Androgens

- **<u>Role in males similar to the of estrogens in females</u>**
  - development of male sexual characteristics
  - stimulating protein synthesis, growth of bones
  - cell differenciation, spermatogenesis
  - male type of behaviour

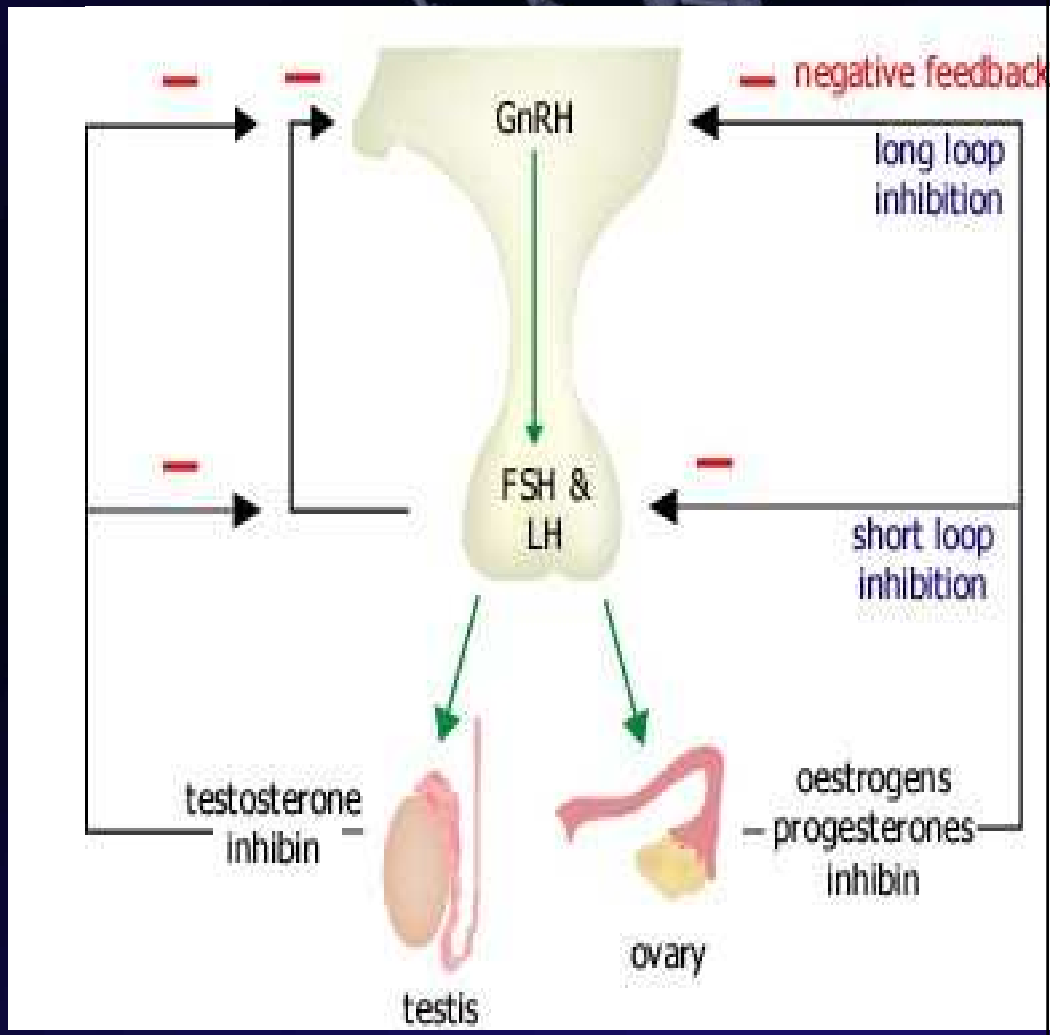
Androgens

- Endogenous ligands – androgen hormones
- testosterone
- dihydrotestosterone (DHT)
- androstanediol
- dehydroepiandrosterone
- androstenedione

