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Nuclear Receptors as Drug Targets for Metabolic Disease

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Abstract

Nuclear hormone receptors comprise a superfamily of ligand-activated transcription factors that control development, differentiation, and homeostasis. Over the last 15 years a growing number of nuclear receptors have been identified that coordinate genetic networks regulating lipid metabolism and energy utilization. Several of these receptors directly sample the levels of metabolic intermediates including fatty acids and cholesterol derivatives and use this information to regulate the synthesis, transport, and breakdown of the metabolite of interest. In contrast, other family members sense metabolic activity via the presence or absence of interacting proteins. The ability of these nuclear receptors to impact metabolism will be discussed and the challenges facing drug discovery efforts for this class of targets will be highlighted.

Keywords

Nuclear Receptors; Metabolic Disease; Diabetes; Atherosclerosis; Cholesterol; Fatty acids; Transcription

1. Introduction

There is currently a worldwide epidemic of metabolic disease characterized by obesity, type II diabetes, hypertension, and cardiovascular disease. The factors behind this epidemic appear to be a combination of genetic predisposition, high caloric diets, and our increasingly sedentary lifestyles. Although a number of drugs are currently available to treat this constellation of metabolic ailments, the growing epidemic indicates that we still require a better understanding of the genetic networks and signal transduction pathways that underlie the pathogenesis of these conditions. Further definition of the factors responsible for metabolic control may pave the way toward new drug targets with novel mechanisms of action for the treatment of human disease.

Nuclear receptors comprise a superfamily of ligand-dependent transcription factors that regulate genetic networks controlling cell growth, development, and metabolism. Consisting of 48 members in the human genome the superfamily includes the well-known receptors for steroids, thyroid hormones and vitamins. Members of the nuclear receptor superfamily are characterized by a conserved structural and functional organization consisting of a

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heterogeneous amino terminal domain, a highly conserved central DNA binding domain (DBD), and a functionally complex carboxy terminal ligand binding domain (LBD). The LBD mediates ligand binding, receptor homo- and heterodimerization, repression of transcription in the absence of ligand, and ligand-dependent activation of transcription when agonist ligands are bound.

Classic experiments that defined the effects of glucocorticoids and thyroid hormone on metabolic control provided the foundation for the endocrine regulation of metabolism. Similar to these classical endocrine receptors, a number of orphan receptors first cloned based on homology to the well conserved receptor DNA binding domain have subsequently been shown to regulate genetic networks that control important metabolic pathways. In many cases these same pathways are deranged in instances of metabolic disease. Several receptors including the peroxisome proliferator activated receptors (PPARs), the liver X receptors (LXRs), the farnesoid X receptor (FXR) and the retinoid-related orphan receptors (RORs) appear to function by directly sampling the levels of fatty acids and cholesterol derivatives via the receptor ligand binding domain and regulating genetic networks that control the synthesis, transport, and breakdown of the cognate ligand. Importantly, these fatty acid- and cholesterol-derived natural ligands bind to receptors with K_ds close to the physiological concentrations known to exist for these metabolites. Thus these “metabolic receptors” are poised to sense and respond to small changes in the flux through the metabolic pathways that they control. The estrogen related receptors (ERRs) comprise an additional sub-family of nuclear receptors that also appear to play important roles in the control of energy utilization and mitochondrial function. In contrast to the ligand-activated receptors mentioned above, the activity of the ERRs appears to be controlled by the presence or absence of interacting proteins instead of lipid-derived ligands.

Proper metabolic control requires a delicate balance among a number of opposing pathways (e.g. glycolysis and gluconeogenesis; fatty acid synthesis and oxidation) and this balance is often disturbed in pathological states. Small molecules that regulate nuclear receptors can function to reset this unbalance providing therapeutic benefits for patients with metabolic disease. The ability of individual nuclear receptors to regulate multiple genetic networks in different tissues, however, many times results in unwanted side effects making drug discovery a challenging process for this class of potential drug targets. Thus the next generation of nuclear receptor-based drugs for metabolic disease will most likely need to be tuned to maximize activity in a tissue- and gene-specific manner. To explore these challenges this review will focus on the PPARs and the LXRs as representatives of established and emerging drug targets respectively.

