VIII[th] International Meeting on Cholinesterases

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# BIOCHEMICAL CHARACTERIZATION OF ACETYLCHOLINESTERASE IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELL MEMBRANES

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## ABSTRACT

Acetylcholinesterase (AChE) is an enzyme whose primary function is to hydrolyse the neurotransmitter acetylcholine (ACh). It is known that AChE is expressed in several non-cholinergic tissues. In this study we proposed to identify and characterize AChE in vascular endothelial cell membranes. We used cultured human umbilical vein endothelial cells (HUVECs) from passage 2 and 3. HUVECs were lysed and membranes isolated, that were further solubilized with Triton X-100 to obtained protein extracts. With *Ellman's* method we analysed AChE activity on HUVECs in terms of substrate specificity and inhibition (with BW284c51 10mM and eserine 10mM) and the studies revealed that there was a predominance of the AChE under other esterases that was significant inhibited by the above inhibitors. These results were complemented with polyacrylamide gel electrophoresis studies, with Triton X-100 and under non-denaturation conditions, with staining for cholinesterases and acetylcholinesterase activity with selective substrates and inhibitors. The gels revealed one enzimatically AChE active band in HUVECs membranes extracts that disappeared when the gel staining with acetylthiocholine substrate was in presence of eserine or BW284c51. To characterize the molecular weight of this AChE band we performed both SDS-PAGE electrophoresis stained with Coomassie blue and Western blot analysis with anti-AChE antibodies and we identified a single protein band of approximately 70 kDa (with C-terminal anti-AChE antibody), characterized of AChE.


In this study we demonstrated and biochemically characterized the existence of a membrane-bounded AChE in HUVECs. In future studies we will investigate the AChE function on the endothelial vascular system.

## OBJECTIVE

The aim of this work was the biochemical characterization of the AChE in the membranes of HUVECs.

## EXPERIMENTAL DESIGN

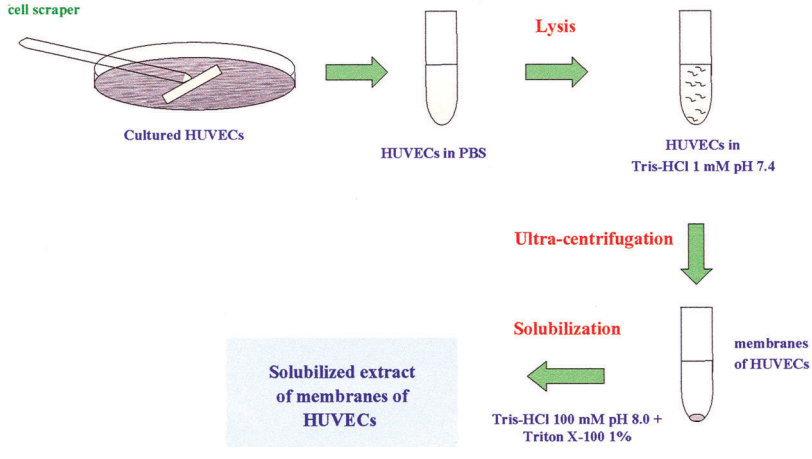


**Figure 1:** Isolation and Solubilization of membranes of HUVECs

Endothelial cells were isolated from human umbilical cords obtained from St. Maria's Hospital Obstetrics Service. Cultured HUVECs were detached with a cell scraper and washed two times with PBS buffer. After that, the HUVECs are lysed during 1 h of cells incubation with Tris-HCl 1 mM pH 7.4 buffer. After the total cell lyses, the membranes of HUVECs are separated by multiple ultra-centrifugations. The membranes are solubilized with Triton X-100 1 % in Tris-HCl 100 mM pH 8.0 buffer and solubilized extracts of membranes of HUVECs are obtained. Before the lyses of the HUVECs, the cells concentration is 4x106cells/sample.

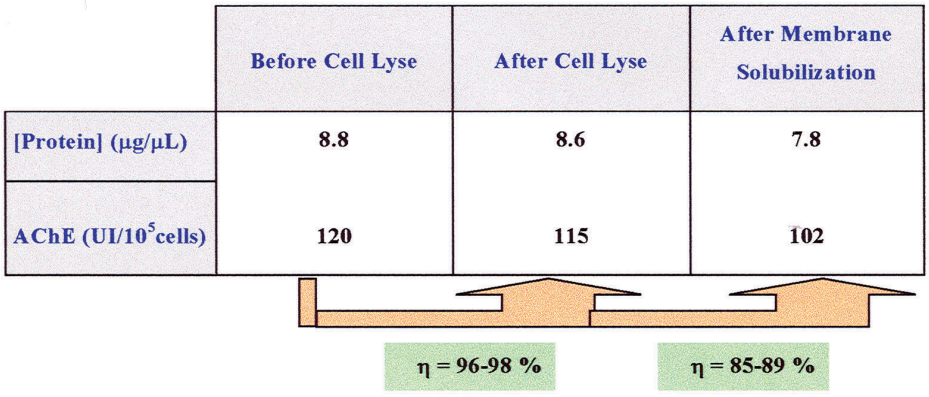


**Figure 2 —** Evaluation of AChE activity or Protein concentration in different phases of membrane of HUVECs solubilization process.

