CIGUATOXINS AND METHODS OF DETECTION

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INTRODUCTION

Ciguatoxins (CTXs), produced by dinoflagellates of the genus *Gambierdiscus* and *Fukuyoa,* are components of one of the most frequent foodborne illness disease known as ciguatera fish poisoning (CFP) that causes gastrointestinal, cardiovascular and neurological disturbances [1, 2]. The spread of the dinoflagellates producers of ciguatoxins, the lack of certified CTX standards, the variability among CTX analogues and the absence of cytotoxic effect by themselves in some cell cultures highlight the need to revaluate the detection methods for these toxins [3]. Currently, the most employed method of detection of CTXs is the cell viability measurement in mouse neuroblastoma neuro2a cell line sensibilized with ouabain and veratridine (O/V) by using 1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan (MTT assay) [4]. However, this cell line does not express a significant number of sodium channels, the main CTXs cellular target. In order to find a more suitable method of detection, HEK293 cell line stably transfected with the 1.6 α subunit was tested.

MATHERIAL AND METHODS

N2a neuroblastoma cell line

N2a cell line was obtained commercially from the American Type Culture Collection (CCL-131). Cells were cultured in in DMEM/F12 medium supplemented with L-glutamine, fetal bovine serum (10%) and penicillin and streptomycin antibiotics (1%).

HEK293 cells expressing the human $Na_v1.6$ sodium channel subunit

HEK293 cells transfected with the human Na_v 1.6 alpha subunit of the sodium channels were used under an MTA with Dr Andrew Powell (GlaxoSmithKline R&D, UK). Cells were cultured in DMEM/F12 medium with L-glutamine supplemented with 1% non essential aminoacids, 10% fetal bovine serum and 400 µg/ml geneticin and placed at 30 °C the 24–36 h prior to electrophysiological measurements.

Electrophysiological recordings

Electrophysiological recordings of sodium currents were obtained in whole-cell configuration applying voltage steps from −80 to +80 mV in 10 mV step increases and 0.15 seconds duration. A computer-controlled current and voltage clamp amplifier (Multiclamp 700B, Molecular Devices) was used. Signals were recorded and analyzed using a computer equipped with a Digidata 1440 data acquisition system and the pClamp10 software.

Ciguatoxins

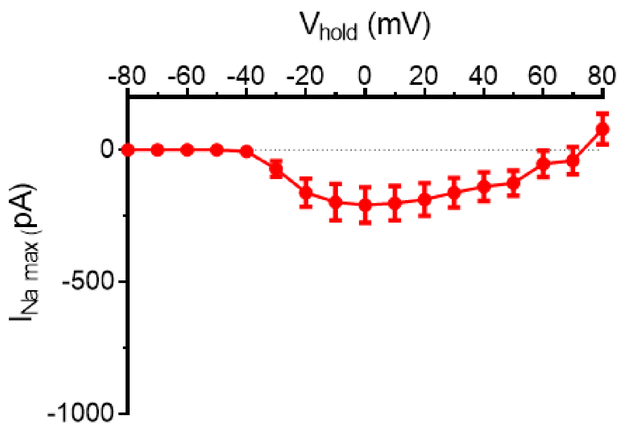
CTX3C were purchased from WAKO (FUJIFILM Wako Chemicals Europe GmbH, Neuss, Germany). Toxins were dissolved in DMSO (maximum concentration of 1%) and subsequent toxin dilutions were performed in Locke's buffer solution.

$DiBAC_4(3)$

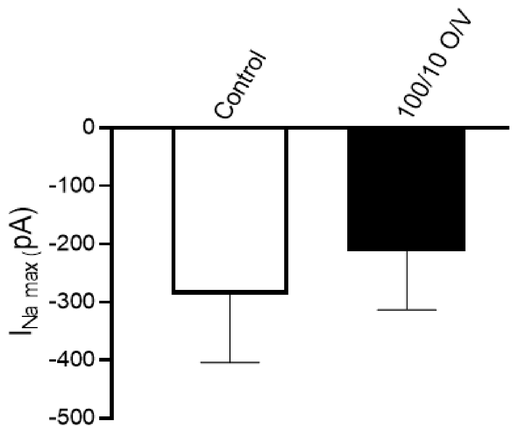
Bis-(1,3-Dibutylbarbituric Acid) Trimethine Oxonol (DiBAC4(3)) were purchased from ThermoFisher. Cells were charged with the membrane potential indictor at a concentration of 100 nM for 10 minutes at room temperature. Fluorescent images were collected and analysed using a Nikon confocal laser scanning microscope.

RESULTS

ELECTROPHYSIOLOGICAL RECORDINGS IN N2a CELL LINE



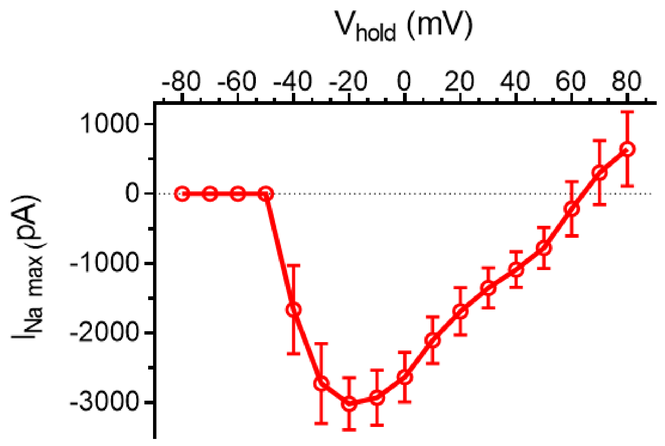
Current-voltage relationship of sodium current amplitude on N2a neuroblastoma cell line with a maximum peak inward current of by about –300 pA representing a low expression of functional sodium channels, the main CTXs target.



