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Ciguatera: Australian perspectives on a global problem

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Abstract

Ciguatera is a global disease caused by the consumption of certain warm-water fish that have accumulated orally effective levels of sodium channel activator toxins (ciguatoxins) through the marine food chain. Symptoms of ciguatera arising from the consumption of ciguateric fish include a range of gastrointestinal, neurological and cardiovascular disturbances. This review examines progress in our understanding of ciguatera from an Australian perspective, especially the laboratory-based research into the problem that was initiated by the late "Bob" Endean at the University of Queensland.

Keywords: Fish poisoning; Ciguatoxin; Gabierdiscus toxicus; Polyether; Sodium channel toxin

1. Introduction

Ciguatera is a form of marine fish poisoning that has complex environmental origins, and causes diverse and often long-lasting human health effects. To address the complexities of ciguatera, in the late 1950s "Hank" Banner in Hawaii initiated multi-disciplinary research into the challenging question of the nature and cause of ciguatera. Together with Paul Scheuer, Jack Randall and other colleagues in Hawaii, and Raymond Bagnis in French Polynesia who became interested in ciguatera after meeting with Banner, this early research laid the foundation for many of the important advances in our understanding of ciguatera achieved to-date. This review extends and updates an earlier review of ciguatera (Lewis, 2001). A number of additional reviews provide more detailed accounts of the origin (Lewis and Holmes, 1993), socioeconomic impact (Lewis, 1992a), clinical effects (Lewis and Ruff, 1993), pharmacology (Lewis et al., 2000), risk analysis (Lehane and Lewis, 2000) and detection (Lewis, 1995) of ciguatera. Reviews by Hasimoto (1979) on the chemistry and Banner (1976) on the ecology of ciguatera cover earlier research in more detail.

2. Ciguatera research in Australia

The earliest accounts of ciguatera in Australia date back to Captain Cook's experiences in the 18th century. More recent accounts describing the signs and symptoms and the species implicated have been published by Whitley (1934), Cleland (1942), Broadbent (1968), Pearn et al. (1982), Edmonds (1974) and Gillespie et al. (1986). Research on ciguatera conducted at James Cook University in the 1970s were not published. In the mid-1970s, Endean's interest in ciguatera was sparked by a review of ciguatera by Banner (1976) that was published in

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the "Biology and Geology of Coral Reefs" series he edited. In 1978, I approached Endean for a Master's project where he suggested I should characterise ciguatera in Queensland fish. Because of my interest in marine chemistry and pharmacology, I chose not to undertake a second and equally insightful project to examine the genetic differences between reef fish populations.

Research on ciguatera with Endean proved extremely rewarding, and not just for the science it produced. Endean's bigger than life character (he was an ex-boxer) was never far from an adventure. Stories of Bob getting into a scuffle were common. His friend Dr. Peter James recounts that when angered, Bob would throw a half-full glass of beer from hand to hand before throwing it at his opponent, who usually caught it with two hands, exposing themselves to a deadly uppercut (those who new this move could impress Bob by catching the glass in one hand and defend with the other). I also remember a day protecting his gold mine from claim jumpers, and the sense of satisfaction and relief when that day passed without a shot.

The early research on ciguatera with Endean yielded five co-authored papers on the chemical and pharmacological characterisation of the toxins in Queensland Spanish mackerel and barracuda (Lewis and Endean, 1983a, b, 1984a, b, 1986). After completing my PhD, Noel Gillespie offered a position at the Queensland Department of Primary Industries where I continued ciguatera research. Here I started to analyse the ciguatera database initiated by Noel, and together with Noel and a PhD student Mike Holmes investigated the origins of ciguatera. Noel had also attracted Australian Fishing Industry Research and Development funding to develop an antibody-based test for ciguatera, providing an opportunity to extract ciguatoxins (CTXs) from highly toxic moray eel viscera collected in the Republic of Kiribati. While this work never resulted in a test, two new CTXs were identified (see Fig. 1; Lewis et al., 1991, 1993a, b). Endean continued researching the origin of ciguatera, proposing that water-soluble toxins identified in Spanish mackerel arose from *Oscillatoria* (Endean et al., 1993) in an extention of the work of Hahn and Capra (1992) who isolated undefined toxins from this blue-green algae. However, the role of *Oscilatoria* remains to be confirmed. Another important Australian researcher in the field, and a friend of Endean, John Pearn contributed to our understanding of clinical aspects and treatments for ciguatera (Pearn et al., 1989; Pearn, 2001).

