

ACETYLCHOLINE

ACETYLCHOLINE

STRUCTURE

VOIES CHOLINERGIQUES

INTERET PHYSIOPATHOLOGIQUE ET PHARMACOLOGIQUE

SYNAPSE CHOLINERGIQUE

SYNTHESE

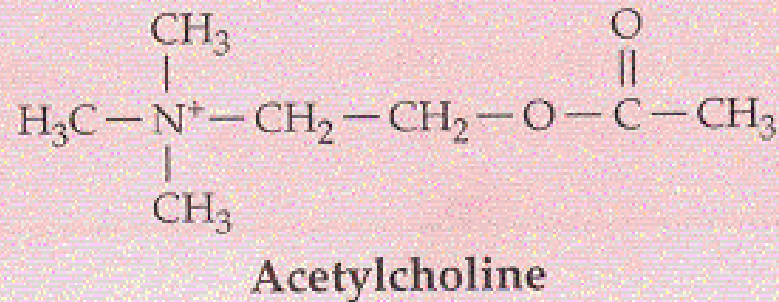
CAPTURE

DEGRADATION

INTERACTION LIGAND/RECEPTEUR CHOLINERGIQUE

INTERACTION ENTRE RECEPTEURS

ACETYLCHOLINE - STRUCTURE



1907: identification de l'acétylcholine (Hunt)

1914: reconnaissance de l'effet vasodilatateur de l'acétylcholine (Dale)

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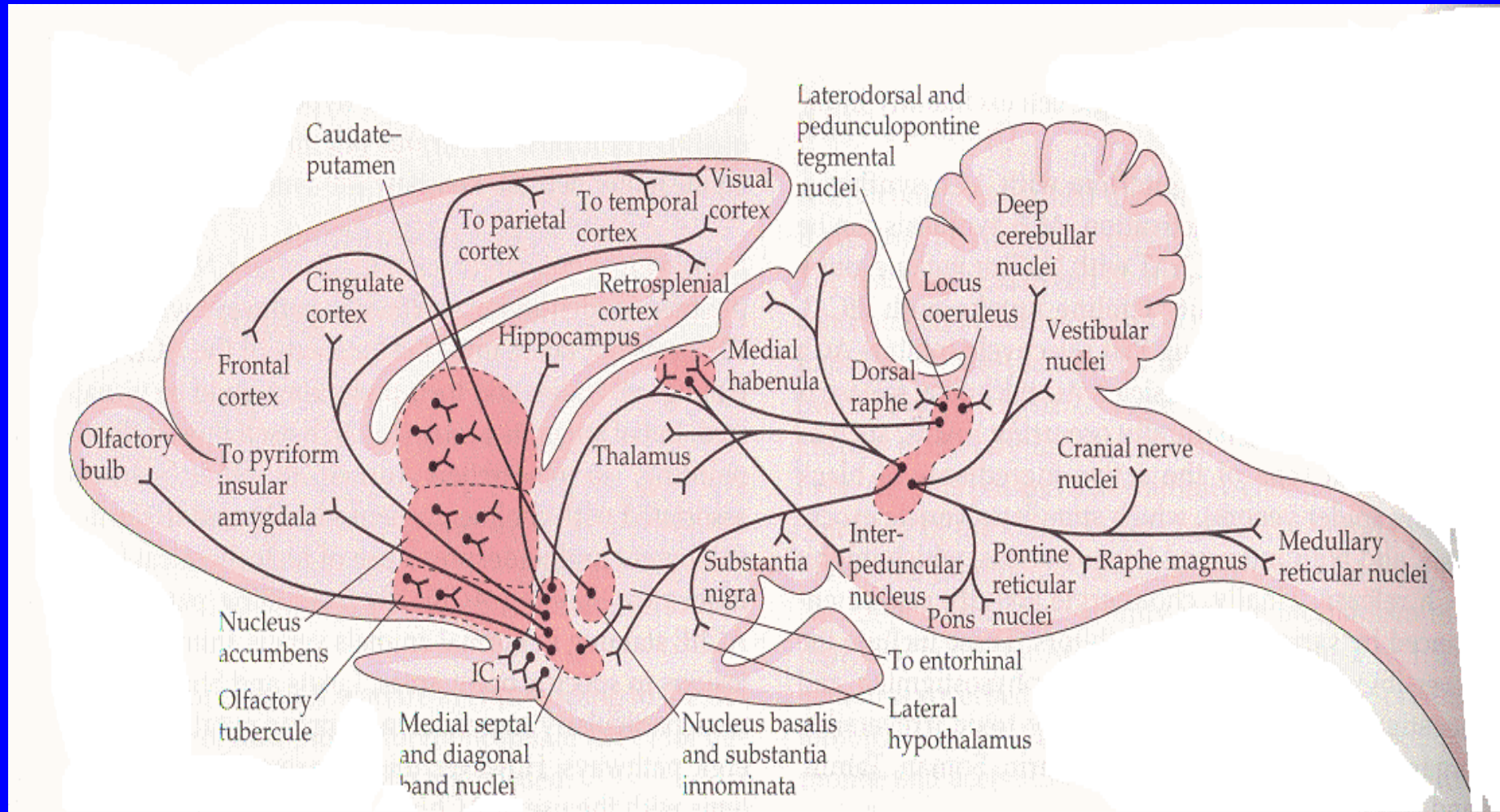
CAPTURE

DEGRADATION

INTERACTION LIGAND/RECEPTEUR CHOLINERGIQUE

INTERACTION ENTRE RECEPTEURS

NOYAUX CHOLINERGIQUES - LOCALISATION



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ACETYLCHOLINE - PHYSIOPATHOLOGIE

Maladie de Parkinson

Signes cliniques (bradykinésie, rigidité des mouvements)



Anticholinergiques

Maladie d'Alzheimer

Signes cliniques (pertes des fonctions cognitives)



Cholinergiques

ALZHEIMER - PHYSIOPATHOLOGIE

- **Atrophie du cortex cérébral**
- **Diminution du nombre de noyaux corticaux et sous corticaux**
- **Plaques séniles (peptide β -amyloid)**
- **Enchevêtrement neuro-fibrillaire (proteine Tau)**

ALZHEIMER - HYPOTHESE CHOLINERGIQUE

Chez les patients décédés de la Maladie d'Alzheimer:

- Baisse de l'activité de la choline acetyltransferase dans le cortex et l'hippocampe
- Surexpression du transporteur à haute affinité de la choline dans le cortex
- Altération des performances cognitives par des agents antimuscariniques

ALZHEIMER - NEUROCHIMIE

Disease stage ^b	EAA-containing neurones			ACh-containing neurones	5-HT-containing neurones	NA-containing neurones	GABA-containing cortical interneurones
	Entorhinal cortex	Hippocampus	Association cortex				
I	++						
II	++ +	++	++	++	++	+	
III	+++ + +	++ + +	++ ++	++ ++	++ ++	++	
IV	+++ + + +	+++ + + +	+++ + + +	+++ + + +	+++ + + +	+++ + + +	+
V	+++ + + +	+++ + + +	+++ + + +	+++ + + +	+++ + + +	+++ + + +	++
VI	+++ + + +	+++ + + +	+++ + + +	+++ + + +	+++ + + +	+++ + + +	+++

^aAbbreviations: Aβ, amyloid-β peptide; ACh, acetylcholine; EAA, excitatory amino acid; NA, noradrenaline.

Perte neuronale ~ diminution en neurotransmetteurs

Déficit majeur en **acétylcholine**

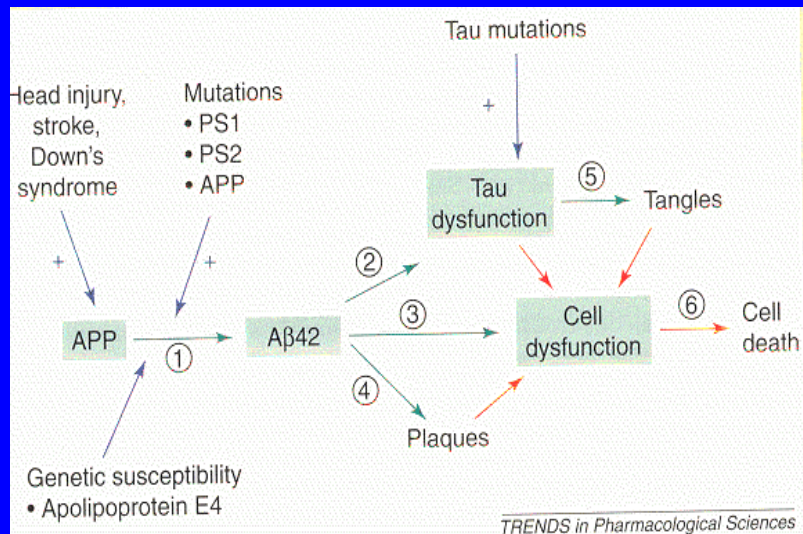
Déficit en sérotonine, glutamate et neuropeptides

