MEDCHEM 562

Drug Targets: Lecture 3; Kent Kunze

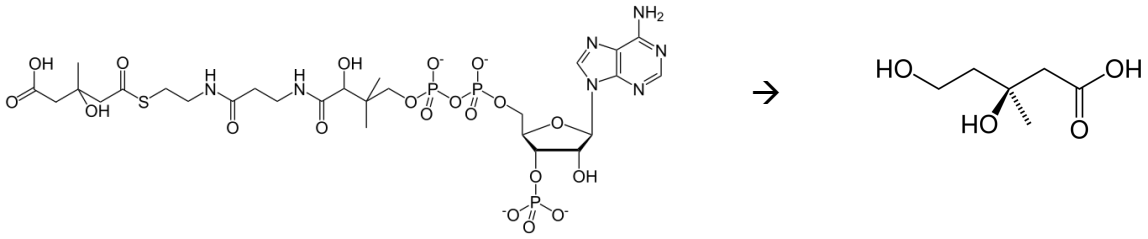
I. Enzymes as Targets:

- Many drugs are inhibitors of enzymes that catalyze biologically important reactions.
- Generally these inhibitors are struturally similar to the endogenous substrate however in some cases only a portion of the substrate structure is found in the inhibitor.
- Most inhibitors are competitive inhibitors which bind reversibly to the enzyme and compete with the natural substrate. The goal of drug design is to increase affinity (reduce IC_{50}) for the enzyme.
- Other inhibitors react covalently with a critical amino acid residue and inhibit the enzyme irreversibly. Here the reversible affinity is less important as the substrate cannot be processed by the inhibited enzyme.

1. Reversible Inhibitors: Statins and HMG CoA Reductase

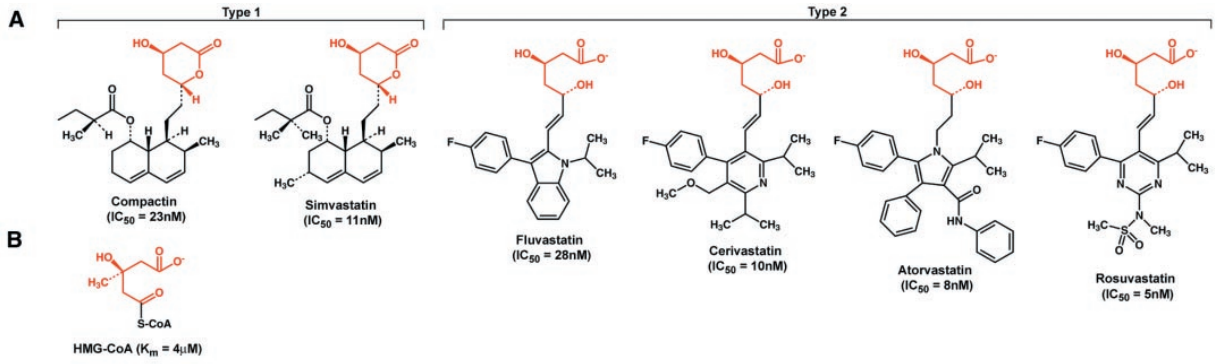
The reaction catalyzed by the enzyme is the production of mevalonic acid from HMG CoA: This is the rate-limiting step in the production of cholesterol. Inhibition of this enzyme by the statins decreases LDL (bad cholesterol) and increases (HDL)