3. Distribution of ciguatera

Ciguatera is caused by the consumption of tropical and sub-tropical fishes (Bagnis, 1981). It is estimated to affect more than 25,000 persons annually. While ciguatera is a global phenomena, it is mostly confined to discrete regions of the Pacific Ocean and western Indian Ocean, and the Caribbean Sea. In the Pacific ciguatera affects most of the island nations (Banner and Helfrich, 1964). Despite this wide distribution, there are many areas that are relatively free of ciguatera, occasionally immediately adjacent to areas of high risk. For example, the southern reef of Tarawa and the western reef of Maraki in The Republic of Kiribati, have a high risk of ciguatera, whereas the remaining reef of these atolls are low-risk areas. An explanation for such patchiness in ciguatera remains elusive. Current difficulties to predict, detect and treat ciguatera (discussed below) mean that this form of fish poisoning will continue to have large socioeconomic impacts, particularly in developing countries. Nowhere are these impacts greater than in regions where fish is the principal source of protein, such as the atoll island communities of the Pacific (Lewis, 1992a).

In Australia, ciguatera is restricted to the tropical waters off Queensland and to the western part of the Gulf of Carpentaria in the Northern Territory (Nhulunbuy). Most cases of ciguatera presently come from reef fish caught on the Great Barrier Reef, and from Spanish mackerel caught inside the reef north of Fraser Island, where it is believed they first accumulate the toxin (Holmes et al., 1994).

4. Symptoms of ciguatera

Globally, the symptoms of ciguatera are well described. In a comprehensive study of ciguatera in the eastern Pacific, Bagnis et al. (1979) described over 3000 cases in French Polynesia. A similar syndrome is observed in Australia and the central Pacific (Gillespie et al., 1986). The onset of the first symptoms can be as short as 30 min for severe intoxications, while in milder cases may be delayed for up to 24 to occasionally 48 h after consumption of fish. The first symptoms can be either gastrointestinal or neurological in nature (e.g. circumoral tingling). Gastrointestinal symptoms usually last

