A Toxicological focus for a final year Molecular and Cellular Biology Reading Party

Matt Wright

ICM

Newcastle University, UK.

Professor of Toxicology

Main task is as a Academic researcher:

Toxicity mechanisms in the liver

Fibrosis – clinical liver disease

In vitro systems for investigating toxic mechanisms

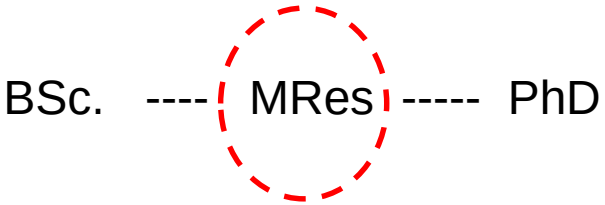
Environmental toxins and liver disease

Medical School (ICM)

I am also the co-ordinator for the MRes in Toxicology course at Newcastle University.

MRes = Masters in Research

6 months taught modules + 4 moth research project



BSc

PhD

Post doc1

Post doc 2

?

The real world!!

Lecturer

Academia


UK
North Sea
North Atlantic Ocean
SCOTLAND
Edinburgh
Glasgow
Newcastle upon Tyne
Tyne
N. IRELAND
Lough Neagh
Belfast
Irish Sea
Liverpool
Manchester
IRELAND
WALES
Trent
Birmingham
ENGLAND
Great Ouse
River Severn
Thames
Cardiff
Bristol
London
English Channel
FRANCE

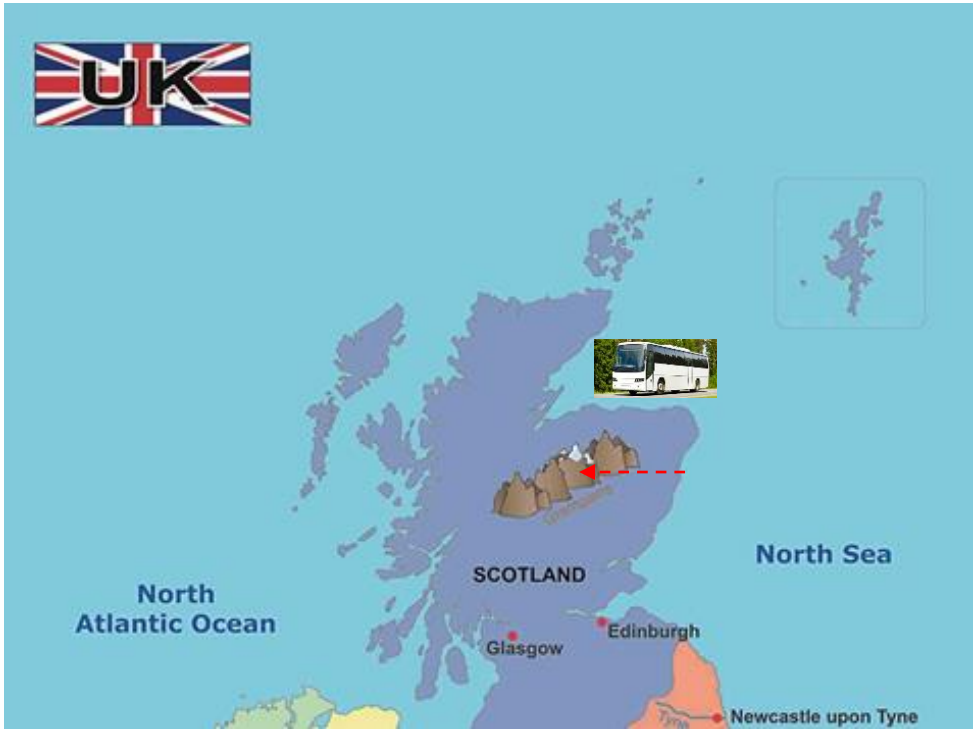
Dept Molecular and Cell Biology

Yeast
Ecoli transporters
Estrogen receptors
Diabetes
Lipid rafts
mRNA Translation
C Elegans (miRNA)