ALZHEIMER - THERAPEUTICAL STRATEGIES

Acétylcholine

PRECURSEURS D'ACETYLCHOLINE

INHIBITEURS D'ACETYLCHOLINE ESTERASES



INHIBITORS OF β/γ SECRETASES

INHIBITORS OF TAU PHOSPHORYLATION

INHIBITOR OF TILTED PEPTIDE

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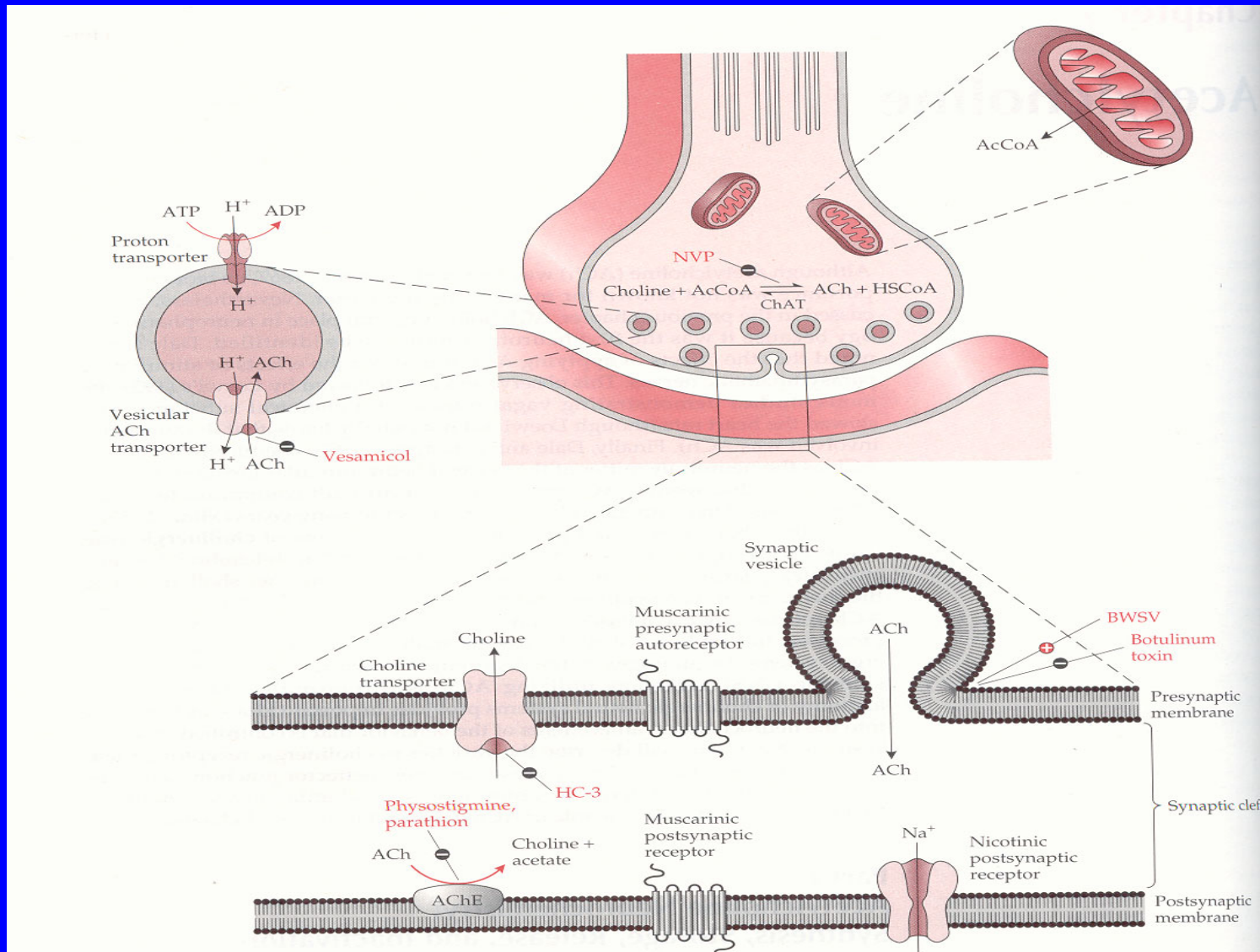
CAPTURE

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INTERACTION LIGAND/RECEPTEUR CHOLINERGIQUE

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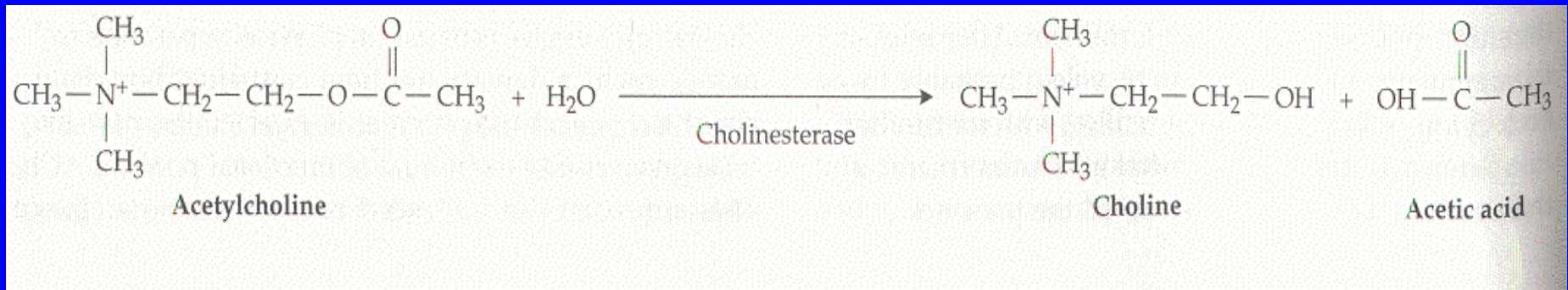
SYNTHESE

DEGRADATION

INTERACTION LIGAND/RECEPTEUR CHOLINERGIQUE

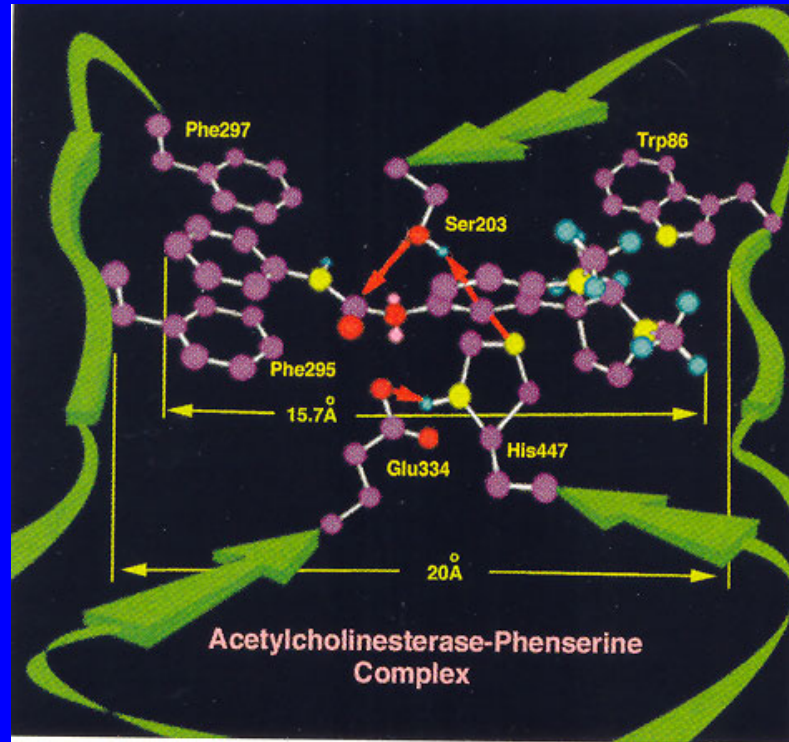
INTERACTION ENTRE RECEPTEURS

ACETYLCHOLINE - DEGRADATION



- 1 molécule d'enzyme peut inactiver 5000 molécules d'acétylcholine par sec
- Temps d'inactivation: 100 msec pour une molécule d'acétylcholine

ACETYLCHOLINE ESTERASE



An acyl pocket:

- Defines the active center involved in the catalysis of acetylcholine
- Is centered around an active serine residue Ser 203

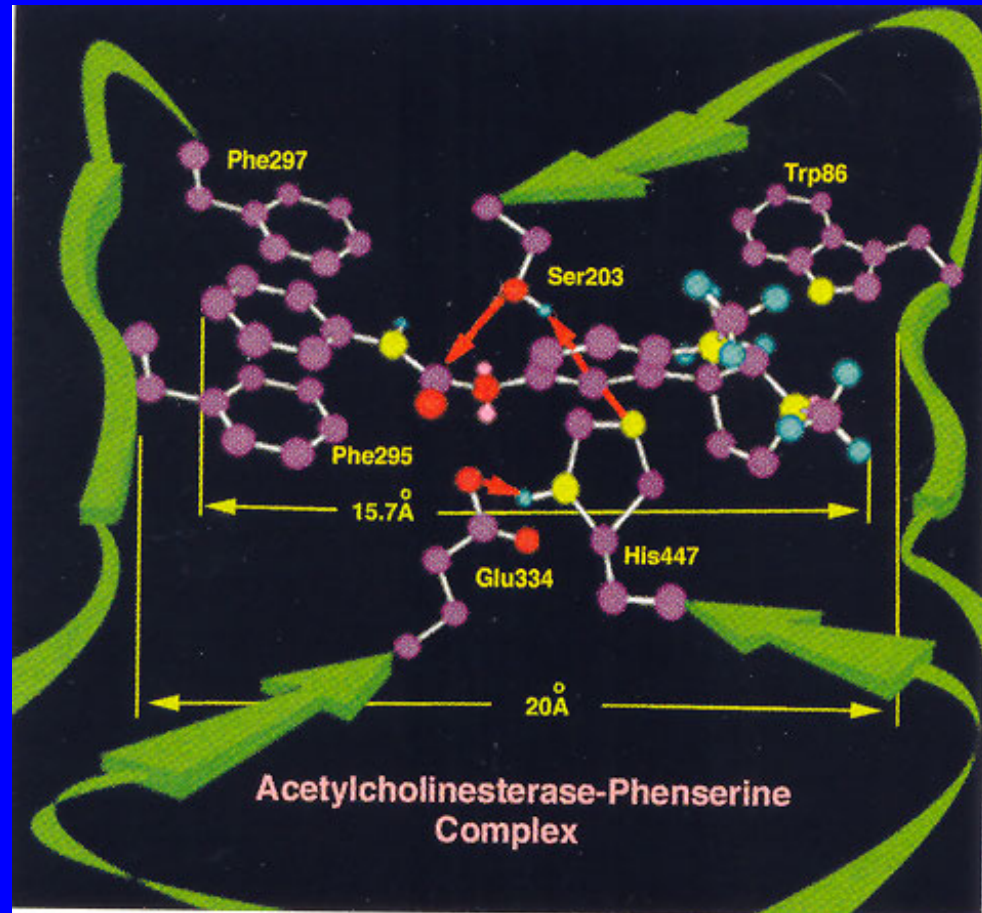
An active center choline substrate:

- Involved in the attraction and binding of the quaternary ammonium of the choline moiety of acetylcholine

A peripheral anionic site:

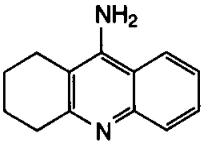
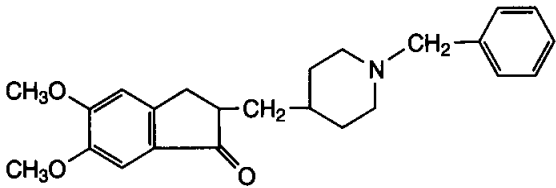
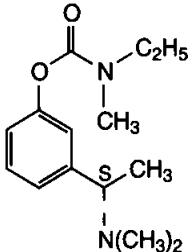
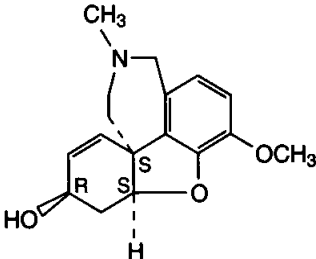
- Uninvolved in the acetylcholine hydrolysis
- Is the binding site of acetylcholinesterase inhibitors

ACETYLCHOLINE ESTERASE



Phe 295 and Phe 297 are replaced by Val and Leu in butyrylcholinesterase

INHIBITEURS DE L'ACETYLCHOLINE ESTERASES

Structure	Drug	Trade name	Efficacy	Side-effects	Comment
	Tacrine	Cognex	Improvements in ADAS-cog and MMSE	Hepatotoxicity and gastrointestinal side-effects	AChE inhibitor with weak antagonist properties at K ⁺ channels
	Donepezil	Aricept	Improvements in ADAS-cog, MMSE CIBIC and global clinical state	Nausea, vomiting and diarrhoea in some patients given 10 mg day ⁻¹	Highly selective, reversible inhibitor of AChE with high bioavailability, almost total protein binding and a half-life of 70 h
	Rivastigmine	Exelon	Improvements in ADAS-cog and global clinical state	Nausea, diarrhoea and anorexia; probably less well tolerated than donepezil	Pseudoirreversible inhibitor of AChE; although the half-life is low (2 h), it inactivates AChE for ~10 h
	Galanthamine	Reminyl	Improvements in ADAS-cog, global impression and assessment of daily living	Nausea, diarrhoea and anorexia occurred acutely at higher doses (24–32 mg day ⁻¹)	Alkaloid extracted from daffodil bulbs; in addition to inhibiting AChE, it modulates neuronal nicotinic acetylcholine receptors; it has a half-life of 8 h and is highly bioavailable

*Abbreviations: AChE, acetylcholinesterase; ADAS-cog, Alzheimer's disease assessment scale; CIBIC, clinician's interview-based impression of change scale, cognitive subscale; MMSE, mini mental state examination.

