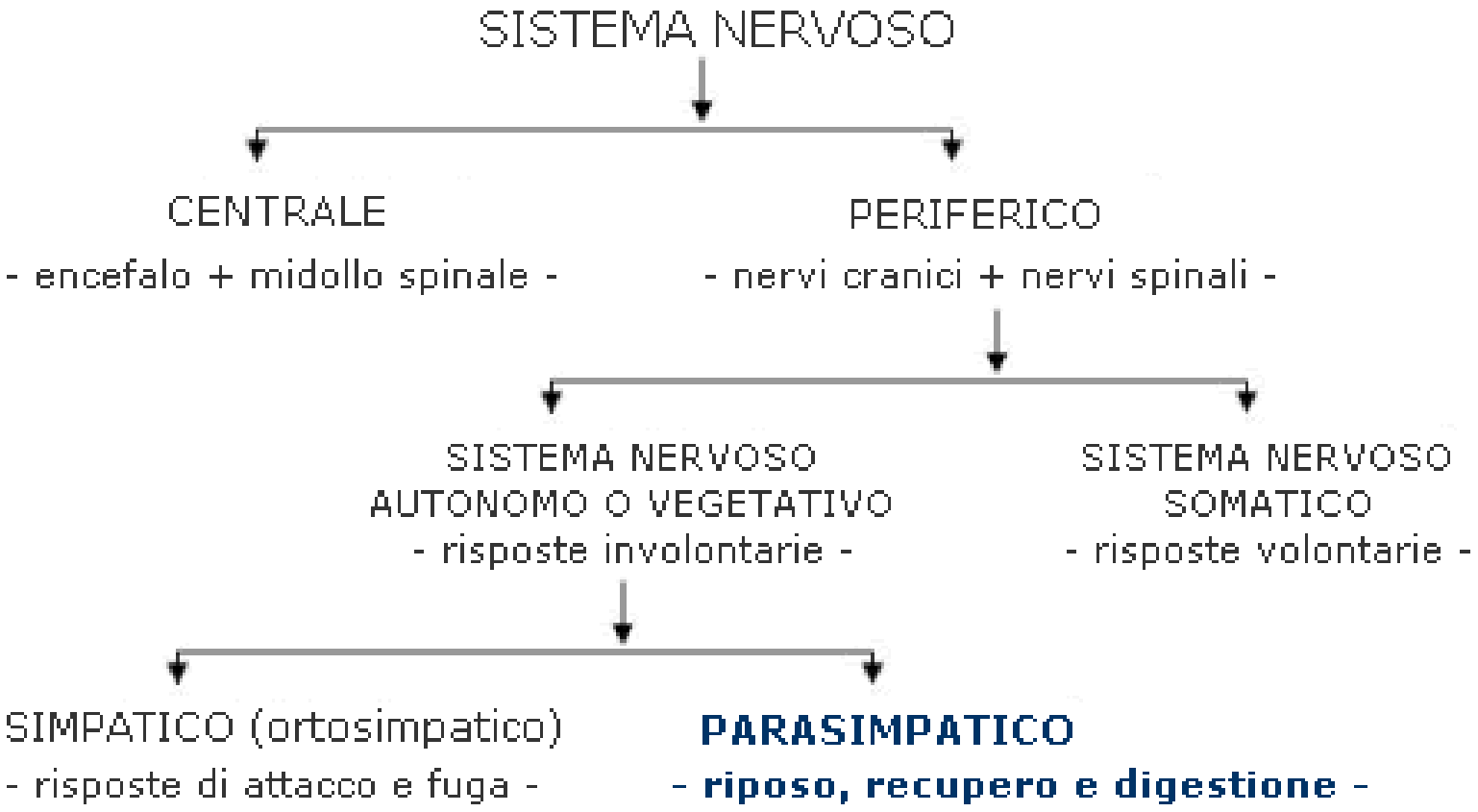
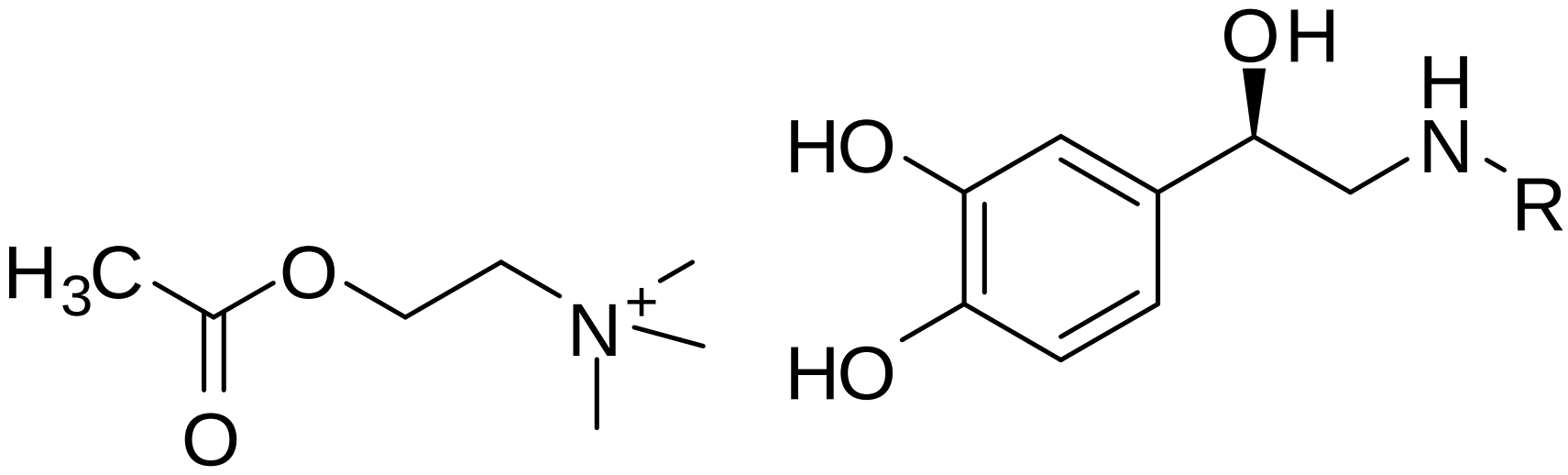
Farmaci adrenergici e colinergici attivi sul SNC ed SNP



- Adrenalina (epinefrina) (R = CH3)
- Noradrenalina (norepinefrina) (R = H)



- Acetilcolina (Ach)

Ach, A e NA hanno un ruolo importante sia nel sistema nervoso
periferico che in quello centrale

FUNZIONI DEL SISTEMA NERVOSO PERIFERICO AUTONOMO

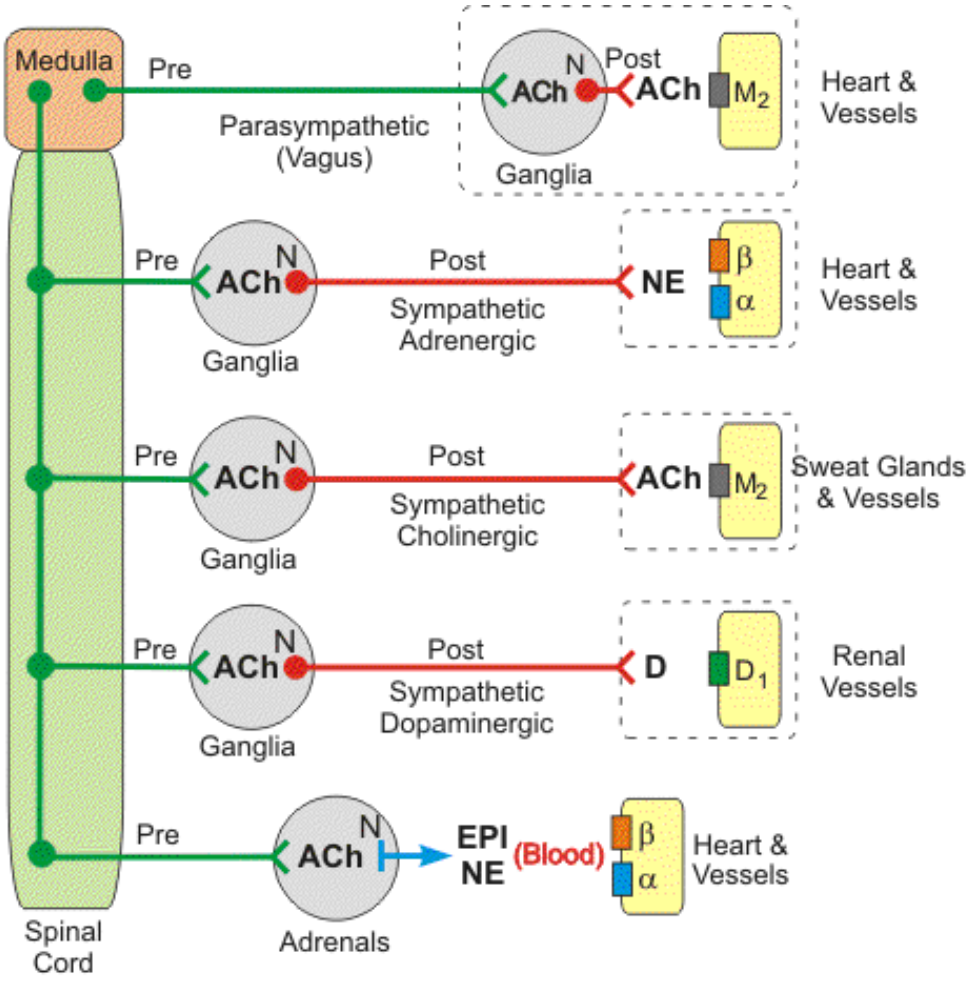
SN PARASIMPATICO

Il sistema parasimpatico stimola la quiete, il rilassamento, il riposo, la digestione e l'immagazzinamento di energia; "rest and digest" (riposo e digestione). In seguito agli stimoli del sistema parasimpatico, aumentano le secrezioni digestive (salivari, gastriche, biliari, enteriche e pancreatiche), l'attività peristaltica viene esaltata, la pupilla si restringe, diminuisce la frequenza cardiaca, si **costringono i bronchi** e viene favorita la minzione. Il sistema parasimpatico si contrappone, in tal senso, all'altra branca del sistema nervoso autonomo, denominata sistema simpatico, che favorisce l'eccitazione e l'attività fisica. Il più delle volte l'azione dei due sistemi è finemente bilanciata, senza una netta prevalenza dell'uno rispetto all'altro (concetto di omeostasi).

SN simpatico

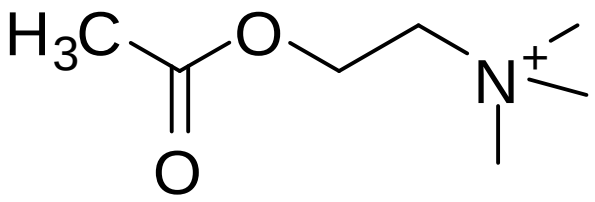
- Gli effetti del sistema nervoso simpatico sono facilmente comprensibili se interpretati in relazione alla "reazione di attacco o difesa" (stress fisico e psichico): **aumentano della frequenza cardiaca, della pressione arteriosa**, della ventilazione (con **dilatazione dei bronchi**), del tono dei muscoli scheletrici, dilatazione delle arterie coronariche e dei vasi dei muscoli scheletrici, **contrazione dei vasi sanguigni periferici** (cute) e degli organi viscerali (tranne cuore e polmoni), dilatazione delle pupille e accomodamento per la visione lontana (tramite rilassamento del muscolo ciliare), rallentamento dei processi digestivi e dell'attività del sistema immunitario, "annebbiamento" dell'attività cognitiva ecc. (schema reazione di stress). Il sistema nervoso parasimpatico è suo antagonista contrastandone e bilanciandone gli effetti.

SNP autonomo

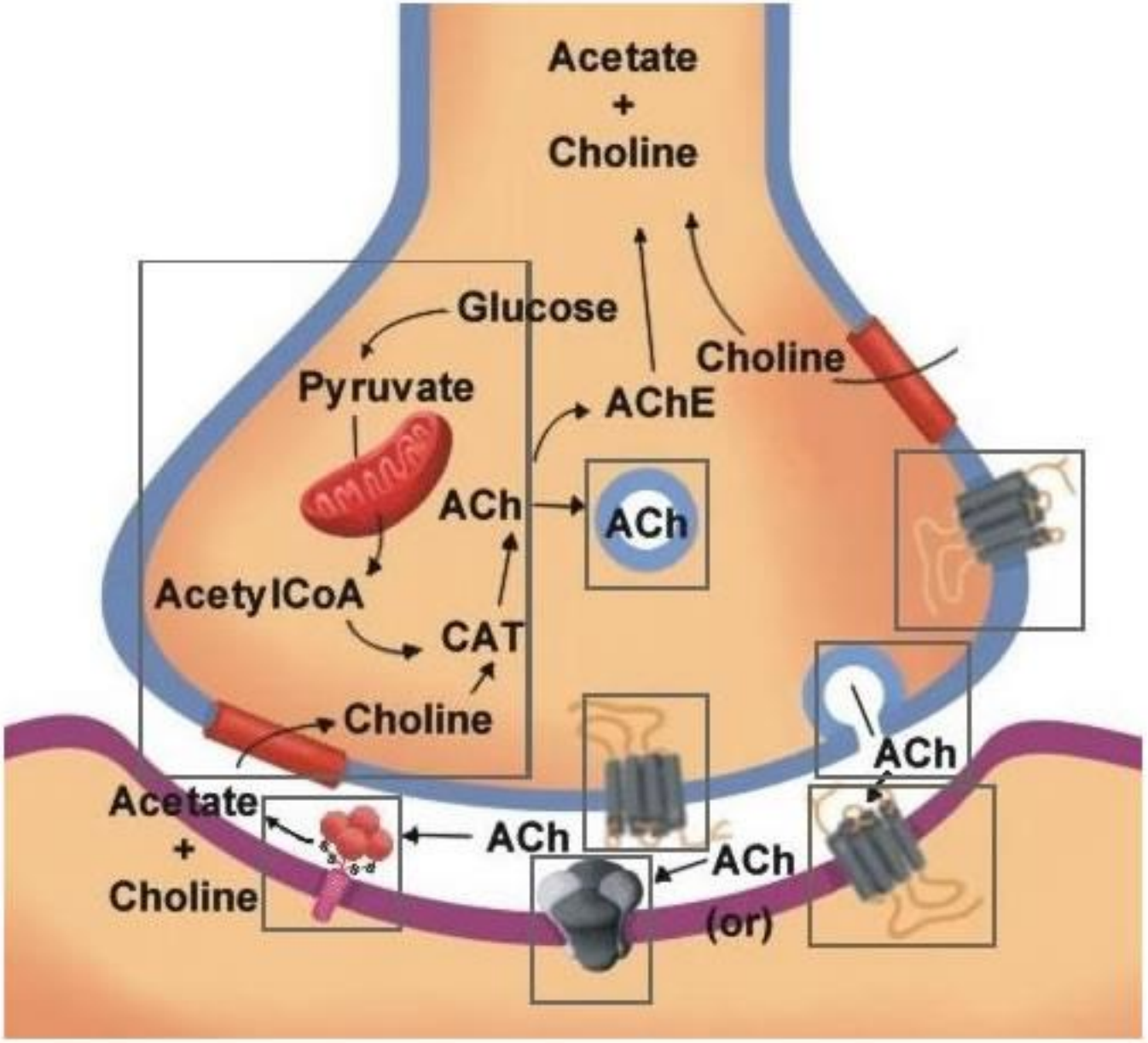


CNS = central nervous system; Pre = preganglionic; Post = postganglionic;
ACh = acetylcholine; N = nicotinic receptor; NE = norepinephrine; EPI = epinephrine;
D = dopamine; M_2 = muscarinic receptor; β = β-adrenoceptor; α = α-adrenoceptor;
D_1 = dopaminergic receptor

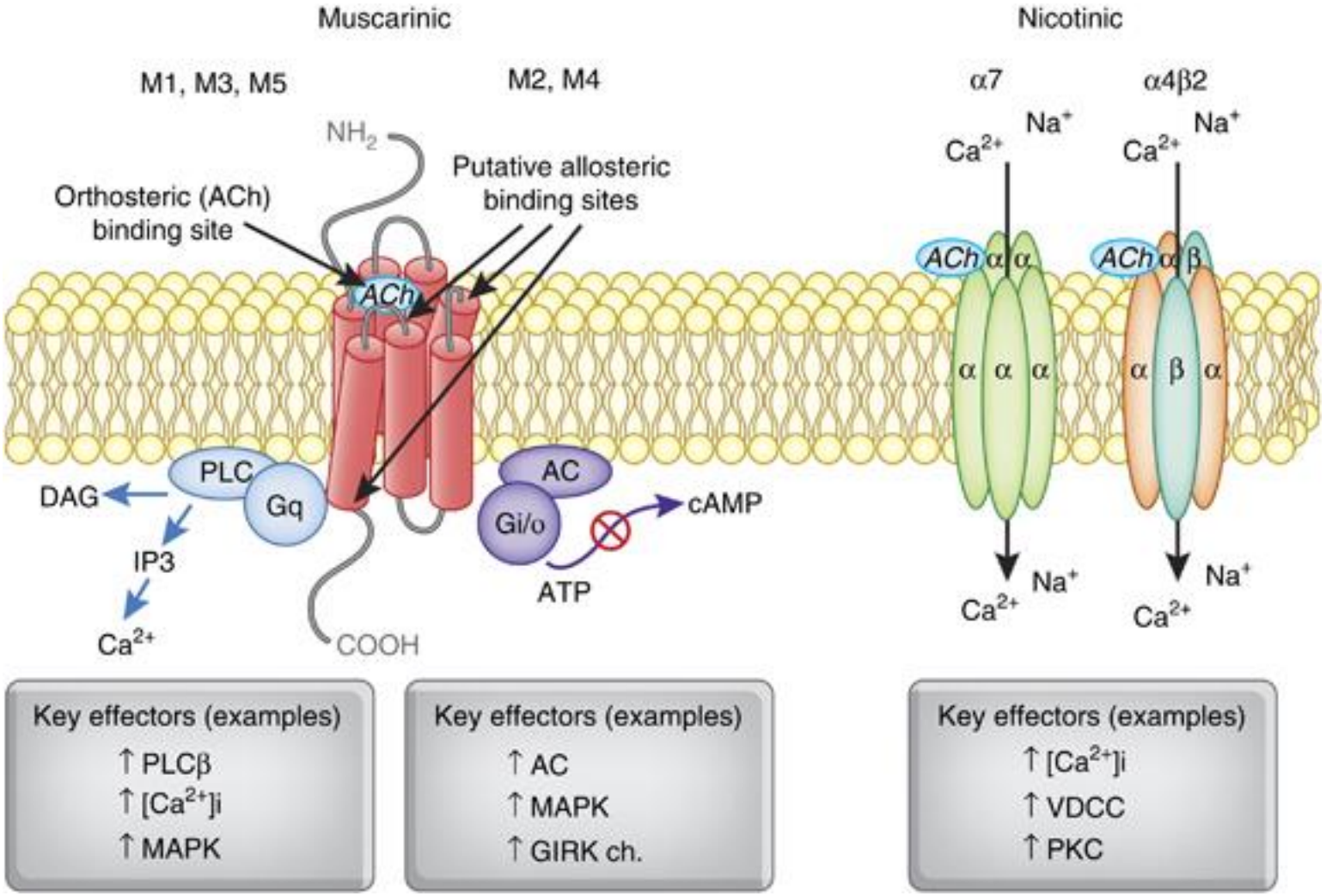
The acetylcoline (Ach)



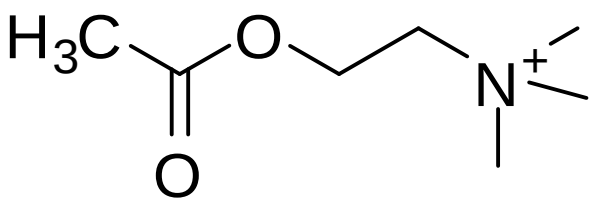
Acetylcholine (ACh) was one of the first neurotransmitters to be discovered and is one of the most important in the central nervous system (CNS) and peripheral nervous system (PNS). ACh is produced by the enzyme choline acetyltransferase and its actions are mediated through two types of acetylcholine receptors (AChRs) — the G protein-coupled muscarinic AChRs and the nicotinic AChRs (nAChRs).

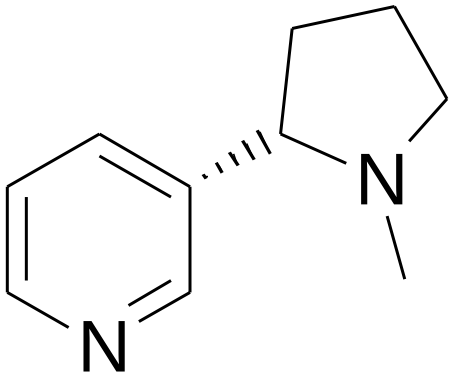

Acetate
+
Choline
Glucose
Pyruvate
AChE
Choline
ACh
ACh
AcetylCoA
CAT
Choline
ACh
Acetate
+
Choline
ACh
ACh
(or)

- Nel 1914 vennero definiti i sottoinsiemi del sistema parasimpatico: la acetilcolina aveva gli stessi effetti della muscarina e della nicotina.
- Recettori muscarinici e nicotinici

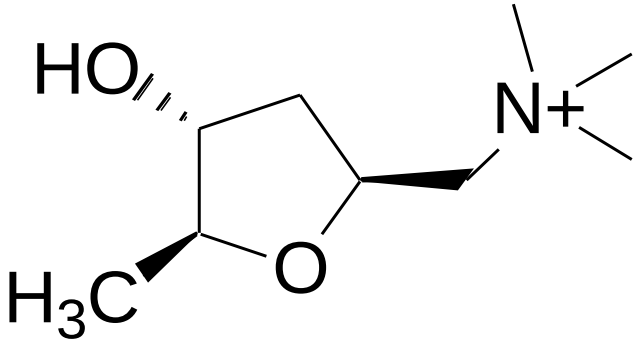


- The muscarinic receptors are now known to be G protein-coupled receptors (GPCRs) and the nicotinic receptor a ligand-gated ion channel.





nicotina



muscarina

The muscarinic acetylcholine receptor (mAChR)

As a consequence of their roles in both the central and parasympathetic nervous systems, muscarinic receptors are targets for treatment of a spectrum of disorders including **Alzheimer's disease, schizophrenia and Parkinson's disease, and chronic obstructive pulmonary disease (COPD)**.

However, developing highly **subtype selective orthosteric drugs** for muscarinic receptors has been challenging and thus far largely unsuccessful.

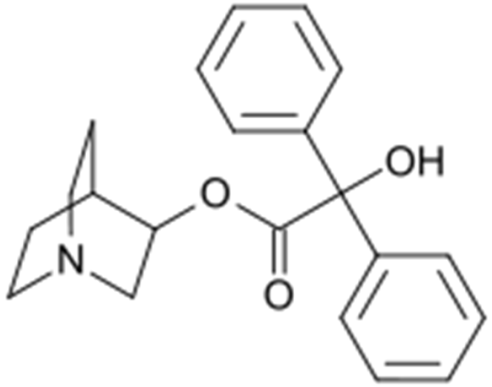
mAChR is a target in the following CNS pathologies

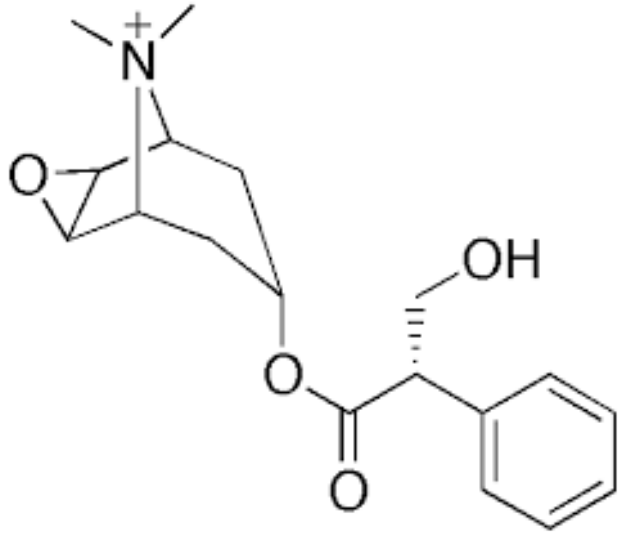
- Based on behavioural, pharmacological, anatomical and neurochemical evidence, **M1 receptor-selective agonists** seem to have the potential to ameliorate the symptoms **of Alzheimer's** disease and related cognitive disorders1.
- In fact, several recent studies support the concept **that M1 receptor-selective agonists** or positive allosteric modulators (PAMs) may prove to be useful as **cognition-enhancing drugs**.
- **Schizophrenia**: two clinical trials demonstrated that **xanomeline, an M1- and M4-preferring muscarinic receptor agonist2**1, was effective in ameliorating psychosis-like symptoms
- **Drug addiction**: recent work demonstrated that **allosteric M1 receptor agonists and xanomeline** can attenuate the reinforcing and discriminative stimulus effects of cocaine

The muscarinic receptors

- The muscarinic receptors constitute a family with five subtypes M1-M51. M1, M3, and M5 subtypes couple with the Gq family of G proteins, and M2 and M4 subtypes with the Gi/Go family of G proteins.
- The muscarinic acetylcholine receptors were originally defined as a functional concept on the basis of the work by Dale and others showing that the muscarinic action by a series of choline esters and other substances in various tissues could be differentiated from their nicotinic action.

- Muscarinic receptors were initially defined biochemically as proteins that specifically bound 3-quinuclidinyl-benzilate (QNB) and N-methylscopolamine (NMS).

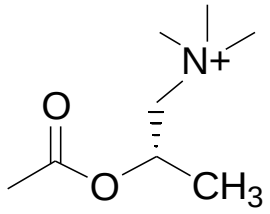




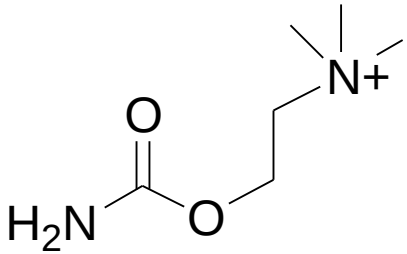
- The M1 receptor together with the β2 adrenergic receptor were the first neurotransmitter-activated GPCRs to be cloned, revealing the seven transmembrane segment (TM) topology initially observed for rhodopsin, and subsequently found to be common to all members of the GPCR family

L’acetilcolina non è un buon farmaco perché si idrolizza rapidamente: agonisti muscarinici

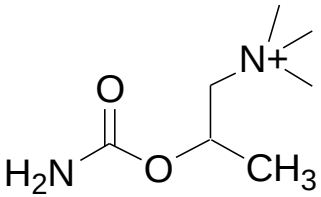
- I gruppi achilici su N non possono essere sostituiti tutti e 3 con omologhi superiori: regola dei 5 legami tra H term ed N per avere attività colinergica
- Sostituzione isosterica dell’acetil con carbamoil porta al carbacolo, attivo per via orale, trattamento del glaucoma.
- Betanecolo, con metile in beta rispetto all’azoto, ha notevole selettività muscarinica vs nicotinica (come beta-colina)



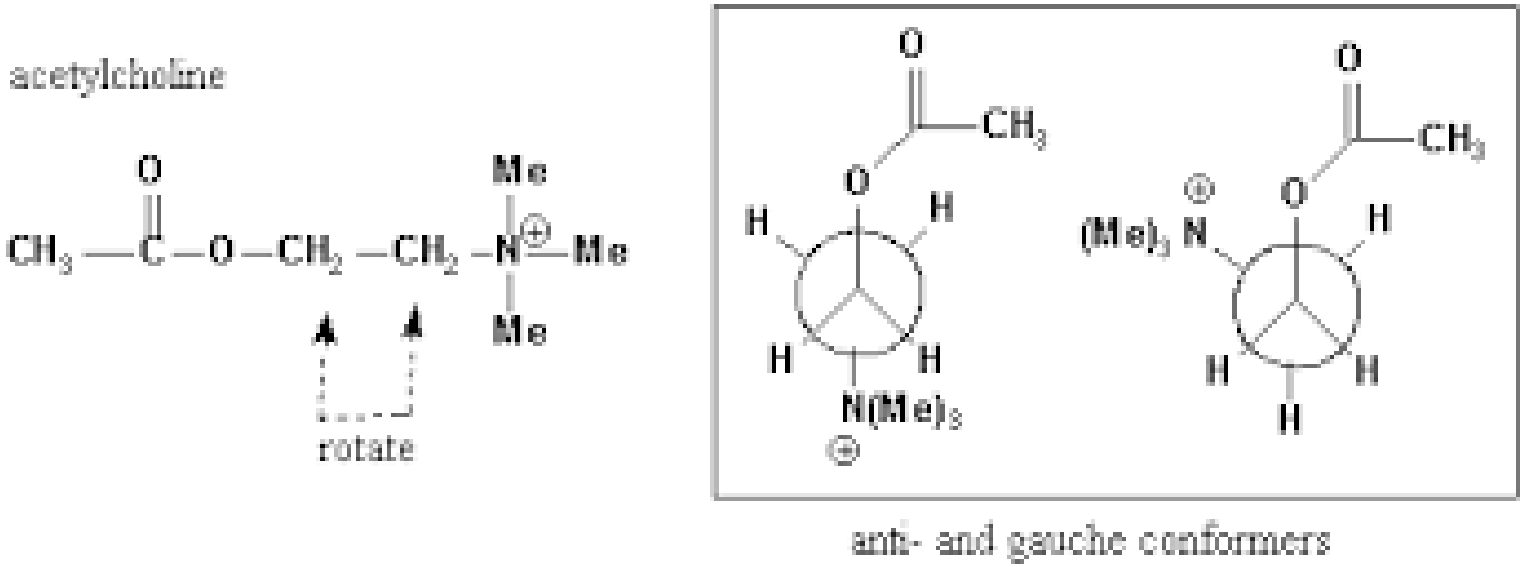
metacolina



carbacolo

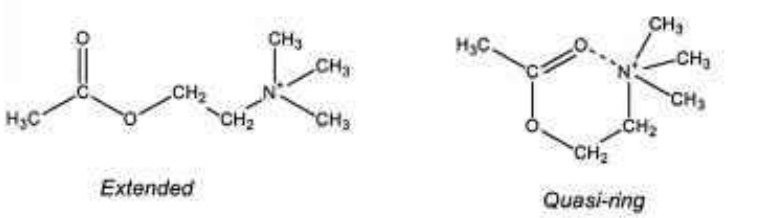


betanecolo

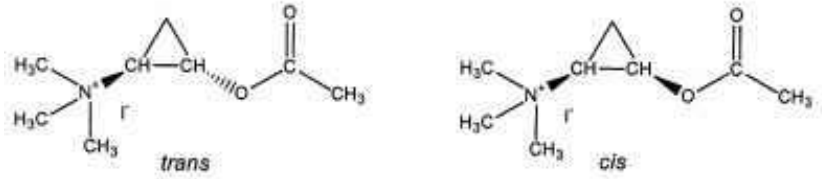


- It has been proposed that the conformational change of M2 receptor upon activation might be accompanied by conformational change of acetylcholine from the gauche to trans form of the O-C2-C1-N dihedral angle26

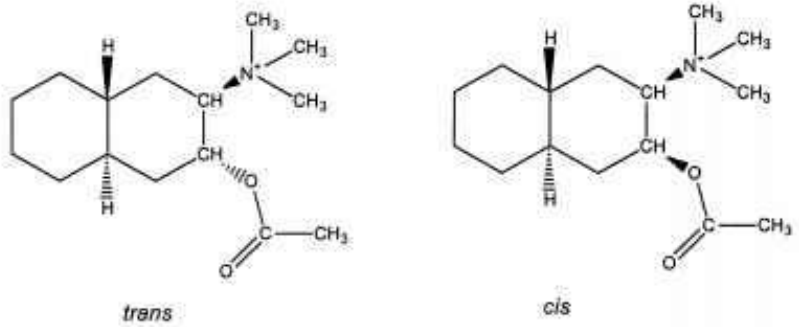
Composti in cui si blocca la conformazione dell'acetilcolina



Acetylcholine

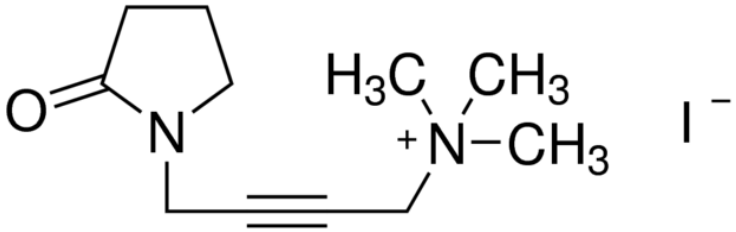


2-Acetoxycyclopropyl trimethylammonium Iodide



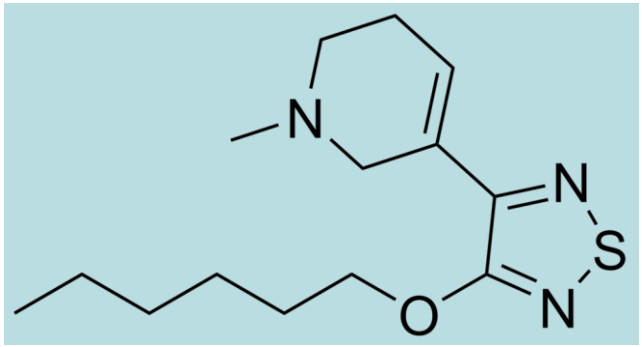
3-Trimethylammonium-2-acetoxydecalins

- Ossotremorina e xanomielina, AGONISTI interessanti esempi di bioisosteria, promettenti risultati contro Alzheimer (aumentare il tono colinergico è solo una teoria nella lotta all'Alzheimer)

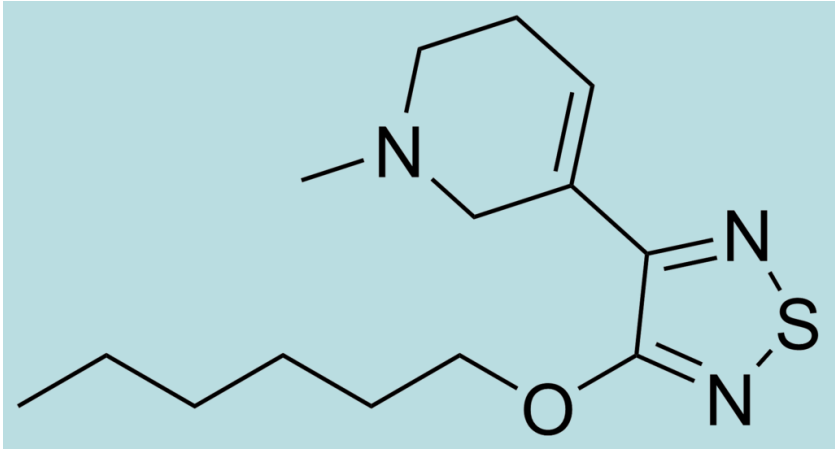


ossotremorina

Xanomielina
Agonista M1-M4
Alzheimer disease

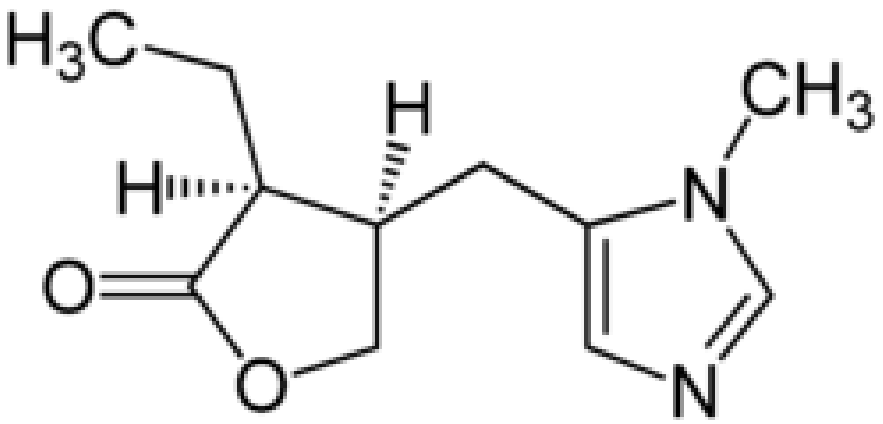


xanomieline



Xanomeline (LY-246,708; Lumeron, Memcor) is a muscarinic acetylcholine receptor agonist with reasonable selectivity for the M1 and M4 subtypes, though it is also known to act as a M5 receptor antagonist. It has been studied for the treatment of both **Alzheimer's disease and schizophrenia**, particularly the cognitive and negative symptoms,

- Agonista ortosterico non-classico: la pilocarpina, concetto di bioisosteria in senso lato. Composto naturale, dalle foglie della pianta del *pilocarpus*. Trattamento del glaucoma.



