

Androgens

- Role in males similar to the of estrogens in females

- development of male sexual characteristics
- stimulating protein synthesis, growth of bones
- cell differentiation, spermatogenesis
- male type of behaviour

Androgens

- Endogenous ligands – androgen hormones

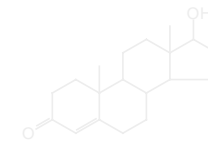
- testosterone

- dihydrotestosterone (DHT)

- androstanediol

- dehydroepiandrosterone

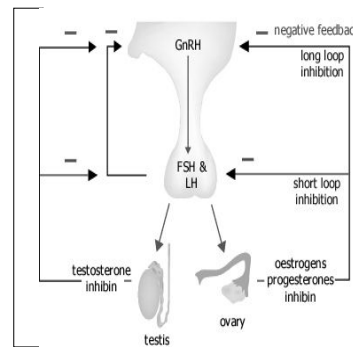
- androstenedione



Testosterone

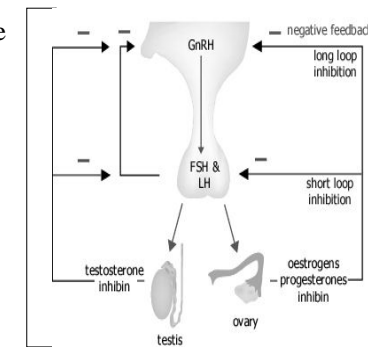
Hypothalamo-pituitary axis

- Regulation of testosterone synthesis
- Hypothalamus – Gonadotropin releasing hormone
- Pituitary – follicle stimulating and luteinising hormone



Hypothalamo-pituitary axis

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



Testosterone

Testicular Biosynthetic Pathway of Testosterone

Cholesterol
 ↓ P450sc
 Pregnenolone
 ↓ 17α-Hydroxylase
 17α-Hydroxypregnenolone
 ↓ C17,20-Lyase
 Dehydroepiandrosterone
 ↓ 17β-Hydroxysteroid dehydrogenase
 Androstenediol
 ↓ 3β-Hydroxysteroid dehydrogenase
TESTOSTERONE

Progesterone
 ↓ 17α-Hydroxylase
 17α-Hydroxyprogesterone
 ↓ 17β-Hydroxysteroid dehydrogenase
 Androstenedione
 ↓ 3β-Hydroxysteroid dehydrogenase
TESTOSTERONE

Major Pathways in Steroid Biosynthesis

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

Dihydrotestosterone

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

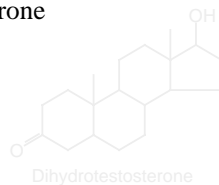
Metabolism of Testosterone to 5α-Dihydrotestosterone

TESTOSTERONE + NADPH + H⁺ → 5α-DIHYDROTESTOSTERONE + NADP

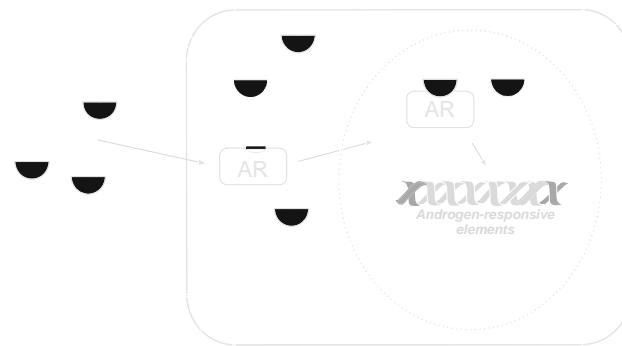
pH optimum:	acidic
apparent Km for Testosterone:	4 - 50 nM
apparent Km for NADPH:	3 - 10 μM

Dihydrotestosterone

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



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 fungicide 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 P450_{scc} 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 μ M)

Compound	IC ₅₀ (μ M)
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 0,1-10 μ M
Bisphenol A	5
vinclozolin metabolites	9,7
hydroxyflutamide	5
Aroclor typical values	0,25-1,11
Individual PCBs typical values	64 - 87
<i>tris</i> -(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)

In vitro antiandrogenicity assessment

GFP

- Possibility of nondestructive measurement (fluorescence of intact cells)

X

Less sensitive – lack of enzymatic amplification

- Human prostatic cell lines

AR-binding - potencies

(Ref: DHT EC50 ~ 0.1 μ M = 100nM)

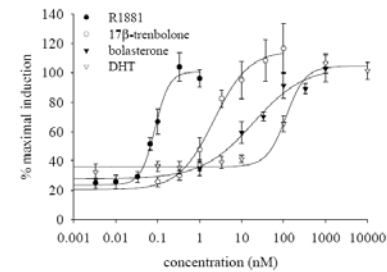


Figure 2 Luciferase induction in AR-LUX cells by various known androgens relative to the calculated maximum of R1881 (n=3, avg. +/- SD). Cells were dosed with the compounds for 24 hr. EC₅₀ R1881: 86.40 pM; EC₅₀ 17β-trenbolone: 2.18 nM; EC₅₀ bolasterone: 18.88 nM; EC₅₀ DHT: 115 nM.

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