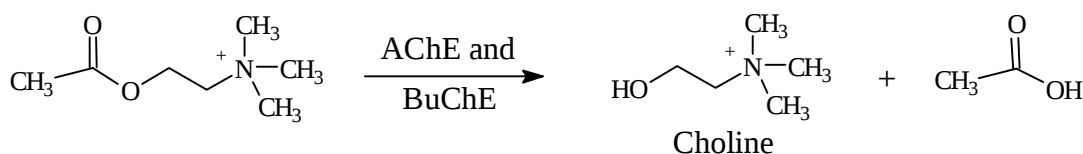


ACETYLCHOLINE AND CHOLINERGIC AGONISTS: STRUCTURE-ACTIVITY RELATIONSHIPS (SARs)

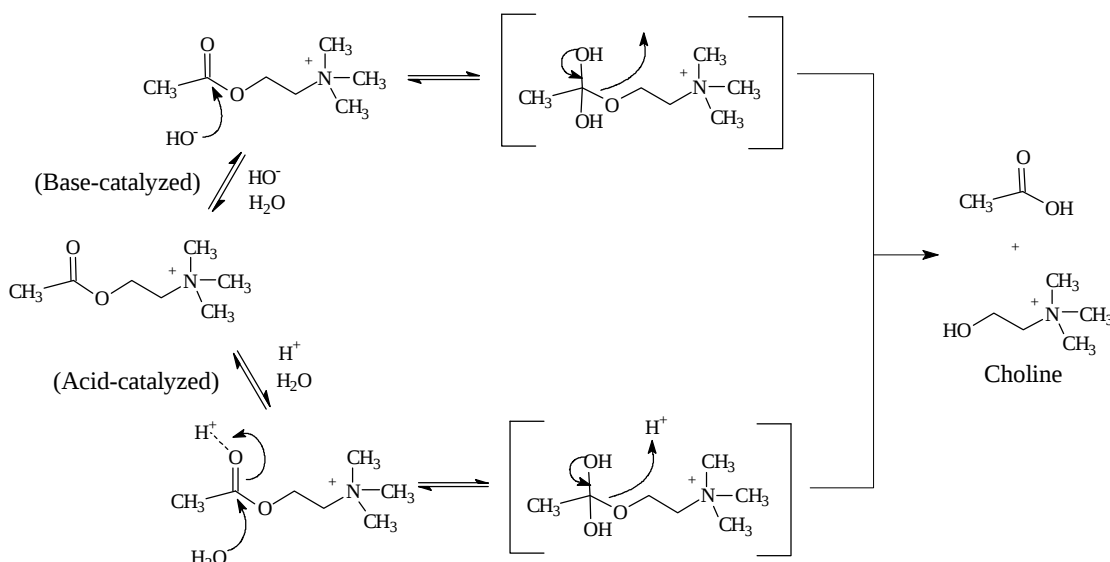
PC/MC Objective: For which disease states/pathologies would ACh be a useful drug?

PC/MC Objective: Why does ACh display limited efficacy as a therapeutic agent?

- Does ACh display adequate cholinergic receptor selectivity (muscarinic and nicotinic receptors and receptor subtypes) to be a safe therapeutic agent?
- Would ACh have an adequate half-life to be an effective therapeutic agent?



- Would ACh be orally bioavailable?
- Is ACh chemically stable?



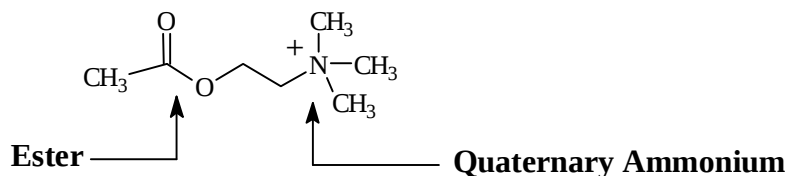
PC/MC Objective: How could the structure of ACh be modified to yield derivatives with enhanced therapeutic potential?

- Selective agonist activity?
- Chemical and metabolic stability?

Global Objectives:

- What is an “Agonist”? What two properties define an agonist?
- What is an “Antagonist”? what properties define an antagonist?
- How are agonists and antagonists similar?
- How are agonists and antagonists different?

MC Objective: Which ACh structural features appear to be required or at least contribute to cholinergic receptor binding.



MC Objective: What chemical properties are imparted by the ester and quaternary ammonium group of ACh?

- Chemical stability?
- Metabolic stability?
- Solubility?