Research

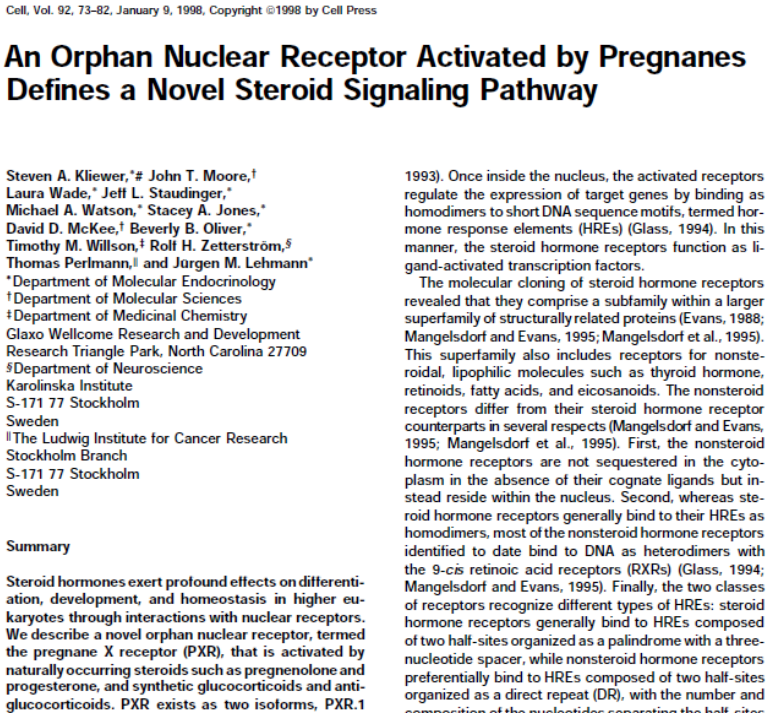
Teaching

Reading party



Reading party

Each student would present a research paper to a group of students and staff



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An Orphan Nuclear Receptor Activated by Pregnanes Defines a Novel Steroid Signaling Pathway

Steven A. Kliewer,*# John T. Moore,†
Laura Wade,* Jeff L. Staudinger,*
Michael A. Watson,* Stacey A. Jones,*
David D. McKee,† Beverly B. Oliver,*
Timothy M. Willson,‡ Rolf H. Zetterström,§
Thomas Perlmann,‖ and Jürgen M. Lehmann*
*Department of Molecular Endocrinology
†Department of Molecular Sciences
‡Department of Medicinal Chemistry
Glaxo Wellcome Research and Development
Research Triangle Park, North Carolina 27709
§Department of Neuroscience
Karolinska Institute
S-171 77 Stockholm
Sweden
‖The Ludwig Institute for Cancer Research
Stockholm Branch
S-171 77 Stockholm
Sweden

Summary

Steroid hormones exert profound effects on differentiation, development, and homeostasis in higher eukaryotes through interactions with nuclear receptors. We describe a novel orphan nuclear receptor, termed the pregnane X receptor (PXR), that is activated by naturally occurring steroids such as pregnenolone and progesterone, and synthetic glucocorticoids and antiglucocorticoids. PXR exists as two isoforms, PXR.1

1993). Once inside the nucleus, the activated receptors regulate the expression of target genes by binding as homodimers to short DNA sequence motifs, termed hormone response elements (HREs) (Glass, 1994). In this manner, the steroid hormone receptors function as ligand-activated transcription factors.

The molecular cloning of steroid hormone receptors revealed that they comprise a subfamily within a larger superfamily of structurally related proteins (Evans, 1988; Mangelsdorf and Evans, 1995; Mangelsdorf et al., 1995). This superfamily also includes receptors for nonsteroidal, lipophilic molecules such as thyroid hormone, retinoids, fatty acids, and eicosanoids. The nonsteroid receptors differ from their steroid hormone receptor counterparts in several respects (Mangelsdorf and Evans, 1995; Mangelsdorf et al., 1995). First, the nonsteroid hormone receptors are not sequestered in the cytoplasm in the absence of their cognate ligands but instead reside within the nucleus. Second, whereas steroid hormone receptors generally bind to their HREs as homodimers, most of the nonsteroid hormone receptors identified to date bind to DNA as heterodimers with the 9-*cis* retinoic acid receptors (RXRs) (Glass, 1994; Mangelsdorf and Evans, 1995). Finally, the two classes of receptors recognize different types of HREs: steroid hormone receptors generally bind to HREs composed of two half-sites organized as a palindrome with a three-nucleotide spacer, while nonsteroid hormone receptors preferentially bind to HREs composed of two half-sites organized as a direct repeat (DR), with the number and

