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Maitotoxin induces two dose-dependent conductances in *Xenopus* oocytes. Comparison with nystatin effects as a pore inductor ☆

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Abstract

Maitotoxin (MTX)-induced conductances in *Xenopus* oocytes were thoroughly characterized using the two-electrode voltage clamp technique with a hyperpolarizing voltage protocol. MTX 5–100 pM induced an inward current with maximal amplitude between 0.1 and 10 μA. The kinetics of this current had rising and decaying phases, which were non-voltage dependent. Its reversal potential (E_{rev}) was close to 0 mV in high K^+ or Na^+ external solution, indicating the participation of non-selective cation channels (NSCC). A second conductance was developed at MTX doses higher than 200 pM whose amplitude increased continuously. This current showed a large instantaneous component and a voltage-independent decay, as well as similar selectivity for Na^+ and K^+ ions ($E_{rev} \approx 0$ mV). Moreover, the maximal current amplitude was about 34% bigger in high K^+ than in high Na^+. The MTX effect was reversible at all doses in pM range. All the properties found are similar to those of NSCC. The differences in the current kinetics suggest that the MTX-elicited currents reflect the activation of two sets of voltage-independent NSCC. As MTX has been proposed to act by forming pores directly into the plasma membrane, we compared its effects with those of nystatin, a well-known membrane pore inductor. We found strong differences between the effects of both substances suggesting different mechanisms for these drugs.

Keywords: Maitotoxin; Non-selective cation channels; Nystatin; *Xenopus laevis* oocytes

1. Introduction

Maitotoxin (MTX), the largest non-polymeric natural product (3422 Da) (Gallimore and Spencer, 2006), is the most potent marine toxin known. MTX was discovered in the surgeonfish *Ctenochaetus striatus* and was named due to the Tahitian name

☆ *Ethical statement*: The acquisition, care, housing, use and disposition of animals were performed in compliance with international laws and institutional guidelines. Besides, an appropriate institutional committee has approved the experimental protocol.

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assigned to this fish, *Maito* (Yasumoto et al., 1976). This toxin is isolated from the dinoflagellate *Gambierdiscus toxicus* besides other 20 toxins known as gambiertoxins, which are the causative toxins of ciguatera, a type of seafood poisoning (Lehane and Lewis, 2000).

A wide variety of MTX effects have been reported in many cell types (Gusovsky and Daly, 1990; Escobar et al., 1998). At nanomolar concentrations MTX induces an increase in $[Ca^{2+}]_i$ in different cell types such as pheochromocytoma PC12 cells (Takahashi et al., 1983), pituitary GH_3 cells (Gusovsky and Daly, 1990), hypothalamic synaptosomes (Taglialatela et al., 1990) and others (Escobar et al., 1998; Gusovsky and Daly, 1990). Several attempts have been made to understand the effects of MTX at molecular level. However, the action mechanisms of this powerful toxin are still unclear. It is currently accepted that its main effect is the activation of non-selective cation channels (NSCC) (Musgrave et al., 1994; Dietl and Völkl, 1994; Worley et al., 1994; Estacion et al., 1996; Bielfeld-Ackermann et al., 1998; Schilling et al., 1999; Estacion and Schilling, 2001; Wisnoskey et al., 2004). Recently, it was reported that MTX activates NSCC as a first response in bovine aortic endothelial cells (Estacion and Schilling, 2001; Wisnoskey et al., 2004) and Chinese hamster ovary cells (Cataldi et al., 1999). Furthermore, it was also proposed that another channel is activated secondarily (Estacion and Schilling, 2001; Wisnoskey et al., 2004; Cataldi et al., 1999). This second channel shows the characteristics of a membrane pore with a huge conductance, allowing the exchange of large organic molecules that finally produces cellular death in a way similar to the polyene antibiotic ionomycin (Estacion and Schilling, 2001). This effect could be responsible for the deleterious consequences found in every cell type where MTX has been tested. However, this hypothesis has not been thoroughly investigated. Additionally, it has been reported that MTX activates two different ionic conductances in *Xenopus* oocytes: a calcium-dependent chloride current (Martínez et al., 1999) and a NSCC (Bielfeld-Ackermann et al., 1998), both at picomolar concentrations. The reported chloride current is activated at depolarizing potentials from a negative holding potential and its activation is voltage- and $[Ca^{2+}]_i$-dependent (Martínez et al., 1999). The MTX-activated NSCC has been only partially characterized (Bielfeld-Ackermann et al., 1998). MTX (50 pM–1 nM) induced a current that was recorded continuously at a constant membrane potential for long time periods and it was not possible to observe other conductances (Bielfeld-Ackermann et al., 1998). In the present study, the currents activated by MTX on the oocyte were characterized widely for the first time. Besides, we compared these currents with those activated by nystatin, a pore-forming molecule (Bolard, 1986; Kasumov et al., 1979; Katsu et al., 2007). Finally this comparison let us conclude that both substances act through different mechanisms and that the toxin could act by activating a native channel present in the oocyte membrane. We propose that this endogenous channel could be a member of the transient receptor potential (TRP) channels family as has been suggested for several cell types (Trevino et al., 2006; Chen and Barrit, 2003; Brereton et al., 2000, 2001).

2. Materials and methods

2.1. Oocytes

Xenopus laevis female frogs were purchased from NASCO (Biology Div., Fort Atkinson, WI, USA). Defolliculated oocytes were obtained according to Martínez et al. (1999). Briefly, frogs were anaesthetized by immersion in a 0.17% tricain solution for 30 min and small pieces of ovarian lobe were dissected out and gently shaken at room temperature (19–25 °C) for 90 min in ND96 solution supplemented with 2 mg/ml of collagenase type IA. The ND96 solution contains in mM: 96 NaCl, 2.5 KCl, 1 $MgCl_2$, 5 HEPES, pH 7.4. Only healthy-looking oocytes of V–VI stages were selected. The oocytes were incubated at 18 °C for 1–5 days in ND96 solution supplemented with 2.5 mM pyruvic acid, 20 U/ml gentamycin and 20 mg/ml streptomycin. The incubation solution was changed daily.

2.2. Electrophysiological recordings

For electrophysiological measurements, oocytes were placed in a 1 ml experimental recording chamber. In each experiment the oocyte was impaled in Ringer solution at room temperature (19–25 °C). Ringer solution contains in mM: 117 NaCl, 2.5 KCl, 1.8 $CaCl_2$, 5 HEPES, pH 7.4. Electrodes showed resistances of 0.5–1 MΩ (3 M KCl). Using the two-electrode voltage-clamp technique, currents resulting from applying a hyperpolarizing and depolarizing voltage step protocol