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In vivo effects of cnidarian toxins and venoms

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ABSTRACT

Cnidarians (Coelenterates), a very old and diverse animal phylum, possess a wide variety of biologically active substances that can be considered as toxins. Anthozoan toxins can be classified into two chemically very different groups, namely polypeptide toxins isolated from sea anemones and diterpenes isolated from octocorals. Cubozoan and scyphozoan protein toxins have been the most elusive cnidarian toxins to investigate – despite a tremendous effort in the past few decades, very few of these large, relatively unstable protein toxins were isolated, but recently this has been achieved for cubozoan venoms. Hydrozoans mainly contain large proteins with physiological mechanisms of action similar to the sea anemone and jellyfish pore-forming toxins. This article will focus on the *in vivo* physiological effects of cnidarian toxins and venoms; their actions at the cellular level will only be considered to understand their actions at the organ and whole animal levels. An understanding of mechanisms underlying the *in vivo* toxic effects will facilitate the development of more effective treatments of cnidarian envenomations.

1. Introduction

Searching the recent literature for the effects of cnidarian toxins *in vivo* and on isolated organs revealed a striking lack of data as compared to a large body of data from studies at molecular and cellular levels. Many of these studies focused on their neurotoxic effects on ion channels in excitable membranes, which have been extensively reviewed (Benoit, 1998; Diochot et al., 2007; Smith and Blumenthal, 2007; Wanke and Restano-Cassulini, 2007; several papers in this issue). Toxins, tissue extracts and venoms have also been investigated for their possible enzymatic activity. Phospholipases as constituents of cnidarian venoms have been recently described in detail (Nevalainen et al., 2004). Phospholipase A_2 (PLA_2) has been found in Anthozoa, Cubozoa, Hydrozoa and Scyphozoa, but in highest concentrations in the hydrozoan fire coral *Millepora* sp., the anthozoan stony coral *Pocillopora damicornis*, in the anthozoan sea anemone *Adamsia carciniopados* and in the tentacles of the cubozoan box jellyfish *Chironex fleckeri*. Some of the PLA_2s possess haemolytic and cytolytic activities (Nevalainen et al., 2004), but no study of the *in vivo* effects of these phospholipases in mammals has yet been published.

2. Anthozoa

2.1. Sea anemone toxins

Substances isolated from sea anemones have served as useful molecular models or probes in biomedical research and will continue to do so. Sea anemone and hydrozoan extracts were used by Portier and Richet to unravel some important and general aspects of the phenomenon of anaphylaxis (see initial article by the editors). The sodium channel modulating peptides were originally studied as putative cardiac inotropes and are currently used to investigate cardiac arrhythmias. Peptides blocking potassium and other ion channels are described by others in this issue.

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Another ubiquitous and well-studied class of sea anemone toxins are the cytolytic polypeptides (Reviews: Maček et al., 1994; Bernheimer, 1996). There are four known groups of cytolysins, based on their differing molecular properties and modes of action. The first group comprises small cysteine containing 5–8 kDa peptides that bind to phosphatidylcholine in cellular membranes and form pores; it also has been shown that they also can inhibit histamine-induced contractions of the guinea-pig ileum (Aldeen et al., 1981). The second group consists of 20 kDa proteins with isoelectric points above 9, and are referred to as actinoporins (Kem, 1988). Sphingomyelin is the membrane lipid acceptor for these cytolysins, which are stable proteins devoid of cysteine and form cation selective pores by oligomerization within the membrane. The third group comprises heterogeneous 28–45 kDa proteins which may or may not possess phospholipase A2 (PLA2) activity (Cline et al., 1995; Grotendorst and Hessinger, 2000). A member of this group, Up I, isolated from *Urticina piscivora*, was haemolytic and cytolytic to several tumour cells. Its cytolytic action can be inhibited by sphingomyelin (Cline et al., 1995; for a related Urticina cyltolysin, see Razpotnik et al., 2009). A cytolysin from *Metridium senile* is the only known member of the fourth group. It is a lethal 80 kDa protein whose haemolytic activity is inhibited by cholesterol (Bernheimer et al., 1979). Several recent reviews on cytolytic toxins from sea anemones have been published describing the structure (Anderluh and Macek, 2002; Kristan et al., 2009; Alvarez et al., 2009) and mechanisms of channel formation by actinoporins in lipid membranes (Alegre-Cebollada et al., 2007).

