



Acetylcholinesterase activity in *Clytia hemisphaerica* (Cnidaria)

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ABSTRACT

Cholinesterase activity is known in representatives of all living organisms phyla but the origin of the cholinergic system as known in bilaterian animals is still undeciphered. In particular the implication of cholinesterases in the nervous system of non-bilaterian Metazoa is not well known. We thus chose to investigate this activity in the *Clytia hemisphaerica* (Cnidaria) medusa. *In toto* histochemical staining revealed an acetylcholinesterase activity in the tentacle bulbs but not in the nervous system. Sequences homologous to acetylcholinesterase were searched within *Clytia* ESTs and compared to other sequences found in public databases.

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1. Introduction

Acetylcholine (ACh) is well known as an important neurotransmitter in the nervous systems of several groups of bilaterians (bilaterally symmetrical organisms, corresponding to all animals except sponges, cnidarians and ctenophores) and is especially studied in insects and in vertebrates [1]. ACh and the cholinergic pathway have also been detected in various life forms, such as bacteria, plants, fungi and various other metazoans [1,2]. These findings suggest that ACh has been used by organisms well before the appearance of the first nervous systems. Moreover, ACh has been detected in several types of non-neuronal cells in mammalian species [4–6] perhaps representing a part of a legacy from an ancestor without a nervous system. More generally, the integration of the cholinergic pathway in the nervous system is a very relevant topic in order to decipher how the nervous system appeared. From this point of view, cnidarians and ctenophores are very important because they are the only non-bilaterian animals with a nervous system. In cnidarians (jellyfishes, corals, anemones, hydras, etc.) acetylcholinesterase activity was only localized in

Hydra in the cell bodies and neurites of putative ganglion cells, nematocytes and also in epitheliomuscular and digestive cells [7]. Despite the histochemical evidence for this activity in *Hydra* and more generally in cnidarians the implication of ACh as a neurotransmitter remains equivocal [8–10]. We chose to investigate the cholinesterase activity in medusae of *Clytia hemisphaerica* (Cnidaria, Hydrozoa) and to look for potential candidate genes by sequence comparison.

2. Materials and methods

For all experiments we used *C. hemisphaerica* medusae cultured in Paris as described in [11], except that artificial seawater was used (36 g/l Reef Crystals®, Aquarium Systems).

Whole-mount histochemical staining was obtained on medusae and gonozooids, fixed in 4% paraformaldehyde at 24 °C for 30 min. The staining was obtained by the Karnovsky and Roots [12] method, using acetylthiocholine iodide as substrate. Specific inhibition of AChE was obtained with eserine (10^{−4} M).

Enzyme activity was measured according to the method of Ellman et al. [13] (0.1 M potassium phosphate buffer pH 7.0, 0.5 mM DTNB 1 mM acetylthiocholine) on homogenates of 20 medusae extracted in 10 volumes of

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ice cold extraction buffer (50mM potassium phosphate, pH 7.5) in glass–glass potter. Samples were incubated in dilutions of 1 nM to 1 mM eserine (Sigma). The remaining activity was expressed relative to the initial activity.

The *Clytia* genes were retrieved by BLAST searches (expect value: 0.0001) with known bilaterian AChE on an unpublished EST collection sequenced by the Genoscope (Evry, France) from a *Clytia* normalized cDNA