Published in final edited form as:
Nature. ; 482(7386): 547–551. doi:10.1038/nature10753.

Structure of the human M_2 muscarinic acetylcholine receptor bound to an antagonist

Kazuko Haga[1,*], **Andrew C. Kruse**[2,*], **Hidetsugu Asada**[3,4,*], **Takami Yurugi-Kobayashi**[3,4], **Mitsunori Shiroishi**[4,5], **Cheng Zhang**[2], **William I. Weis**[2,6], **Tetsuji Okada**[1], **Brian K. Kobilka**[2], **Tatsuya Haga**[1], and **Takuya Kobayashi**[3,4]

Here we report the structure of antagonist-bound M2 receptor, the first human acetylcholine receptor to be characterized structurally.
The antagonist QNB binds in the middle of a long aqueous channel extending approximately two-thirds through the membrane.

Muscarinic receptors are G protein coupled receptors (GPCRs) that mediate the response to acetylcholine released from parasympathetic nerves.

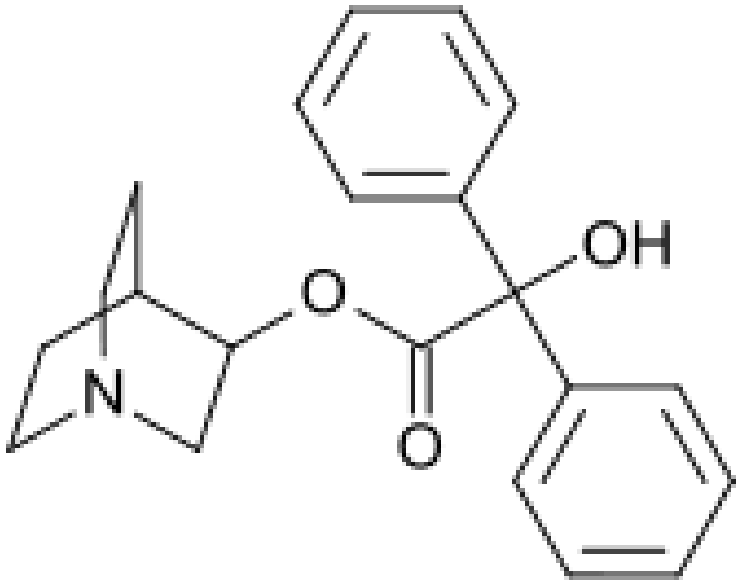
Their role in the unconscious regulation of organ and central nervous system function makes them potential therapeutic targets for a broad spectrum of diseases.

The M2 muscarinic acetylcholine receptor (M2 receptor) is essential for the physiologic control of cardiovascular function through activation of G protein-coupled inwardly-rectifying potassium channels, and is of particular interest because of its extensive pharmacological characterization with both **orthosteric and allosteric ligands**.

Switch da agonismo ad antagonismo

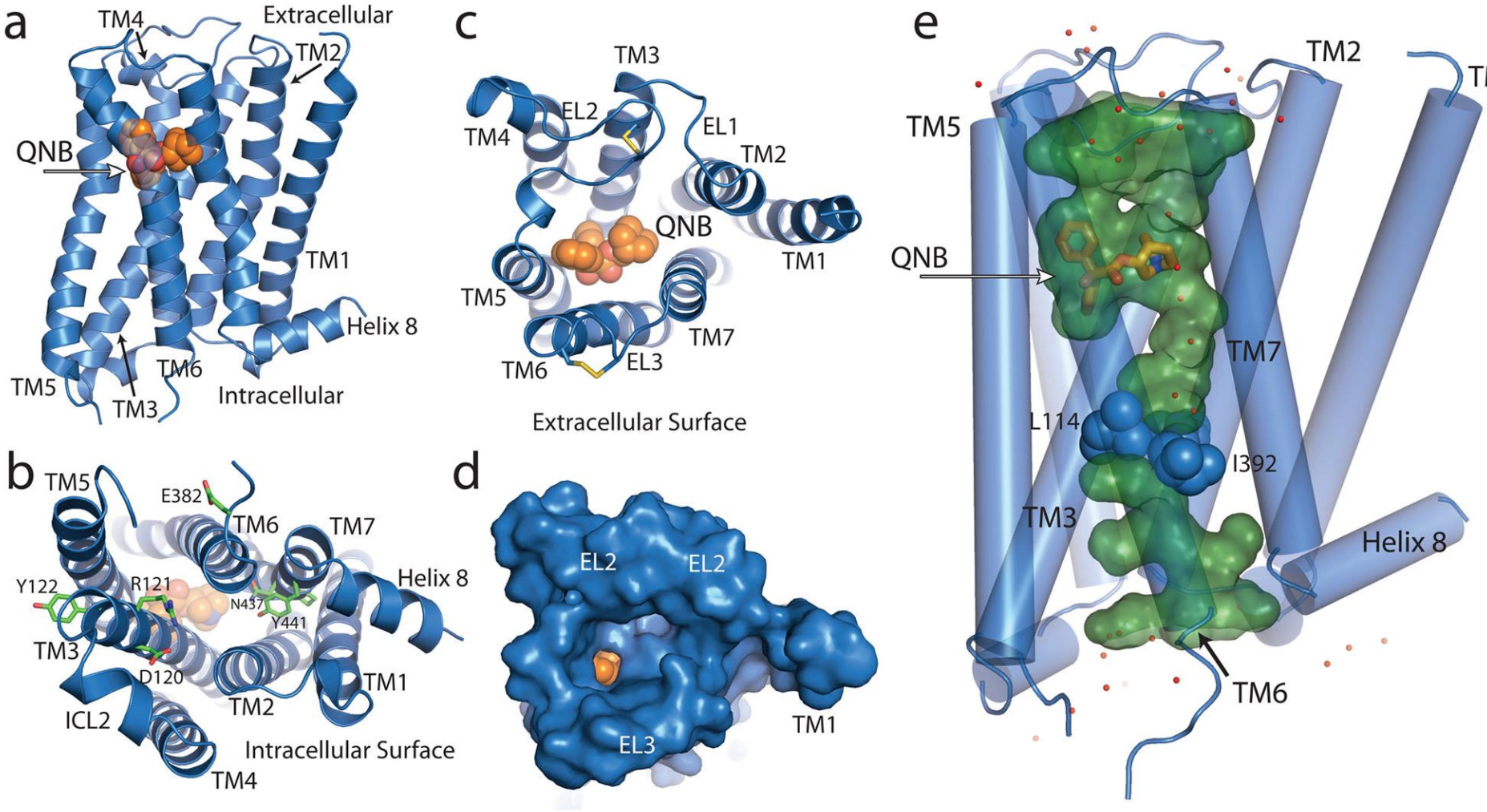
- The natural agonist acetylcholine is much smaller than the bulky antagonist QNB. As described in agonist-bound structure of the β2 adrenergic receptor, the contraction of ligand binding pocket is expected as a result of an inward shift of TM5.
- This result is consistent with the previous mutation studies showing that Thr187 and Thr190 in TM5 (Fig. 2) alter binding of most agonists but not of antagonists.
- Bulky compounds capable of blocking activation-related contraction of the pocket would be very efficient in locking M2 receptor in an inactive conformation as is exemplified here by the antagonist QNB.

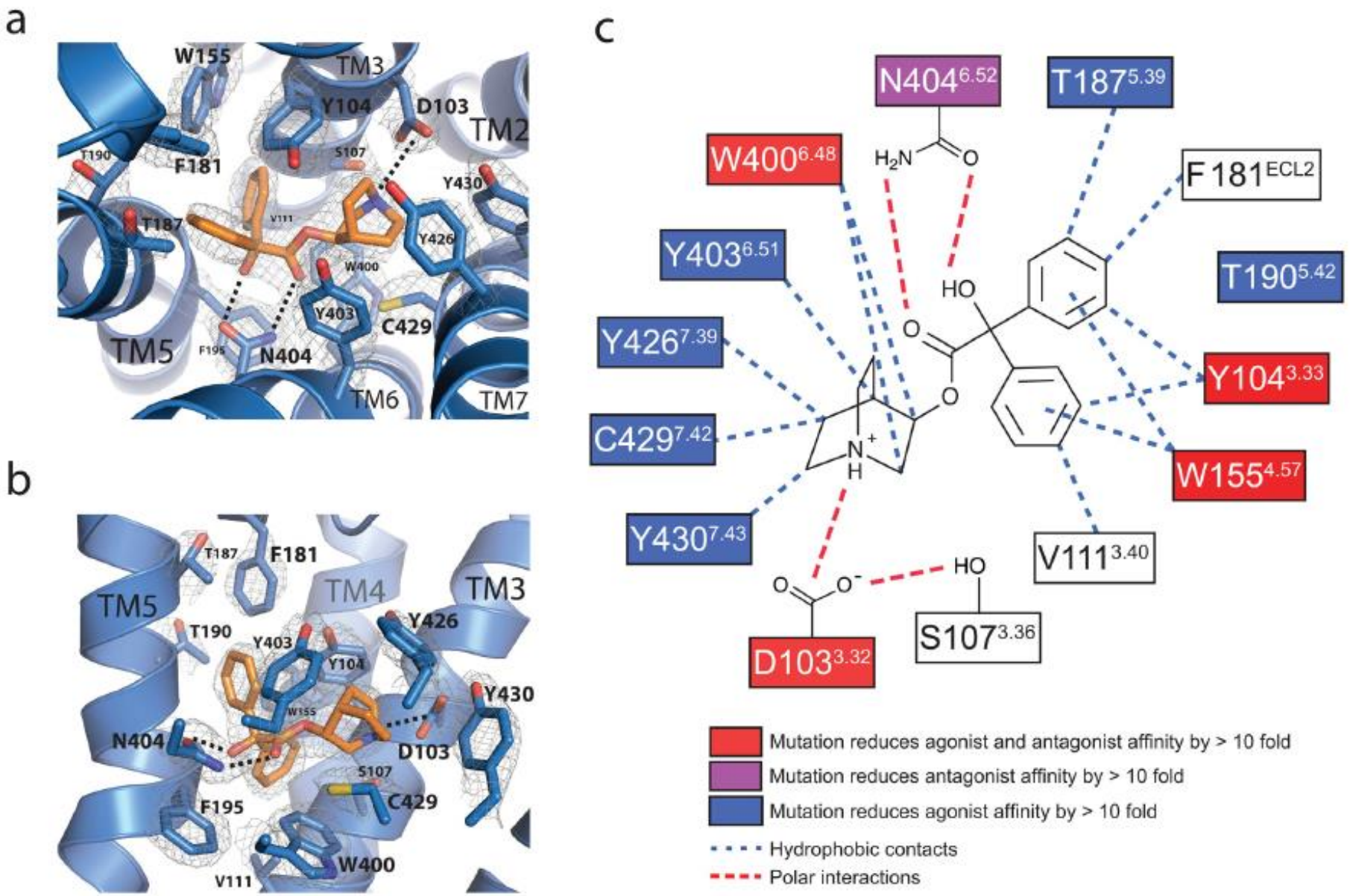
The 3-QNB antagonist



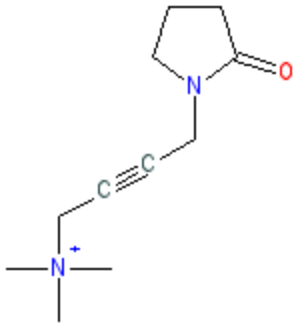
Binding mode

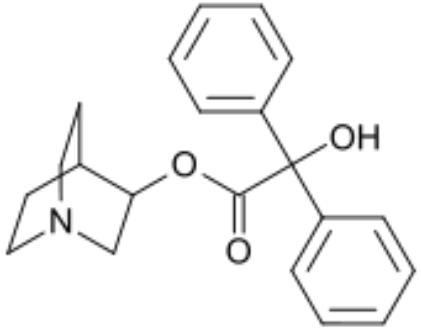
- The ligand QNB binds within a deeply buried pocket defined by side chains of TM3, 4, 5, 6 and 7.
- An aromatic cage encloses the amine and forms a lid over the ligand, separating the **orthosteric site** from the extracellular vestibule


a
TM4
Extracellular
TM2
QNB
TM1
Helix 8
TM6
TM5
TM3
Intracellular
b
TM5
E382
TM6
TM7
Helix 8
Y122
R121
N437
Y441
TM3
D120
TM1
TM2
ICL2
Intracellular Surface
TM4
c
TM3
EL2
EL1
TM4
TM2
QNB
TM1
TM5
TM7
TM6
EL3
Extracellular Surface
d
EL2
EL2
TM1
EL3
e
TM2
TM1
TM5
QNB
TM7
L114
I392
TM3
Helix 8
TM6


a
W155
TM3
Y104
D103
TM2
T190
F181
S107
Y430
T187
V111
Y426
W400
Y403
C429
TM5
F195
N404
TM6
TM7
b
T187
F181
TM4
TM3
TM5
T190
Y403
Y426
Y104
Y430
W155
N404
D103
S107
C429
F195
W400
V111
c
N404 6.52
T187 5.39
W400 6.48
F181 ECL2
Y403 6.51
T190 5.42
Y426 7.39
Y104 3.33
C429 7.42
W155 4.57
Y430 7.43
V111 3.40
D103 3.32
S107 3.36
Mutation reduces agonist and antagonist affinity by > 10 fold
Mutation reduces antagonist affinity by > 10 fold
Mutation reduces agonist affinity by > 10 fold
Hydrophobic contacts
Polar interactions

iperoxo agonist

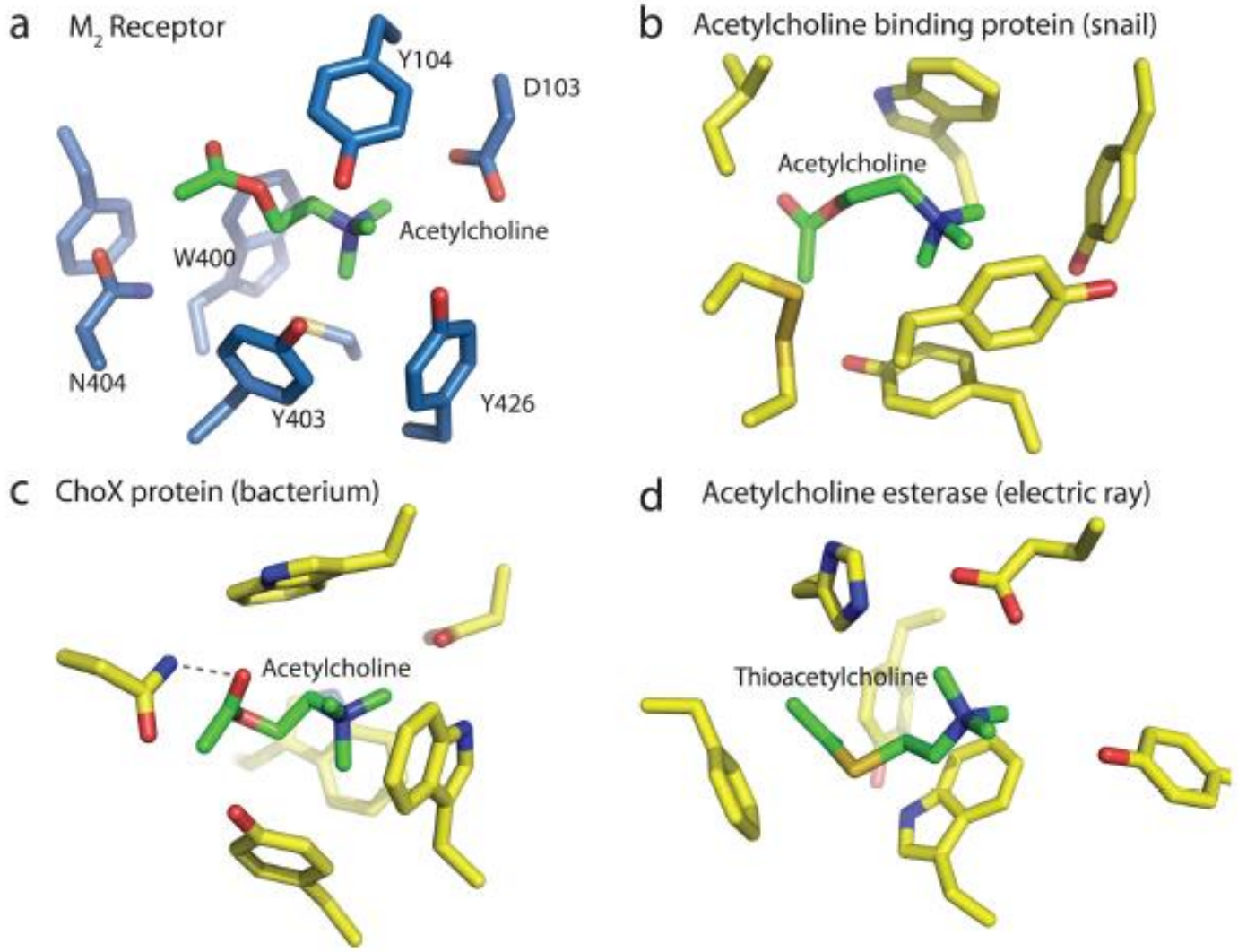




QNB antagonist

- In the active state of the M2 receptor, the small orthosteric agonist iperoxo engages Asp1033.32 with its cationic head group, whereas the polar isoxazoline tail contacts Asn4046.52 with a single hydrogen bond, paralleling the interactions of the inactive receptor with the antagonist QNB). These two interactions are bridged by an acetylene moiety in iperoxo, which runs perpendicular to transmembrane domain 3. Within the binding site, iperoxo exhibits a high degree of shape complementarity to the active conformation, filling the pocket almost entirely (FIG. 4d). By contrast, in the inactive conformation the M2 receptor presents a large orthosteric site that is better suited to the binding of larger antagonists and inverse agonists. The exceptional complementarity of iperoxo with the active — but not inactive — orthosteric site of the receptor may account for its agonistic activity.

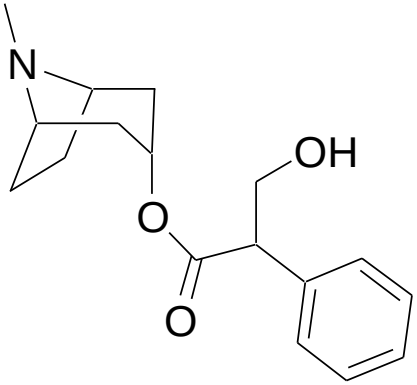
Convergent evolution of acetylcholine binding sites.



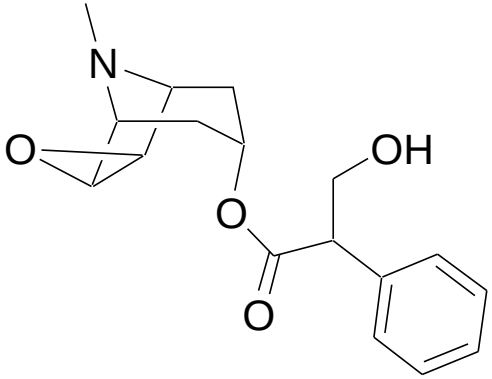
Antagonisti del recettore muscarinico che rimangono nel SNP: anticolinergici, antimuscarinici, parasimpaticolitici

- Dilatazione pupilla
- Riduzione motilità tratto GI ed urinario
- Riduzione secrezione salivare e gastrica
- Utilità farmacologica:
- ANTIASMATICI
- DILATAZIONE PUPILLA
- SPASMOLITICI
- ANTI ULCERE GASTRCHE
- CONTROLLO DISTURBI MOTORI DEL PARKINSON

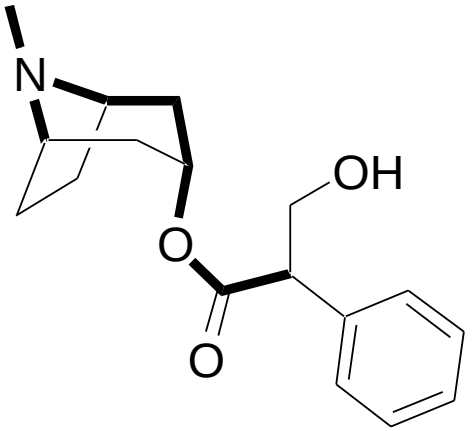
- Nelle visite oculistiche, dilatazione pupilla, ma possono indurre aumento pressione dell'occhio, controindicati in pazienti con glaucoma.
- L'**atropina** si ricava dalle radici di *Atropa belladonna* (pianta velenosa della famiglia delle solanacee).
- La **ioscina** (scopolamina) si ottiene dallo *Hyoscyamus niger* e *Datura stramonium* (stramonio) ed ha struttura simile (epossido)
- Perché queste molecole si legano al recettore muscarinico bloccandolo?



atropina



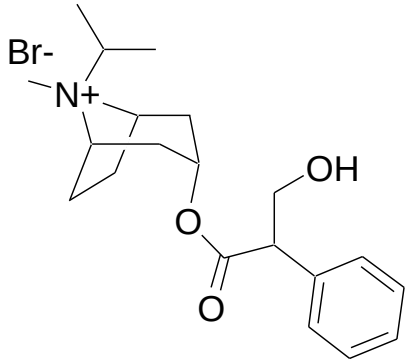
ioscina



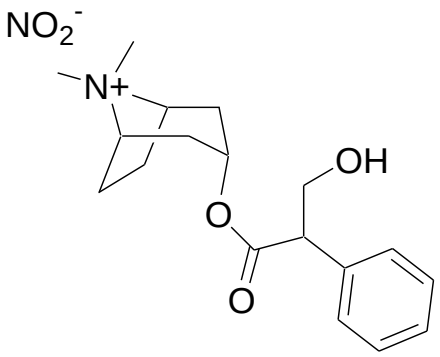
- A pH fisiologico l’atomo di azoto di atropina e scopolamina è protonato e dunque carico.
- Atropina ha pKa = 9.8, mentre scopolamina o ioscina ha pKa =7.6
- La scopolamina, a pH fisiologico è in larga parte neutra, non avendo una carica netta penetra nel SNC con importanti effetti collaterali.
- E’ dunque un allucinogeno se usati in dosi massicce: dalle streghe nel medioevo (estratti di stramonio).
- “Siero della verità”, inducono perdita di memoria.

Analoghi strutturali carichi dell'atropina

- Per ridurre gli effetti collaterali sul SNC: sali di ammonio quaternario (ipratropio, atropina metonitrato, rispettivamente broncodilatatore e riduzione motilità tratto GI).
- La carica del metonitrato ne sfavorisce anche l'assorbimento intestinale, questo favorisce l'effetto sulla muscolatura liscia intestinale

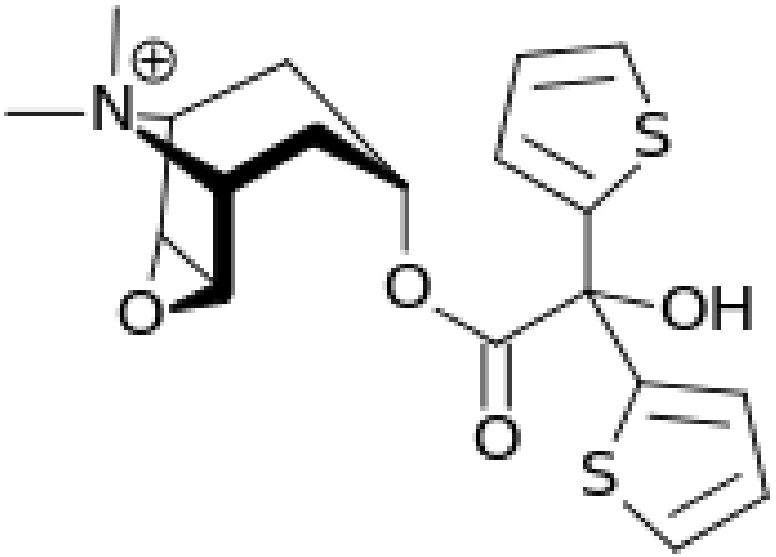


ipratropio

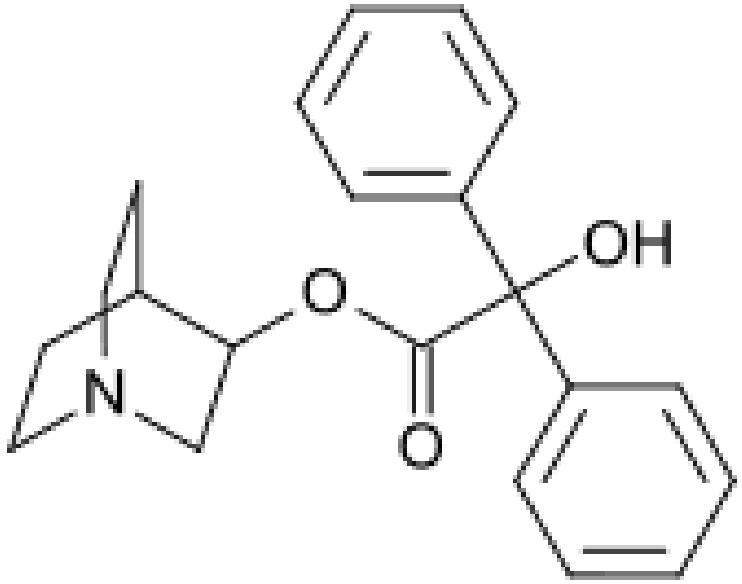


atropina metonitrato

Tiotropium and QNB (antagonists on M3 and M2, respectively)



tiotropium

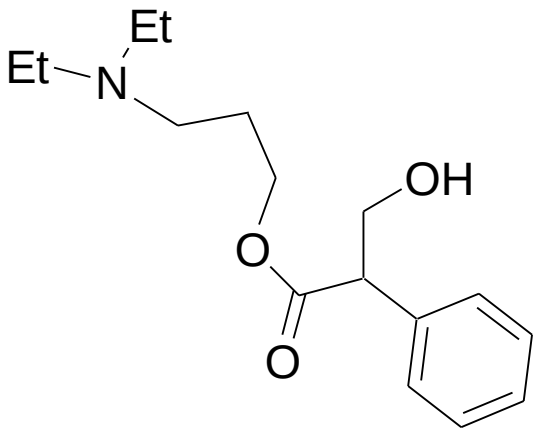


QNB

SAR per antagonisti sintetici: semplificazione della struttura

- Generalizzazione della SAR degli antagonisti muscarinici:
- Il sistema ciclico presente nell'atropina non è strettamente necessario, caso dell'Amprotropina e della Propantelina .
- E' preferibile una ramificazione in alfa all'estere con due sostituenti ciclici e lipofili, in genere un aromatico ed un alifatico.
- Vedere varietà chimica delle strutture degli antagonisti noti ed in uso.

- Glicopirrolato
- Propantelina
- Clidinio
- Ipratropio
- Flavoxato
- Oxifenciclimina



amprotropina

- Struttura generale di un antagonista della acetilcolina:
- Il gruppo alchilico sull'azoto può essere più grande del metile (a differenza degli agonisti)
- L'atomo di azoto può essere terziario o quaternario
- Gruppo acilico grande e ramificato (siti aggiuntivi di binding).
- Queste strutture hanno affinità maggiore per il recettore rispetto all'ACh perché aumentano il numero di interazioni idrofobiche. I gruppi aromatici si legano in zone idrofobiche al di fuori del sito di legame per ACh.
- Maggiore affinità, ma non provocano il cambiamento conformazionale nel recettore che è necessario per la trasmissione del segnale (interazione con la G-protein nella cellula).

Orthosteric and allosteric ligands

- In biochemistry and pharmacology, an allosteric modulator (allo- from the Greek meaning "other") is a substance which indirectly influences (modulates) the effects of a receptor agonist or inverse agonist at its receptor protein target.
- Allosteric modulators bind to a site distinct from that of the orthosteric binding site where a receptor agonist would normally bind. Usually they induce a conformational change within the protein structure.

The orthosteric binding pocket is formed by amino acids that are identical in all 5 muscarinic receptor subtypes, and shares structural homology with other functionally unrelated acetylcholine binding proteins from different species.

A layer of tyrosine residues forms an aromatic cap restricting dissociation of the bound ligand. A binding site for allosteric ligands has been mapped to residues at the entrance to the binding pocket near this aromatic cap.

The M2 receptor structure provides insights into the challenges of developing subtype-selective ligands for muscarinic receptors and their propensity for allosteric regulation.

Muscarinic acetylcholine receptors: novel opportunities for drug development

Andrew C. Kruse[1,2], Brian K. Kobilka[1], Dinesh Gautam[3], Patrick M. Sexton[4], Arthur Christopoulos[4] and Jürgen Wess[3]

Abstract | The muscarinic acetylcholine receptors are a subfamily of G protein-coupled receptors that regulate numerous fundamental functions of the central and peripheral nervous system. The past few years have witnessed unprecedented new insights into muscarinic receptor physiology, pharmacology and structure. These advances include the first structural views of muscarinic receptors in both inactive and active conformations, as well as a better understanding of the molecular underpinnings of muscarinic receptor regulation by allosteric modulators. These recent findings should facilitate the development of new muscarinic receptor subtype-selective ligands that could prove to be useful for the treatment of many severe pathophysiological conditions.

Novel mAChR pharmacology (CNS)

A major shift in drug discovery has been the recognition that most — if not all — GPCRs possess spatially distinct **allosteric sites** that can be exploited by small molecules to modulate the activity of orthosteric ligands.

The mAChRs are arguably the pre-eminent models for contributing to our understanding of GPCR allostery

Definitions: PAM and NAM

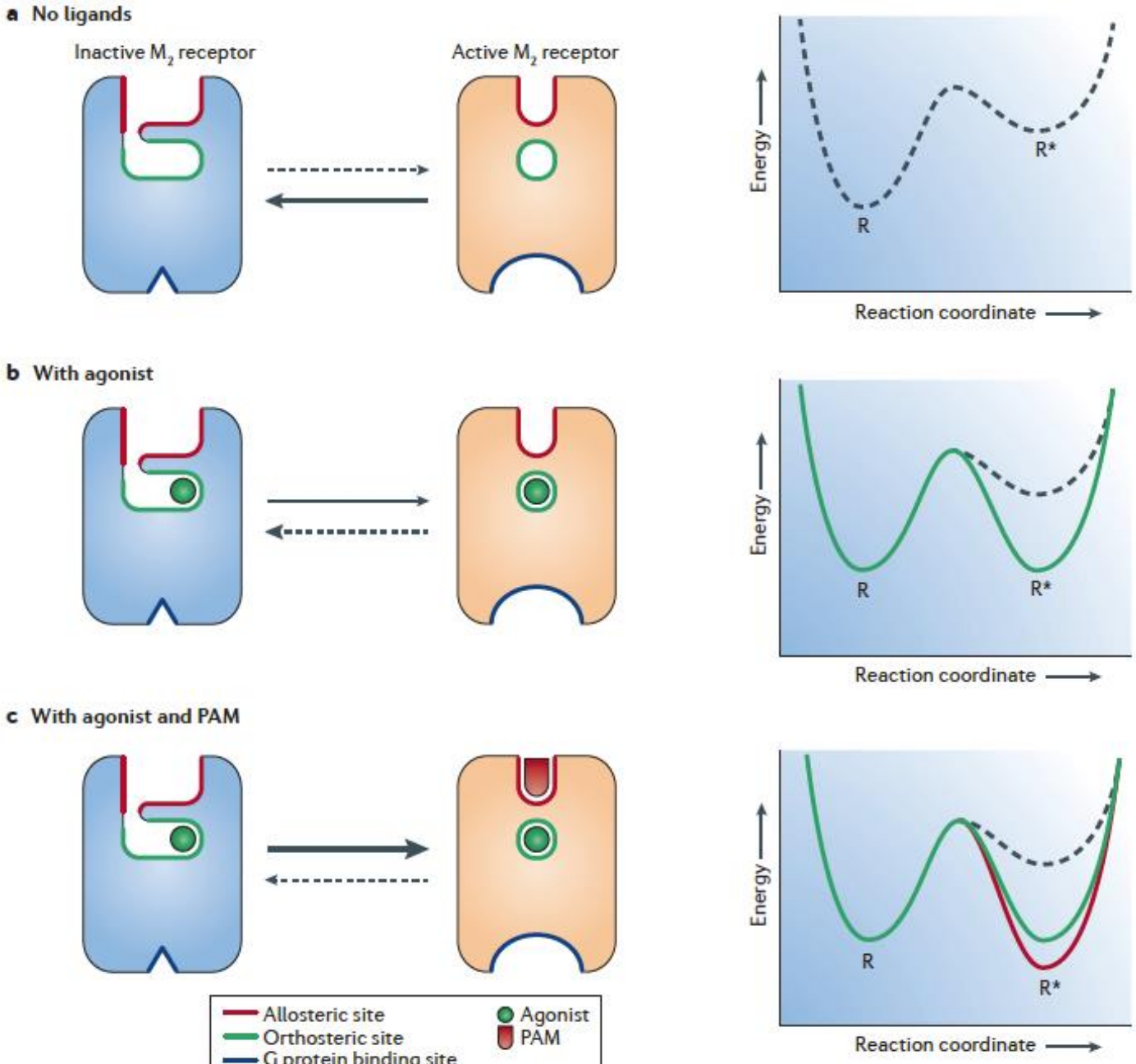
- Positive allosteric modulators (PAMs) enhance orthosteric activity, negative allosteric modulators (NAMs) inhibit it.

Molecular mechanism of allostery

- The simplest mechanism to account for allosteric behaviour is the Monod–Wyman–Changeux (MWC) model which postulates that receptors exist in an equilibrium between different states, and that orthosteric and allosteric ligands select one or more of these states over others.

- Because this occurs via topographically distinct sites, the state that is stabilized by one class of ligand can present a modified binding surface for the other class, which is manifested as the allosteric interaction.