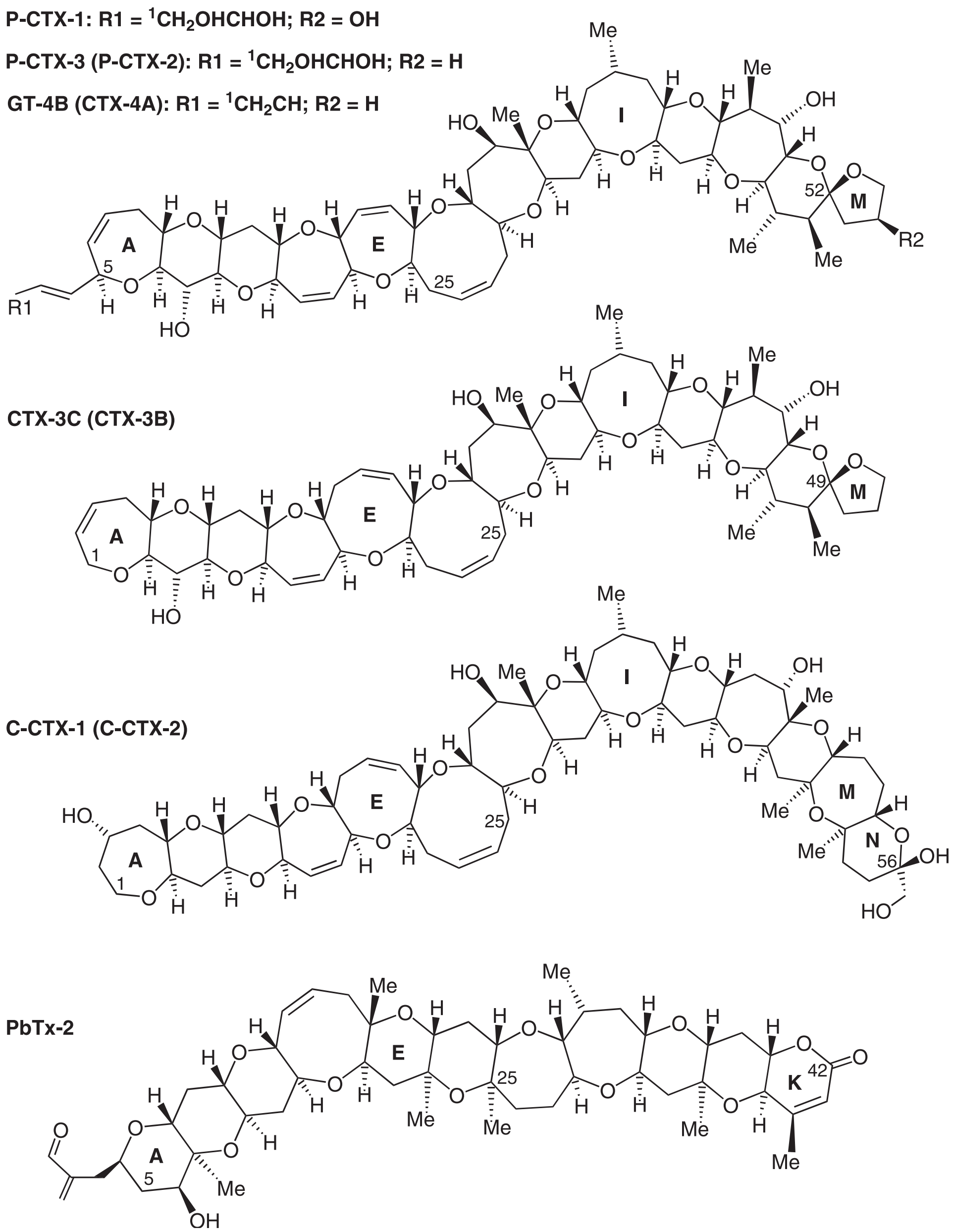

Fig. 1. Structure of Pacific and Caribbean ciguatoxins (CTXs). Shown are P-CTX-1 (Murata et al., 1990), P-CTX-3 (Lewis et al., 1991, 1993a, b), P-CTX-4B (Murata et al., 1990), P-CTX-3C (Satake et al., 1993), and C-CTX-1 (Lewis et al., 1998). Also shown is the structure of PbTx-2 for comparison. The energetically less favoured epimers, P-CTX-2 (52-epi P-CTX-3; Lewis et al., 1993a, b), P-CTX-4A (52-epi P-CTX-4B; Satake et al., 1997), and C-CTX-2 (56-epi C-CTX-1; Lewis et al., 1998), are indicated in parenthesis. 2,3-dihydroxyP-CTX-3C and 51-hydroxyP-CTX-3C have also been isolated from Pacific fish (Satake et al., 1998). Additional minor P-CTXs structurally characterised by mass spectroscopy are reported in Yasumoto et al. (2000).

only a few days, while some neurological symptoms can take several days to develop. Ciguatera typically lasts for several weeks to several months. In a small percentage of cases (estimated $<5\%$) certain symptoms may persist for a number of years.

