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FINAL REPORT

CIGUATERA POISONING: PHARMACOLOGY AND PATHOLOGY

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JOHN CAMERON MBBS FRACP PhD

Queensland Institute of Technology

A FINAL REPORT

ON

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"The Pharmacology and Pathology of Ciguatera Poisoning in Mammals and Studies on the Possible Effects of Ciguatoxin on Fish."

FINAL REPORT

CIGUATERA POISONING: PHARMACOLOGY AND PATHOLOGY

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I. GENERAL INTRODUCTION AND REVIEW OF CIGUATERA POISONING

Ciguatera poisoning is a form of seafood poisoning that occurs in humans after the ingestion of particular specimens of a variety of tropical marine fish species. Ciguatera poisoning is caused by the ingestion of small quantities of a very powerful toxin, ciguatoxin (CTX), that occurs in the tissues of the offending fish.

The distribution of human ciguatera poisoning is confined mainly to the tropics where it is a considerable cause of morbidity. Over 50,000 people may be afflicted each year in the tropical regions of the world (Ragelis, 1984). It is the most frequently reported foodborne disease of a chemical nature within the USA with most cases emanating from tropical regions of southeastern Florida and Hawaii (Withers, 1982).

In the small Pacific Island nations where fish forms a considerable component of the diet, ciguatera poisoning can cause a significant public health problem. South Pacific Commission figures over the five year period 1977 to 1981 report incidences as low as 2.5/100,000 population in the Solomon Islands, to as high as 570/100,000 in French Polynesia. Estimates of the annual incidences of ciguatera poisoning in two Australia communities (Capra, 1985; Capra and Cameron 1985) were 24/100,000 in Maryborough - Hervey Bay and 34/100,000 in Cairns.

The Chemistry and Pharmacology of CTX

A heat stable, non proteinaceous compound, CTX, with a MW of approximately 1,100 Daltons can be extracted from the tissues of fish that have caused human ciguatera poisoning. The extraction and purification of CTX is both difficult and expensive as only minute quantities of the toxin, 0.5 to 10.0 ppb, occur in the tissues of poisonous fish (Yasumoto et al, 1984). A yield of less than 300 µg of purified CTX from 100 kg of toxic fish flesh was reported by Yasumoto et al (1984).

As yet there has been no full structural elucidation of CTX, however the molecule is known to be a polycyclic lipid with a number of ethereal oxygen groups (Tachibana, 1980.) CTX is believed to be structurally similar to another marine compound, okadaic acid, a polycyclic ether produced by sponges (Tachibana et al, 1981) and dinoflagellates (Murakami et al, 1982).

The symptomatology of ciguatera poisoning is not always constant: individual symptoms or groups of symptoms may be absent or exaggerated in individual victims of the toxin. Variation in the symptomatology may reflect individual differences in the physiological responses to the same toxin or quantitative differences due to the actual amount of toxin consumed. A possibility that cannot be excluded, at present, is that CTX is not a single entity but as family of related compounds each with slightly different pharmacological actions. (Miyahara et al 1987).

Purified CTX is an extremely toxic compound with an LD_{50} of 0.45 µg/kg ip in mice. It has long been recognised that CTX has profound effects upon the nervous system and Li (1965) suggested that CTX acted primarily as an anticholinesterase. However, Li's studies appeared to be based on impure extracts contaminated by other compounds and his assertion of an anticholinesterase activity was subsequently rejected (Ogura et al, 1968; Rayner, 1969).

Rayner (1969) was the first to suggest that CTX caused a widespread effect on excitable tissues by increasing Na^+ permeability. There is now ample evidence to support the view that CTX acts on the Na^+ channels of excitable membranes and increases the permeability of these membranes to Na^+ (Boyarsky and Rayner, 1970; Rayner, 1972; Bidard et al, 1984; Capra and Cameron, 1985; Lewis, 1985).

In cultured neuroblastoma cells CTX induces a membrane depolarization that is antagonized by tetrodotoxin (Bidard et al, 1984). The widespread neural effects of CTX would appear to be due to this fundamental action of CTX on Na^+ channels within excitable membranes. The autonomic response to CTX may be due to both presynaptic effects producing transmitter release and postsynaptic effects of CTX on Na^+ channels in effectors (Lewis, 1985).

The Origin and Transmission of CTX in the Biota

Humans can fall victim to ciguatera poisoning after eating an individual specimen of any one of a number of marine tropical fish. Over 400 species of fish have been listed worldwide as potential carriers of CTX and so possible sources of human ciguatera poisoning (Halstead, 1978). Halstead's figures are considered as an overestimation and the number of potential carrier species may be much lower; data collected by Bagnis et al (1985) over a 20 year period in French Polynesia suggest that some 100 species are carriers of CTX.

In Australia several species of primarily carnivorous fish are known to have caused human intoxication; included in these are:- barracuda, Sphyraena jello; chinaman-fish, Symphorus nematophorus; coral trout, Plectropomus maculatus; coronation trout, Variola louti; narrow barred Spanish mackerel, Scomberomorus commersoni; paddletail, Lutjanus gibbus and red bass, Lutjanus bohar.

In Queensland waters, three species of fish, chinaman-fish, paddletail and red bass are considered by many to be high risk species. Studies on the toxicity of these species in the Cairns region affirm such an assertion and suggest the consumption of the three species should be avoided (Table 1), (Capra, 1985).

Table 1: Estimated incidence of toxicity in three species of fish from the Cairns region.

Species (# assayed)	Incidence Toxic Fish/1000
Red Bass Lutjanus bohar (93)	43
Paddle tail Lutjanus gibbus (11)	182
Chinaman-fish Symphorus nematophorus (44)	68

Most cases of ciguatera poisoning in Australia are caused by eating carnivorous species, as Australian fish consumption patterns tend to favour such fish. Where herbivorous fish such as the surgeon fish Ctenochaetus are eaten they too can cause human ciguatera poisoning.

Although it has long been known that ciguatera poisoning may follow the ingestion of tropical fish, the actual origin of the toxin remained obscure until quite recently when the diets of carrier species were closely examined in endemically toxic areas of French Polynesia (Yasumoto et al, 1977a). Large number of dinoflagellate Diplopsalis sp were found in a toxic fraction of detritus from the Gambier Islands and extracts from these dinoflagellate rich samples had similar chromatographic, pharmacological and immunological properties to CTX derived from fish tissues (Yasumoto et al, 1977b). The dinoflagellate, initially classified as Diplopsalis, was subsequently described as Gambierdiscus toxicus (Adachi and Fukuyo, 1979). Gambierdiscus, an epiphyte found on a number of species of macroalgae, has now been identified in Hawaii (Taylor, 1979), the Caribbean (Miller et al, 1982), areas of the Pacific other than French Polynesia (Yasumoto et al, 1984) and Australia (Gillespie et al, 1985). Cultures of Gambierdiscus have produced maitotoxin (MTX) (Gillespie et al, 1985) and mixtures of both MTX and CTX (Durand et al, 1985). MTX is a large (MW > 10,000 Daltons) non proteinaceous compound found in the viscera of fish that are herbivores or benthic grazers. It has been suggested that MTX may be a precursor of CTX (Bagnis, 1981). However, Gambierdiscus toxicus may not be the sole originator of toxins responsible for the ciguatera syndrome. In the Caribbean the dinoflagellate Prorocentrum concavum produces a number of compounds that may cause ciguatera-like diseases (Tindall et al, 1984).

CTX is believed to enter the food chain via browsing and grazing fish such as the surgeon fish, Ctenochaetus, and then move through various trophic levels. It would seem that CTX moves from one trophic level to the next in fish without apparently harming either predator or prey.

Detection of Toxic Fish

As noted, many species of fish are potential carriers of CTX, however the probability of any one single specimen from a potential carrier species having CTX in its tissues is often very small. Thousands of coral trout may be sold in an area before a toxic specimen is found by unwitting human bioassay. Although CTX is an extremely potent toxin, it occurs only in minute traces in the tissues of fish and hence there are no simple chemical or physical means available to detect its presence.

Currently, most techniques for the detection of CTX are based on some form of bioassay: mongeese and cats can be directly fed suspect fish (Banner et al, 1960), while rapid and crude extractions from fish can be made and injected into mice (Pompon and Bagnis, 1984) or mosquitoes (Chungue et al, 1984). All bioassay techniques are labour intensive, time consuming and difficult to quantify.

Significant developments that may allow a rapid, simple and cost effective methods of CTX detection, based upon enzyme immunoassay techniques and the raising of monoclonal antibodies are currently in progress at the University of Hawaii (Hokama et al, 1984; Hokama et al, 1985) and in the laboratories of the Queensland Department of Primary Industries.

Symptoms of Ciguatera Poisoning

There have been many reports on the clinical manifestations and symptomatology of ciguatera poisoning in humans (Russell, 1975; Bagnis et al, 1979; Lawrence et al, 1980; Capra and Cameron, 1985a). After the ingestion of a toxic fish, the course of the affliction usually follows a reasonably predictable pattern. The initial symptoms are gastrointestinal and usually develop at an early stage some three to twelve hours after a meal of fish. These symptoms can include nausea, vomiting, diarrhoea and abdominal cramps.

The usual time of onset for the gut symptoms is around six hours after the meal; the severity of the symptoms is variable and may depend upon the toxicity and quantity of ingested fish. Following the gastrointestinal dysfunction, neurological symptoms usually begin to appear twelve to eighteen hours after the fish was eaten. The neurological symptoms can include, paraesthesia, arthralgia, myalgia, dental pain, severe pruritus, convulsions, muscular paralysis, audio and visual hallucinations, vertigo, severe headache, diaphoresis, loss of short term memory and temperature perception reversals. Some patients have shown psychological disturbances, manifested as anxiety states and depression for several months after intoxication. Skin rashes on the limbs, neck and trunk often occur within a few days to a few weeks of the ingestion of toxic fish. Cardiovascular symptoms including bradycardia, tachycardia, arrhythmias and hypotension are also noted in a number of victims of ciguatera poisoning.

A striking feature of ciguatera poisoning is the pattern of neurological disturbance that may vary from mild discomfort for a few days to more severe symptoms that can last for many weeks. In some individuals the neurological symptoms may persist for months (Lawrence et al, 1980) or even years (Capra and Cameron, 1985a).

Intoxication with CTX confers no immunity for further exposure to the toxin: quite the contrary situation exists in which a second or subsequent intoxication leads to more severe symptoms (Bagnis, 1967; Bagnis et al, 1979). Also ingestion of alcohol can exacerbate the symptoms and can reintroduce symptoms in individuals who have apparently recovered from an episode of intoxication. Likewise the ingestion of non tropical fish or even unrelated protein e.g., poultry can re-establish the symptoms of the poisoning. The mechanisms underlying these re-establishments of symptoms remain obscure, however the immune system may be implicated in some instances.

Although the symptoms of ciguatera poisoning are often severe and the course of the illness can be prolonged, the incidence of mortality is low. A comprehensive study of some three thousand victims of ciguatera poisoning in the Pacific by Bagnis et al (1979) reveal a mortality rate of less than 0.1%. In Australia there has been only one well documented death attributable to a severe CTX intoxication (Tonge et al, 1967).

The Impact of Ciguatera Poisoning on the Australian Community

The symptomatology of ciguatera poisoning particularly those of neural origin sets it apart from some of the more common but less severe forms of food poisoning. Outbreaks of ciguatera poisoning are thus more likely to generate media attention than an outbreak of a less severe food poisoning. Since the early 1980's the printed and electronic media have from time to time featured the cause, symptoms and sequelae of ciguatera poisoning. The topic was covered by Four Corners in 1981 and then featured on the 0-10 network documentary "Australian Killers" in 1982. Within the last twelve months items on ciguatera poisoning have been presented on the ABC Quantum programme and on Beyond 2000 on the Seven network. Many reports of ciguatera poisoning have appeared in newspapers throughout Australia and as these reports often detail outbreaks of ciguatera poisoning following the sale of toxic fish they tend to be of a sensational nature.

Considerable press interest was focused on the 1987 outbreak of ciguatera poisoning in Sydney in which sixty three people were poisoned (Cameron and Capra, 1987). The 1987 Sydney ciguatera outbreak is, to date, the largest single outbreak on record in Australia. Litigation, both by the N.S.W. State Health Department and individual victims is still pending as a result of the sale of toxic mackerel from Hervey Bay, Queensland by a Sydney wholesale vendor. The litigation in Sydney will again draw media coverage and if successful may severely compromise the exportability of fish caught in tropical waters.

II. OBJECTIVES OF THE PROJECT

In the initial application for funds in 1983/84 the following objectives were detailed:-

(i) Isolation and purification of CTX from toxic fish.

(ii) Testing the hypothesis "that CTX as a fundamental action causes opening of Na^+ channels in excitable tissues".

(iii) An examination of the electrophysiological and neuropathological effects of CTX on in vivo and in vitro nerve muscle preparations in laboratory mammals.

(iv) An assessment of functional and structural damage to peripheral nerves in the victims of chronic CTX intoxication.

(v) Examination of the effects of CTX on excitable membranes in "carrier" and "non-carrier" species of fish.

In renewals of the project two additional objectives were included as follows:-

(vi) To examine the histopathological effects of CTX on non nervous tissues.

(vii) To assess the effects of CTX on survival in fish.

Each of the above objectives were addressed during the duration of the project and will be discussed in the following sections.

III EXTRACTION AND PURIFICATION OF CTX

Introduction

Central to any successful study of the actions of CTX is a supply of toxic fish. Toxic fish or suspected toxic fish were obtained from three sources:-

a) Toxic material obtained in an early FIRTA project "A survey of the incidence of ciguatoxin in the "high risk" fish from the Cairns region", FIRTA 82/89.

b) As donations from victims of ciguatera poisoning.

c) Purchased from commercial fishermen from Gove in the Northern Territory.

Suspected toxic fish was assessed by animal bioassay and if toxicity was confirmed the fish was extracted by a modification of the method of Nukina et al (1984).

Methods

a) Bioassay Techniques

At the beginning of the programme all suspected fish were screened for toxicity by feeding flesh to juvenile and adult cats (450g - 3.1 kg). Cats were fed fish at 5% or 10% of body weight and observed for 48 hours for signs and symptoms of ciguatera poisoning such as vomiting, diarrhoea and ataxia. A review of animal ethics by the R.S.P.C.A. in 1985 stopped the use of cats for research and consequently mice were used for screening from mid 1985.

The mouse bioassay involves the intraperitoneal injection of an extract of 10g of suspect fish. The extract was made by a modification of the method of Pompon and Bagnis (1984) and involves an acetone extraction and subsequent purification on columns of diatomaceous earth and silicic acid. Mice injected with the fish extract were then carefully monitored for signs of ciguatera poisoning. The degree of toxicity of a sample could be estimated by the severity and duration of symptoms or the death time if the extract was potent.

b) Extraction and Purification of CTX

Fish giving a positive bioassay result were extracted as detailed below and as summarized in the flow diagrams (Figs. 1 & 2).

Figure 1

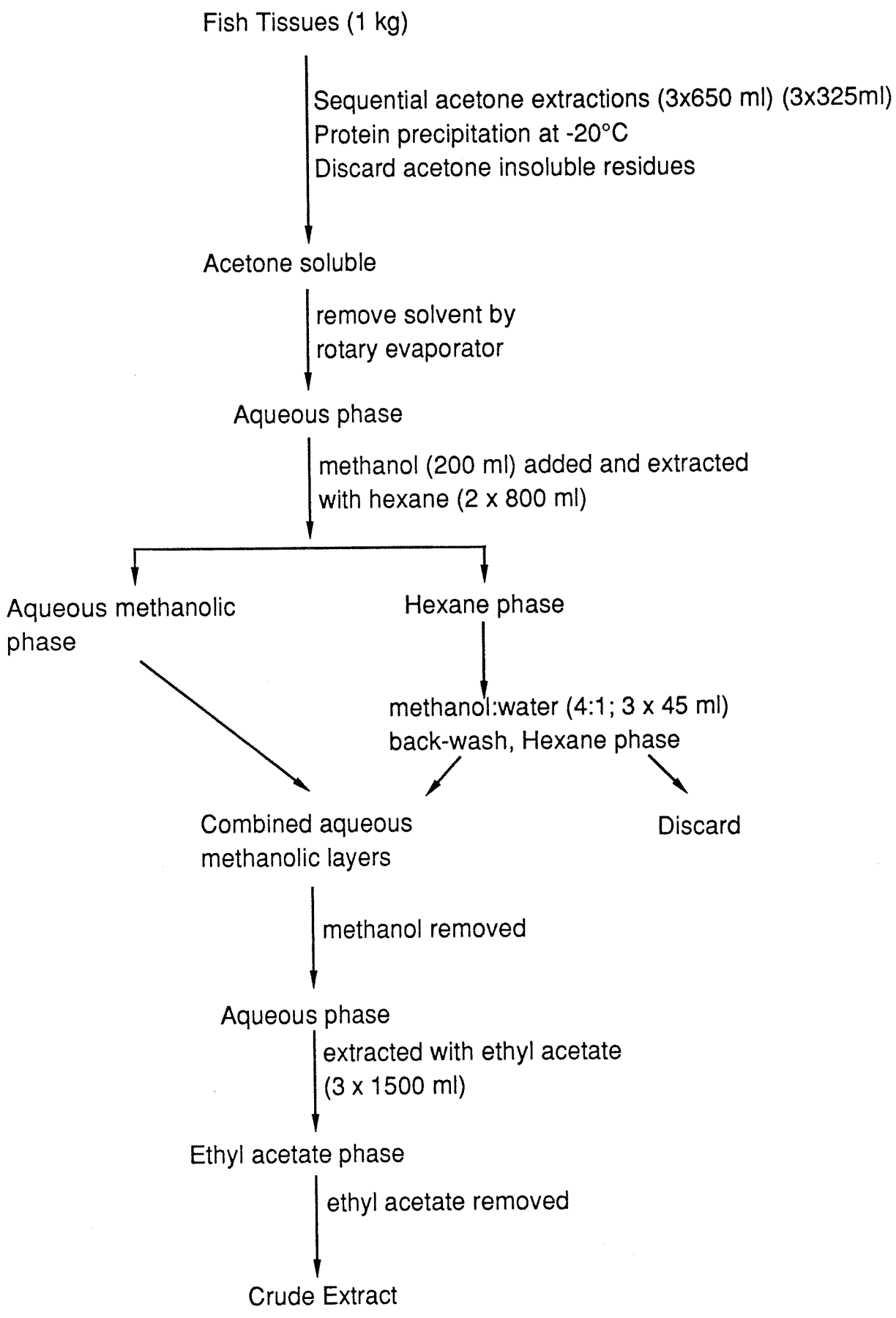
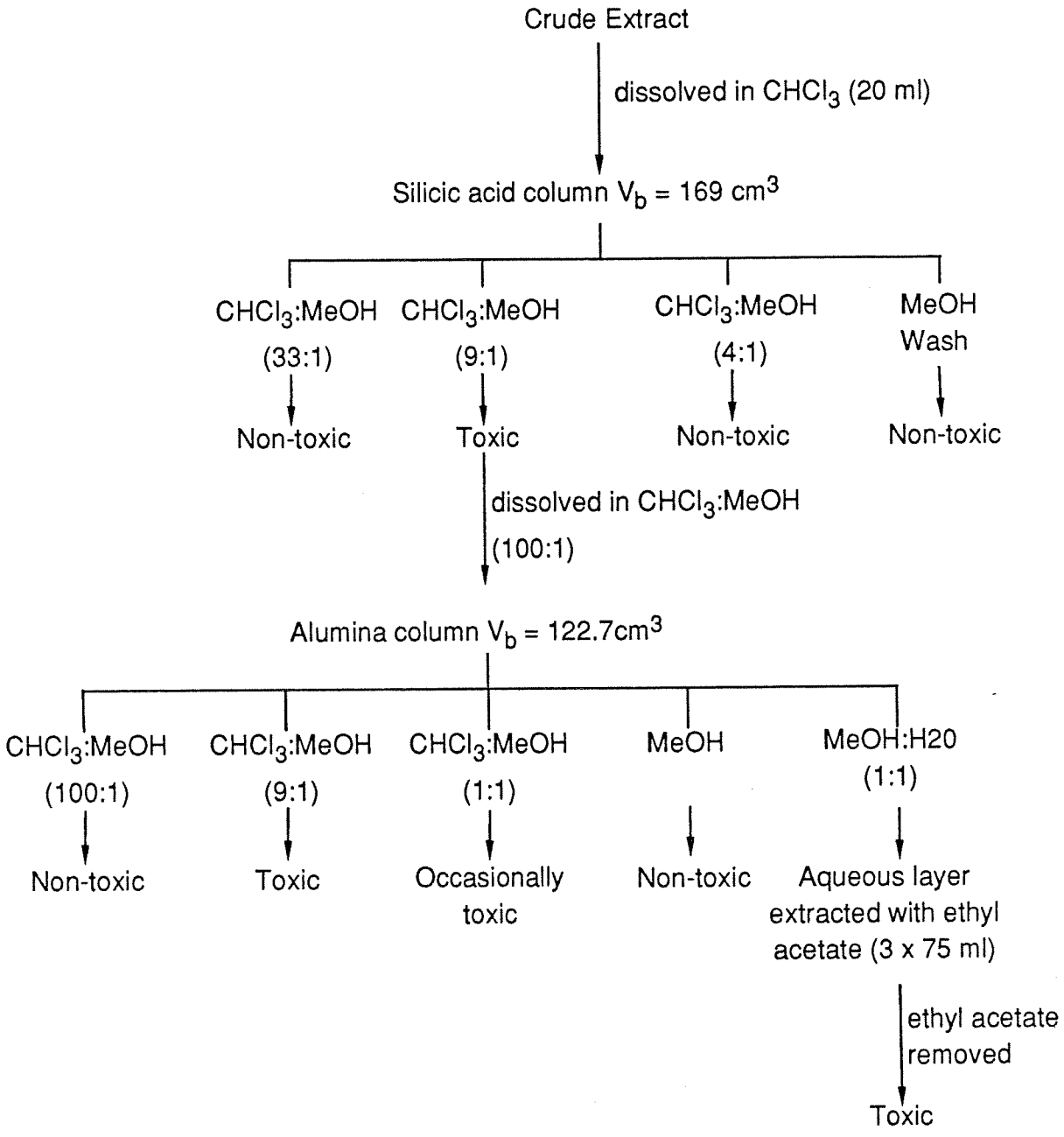

Fish Tissues (1 kg)
Sequential acetone extractions (3x650 ml) (3x325ml)
Protein precipitation at -20°C
Discard acetone insoluble residues
Acetone soluble
remove solvent by
rotary evaporator
Aqueous phase
methanol (200 ml) added and extracted
with hexane (2 x 800 ml)
Aqueous methanolic
phase
Hexane phase
methanol:water (4:1; 3 x 45 ml)
back-wash, Hexane phase
Discard
Combined aqueous
methanolic layers
methanol removed
Aqueous phase
extracted with ethyl acetate
(3 x 1500 ml)
Ethyl acetate phase
ethyl acetate removed
Crude Extract

Figure 2


Crude Extract
dissolved in $CHCl_3$ (20 ml)
Silicic acid column V_b = 169 cm^3
$CHCl_3$:MeOH (33:1)
Non-toxic
$CHCl_3$:MeOH (9:1)
Toxic
$CHCl_3$:MeOH (4:1)
Non-toxic
MeOH Wash
Non-toxic
dissolved in $CHCl_3$:MeOH (100:1)
Alumina column V_b = 122.7cm^3
$CHCl_3$:MeOH (100:1)
Non-toxic
$CHCl_3$:MeOH (9:1)
Toxic
$CHCl_3$:MeOH (1:1)
Occasionally toxic
MeOH
Non-toxic
MeOH:H20 (1:1)
Aqueous layer extracted with ethyl acetate (3 x 75 ml)
ethyl acetate removed
Toxic

Solvent Extraction

1.0 kg of flesh and/or viscera from a toxic fish was homogenised and placed in glass conical flasks (3 x 3000ml) to which acetone (3 x 650ml) was added and then left for 1 day at 20°C. The acetone was filtered and kept at - 20°C. Fresh acetone (3 x 325ml) was added to solid residue and left for 2 days after which the acetone was filtered and combined with the initial acetone filtrate and stored overnight at -20°C.

The combined extracts were filtered and again the acetone filtrate kept in a freezer at -20°C for 3 days. The acetone residue was filtered and the acetone removed using a rotary evaporator (40°C, 0.2 atm, Rotavapor R110, Büchi), methanol (200ml) was added to the aqueous residue and back-extracted with hexane (2 x 800ml). The hexane phase was extracted with the methanol water (4:1,3 x 45ml) and methanolic aqueous phases combined and the methanol removed by rotary evaporation (40°C, 0.2 atm, Rotavapor R110, Büchi). The aqueous residue left was then extracted with ethyl acetate (3 x 1500ml) and again the solvent removed by vacuum and finally N_2. The crude extract was stored under N_2 at -20°C until further purification.

Silicic Acid Chromatography

Silicic acid (Bio-sil A, Bio-Rad, Richmond, CA., 200-400 mesh, 60g) was placed in a large column (6cm x 6cm) and chloroform was added until the silicic acid formed a air-free wet column. The crude extract was dissolved in chloroform (20ml) and carefully applied to the column which was then quickly eluted with the following mixtures (a) chloroform : methanol (33:1), (b) chloroform : methanol (9:1), (c) chloroform : methanol (4:1), (d) methanol. After the solvents were removed by vacuum and N_2 they gave four residues. The toxicity of each fraction was determined by mouse bioassay.

Alumina Chromatography

Alumina (alumina Woelm B, activity I, Woelm Pharma GmbH, Eschwege, 50g) was made into a slurry with chloroform and placed in a column (25cm x 2.5cm). Fraction (ii) from the silicic acid chromatography was dissolved in chloroform:methanol (100:1) and eluted with the following solvent system; (a) chloroform:methanol (100:1), (b) chloroform:methanol (9:1), (c) chloroform:methanol (1:1), (d) methanol and (e) methanol:water (1:1). In the cases of (a) and (d) the solvents were removed by vacuum to dryness and stored under N_2 at -20°C, in the case of (e) the methanol was removed by vacuum and the residual aqueous fraction was extracted by ethyl acetate (3 x 100ml). The solvent was removed by vacuum and stored under N_2 at -20°C. Mouse bioassay established that fractions (b) and (e) were usually toxic. In some cases fraction (c) also showed toxicity.

(c) Extraction of Non Toxic Fish

Control extractions were performed on non toxic fish using the methodology detailed above. These control extracts (CE) were then used in all physiological, pharmacological and histopathological studies to eliminate any possibility of a toxic reaction being due to the extraction procedure rather than inherent toxicity.

(d) Toxicity of Extracted Material

All extracts derived from toxic and non toxic fish were assessed by mouse bioassay. Two mice at least were used in each determination, they were weighed and given an amount of extract made up in saline and 0.1% Tween 80 to 300µl and injected into the intraperitoneal cavity. The behaviour of the mice was observed till death or for at least 48 hours. The observed death times were compared with those in the literature (Hoffman et al., 1983; Nukina et al., 1984; Lewis, 1985). Death times were then used to estimate the approximate concentration of CTX in each of the toxic fractions.

Results

Twenty toxic fish and six non toxic fish were extracted during the course of the programme. The toxin obtained has been used in the physiological, pharmacological and histopathological studies detailed in the following sections.

IV ACTION OF CTX ON NON-HUMAN MAMMALIAN NERVES

Introduction

A fundamental aspect of the initial proposal was to develop a nerve preparation from a mammal that could be used as a model for the peripheral nerve dysfunction reported in humans.A nerve preparation was developed in intact rats that closely approximated the human clinical situation in that the electrophysiology of the nerve was examined in situ without surgical intervention. Prior studies on the effects of ciguatoxin on nerves had been performed on nerves isolated from the body and thus under abnormal physiological conditions. Many neurotoxins are known to produce subtle changes in a variety of nerve conduction parameters including conduction velocity, latency, refractory periods and supernormality. All of these parameters can be measured by standard neurological techniques. The parameter of most significance and therefore the greatest interest is supernormality.

Supernormality is a period of increased sensitivity that occurs in normal nerves for 6-40 m sec after an impulse has been conducted. This supernormal period is believed to reflect an increased capacity for conductance in Na^+ channels (Narahashi and Anderson, 1967). Compounds that alter Na^+ channel conductance produce changes in the length of the supernormal period (Parkin and Le Quesne, 1982).

Functional changes reflected by alterations in various nerve conduction parameters may reflect structural changes within nerve fibers. Examination at the electron microscope level has been undertaken on the peripheral nerves of rats exposed to CTX.

Methods

a) Neurophysiology

Nerve conduction studies were performed on male Wistar rats of 120-150 g. Rats were given a non-lethal dose of CTX extract in a small volume of 0.9% saline and 1% Tween 80 by intraperitoneal injection. Thirty minutes after the CTX injection the rats were anaesthetised by intramuscular injection of Innovar Vet (420 µl/kg) (Smith Cline and French). Within five minutes of the induction of anaesthesia the rats were placed in a supine position with the ventral surface uppermost in a Faraday cage. The tails were shaved, cleaned and then two flexible stainless steel surface stimulating electrodes were placed about 1 cm apart in the distal third of the tail. A flexible metal foil strip served as an earth near the centre of the tail. Stainless steel needle recording electrodes were positioned subcutaneously, about 1 cm apart in the proximal third of the ventral surface of the tail. After the electrodes had been positioned the tail was placed on a heated pad (35-37°C).

Nerve fibers in the ventral coccygeal nerve of the tail were stimulated using a Medelec MS92a electromyography unit and a purpose built stimulator. The evoked compound nerve action potentials were recorded after 4 - 8 averages on the same unit. A paper printout of each averaged evoked action potential was obtained for subsequent analysis

Single and paired supramaximal stimuli (square wave pulse: 150-200 v, 0.1 ms) were given to determine amplitude, latency, conduction, velocity, absolute refractory period (A.R.P.), relative refractory period (R.R.P) and absolute-relative refractory period (A.R.R.P.)

The refractory periods indicate the state of activity of voltage dependent Na^+ channels following the conduction of a nerve action potential. For a short period after the conduction of an impulse a nerve becomes refractory and will not conduct a further impulse. For a period of approximately 0.6 ms the compound nerve is in absolute refractoriness and will not conduct a further impulse. In this period the Na^+ gates will not open no matter what magnitude of stimulus is applied. For a further period of 1 - 15 ms, in mammals, the nerve is in relative refractoriness in that when a supramaximal stimulus is given the resultant compound nerve action potential will be diminished indicating that some fibers are not responsive. A further period, the absolute-relative refractory period (A.R.R.P) can be derived by the subtraction of the A.R.P. from the R.R.P.

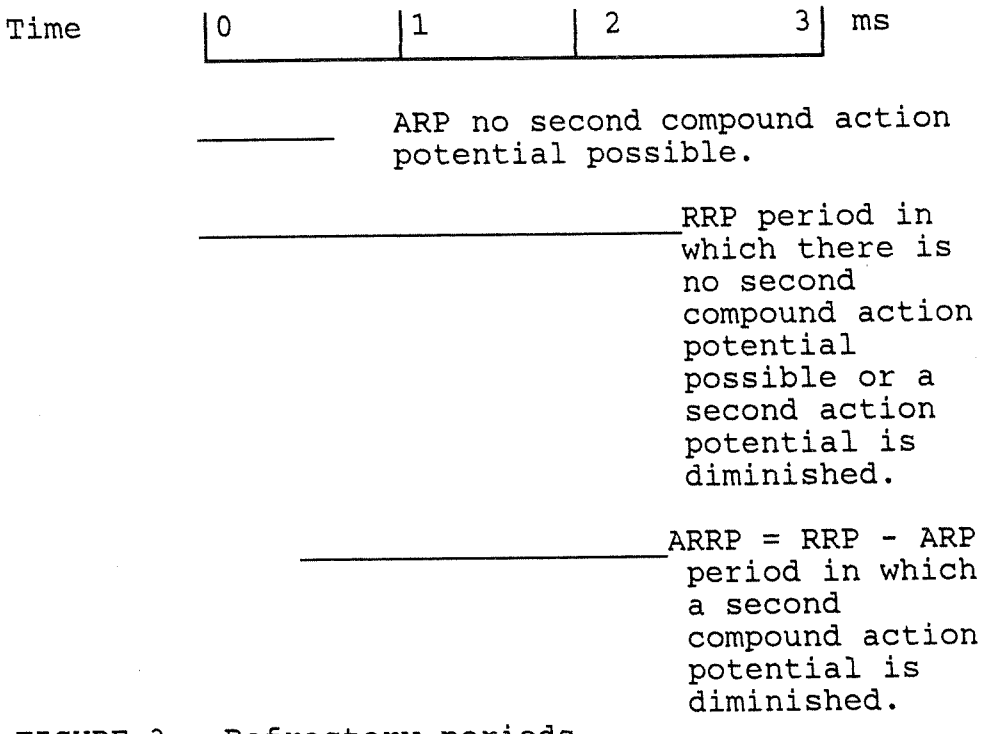


FIGURE 3. Refractory periods.

