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As pharmacologists we have a good working knowledge of the principal families of membrane receptors such as those of the 7TM family but we have sometimes neglected other major groupings and one prominent example is that family of intracellular receptors generally known as the *nuclear receptors*. The biology of these receptors has nevertheless been brilliantly exploited by other life scientists, especially by our endocrinological colleagues, and the fruits of their labours may be seen in the widespread use of such drugs as the synthetic glucocortico steroids and anti-estrogens such as tamoxifen.

The nuclear receptors constitute a large family of structurally-related proteins (m.ws. generally within the range 40-100kDa) which includes receptors for glucocorticoids, mineralocorticoids, sex steroids, thyroid hormones, vitamins and fatty acids as well as some 'orphan receptors. All members of the family have highly conserved domains which enable them, when activated, to bind to *hormone response elements* (HREs) contained within the promoters of target genes. Whilst maintaining a high degree of homology within the DNA and ligand binding domains other, more variable, regions of the receptor protein particularly at the N- and C-terminus govern their differential affinity for particular ligands and confer other individual properties.

Entry into cells of ligands for nuclear receptors is probably through a process of passive diffusion as many are lipophilic, although they have been sporadic reports in the literature of carrier or active uptake processes. The term 'nuclear receptors' is a little confusing because whilst acting within the nucleus, some members of the family (eg. glucocorticoid receptors) are found in the cytoplasm when unliganded, whereas other members of the family (eg. the estrogen receptor) are thought to be retained largely within the nucleus.

When unactivated, the intracellular nuclear receptors are present normally as a multimeric complex with other proteins including members of the heat shock protein (HSP) family. Following ligand binding, there are often changes in the phosphorylation and allosteric conformation of the receptor which then dissociates from the complex and translocates to HRE binding sites where, generally in the form of a dimer, it modulates the transcription of target genes thus ultimately bringing about the biological effect. In the case of the anti-inflammatory glucocorticosteroids for example, many genes which code for cytokines and other pro-inflammatory signals have been shown to contain *GREs* (glucocorticoid response elements) and thus to be directly down-regulated by the activated glucocorticoid receptor complex, whilst other, anti-inflammatory substances, can be shown to be elevated.

Whilst many nuclear receptors act as *homodimers* form to produce gene transcription events, there is increasing evidence that others can form *heterodimers* (eg. one glucocorticoid plus one mineralocorticoid receptor molecule) and it is quite likely that the effects on gene transcription produced by homodimers of (e.g.) GR is different from that seen with heterodimers of GR/MR.

Another exciting growth area is the putative role of the receptor itself in producing *non-genomic* actions. It has been demonstrated, for example, that the binding of glucocorticoids to the GR may alter the affinity of the complex for some transcription factors and, in some cases, may be followed by activation of intracellular phosphatases (such as calcineurin). This means that some hormones may also produce biological actions in a genome-independent, receptor-dependent fashion, thus broadening the scope of potential hormone and drug effects. Another interesting development concerns the possibility that some steroid hormones may have other non-genomic effects by direct action on separate membrane-bound receptors or on ion channels or signal transduction systems.

Some Key Nomenclature.

AR: androgen receptor
GR: glucocorticoid receptor.
MR: mineralocorticoid receptor.
PR: progesterone receptor.
ER: estrogen receptor.
TR: thyroid hormone receptor.
RAR, VDR: vitamin receptor.
PPAR: peroxisome proliferator receptor.
HREs: hormone response elements.
GREs: glucocorticoid response elements.
HSP: heat shock protein.

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146P TRANSCRIPTIONAL ACTIVATION BY THE OESTROGEN RECEPTOR

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Oestrogen receptors, in common with other members of the nuclear receptor family, are ligand dependent transcription factors that regulate the proliferation and differentiation of many target cells.

As a consequence of ligand binding, nuclear receptors bind directly to response elements in the vicinity of hormone responsive genes and undergo a conformational change which results in the recruitment of additional proteins.

A surprising number of proteins have been reported to interact with activated receptors and their roles are now being investigated. We have focused on two groups that appear to be essential for hormone dependent transcription.