- If this mechanism were restricted to two states, it would be expected that any ligand favouring the active state would display some degree of agonism, whereas compounds stabilizing the inactive state would show some degree of inverse agonism. Moreover, allosteric ligands that favoured the active state would be PAMs for agonists but NAMs for inverse agonists; this is the simplest molecular explanation for probe dependence.


a No ligands
Inactive M2 receptor
Active M2 receptor
Energy
R
R*
Reaction coordinate
b With agonist
Energy
R
R*
Reaction coordinate
c With agonist and PAM
Energy
R
R*
Reaction coordinate
Allosteric site
Orthosteric site
G protein binding site
Agonist
PAM

agonism, inverse agonism, antagonism

- In the field of pharmacology, an inverse agonist is an agent that binds to the same receptor as an agonist but induces a pharmacological response opposite to that agonist. A neutral antagonist has no activity in the absence of an agonist or inverse agonist but can block the activity of either. Inverse agonists have opposite actions to those of agonists but the effects of both of these can be blocked by antagonists

Example of PAM

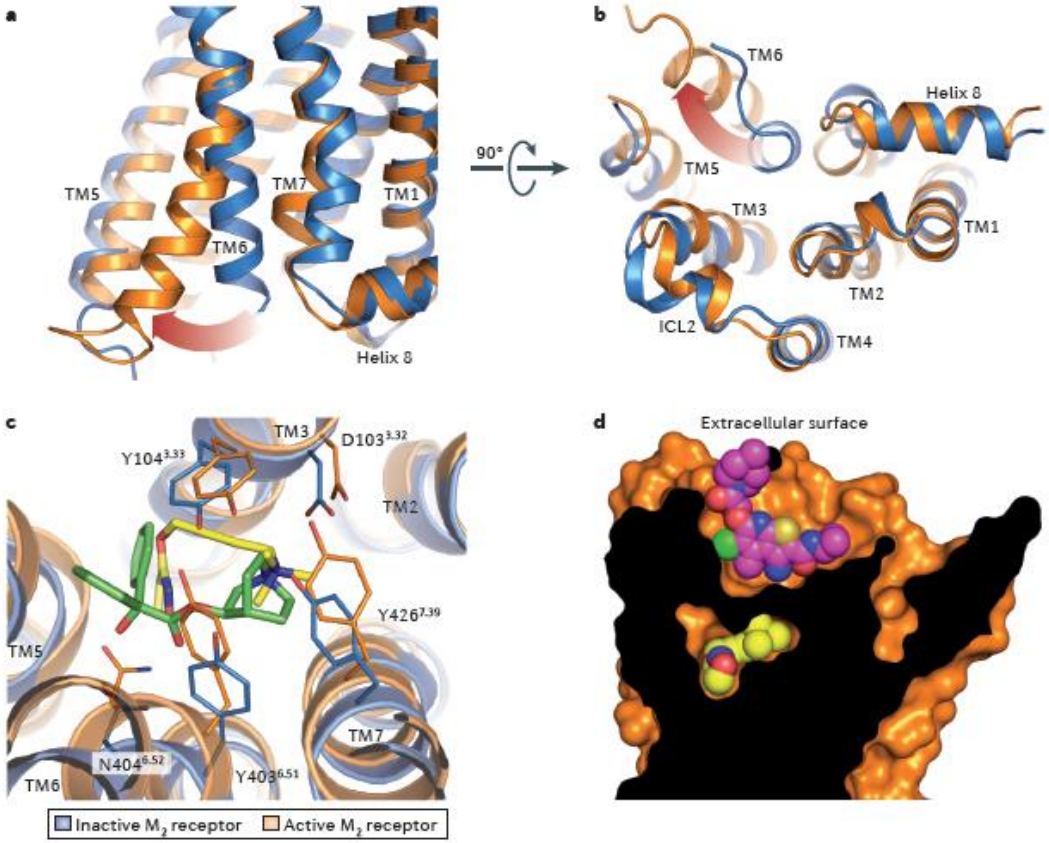
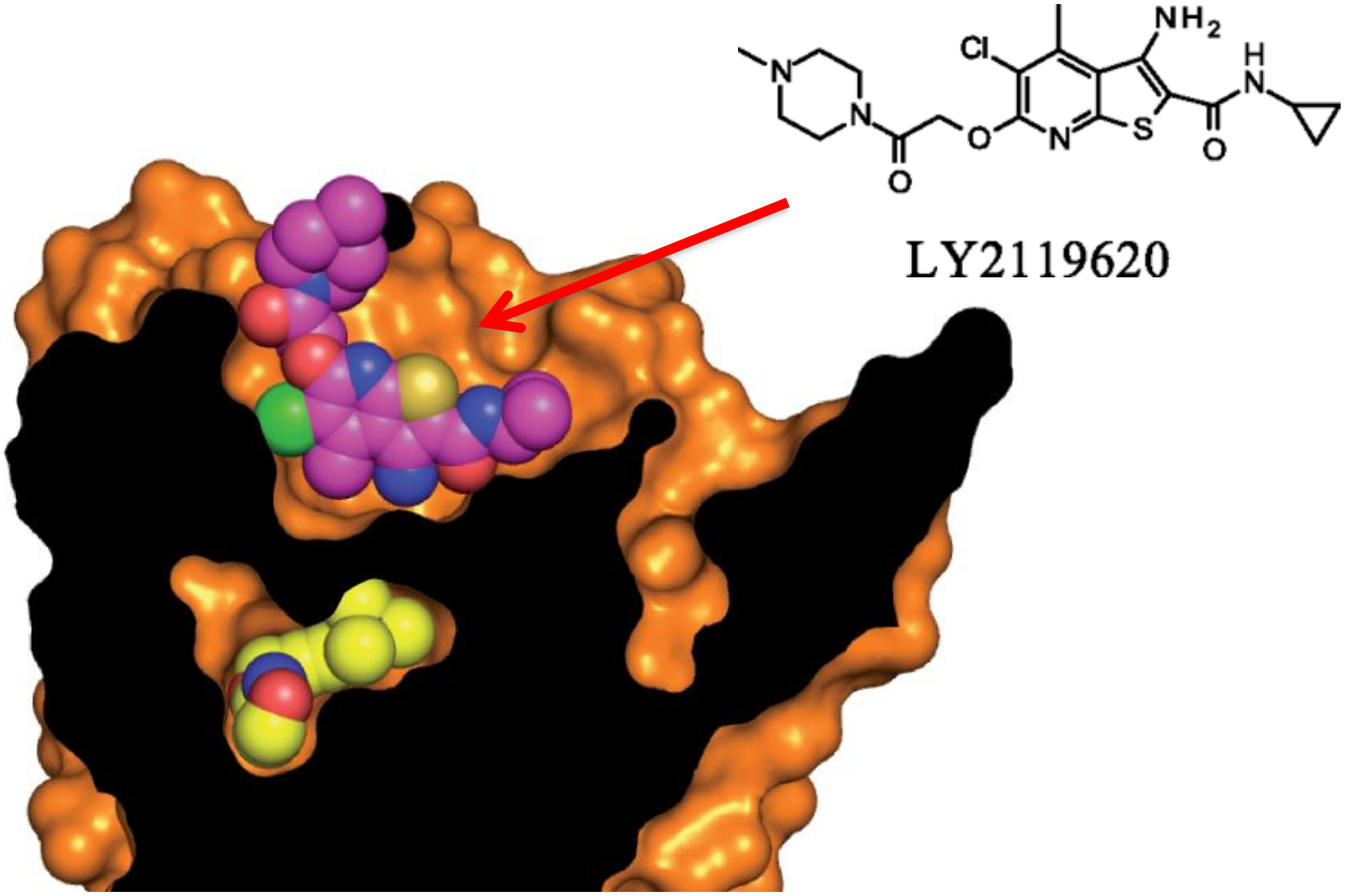


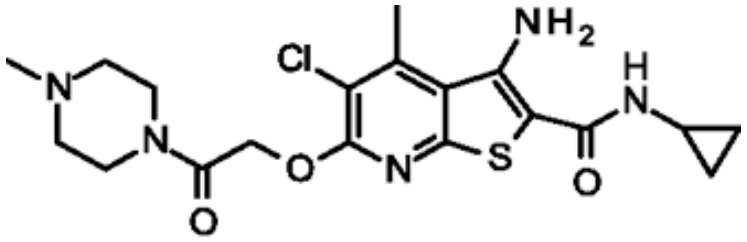
Figure 4 | **Activation and allosteric modulation of the M_2 receptor.** As shown in part **a** and part **b**, the intracellular tip of transmembrane domain 6 (TM6) rotates outwards in the active M_2 receptor structure (orange) relative to the inactive state (blue). As shown in part **c**, the orthosteric binding site contracts upon M_2 receptor activation, enclosing the agonist iperoxo (yellow) in a smaller binding site, as compared to the antagonist (QNB; green) binding cavity. Residues are numbered according to the human M_2 receptor sequence. As shown in part **d**, LY2119620 (magenta), a muscarinic positive allosteric modulator, binds to the extracellular vestibule of the M_2 receptor directly above the orthosteric agonist iperoxo (yellow). ICL2, intracellular loop 2.

In the active state of the M2 receptor, the small orthosteric agonist iperoxo engages Asp1033.32 with its cationic head group, whereas the polar isoxazoline tail contacts Asn4046.52 with a single hydrogen bond, paralleling the interactions of the inactive receptor with the antagonist QNB

Agonista allosterico LY2119620



LY2119620



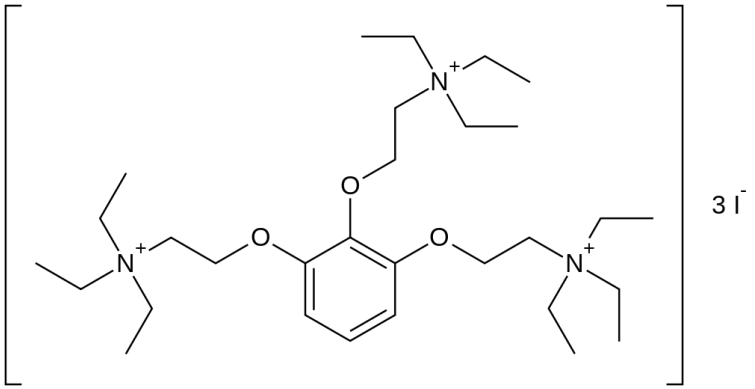
LY2119620

- Strikingly, structures of the active M2 receptor with and without LY2119620 were highly similar, even in the extracellular vestibule. Indeed, the only substantial change in this pocket was a reorientation of Trp4227, which directly interacts with the bound LY2119620.

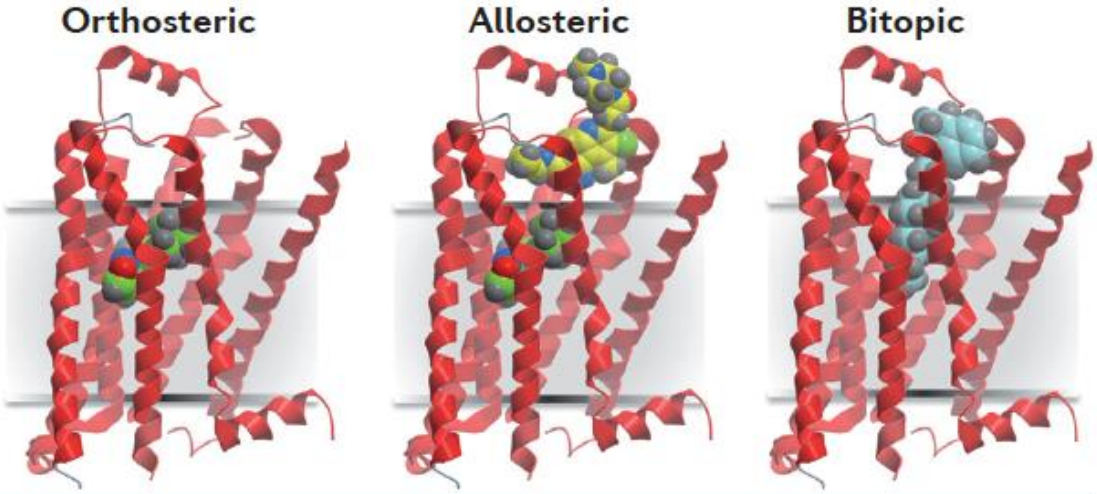
...exceptions to the current model of allosteric modulation

- However, this simple model does not explain the probe dependence observed with compounds such as LY2033298 at the M2 receptor, which can show PAM or NAM activity with different agonists. It also does not explain biased allosteric modulation, or why compounds such as gallamine are NAMs for agonists and antagonists.
- Thus, additional mechanisms seem to be involved, most obviously involving multiple active receptor states.

gallamine iodide: NAM for both agonists ad antagonists



Gallamine triethiodide (Flaxedil) is a non-depolarising muscle relaxant. It acts by combining with the cholinergic receptor sites in muscle and competitively blocking the transmitter action of acetylcholine. Gallamine triethiodide has a parasympatholytic effect on the cardiac vagus nerve which causes tachycardia and occasionally hypertension. Very high doses cause histamine release.



	Orthosteric	Allosteric	Bitopic
Well-defined SAR	✓	✗	✗
High affinity	✓	✗	✓
Subtype selectivity	✗	✓	✓
Spatial and temporal selectivity of endogenous signalling	✗	✓	✗

Figure 1 | **Modes of targeting mAChRs (GPCRs) by different classes of ligands.** Orthosteric ligands (green) bind to the site recognized by the endogenous agonist (acetylcholine) for the receptor. Allosteric ligands (yellow) bind to a topographically distinct site. Bitopic ligands (blue) concomitantly interact with both orthosteric and allosteric sites. The key properties generally associated with each mode of receptor targeting are also indicated. GPCR, G protein-coupled receptor; mAChR, muscarinic acetylcholine receptor; SAR, structure–activity relationship.

bitopic ligands

The bitopic muscarinic receptor antagonist THRX-160209 demonstrated remarkable gains in both affinity and selectivity for the M2 receptor, which highlights that the appropriate combination of an orthosteric antagonist, NAM and linker can yield ligands with the desired pharmacological properties.

THRX-160209

A Novel Multivalent Ligand That Bridges the Allosteric and Orthosteric Binding Sites of the M_2 Muscarinic Receptor

Tod Steinfeld, Mathai Mammen, Jacqueline A. M. Smith, Richard D. Wilson, and Jeffrey R. Jasper[1]

Theravance, Inc., South San Francisco, California

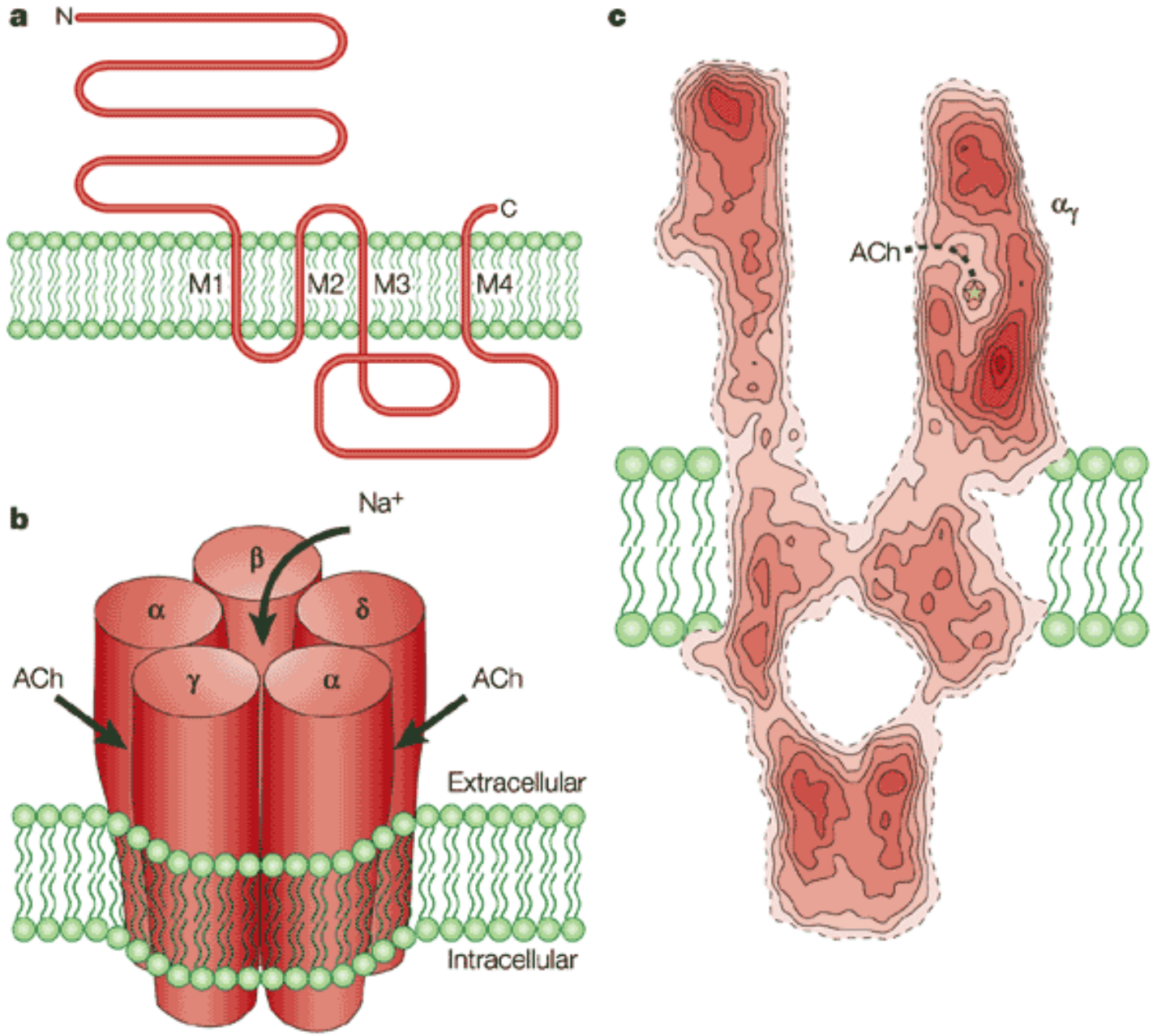
Received January 8, 2007; accepted May 3, 2007

Antagonisti del recettore colinergico nicotinico nel SNP: bloccanti neuromuscolari

- Recettore nicotinico: nelle sinapsi tra due cell. nervose (sistema simpatico e parasimpatico) e nella sinapsi tra fibra nervosa e muscolo scheletrico.
- Bloccanti neuro-muscolari
- Come nel caso della atropina per i muscarinici, la prima sostanza antagonista dei nicotinici, la tubocurarina è di origine naturale.
- Anche in questo caso abbiamo un processo di semplificazione strutturale quando vengono progettati composti di tipo sintetico

Il recettore nicotinico

- Recettore a canale ionico con accesso controllato dal ligando
- Cinque subunità disposte attorno ad un poro centrale: pentamero $\alpha_2\beta\gamma\delta$
- Ciascuna subunità è costituita da 4 α-eliche parallele M_1, M_2, M_3, M_4
- Due siti di binding per l'Ach, uno su ogni subunità α, all'interfaccia α–δ ed α–γ



Nature Reviews | Neuroscience

Nicotinic receptors: allosteric transitions and therapeutic targets in the nervous system

Antoine Taly, Pierre-Jean Corringer‡, Denis Guedin§, Pierre Lestage§ and Jean-Pierre Changeux||*

Abstract | Nicotinic receptors — a family of ligand-gated ion channels that mediate the effects of the neurotransmitter acetylcholine — are among the most well understood allosteric membrane proteins from a structural and functional perspective. There is also considerable interest in modulating nicotinic receptors to treat nervous-system disorders such as Alzheimer's disease, schizophrenia, depression, attention deficit hyperactivity disorder and tobacco addiction. This article describes both recent advances in our understanding of the assembly, activity and conformational transitions of nicotinic receptors, as well as developments in the therapeutic application of nicotinic receptor ligands, with the aim of aiding novel drug discovery by bridging the gap between these two rapidly developing fields.

nAchRs

Knowledge of the atomic structure, functional organization and conformational transitions of the nAChRs has recently progressed to the extent that these receptors are now among the most well understood allosteric membrane proteins (that is, proteins that mediate signal transduction between topographically distinct sites, through a conformational change). This makes them an exceptional model to investigate the molecular mechanisms of drug–receptor interactions. The acute effect of ACh consists of the fast opening (microsecond to millisecond range) of a cationic channel that is permeable to Na+, K+ and sometimes Ca2+ ions

Pharmacological interest of nAChRs

There is considerable interest in modulating nAChRs to treat various nervous-system disorders, such as Alzheimer's disease, schizophrenia, depression, attention deficit hyperactivity disorder (ADHD) and tobacco addiction

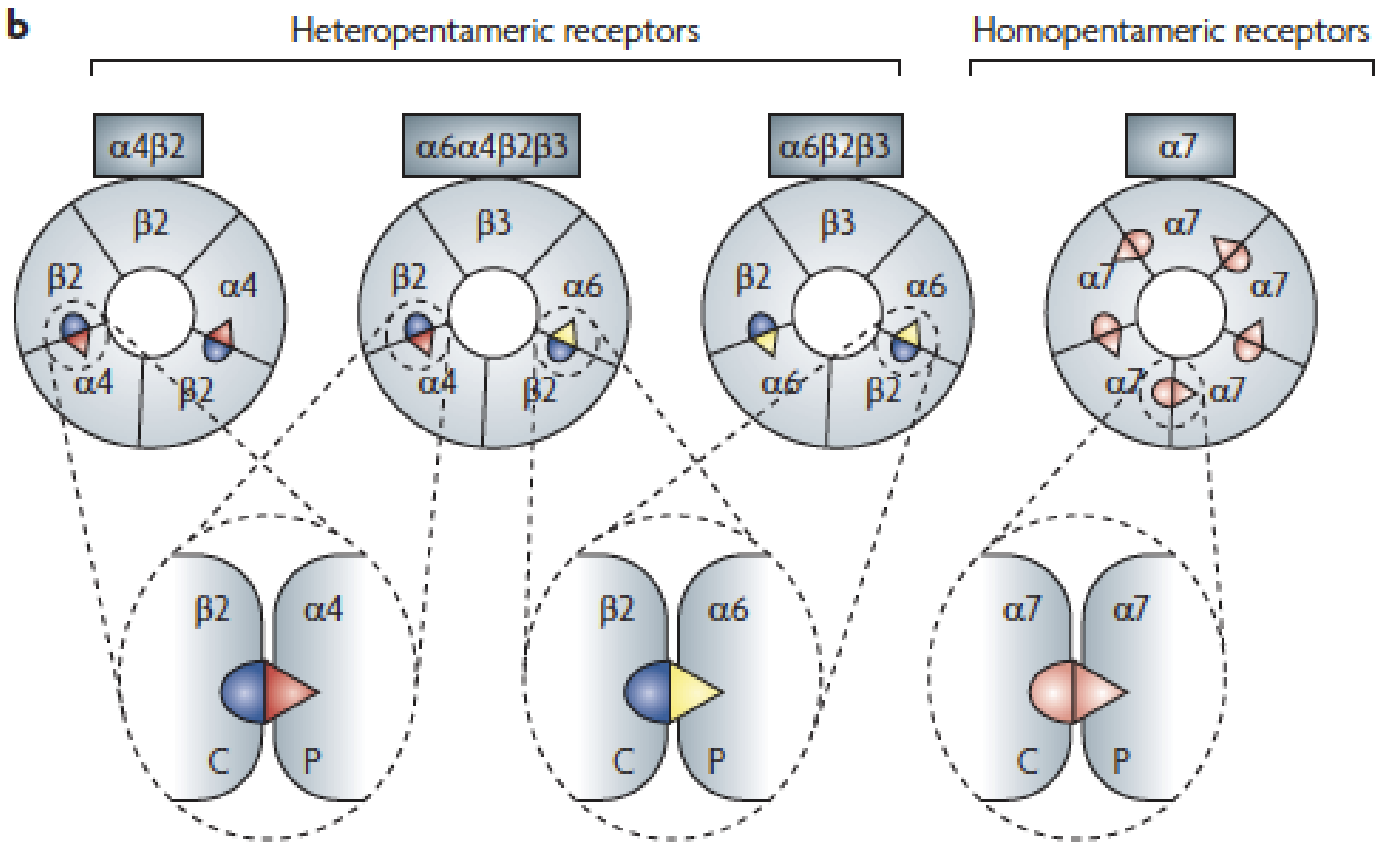
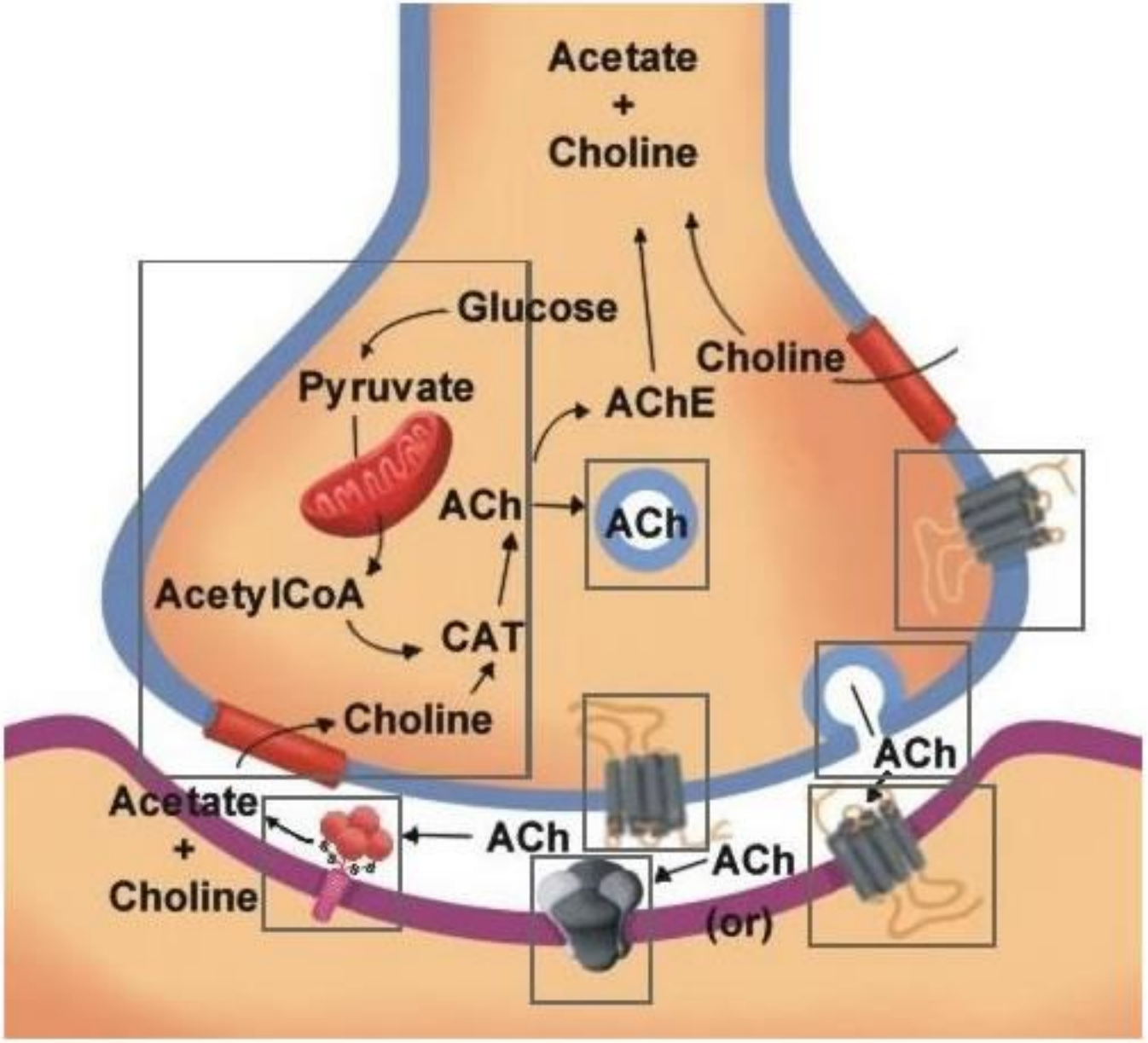


Figure 1 | **Variability of nicotinic binding sites and receptor subunits. a** | The various assemblies of nicotinic acetylcholine receptor subtypes are broadly distributed in the brain. **b** | Variability in pharmacological specificity of the nicotinic binding site at subunit interfaces results from the association of nicotinic receptor subunits into multiple oligomers. Triangles represent the 'principal' (P) side of the binding site, and semi-circles represent the 'complementary' (C) side. The receptors are named according to the subunits that form the binding site. Figure is reproduced, with permission, from REF. 280 © (2006) Elsevier.


Acetate
+
Choline
Glucose
Pyruvate
AChE
Choline
ACh
ACh
AcetylCoA
CAT
Choline
ACh
Acetate
+
Choline
ACh
ACh
(or)

Agonisti colinergici (ortosterici)

Trattamento del glaucoma

I meccanismi attraverso i quali si sviluppa un glaucoma, sono ancora in parte sconosciuti, ma sono stati individuati numerosi fattori di rischio, che si associano alla malattia, tra cui si segnalano in particolare: pressione oculare elevata, età, razza, familiarità, miopia, spessore corneale centrale, e fattori vascolari.

Reintegro funzioni gastrointestinali

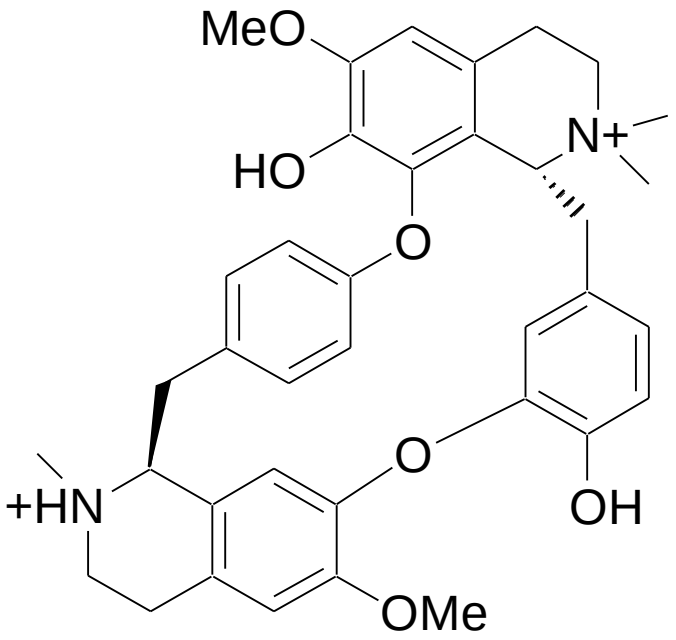
Trattamento di difetti cardiaci per i quali sia necessario deprimere il muscolo cardiaco

Agonisti nicotinici: trattamento della *myastenia gravis*.

La **miastenia gravis** è una malattia neuromuscolare caratterizzata da debolezza muscolare fluttuante e affaticabilità. È una delle malattie autoimmuni meglio conosciute e gli antigeni e i meccanismi della malattia sono stati identificati con precisione. La debolezza muscolare è causata da anticorpi circolanti che bloccano i recettori colinergici postsinaptici o le proteine MuSK (muscle-specific tyrosine kinases) della giunzione neuromuscolare, inibendo l'effetto stimolante del neurotrasmettitore acetilcolina.

La tubocurarina

- E’ il principio attivo del curaro, estratto grezzo essiccato dalla pianta di *Chondrodendron tomentosum* usato dagli indigeni del Sud America come veleno da freccia. Causa paralisi muscolare.



- Sono 10 atomi che separano due azoti carichi, uno dei due azoti ha due metili ed è quaternario, l’altro è terziario, ma protonato a pH fisiologico.

- Nel recettore del muscolo, due molecole di ACh si legano alle due subunità α (nella parte N-terminale) e ciascuna delle subunità che formano il pentamero presenta all'interno l'elica trans-membrana M2.
- Le alfa eliche M2 formano un canale e la parte di elica che forma la parete del canale ha residui carichi negativamente (aspartati, glutammati).
- Il legame dell'acetilcolina con le subunità alfa genera un cambiamento conformazionale delle eliche che "aprono" il canale, il sodio entra e la membrana si depolarizza.
- L'acetilcolina è un neurotrasmettitore **eccitatore** e causa un potenziale d'azione con l'entrata nella cellula di ioni positivi.

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 277, No. 44, Issue of November 1, pp. 42249–42258, 2002

Printed in U.S.A.

Orientation of *d*-Tubocurarine in the Muscle Nicotinic Acetylcholine Receptor-binding Site*

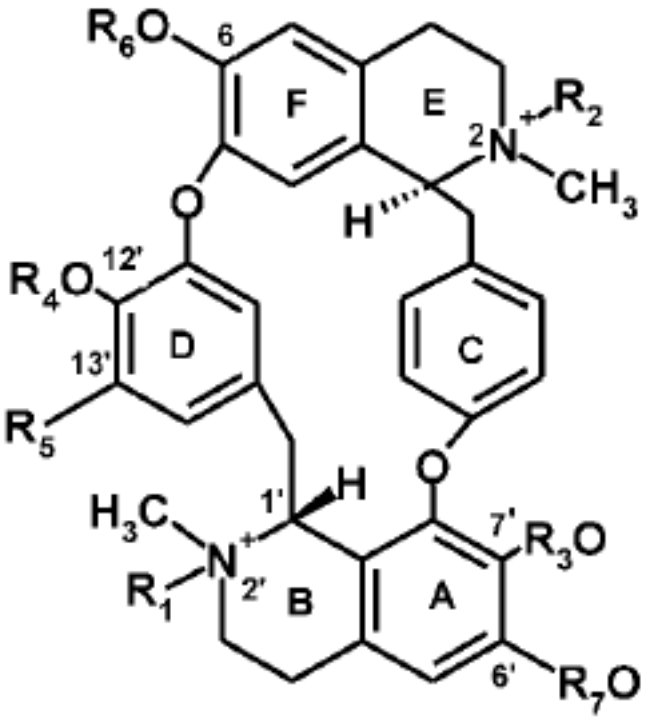
Received for publication, May 31, 2002, and in revised form, August 20, 2002
Published, JBC Papers in Press, August 23, 2002, DOI 10.1074/jbc.M205383200

Irmgard Ursula Willcockson‡, Ailing Hong§, Robert P. Whisenant¶, Jonathan B. Edwards, Haijun Wang, Hemanta K. Sarkar‖, and Steen E. Pedersen**

From the Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, Texas 77030

- The muscle nicotinic acetylcholine receptor (AChR)1 is a member of the ligand-gated ion channel superfamily. It is a pseudo-symmetric pentamer □with a subunit stoichiometry of the agonist-binding sites, which are responsible for activating channel opening, lie at the interfaces between the α- and γ- subunits and between the α- and δ- subunits.

- Ligand modification and receptor site-directed mutagenesis were used to examine binding of the competitive antagonist, d-tubocurarine (dTC), to the muscle type nicotinic acetylcholine receptor (AChR).
- By using various dTC analogs, we measured the interactions of specific dTC functional groups with amino acid positions in the AChR -subunit.

