23

Maitotoxin: An Enigmatic Toxic Molecule with Useful Applications in the Biomedical Sciences

Juan G. Reyes, Claudia Sánchez-Cárdenas, Waldo Acevedo-Castillo, Patricio Leyton, Ignacio López-González, Ricardo Felix, María A. Gandini, Marcela B. Treviño, and Claudia L. Treviño

CONTENTS

23.1 Introduction

Many marine invertebrates produce potent toxins, turning themselves poisonous as a defense strategy against predators. In contrast, other organisms can become poisonous by accumulating toxins from their own prey. Dinoflagellates are aquatic photosynthetic microbial eukaryotes, and some species produce highly toxic metabolites. These dinoflagellate toxins bioaccumulate up the food chain in various consumer organisms. Many filter-feeding organisms such as bivalves accumulate such toxins with no apparent adverse effects on them[1] but causing intoxication when ingested by their predators, including fish and marine mammals, and ultimately also when humans consume contaminated seafood.[2]

Four major groups of dinoflagellate toxins have been described, namely, saxitoxins, ladder-shaped polyether compounds, long-chain polyketides, and macrolides.[2] The dinoflagellate species *Gambierdiscus toxicus* produces several potent polyether toxins, some of which were initially identified in connection with a common type of food poisoning called ciguatera, caused by consumption of certain contaminated tropical and subtropical fish. Ciguatera involves a combination of gastrointestinal, neurological, and cardiovascular disorders. The two most common toxin classes associated with ciguatera are ciguatoxin (CTx) and maitotoxin (MTx), and they are among the most lethal natural substances known to man.[2]

Most of the neurological symptoms of ciguatera are caused by CTx, which exert their effects due primarily to the activation of voltage-gated sodium channels, causing cell membrane depolarization.[1] MTx displays diverse pharmacological activities, which seem to be derived from its ability to activate Ca^{2+}-uptake processes in a variety of cell types.[3] MTx is the largest and most toxic known nonbiopolymeric toxin, with a molecular weight of 3422 Da. MTx is a very interesting compound given its extremely potent biological activity, and it has been used as a powerful pharmacological tool for the elucidation of Ca^{2+}-dependent cellular processes.

23.2 History

The discovery of MTx is closely related to the characterization of ciguatera, a food-borne illness caused by the consumption of fish contaminated with certain dinoflagellate toxins. Ciguatera symptoms can vary with the geographic origin of the contaminated fish. Gastrointestinal symptoms—such as diarrhea, vomiting, and abdominal pain—occur first, usually within 24 h of eating implicated fish. Neurological symptoms may occur at the same time or may follow several days later and include thermal sense inversion, characterized by the feeling of receiving an electric shock when touching cold water, pain and weakness in the lower extremities, and circumoral and peripheral paresthesia.

This mode of poisoning was called "ciguatera" after *cigua*, a snail commonly occurring in the Caribbean Sea.[4,5] Ciguatera occurs worldwide in tropical and subtropical regions causing 20,000–50,000 victims per year. The agents causing this condition bioaccumulate in fish as they are transferred up the food chain, to be finally consumed by humans. Ancient references to toxic diseases conditions similar to "ciguatera" are found in Homer's Odyssey (ca. 800 BC), in reports of a pandemic occurring in China (600 BC), and in the chronicles written by Pedro Martir de Anglería in 1555.[6]

MTx was discovered in 1965 by Bagnis, who reported that the human symptoms caused by ingestion of contaminated herbivorous fish were different from those caused by carnivorous fish, primarily involving gastrointestinal discomfort and less neurological disorders. The molecule responsible for these symptoms was discovered upon examination of the toxic constituents in the surgeonfish *Ctenochaetus striatus* and was named after the Tahitian namesake of this species—"maito."[7] MTx is a polyketide-derived polycyclic ether consisting of four extended fused-ring systems termed polyether ladders (molecular formula $C_{164}H_{256}O_{68}S_2Na_2$) (Figure 23.1).

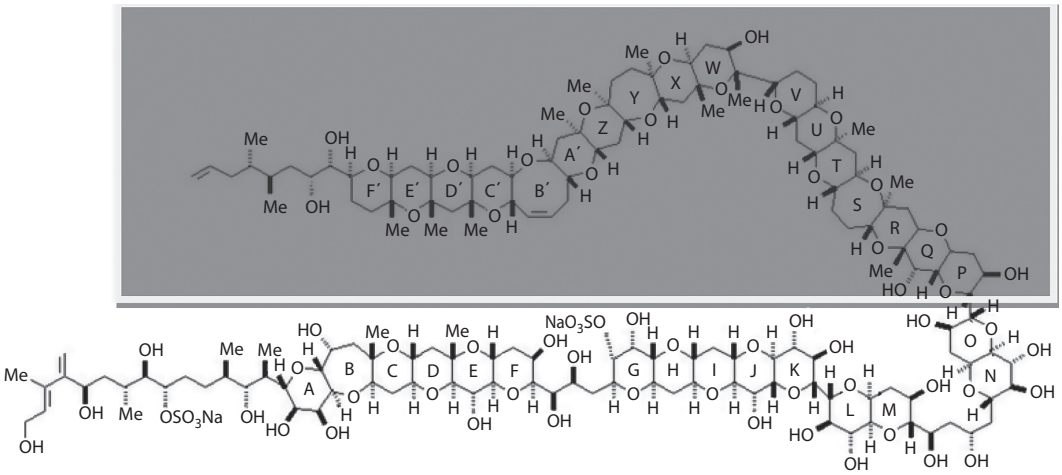

FIGURE 23.1 Structure of MTx. (Taken from Treviño, C.L. et al., Maitotoxin: A unique pharmacological tool for elucidating Ca^{2+}-dependent mechanisms, in: Botana, L.M. (ed.), *Seafood and Freshwater Toxins: Pharmacology, Physiology and Detection*, 2nd edn., CRC Press, Boca Raton, FL, pp. 503–516, 2008.) The grey region was utilized for the theoretical molecular dynamics calculations in the section *MTx interaction with membranes*.

23.3 Natural Origin

The marine dinoflagellate *G. toxicus* is a single-celled phytoplanktonic organism, and it may be found on the surface of algae in tropical waters worldwide. Among other toxins, it produces CTx and MTx, which accumulate through the food chain as carnivorous fish consume contaminated herbivorous reef fish. MTx toxin accumulates primarily in the liver and viscera of fishes, but not in their flesh.[8] Higher concentrations of toxins can be found in large predatory fish such as barracuda, grouper, amberjack, snapper, and shark. Because the fish industry has no borders and marine products are shipped to many countries, ciguatera fish poisoning can occur almost anywhere. People who live in or travel to endemic areas should avoid consumption of barracuda or moray eel, should be cautious with grouper and red snapper, and are advised to enquire about local fish associated with ciguatera. Since there is no reliable way to "decontaminate" or even to distinguish contaminated fish by smell or appearance, it is wise to avoid eating the viscera of any reef fish and to prevent consumption of large predacious reef fish.[9,10].

The temperatures of the northern Caribbean and extreme southeastern Gulf of Mexico have been predicted to increase 2.5°C–3.5°C during the next years.[11] Higher temperatures favor *G. toxicus* growth[12] and are also likely to alter fish migration patterns. Ciguatera outbreaks have been correlated with sea-surface temperature increases in the south Pacific Ocean.[13]

After Yasumoto discovered in 1977 that the dinoflagellate *G. toxicus* was responsible for producing MTx, he cultivated this organism for 10 years in order to have enough material to isolate this toxin and to determine its structure.[14,15] For some years, MTx was commercially available for experimentation and was used to study Ca^{2+} dynamics in diverse cell types (see Section 23.7). Having this tool commercially available again would certainly continue to be useful for research purposes.

23.4 Toxicology

Human ingestion of seafood contaminated with toxins produced by marine phytoplankton[10,16,17] can cause a variety of diseases. These toxins can have a wide range of acute and chronic health effects not just in humans but also in other animal species. Given that these compounds are tasteless, odorless, and heat and acid stable, conventional food testing methods fail to detect and destroy them in contaminated seafood.

Ciguatera can cause mild to severe symptoms lasting from a few days and up to years. Around 400 species of fish are considered to be ciguateric and contain distinct combinations and quantities of toxins. Classical ciguateric symptoms include gastrointestinal and neurological disorders, abdominal cramps, diarrhea, nausea, vomiting, temperature reversal, and itching. The toxins can even be passed on to a fetus or to a newborn child, via placental or breast milk transmission, respectively.[9,10]

Most of the neurological symptoms of ciguatera are due to CTx, while MTx is considered to be less important in the generation of ciguatera symptoms given that it is less concentrated in fishes. However, it should be noted that the observed toxicity differences could also be the result of chemical modifications of the toxins possibly occurring as they pass through the food chain, and it is therefore difficult to establish a direct relationship between the various symptoms and a particular toxin. Diagnosis of ciguatera is based solely on the presence of the general symptoms in correlation to patients with a recent history of fish ingestion.

In contrast to the lipid-soluble CTxs, MTx is water soluble, and it apparently does not accumulate in the flesh of fishes but rather in organs such as the liver.[18] MTx has a very low oral potency as compared to its high lethality when injected intraperitoneally (i.p.), and pure MTx is even more toxic than CTx. For example, in mice, CTx is lethal at 0.45 μg/kg i.p. and MTx at a dose of 0.15 μg/kg i.p. However, the precise lethal dose depends on the mouse strain, the sample source, and even the sample preparation procedure, as MTx binds to glass and plastic and thus its exact concentration may be underestimated. Mice injected i.p. with MTx display reduced body temperature, piloerection, dyspnea, progressive paralysis, slight tremors or convulsions, and long death times. High doses of MTx produce CTx-like symptoms, such as gasping with convulsions and shorter death times.