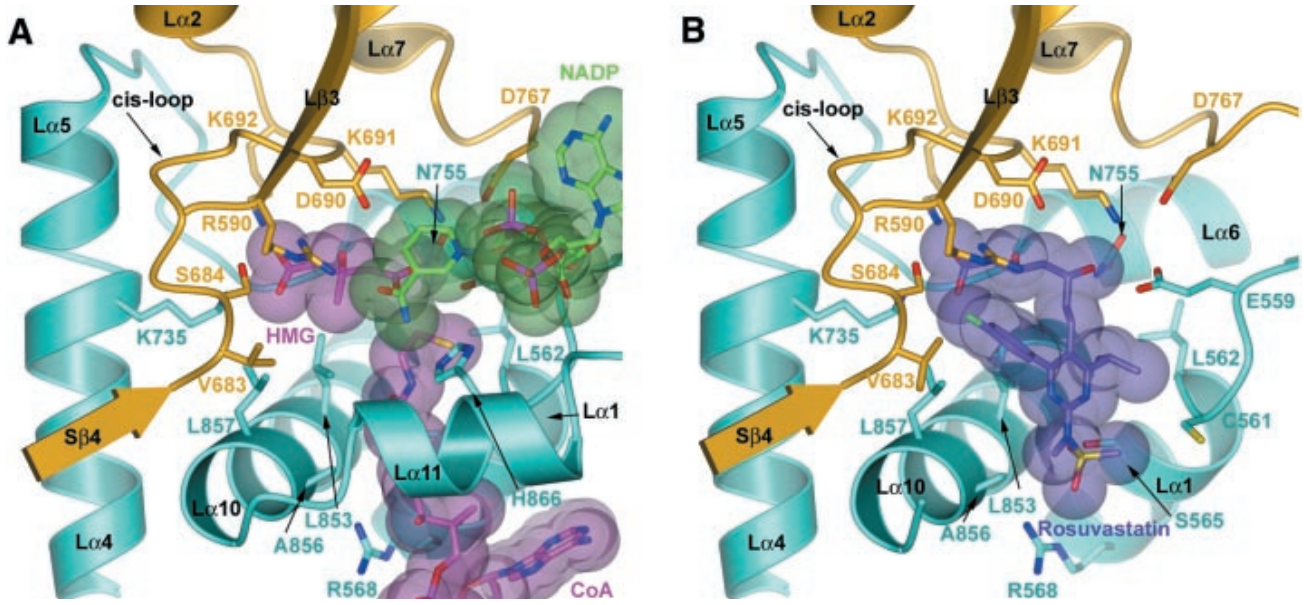
NADPH + HMG CoA ->-> Mevalonate + CoASH -> -> -> Cholesterol



HMG-CoA Mevalonic Acid

The statin drugs are tight binding (IC_{50}'s are low nM) competitive inhibitors of HMG CoA reductase. They bind in the active site to prevent the binding of HMG CoA. Note the statins only occupy a portion of the binding site for HMG CoA. The affinity of HMG CoA for the enzyme (Km = 4 μM) is much poorer than the inhibitors.





<u>The structures of some of the common statins are similar to each other.</u>

-The CoA portion of HMG CoA lies outside of the binding area for the statins.
-The statins have a rigid core.
-Successful drugs of the Type 2 class contain the para-fluoro phenyl group which associates with an arginine side chain.
-The ring open form binds to the enzyme (see simvastatin which must undergo hydrolysis to be active).
-Note that the inhibitors do not have a methyl group in the 3 position.

2. <u>Irreversible Inhibitors: Acetylcholine Esterase and Sarin</u>

Acetylcholine is a neurotransmitter that is released from presynaptic neurons and binds to postsynaptic receptors opening a chloride channel.

