

***C'E' DEL NUOVO NEGLI INIBITORI  
DELLA COLINOESTERASI?***

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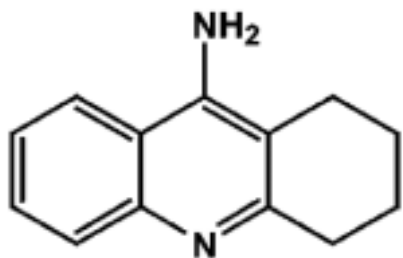
**Dipartimento di Scienze Farmacologiche**

**Universita' di Milano**

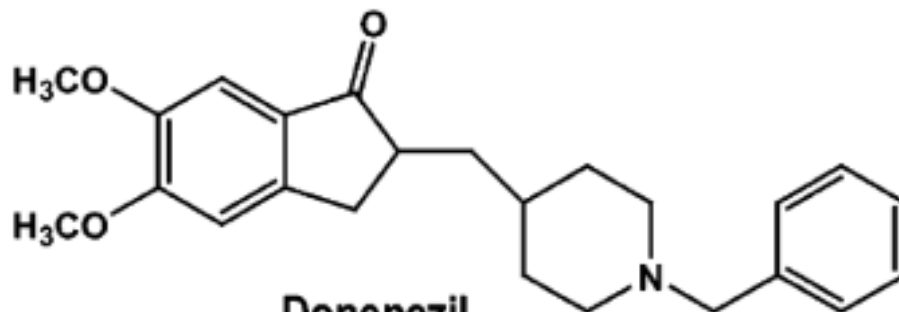
**52^ Congresso S.I.G.G.**

**Firenze 30.11.2007**

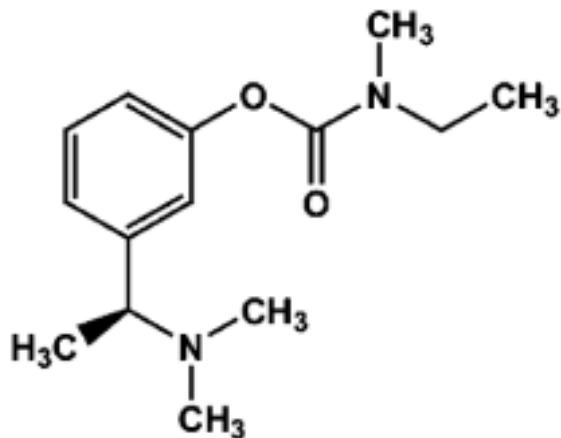
# Gli inibitori della AChE in uso clinico oggi



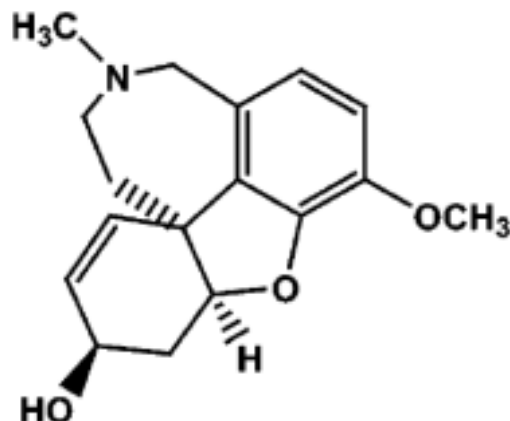
Tacrine



Donepezil



Rivastigmine



Galantamine

## Non solo aumento di Acetilcolina: evidenze cliniche

- i farmaci sono attivi (anche se non sempre lo sono) in pazienti AD moderati, cioè quando il deficit colinergico non è ancora evidente.
- l'effetto clinico è presente per un certo periodo di tempo dopo la sospensione del trattamento quando l'effetto sulla colinesterasi è svanito.
- l'efficacia dei farmaci si osserva anche con trattamenti prolungati, oltre le trenta settimane, quando, teoricamente, si dovrebbero verificare fenomeni di tolleranza in seguito all'aumento di acetilcolina.

# Non solo aumento di Acetilcolina: evidenze di altri ruoli della AChE

AChE e' presente prima della formazione di sinapsi colinergiche

AChE e' presente anche in neuroni *non* colinergici

Influenza la neuritogenesi e la sinaptogenesi

Favorisce la formazione di fibrille di A $\beta$

Ruolo nell'ematopoiesi e trombopoiesi

# **EFFETTI DEGLI INIBITORI DELLE CHOLINOESTERASI DOVUTI ALL'AUMENTO DI ACETILCOLINA**

- EFFETTI CLINICI SINTOMATICI**
  
- EFFETTI SUL METABOLISMO DI APP TRAMITE RECETTORI  
MUSCARINICI**
  
- EFFETTI DI NEUROPROTEZIONE MEDIATI DA RECETTORI  
NICOTINICI**

## EVIDENZE DELL'AZIONE SUL METABOLISMO DI APP

- sAPP $\alpha$  release is increased by stimulation of PKC and MAPK through muscarinic agonists or glutamate

in cell lines: Nitsch et al., 1992; Caporaso et al., 1992; Hung et al., 1993; Buxbaum et al., 1993; Racchi et al., 1999; Svensson and Nordberg, 1998; Lahiri et al., 2000

in brain slices: Caputi et al., 1997; Pakasky et al., 2001

- AChE cause selective muscarinic activation of alpha-secretase through MAPK signalling pathway  
Yogev-Falach et al., 2002