CLINICAL TRIALS OF CHOLINE ESTERASE INHIBITORS

		6 months mean change (95% CI)	12 months mean change (95% CI)	18 months mean change (95% CI)	24+ months mean change (95% CI)
MMSE	AChE-Is	2.67 (0.19 to 5.14)	-1.34 (-7.88 to 5.20)	-3.67 (-9.91 to 2.58)	-2.12 (-5.87 to 1.63)
	Nootropics	1.03 (-0.74 to 2.79)	-0.17 (-1.57 to 1.23)	-2.75 (-5.17 to -0.32)	-1.76 (-3.92 to 0.40)
CAMCOG	AChE-Is	5.00 (-1.31 to 11.31)	1.00 (-17.21 to 19.21)	-14.00 (-25.31 to -2.70)	-13.75 (-20.92 to -6.58)
	Nootropics	1.60 (-3.69 to 6.89)	-4.14 (-11.52 to 3.24)	-11.00 (-22.75 to 0.75)	-5.00 (-16.28 to 6.28)
FRSSD	AChE-Is	-4.08 (-6.93 to -1.24)	6.67 (-4.65 to 17.99)	5.00 (-9.79 to 19.79)	9.56 (2.25 to 16.87)
	Nootropics	-0.97 (-3.98 to 20.4)	3.27 (-0.27 to 6.81)	4.31 (-1.74 to 10.36)	12.74 (9.12 to 16.36)
GDS	AChE-Is	-0.50 (-5.39 to 4.39)	-4.50 (-7.44 to -1.56)	-2.00 (-0.41 to 9.41)	NA
	Nootropics	-0.09 (-1.72 to 1.54)	-1.45 (-3.68 to 0.81)	4.50	NA

AD, Alzheimer's disease; CAMCOG, Cambridge Cognitive Examination for the Elderly; FRSSD, Functional Rating Scale for Symptoms of Dementia; GDS, Geriatric Depression Scale; MMSE, Mini-Mental State Examination. CI, confidence interval; NA, not applicable as no patient scored on the GDS at the 24+-month time-point. $P > 0.05$ at each time-point.

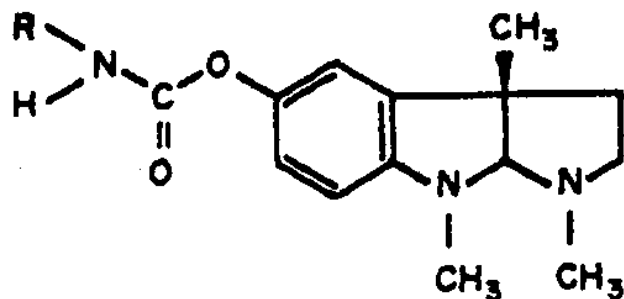
INHIBITEURS DE L'ACETYLCHOLINE ESTERASES

- **Tacrine (Cognex^R)**
(1996 - Warner Lambert)
- **Donezepil (Aricept^R)**
(1997 – Eisai/Pfizer)
- **Rivastigmine (Exelon^R)**
(1998 – Novartis)
- **Galantamine (Reminyl^R)**
(2000 – Shire/Janssens)

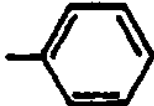
INHIBITEURS DE L'ACETYLCHOLINE ESTERASES EN DEVELOPPEMENT

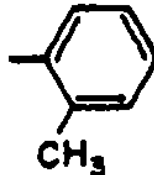
- **Phenserine Phase II (Axonyx)**
 - **↑ de la concentration en acétylcholine**
 - **↓ de la quantité d'APP et in fine de la quantité de peptide β -amyloïde**
- **Cymserine MF 8622 Preclinical (Axonyx)**
 - **Inhibe l'action de la butyrylcholinestérase**
 - **↓ la synthèse de l'APP**