One group, with a molecular weight of 160kDa, consists of three related proteins, encoded by distinct genes, called SRC1, TIF2 and p/CIP, while the other group consists of two proteins CBP/p300. The p160 family members are recruited to activated to receptors directly by means of short LXXLL motifs. Their primary role may be to recruit CBP/p300, which do not seem to interact directly with receptors, but they do contain other functional domains including an activation domain and histone acetyl transferase activity. CBP/p300 also encodes intrinsic histone acetyl transferase activity but is also capable of recruiting p/CAF and contacting the basic transcription machinery. P/CAF is also a histone acetyl transferase.

We are now in the process of determining whether the recruitment of all of these proteins is necessary to mediate hormonal responses, and, if so, determining their individual roles.

147P MECHANISMS OF GENE ACTIVATION AND REPRESSION BY THE GLUCOCORTICOID RECEPTOR

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The glucocorticoid receptor (GR) is a member of the steroid receptor super-family which modulates the expression of genes, activating some while repressing others, in a tissue and cell type specific manner. We have sought to define the mechanisms by which glucocorticoid receptors regulate gene expression in three model systems.

As a phosphoprotein the glucocorticoid receptor is potentially subject to regulation by cellular kinases and phosphatases. Mutagenesis of all the known phosphoamino acids within GR phosphorylation does not affect receptor expression, ligand binding or nuclear translocation. In contrast, both transcriptional activation and ligand dependent desensitization areinfluenced by phosphorylation. Together these data suggest that glucocorticoid receptor function can be modulated by small nonsteroidal molecules that alter cellular phosphorylation.

Glucocorticoid receptors also have profound suppressor actions on inflammatory responses which are induced by the transcription factor NFKB. We have previously shown that a mutual antagonism exists between NFKB and hGRa due to transcriptional repression that involves direct association of specific domains of each protein.

To clarify the mechanism by which p65 NFKB and hGRa interact we have employed the p65 Rel Homilogy Domain (p65RHD), a dominant negative regulator of p65 transactivation. p65RHD efficiently blocks gene transactivation of an NFKB responsive reporter but it neither interferes with p65 repression of GR transactivation nor represses GR transactivation alone. Interestingly CREB binding protein (CBP) rescues hGRa from repression by NFKB and augments GR repression of p65 signalling. These data suggest that although p65 and GR do physically interact, competition for cofactors modulate the efficacy of mutual antagonism and thus modify the anti-inflammatory actions of steroids.

Finally, we have recently demonstrated that alternative splicing of the hGR gene produces a second form of glucocorticoid receptor called hGRb. hGRb does not bind hormone but inhibits the transcriptional activity of ligand occupied hGRa.

Repression by hGRb is specific to hGRa since activation of the same promoter by progesterone receptor or androgen receptor is not compromised by hGRb. Repression of hGRa function by hGRb appears to involve hGR a/b heterodimerization. Unlike the ubiquitous tissue distribution observed for hGRa, hGRb protein is selectively expressed in specific cell types including lung, liver, kidney, thymus and peripheral immune cells. Inflammatory activation of cells leads to selective up-regulation of hGRb and development of a state glucocorticoid resistance. The tissue specific localization of hGRb makes it a good target for the development of therapeutics.

148P RETINOIC ACID RECEPTORS

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Retinoids, derivatives of vitamin A, influence many aspects of cell and development biology, and may be important intracellular regulators or signalling molecules. The mechanism of action of these compounds is thought to be largely via nuclear receptors for retinoic acid, acting as ligand-dependent transcriptional regulators.

There are two main families of retinoic acid receptor: the retinoic acid receptors (RARs) which bind all-trans and 9-cis retinoic acid, and the retinoid X receptors (RXRs) which apparently bind only 9-cis retinoic acid. These receptors function as heterodimers (RAR-RXR) or homodimers (RXRs), with the ability to regulate gene transcription through different DNA-response elements.

The existence of different RAR and RXR types, and isoforms within each type, results in a baffling complexity of possible regulatory mechanisms. Transcriptional regulation by these receptors is also influenced by co-repressors and co-activators which may inhibit or mediate, respectively, the interaction of receptor dimers with the pre-initiation complex. RXRs can form heterodimers with other nuclear receptors, and retinoids thus have the potential to influence a wide variety of cellular regulatory pathways. This has important implications for the design and use of RXR-specific ligands.

To develop a rational basis for the clinical use of retinoids, it is essential to characterise the functions of RARs and RXRs in the context of specific biological responses to receptor ligands.