Three different MTx molecules have been isolated from a variety of strains of *G. toxicus*. Injection of MTx-1 and MTx-2 in mice exerted similar symptoms, except that MTx-2 exhibited shorter death times. MTx-3 induced additional symptoms such as intense gasping that ameliorated near death; however, further purification of MTx-3 by HPLC reduced the gasping symptoms, suggesting that additional bioactive components may be present in the crude preparation.[19] The death times produced by MTx-1 and MTx-3 were very similar. Desulfonation of MTx (solvolysis) reduces the toxicity of all three forms about 200-fold.[19,20]

Efforts to develop a radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA) to detect CTx have been made over the past few years, such as the Hokama enzyme immunoassay stick test.[21] There is a commercial kit called Cigua-Check® that may be used by fisherman or restaurants to prevent ingestion of contaminated fish. Unfortunately, this kit only detects CTx. Detection can be confirmed by finding CTx and MTx in contaminated fish samples by high-performance liquid chromatography and mass spectrometry, although this process is costly and not widely available in high risk areas, such as small islands.

To date, there is no antidote for ciguatera, but medications such as amitriptyline have been used to diminish some of the symptoms of chronic ciguatera, including fatigue and paresthesias. There are several palliative remedies including medicinal teas used in both the Indo-Pacific and West Indies regions. However, none of these treatments have been properly standardized to provide effective treatment.[9,10] Patients are also advised to avoid alcohol, nuts, and nut oil for at least 6 months after the intoxication in order to avoid reappearance of symptoms.

23.5 Synthesis

23.5.1 Biosynthesis of Ladder-Shaped Polyether Compounds

The structural similarities among dinoflagellate-produced polyether ladder toxins including brevetoxins, CTxs, yessotoxins, and MTxs strongly suggest that their biosynthetic pathways may share similar strategies. In the case of brevetoxins, experimental studies on their biosynthetic pathways are starting to unveil the biochemical reactions involved in the production of such complex polyethers. Based on tracing studies using radiolabeled carbon precursors, it is now clear that polyketides in general are assembled from acetyl-CoA or malonyl-CoA precursors; they also contain carbon units derived from methionine and from some larger carbon unit precursors, such as glycolate.[22] The carbon chain formed from the aforementioned precursors appears to be interrupted by C-1 deletions of acetate-derived carbon atoms. Although a Favorskii-like rearrangement has been suggested to be involved in the ring size reduction observed in polyether ladder toxins, such proposed catalytic mechanism (which in vitro takes place with halogenated compounds and under strong basic conditions) has not yet been unequivocally demonstrated. In this biosynthetic pathway, and similarly to bacterial polyether synthesis, polyketide synthases appear to play an important role. This family of enzymes catalyzes the synthesis of complex natural products from precursors such as acetyl-CoA, propionyl-CoA, and methylmalonyl-CoA through a biosynthetic strategy resembling the one used for fatty acid (FA) biosynthesis.[23] Thus, polyketides are built by a series of successive condensations of simple precursors, decarboxylations, reductions, and rearrangements. However, unlike FA biosynthesis, in which the FA chain is subjected to the whole series of intermediate reactions, the partial chemical processing of intermediaries in polyketide biosynthesis can give rise to a complex pattern of functional groups associated to the polyether chain. Furthermore, different dinoflagellate species can use different combinations of starter CoA-activated precursors and chain-extension substrates during polyketide biosynthesis, which at least in some cases involve the generation of specific chiral centers and cyclization. Despite the proposed existence of similar polyether ladder biosynthetic pathways in different dinoflagellate species, suggested by their products' structural similarities.[22,24] Defining the specific reactions of any such pathway has proven a very difficult task. This challenge stems from the diversity of chemical structures found in this family of compounds, from the diversity of species within this phylum of alveolate eukaryotes, and from the particularly complex genetic organization

observed in these organisms, including multigene phylogenies, diverse plastid endosymbiotic relationships, and chromatin configuration.[22,25–28] It has been suggested that for polyether ladder toxins, the participating acetate units can enter the tricarboxylic acid (TCA) cycle, and some TCA intermediate could be the actual precursor involved in the condensation process.[29] In fact, to the best of our knowledge, there are thus far no reports of specific studies on MTx biosynthesis. Furthermore, it is likely that the toxins isolated from the organs of herbivorous fishes represent a chemically modified version of the toxins originally synthesized by the dinoflagellates.[30–32]

The cyclization process in these compounds is probably associated to the formation of an epoxide intermediate presumably catalyzed by epoxidases and epoxide hydrolases.[33,34] The accompanying polyepoxide process proposed for marine polyether ladder toxins has clear precedents in several reports on epoxide and cycle formation during antibiotic polyether biosynthesis.[35] Although the process whereby 10 or more epoxides are formed and then coordinated to a polyepoxide cascade to yield polyether ladder toxins still remains unclear, an oxidase-catalyzed tandem epoxide formation and epoxide protection, followed by a hydrolase-catalyzed epoxide opening and cycle rearrangement, is likely involved.

23.5.2 Chemical Synthesis of Maitotoxin

The chemical structures of marine toxins in general—and MTx in particular—are very interesting and represent a formidable challenge for organic synthesis.[36] The appearance in the literature of the structure of brevetoxin-B (the first marine polycyclic ether to be isolated and characterized[37]) awoke a large interest in synthetic organic chemists. The structure of brevetoxin-B is characterized by several fused polycyclic ethers containing ether rings with 6, 7, and 8 units, in addition to 23 chiral centers, certainly a daring task for chemical synthesis. It contains 32 fused ether rings, 28 hydroxyl groups, 21 methyl groups, 98 chiral centers, and 2 sulfates.[3,38–40]

The efforts of chemical synthesis have been directed toward developing new methods that allow synthesis of only part of these molecules by initially building structural fragments of these ladder-shaped polyether toxins. Great progress in the synthesis of several marine polycyclic ethers has been accomplished since the year 2000.[41,42] They designed a remarkable synthetic strategy involving an efficient iterative method for the stereoselective construction of transfused polycyclic ethers based on induced reductive cyclization of β-alcoxy-acrylate by samarium iodide (SmI_2). In the case of the synthesis of the ring systems of MTx, the stereoselectivity was accomplished by state transition chelation (Figure 23.2).[42–46]

Oxepane ring formation also was stereoselectively synthesized through this reaction generating a single product with an 84% yield (Figure 23.3).

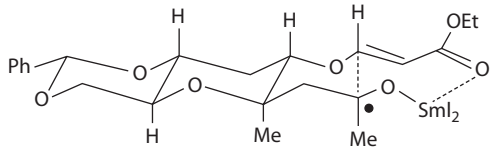

FIGURE 23.2 Transition state of the cyclization induced by SmI_2. (From Sakamoto, Y. et al., *Org. Lett*., 3, 2749, 2001; Nakata, T., *Chem. Rec*., 10, 159, 2010.)

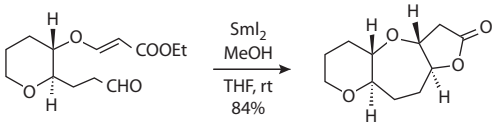

FIGURE 23.3 Synthesis of polycyclic ethers based on cyclization induced by SmI_2. (From Nakata, T., *Chem. Rec*., 10, 159, 2010.)

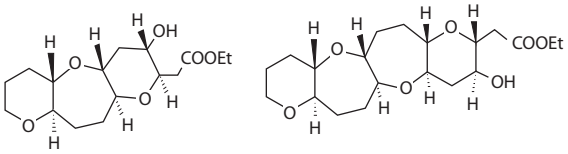

FIGURE 23.4 Examples of ethers synthesized by iterative strategy induced cyclization 6,7,6 and 6,7,7,6 membered polycyclic ethers. (From Nakata, T., *Chem. Rec*., 10, 159, 2010.)

Moreover, the trans-membered fused polycyclic 6,7,6 and 6,7,7,6 ethers were iteratively synthesized using this cyclization induced by SmI_2 strategy (Figure 23.4).[42]

Additionally, in 1998, Dr. Nicolaou's research group (La Jolla, CA) reported the complete synthesis of brevetoxin-A, another ladder-shaped polyether compound, the first stereoselective synthesis of pyrans involving the opening of epoxides with a hydroxyl group. This synthesis route has the particularity of overcoming the natural preference for cyclization to an unwanted product 5-exo by placing one C−C bond adjacent to the epoxy fragment. Thus, under these conditions, the structure shown in Figure 23.5 exclusively undergoes ring closure to produce a 6-endo by pyran system instead of 5-exo product. The selectivity observed is attributed to the π orbital stabilization generated by a carbon atom next to the electron-deficient transition state provoking an endo attack; this effect would be absent during the exo attack.[47]

Using similar synthetic strategies, this same research group has accomplished the stereoselective synthesis of ladder-shaped portions of a large part of MTx.[40,41,48–50] Altogether, between Dr. Nakata's and Dr. Nicolaou's group, 29 out of the 32 rings of MTx have been chemically synthesized.

Although thus far no complete chemical synthesis has been achieved for MTx, complete synthesis has been successful for some of the other ladder-shaped polycyclic ester toxins. New methods such as biomimetic cascades, cross-coupling reactions catalyzed by palladium, and radical reactions could provide additional tools for approaching the laboratory synthesis of this type of compounds.