Paired stimuli were also used in each animal to determine the amplitude and duration of the supernormal period. The paired stimuli used in the determination of supernormality are denoted as S1 the conditioning stimulus and S2 the conditioned stimulus or unconditioned stimulus (Parkin and Le Quesne, 1982). S1 is set so as to produce a supramaximal response in the ventral coccygeal nerve. In the absence of S1, S2 is set to evoke an approximate one third maximal response. S2 is a conditioned stimulus when preceded by S1; S2 given without S1 is an unconditioned stimulus. S2 is presented alternatively conditioned and unconditioned. If necessary S2 was adjusted periodically to maintain a one third maximal response. The temporal interval between S1 and S2 was adjusted to discrete steps from 6 to 1,000 ms. The amplitude of S2 conditioned was expressed as a percentage of the response of S2 unconditioned.

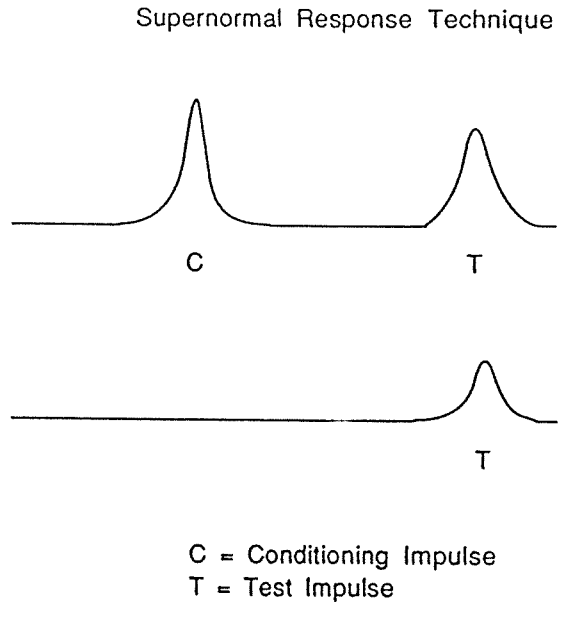


FIGURE 4. Supernormality.

All nerve conduction parameter studies were performed on a minimum of ten animals for each determination. Statistical analysis was performed on the data by one way and multifactorial analysis of variance.

(b) Antagonistic and Synergistic Experiments

The nerve preparation developed by the group has been used to examine possible antagonist compound. Antagonists were injected intraperitoneally either alone or after an animal had been exposed to CTX. The

effects of the antagonists alone or in combination with CTX were assessed on a variety of nerve conduction parameters. As ethanol has been reported to potentiate the effects of CTX in human ciguatera victims a series of experiments was performed on the actions of ethanol and ethanol plus CTX on nerve conduction in the rat. Sufficient ethanol was administered to rats to achieve a blood alcohol level of 0.05%.

c) Ultrastructural Studies on Rat Nerves

Male Wistar rats (150 -200g) were injected intraperitoneally with a dose of CTX approximately the LD_{50}. These rats were euthanased at 1.5 and 3.0 hours after the CTX injection. After euthanasia the thoracic and abdominal cavities were quickly opened, the left ventricle was cannulated and all major abdominal vessels clamped. A solution of glutaraldehyde (4%) in cacodylate buffer (pH 7.4) was infused via the left ventricle until the blood was replaced and the perfusate flowed freely from the right atrium. Sections of the Sciatic and Sural nerves were dissected from the animal and prepared for transmission electron microscopy.

Results

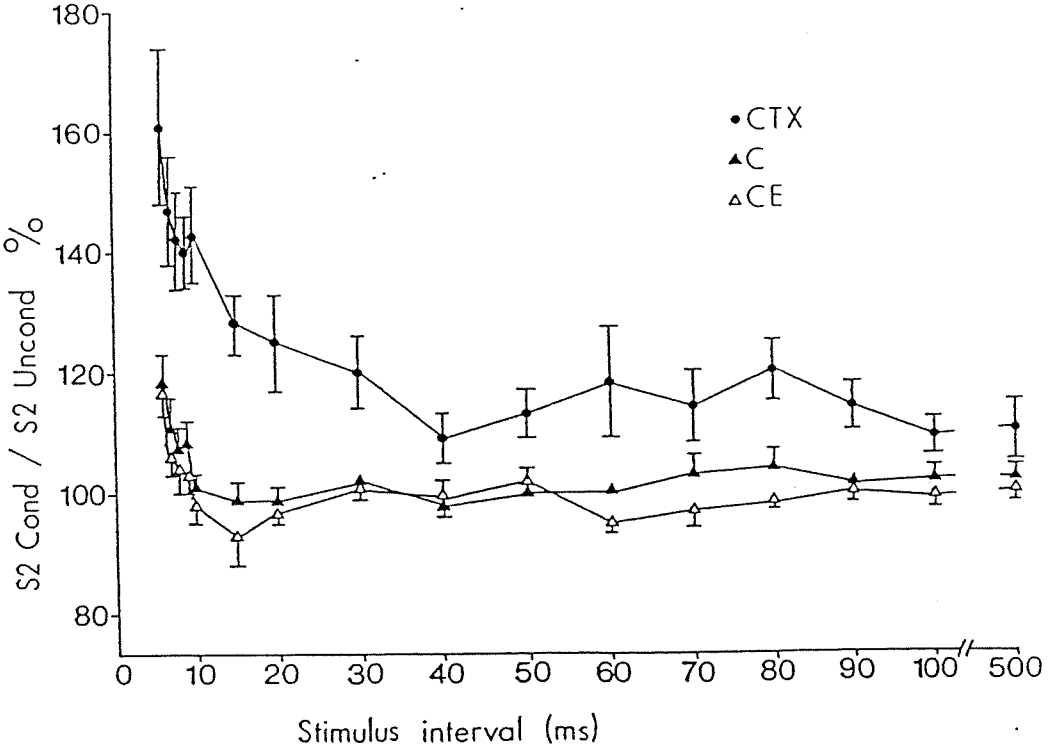
a) Neurophysiology

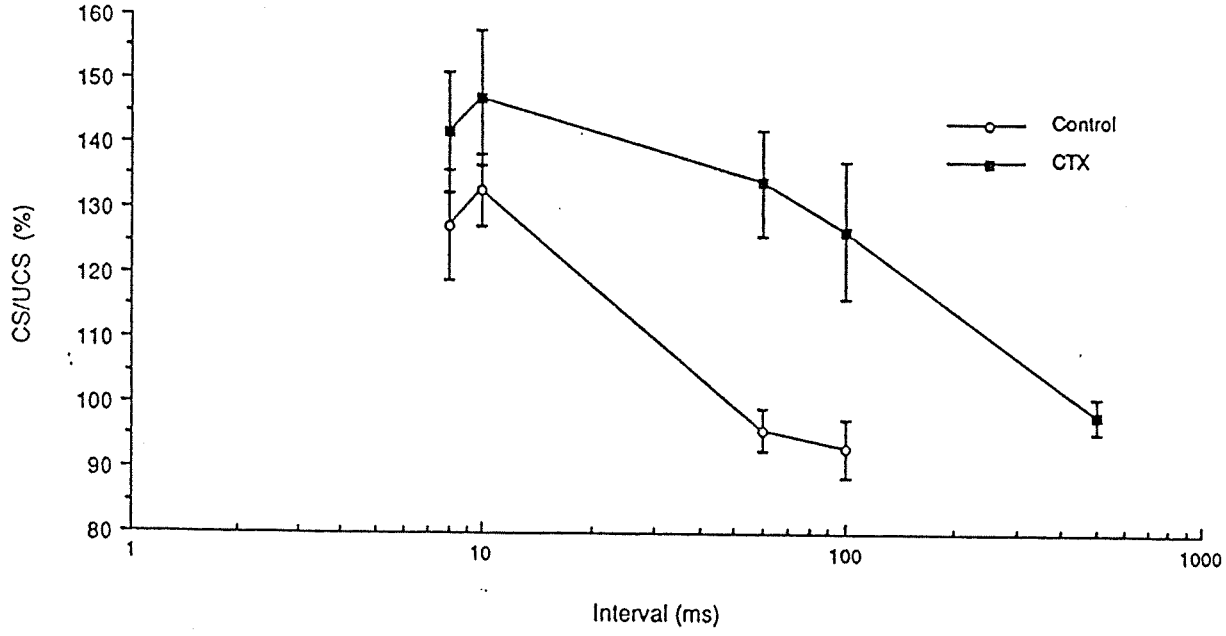
The intact rat, ventral coccygeal nerve preparation allowed an examination of the effects of CTX in conditions approaching those of clinical presentation. As most cases of human intoxication involve the consumption of relatively small amounts of toxic fish and are usually non-fatal, dose levels of CTX were chosen that produced symptoms of moderate ciguatera poisoning without being lethal. As the extraction procedure for CTX involves the possibility of concentrating a toxic agent other than CTX a control extract (CE) obtained from a non-toxic fish was given to a group of control mice.

The results of a detailed study of supernormality are shown in Figure 5 (Capra and Cameron, 1985). Ciguatoxin has significantly prolonged the duration and increased the magnitude of the supernormal period. There was, however, no significant difference between the group of ten rats exposed to an intraperitoneal injection of control extract and the group of ten rats injected only with the saline vehicle. The results presented in Figure 5 represent a very comprehensive study of supernormality with determinations at 10 ms intervals. In all subsequent experiments supernormality was determined at fewer points so that studies could be accomplished more easily. Figure 6 shows the increased magnitude and duration of supernormality still evident with fewer determinations in ten animals exposed to CTX when compared to a control group.

Figure 5. A detailed study of the effects of CTX on the magnitude and duration of the supernormal period in male Wistar rats. Rats exposed to CTX show a marked increase in the magnitude and duration of supernormality ie. ratio of S2 conditioned to S2 unconditioned. Note that rats exposed to a control extract derived from a non toxic fish have no difference in their supernormal response to rats injected with the vehicle of saline and Tween 80. Each point represents data derived from ten animals. Error bars indicate the standard error of the mean.

Figure 6. The effects of CTX (0.05 µl/g of $5x10^{-9}M$) on the magnitude and duration of the supernormal period in a different group on male Wistar rats. Control animals were given only the vehicle of saline and Tween 80. Each point represents data derived from ten animals. Standard errors of means are shown.


180
160
140
120
100
80
S2 Cond / S2 Uncond %
•CTX
▲C
△CE
0
10
20
30
40
50
60
70
80
90
100
500
Stimulus interval (ms)


160
150
140
130
120
110
100
90
80
CS/UCS (%)
Control
CTX
1
10
100
1000
Interval (ms)

(b) Antagonists and Synergists of CTX

The ventral coccygeal nerve preparation of the rat has been used to test the efficacy of possible antagonists to CTX. Two potentially useful therapeutic agents, the calcium channel antagonist, verapamil and the local anaesthetic, lignocaine were tested. Verapamil alone increases the magnitude and duration of supernormality above control levels (Fig. 7) whereas lignocaine decreases both the magnitude and duration below control values (Fig. 9). While verapamil and lignocaine have opposing effects on supernormality where given alone, they both bring about a reduction in the increased magnitude and duration of supernormality induced by CTX (Figs. 8 and 10).

Ethanol, on the other hand, produces only small changes in the supernormal period when given alone. Ethanol, however, when given after pretreatment with CTX increases the duration of the supernormal period (Fig. 12).

The effects on supernormality of lignocaine, verapamil and ethanol with or without CTX are summarised in Figures 13 and 14. Both lignocaine and verapamil significantly reduce the increased magnitude and duration of supernormality induced by CTX (Fig. 13 and 14).

While supernormality is a very important nerve condition parameter in terms of assessing the operation of the Na^+ channel and Na^+ gating mechanism other parameters also give information on the state of nervous function. The ventral coccygeal nerve is a mixed sensory and motor nerve. When the nerve is stimulated distally and recorded from proximally a mix sensory/motor action potential is recorded. The amplitude, latency and conduction velocity of this compound action potential under various treatments is shown in Figures 15, 16 and 17. It can be clearly seen that CTX significantly decreases the amplitude (Fig. 15) and conduction velocity (Fig. 17) of the action potential while increasing the latency (Fig 16). Lignocaine, verapamil and ethanol all significantly alter amplitude from control values. Lignocaine in the presence of CTX increases the amplitude towards control values while ethanol and verapamil in the presence of CTX cause further decreases in amplitude that are below the value of CTX alone. CTX significantly increases the latency and reduces the conduction velocity from control values (Figs. 16 and 17). All treatments with the exception of lignocaine alone increase the latency. Ciguatoxin and verapamil appear to act synergistically in increasing the time of latency.

Another useful indicator of nerve function is the state of refractoriness of nerves to stimulation in the period immediately following the transmission of nerve action potential. CTX produces a very significant increase in the absolute refractory period (Fig. 18) over control nerves. The relative refractory period is also prolonged but to a lesser degree (Fig. 19). The absolute - relative refractory period is however reduced in the presence of CTX (Fig. 20). While lignocaine and ethanol tend to prolong all refractory periods, verapamil shortens the absolute refractory period, has little effect on the relative refractory period and leads to a prolongation of the absolute-relative refractory period (Figs 18,19 & 20). Both lignocaine and ethanol appear to have little influence on the prolongation of the absolute refractory period while verapamil in the presence of ciguatoxin reduces the duration of the absolute, relative and absolute-relative refractory periods (Figs. 18, 19 & 20).

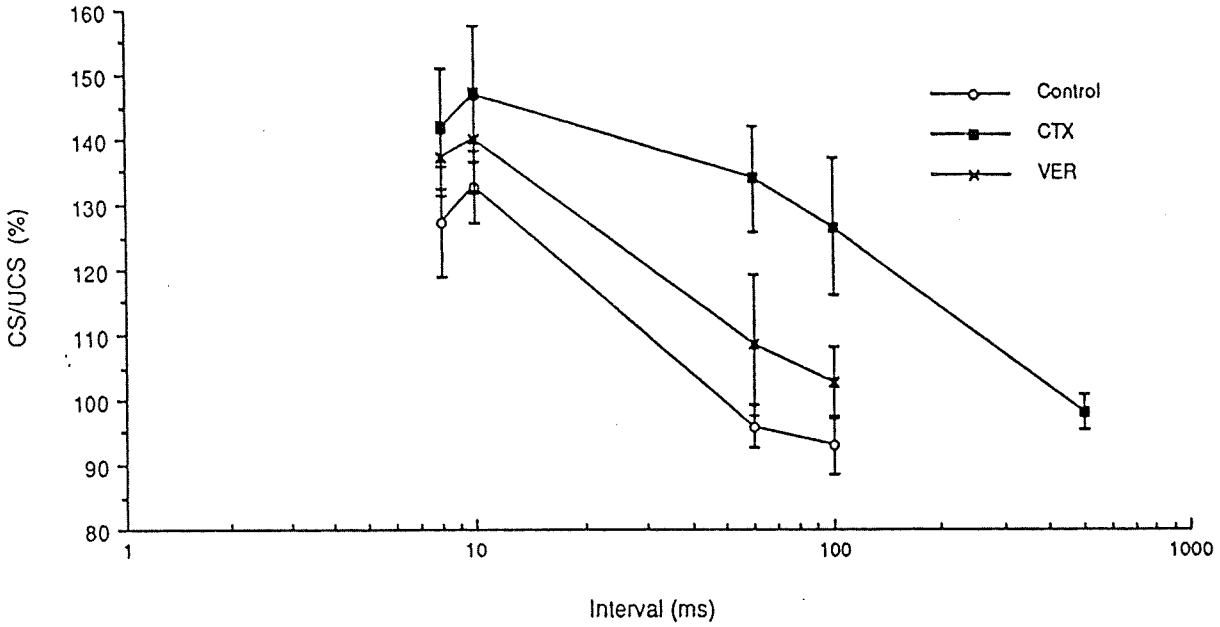
When the ventral coccygeal nerve is stimulated proximally the motor fibers are stimulated anterogradely and will evoke muscle action potentials and muscle contraction. The evoked muscle potentials can be recorded and the time interval between stimulation and the muscle action potential can be used with the distance between stimulating and recording electrodes to calculate motor conduction velocity. Motor conduction velocity and the amplitude of the motor action potential are shown for various treatments in Figures 21 and 22. CTX produces highly significant decreases in both motor conduction velocity and the amplitude of the motor action potential. Ethanol and verapamil in the presence of CTX also produce a diminution in motor conduction velocity and motor amplitude while lignocaine in the presence of CTX tends to restore conduction velocity and motor amplitude towards control values.

(c) Structural Effects of CTX on Mammalian Nerves

Structural changes are seen in the myelinated nerve fibers of rats exposed to CTX. Figure 23 shows the normal appearance of myelinated mammalian nerve fibers with the axoplasm completely filling the space surrounded by the myelin sheath. In the nerves of rats exposed to CTX (Fig. 24) the fluid accumulates within the periaxonal space and displaces and indents the axon.

Figure 7. A comparison of the effects of CTX (0.05 µl/g of $5x10^{-9}M$) and verapamil (0.1mg/kg) on the magnitude and duration of the supernormal period compared to control levels. Each point represents data derived from 10 animals. Standard errors of the means are shown.

Figure 8. A comparison of the effects of CTX (0.05 µl/g of $5x10^{-9}M$) and CTX (0.05 µl/g of $5x10^{-9}M$) in the presence of veropamil (0.1 mg/kg). The magnitude of supernormality is reduced however. The duration of the supernormal period is prolonged in the presence of veropamil. Each point represents data derived from ten animals. Standard errors of the means are shown.


160
150
140
130
120
110
100
90
80
CS/UCS (%)
1
10
100
1000
Interval (ms)
Control
CTX
VER

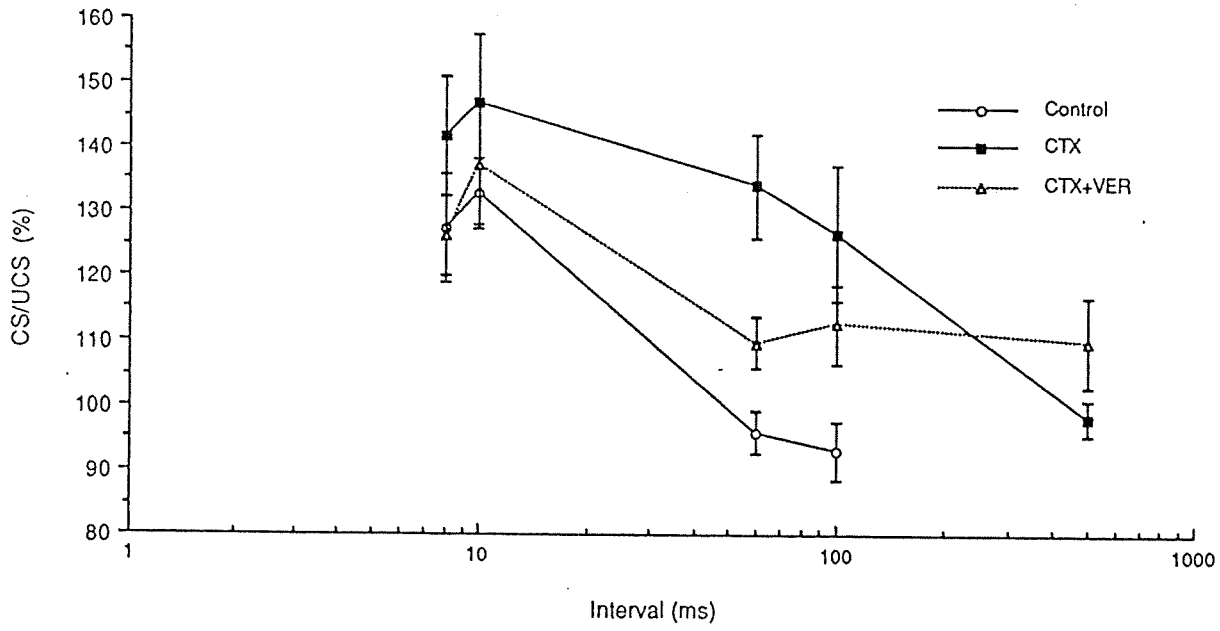
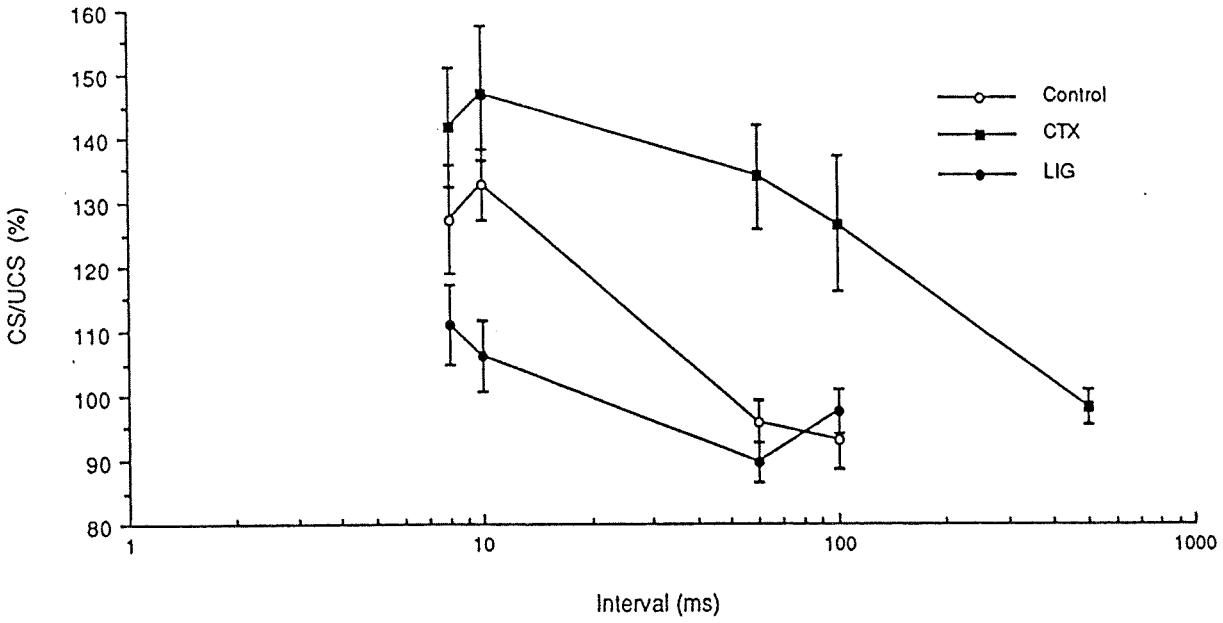

160
150
140
130
120
110
100
90
80
CS/UCS (%)
1
10
100
1000
Interval (ms)
Control
CTX
CTX+VER

Figure 9. A comparison of the effects of CTX (0.05 µl/g of $5x10^{-9}M$) and lignocaine (1mg/kg) on the magnitude and duration of the supernormal period. CTX increases the magnitude and duration of the supernormal period while lignocaine decreases the magnitude and duration of the supernormal period. Each point represents data derived from ten animals. Standard errors of the means are shown.

Figure 10. A comparison of the effects of the CTX (0.05 µl/g of $5x10^{-9}M$) in the presence of lignocaine (1mg/kg). The magnitude and duration of the supernormal period are reduced in the presence of lignocaine. Each point represents data derived from ten animals. Standard errors of the means are shown.


160
150
140
130
120
110
100
90
80
CS/UCS (%)
1
10
100
1000
Interval (ms)
Control
CTX
LIG

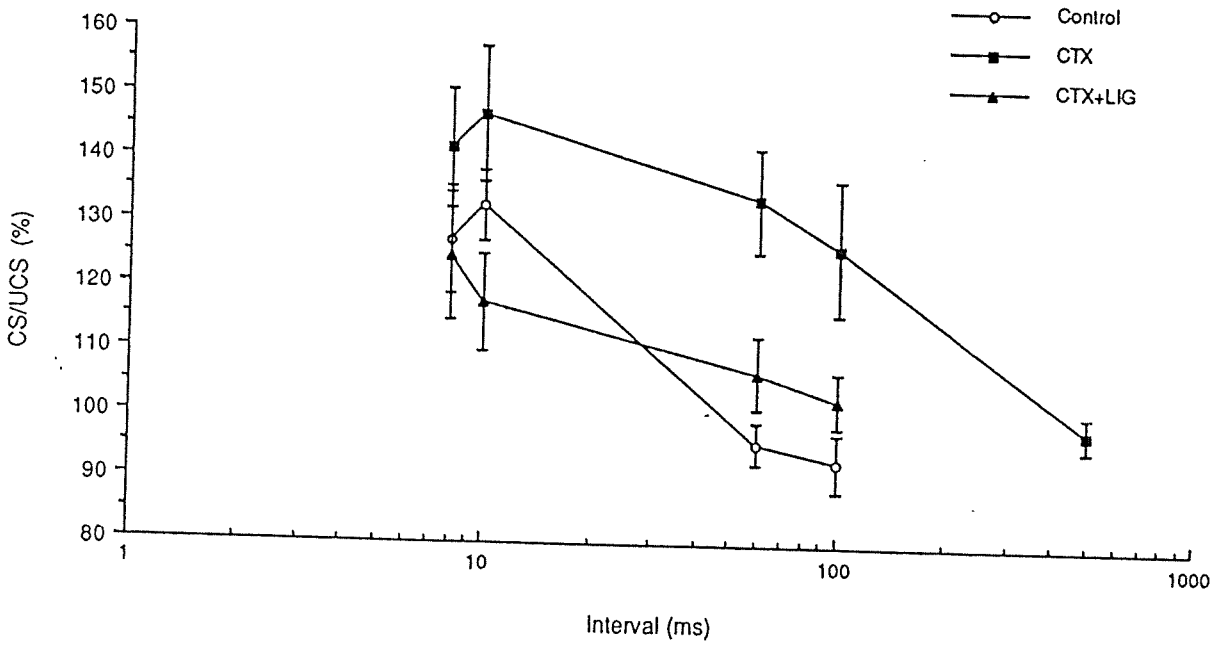
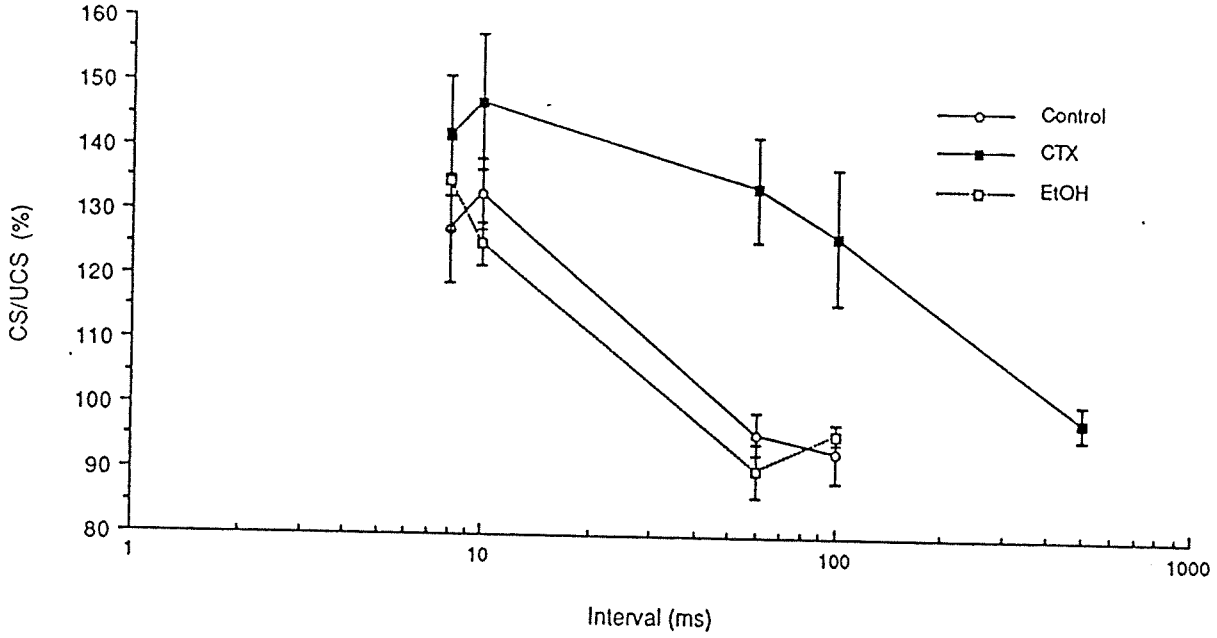

160
150
140
130
120
110
100
90
80
CS/UCS (%)
1
10
100
1000
Interval (ms)
Control
CTX
CTX+LIG

Figure 11. A comparison of the effects of CTX (0.05 µl/g of $5x10^{-9}M$) and ethanol (blood conc. 0.05%) on the magnitude and duration of the supernormal period. CTX increases both magnitude and duration of the supernormal period while ethanol has little effect on either magnitude or duration. Each point represents data derived from ten animals. Standard errors of the means are shown.

Figure 12. A comparison of the effects of CTX (0.05 µl/g of $5x10^{-9}M$) and CTX (0.05 µl/g of $5x10^{-9}M$) and ethanol (blood conc. 0.05%). The magnitude of the supernormal period is not increased in the presence if Ethanol plus CTX, however the duration of the supernormal period is prolonged. Each point represents data derived from ten animals. Standard errors of the means are shown.


