Nuclear Receptors as Therapeutic Targets: Society for Medicines Research Symposium

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A large proportion of drug-discovery programmes target receptors localised on the plasma membrane. This meeting focused on the possibilities of targeting receptors that are present in the cytosol, which, when activated, move to the nucleus and modulate gene expression. A number of these nuclear receptors were considered in this meeting, including receptors for oestrogen, retinoic acid, vitamin D, glucocorticoids and thyroid hormone; the peroxisome proliferator-activated receptor (PPAR) was also considered. The biochemistry, physiology and clinical relevance of these receptors were described and prospects for new therapies considered.

Introduction

This symposium, organised by the Society for Medicines Research and chaired by Drs Mark Giembycz (Imperial College, London), Alan Palmer (Cerebrus Ltd, Winnersh) and Roger Horton (St George's Hospital Medical School, London), was attended by 60 participants from both industry and academia. The meeting focused on nuclear receptors and the new drug development possibilities they represent, together with the aspects of mechanism of action of nuclear receptors and their role in both normal function and in certain disease states.

Nuclear receptors are members of a family of ligand-inducible transcription regulators for steroids and steroid hormones, as well as retinoids, vitamin D and certain drugs (such as the fibrate group of lipid-lowering drugs, which bind to the peroxisome proliferato-activated receptor). In addition, there are a large number of `orphan' nuclear receptors for which the ligands have not yet been established.

Oestrogen receptors

The biology of the oestrogen receptor was described by Dr Malcolm Parker (Imperial Cancer Research Fund, London) in order to illustrate the general principles underlying the mechanism of action of nuclear receptors. The classical oestrogen receptor (ER□), like most other nuclear receptors, has both a DNA-binding domain and a ligand-binding domain. The receptor dimerises before binding to target genes and modulating transcription (transactivation). Fine adjustments to gene expression are effected by transcriptional control of the expression of other transcription factors, such as activator protein-1 (AP-1) or nuclear factor-□B (NF-□B). Thus ER□ can transactivate AP-1- or NF-□B-responsive genes.

The role of oestrogen receptor variants in breast cancer was reviewed by Dr Valerie Speirs (University of Hull). A number of variant forms of the classic oestrogen receptor (ERα) have been identified in human breast tumors and breast cancer cell lines. Exon 7 and exon 4 deletion variants are common and over-expression or altered expression of such variants has been correlated with carcinogenesis and tumour progression. A second ER, ERβ, was cloned in 1996 and recent studies have also indicated variant forms of this receptor in normal and malignant breast tissue. Using nested reverse-transcription polymerase chain reaction (RT-PCR) with oligo-nucleotide primers spanning exons 4 to 7, 82 breast tumours were analysed for the presence of wild-type and variant ERβ. Co-expression of ERβ-deletion variants with wild-type ERβ was common; ERβ was detected in over 50% of samples. Sequence analysis of the deletion variants revealed that the deleted portion corresponds to the entire exon 5 of human ERβ. This corresponds to a portion of the ligand-binding domain. No direct associations were observed between expression of this deletion variant and clinical prognostic factors including tumour grade, node status and expression of ERα. It appears that, like ERα variants, ERβ variants are also common in breast tumours, although their functional significance remains unclear.

Retinoid receptors

The biology of retinoic acid receptors (RARs) was reviewed by Dr Christopher Redfern (University of Newcastle upon Tyne). Retinoids have been used since the 1960s for the treatment of acne, psoriasis and premalignant skin lesions. Toxicity has limited their use, emphasising the need for new therapies. Retinoic acid receptors were discovered over 12 years ago as a mechanism-mediating cellular responses to retinoic acid (RA). These receptors are closely related to thyroid hormone receptors and function as heterodimers with related receptors called retinoid-X receptors (RXRs). Three types of RAR (RARα, β and γ), and three