Testosterone

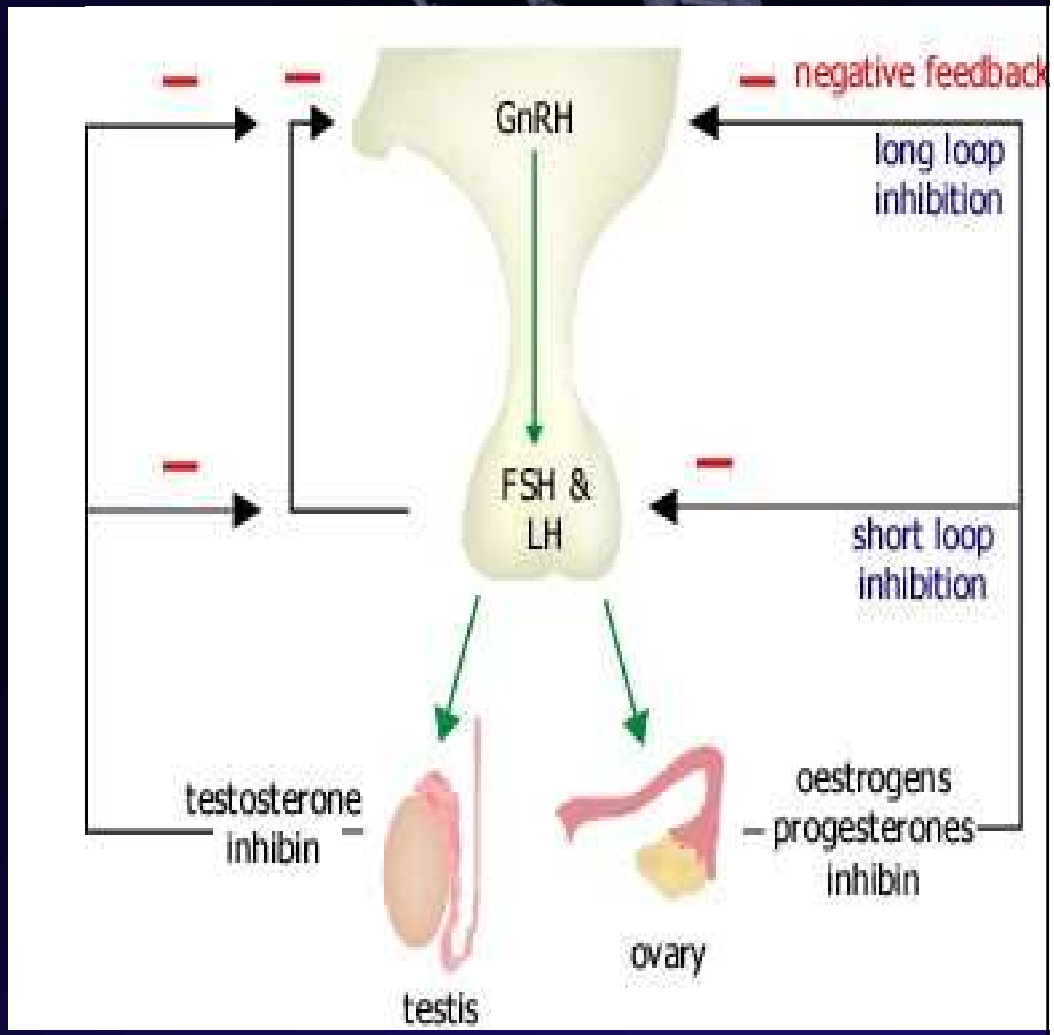
Hypothalamo-pituitary axis

- Regulation of testosterone synthesis
- Hypothalamus – Gonadotropin releasing hormone
- Pituitary – folicle stimulating and luteineising hormone