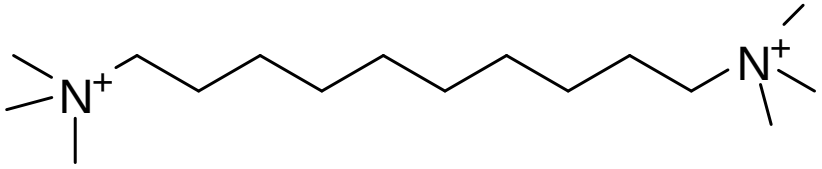


R_1	R_2	R_3	R_4	R_5	R_6	R_7	NAME
CH_3	H	H	H	H	CH_3	CH_3	*d*-Tubocurarine (dTC)
CH_3	H	$COCH_3$	$COCH_3$	H	CH_3	CH_3	Diacetyl-*d*-tubocurarine
CH_3	H	H	H	H	H	H	Di-demethyl-*d*-tubocurarine
H	H	H	H	H	CH_3	CH_3	Tubocurine
CH_3	CH_3	H	H	H	CH_3	CH_3	Chondocurarine
H	H	CH_3	CH_3	H	CH_3	CH_3	O,O-dimethyl-tubocurine
CH_3	CH_3	CH_3	H	H	CH_3	CH_3	7'-O-methyl-chondocurarine
CH_3	CH_3	H	CH_3	H	CH_3	CH_3	12'-O-methyl-chondocurarine
CH_3	CH_3	CH_3	CH_3	H	CH_3	CH_3	Metocurine
CH_3	H	H	H	I	CH_3	CH_3	Iodo-*d*-tubocurarine
CH_3	CH_3	H	H	I	CH_3	CH_3	Iodo-chondocurarine
CH_3	H	H	H	SO_3^-	CH_3	CH_3	Sulfo-*d*-tubocurarine

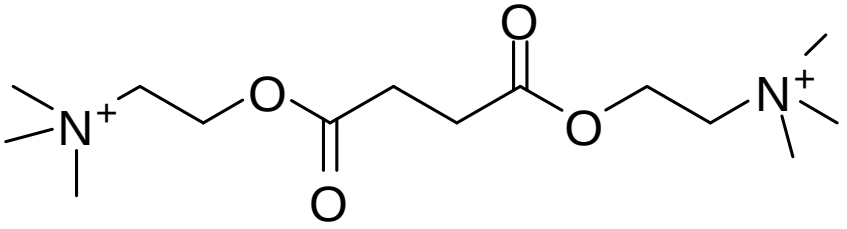
- Come interagisce la tubocurarina col recettore nicotinico?
- Distanza di 1.15 nm cioè 11.5 A tra i due azoti carichi. Si lega contemporaneamente ai due siti dibinding sulle subunità α?
- Si lega a due recettori nicotinici che vengono fatti dimerizzare?
- Si lega al sito di binding + un altro sito di interazione.
- La distanza tra gli azoti sembra importante: composti bis-ammonici quaternari

Bloccanti neuromuscolari , decametonio e succinilcolina

- Il decametonio si lega troppo fortemente al recettore generando un effetto troppo prolungato.
- Inoltre non viene metabolizzato in nessun modo. E' stato progettato il suxametonio: maggiore instabilità metabolica e chimica.
- Sono entrambi coadiuvanti degli anestetici generali, permettono di usarli in dosaggi ridotti.
- Nell'ordine bloccano i muscoli facciali, del collo e degli occhi, poi torace, addome, arti. Il recupero in ordine inverso.



decametonio



Succinil colina o suxametonio

Metocurina ioduro

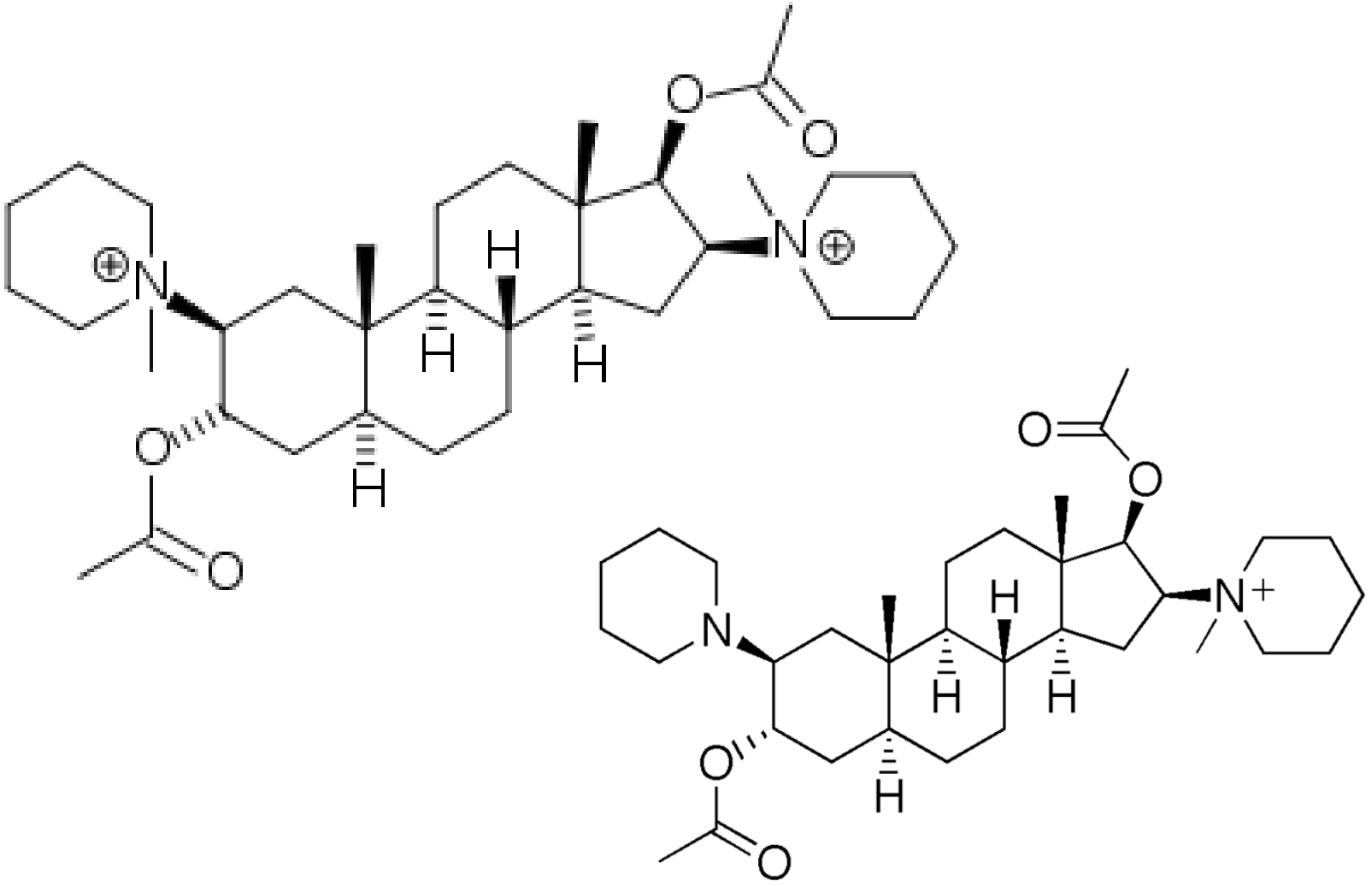
- Ottenuta per semplice trattamento della d-tubocurarina con ioduro di metile, ha entrambi gli azoti sotto forma di sali quaternari di dimetilammonio.
- E’ 4 volte più attiva della tubocurarina.
- Bloccante neuromuscolare, non viene metabolizzato e viene escreto invariato.

Pancuronio (Pavulon) e vecuronio (Norcuron)

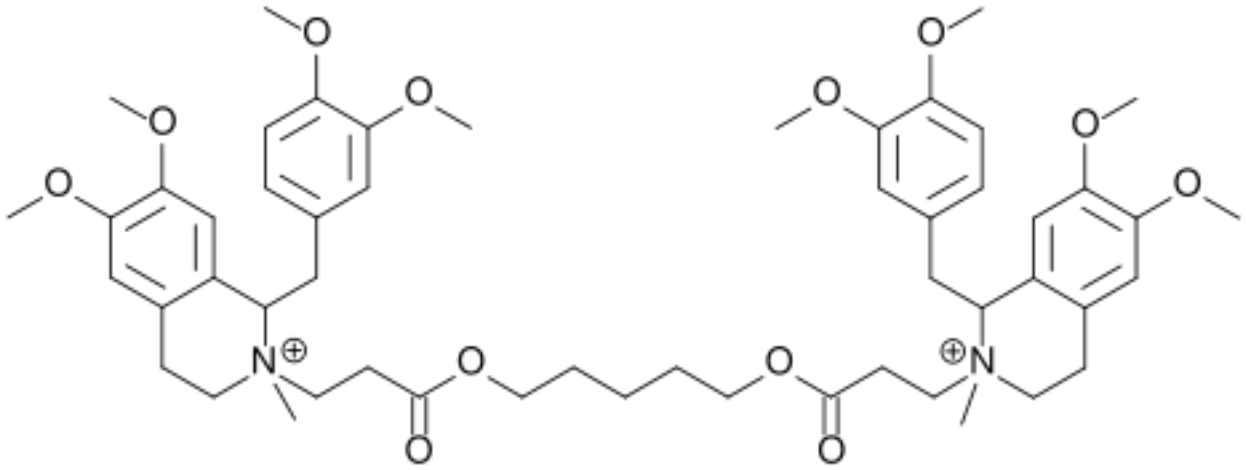
- Nucleo steroideo come spaziatore e scaffold. E' presente anche il gruppo estere acetico per rafforzare il legame col recettore. **Concetto di scaffold**

- ATRACURIO: dopo un po' si autodistrugge per eliminazione di Hoffmann.

Pancuronio, vecuronio



Atracurio, si disattiva per eliminazione di Hofmann

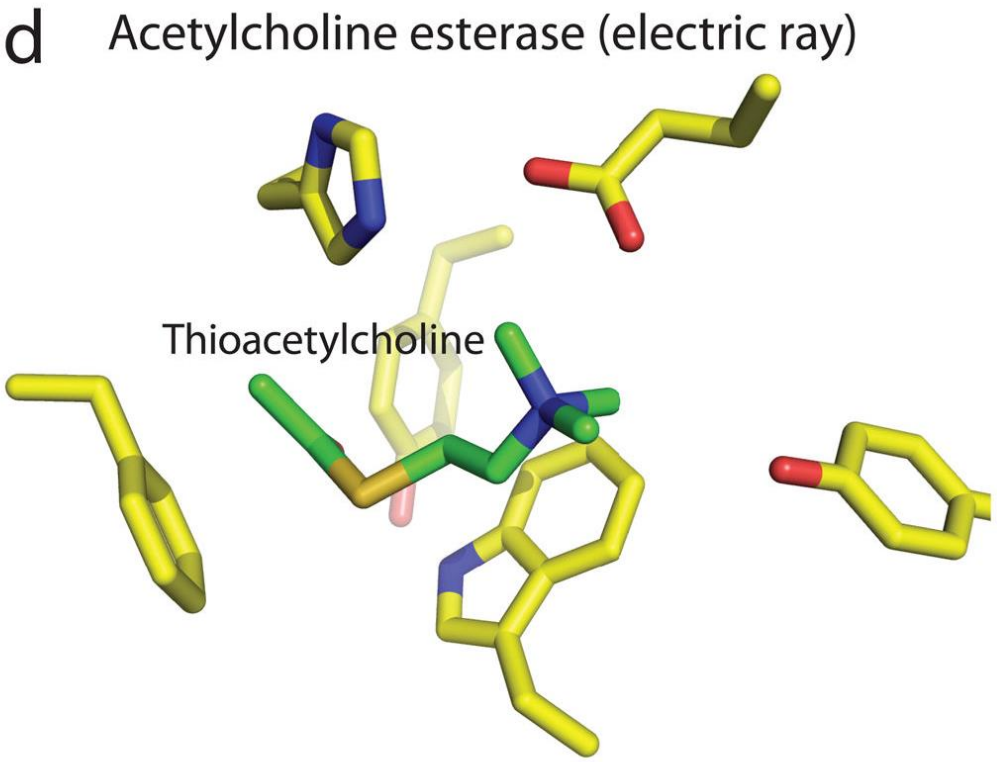


Agonisti colinergici indiretti: inibitori della acetilcolina esterasi (effetto su SNP e SNC)

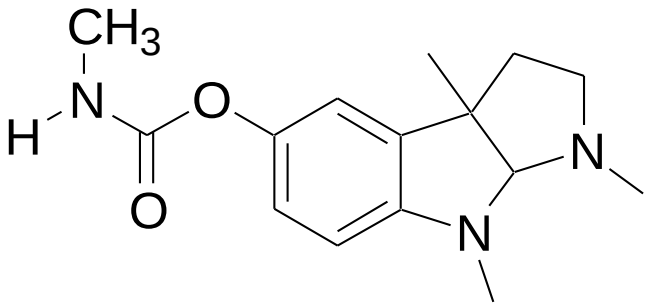
- Inibiscono l'acetilcolina esterasi che è responsabile dell'idrolisi dell'acetilcolina dallo spazio sinaptico in AcOH e colina.
- Quindi questi composti hanno un effetto agonistico (parasimpaticomimetici).
- Meccanismo di idrolisi dell'estere dell'acetilcolina attraverso la formazione di acetil-enzima intermedio che poi viene idrolizzato da acqua per ripristinare l'enzima.
- Gli inibitori formano un intermedio covalente stabile con l'enzima.

Legame dell'ACh al sito di legame dell'AChE

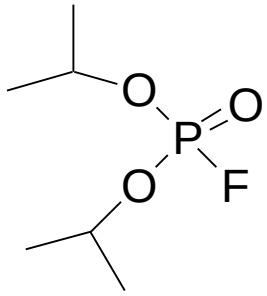
- Immagine, interazione con Ser, His che stabilizza carica neg dell'intermedio tetraedrico.
- La parte carica pos dell'N va in una zona con residui carichi negativamente



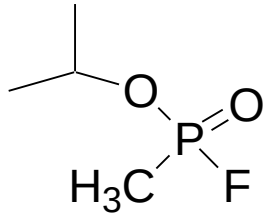
- Inibitori reversibili, sono farmaci, arilcarbammati
- Inibitori irreversibili, sostanze tossiche (gas nervini, insetticidi), formano fosfati o fosfinati con l’enzima



Fisostigmina, reversibile



DYFLOS

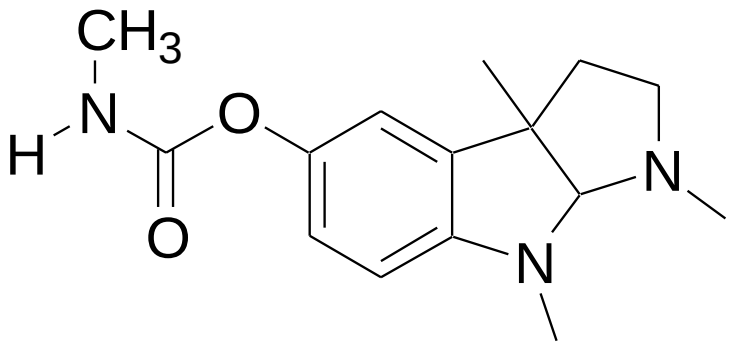


SARIN

irreversibili

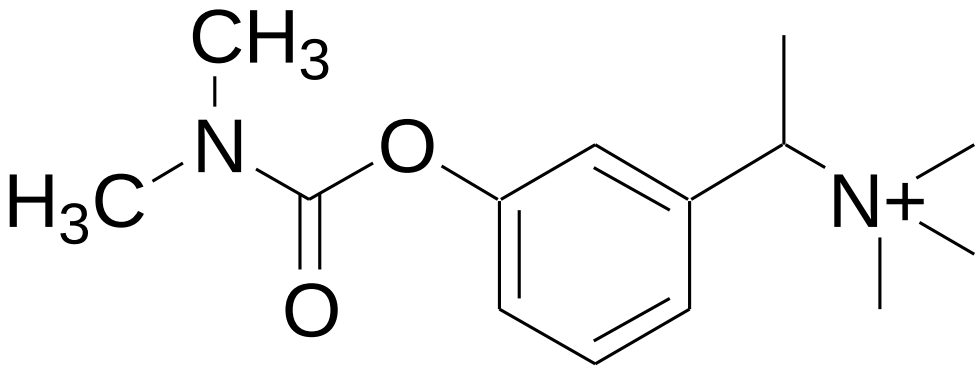
Inibitori reversibili: la Fisostigmina

- E’ un carbammato o uretano, estratto dal *Physostigma venenosum* o fava del Calabar (veleno).
- Usato in oftalmologia per la cura del glaucoma (danneggiamento progressivo del nervo ottico, porta a cecità, correlato a pressione alta del fondo dell’occhio)
- Genera un intermedio carbammico stabile con l’enzima, ha effetti collaterali gravi.
- Si idrolizza facilmente eliminando metilammina e CO2, dando la eserolina, inattiva.



Neostigmina

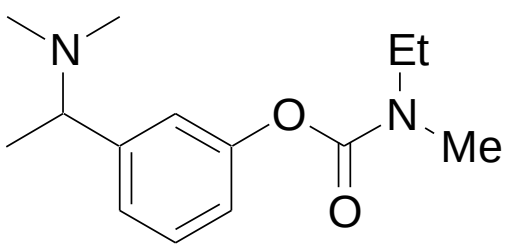
- Ha una azoto quaternario sempre carico, non interferisce col SNC poiché è troppo polare per penetrare la barriera ematoencefalica. Inoltre il secondo metile sull'azoto (ammide terziaria), inibisce ulteriormente il meccanismo di idrolisi dell'intermedio carbammico



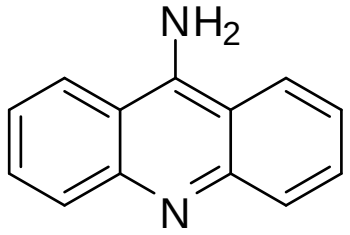
- Attiva oralmente, viene utilizzata per combattere la miastenia gravis.

Tre AChEI reversibili sono entrati in clinica come anti-Alzheimer

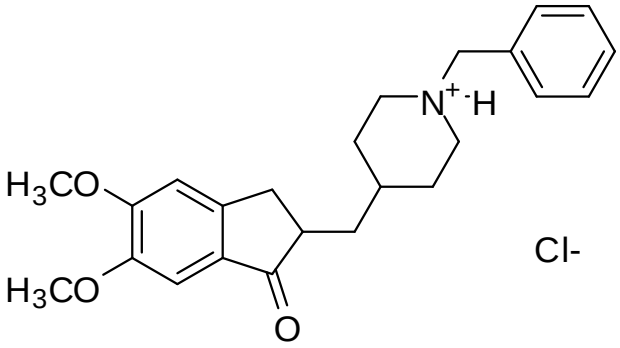
- Rivastigmina tartrato (Exelon)
- tacrina (AChEI non classico, il primo approvato, epatotossico)
- Donepezil meno tossico e più selettivo della tacrina, più recente, AChEI non classico



rivastigmina (Novartis)



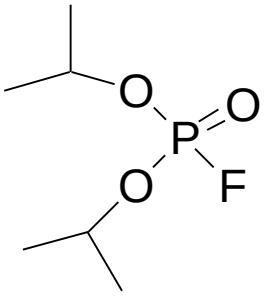
tacrina



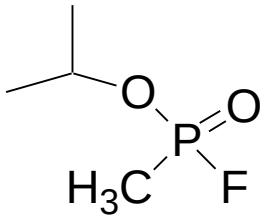
Donezepil cloridrato

AChEI irreversibili: i gas nervini, formano esteri dell'acido fosforico

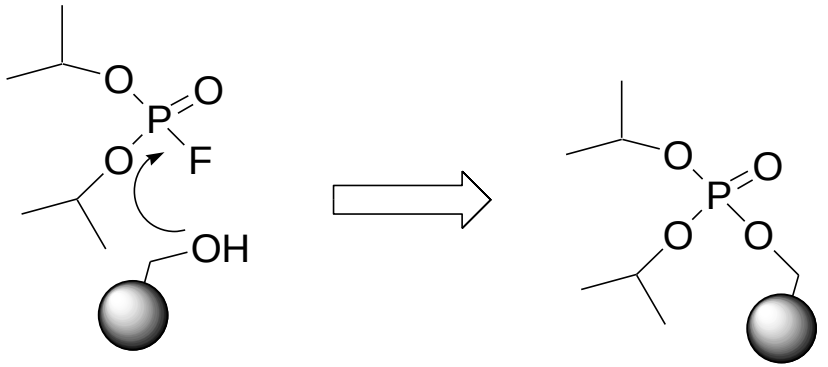
- Inibiscono irreversibilmente l'acetilcolina esterasi attraverso la formazione di un legame covalente con la Ser, un estere dell'acido fosforico: fosfato, fosfonato o fosfinato.
- . Sviluppati durante la II guerra mondiale.
- Il muscolo scheletrico si contrae in modo irreversibile fino alla morte.



DYFLOS

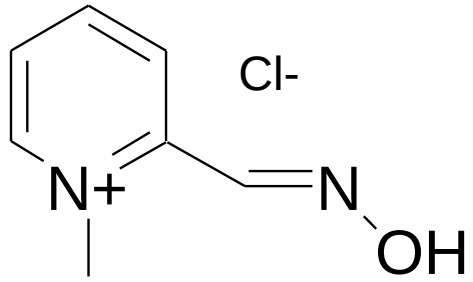


SARIN



- Meccanismo di azione: formano un estere fosforico con la Ser del sito attivo dell'AChE.
- Esiste un processo di invecchiamento dell'enzima fosforilato, da estere fosforico ad acido fosforico. A questo punto il legame covalente è davvero irreversibile.
- Gli antidoti riescono ad idrolizzare l'estere fosforico ma devono essere somministrati prima della trasformazione in acido

Pralidossima: un antidoto per gli avvelenamenti da organofosforici

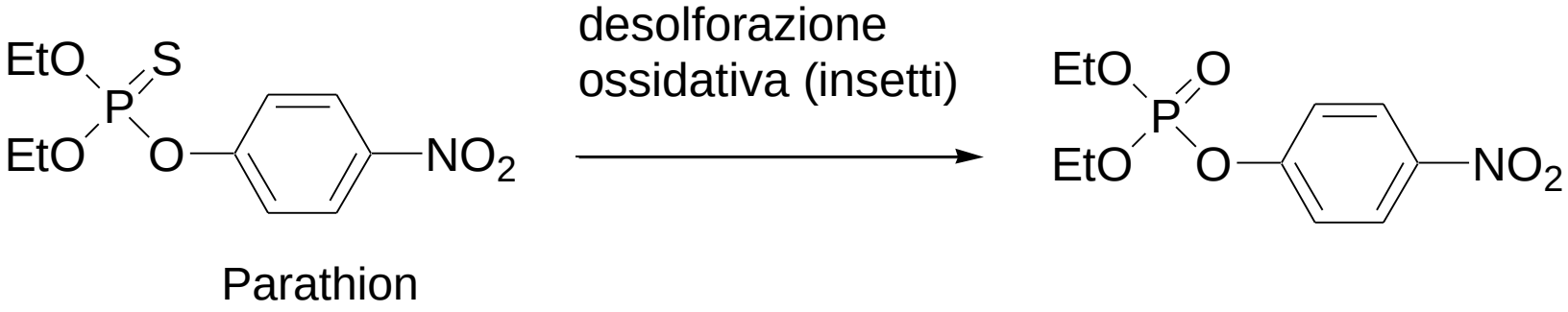


Pralidossima cloruro (2-PAM)

- La funzione ossima, come la idrossilammina, riesce a spostare l'ossidrile della Ser dal legame col fosforo e a far tornare indietro la fosforilazione dell'enzima.
- Deve essere però somministrata a distanza di poche ore dall'avvelenamento, altrimenti l'enzima invecchia.

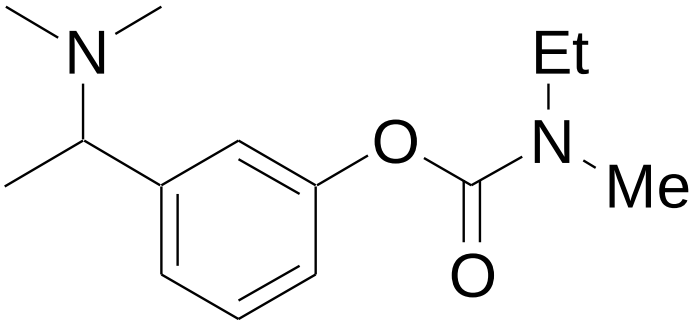
Agenti specifici, insetticidi AChEI

- Gli insetticidi Parathion e Malathion sfruttano la diversità delle vie biosintetioche tra uomo e insetti.
- Sono dei pro-xenobiotici che , solo negli insetti, vengono trasformati in sostanze capaci di fosforilare in modo irreversibile l'acetilcolina esterasi provocando la morte.
- Solo negli insetti avviene la trasformazione del legame P=S in P=O.



Parathion

- Gli anticolinesterasici come smart-drugs: realtà o fantascienza?
- Trattamento sintomatico della demenza di Alzheimer da lieve a moderatamente grave.
- Oltre alle proprietà anti-Alzheimer, la rivastigmina è il primo farmaco approvato da tutti i paesi della CEE che ha mostrato effetti benefici sulla cognizione, sulla memoria e sulle abilità funzionali.



rivastigmina (Novartis)

Farmaci adrenergici

- L’azione dell’adrenalina e della noradrenalina sono opposte a quella della Ach
- Meccansimo di “lotta o fuga”:
- Stimolazione cardiaca
- Vasodilatazione
- Soppressione attività muscolatura liscia GI

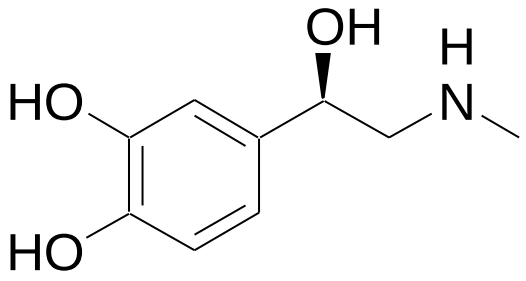
Recettori adrenergici (7TM)

- Due tipi: α e β adrenergico, acoppiati rispettivamente alle proteine G_0 e G_s
- Sottotipi dell'α: α_1 ed α_2 che differiscono per la struttura ed il secondo messaggero (gli α_1 producono IP_3 o diacilglicerolo, mentre gli α_2 c-AMP).
- Sottotipi del β: β1, β2, β3. Tutti attivano c-AMP.
- Tutti questi recettori sono attivati da adrenalina e noradrenalina, ma hanno strutture leggermente diverse. Questo permette di progettare agonisti ed antagonisti selettivi.

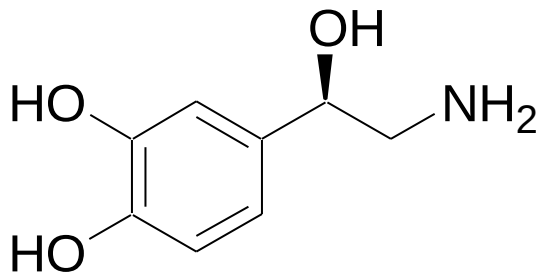
Agonisti-antagonisti adrenergici

- Uso clinico principale degli agonisti β-adrenergici è il trattamento dell'asma. **L'attivazione dei recettori β_2-adrenergici causa il rilassamento** della muscolatura liscia dei bronchi, dilatando così le vie respiratorie.
- **L'attivazione dei β1 causa la contrazione** dei muscoli cardiaci e dei vasi sanguigni, aumento di pressione.
- Farmaci : agonisiti β2, antiasmatici
- Antagonisti β1, antianginosi, antiaritmici, antiipertensivi.

Struttura delle catecolammine



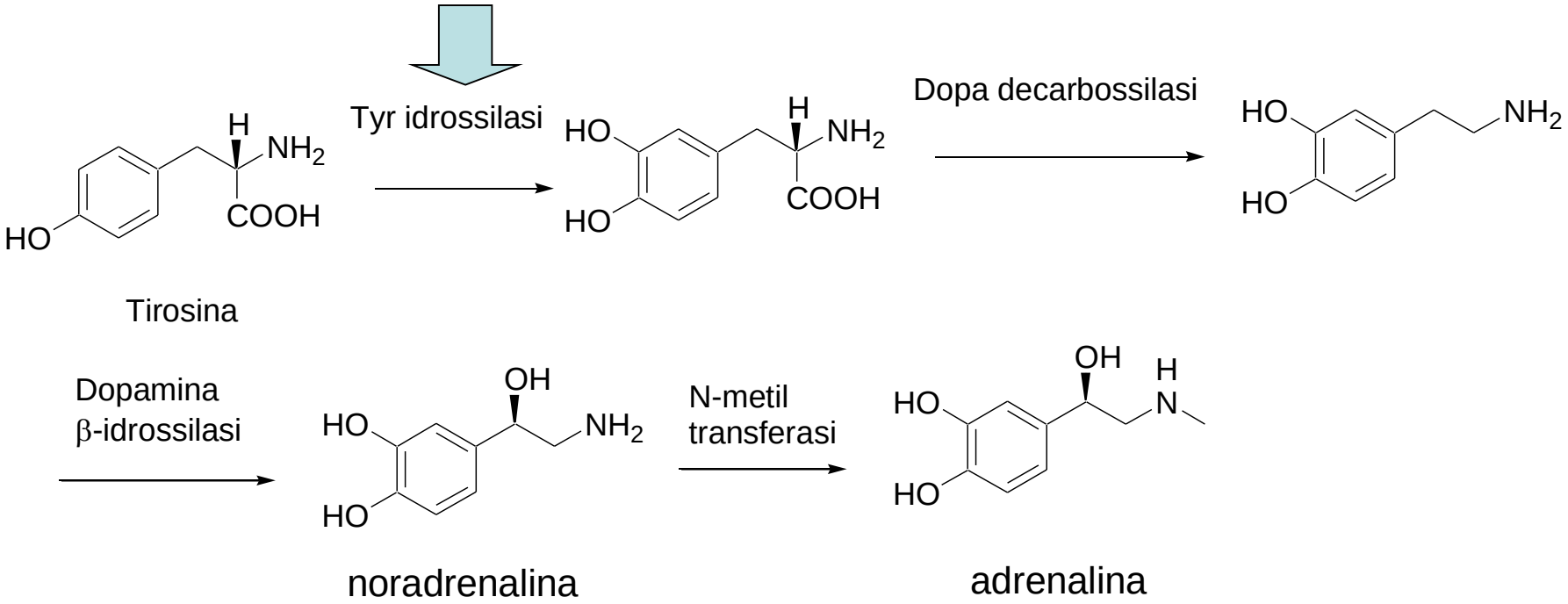
adrenalina



noradrenalina

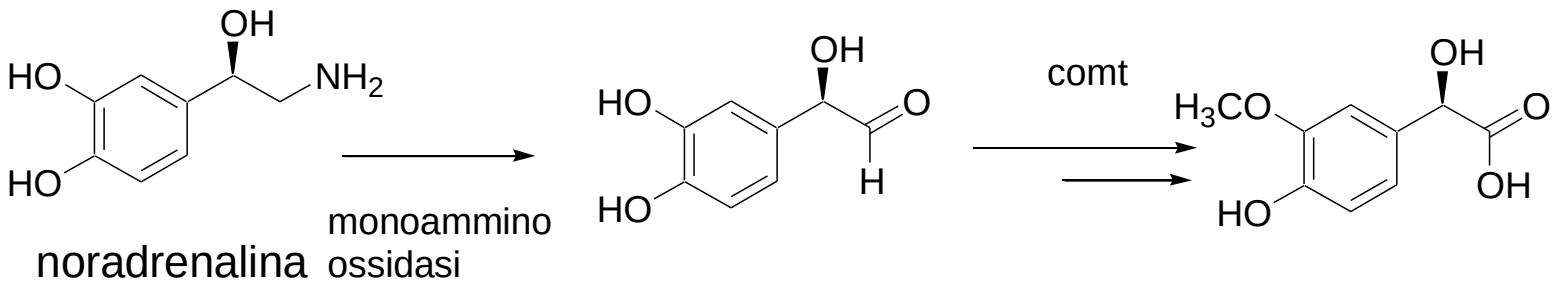
Biosintesi

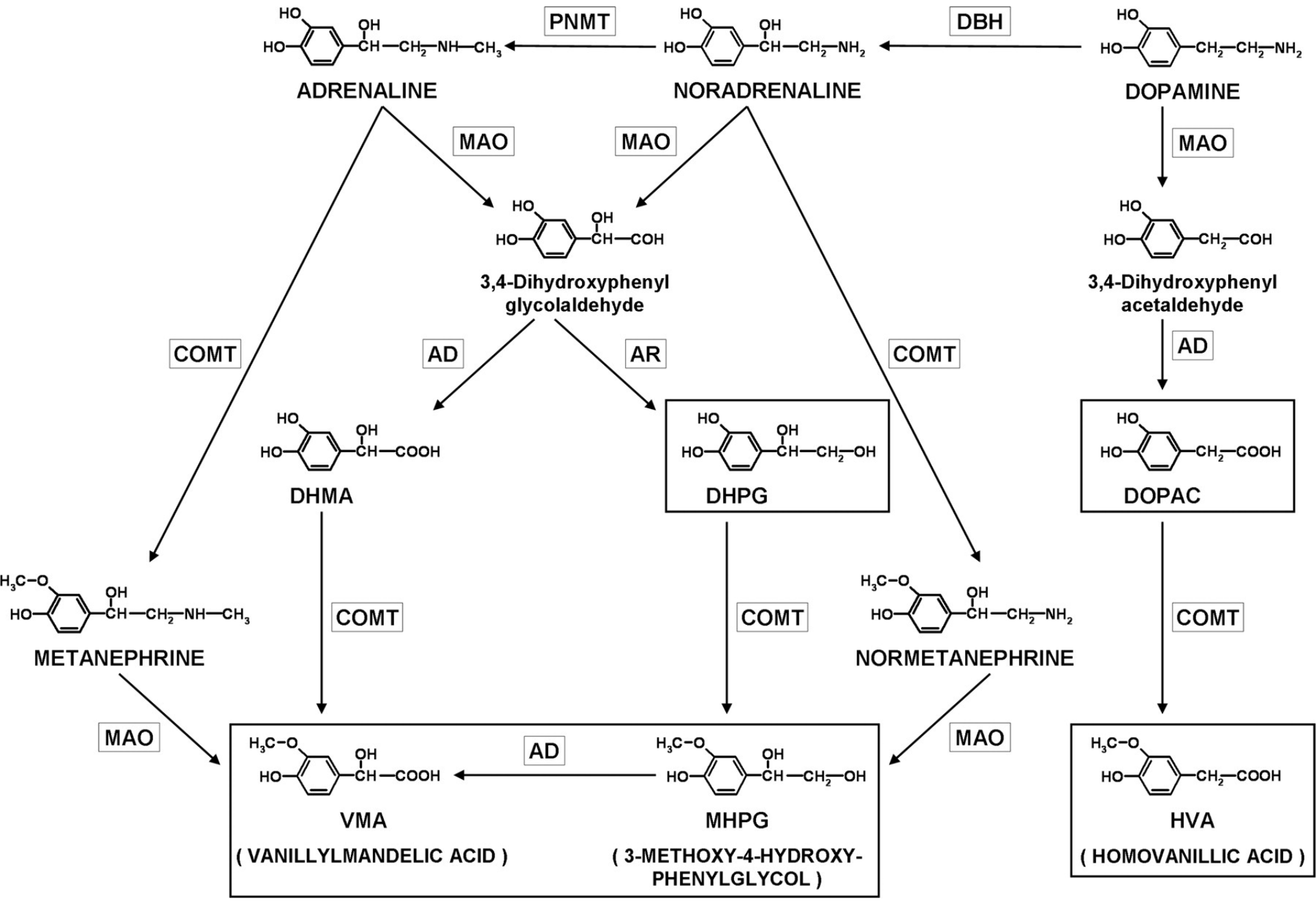
regolazione



Metabolismo

- Monoammino ossidasi (MAO)
- Catecol-O-metiltransferasi
- L’acido carbossilico finale è escreto nelle urine.