of RXR (RXRα, β and γ), each encoded by separate genes, have been identified. The two classes of retinoid receptors (RARs and RXRs) have different ligand-binding properties: RARs bind all-trans and 9-cis RA, whereas RXRs bind only 9-cis RA. RARs function as ligand-dependent transcription factors in the context of RAR/RXR heterodimers binding to recognition sequences predominantly consisting of two direct repeats (DR) or half-sites separated by five base pairs, and referred to as a DR5 RARE. As RAR heterodimer partners, RXRs play an important role in mediating RA responses at the level of gene expression, but may also affect other hormone-response pathways since they are able to form heterodimers with other nuclear receptors. In addition, RXR homodimer formation may be induced in response to 9-cis RA and, since RXR homodimers are reported to control transcription via DR1 RARE, this is an additional mode of gene regulation by RXRs. The discovery of different classes of retinoid receptors and different types within each class has prompted a fruitful search for synthetic retinoid receptor type-specific and class-specific retinoids. A range of compounds which bind specifically to different RARs has been identified, and may act as RAR agonists or antagonists. These compounds are useful as tools to elucidate the molecular pathways of retinoid action and may have clinical applications as biological-response modifiers. In addition, compounds which show specifically for RXRs and act as RXR agonists or RXR antagonists have also been developed and show considerable clinical potential for disease therapy. However, as with many synthetic agonists/antagonists, many of these retinoid-receptor specific compounds are active at relatively high concentrations, and may have effects unrelated to their ability to bind RARs or RXRs. This is illustrated by recent work on a RARβ/γ-specific retinoid fenretinide (McNeil Pharmaceuticals Inc). Fenretinide is an effective inducer of apoptosis in neuroblastoma cells, a property not shown (at least to the same extent) by RA. Although RARβ/γ antagonists block the ability of fenretinide to induce apoptosis, this may result from effects other than inhibition at a receptor level. Clearly, empirical studies on synthetic retinoids are important to elucidate the mechanisms of biological responses.

The role of retinoic acid in the formation of pulmonary alveoli was discussed by Dr Donald Massaro (Georgetown University, Washington DC). Pulmonary alveoli are formed, in part, by the developmentally regulated subdivision (septation) of the large saccules that constitute the gas-exchange region of the architecturally immature lung. Administra-tion of dexamethasone (DEX), a synthetic gluco-corticosteroid to rats in the early post-natal period prevents septation; `catch-up' septation does not spontaneously occur after treatment with DEX is stopped. The molecular signals responsible for septation are poorly understood. On the basis that retinoids are key signalling ligands, all-trans retinoic acid (RA) was administered daily to rats from postnatal day three to day 13, the usual period of septation, and a 50% increase in the number of alveoli without an increase in lung volume was observed; treatment with RA also prevented the low number of alveoli and low body mass-specific alveolar surface area caused by treatment with DEX. Instilling elastase into the trachea of adult rats resulted in lowered lung elastic recoil, larger but fewer alveoli, and diminished volume-corrected alveolar surface area. Treatment with RA reversed these changes. These findings support the possibility that, in individuals with too few alveoli for adequate gas-exchange, treatment with a pharmacological agent might provide remedial therapy.

Vitamin-D receptors

The molecular mechanisms of the selective action of vitamin-D analogues together with their therapeutic implications were reviewed by Dr Carsten Carlberg (University of Düsseldorf, Germany). The nuclear hormone 1α,25-dihydroxyvitamin D_3 (VD), which is the physiologically active form of vitamin D_3, plays a key role in calcium homeostasis and bone formation. However, its ability to induce cellular differentiation and apoptosis and to inhibit cellular proliferation makes VD and its synthetic analogues a potential therapy of hyperproliferative diseases, such as psoriasis and different types of cancer. VD binds with high affinity to the nuclear vitamin D_3 receptor (VDR), which is a member of a super family of structurally related nuclear receptors that can act as ligand-inducible transcription factors. Thus, VD directly modulates transcription of those genes that have a functional building site for the VDR, referred to as a VD response element (VDRE), in their regulatory region. Present evidence strongly suggests that VDR-RXR heterodimers are the major components in VD signalling, but this single type of heterodimer complex binds to several different VDRE types. These protein-DNA complexes are the molecular switches in VD signalling. The sharp biological profile of the model VD analogs EB-1089 (Leo Pharmaceutical Products Ltd A/S), namely, its high antiproliferative effect combined with low calcemic actions, has been correlated with the selectivity of EB-1089 to activate VDR-RXR heterodimers on VDREs that are formed by a inverted palindromic arrangement of two hexameric core-binding motifs spaced by nine nucleotides (IP9-