160
150
140
130
120
110
100
90
80
CS/UCS (%)
1
10
100
1000
Interval (ms)
Control
CTX
EtOH

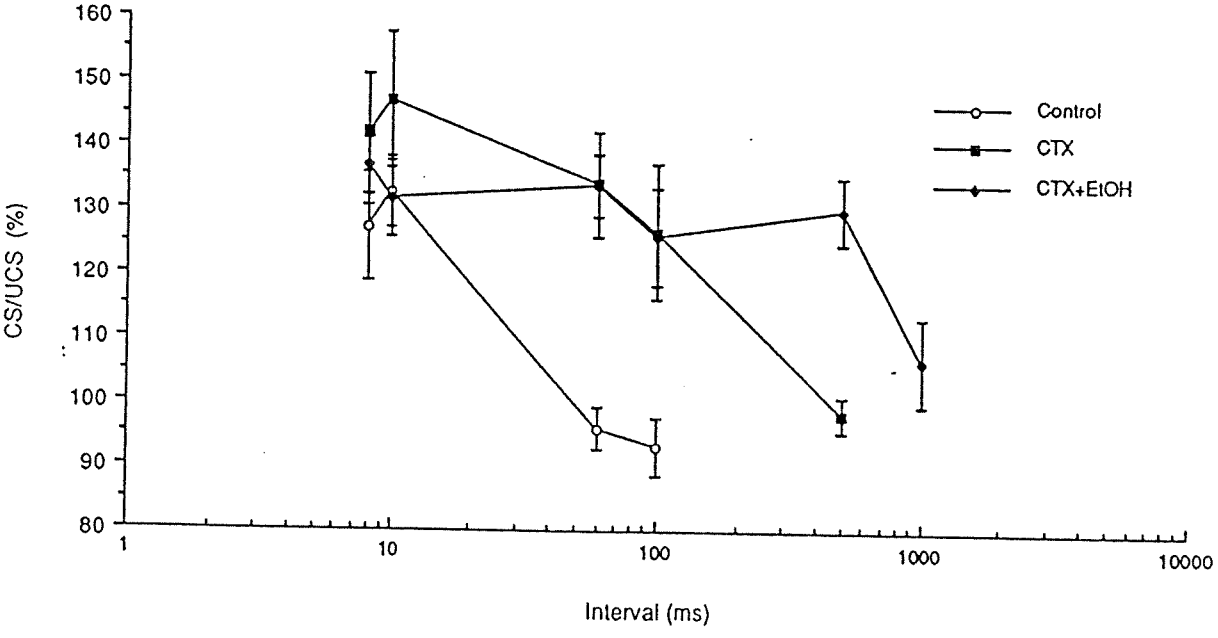
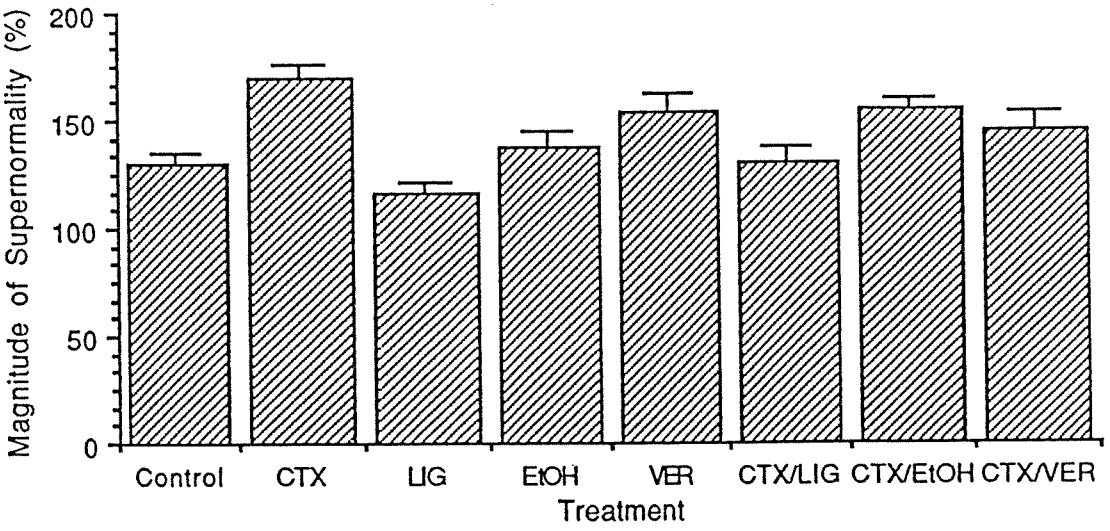

160
150
140
130
120
110
100
90
80
CS/UCS (%)
1
10
100
1000
10000
Interval (ms)
Control
CTX
CTX+EtOH

Figure 13. A comparison of the magnitude of supernormality in the rat following various treatments. Dose rates are as given in previous figures. Each histogram represents data derived from ten animals. Standard errors of means are shown.

CTX	Ciguatoxin
LIG	Lignocaine
EtOH	Ethanol
VER	Veropamil

Figure 14. A comparison of the duration of supernormality in the rat following various treatments. Dose rates are as given in previous figures. Note the long prolongation due to synergistic effects of ethanol and CTX. Each histogram represents data derived from ten animals. Standard errors of means are shown


Magnitude of Supernormality (%)
200
150
100
50
0
Control
CTX
LIG
EtOH
VER
CTX/LIG
CTX/EtOH
CTX/VER
Treatment

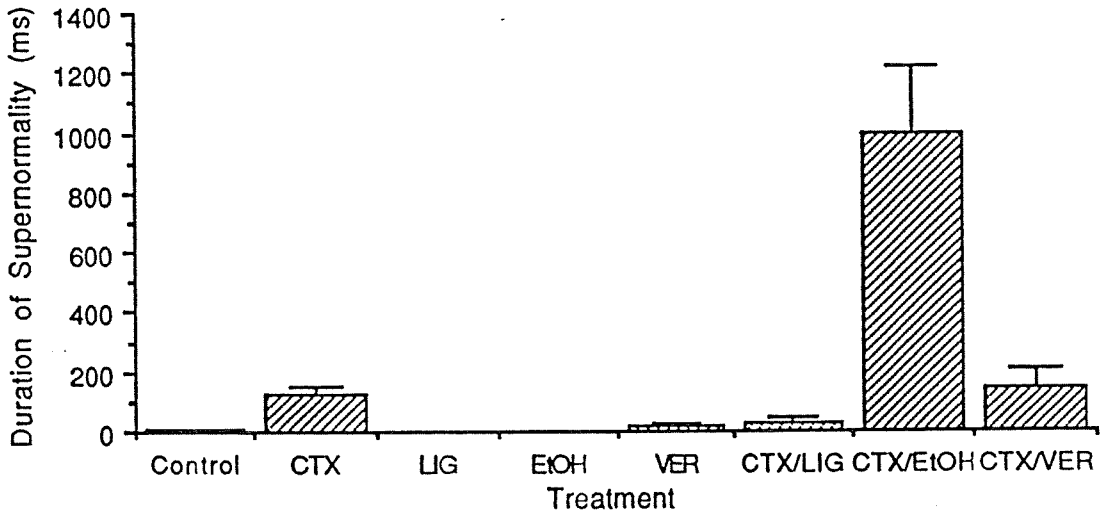
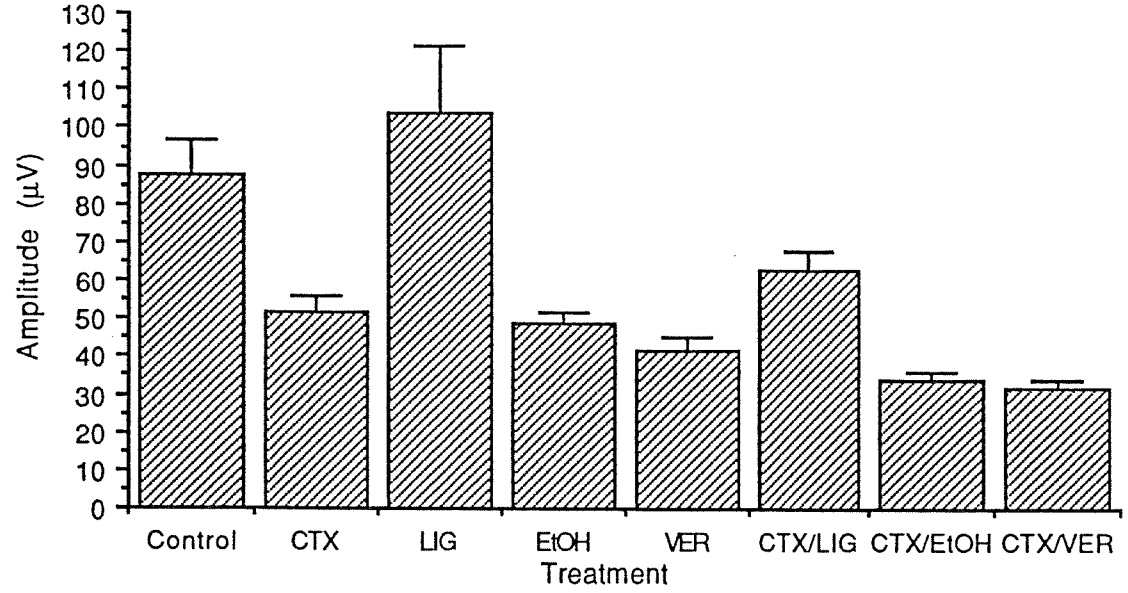
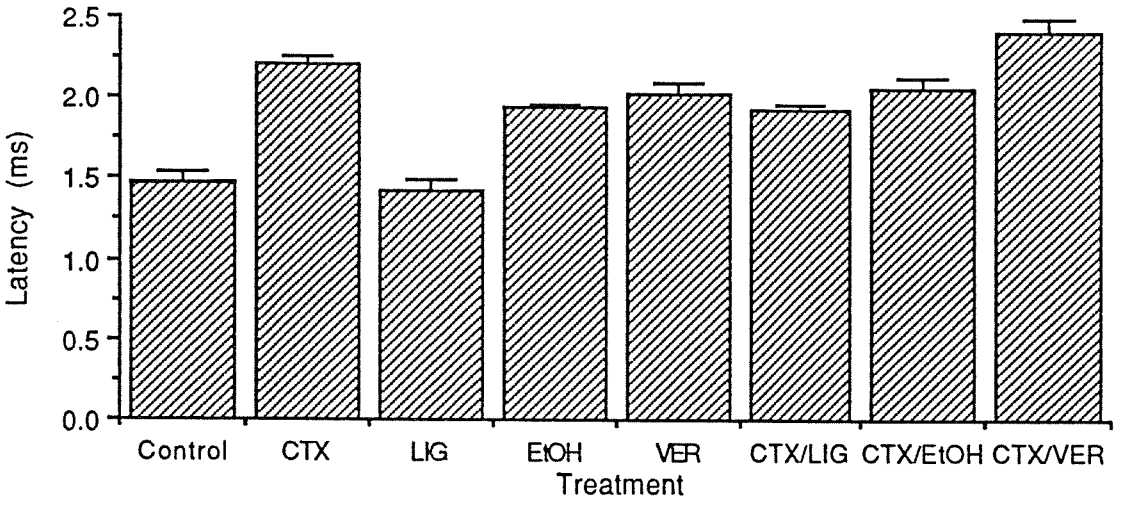

Duration of Supernormality (ms)
1400
1200
1000
800
600
400
200
0
Control
CTX
LIG
EtOH
VER
CTX/LIG
CTX/EtOH
CTX/VER
Treatment

Figure 15. A comparison of the effects of various treatments on the amplitude of the compound nerve action potential in the ventral coccygeal nerve of the rat. Each histogram represents data derived from ten animals. Standard errors of means are shown.

Figure 16. A comparison of the effects of various treatments on the latency of the compound nerve action potential in the ventral coccygeal nerve of the rat. Each histogram represents data derived from ten animals. Standard errors of means are shown.

Figure 17. A comparison of the effects of various treatments on the conduction velocity of the compound nerve action potential in the ventral coccygeal nerve of the rat. Each histogram represents data derived from ten animals. Standard errors of means are shown.


Amplitude (μV)
130
120
110
100
90
80
70
60
50
40
30
20
10
0
Control
CTX
LIG
EtOH
VER
CTX/LIG
CTX/EtOH
CTX/VER
Treatment


Latency (ms)
2.5
2.0
1.5
1.0
0.5
0.0
Control
CTX
LIG
EtOH
VER
CTX/LIG
CTX/EtOH
CTX/VER
Treatment

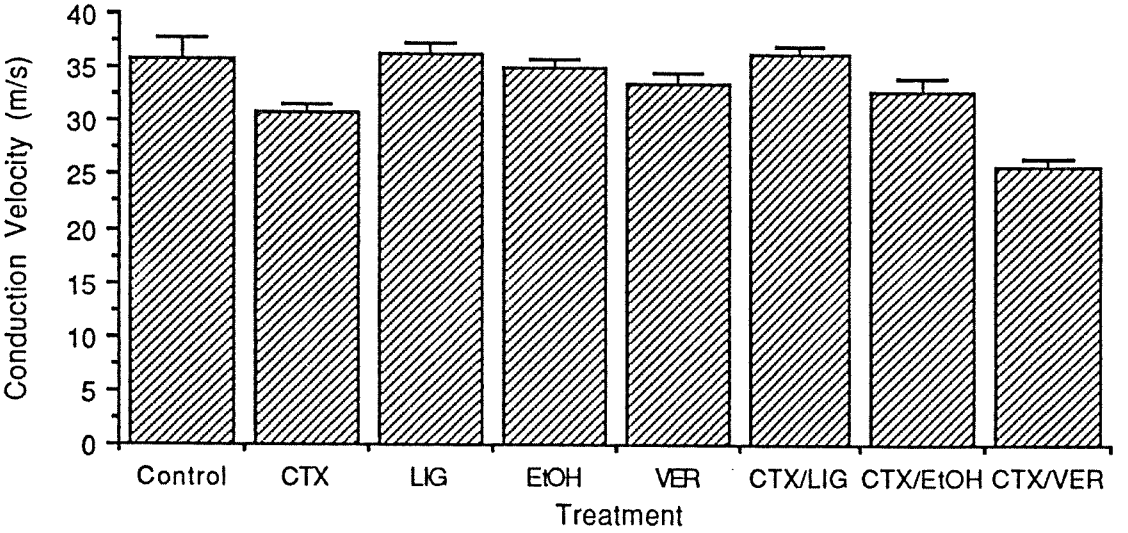
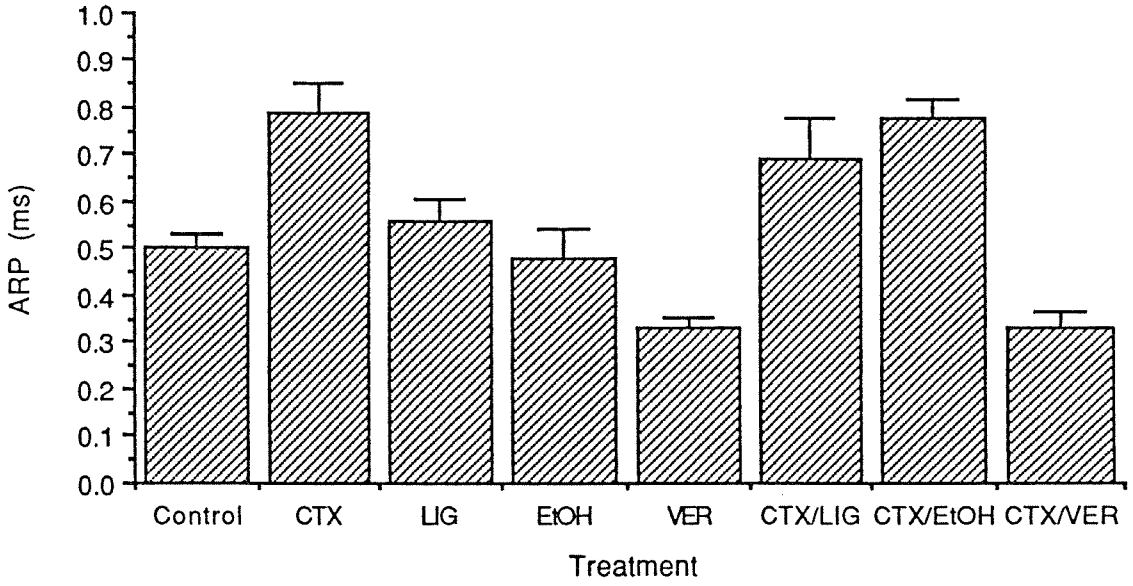
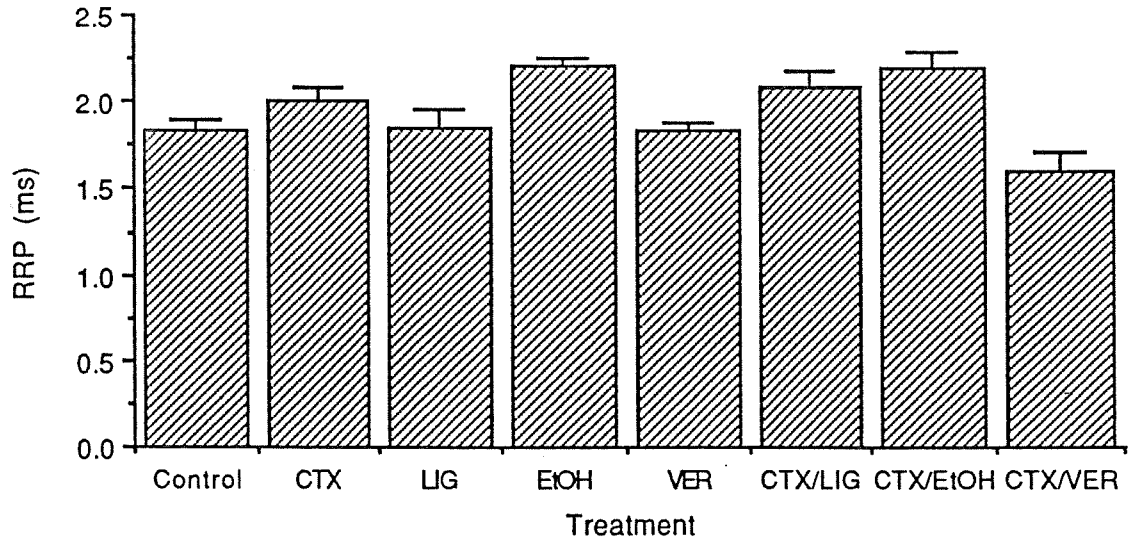

Conduction Velocity (m/s)
40
35
30
25
20
15
10
5
0
Control
CTX
LIG
EtOH
VER
CTX/LIG
CTX/EtOH
CTX/VER
Treatment

Figure 18. A comparison of the effects of various treatments on the absolute refractory period in the ventral coccygeal nerve of the rat. Each histogram represents data derived from ten animals. Standard errors of the means are shown.

Figure 19. A comparison of the effects of various treatments on the relative refractory period in the ventral coccygeal nerve of the rat. Each histogram represents data derived from ten animals. Standard errors of the means are shown.

Figure 20. A comparison of the effects of various treatments on the absolute relative refractory period in the ventral coccygeal nerve of the rat. Each histogram represents data derived from ten animals. Standard errors of the means are shown.


ARP (ms)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Control
CTX
LIG
EtOH
VER
CTX/LIG
CTX/EtOH
CTX/VER
Treatment


RRP (ms)
2.5
2.0
1.5
1.0
0.5
0.0
Control
CTX
LIG
EtOH
VER
CTX/LIG
CTX/EtOH
CTX/VER
Treatment

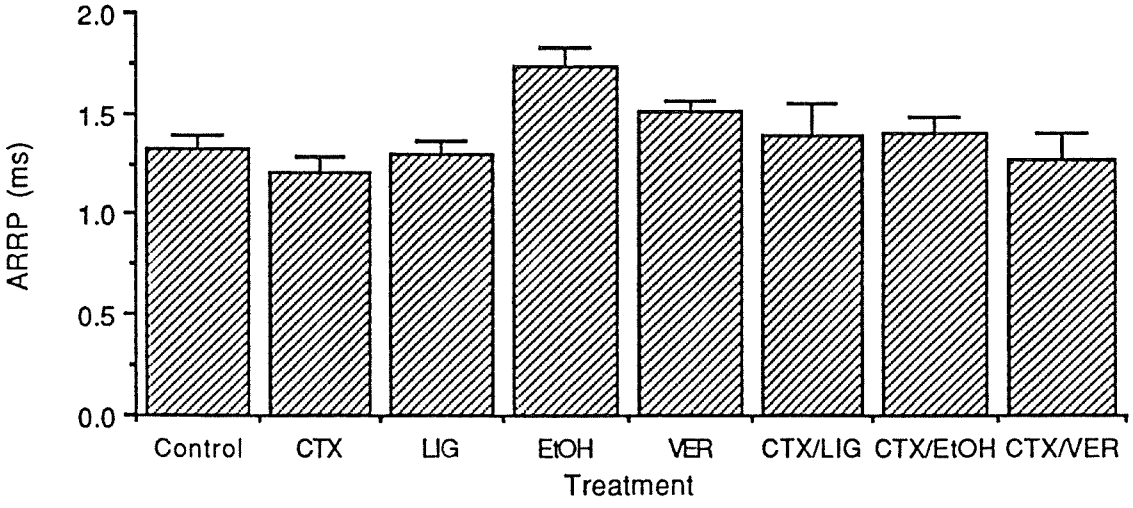
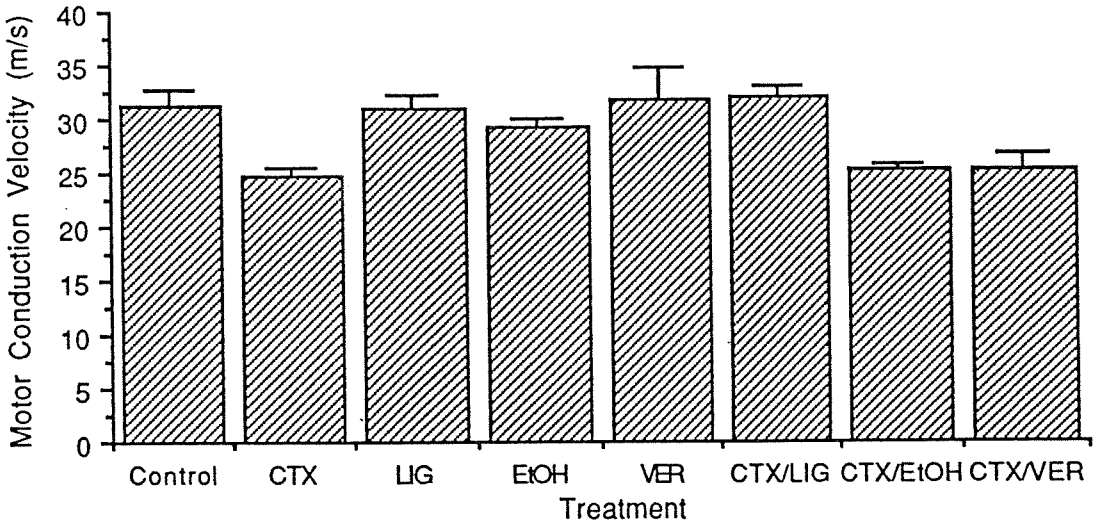
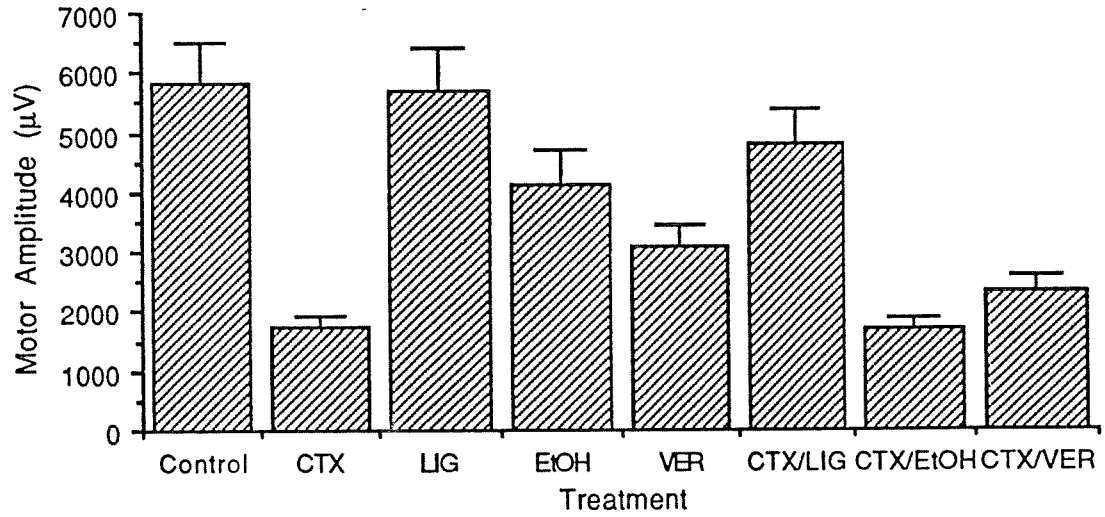

ARRP (ms)
2.0
1.5
1.0
0.5
0.0
Control
CTX
LIG
EtOH
VER
CTX/LIG
CTX/EtOH
CTX/VER
Treatment

Figure 21. A comparison of the effects of various treatments on the motor conduction velocity in the ventral coccygeal nerve of the rat. Each histogram represents ten animals. Standard errors of the means are shown.

Figure 22. A comparison of the effects of various treatments on the amplitude of the muscle action potential in the tail of the rat after anterograde stimulation of the motor components of the ventral coccygeal nerve. Each point represents data derived from ten animals. Standard errors of the means are shown.


Motor Conduction Velocity (m/s)
40
35
30
25
20
15
10
5
0
Control
CTX
LIG
EtOH
VER
CTX/LIG
CTX/EtOH
CTX/VER
Treatment


Motor Amplitude (μV)
7000
6000
5000
4000
3000
2000
1000
0
Control
CTX
LIG
EtOH
VER
CTX/LIG
CTX/EtOH
CTX/VER
Treatment

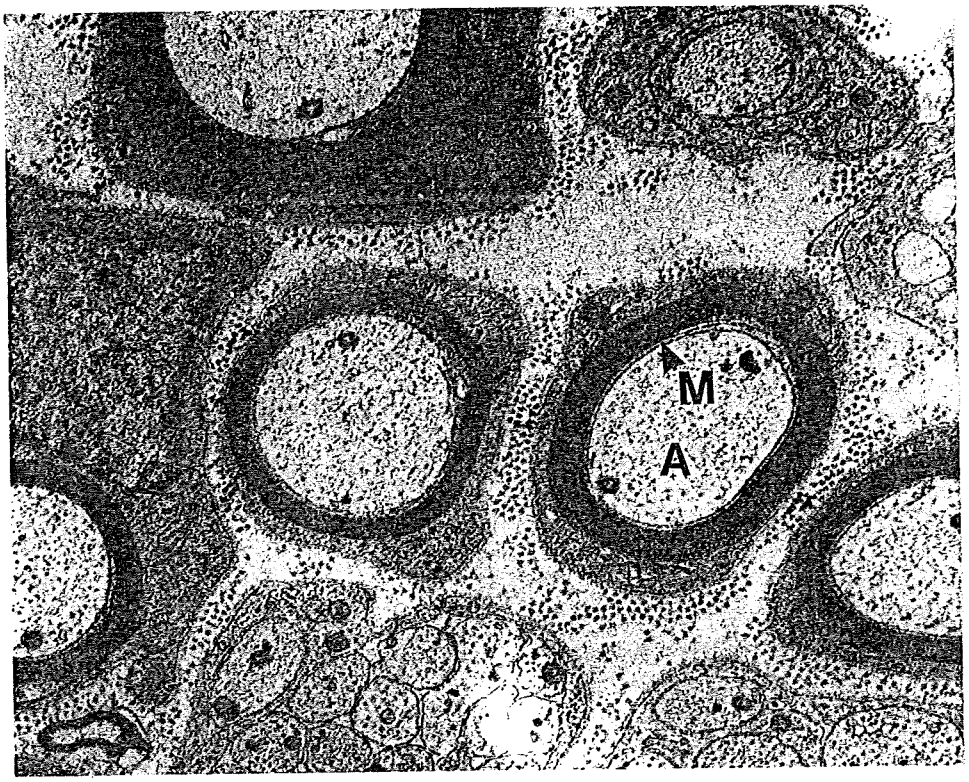


Figure 23. Transmission electron micrograph of the normal appearance of the axoplasm A within the myelin sheath M of individual mammalian nerve fibres.

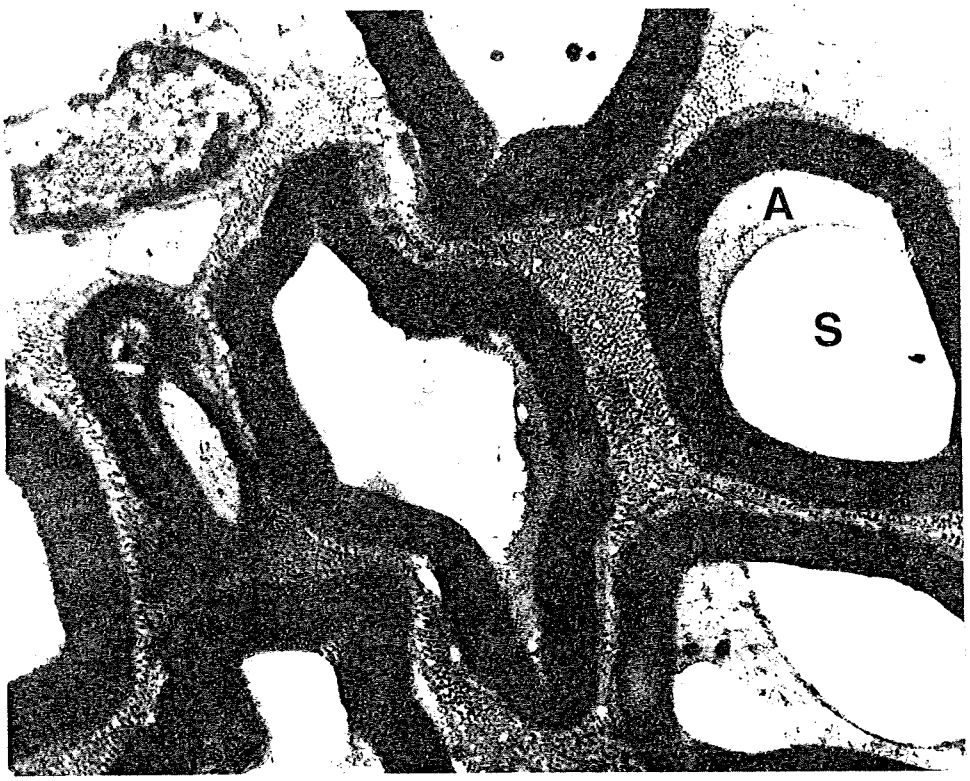


Figure 24. Transmission electron micrograph of the nerve of a rat exposed to a lethal dose of CTX. Note the abnormal accumulation of fluid in the periaxonal spaces displaces and indents the axon A.

Discussion

The results presented indicate that CTX alters several nerve conduction parameters. The actions of CTX on the supernormal and refractory periods are consistent with the hypothesis that CTX modifies the sodium channel and the sodium gating mechanism of nerve fibers. For a nerve to operate normally, voltage dependant Na^+ gates open at the onset of an action potential and the rapid influx from the extracellular to intracellular fluid of Na^+ via Na^+ channels carries a positive charge into the axon producing a brief period of depolarisation. This depolarisation is propagated along the nerve and constitutes the nerve action potential. Nerve action potentials are the basic units of information transfer with the nervous system. Many toxins have fundamental actions on the normal functioning of the nerve action potential: CTX appears to be such a toxin. The increased magnitude and duration of the supernormal period strongly suggests that CTX acts on the Na^+ gates and channels to cause increased opening. In normal nerves most Na^+ gates are closed except when the nerve is carrying information in the form of a nerve action potential. Some toxins including the marine toxin Tetrodotoxin (TTX) block Na^+ channels and stop the passage of Na^+ into the nerve thus blocking nerve action potentials and information transfer. Blocking toxins such as TTX decrease the magnitude and duration of the supernormal period from control values. Toxins with an opposing action increase the magnitude and duration of supernormality as was clearly seen for CTX in this study.

The rat ventral coccygeal nerve preparation developed during the FIRTA funded programme has not only been useful in assessing the fundamental actions of CTX on nerves but it has provided a useful tool for the testing of compounds that may alleviate or exacerbate the effects of CTX. Two compounds commonly used in general medicine, lignocaine and verapamil have been examined for their effects on the response of CTX. Doses of these compounds were chosen so that their concentrations in the animal model approximated those used in human drug therapy. Both compounds antagonised the CTX induced changes in supernormality with lignocaine being more effective. Lignocaine was likewise more effective in the antagonism of the effects of CTX on action potential amplitude, latency and conduction velocity. However, verapamil was more effective in the reduction of the CTX prolonged absolute refractory period. The developed animal model allows the assessment of the actions of antagonists on a variety of nerve conduction parameters and would thus be very useful in a detailed screening of potential therapeutic agents for human ciguatera intoxication.

The ultrastructural studies on rat nerves have shown that CTX is not only a functional neurotoxin but it may also have structural effects. The axon within the myelin sheath is compressed and pushed away from the surrounding myelin by what appears to be a fluid build up within the periaxonal space. CTX acts on Na^+ channels and hence must disrupt the normal distribution of Na^+ ions across excitable membranes. Any abnormal movement of Na^+ is likely to induce a concomitant osmotic movement of water and this is reflected by the large increase in periaxonal fluid.

V ACTION OF CTX ON THE MAMMALIAN GUT

Introduction

Ciguatera poisoning characteristically presents with an initial gastro-intestinal dysfunction followed by secondary neurological symptoms (Capra, 1986). Much attention has been focussed on the latter symptoms, (Capra and Cameron, 1985; Chetian et al, 1981), while little is reported for the former. This section describes observations on the pathological changes in the small and large intestines of laboratory mice exposed to CTX.