The symptoms of ciguatera comprise a combination of a few to more than 30 gastrointestinal, neurological and/or generalised disturbances (Gillespie et al., 1986). Gastrointestinal symptoms, involving vomiting, diarrhoea, nausea and abdominal pain (>~50% of cases), typically occur early in the course of the disease, and often, but not always, accompany the neurological disturbances. Neurological disturbances invariably occur in ciguatera, and include tingling of the lips hands and feet, unusual temperature perception disturbances where cold objects give a dry-ice sensation, and a severe localised itch of the skin (>~70% of cases). These symptoms and a profound feeling of fatigue (90% of cases) can occur throughout the illness. Muscle (>80%), joint (>70%) and teeth aches (>30%) occur to varying extents, and mood disorders including depression and anxiety (50%) occur less frequently. Severe cases of ciguatera can involve hypotension with bradycardia, respiratory difficulties and paralysis, but deaths are uncommon. The low fatality rate appears to arise because fish rarely accumulate sufficient levels of CTX to be lethal at a single meal, perhaps because fish succumb to the lethal effects of higher CTX levels (Lewis, 1992b). Some fatalities may be avoided with the introduction of better clinical management practices.

Some sufferers of ciguatera develop adverse reactions to the consumption of certain foods (estimated to be <2%), or while affected by alcohol (>20%) which are reminiscent of the original symptoms experienced. Implicated foods include fish, peanuts, chicken and pork. These unusual sensitivities can last long after the victim feels otherwise recovered, and can contribute to the long convalescence periods occasionally observed in ciguatera. The pathophysiological basis of these sensitivities, which appear allergy-like in nature, remains to be determined.

5. Comparison of ciguatera from different oceans

The severity, number and duration of ciguatera symptoms reflect a combined influence of dose, toxin profile and individual susceptibility. In the Pacific Ocean neurological symptoms predominate, while in the Caribbean Sea it is the gastrointestinal symptoms that dominate. These quantitative differences in symptoms provide a clue that different CTXs may underlie ciguatera in Pacific and Caribbean waters. This has now been confirmed following the isolation of different CTXs in each region, and determination of the structures of the major CTXs involved in the Pacific and Indian Oceans (see Fig. 1). Four Indian Ocean CTXs (I-CTX-1–4) have been isolated and characterised as site 5 sodium channel toxins (Hamilton et al., 2002a, b). However, the I-CTXs have defied structural characterisation due to their instability during the late stages of purification (Hamilton et al., 2002b). These toxins give rise to symptoms that are typical of ciguatera in the Pacific, but in addition cause a cluster of symptoms reminiscent of hallucinatory poisoning, including lack of coordination, loss of equilibrium, hallucinations, mental depression and nightmares (Quod and Turquet, 1996). Ciguateric fish in the Indian Ocean are also more frequently contaminated by lethal levels of CTX (Habermehl et al., 1994). A number of additional C-CTXs have been identified but not structurally characterised beyond their mass spectrometry and HPLC profile (Pottier et al., 2002a, b, 2003). Exactly how the different CTXs influence the pattern of symptoms is not known, despite recent advances in our understanding of their chemistry and pharmacology. Pharmacokinetic and subtle pharmacological differences are likely to be the key determinants.

Across the western, central and eastern Pacific Ocean the pattern of symptoms of ciguatera from carnivorous fish is similar, suggesting that a similar class of CTXs are involved. Differences in the frequency of certain symptoms have been observed for herbivorous and carnivorous species from French Polynesia (Bagnis et al., 1992), with *Ctenochaetus striatus* causing fewer symptoms than carnivorous species. However, this may simply reflect the lower levels of CTXs that are accumulated by *C. striatus* than by carnivorous species in this region, and thus the number (and severity) of symptoms is less. Maitotoxins, a class of polyether toxins that enhance calcium entry into cells, also accumulate in the viscera but not the flesh of herbivorous species. However, if maitotoxins are involved, which is unlikely given their low oral toxicity, qualitative (not just quantitative) differences in symptomology are expected, given that maitotoxins have quite different pharmacology to the CTXs (in French Polynesia the viscera of *C. striatus* is consumed without the appearance of new symptoms). An unusual delayed ataxia has been observed briefly for ciguatera from Scaridae in the Gambier Islands (Bagnis et al., 1974). While scaritoxin was isolated at the time, and later reisolated and identified as P-CTX-4A (Satake et al., 1997), delayed ataxia is no longer reported in this region, and the chemical entity responsible for this syndrome may still be uncharacterised.