PNMT
DBH
ADRENALINE
NORADRENALINE
DOPAMINE
MAO
MAO
MAO
3,4-Dihydroxyphenyl glycolaldehyde
3,4-Dihydroxyphenyl acetaldehyde
COMT
AD
AR
COMT
AD
DHMA
DHPG
DOPAC
METANEPHRINE
NORMETANEPHRINE
COMT
COMT
COMT
MAO
MAO
AD
VMA
(VANILLYLMANDELIC ACID)
MHPG
(3-METHOXY-4-HYDROXY-PHENYLGLYCOL)
HVA
(HOMOVANILLIC ACID)

Binding con il recettore

- I recettori adrenergici sono 7-TM, 3 di queste eliche sono coinvolte nel binding dell’agonista.
- Gruppi importanti sul ligando per l’interazione col recettore:
- OH meta, OH para, configurazione R dell’ossidrile, gruppo amminico protonabile

Il sito di legame del recettore β2 adrenergico

Adrenaline-activated structure of the β_2-adrenoceptor stabilized by an engineered nanobody

Aaron M. Ring[#1,2], Aashish Manglik[#1], Andrew C. Kruse[#1], Michael D. Enos[1,2], William I. Weis[1,2], K. Christopher Garcia[1,2,3], and Brian K. Kobilka[1]

The activation mechanism of the β_2AR is highly similar for all agonists

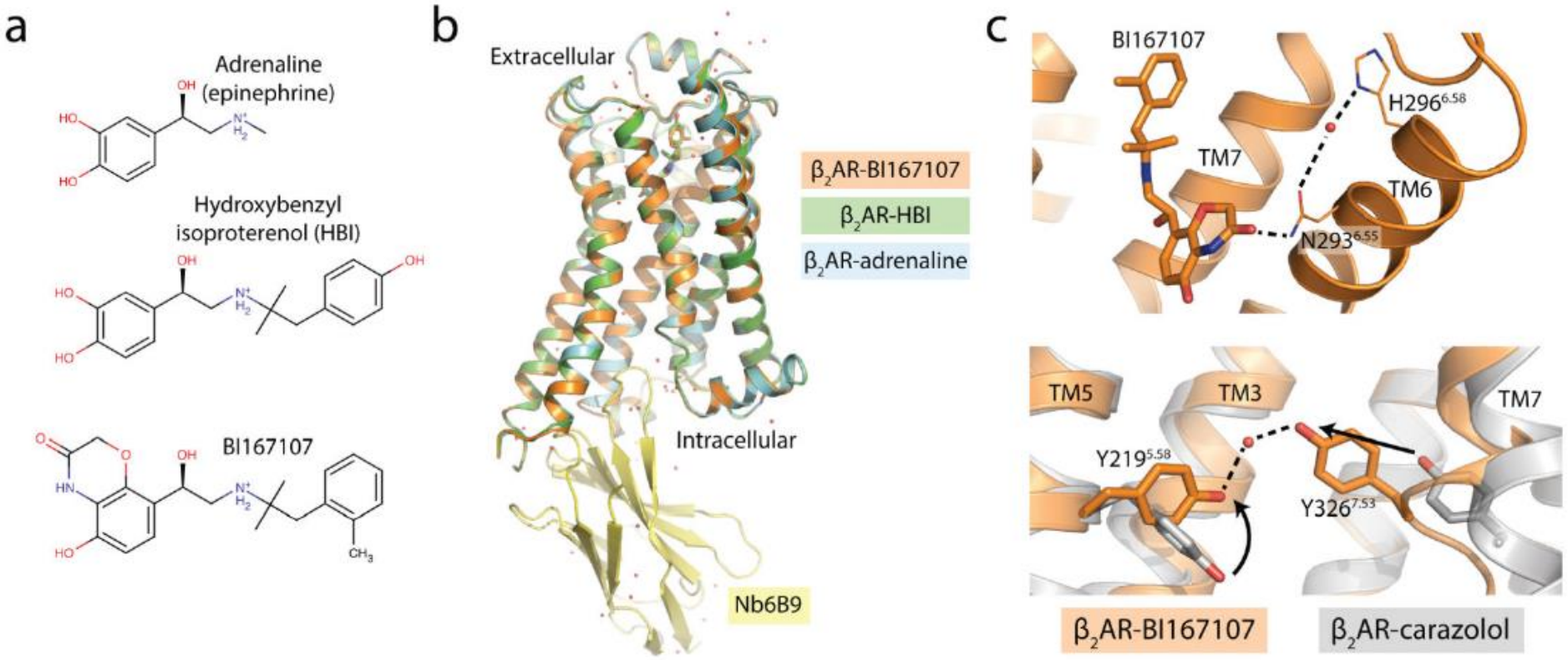
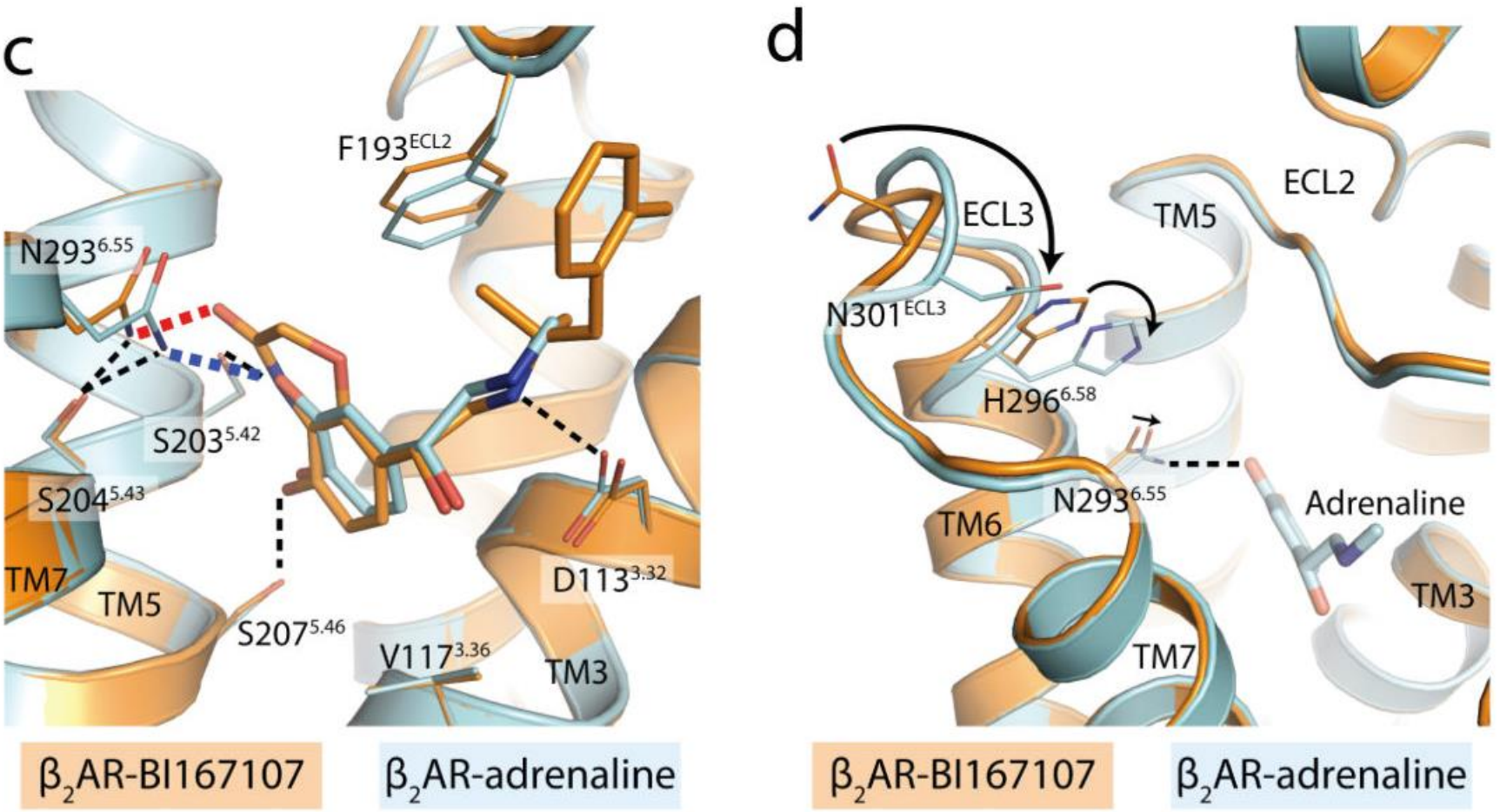


Figure 2. Structure of the activated β_2AR in complex with three agonists

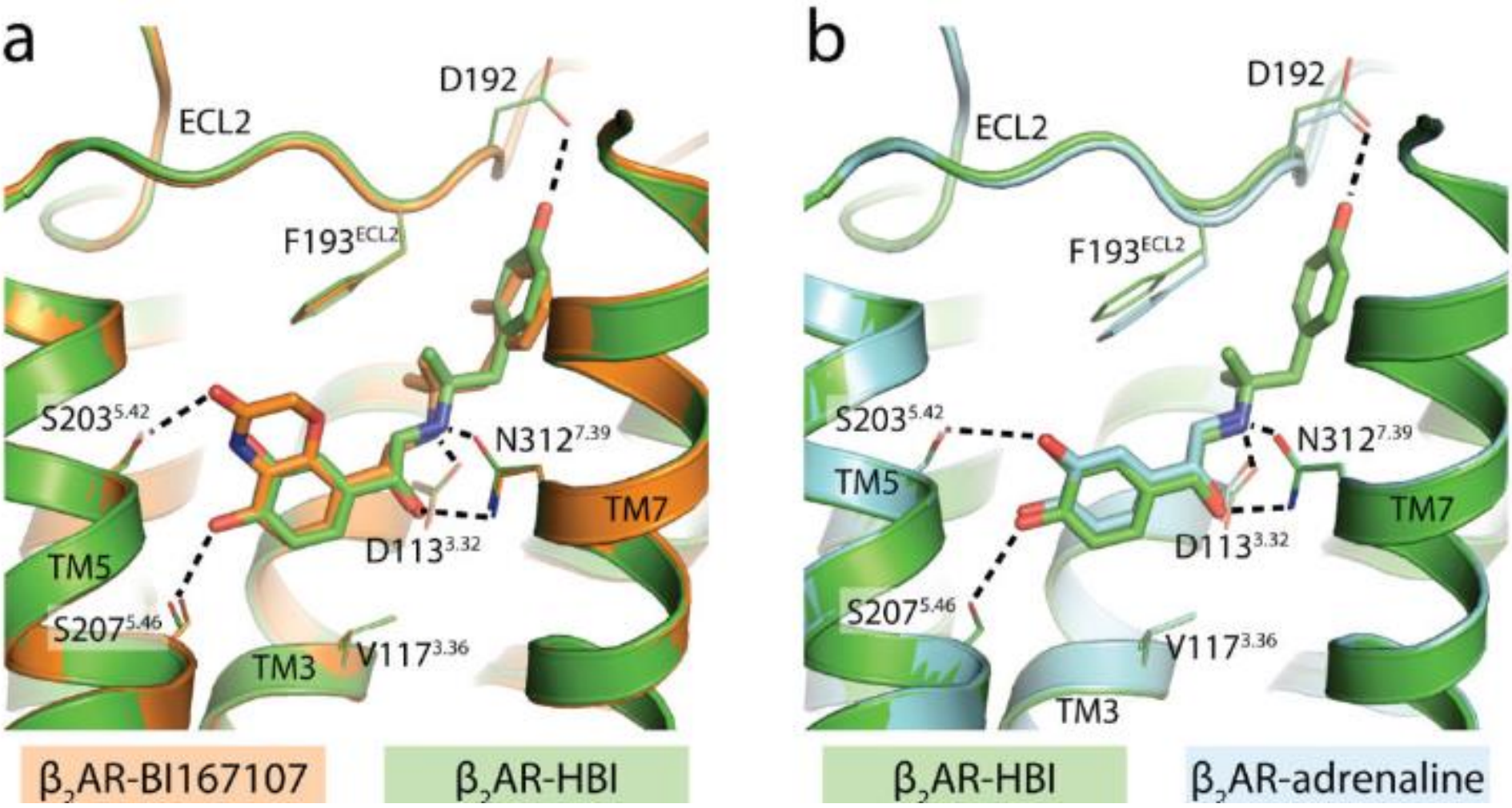
a, Chemical structures of the three ligands used for crystallization trials. **b,** All three active-state structures, showing remarkable similarity in overall receptor conformation. **c,** The 2.8 Å resolution structure of BI167107-bound β_2AR reveals active-state water molecules: a bridging water molecule participates in a polar network at the ligand binding site (top) and a second water molecule mediates a hydrogen bond between two highly conserved tyrosines. Such an interaction is possible in the active state (orange) but not the inactive state (gray).



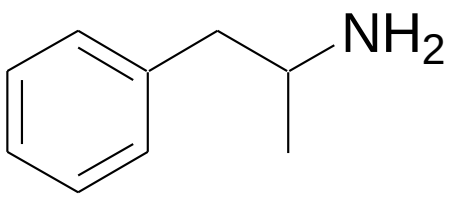
c, An analogous comparison of BI167107-bound β2AR (orange) with adrenaline-bound receptor (cyan) shows the similar polar networks for the two ligands (black dotted lines) with a notable difference in the hydrogen bonding of Asn2936.55 to the amide proton in BI167107 (red dotted line) or the *meta* hydroxyl of adrenaline (blue dotted line) **d,** Due to this difference, Asn2936.55 and TM6 shift inward in the adrenaline-bound structure,, leading to a cascade of changes culminating in a rearrangement of ECL3.

Important interactions with ligands

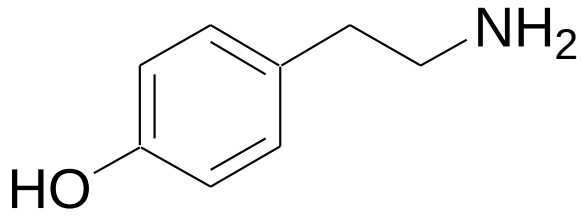
- Consistent with prior mutagenesis studies, Ser203 and Ser207 make hydrogen bonds with the catecholamine phenoxy moieties.


a
D192
ECL2
F193ECL2
S2035.42
N3127.39
TM7
D1133.32
TM5
S2075.46
TM3
V1173.36
β2AR-BI167107
β2AR-HBI
b
D192
ECL2
F193ECL2
S2035.42
TM5
N3127.39
TM7
D1133.32
S2075.46
V1173.36
TM3
β2AR-HBI
β2AR-adrenaline

- I gruppi fenolici sono molto importanti:
- Composti come l’amfetamina, la tiramina e l’efedrina non hanno affinità per i recettori adrenergici.
- Il gruppo meta può essere sostituito con altri gruppi capaci di dare legame ad H senza perdita totale di attività.



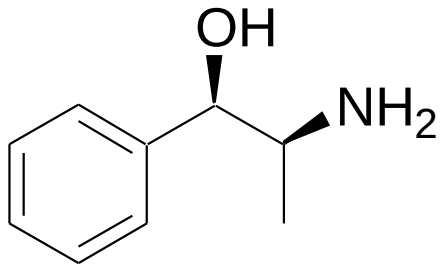
amfetamina



tiramina

- Sostituzione *N*-alchilica:
- Mentre l'adrenalina ha la stessa potenza sui due tipi di recettori adrenergici, la noradrenalina è più potente sugli α.
- La sostituzione di un alchile sull'azoto aumenta la selettività rispetto ai β.
- Il composto di sintesi isoprenalina (con il gruppo NH-iPr)è un potente agonista β-adrenergico, privo di attività α-agonista.

- I gruppi fenolici sono importanti per il recettore β-adrenergico + che per l'α adrenergico.
- L'introduzione di un metile in α, aumenta la selettività per il recettore α_2-adrenergico.

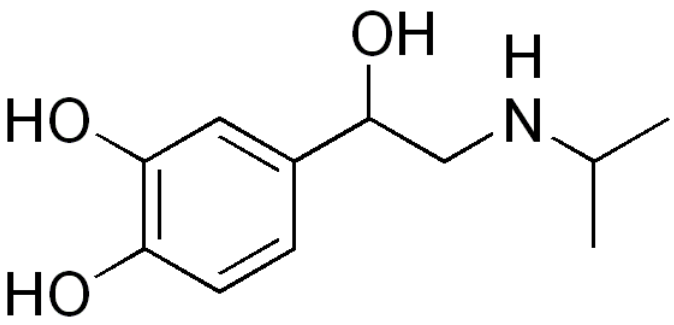


α-metilnoradrenalina

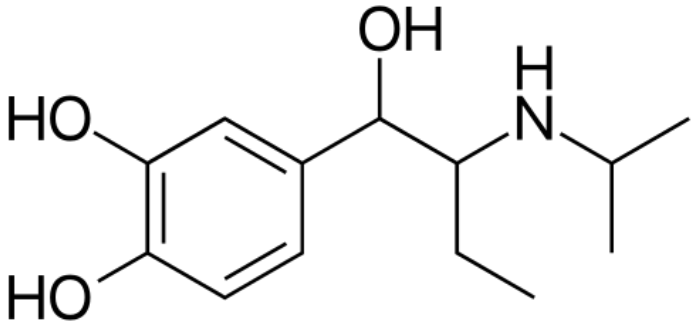
Agonisti adrenergici

- Non c'e' grande interesse farmacologico a fare agonisti α-adrenergici, invece sono stati sviluppati agonisti β_2-adrenergici con le seguenti funzioni:
- Rallentare le contrazioni uterine per prevenire il parto prematuro
- Antiasmatici, rilassare la muscolatura liscia dei polmoni

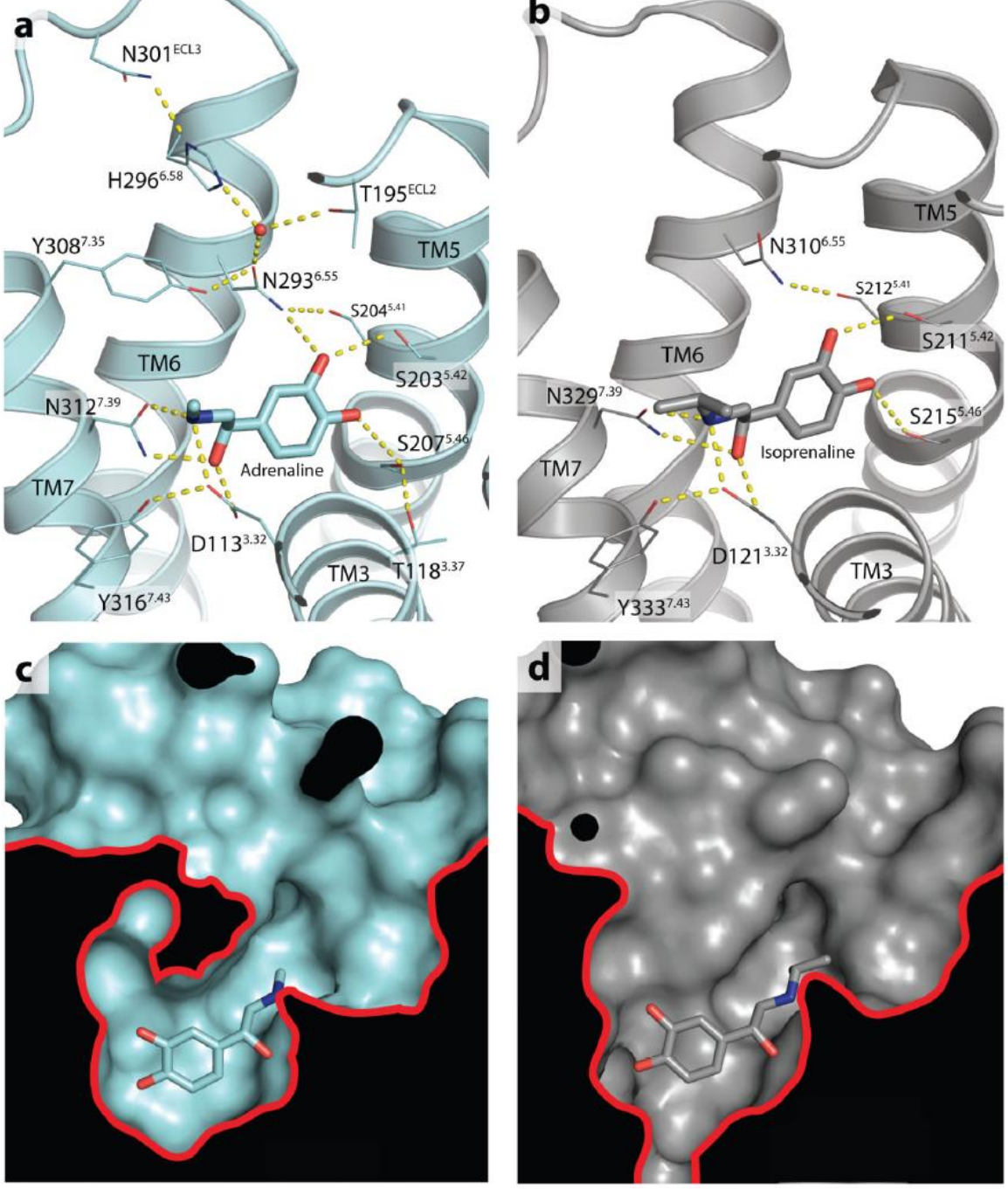
- L’isoprenalina è un antiasmatico, agonista con **selettività beta vs alfa**, ma siccome non è del tutto inattiva sui β_1 cardiaci determina effetti collaterali cardiovascolari non desiderati.
- Isoetarina: **selettiva β_2** ma rapidamente metabolizzata dalle COMT sull'ossidrile fenolico in meta....evoluzione R-Soterenolo
- Il SALBUTAMOLO è attivo sui β_2 adrenergici dei polmoni come l’isoprenalina, ma è 2000 volte meno attivo sui β_1 del cuore.



isoprenalina o isoproterenolo



isoetarina



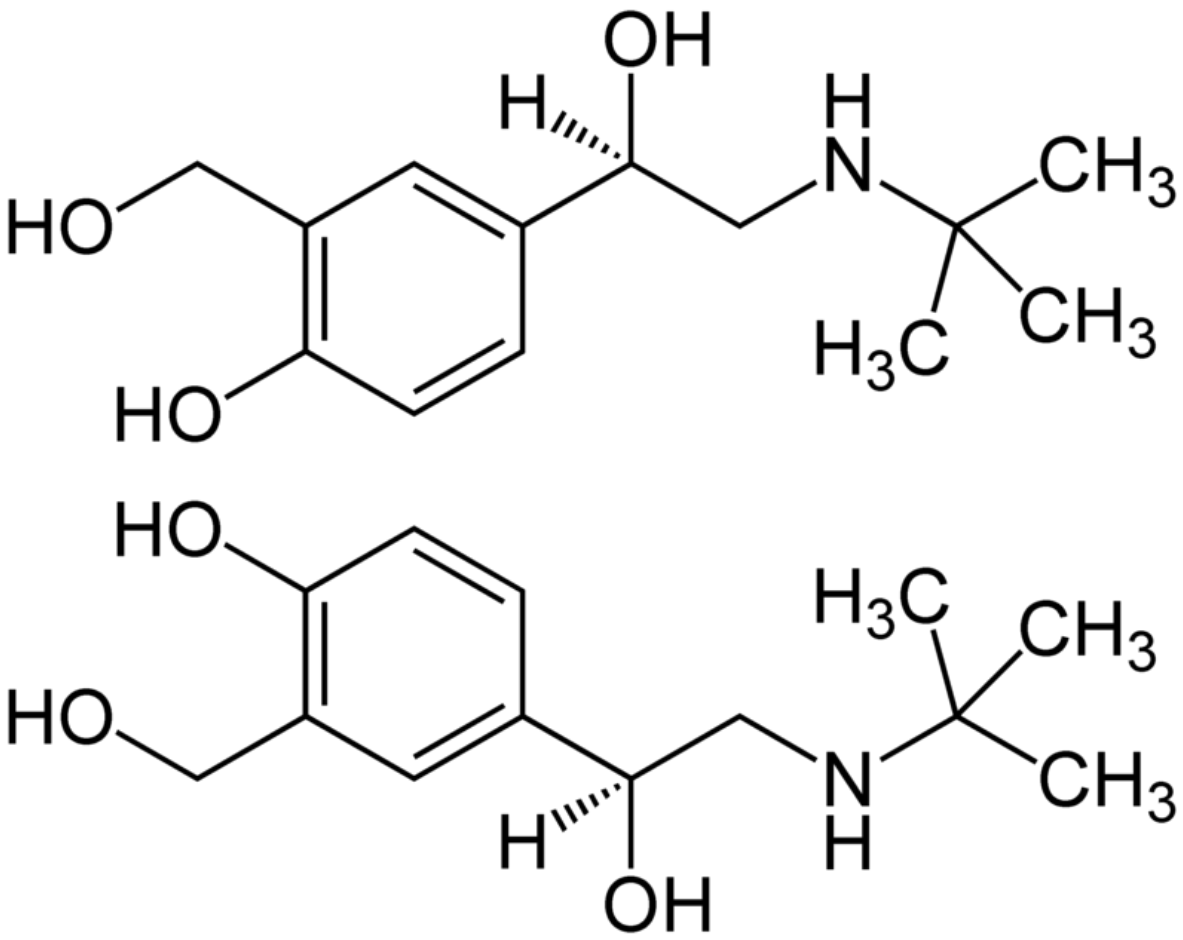
β_2AR-adrenaline

β_1AR-isoprenaline

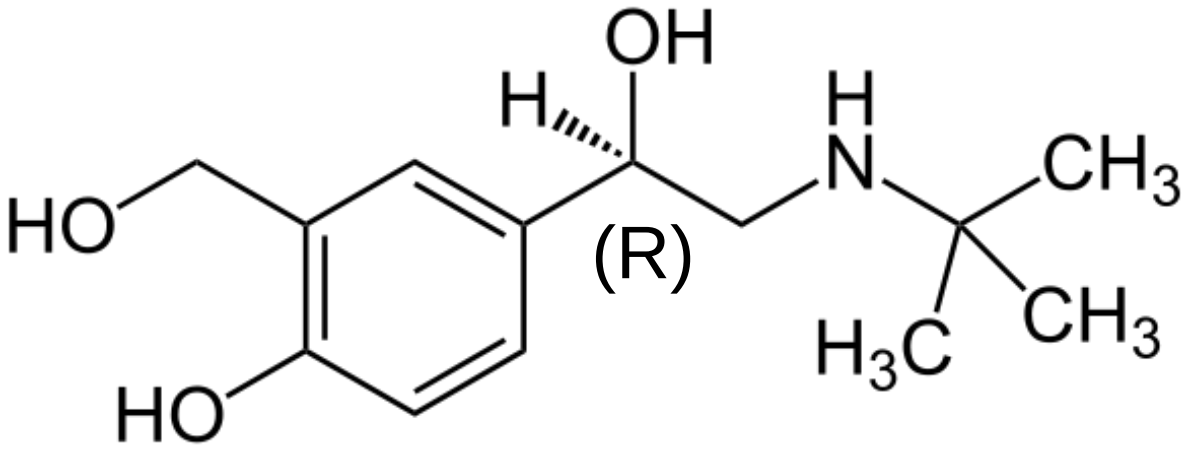
Crystal structure (2014)

- In contrast to the striking similarity in receptor conformation for all three agonists crystallized here, far more substantial conformational differences are seen relative to a previously reported structure of the thermostabilized turkey β1 adrenoceptor (β1AR) bound to the catecholamine agonist isoproterenol. Likely due to the thermostabilization procedure, the overall receptor conformation of isoprenaline-bound β1AR closely resembles that of the antagonist-bound, inactive β1AR, as well as that of covalent agonist-bound, inactive β2AR.

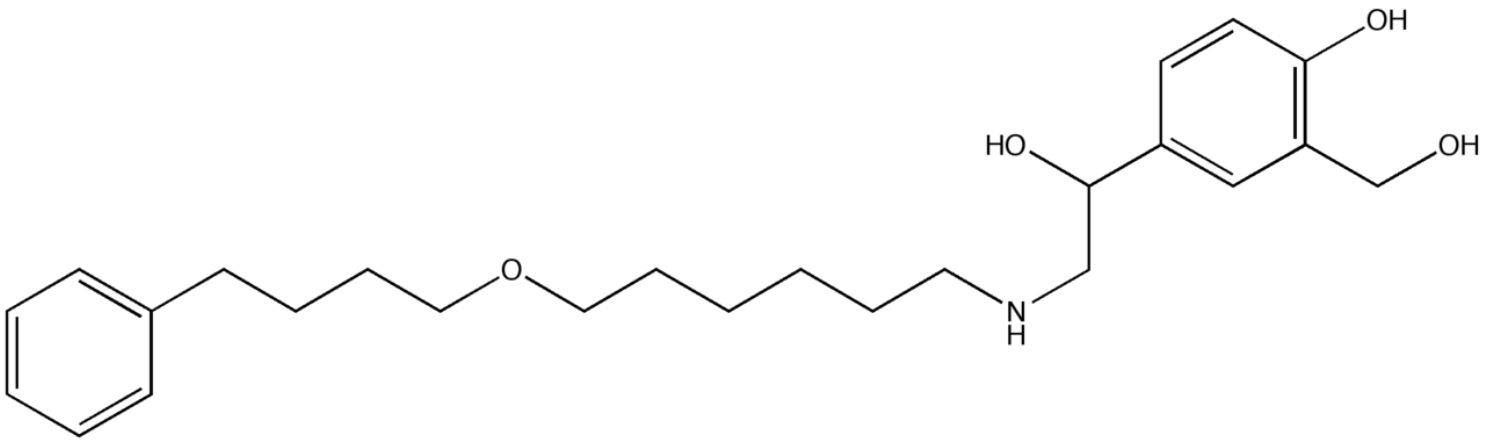
salbutamolo o albuterolo, prodotto come racemo



- Il salbutamolo ha un gruppo idrossimetilenico in meta ed un t-Bu sull’azoto.
- Può essere sintetizzato a partire dall’aspirina
- E’ il principio attivo del Ventolin (Glaxo)
- Enantiomero R è 68 volte più attivo dell'S che si accumula nell'organismo
- Il levalbuterolo (R puro)...commutazione chirale..nuovo farmaco (vedi la storia dell'omeprazolo)

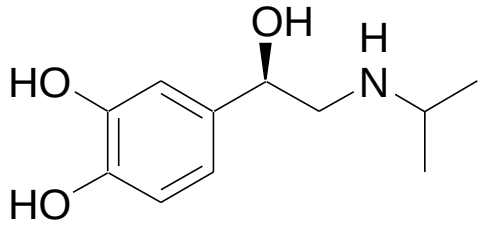


Il salmeterolo: durata prolungata grazie alla lunga coda lipidica che ne aumenta la permanenza nell'organismo

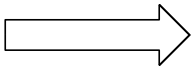


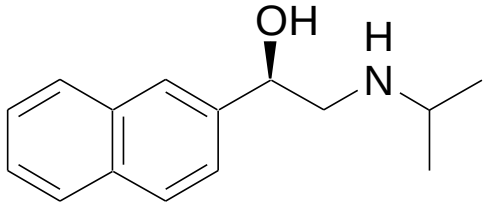
Antagonisti adrenergici

- Gli α-antagonisti non sono molto utili, i β-bloccanti sono molto più importanti.
- Il primo punto degli antagonisti β è quello che dovevano essere selettivi rispetto agli α.
- Punto di partenza: isoprenalina (agonista dei recettori β e non degli α). Se si rafforza questo legame selettivo col recettore si può pensare di passare da un agonista ad un antagonista.
- In realtà i primi antagonisti sono degli agonisti parziali. Il pronetalolo è il primo β-bloccante usato clinicamente nel trattamento dell'angina, pressioni elevate ed aritmie.



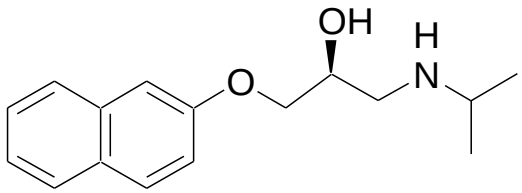
Isoprenalina (agonista selettivo)



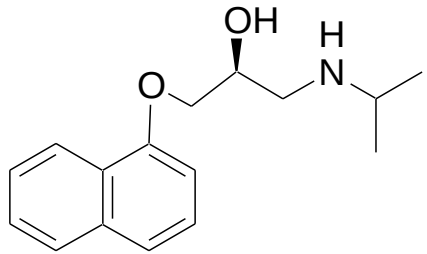


Pronetalolo (antagonista selettivo)

- Da isoprenalina a pronetalolo: eliminazione degli ossidrili!
- Si è poi variata la lunghezza della catena che congiunge l'entità aromatica (β-naftile) con l'ammina, ottenendo per casousanod l'alfa-naftile un β-antagonista (bloccante) puro, il propranololo.
- Il propranololo è usato come racemo in clinica ma l'enantiomero S è il più attivo



prodotto progettato



propranololo

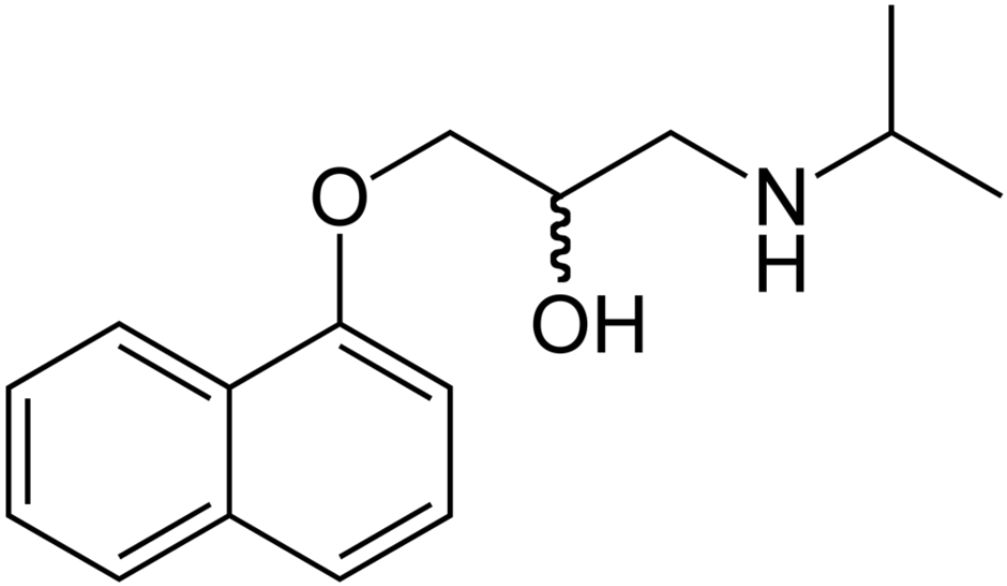
Le arilossipropanolammine

- I primi β-bloccanti puri come il propranololo non sono b1 selettivi, sono attivi anche sui b2:
- Riduzione della gittata cardiaca
- Riduzione di rilascio di renina dai reni (anti-ipertensivi)
- Riduzione del sistema nervoso simpatico

- Effetti collaterali:
- Broncocostrizione (pazienti asmatici!!!)
- Effetti sul SNC
- Inibizione del rilascio di NA nelle sinapsi

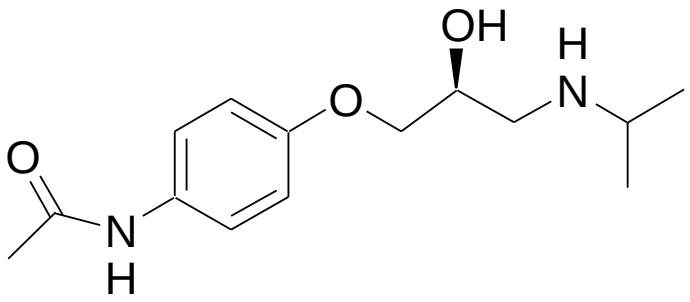
il propranololo

- Secondo beta-bloccante in clinica dopo il pronetalolo
- Premio Nobel a James Black nel 1988
- non totalmente β1 selettivo (problema per i pazienti asmatici)...seconda generalzione

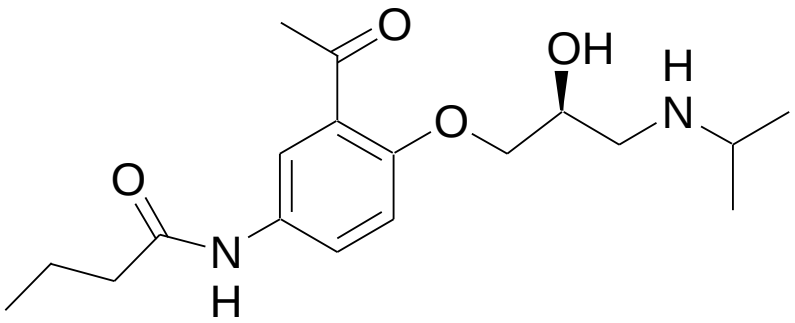


β_1-bloccanti selettivi di II generazione

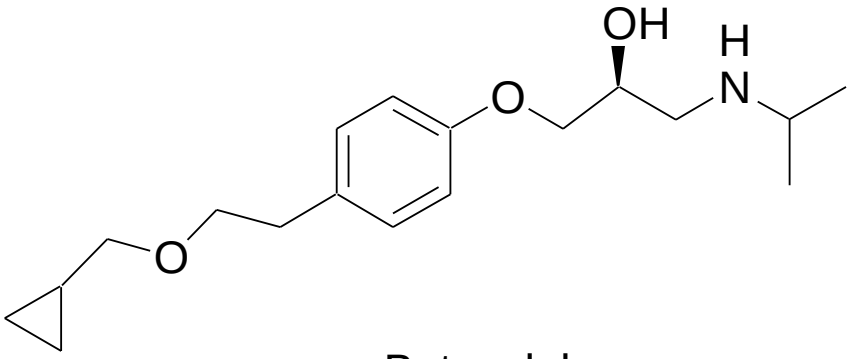
- Il practololo non è potente come il propranololo ma è selettivo ed agisce solo sui β_1 cardiaci.
- Il practololo è stato lanciato sul mercato come primo β_1-bloccante selettivo ma poi è stato ritirato per effetti collaterali dannosi. Derivati ulteriormente funzionalizzati come il betaxololo o l'acebutololo sono privi di questi effetti collaterali.
- I β-bloccanti hanno anche altre applicazioni, per ridurre gli stati d'ansi, l'emicrania, alleviare i sintomi di astinenza da alcol.