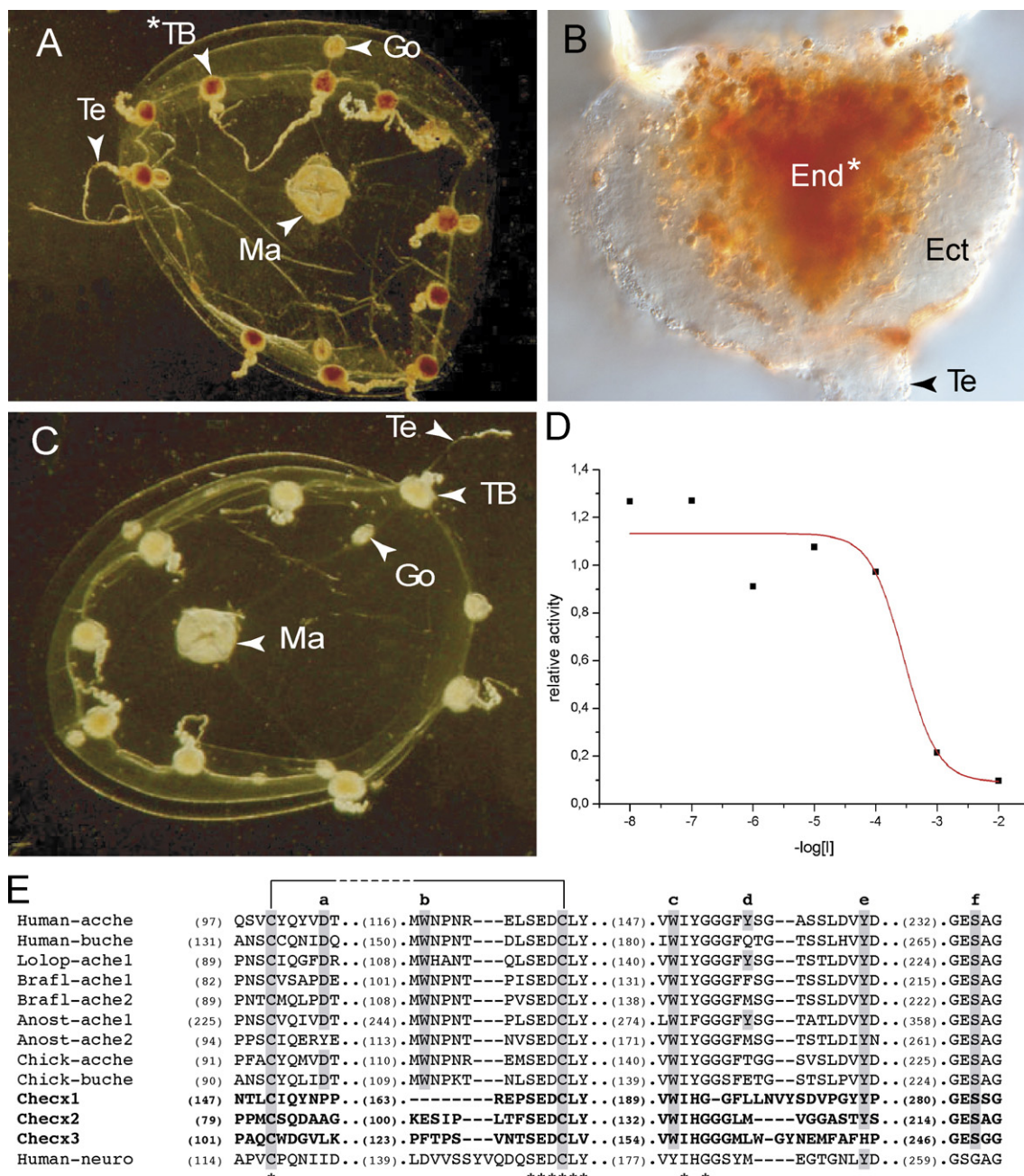


Fig. 1. (A–B) Whole-mount histochemical staining of AChE activity. Asterisk (*) indicates specific staining; TB: tentacle bulb; Ect: bulb ectoderm; End: bulb endoderm; Go: Gonad; Ma: manubrium; Te: tentacle. (A) Whole medusa without eserine; (B) detail of bulb without eserine; (C) whole medusa with eserine; (D) extent of inhibition of acetylthiocholine hydrolysis by increasing concentration of eserine (remaining activity is expressed relative to the initial activity without eserine). (E) Alignment of partial sequences of *Clytia* genes candidates for cholinesterase activity (Che-Cx1, -2, and -3; *Clytia hemisphaerica* putative carboxylesterases 1, -2, and -3). Informative positions are indicated. (a) Peripheral anionic site; (b) choline binding site; (c–e) aromatic residues in catalytic gorge; (f) active site serine. Human-AChE: human acetylcholinesterase (AAH94752); Human-BuChE: human butyrylcholinesterase (EAW78592); Chick-AChE: chicken acetylcholinesterase (P36196); Chick-BuChE: chicken butyrylcholinesterase (NP.989977); Lolop-AChE1: opal squid acetylcholinesterase (AAD15886); Brafl-AChE1 and -2: florida lancelet acetylcholinesterase 1 (AAD05373) and -2 (AAD05374); Anoga-AChE1 and -2: *Anopheles gambiae* mosquito acetylcholinesterase 1 (XP.321792) and -2 (XP.310628); Human-neuro: human neuroigin 1 (NP.055747). Asterisk (*) indicates conserved position. Disulfide bonds are indicated by a line. Numbers in parenthesis indicate the amino acid position starting from the beginning of sequences.

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