While most toxins produced by sea anemones are polypeptides, other toxic substances have also been found. Interestingly, palytoxin, first isolated from Zoanthids (Palythoa), has also been found in the sea anemone *Radianthus macrodactylus* (Mahnir et al., 1992). Palytoxin is one of the most potent known toxins, and its *in vivo* effects in several mammals, including man, include neurotoxicity, rhabdomyolysis, and cardiovascular collapse (Hoffmann et al., 2008). Another toxin, caissarone, is an iminopurine isolated and purified from the sea anemone *Bunodosoma caissarum*. It has been shown that the increased intestinal motility observed in experimental animals after administration of caissarone is due to its competitive antagonism at an adenosine receptor (Cooper et al., 1995). A crude extract from *B. caissarum* caused convulsions in mice. It seems that the convulsions are not caused by a putative presence of a cytolysin in the venom or by an increase in glutamate release. The experimental data suggest that a peptide toxin directly interacting with NMDA receptors may cause this effect (Gondran et al., 2002).

An assessment of the pharmacological actions of sea anemone venom or crude extracts *in vivo* and on isolated organs is missing for most of the described toxins. The fact that several types of toxins may coexist in the venom of a single sea anemone further complicates the study of their effects *in vivo* and *in vitro,* especially when taking into account that it is difficult to isolate undischarged nematocysts, the presumed source of toxins, in quantity sufficient to obtain enough pure venom in most species. Here, *in vivo* and isolated organs effects of purified sea anemone toxins on mammals are presented with emphasis on studies carried out during the last two decades.

2.1.1. *Cellular and* in vivo *effects of sea anemone neurotoxins*

Several sea anemones produce potent neurotoxins. The best known neurotoxins are the sodium channel toxins. All of these toxins prevent inactivation of sodium channels by stabilizing the open state conformations, and their structures (Norton et al., 2004; Norton, 2009) structure–activity properties (Moran et al., 2009) and actions on various mammalian sodium channels (Wanke et al., in press) are discussed in detail in other parts of this issue of Toxicon. Potassium channel blockers from sea anemones, especially the Kv 1(Shaker type channel) blockers and other toxins have become valuable experimental tools and their immunosupressive actions (Kalman, 1998; Pennington, 2009) may be the basis for their drug development. Reversible block of potassium current by a sea anemone toxin was first described for equinatoxin from *Actinia equina*; it reversibly decreased potassium current in single myelinated nerve fibres from frog (Šuput et al., 1986). Later it was shown that *A. equina* contains a smaller peptide, a ShK like channel blocker (Minagawa et al., 1998). Therefore, it is possible that the previously described effect of equinatoxin on the potassium current was due to the contamination of equinatoxin with this smaller peptide. So far several well-defined potassium channel toxins from a number of sea anemones have been isolated and well characterized, which is reviewed elsewhere in this volume (Castenada and Harvey, in press). An inhibitor of ether-a-go-go-related gene potassium channels APETx1, has also been isolated from *Anthopleura elegantissima* (Diochot et al., 2003; Wanke and Restano-Cassulini, 2007). APETx2 from the same sea anemone is a blocker of acid-sensing ion channels (Diochot et al., 2007), which are permeable to several cations.

Granulitoxin (GRX) is a lethal (LD_{50} 400 μg/kg) neurotoxic ≈5 kDa peptide isolated from *Bunodosoma granulifera*. Intraperitoneal (i.p.) injection of the toxin in mice caused circular movements, aggressive behaviour, convulsions and death (Santana et al., 1998). Intrahippocampal injection in rats of granulitoxin caused seizure-type brain activity that began in the hippocampus and spread rapidly to the occipital cortex, as shown by EEG measurements. During that period akinesia presented as the most prominent symptom, but then facial automatisms, head tremor, salivation, rearing, jumping, barrel-rolling, wet dog shakes and forelimb clonic movements were also observed. Those effects developed further into the status epilepticus, and eventually the experimental animals died (Santana et al., 2001).

2.1.2. *Sea anemone toxins acting on the cardiovascular system*

Many sea anemone toxins including cytolysins and neurotoxins have been investigated for their putative cardiotonic actions. A positive inotropic effect (increase in contractile force of myocardium) has been described for most purified sea anemone neurotoxins that have been tested. Unfortunately, this potentially useful effect is transient and is rapidly followed by cardiotoxic effects that