Practololo

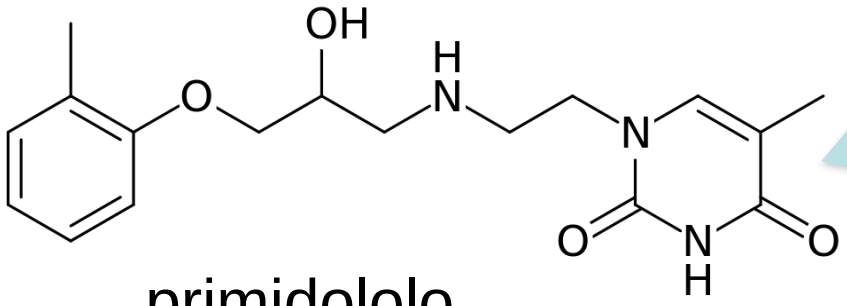


Acebutolo

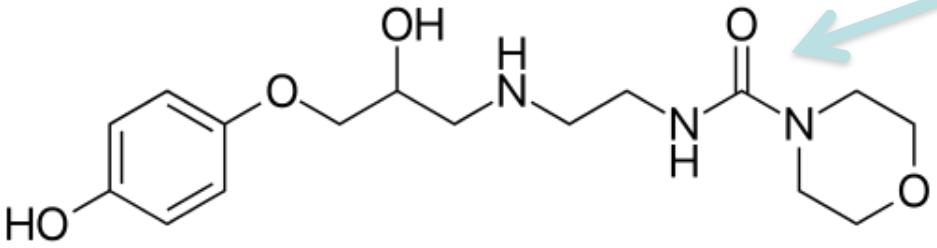


Betaxololo

β-bloccanti di terza generazione: omologazione o complicazione strutturale



primidololo



xamoterolo

legami ad H
aggiuntivi
con il recettore β1

The structural basis for agonist and partial agonist action on a β1-adrenergic receptor *Nature 2011*

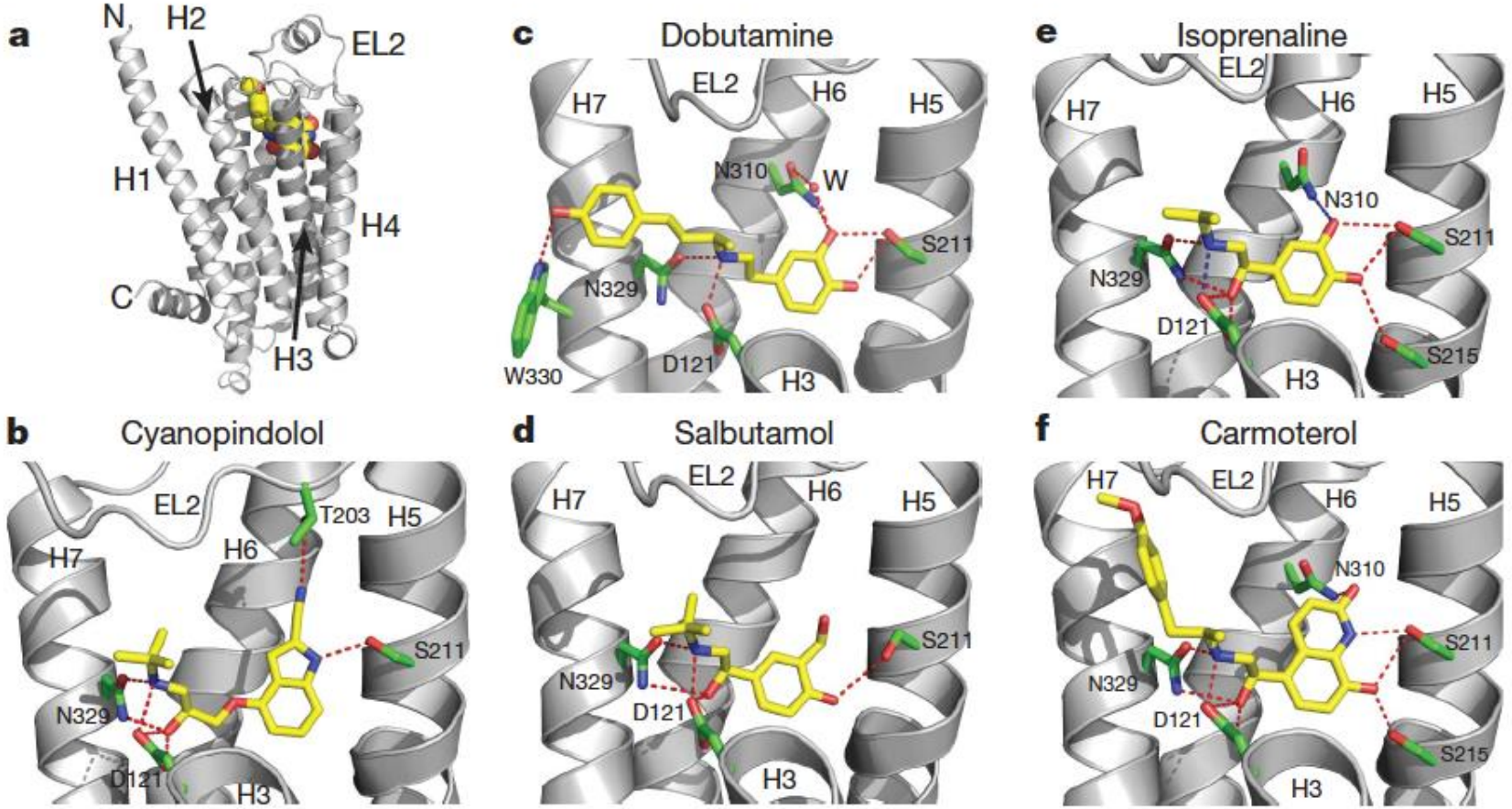
- Determining how agonists and antagonists bind to the β receptors has been the goal of research for more than 20 years.
- Although the structures of the homologous β1 and β2 receptors show how some antagonists bind to receptors in the inactive state, structures with agonists bound are required to understand subsequent structural transitions involved in activation.
- GPCRs exist in an equilibrium between an inactive state (R) and an activated state (R*) that can couple and activate G proteins.

- Native turkey β1AR is unstable in detergent, so crystallization and structure determination relied on using a thermostabilized construct (β1AR-m23) that contained six point mutations, which dramatically improved its thermostability.
- In addition, the thermostabilizing mutations altered the equilibrium between R and R*, so that the receptor was preferentially in the R state.

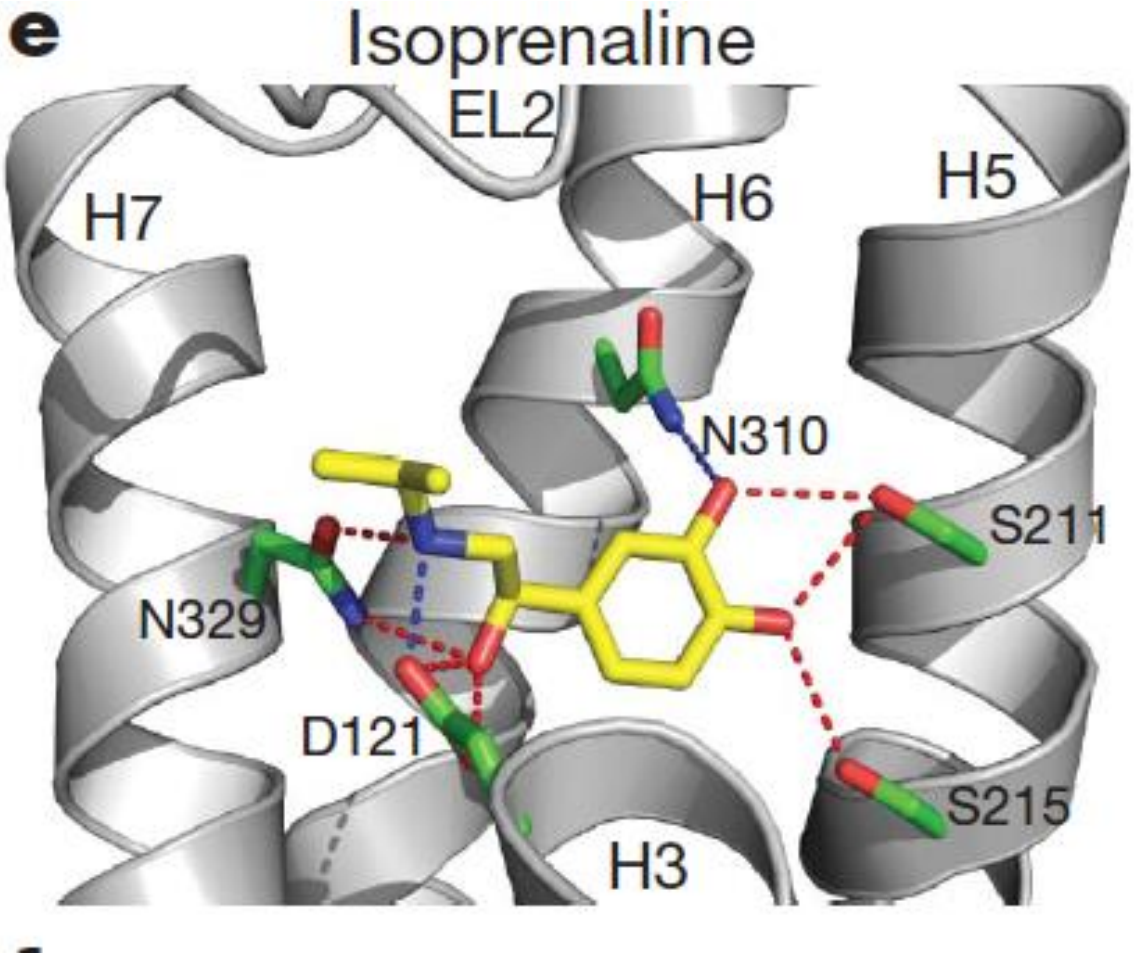
- As a first step towards understanding how agonists activate receptors, we have determined the structures of β1AR bound to four different agonists.

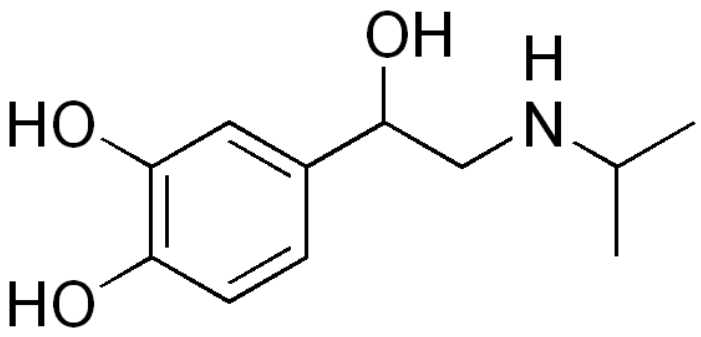
Agonists binding to β1 receptor

- All four agonists bind in the catecholamine pocket in a virtually identical fashion.
- The **secondary amine and β-hydroxyl** groups shared by all the agonists form potential hydrogen bonds with **Asp 121 and Asn 329**, whereas the **hydrogen bond donor/ acceptor group equivalent to the catecholamine meta-hydroxyl (m-OH)** generally forms a hydrogen bond with **Asn 310**.
- In addition, all the agonists can form a hydrogen bond with **Ser 211**, and **Ser 215** and **Asn 310**.



the structures represent an inactive, non-signalling state of the receptor formed on initial agonist binding.

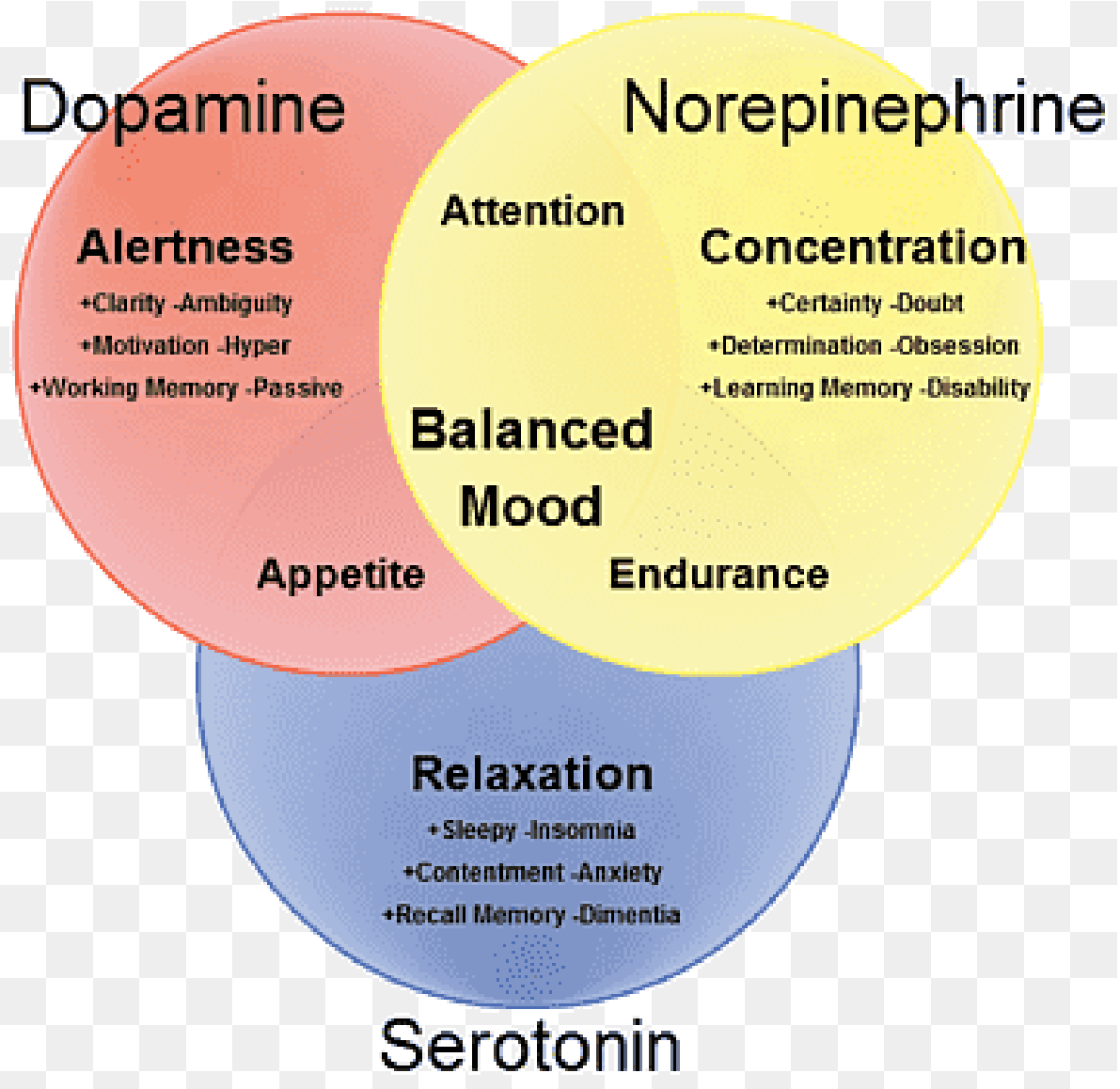

e
Isoprenaline
EL2
H7
H6
H5
N310
S211
N329
D121
S215
H3

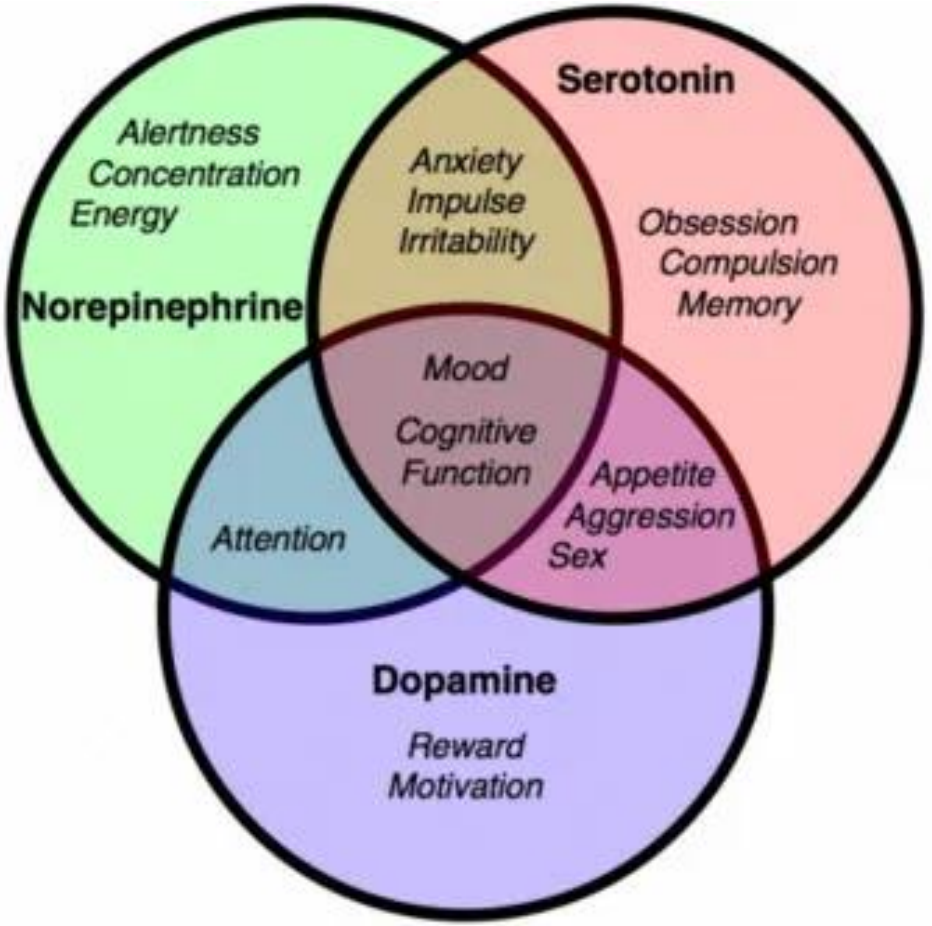

OH
H
N
HO
HO

Adrenaline-activated structure of the β_2-adrenoceptor stabilized by an engineered nanobody

Aaron M. Ring[#1,2], **Aashish Manglik**[#1], **Andrew C. Kruse**[#1], **Michael D. Enos**[1,2], **William I. Weis**[1,2], **K. Christopher Garcia**[1,2,3], and **Brian K. Kobilka**[1]

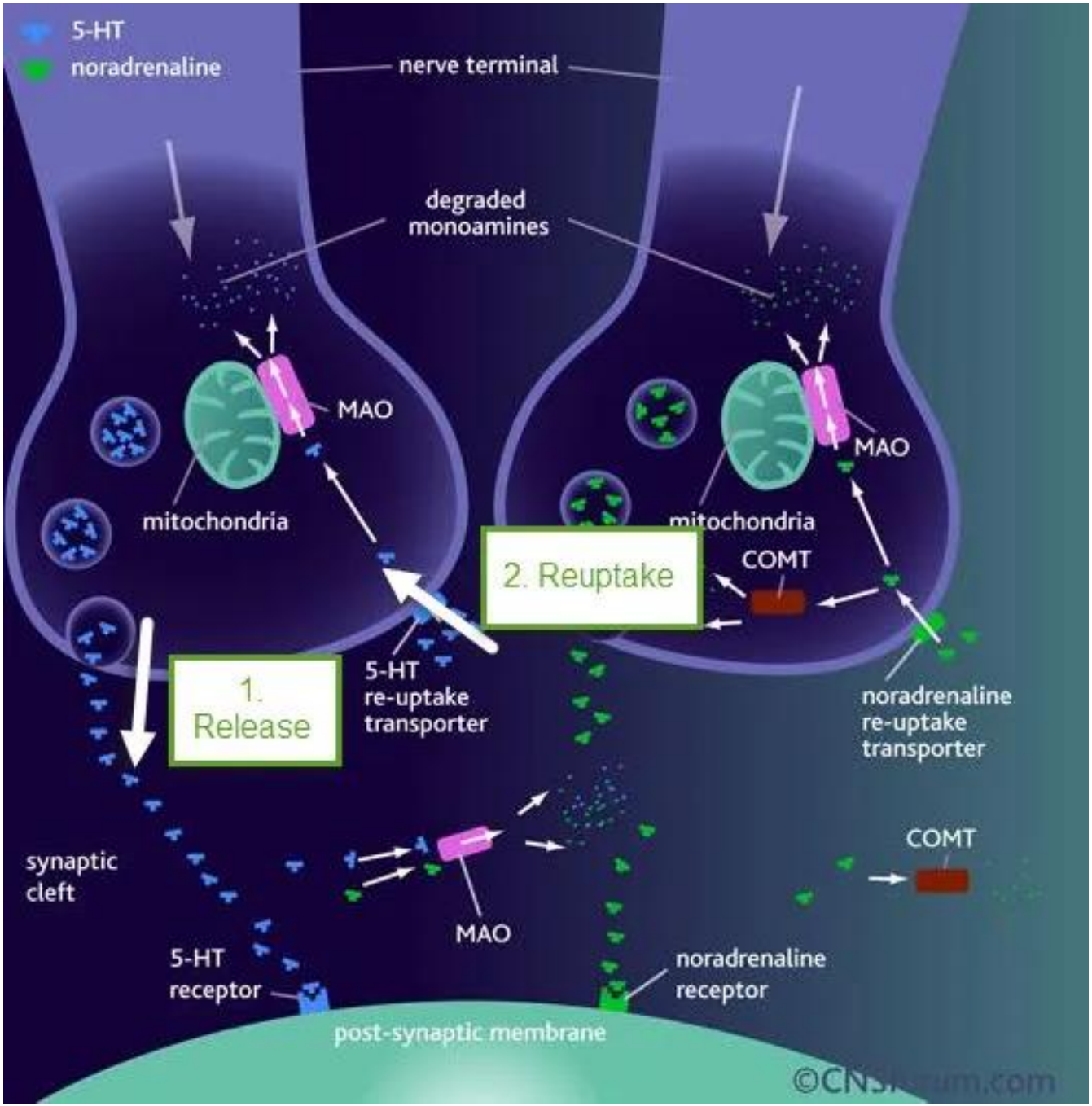
Farmaci attivi sul SNC




Serotonin
Alertness
Concentration
Energy
Anxiety
Impulse
Irritability
Obsession
Compulsion
Memory
Norepinephrine
Mood
Cognitive
Function
Appetite
Aggression
Sex
Attention
Dopamine
Reward
Motivation

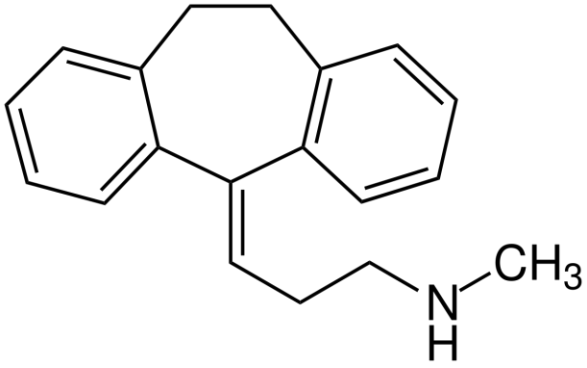
Antidepressants and Monoamine Neurotransmitters

- In the very simplified drawing to the left you can see that when a signal comes to the top neuron that signal leads to the release of serotonin (transmitting the signal to the next brain cell or neuron). The signal ends when the serotonin is either taken back into the releasing neuron or is broken down by enzymes in the gap between the two neurons (the synaptic cleft).
- Reuptake inhibitors interfere with the reuptake of the neurotransmitter and thus should lead to increased brain signalling.
- This process occurs in the synapses of all of the monoamine neurotransmitters, so there can be agents that inhibit norepinephrine reuptake (norepinephrine reuptake inhibitors or NRIs), dopamine reuptake (DRIs), etcetera. However, it turns out that most of the medications we routinely use primarily affect serotonin.

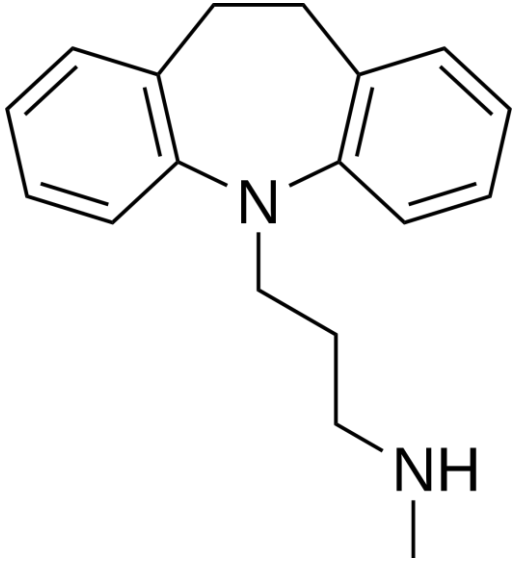

5-HT
noradrenaline
nerve terminal
degraded monoamines
MAO
mitochondria
MAO
mitochondria
COMT
2. Reuptake
1. Release
5-HT re-uptake transporter
noradrenaline re-uptake transporter
synaptic cleft
MAO
COMT
5-HT receptor
noradrenaline receptor
post-synaptic membrane

Inibitori della ricaptazione della NA
Prima generazione: antidepressivi tricilici (anni '60-'80)

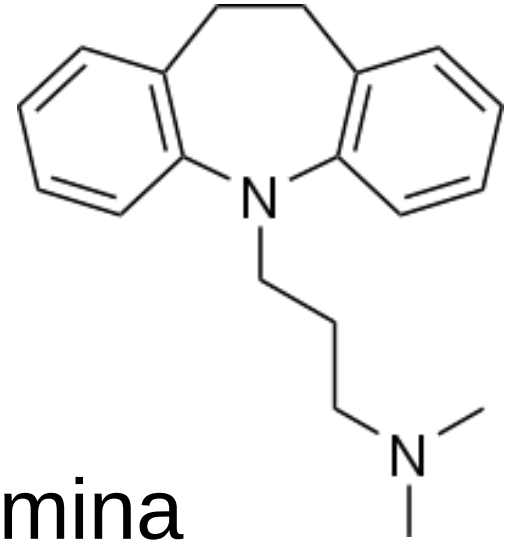
- desipramina, imipramina
- struttura a "v", conformazione bloccata
- inibitori della ricaptazione post-sinaptica della NA e della serotonina, inibizione dei canali ionici del sodio e del calcio a livello cardiaco, effetti collaterali cardiaci!!!



nortriptilina



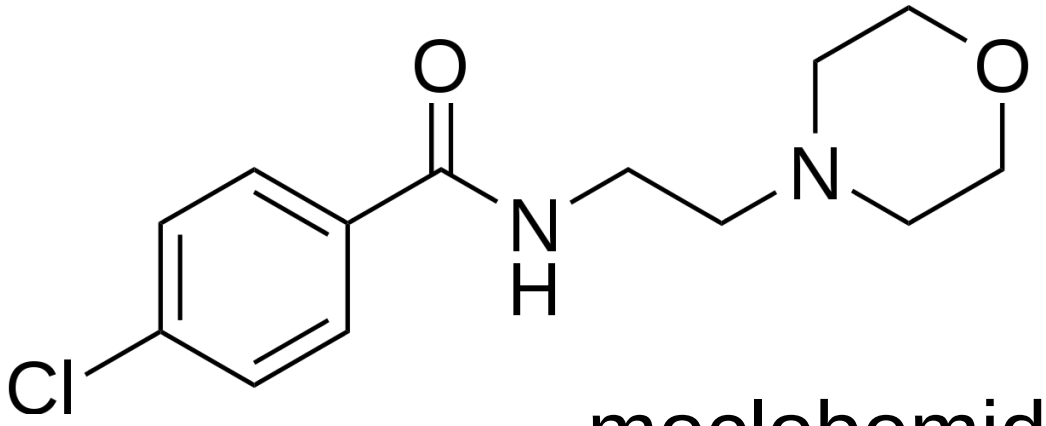
desipramina



imipramina

I generazione
inibitori delle MAO

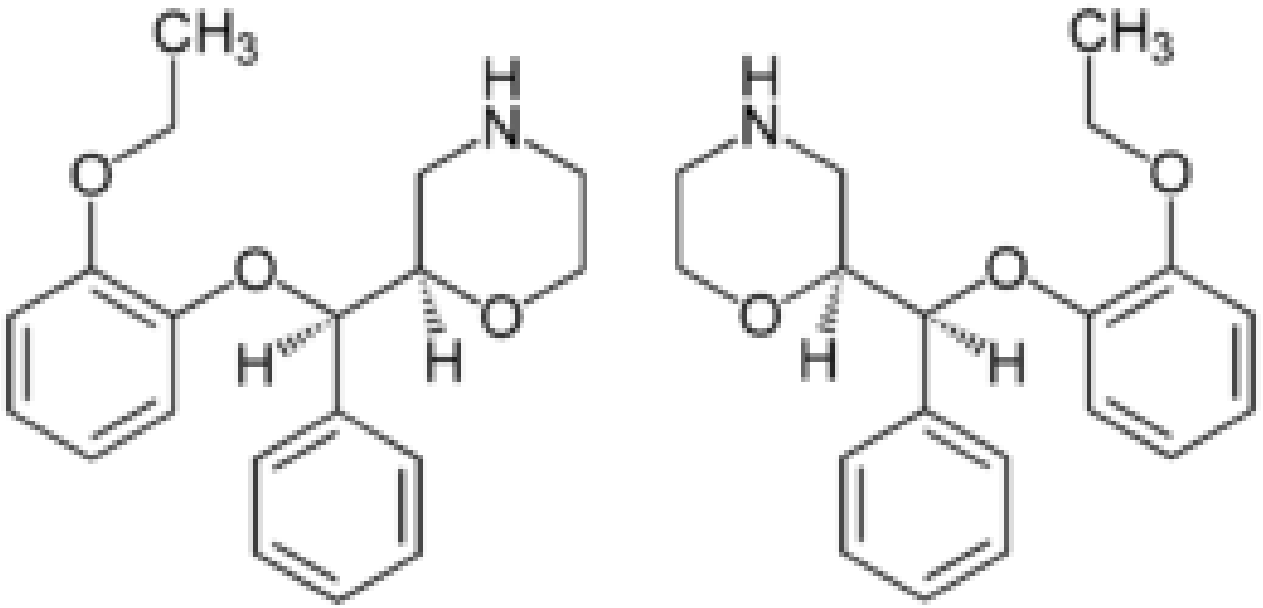
- fenelzina, iproniazide, tranilcipromina, scarsa selettività, inibiscono tutte le MAO
- moclobemide più selettiva



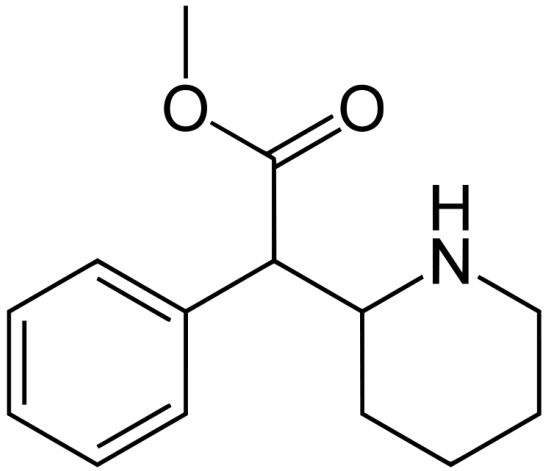
moclobemide

seconda generazione di antidepressivi: i NRI

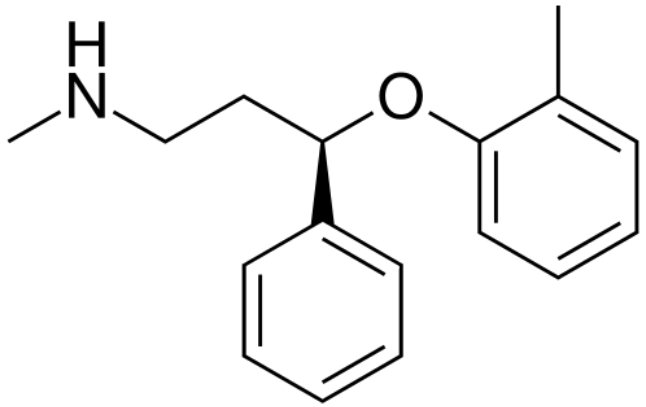
- I NRI hanno maggiore selettività sui trasportatori della NA: Reboxetina (commercializzata nel 2003)



Metilfenidato ed atomoxetina: NRI usati per disturbi da iperattività e dell'attenzione (ADHD), aumentano NA e dopamina nel cervello.



Metilfenidato (Ritalin)



atomoxetina

ADHD

Il Disturbo da deficit di attenzione/iperattività (DDAI in italiano o ADHD in inglese), prevalente in età infantile, è caratterizzato da sintomi di distrazione, iperattività e impulsività che sono incompatibili con il livello di sviluppo e hanno un impatto negativo diretto sulle attività sociali e scolastiche/lavorative.

Con il libro *Driven to distraction (revised): recognizing and coping with attention deficit disorder* gli autori mettono in luce che l'ADHD non è solo una malattia dell'infanzia ma colpisce anche gli adulti.

Infatti, indagini sulla popolazione suggeriscono che l'ADHD si verifica nella maggior parte delle culture in circa il 5% dei bambini in età scolare e circa il 2,5% degli adulti ed è più frequente nei maschi rispetto alle femmine nella popolazione generale, con un rapporto di circa 2:1 nei bambini e 1,6:1 negli adulti.

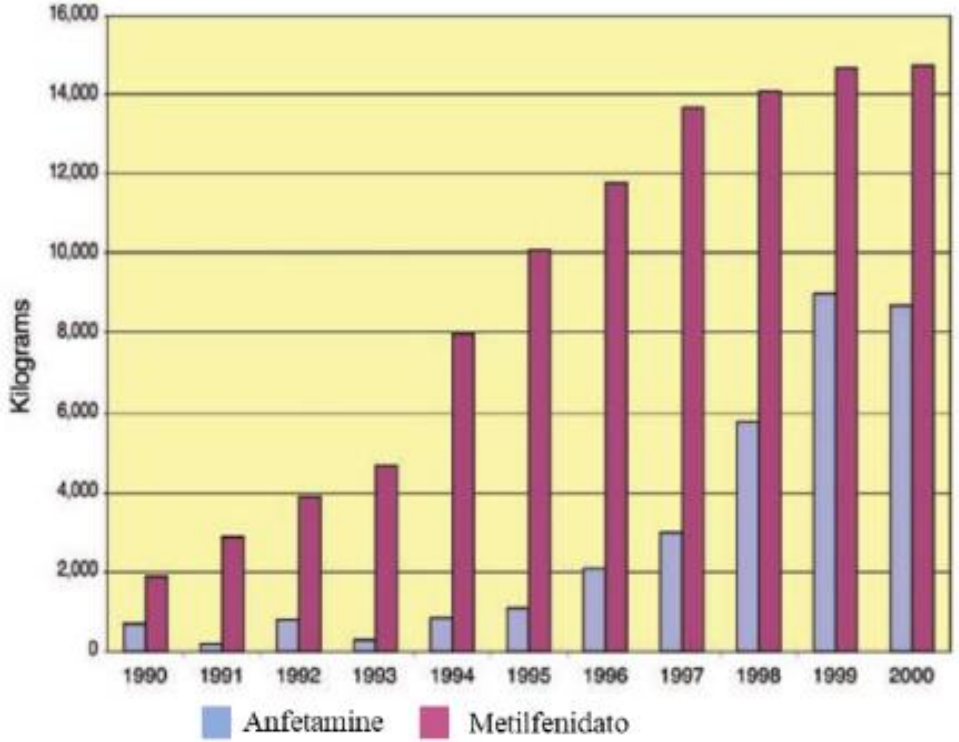


Figura 1: Produzione globale di anfetamine e metilfenidato. I numeri mostrati indicano la quantità di farmaco in kilogrammi. Sebbene un po' di MP veniva usato per trattare la depressione e il deterioramento mentale e neurologico associato all'AIDS, la maggior parte è stata usata per trattare l'ADHD.