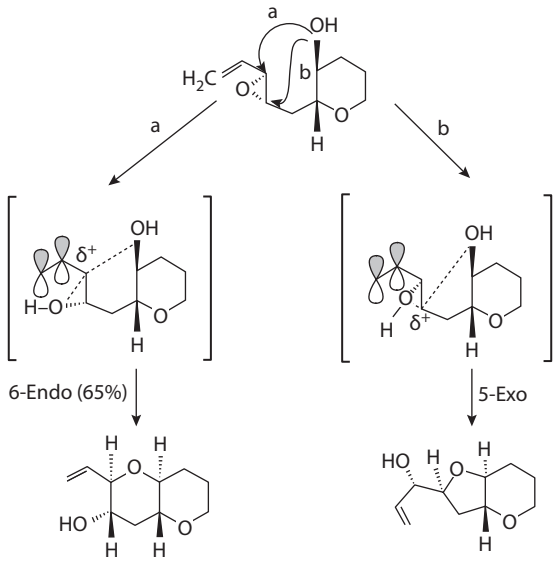

FIGURE 23.5 Reactions involved for the formation of cyclic ethers. (From Nicolaou, K. et al., *J. Am. Chem. Soc*., 108, 2468, 1986; Nicolaou, K.C. et al., *J. Am. Chem. Soc*., 130, 7466, 2008.)

23.6 Mechanisms of Action

23.6.1 MTx and Plasma Membrane Channel Activation

After the early work of Dr. Yasumoto's group,[51–53] it became clear that MTx acted on mammalian cells inducing entry of external Ca^{2+}, and based on pharmacological criteria, it was proposed that MTx activated voltage-gated Ca^{2+} (Cav) channels.[54–57] Consistent with this proposal, MTx-induced Ca^{2+} entry was absent in fibroblast that lacked these channels.[55] However, even this early work on the mechanisms of action of MTx pointed out some peculiar aspects of the toxin's effects, such as a delay of about 2 min in the induction of Ca^{2+} entry observed in certain cell types[55] or the reported inhibition of Na^+-K^+-ATPase induced by the toxin.[58] Furthermore, it was soon reported that MTx was able to induce inositol triphosphate (IP_3) release independently of its action on Ca^{2+} channels.[59] These results were corroborated by Gusovsky et al.[60–63] in several cell lines, showing also that MTx activated phosphoinositide (PI) breakdown in a Ca^{2+}-dependent manner but it was not inhibited by Cav channel blockers, suggesting the activation of phospholipase C (PLC) or the activation of other Ca^{2+} channels. The fact that MTx action on PI breakdown was not mimicked by Ca^{2+} ionophores supported the notion that MTx activated PLC directly. However, in the work of Gusovsky et al.[64] using HL60 cells, it became clear that MTx action on PI turnover was associated to a Ca^{2+}-induced activation of PLC, rather than a direct PLC activation by MTx. Furthermore, the work of Pin et al.[65] suggested that the effects of MTx on gamma-aminobutyric acid (GABA) release in striatal neurons were associated to a Ca^{2+}-dependent Na^+ influx, introducing the notion of an additional cellular target for MTx. Similar results of a Ca^{2+}-dependent Na^+ influx was reported by Sladescek et al.[66] The pleiotropic effects and action mechanisms of MTx on different cells was reviewed by Hamilton and Perez.[67]

Although the effects of MTx were pleiotropic, they all seemed to involve an intracellular Ca^{2+} increase. The fact that MTx activated cation channels was clear from direct voltage-clamp current measurements (e.g., [38,68–81]). Some of the proposed channels were Cav and also nonselective cation channels such as store-operated channels (SOCs). Furthermore, in some cells such as skin fibroblasts, MTx seems to activate large conducting channels[82,83] leading to cell lysis. Hence, the action of MTx would be highly dependent on the channel expression profile of each cell type and also on the MTx concentration used (e.g., [84]). Due to scope limitations in this review, we have excluded a discussion of all cellular effects downstream of the MTx interaction with membranes, on which a wealth of data is undoubtedly available in the literature.

The ability of MTx to activate Cav and other cationic channels posed the question as to whether the toxin was activating channels by inserting itself in the membrane and perturbing the phospholipid membrane structure (see MTx interaction with membranes in the following texts) or whether it had specific interactions with the proteins forming the channels. Murata et al.[85] reported that either a removal of the sulfated residues from MTx or a hydrogenation of the molecule decreased (by several orders of magnitude) its ability to induce Ca^{2+} entry or PI breakdown in insulinoma or glioma cells. When these MTx derivatives were used together with intact MTx, they acted as blockers of the MTx effect. The work of Konoki et al.[86] describing inhibition of MTx-induced Ca^{2+} entry in glioma C6 cells by brevetoxin and synthetic fragments of MTx (corresponding to a sulfated portion, rings EF-GH, and rings LM-NO) strongly suggested that MTx acted on specific sites to activate Ca^{2+} entry in these cells. Interestingly, the sulfated fragment of MTx (EF-GH rings, and hence, a portion expected to remain in the aqueous phase or at the lipid–water interphase) was more potent in inhibiting MTx effects on Ca^{2+} uptake than the LM-NO rings. Recent work by Oishi et al.[87] clearly showed that an artificial ladder-shaped heptacyclic polyether was the most potent substance described to date capable of inhibiting MTx-induced Ca^{2+} influx in glioma C6 cells, strongly suggesting the existence of a specific MTx binding site in these cells. However, this work did not give insight into the molecular mechanisms of action of MTx.

23.6.2 MTx Interaction with Membranes

The insertion of MTx in membranes was first suggested by Konoki et al.,[88] with rings RSTUVWXYZA′B′C′D′E′F′ inserted in and spanning the phospholipid bilayer and rings ABCDEFGHIJKLMNOPQ—containing the sulfated parts of the molecule—staying in the aqueous

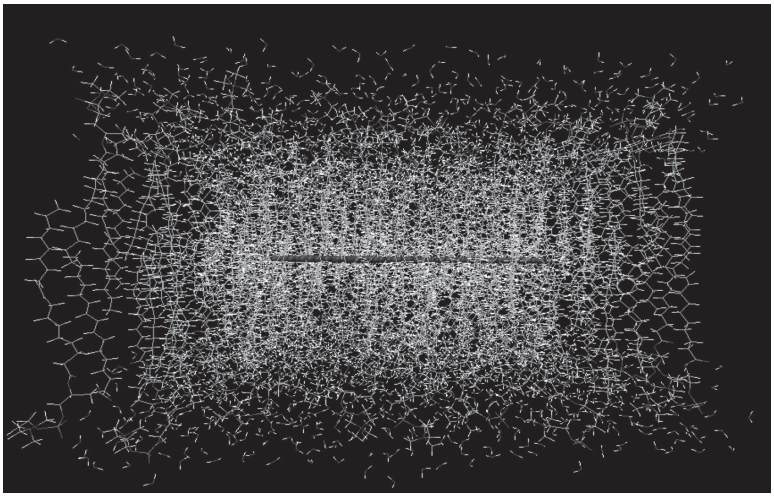

FIGURE 23.6 The initial position of MTx in the 1-palmitoyl-2-oleoyl-*sn*-glycero-3- phosphocholine (POPC) membrane was set with the plane of the molecule parallel to the POPC/water interphase. This position and conformation as well as the environment were then allowed to drift with molecular dynamics calculations toward steady states with minimal energy conformations. Water molecules: oxygen = red, hydrogen = white. Phospholipids: light blue large chains. MTx: darker blue at the center of the bilayer. (http://www.ibt.unam.mx/server/PRG.base?tipo:doc,dir:PRG.curriculum,par:ctrevino)

environment. This work was later cited by Murata et al.,[89] Nicolau et al.,[90] and Nicolau and Aversa[91] to discuss the properties of MTx. This association of MTx with phospholipid membranes was deduced from the structural properties of MTx and by analogy to the properties of other ladder-shaped polyether toxins.[89] However, to the best of our efforts, we were unable to find the specific model or program used to estimate MTx distribution in phospholipid membranes. Thus, in order to theoretically estimate the stability of the relatively hydrophobic portion of MTx in a phosphatidylcholine phospholipid membrane, we performed force field parameterization of rings P–F′ and molecular dynamics as shown in the following texts. The molecular structure of MTx was taken from Nicolau and Frederic[40] (Figure 23.1). Partial parameterization with the CHARMM force field was accomplished taking different sections of the molecule from ring P to F′ using the CCPN web applications (http://www.ccpn.ac.uk/). The 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (DOPC) bilayer was built with visual molecular dynamics (VMD 1.9).[92] The mentioned fragment of MTx was distributed in the center of the bilayer, and the program not (just) another molecular dynamics (NAMD) allowed to iteratively locate the molecule to acquire a conformation giving steady values of potential energy parameters (http://www.ks.uiuc.edu/Research/vmd/). This modeling clearly shows that rings P–F′ and tail were stable within the lipid bilayer (Figures 23.6 and 23.7), and this conformation is likely restricted and modified by the more hydrophilic portion of MTx (see also Murata et al.[89]). This distribution of the hydrophobic portion of MTx is consistent with the proposed role of this part of MTx (or similar portions of ladder-shaped polyether toxins) in the interaction with integral membrane α-helices.[89,93] However, these results do not exclude that the hydrophilic portion of MTx, anchored in the membrane by the hydrophobic portion, could also interact externally with channels[86] or, as we could cautiously propose, that both mechanisms could be responsible for activation of different types of channels in biological membranes.

23.7 MTx Bioactivity and Applications

Ca^{2+} is an ubiquitous secondary intracellular messenger responsible for mediating a multitude of cellular responses as diverse as proliferation, development, contraction, secretion, and fertilization. Ca^{2+} action is quite simple: cells at rest have an intracellular concentration of ~100 nM but are activated when this level rises to ~1 μM. However, the universality of Ca^{2+} as an intracellular messenger depends on its remarkable capacity to create a wide range of spatial and temporal signals.

