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Ciguatera research at the Queensland University of Technology

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An active ciguatera research group has existed at the Queensland University of Technology (QUT) since 1985. This group has worked on various aspects of ciguatera research including the effects of ciguatoxin on vertebrate nerves, the symptomatology of ciguatoxin in humans and the response of fish to ciguatoxin. The group has been led by myself (M. Capra) and a medical colleague (J. Cameron) from the Princess Alexandra Hospital in Brisbane.

Since 1985 three students have completed and been awarded higher degrees for work on ciguatera research (A. Flowers and C. Blanton - Masters degrees: S. Hahn - Ph.D. degree). Currently another student (C. Purcell) is completing a Ph.D. research program. Aspects of our work on ciguatera at QUT are briefly reviewed below.

The effects of ciguatoxin on nerves

Although there have been many clinical reports describing the neurological signs and symptoms of ciguatera, very little has been documented as to the electrophysiological disturbance ciguatoxin causes in the peripheral nervous symptom.

The initial electrophysiological studies undertaken at QUT were on nerves in anaesthetised rats. The nerve chosen for study was the ventral coccygeal nerve of the rat tail. This nerve was electrically stimulated by subcutaneous needle electrodes and the elicited compound nerve action potentials were recorded by a second set of subcutaneous needle electrodes placed proximally to the stimulating electrodes. This rat tail preparation has been used to gain some insight into the mode of action of ciguatoxin on peripheral nerves.

A number of nerve conduction parameters were measured, the most useful of which were nerve conduction velocity, the duration of the refractory periods and the magnitude and duration of the supernormal period.

The refractory periods and the supernormal period give some indication of fundamental ionic and molecular processes that occur during nervous transmission. When a nerve carries an impulse there is a brief period after that impulse in which the nerve is refractory (0.5 - 4 msec). In the first part of this period (absolute refractory period) the nerve cannot carry a second impulse while in the latter part of the period (relative refractory period) a greater stimulus will elicit a second impulse.

The refractory period is related to the physiological processes controlling the movement of sodium ions (Na^+) across the nerve membrane. The regulated movement of Na^+ is the basis of normal nerve function. After the refractory periods, the supernormal period (6 - 30 msec), occurs in which

the nerve becomes more excitable and can be more easily stimulated to carry another impulse. During the supernormal period the movement of Na^+ through the Na^+ channels of the nerve membrane becomes easier.

In our studies of rat nerves it has been found that when rats are given a sublethal dose of ciguatoxin the conduction velocity is decreased, the refractory period is extended and the magnitude (Figure 1) and duration of the supernormal period are both increased. These studies on alterations in nerve conduction parameters, especially the changes in the supernormal period, indicate that ciguatoxin is acting on the Na^+ channels in the peripheral nerves and producing an increase in the ease of Na^+channel opening or in the time course of Na^+ channel opening.

In 1987 two major outbreaks of ciguatera poisoning occurred in Australia – one in Sydney, New South Wales and the other in Maryborough, Queensland. Conduction velocity, refractory period and supernormal period (Figure 1) studies were performed on the Sural nerve of 15 of these victims who were showing acute signs and symptoms of ciguatera poisoning. Control studies were performed on 15 age-matched non-poisoned individuals with no obvious neurological dysfunction. In the 15 people who had ingested toxic fish there was a significant decrease in conduction velocity, increase in the refractory period and increase in the magnitude and duration of the supernormal period. The nerves of the human

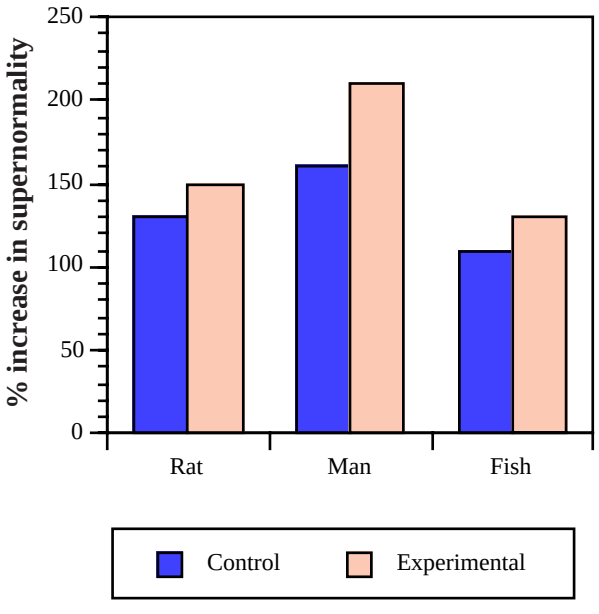

Figure 1: The effects of ciguatoxin on the magnitude of the supernormal period at an interstimulus separation of 10 ms in laboratory rats, humans and coral trout.

victims of ciguatera poisoning were affected in an identical manner to the nerves of rats exposed to ciguatoxin under controlled conditions. Hence, the rat nerve preparation has become a very useful model to study potential therapies for ciguatera poisoning and is currently being used by our group to assess potential therapeutic benefits of mannitol on peripheral nerve conduction.