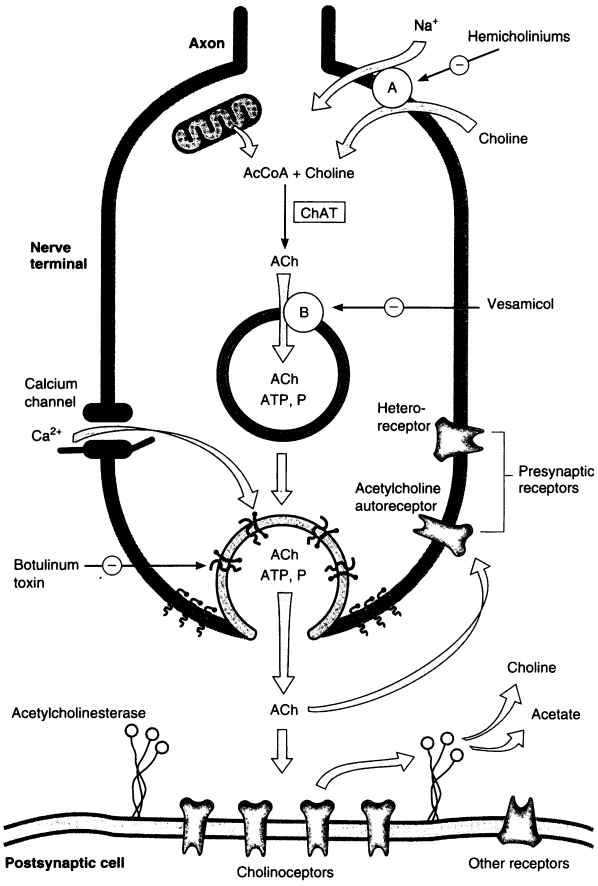
-There are two major types of receptors referred to as nicotinic and muscarinic.

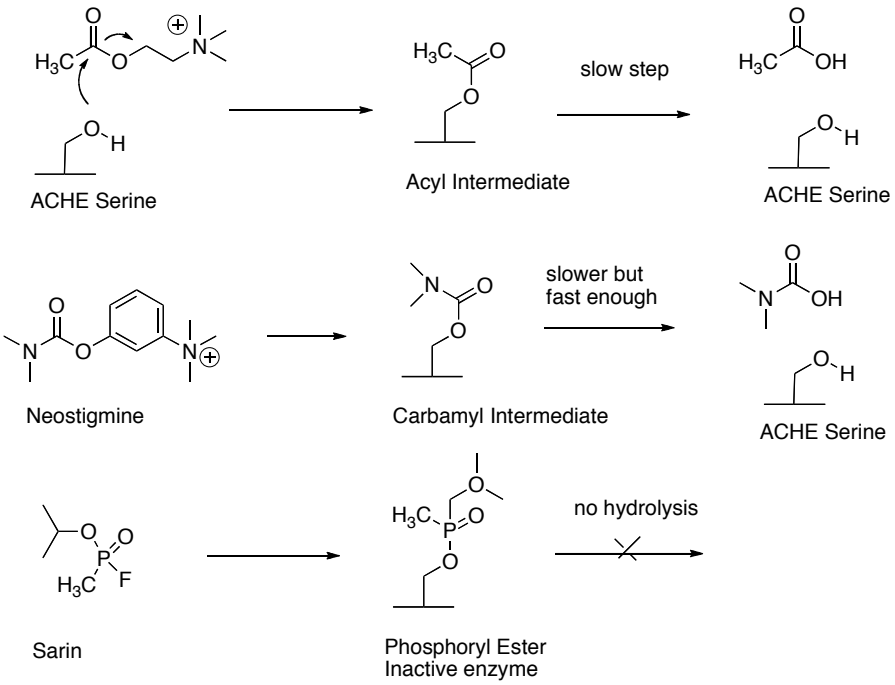
-Cholinergic nerve transmission is modified by a wide range of drugs and other agents that bind to receptors, enzymes and transporters. "<u>*Cholinergic" side effects of drugs are very common.*</u>

-<u>Importantly the action of acetylcholine is terminated by an esterase located on the post-synaptic membrance (ACHE). Many drugs, insecticides and nerve gases inhibit the enzyme which increases the acetylcholineric effect.</u>

Neostigmine is a reversible inhibitor of ACHE and is also a substrate

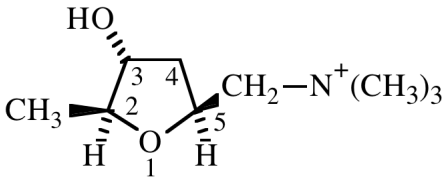
Sarin is an irreversible inhibitor which irreversibly phosphorylates the active site serine.