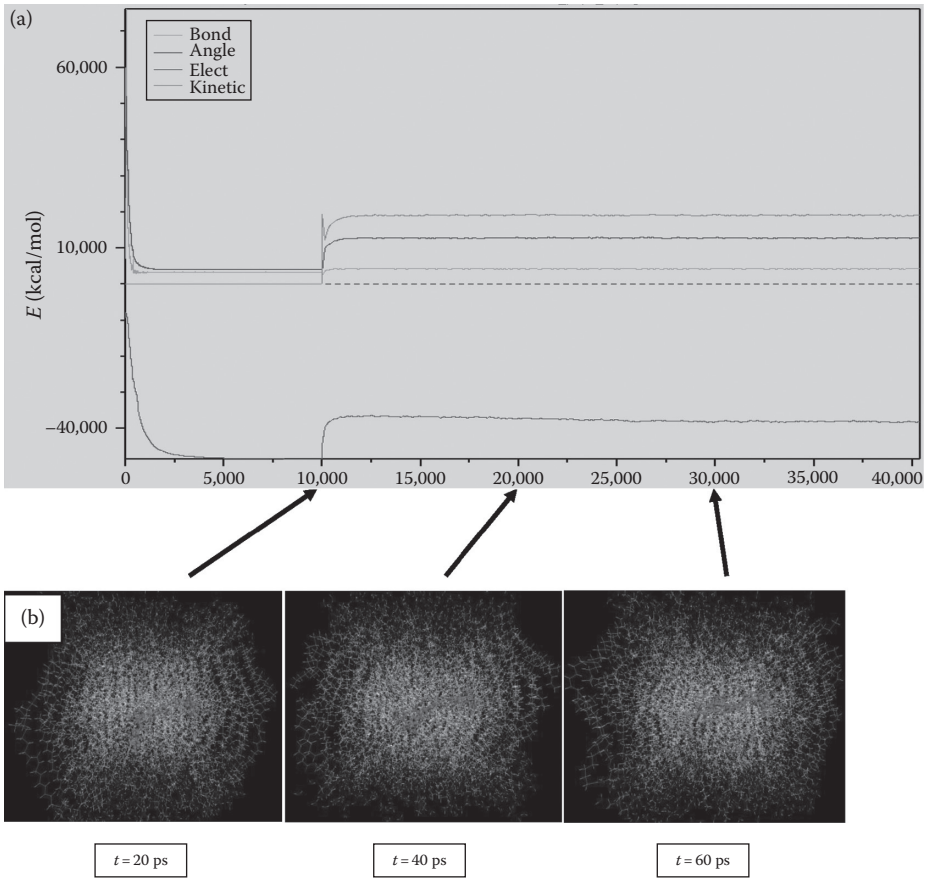

FIGURE 23.7 (a) Plot of energy (kcal/mol) versus the molecular simulation steps (1 ps corresponds to 500 steps). Each curve corresponds to the different components of potential energy: binding energy (blue line), angles (red line), electrostatic energy (green line), and kinetic energy (orange line). (b) Spatial distribution of MTx in the 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) membrane at 20, 40, and 60 ps. From the potential energy curves and the molecular images, it can be seen that this portion of MTx is stably located within the hydrophobic portion of the phospholipid bilayer at 40 ps, with a location and dynamics that certainly would be restricted by the hydrophilic portion that is expected to be located at the water/phospholipid interface. (See also Murata, M. et al., *Bull. Chem. Soc. Jpn*., 81, 307, 2008.)

MTx has attracted much attention given its powerful bioactivity involving disruption of Ca^{2+} homeostasis. MTx is not only one of the most potent toxins, but it also possesses multiple activities that appear to be linked to elevation of intracellular Ca^{2+} concentration. Thus, the toxin serves as a versatile tool for studies on cellular events associated with intracellular Ca^{2+} changes that are of particular interest, including hormone secretion, programmed cell death activation, and fertilization.

23.7.1 Insulinotropic Actions of MTx

Although some hormones such as insulin-like growth factor and adiponectin have hypoglycemic effects,[94] insulin has long been considered the only hypoglycemic agent in mammals. Insulin is synthesized and secreted by pancreatic β-cells located in specialized structures, the islets of Langerhans. In general, β-cells adjust insulin secretion to the prevailing blood glucose levels by a process called glucose-stimulated insulin secretion (GSIS). Inside pancreatic β-cells, glucose metabolism induces insulin secretion by altering the cellular array of messenger molecules. ATP is particularly important given its role in regulating cation channel activity dependent upon its hydrolysis.

ATP-dependent K^+ (K_{ATP}) channels play a key role in insulin secretion. Under euglycemic conditions, K_{ATP} channels are maintained in an open state, resulting in K^+ efflux and thus clamping the resting membrane potential close to −70 mV. When glucose is elevated, ATP levels increase and displace bound ADP on K_{ATP} channels, which results in channel closure. These events lead to a small membrane depolarization that activates voltage-dependent Ca^{2+} channels, which trigger Ca^{2+} influx and raise the intracellular Ca^{2+} concentration, thus promoting insulin secretion.[95,96]

Several reports have shown that some members of the transient receptor potential (TRP) channel family, which mediate nonselective cationic currents (NSCCs), are expressed, and might contribute to pancreatic β-cell function. Although the role of TRP channels in β-cells remains largely enigmatic, these channels may provide an alternative for the depolarizing background membrane conductance required for the cells to depolarize upon K_{ATP} channel closure.[96] Indeed, it has been reported that thermosensitive TRPM2, TRPM4, and TRPM5 channels control insulin secretion levels by sensing intracellular Ca^{2+} increase, NAD^+ metabolites, or hormone receptor activation.[97]

In addition to glucose, insulin secretion may be regulated by diverse chemical messengers such as neurotransmitters and hormones,[96] as well as by exogenous substances such as toxins that act on ion channels. Hence, some peptide toxins present in the venom of marine organisms may affect NSCCs and serve as potential insulinotropic agents. For example, it has been shown that the activity of TRPV1, a channel that modulates insulin secretion in β-cells, is affected by crude cell-free extracts obtained from marine invertebrates.[98,99] Interestingly, one of these extracts has shown insulinotropic activity.[100]

By activating NSCCs, MTx has also shown insulinotropic activity in insulinoma cells. The time course of these currents is very similar to that evoked by incretin hormones such as glucagon-like peptide-1 (GLP-1), which stimulate glucose-dependent insulin secretion by activating cAMP-mediated signaling pathways.[101] Likewise, NSCCs in insulinoma cells can be attenuated by application of a Ca^{2+} SOC blocker SKF 96365, suggesting a contribution of the mammalian TRP-related channels in these currents.[102] The ability to activate NSCCs in insulin-secreting cells stresses the role of MTx as a helpful tool for the analysis of ion channels and insulin secretion.[103] Likewise, the role of MTx as a novel blood glucose-lowering agent remains an interesting topic for future research.

23.7.2 MTx as Interleukin-1β Secretagogue and Oncotic Death Inducer

Most inflammatory reactions are mediated by cytokines, including IL-1, IL-6, TNF-α, and TGF-β. The term interleukin-1 (IL-1) refers to two cytokines, IL-1α and IL-1β, which are the master cytokines of local and systemic inflammation.[104,105] In particular, IL-1β is primarily synthesized in activated macrophages as an immature protein that remains cytosolic until converted through proteolytic cleavage by caspase-1 into its mature active form, which can then be exported outside the cell.

Given its ability to induce cell death secondary to its disruption of Ca^{2+} homeostasis, MTx is likely to trigger innate immune responses and inflammation in vivo. Indeed, it has been suggested that the toxic effect of MTx during shellfish seafood poisoning may involve a component mediated by secretion of proinflammatory cytokine IL-1β. In line with this, Verhoef and coworkers[106] reported that MTx induces a biphasic release of IL-1β from bacterial lipopolysaccharide-primed macrophages. At subnanomolar concentrations, MTx induced mature IL-1β release via a mechanism that can be blocked by high extracellular K^+ or nominally zero extracellular Ca^{2+}. MTx may therefore represent an exceptional tool for studying specific components of the innate immune response and/or the physiology of inflammatory effector cells such as monocytes, macrophages, and neutrophils. One representative example of this type of application is the work by Mariathasan and colleagues.[107] These authors found that cryopyrin is responsible for assembly of the so-called inflammasome, a cytosolic complex of proteins that activates caspase-1 to process the proinflammatory cytokine IL-1β. Cryopyrin is essential for inflammasome activation in response to signaling pathways triggered by specific bacterial infections as well as by MTx.

It is worth mentioning here that there are several human diseases caused by different mutations in the cryopyrin gene, including familial cold autoinflammatory syndrome, Muckle–Wells syndrome, as well as chronic infantile neurological cutaneous and articular syndrome.[108] Mutations in the cryopyrin gene are associated with gain of function leading to an enhanced and faster production of IL-1β. In this scenario, MTx could be used as a probe to study possible mechanisms of release and implications of IL-1β overproduction.

Likewise, the second phase of IL-1β release induced by MTx from macrophages occurs at nanomolar concentrations.[106] In this case, MTx produces secretion of unprocessed IL-1β, which is indicative of cell lysis. Interestingly, cell death induced by MTx shares some elements involved in the signaling cascade activated by stimulation of purinergic receptors of the P2Z/$P2X_7$ type.[82,83,109] As discussed earlier, MTx initially activates Ca^{2+}-permeable channels and then induces the formation of large cytolytic/oncotic pores (COPs) that allow molecules <800 Da to enter the cell. These effects are similar to those observed upon activation of P2Z/$P2X_7$ receptors in a variety of cell types, raising the intriguing possibility that MTx and P2Z/$P2X_7$ receptor stimulation activate a common cytolytic pore.