149P THE ANDROGEN RECEPTOR

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Androgens play a crucial role in several stages of male development and in the maintenance of the male phenotype. Androgens act in their target cells via an interaction with the androgen receptor, resulting in direct regulation of gene expression. The androgen receptor is a phosphoprotein and modulation of the phosphorylation status of the receptor influences ligand binding and consequently transcription activation of androgen responsive genes. Androgen binding induces a conformational change in the ligand-binding domain, accompanied by additional receptor phosphorylation, and the liganded androgen receptor subsequently interacts with specific androgen response elements in the regulatory regions of androgen target genes, resulting in stimulation of gene expression.

Anti-androgens induce a different conformational change of the ligand-binding domain, which does not or only partially result in stimulation of transactivation. Interestingly different anti-androgens can induce different inactive conformations of the androgen receptor ligand binding domain. Recent evidence strongly support a ligand dependent functional interaction between the ligand-binding domain and the N-terminal transactivating domain of the androgen receptor. Two regions in the N-terminal domain are involved in this interaction, while in the ligand binding domain the AF-2 AD core region is involved.

At least three pathological situations are associated with abnormal androgen receptor structure and function: (1) androgen insensitivity syndrome (AIS), (2) spinal and bulbar muscular atrophy (SBMA, or Kennedy's disease) and (3) prostate cancer.

In the X-linked AIS, a large variety of amino acid substitutions in the DNA- or ligand binding domain, or mutations resulting in premature stopcodons, in the androgen receptor gene have prevented the normal development of both internal and external male structures in 46,XY individuals.

SBMA is a rare, X-linked motor neuron disease, with a late onset and characterized by a progressive spinal and bulbar muscular atrophy associated with signs of androgen insensitivity and infertility. The CAG-repeat in exon 1 of the androgen receptor gene, coding for a polyglutamine stretch, is expanded in all investigated SBMA patients.

In prostate cancer the initial tumor growth depends on the presence of an activated androgen receptor. In this stage cancer patients benefit temporarily from androgen ablation therapies. However, most prostate tumors develop into an androgen-independent stage. In most of these apparently hormone independent tumors high nuclear androgen receptor expression exists. Additionally in part of the hormone refractory prostate cancers androgen receptor gene amplification occurs or androgen receptor mutations are found, which can modify the ligand specificity.

150P SIGNALLING BY PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS

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Peroxisome Proliferator-Activated Receptors (PPARs) are nuclear hormone receptors controlling the expression of genes involved in lipid homeostasis. PPARs activate gene transcription in response to a variety of compounds including hypolipidemic drugs as well as natural fatty acids. Transcriptional regulation by PPARs is achieved through PPAR-RXR heterodimers which bind to DNA motifs termed PPAR-response elements (PPRE) in the promoter of their target genes. PPREs consist of a direct repeat of the AGGTCA hexamer with one base pair between the two half-sites and are thus referred to as DR1 elements. The 5' half-site is extended and binds the PPAR molecule, while RXR occupies the 3' half-site.

Based on the hypothesis that ligand binding to PPAR would induce interactions of the receptor with transcriptional co-activators, we have developed a ligand sensor assay, termed Co-Activator-dependent Receptor Ligand Assay (CARLA). With CARLA, we have screened several natural and synthetic candidate ligands and we have identified naturally occurring fatty acids and metabolites as well as hypolipidaemic drugs as bona fide ligands of the three PPAR subtypes from Xenopus laevis. Our results suggest that PPARs, by their ability to interact with a number of structurally diverse compounds, have acquired unique ligand binding properties among the superfamily of nuclear receptors that are compatible with their biological activity.

Furthermore, we identified some structure-function relationships in PPARa, by using the species-specific responsiveness to the two hypolipidaemic agents, Wy 14,643 and 5,8,11,14-eicostetraenoic acid (ETYA). These two drugs are ligands of PPARa and species-specific differences are indeed mediated primarily via the ligand binding domain of the receptor. By mutagenesis analyses, we have identified amino acid residues in the ligand binding domains of Xenopus, mouse and human PPARa, that confer preferential responsiveness to ETYA and Wy 14,643. These findings may aid in the development of new PPARa ligands as effective therapeutics for lipid-related diseases and inflammatory disorders.

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