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Modulation of voltage-gated Na^+ and K^+ channels by pumiliotoxin **251D**: A "joint venture" alkaloid from arthropods and amphibians ☆

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Abstract

Certain amphibians provide themselves with a chemical defense by accumulating lipophilic alkaloids into skin glands from dietary arthropods. Examples of such alkaloids are pumiliotoxins (PTXs). In general, PTXs are known as positive modulators of voltage-gated sodium channels (VGSCs). Unlike other PTXs, PTX **251D** does not share this characteristic. However, mice and insect studies showed that PTX **251D** is highly toxic and to date the basis of its toxicity remains unknown.

In this work, we searched for the possible target of PTX **251D**. The toxin was therefore made synthetically and tested on four VGSCs (mammalian $rNa_v1.2/\beta_1$, $rNa_v1.4/\beta_1$, $hNa_v1.5/\beta_1$ and insect *Para/tipE*) and five voltage-gated potassium channels (VGPCs) (mammalian $rK_v1.1$-1.2, $hK_v1.3$, $hK_v11.1$ (hERG) and insect *Shaker IR*) expressed heterologously in *Xenopus laevis* oocytes, using the two-electrode voltage clamp technique.

PTX **251D** not only inhibited the Na^+ influx through the mammalian VGSCs but also affected the steady-state activation and inactivation. Interestingly, in the insect ortholog, the inactivation process was dramatically affected. Additionally, PTX **251D** inhibited the K^+ efflux through all five tested VGPCs and slowed down the deactivation kinetics of the mammalian VGPCs. $hK_v1.3$ was the most sensitive channel, with an IC_{50} value $10.8 \pm 0.5\,\mu M$. To the best of our knowledge this is the first report of a PTX affecting VGPCs.

Keywords: PTX **251D**; PTXs; VGSC; VGPC; Two-electrode voltage clamp technique

1. Introduction

A remarkable diversity of biologically active alkaloids has been discovered in amphibian skin (Daly et al., 2005). During evolution, certain amphibians have developed an efficient system to accumulate some of these toxic compounds from

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dietary alkaloid-containing arthropods into their skin. Together with the bright warning skin coloration, the accumulation of toxins offers an extreme evolutionary advantage against predators. Examples of such toxins are the lipophilic alkaloids pumiliotoxins (PTXs) that are very widely distributed in alkaloid-containing anurans from the neotropics (*Dendrobates*, *Epipedobates*, *Minyobates*, *Phylobates*, *Oophaga*, *Ameerga* and *Ranitomeya*), semi-temperate South America (*Melanophryniscus*), Madagascar (*Mantella*) and Australia (*Pseudophryne*). The presence of PTX **307A** and **323A** (Fig. 1) has also been reported in formicine ants of the genera *Brachymyrmex* and *Paratrechina* (Saporito et al., 2004). Recently, in the extracts of scheloribatid mites PTX **251D** (Fig. 1) was found together with PTX **237A** and 8-deoxyPTX **193H** (Takada et al., 2005), while PTXs **251D**, **307F** and **307A** and a homoPTX **251R** were found in oribatid mites from Costa Rica and Panama (Saporito et al., 2007). It has been proposed that mites are the major source of PTXs and other frog skin alkaloids with branches in their carbon skeletons.

PTXs are known to be toxic. They are described as potent cardiotonic agents with positive modulatory effects on voltage-gated sodium channels (VGSCs) (Daly et al., 1985). Like other PTXs, PTX **251D** is highly toxic, inducing convulsions and death to mice and insects (LD_{50} being, respectively, 10 mg/kg and 150 ng/larvae) (Bargar et al., 1995; Daly et al., 2003). In addition, this toxin seems to repel predatory and ectoparasitic arthropods, and hence, its function in anuran chemical defense is proved (Weldon et al., 2006). However, earlier studies in brain synaptoneurosomes showed that PTX **251D** only weakly stimulates the sodium flux at low concentrations (10 μM) and has inhibitory effects on VGSCs at higher concentrations (100 μM) (Daly et al., 1985, 1990). Therefore, the observed effects in mice and insects cannot be explained by a modulation of VGSCs alone, making the possible physiological target(s) for this toxin unknown.

