CONffIDENCE: Contaminants in food and feed: Inexpensive detection for control of exposure

Spirolides and gymnodimine target human muscarinic acetylcholine receptors

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Abstract

Spirolides are a group of cyclic imine marine toxins recently described. Although no human intoxication has been related to their presence in shellfish yet , the possible toxicological consequences to human health are actually unknown. In this work the effects of the 13-desmethyl and 13,19-didesmetyl C spirolides on the activity and the expression of muscarinic acetylcholine receptors (mAChR) were analyzed using a human neuroblastoma cell model. Both spirolides inhibited the acetylcholine-induced calcium signal with a reduction of the maximum response to acetylcholine in the presence of the toxin. The effect of the spirolides persisted after toxin removal suggesting a mAChR irreversible antagonism.

Introduction

The 13-desmethyl and 13,19-didesmetyl C spirolides belong to a group of marine toxins with a macrocyclic structure that was designated as spirolide due to the presence of a spiro ring [1]. The spirolides are produced by phytoplankton (*Alexandrium ostenfeldii* and *Alexandrium peruvianun)* [2, 3], and can reach human consumers by accumulation in filter-feeding mollusks. Spirolide-toxic, algal blooms have been described mainly in America and Europe [4], but their toxicity to humans is still unknown. The mechanism of action of these toxins is still not fully elucidated. Toxicological studies in vivo demonstrated that the toxin induces neurological symptoms [5] and the mRNA levels of nicotinic and muscarinic acetylcholine recepotors in brain tissue were altered suggesting targeting of both types of acetylcholine receptors. Very recently, the spirolides have been demonstrated to bind and inhibit nAChRs [6, 7]. However, there is no direct evidence of mAChRs being targeted by spirolides. The aim of this study was to elucidate the pharmacologic effect of the spirolides on human mAChRs.

Methods

Cell Line Culture: Human neuroblastoma BE(2)-M17 cell line (European Collection of Cell Cultures) was cultured in EMEM: Ham's F12 (1:1).

$[Ca^{2+}]_i$ measurements: The cells were loaded with fura-2-AM (0.5 μM, Molecular Probes). Fluorescent images were collected with dual excitation 340/380 nm, and an emission of 530 nm using a Nikon Diaphot microscope equipped with epifluorescence optics. The calculation of $[Ca^{2+}]_i$ was carried out as in [8].

Western blot analysis was performed with an anti-M3 muscarinic receptor (Santa Cruz) .Images were acquired by a Diversity Imaging System (Syngene, Cambridge, United Kingdom) and band density was quantified with the Gene Tools 3.08 software.

Statistical Analysis: Statistical significance was determined by using the paired t – test ($p < 0.05$).

Results

Acetylcholine- elicited response in BE(2)-M17 neuroblastoma cells is dependent on mAChR stimulation, mainly the M3 subtype

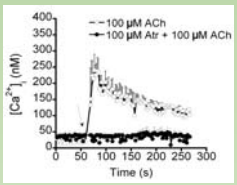

Figure 1: The calcium signal elicited by acetylcholine in this neuroblastoma cell line is atropine dependent.

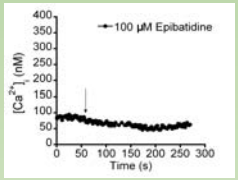

Figure 2: Epibatidine did not induce a $[Ca^{2+}]_i$ response in this cell line.

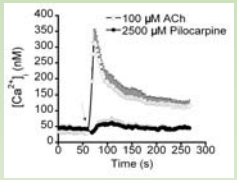

Figure 3: Pilocarpine did not induce a $[Ca^{2+}]_i$ response in this cell line.

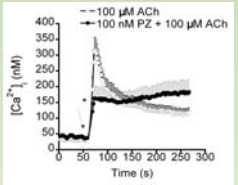

Figure 4: Pirenzepine did not inhibit substantially the acetylcholine-induced $[Ca^{2+}]_i$ response in neuroblastoma cells.