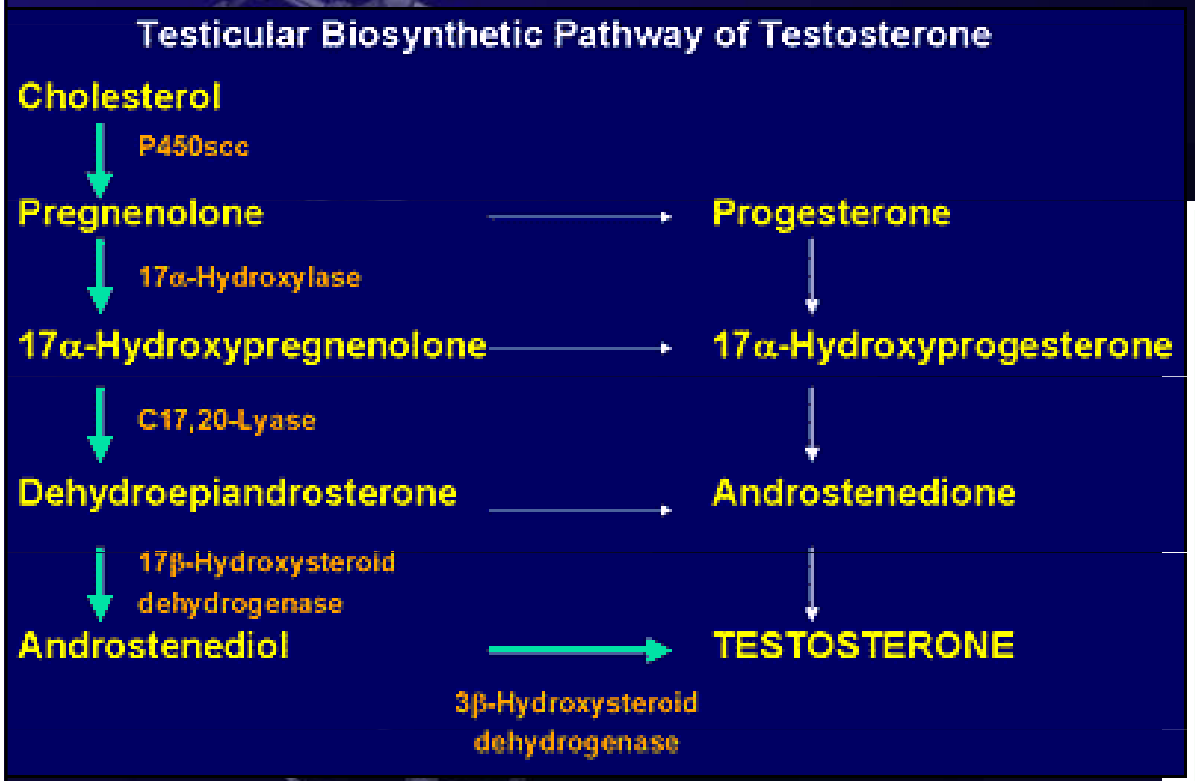


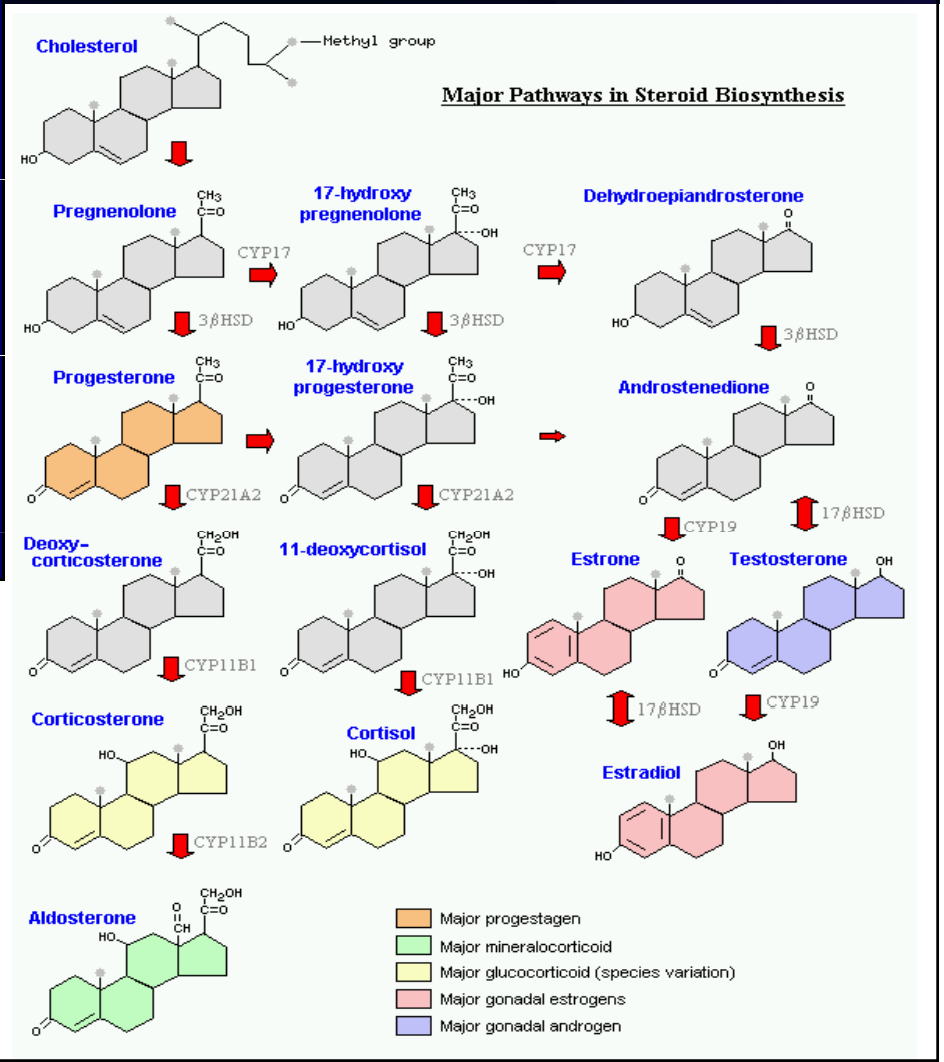
Hypothalamo-pituitary axis

- Folicle stimulating hormone
  - Stimulates synthesis of androgen binding proteins and spermatogenesis in Sertoli cells (testis)
- Luteineizing hormone
  - Stimulates testosterone production in Leydig cells