6. Differential diagnosis, treatment and convalescence

Effective treatment of ciguatera requires accurate diagnosis of the syndrome. Despite its key role in clinical management, differential diagnosis of ciguatera remains problematic. Presently, ciguatera is a clinically determined disease associated with the recent consumption of a potentially ciguateric fish. Usually at least one neurological plus one other typical symptom are required for diagnosis, and each person exposed to the fish of moderate and high toxicity are likely to be affected with symptoms consistent with ciguatera. It is important to exclude other marine poisonings such as palytoxin poisoning and other unrelated illnesses. Ciguatera is often misdiagnosed as illnesses such as flu. In Queensland it is estimated that $<20\%$ of cases are reported. The Queensland ciguatera database established originally by Dr. Gillespie at the Queensland Department of Primary Industries more than 20 years ago, presently contains detailed record of ~1000 cases (0.16 cases per 10,000 Queensland residents per annum).

In an important advance, intravenous mannitol was introduced as a treatment for ciguatera in the late 1980s (Palafox et al., 1988; Pearn et al., 1989) following its chance use in the Marshall Islands on a coma victim who was later diagnosed to be severely affected by ciguatera (Palafox et al., 1988). Correctly diagnosed and adequately hydrated cases of ciguatera often respond to an i.v. infusion of mannitol, given at 1 g/kg over ~30 min. In instances where symptoms recur within the first 24 h after treatment, a second infusion is usually effective. Mannitol is not consistently beneficial, however, and appears best when used in the acute phase of more severe intoxications. Reasons for a poor response are not known. A recent double-blind clinical trial of i.v. mannitol indicated no significant benefit over i.v. saline, with both interventions producing a significant improvement in symptoms (Schnorf et al., 2002). The apparent beneficial effect of saline suggests that symptomatic and supportive therapies still have a role in managing more severe cases, especially the control of fluid and electrolyte balance. Local anaesthetics and antidepressants may also be useful in some instances. During the recovery phase it is recommended that victims avoid fish and alcohol for 3–6 months, and long-term sufferers should question if certain foods are contributing to recurrences of symptoms and attempt to replace them.

7. Origin of and transfer of toxins involved in ciguatera

Since the food chain hypothesis for ciguatera proposed by Randall (1958), research on the origin of ciguatera has focussed on identifying a small benthic organism as the origin of "ciguatera toxins". Twenty years later, a Japan–France collaboration located a bloom of a benthic dinoflagellate in the ciguatera endemic Gambier Islands which produced CTX-like toxins (Yasumoto et al., 1977). This epiphytic, benthic dinoflagellate, named *Gambierdiscus toxicus* (Adachi and Fukuyo, 1979), has now been confirmed to indeed be the origin of Pacific CTXs by Yasumoto's group. Since its discovery, circumtropical surveys have confirmed that *G. toxicus* is present wherever ciguatera occurs (see Lewis and Holmes, 1993). However, as might be expected from the patchy distribution of ciguateric fishes, *G. toxicus* has rarely been found naturally in bloom numbers, making difficult to conduct research into the nature of the CTXs produced in the field. This obstacle was circumvented by laboratory culture studies, but not until it was recognised that only low percentage of culturable, clonal strains of *G. toxicus* were capable of producing detectable CTX (Holmes et al., 1991). Interestingly, different strains of *G. toxicus* also produce chemically distinct, though pharmacologically related, water-soluble toxins, named maitotoxins (Murata et al., 1993; Holmes and Lewis, 1994). While the maitotoxins characterised have slightly higher potency than CTX when injected i.p. in mice, their low oral potency and poor ability to accumulate in fish flesh suggests they are unlikely to play a significant role in causing human illness. To-date, no compelling evidence has been provided for a role in ciguatera for water-soluble toxins of any kind, or for toxins from other benthic dinoflagellate species, including okadaic acid.