N2a cell exposure to a non-destructive O/V concentration of 100/10 µM for 24 h did not increase sodium channel expression.

RESULTS

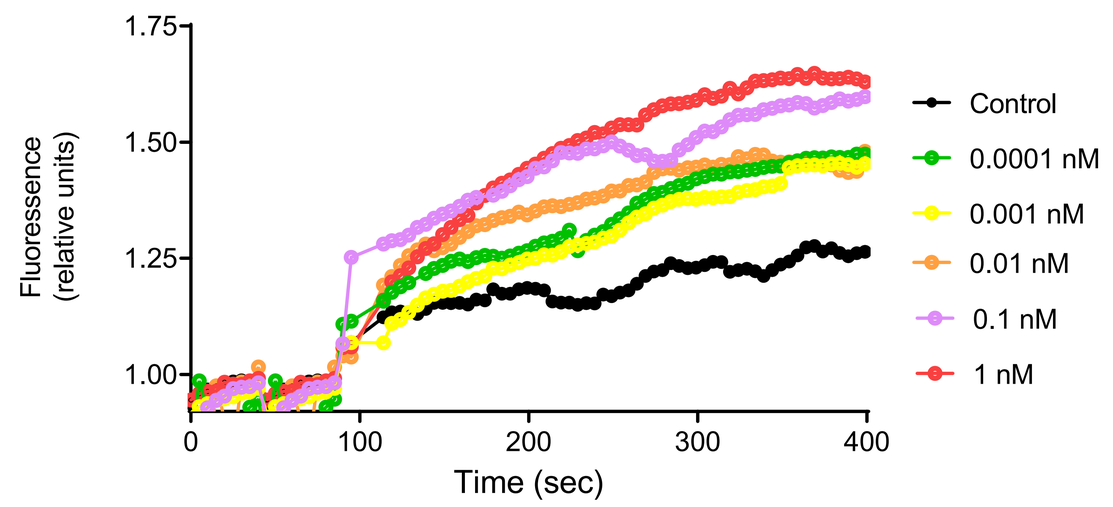
ELECTROPHYSIOLOGICAL RECORDINGS IN HEK293 Na_v 1.6 CELL LINE



Current-voltage relationship of sodium current amplitude on HEK293 $Na_v1.6$ cell line with a maximum peak inward current of by about –3000 pA illustrating a high expression of functional sodium channels

RESULTS

CONFOCAL DETECTION OF THE FLUORESCENT RESPONSE TO DEPOLARIZATION CAUSED BY CTX3C IN HEK293 Na_v 1.6 CELL LINE



Fluorescent response to the depolarization elicited by CTX3C addition in HEK293 Na_v 1.6 cell line. Cells incubated with the membrane potential indicator DiBAC4(3) were exposed in sec 100 to increasing CTX3C concentrations and a significant concentration dependent increase in fluorescent response was measured in contrast to control cells.

CONCLUSIONS

N2a is not suitable for the detection of ciguatoxins since they do not express sodium channels as demonstrated by electrophysiology. N2a cell exposure to O/V to increase their sensitivity to ciguatoxins shows that it does not increase the expression of sodium channels. Therefore, an alternative suitable cell line is proposed. Electrophysiological recordings of HEK293 cell line stably expressing Na_v 1.6 alpha subunit of sodium channels demonstrated a high availability of the main CTXs target. As a consequence of the large number of sodium channels, a significant depolarization response was detected after the addition of ciguatoxins, not requiring the addition of any other compound to the cells, allowing their detection by increasing in cell fluorescence as a consequence of membrane depolarization.

AKNOWLEDGEMENTS

The research leading to these results has received funding from the following FEDER cofunded-grants.. From Conselleria de Cultura, Educacion e Ordenación Universitaria, Xunta de Galicia, GRC (ED431C 2021/01). From Ministerio de Ciencia e InnovaciónGrant CPP2021-008447 funded by MCIN/AEI/10.13039/501100011033 and by The European Union NextGenerationEU/PRT. From European Union Interreg Agritox EAPA-998-2018, H2020 778069-EMERTOX and HORIZON-MSCA-2022-DN-01-MSCA Doctoral Networks 2022-101119901-BIOTOXDoc.

REFERENCES

1. Friedman, M.A., et al., *An Updated Review of Ciguatera Fish Poisoning: Clinical, Epidemiological, Environmental, and Public Health Management.* Mar Drugs, 2017. **15**(3).
2. Soliño, L. and P.R. Costa, *Global impact of ciguatoxins and ciguatera fish poisoning on fish, fisheries and consumers.* Environmental research, 2020. **182**: p. 109111.
3. Organization, W.H., *Report of the expert meeting on ciguatera poisoning: Rome, 19-23 November 2018*. Vol. 9. 2020: Food & Agriculture Org.
4. EFSA, *Scientific Opinion on marine biotoxins in shellfish–Emerging toxins: Ciguatoxin group.* EFSA Journal, 2010. **8**(6): p. 1627.