Testosterone

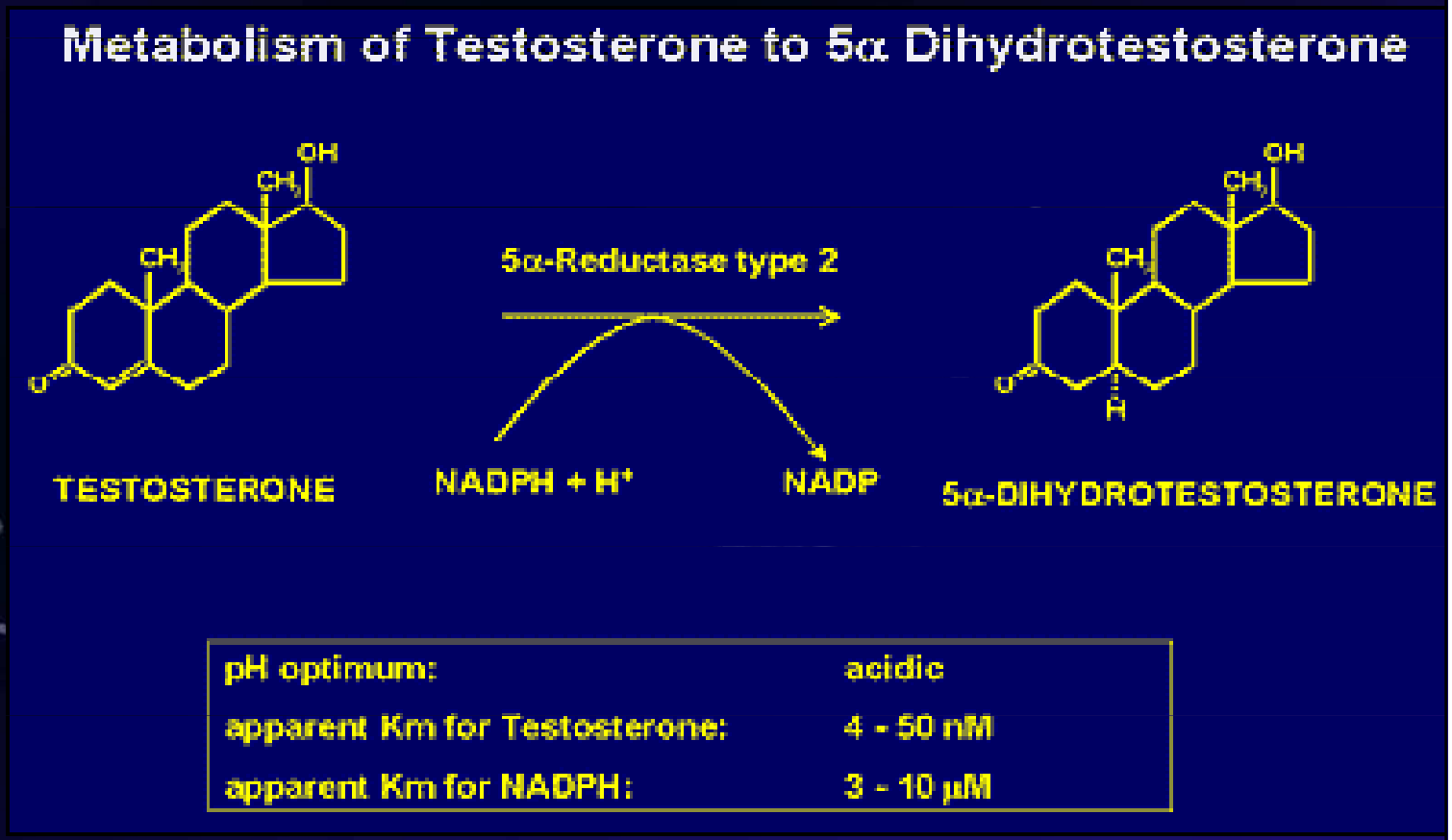




- synthetized in testis (Leydig cells)
- in lesser extent in adrenals

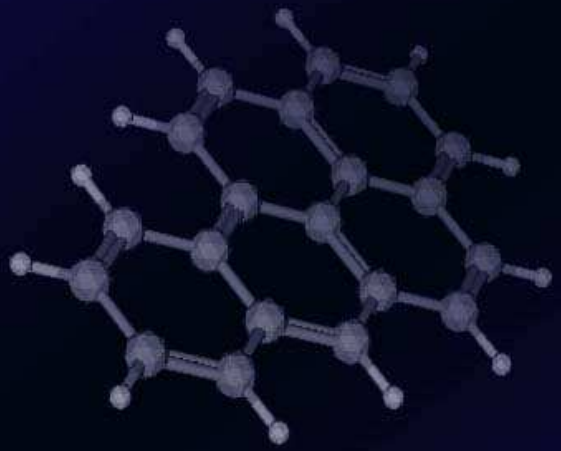
Dihydrotestosterone

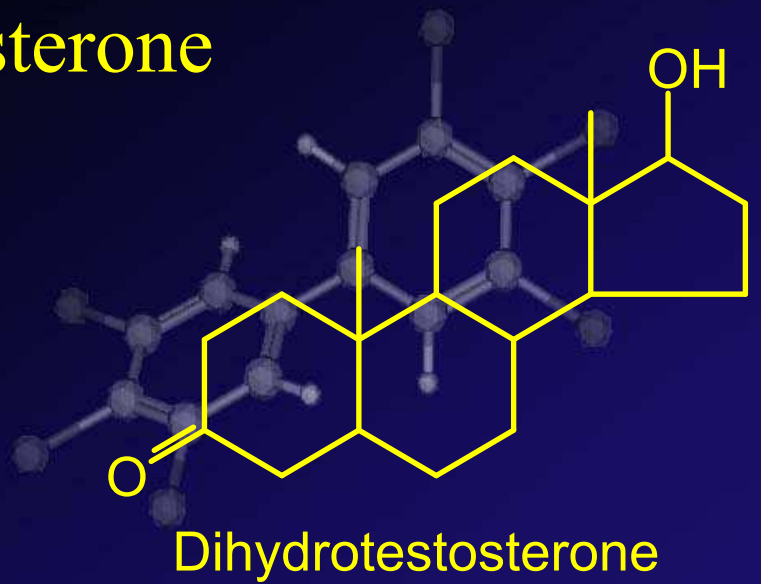
- The most important derivative of testosterone
- Formed **extratesticulary** from testosterone
- 5α-reductase



