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Ciguatoxin-induced catecholamine secretion in bovine chromaffin cells: Mechanism of action and reversible inhibition by brevenal

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ABSTRACT

Ciguatoxin (P-CTX-1B) from the dinoflagellate *Gambierdiscus toxicus*, belongs to the family of polyether neurotoxins responsible for the neurological poisoning disorder ciguatera. Although it is the most widespread marine-borne disease affecting humans, there is no current FDA-approved treatment available except for symptomatic therapies. In this paper, we report that P-CTX-1B promotes catecholamine secretion from bovine chromaffin cells, an effect that is insensitive to concomitant activation of capacitative Ca^{2+} entry. Moreover, we confirm that brevenal, a polyether from the dinoflagellate *Karenia brevis*, blocks P-CTX-1B-induced catecholamine secretion. This effect is partially reversible. Our results therefore raise the prospect of finding functional antagonists for P-CTX-1B that could be useful for the treatment of ciguatera.

1. Introduction

Ciguatera is a marine-borne poisoning that occurs following the consumption of tropical and subtropical ciguateric fishes containing the neurotoxin ciguatoxin (Achaibar et al., 2007; Isbister and Kiernan, 2005). The disease's symptoms are characterised by an acute gastroenteritis (vomiting, diarrhoea, nausea and abdominal pain) followed by neurological disturbances, musculoskeletal symptoms, and paradoxical dysaesthesia (Friedman et al., 2008). Ciguatera is considered one of the most widespread seafood-related diseases affecting human with at least 50,000 cases annually (Lewis, 2001). Moreover, the concern related to ciguatera is growing due to the touristic nature of South Pacific Ocean, main endemic areas, and the globalisation of the fishing industry, which allow fish from endemic areas to be distributed around the world (Achaibar et al., 2007). Unfortunately, there is no currently approved treatment available except for symptomatic therapies (Achaibar et al., 2007; Pearn, 2001). One of the most commonly applied treatment is the intravenous injection of mannitol (Achaibar et al., 2007). However, a randomised trial failed to show beneficial effects (Schnorf et al., 2002).

Ciguatoxin (P-CTX-1B), and brevetoxin are lipid-soluble cyclic polyether compounds produced by the benthic dinoflagellates *Gambierdiscus toxicus* and *Karenia brevis*, respectively, that bind competitively to the site 5 of voltage-sensitive

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Na^+ channels (VSSC) (Dechraoui et al., 2006; Lombet et al., 1987; Poli et al., 1986). In both cases, the toxin's interaction with VSSC is believed to promote an increase in Na^+ permeability of excitable cells (Mattei et al., 1999). More specifically, P-CTX-1B was shown to shift VSSC activation to more negative membrane potentials, and to inhibit inactivation (Benoit et al., 1996). One important consequence of such an influx of Na^+, is the significant increase in asynchronous neurotransmitter release from presynaptic nerve terminals (Molgó et al., 1990). This is accompanied by impairments of synaptic vesicle recycling leading to a dramatic depletion of synaptic vesicles, believed to contribute to some of the symptoms associated with ciguatera (Molgó et al., 1990; Pearn, 2001). The precise mechanism by which Na^+ influx leads to an increase in neuroexocytosis remains unclear. One hypothesis put forward is that ciguatoxin promotes Ca^{2+} entry either through the reverse mode activation of the Na^+/Ca^{2+} exchanger (Gaudry-Talarmain et al., 1996; Hidalgo et al., 2002; Meunier et al., 1997; Molgó et al., 1993a,b) or via direct mobilisation of Ca^{2+} from internal stores, as shown in neuroblastoma cells and rat myotubes (Hidalgo et al., 2002; Molgó et al., 1993a). Such Ca^{2+} rise could in turn stimulate exocytosis (Mattei et al., 2008). The contribution of internal Ca^{2+} stores in P-CTX-1B stimulatory effect was also suggested by the use of caffeine pre-treatment and was found to reduce both Ca^{2+} release from the Ca^{2+} stores and subsequent stimulatory effect (Mattei et al., 2008). Interestingly, ciguatoxins have been described as potent K^+ channels inhibitors (Hidalgo et al., 2002). Brevetoxin (PbTx) has also recently been shown to allosterically modify TRPV1 channel (Cuypers et al., 2007), suggesting that ciguatoxins and brevetoxins not only act on voltage-gated Na^+ channels, but also on different molecular targets. This raises the possibility that ciguatoxins may interfere with the Ca^{2+} homeostasis through distinct mechanisms (Heiner et al., 2003).