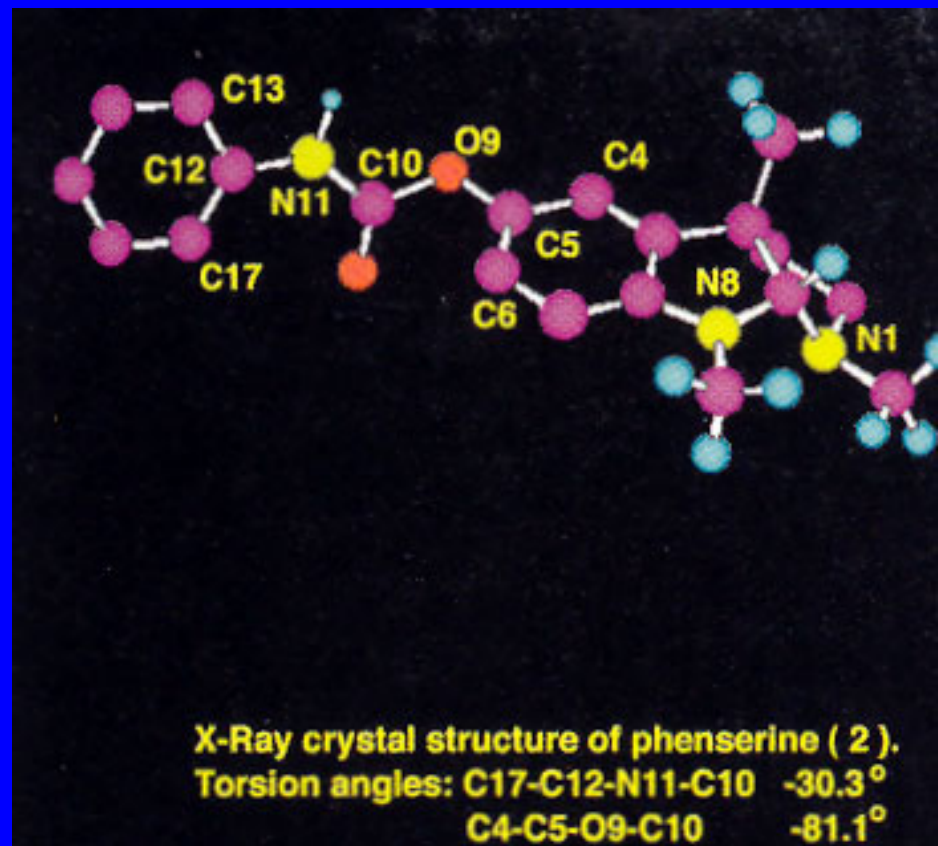
NOUVEL INHIBITEURS OF ACETYLCHOLINE ESTERASES



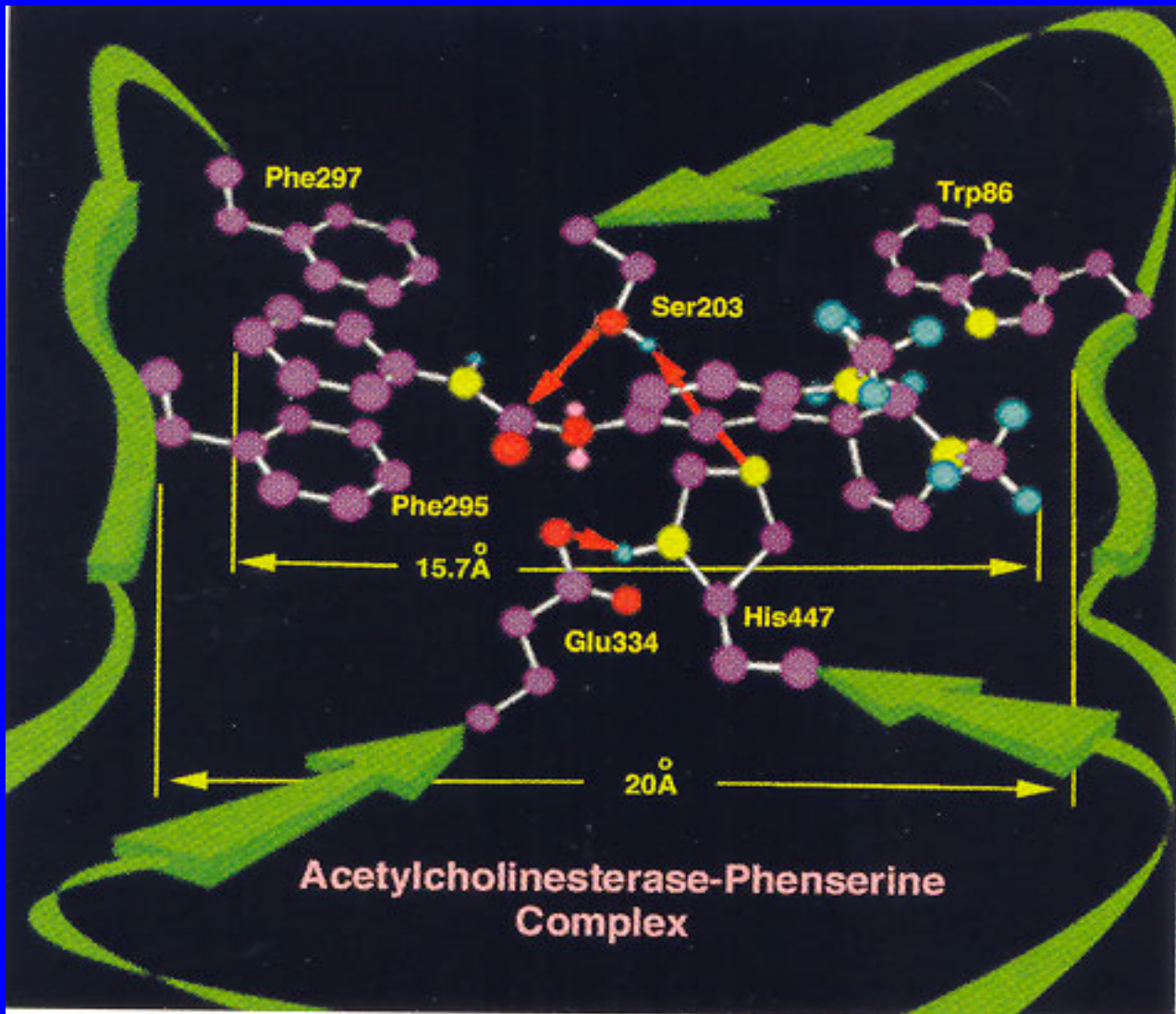
R = —CH₃ Physostigmine

R =  Phenserine

R =  Tolserine

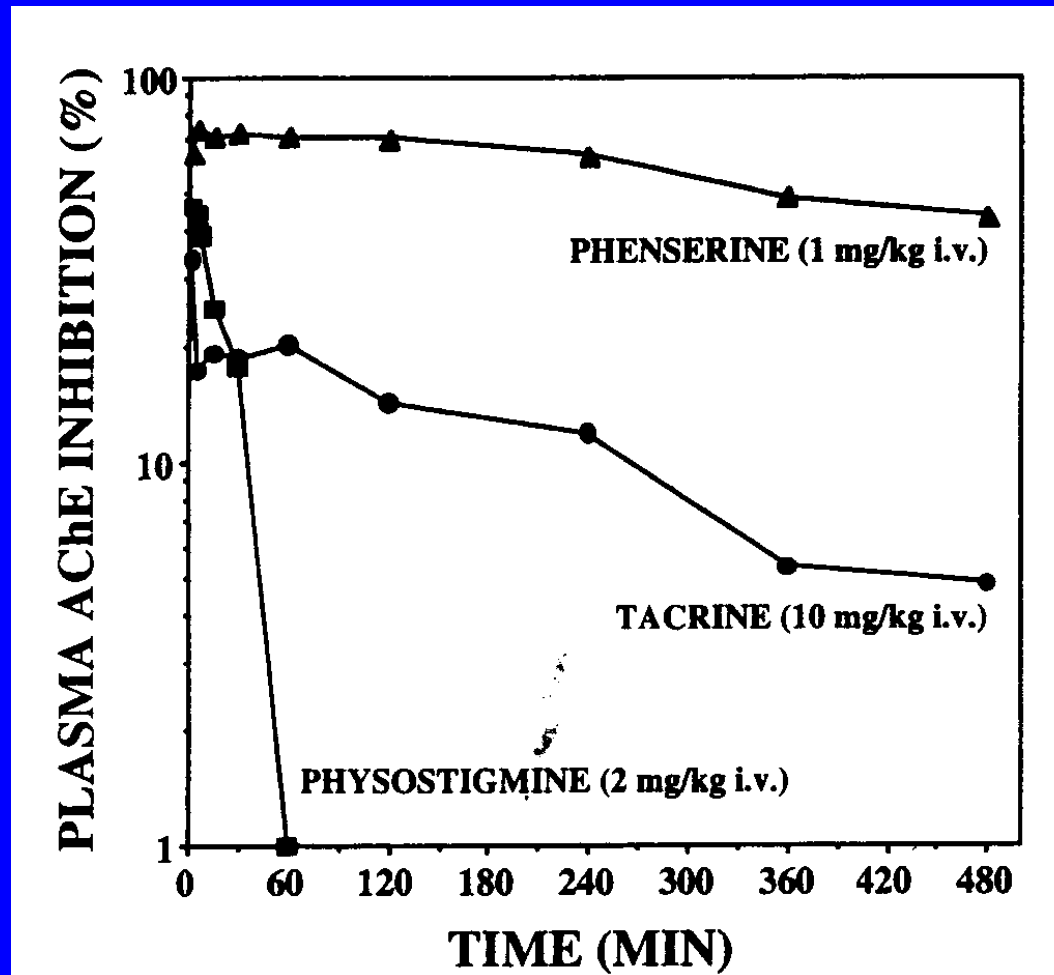


ACETYLCHOLINEESTERASE - PHENSERINE COMPLEX



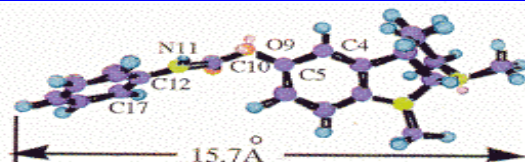
- The carbamylated enzyme only slowly hydrolyzes
- Phenserine is further stabilized by both hydrophobic and π electron interaction due to π - π stacking of the phenyl group of phenylcarbamate between the flanking phenyl moieties of Phe 295 and Phe 297

EFFECT OF PHENSERINE ON BRAIN EXTRACELLULAR FLUID LEVELS OF Ach IN STRIATUM



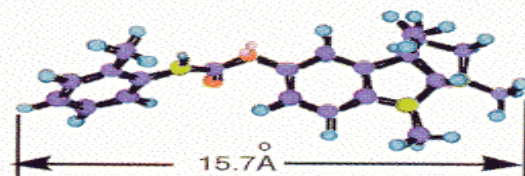
Greig et al, 2000. *Acta Neurologica Scandinavica*. 176: 74-84

PHENSERINE DERIVATIVES



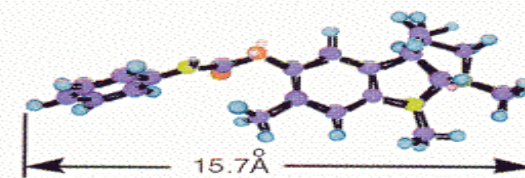
2, Phenserine

C17-C12-N11-C10 2.170
C10-O9-C5-C4 159.789
E1=15.1982
E2=15.2067



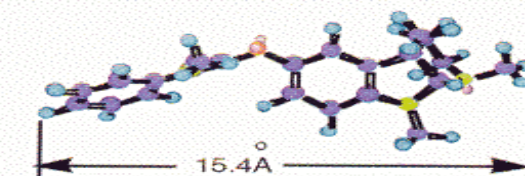
3, 2'-Methylphenserine

C17-C12-N11-C10 3.364
C10-O9-C5-C4 159.301
E1=15.1626
E2=15.1626



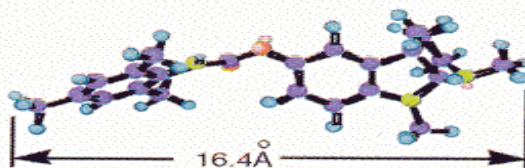
13, 6-Methylphenserine

C17-C12-N11-C10 0.033
C10-O9-C5-C4 145.212
E1=16.1105
E2=16.7170



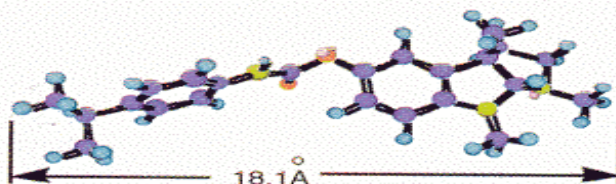
12, N-Methylphenserine

C17-C12-N11-C10 23.297
C10-O9-C5-C4 160.281
E1=29.1270
E2=29.8127



14, 2',4',6'-Trimethylphenserine

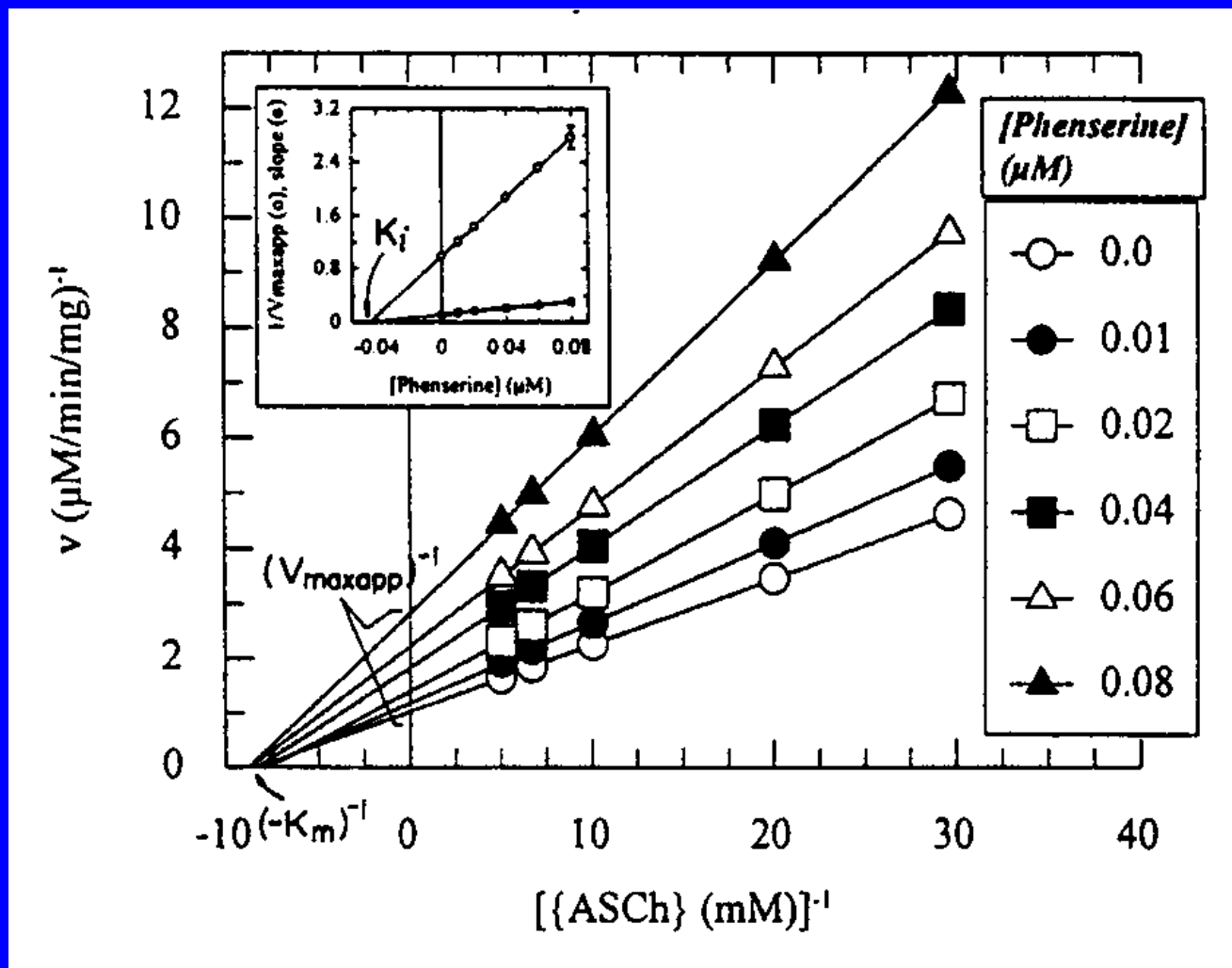
C17-C12-N11-C10 5.637
C10-O9-C5-C4 149.826
E1=19.8350
E2=19.8829