Dihydrotestosterone

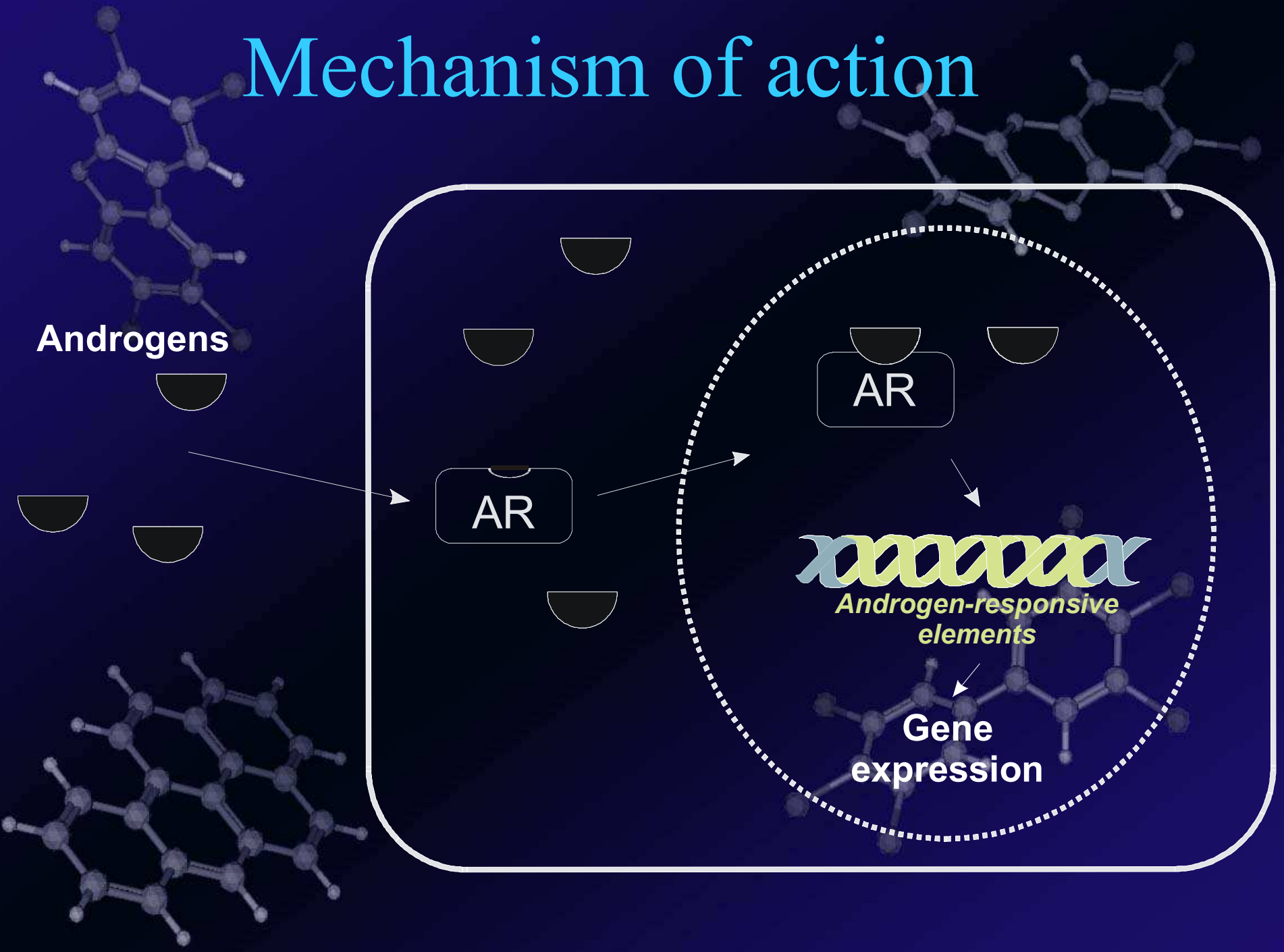
- In several tissues (seminal vesicles, prostate, skin) higher affinity to androgen receptor than testosterone
- Daily production 5-10% of testosterone





Dihydrotestosterone

Mechanism of action



Mechanisms of **androgen signalling disruption**

- Illegitimate activation of AR
- Binding to AR without activation
- Decrease of AR cellular levels
- FSH/LH signalling disruption
- Changes in androgen metabolism

Mechanisms of androgen signalling disruption

Binding to AR

- Mostly competitive inhibition – xenobiotics do mostly NOT activate AR-dependent transcription

- Few compounds are able to activate AR in absence of androgen hormones x in presence of T/DHT antiandrogenic (**metabolites of funghicide vinclozoline**, some PAHs)

Mechanisms of androgen signalling disruption

Decrease of AR levels

- Under normal circumstances, DHT treatment leads to increase of AR level

BUT no effect observed during co-treatment with Cyproterone acetate or hydroxyflutamide (drugs/pesticides)

Mechanisms of androgen signalling disruption

FSH/LH (gonadotropins) signalling disruption

- FSH/LH expression - regulation via negative feedback by testosterone
- Suppressing leads to alterations of spermatogenesis

Mechanisms of androgen signalling disruption

Alterations of testosterone synthesis

- Inhibition of P450scc needed for side chain cleavage of cholesterol (fungicide **ketoconazol**)
- Inhibition of 17- α-hydroxylase and other CYPs - – enzymes needed for testosterone synthesis (**ketoconazol**)