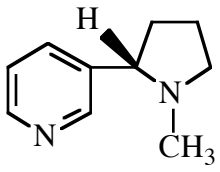


II Extracellular Receptors as Targets

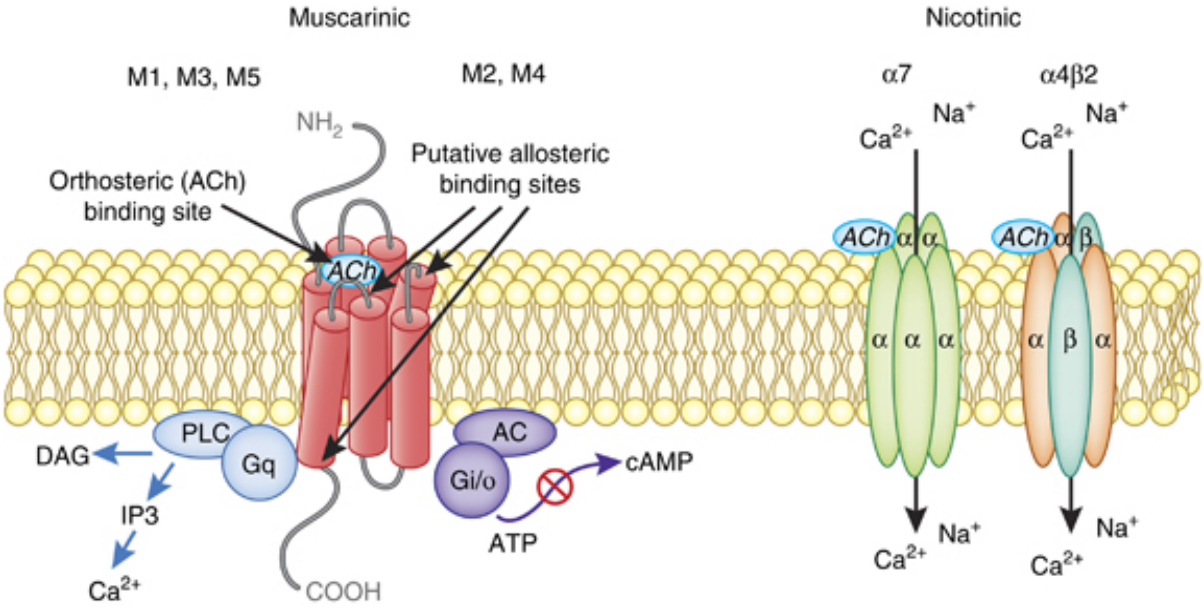
Acetylcholine binds to cholinergic receptors. There are two major types of cholinergic receptors which are classified as nicotinic (nAChR) or muscarinic (mAChR) due to the ability of these two compounds to act as selective agonists for the receptor in question. The two major classes have a number of subclasses…. so it is complicated. We note right away that muscarine has a quaternary nitrogen (always positively charged just like acetylcholine) so we wouldn't expect it to cross membranes particularly well. Nicotine has two basic ionizable groups (pyridine pKa around 5) and an alkylamine (pKa around 9). Both compounds have stereochemistry.



Muscarine



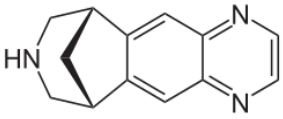
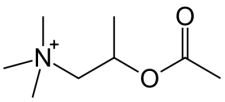
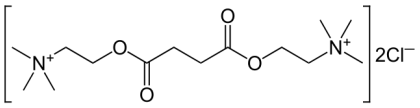
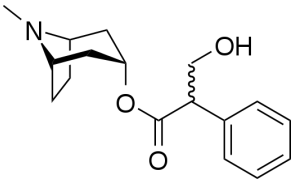
(-)-S-Nicotine



Note that there are 5 types of muscarinic receptors that are all G-coupled receptors. M1, M3 and M5 control intracellular free calcium concentrations via the phosphoinisitol pathway while binding to M2 and M4 inhibits the formation of the second messenger of the adrenergic system cAMP.