Given the high permeability of the MTx-induced channels for Ca^{2+} transport and the structural similarity of MTx with palytoxin—a marine peptide toxin that converts the plasmalemmal Na^+/K^+-ATPase (NKA) pump into a channel—it has been proposed that MTx may activate another member of the P-type ATPase family, specifically the plasmalemmal Ca^{2+}-ATPase (PMCA) pump. The results obtained by Sinkins and colleagues[110] are consistent with this idea and suggest that MTx binds to PMCA and converts the pump into a Ca^{2+}-permeable nonselective cation channel. Therefore, MTx could be used as a cell death inducer to unveil some of the molecular mechanisms involved in this process. For instance, whether or not the channel mode of operation of the PMCA plays a role in pathological cell death could be an interesting possibility for future investigations.

23.7.3 MTx and Sperm Physiology

Fertilization is fundamental for the preservation of life by sexual reproduction. The ability of the sperm and the oocyte to recognize, adhere to, and fuse with each other is a crucial aspect of fertilization. All these processes are largely determined by nicely orchestrated ionic fluxes.[111] Hence, it is well known that raises in intracellular Ca^{2+} play crucial roles in sperm functions such as capacitation, motility, and the acrosome reaction.[111]

The acrosome reaction is a secretory process triggered in sperm by components of the outer layer of the egg, and in many species it must occur before the sperm can fertilize the egg. However, the mechanisms responsible for increasing intracellular Ca^{2+} and resulting in the biochemical events that trigger the acrosome reaction are not fully understood. Two different types of Ca^{2+} channels have been proposed to participate in mammalian sperm acrosome reaction: one necessary for a fast transient change in Ca^{2+} levels and another needed to sustain an elevated intracellular Ca^{2+} concentration. The membrane pathway responsible for the first phase of Ca^{2+} entry seems to belong to the Cav channel family, while the sustained Ca^{2+} influx may be carried through a store depletion-operated pathway.[112,113] Interestingly, it has been reported that several TRP channels are expressed in sperm and may be important for the sustained Ca^{2+} entry that drives the acrosome reaction.[113–115]

Evidence obtained in our laboratory indicated that MTx activates a Ca^{2+} influx that induces the mammalian acrosome reaction. The data initially suggested that the actions of MTx were comparable to those of other agents that promote a sustained increase in intracellular Ca^{2+} and drive the mammalian sperm acrosome reaction, including the physiological ligands of the *zona pellucida* (ZP).[116] More recently, however, we found differences in the acrosome reaction induced by MTx and the ZP in human and mouse sperm. Our data indicated that the acrosome reaction induced by the physiological ligands and by MTx occurred through distinct pathways.[117] By using specific PLC antagonists, the participation of a PLC-dependent signaling pathway in the ZP-induced acrosome reaction was confirmed. In contrast, the use of PLC inhibitors blocked the acrosome reaction induced by MTx in mouse but not in human sperm, unveiling species-specific variants of the acrosome reaction induced by the toxin.

Lastly, MTx has also been instrumental in unveiling of some of the mechanisms of the spermatogenic cell regulation exerted by Sertoli cells. We have previously shown that glucose and lactate, two substrates secreted by Sertoli cells toward the adluminal compartment in the seminiferous tubules, can modulate the activity of MTx-sensitive Ca^{2+} channels in enzymatically dissociated rat spermatocytes and spermatids.[118] By inducing changes in intracellular Ca^{2+}, both substrates can activate a Ca^{2+}/calmodulin-dependent protein kinase that results in the phosphorylation of MTx-sensitive channels. We have recently developed in our laboratory a methodology to study Ca^{2+} signaling in spermatogenic

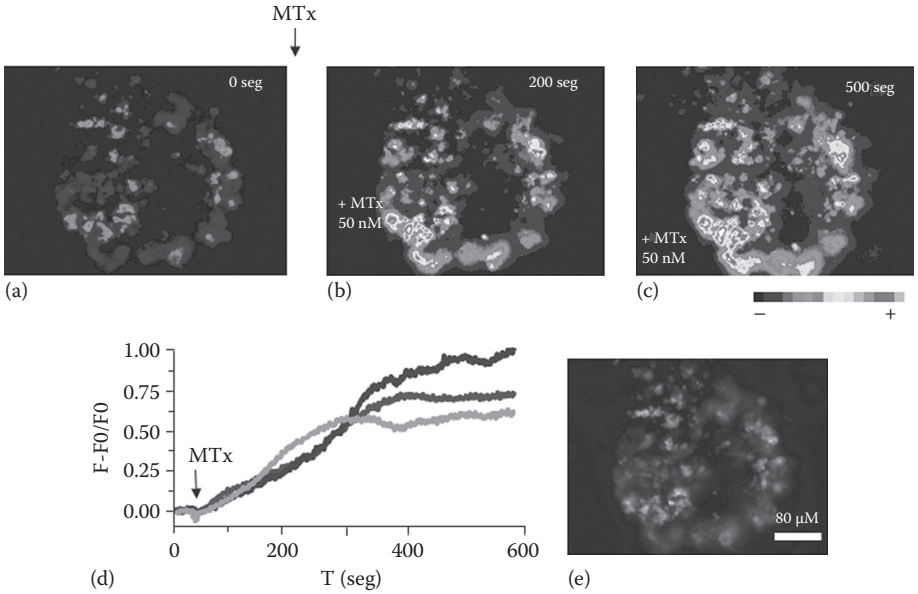

FIGURE 23.8 STSs are obtained as reported. Briefly, the *Tunica albuginea* is removed from mice testis and the seminiferous tubules are mechanically dispersed with tweezers in a Petri dish containing Ringer solution (in mM: 125NaCl, 2.5KCl, 2$CaCl_2$, 1$MgCl_2$, 1.25NaH_2PO_4, 26$NaHCO_3$, 12 glucose, gassed with 5% CO_2, 95% O_2, adjusted to pH 7.4). The dispersed tubules are embedded in agar (low melting point, 3%) to form a cube that is mounted on the plate of a vibratome, and 160 µM thick slices are obtained. STSs were loaded with fluo 4-AM (20 µM) immobilized with a nylon mesh, placed on the stage of a microscope, and continuously perfused (2 mL/min) with gassed physiological solution at room temperature. Fluorescence images were acquired (for equipment details, see [119]) every second with an exposure/illumination time of 10 ms for a total of 10 min (600 images). Pseudocolored fluorescence image obtained from the recording of STS before (a) and after addition of 50 nM MTx (b and c). Fluorescence traces obtained from three different cells in the STS shown in (a) and (d). Fluorescence image (black and white) of the same STS after incubation with fluo 4-AM (e). (From Sánchez-Cárdenas, C. et al., *Biol. Reprod*., 87, 92, 2012.)

cells by preparing slices of seminiferous tubules. This methodology has the advantage of preserving tissue architecture and intercellular connections,[119] and we demonstrated that Ca^{2+} signaling differs in dissociated spermatogenic cells compared to spermatogenic cells inside the tubules. Here, we show that MTx induces a generalized Ca^{2+} increase when applied to this seminiferous tubule slice (STS) preparation, which can be used to further study spermatogenic cell Ca^{2+} dynamics in a physiological environment that preserves cell interactions within the tubule (Figure 23.8). The knowledge generated using this approach could have relevant implications for the understanding of the physiological process of spermatogenic cell regulation by Sertoli cells, as well as the hormonal control that they may exert on spermatogenesis, which is not possible to study in vitro.

23.8 Final Remarks

MTx has inspired vast experimentation by organic and biological researchers due to its structural complexity and intricate mode of action. However, knowledge of both its organic and biochemical synthesis, as well as of its biological target(s), remains incomplete. The current lack of commercially available MTx underscores the importance for achieving its organic synthesis, so that it can become readily available again for studies on Ca^{2+} dynamics in different systems and also to help identify its putative receptor(s). This would lead to a better understanding of the molecular mechanisms involved in MTx action, which in turn may help explain the apparent discrepancies in its functional modalities. Knowledge in this area could also help to find appropriate treatment or an antidote for ciguatera.

ACKNOWLEDGMENTS

The authors would like to thank José Luis de la Vega, Yoloxochitl Sánchez, and Shirley Ainsworth for technical assistance. This work was supported by grants DGAPA-UNAM (IN202212 to CT, IN217210 to ILG) and CONACyT (99333 to CT, 84362 to ILG) and Fondecyt (1110267 to JGR).

