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Mini review

Angiotensin converting enzymes in fish venom

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ABSTRACT

Animal venoms are multifaceted mixtures, including proteins, peptides and enzymes produced by animals in defense, predation and digestion. These molecules have been investigated concerning their molecular mechanisms associated and possible pharmacological applications. *Thalassophryne nattereri* is a small venomous fish inhabiting the northern and northeastern coast of Brazil, and represents a relatively frequent cause of injuries. Its venom causes severe inflammatory response followed frequently by the necrosis of the affected area. *Scorpaena plumieri* is the most venomous fish in the Brazilian fauna and is responsible for relatively frequent accidents involving anglers and bathers. In humans, its venom causes edema, erythema, ecchymoses, nausea, vomiting, and syncope. Recently, the presence of a type of angiotensin converting enzyme (ACE) activity in the venom of *Thalassophryne nattereri* and *Scorpaena plumieri,* endemic fishes in northeastern coast of Brazil, has been described. The ACE converts angiotensin I (Ang I) into angiotensin II (Ang II) and inactivates bradykinin, there by regulating blood pressure and electrolyte homeostasis, however, their function in these venoms remains an unknown. This article provides an overview of the current knowledge on ACE in the venoms of *Thalassophryne nattereri* and *Scorpaena plumier.*

1. Introduction

Research on animal venoms has mainly sought to understand their deleterious effects in humans, with the aim of developing specific treatments. In addition, the venom of several animals consists of substances with therapeutic potential. These toxins have a great affinity for the physiological targets of preys/predators and are involved in immobilization, necrosis, or death. Among studies investigating animal venom, fish are the least explored because venomous terrestrial animals cohabit in our environment, leading to a larger number of interactions and accidents involving human beings (Church and Hodgson, 2002). Studies with toxins have demonstrated their large pharmacological diversity and activity; these molecules have been investigated regarding molecular mechanisms associated with their physiological actions and possible pharmacological applications (Seino et al., 1988; Olivera, 2002; Terlau and Olivera, 2004; Nakamura et al., 2016).

Scorpaena plumieri, known as scorpionfish, is the most venomous fish in the Brazilian fauna (Haddad et al., 2003). Its inoculating apparatus is constituted of venom glands extended with sheaths in the anterior portion of the dorsal, pelvic, and anal fins, without excretory duct or well-developed paired longitudinal glands, with extensions similar to ducts, as observed in stonefish (Haddad et al., 2003). Mice envenomed by *S. plumieri* present either local effects, such as nociception, edema, and erythema, following the pattern of a supported inflammatory response (Menezes et al., 2012), or systemic effects, such as cardiovascular symptoms (Gomes et al., 2010) and acute lung injury (Boletini-Santos et al., 2008). A collagenase with gelatinolytic activity has been isolated from the *S. plumieri* venom, with an estimated mass of 80 kDa in the SDS-PAGE, in reducing conditions, and 72 kDa, in non-reducing conditions (Carrijo et al., 2005). A cytolysin named Sp-CTx was also isolated, with mass estimated through exclusion chromatography in 121 kDa, being a dimer of glycoprotein character. This toxin causes a biphasic response in aortic annuli of phenylephrine precontracted in rats, characterized by an initial endothelium dependent relaxation response followed by a contraction (Andrich et al., 2010). It was subsequently demonstrated that this toxin has no phospholipase A-2 activity (Gomes et al., 2013). A type-C isolectins, from the hemagglutinating fraction of S. *plumieri* venom, have also been isolated, with five fractions (Sp-CL 1e5) with similar

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chromatographic profiles of elution, suggesting that they are isoforms of a single protein (Andrich et al., 2015).

Thalassophryne nattereri belongs to the family *Batrachoididae*; fish of this family are named toadfish for their appearance. Also known as *niquim* on the Brazilian coast, this fish is a venomous animal living in lagoon or estuarine areas. *Thalassophryne nattereri* is small (12–15 cm) and has big, bulging eyes, a large head, and a wide mouth. It inhabits shallow waters and stands still most of the time. This fish possesses an inoculating hollow thorn of venom in the dorsal region; usually accidents occur when fishermen or unadvised bathers step on the fish and try to grab them using their hands. It is a carnivorous fish that consumes particularly crabs, mollusks, and small fish; it lives in groups and is extremely resistant, being able to remain out of water for up to 12 h (Lopes-Ferreira et al., 2014).

T. nattereri venom does not present either hemorrhagic or neurotoxic action, but it causes severe local inflammation, rupturing of blisters with serous content followed by a necrosis that lingers for several days (Haddad et al., 2003). A significant factor in the evolution of the lesion is an inefficient cure without either specific treatment or drug therapy. This venom also causes hemolytic, myotoxic and proteolytic activities (Lopes-Ferreira et al., 1998). In the mouse leg injected with *T. nattereri* venom, was observed through histopathological analysis the presence of edema, mionecrosis, congestion of veins and venules, the presence of thrombi, in addition to inflammatory cellular infiltration (Lima et al., 2003). Natterins, proteolytic enzymes, are able to induce edema and nociception and hydrolyze human kininogen and synthetic peptides derived from kininogen releasing Lys-BK and calidine (Magalhães et al., 2005). More recently, the presence of a proteolytic activity capable of converting Ang I into Ang II has been described in *T. nattereri* and *S. plumieri* venom (Tenório et al., 2015 and Tenório et al., 2016).