**MA CI SONO ANCHE EFFETTI**

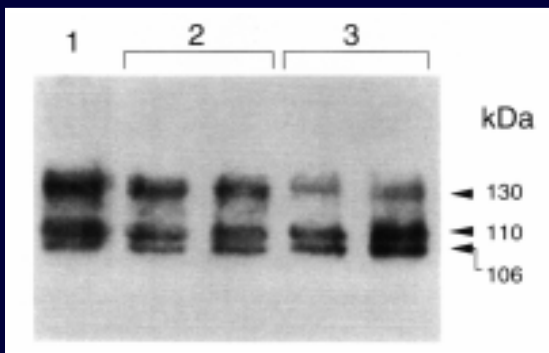
**DOVUTI AD UN RUOLO**

**NON CATALITICO DELLA AChE**

# Platelet APP Forms Ratio from Control Subjects and AD Patients

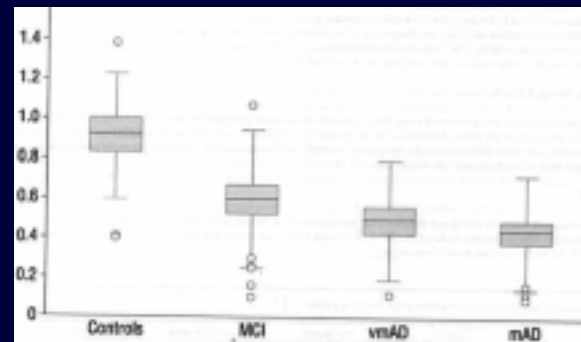


Control    AD Patients



*Di Luca et al., Arch Neurol 1998*

APP ratios correlate with the severity



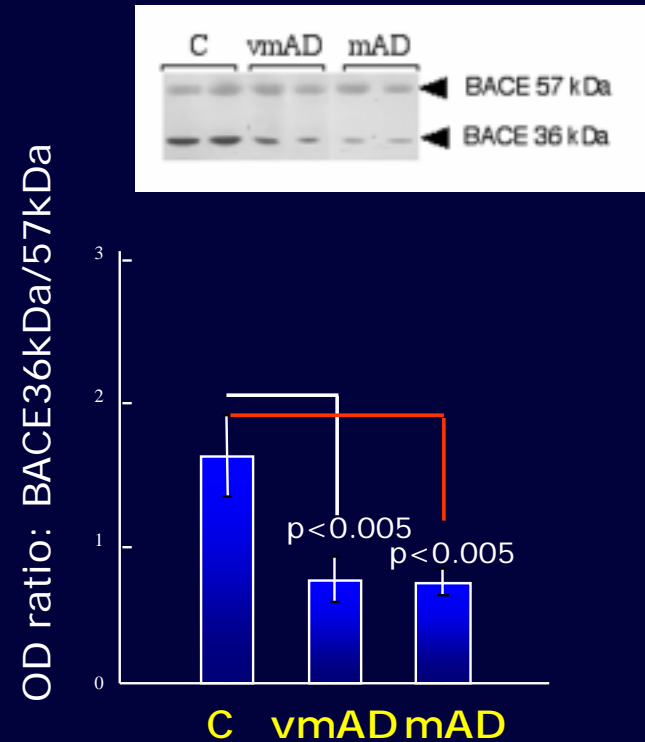
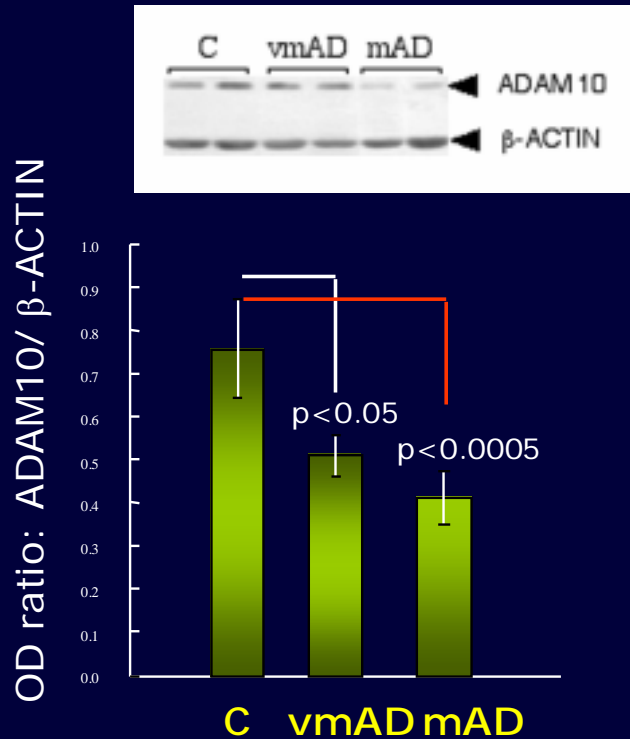
*Padovani et al, Arch. Neurol. 2002*

# APPr in platelets identifies sporadic AD patients and correlates with the severity of the disease

SUBJECTS	MMSE(Score±S.D.)	APPr
<b>Controls</b>		
Non-demented	28.9 ± 1.8	0.97 ± 0.46
Demented non-AD	17.3 ± 0.7	0.94 ± 0.38
Very mild AD	25.7 ± 0.78	0.45 ± 0.16°
Mild AD	20.4 ± 3.6	0.45 ± 0.12°
Moderate AD	13.8 ± 2.5	0.31 ± 0.17° *
Severe AD	5.3 ± 0.7	0.22 ± 0.1° * \$
<b>Mild Cognitive Imp.</b>		
stable	28.7 ± 1.6	0.73 ± 0.32
non-AD converting	27.7 ± 2,3	0.83 ± 0.27
AD converting	26.4 ± 1.5	0.36 ± 0.28§

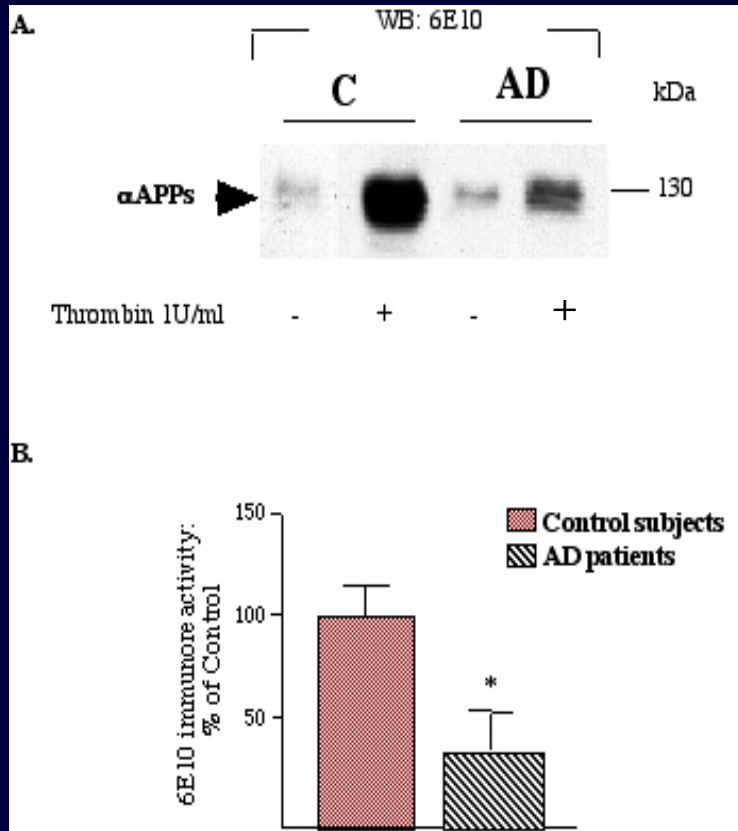
Adapted From: Borroni et al. *Arch.Neurol.* 2003; Colciaghi et al. *Neurology* 2004