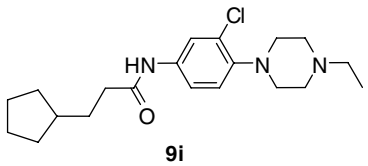
Drugs that inhibit acetylcholine esterase cause agonist like effects however receptor selectivity will only be imparted by distribution of the inhibitors (e.g. the blood brain barrier).

We have developed drugs that are selective agonists and antagonist of the acetylcholine receptors.

	Receptor Type	
Type of Action	Nicotinic (nAChR) Na/Ca channel Multiple subtypes	Muscarinic (mAChR) G-coupled receptor Five Subtypes
Agonist	 Chantix (α4β2 subtype Smoking cessation Issues with bizarre behavior	 Methanocholine Diagnostic for bronchial hypersensitivity in asthma (S) enantiomer more potent
Antagonist	 Succinyl Choline Depolarizing neuromuscular blocking agent	 Atropine Classic antagonist (S)-enantiomer 120 times more potent

Extensive structure activity relationship (SAR) have been carried out for antagonists and antagonists. One goal of these kinds of studies is to develop ligands that differentiate between the various types of receptors. Below we see the results of a small study that seeks to differentiate between the five mAChR receptor subtypes by developing selective antagonists. Results are shown for the most selective inhibitor in the displacement of a radioactive ligand. Overall selectivity for M1 is significantly improved over the other receptor subtypes and over atropine.

Table 2. K_i determinations and binding fold selectivity for **9i**



9i

mAChR	**9i** K_i[a] (nM)	Fold selectivity (vs M1)	Atropine K_i (nM)[a]
M1	12.7 ± 1.7		0.88 ± 0.04
M2	338.0 ± 13.5	27	2.69 ± 0.20
M3	74.8 ± 4.3	6	0.96 ± 0.03
M4	445.1 ± 23.8	35	0.56 ± 0.01
M5	85.7 ± 15.9	7	1.80 ± 0.11

[a] K_is are an average of three independent experiments using rat mAChR (CHO) cell lines.

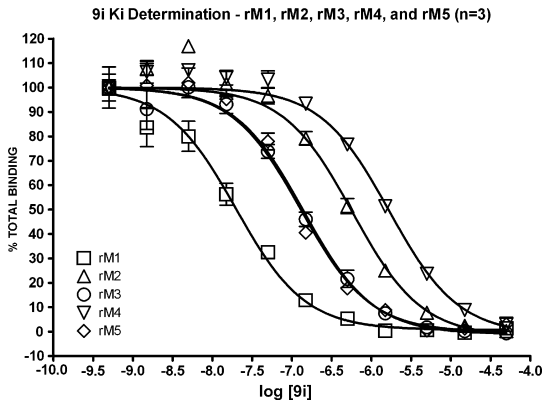
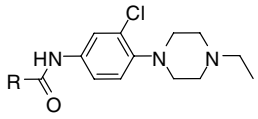


Figure 5. [^{3}H]-NMS competition binding experiments for **9i** on rat M1–M5. Compound **9i** displays 27-fold selectivity versus M2, 6-fold selectivity versus M3, 35-fold selectivity versus M4, and 7-fold selectivity versus M5. Curves represent the average of three separate experiments.

Note the other analogs that were tested against the receptor subtypes to arrive at 9i. In these cases size and lipophillicity were varied often with dramatic and suprising effect on binding affinity.