REFERENCES

1. Plakas, S.M. and Dickey, R.W. 2010. Advances in monitoring and toxicity assessment of brevetoxins in molluscan shellfish. *Toxicon: Official Journal of the International Society on Toxinology*, 56, 137–149.
2. Fusetani, N. and Kem, W. 2009. Marine toxins: An overview. *Progress in Molecular and Subcellular Biology*, 46, 1–44.
3. Yasumoto, T. 2001. The chemistry and biological function of natural marine toxins. *The Chemical Record*, 1, 228–242.
4. Halstead, B.W. 1988. *Poisonous and Venous Marine Animals of the World*. Darwin Press, Princeton, NJ, p. 1168.
5. Hashimoto, Y. 1979. Marine organisms which cause food poisoning. In *Marine Toxins and Other Bioactive Marine Metabolites*. Japan Scientific Society Press, Tokyo, Japan, pp. 91–105.
6. Rey, J. 2007. Ciguatera. Available at: http://edis.ifas.ufl.edu/ENY-741 (accessed October 2007).
7. Yasumoto, T., Bagnis, R., and Vernoux, J. 1976. Toxicity of the surgeonfishes-II properties of the principal water soluble toxin. *Bulletin of the Japanese Society of Scientific Fisheries*, 42, 359–366.
8. Yasumoto, T. 1971. Toxicity of the surgeonfishes. *Bulletin of the Japanese Society of Scientific Fisheries*, 37, 724–734.
9. Friedman, M.A., Fleming, L.E., Fernandez, M., Bienfang, P., Schrank, K., Dickey, R., Bottein, M.-Y. et al. 2008. Ciguatera fish poisoning: Treatment, prevention and management. *Marine Drugs*, 6, 456–479.
10. Skinner, M.P., Brewer, T.D., Johnstone, R., Fleming, L.E., and Lewis, R.J. 2011. Ciguatera fish poisoning in the Pacific Islands (1998 to 2008). *PLoS Neglected Tropical Diseases*, 5, e1416.
11. Sheppard, C. and Rioja-Nieto, R. 2005. Sea surface temperature 1871–2099 in 38 cells in the Caribbean region. *Marine Environmental Research*, 60, 389–396.
12. Chateau-Degat, M., Chinain, M., Cerf, N., Gingras, S., Hubert, B., and Dewailly, E. 2005. Seawater temperature, *Gambierdiscus* spp. variability and incidence of ciguatera poisoning in French Polynesia. *Harmful Algae*, 4, 1053–1062.
13. Hale, S., Weinstein, P., and Woodward, A. 1999. Ciguatera (fish poisoning), el niño and the pacific sea surface temperatures. *Ecosystem Health*, 5, 20–25.
14. Yokoyama, A., Murata, M., Oshima, Y., Iwashita, T., Yasumoto, T., and Chemistry, F. 1988. Some chemical properties of maitotoxin, a putative calcium channel agonist isolated from a marine dinoflagellate. *Biochemistry Journal*, 187, 184–187.
15. Murata, M. and Yasumoto, T. 2000. The structure elucidation and biological activities of high molecular weight algal toxins: Maitotoxin, prymnesins and zooxanthellatoxins. *Natural Product Reports*, 17, 293–314.
16. Falkoner, I. 1993. *Algal Toxins in Seafood and Drinking Water*. Academic Press, San Diego, CA, p. 224.
17. Baden, D., Fleming, L.E., and Bean, J.A. 1995. Marine toxins. In *Handbook of Clinical Neurology: Intoxications of the Nervous System Part II. Natural Toxins and Drugs*. F.A. de Wolff (Ed). Elsevier Press, Amsterdam, the Netherlands, pp. 141–175.
18. Lewis, R. 2006. Ciguatera: Australian perspectives on a global problem. *Toxicon*, 48, 799–809.
19. Holmes, M. and Lewis, R. 1994. Purification and characterization of large and small maitotoxins from cultured *Gambierdiscus toxicus. Natural Toxins*, 2, 64–72.
20. Holmes, M., Lewis, R., and Gillespie, N. 1990. Toxicity of Australian and French Polynesian strains of *Gambierdiscus toxicus* (Dinophyceae) grown in culture: Characterization of a new type of maitotoxin. *Toxicon*, 28, 1159–1172.
21. Hokama, Y. 1985. A rapid, simplified enzyme immunoassay stick test for the detection of ciguatoxin and related polyethers from fish tissues. *Toxicon*, 23, 939–946.
22. Kellmann, R., Stüken, A., Orr, R.J.S., Svendsen, H.M., and Jakobsen, K.S. 2010. Biosynthesis and molecular genetics of polyketides in marine dinoflagellates. *Marine Drugs*, 8, 1011–1048.

23. Khosla, C., Gokhale, R.S., Jacobsen, J.R., and Cane, D.E. 1999. Tolerance and specificity of polyketide synthases. *Annual Review of Biochemistry*, 68, 219–253.
24. Hotta, K., Chen, X., Paton, R.S., Minami, A., Li, H., Swaminathan, K., Mathews, I.I. et al. 2012. Enzymatic catalysis of anti- Baldwin ring closure in polyether biosynthesis. *Nature*, 483, 355–358.
25. Murray, S.A., Garby, T., Hoppenrath, M., and Neilan, B.A. 2012. Genetic diversity, morphological uniformity and polyketide production in dinoflagellates (*Amphidinium*, *Dinoflagellata*). *PloS One*, 7, e38253.
26. Van Wagoner, R.M., Deeds, J.R., Tatters, A.O., Place, A.R., Tomas, C.R., and Wright, J.L.C. 2010. Structure and relative potency of several karlotoxins from *Karlodinium veneficum. Journal of Natural Products*, 73, 1360–1365.
27. Monroe, E.A. and Van Dolah, F.M. 2008. The toxic dinoflagellate *Karenia brevis* encodes novel type I-like polyketide synthases containing discrete catalytic domains. *Protist*, 159, 471–482.
28. Kubota, T., Iinuma, Y., and Kobayashi, J. 2006. Cloning of polyketide synthase genes from amphidinolide-producing dinoflagellate *Amphidinium* sp. *Biological & Pharmaceutical Bulletin*, 29, 1314–1318.
29. Shimizu, Y., Yorimitsu, A., Maruyama, Y., Kubota, T., Aso, T., and Bronson, R.A. 1998. Prostaglandins induce calcium influx in human spermatozoa. *Molecular Human Reproduction*, 4, 555–561.
30. Kwong, R.W.M., Wang, W.-X., Lam, P.K.S., and Yu, P.K.N. 2006. The uptake, distribution and elimination of paralytic shellfish toxins in mussels and fish exposed to toxic dinoflagellates. *Aquatic Toxicology* (Amsterdam, the Netherlands), 80, 82–91.
31. Monteiro, A. and Costa, P.R. 2011. Distribution and selective elimination of paralytic shellfish toxins in different tissues of *Octopus vulgaris. Harmful Algae*, 10, 732–737.
32. Costa, P.R., Pereira, P., Guilherme, S., Barata, M., Nicolau, L., Santos, M.A., Pacheco, M., and Pousão-Ferreira, P. 2012. Biotransformation modulation and genotoxicity in white seabream upon exposure to paralytic shellfish toxins produced by *Gymnodinium catenatum. Aquatic Toxicology* (Amsterdam, the Netherlands), 106–107, 42–47.
33. Liu, T., Cane, D.E., and Deng, Z. 2009. The enzymology of polyether biosynthesis. *Methods in Enzymology*, 459, 187–214.
34. Minami, A., Migita, A., Inada, D., Hotta, K., Watanabe, K., Oguri, H., and Oikawa, H. 2011. Enzymatic epoxide-opening cascades catalyzed by a pair of epoxide hydrolases in the ionophore polyether biosynthesis. *Organic Letters*, 13, 1638–1641.
35. Minami, A., Shimaya, M., Suzuki, G., Migita, A., Shinde, S.S., Sato, K., Watanabe, K., Tamura, T., Oguri, H., and Oikawa, H. 2012. Sequential enzymatic epoxidation involved in polyether lasalocid biosynthesis. *Journal of the American Chemical Society*, 134, 7246–7249.
36. Yasumoto, T. 2001. The chemistry and biological function of natural marine toxins. *Chemical Record* (New York), 1, 228–242.
37. Nakanishi, K. 1985. The chemistry of brevetoxins: A review. *Toxicon*, 23, 473–479.
38. Murata, M., Sasaki, M., Yokoyama, A., Iwashita, T., Gusovsky, F., Daly, J., and Yasumoto, T. 1992. Partial structures and binding studies of maitotoxin, the most potent marine toxin. *Bulletin de la Societe de Pathologie Exotique*, 85, 470–473.
39. Murata, M. and Yasumoto, T. 2000. The structure elucidation and biological activities of high molecular weight algal toxins: Maitotoxin, prymnesins and zooxanthellatoxins. *Natural Product Reports*, 17, 293–314.
40. Nicolaou, K.C. and Frederick, M.O. 2007. On the structure of maitotoxin. *Angewandte Chemie* (International ed. in English), 46, 5278–5282.
41. Nicolaou, K.C., Frederick, M.O., Burtoloso, A.C., Denton, R.M., Rivas, F., Cole, K.P., Aversa, R.J., Gibe, R., Umezawa, T., and Suzuki, T. 2008. Chemical synthesis of the GHIJKLMNO ring system of Maitotoxin. *Journal of the American Chemical Society*, 130, 7466–7476.
42. Nakata, T. 2010. SmI2-induced cyclizations and their applications in natural product synthesis. *Chemical Record* (New York), 10, 159–172.
43. Sakamoto, Y., Matsuo, G., Matsukura, H., and Nakata, T. 2001. Stereoselective syntheses of the C′D′E′F′-ring system of maitotoxin and the FG-ring system of gambierol. *Organic Letters*, 3, 2749–2752.
44. Morita, M., Haketa, T., Koshino, H., and Nakata, T. 2008. Synthetic studies on maitotoxin. 2. Stereoselective synthesis of the WXYZA′-ring system. *Organic Letters*, 10, 1679–1682.
45. Morita, M., Ishiyama, S., Koshino, H., and Nakata, T. 2008. Synthetic studies on maitotoxin. 1. Stereoselective synthesis of the C′D′E′F′-ring system having a side chain. *Organic Letters*, 10, 1675–1678.