# ADAM 10 and BACE in platelets of mild AD

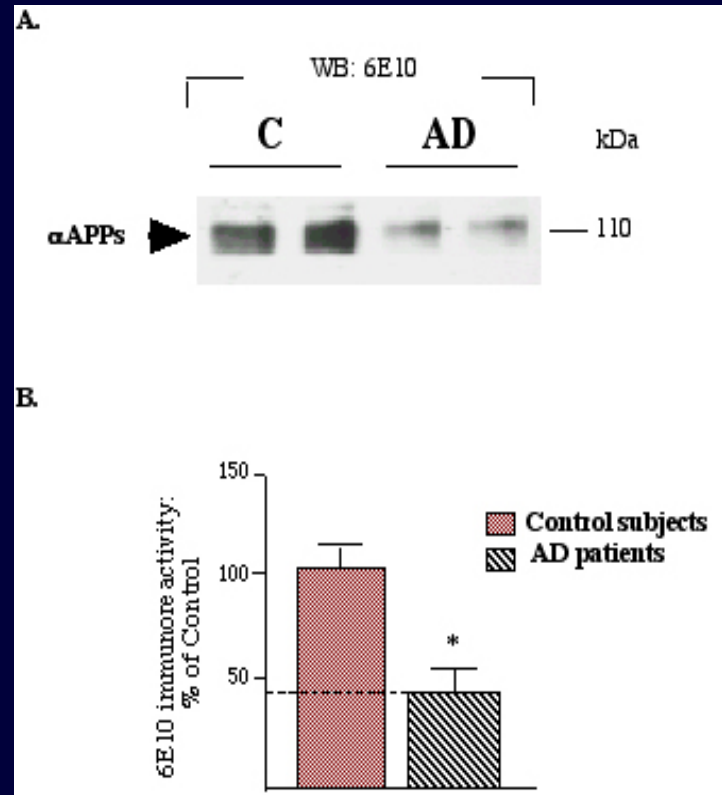


# $\alpha$ -secretase activity is decreased in AD patients

## Platelets

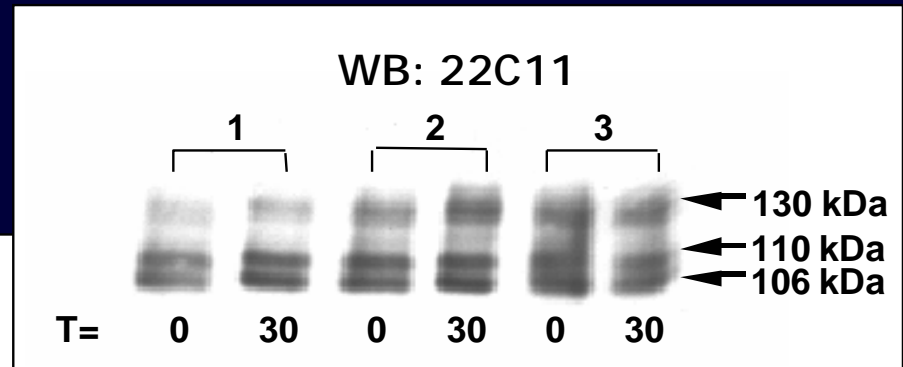
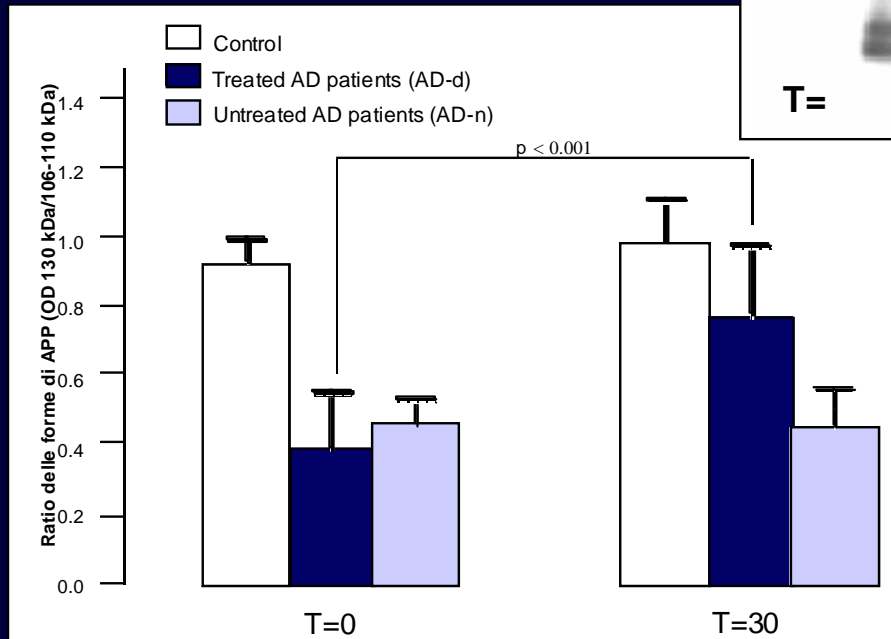


## CSF

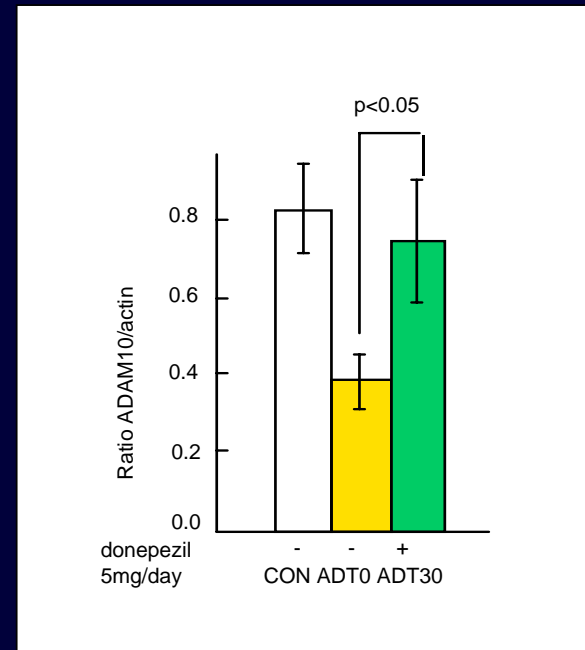
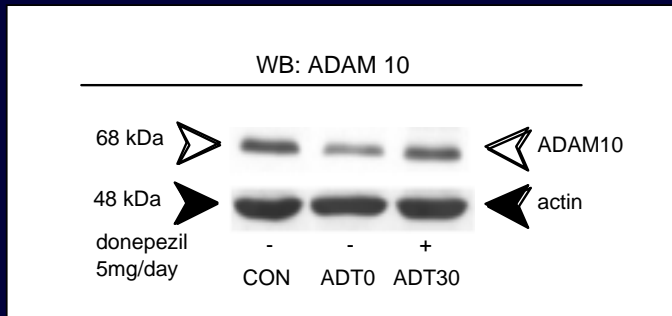


Colciaghi, et al., 2002 Mol. Med.

# Donepezil effect on platelet APP forms



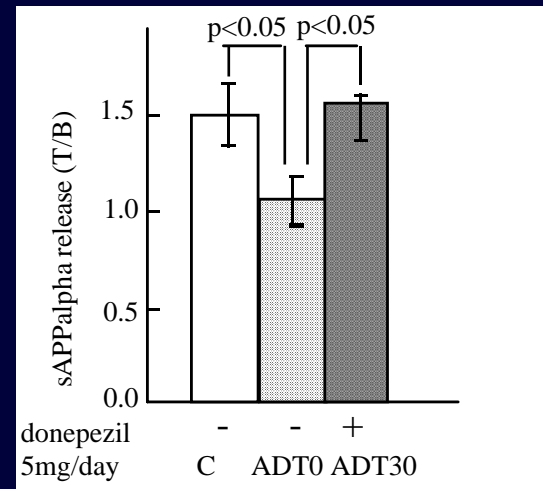
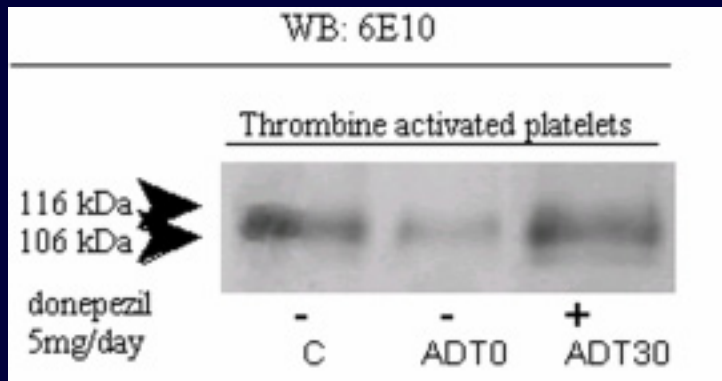
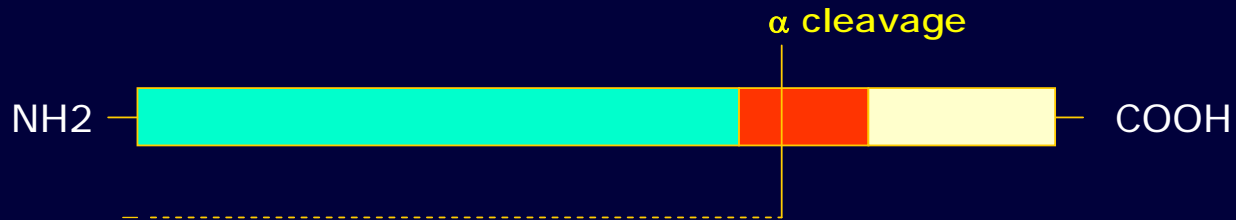
# Donepezil increases levels of ADAM 10