Methods

Lethal intraperitoneal doses of CTX were given to 17 to 20 g Quackenbush mice. The mice were divided into three groups and given three different doses of CTX. Four "high" dose mice were given a dose of CTX that produced a mean death time of 65 minutes, (range: 52-79 minutes). Four "medium" dose mice were given CTX that produced a mean death time of 2 hr. 43 min. (range: 2 hr. 30min. - 2 hr. 58 min.) while three "low" dose mice had a mean death time of 7 hr. 15min. (range: 6hr. 36 min. - 8 hr. 10 min.). One group of control mice were injected with the vehicle of 0.9% saline and 1% Tween 80 while a second control group was injected with an extract obtained from a non toxic fish and the vehicle.

Intestinal tissue was taken at the time of death in the experimental group, fixed in 10% buffered formalin and prepared for Haematoxylin and Eosin (H&E) straining and Periodic Acid Schiff (PAS). Both groups of control mice were sacrificed at a time corresponding to the death time of the experimental mice.

Results

All mice exposed to CTX exhibited the now well documented signs of ciguatera poisoning including diarrhoea, ataxia, hypersalivation, piloerection, loss of activity and respiratory distress prior to death (Hoffman et al, 1983). Mice exposed to the saline vehicle alone or the saline vehicle plus the control extract showed no overt pathology.

(a) Small intestine

Damage to the small intestine was apparent in all mice exposed to CTX. The appearance of a normal small intestine showing villi and the lamina propria is shown in Figure 25. The severity of damage appears to be related to the death time.

CTX produces several pathological changes in the small intestine. There is a characteristic expansion of the lamina propria within the tips of the villi of the small intestine (Fig. 26). This expansion appears to occur concomitantly with vascular and lymphatic degeneration and a general disruption of the structure of the lamina. The expansion of the lamina at the villi tips is greater in mice with a longer death time. As the expansion increases the villi tips rupture and the laminal contents are lost into the lumen of the intestine (Fig. 27). CTX also exerts an effect on the columnar epithelium of the small intestine. Nuclear degeneration and cytoplasmic loss can be seen in mice exposed to each of the concentrations of CTX (Figs. 26, 27 and 28). In "high dose", short death time animals, the epithelial damage appears to be nuclear degeneration. The nuclear degeneration is then followed by cytoplasmic loss and vacuolation (Fig. 27). Associated with the onset of nuclear degeneration and cytoplasmic disruption of the epithelial cells of the villus there is a degeneration and stripping of the brush border (Fig. 28).

The type of damage apparent in experimental animals was not found in the control groups.

(b) Large Intestine

The appearance of normal control large intestine in mice not exposed to CTX is shown in Figures 29 and 31. Pathological changes are apparent in the large intestine of the mice with the longest death time ("low" dose group). These show surface disruption of the columnar epithelium with the extrusion of many epithelial cells into the lumen (Fig. 30). Such changes were not evident in control, "high" dose and "medium" dose groups. PAS staining of the large intestine has shown (Fig. 32) an altered pattern of mucin distribution within the walls of the large intestine as well as a large quantity of mucin in the lumen. Normal mucin distribution is shown in (Fig. 31).

Discussion

While much attention has been devoted to the functional pharmacological effects of CTX on a variety of preparations (Gillespie et al, 1986) there have been very few studies on structural changes associated with intoxication. Histological changes in human peripheral nerve with a striking oedema of the adaxonal Schwann cell cytoplasm has recently been described (Allsop et al, 1986).

The experiments in this study indicate that CTX produces pathological changes in the structure of the small and large intestines. The severity of damage appears to be inversely related to the death time, suggesting that prolonged exposure to the toxin is more disruptive. It can be inferred that animals exposed to high doses of CTX succumb to the neurotoxin effects of the toxin before major structural damage is manifest. The gut symptomatology in human victims of CTX may not only be due to the functional actions of this toxin on membrane channels but may also reflect gross pathological changes in the intestinal lining. Endoscopy and small intestine biopsy of ciguatera victims may confirm this suggestion.

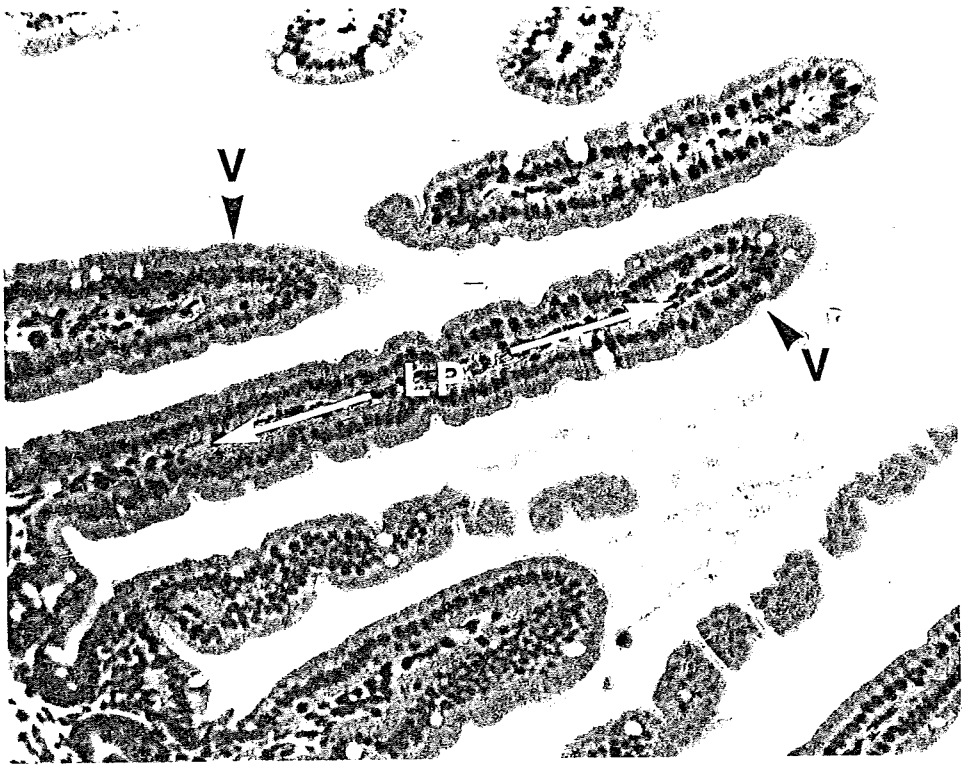


Figure 25. A H&E stained portion of the small intestine from a control mouse shown normal appearance of villi V and lamina propria LP.

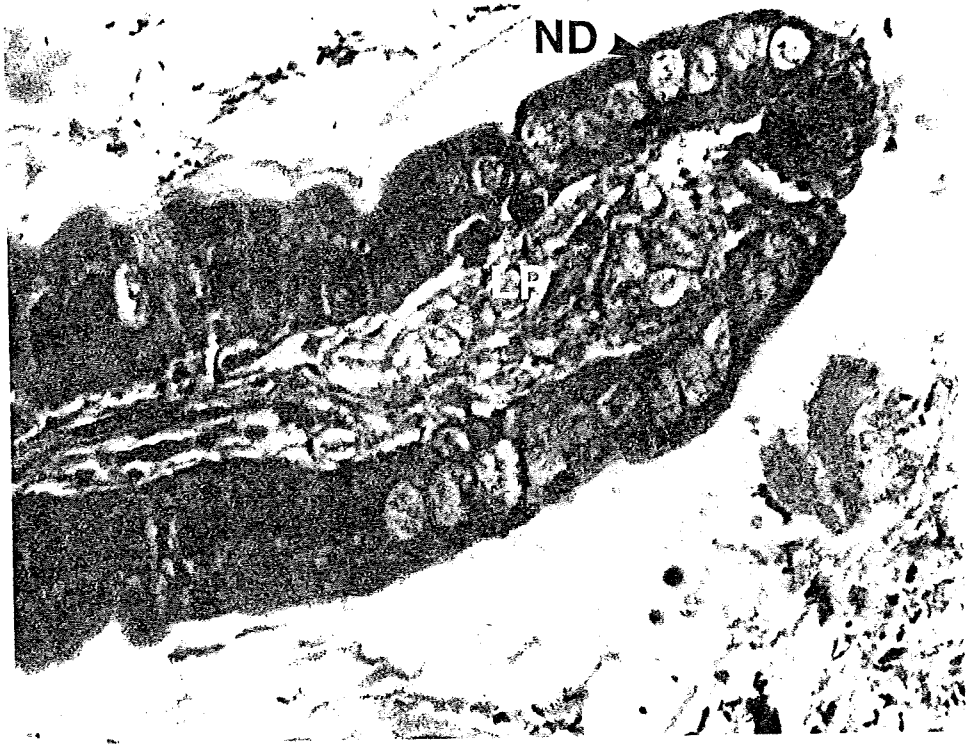


Figure 26. H&E stained section of "high" dose small intestine. Note expansion of lamina propria LP and nuclear degeneration ND of epithelial cells.

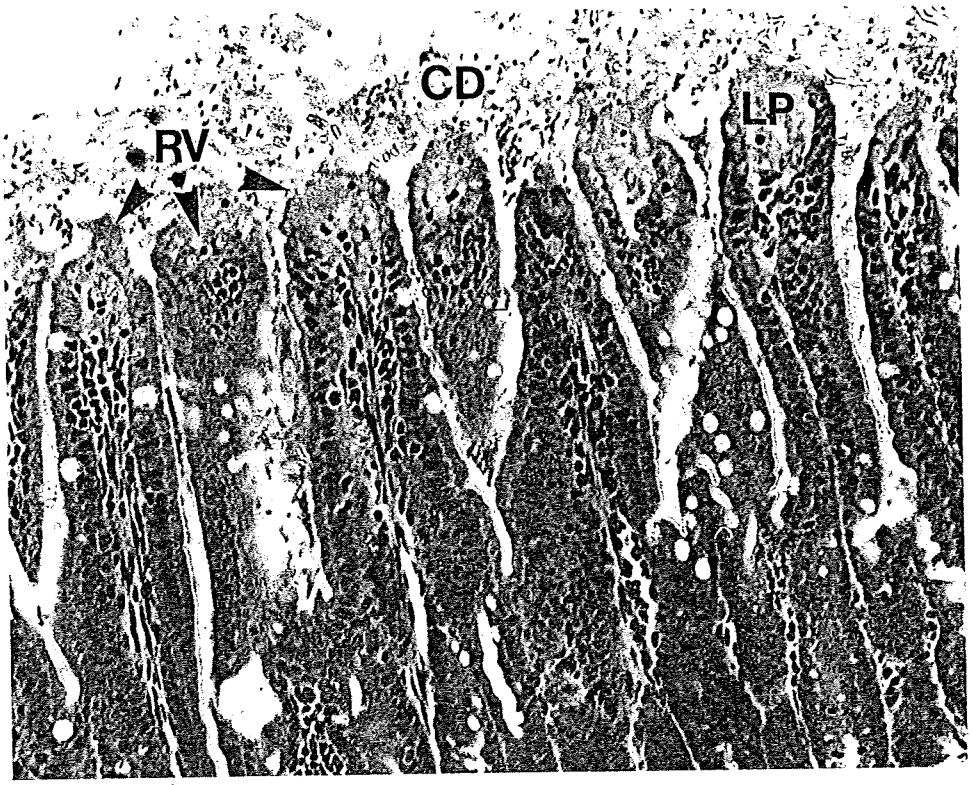


Figure 27. A H&E stained section of the small intestine of a mouse exposed to a "medium" dose of CTX. Note the expansion of the lamina propria LP and the rupture of the villi tips RV into the lumen of the gut. Cellular debris CD can be seen in the lumen.

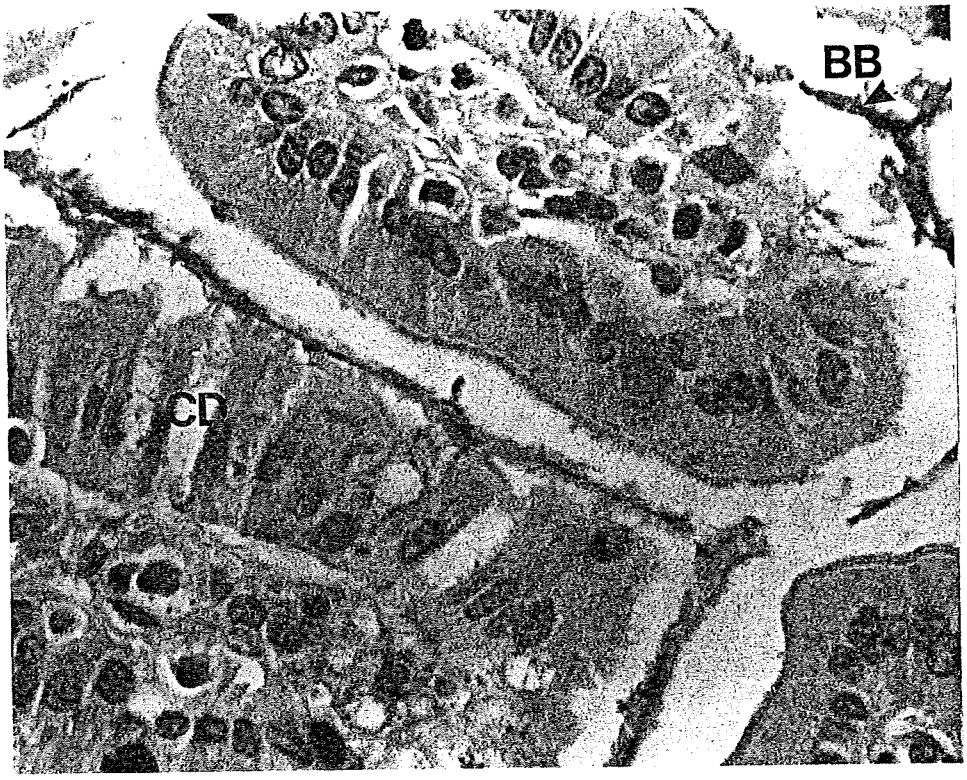


Figure 28. PAS stained section from "low" dose small intestine showing stripping of brush border BB from villus and cellular degeneration CD of the epithelial cells.

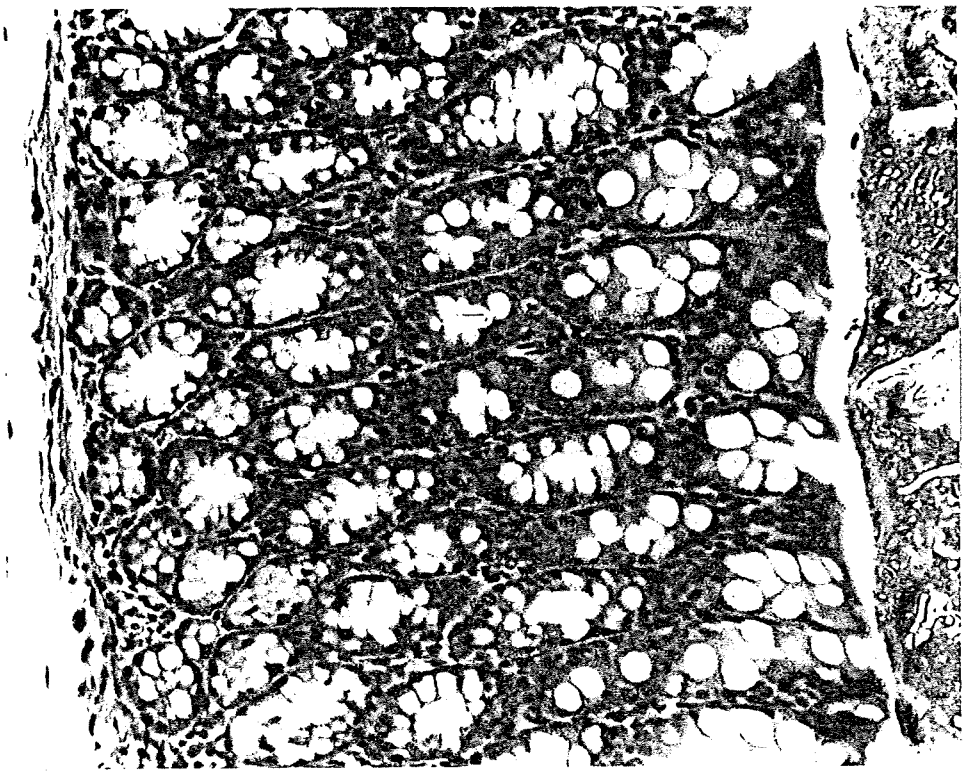


Figure 29: H&E stained large intestine from a control mouse.

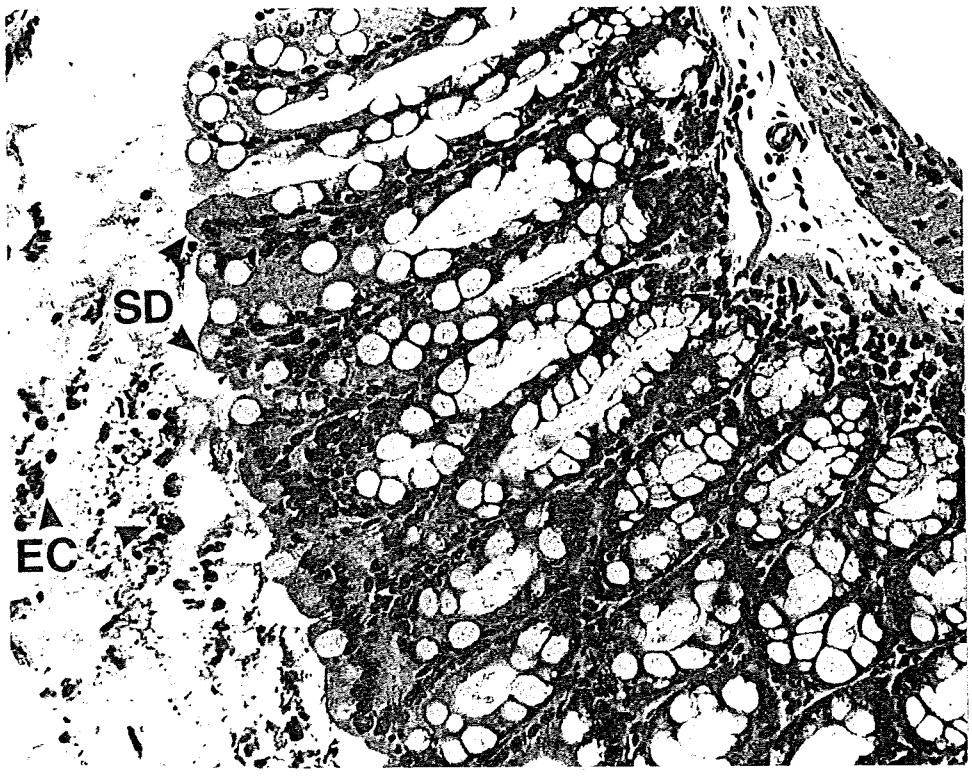


Figure 30. H&E stained large intestine from a "low" dose mouse showing surface disruption SD and free epithelial cells EC in the lumen.

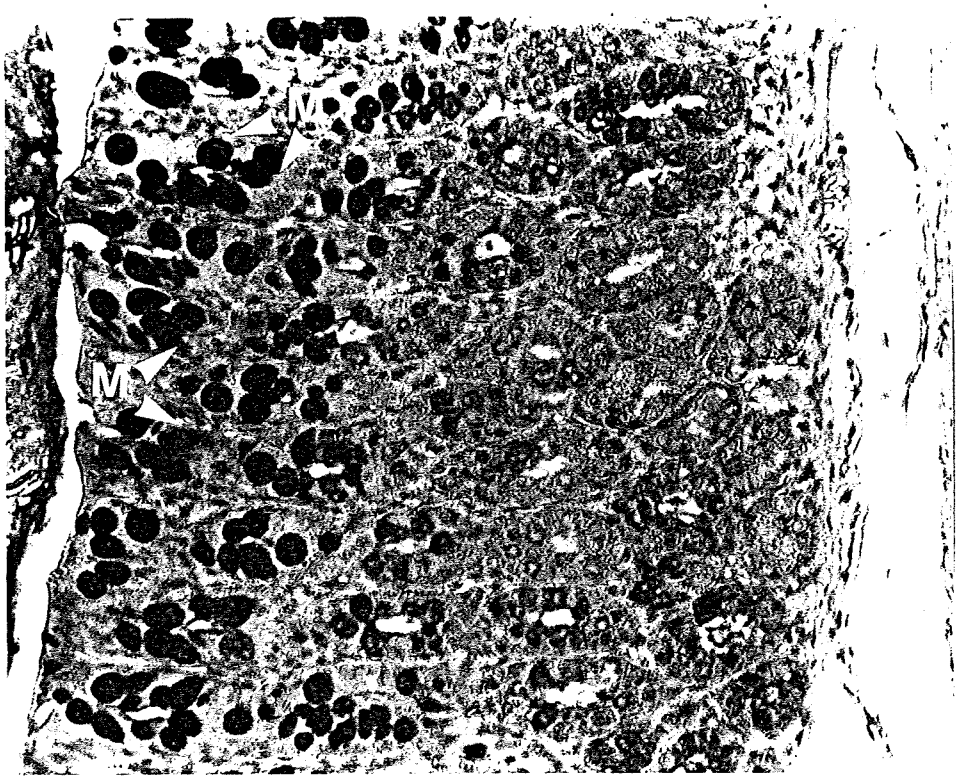


Figure 31. PAS stained control large intestine showing normal mucin distribution M.

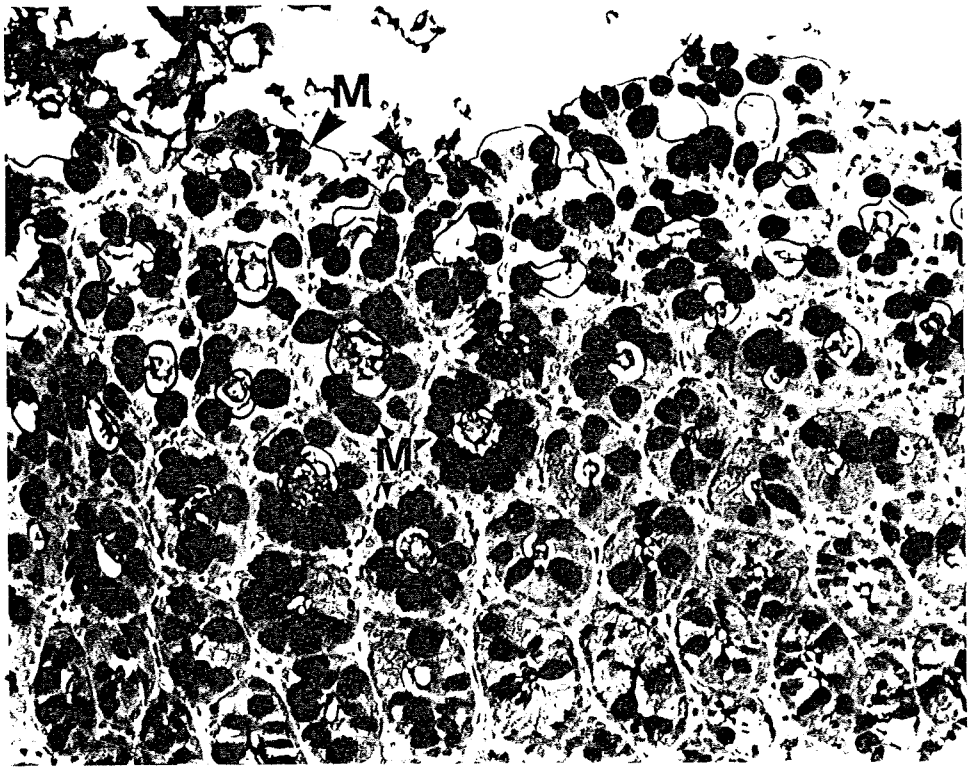


Figure 32. PAS stained"low" dose large intestine. Note the excessive production of mucin M.

VI ACTION OF CTX ON FISH

General Introduction

CTX can, upon ingestion by humans, produce an often severe episode of gastrointestinal and neurological dysfunction. An individual tropical fish can carry sufficient CTX within its tissues to poison several humans (Capra and Cameron, 1985), yet there is no evidence to suggest that such fish display any overt pathology.

There is surprising paucity of information on the effects of CTX on fish or fish tissues. CTX added to the ambient water of the guppy, Lebistes reticulatus produced death within a short time (Bagnis et al, 1980). Presumably, in this situation the toxin is absorbed via the gills, a route that would not be encountered under normal conditions. When the normally non-toxic surgeon fish Acanthurus xanthopterus of Hawaii were fed toxic fish flesh, these fish became toxic, but apparently showed no signs of intoxication (Helfrich and Banner, 1963). It has also been established that the normally toxic fish Lutjanus bohar maintain their toxicity even after eighteen months of captivity in which the diet was totally lacking in CTX (Banner et al, 1966). Hence reports on the toxicity of CTX to fish differ.

It is now established that CTX exerts a fundamental action on the excitable tissues of mammals by altering the permeability of Na^+ channels to Na^+ ions (Capra and Cameron, 1985; Lewis, 1985). The voltage-dependent sodium channels in amphibian nerves are opened at resting membrane potential by CTX (Benoit et al, 1986). Even in molluscan nerves CTX would appear to alter function by effecting Na^+ movement across the nerve membrane (Boyarsky and Rayner, 1970).

As fish which carry CTX show no overt pathology it is either possible that the Na^+ channels of such fish are structurally different to other Na^+ channels or that carrier fish have evolved mechanisms to detoxify CTX or to partition it away from target sites on excitable tissues.

This section examines:-

a) the effects of CTX on mortality in tropical fish,

b) the effects of CTX on movement of Na^+ across fish nerves and

c) the effects of CTX on the electrophysiology of teleost nerves.

a) CTX and Mortality in Tropical Fish

Introduction

Two small reef dwelling pomacentrid fish Pomacentrus wardi and Chromis nitida were selected for study during field work at the Heron Island Research Station. P. wardi is a browsing fish that feeds around coral heads and macroalgae while C. nitida is planktivorous and feeds in the water column. As P. wardi feeds in a niche in which G. toxicus is normally found it is conceivable that this fish may be an early link in the food chain that produces toxic predatory fish of the reef environments. Coral trout have been observed by graduate students feeding on P. wardi. C. nitida on the other hand is a midwater planktivore and such a feeding behaviour would isolate it from consumption of G. toxicus which is a benthic epiphyte. P. wardi and C. nitida were chosen for study because of their different feeding regimes.

Methods

Specimens of both species were collected by scuba diving and the use of the fish anaesthetic quinaldine. When a group of the desired species are located near a coral head a suspension of quinaldine is sprayed into the surrounding water from a wash bottle. The fish are momentarily anaesthetized and can be collected in small nets or by hand. Fish collected by this method were transported to the Heron Island Research station and held in glass aquaria with continually replenished sea water.

Members of each species were selected, lightly anaesthetized in a solution of MS222 and then injected intraperitoneally with a small volume of CTX, control extract or the saline vehicle. Fish were weighed prior to injection and then given doses of CTX equivalent to the mouse LD_{50} x 4, LD_{50} x 2 and LD_{50} x 1. Control fish were given equivalent volumes of a comparably purified control extract. Fish used in this study were small (0.7 - 2.5g) and special care was needed in the intraperitoneal injection. Solutions were injected between scales, just to one side of the midline of the ventral surface of the abdominal cavity from a glass micropipette attached to the needle of a micro-syringe. After injection the fish were placed in individual containers and watched for symptoms of ciguatera poisoning at 15 - 30 minute intervals until death.

Results

Fish injected with LD_{50} x 4 and LD_{50} x 2 doses of CTX showed signs of intoxication. Fish produced excessive faeces compared to control groups, became ataxic and disoriented, had increased or irregular respiratory movements and frequently displayed convulsive movements prior to death. Fish exposed to LD_{50} x 4 and LD_{50} x 2 doses of CTX were monitored until death and the death times recorded. Fish given LD_{50} x 1 showed signs of intoxication but did not die. Two series of experiments were performed, one in January 1987 and the other in January 1988. Death times for each set of experiments are shown in Tables 2 and 3.

Table 2

Death times of P. wardi and C. nitida exposed to CTX in 1987.

Species and treatment	Number of fish	Mean death time	Standard error
		Min.	Min
P. wardi $LD_{50}x2$	2	265	-
P. wardi $LD_{50}x4$	5	288	13
C. nitida $LD_{50}x2$	8	235	25
C. nitida $LD_{50}x4$	9	183	3

Table 3

Death times of P. wardi and C. nitida exposed to CTX in 1988.

Species and treatment	Number of fish	Mean death time	Standard Error
		Min	Min
P. wardi $LD_{50}x2$	5	323	32
P. wardi $LD_{50}x4$	5	413	88
C. nitida $LD_{50}x2$	5	119	12
C. nitida $LD_{50}x4$	6	107	5

In 1987 the death time of P. wardi exposed to LD_{50} x 4 dose of CTX is significantly longer (5% level) than the death time of C. nitida exposed to the same dose. Likewise in 1988 the death times of P. wardi were significantly longer than those of C. nitida for each of the lethal doses of CTX. There was however no significant difference between the death times of either species for the high ($LD_{50}x4$) or low ($LD_{50}x2$) dose of CTX.

Tissues were taken from both the 1987 and 1988 groups. Gill, gut and liver tissues all displayed some disruption and are now being studied in more detail in the current FIRTA funded project.

Discussion

Both species of fish examined were susceptible to CTX but at a dose greater than that required to kill a mouse of comparable size. Fish given a mouse LD_{50} dose of CTX displayed symptoms but did not die. However, two and four times the mouse LD_{50} proved to be lethal. The death time differences between the browser, P. wardi and the planktivore C. nitida may reflect the niche occupied by each species. A planktivore is less likely to encounter CTX from G. toxicus in its mode of feeding than is the browser. Fish normally exposed to ingestion CTX might be expected to have a higher tolerance to the toxin than fish not likely to encounter the toxin in their normal mode of feeding. The route of administration of CTX in this study differs from the normal mode of ingestion and may account for the lethality of the toxin.

CTX in nature would be absorbed through the gut and partitioning or detoxification mechanisms may be available in the gut or the liver before the toxin can exert a systemic effect. There are however conflicting reports on the actions of CTX on fish even when the toxin is administered with food. Surgeon fish fed toxic flesh absorbed and held the toxin with no overt signs of pathology (Helfrich and Banner, 1963) while the bluehead when fed an extract of G. toxicus displayed a number of pathological signs (Davin et al, 1986).

b) The Effects of CTX on the Movement of Na^+ Across Fish Nerves.

Introduction

CTX is known to alter the Na^+ channel in mammals in a manner that suggests a prolongation of the opening time of the Na^+ gates. Fish that carry CTX may have evolved nerves that have CTX insensitive Na^+ channels. In the series of experiments reported in this section nerves from species that are known to carry CTX ("carrier" fish) and nerves from fish that never carry CTX ("non-carrier" species) have been exposed to CTX and the status of the Na^+ channel examined using the movement of radioactive $^{22}Na^+$ as an indicator of Na^+ flux across the nerve membrane.

Methods.

Two groups of fish were used in this study: one, a "non-carrier" group that never carries CTX in their tissues. The "non-carrier" fish were whiting, Sillago ciliata obtained from Sea World Marine Park, 80 km from Brisbane.

Two species of carrier fish, the coral trout, Plectropomus sp, and the sweetlip, Lethrinus sp, were obtained and used on a visit to the Heron Island Research Station located on the southern fringe of the Great Barrier Reef.

In each experiment fifteen olfactory nerves were carefully dissected from either "carrier" or "non-carrier" fish and held at 4°C in oxygenated Teleost Ringer. Each nerve was firmly tied at each end with fine surgical silk (6.0). Upon completion of dissection the nerves were divided into three groups and transferred to three vials of an incubation medium. Each incubation vial contained a Teleost Ringer (2ml), ouabain (2.05×10^{-4}M) and carrier free $^{22}Na^+$(145 µCi) (Amersham). Experimental vials contained either CTX, tetrodotoxin (TTX) (Sigma Chem. Co.) or veratridine (VTD) (Sigma Chem. Co.). Two control groups were used. In one group the nerves were exposed only to the fish Ringer, the ouabain and the isotope while in the other control group a volume of CE equivalent to the Volume of the CTX extract was present in addition to incubation medium.