The environmental factors responsible for blooms of *G. toxicus* have not been clarified, despite the critical role that an understanding of the biogenesis of ciguatera could play in the mitigation of ciguatera. Several studies have linked anthropogenic effects to upsurges of ciguatera. However, once again the specific factors involved have not been identified, and many human (and natural) disturbances occur without triggering an increase in ciguatera risk. An added complexity to the origin of ciguatera is the strain-dependent nature of CTX production. It is possible that "super-producing" strains may underlie ciguatera but be difficult to isolate or culture (Holmes et al., 1994). An influence of environmental factors on toxin production, and any differential effect of environmental factors on low and high CTX producing strains has not been established. Production of antifungal polyether compounds is also strain dependent. Dinoflagellates with different capacities to produce antifungals may respond differently to changed environmental conditions (Lewis et al., 1997).

It is well-established in the Pacific that *G. toxicus* produces less polar (less oxidised) CTX (Fig. 1) that undergo varying extents of biotransformation as they pass through the marine food chain (Lewis and Holmes, 1993). Interestingly, the more oxidised forms, especially P-CTX-1 the dominant CTX found in carnivorous fish in the Pacific, are up to 10-fold more toxic than the CTXs produced by *G. toxicus*. This increase in potency amplifies the effective levels of CTX accumulated by fish, and increases the risk these toxins pose to humans. Little is known about the origin of CTXs in the Caribbean Sea and Indian Ocean, and the extent these toxins are biotransformed as they pass through their respective marine food chains. The recent identification of a small (809 and 857 Da) fast acting toxin from barracuda adds to the complexity of toxins involved in this region (Pottier et al., 2003).

8. Fish involved

Many species and many families of reef fish are involved in ciguatera globally. These include the herbivorous Acanthurids and corallivorous Scarids, which are considered key vectors in the transfer of CTXs from *G. toxicus* to carnivorous fish. However, herbivorous species not normally caught for food are also likely to play important roles as toxin vectors. In the Caribbean, herbivorous fishes are not normally associated with ciguatera and the herbivore vectors in this region are not obvious and perhaps include invertebrates. In contrast, many more species of carnivorous fish cause ciguatera. These include the Muraenids and Lutjanids (e.g. red bass), which are notorious in the Pacific, Serranids including coral trout from the Great Barrier Reef, Epinephelids, Lethrinids, Scombrids, Carangids and Sphyraenids. The latter two families are a particular problem in the Caribbean. In some regions, the first herbivore may be an invertebrate species. This may be the case in Platypus Bay inside Fraser Island, Queensland, where Alpheid shrimps appear to the important vector transferring CTXs to the small carnivore *Pomadasys maculatus. P. maculatus* probably then passes these toxins to the large mackerel (*Scomberomerus commersoni*) which are notorious in this region (Lewis and Endean, 1983a; Lewis and Holmes, 1993). Given the diversity of prey preferences among these families of carnivores, it seems likely that additional herbivore vectors of CTXs will be identified in the future. Differences in digestive strategies among different herbivores and carnivores are likely to influence the degree and nature of CTX biotransformation through the marine food chain (Lewis and Holmes, 1993).

9. Chemical features of CTXs

CTXs are a family of heat-stable, lipid-soluble, highly oxygenated, cyclic polyether molecules (Fig. 1) that have a structural framework reminiscent of the brevetoxins (PbTx-1–10) produced by the marine dinoflagellate *Gymnodinium breve* (= *Ptychodiscus brevis*; Baden, 1989). Modern NMR has proved an invaluable technique for the elucidation of structures from the microgram quantities of CTX typically isolated from fish and *G. toxicus* (Murata et al., 1989, 1990; Lewis et al., 1991, 1993a, b, 1998; Satake et al., 1993, 1997, 1998). The source and potency of structurally defined CTXs are shown in Table 1. Many additional minor toxins have been detected using mass spectrometry (MS), and liquid chromatography/MS (Lewis and Jones, 1997; Vernoux and Lewis, 1997; Pottier et al., 2002a, b, 2003). Yasumoto et al. (2000) described the use of high-energy MS/MS methods to resolve the structures of 16 CTXs from samples containing as little as a few μg of mixed CTXs.