ADAM10/actin Ratio

*...Does it affect also alpha-secretase activity?*

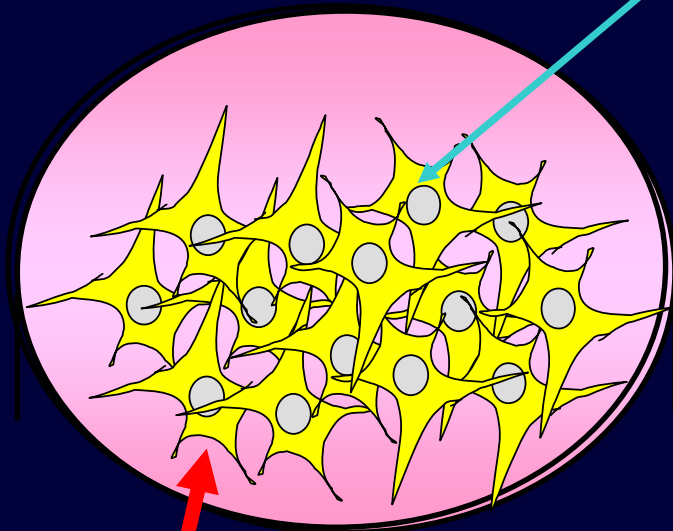
# $\alpha$ -secretase activity in platelets of AD patients