Nerves were incubated for a period of four hours at 22-24°C with gentle agitation. After which the nerve was removed from the incubation mixture and washed in a 10ml volume of Ringer. The nerves were then transferred into separate vials of Ringer (2ml). The efflux rate of $^{22}Na^+$ from each nerve was then determined by transferring the nerves successively to a series of vials each containing 2ml of Ringer. After nine transfers of 10 minutes each a sample (1 ml) of the solution from each washout vial was added to a scintillation vial and 10 ml of an aqueous counting scintillant (ACS II, Amersham) was added. Each nerve was dried with a paper tissue and then weighed. Tubes were counted for two minutes each in either a Konton (W and W Electronics) or a Beckman 6800 liquid scintillation counter.

The efflux rate was weight adjusted and is presented as counts per minute (cpm) per mg of nerve. Efflux rates between treatments were compared statistically using a two way analysis of variance.

Results

The efflux rate of $^{22}Na^{+}$ was significantly increased from the nerves of both "carrier" (Fig. 33) and "non-carrier" (Fig. 34) species of fish by the presence of CTX in the incubation medium. The presence of a control extract in the incubation medium produced an efflux pattern that was not significantly different from the saline control (Fig. 34). Exposure of nerves to tetrodotoxin (Fig. 35) resulted in a significant decrease of $^{22}Na^{+}$ efflux from control values. Veratridine when present in the incubation increased the $^{22}Na^{+}$ efflux in a dose dependent manner (Fig. 36). The effects of veratridine are similar to those of CTX. CTX in a high dose (Fig. 37) produced a very rapid efflux of $^{22}Na^{+}$ within the first ten minutes of exposure and thereafter an efflux rate that was below control values.

Discussion

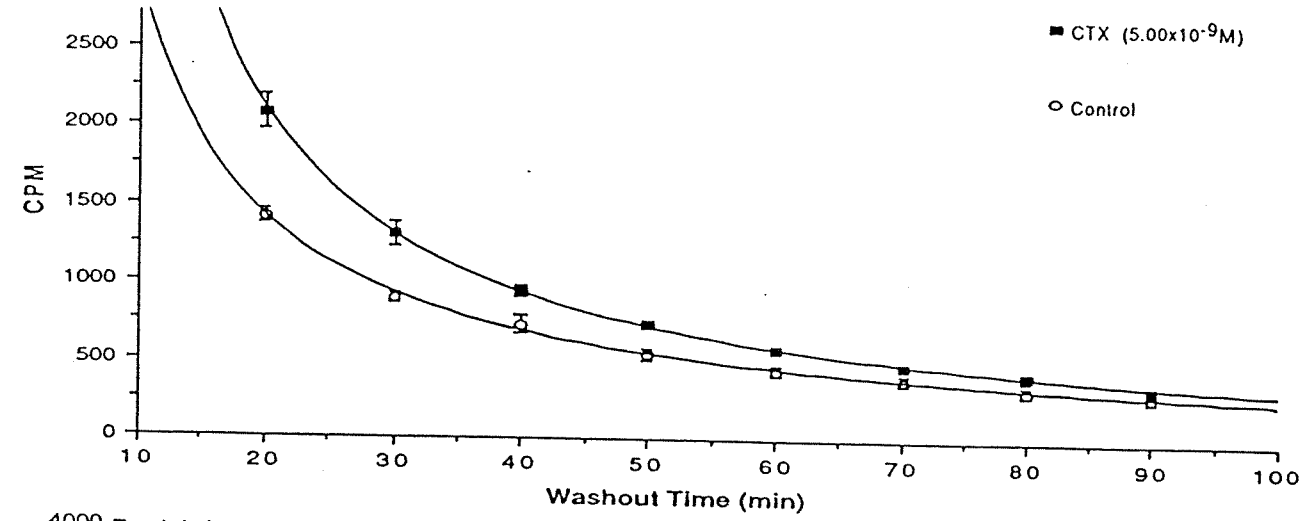
Ciguatoxin produces a significant increase in the $^{22}Na^{+}$ efflux from ouabain treated olfactory nerves in both "carrier" and "non-carrier" species of fish. Work on mammalian preparations suggests that ciguatoxin acts on Na^{+} channels causing an increased opening (Capra and Cameron, 1985; Lewis, 1985). The data presented suggests that the Na^{+} channel of fish nerves are influenced by ciguatoxin in a similar manner to mammalian nerves. Fish, then, that carry ciguatoxin have not evolved a fundamentally different type of Na^{+} channel as have the puffer fish which carry tetrodotoxin, (Kao and Fuhrman, 1967). Veratridine, a compound that has been shown to increase Na^{+} channel opening, (Ulbricht, 1969), also produces a significant increase in the $^{22}Na^{+}$ efflux from fish nerves. Tetrodotoxin a compound that produces closure of Na^{+} channels, (Namakura et al, 1965) has an opposite effect to ciguatoxin and veratridine and decreases the rate of $^{22}Na^{+}$ from fish nerves. The high dose of CTX appears to have opened Na^{+} channels so completely that most of the $^{22}Na^{+}$ is washed out of the nerve in the first ten minutes of incubation.

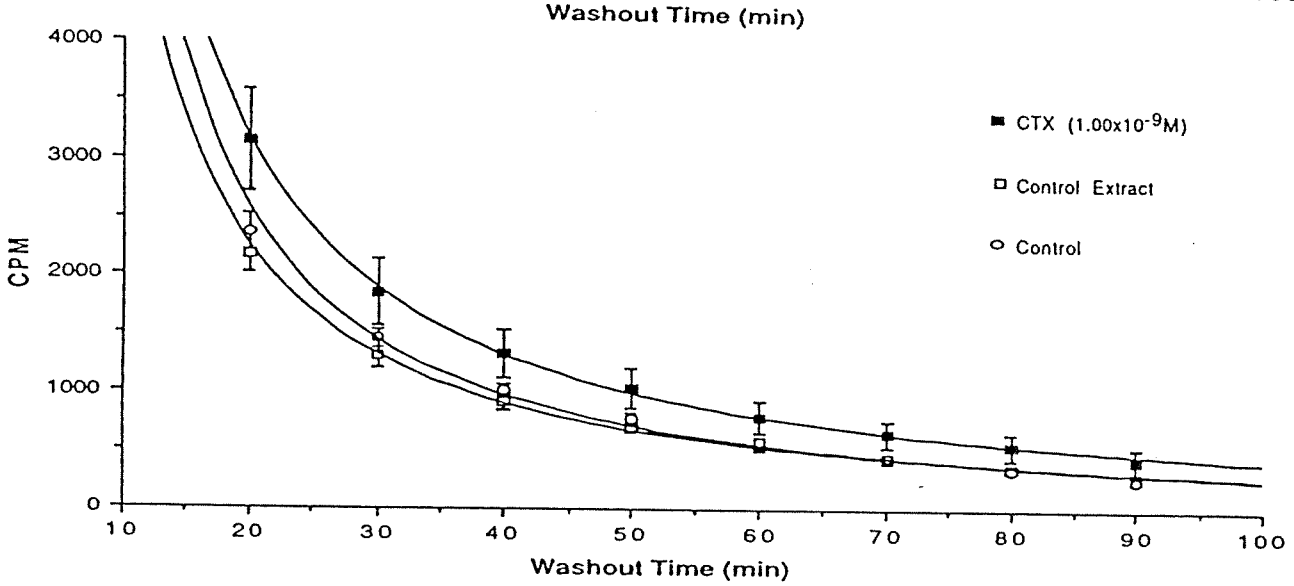
As fish which carry CTX show no overt pathology it is either possible that the Na^{+} channels of such fish are structurally different to other Na^{+} channels or that carrier fish have evolved mechanisms to detoxify CTX sensitivity of Na^{+} channels in fish to ciguatoxin yet there is no indication that "carrier" fish show any overt pathology.

Figure 33. The $^{22}Na^{+}$ efflux with time from nerves of the "carrier" species Plectropompus and Lethrinus exposed to CTX and saline controls. Each point represents data derived from five nerves. Standard errors of the means are shown.

Figure 34. The $^{22}Na^{+}$ efflux with time from the nerves of the "non-carrier" species, Sillago ciliata, exposed to CTX a control extract (CE) and a saline control. Each point represents data derived from five nerves. Standard errors of the means are shown.

Figure 35. The $^{22}Na^{+}$ efflux with time from the nerves of the "non-carrier" species, Sillago ciliata exposed to CTX, tetrodotoxin (TTX) and control saline. Each point represents data derived from five nerves. Standard errors of the means are shown.


CTX (5.00x10-9M)
Control
CPM
2500
2000
1500
1000
500
0
10
20
30
40
50
60
70
80
90
100
Washout Time (min)


CTX (1.00x10-9M)
Control Extract
Control
CPM
4000
3000
2000
1000
0
10
20
30
40
50
60
70
80
90
100
Washout Time (min)

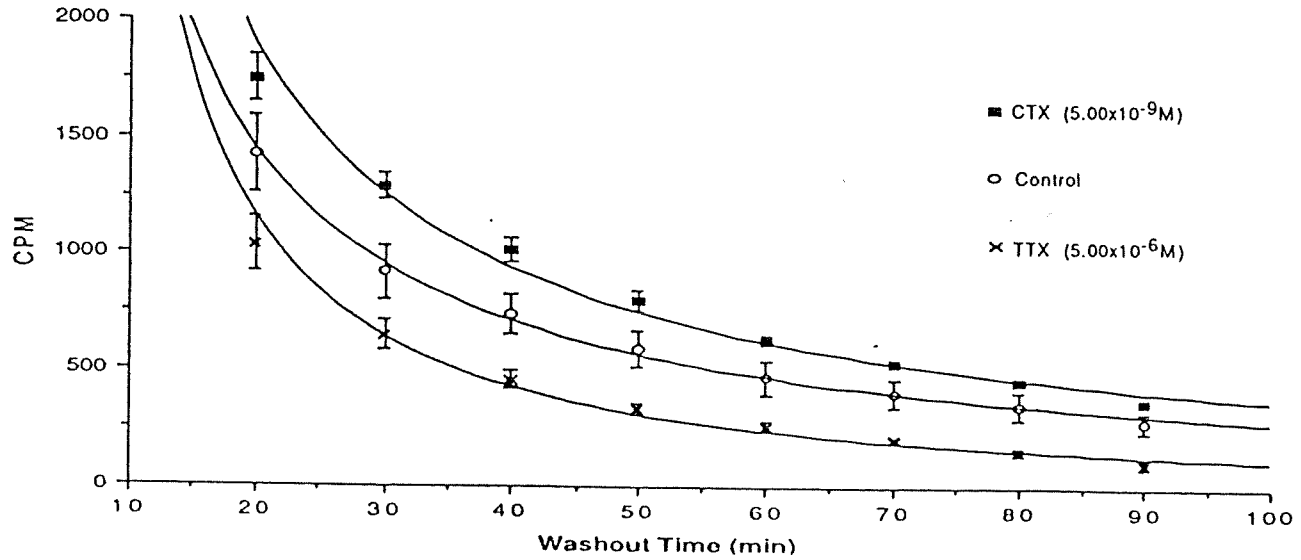
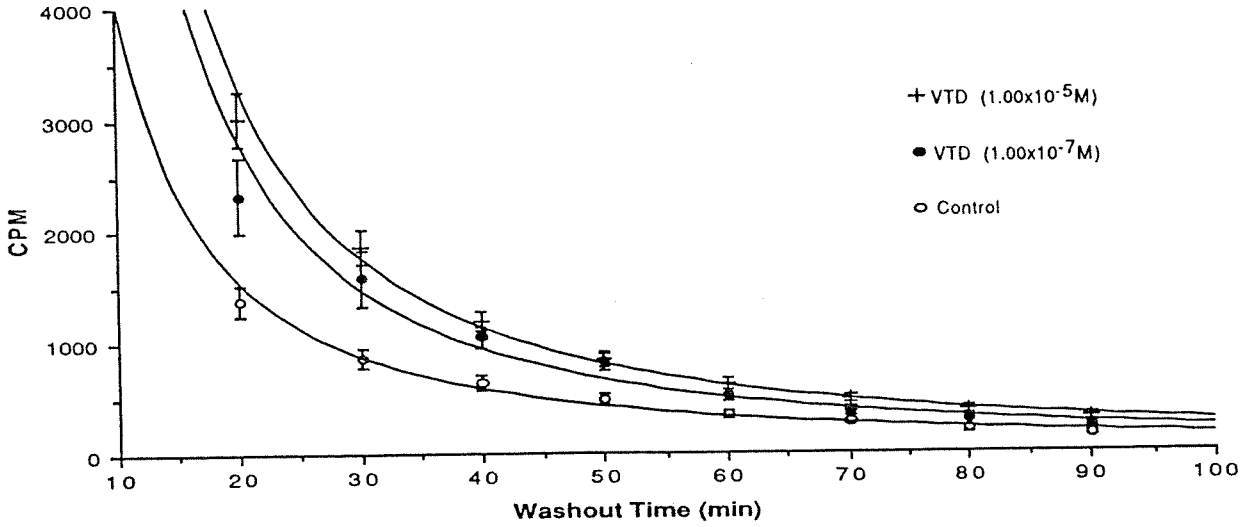
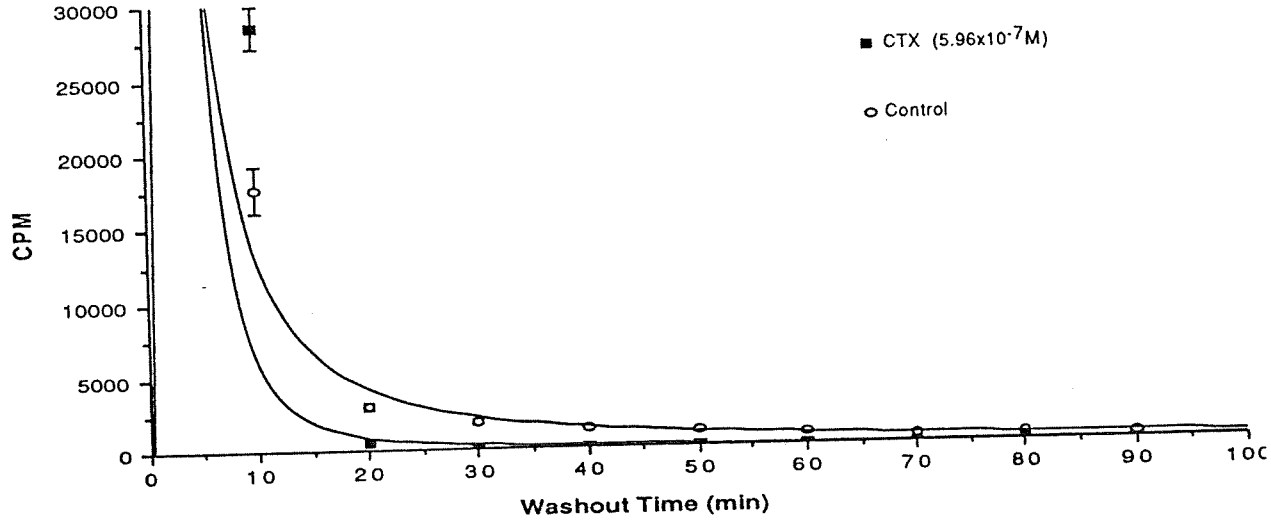

CTX (5.00x10-9M)
Control
TTX (5.00x10-6M)
CPM
2000
1500
1000
500
0
10
20
30
40
50
60
70
80
90
100
Washout Time (min)

Figure 36. The $^{22}Na^{+}$ efflux with time from the nerves of the "non-carrier" species, Sillago ciliata exposed to two doses of veratridine (VTD) and control saline. Each point represents data derived from five nerves. Standard errors of the means are shown.

Figure 37. The $^{22}Na^{+}$ efflux with time from the nerves of the "non-carrier" species, Sillago ciliata exposed to a high dose of CTX and control saline. Each point represents data derived from five nerves. Standard errors of the means are shown.


4000
3000
2000
1000
0
CPM
10
20
30
40
50
60
70
80
90
100
Washout Time (min)
+ VTD (1.00x10-5M)
• VTD (1.00x10-7M)
o Control


30000
25000
20000
15000
10000
5000
0
CPM
0
10
20
30
40
50
60
70
80
90
Washout Time (min)
■ CTX (5.96x10-7M)
o Control

(c) The Effects of CTX on the Electrophysiology of Teleost Nerves

Introduction

In the past much research has been directed towards the elucidation of the actions of CTX on excitable tissues of mammalian origin (Lewis and Endean, 1984; Capra and Cameron, 1985; Legrand et al, 1985 and Lewis, 1985). The basis at least, of the peripheral neurological symptoms appears to be an alteration in the state of the Na^+ channels of the nerve membrane (Capra and Cameron, 1985). Voltage clamp studies in amphibian nerves indicate that CTX opens the voltage dependent sodium channels at resting membrane potential (Benoit et al, 1986). CTX has been shown to bind to a unique site on the Na^+ channel in cultured mammalian cells (Bidard et al, 1984).

While attention has been focussed on the actions of CTX on mammalian nerves there has been no report on how this toxin effects fish nerves. This section describes the actions of CTX on the nerves of two groups of teleost fish: those that never carry the toxin ("non-carrier" species) and those that can, at times, carry CTX ("carrier" species).

Method

The fish used as the "non-carrier" was the whiting, Sillago ciliata while coral trout, Plectropompus sp. and sweetlip, Lethrinus sp were used as carrier fish. Electrophysiological studies were performed on isolated segments of the lateral line branch of the vagus nerve and on spinal nerves supplying the musculature of the tail. Nerve segments were mounted across a carrier consisting of two silver stimulating electrodes, an earth and two silver recording electrodes. The carrier with the nerve was immersed in a temperature controlled paraffin bath (22-24°C for "non-carrier" fish and 24-26°C for "carrier" fish). Nerves were stimulated by custom built square wave stimulators triggered by a TTL output from a discretely variable interval generator which also triggered a Medelec MS92a electromyography unit. The electromyography unit was used to average and record the evoked compound nerve action potentials.

Prior to mounting in the electrode assembly, nerves were exposed to test solutions of CTX, CE, tetrodotoxin (TTX), veratridine (VTD), verapamil (VER) and lignocaine (LIG) for a period of one minute. In antagonist experiments the nerves were incubated in a CTX solution for one minute and then placed for a further one minute period into a solution of the antagonist.

Single and paired supramaximal stimuli (square wave 3-3.5 V; 0.1 ms duration) were given to determine amplitude, conduction velocity and absolute and relative refractory periods for each of the treatments studied. Paired stimuli were also used in each nerve to determine the amplitude and duration of the supernormal period.

Results

(i) "Non-carrier" fish.

Exposure of whiting nerves to CTX produced a significant increase in the magnitude and duration of the supernormal period while the control extract caused no significant alteration from nerves exposed to saline alone (Figs. 38, 41 and 42). Tetrodotoxin significantly decreased magnitude and duration of supernormality from control values (Figs. 39, 41 and 42) while vertridine increased values above control levels (Figs. 40, 41 and 42).

The effects of a variety of other compounds on supernormality are shown in Figures 41 and 42. Tetrodotoxin, lignocaine and verapamil all antagonise the effects of CTX on magnitude and duration of supernormality.

Refractory studies are also given for whiting nerves exposed to CTX and various other compounds (Figs. 43 and 44). CTX significantly prolongs both the relative and absolute refractory periods while there is no difference in the duration of these periods between nerves exposed to saline and those exposed to saline plus the control extract. The effects of CTX on both the absolute and relative refractory periods are antagonised by tetrodotoxin, verapamil and lignocaine (Figs. 43 and 44). Veratridine induces changes in the refractory periods that are comparable to those induced by CTX (Figs. 43 and 44).

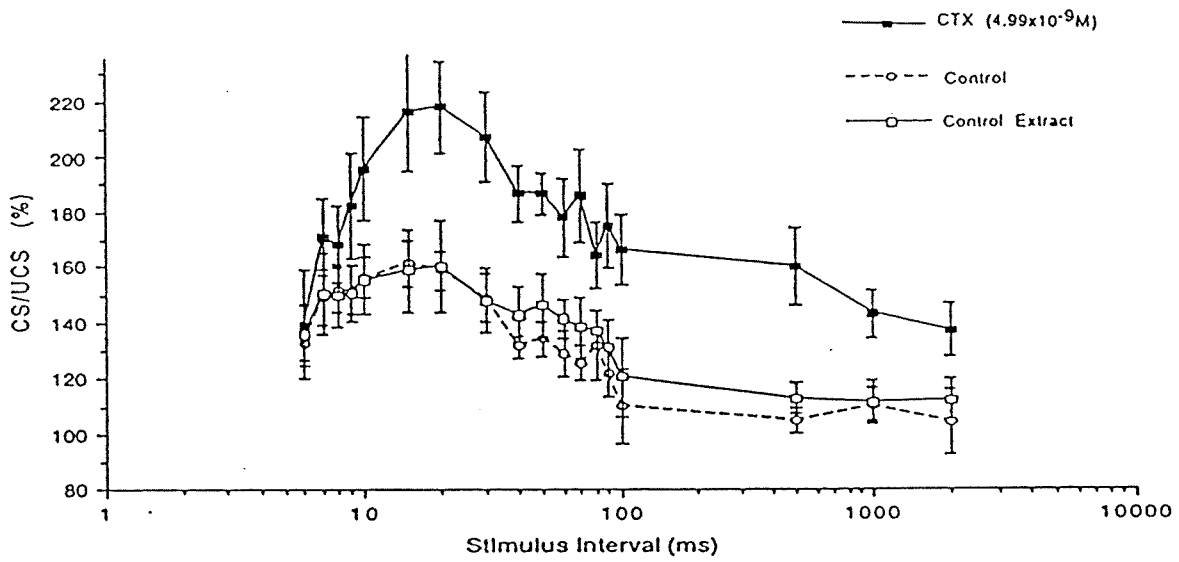
(ii) "Carrier fish"

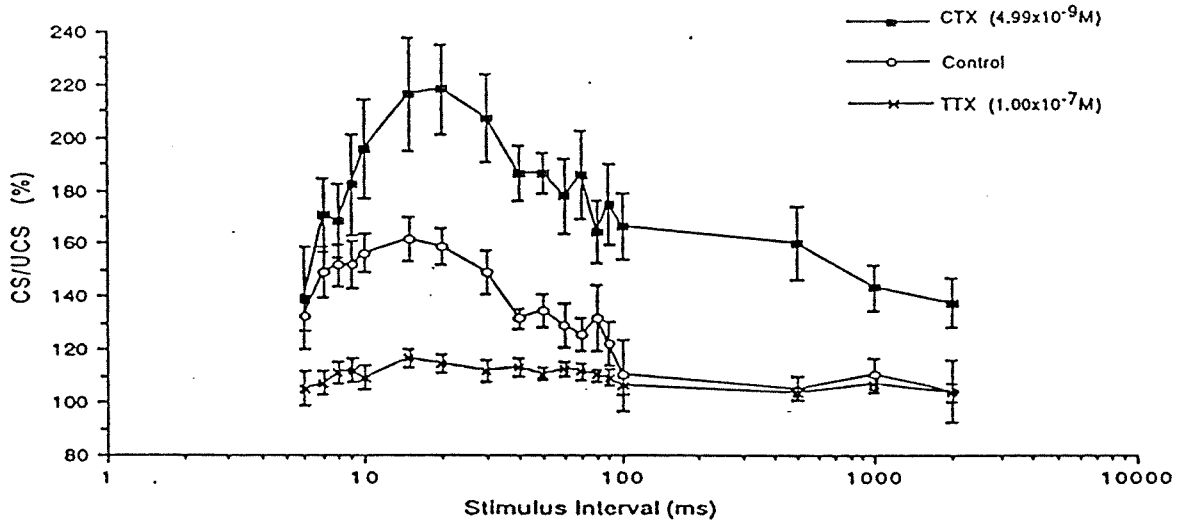
Exposure of nerves from both species of "carrier" fish to CTX produced significant changes to the supernormal period (Figs. 45, 46 and 47). In the coral trout both the magnitude and duration of the supernormal period were significantly prolonged while in the sweetlip the prolongation of supernormality was significant. The sample size for the sweetlip was small (n=5) and may account for a lack of significance in the magnitude of supernormality. Several other nerve conduction parameters were measured for each of the carrier species. Latency, absolute refractory period and absolute-relative refractory period were all significantly altered from control values in the coral trout (Fig. 47). In the sweetlip no significant differences were shown in the above parameters, however the sample size was small with large variations in measured values.

Figure 38. The effects of CTX and control extract (CE) on the supernormal period of whiting nerves. Each point represents data derived from at least ten nerves. Standard errors of the means are shown.

Figure 39. The effects of CTX and tetrodotoxin on the supernormal period of whiting nerves. Each point represents data derived from at least ten nerves. Standard errors of the means are shown.

Figure 40. A comparison of the effects of CTX and veratridine VTD on the supernormal period of whiting nerves. Each point represents data derived from at least ten nerves. Standard errors of the means are shown.


CTX (4.99x10-9M)
Control
Control Extract
CS/UCS (%)
220
200
180
160
140
120
100
80
1
10
100
1000
10000
Stimulus Interval (ms)


CTX (4.99x10-9M)
Control
TTX (1.00x10-7M)
CS/UCS (%)
240
220
200
180
160
140
120
100
80
1
10
100
1000
10000
Stimulus Interval (ms)

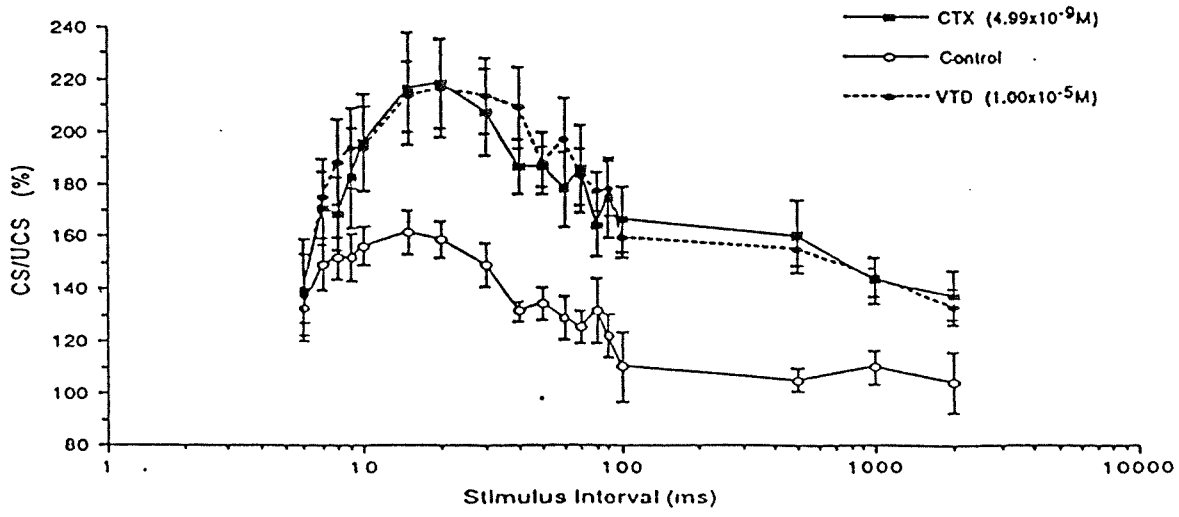
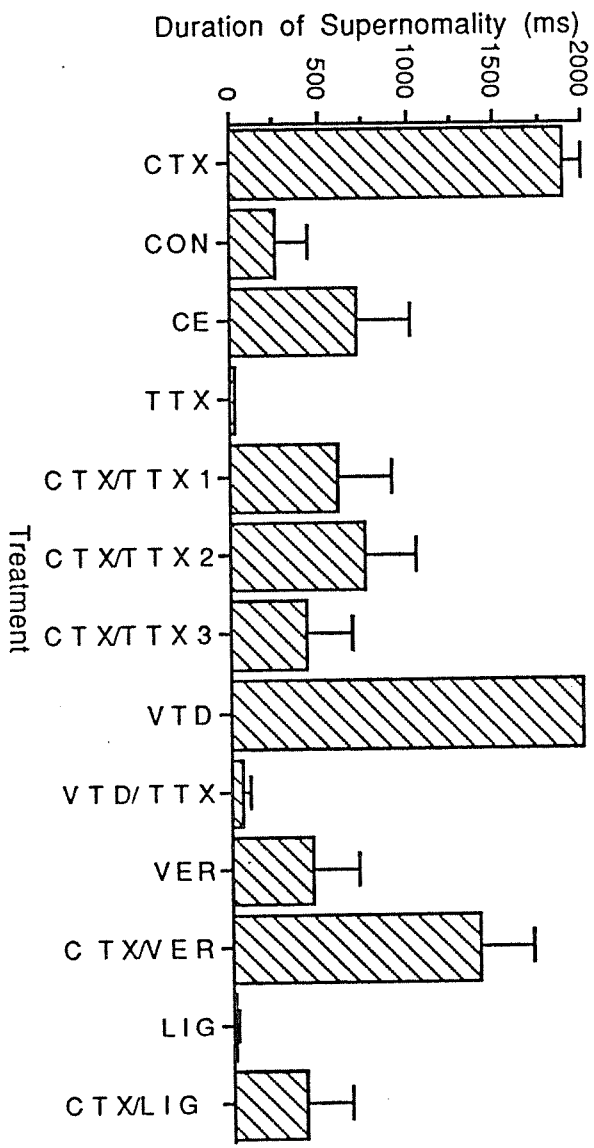

CTX (4.99x10-9M)
Control
VTD (1.00x10-5M)
CS/UCS (%)
240
220
200
180
160
140
120
100
80
1
10
100
1000
10000
Stimulus Interval (ms)

Figure 41. The effects of various compounds and combinations of these compounds on the magnitude of the supernormal period in nerves from the whiting. Concentrations of these compounds for this Figure and Figures 42, 43, and 44 are as follows: CTX, $5x10^{-9}M$; TTX, $10^{-7}M$; TTX1, $5x10^{-10}M$; TTX2, $5x10^{-9}M$; TTX3, $5x10^{-10}M$; VTD, $10^{-5}M$; VER, $5x10^{-7}M$; LIG, $4x10^{-5}M$. Each histogram represents data derived from at least ten nerves. Standard errors of the means are shown.

Figure 42. The effects of various compounds and combinations of compounds on the duration of the supernormal period in whiting nerves. Each histogram represents data derived from at least ten nerves. Standard errors of the means are shown.