2. The PPARs

Three distinct members of the peroxisome proliferator activated receptor (PPAR) sub-family each encoded by a distinct gene have been identified and well characterized. PPAR α (NR1C1) is highly expressed in liver, kidney, and muscle. PPAR γ (NR1C3) is enriched in adipose tissue and PPAR β/δ (NR1C2; referred to as PPAR δ in this review) appears to be ubiquitously expressed. All three PPARs bind to DNA as heterodimers with retinoid X receptors (RXR; NR2B sub-group) and prefer to bind to direct repeats of the nuclear receptor half site AGGTCA separated by 1 nucleotide (DR1). Each sub-type appears to have unique functions and PPAR α and PPAR γ are the targets of the fibrate and thiazolidinedione (TZD) classes of drugs respectively.

2.1 PPAR α

PPAR α is the molecular target of the fibrate class of drugs used for the treatment of hypertriglyceridemia. Studies *in vitro* and *in vivo* demonstrate that PPAR α directly regulates

a network of genes encoding the proteins required for the uptake of fatty acids, enzymes required of the oxidation of fatty acids (β oxidation), and enzymes required for ketogenesis by binding to control regions in the promoter of these genes. Thus activation of PPAR α promotes the utilization of fat as an energy source. Mice lacking PPAR α accumulate triglycerides in the liver and become hypoketotic and hypoglycemic during fasting or starvation. Recent studies indicate that fibroblast growth factor 21 (FGF21) functions as an endocrine hormone that mediates many of the effects of PPAR α . The gene encoding FGF21 is directly induced by PPAR α in response to fasting via a binding site in the promoter. FGF21 in turn stimulates lipolysis in adipose tissue and ketogenesis in the liver. Taken together PPAR α appears to function as a sensor of the fed/starved state.

In vitro studies have demonstrated that fatty acids can bind to PPAR α and functions as ligands. More recently, affinity purification of PPAR α from mouse livers followed by mass spectrometry identified 1-palmitoyl-2-oleoyl-*sn*-glycerol-3-phosphocholine (16:0/18:1-GPC) as a selective PPAR α agonist. Interestingly, the production of 16:0/18:1-GPC requires the activity of fatty acid synthase (FAS) suggesting that PPAR α -dependent fat oxidation serves as a counter balance to FAS to insure that fatty acids levels stay within an optimum range.

2.2 PPAR γ

PPAR γ is the master transcriptional regulator of adipogenesis and plays an important role in the process of lipid storage. Thus PPAR α and PPAR γ have opposing functions in the regulation of fat metabolism; PPAR α promotes utilization while activation of PPAR γ promotes storage. A number of naturally occurring fatty acids and prostanoids have been shown to act as PPAR γ agonists, however, perhaps most importantly was the identification that the TZD class of insulin sensitizing drugs including rosiglitazone (Avandia) and pioglitazone (Actos) are PPAR γ agonists. Fatty acid accumulation in insulin sensitive tissues such as liver and skeletal muscle has been shown to promote insulin resistance. Activation of PPAR γ in adipose has been proposed to increase the number of adipocytes and promote the relocation and storage of fat in adipose, protecting peripheral tissues from lipotoxicity. Consistent with this idea is the observation that selective knockout of PPAR γ in adipose eliminates the therapeutic activity of TZDs in mice and that a common side effect of TZD in treatment in humans is weight gain due to an increase in adipose mass.

The TZDs have proven to be effective drugs for improving insulin sensitivity and treating type II diabetes. Nevertheless, they are not without problems. Troglitazone, the first TZD in the clinic, was taken off the market because of cases of drug-induced liver damage. Additionally recent meta-analyses have indicated that treatment with rosiglitazone is associated with increased risk of myocardial infarction and deaths due to cardiovascular events. Pioglitazone treatment was also shown to be associated with an increase in serious heart failure although a significantly lower risk of myocardial infarction and death was observed in this patient population. Finally, muraglitazar an investigational drug that is a dual agonist of PPAR α and PPAR γ was found to be associated with an increase in major cardiovascular events and increased incidence of death. The increases in cardiovascular events and mortality seen with these drugs are relatively small. Nevertheless, the wide scale use of PPAR γ agonists in the type II diabetic population has raised serious concerns about the safety of these drugs for long term therapy (see section 4).

The molecular basis underlying the increase in cardiovascular events is not clear. Changes in energy metabolism mediated by PPARs could significantly influence cardiac function. Several studies have also identified edema as a side effect of TZD treatment that could impact heart function. Regulation of the epithelial sodium channel (ENaC γ) in the kidney by PPAR γ has been suggested as potential mechanism behind the TZD-dependent edema but it

remains to be seen if inhibiting this channel will decrease the cardiovascular events associated with TZD treatment.