# How can we explain the effect of donepezil on ADAM 10 in human platelets?

A follow up **in vitro** RETINOIC ACID

Neuroblastoma cell line



SH-SY5Y

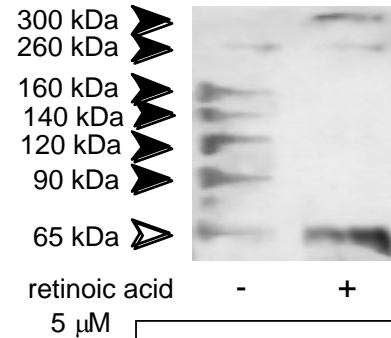
**DONEPEZIL**

1.5  $\mu$ M

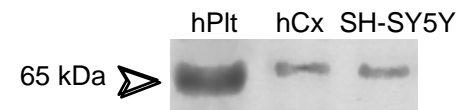
## Cholinergic phenotype

- ACh
- mAChR, nAChR
- AChE

### WB: AChE in SH-SY5Y cells

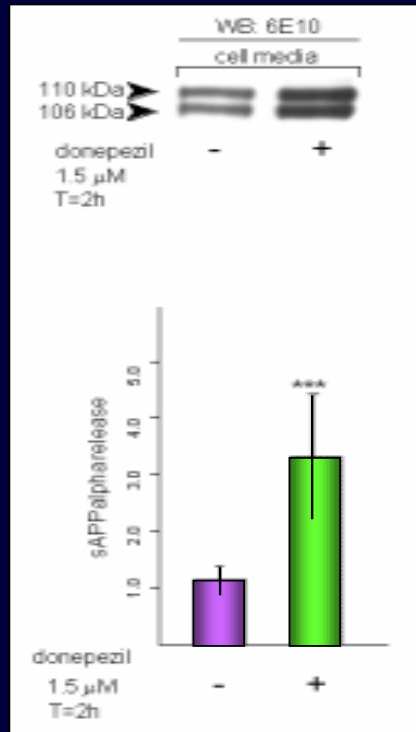


### WB: AChE

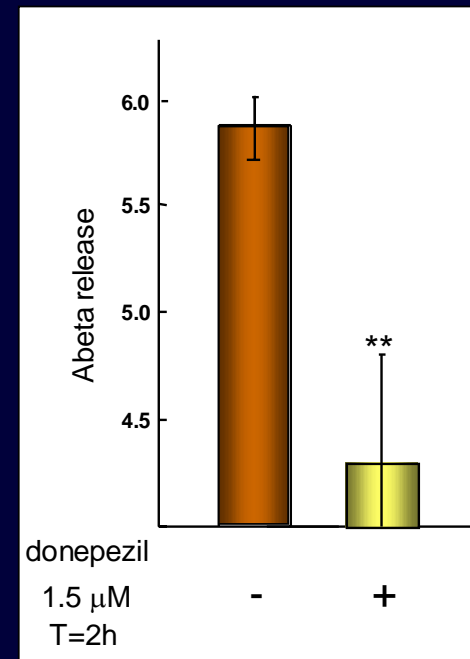


# Donepezil effect in neuroblastoma cell line SH-SY5Y

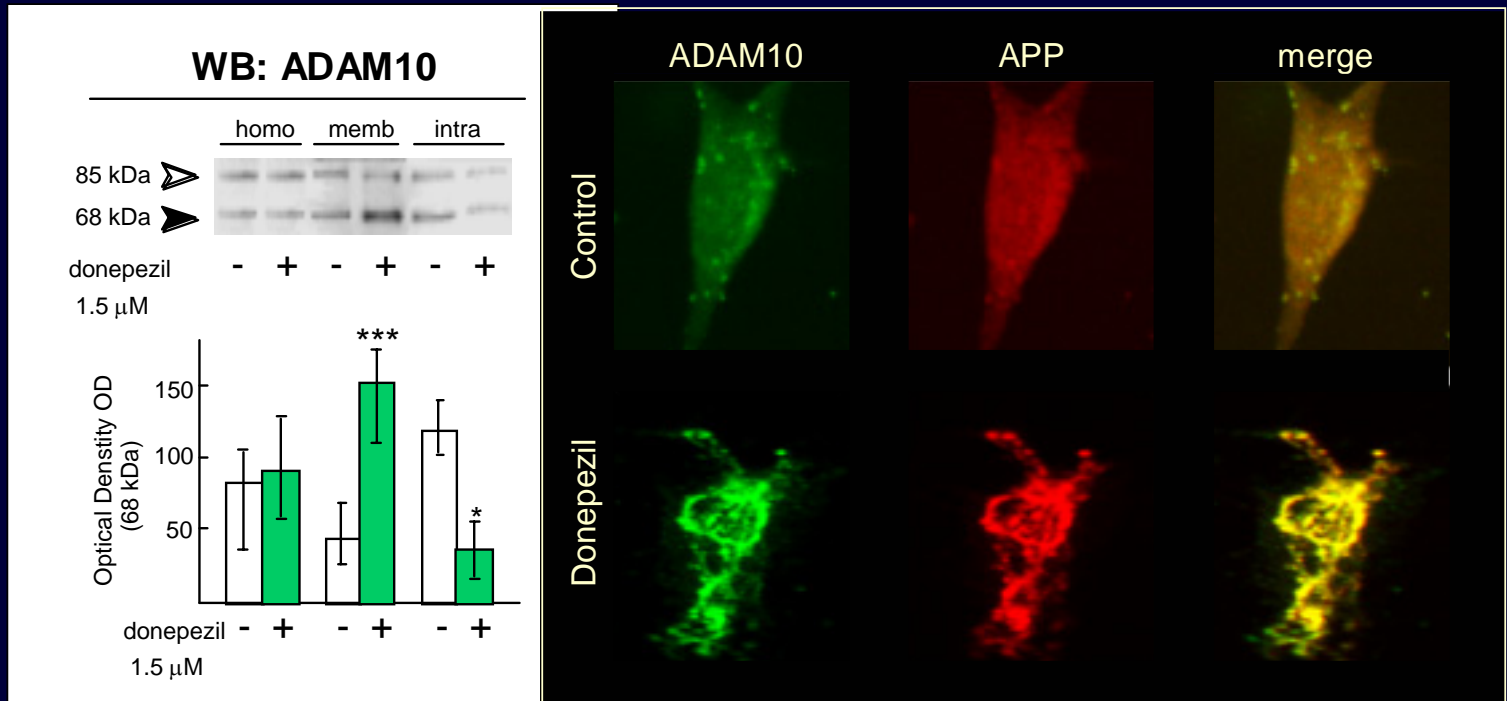