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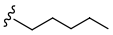
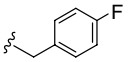
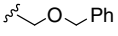
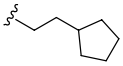
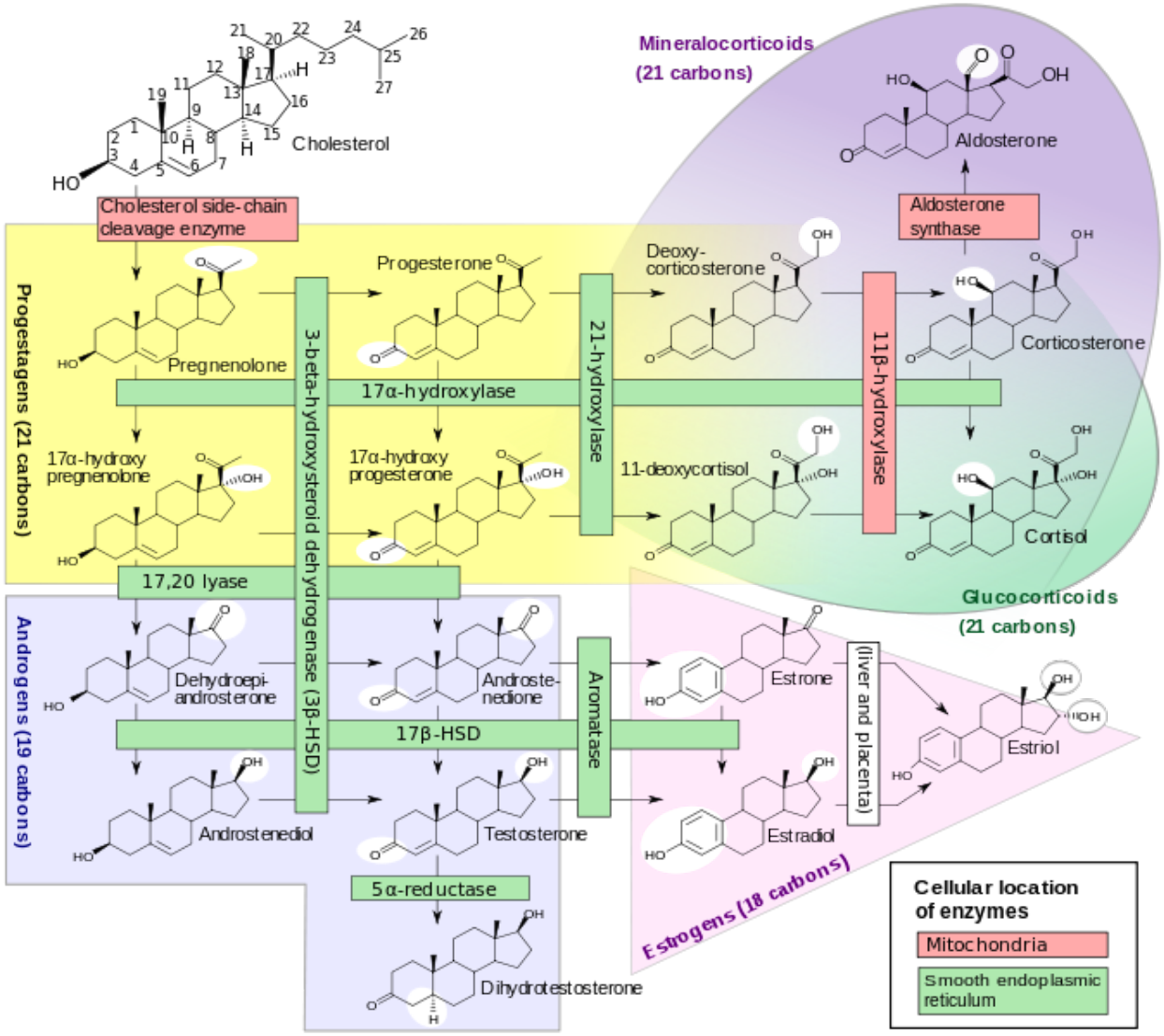
Compound	R	M1 IC_{50}[a] (μM)	M2 IC_{50}[a] (μM)	M3 IC_{50}[a] (μM)	M4 IC_{50}[a] (μM)	M5 IC_{50}[a] (μM)
5		13.2	>150	>150	>150	>150
9a		>150	>150	>150	>150	>150
9b		4.6	>150	>150	>150	>150
9c		5.0	>150	>150	66	>150
9d		5.6	>150	>150	>150	>150
9e		1.15	29	24	20	13
9f		1.1	52	70	18	7.6
9g		3.3	>150	>150	>150	>150
9h		18.8	>150	>150	>150	>150
9i		0.44	3.5	3.1	>150	1.1
9j		>150	>150	>150	>150	>150