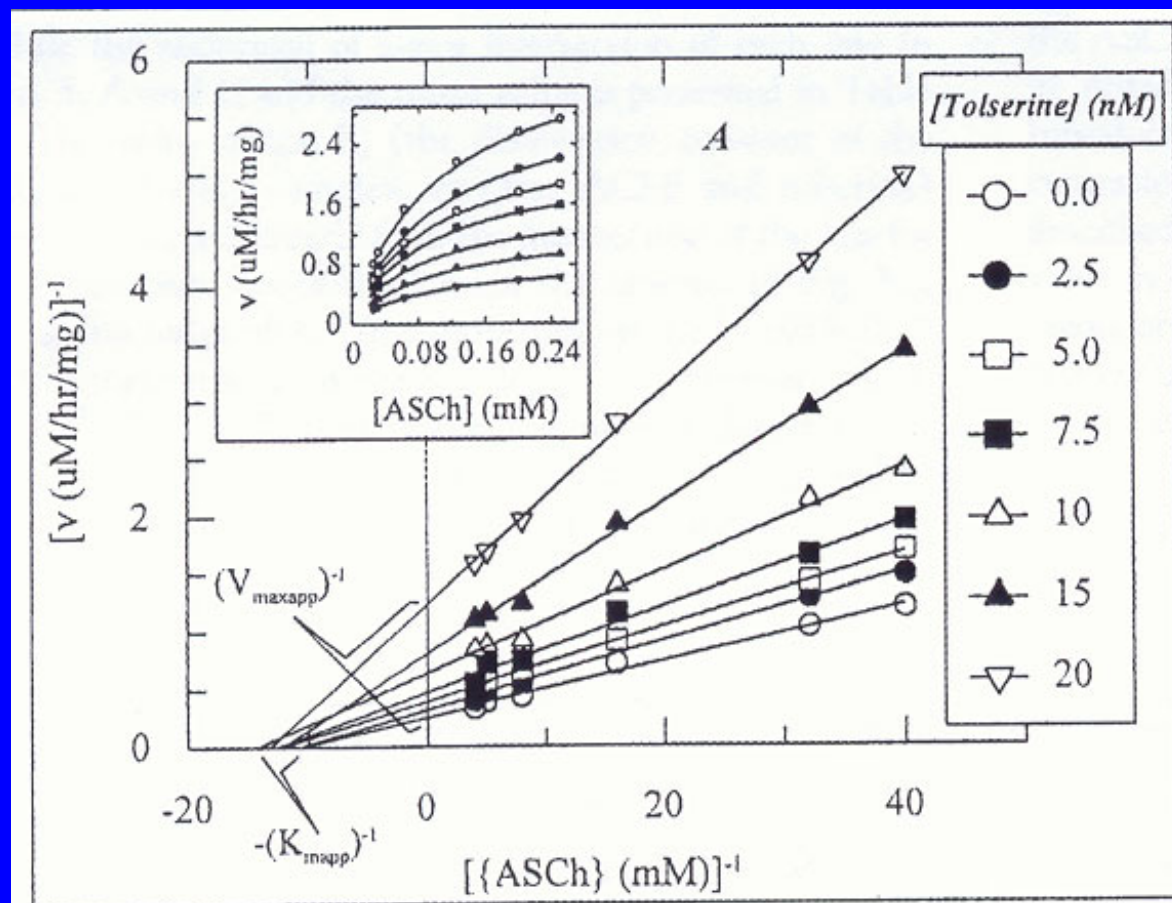
18, 4'-Isopropylphenserine

C17-C12-N11-C10 1.306
C10-O9-C5-C4 159.809
E1=17.3769
E2=17.3806

EFFECT OF PHENSERINE ON THE K_m AND V_{max} ERYTHROCYTE AChE



EFFECT OF TOLSERINE ON THE K_m AND V_{max} OF AChE



Kamal et al, 2000. *Biochem.Pharmacol.* 60: 561-570

EFFECT OF PHENSERINE DERIVATIVES ON AChE AND BChE

compounds	IC ₅₀ (nM)		selectivity
	AChE	BChE	
physostigmine	28 ± 2	16 ± 3	2-fold BChE
phenserine	22 ± 1	1560 ± 270	70-fold AChE
2'-methylphenserine	10 ± 2	1950 ± 240	195-fold AChE
2'-ethylphenserine	10 ± 1	2915 ± 535	290-fold AChE
2'-isopropylphenserine	15 ± 1	650 ± 45	43-fold AChE
3'-methylphenserine	28 ± 4	165 ± 41	12-fold AChE
4'-methylphenserine	140 ± 4	250 ± 8	2-fold AChE
4'-isopropylphenserine	760 ± 20	50 ± 1	15-fold BChE
2',3'-dimethylphenserine	23 ± 6	170 ± 32	13-fold AChE
2',4'-dimethylphenserine	14 ± 1	1820 ± 560	130-fold AChE
2',5'-dimethylphenserine	26 ± 1	490 ± 79	19-fold AChE
2',6'-dimethylphenserine	785 ± 140	290 ± 50	3-fold BChE
2',6'-diethylphenserine	1500 ± 50	1100 ± 50	none
3',4'-dimethylphenserine	31 ± 7	66 ± 7	2-fold AChE
3',5'-dimethylphenserine	78 ± 17	798 ± 147	10-fold AChE
2',4',6'-trimethylphenserine	1300 ± 75	3290 ± 885	3-fold AChE
N-methylphysostigmine	210 ± 40	420 ± 120	2-fold AChE
N-methylphenserine	690 ± 50	>10000	15-fold AChE
6-methylphysostigmine	>10000	8530 ± 235	none
6-methylphenserine	260 ± 130	5020 ± 180	19-fold AChE
6-dimethylaminoethylenphysostigmine	2500 ± 100	3890 ± 1500	none

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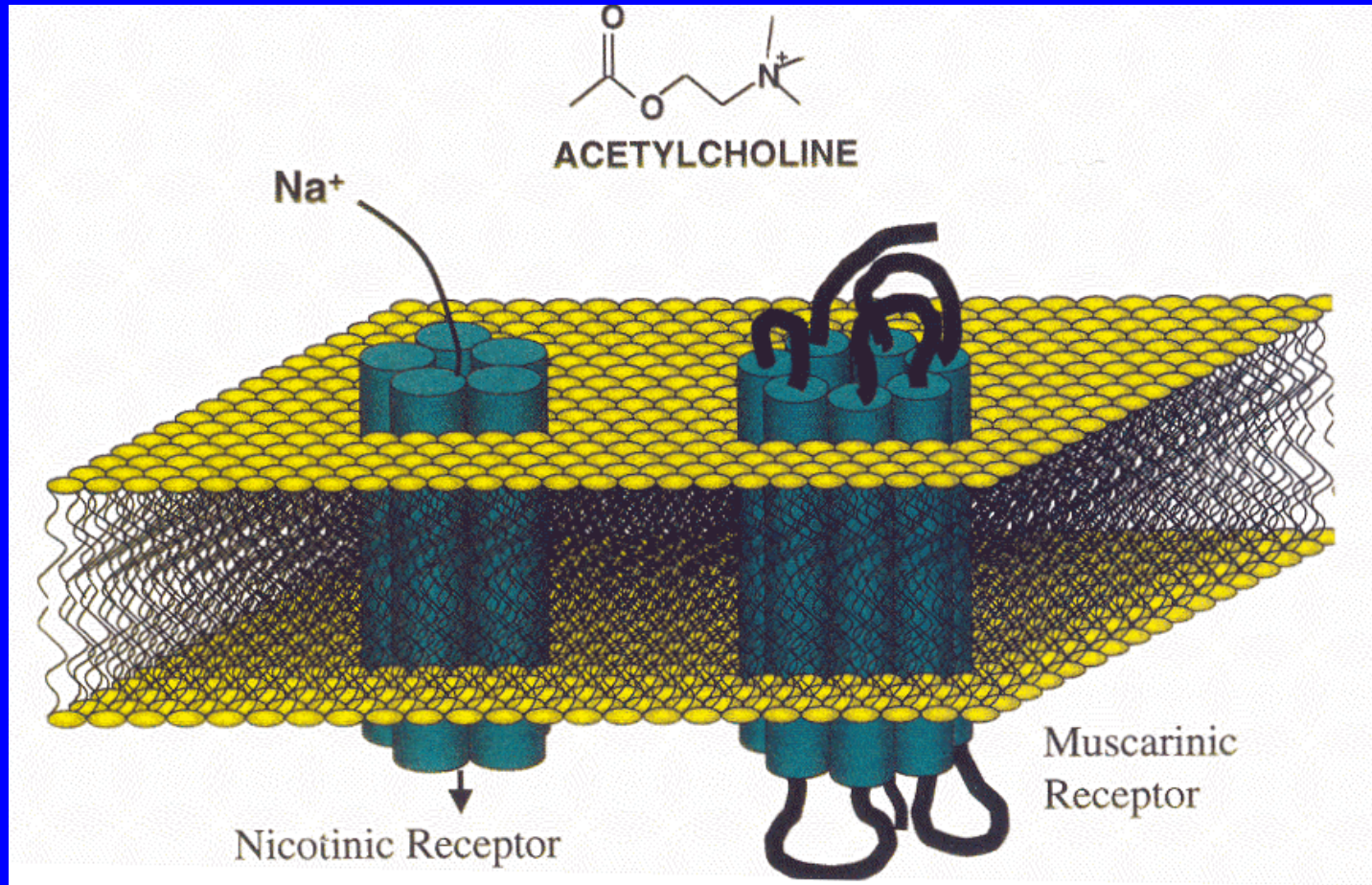
INTERACTION LIGAND/RECEPTEUR CHOLINERGIQUE