## $\alpha$ APPs release



## $A\beta_{42}$ release

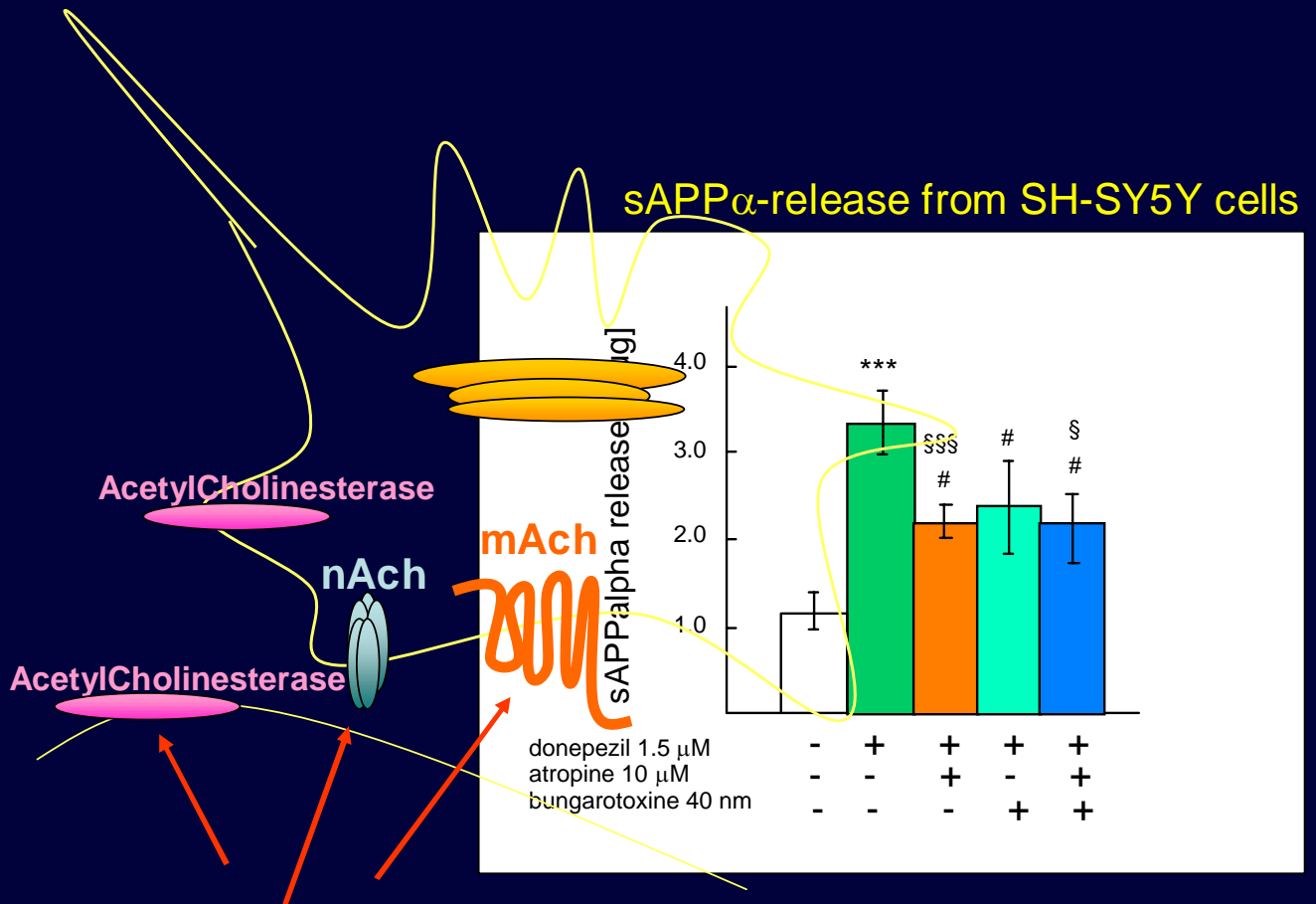


# Donepezil increases levels of key players in APP metabolism in cell membranes



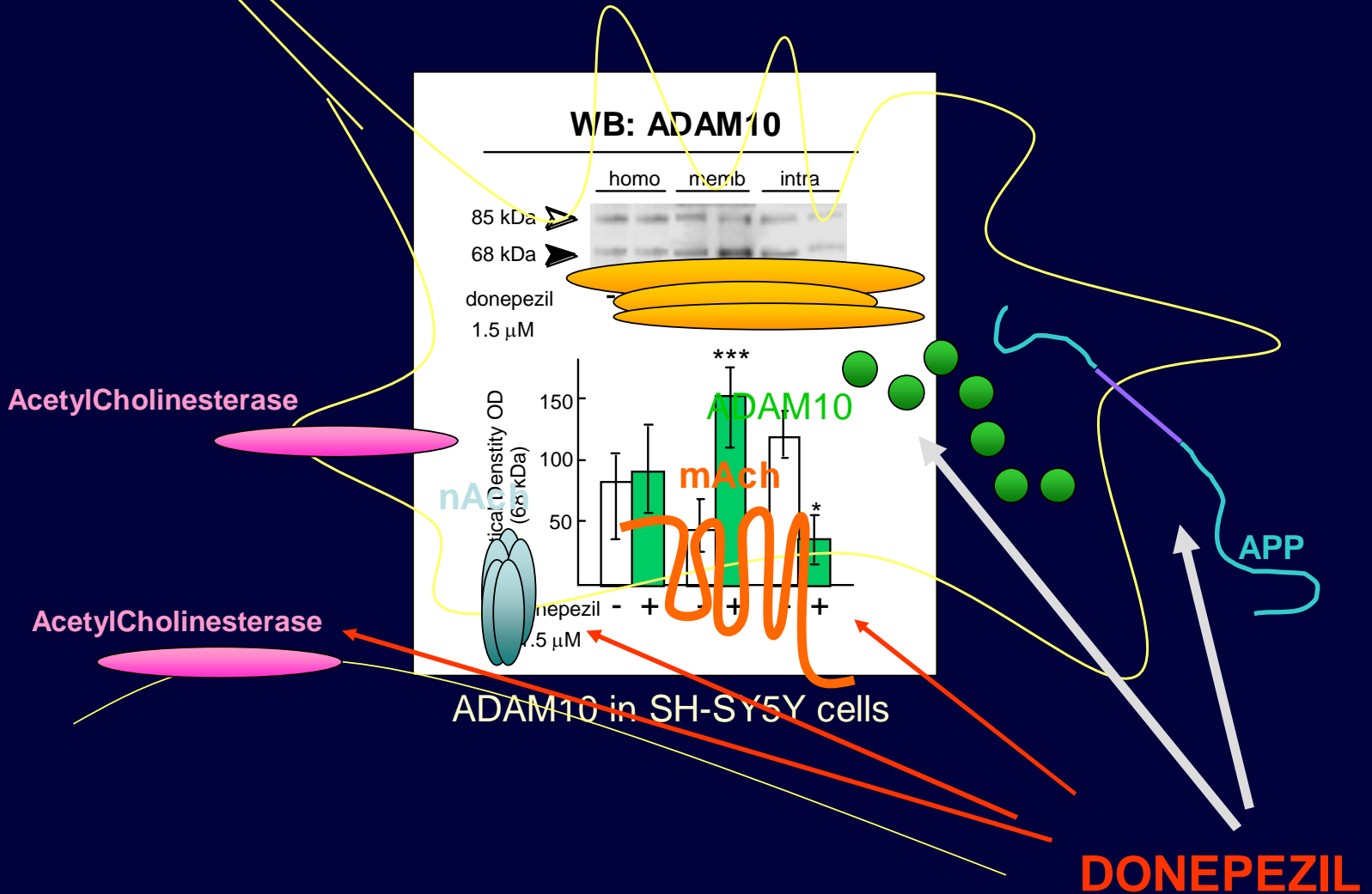
ADAM10 in SH-SY5Y cells

# How can Donepezil exert this effect?



**Donepezil receptor-independent manner!**

# Donepezil - an influence on protein trafficking?

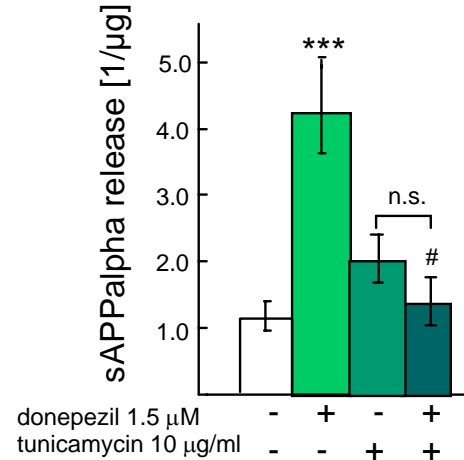
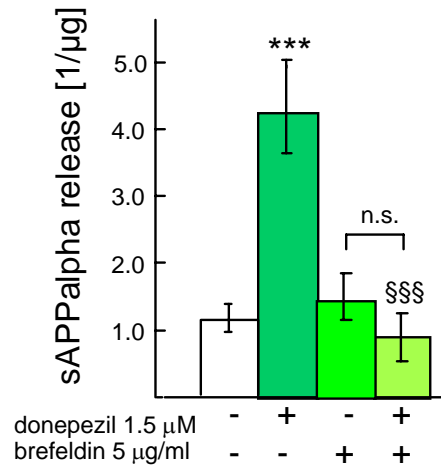