Duration of Supernomality (ms)
0
500
1000
1500
2000
Treatment
CTX
CON
CE
TTX
CTX/TTX1
CTX/TTX2
CTX/TTX3
VTD
VTD/TTX
VER
CTX/VER
LIG
CTX/LIG

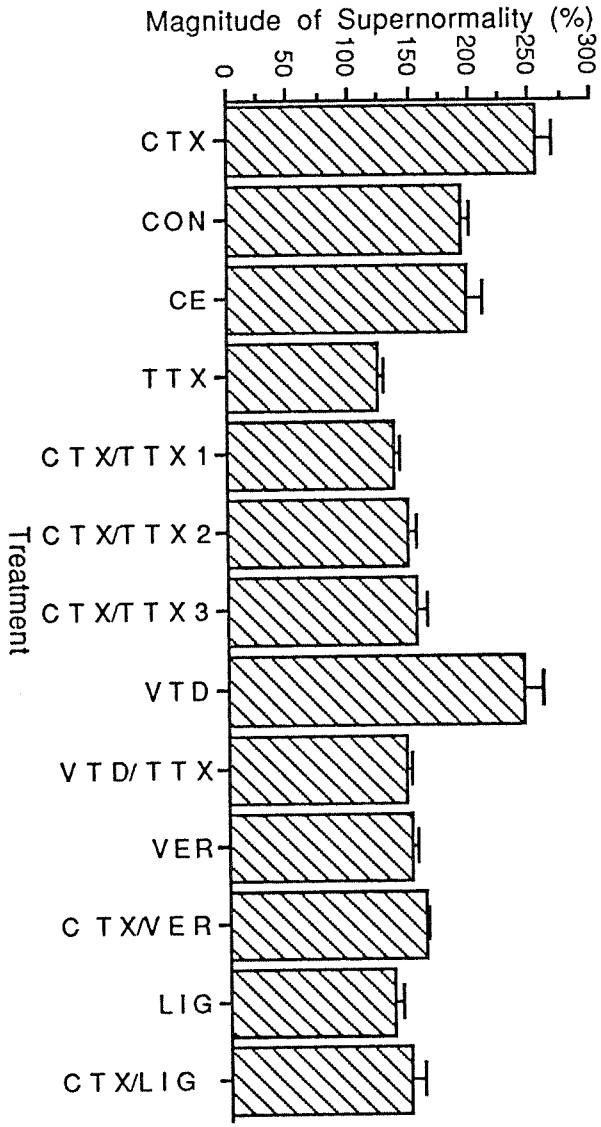
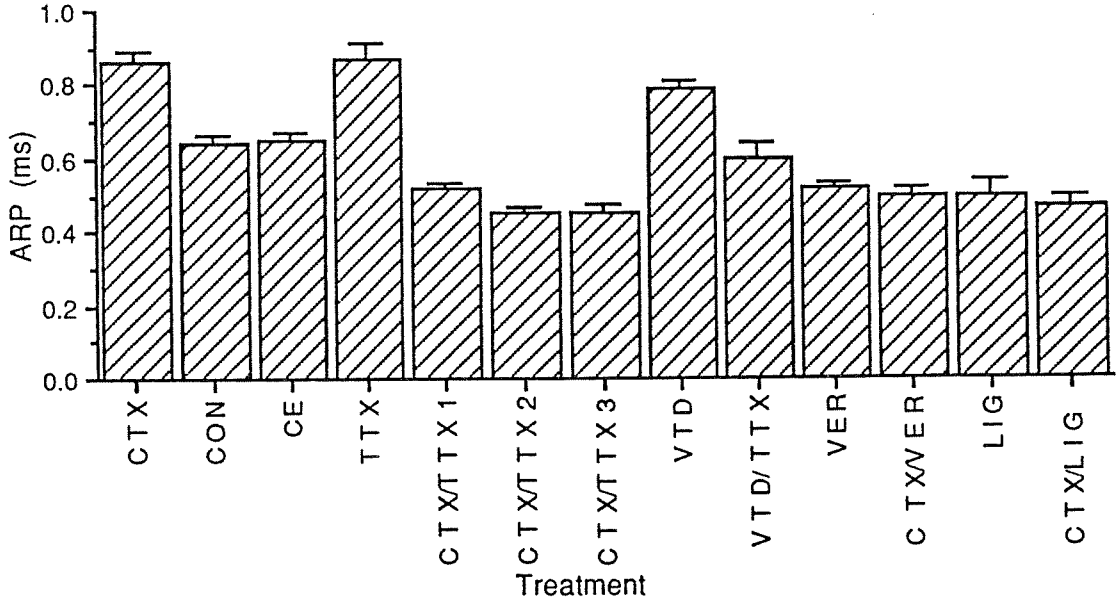

Magnitude of Supernormality (%)
0
50
100
150
200
250
300
Treatment
CTX
CON
CE
TTX
CTX/TTX1
CTX/TTX2
CTX/TTX3
VTD
VTD/TTX
VER
CTX/VER
LIG
CTX/LIG

Figure 43. The effects of various compounds and combinations of these compounds on the absolute refractory period in whiting nerves. Each histogram represents data derived from at least ten nerves. Standard errors of the means are shown.

Figure 44. The effects of various compounds and combinations of these compounds on the relative refractory period in whiting nerves. Each histogram represents data derived from at least ten nerves. Standard errors of the means are shown.


ARP (ms)
1.0
0.8
0.6
0.4
0.2
0.0
CTX
CON
CE
TTX
CTX/TTX1
CTX/TTX2
CTX/TTX3
VTD
VTD/TTX
VER
CTX/VER
LIG
CTX/LIG
Treatment

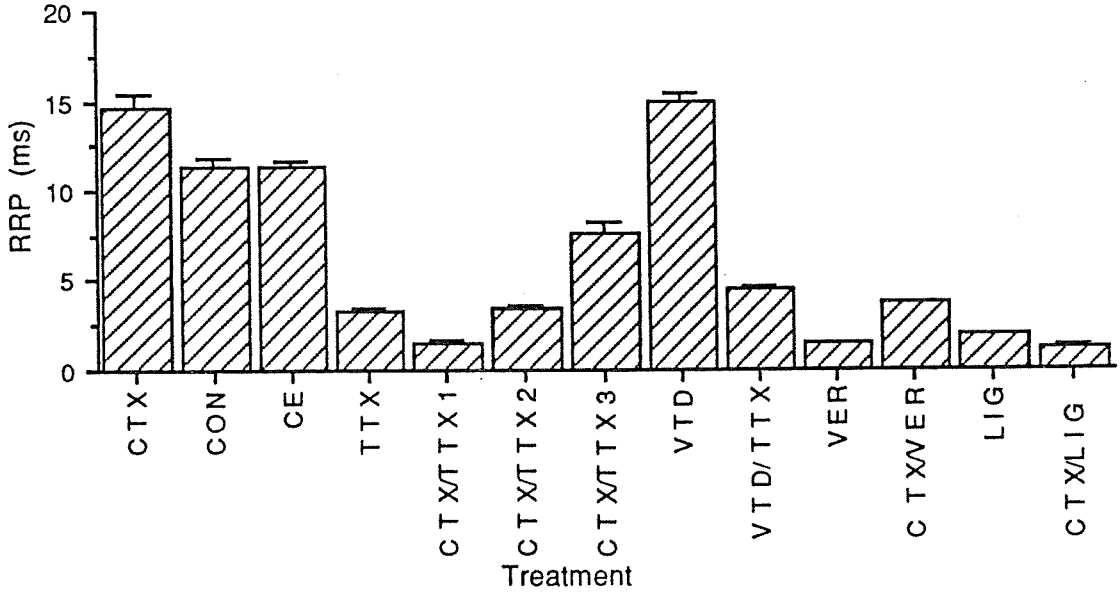
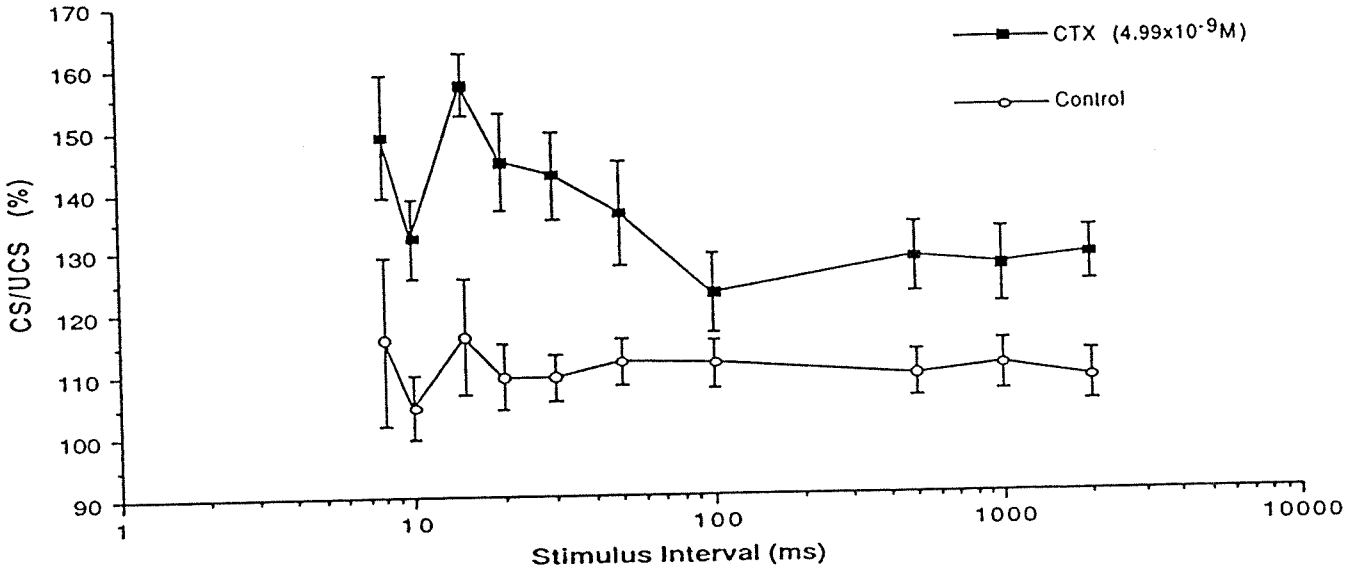

RRP (ms)
20
15
10
5
0
CTX
CON
CE
TTX
CTX/TTX1
CTX/TTX2
CTX/TTX3
VTD
VTD/TTX
VER
CTX/VER
LIG
CTX/LIG
Treatment

Figure 45. The effects of CTX on the supernormal period of coral trout nerves. Each point represents data derived from fifteen nerves. Standard errors of the means are shown.

Figure 46. The effects of CTX on the supernormal period of sweet lip nerves. Each point represents data derived from five nerves. Standard errors of the means are shown.


CTX (4.99x10-9M)
Control
CS/UCS (%)
170
160
150
140
130
120
110
100
90
1
10
100
1000
10000
Stimulus Interval (ms)

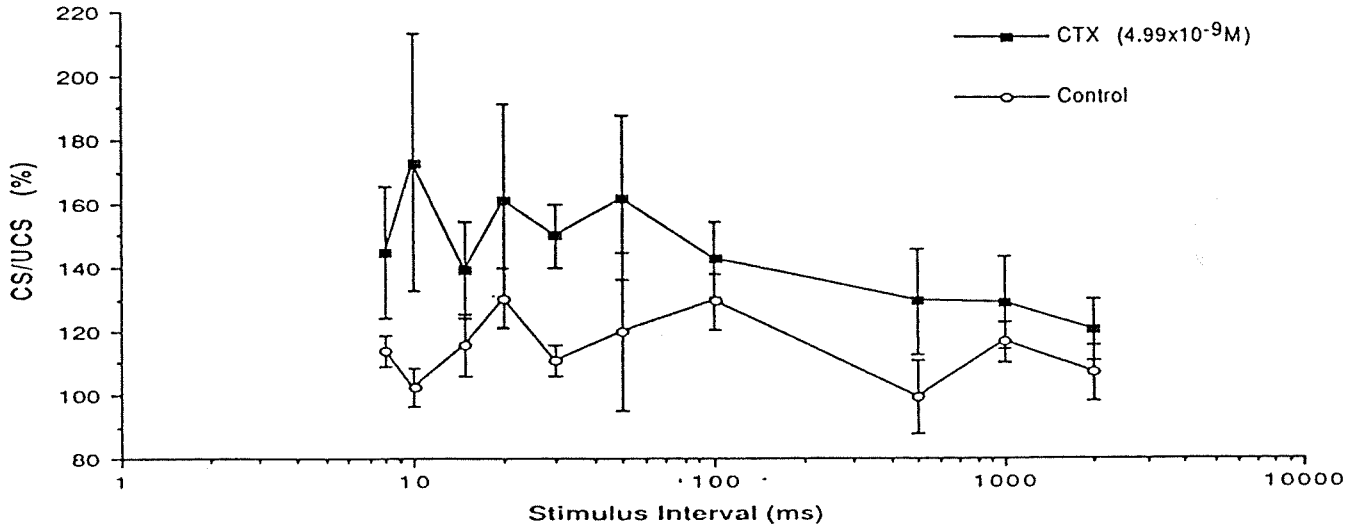

CTX (4.99x10-9M)
Control
CS/UCS (%)
220
200
180
160
140
120
100
80
1
10
100
1000
10000
Stimulus Interval (ms)

Figure 47.

Histogram showing the effects of CTX (10^{-9}M) (n=15) compared to a Control of fish Ringer (n=17) on the Latency of Nerves from the Carrier species Plectropomus.

Histogram showing the effects of CTX (10^{-9}M) (n=15) compared to a Control of fish Ringer (n=17) on the Absolute Refractory Period of Nerves from the Carrier species Plectropomus.

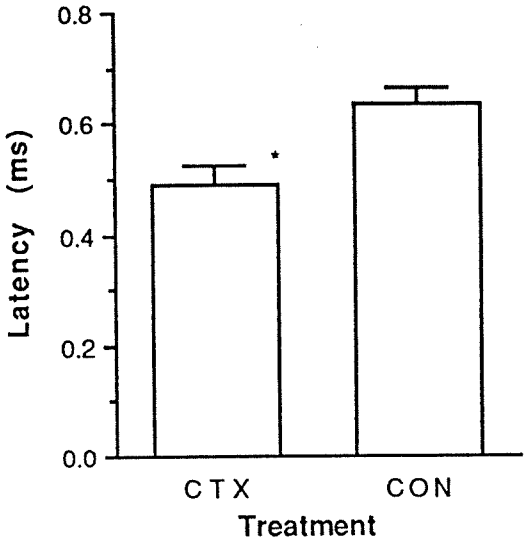
Histogram showing the effects of CTX (10^{-9}M) (n=15) compared to a Control of fish Ringer (n=17) on the Relative Refractory Period of Nerves from the Carrier species Plectropomus.

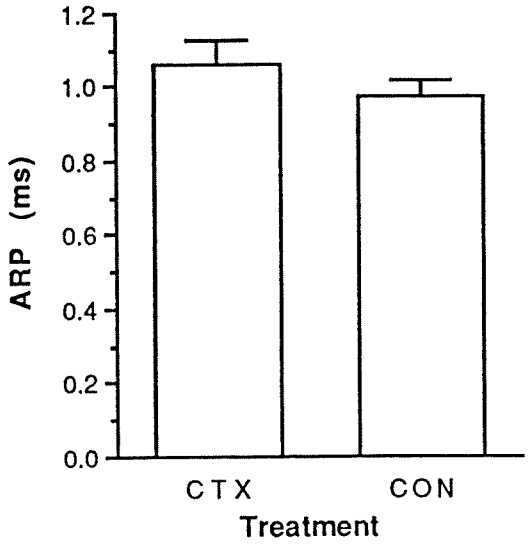
Histogram showing the effects of CTX (10^{-9}M) (n=15) compared to a Control of fish Ringer (n=17) on the Absolute Relative Refractory Period of Nerves from the Carrier species Plectropomus.

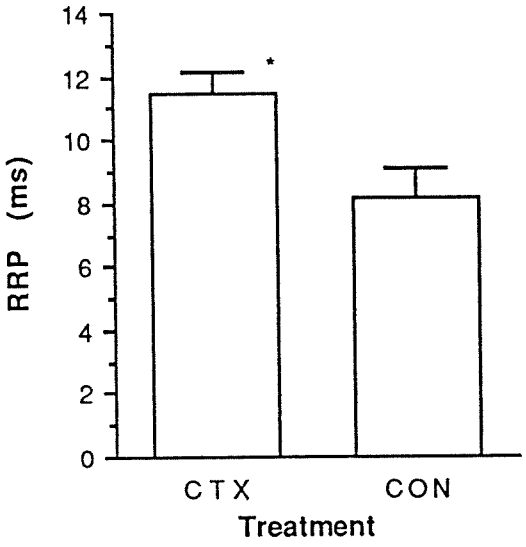
Histogram showing the effects of CTX (10^{-9}M) (n=15) compared to a Control of fish Ringer (n=17) on the Magnitude of Supernormality of Nerves from the Carrier species Plectropomus.

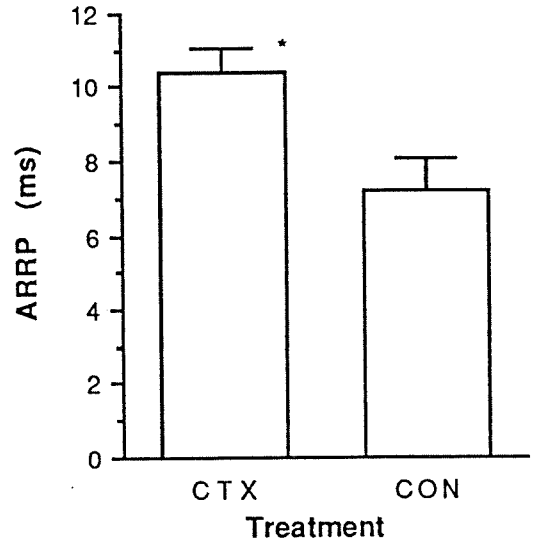
Histogram showing the effects of CTX (10^{-9}M) (n=15) compared to a Control of fish Ringer (n=17) on the Duration of Supernormality of Nerves from the Carrier species Plectropomus.

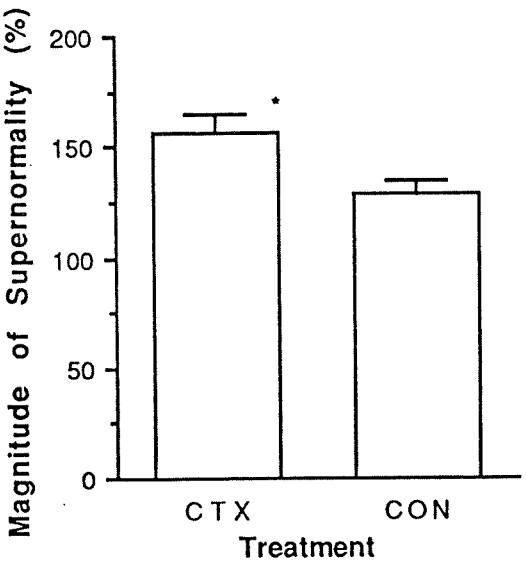
Error bars indicate the Standard errors of the means.
Significantly different results are shown by an asterisk.*

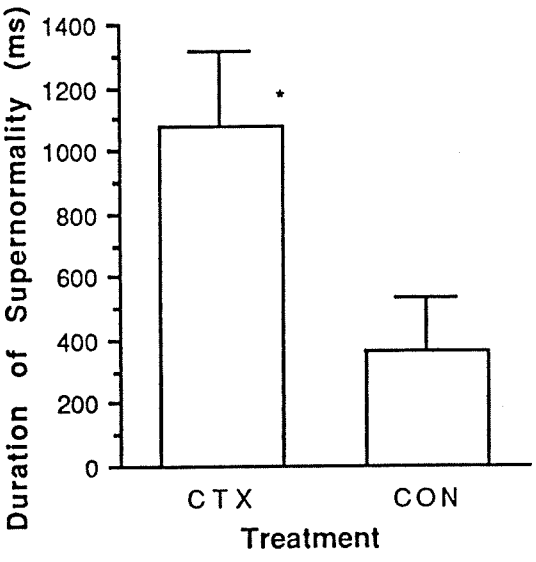

Latency (ms)
0.8
0.6
0.4
0.2
0.0
*
CTX
CON
Treatment


ARP (ms)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
CTX
CON
Treatment


RRP (ms)
14
12
10
8
6
4
2
0
*
CTX
CON
Treatment


ARRP (ms)
12
10
8
6
4
2
0
*
CTX
CON
Treatment


Magnitude of Supernormality (%)
200
150
100
50
0
*
CTX
CON
Treatment


Duration of Supernormality (ms)
1400
1200
1000
800
600
400
200
0
*
CTX
CON
Treatment

Discussion

CTX produces changes in a number of conduction parameters in teleost nerves in a manner similar to its actions on mammalian nerves (Capra and Cameron, 1985). The enhancement and prolongation of the supernormal period by ciguatoxin is indicative of the action of this toxin in producing prolonged opening of Na^+ channels. Increases in amplitude and duration of supernormality in peripheral nerves have been associated with increased opening of Na^+ channels (Parkin and Le Quesne, 1982). Veratridine, a lipid soluble alkaloid, that has been shown to increase the permeability of Na^+ channels after a nerve action potential (Ulbricht and Flacke, 1965) also prolonged the amplitude and duration of the supernormal period in fish nerves.

Tetrodotoxin a compound that closes Na^+ channels has the opposite effect to both CTX and veratridine and produces a decrease in both the amplitude and duration of the supernormal period in teleost nerves. Tetrodotoxin blocks the action of CTX on fish nerves in a manner that is not apparently correlated to tetrodotoxin concentration.

Tetrodotoxin has been shown to reverse the actions of CTX on atrial and papillary muscles of the guinea pig (Lewis and Endean, 1986). Stimulation of $^{22}Na^+$ flux by CTX in cultured neuroblastoma cells is also abolished by tetrodotoxin (Bidard, et al, 1984).

The actions of CTX on refractory periods also indicates a fundamental action on Na^+ channel and Na^+ gating mechanisms in fish nerves. Again the action of veratridine parallels that of CTX on refractory periods. The actions of tetrodotoxin on the absolute refractory period are some what surprising and are still being reviewed in our laboratory.

VII ACTIONS OF CTX ON HUMANS

Introduction

Many reports have appeared in the literature (General Introduction) on the symptomatology of acute ciguatera poisoning in humans. Much interest has been focussed on the neurological symptoms of CTX ingestion, however there have been no detailed studies on how CTX effects nerve conduction in human victims. A major difficulty in any such study is the fact that ciguatera poisoning in humans is a sporadic phenomenon with geographic and temporal discontinuities in its occurrence. Since the inception of this programme an important objective has been to undertake a detailed study of the effects of CTX on nerve conduction parameters in humans. In 1987 there were, quite fortuitously, two large outbreaks of human ciguatera poisoning in Australia. In the period of March to May, 1987 the largest single outbreak of ciguatera poisoning ever recorded in Australia, occurred in Sydney, when sixty three people became ill after eating Spanish mackerel that had originated in Hervey Bay, Queensland. In August, 1987 approximately twenty people became ill after consuming a sweet and sour fish dish at a football function in Maryborough. In each case the symptomatology of the illness in the victims were completely consistent with ciguatera poisoning. Each of these outbreaks were investigated by the Queensland Institute of Technology group and invaluable electrophysiological data was obtained from groups of victims in both Sydney and Maryborough. A detailed study of the symptomatology of the Sydney outbreak was initiated in May, 1987 and completed in December, 1987. The results of these studies are presented below.

Methods

(a) Electrophysiological Studies

A variety of nerve conduction parameters were recorded from the sural and the lateral popliteal nerves in the lower limbs of seven patients in Sydney and eight patients in Maryborough. A control group of fifteen normal subjects was also utilised. The detailed studies performed on each individual take 30 to 45 minutes and cause some discomfort to the patient. Surface, stimulating earth and recording electrodes were positioned over the sural and lateral popliteal nerves of ciguatera victims and volunteer control subjects. The stimulating electrodes were connected to the stimulator of a Medelec MS92a electromyography unit and a purpose built human nerve stimulator. Recording electrodes were connected via a preamplifier to the oscilloscope input of the MS92a. Single and paired stimuli of 200 volts maximum amplitude and 0.1 ms duration were applied at a frequency of 1 Hz or less across the skin of patients.

Such stimulus parameters initiated action potentials in underlying nerves and these evoked action potentials were then recorded across the skin from a point distant to the stimulating electrodes. Each measurement made was based on an electronically averaged group of 4-8 action potentials. Single stimuli were used to determine sensory and motor conduction velocities and amplitudes while paired stimuli were used to determine refractory and supernormal periods. Skin temperature was monitored from a calibrated thermistor probe positioned on the skin adjacent to the recording electrodes.

(b) Epidemiological Studies

In May, 1987 thirty seven (37) of a total of sixty three (63) victims of the Sydney ciguatera poisoning outbreak were interviewed. Each victim was given a series of questions on the type and severity of symptoms experienced. Victims were interviewed in their own homes or at the Prince Alfred Hospital, Sydney after nerve conduction studies had been performed. The results of this study were presented as an Interim report to the Fishing Industry Research Committee and a copy of the Interim report is appended to this report. In early December, 1987 forty (40) of the ciguatera poisoning victims were again interviewed in their homes in Sydney at which time a history of the type and duration of symptoms was taken.

Results

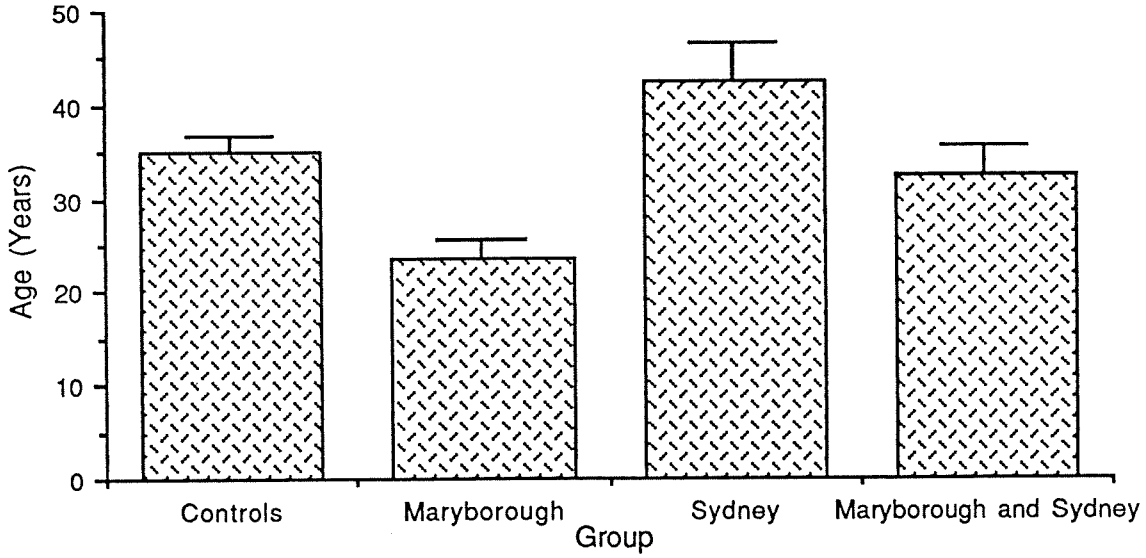
(a) Electrophysiological Studies

Preliminary results of electrophysiological and neurological examinations of seven victims of the Sydney outbreak were forwarded to the Committee in June, 1987 and are appended to this report. The electrophysiological studies from Sydney have now been fully analysed and are presented below with results form eight victims of the Maryborough outbreak.

The age structure and skin temperatures for each group are shown in Figures 48 and 49. There are no significant differences in the skin temperature at which conduction determinations were made between the groups. The age distribution of the combined Sydney and Maryborough groups does not differ significantly from the control group.

Figure 48. The age structure of controls, Sydney and Maryborough victims of ciguatoxin poisoning. A combined Maryborough-Sydney group is shown. There is no significant difference between the control group and the combined group. Standard errors of the means are shown.

Figure 49. Skin temperatures in the vicinity of recording electrodes from each group.


50
40
30
20
10
0
Age (Years)
Controls
Maryborough
Sydney
Maryborough and Sydney
Group

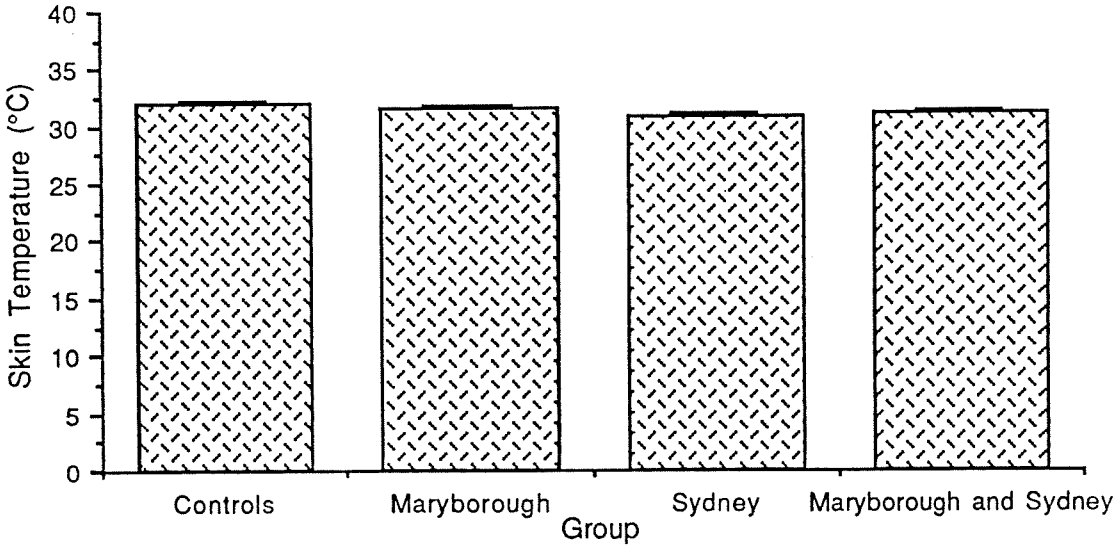
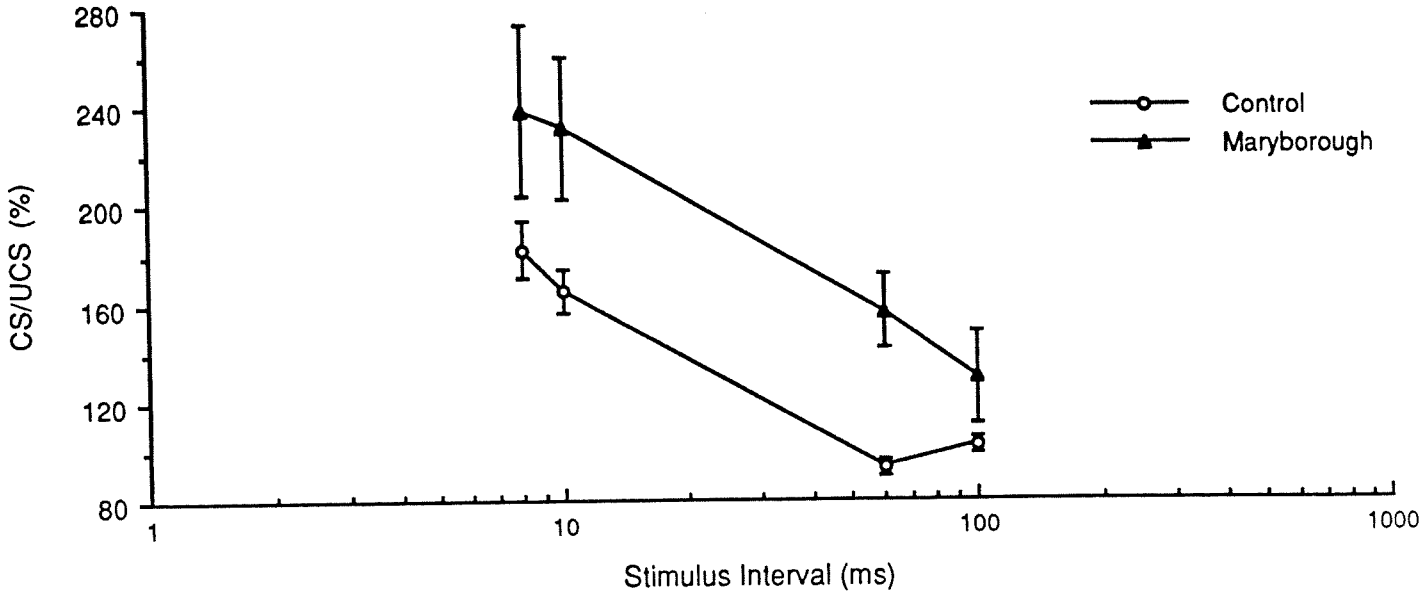
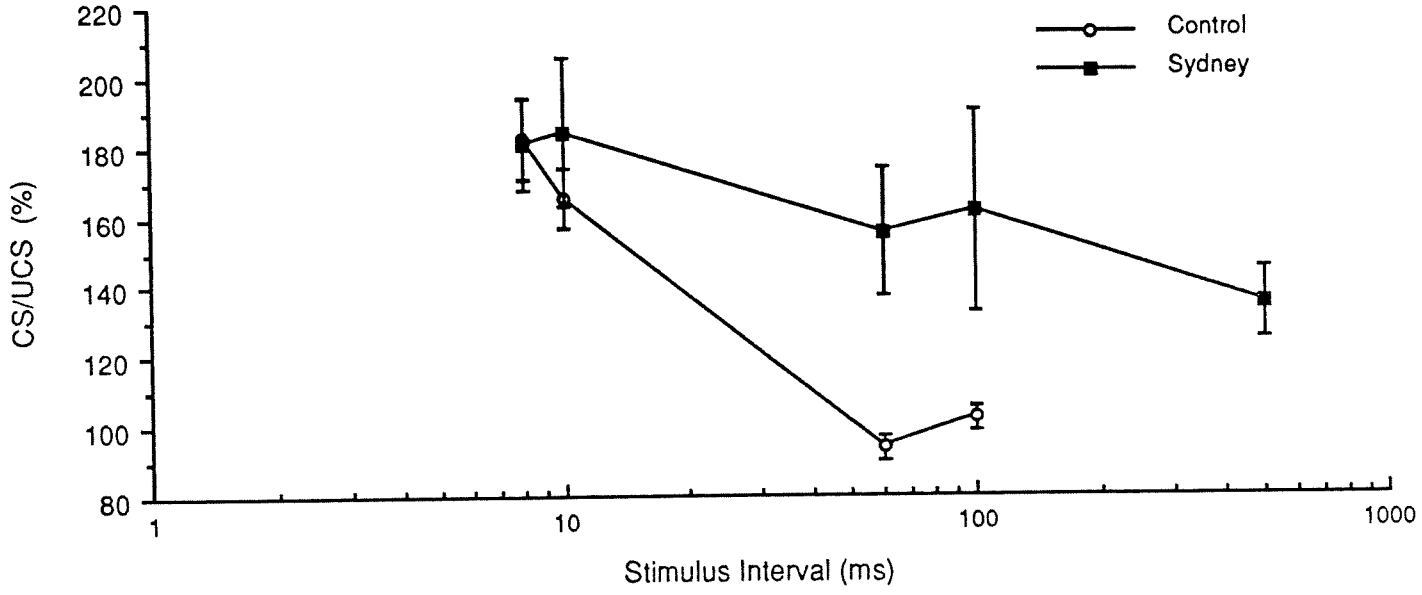

40
35
30
25
20
15
10
5
0
Skin Temperature (°C)
Controls
Maryborough
Sydney
Maryborough and Sydney
Group

Figure 50. A comparison of supernormality between a group of eight victims of ciguatera poisoning and a control group of fifteen people. Standard errors of the means are shown.

