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Dual action of a dinoflagellate-derived precursor of Pacific ciguatoxins (P-CTX-4B) on voltage-dependent K^+ and Na^+ channels of single myelinated axons

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ABSTRACT

The effects of Pacific ciguatoxin-4B (P-CTX-4B, also named gambiertoxin), extracted from toxic *Gambierdiscus* dinoflagellates, were assessed on nodal K^+ and Na^+ currents of frog myelinated axons, using a conventional voltage-clamp technique. P-CTX-4B decreased, within a few minutes, both K^+ and Na^+ currents in a dose-dependent manner, without inducing any marked change in current kinetics. The toxin was more effective in blocking K^+ than Na^+ channels. P-CTX-4B shifted the voltage-dependence of Na^+ conductance by about 14 mV towards more negative membrane potentials. This effect was reversed by increasing Ca^{2+} in the external solution. A negative shift of about 16 mV in the steady-state Na^+ inactivation-voltage curve was also observed in the presence of the toxin. Unmodified and P-CTX-4B-modified Na^+ currents were similarly affected by the local anaesthetic lidocaine. The decrease of the two currents by lidocaine was dependent on both the concentration and the membrane potential during pre-pulses. In conclusion, P-CTX-4B appears about four times more effective than P-CTX-1B to affect K^+ channels, whereas it is about 50 times less efficient to affect Na^+ channels of axonal membranes. These actions may be related to subtle differences between the two chemical structures of molecules.

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1. Introduction

Ciguatoxins are complex lipid-soluble, highly oxygenated cyclic polyether compounds (Fig. 1; Murata et al., 1990; Lewis et al., 1991), responsible for a distinctive and common form of human seafood poisoning known as ciguatera, acquired by eating certain contaminated species of tropical and subtropical coral reef fishes. The origin of ciguatera fish poisoning has been linked to the benthic dinoflagellate *Gambierdiscus toxicus*, believed to elaborate the toxins which are transmitted to fish through the marine food chain and ultimately to man (reviewed by Swift and Swift, 1993; Lehane and Lewis, 2000).

Pacific ciguatoxin-1B (P-CTX-1B), extracted from the moray eels *Gymnothorax javanicus* (Murata et al., 1990) and *Lycodontis javanicus* (Lewis et al., 1991), is considered as the principal ichthyotoxin involved in ciguatera fish poisoning. Pharmacological studies revealed that P-CTX-1B, in the nanomolar range, specifically activates voltage-dependent Na^+ channels in various preparations (reviewed by Molgó et al., 1992). In particular, the toxin has been reported to evoke spontaneous action potential firing in myelinated nerve fibres, due to the modification of a fraction of Na^+ channels. The biophysical properties of P-CTX-1B-modified Na^+ channels as well as some of their pharmacological properties have been previously investigated (Benoit et al., 1986, 1996; Benoit and Legrand, 1992; Mattei et al., 2008).

Chemical structural evidence suggests that P-CTX-1B results from the biotransformation (i.e. oxidative modification) of P-CTX-4B, the toxin produced by the dinoflagellate *Gambierdiscus toxicus* also named gambiertoxin

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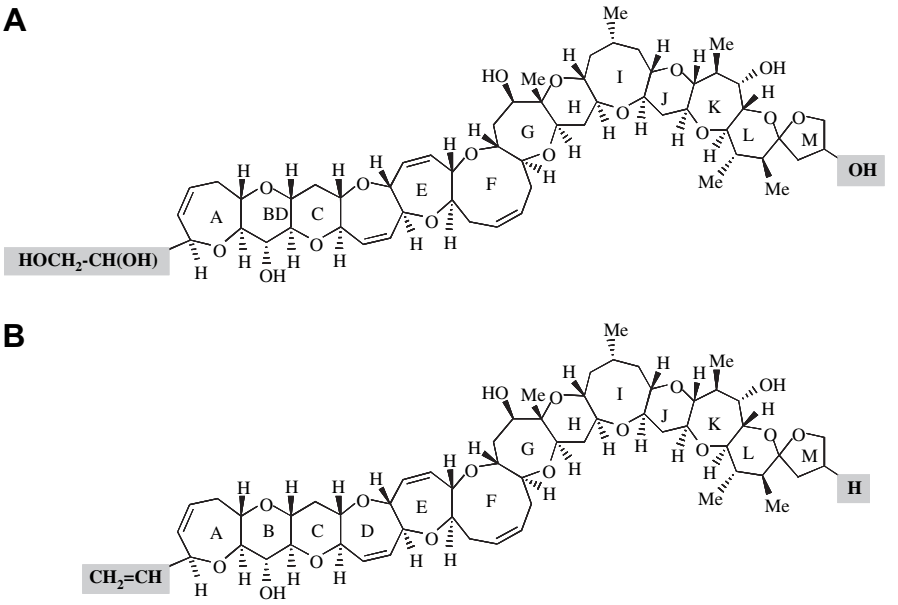