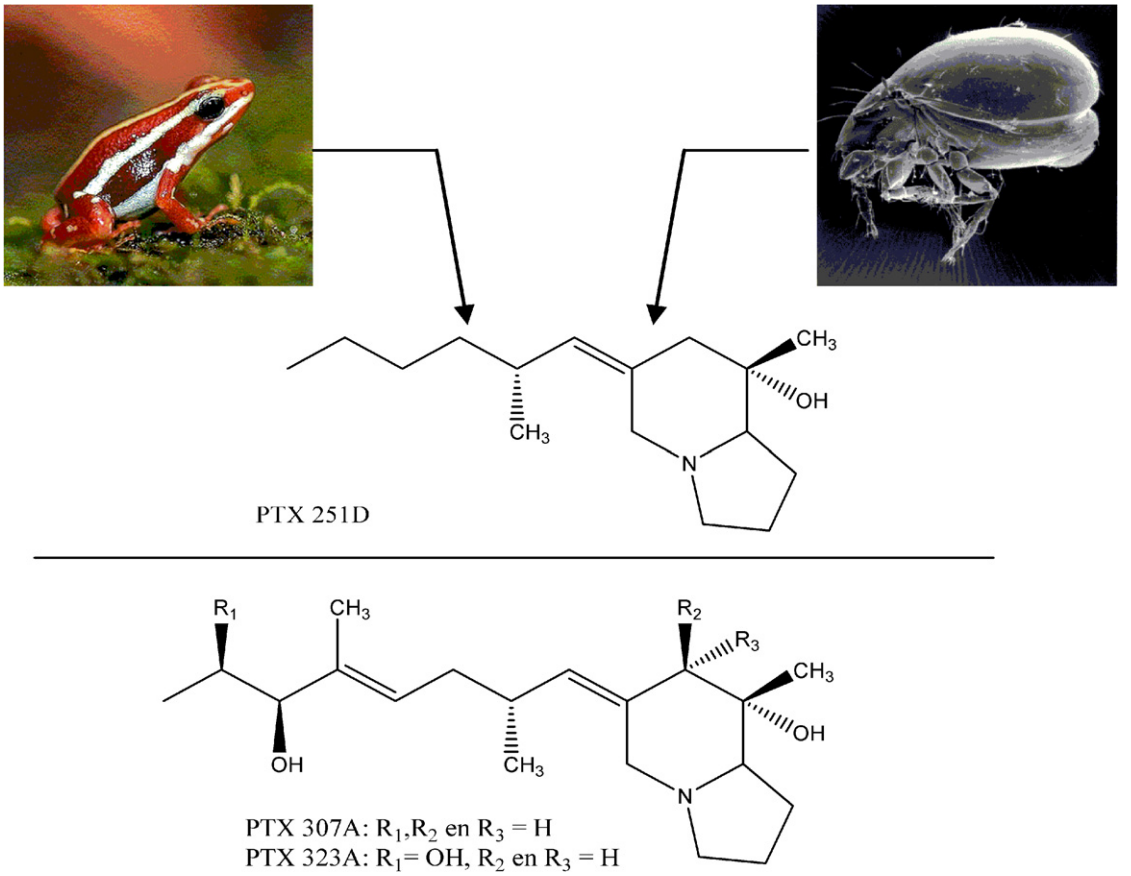

Fig. 1. Structure of PTX **251D**, a lipophilic alkaloid isolated from the skin of some anurans (left *Epipedobates tricolor*) and from Scheloribatid mites (right). Below, structures of related pumiliotoxines PTX **A** (307A) and **B** (323A).