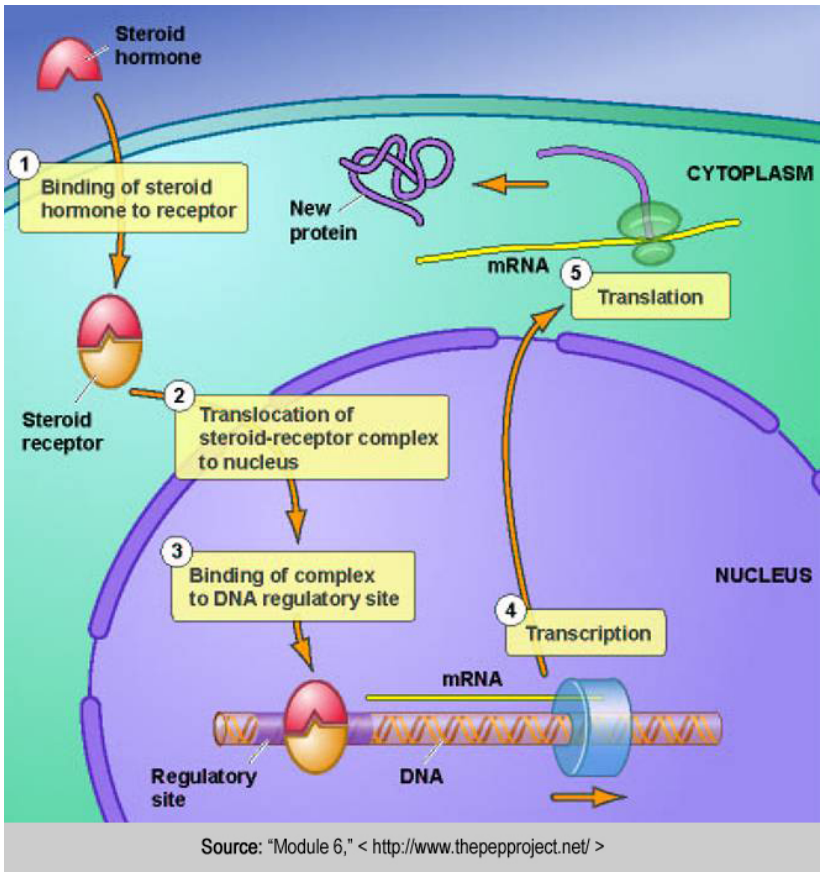
III. Intracellular Receptors as Targets (example Glucocorticoids)



Steroids readily enter cells and bind to receptors in the cell cytosol. They exert their effects by subsequent translocation of the receptor complexes into the nucleus and binding to regulatory regions of DNA called response elements (SRE, GRE, MRE). Generally, but not always, binding activates transcription and the product mRNA is translated to protein. However, binding to a response element can also inhibit transcription. For instance RU-486 is an antagonist of the progesterone response system (an SRE) and has no effect on the mineralcorticoid response system (GRE). Typically steroid antagonist-receptor complexes enter the nucleus, they are just not active.

Steroid type drugs may be either receptor agonists or antagonists.
Note the differences between the glucocorticoids, mineralocorticoids and the estrogen and androgens.