10. Pharmacology and mode of action of CTX

In mammals, the CTXs are the most potent sodium channel toxins known (Table 1), being similarly potent in mice by the oral or i.p. route. Pharmacological studies have revealed that CTXs activate voltage-sensitive sodium channels (VSSCs) at nM and pM concentrations (reviewed by Lewis et al., 2000). Specifically, CTXs cause a hyperpolarising shift in the voltage dependence of channel activation, causing the opening of sodium channels

Table 1
Source (*G. toxicus*, herbivorous or carnivorous fish) and potency of ciguatoxins from the Pacific Ocean (P-CTXs), Indian Ocean (I-CTXs) and Caribbean Sea (C-CTXs)

Ciguatoxin	Source	$[M+H]^+$	Potency (μg/kg)	Reference
P-CTX-1 (CTX)	Carnivore	1111	0.25	Murata et al. (1990), Lewis et al. (1991)
P-CTX-2	Carnivore	1095	2.3	Lewis et al. (1991)
P-CTX-3	Carnivore	1095	0.9	Lewis et al. (1991)
P-CTX-3C	*G. toxicus*	1045	2	Satake et al. (1993)
2,3-DihydroxyP-CTX-3C	Carnivore	1057	1.8	Satake et al. (1998)
51-HydroxyP-CTX-3C	Carnivore	1039	0.27	Satake et al. (1998)
P-CTX-4A	*G. toxicus*/herbivore	1061	2	Satake et al. (1997)
P-CTX-4B	*G. toxicus*/herbivore	1061	4	Murata et al. (1990)
I-CTX-1	Carnivore	1141	~0.5	Hamilton et al. (2002b)
I-CTX-2	Carnivore	1141	~0.5	Hamilton et al. (2002b)
C-CTX-1	Carnivore	1141	3.6	Vernoux and Lewis (1997)
C-CTX-2	Carnivore	1141	1	Vernoux and Lewis (1997)

Protonated molecular mass ($[M+H]^+$) and intraperitoneal (i.p.) potency to mice are indicated. I-CTX potency is estimated from potency to displace ^{3}H-brevetoxin from rat brain membrane relative to P-CTX-1 (Hamilton et al., 2002b).

at resting membrane potentials. These effects lead to a TTX-sensitive, Na^+-dependent cell depolarisation (Rayner, 1972), spontaneous firing of a variety of nerve types (Bidard et al., 1984; Molgó et al., 1990; Hamblin et al., 1995; Brock et al., 1995; Hogg et al., 1998), an elevation of $[Ca^{2+}]_i$ (Lewis and Endean, 1986; Molgó et al., 1993), and oedema of Schwann cells (Allsop et al., 1986) and axons (Benoit et al., 1996; Mattei et al., 1999). CTXs and brevetoxins selectively target a common binding site ("site 5") on the neuronal VSSC protein (Bidard et al., 1984; Sharkey et al., 1987; Lombet et al., 1987; Lewis et al., 1991; Poli et al., 1986, 1997). Using a photolabelled derivative of PbTx-3 and site-directed antibody mapping, "site 5" of rat brain VSSCs includes segments S6 and S5 of domains I and IV, respectively (Trainer et al., 1994).

Strachan et al. (1999) studied the effect of P-CTX-1 on TTX-sensitive and TTX-resistant VSSCs in sensory ganglia. P-CTX-1 (0.2–20 nM) causes modest hyperpolarising shifts in the activation and steady-state inactivation of TTX-sensitive VSSCs. Interestingly, at the TTX-resistant VSSC the major effect of P-CTX-1 is an increase in the rate of recovery from inactivation. P-CTX-1 also induces a leakage current, but only through TTX-sensitive channels. Studies of P-CTX-1 at the level of single VSSCs show effects consistent with these alterations to whole cell current (Hogg et al., 1998). From single channel studies, it appears that the leakage current arises from a proportion of VSSCs that open spontaneously at normal and hyperpolarised cell membrane potentials. Recently, Hogg et al. (2002) described Na^+- and voltage-dependent membrane oscillations produced by CTX-1 but not brevetoxin. These oscillations led to the development of spontaneous action potentials in nerves.