46. Satoh, M., Koshino, H., and Nakata, T. 2008. Synthetic studies on maitotoxin. 3. Stereoselective synthesis of the BCDE-ring system. *Organic Letters*, 10, 1683–1685.
47. Nicolaou, K., Yang, Z., Shi, G., Gunzner, J., Agrios, K., and Gärtner, P. 1998. Total synthesis of brevetoxin A. *Nature*, 392, 264–269.
48. Nicolaou, K.C., Gelin, C.F., Seo, J.H., Huang, Z., and Umezawa, T. 2010. Synthesis of the QRSTU domain of maitotoxin and its 85-epi- and 86-epi-diastereoisomers. *Journal of the American Chemical Society*, 132, 9900–9907.
49. Nicolaou, K.C., Aversa, R.J., Jin, J., and Rivas, F. 2010. Synthesis of the ABCDEFG ring system of Maitotoxin. *Journal of the American Chemical Society*, 132, 6855–6861.
50. Nicolaou, K.C., Seo, J.H., Nakamura, T., and Aversa, R.J. 2011. Synthesis of the c′d′e′f′ domain of maitotoxin. *Journal of the American Chemical Society*, 133, 214–219.
51. Miyahara, J., Akau, C., and Yasumoto, T. 1979. Effects of ciguatoxin and maitotoxin on the isolated guinea pig atria. *Research Communications in Chemical Pathology and Pharmacology*, 25, 177–180.
52. Takahashi, M., Ohizumi, Y., and Yasumoto, T. 1982. Maitotoxin, a Ca^{2+} channel activator candidate. *The Journal of Biological Chemistry*, 257, 7287–7289.
53. Ohizumi, B.Y.Y. and Yasumotot, T. 1983. Contractile response of the rabbit aorta TO maitotoxin, the most potent marine toxin. *Journal of Physiology*, 337, 711–721.
54. Miyamoto, T., Ohizumi, Y., Washio, H., and Yasumoto, Y. 1984. Potent excitatory effect of maitotoxin on Ca channels in the insect skeletal muscle. *Pflugers Arch*, 400, 439–441.
55. Freedman, S.B., Miller, R.J., Miller, D.M., and Tindall, D.R. 1984. Interactions of maitotoxin with voltage-sensitive calcium channels in cultured neuronal cells. *Proceedings of the National Academy of Sciences of the United States of America*, 81, 4582–4585.
56. Schettini, G., Koike, K., Login, I., Judd, A., Cronin, M., Yasumoto, T., and MacLeod, R. 1984. Maitotoxin stimulates hormonal release and calcium flux in rat anterior pituitary cells in vitro. *American Journal of Physiology Endocrinology and Metabolism*, 247, E520–E525.
57. Kobayashi, M., Ohizumi, Y., and Yasumoto, T. 1985. The mechanism of action of maitotoxin in relation to Ca^{2+} movements in guinea-pig and rat cardiac muscles. *British Journal of Pharmacology*, 86, 385–391.
58. Legrand, A. and Bagnis, R. 1984. Effects of highly purified maitotoxin extracted from dinoflagellate *Gambierdiscus toxicus* on action potential of isolated rat heart. *Journal of Molecular and Cellular Cardiology*, 16, 663–666.
59. Berta, P., Sladeczek, F., Derancourt, J., Durand, M., Travo, P., and Haiech, J. 1986. Maitotoxin stimulates the formation of inositol phosphates in rat aortic myocytes. *FEBS Letters*, 197, 349–352.
60. Gusovsky, F., Yasumoto, T., and Daly, J. 1987. Maitotoxin stimulates phosphoinositide breakdown in neuroblastoma hybrid NCB-20 cells. *Cellular and Molecular Neurobiology*, 7, 317–322.
61. Gusovsky, F., Daly, J.W., Yasumoto, T., and Rojas, E. 1988. Differential effects of maitotoxin on ATP secretion and on phosphoinositide breakdown in rat pheochromocytoma cells. *FEBS Letters*, 233, 139–142.
62. Gusovsky, F., Yasumoto, T., and Daly, J.W. 1989. Maitotoxin, a potent, general activator of phosphoinositide breakdown. *FEBS Letters*, 243, 307–312.
63. Gusovsky, F., Yasumoto, T., and Daly, J. 1989. Calcium-dependent effects of maitotoxin on phosphoinositide breakdown and on cyclic AMP accumulation in PCi 2 and NCB-20 cells. *Molecular Pharmacology*, 36, 44–53.
64. Gusovsky, F., Bitran, J.A., Yasumoto, T., and Daly, J.W. 1990. Mechanism of maitotoxin- stimulated phosphoinositide breakdown in HL-60 cells. *The Journal of Pharmacology and Experimental Therapeutics*, 252, 466–473.
65. Pin, J., Yasumoto, T., and Bockaert, J. 1988. Maitotoxin-evoked gamma-aminobutyric acid release is due not only to the opening of calcium channels. *Journal of Neurochemistry*, 50, 1227–1237.
66. Sladeczek, F., Schmidt, B.H., Alonso, R., Vian, L., Tep, A., Yasumoto, T., Cory, R.N., and Bockaert, J. 1988. New insights into maitotoxin action. *European Journal of Biochemistry*, 174, 663–670.
67. Hamilton, S. and Perez, M. 1987. Toxins that affect voltage-dependent calcium channels. *Biochemical Pharmacology*, 36, 3325–3329.
68. Kobayashi, M., Ochi, R., and Ohizumi, Y. 1987. Maitotoxin-activated single calcium channels in guinea-pig cardiac cells. *British Journal of Pharmacology*, 92, 665–671.
69. Yoshii, M., Tsunoo, A., Kuroda, Y., Wu, C.H., and Narahashi, T. 1987. Maitotoxin-induced membrane current in neuroblastoma cells. *Brain Research*, 424, 119–125.
70. Murata, M., Gusovsky, F., Yasumoto, T., and Daly, J. 1992. Selective stimulation of Ca^{2+} flux in cells by maitotoxin. *The European Journal of Pharmacology*, 227, 43–49.

71. Xi, D., Van Dolah, F.M., and Ramsdell, J.S. 1992. Maitotoxin induces a calcium-dependent membrane depolarization in GH4C1 pituitary cells via activation of type L voltage-dependent calcium channels. *The Journal of Biological Chemistry*, 267, 25025–25031.
72. Nishio, M., Kigoshi, S., Muramatsu, I., and Yasumoto, T. 1993. $Ca^{(2+)}$- and $Na^{(+)}$- dependent depolarization induced by maitotoxin in the crayfish giant axon. *General Pharmacology*, 24, 1079–1083.
73. Worley III, J.F., Mcintyre, M.S., Spencer, B., and Dukes, I.D. 1994. Depletion of intracellular Ca^{2+} stores activates a maitotoxin-sensitive nonselective cationic current in beta-cells. *The Journal of Biological Chemistry*, 269, 32055–32058.
74. Musgrave, I.F., Seifert, R., and Schultz, G. 1994. Maitotoxin activates cation channels distinct from the receptor-activated non-selective cation channels of HL-60 cells. *The Biochemical Journal*, 301 (Pt 2), 437–441.
75. Dietl, P. and Volkl, H. 1994. Maitotoxin activates a nonselective cation channel and Ca^{2+} entry in MDCK renal epithelial cells. *Molecular Pharmacology*, 45, 300–305.
76. Young, R., McLaren, M., and Ramsdell, J. 1995. Maitotoxin increases voltage independent chloride and sodium currents in GH4C1 rat pituitary cells. *Natural Toxins*, 3, 419–427.
77. Nishio, M., Muramatsu, I., and Yasumoto, T. 1996. $Na^{(+)}$-permeable channels induced by maitotoxin in guinea-pig single ventricular cells. *European Journal of Pharmacology*, 297, 293–298.
78. Estacion, M., Nguyen, H.B., Gargus, J.J., Estacion, M., and Bryant, H. 1996. Calcium is permeable through a maitotoxin-activated nonselective cation channel in mouse L cells Calcium is permeable through a maitotoxin-activated nonselective cation channel in mouse L cells. *The American Journal of Physiology-Cell Physiology*, 270, 1145–1152.
79. Bielfeld-Ackermann, A., Range, C., and Korbmacher, C. 1998. Maitotoxin (MTX) activates a nonselective cation channel in *Xenopus laevis* oocytes. *Pflügers Archiv: European Journal of Physiology*, 436, 329–337.
80. Cataldi, M., Secondo, A., D'Alessio, A., Taglialatela, M., Hofmann, F., Klugbauer, N., Di Renzo, G., and Annunziato, L. 1999. Studies on maitotoxin-induced intracellular $Ca^{(2+)}$ elevation in Chinese hamster ovary cells stably transfected with cDNAs encoding for L- type $Ca^{(2+)}$ channel subunits. *The Journal of Pharmacology and Experimental Therapeutics*, 290, 725–730.
81. Martínez-françois, J.R., Morales-tlalpan, V., and Vaca, L. 2002. Characterization of the maitotoxin-activated cationic current from human skin fibroblasts. *Journal of Physiology*, 538, 79–86.
82. Schilling, W.P., Sinkins, W.G., and Estacion, M. 1999. Maitotoxin activates a nonselective cation channel and a P2Z/$P2X_7$-like cytolytic pore in human skin fibroblasts Maitotoxin activates a nonselective cation channel and a P2Z/$P2X_7$-like cytolytic pore in human skin fibroblasts. *The American Journal of Physiology*, 277, C755–C765.
83. Schilling, W.P., Wasylyna, T., Dubyak, G.R., Humphreys, B.D., and Sinkins, W.G. 1999. Maitotoxin and P2Z/$P2X_7$ purinergic receptor stimulation activate a common cytolytic pore. *The American Journal of Physiology*, 277, C766–C776.
84. Egido, W., Castrejón, V., Antón, B., and Martínez, M. 2008. Maitotoxin induces two dose-dependent conductances in *Xenopus oocytes*. Comparison with nystatin effects as a pore inductor. *Toxicon: Official Journal of the International Society on Toxicology*, 51, 797–812.
85. Murata, M., Gusovsky, F., Sasaki, M., Yokoyama, A., Yasumoto, T., and Daly, J. 1991. Effect of maitotoxin analogues on calcium influx and phosphoinositide breakdown in cultured cells. *Toxicon*, 29, 1085–1096.
86. Konoki, K., Hashimoto, M., Nonomura, T., Sasaki, M., Murata, M., and Tachibana, K. 1998. Influx in rat glioma inhibition of maitotoxin-induced Ca^{2+} influx in rat glioma C6 cells by brevetoxins and synthetic fragments of maitotoxin. *Journal of Neurochemistry*, 70, 409–416.
87. Oishi, T., Konoki, K., Tamate, R., Torikai, K., Hasegawa, F., Matsumori, N., and Murata, M. 2012. Artificial ladder-shaped polyethers that inhibit maitotoxin-induced Ca^{2+} influx in rat glioma C6 cells. *Bioorganic & Medicinal Chemistry Letters*, 22, 3619–3622.
88. Konoki, K., Hashimoto, M., Murata, M., and Tachibana, K. 1999. Maitotoxin-induced calcium influx in erythrocyte ghosts and rat glioma C6 cells, and blockade by gangliosides and other membrane lipids. *Chemical Research in Toxicology*, 12, 993–1001.
89. Murata, M., Matsumori, N., Konoki, K., and Oishi, T. 2008. Structural features of dinoflagellate toxins underlying biological activity as viewed by NMR. *Bulletin of the Chemical Society of Japan*, 81, 307–319.