# Disruption of protein trafficking and maturation causes decreased ADAM10 levels at cell membrane

## WB: ADAM10 in SH-SY5Y cells

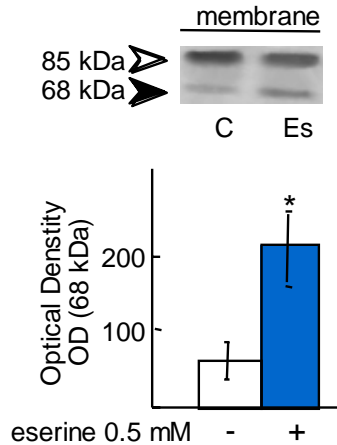


## sAPPalpha release from SH-SY5Y cells

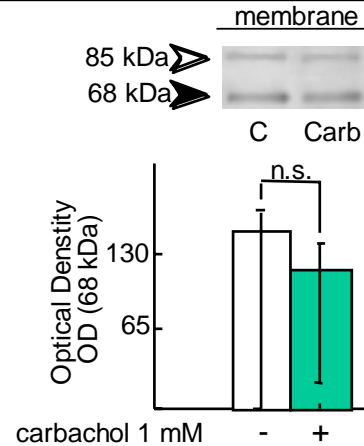


# The Donepezil mediated effect is a characteristic of AChEIs

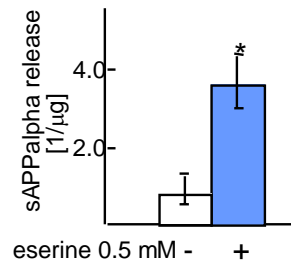
## WB: ADAM10 in SH-SY5Y cells



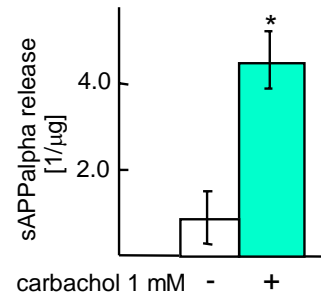
## WB: ADAM10 in SH-SY5Y cells



## sAPP $\alpha$ release from SH-SY5Y cells



## sAPP $\alpha$ release from SH-SY5Y cells



Eserine

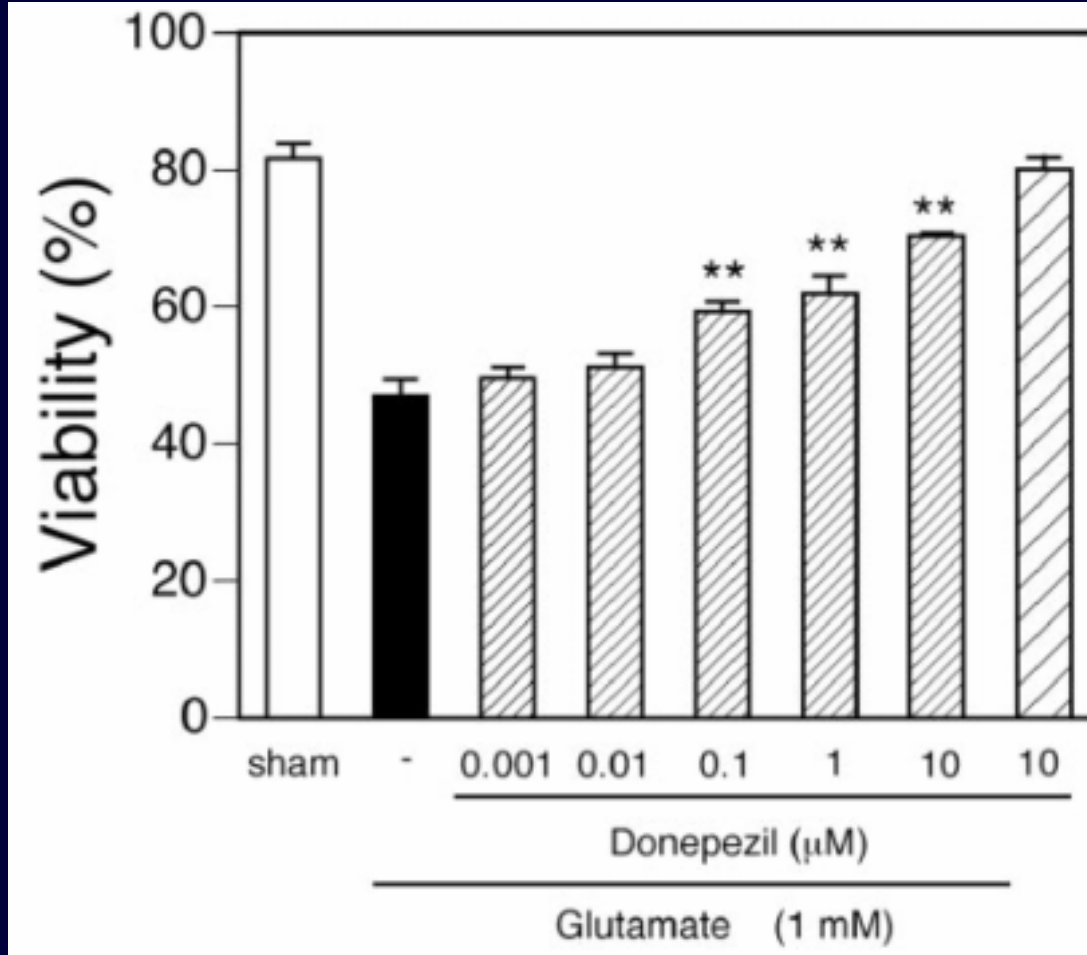
Carbachol

**ALTRI EFFETTI**

**DOVUTI AD UN RUOLO**

**NON CATALITICO DELLA AChE**

# Donepezil protegge in modo dose-dipendente dalla neurotossicità da Glu in culture neuronali



*Takada et al., 2003*

...ma non tutti gli inibitori sono efficaci

..e non e' correlato alla azione sulla estersasi

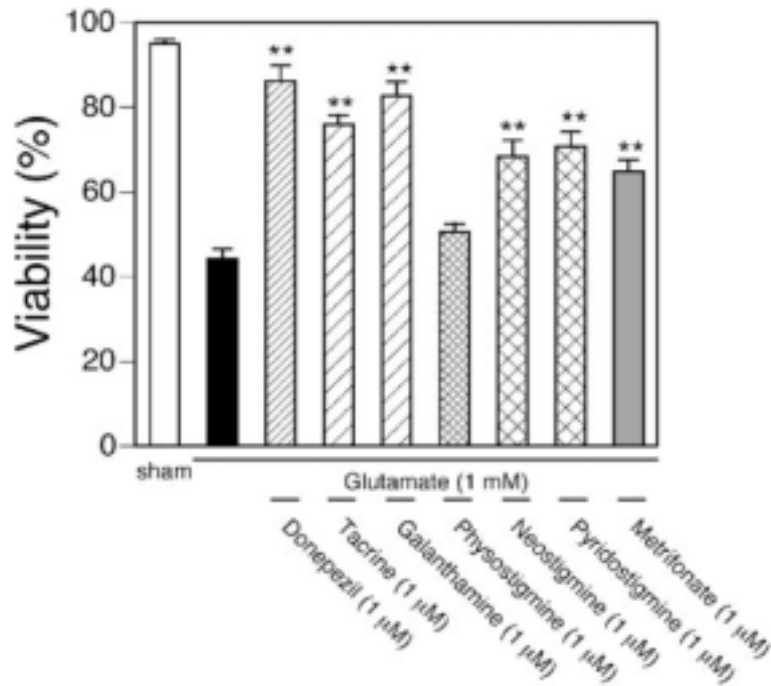


TABLE 1  
Comparison of IC<sub>50</sub> of AChE inhibitors

Drug	IC <sub>50</sub> <sup>a</sup>	IC <sub>20</sub> <sup>b</sup>	IC <sub>50</sub> <sup>c</sup>	IC <sub>50</sub> <sup>d</sup>
Donepezil	6.7 ± 0.35		13.6	
Tacrine	77 ± 1.4	450	93.0	80
Galanthamine			1995	
Physostigmine	0.67 ± 0.015	18	251	60
Neostigmine		25		15
Pyridostigmine				300
Metrifonate		1080		

<sup>a</sup> IC<sub>50</sub> values for AChE in the rat brain are taken from Ogura et al. (2000).

<sup>b</sup> IC<sub>50</sub> values for AChE in human cortex are taken from Rakonczay et al. (1998).

<sup>c</sup> IC<sub>20</sub> values for AChE in the rat cerebral cortex are taken from Tang (1996).

<sup>d</sup> IC<sub>20</sub> values for AChE in the mouse brain are taken from Arnal et al. (1990).

Takada et al., 2003

# ALTRI EFFETTI DOVUTI AD UN RUOLO NON CATALITICO DELLA AChE

Neuron, Vol. 16, 881-891, April, 1996, Copyright ©1996 by Cell Press

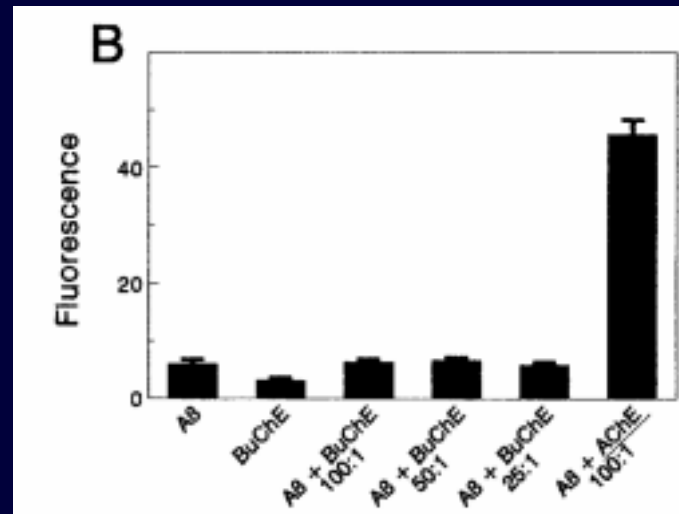
## **Acetylcholinesterase Accelerates Assembly of Amyloid- $\beta$ -Peptides into Alzheimer's Fibrils: Possible Role of the Peripheral Site of the Enzyme**

**Nibaldo C. Inestrosa, Alejandra Alvarez, Cristian A. Perez, Ricardo D. Moreno,  
Matias Vicente, Claudia Linker, I. Casanueva, Claudio Soto,  
Jorge Garrido**

