, characterization and action of two insect toxins from the venom of the scorpion *Buthotus judaicus*. *Biochim Biophys Acta* 701:370–381 (1982).
- 5 Eitan M, Fowler E, Herrmann R, Duval A, Pelhate M and Zlotkin E, A scorpion venom neurotoxin paralytic to insects that affects sodium current inactivation: purification, primary structure, and mode of action. *Biochemistry* 29:5941–5947 (1990).
- 6 Pelhate M, Stankiewicz M and Ben Khalifa R, Anti-insect scorpion toxins: historical account, activities and prospects. *CR Soc Biol* 192:463–484 (1998).

- 7 Lapied B, Stankiewicz M, Grolleau F and Pelhate M, Background sodium channels are necessary for maintenance of beating pacemaker activity in insect neurosecretory cells. *J Physiol (Lond)* **504**:27P (1997).
- 8 Lapied B, Malécot CO and Pelhate M, Patch clamp study of the properties of the sodium current in cockroach single isolated adult aminergic neurones. *J Exp Biol* **151**:387–403 (1990).
- 9 Lapied B, Malécot CO and Pelhate M, Ionic species involved in the electrical activity of single adult aminergic neurones isolated from the sixth abdominal ganglion of the cockroach *Periplaneta americana*. *J Exp Biol* **144**:535–549 (1989).
- 10 Grolleau F and Lapied B, Separation and identification of multiple potassium currents regulating the pacemaker activity of insect neurosecretory cells (DUM neurones). *J Neurophysiol* **73**:160–171 (1995).
- 11 Hamill OP, Marty A, Neher E, Sakmann B and Sigworth FJ, Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch* **391**:85–100 (1981).
- 12 Pelhate M and Zlotkin E, Actions of insect toxin and other toxins derived from the venom of the scorpion *Androctonus australis* on isolated giant axons of the cockroach (*Periplaneta americana*). *J Exp Biol* **97**:67–77 (1982).
- 13 Strichartz G Rando T and Wang GK, An integrated view of the molecular toxicology of sodium channel gating in excitable cells. *Ann Rev Neurosci* **10**:237–267 (1987).
- 14 Borchani L, Stankiewicz M, Kopeyan C, Mansuelle P, Kharrat R, Cestèle S, Karoui H, Rochat H, Pelhate M and El Ayeb M, Purification, structure and activity of three insect toxins from *Buthus occitanus tunetanus* venom. *Toxicon* **35**:365–382 (1997).
- 15 Nagy K, Mechanism of inactivation of single sodium channels after modification by chloramine-T, sea anemone toxin and scorpion toxin. *J Membr Biol* **106**:29–40 (1988).
- 16 Kirsch GE, Skattebol A, Possani LD and Brown AM, Modification of Na channels gating by an α -scorpion toxin from *Tityus serrulatus*. *J Gen Physiol* **93**:67–83 (1989).
- 17 Castillo C, Piernavieja C and Recio-Pinto E, Anemone toxin II unmasks two conductance states in neuronal sodium channels. *Brain Res* **733**:231–242 (1996).
- 18 Hunter M and Giebish G, Multi-barrelled K channels in renal tubules. *Nature (London)* **327**:522–524 (1987).
- 19 Meves H and Nagy K, Multiple conductance states of the sodium channel and of other ion channels. *Biochim Biophys Acta* **988**:99–105 (1989).
- 20 McCormick DA and Pape HC, Properties of a hyperpolarization-activated cation current and its role in rhythmic oscillation in thalamic relay neurones. *J Physiol (Lond)* **431**:291–318 (1990).
- 21 Turrigiano G, Abbott LF and Marder E, Activity-dependent changes in intrinsic properties of cultured neurons. *Science (Washington)* **264**:974–977 (1994).
- 22 Mantegazza M, Franceschetti S and Avanzini G, Anemone toxin (ATX II)-induced increase in persistent sodium current: effects on the firing properties of rat neocortical pyramidal neurones. *J Physiol (Lond)* **507**:105–116 (1998).
- 23 Burrows M, *The Neurobiology of an Insect Brain*, Oxford University Press, Oxford. pp 1–669 (1996).
- 24 Gordon D, Sodium channels as target for neurotoxins. Mode of action and interaction of neurotoxins with receptor sites on sodium channels, in *Toxins and Signal Transduction*, ed by Lazarovici P and Gutman Y, Harwood Academic Publishers, The Netherlands, pp 119–149 (1997).
- 25 Park Y, Taylor MFJ and Feyereisen R, A valine 421 to methionine mutation in IS6 of the *hscp* voltage-gated sodium channel associated with pyrethroid resistance in *Heliothis virescens* F. *Biochem Biophys Res Com* **239**:688–691 (1997).
- 26 Stewart LMD, Hirst M, Ferber ML, Merryweather AT, Cayley PJ and Possee RD, Construction of an improved baculovirus insecticide containing an insect-specific toxin gene. *Nature (London)* **352**:85–88 (1991).
- 27 Chejanovski N, Zilberberg N, Rivkin H, Zlotkin E and Gurevitz M, Functional expression of an alpha anti-insect scorpion neurotoxin in insect cells and lepidopterous larvae. *FEBS Lett* **376**:181–184 (1995).
- 28 Gersburg E, Stockholm D, Froy O, Rashi S, Gurevitz M and Chejanovsky N, Baculovirus-mediated expression of a scorpion depressant toxin improves the insecticidal efficacy achieved with excitatory toxins. *FEBS Lett* **422**:132–136 (1998).