Encourage the student to read research literature
Broaden their academic outlook
Comprehend and describe the work
Presentation skills
Critically evaluate within the group



Provided with pre-clinical toxicology data for 4 potential anti HCV drugs (W, X, Y and Z)

Therapeutic data, HCV infected human hepatocyte data)

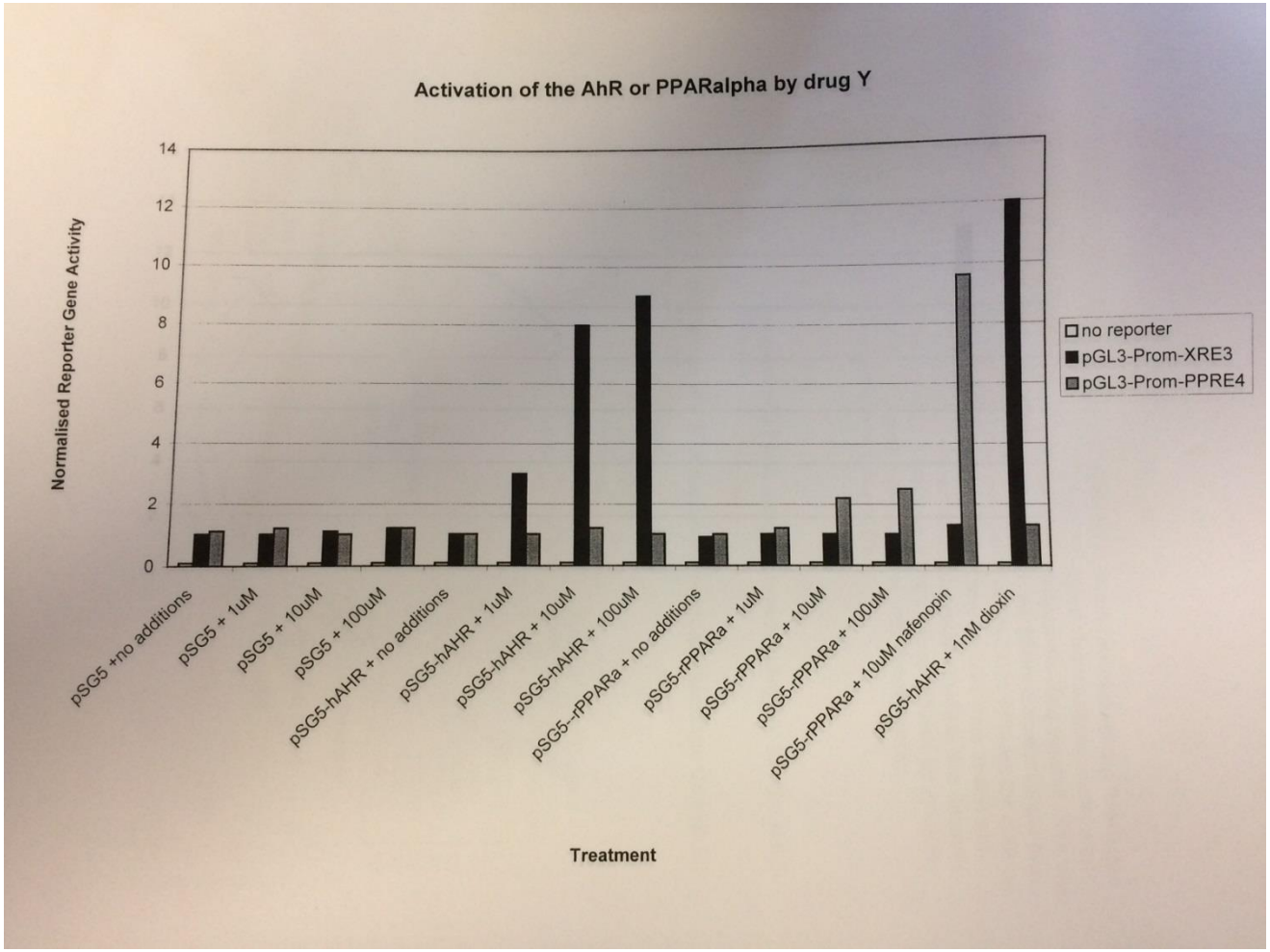
ADME data
Acute toxicity
Sub-chronic toxicity
Genotoxicity
Chronic toxicity and carcinogenicity