Fig. 1. Chemical structures of P-CTX-1B (A) and P-CTX-4B (B) purified from *Gymnothorax javanicus* and *Gambierdiscus toxicus*, respectively (adapted from Murata et al., 1990).

(see Fig. 1; Murata et al., 1990). Relatively little work has been done with P-CTX-4B mainly due to difficulties in obtaining consistent amounts of purified toxin either from wild or cultured ciguatoxic *G. toxicus* dinoflagellates. However, preliminary studies revealed that, in myelinated nerve fibres, P-CTX-4B induced spontaneous action potentials although it was about 50 fold less effective than P-CTX-1B (Benoit and Legrand, 1994). In addition, like P-CTX-1B, P-CTX-4B was reported to increase the relative volume of both nodes of Ranvier and nerve terminals (Benoit et al., 1996; Mattei et al., 1997). Therefore, P-CTX-4B may act as an agonist of Na^+ channels as does P-CTX-1B. However, in contrast to P-CTX-1B, P-CTX-4B was found to decrease the amplitude and to increase the duration of spontaneous action potentials, as compared to control conditions. These results suggest that P-CTX-4B affects both Na^+ and K^+ channels.

The aim of the present work was to characterize the effects of P-CTX-4B on ionic channels. In this study, the effects of the toxin were assessed on nodal Na^+ and K^+ currents of single frog myelinated nerve fibres using a conventional voltage-clamp technique. Some experiments were also performed with P-CTX-1B to further investigate its mode of action on the nodal K^+ current.

2. Materials and methods

2.1. Preparations

The experiments were carried out on nodes of Ranvier from single myelinated axons, isolated from the sciatic nerve removed from adult male frogs (*Rana esculenta*) weighing 20–25 g. All efforts were made to minimize the suffering of frogs (i.e. they were rapidly decapitated and demedullated), and a minimal number of animals was used. All animal experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.2. Electrophysiological recordings in single myelinated axons

Membrane currents of single myelinated nerve fibres were recorded under voltage-clamp conditions using the method of Nonner (1969). The node of Ranvier under investigation was stimulated at a frequency of 0.5–2.5 Hz. The normal resting potential of fibres was assumed to be −70 mV, corresponding to 30% inactivation of peak Na^+ current (Stämpfli and Hille, 1976). The membrane was maintained at a holding potential of −120 mV between pulses. Membrane currents were calculated assuming an axoplasmic resistance of 10 MΩ. Linear leakage and capacitative currents were subtracted electronically from the total current. The series resistance was not compensated (for details, see Benoit et al., 1985). Experiments were performed at 13–16 °C.

To describe the activation state of Na^+ channels, experimental values of conductance (g) were calculated using the following equation:

$$g = I/(V - V_{eq}) \quad (1)$$

where I is the current amplitude, V is the test voltage, and V_{eq} is the equilibrium potential of a given ion.

2.3. Solutions, drugs and toxins used

The standard physiological Ringer's solution had the following composition (in millimolar): NaCl, 111.5; KCl, 2.5; $CaCl_2$, 1.8; HEPES, 10 (pH 7.4). When recording K^+ current,

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