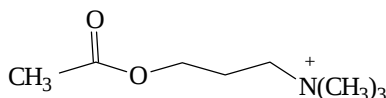
MC Objective: How does modification of the quaternary ammonium group of ACh influence “agonist” activity?

- How does sequential removal on the ammonium methyl groups of ACh influence cholinergic receptor affinity?
- How does sequential replacement of the ammonium methyl groups of ACh with ethyl (Et) groups influence cholinergic receptor affinity?

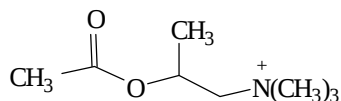
ACTIVITY OF ACETOXYETHYL
ONIUM SALTS: EQUIPOTENT MOLAR RATIOS
RELATIVE TO ACETYLCHOLINE

$\text{CH}_3\text{COOCH}_2\text{CH}_2$	Cat Blood Pressure	Intestine	Frog Heart
NMe_3^+	1	1 (Rabbit)	1
NMe_2H^+	50	40	50
NMeH_2^+	500	1000	500
NH_3^+	2000	20,000	40,000
NMe_2Et^+	3	2.5 (Guinea pig)	2
NMeEt_2^+	400	700	1500
NEt_3^+	2000	1700	10000 ^a
PMe_3^+	13	12 (Rabbit)	12
AsMe_3^+	66	90	83
SMe_2^+	50	30 (Guinea pig)	96

MC Objective: Why does increasing the length of the ethylene side chain of ACh cause a decrease in “agonist” activity? What is “Ing’s rule of five”?

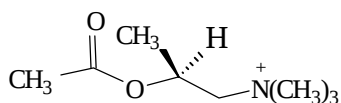


MC Objective: How does methyl substitution on the beta-carbon of the ethylene side chain of ACh influence “agonist” activity as in acetyl-beta-methylcholine (methacholine, Mecholyl[®])?

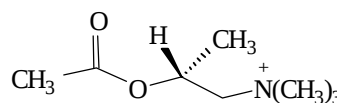


Acetyl-beta-methylcholine

- What influence does beta-methyl substitution have on cholinergic receptor selectivity? $M > N$ (ACh: $M = N$)? Why is this important?
- Beta-methyl substitution introduces a center of chirality. Do muscarinic receptors display selectivity for one enantiomer of acetyl-beta-methylcholine: (*S*200X > *R*)

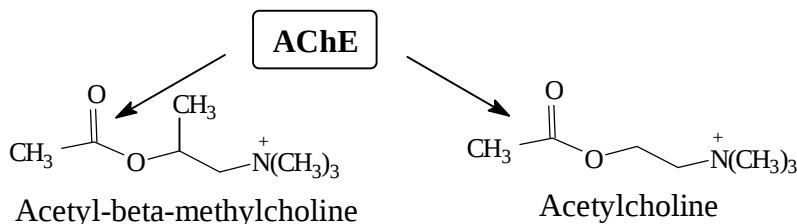


(*S*)-Acetyl-beta-methylcholine



(*R*)-Acetyl-beta-methylcholine

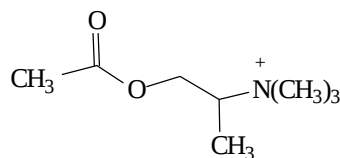
- How does (*S*)-acetyl-beta-methylcholine compare to ACh in terms of MACHR agonist activity: (Equivalent)
- The half-life of ACh is limited by rapid hydrolytic metabolism and inactivation mediated by acetylcholinesterase (AChE) and plasma esterases (BuChE). How does (*S*)-acetyl-beta-methylcholine compare to ACh in terms of rate of hydrolysis by AChE?



Rate of AChE-catalyzed hydrolysis: ACh > *S*-isomer > *R* (AChE inhibitor)
Why is ACh hydrolyzed more rapidly than acetyl-beta-methylcholine?

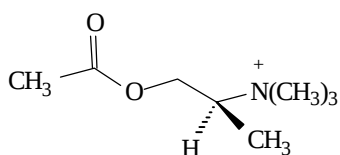
- Is the ester group of acetyl-beta-methylcholine acid stable? Why/why not?
- Is acetyl-beta-methylcholine orally effective? Why/why not?
- Is this a therapeutically useful compound? If so, what is its use?
- Why should methacholine be avoided in patients with asthma?

MC Objective: How does methyl substitution on the alpha-carbon of the ethylene side chain of ACh influence “agonist” activity?