Récepteurs nicotiniques

Récepteurs muscariniques

INTERACTION ENTRE RECEPTEURS

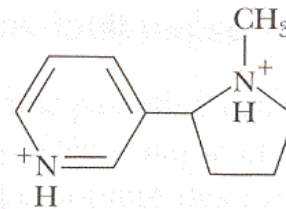
ACETYLCHOLINE - MUSCARINIC AND NICOTINIC RECEPTORS



ACETYLCHOLINE - MUSCARINIC AND NICOTINIC RECEPTORS



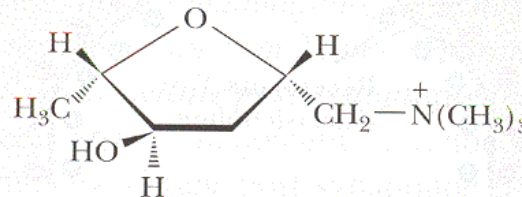
Nicotiana tabacum



Nicotine

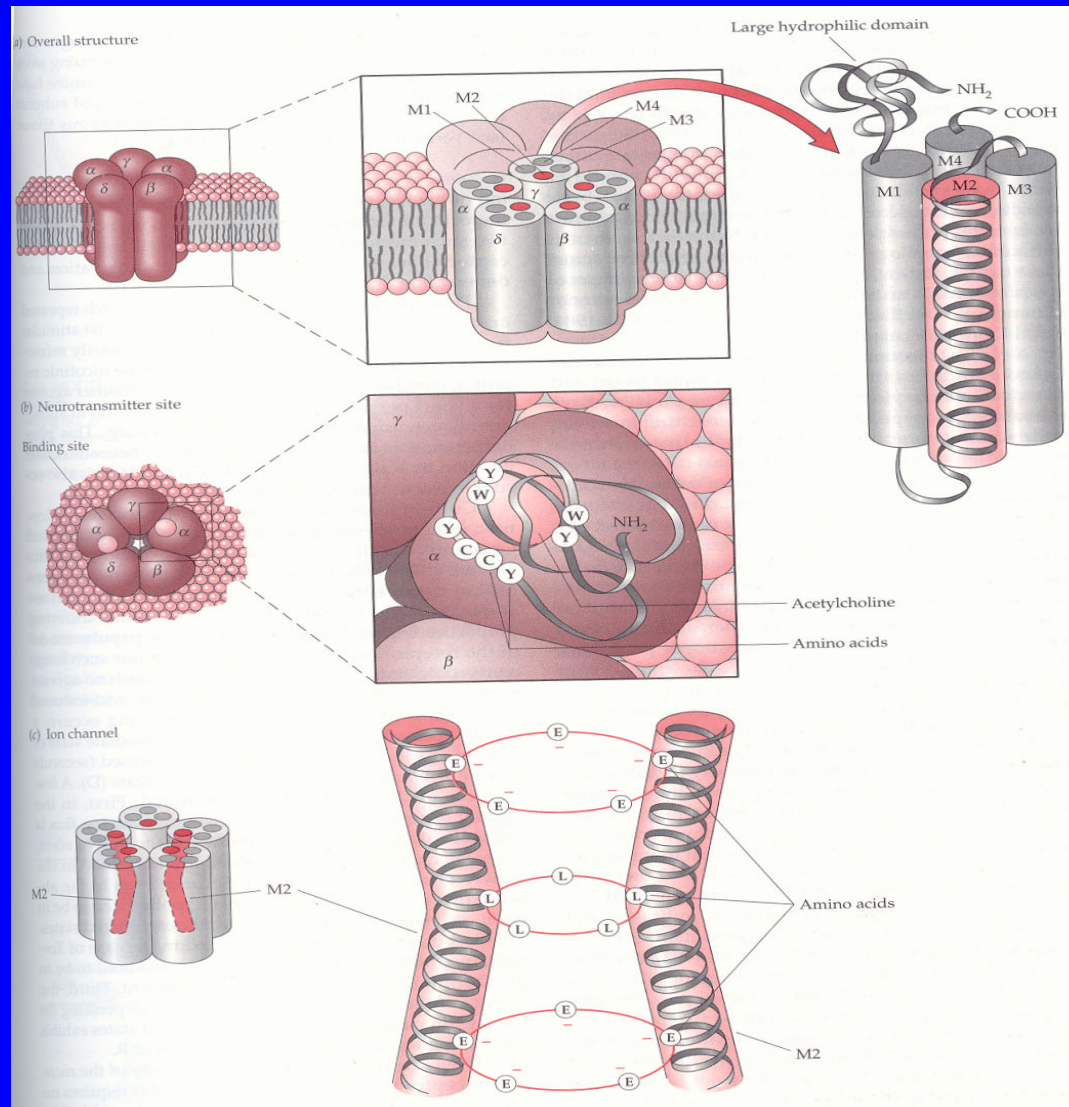


Amanita muscaria

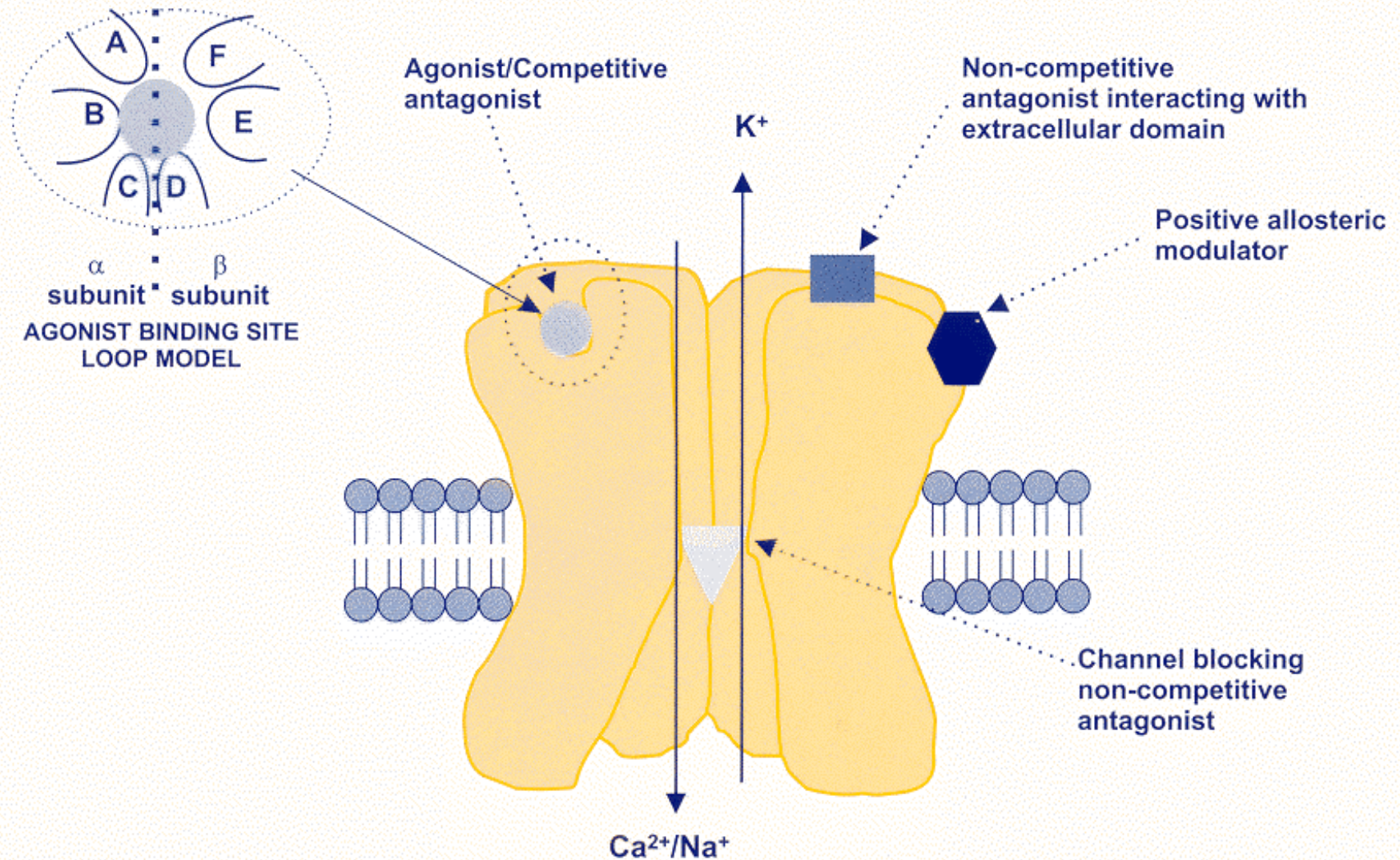


Muscarine

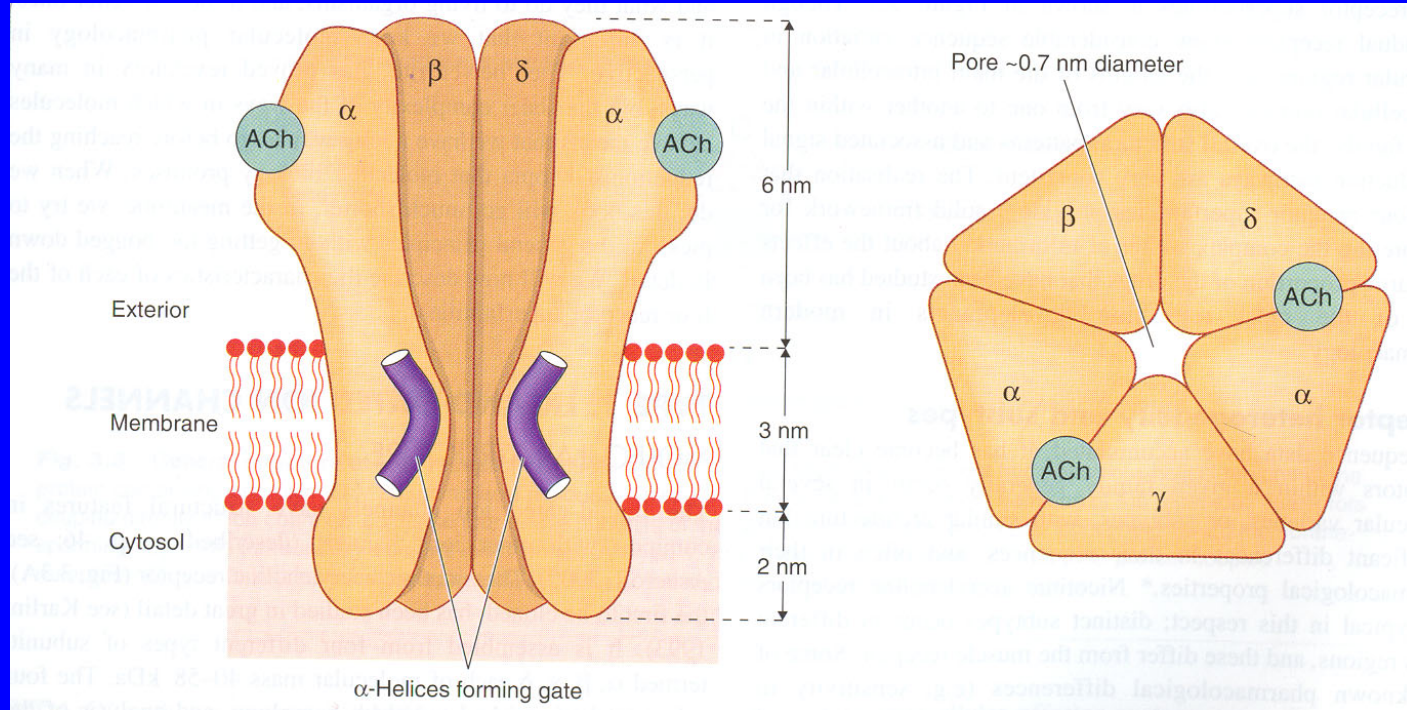
ACETYLCHOLINE - NICOTINIC RECEPTOR



ACETYLCHOLINE - NICOTINIC RECEPTOR



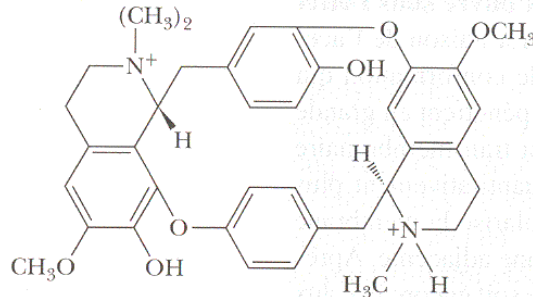
ACETYLCHOLINE - NICOTINIC RECEPTOR



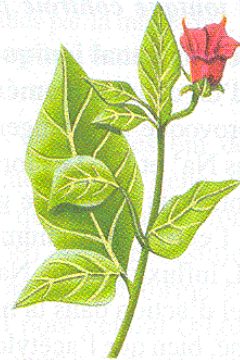
ACETYLCHOLINE - NICOTINIC RECEPTOR ANTAGONISTS