## RESULTS

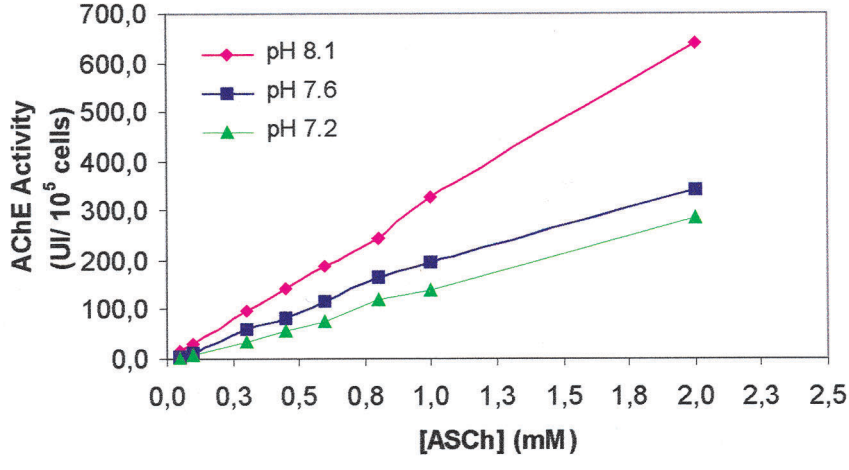


**Figure 3 —** Determination of AChE activity of membranes of HUVECs *vs* ASCh substrate concentration at different pH values.

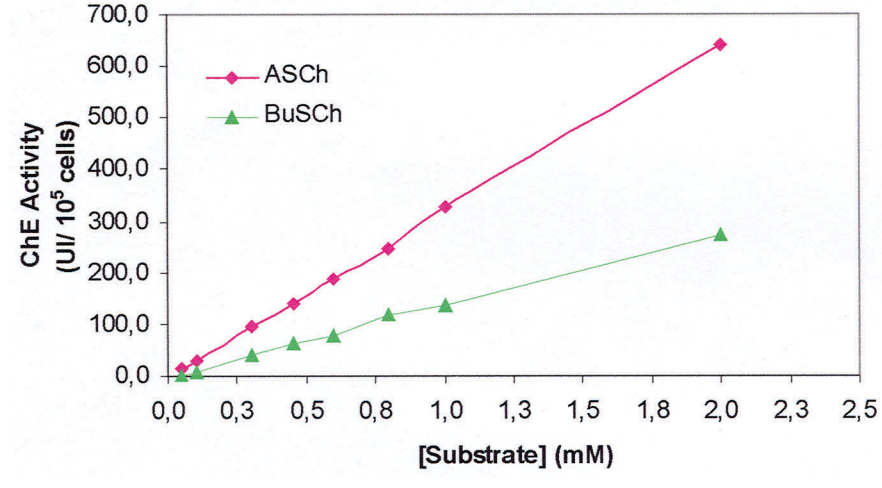


**Figure 4 —** Determination of ChE activity of membranes of HUVECs *vs* different substrate concentration at pH = 8.1.

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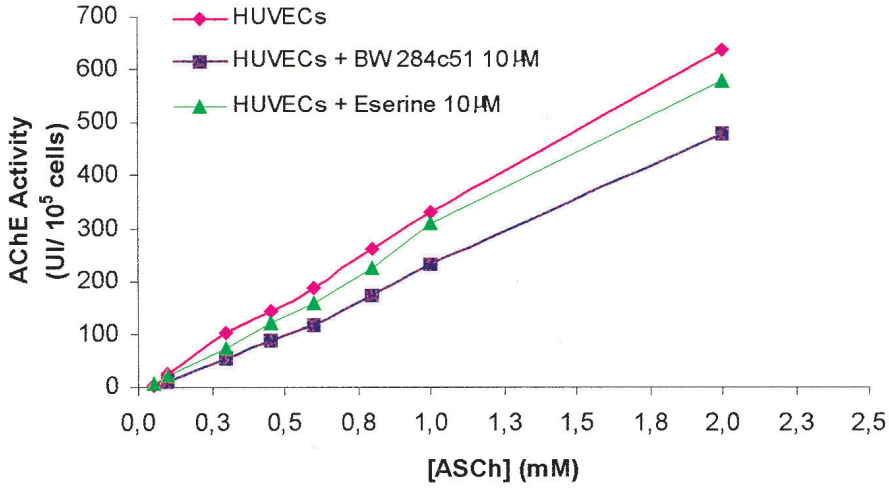