In this paper, we show that P-CTX-1B stimulatory effect is not affected by thapsigargin-induced capacitative Ca^{2+} entry in bovine chromaffin cells, therefore suggesting that the effect of P-CTX-1B on internal Ca^{2+} stores is independent from capacitative Ca^{2+} entry. We further show that brevenal, a natural inhibitor of brevetoxin, reversibly blocks ciguatoxin-induced catecholamine release.

2. Materials and methods

2.1. Cell culture

Bovine chromaffin cells were used as primary culture. Cells were prepared from adrenal glands, obtained from Churchill Abattoir (Brisbane, Australia), as previously described (Meunier et al., 2002, 2005; Osborne et al., 2008). In brief, medulla of adrenal glands was extracted using protease (Sigma) and then chromaffin cells were obtained using collagenase (Sigma). Cell pellets were centrifuged and filtered several times, before being resuspended in DMEM and plated in 96-well plates.

2.2. Drugs

P-CTX-1B, brevenal and β-naphtoyl-brevetoxin were kept dry at −20 °C and diluted with 100% ethanol immediately before experiments. Thapsigargin was purchased from Sigma and dissolved in DMSO, aliquoted and stored at −20 °C until use.

2.3. Measurement of catecholamine secretion

The catecholamine secretion was quantified as previously described (Meunier et al., 2000). Briefly, chromaffin cells were washed once with Buffer A (145 mM NaCl, 5 mM KCl, 1.2 mM Na_2HPO_4, 10 mM glucose and 20 mM HEPES–NaOH, pH 7.4) and processed as stated in the figure legends. Aliquots of the supernatant were taken at the end of each experiment, and cells were lysed with Triton X-100 (1% v/v). Both set of samples were assayed fluorimetrically for catecholamine content. Amounts released are expressed as percentage of the total content of catecholamines present in the cells (Burgoyne, 1991). Plotted data are representative of experiments carried out in octoplicate and performed at least twice.

2.4. Statistical analysis

Data analysis was carried out using the Student's *t* test. All experiments were performed at least 3 times. Values are expressed as mean ± SEM, and data were considered significant at $^*p < 0.05$ or $^{**}p < 0.01$.

3. Results

We have identified key steps implicated in P-CTX-1B-induced catecholamine release from bovine chromaffin cells (Mattei et al., 2008). P-CTX-1B was shown to act on VSSC by promoting a slow albeit marked rise in intracellular Na^+. The resulting membrane depolarisation in turn elicits a tetrodotoxin (TTX)-sensitive increase of intracellular Ca^{2+} that originates from both external Ca^{2+} influx and Ca^{2+} release from internal stores. Such rise in intracellular Ca^{2+} in turn triggers a robust catecholamine secretion (Mattei et al., 2008).

3.1. Effect of capacitative Ca^{2+} entry on P-CTX-1B-induced catecholamine secretion

Because stored Ca^{2+} was shown to potentiate P-CTX-1B-induced catecholamine secretion (Mattei et al., 2008), we determined whether capacitative Ca^{2+} entry could contribute to P-CTX-1B-induced catecholamine release in chromaffin cells. Chromaffin cells were pre-treated with thapsigargin (2 μM) in the absence of external Ca^{2+}, and then stimulated in the presence or absence of P-CTX-1B (50 nM) and external Ca^{2+}.

Thapsigargin-induced capacitative Ca^{2+} entry promoted a limited albeit significant catecholamine secretion. In the presence of thapsigargin, P-CTX-1B evoked a marked catecholamine secretion (Fig. 1). However, this secretion was not significantly different from that promoted by P-CTX-1B alone (Fig. 1) suggesting that capacitative Ca^{2+} entry did not contribute to P-CTX-1B stimulatory effect. Importantly, this experiment shows that external Ca^{2+} is a major contributor to CTX-induced catecholamine secretion as previously reported (Mattei et al., 2008). This suggests that activation of voltage-activated Ca^{2+}