11. Avoiding ciguateric fish

Several approaches can be taken to minimise the impact of ciguatera. At the distributor/individual level, the risk of ciguatera can be minimised or avoided by excluding the purchase or sale of warm-water fish, allowing only small servings of individual risk fish ($\leqslant 50$ g), or in the future by testing risk fish prior to consumption. At the Industry/Government level, ciguatera could be minimised through the introduction of bans on high-risk fish, restrictions on fish captured in high-risk areas, or in the future the large-scale screening of high risk. A number of in vivo methods have been established to detect CTXs by oral dosing of flesh (meat-eating fly larvae and chicken) or i.p. injection of crude extracts of fish (mouse) (see Lewis, 1995). Alternative in vitro assays offer much potential to replace such assays, particularly sodium channel and antibody assays. Unfortunately, the simple detection of ciguateric fish is made difficult by a number of factors, including the low levels of CTXs present in ciguateric fish (<0.05 ppb for P-CTX-1), the meagre quantities of CTX available for research, the multiple structural forms that are present even within a single fish, the absence of any useful chromophore, and the difficulties synthesising even fragments of these molecules (Sasaki et al., 1994; Eriksson et al., 1999).

A membrane immunobead assay, originally developed by Hokama's group as a simple, rapid, sensitive, and specific detection method for CTX and its related polyethers (Hokama et al., 1998), is being sold by Oceanit as Cigua-Check. While no false-negative results are reported, further validation of the test is required to confirm that the method is robust and accurate. Recently, antibodies were produced to a synthetic fragment of P-CTX-1 that may prove suitable for the simple detection of CTXs in fish (Pauillac et al., 2000; Oguri et al., 2003). Unfortunately, affinity for the IJKLM antibody of CTX-3C was relatively poor and the ABCDE antibody would not be expected to interact with the major pacific CTX (P-CTX-1). The recent synthesis of the ABCDE rings of P-CTX-1 (Kobayashi et al., 2004) provides an opportunity to improve the targeting of the sandwich assay. Synthesis of fragments of C-CTX are needed before antibodies suitable for detecting the C-CTX can be developed (see Fig. 1).

Sodium channel assays have also been developed to detect CTXs using cytotoxicity (Manger et al., 1993, 1995) or displacement of [^{3}H]PbTx-3 binding to brain VSSCs (Lombet et al., 1987; Lewis et al., 1991; Poli et al., 1997) as measures of CTX content. These assays can detect subpicogram levels of P-CTX-1, give an estimate that reflects potency, and have the potential to be developed into high-throughput assays. As with the antibody-based assays, rigorous validation is required to confirm the potential of such assays to be developed into rapid, cost-effective screens for public health protection. Analytical MS methods have been developed with the required sensitivity, but are unlikely to be cost-effective for routine screening (Lewis et al., 1999). Tests that detect the presence of CTX in patients are needed to overcome the present limitations of differential diagnosis.

12. Future directions for ciguatera research

Many important and challenging questions remain to be answered in relation to ciguatera. These include: (i) determining the specific environmental and genetic factors that influence the proliferation and toxin production of *G. toxicus*; (ii) determining the origin and food chain transfer of Caribbean CTXs; (iii) determining the origin and structure of Indian Ocean CTX(s); (iv) developing effective (oral) therapies for ciguatera; (v) developing rapid methods for polyether synthesis; (vi) developing a validated, cost-effective screen (e.g. antibody or VSSC based) for detecting toxic levels of CTXs in fish; and (vii) developing improved analytical methods (e.g. LC/MS) that can rapidly quantify CTXs present in fish, humans and dinoflagellates. To answer them will require well-funded multidisciplinary research programs, with a greater emphasis on inter-laboratory collaboration.

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