The Steroid and Xenobiotic Receptor SXR: A Key Regulator of Drug and Xenobiotic Metabolism

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Nuclear Receptors - A Large Family of Ligand Modulated Transcription Factors

DNA-binding
AGGTCA

half-site recognition
half-site spacing
dimerization

ligand-binding
transcriptional activation
dimerization

DNA LIGAND
Nuclear Receptors

• Bind to specific DNA targets - hormone response elements
• Most activate transcription upon ligand binding
  – Some are constitutive
  – A few are deactivated by ligand binding
• Ligands are small lipophilic molecules that freely enter cells
  – Diffuse from source
  – Penetrate to a target
• Typically respond to low levels of hormone ~3 ppb (10^{-8} M)
  – Regulation of levels
  – Environmental agents
Mithridates VI Eupator
The Royal Toxicologist

(120-63 BC) King of Pontus
aka Mithridates the Great
Long Standing Questions

- Mithridatum - generalized tolerance to poison

- Adaptive hepatic response (Hans Selye)
  - Exposure to certain “catatoxic” chemicals elicits protection against later exposure to others
  - Apparently mediated via CYP upregulation

- What is the mechanism?
SXR and Close Relatives

- **hSXR**
  - DNA: 141
  - LIGAND: 323

- **mPXR**
  - DNA: 105
  - LIGAND: 357

- **xBXR**
  - DNA: 102
  - LIGAND: 284

- **CXR**
  - DNA: 97
  - LIGAND: 284

- **hCAR**
  - DNA: 155
  - LIGAND: 284

- **hVDR**
  - DNA: 89
  - LIGAND: 338
GAL-SXR Responds to Many Steroids

The graph shows the fold induction of GAL-SXR for various steroids. The x-axis represents different steroids, including solvent, corticosterone, pregnenolone, dihydrotestosterone, dehydroepiandrosterone, progesterone, dexamethasone, estradiol, cortisol, and cortisone. The y-axis represents the fold induction, ranging from 0 to 14. The data is presented as a bar chart with error bars, indicating the variability in fold induction for each steroid.
The Steroid Sensor Hypothesis

- Removal of bioactive steroids and xenobiotics is required for physiologic homeostasis

- Steroid production is regulated, why not catabolism?

- Hundreds of steroid metabolites make it unreasonable to have individual regulation

- Hypothesize a broad specificity sensor that monitors steroid levels and regulates the expression of degradative enzymes, e.g. P450s
  - Broad specificity probably necessitates low-affinity
Predictions and Requirements of the Model

- Sensor should be expressed in tissues that catabolize steroids and xenobiotics
- Catabolic enzymes should be targets for the sensor
- Compounds known to induce catabolic enzymes should activate the sensor
- Partially metabolized (reduced) steroids should activate sensor
Expression of SXR mRNA

heart | brain | lung | placenta | liver | muscle | kidney | pancreas |
------|-------|-----|----------|-------|--------|--------|----------|

adrenal medulla | thyroid | adrenal cortex | testis | thymus | small intestine | stomach

9.0 | 9.5
6.5 | 7.5
5.2 | 4.4
3.5 | 2.4
1.4
SXR DNA-binding Specificity

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SXR

hRXRα

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<th>βDR-2</th>
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<th>βDR-4</th>
<th>βDR-5</th>
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<th>MMTV</th>
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α
Candidate SXR Response Elements in Genes Encoding Steroid Degradative Enzymes

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SXR Activates Transcription Through βDR Elements

- DR-1
- DR-2
- DR-3
- DR-4
- TREp
- βDR-1
- βDR-2
- βDR-3
- βDR-4
- βDR-5

Fold Induction

- solvent
- corticosterone
- pregnenolone
- DHEA
- testosterone
- progesterone
- PCN
- estradiol
- cortisol
- cortisone
Classes of SXR Activators

- Steroids
- Phytoestrogens
- Xenobiotics

Graph showing fold induction for various activators:
- Solvent
- Corticosterone
- Diethylstilbestrol
- Spironolactone
- Tamoxifen
- Cyproterone
- Coumestrol
- Badzen
- Genistein
- Equol
- Quercetin
- Luteolin
- Cyclosporine A
- Rifampicin
- Nifedipine
- Erythromycin
SXR Senses Total Steroid Concentration

- 10 µM individual steroids
- 100 µM total in cocktail
Predictions and Requirements of the Model

- Sensor should be expressed in tissues that catabolize steroids and xenobiotics
  - Expressed in liver, small and large intestine

- Catabolic enzymes should be targets for the sensor
  - CYP genes are known targets of SXR, in vivo

- Compounds known to induce catabolic enzymes should activate the sensor
  - Majority of known CYP3 inducers activate SXR

- Partially metabolized (reduced) steroids should activate sensor
  - Mixture of steroids, each at concentrations below that required to activate SXR, will collectively activate SXR-mediated gene expression
CYP3A4 and Human Steroid Metabolism

- Steroid levels are tightly regulated. Increased catabolism will lead to ACTH release and upregulated adrenal synthesis
  - Observation is elevated ACTH, slightly increased circulating steroids
  - Decreased circulating steroid metabolites

- Increased catabolism will be reflected by urinary metabolites
  - Large increases in urinary steroids caused by rifampicin therapy have led to misdiagnosis of Cushing’s syndrome
  - VP16 SXR transgenic mice have drastically elevated urinary steroid metabolite levels