Figure 51. A comparison of supernormality between a group of seven victims of ciguatera poisoning from Sydney and a control group of fifteen people. Standard errors of the means are shown.

Figure 52. A comparison of supernormality between a combined Maryborough-Sydney group of ciguatera victims (15) and a control group of fifteen people. Standard errors of the means are shown.


280
240
200
160
120
80
CS/UCS (%)
1
10
100
1000
Stimulus Interval (ms)
Control
Maryborough


220
200
180
160
140
120
100
80
CS/UCS (%)
1
10
100
1000
Stimulus Interval (ms)
Control
Sydney

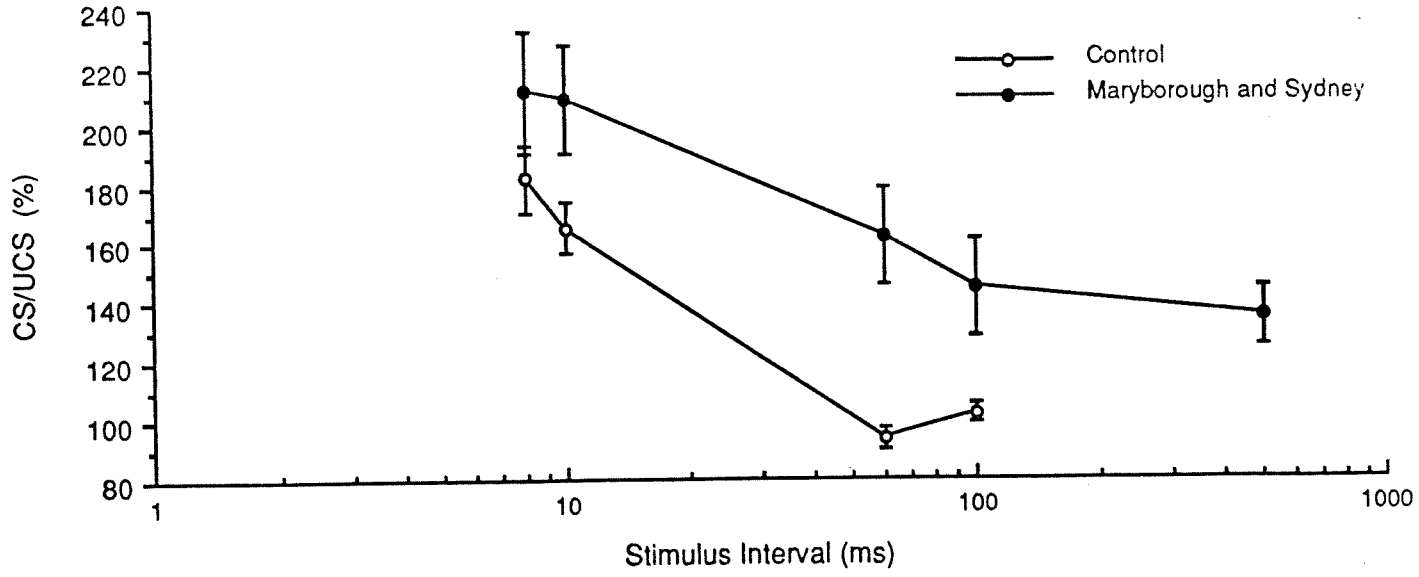
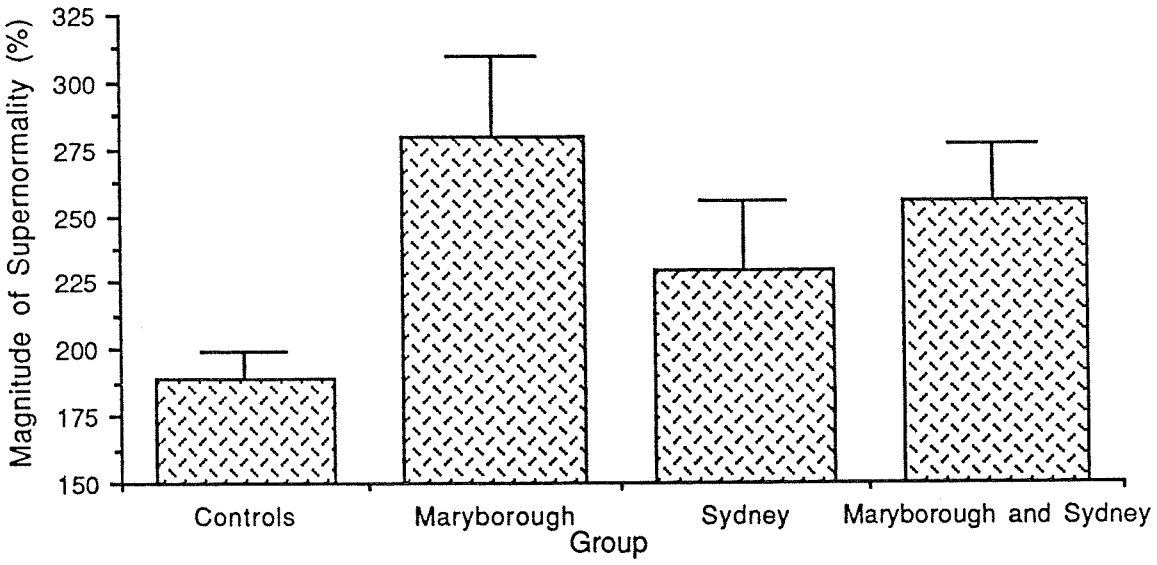

240
220
200
180
160
140
120
100
80
CS/UCS (%)
1
10
100
1000
Stimulus Interval (ms)
Control
Maryborough and Sydney

Figure 53. A comparison of the magnitude of supernormality in human control subjects (15), victims of ciguatera poisoning from Maryborough (8), victims of ciguatera poisoning from Sydney (7), and a combined group of Maryborough-Sydney victims (15). Standard errors of the means are shown.

Figure 54. A comparison of the duration of supernormality in human control subjects (15), victims of ciguatera poisoning from Maryborough (8), victims of ciguatera poisoning from Sydney (7) and a combined group of Maryborough-Sydney victims (15). Standard errors of the means are shown.


Magnitude of Supernormality (%)
325
300
275
250
225
200
175
150
Controls
Maryborough
Sydney
Maryborough and Sydney
Group

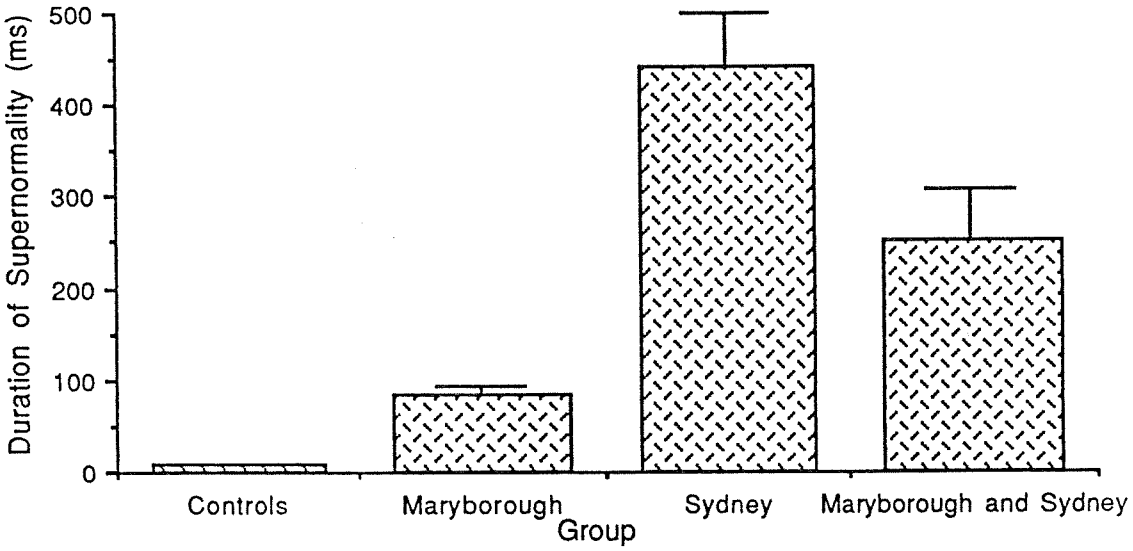
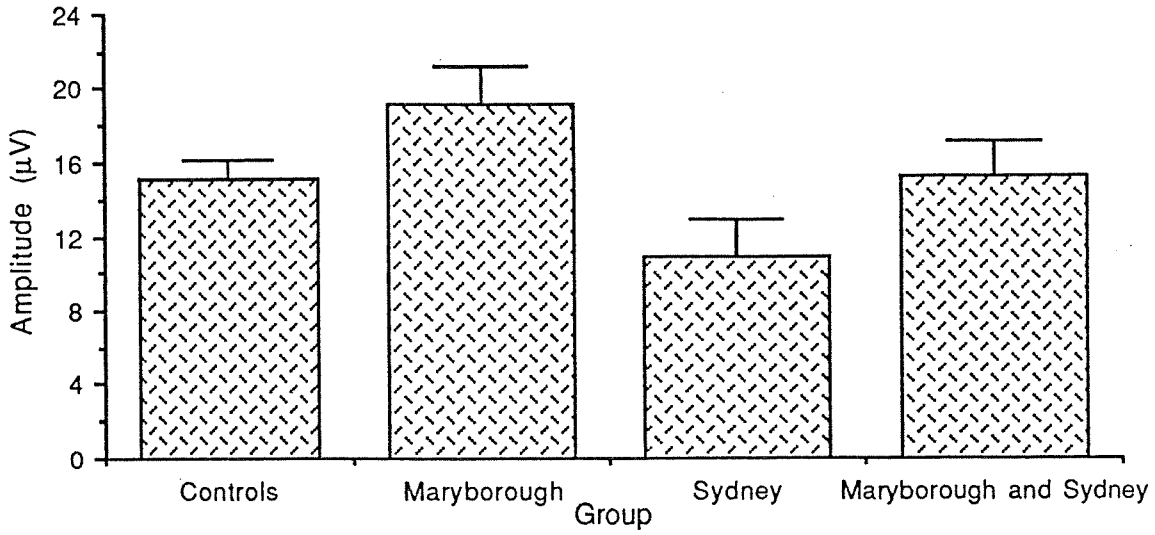
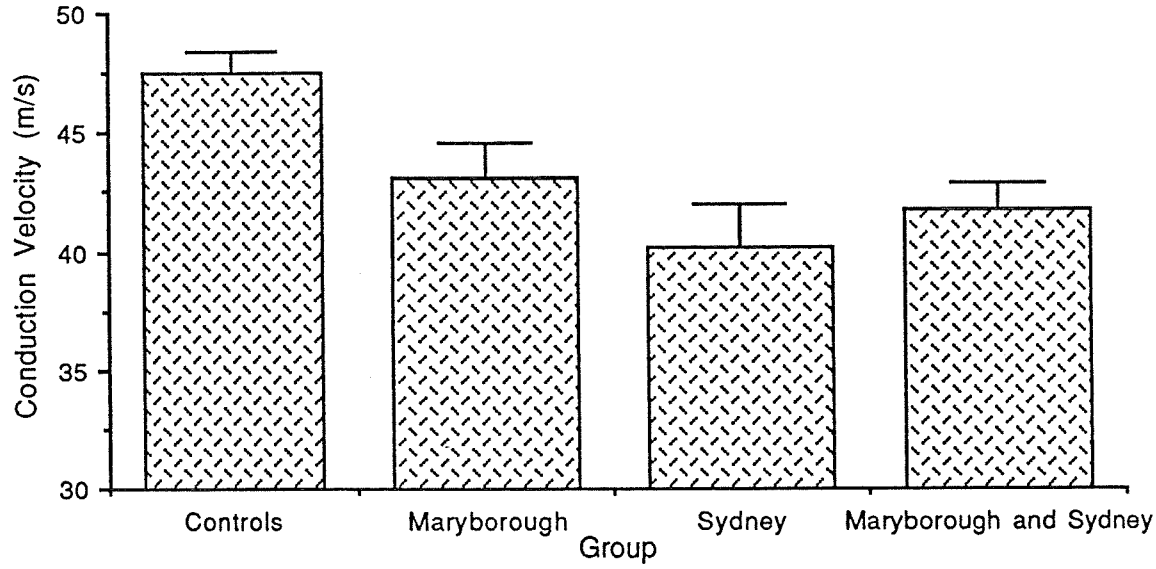

Duration of Supernormality (ms)
500
400
300
200
100
0
Controls
Maryborough
Sydney
Maryborough and Sydney
Group

Figure 55. A comparison of the sural nerve compound action potential amplitude in human control subjects (15), victims of ciguatera poisoning from Maryborough (8), victims of ciguatera poisoning from Sydney (7), and a combined group of Maryborough-Sydney victims (15). Standard errors of means are shown.

Figure 56. A comparison of the sural nerve conduction velocity in human control subjects (15), victims of ciguatera poisoning from Maryborough (8), victims of ciguatera poisoning from Sydney (7), and a combined group of Maryborough-Sydney victims (15). Standard errors of means are shown.

Figure 57. A comparison of the sural nerve latency in human control subjects (15), victims of ciguatera poisoning from Maryborough (8), victims of ciguatera poisoning from Sydney (7), and a combined group of Maryborough-Sydney victims (15). Standard errors of the means are shown.


Amplitude (μV)
24
20
16
12
8
4
0
Controls
Maryborough
Sydney
Maryborough and Sydney
Group


Conduction Velocity (m/s)
50
45
40
35
30
Controls
Maryborough
Sydney
Maryborough and Sydney
Group

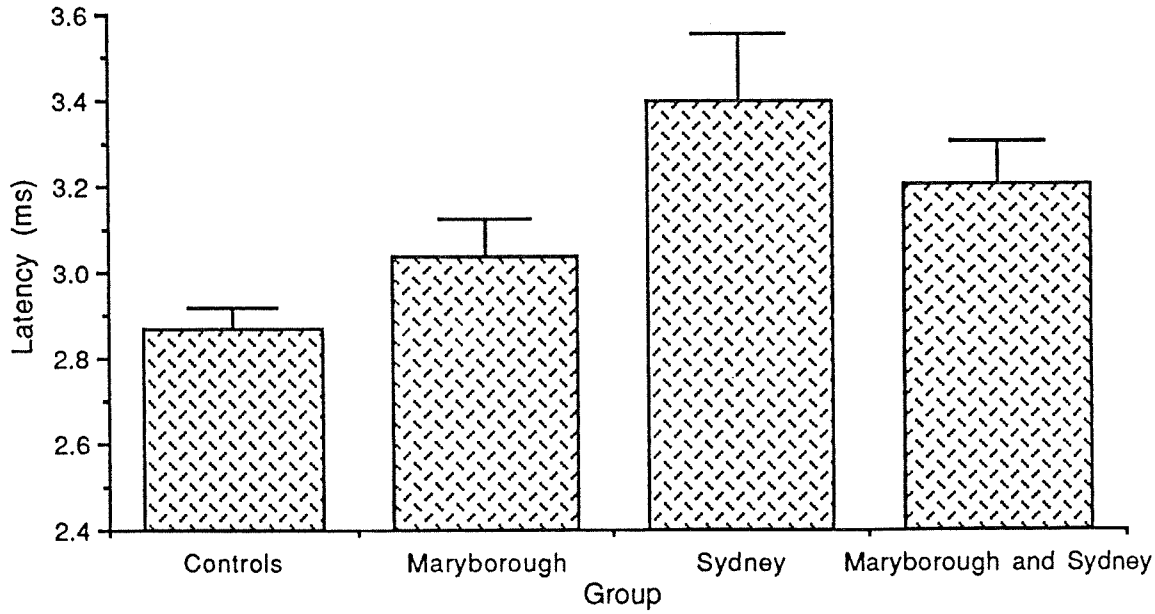
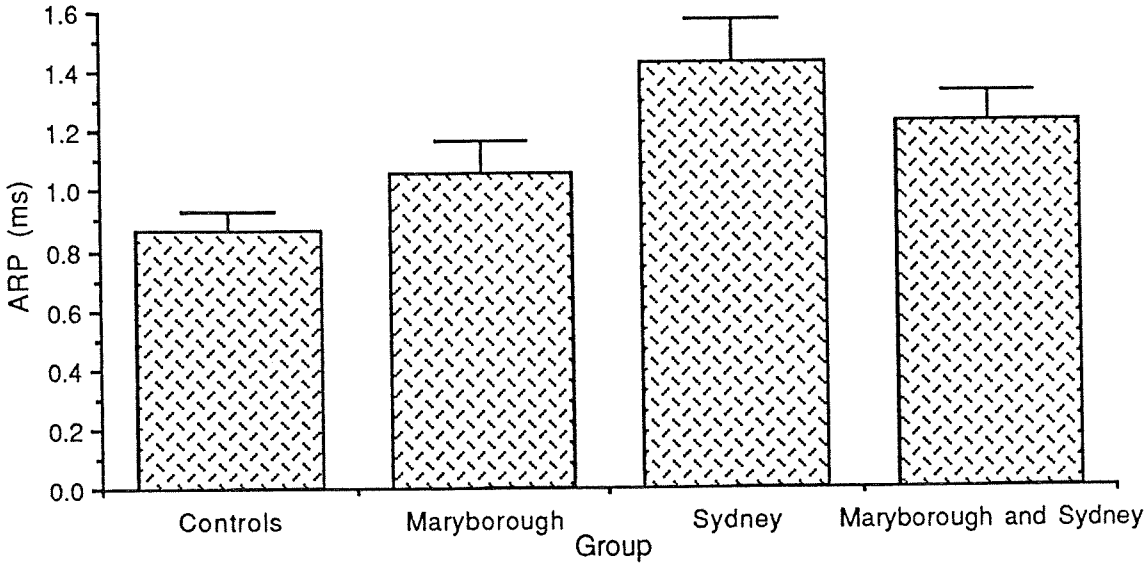
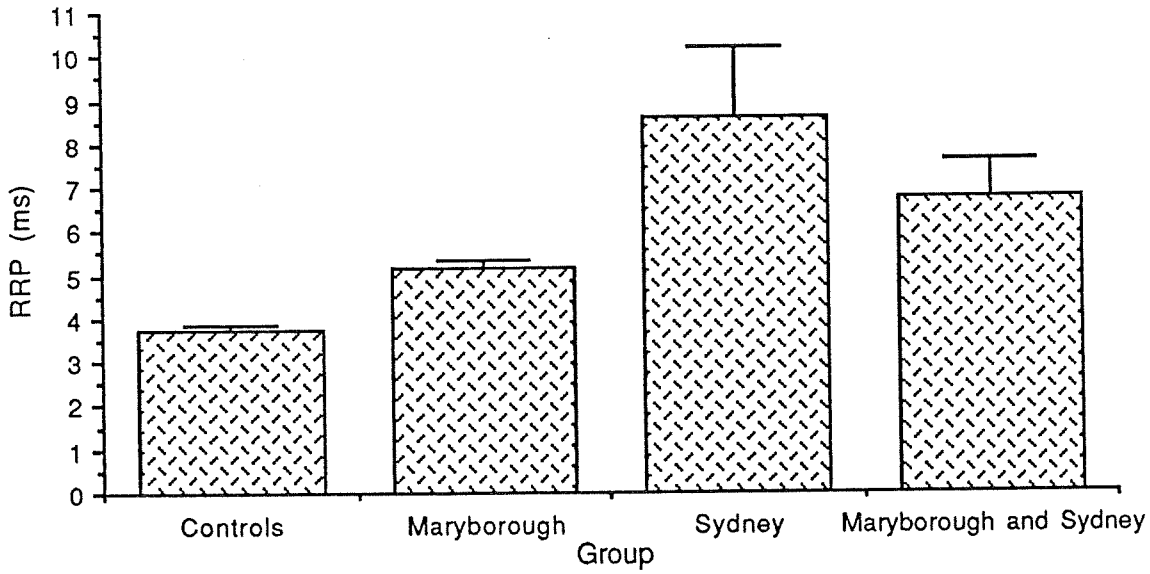

Latency (ms)
3.6
3.4
3.2
3.0
2.8
2.6
2.4
Controls
Maryborough
Sydney
Maryborough and Sydney
Group

Figure 58. A comparison of the sural nerve absolute refractory period in human control subjects (15), victims of ciguatera poisoning from Maryborough (8), victims of ciguatera poisoning from Sydney (7), and a combined group of Maryborough-Sydney victims (15). Standard errors of the means are shown.

Figure 59. A comparison of the sural nerve relative refractory period in human control subjects (15), victims of ciguatera poisoning from Maryborough (8), victims of ciguatera poisoning from Sydney (7), and a combined group of Maryborough-Sydney victims (15). Standard errors of the means are shown.

Figure 60. A comparison of the sural nerve absolute-relative refractory period in human control subjects (15), victims of ciguatera poisoning from Maryborough (8), victims of ciguatera poisoning from Sydney (7), and a combined group of Maryborough-Sydney victims (15). Standard errors of the means are shown.


ARP (ms)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Controls
Maryborough
Sydney
Maryborough and Sydney
Group


RRP (ms)
11
10
9
8
7
6
5
4
3
2
1
0
Controls
Maryborough
Sydney
Maryborough and Sydney
Group

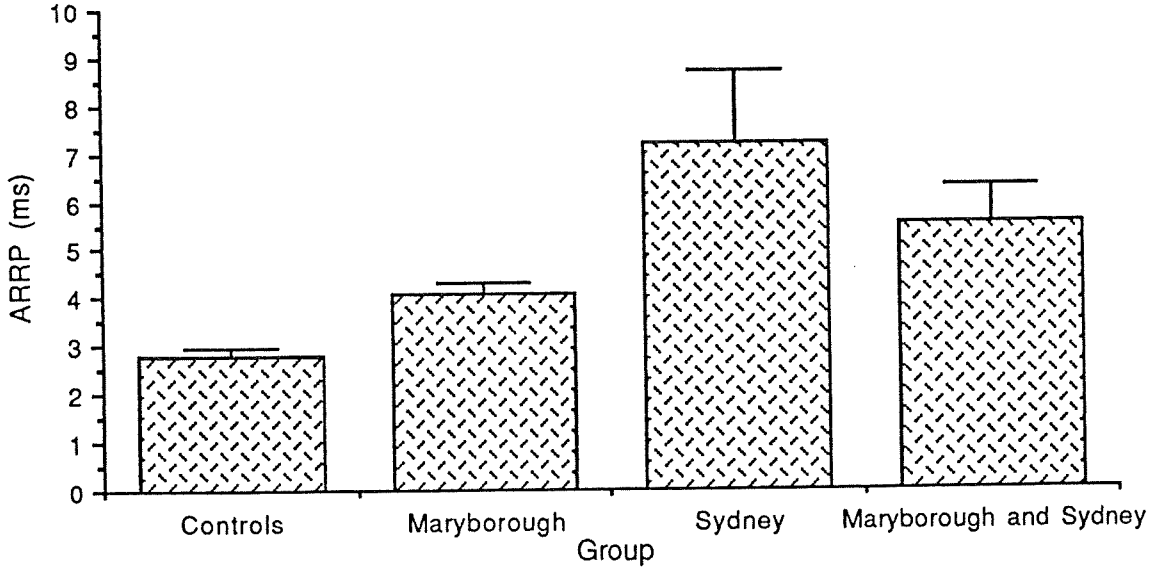
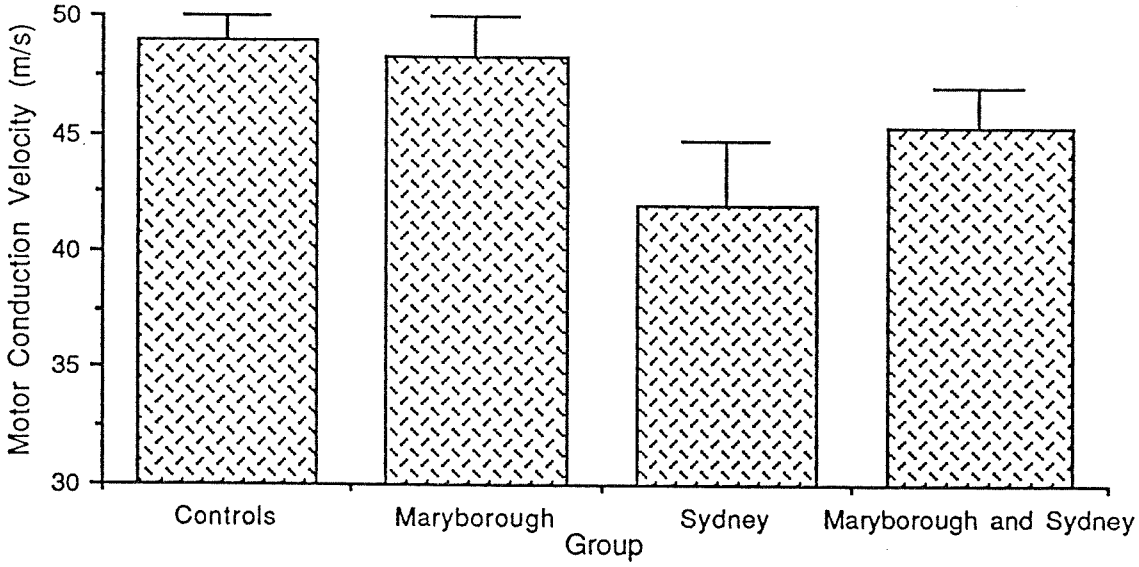
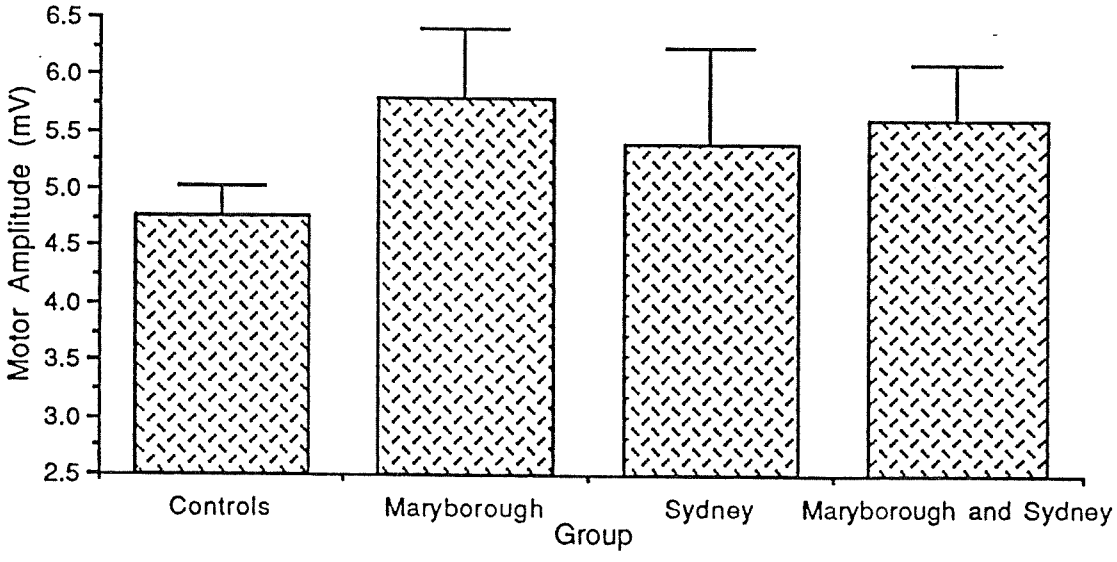

ARRP (ms)
10
9
8
7
6
5
4
3
2
1
0
Controls
Maryborough
Sydney
Maryborough and Sydney
Group

Figure 61. A comparison of the popliteal nerve conduction velocity in human control subjects (15), victims of ciguatera poisoning from Maryborough (8), and victims of ciguatera poisoning from Sydney (7), and a combined group of Maryborough-Sydney victims (15). Standard errors of means are shown.

Figure 62. A comparison of motor amplitude following popliteal nerve stimulation in human control subjects (15), victims of ciguatera poisoning in Maryborough (8), victims of ciguatera poisoning in Sydney (7), and a combined group of Maryborough-Sydney victims (15). Standard errors of means are shown.


Motor Conduction Velocity (m/s)
50
45
40
35
30
Controls
Maryborough
Sydney
Maryborough and Sydney
Group


Motor Amplitude (mV)
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
Controls
Maryborough
Sydney
Maryborough and Sydney
Group

Supernormal periods determined from the sural nerve of the Maryborough group (Fig. 50), the Sydney group (Fig. 51) and the combined Maryborough-Sydney groups (Fig. 52) differ significantly from control values. Both the magnitude (Fig. 53) and the amplitude (Fig. 54) of supernormality for the individual and combined groups of ciguatera poisoning victims differ significantly from the control values.

The amplitude of the sural nerve compound nerve action potential is elevated above control values in the Maryborough group while in the Sydney group the amplitude is diminished (Fig. 55). When the combined amplitude values for Maryborough and Sydney are compared to control values there is no significant difference (Fig. 55). The conduction velocity is significantly decreased in all victims of ciguatera poisoning (Fig. 56) in comparison to controls while the latency is significantly prolonged (Fig. 57).

The absolute (Fig. 58), relative (Fig. 59) and absolute-relative refractory (Fig. 60) periods of the sural nerve are all significantly prolonged in the victims of ciguatera poisoning.

Motor conduction velocities and amplitudes of muscle action potentials were recorded from the lateral popliteal nerve and the muscles it innervates. Motor conduction velocities in the Maryborough victims were not significantly different from control values while the conduction velocities of the Sydney and the combined Maryborough-Sydney groups were below control values (Fig. 61). Motor amplitudes were elevated above control values in the individual and combined groups of victims in comparison to controls.

(b) Symptomatological Studies

The type and intensity of acute symptoms in a sample of the Sydney ciguatera victims was submitted in June, 1987 to the committee and is appended to this report.
A follow up study was made of the Sydney victims six months after the ingestion of toxic fish. The frequency of duration of a variety of the symptoms suffered by the victims is given in Table 4.

Table 4

Type and duration of symptoms in forty of the sixty three victims of the 1987 ciguatera poisoning outbreak in Sydney.