type VDREs), rather than for VDREs that are formed by direct repeats with three intervening nucleotides (DR3). On each VDRE, two different functional conformations of the VDR can be differentiated and allow a more differential view on DNA-complexed VDR-RXR heterodimers. The more ligand-sensitive VDR-RXR conformation gains, through EB-1089, a clearly higher affinity for DNA binding and provides a more sensitive activation of an IP9-type VDRE than of a DR3-type VDRE, whereas with the natural hormone VD, no VDRE-type preference is observed. This indicates that a promoter selectivity of VDR ligands is based on their property selectively to increase affinity for VDREs and very sensitively stabilise VDR conformations in VDR-RXR-VDRE complexes. Moreover, the analysis of the conformations of VDR in solution in comparison to those of DNA-complexed VDR-RXR heterodimers allows a differentiation between DNA-dependent and DNA-independent VD-signalling pathways that can be used for the identification of pathway selective VDR agonists. Therefore, analysing the interaction of VD analogues with the VDR-RXR conformation presently appears to be the most informative method for an *in-vitro* evaluation of these analogues.

Peroxisome proliferator-activated receptor

An update of the PPAR was provided by Dr Colin Palmer (University of Dundee). The PPARs are well-known targets for lipid-lowering and antidiabetic drugs. However, knowledge of the spectrum of action of these receptors is in its infancy. Initially, it was found that PPARα was the target for the fibrate group of lipid-lowering drugs. The toxicology of these compounds in rodents has been well characterised; however, their action in humans is quite distinct and poorly characterised. Another receptor, PPARγ, has been widely studied, owing to its interaction with the thiazolidinedione class of insulin-sensitising drugs. PPARγ has been described as a fatty-acid sensor in the formation of adipose tissue from skeletal muscle and fibroblasts and it is thought that this is where the insulin-sensitising function resides.

The tightest binding fatty acids to PPARγ are the ω-3 fatty acids such as the fish oils, all-cis-4,7,10,13,16,19-docosahexanoic acid (DHA) and all-cis-5,8,11,14,17-eicosatetraenoic acid (EPA). DHA is a potent activator of PPARγ; however, EPA does not activate PPARγ and will block the action of DHA or rosiglitazone (SmithKline Beecham) in an adipogenesis assay. This is the first description of a natural antagonist of PPARγ and suggests that the characterisation of PPAR antagonists may be important for the formulation of improved pharmaceuticals.

PPARγ has also been shown to be an important regulator of cancer cell growth. Aromatic fatty acids have been found to bind and activate PPARγ at concentrations required to halt the growth of cancer cell lines. In addition, over-expression of PPARγ in cancer cell lines confers a greater sensitivity to aromatic fatty acids, demonstrating that PPARγ is a molecular target for these drugs. Non-steroid anti-inflammatory drugs (NSAIDs) have been studied in the treatment and prevention of colon cancer and it has shown that several NSAIDs bind weakly to and activate PPARγ; however, the relevance of this to colon cancer remains unclear. It has been shown that certain NSAIDs have a high affinity for PPARγ and that some of these may have antagonistic activities towards PPARγ.