Table 2. AChE Is an Efficient Promoter of Amyloid Formation

Protein	Amyloid Formation (Thioflavine-T Fluorescence)	Relative Enhancement
A $\beta$ alone	14.1 $\pm$ 2.7	1.00
A $\beta$ plus apoE3	17.6 $\pm$ 3.9	1.25
A $\beta$ plus $\alpha_1$ -ACT	19.2 $\pm$ 4.1	1.36
A $\beta$ plus AChE	58.2 $\pm$ 3.7 <sup>a</sup>	4.12

...ma non la BuChE



# Inibizione di aggregazione ed attivita' AChE non sono correlate

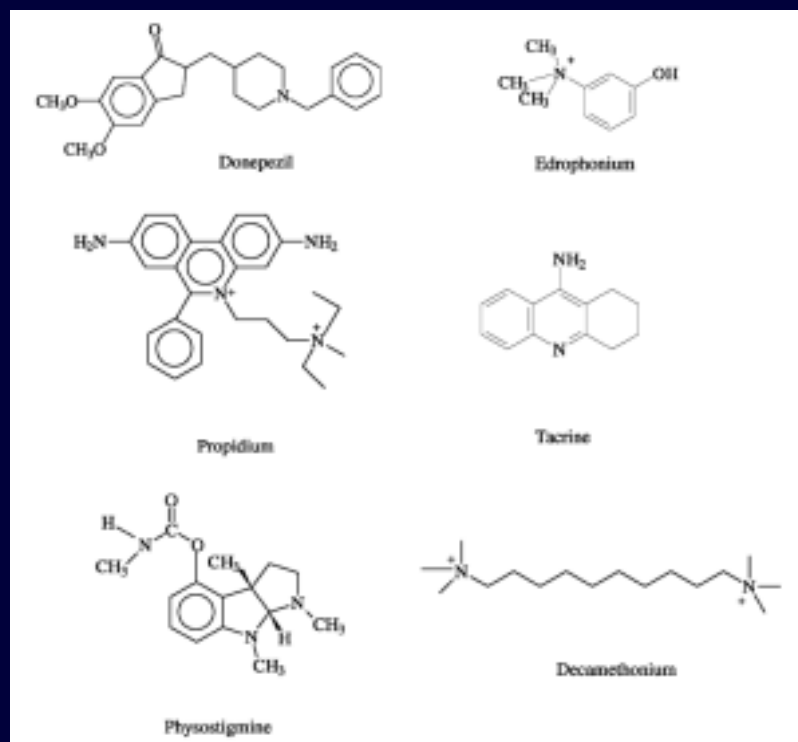


Table 1  
Inhibition of AChE-induced A $\beta$  aggregation produced by the tested compounds at 100  $\mu$ M concentration and  $IC_{50}$  against HuAChE

Compounds	Fibrillogenesis inhibition (%) (SD within 3%)	Inhibition type [25–28]	HuAChE $IC_{50}$ (M)
Propidium	82	Non-competitive	$3.23 \pm 0.22 \times 10^{-5}$
Decamethonium	25	Mixed	$2.05 \pm 0.21 \times 10^{-5}$
Donepezil	22	Mixed	$2.31 \pm 0.48 \times 10^{-8}$
Tacrine	7	Mixed	$4.24 \pm 0.21 \times 10^{-7}$
Edrophonium	0	Competitive	$7.30 \pm 0.35 \times 10^{-6}$
Physostigmine	30	Pseudo-irreversible	$1.34 \pm 0.54 \times 10^{-8}$

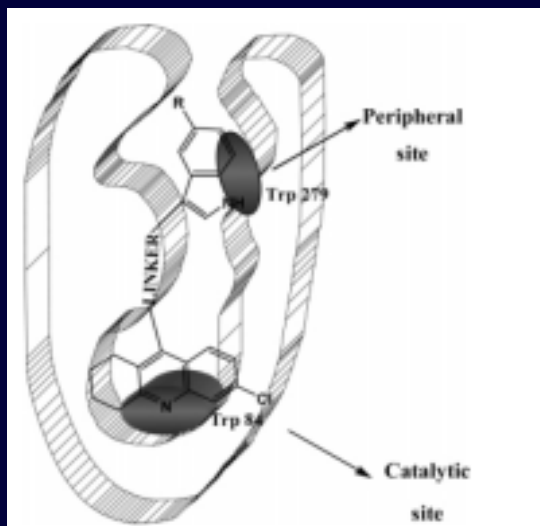
# PER TRARRE VANTAGGIO DALLE AZIONI CATALITICHE E NON- DELLA AChE

*J. Med. Chem.* **2005**, *48*, 7223–7233

7223

## Design, Synthesis, and Biological Evaluation of Dual Binding Site Acetylcholinesterase Inhibitors: New Disease-Modifying Agents for Alzheimer's Disease

Pilar Muñoz-Ruiz,<sup>§</sup> Laura Rubio,<sup>§</sup> Esther García-Palomero,<sup>§</sup> Isabel Dorronsoro,<sup>§</sup> María del Monte-Millán,<sup>§</sup> Rita Valenzuela,<sup>§</sup> Paola Usán,<sup>§</sup> Celia de Austria,<sup>§</sup> Manuela Bartolini,<sup>‡</sup> Vincenza Andrisano,<sup>‡</sup> Axel Bidon-Chanal,<sup>#</sup> Modesto Orozco,<sup>†</sup> F. Javier Luque,<sup>#</sup> Miguel Medina,<sup>§</sup> and Ana Martínez<sup>\*,§</sup>



**Table 3.** Inhibition of A $\beta$ <sub>1–40</sub> Peptide Aggregation (with and without Human AChE) by Some Heterodimers and Reference Compounds

compd	with AChE		without AChE
	inhibition at 100 $\mu$ M $\pm$ SEM (%)	IC <sub>50</sub> $\pm$ SEM ( $\mu$ M)	inhibition at 100 $\mu$ M $\pm$ SEM (%)
<b>5</b>	98 $\pm$ 2	2 $\pm$ 1	49 $\pm$ 7
<b>6</b>	96 $\pm$ 0	6 $\pm$ 2	65 $\pm$ 5
<b>18</b>	84 $\pm$ 1	7 $\pm$ 4	63 $\pm$ 10
<b>20</b>	99 $\pm$ 4	2 $\pm$ 0	63 $\pm$ 4
<b>19</b>	82 $\pm$ 1	15 $\pm$ 3	62 $\pm$ 14
propidium	82 <sup>a</sup>	13 $\pm$ 0	46 $\pm$ 5
decamethonium	25 <sup>a</sup>	nd <sup>b</sup>	0
donepezil	22 <sup>a</sup>	nd <sup>b</sup>	0
tacrine	7 <sup>a</sup>	nd <sup>b</sup>	0
edrophonium	0 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>
physostigmine	30 <sup>a</sup>	nd <sup>b</sup>	nd <sup>b</sup>

<sup>a</sup> SD within 3% (data from ref 43). <sup>b</sup> nd = not determined.

# CONCLUSIONI

- Evidenze biochimiche confermano quanto emerge dalla clinica:  
gli AChEI hanno anche azioni dovute ad effetti non-mediati da Acetilcolina e quindi - e a buon diritto- devono essere considerati farmaci in grado di modificare il decorso della malattia
- Probabilmente in futuro avremo molecole capaci di sfruttare anche gli effetti non legati all'attività catalitica

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Monica Di Luca  
Roberta Epis  
Fabrizio Gardoni  
Lorenza Magno  
Matteo Malinverno  
Elena Marcello  
Daniela Mauceri  
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CEND



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