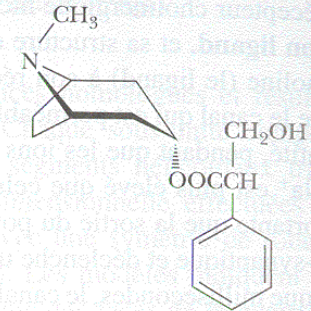
Chondrodendron



Tubocurarine



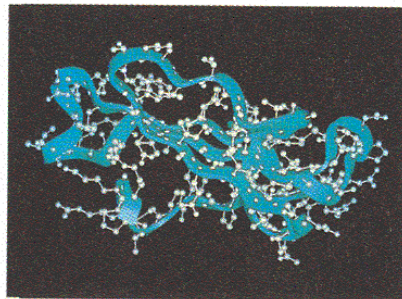
Belladone
(*Atropa belladonna*)



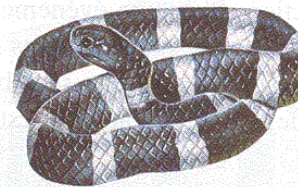
Atropine



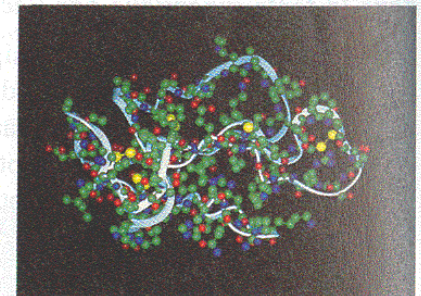
Cobra de l'Inde
(*Naja naja*)



Cobratoxine

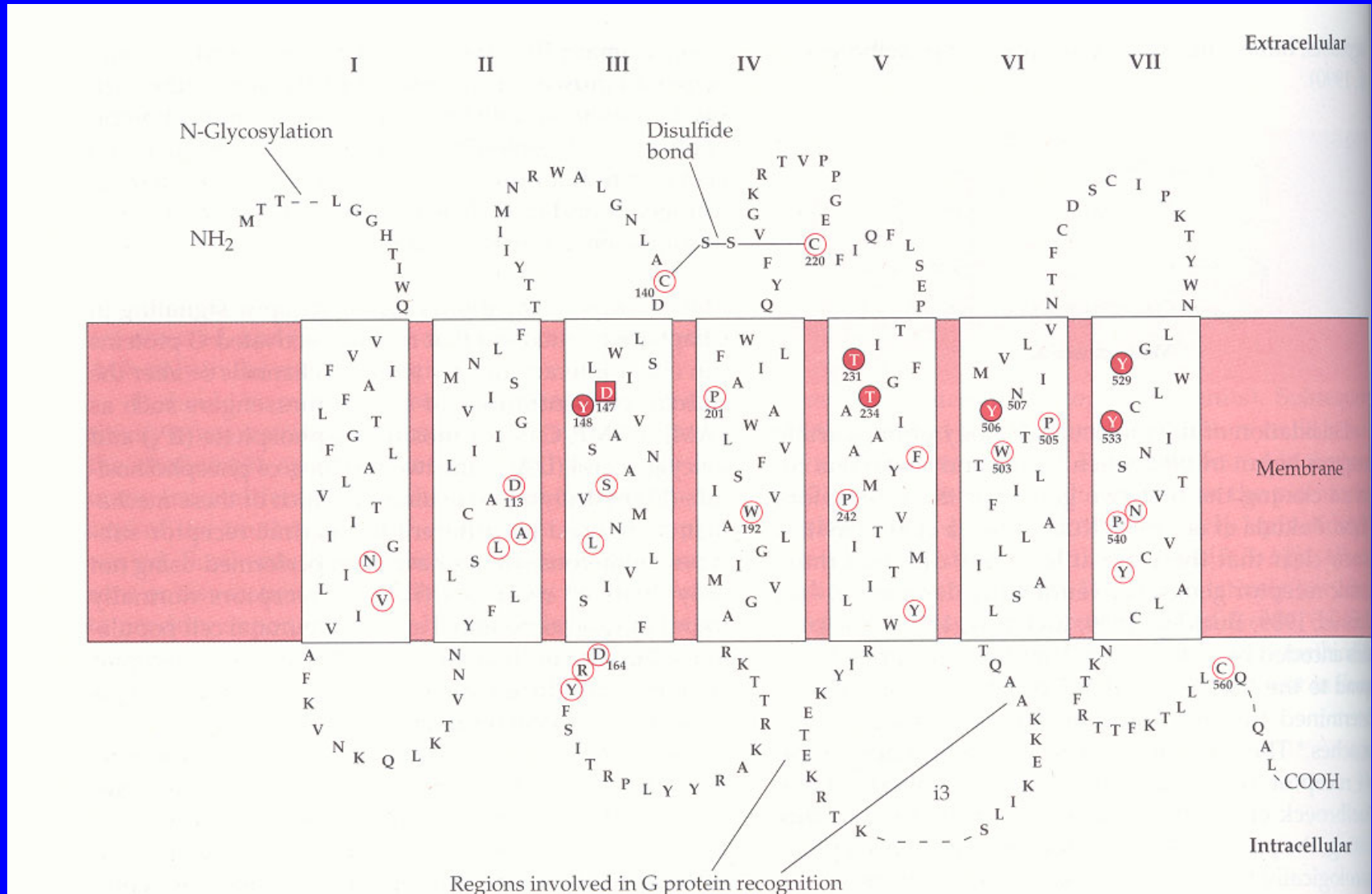


Bungarus multicinctus

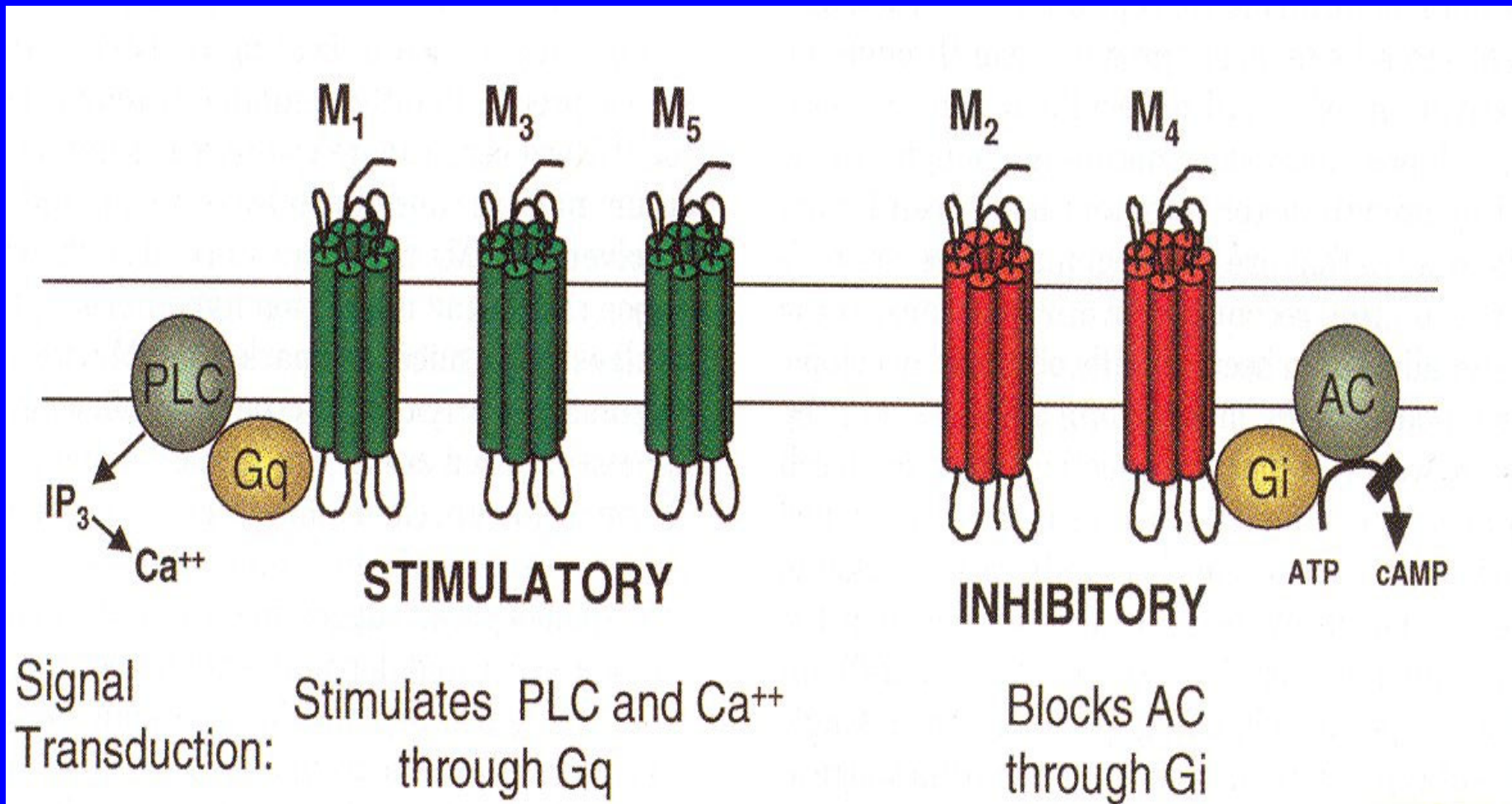


Bungarotoxine α

ACETYLCHOLINE - MUSCARINIC RECEPTOR



ACETYLCHOLINE - MUSCARINIC RECEPTOR



From Felder et al (2000) J. Med. Chem. 43:4333-4353

ACETYLCHOLINE - MUSCARINIC RECEPTOR

Table 7.1 Effector Mechanisms Associated with Specific Muscarinic Receptor Subtypes^a