Concurrently with studies on rats and humans the QUT group has examined the effects of ciguatoxin on fish nerves. The fact that a fish can carry sufficient ciguatoxin to poison several humans yet show no overt signs of intoxication has always intrigued our group. The electrophysiological studies performed on fish nerves have shown that these nerves respond in the same way to ciguatoxin as the nerves of rats and humans. Coral trout, *Plextropomus* sp., nerves respond to ciguatoxin with a decrease in conduction velocity, an increase in the duration of the refractory and supernormal periods and an increase in magnitude of supernormality (Figure 1).

Studies on the flux of Na^+ across fish nerves have also indicated that ciguatoxin acts on fish nerves and increases the opening of Na^+ channels. The actions of ciguatoxin on fish nerves indicate that fish nerves are susceptible to this toxin and have led us to speculate that some protective partitioning mechanism must exist in fish so that exposure of targets within the nervous system is minimalised.

Symptomatology of ciguatera poisoning

Data have been collected on a number of ciguatera victims over the past eight years. A number of patients have had prolonged expression of signs and symptoms and these are currently being followed by our group. In 1987 Australia's largest single outbreak of ciguatera poisoning, in which 63 people were poisoned, occurred in Sydney. Sydney is well below the latitude at which ciguatoxic fish are captured and the offending fish was caught in a ciguatera-endemic region of Hervey Bay in Queensland.

The victims of the Sydney outbreak were followed for six months after ingestion of toxic fish and the mean duration of symptoms was determined (Table1). Even six months after the ingestion of toxic fish several victims suffered from one or more symptoms (Table2). One woman who consumed 1kg of the toxic fish in two 500 g portions over a three-day period had eight symptoms and was debilitated to the extent that she was unable to resume employment six months after her initial poisoning. Currently the victims of the Sydney outbreak are being contacted to document any persistent or recurrent symptoms.

Table 1: Type and duration of symptoms in 40 of the 63 victims of the ciguatera poisoning outbreak in 1987 in Sydney

Symptom	% with symptom	Duration of symptom (days mean ± SE)
Nausea	83	17±7
Vomiting	50	11±9
Abdominal pain	68	9±2
Diarrhea	78	5±1
Headache	85	32±9
Vertigo	65	24±9
Memory disturbance	43	121±18
Anxiety	60	60±13
Depression	63	54±11
Joint pain	83	59±10
Paresthesia, hands	88	50±8
Paresthesia, lips	78	35±8
Temperature perception reversals	80	45±7
Muscle pain	93	40±8
Loss of energy	83	49±8
Shortness of breath	38	16±6
Sweating	48	12±4
Salivation	15	12±4
Pruritus	88	36±7
Skin rash	25	17±3

Table 2: Persisting symptoms in the victims of the 1987 ciguatera outbreak in Sydney 6 months after ingestion of toxic fish

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

Response of fish to ciguatoxin

A series of toxicological experiments was undertaken with two species of small Pomacentrid fish, *Chromis nitida* and *Pomacentrus wardi.* These fish (1to8 g) were anaesthetised in MS222, then microinjected in the peritoneal cavity with varying doses of ciguatoxin. The fish were allowed to recover from anaesthesia and monitored for several days for signs of toxicity. Each species of fish was susceptible to ciguatoxin but at much higher doses than those for mammals. Interestingly, *C. nitida,* which is a planktivore, was significantly more susceptible to ciguatoxin than was *P. wardi,* a browser, that feeds in areas where *G. toxicus* is found.

The results of this study suggest that the feeding niche of tropical fish may influence their susceptibility to intoxication. Fish that naturally come into contact with *G. toxicus* may have evolved partitioning strategies to reduce the likelihood of ciguatoxin acting on binding sites in nerve fibres. Histopathological studies have also been performed on pomacentrids injected with ciguatoxin. Cytological changes have been observed in the gut, gills and livers of fish injected with ciguatoxin. Studies are continuing on the resistance of fish to ciguatoxin with particular emphasis on partitioning mechanisms.

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