Mechanisms of androgen signalling disruption

Testosterone metabolic clearance

- Induction of UDP-glucuronosyltransferase or monooxygenases CYP1A, 1B involved in androgen catabolism

- Pesticides **Endosulfan, Mirex, o-p´-DDT**

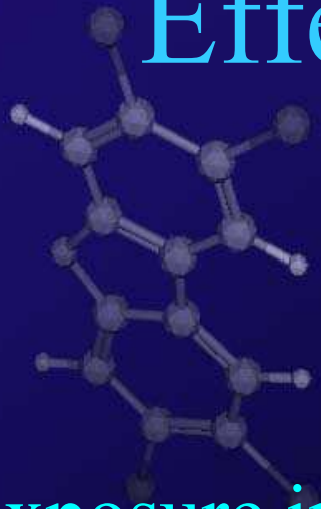
Effects of male exposure to antiandrogens

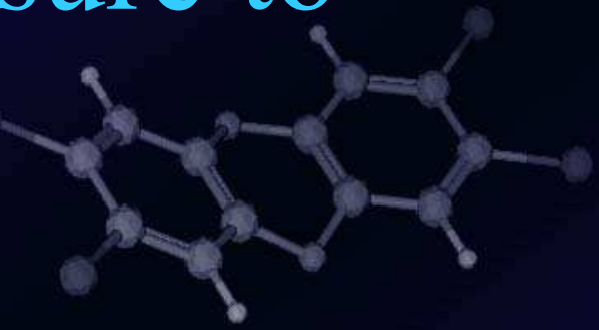
Exposure **during prenatal** development:

-malformations of the reproductive tract

- reduced anogenital distance
- hypospadias *(abnormal position of the urethral opening on the penis)*
- vagina development
- undescendent ectopic testes
- atrophy of seminal vesicles and prostate gland

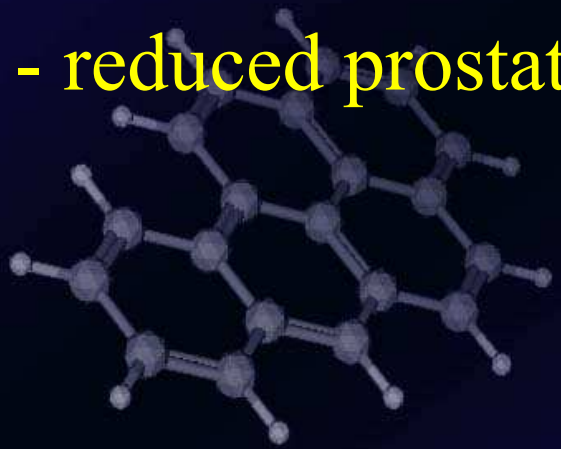
Effects of male exposure to antiandrogens





Exposure in prepubertal age:

- delayed puberty
- reduced seminal vesicles
- reduced prostate



Exposure in adult age:

- oligospermia
- azoospermia
- libido diminution

AR-binding - potencies

(Ref: DHT EC50 ~ 0.1 uM)

Compound	IC_{50} (µM)
Benz[a]anthracene	3,2
Benzo[a]pyrene	3,9
Dimethylbenz[a]anthracene	10,4
Chrysene	10,3
Dibenzo[a,h]anthracene	activation in range 0,1-10µM
Bisphenol A	5
vinclozolin metabolites	9,7
hydroxyflutamide	5
Aroclor typical values	0,25-1,11
Individual PCBs typical values	64 - 87
tris-(4-chlorophenyl)-methanol	0,2

Antiandrogenic compounds

tris-(4-chlorophenyl)-methanol

- Ubiquitous contaminant of uncertain origin
- Probable metabolite of DDT-mixture contaminant
- Levels in human blood serum cca. 50nM
- EC50 – cca. 200nM

In vivo antiandrogenicity assessment

Hershberger assay

- castrated rats treated with substance examined

- Endpoint – after 4-7 days – seminal vesicles and ventral prostate weight

Measurement of testosterone concentration in serum

In vitro
antiandrogenicity assessment

Most often employed – prostatic cell lines

Cell proliferation assays – cell lines with androgen-dependent growth;

- Treatment with tested chemical only (androgenicity) or cotreatment with DHT (antiandrogenicity)