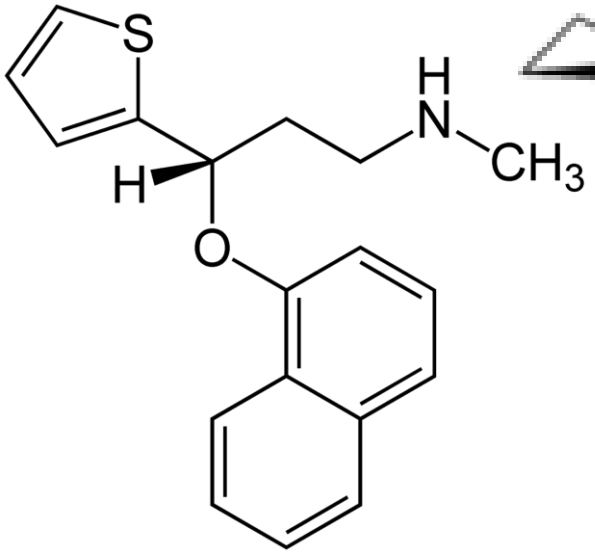
Figura 2:
Copertina del New York Times, anno 1994.

SCOPERTA E ORIGINE DEL NOME: il METILFENIDATO (vedi Figura 19)

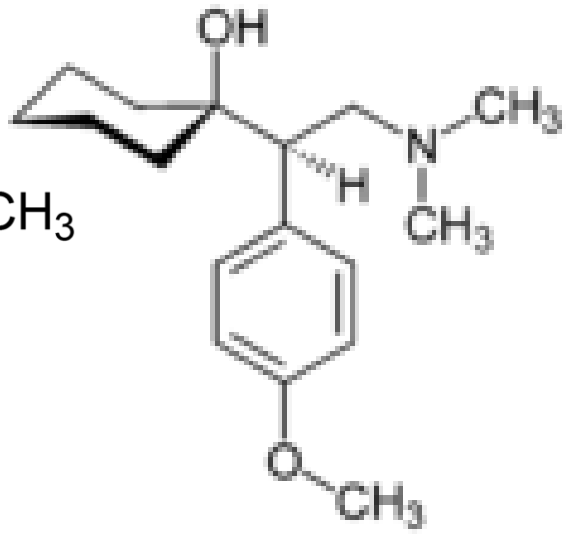
Il metilfenidato fu scoperto nel 1944 dal chimico di Basilea Leandro Panizzon (Figura 3) all'età di 37 anni, presso i laboratori di Ciba a Basilea, attuale Novartis.

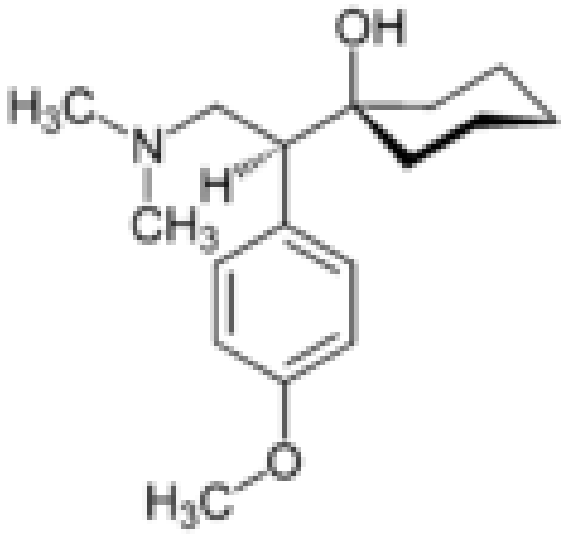
NSRI

- NSRI: Inibitori duplici della ricaptazione della NA e della serotonina: duloxetina e venlafaxina



duloxetina

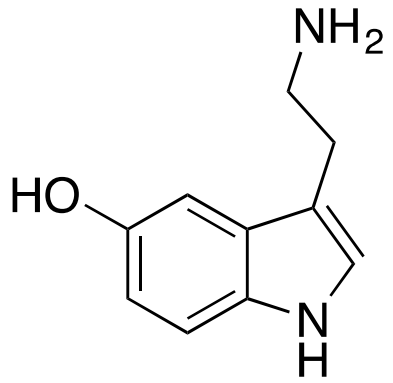




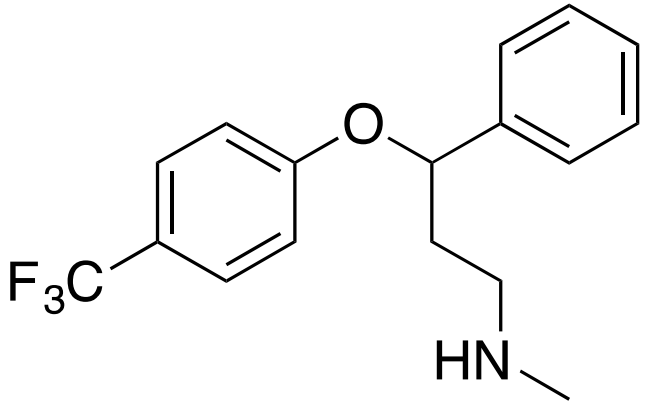
venlafaxina

SRI

- ipotesi delle monoammine: deficit di neurotrasmettitori monoamminici dopamina, NA, serotonina (5-HT)
- Dagli anni '80: inibitori selettivi della ricaptazione della serotonina (SSRI)



serotonina



fluoxetina (Prozac)

The opioid receptor (OR)

- Opioid receptors (OR), members of the GPCRs superfamily, are crucial in pain management, drug addiction, and mood disorders.
- There are four OR subtypes: μ-opioid receptor (μ-OR), δ-opioid receptor (δ-OR), κ-opioid receptor (κ-OR) and the opioid receptor-like 1 [OLR-1 or nociceptin receptor (NOP)].

OR agonists action and side effects

- Activation of μ-OR inhibits severe acute pain, modulating mechanical, chemical and supraspinally controlled thermal nociception. Given that μ-OR mediates rewarding properties of nonopioid drugs of abuse (i.e., cannabinoids, alcohol and nicotine), these receptors represent a molecular target for reward processing, contributing to the initiation of addictive behaviors.
- Adverse effects of μ-OR agonists include nausea, vomiting, gastrointestinal constipation, respiratory depression, rewarding effects, tolerance, and depen-dence.

Morphine: strong stimulation of μ-OR

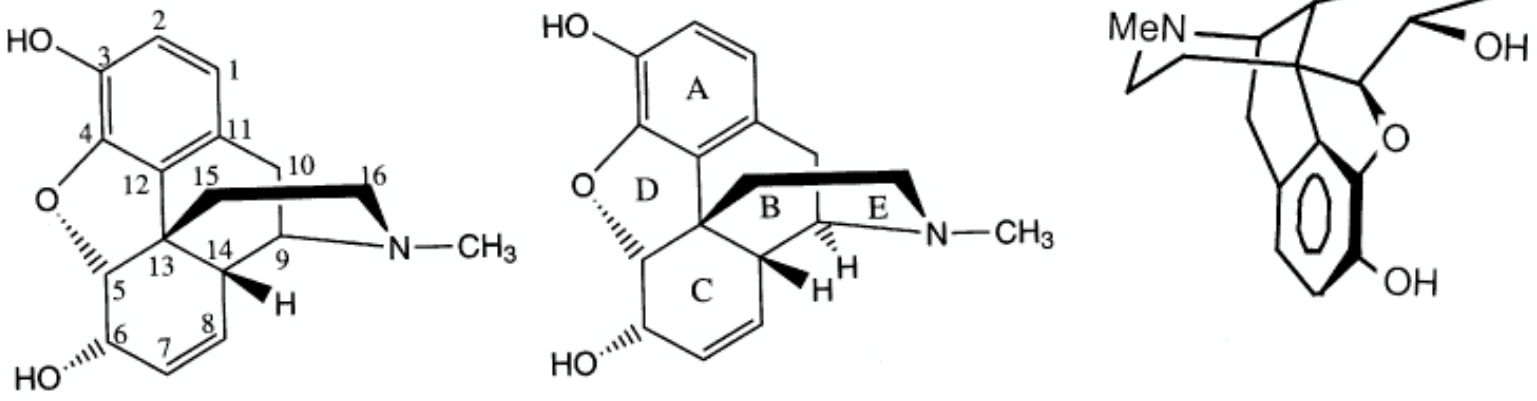
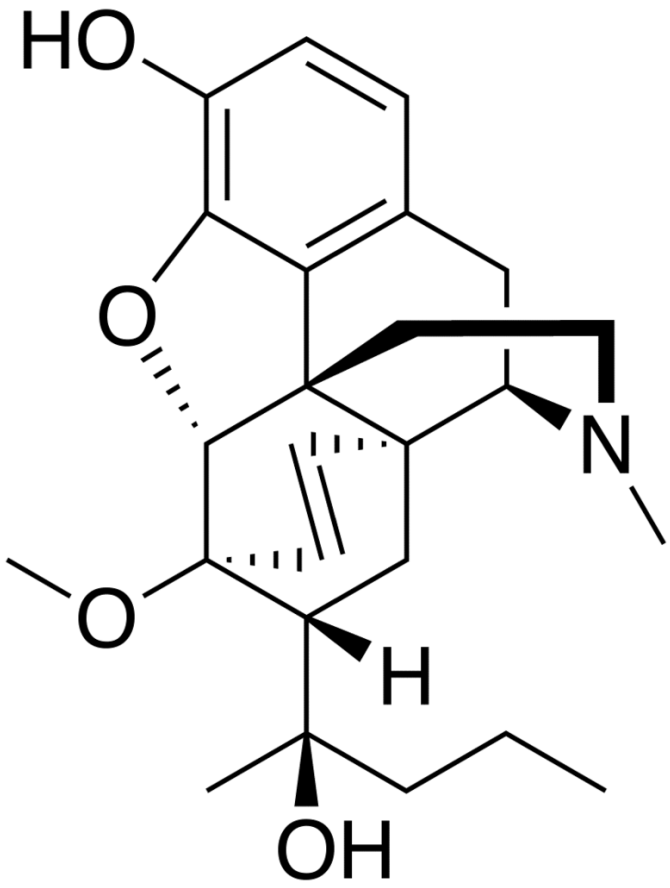


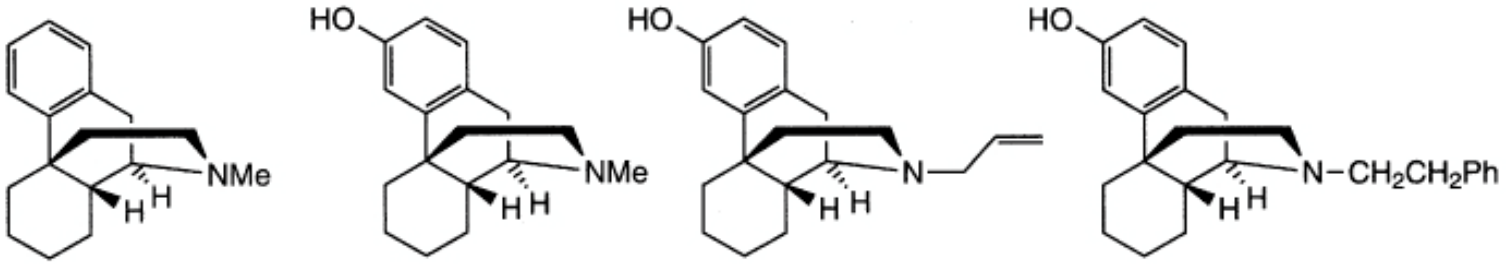
Fig. 17.2 Struttura della morfina

Incremento di attività per omologazione e per rigidificazione strutturale



etrofina e oripavine: 10.0000 volte più attive della morfina:
1) rigidificazione
2) log p maggiore

Semplificazione strutturale : i morfinani



N-METIL MORFINANO
(20% dell'attività della morfina)

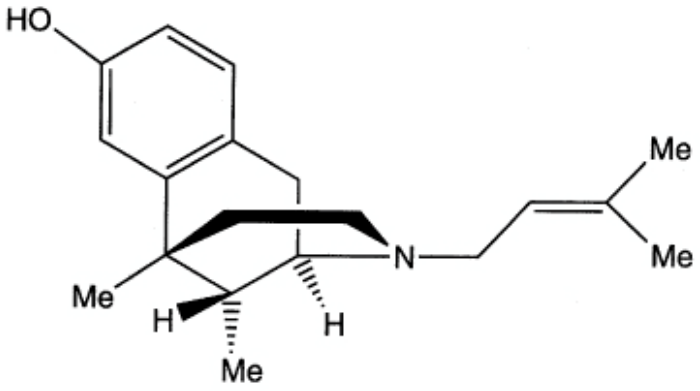
LEVORFANOLO
(5x più potente
della morfina)

LEVALLORFANO
(Antagonista 5x più potente
della nalorfina)

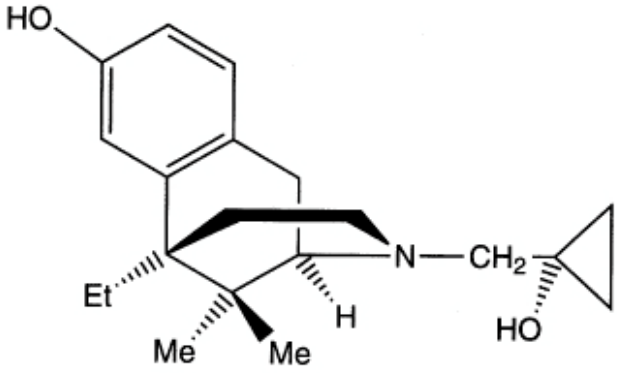
(15x più potente della morfina)

Fig. 17.19 Esempi di morfinani

I benzomorfani

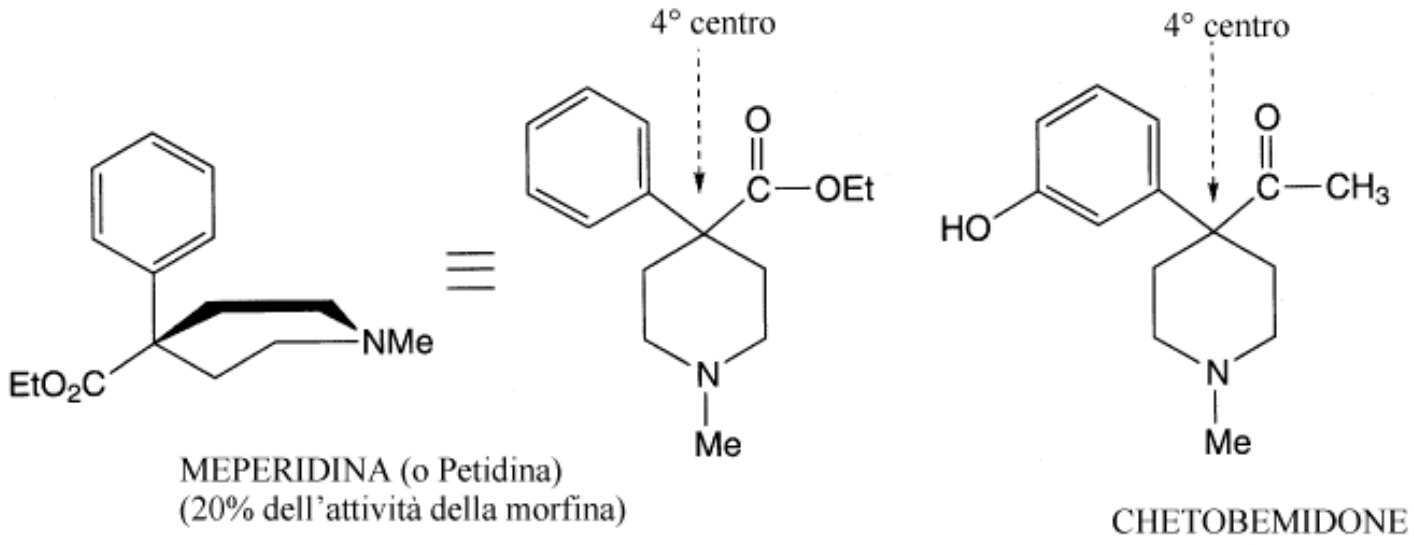


PENTAZOCINA
(33% dell'attività della morfina,
breve durata d'azione,
bassa predisposizione alla dipendenza)



BREMAZOCINA

La meperidina



MEPERIDINA (o Petidina)
(20% dell'attività della morfina)

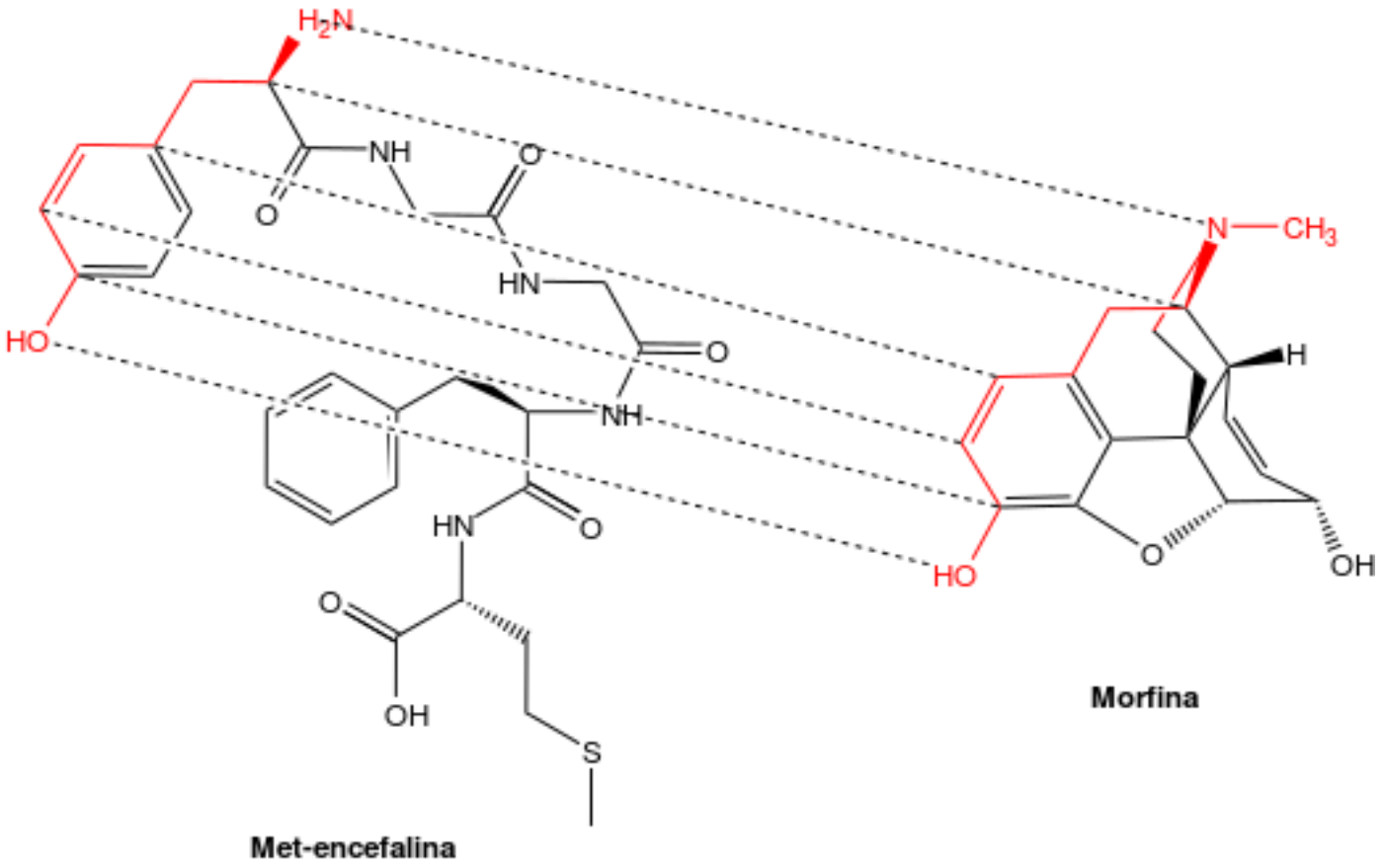
CHETOBEMIDONE

Fig. 17.22 4-Fenilpiperidine

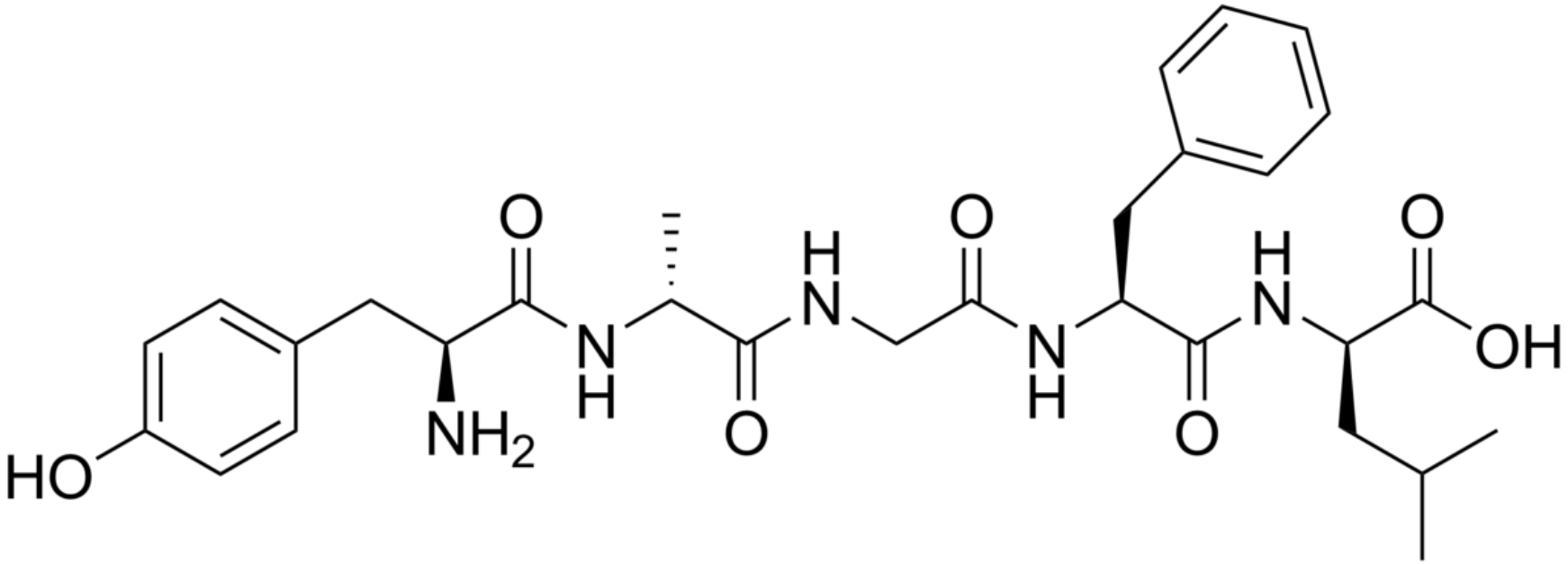
Enkephalins and endorphins: endogenous μ-OR ligands

- Endogenous peptides represent relevant μ-OR ligands with a key role in functional selectivity of several GPCRs as well as opioid receptors. Notably, μ-OR endogenous peptides (i.e., enkephalins, endorphins, dynorphins, and neoendorphins) and putative endogenous peptides (endomorphines) have shown promising analgesic effects compared with opiates , despite their high liability for proteolysis.
- Efforts in the development of endomorphin analogs have been conducted

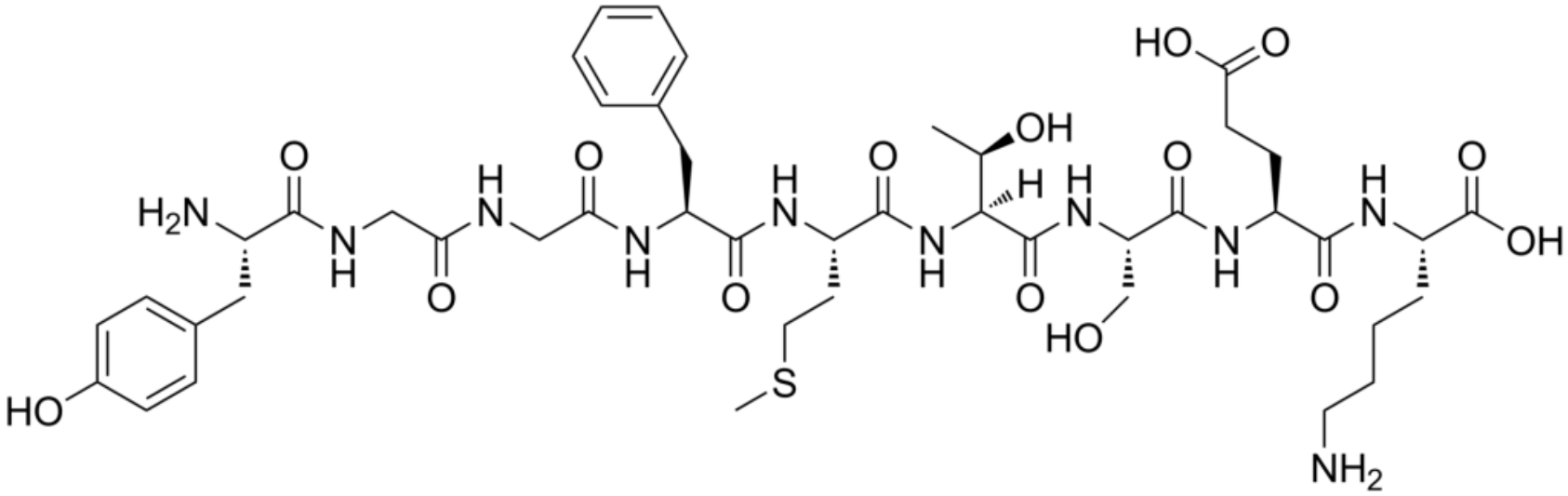
La Met-encefalina



La Leu-encefalina

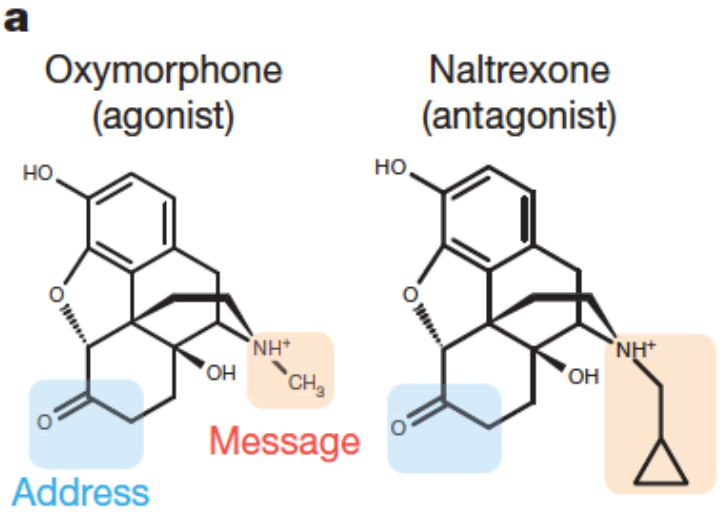


Endorphins



- From a pharmacological point of view, the use of positive allosteric modulators (PAM) has resulted in an increased potency and/or efficacy of the ligands binding to the orthosteric site.
- Although the development of allosteric modulators is still in the early drug discovery stage, it shows promise in the development of analgesics with **fewer adverse effects**. Alternatively, agonists that activate both μ-OR/δ-OR and NOPR/μ-OR have demonstrated an increase in antinociceptive efficacy.
- By contrast, molecules with dual but opposite effects acting as **μ-OR agonists and δ-OR antagonists** preferentially prevent or reduce tolerance and physical dependence.

- Following this mechanism, opioid combination drugs [i.e., morphine (agonist)/naltrexone (antagonist) and oxycodone (agonist)/naloxone (antagonist)] have been developed and marketed for the treatment of moderate to severe pain.

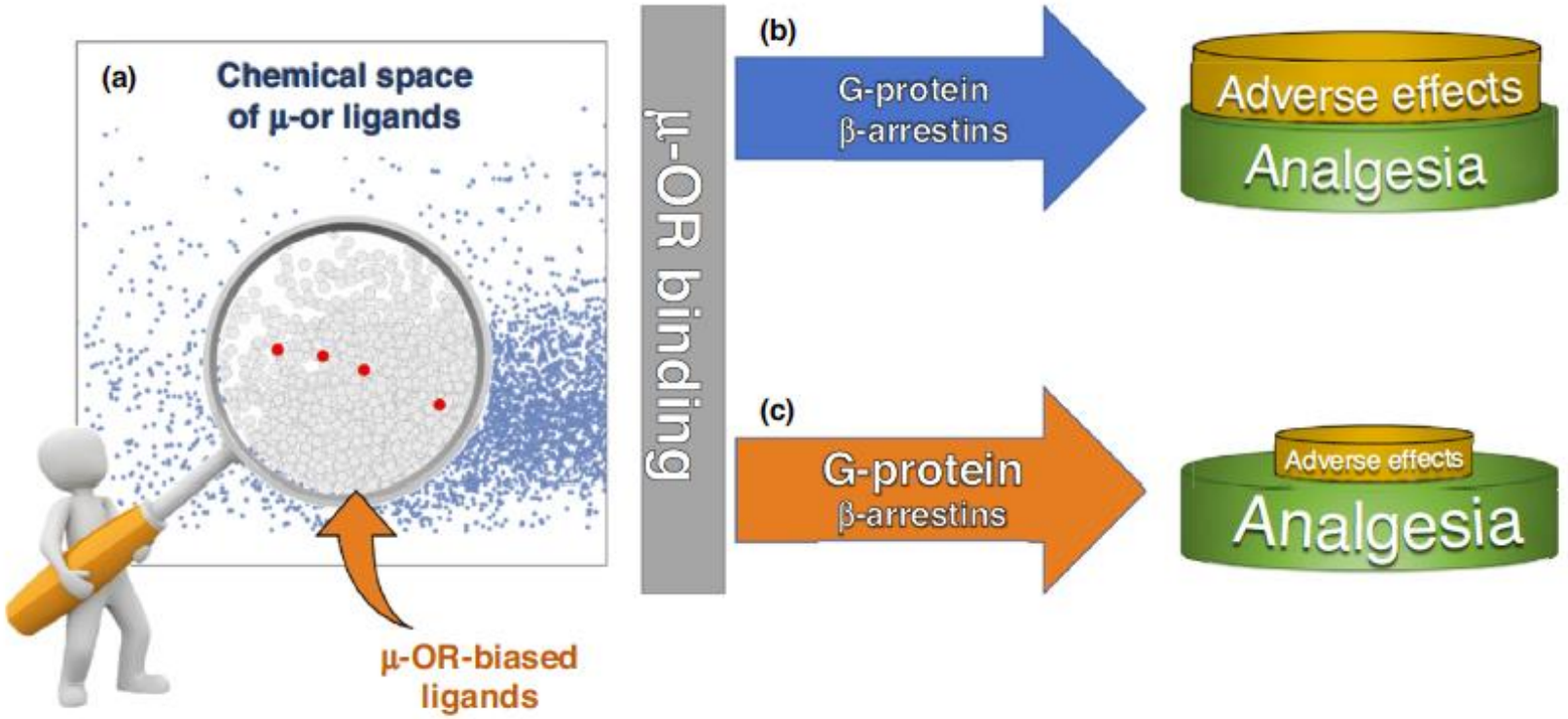


GPCR and β-arrestin activation

- Upon coupling to GPCRs, β-arrestins hinder the G protein interaction with GPCRs at the ICL2 , resulting in receptor desensitization. Consistently with a cellular study, β-arrestin-2 knockout mice are characterized by an elevated and prolonged morphine analgesia with impaired desensitization to elevated and prolonged morphine analgesia, as well as the attenuation of respiratory depression and acute constipation

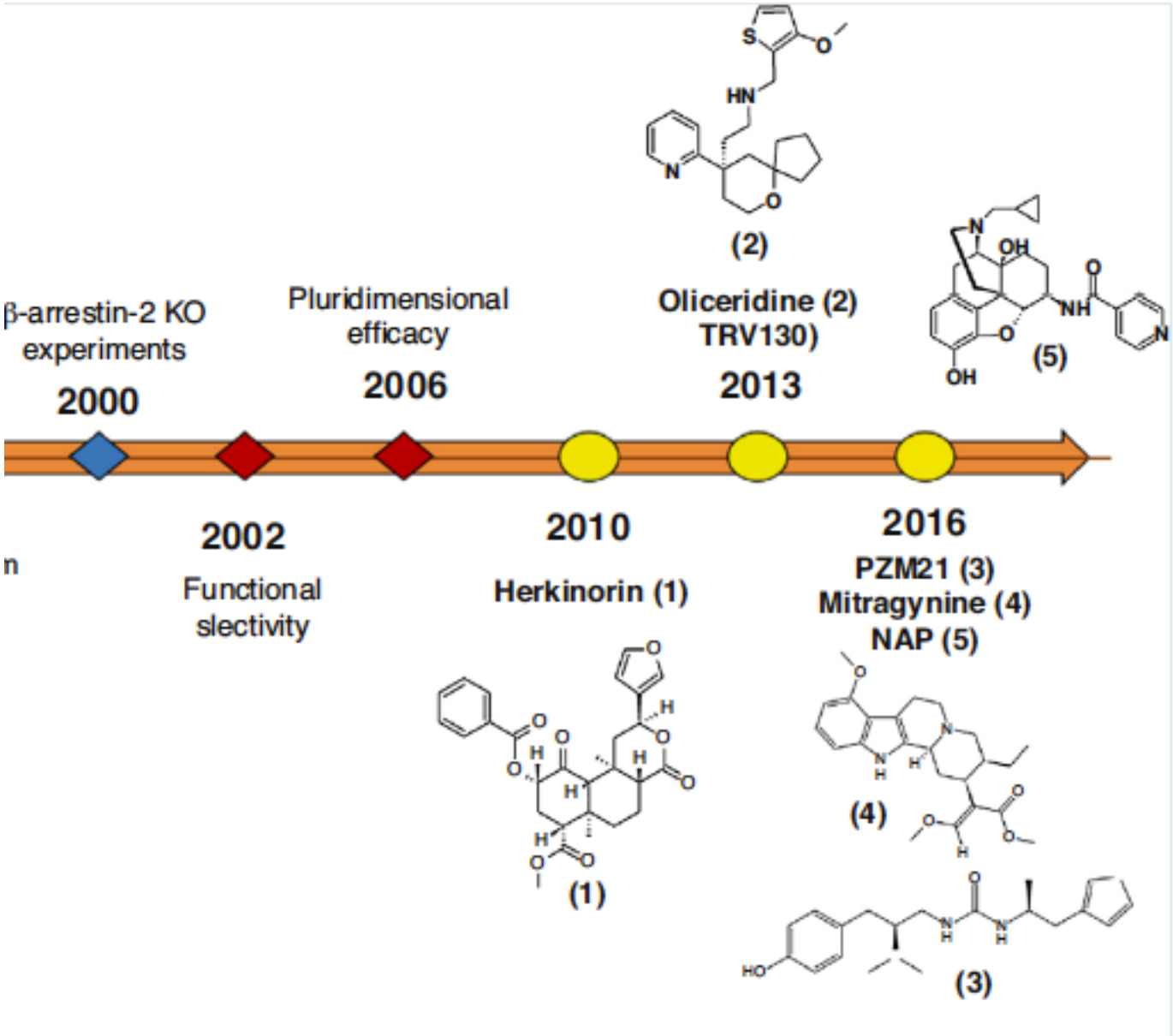
- Classical opiates, such as morphine, bind to the μ-OR and produce analgesia (mainly via the G-protein pathway) with the concomitant triggering of parallel signaling cascades (β-arrestin pathway) responsible for the adverse effects.
- Biased agonists that are capable of preferentially activate the G-protein signaling pathway over the arrestin pathway produce analgesia with diminished adverse effects.