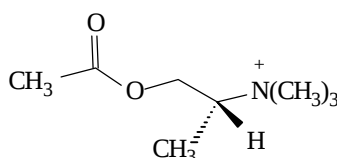


Acetyl-alpha-methylcholine

- What influence does alpha-methyl substitution have on cholinergic receptor selectivity? $N > M$ (ACh: $M = N$)
- Alpha-methyl substitution introduces a center of chirality. Do muscarinic receptors display selectivity for one enantiomer of acetyl-beta-methylcholine: No: $S = R$



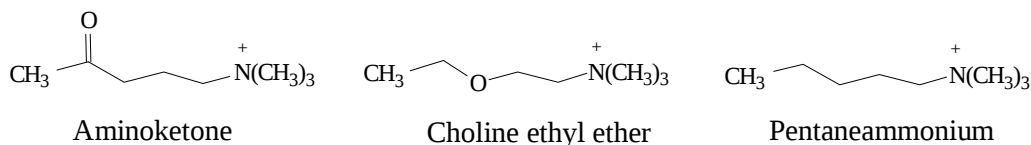
(S)-Acetyl-alpha-methylcholine



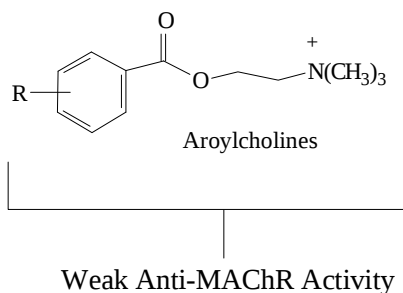
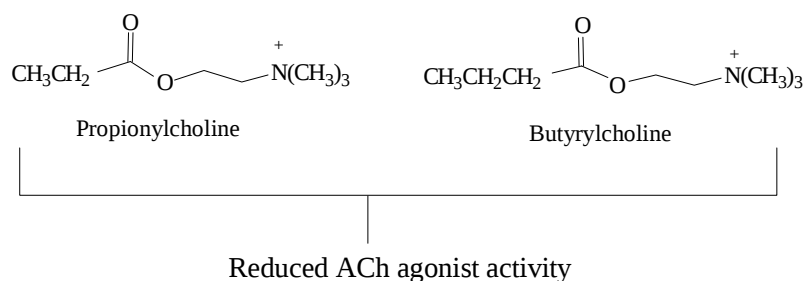
(R)-Acetyl-alpha-methylcholine

- How does acetyl-alpha-methylcholine compare to ACh in terms of NACHR agonist activity: $ACh > \text{acetyl-alpha-methylcholine}$
- How does acetyl-alpha-methylcholine compare to ACh in terms of rate of hydrolysis by AChE? Rate of hydrolysis: $ACh > S\text{-acetyl-alpha-methylcholine} = R\text{-acetyl-alpha-methylcholine}$. Explain the observation that acetyl-alpha-methylcholine is hydrolyzed more slowly than ACh by AChE
- Which compound would be hydrolyzed more readily by AChE? Acetyl-alpha-methylcholine or Acetyl-beta-methylcholine? Why?
- Is the ester group of acetyl-alpha-methylcholine acid stable? Why/why not?
- Is acetyl-alpha-methylcholine orally active? Why/why not?
- Is this a therapeutically useful compound? If so, what is its use?

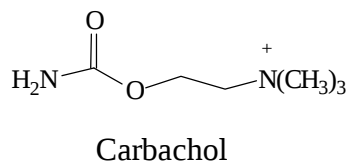
MC Objective: How does modification of the acetoxy group of ACh influence “agonist” activity? Why do these derivatives have reduced agonist activity?



MC Objective: How does alkyl or aryl substitution on the acetyl methyl carbon of ACh influence “agonist” activity?

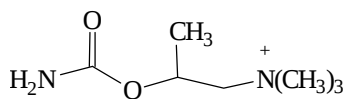


MC Objective: How does amino substitution for the acetyl methyl group of ACh influence “agonist” activity? This substitution results in carbamate formation as in carbachol (Doryl⁰, IsoptoCarbachol⁰, Miostat⁰)



- Carbachol shows no cholinergic receptor selectivity ($M=N$). Why?
- Carbachol is much less susceptible to AChE-mediated hydrolysis and, in fact, functions as an AChE inhibitor. Why?
- Carbachol is significantly more acid-stable than ACh. Why?
- Is carbachol orally active or not? Why?
- Is this a therapeutically useful compound? If so, what is its use?
- Explain why carbachol has a substantially longer duration of action than ACh?

MC Objective: What effect does *BOTH* amino substitution for the acetyl methyl group of ACh (carbamate formation) AND beta-methyl substitution have on agonist activity (as in Bethanechol, Urecholine[®])?