Symptom	Percent with Symptom	Duration of Symptom (Days) Mean ± SE
Nausea	83	17 ± 7
Vomiting	50	11 ± 9
Abdominal pain	68	9 ± 2
Diarrhoea	78	5 ± 1
Headache	85	32 ± 9
Dizziness	65	24 ± 9
Memory disturbance	43	121 ± 18
Anxiety	60	60 ± 13
Depression	63	54 ± 11
Dental pain	48	18 ± 4
Joint pain	83	59 ± 10
Paraesthesia of hands	88	50 ± 8
Paraesthesia of lips	78	35 ± 8
Temperature perception reversals	80	45 ± 7
Muscle pain	93	40 ± 8
Weakness	83	49 ± 8
Difficulty walking	65	24 ± 7
Shaking	30	20 ± 7
Shortness of breath	38	16 ± 6
Sweating	48	12 ± 4
Watery eyes	33	15 ± 3
Salivation	15	12 ± 4
Itchy skin	88	36 ± 7
Skin rash	25	17 ± 3
Chills	65	9 ± 2
Neck stiffness	50	44 ± 14
Pain on urination	23	24 ± 9
Abnormal sensations	13	± 11
Hallucinations	13	46 ± 34
Visual defects	18	84 ± 34

Even after six months a number of people (Table 5) still suffered from one or more of the symptoms listed in Table 4. One woman who consumed 1kg of toxic fish in two 500g portions over a three day period still had eight of the symptoms and was debilitated to the extent in December, 1987 that she was unable to continue in employment.

Table 5

Symptoms persisting six months after ingestion of toxic Mackerel.

Number of persistent symptoms	Number of victims with symptoms
1	11
2	5
3	1
4	2
8	1

Discussion

The opportunity to assess the changes in nerve conduction parameters in the human victims of ciguatera poisoning has been of invaluable benefit to the programme. For the three year period before significant numbers of human intoxicated by CTX became available for study all nerve conduction studies were performed on an animal model, the laboratory rat. The results from the human experiments have shown that most of the parameters assessed in humans exposed to CTX are influenced in the same manner as that described in rats. In particular there are significant increases in both the magnitude and duration of the supernormal period in both humans and rats. Changes in other parameters are essentially similar to those described in the rat, exceptions occur in the motor amplitude and the absolute-relative refractory period. These may reflect differences between species or the variability of the human population as compared to genetically similar individuals in a strain of laboratory animals. The differences in some parameters between the species may of course be due to the different amounts and composition of toxins present in fish used for extraction and fish eaten by ciguatera victims.

The essential action of CTX is however believed to one in which the toxin acts on Na^+ channels to produce a prolonged opening time. Toxins that act in this manner on Na^+ channels are known to increase both magnitude and duration of the supernormal period. Such changes in supernormality occurred consistently in the rat and the human. The results obtained from humans have vindicated the extensive work done on the animal model and suggest that the rat ventral coccygeal nerve is an appropriate animal model for the testing of antagonists to CTX and hence the testing of therapeutic agents.

VIII GENERAL CONCLUSIONS

Considerable data on the pharmacological and pathological actions of CTX on both fish and mammalian tissues has been collected over the four year period of the Fishing Industry Research Grant. The most significant results have been summarised within this report however it is anticipated that eight to ten scientific papers will emanate from this study. To date four written research papers have been published , one in the Proceedings of the International Coral Reef Symposium of 1985 and three in the Proceedings of an Asian-Pacific Meeting of the International Society of Toxicologists in 1987. The considerable data accumulated is now being prepared for publication and copies of published work will be forwarded to the committee when available.

Aspects of all of the objectives detailed in Section II have been addressed and the most significant findings are summarised below.

(i) Electrophysiological studies especially the measurement of supernormality have shown that CTX acts on the Na^+ channel and/or Na^+ gating mechanisms in the nerves of fish, laboratory mammals and man.

(ii) Unique electrophysiological studies have been performed on a significant number of human victims of CTX.

(iii) The results of the human studies are essentially the same as those obtained from the animal model and thus vindicate the comprehensive study of the model and indicate the validity of using the model to assess the actions of potentially useful therapeutic agents.

(iv) Ultrastructural studies on mammalian nerves have shown that histopathological changes occur in the nerves of animals exposed to CTX.

(v) Studies on the actions of CTX on the mammalian gut have shown for the first time that this toxin can produce histopathological changes in mammalian tissue other than nervous tissue. Preliminary results that are now being pursued rigorously in a later FIRTA grant indicate that CTX also induces histopathological changes in fish tissues.

(vi) Fish are susceptible to CTX but at doses higher than those that cause death in mammals.

(vii) The Na^+ channels and/or Na^+ gates of both "carrier" and "non-carrier" fish are sensitive to CTX being opened by this toxin.

(viii) The sensitivity of fish nerves to CTX and the lack of overt pathology in toxic fish suggests that carrier fish have a partitioning or detoxification mechanism available to keep the toxin away from target sites. Any elucidation of such a mechanism may suggest new and effective therapies for human ciguatera poisoning.

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Interim Report on Clinical and Symptomatological studies performed on the Victims of Ciguatera Poisoning in Sydney on 26th and 27th May 1987.

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INTRODUCTION

In late March 1987, several cases of ciguatera poisoning were reported to the NSW Health Authorities. The offending fish in the March ciguatera poisoning episode appeared to come from a single vendor, the De Costi's at the Pyremont Fish Market. In early to mid May a much greater number of people again became ill after eating fish carrying ciguatoxin. The May outbreak was traced by the NSW Health Authorities to the De Costi outlet at Pyremont and to a fish shop at the Miranda Fair Shopping Centre on the Southern outskirts of Sydney. This shop was also supplied by De Costi's. Isolated cases were also reported from a restaurant in the Queanbeyan district of NSW. By mid June the NSW Health Department had investigated approximately sixty cases of ciguatera poisoning in the period beginning in late March. Due to the unique features of ciguatera poisoning it is possible that more cases remain unreported and undiagnosed. The cause of the poisoning was traced by the NSW Health Department to large spanish mackerel which had been caught in the Hervey Bay region of Queensland and apparently marketed through the Brisbane Fish Markets at Morningside before being offered for retail sale in Sydney. The number of fish involved is unknown however the outbreaks may have been due to just two fish one in March and one in May. There was a widespread news media coverage of the May outbreak in the metropolitan press of both Sydney and Brisbane and it was this coverage that initially alerted members of our research group in Brisbane to the cases of ciguatera poisoning in Sydney. John McMahon, the Deputy Chief Food Inspector in the NSW Department of Health was contacted and a request was made to see, it possible, some of the more severely affected cases still manifesting neurological symptoms and disability. Mr McMahon was very sympathetic to our requests and provided a list of some ten severe cases. He also provided our group with the names, addresses and telephone numbers of all recorded cases. The ten cases with moderately severe and persisting symptoms were contacted from Brisbane on 25/5/87 and seven agreed to undergo a comprehensive neurological examination and an electrophysiological investigation of peripheral nerve function in Sydney the following day. The patients were reviewed at the Royal Prince Alfred Hospital in the Department of Neurology and the studies were performed in one of the electrophysiological laboratories of the hospital that was kindly provided by Dr John Walsh, Staff Neurologist, a colleague of John Cameron.

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RESULTS

I. Clinical Studies at Royal Prince Alfred Hospital.

a) History

The seven patients interviewed presented with a classical history of ciguatera poisoning. All had eaten mackerel steaks and all had developed very severe diarrhoea and vomiting within a 4 hour period following ingestion. All cases had developed quite severe senory disturbance in the arms, legs and perioral region within 12 hours of eating the fish. All had complained of temperature reversal manifestations, myalgia and two had complained of central disturbance as evident by disturbed sleep patterns, confusion, depression and hallucinations. Two patients, both women, were still very severely affected to such an extent they remained virtually bedridden despite some 7 days since the onset of their symptoms.

b) Examination

All patients examined demonstrated impaired vibration appreciation about the feet and hands. In three of these cases this was quite prominent. Light touch and pain appreciation was also affected but to a less severe degree in all cases. Reflexed were preserved although the ankle jerks in two cases were somewhat depressed. No weakness was detectable and otherwise neurological examination in each case was normal.

c) Electrophysiological Studies

Sensory action potentials were recorded from the sural nerve of the leg. The nerve was stimulated using surface electrodes and the elicited potentials were also recorded from surface electrodes using a Medelec MS 92a, Electromyography Unit. There was a slight but significant delay in the latency period (3.4±0.3ms) compared to the control range (2.3±0.4ms). The absolute refractory period was also significantly increased from the control value of 0.5 to 1.0ms to 1.4±0.39ms. The relative refractory period (8.6±4.0) in the affected people was also significantly increased from control values (1.5 to 3.0ms). All cases demonstrated significantly elevated and prolonged supernormal periods. A

period of increased excitability, the supernormal period occurs in all nerves for a short period after they have conducted an impulse. The magnitude of the supernormal response was elevated above control values in all seven subjects. Supernormality was evident at 8 ms, 60 ms, 100 ms and 500 ms in all seven subjects whereas supernormality was not evident in controls after 60 ms.

II. Symptomatological Studies

In addition to the seven patients seen at the Royal Prince Alfred Hospital, thirty victims of the outbreaks were interviewed in their homes. In all, thirty seven victims of the March and May outbreaks of ciguatera poisoning were asked a series of questions related to the type and severity of manifested symptom. The results of this questionairre are presented as a series of histograms at the back of this report.

DISCUSSION

Large outbreaks of ciguatera poisoning are rare and infrequent in the Australian Community. It is more usual for isolated cases to be reported.

It would appear from our study and the information supplied to us from the NSW Health Department that the recent outbreaks in Sydney were due to very toxin fish. Several patients were hospitalised and many more experienced moderately severe symptoms. The symptomatological data presented in the histograms (Fig 1) shows that the majority of patients had gastrointestinal dysfunction with many having severe symptoms. Nearly 50% of the poisoned patients reported severe diarrhoea. Most patients also reported a variety of neurological symptoms with many of these being severe e.g. muscle pain, Joint pain, numbness and tingling of hands and itch. Over the past four years we have become increasingly aware of a number of long term effects of ciguatera poisoning including persistent peripheral neurological dysfucntion and severe central symptoms including depression, anxiety and memory disturbances. It is our intention to follow up this Sydney group in six months time so as to gain some estimate of the frequency and type of long term effects of ciguatoxin.

This outbreak in Sydney has provided the much needed clinical material to correlate with our laboratory work and to increase our understanding of the mechanisms of action of ciguatoxin. Electrophysiological studies on rats (Capra and Cameron, 1985) demonstrated significant changes in the refractory periods of peripheral nerves. The present study has confirmed that similar changes occur in human nerves. Our rat studies also demonstrated that ciguatoxin has profound effects on the supernormal period of peripheral nerves (Fig 2). In rats, ciguatoxin increases both the amplitude and duration of the supernormal period. These effects on supernormality are indicative of compounds that increase the Na^+ flux across excitable membranes (Parkin and Le Quesne, 1982). These studies on humans in Sydney have validated our previous studies on the rat and mouse animal models and confirm the value of using animal models to study the actions of ciguatoxin on mammalian nerves. It is now our intention to continue studies on the animal preparations we have developed and use such preparation to examine antagonistic agents for the treatment of human ciguatera poisoning. Prior to our study in Sydney there has only been one study of the effects of ciguatera poisoning on the electrophysiology of human nerves and in that study only basic electrophysiological data were recorded from one patient (Allsop et al, 1986).

Our brief visit to Sydney allowed us to gain valuable information on the symptomatology and electrophysiological manifestations of ciguatera poisoning. Episodes such as the one in Sydney attract a large media coverage and no doubt have serious effects on the fishing industry. One Sydney newspaper carried a full front page headline on "Poisonous fish" on sale in Sydney. We were recently in contact (15/6/87) with the NSW Fish Marketing Authority and were told that prices were depressed from 20 to 30% since the adverse publicity in May. Many of the people interviewed by us who were quite seriously effected had a strong determination to begin legal proceedings against the vendor, the supplier and the initial source. It would seem that the publicity relating to the Sydney outbreak of ciguatera poisoning has not yet been laid to rest.

ACKNOWLEDGEMENTS

Our thanks go to the NSW Health Authorities for their assistance and for the provision of much useful information. In particular we were most appreciative of extent of assistance given by Mr John McMahon the Deputy Chief Food Inspector.

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Thanks are also given to his superior Mr Des Sibraa, the Chief Food Inspector and to Dr David Fox, the Director of the NSW Institute of Public Health and Biosciences. Our Thanks are also given to the Fishing Industry Research Committee without whose assistance this study would not have been possible.

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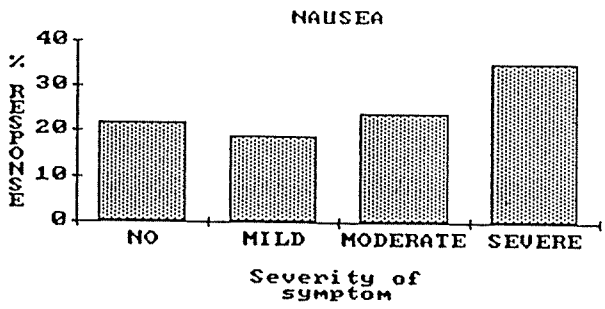
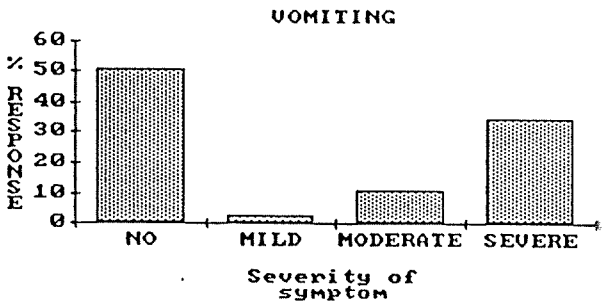
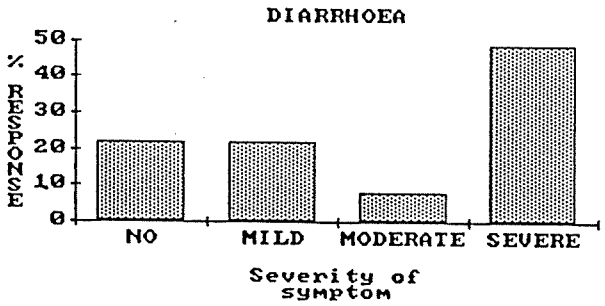
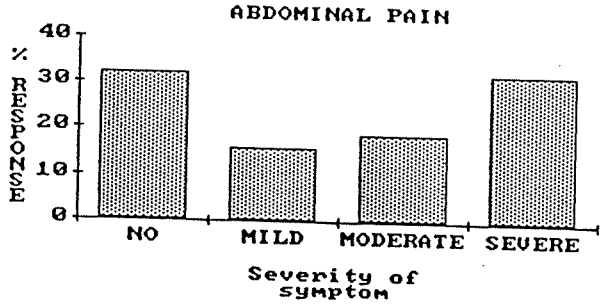
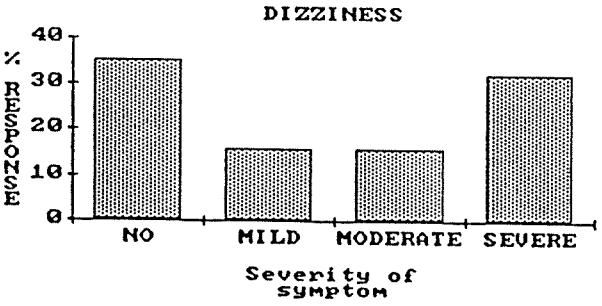
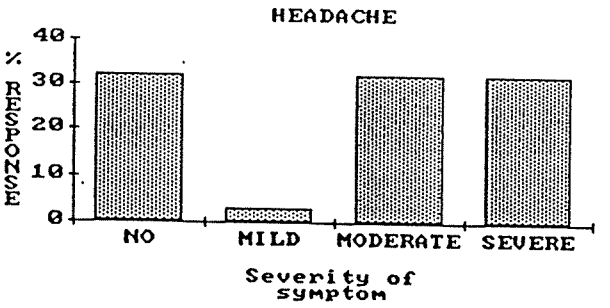


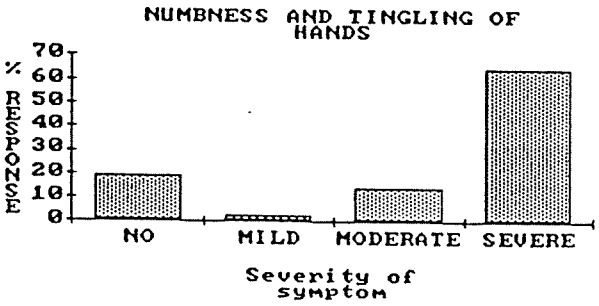












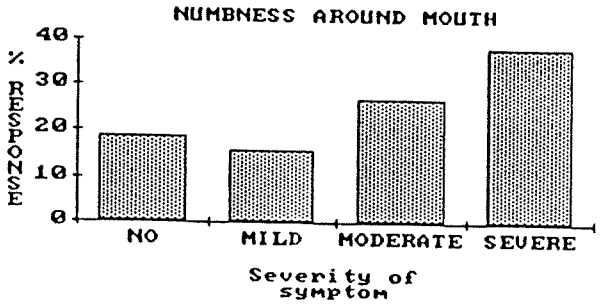
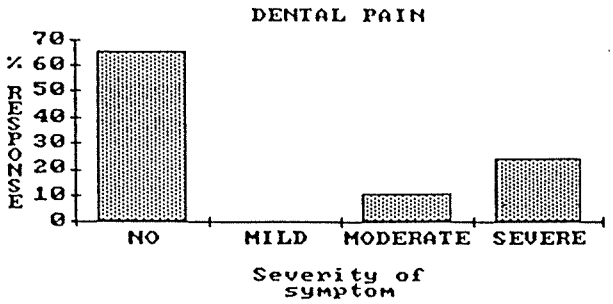
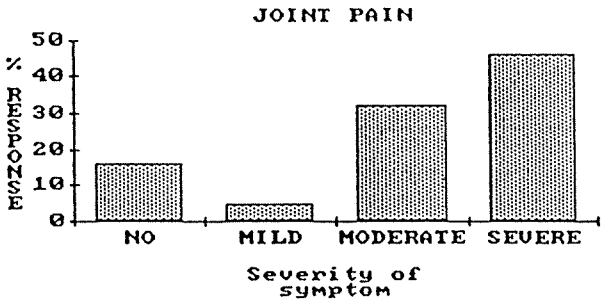
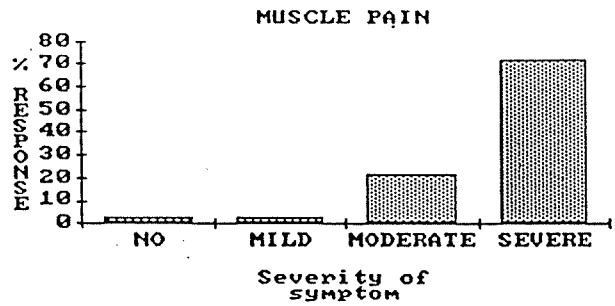


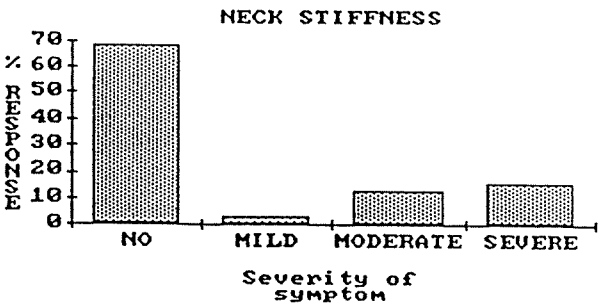
FIGURE 1

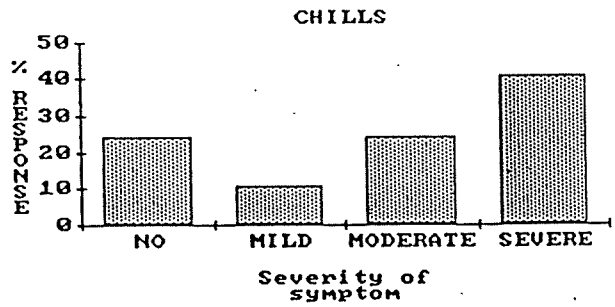
Symptomatology of 1987 Sydney Ciguatera outbreak.

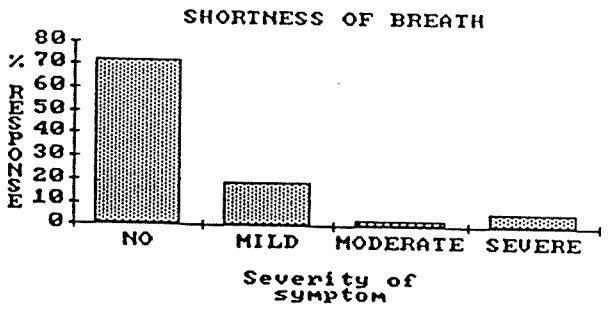


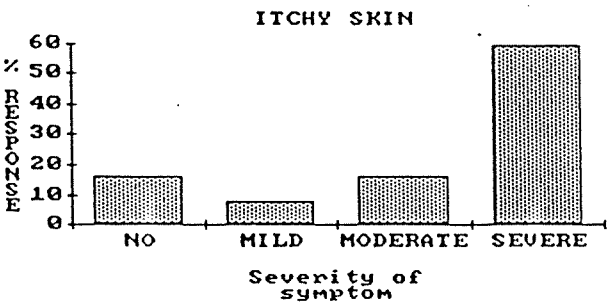












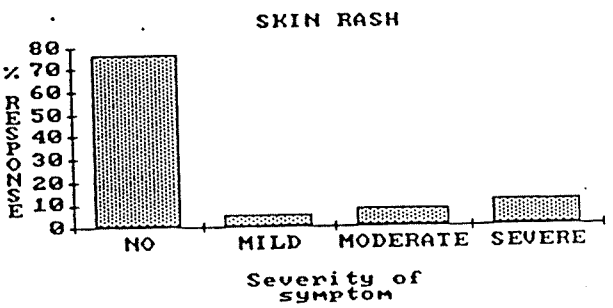
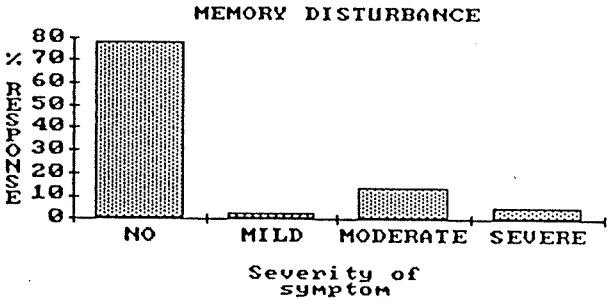
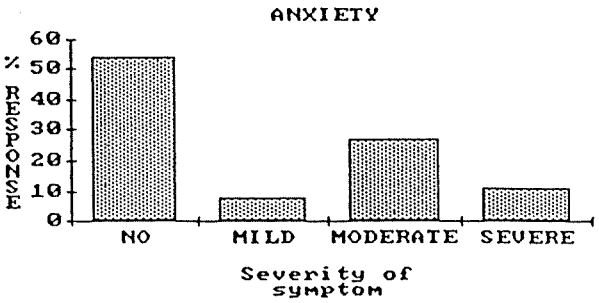
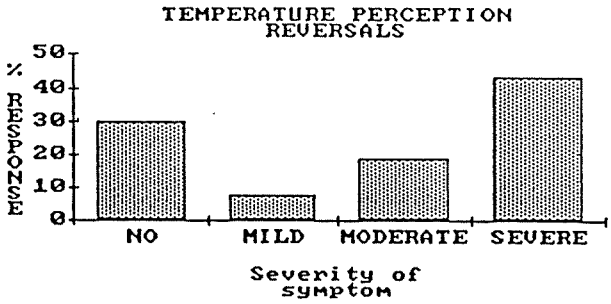
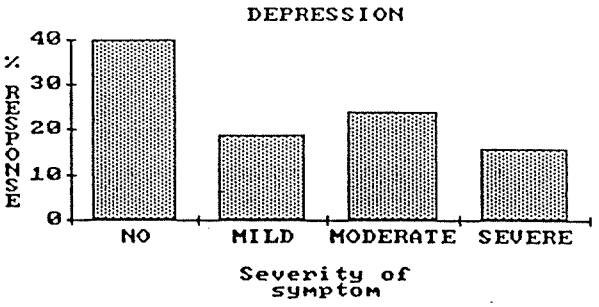


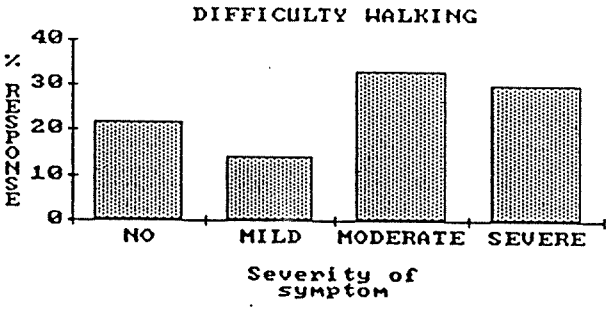
FIGURE 1 (CONTINUED)

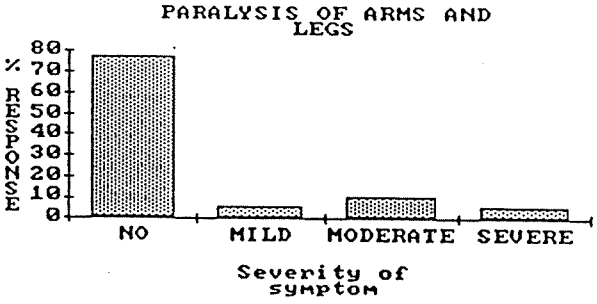

MEMORY DISTURBANCE
% RESPONSE
0
10
20
30
40
50
60
70
80
NO
MILD
MODERATE
SEVERE
Severity of symptom

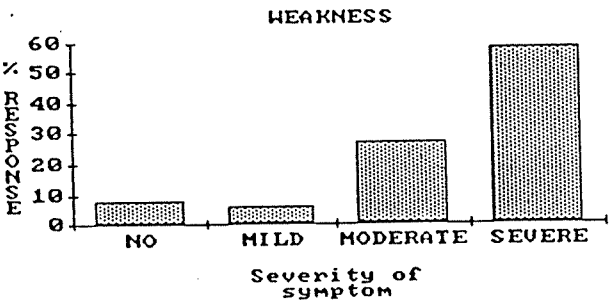

ANXIETY
% RESPONSE
0
10
20
30
40
50
60
NO
MILD
MODERATE
SEVERE
Severity of symptom


TEMPERATURE PERCEPTION REVERSALS
% RESPONSE
0
10
20
30
40
50
NO
MILD
MODERATE
SEVERE
Severity of symptom


DEPRESSION
% RESPONSE
0
10
20
30
40
NO
MILD
MODERATE
SEVERE
Severity of symptom


DIFFICULTY WALKING
% RESPONSE
0
10
20
30
40
NO
MILD
MODERATE
SEVERE
Severity of symptom


PARALYSIS OF ARMS AND LEGS
% RESPONSE
0
10
20
30
40
50
60
70
80
NO
MILD
MODERATE
SEVERE
Severity of symptom


WEAKNESS
% RESPONSE
0
10
20
30
40
50
60
NO
MILD
MODERATE
SEVERE
Severity of symptom

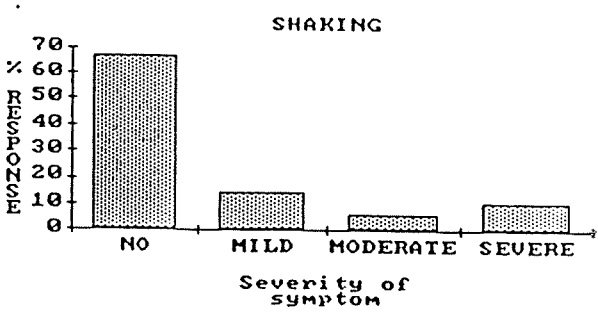

SHAKING
% RESPONSE
0
10
20
30
40
50
60
70
NO
MILD
MODERATE
SEVERE
Severity of symptom

FIGURE 1

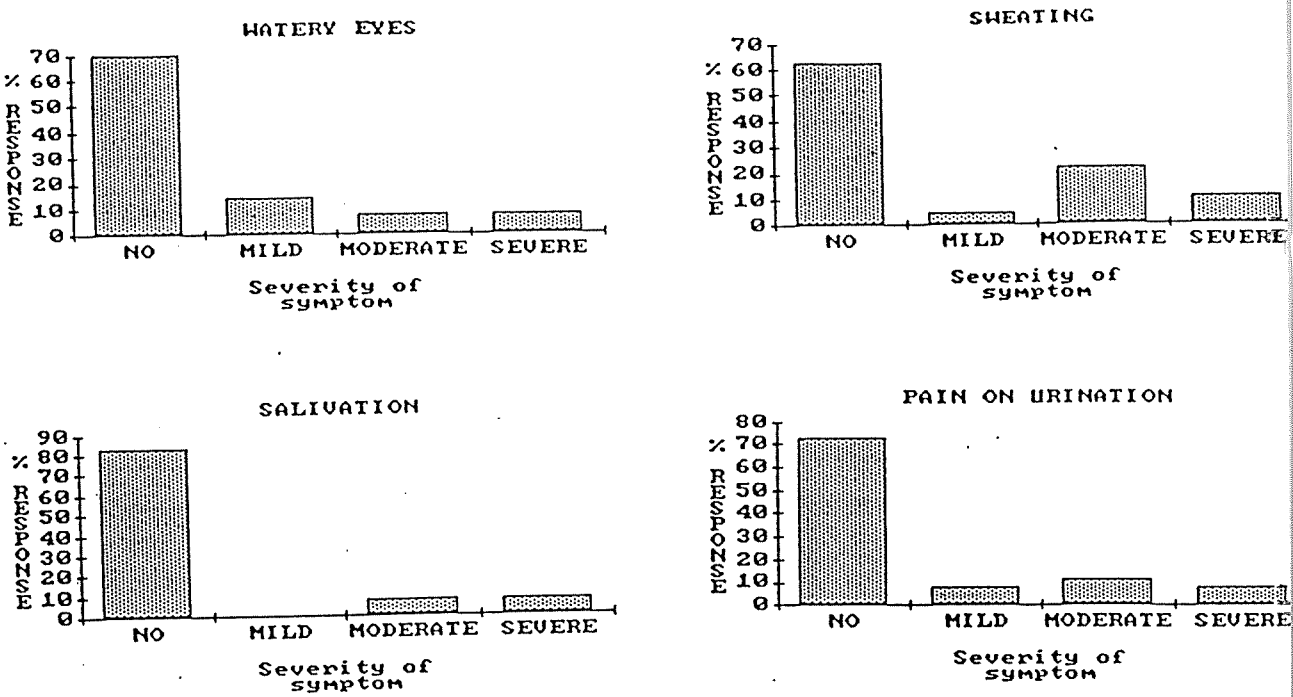


FIGURE 1

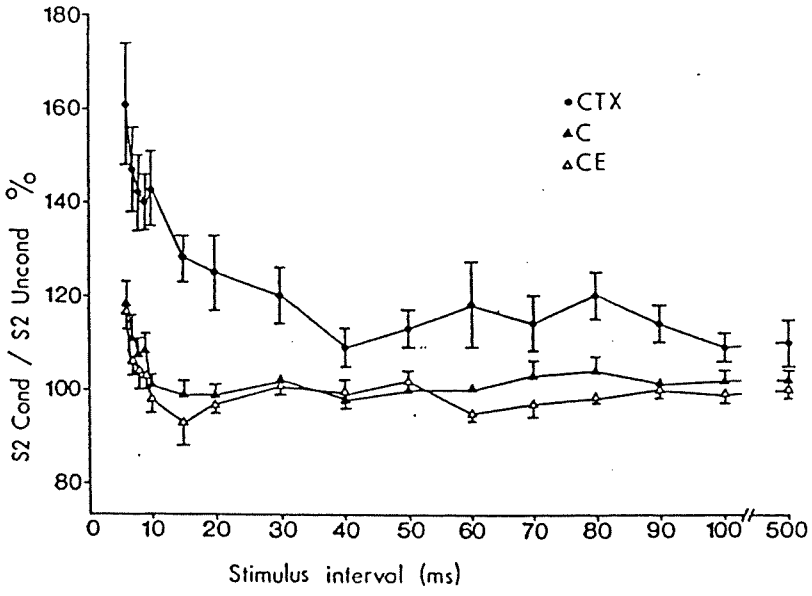


FIGURE 2

Elevation and prolongation (upper trace) of the supernormal period of a peripheral nerve in rats exposed to sub-lethal doses of ciguatoxin. Lower traces show results from control animals (Capra and Cameron 1985).