Biased agonists activate G-protein and not β-arrestin



Drug Discovery Today

μ-OR receptor-biased agonists

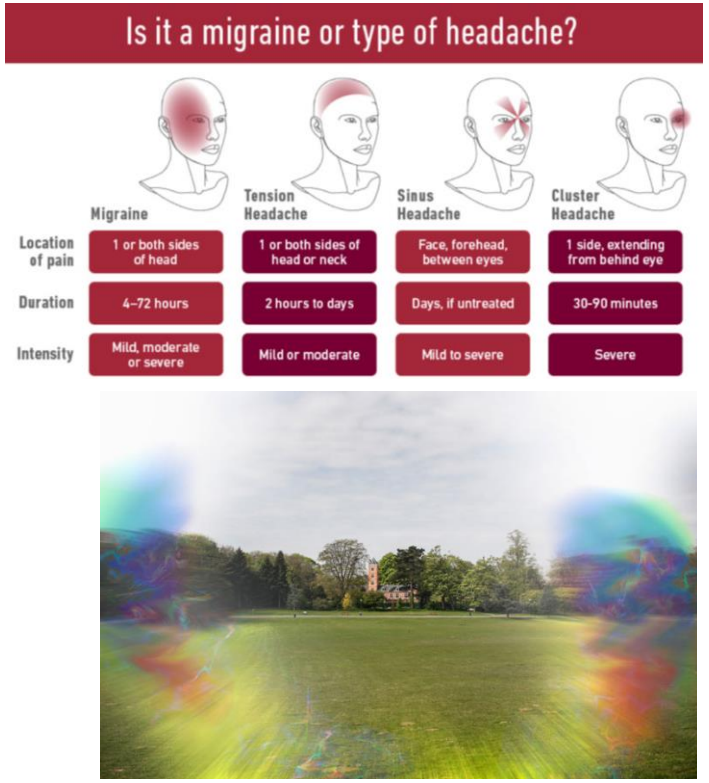


EMICRANIA

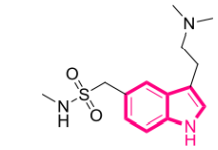
Sintomi: mal di testa, nausea, vomito, fotofobia, fonofobia, aura

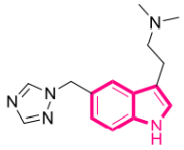
Trattamento: analgesico; triptani o ergotamina; gepant o anticorpi monoclonali

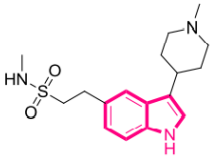
Costo per pazienti: 8924$ annui in più rispetto chi senza emicrania (nel 2014).

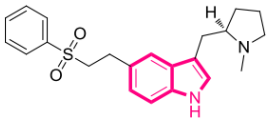


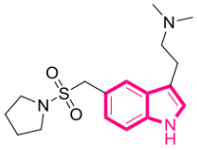
TRATTAMENTO FARMACOLOGICO

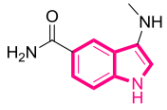

Sumatriptan

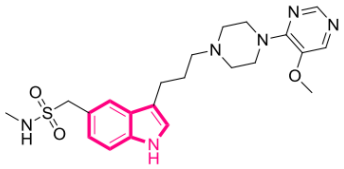

Rizatriptan

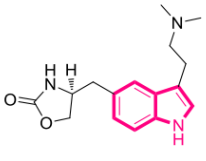

Naratriptan


Eletriptan

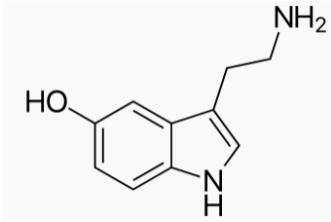

Almotriptan


Frovatriptan


Avitriptan


Zolmitriptan

- Antiinfiammatori non steroidei.
- Ergotamina. Agonista di diversi recettori. Effetti collaterali e PK lenta. Non più usata.
- Triptani (gold standard). Agonisti recettore serotonina. Core: indolo. Effetti collaterali se problemi cardiovascolari.


Serotonina

EMICRANIA: FISIOPATOLOGIA

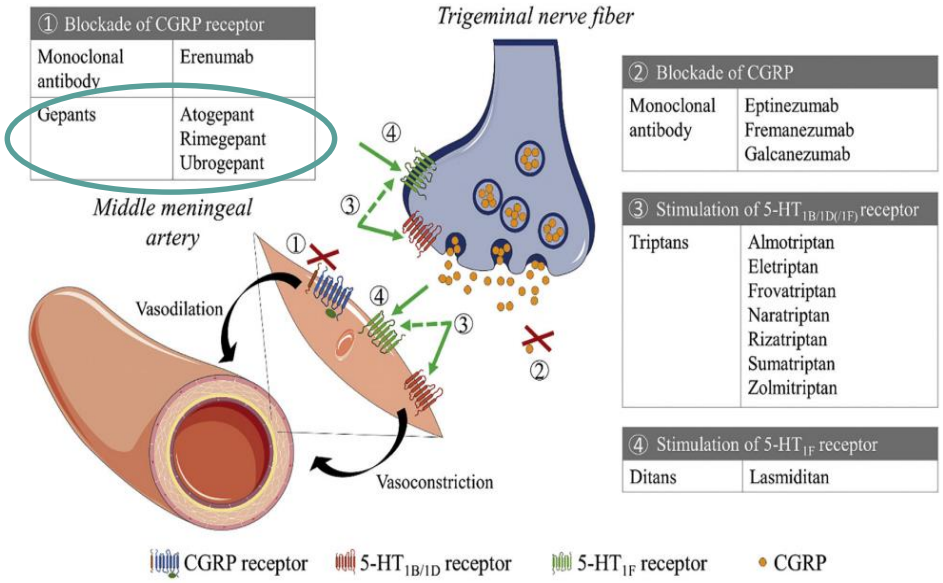


Fig. 1. Overview of migraine-specific medications and their possible targets. Migraine drugs can act through blockade of CGRP or its receptor or by stimulation of 5-$HT_{1B/1D/1F}$ receptors.

- Attivazione sistema trigemino vascolare
- Rilascio CGRP
- Degranulazione mastociti
- Vasodilatazione
- Attivazione nocicettori

PRINCIPALI COMPOSTI SELETTIVI

Medications targeting CGRP or the CGRP receptor.

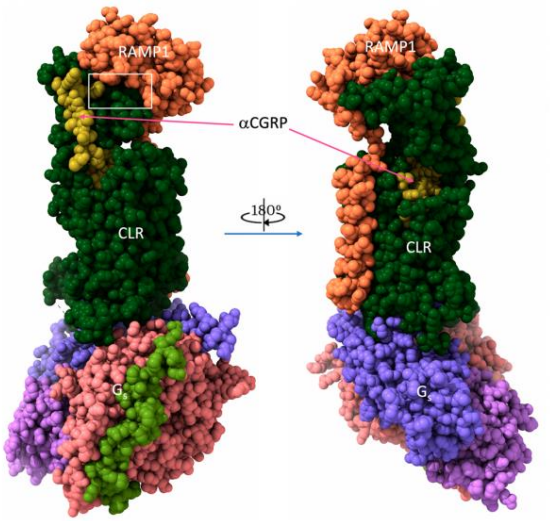
Compound	Type of treatment	Stage of development	Route of administration	Type of medication and target
Gepants				
Olcegepant	Acute	Phase II - Discontinued	Intravenous	Small molecule CGRP receptor antagonist
Telcagepant	Acute	Phase III - Discontinued	Oral	Small molecule CGRP receptor antagonist
MK3207	Acute	Phase II - Discontinued	Oral	Small molecule CGRP receptor antagonist
BI44370TA	Acute	Phase II - Discontinued	Oral	Small molecule CGRP receptor antagonist
Ubrogepant	Acute	FDA approved	Oral	Small molecule CGRP receptor antagonist
Rimegepant	Acute (prophylactic)	FDA approved	Oral	Small molecule CGRP receptor antagonist
Atogepant	Prophylactic	Phase III	Oral	Small molecule CGRP receptor antagonist
Monoclonal antibodies				
Eptinezumab	Prophylactic	FDA approved	Intravenous	Antibody against CGRP
Erenumab	Prophylactic	FDA and EMA approved	Subcutaneous	Antibody against CGRP receptor
Fremanezumab	Prophylactic	FDA and EMA approved	Subcutaneous	Antibody against CGRP
Galcanezumab	Prophylactic	FDA and EMA approved	Subcutaneous	Antibody against CGRP

*Atogepant approvato da FDA 28 settembre 2021

CGRP, RECETTORE e MECCANISMO DI BINDING

αCGRP H_2N-ACDTATCVTHRLAGLLSRSGGVVKNNFVPTNVGSKAF-NH_2

βCGRP H_2N-ACNTATCVTHRLAGLLSRSGGMVKSNFVPTNVGSKAF-NH_2



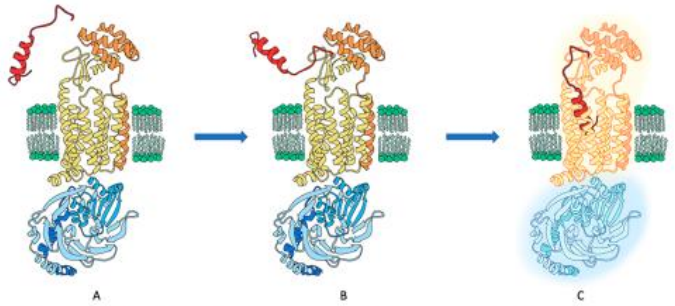


Figure 3. Proposed two-step mechanism of CGRP ligand (red) activation of the human CGRP receptor (CLR/RAMP1) involving initial C-terminal binding at the CLR (yellow)/RAMP1 (orange) interface (A to B), followed by insertion of the N-terminal macrocyclic loop and helix within the CLR, causing signaling through cytosolic G-proteins (light blue) (B to C).[33]

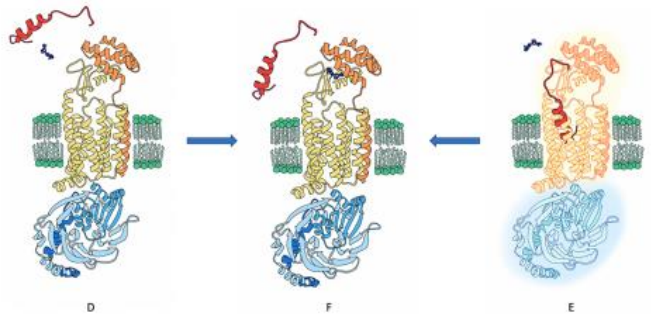
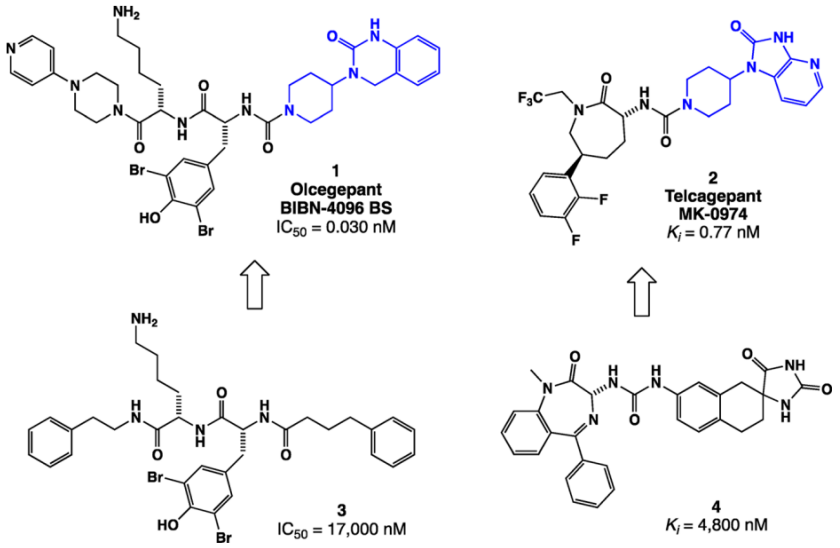


Figure 4. Mechanisms of CGRP blockade by a small molecule CGRP RA (dark blue): competition with the initial CGRP ligand (red) C-terminal binding event, preventing N-terminal agonist insertion (D to F), and displacement of bound CGRP ligand (E to F).

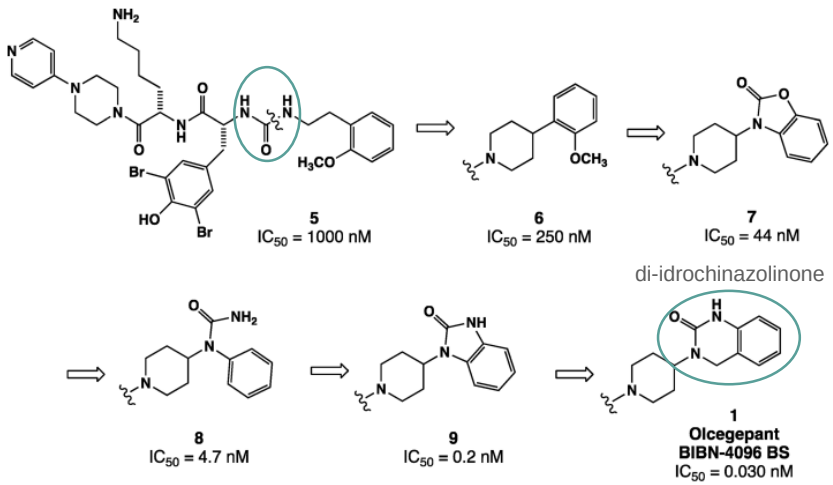
FIRSTS IN CLASS

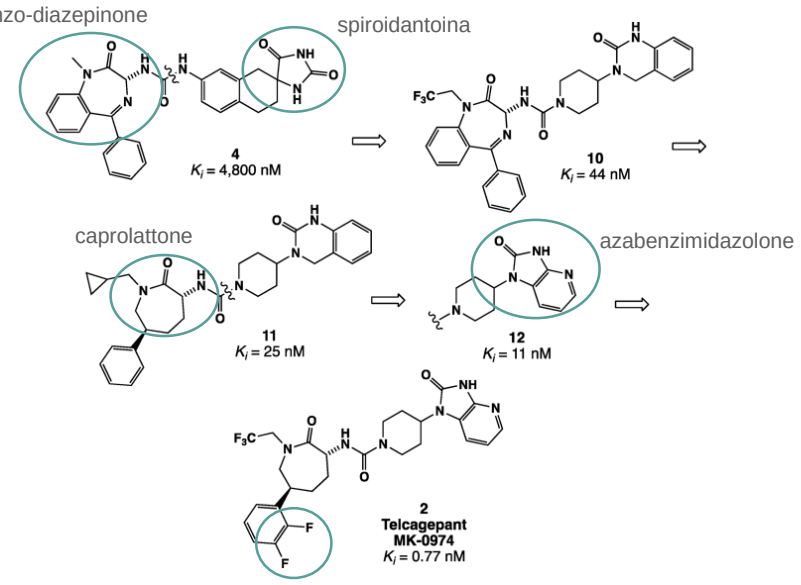
- Olcegepant: grande (MW 869.65); molto polare; no biodisponibilità; peptide-like; alta plasma free-fraction
- Telcagepant: più piccolo (MW 566.53); più lipofilo; buona biodisponibilità; Ki minore

Olcegepant mantiene core Lys e di-Br-Tyr



FIRSTS IN CLASS: SAR





- maggiore stabilità (5)
- maggiore lipofilia (6)
- ottimizzazione H-bond; mimo Phe-NH2 (7), (8) e (9)

- maggiore binding (10)
- minore dimensione e PM (11) e (12)
- no ossidazione (2)

ASPETTI DI PK, PD e TRIAL CLINICI

OLCEGEPANT: intravenoso

- In ratti maggiore IC_{50} (6.4 nM)
- Azione in PNS (arterie cerebrali), no CNS (arterie piali)

Trial clinici

- Successi in fase 1 e 2 (66% vs 27 % riduzione emicrania)
- Effetto collaterale: 8% parestesia
- No side effect cardiovascolari
- Problema: intravenoso. Sviluppo interrotto

TELCAGEPANT: orale

- F_{PO} 22% in topi, 35% in cani, non lineare e basso in scimmie (metabolismo first-pass)
- Alta frazione protein-bound (aumento della IC50=10,9 nM)

Triali clinici

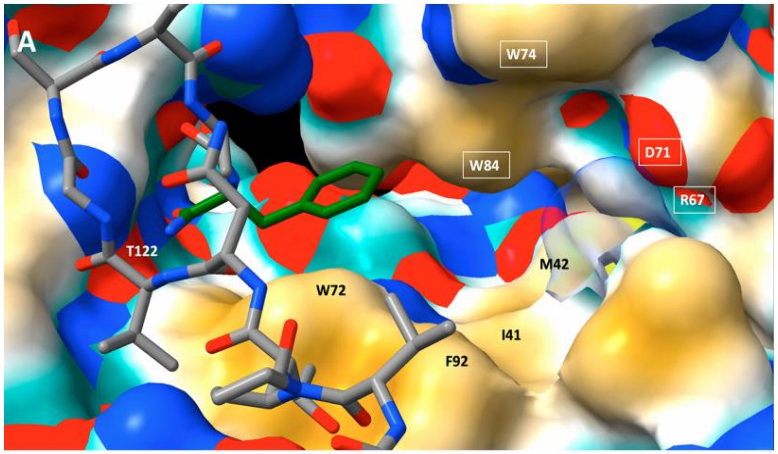
- Confronto con Rizatriptan: risposta simile, minori effetti collaterali
- Fase 3: sicuro, sollievo dal dolore in 2h e riduzione sintomi «fastidiosi»
- Fase 2: livelli elevati alanina-amminotransferasi nel fegato. Sviluppo interrotto

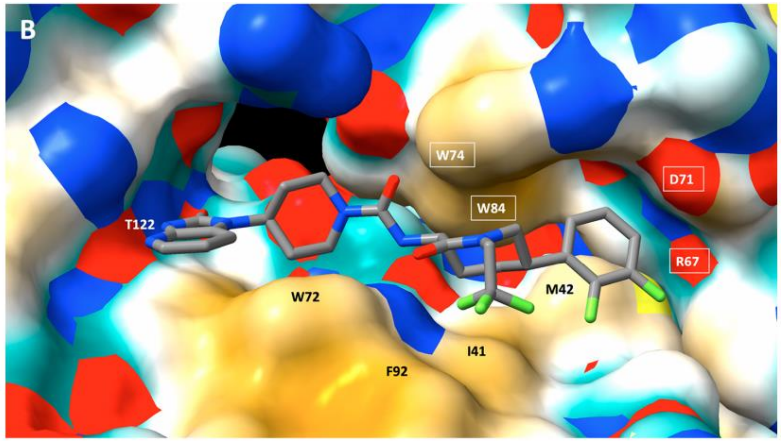
STUDI STRUTTURALI DI BINDING

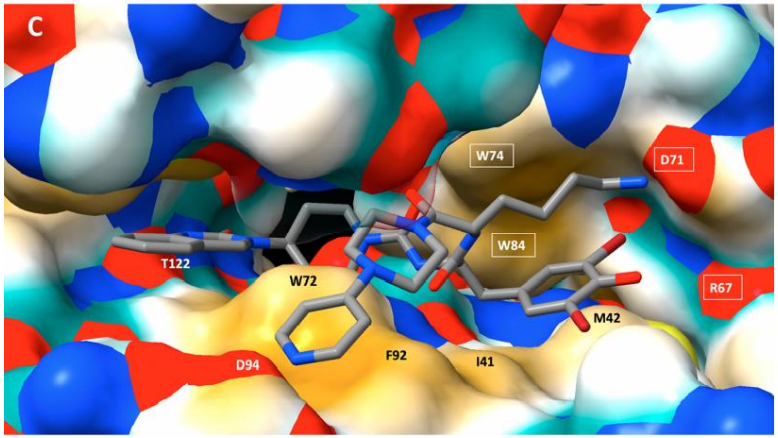
A. **CGRP**. PheNH2 – O=C e NH T122 (CLR); Phe – indolo W72 (CLR); Phe – indolo W84 (RAMP1); tasca vuota.

B. **Telcagepant**. Interazioni idrofobiche tasca; O=C caprolattone – NH W72 (CLR); di-fluorofenile – O=C R67 (RAMP1)

C. **Olcegepant**. OH - H2O – O=C e NH R67 (RAMP1); Lys protonata- D71 (RAMP1); Lys – indolo W74 (RAMP1); piridina – F92 (CLR) e O di D94 (CLR).



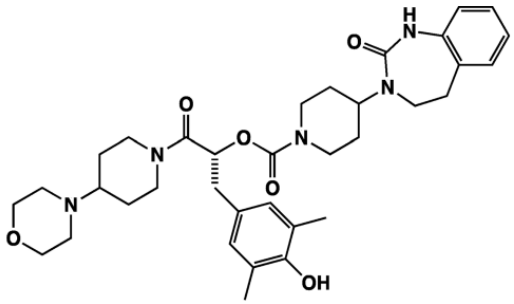




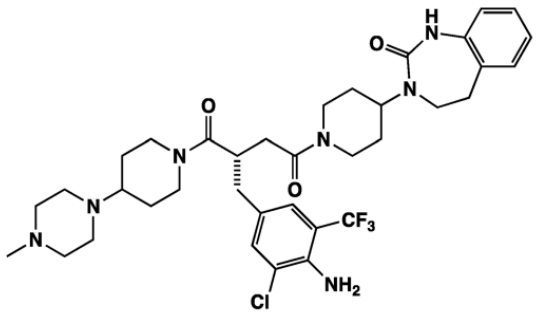
OLCEGEPANT ORALE

- Rimosso Lys
- Sostituito NH di urea con O (23) e CH2 (24)
- Benzodiazepinone come PS

Problema: alte fosfolipidosi



23
BI 44370
IC_{50} = 0.3 nM



24
BIBP 5371
IC_{50} = 0.14 nM

Figure 13. BI clinical lead BI 44370 and its predecessor BIBP 5371.

UBROGEPANT e ATOGEPANT

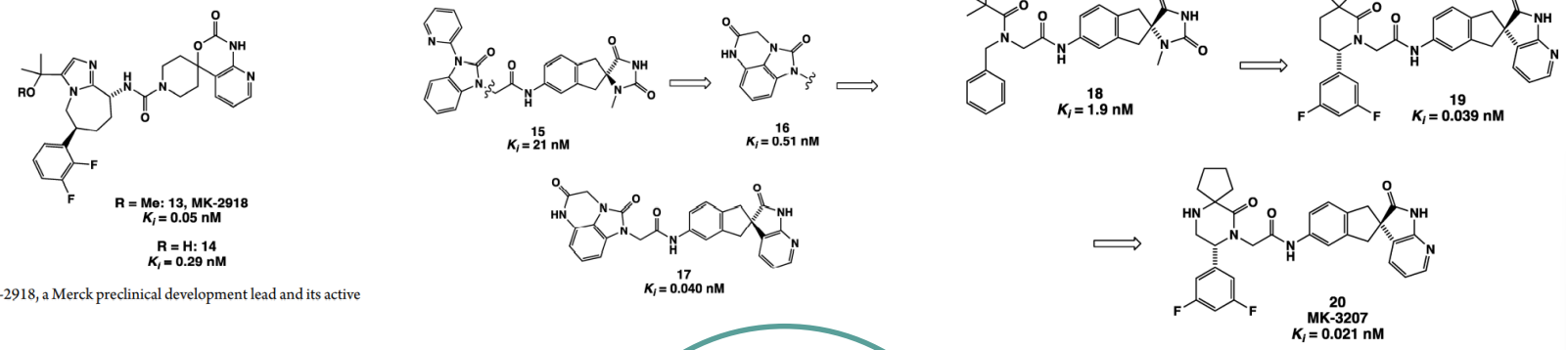


Figure 9. MK-2918, a Merck preclinical development lead and its active metabolite.

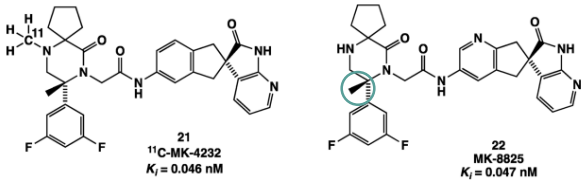


Figure 12. CGRP RA tool compounds from Merck: ^{11}C-MK-4232, a CNS-penetrant PET ligand, and MK-8825, an antagonist useful in rat model testing.

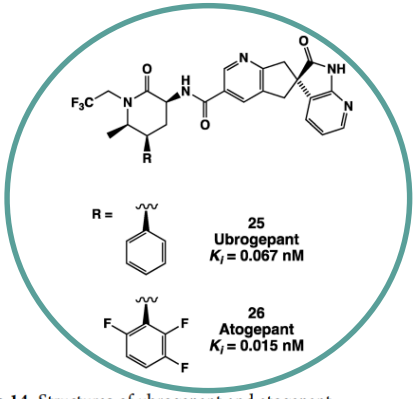
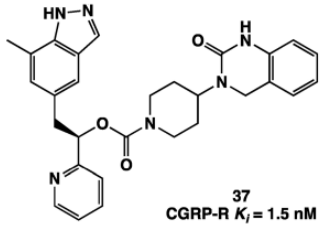


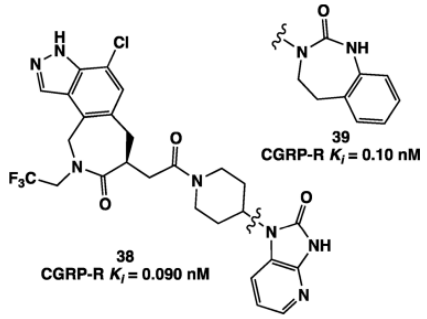
Figure 14. Structures of ubrogepant and atogepant.

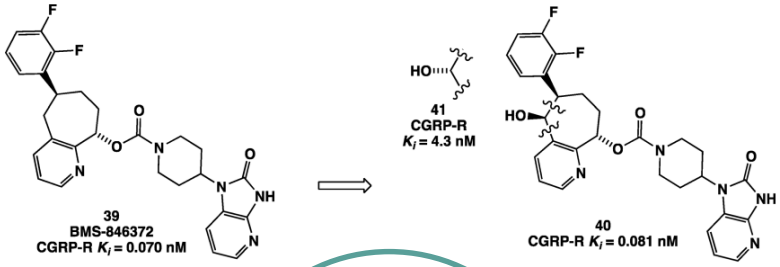
- 13 ha metabolita attivo: 14
- Aumento polarità e affinità (da 4 a 20)
- 20 alto valori alanina-transaminasi in fegato (fase I).
- 22. CH3 attraversa CNS (efflux ratio 25 vs 1.7)
- Ubrogepant e Atogepant: differenze in R

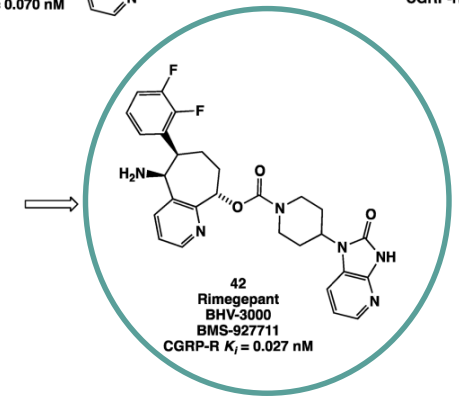
RIMEGEPANT: SAR

- 37. diminuire legami H mantenendo solubilità, polarità e estensione
- 38 e 39 più vincolato ma problemi ADME









- 39 migliore affinità ma bassa plasma free-fraction e solubilità
- 40, 41 e 42 migliore solubilità.

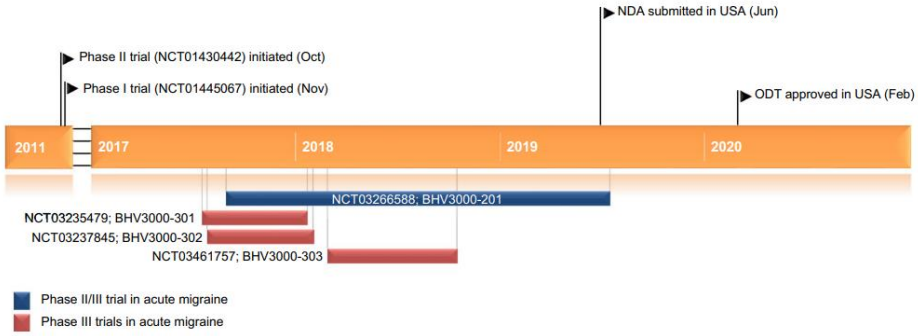
RIMEGEPANT: PK, PD e TRIAL CLINICI

Studi pre-clinici
- Arterie coronarie e cerebrali ex vivo: confronto con Sumatriptan. Non è vasocostrittore.
- No effetti collaterali cardiovascolari.

PK
- Tempi assorbimento brevi: 1,48 vs 1,92h (ODT vs originale) .
- Frazione legata a proteine plasma= 96%
- Metabolismo: CYP3A4 e CYP2C9. Eliminazione: 77% inalterato. No metaboliti rilevanti.

Trial clinici (75mg)
- Fase 3: sollievo dolore (21,2 vs 10,9 % placebo); sollievo sintomi fastidiosi (35,1 vs 26,8%) dopo 2h.

Eventi avversi
- Tollerabilità: Nausea (2 vs 1%); infezione vie urinarie (1,5 vs 1,1%). Eventi avversi seri (0,4 vs 0,2%).



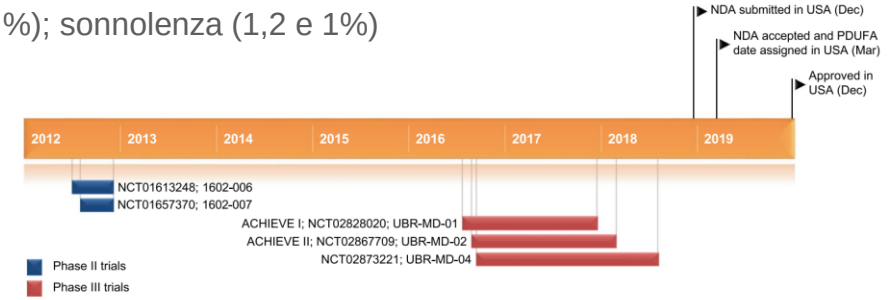
UBROGEPANT: PK, PD e TRIAL CLINICI

Studi pre-clinici
- In vitro: no vasocostrittore arterie coronarie.
- No effetti avversi cardiovascolari.

PK
- Tempi assorbimento brevi: Cmax a 1,5h.
- Frazione legata a proteine plasma= 87%
- Metabolismo: CYP3A4. Metaboliti principali: coniugati dell'acido glucoronico.

Trial terapeutici
- Fase 3: 50 o 100 mg. Per 50 mg: sollievo dolore (21,8 vs 14,3 % placebo); sollievo sintomi fastidiosi (38 vs 27,4%) dopo 2h.

Eventi avversi
- 50 e 100 mg. Nausea (2,4 e 2 %); sonnolenza (1,2 e 1%)



Key milestones in the development of ubrogepant for the acute treatment of migraine. *NDA* New Drug Application, *PDUFA* Prescription Drug User Fee Act

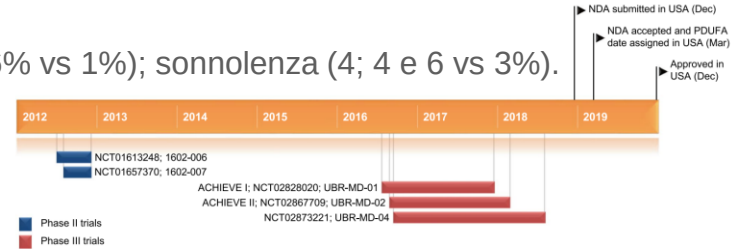
ATOGEPANT: PK, PD e TRIAL CLINICI

Studi pre-clinici
- In vitro: no vasocostrittore arterie coronarie. Maggiore efficacia inibizione vasodilatazione arterie craniche.
- No alanina-amminotransferasi sopra limiti.

PK
- Frazione legata a proteine plasma= 95.3%
- Metabolismo: CYP3A4. Metabolita principale: coniugato dell'acido glucoronico. 47% eliminato inalterato.

Trial terapeutici (10, 30 e 60 mg)
- Fase 3: 10, gironaliera per 12-week: riduzione giorni con emicrania al mese (-3,7; -3,9 e -4,2% vs -2,5% placebo). Benefici dal primo giorno: 10,8-14,1% vs 25,2 % placebo.
- Fase 3 efficacia a lungo termine (52-week): 60 mg. da 7,4 a -3,8 tra 1-4 week e -5,2 tra 49-52week. Riduzione emicrania: 50%, ≥ 75% o ≥ 100% rispettivamente in 60.4%; 37.2 % e 20.7 % per 1-4 week; aumenta tra 49-52 di: 84.2%, 69.9% e 48.4%.

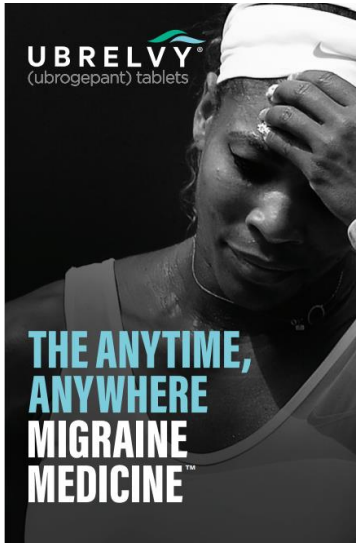
Eventi avversi
- Nausea (5; 6 e 9% vs 3%); costipazione (6% vs 1%); sonnolenza (4; 4 e 6 vs 3%).

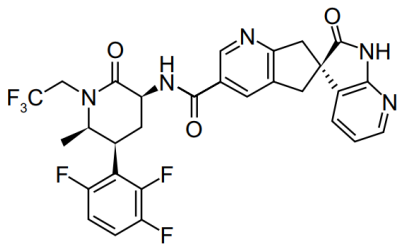


Key milestones in the development of ubrogepant for the acute treatment of migraine. *NDA* New Drug Application, *PDUFA* Prescription Drug User Fee Act

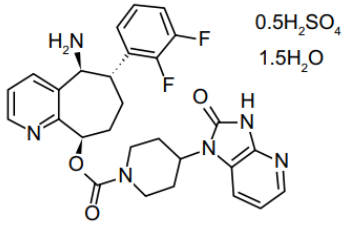
CONCLUSIONI

- I triptani sono stati per molto tempo i «gold standard» per trattamento emicrania. Problema legato a side effects.
- Sviluppo farmaci selettivi per recettore CGRP. Necessità di bilanciare diversi fattori: solubilità, polarità, dimensione, plasma free-fraction, affinità di binding, tempi di azione e tossicità.
- Solo gepant di ultima generazione sono in grado di bilanciare tutti questi fattori. Approvazione FDA di Atogepant, Rimegepant e Ubrogepant segna nuova era nel mondo dei farmaci contro emicrania.

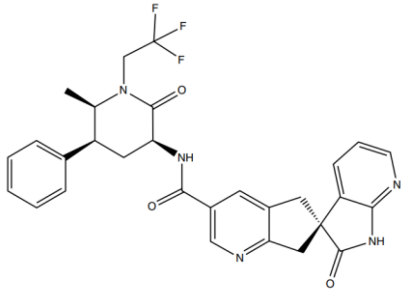




Chemical structure of atogepant



Chemical structure of rimegepant



Chemical structure of ubrogepant

BIBLIOGRAFIA

- Scott L. (2020). *Ubrogepant: First Approval*. Springer Nature
- Scott L. (2021). *Atogepant: First Approval*. Springer Nature
- Scott L. (2020). *Rimegepant: First Approval*. Springer Nature
- Leung L.; liao S.; Wu C. (2021). *To Probe the Binding Interactions between Two FDA Approved Migraine Drugs (Ubrogepant and Rimegepant) and Calcitonin-Gene Related Peptide Receptor (CGRPR) Using Molecular Dynamics Simulations* . ACS Chemical Neuroscience
- De Vries T.; Villalòn C.M.; MaassenVanDenBrink A. (2020). *Pharmacological treatment of migraine: CGRP and 5-HT beyond the triptans*. Pharmacology and Therapeutics
- Chadha N.; Silakari O. (2017). *Indoles as therapeutics of interest in medicinal chemistry: Bird's eye view*. European Journal of Medicinal Chemistry
- Dubowchik G. M.; Conway C. M.; Xin A. W. (2020). *Blocking the CGRP Pathway for Acute and Preventive Treatment of Migraine: The Evolution of Success*. Journal of Medicinal Chemistry