Subtype	Mechanism
m1, m3, m5	Stimulate PI hydrolysis Increase intracellular Ca^{2+} Increase cAMP levels Release arachidonic acid Inhibit M-current (K^+) Activate Ca^{2+} -dependent K^+ and Cl^- currents
m2, m4	Inhibit adenylyl cyclase Stimulate inward-rectified K^+ current Inhibit Ca^{2+} currents

^aNot all reported effects of muscarinic receptor activation are shown here (see text).

Source: After Richards, 1991; Hulme, Birdsall, and Buckley, 1990.

ACETYLCHOLINE - MUSCARINIC RECEPTOR

Table 7.3 Distribution of Muscarinic Receptor Subtypes in Rat Brain: Comparative Receptor Protein Immunoreactivity, mRNA Levels, and Pharmacological Binding Sites^a

Brain region	Receptor protein (immunohistochemistry) ^b				Receptor mRNA levels (in situ hybridization) ^b				Receptor binding (autoradiography) ^c	
	m1	m2	m3	m4	m1	m2	m3	m4	M1	M2
Neocortex	+++	++	+	++	+++	++	++	+	+++	++
Hippocampus	+++	++	+	++	+++	+	++	++	+++	++
Striatum	+++	++	+	+++	+++	++	-	+++	+++	+
Basal forebrain	-	+++	ND	-	-	+++	+	+	-	+++
Thalamus	+	+++	+	+	+	+++	++	+	+	++
Motor neurons	-	+++	ND	-	-	+++	-	-	-	+++

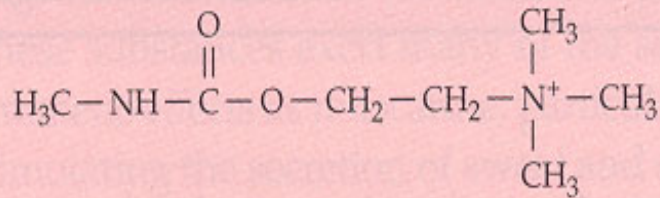
^aIndices of relative density: -, extremely low or undetectable; +, low; ++, moderate; +++, high; ND, not determined.

^bm5 immunoreactivity and mRNA are undetectable or low in all of the listed areas.

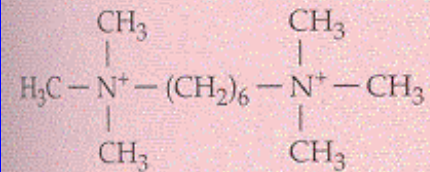
^cNeither M3 nor M4 receptor binding are shown because of uncertainties in the distribution of these pharmacologically defined subtypes.

Source: After Levey et al., 1991, based on data from the references cited in the text.

ACETYLCHOLINE RECEPTORS - AGONISTS AND ANTAGONISTS

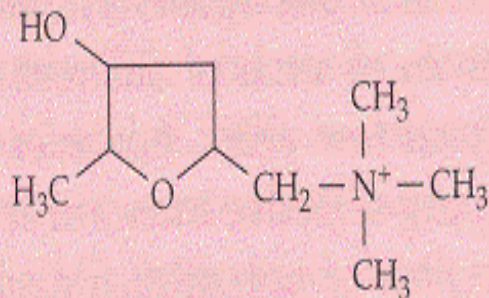


Methylcarbachol

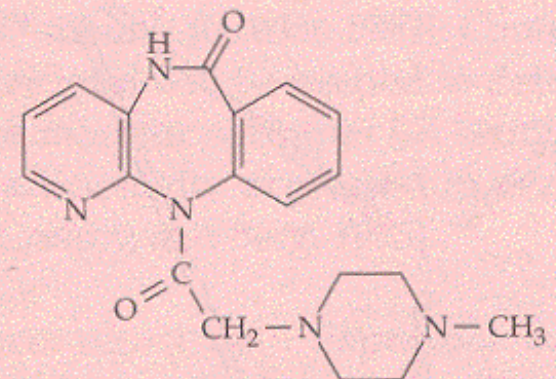


Hexamethonium

Receptor subtype	Agonists	Antagonists
Nicotinic	Nicotine, methylcarbachol, DMPP	D-Tubocurarine, gallamine, mecamylamine, hexamethonium
Muscarinic	Muscarine, pilocarpine, oxotremorine	Atropine, scopolamine, pirenzepine (M1-selective), AF-DX 116 (M2-selective)



Muscarine



Pirenzepine

ACETYLCHOLINE

STRUCTURE

VOIES CHOLINERGIQUES

INTERET PHYSIOPATHOLOGIQUE ET PHARMACOLOGIQUE

SYNAPSE CHOLINERGIQUE

DEGRADATION

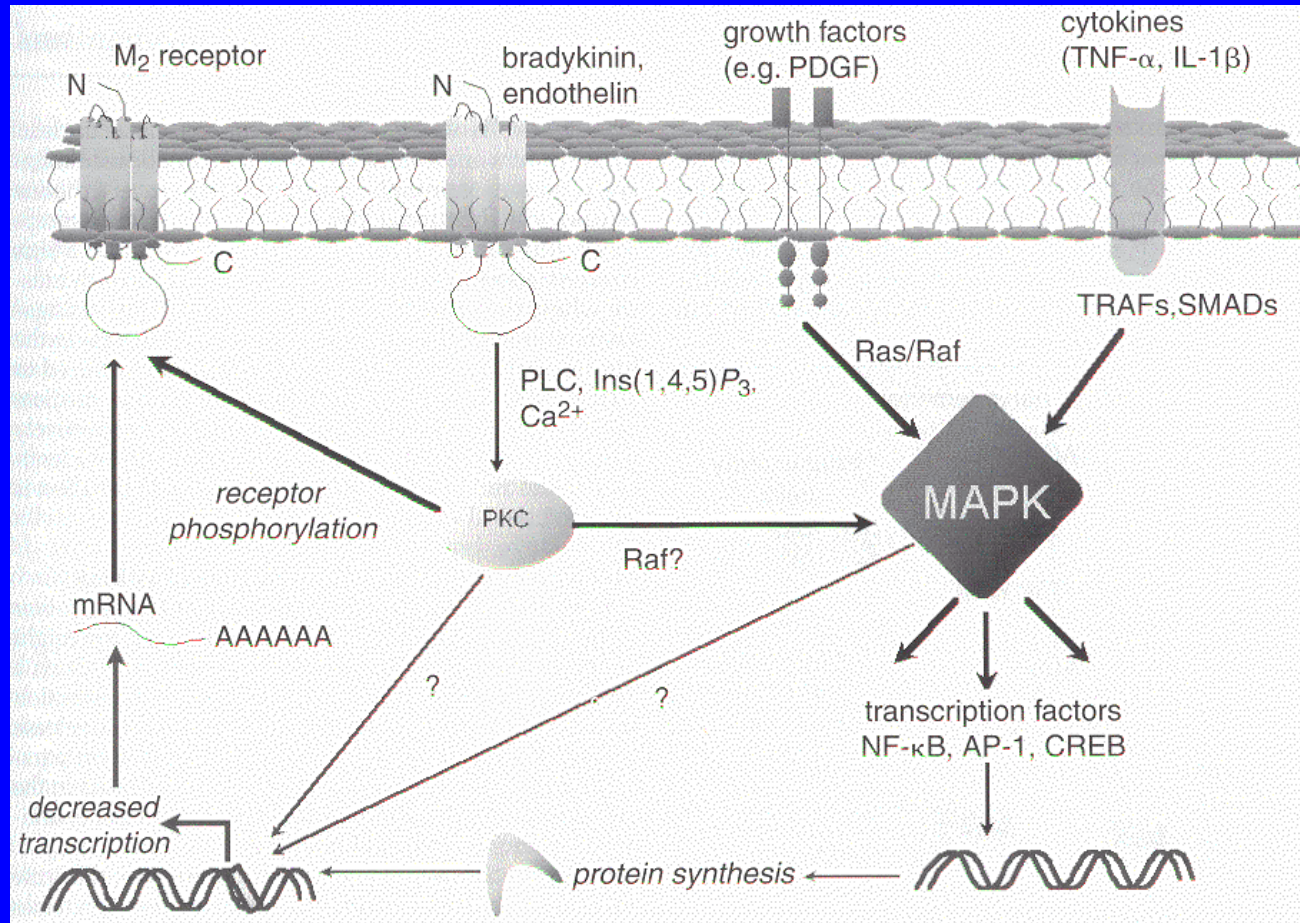
INTERACTION LIGAND/RECEPTEUR CHOLINERGIQUE

Récepteurs nicotiniques

Récepteurs muscariniques

INTERACTION ENTRE RECEPTEURS

ACETYLCHOLINE - ACTIVATION OF M2 RECEPTOR AND TRANSDUCTION OF SIGNAL



INTERACTIONS BETWEEN DOPAMINERGIC RECEPTORS AND METABOTROPIC RECEPTORS, ION CHANNELS AND ACCESSORY PROTEINS

Following activation of muscarinic acetylcholine receptors or mGlu1 receptors, stimulation of D1-like receptors by an agonist evokes the release of intracellular Ca^{2+} . Involvement of dopamine receptor-interacting proteins (DRIPs) like calcyon

PSD: accessory proteins located on or near the postsynaptic density of neocortical dendritic spines