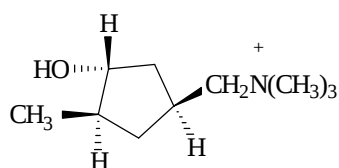


Bethanechol

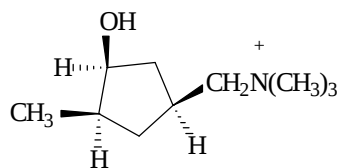
- This compound displays cholinergic receptor selectivity ($M \gg N$) comparable to acetyl-beta-methylcholine. Why?
- Bethanechol is chiral and MACH receptors display stereoselectivity with $S > R$. How does this compare to acetyl-beta-methylcholine?
- Bethanechol is much less susceptible to AChE-mediated hydrolysis and, in fact, functions as an AChE inhibitor. Why?
- Bethanechol is significantly more acid-stable than ACh. Why?
- Is bethanechol orally active? Why/why not?
- Is this a therapeutically useful compound? If so, what is its use?
- Explain why carbachol has a substantially longer duration of action than ACh?

MC Objective: The natural product muscarine is an experimental agent. Used originally in the subclassification of cholinergic receptors (“muscarinic receptors”).

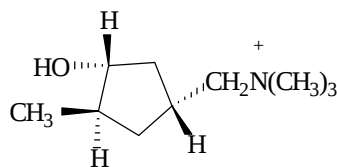
- What is the natural source of muscarine?
- How many stereoisomeric forms of muscarinic could exist?
- Which stereoisomeric form(s) is/are active muscarinic ligands?
- Which muscarine functional groups are important for MACHR binding? How does muscarine bind to MACH relative to ACh?
- Why is muscarine not used as a therapeutic agent?



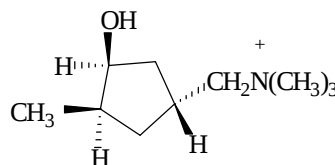
Muscarine



Epimuscarine



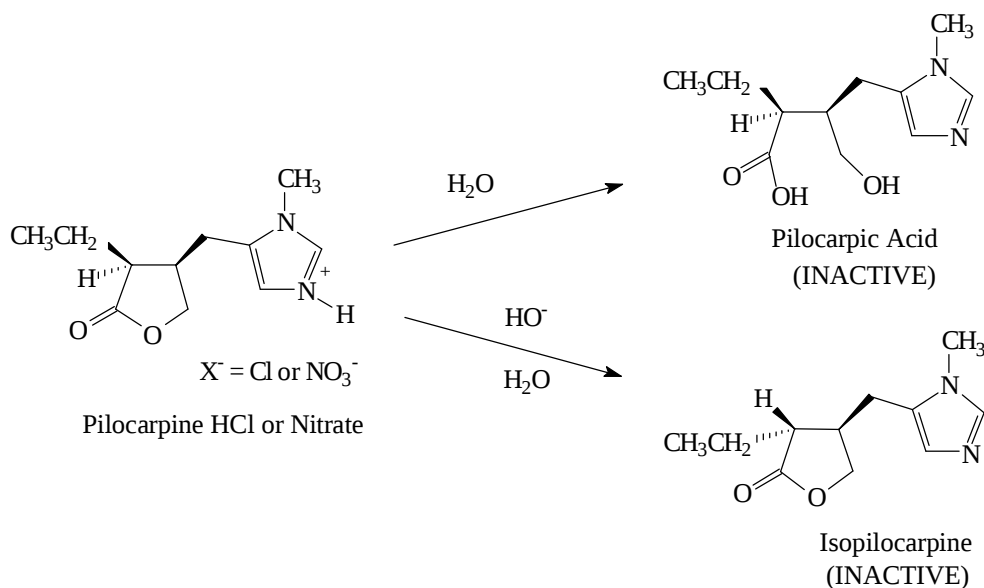
Allomuscarine



Epiallomuscarine

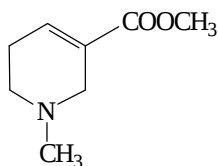
MC/PC Objectives: The natural product pilocarpine (Isoptocarpine, Ocusert, Pilo, Pilocar) functions as a muscarinic receptor agonist (MACHR).