Glucocorticoid receptors

The mechanism of action of glucocorticoids was reviewed by Dr Ian Adcock (Imperial College School of Medicine, London). Glucocorticoids are the most effective anti-inflammatory drugs used in the treatment of chronic inflammatory diseases, such as asthma. They act by binding to a specific receptor that, upon activation, translocates to the nucleus and either increases (transactivates) or decreases (transrepresses) the expression of responsive genes. In order to investigate the relative roles for transactivation and transrepression in the control of asthmatic inflammation, the ability of dexamethasone (DEX) to regulate interleukin (IL)-1β-induced gene expression, histone acetyltransferase (HAT) and deacetylase (HDAC) activity was investigated. Low concentrations of DEX (10^{-10} M) repress IL-1β-stimulated granulocyte macrophage colony-stimulating factor (GM-CSF) expression and fail to stimulate secretory leukocyte proteinase inhibitor (SLPI) expression. DEX (10^{-7} M) and IL-1β stimulated HAT activity, but showed a different pattern of histone H4 acetylation. DEX targeted lysines K5 and K16, whereas IL-1β targeted K8 and K12. Low concentrations of DEX (10^{-10} M), that do not transactivate, repressed IL-1β-stimulated K8 and K12 acetylation. In contrast, RU-486 (Hoechst Marion Roussel AG) repressed IL-1β-stimulated HAT activity without upregulating K5 and K16 acetylation. The activated GR complex acts both as a direct inhibitor of cyclic AMP response-element binding-protein (CREB)-binding protein (CBP)-associated HAT activity and also by recruiting HDAC2 to the p65/CBP HAT complex. This action does not involve *de-novo*

synthesis of HDAC protein or altered expression of p300/CBP-associated factor (PCAF). This mechanism for glucocorticoid repression is novel and establishes that repression of histone acetylation is an additional level of control of inflammatory gene expression. This further suggests that pharmacological manipulation of specific histone acetylation status is a potentially useful approach for the treatment of inflammatory diseases. Identification of the precise mechanism by which activated GR recruits HDAC2 may reveal new targets for the development of drugs that may dissociate the anti-inflammatory actions of glucocorticoids from their side-effects that are largely due to gene induction.

Thyroid hormone receptor

Dr Björn Vennström (Karolinska Institute, Stockholm) described how gene knock-out studies have helped elucidate the function of thyroid hormone receptor action. Thyroid hormone (T3) has widespread functions in development and homeostasis, although the receptor pathways by which this diversity arises are unclear. Deletion of the T3 receptors TRαl or TRβ individually reveals only a small proportion of the phenotypes that arise in hypothyroidism. For instance, TRαl-deficient mice have cardiac-function abnormalities and low body temperature, whereas TRβ -/- mice have impaired hearing, a dysfunctional pituitary-thyroid axis of hormone control and abnormal regulation of 7-α-hydroxylase, a key enzyme in lipid metabolism. However, analyses of mice lacking both TRαl and TRβ (TRαl -/-β-/-) identified an array of phenotypes not found in single receptor-deficient mice, including an extremely hyperactive pituitary-thyroid axis, low female fertility, intolerance to cold and retarded growth and bone maturation. These results establish that major T3 actions are mediated by common pathways in which TRαl and TRβ co-operate with, or substitute for, each other in some tissues. Thus, varying the balance of use of TRαl and TRβ individually or in combination facilitates control of an extended spectrum of T3 actions. Compared to the debilitating symptoms of severe hypothyroidism, the milder overall phenotype of TRαl -/-β-/- mice, lacking all known T3 receptors, suggests that T3-independent actions of T3 receptors, previously demonstrated *in vitro*, have a significant physiological role.

Summary

This meeting illustrated the increased understanding of nuclear receptors that has occurred in the last decade and highlighted a number of disorders that are associated with dysfunction of nuclear receptors. Linking structure to function has been greatly assisted by gene knock-out studies, illustrated here by studies of the thyroid hormone receptor. A clear link between nuclear receptors and human disease was illustrated by studies showing that oestrogen receptor variants are common in breast tumours. The increased understanding of the biology of nuclear receptors, together with a more detailed appreciation of the link between nuclear receptors and human disease serves to increase the opportunity for the development of new therapies based on the modulation of nuclear receptors. Other exciting possibilities for new therapies derive from the number of orphan receptors that have been identified, the oldest of which is RXR. The development of RXR-specific ligands for its various heterodimeric partners shows considerable potential for the development of new medicines.