CELLULAR ACTIVITY OF PARALYTIC SHELLFISH SAMPLES EXTRACTED FROM *SEMELE PROFICUA* AND *SENILIA SENILIS*

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INTRODUCTION

Paralytic shellfish poisoning (PSP) is a worldwide affection caused by consumption of shellfish that accumulated neurotoxins produced by toxicogenic dinoflagellates. The PSP syndrome, characterized by neurological symptomatology, is due to their mechanism of action blocking the voltage-gated sodium channels in excitable membrane cells [1] .The syndrome is associated to three groups of hydrophilic analogues of Saxitoxin: N-sulfocarbamoyl, carbamate and decarbamoyl, produced mainly by dinoflagellates of the genus *Gymnodinium*, *Alexandrium* and *Pyrodinium* [2]. In Angola, between 2007 and 2008 episodes of PSPs contaminations in bivalves were reported [3]. Ten samples collected and extracted from the clamps *Semele proficua* captured in Luanda Bay and *Senilia senilis* collected in Mussulo Bay were analysed using high performance liquid chromatography (HPLC) to determine the amount of toxin present. Moreover, functional electrophysiology assays in cerebellar neurons were performed to elucidate the activity of these new compounds on voltage-gated sodium channels to determine their contribution to the PSP intoxication and assess the risk they pose to consumers.

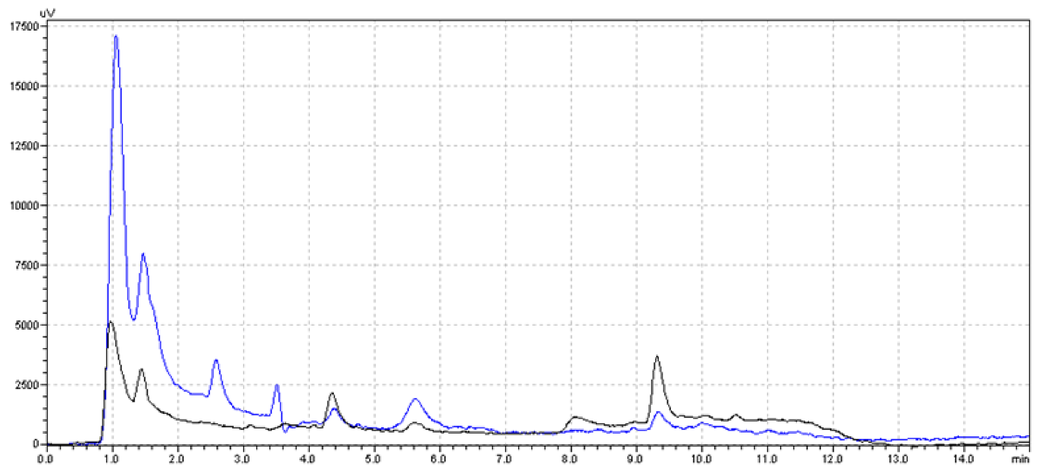
MATERIAL AND METHODS

Cell culture: Cerebellar granule cells were obtained from 7-day-old mice seeded in 18 mm glass coverslips precoated with poly-D-lysine and incubated in 12 multiwell plates for 6-11 days in a 5% CO_2/95% O_2 humidified incubator at 37 ˚C and kept with cytosine arabinoside to prevent glial proliferation.
Toxins: Purified STX was purchased from National Research Council Canada (NRCC) and dissolved in hydrochloric acid at a final concentration of 63.3 µM, subsequent solutions were performed in Locke´s buffer containing (in mM): 154 NaCl, 5.6 KCl, 3.6 $NaHCO_3$, 1.3 $CaCl_2$, 1 $MgCl_2$, 5 glucose and 10 HEPES (pH 7.4). To perform oxidation of the extracts and quantitation, toxin standard mixes (I-IV) were prepared as recommended (Horwitz and Latimer, 2006).
Electrophysiology: Sodium currents were recorded in the perforated patch-clamp mode using gramicidin, with a Multiclamp 700B, Molecular Devices. Signals were recorded and analyzed using a Pentium computer equipped with a Digidata 1440 acquisition system and the pClamp10 software (Molecular Devices, Sunnyvale, CA), prefiltered at 10 kHz and digitized at 20 µs intervals. Recording electrodes fabricated from borosilicate glass had a tip resistance of 5-10 MΩ. The internal pipette solution contained (in mM): 108 Cs gluconate, 1.7 NaCl, 0.9 EGTA, 9 HEPES, 1.8 $MgCl_2$, 4 Na_2ATP and 0.3 NaGTP, pH 7.2. The extracellular medium was Locke´s buffer containing 20 mM TEA and 1 mM 4-AP to block voltage-dependent potassium currents. Sodium currents were elicited by applying series of 25 ms depolarizing pulses, in 5 mV increments, from a holding potential of –100 mV.
Sample preparation: Samples of *Semele proficua* and *Senilia senilis* were dissolved in 50 µl 0.03 M acetic acid and after dilution 1/100 with extracellular medium.
HPLC: A LC-18 Supelco reversed-phase column of 15 cm x 4,6 mm and 5 µm particle size with a TR-C-160-1 Teknokroma guard and a Shimadzu fluorescence detector (model RF-10AXL), were used. Mobile phases were: (A): ammonium phormiate 0,1 M (pH = 6 ± 0,1); (B): ammonium phormiate 0,1 M in 5% acetonitrile (pH= 6 ± 0,1) were pumped through two Shimadzu pumps (model LC-10AD) at a flow rate of 2 mL/min. Gradients were: 0-5% of B for 5 minutes, 5-70% of B for the next 4 minutes and 0% of B the following 6 minutes to equilibrate the column. Individual toxins and mixtures were oxidized using either peroxide or periodate, injecting 25 and 100 µL respectively. Individual toxin concentrations were reported as µg STX dihydrochloride eq/kg.