  - mammary carcinoma cell lines

  - prostatic carcinoma cell lines

In vitro antiandrogenicity assessment

Receptor-reporter assays

- Gene for luciferase or GFP synthesis under transcriptional control of AR

- Luciferase:

- AR-Calux (human breast carcinoma T47D)

- PALM (human prostatic carcinoma PC-3)

- CHO515 (Chinese hamster ovary CHO)

AR-binding - potencies

(Ref: DHT EC50 ~ 0.1 uM = 100nM)

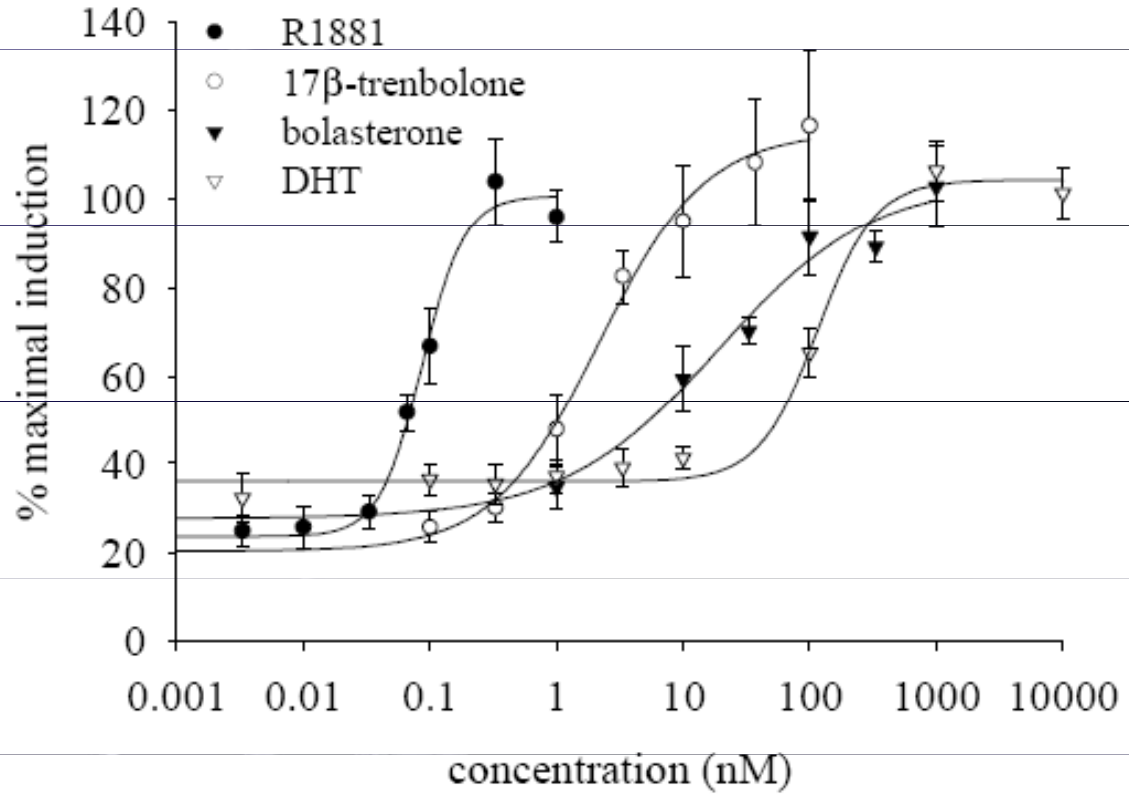


Figure 2 Luciferase induction in AR-LUX cells by various known androgens relative to the calculated maximum of R1881 (n=3, avg. +/- SD). Cells were dosed with the compounds for 24 hr. EC_{50} R1881: 86.40 pM; EC_{50} 17β-trenbolone: 2.18 nM; EC_{50} bolasterone: 18.88 nM; EC_{50} DHT: 115 nM.

In vitro antiandrogenicity assessment

GFP

- Possibility of nondestructive measurement (fluorescence of intact cells)

X

Less sensitive – lack of enzymatic amplification

- Human prostatic cell lines

In vitro antiandrogenicity assessment

Yeast assays

- Mostly **β-galactosidase** as reporter enzyme

- Easy cultivation and experimental design

X

- Cell wall may obstruct transport of chemical into cell=>

=> false negatives