**Figure 5 —** Determination of AChE activity of membranes of HUVECs with different substrate inhibitors at pH = 8.1.

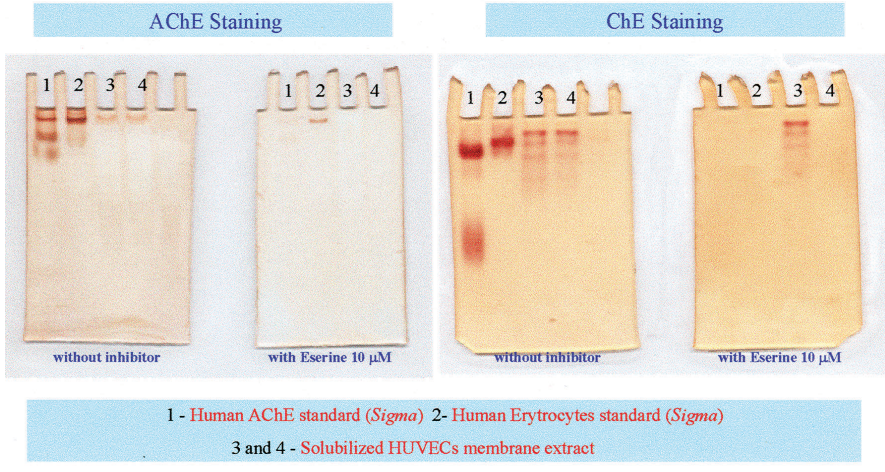



**Figure 6 —** Results of electrophoresis of PAGE 7.5% with Triton X-100 0.5% (non denaturating conditions) of solubilized HUVECs membrane extracts and human AChE standards The gels are incubated with or without eserine 10 mM and staining with the AChE or ChE staining.

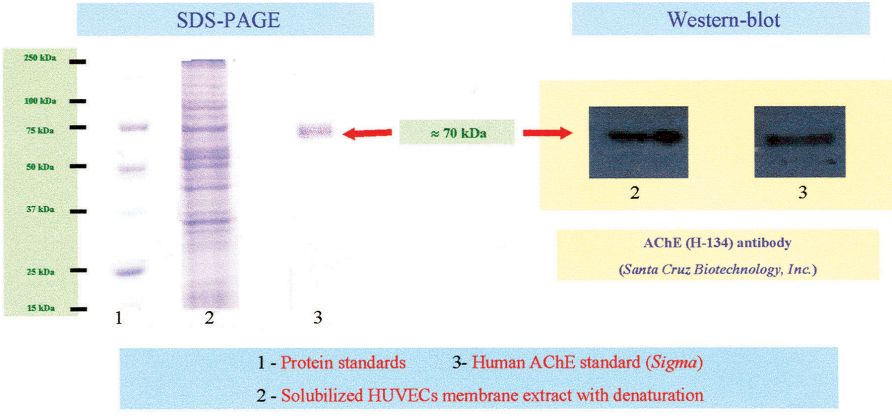
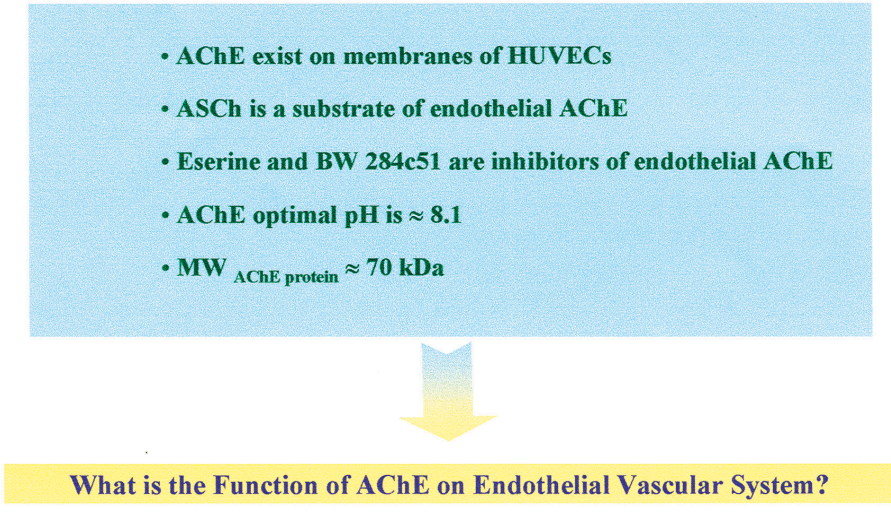



**Figure 2** — Results of electrophoresis of SDS-PAGE 7.5% with SDS 0.5% (denaturating conditions) and Western blot radiographic films of solubilized HUVECs membrane extracts and human AChE standards. The SDS gel is marked with the protein standards.


## ACKNOWLEDGMENS

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