Research literature (covering likely topics new to staff and students)

2-3 hours analysing data provided. Eg Pathology

Substance (species, strain, sex [a], n [b])	Duration	Dosing information	NOAEL [c]	Investigated parameters/effects	Reference
Polysorbate 60 (rat, Sprague–Dawley, m, 12)	14 days	0 or 4 % in the diet	4 %	**4 %:** no effects on body weight gain using a diet with dietary fibre but diarrhoea and decreased body weight without dietary fibre	Ershoff (1976)
Polysorbate 60 (rat, nd, m, nd)	8 weeks	2 or 5 % in the diet, no data about control	na	**≤ 5 %:** no toxic symptoms	Krantz (1943b) [d], cited in JECFA (1974a)
Polysorbate 60 (rat, nd, m&f, 12)	10 weeks	0, 5 or 15 % in the diet	15 %	**≤ 15 %:** no effects on body, no clinical signs; no effects at necropsy and histopathology (normal diet)	Chow et al. (1951, 1953)
Polysorbate 60 (rat, nd, m&f, 12)	10 weeks	0 or 5 % in the diet	na	**5 %:** diarrhoea and body weight ↓, but related to basal casein diet	Chow et al. (1951, 1953)
Polysorbate 60 (rat, nd, m&f, 12)	12 weeks	0 or 25 % in the diet (standard diet)	na	**25 %:** body weight gain in m ↓ (not in f), no effects on food consumption or efficiency	Fitzhugh et al. (1959)
Polysorbate 60 (rat, Sprague–Dawley, m&f, 48 control, 24 test groups)	13 weeks	0, 1.0, 2.0 or 5.0 % in the diet	2 % (m, 1 355 mg/kg bw/day; f, 1 565 mg/kg bw/day; m/f, 1 460 mg/kg bw/day)	**5 %:** diarrhoea, increased water intake, enlarged caecum, haemoglobin slightly decreased	BIBRA (1981) [e]

The request limited additional data to help them with their decision


Activation of the AhR or PPARalpha by drug Y
Normalised Reporter Gene Activity
14
12
10
8
6
4
2
0
no reporter
pGL3-Prom-XRE3
pGL3-Prom-PPRE4
pSG5 +no additions
pSG5 + 1uM
pSG5 + 10uM
pSG5 + 100uM
pSG5-hAHR + no additions
pSG5-hAHR + 1uM
pSG5-hAHR + 10uM
pSG5-hAHR + 100uM
pSG5--rPPARa + no additions
pSG5-rPPARa + 1uM
pSG5-rPPARa + 10uM
pSG5-rPPARa + 100uM
pSG5-rPPARa + 10uM nafenopin
pSG5-hAHR + 1nM dioxin
Treatment

	W	X	Y	Z	
Therapeutic	✓	✓	Variable activity	Weak	
ADME			CYP2D6 metabolised		
Acute	✓	✓	✓	Toxic / below therapeutic window	
Sub Chronic	✓	✓	?	✓	
Genotoxicity	-ve	Ames +ve	-ve	-ve	
Carcinogenicity	+ve in rodents	+ve in rodents			

This task very amenable to IT/online delivery as a Toxicology revision tool with multiple choice testing.

A variety of data could be generated for 10s of drugs, all having different safety data profiles.

These could be provided in a restricted fashion / different profiles provided to reduce opportunities to memorize ”correct” interpretation – pushes students to analyse the data as they see it.

IT-Based International diploma and professional certificates in clinical toxicology

Thank you for your attention

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