The 13-desmethyl and 13,19- didesmethyl C spirolides have similar effects on the calcium signal elicited by ACh, both quantitatively and qualitatively

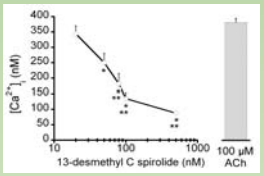

Figure 8: Dose-response curve of the 13-desmethyl C spirolide for the inhibition of the ACh-induced $[Ca^{2+}]_i$ rise.

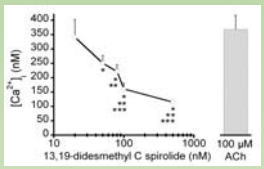

Figure 9: Dose-response curve of the 13, 19-didesmethyl C spirolide for the inhibition of the ACh-induced $[Ca^{2+}]_i$ rise.

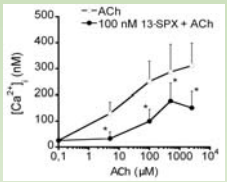

Figure 10: The 13-desmethyl C spirolide reduces the maximum calcium response to ACh in this cell line.

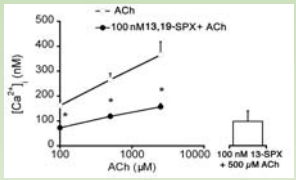

Figure 11: The 13, 19-didesmethyl C spirolide reduces the maximum calcium response to ACh in this cell line, similarly to 13-desmethyl C spirolide.

The 13-desmethyl C spirolide inhibits the calcium signal elicited by acetylcholine stimulation in BE(2)-M17 neuroblastoma cells

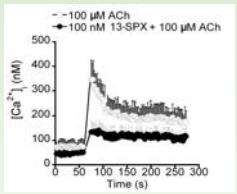

Figure 5: The 13-desmethyl C spirolide inhibits acetylcholine-dependent $[Ca^{2+}]_i$ response in neuroblastoma cells.

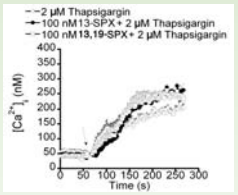

Figure 6: The thapsigargin-induced $[Ca^{2+}]_i$ response in neuroblastoma cells is not affected by the spirolides.

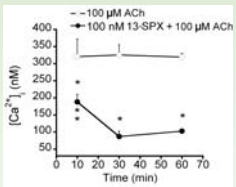

Figure 7: Kinetics of the 13-desmethyl C spirolide-induced inhibition of the ACh-dependent $[Ca^{2+}]_i$ rise.

The 13-desmethyl C spirolide does not alter the levels of M3 mAChR protein

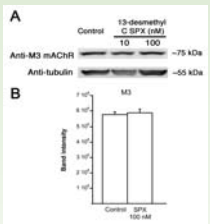

Figure 12: The 13-desmethyl C spirolide does not alter M3 mAChR protein levels in neurobalstoma cells after 12 hours of incubation in the presence of the toxin. (A) Image of a Western blot of whole cell lysates for M3 mAChR (upper panel) and the re-blot of the same membrane for ß-tubulin (lower panel). (B) Quantification of M3 protein levels

Conclusions

- The 13-desmethyl and 13,19-didesmetyl C spirolide inhibit the ACh-elicited $[Ca^{2+}]_i$ response in neuroblastoma cells suggesting that these molecules are antagonists of mAChRs.
- The inhibition is irreversible and dependent on the time of exposure to the toxin.
- The 13-desmethyl and 13,19-didesmetyl C spirolide inhibit the ACh-elicited $[Ca^{2+}]_i$ response with similar potency
- The amount of M3 mAChR protein was not significantly altered by an exposure to 10 or 100 nM 13-desmethyl C spirolide for 12 hours.

References

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The research leading to these results has received funding from the European Community's Seventh Framework Programme (*FP7/2007-2013)* under *grant agreement* n° 211326.