- How many stereoisomeric forms of pilocarpine could exist?
- Which stereoisomeric form(s) is/are active muscarinic ligands?
- Which pilocarpine functional groups are important for MACHR binding? How does pilocarpine bind to MACH relative to ACh?
- Why are the primary therapeutic indications for pilocarpine glaucoma, miosis induction, xerophthalmia and xerostomia?
- What types of pilocarpine dosage forms are available? Is it administered orally for any indication?
- Why does pilocarpine have a slower onset but longer duration of action than ACh?
- Why does pilocarpine interact with drugs such as tricyclic antidepressants, antihistamines and antimuscarinics?
- Why should pilocarpine used with caution in patients with cardiac disease, asthma or biliary tract disease?
- Pilocarpine's lactone (cyclic ester) is susceptible to hydrolysis in acid or base. What is the significance of this reaction?
- Pilocarpine may undergo "epimerization" in basic media. What does "epimerization" mean, and why does this reaction occur hydrolysis with pilocarpine? What is the significance of this reaction?

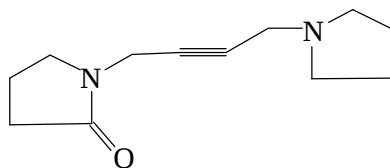


MC Objective: Arecoline and oxotremorine are experimental agents that function as MACHR agonists.

- Which functional groups present in these compounds are important for MACHR binding? How do these compounds bind to MACH relative to ACh?
- Why are these compounds not used therapeutically?

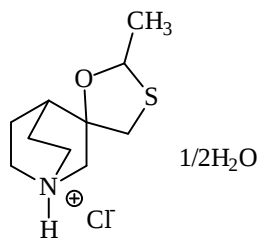


Arecoline



Oxotremorine

MC/PC Objective: In 2000 the drug Cevimeline (Evoxac[®]) was approved for the treatment of Sjorgren's syndrome



Cevimeline HCl (EvoxacTM)

- What is Sjorgren's syndrome and why is a muscarinic agonist useful in the treatment of this disease? Which MACHR receptor subtype is important for therapeutic activity?
- Which cevimeline functional groups are important for MACHR binding? How does cevimeline bind to MACH relative to ACh?
- Clinical pharmacology states that "cevimeline is a partial M1 agonist in the CNS". What does this statement mean?
- Based on its MACHR binding profile, generally what side effects might be expected in patients using cevimeline?
- Explain why cevimeline should be used with caution in patients with cardiovascular disease (angina, MI, arrhythmias, etc), asthma, and a hhistory of biliary stones or nephrolithiasis?
- Why should cevimeline used with caution in patients who drive at night or perform hazardous activities in poorly lit environments?

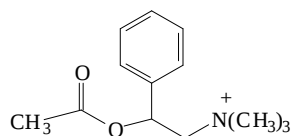
- *Is cevimeline orally effective?*
- *Cevimeline is metabolized by CYP 2D6 and CYP 3A4? What does this mean and which metabolites form?*
- *Why should cevimeline be used with caution in patients receiving amiodarone, fluoxetine, paroxetine, diltiazem, erythromycin or ketoconazole?*
- *Why do some tricyclic antidepressants and phenothiazine antipsychotics antagonize the pharmacologic actions of cevimeline?*
- *What advantages might cevimeline offer over ACh or other MACHR agonists such as pilocarpine?*

REMEDIAL MATERIAL FOR THIS SECTION:

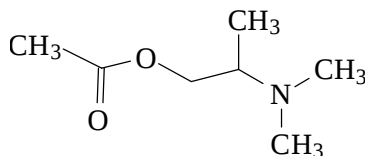
Ester Chemistry Tutorial at <http://www.duc.auburn.edu/~deruija/pdamcli2.html>
Stereochemistry Tutorials at <http://www.duc.auburn.edu/~deruija/pdamcli2.html>

ADDITIONAL QUESTIONS AND PROBLEMS

1. The compound below is resistant to hydrolysis by AChE, but is ineffective as a muscarinic agonist. Using the structure, explain both of these observations.



2. What are the two properties that define a compound as an agonist? Hint: what parameters can you determine from a dose-response profile for a compound that is capable of functioning as an agonist.
3. How are these two properties in question 2 above related?
4. Are the same structural features of an agonist necessarily linked to both properties?
5. Draw the structure of the enantiomer of the compound below that would have highest affinity for muscarinic receptors (Be sure your drawing clearly shows the configuration!). Would this compound have higher, lower or similar muscarinic receptor affinity compared to acetylcholine? Why?



6. Why does pilocarpine epimerize in basic media (what is the chemical mechanism)? Why is the epimeric form inactive as a muscarinic agonist?
7. Draw a 3-D structure that shows how arecoline is capable of binding to muscarinic receptors like acetylcholine (superimpose the structures).