An angiotensin converting enzyme (dipeptidyl carboxypeptidase, EC 3.4.15.1, kininase II) a zinc metalopeptidase is an important enzyme of the renin-angiotensin system (RAS) that modulate fluids and electrolytes regulating the blood pressure (Fig. 1). By removing dipeptide His-Leu from C-terminal, the ACE converts Ang I into Ang II and inactivates bradykinin, the vasodilatory peptide, by sequential removal of two C-terminal dipeptides; consequently, the ACE became a target in the treatment of hypertension, heart failure, and diabetic nephropathy (Zhang et al., 2013). For a long time, RAS was thought to encompass only angiotensin II as an active component, but in the past few years, a vastly different picture has emerged. Despite the central role of the ACE, some tissues have presented the capacity to form Ang II from Ang I without involving ACE (Banegas et al., 2006; Urata et al., 1990; Paula et al., 1998), or from Ang (1–12) (Nagata et al., 2006), through ways considered alternative. Many venoms have ACE inhibitor components (Ferreira et al., 1970; Politi et al., 1985; Higuchi et al., 1999). According to the results of resent studies, however, the venom of marine animals interferes with cardiovascular function via a different mechanism, ACE activity and no ACE inhibition (Tenório et al., 2015 and Tenório et al., 2016).

In human, the ACE gene encodes two isoenzymes (Hubert et al., 1991), the somatic form (sACE) of around 150–180 KDa, with two catalytic sites, and a smaller one, testicular (tACE) (90–110 KDa), with a single active site (Langford et al., 1993; Kondoh et al., 2005). Isoenzyme sACE is expressed in several tissues, including vascular endothelium, epithelial cells of the kidney proximal tubules, intestinal brush edge, epididymal epithelium, surface of pulmonary endothelial cells, glial brain cells, male genital tract epithelium, and Leydig cells (Ryan et al., 1975; Ehlers et al., 1986). In contrast, tACE is expressed exclusively in the development of spermatid and mature spermatozoon (Köhn et al., 1998) both the isoenzymes are ectoproteins anchored in the plasma membrane through a single hydrophobic transmembrane polypeptide near the C-terminal (Hooper, 1991). Most of the enzyme, including its catalytic sites, is exposed to the extracellular medium (Ramchandran et al., 1994; Köhn et al., 1998). Soluble isoforms are largely distributed in body fluids, which suggest a greater involvement of this enzyme in many biological processes (Deguchi et al., 2007).

Studies with ACE have demonstrated the presence of the active sites named domain N and domain C, in reference to, respectively, portions N- and C-terminals of the enzyme molecule catalytically independent (Wei et al., 1992). Despite their high level of homology, particularly in the region of the active site, these domains present differences in specificity for substrates, activation by chloride, and in their inhibition and denaturation profiles (Jaspard et al., 1993; Rousseau et al., 1995). Domain C of the enzyme hydrolyzes substrate Hip-His-Leu and Ang I with higher catalytic efficiency than domain N (Wei et al., 1992) Fuchs and collaborators pointed out that ACE C-domain is the prevailing site in the separation of Ang I *in vivo* (Fuchs et al., 2008). Regarding bradykinin, both catalytic sites convert it into BK (1–7) and BK (1–5) with similar efficiency. Studies on the human genome describe a third isoform, the homologous ACE or ACE 2, which contains a single active site and hydrolyzes angiotensins I and II; however, it does not hydrolyze bradykinin (Crackower et al., 2002). Angiotensin (1–7) is a specific natural substrate for the catalytic N-domain of ACE that can act as a domain C inhibitor (Deddish et al., 1998). This peptide is increased in the plasma of humans and mice treated with ACE inhibitors, and presents a function opposite to Ang II, with antihypertensive action (Luque et al., 1996; Ferrario and Flack, 1996).

2. Review

Enzymes are an important and common component in the venom of many animals frequently involved in toxic action. In this context, some enzymes have been isolated and identified from fish venom, including phosphodiesterase, 5' nucleotidase, phospholipase, hyaluronidase, and those with caseinolytic, gelatinolytic, and fibrinogenolytic activities (Silvan, 2009). Proteases can also act as toxins and may be characterized in the venom of spiders and snakes (Chaim et al., 2011; Serrano, 2013).

Conversion activity of angiotensin, similar to ACE, had been found in the venom of fish, *T nattereri* (Tenório et al., 2015) and *S. plumieri* (Tenório et al., 2016). The main product formed was Ang II, which indicated the presence of a converting enzyme of angiotensin (Skeggs et al., 1956). Furthermore, the converting activities were completely inhibited by captopril and EDTA, in both venoms (Tenório et al., 2015 and Tenório et al., 2016). The venoms did not hydrolyze the chromogenic substrates for the converting serinoproteases chymase and elastase-II, N-succinyl-AAAPF-pNA, and N-Succini-AAAPL-pNa, respectively (Tenório et al., 2015 and Tenório et al., 2016). The presence of this protease producer of vasoconstrictor peptide in these venoms infers the importance of its action since this enzyme is one of the key elements in the activation of the cascade production of bioactive peptides in organisms. The product formed (Ang II) unleashes a series of events when attached to receptors AT1 and AT2. When associated with its receptor AT1, it promotes an increase in blood pressure. In addition, Ang II plays an essential role in the activation of inflammatory responses, being considered a peptide with strong pro-inflammatory effect. Because it is an important cellular growth factor, it is also related to the emergence of hyperplasia, vascular and heart hypertrophy that follow a hypertensive status (Santos et al., 2000; Rigatto et al., 2004). No activity on Ang II was observed (degradation or product formation can be detected) regarding *T. nattereri* (Tenório et al., 2015) and *S. plumieri* (Tenório et al., 2016), possibly favoring the local accumulation of Ang II, which could contribute to