- Induction of CYP3A4 should lead to decreases in administered steroid levels
  - Steroid crisis in Addison’s patients on rifampicin and oral steroids
  - Pregnancy in rifampicin-treated patients on oral contraceptives
Many CYP3A4 Inducers Are SXR Activators

Natural and Synthetic Steroids

Steroid receptor agonists:
- corticosterone (C21)
- estradiol (C18)
- testosterone (C19)

Steroid receptor antagonists:
- spironolactone
- tamoxifen
- PCN

xenobiotic drugs:
- rifampicin
- clotrimazole

phytoestrogens (isoflavones):
- coumestrol
- equol
Pharmacology of Mouse and Human SXR

hSXR

mPXR

hER

r ER
Model Systems

• Central tenet of model system is parallel biochemistry and endocrinology
  – Toxicology: effects on animals predict effects on humans
  – Nuclear receptors behave virtually identically across species

• Different pharmacology of SXR and PXR suggests that there are important differences in metabolism

• These differences may be highly relevant for toxicology, drug interactions and endocrine disruption

• Cross-species extrapolation must account for differences in response of xenobiotic sensors
  – SXR
  – CAR
Drug Interactions

- CYP3A4 is the primary steroid and xenobiotic metabolizing enzyme

- Drugs interactions arise from:
  - Induction or inhibition of CYP3A4 expression
    - rifampicin and oral steroids
    - St John’s Wort and many drugs
  - Modulation of CYP3A4 enzyme activity
    - macrolide antibiotics (e.g. erythromycin) and many drugs (e.g. Seldane)

- Activated SXR mediates induction of CYP3A4
  - SXR activation is a direct molecular test for potential drug interactions

- Pharmacological differences between inducibility of rodent and human CYP3 genes explained by receptor pharmacology
  - Differences suggest rodents may not be an appropriate model for human drug interactions
  - Rabbit CYP3A induction closely parallels human
  - Mouse now exists that expresses human SXR instead of mouse gene
SXRx and Endocrine Disruption

- SXR regulates the P450-mediated breakdown of ingested steroids and xenobiotics

- Activation of SXR may predict effects of suspected EDC
  - SXR activators may be detoxified by CYP3A action and not a human risk
  - But activators may also be toxified by CYP3A action, increasing the risk.
  - EDC may have no effect on SXR and therefore more likely to act on other receptors, e.g. ER

- SXR is a molecular assay for potential activity of EDCs

- Different pharmacology of SXR and PXR suggests that differences in metabolism may exist and be relevant for risk assessment
Approach to Studying EDC Metabolism

• Test potential for metabolism by investigating SXR activation by a panel of known and candidate EDCs
  – Pesticides: DDT, DDE, methoxychlor, endosulfan, dieldrin, alachlor, chlordane, transnonachlor, chlorpyrifos, kepone
  – Plasticizers: bisphenol A, phthalates
  – PCBs: e.g. 184, 196
  – Alkylphenols: 4-nonylphenol
  – xenobiotics: thalidomide, dichlorophenol, triclosan, BHA, BHT

• Extend analysis to SXRs from other model organisms of interest
  – fish - e.g., zebrafish, medaka, fathead minnow
  – reptiles - alligator, sea turtle
  – birds - Japanese quail, zebra finch
  – amphibians - Xenopus, Rana
  – mammals - monkey, canine

• Investigate actual metabolism in animal models
  – model organisms including humanized mouse
  – wild populations
EDCs can activate SXR

- bisphenol A
- PCB 184
- PCB 196
- DDT
- DDE
- Chlorpyrifos
- nonylphenol
- bis-phthalate
SXR and its mammalian homologs

- Human: DNA (35-107), LIGAND (107-434), 73% identity
- Mouse: DNA (38-104), LIGAND (138-431), 95% identity, 73% similarity
- Rat: DNA (38-104), LIGAND (138-431), 95% identity, 76% similarity
- Rabbit: DNA (118-84), LIGAND (118-411), 94% identity, 84% similarity
## EDC activation of SXR

<table>
<thead>
<tr>
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<tr>
<td>bisphenol A</td>
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- rifampicin       | +     | -     | -    | +      |
- PCN               | -     | +     | +    | +      |
Conclusions and prospects SXR and EDC

• Many compounds activate SXR from all four species, suggesting metabolism is the same
  – SXR is a molecular assay for interspecies variations in metabolism

• There are significant differences that suggest metabolism of certain compounds of great interest is different
  – bisphenol A
  – phytoestrogens
  – PCBs

• Animal models used for extrapolation of toxicology and drug interaction testing to humans must be validated for each compound.
  – Is the activation profile of SXR, and by implication metabolism, the same or different?
  – Are the compounds in fact metabolized?
  – What is the nature and fate of the metabolites?
SXR – A steroid and Xenobiotic Sensor

bisphenol A

CYP3A

mdr1

mrp1

SXRx

Target genes
SXR - A Steroid and Xenobiotic Sensor

• SXR has properties predicted for a steroid and xenobiotic sensor
  – Expression
  – Targets
  – Activators
    Endogenous and dietary steroids
    Xenobiotic drugs
    Environmental toxicants
  – Expected responses from induction
    Increased ACTH
    Increased urinary metabolites
    Increased circulating steroids but decreased metabolites

• SXR is an important molecular test for potential species-specific metabolism of drugs and xenobiotics
• Understanding SXR regulation and identifying target genes is an important goal to aid in understanding the xenobiotic response
• SXR must be considered when working with model organisms