2.3 PPAR δ

Genetic knockout of PPAR δ results in a number abnormalities including embryonic lethality resulting from placental defects, decreased adipose mass, myelination deficiencies, altered inflammatory responses, and impaired wound healing. More recent studies utilizing tissue-specific transgenes and synthetic agonists, however, have uncovered important functions for this receptor in the control of metabolism.

In vitro studies indicate that fatty acids as well as eicosanoids including prostaglandin A1 and carbaprostacyclin function as PPAR δ agonists. Very low density lipoprotein (VLDL) particle associated fatty acids have also been demonstrated to induce PPAR δ target genes in a receptor-dependent manner. Further support for a role of PPAR δ in lipoprotein metabolism results from studies exploring the activity of the PPAR δ -specific synthetic agonist GW501516. Treatment of animals, including, non-human primates with GW501516 significantly increases high density lipoprotein (HDL) particles, lowers triglycerides and low density lipoprotein (LDL) particles and decreases fasting insulin levels. Mechanistic studies point to regulation of the gene encoding the ATP binding cassette transporter ABCA1 by PPAR δ as important step in the control of HDL levels. ABCA1 functions as a cholesterol transporter to transfer cholesterol out of cells to HDL particles (see section 3.1). PPAR δ mediated down regulation of intestinal cholesterol absorption via regulation of the gene encoding Niemann-Pick C1-like protein 1 (NPC1L1), a cholesterol transporter that is the target of the cholesterol lowering drug ezetimide (Zetia), has also been suggested to contribute to lipid lowering by PPAR δ .

Transgenic approaches utilizing constitutively active forms of PPAR δ that activate transcription in the absence of agonists (VP16-PPAR δ) have been used to examine the role of PPAR δ in specific tissues. In both adipose and muscle over expression of VP16-PPAR δ produced a dramatic increase in the β -oxidation of fatty acids. In adipose the increase in fat oxidation leads to a decrease in adipose mass and protection from diet-induced obesity and insulin resistance. The protection against diet-induced obesity results, at least in part, from increased thermogenesis in brown fat secondary to the induction of genes involved in β -oxidation and the uncoupling of oxidative phosphorylation from ATP production. Weight loss was also observed in obese mice treated with the synthetic agonist GW501516 suggesting that the same PPAR δ -dependent pathways can be activated pharmacologically. Significant changes in body weight, however, were not detected when obese Rhesus monkeys were treated for 4 months with GW501516. Species-dependent differences in the bioavailability, tissue distribution, and/or efficacy of GW501516 may account for the differences on adipose mass between rodents and primates.

In skeletal muscle over expression of VP16-PPAR δ induces genes involved in β -oxidation, mitochondrial respiration and increases the proportion of slow twitch oxidative muscle fibers. The muscles of these animals become fat burning machines that allow the transgenic mice to run on a treadmill for significantly longer times than controls. In contrast to the result observed with the constitutively active VP16-PPAR δ construct, activation of endogenous PPAR δ with the synthetic agonist GW501516 did not increase endurance. When agonist treatment was coupled with a minimal exercise regimen, however, the combination of drug + exercise produced a significantly larger increase in running time compared to exercise alone. Exercise is known to activate AMP kinase and pharmacologic activation of AMP kinase is sufficient by itself to improve endurance in sedentary mice. Importantly the endurance promoting activity of an AMP kinase activator is lost in *Ppar δ ^{-/-}* mice. In cell culture experiments activated AMP kinase increases the transcriptional activity

of PPAR δ at least in part by phosphorylation of the peroxisome proliferator activated receptor γ coactivator 1 α (PGC-1 α), a transcriptional coactivator that directly interacts with PPAR δ . Taken together these studies suggest that exercise activated AMP kinase increases the transcriptional activity of PPAR δ leading to expression of a genetic network involved in the specification of slow twitch oxidative muscle fibers and improved endurance.

Synthetic PPAR δ ligands have proven to be very effective in preclinical models of diabetes and GW501516 was taken into clinic for the treatment of dyslipidemia in 2006. Results of a 2 week phase II study in patients with dyslipidemia demonstrated that total cholesterol, LDL cholesterol, triglycerides and nonesterified fatty acids were significantly lowered by GW501516. In contrast to the study in Rhesus monkeys, no changes in HDL were observed. Metabolex has also reported positive phase II data with another PPAR δ agonist MBX-8025.