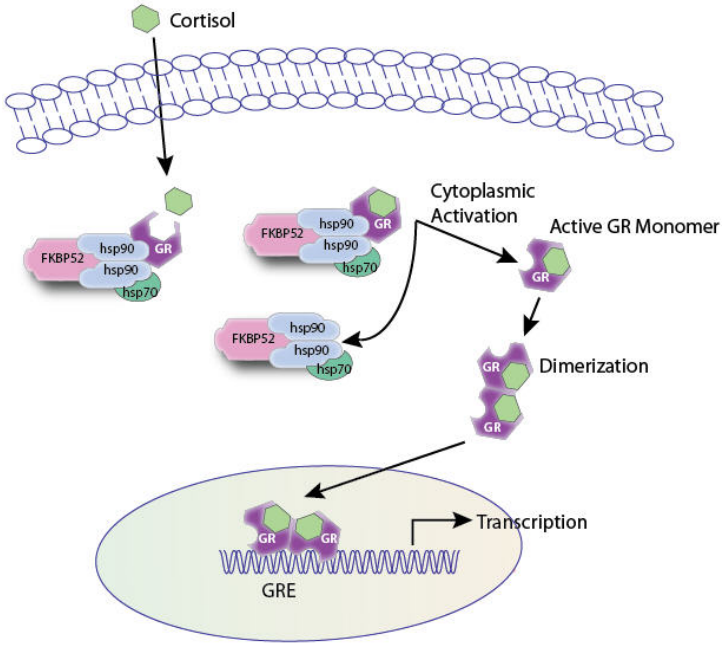
There are a number of different steroid receptors that control the transcription of different genes. Many steroid containing drugs bind to more than one receptor so it is complicated.

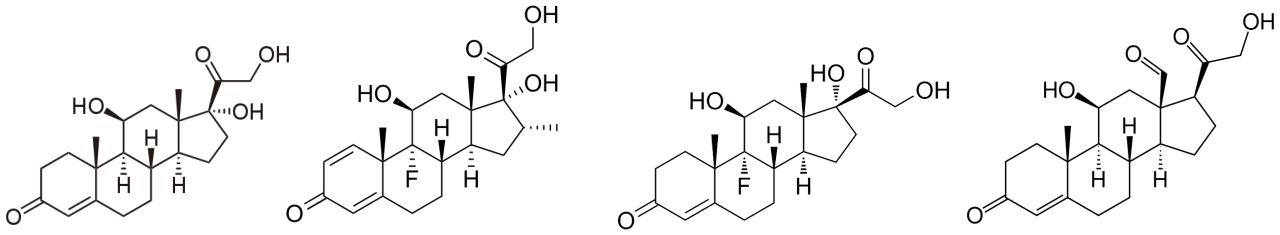


Source: "Module 6," < http://www.thepepproject.net/ >

Glucocorticoids, a major subclass of steroid hormones, modulate a large number of metabolic, cardiovascular, immune, and behavioral functions. The intracellular effects of glucocorticoids are mediated by the glucocorticoid receptor (GR), a 94-kDa intracellular protein belonging to the phylogenetically conserved nuclear hormone receptor superfamily. In the absence of glucocorticoid, the GR is maintained predominantly in the cytoplasm as part of an inactive multiprotein complex that consists of the receptor, two Hsp90 molecules, one molecule each of Hsp70 and Hsp56, and an immunophilin of the FK506- and rapamycin-binding class. When glucocorticoid binds to GR, the receptor undergoes a change in conformation, dissociates from regulatory heat shock proteins, and is hyperphosphorylated. The activated receptor rapidly translocates to the cell nucleus, where it is able to initiate transcriptional regulation.

In the nucleus, hormone-activated GR regulates transcription. During transcriptional regulation, the GR binds as a homodimer directly to short, palindromically arranged DNA sequences located in the promoter regions of glucocorticoid-responsive genes. *Binding to these sequences, known as glucocorticoid response elements, leads to transcriptional induction or repression of target genes.* (Affymetrix web site: http://www.panomics.com/index.php?id=product_94)





Cortisol *GRE* glucocorticoid

Dexamethasone *GRE* (anti-inflammatory)

Fludrocortisone *MRE* (mineralocorticoid)

Aldosterone *MRE* (mineralocorticoid)

Mineralocorticoids bind to a different receptor in the kidney called the mineralcorticoid response element (MRE) to upregulate the expression of the basolateral Na/K pump. The net agonist effect of an MRE agonist is conservation of sodium, secretion of potassium, increased water retention, and increased blood pressure. Generally drugs are designed to be either mineralcorticoid-like or glucocorticoid-like.