RESULTS

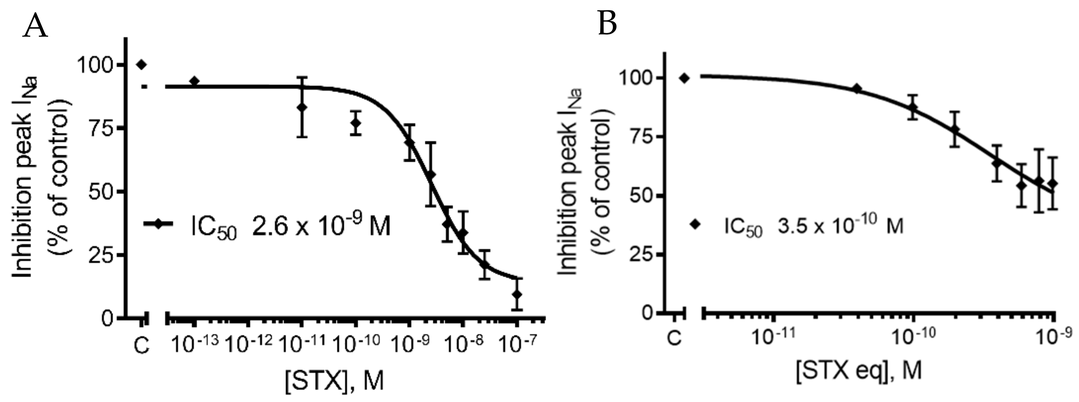
SAMPLE	µg/kg
Sample 12	STX: 8.21 Other: 7.87
Sample 15	STX: 9.78 Other: 12.84
Sample 16	STX: 7.83 Other: 16.86
Sample 17	STX: 7.53 Other: 6.04
Sample 18	STX: 22.0 dcSTX: 4.86 Other: 9.61
Sample 19	STX: 20.37 dcSTX: 5.03 Other: 13.19
Sample 20	STX: 60.22 dcSTX: 11.57 Other: 22.32
Sample 23	STX: 6.99
Sample 24	STX: 11.32 dcSTX: 5.14 Other: 15.05
Sample 25	STX: 7.31 Other: 11.06

HPLC analysis of the PSP content of the samples extracted from Semele proficua and Senilia senilis



HPLC-MS chromatograms obtained after peroxide (black trace) and periodate oxidation (blue trace) of sample 25, the presence of STX and two compounds that appeared at 2.5 and 5.6 min in periodate oxidation.

SAMPLE	IC_{50} M
Pure STX	2.6 x 10^{-9} M
Sample 16	5.3 x 10^{-10} M
Sample 18	1.9 x 10^{-10} M
Sample 20	7.2 x 10^{-10} M
Sample 23	4.0 x 10^{-10} M



Concentration-response graph indicating the effect decreasing the maximum peak inward sodium currents of pure STX (A) and sample 25 (B) containing 7.31 µg/kg STX and two unknown compounds

CONCLUSION: Ten analysed samples contained STX, dcSTX and three unknown compounds in different amounts. No effects on sodium channels or toxicity of the unknown compounds were found to date in the samples of the specimens *Senilia senilis* and *Semele proficua*.

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