90. Nicolaou, K.C., Frederick, M.O., and Aversa, R.J. 2008. The continuing saga of the marine polyether biotoxins. *Angewandte Chemie* (International ed. in English), 47, 7182–7225.
91. Nicolaou, K. and Aversa, R.J. 2011. Maitotoxin: An inspiration for synthesis. *Israel Journal of Chemistry*, 51, 359–377.
92. Humphrey, W., Dalke, A., and Schulten, K. 1996. VMD: Visual molecular dynamics. *Journal of Molecular Graphics*, 14, 33–38, 27–28.
93. Mori, M., Oishi, T., Matsuoka, S., Ujihara, S., Matsumori, N., Murata, M., Satake, M., Oshima, Y., Matsushita, N., and Aimoto, S. 2005. Ladder-shaped polyether compound, desulfated yessotoxin, interacts with membrane-integral alpha-helix peptides. *Bioorganic & Medicinal Chemistry*, 13, 5099–5103.
94. Yamauchi, T., Kamon, J., Waki, H., Terauchi, Y., Kubota, N., Hara, K., Mori, Y. et al. 2001. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nature Medicine*, 7, 941–946.
95. McTaggart, J.S., Clark, R.H., and Ashcroft, F.M. 2010. The role of the KATP channel in glucose homeostasis in health and disease: More than meets the islet. *The Journal of Physiology*, 588, 3201–3209.
96. Rorsman, P. and Braun, M. 2013. Regulation of insulin secretion in human pancreatic islets. *Annual Review of Physiology*, 75, 1–25.
97. Uchida, K. and Tominaga, M. 2011. The role of thermosensitive TRP (transient receptor potential) channels in insulin secretion. *Endocrine Journal*, 58, 1021–1028.
98. Akiba, Y., Kato, S., Katsube, K., Nakamura, M., Takeuchi, K., Ishii, H., and Hibi, T. 2004. Transient receptor potential vanilloid subfamily 1 expressed in pancreatic islet beta cells modulates insulin secretion in rats. *Biochemical and Biophysical Research Communications*, 321, 219–225.
99. Cuypers, E., Yanagihara, A., Karlsson, E., and Tytgat, J. 2006. Jellyfish and other cnidarian envenomations cause pain by affecting TRPV1 channels. *FEBS Letters*, 580, 5728–5732.
100. Diaz-Garcia, C.M., Fuentes-Silva, D., Sanchez-Soto, C., Domínguez-Pérez, D., García- Delgado, N., Varela, C., Mendoza-Hernández, G., Rodriguez-Romero, A., Castaneda, O., and Hiriart, M. 2012. Toxins from *Physalia physalis* (Cnidaria) raise the intracellular Ca^{2+} of beta-cells and promote insulin secretion. *Current Medicinal Chemistry*, 19, 5414–5423.
101. Leech, C.A. and Habener, J.F. 1997. Insulinotropic glucagon-like peptide-1-mediated activation of non-selective cation currents in insulinoma cells is mimicked by maitotoxin. *The Journal of Biological Chemistry*, 272, 17987–17993.
102. Roe, M.W., Worley, J.F., Qian, F., Tamarina, N., Mittal, A.A., Dralyuk, F., Blair, N.T., Mertz, R.J., Philipson, L.H., and Dukes, I.D. 1998. Characterization of a Ca^{2+} release- activated nonselective cation current regulating membrane potential and $[Ca^{2+}]i$ oscillations in transgenically derived beta-cells. *The Journal of Biological Chemistry*, 273, 10402–10410.
103. Holz, G.G., Leech, C.A. and Habener, J.F. 2000. Insulinotropic toxins as molecular probes for analysis of glucagon-like peptide-1 receptor-mediated signal transduction in pancreatic beta-cells. *Biochimie*. 82(9–10), 915–926.
104. Dinarello, C.A., Simon, A., and Van der Meer, J.W.M. 2012. Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. *Nature Reviews: Drug Discovery*, 11, 633–652.
105. Gabay, C., Lamacchia, C., and Palmer, G. 2010. IL-1 pathways in inflammation and human diseases. *Nature Reviews Rheumatology*, 6, 232–241.
106. Verhoef, P.A., Kertesy, S.B., Estacion, M., Schilling, W.P., and Dubyak, G.R. 2004. Maitotoxin induces biphasic interleukin-1beta secretion and membrane blebbing in murine macrophages. *Molecular Pharmacology*, 66, 909–920.
107. Mariathasan, S., Weiss, D.S., Newton, K., McBride, J., O'Rourke, K., Roose-Girma, M., Lee, W.P., Weinrauch, Y., Monack, D.M., and Dixit, V.M. 2006. Cryopyrin activates the inflammasome in response to toxins and ATP. *Nature*, 440, 228–232.
108. Federici, S., Caorsi, R., and Gattorno, M. 2012. The autoinflammatory diseases. *Swiss Medical Weekly*, 142, 13602.
109. Verhoef, P.A., Estacion, M., Schilling, W., and Dubyak, G.R. 2003. $P2X_7$ receptor-dependent blebbing and the activation of Rho-effector kinases, caspases, and IL-1 beta release. *Journal of Immunology* (Baltimore, MD: 1950), 170, 5728–5738.
110. Sinkins, W.G., Estacion, M., Prasad, V., Goel, M., Shull, G.E., Kunze, D.L., and Schilling, W.P. 2009. Maitotoxin converts the plasmalemmal $Ca^{(2+)}$ pump into a $Ca^{(2+)}$-permeable nonselective cation channel. *The American Journal of Physiology Cell Physiology*, 297, C1533–C1543.

111. Darszon, A., Nishigaki, T., Beltran, C., and Trevino, C.L. 2011. Calcium channels in the development, maturation, and function of spermatozoa. *Physiological Review*, 91, 1305–1355.
112. O'Toole, C.M., Arnoult, C., Darszon, A., Steinhardt, R.A., and Florman, H.M. 2000. $Ca^{(2+)}$ entry through store-operated channels in mouse sperm is initiated by egg ZP3 and drives the acrosome reaction. *Molecular Biology of the Cell*, 11, 1571–1584.
113. Jungnickel, M.K., Marrero, H., Birnbaumer, L., Lemos, J.R., and Florman, H.M. 2001. Trp2 regulates entry of Ca^{2+} into mouse sperm triggered by egg ZP3. *Natural Cell Biology*, 3, 499–502.
114. Trevino, C.L., Serrano, C.J., Beltran, C., Felix, R., and Darszon, A. 2001. Identification of mouse trp homologs and lipid rafts from spermatogenic cells and sperm. *FEBS Letters*, 509, 119–125.
115. Sutton, K.A., Jungnickel, M.K., Wang, Y., Cullen, K., Lambert, S., and Florman, H.M. 2004. Enkurin is a novel calmodulin and TRPC channel binding protein in sperm. *Devision of Biology*, 274, 426–435.
116. Treviño, C.L., De la Vega-Beltrán, J.L., Nishigaki, T., Felix, R., and Darszon, A. 2006. Maitotoxin potently promotes Ca^{2+} influx in mouse spermatogenic cells and sperm, and induces the acrosome reaction. *Journal of Cellular Physiology*, 206, 449–456.
117. Chávez, J.C., De Blas, G.A., De la Vega-Beltrán, J.L., Nishigaki, T., Chirinos, M., González-González, M.E., Larrea, F., Solís, A., Darszon, A., and Treviño, C.L. 2011. The opening of maitotoxin-sensitive calcium channels induces the acrosome reaction in human spermatozoa: Differences from the zona pellucida. *Asian Journal of Andrology*, 13, 159–165.
118. Reyes, J., Osses, N., Knox, M., Darszon, A., and Trevino, C. 2010. Glucose and lactate regulate maitotoxin-activated Ca^{2+} entry in spermatogenic cells: The role of intracellular [Ca^{2+}]. *FEBS Letters*, 584, 3111–3115.
119. Sánchez-Cárdenas, C., Guerrero, A., Treviño, C.L., Hernández-Cruz, A., and Darszon, A. 2012. Acute slices of mice testis seminiferous tubules unveil spontaneous and synchronous Ca^{2+} oscillations in germ cell clusters. *Biology of Reproduction*, 87, 92.
120. Treviño, C.L., Escobar, L., Vaca, L., Morales-Tlalpan, V., Ocampo, A.Y., and Darszon, A. 2008. Maitotoxin: A unique pharmacological tool for elucidating Ca^{2+}-dependent mechanisms. In *Seafood and Freshwater Toxins: Pharmacology, Physiology and Detection*, 2nd edn. L.M. Botana (ed.). CRC Press, Boca Ratón, FL, pp. 503–516.
121. Nicolaou, K., Duggan, M., and Hwang, C. 1986. New synthetic technology for the construction of oxocenes. *Journal of American Chemical Society*, 108, 2468–2469.