2.4 PPARs and Inflammation

Pharmacologic or genetic inhibition of pathways that underlie inflammatory responses protect experimental animals from diet-induced insulin resistance as well as atherosclerosis. In both diseases inflammatory responses mediated by macrophages appear to be crucial for disease progression. Anti-inflammatory activity is a property shared by the 3 PPAR subtypes and occurs by inhibition of the transcriptional activity of the pro-inflammatory transcription factors activator protein 1 (AP-1) and nuclear factor kappa B (NF κ B). A number of mechanisms have been proposed for this process, termed transrepression, including direct interactions between PPARs and the p65 subunit of NF κ B, induction of the inhibitor of kappa B alpha (I κ B α), regulation of c-Jun N-terminal kinase (JNK) activity, competition for limiting transcriptional coactivators and corepressors, and inhibition of corepressor clearance from NF κ B regulated promoters. The contribution of chronic inflammation to metabolic disease has led to the idea that the anti-inflammatory activity of the PPARs, particularly in macrophages, may contribute to the beneficial effects of PPAR ligands in animal models of atherosclerosis and insulin resistance. This hypothesis is yet to be tested with either PPAR ligands or PPAR mutants that dissociate the process of activation of transcription from the process transrepression. Nevertheless, recent studies discussed below have indicated that macrophages may be critical sites of action for the activity of PPARs.

Resident macrophages in tissues display significant heterogeneity. In obesity classically activated macrophages, also called M1 macrophages, accumulate in adipose and play a role in mediating an inflammatory response that contributes to insulin resistance. In lean animals most adipose associated macrophages display an alternatively activated or M2a phenotype. Alternatively activated macrophages are less inflammatory and appear to play roles in tissue repair. Energy utilization also differs between these two populations of macrophages. Classically activated macrophage predominately use glucose while a switch to oxidative metabolism is an integral component of alternative activation; linking metabolic control to macrophage phenotype and inflammation. Alternative activation is induced by IL-4 and IL-13 and studies indicating that both PPAR γ and PPAR δ are induced in IL-4/IL-13 treated macrophages promoted a number of investigators to examine the role of macrophage PPARs in models of diet-induced obesity.

Odegaard et al. and Hevener et al. used selective knockouts and bone marrow transplantations to delete PPAR γ in macrophages and observed glucose intolerance and increased insulin resistance in mice exposed to a high fat diet. The Odegaard et al. study specifically explored macrophage phenotype and determined that alternative activation was impaired, suggesting that insulin resistance observed in the absence of PPAR γ results from increased inflammation mediated by M1 type macrophages. Hevener et al. additionally demonstrated that the therapeutic activity of rosiglitazone was compromised when

macrophage PPAR γ was selectively eliminated, indicating that the anti-inflammatory activity of PPAR γ contributes to the therapeutic activity of TZDs.

Using macrophage selective knockouts similar to those described for PPAR γ , Kang et al. and Odegaard et al. demonstrated that PPAR δ is required for regulating the gene expression program specifying alternative activated (M2a) macrophages. The consequences of macrophage PPAR δ deletion are impaired glucose tolerance and an exacerbation of insulin resistance in response to a high fat diet. Based on these studies it appears that both PPAR γ and PPAR δ play important roles in establishing the alternative activated phenotype. It has been suggested that both receptors must have both distinct roles since knockout of either sub-type alone is sufficient to impair alternative activation. Nevertheless, the exact function of each receptor in the alternative activation pathway remains to be determined.

The macrophage-selective knockout experiments described above suggest that the anti-inflammatory activity of the PPARs may contribute to their therapeutic activity. Several investigators have gone a step further and suggested that specifically targeting PPARs in macrophages with tissue-selective small molecules may be a novel and effective method for treating metabolic disease. The enthusiasm for such approaches, however, must be tempered with the realization that other factors including genetic background, diet, and environment may contribute to the knockout phenotypes. In a separate study Marathe et al. used bone marrow transplantation approaches to selectively knockout PPAR γ and PPAR δ in hematopoietic cells either individually or together. These authors concluded that in C57BL/6 mice that the two receptors have little or no impact on the development of diet-induced obesity and insulin resistance and that rosiglitazone is effective in the absence of macrophage PPAR γ . Clearly the contribution of macrophage PPARs to the pathology of metabolic disease and the beneficial activity of PPAR agonists remains to be determined.

3. LXRs

The LXR sub-group of the nuclear receptor superfamily is comprised of two sub-types, LXR α (NR1H3) and LXR β (NR1H2) that are encoded by separate genes. The founding member of the sub-group LXR α , was originally cloned from a liver cDNA library, hence the name liver X receptor, and found to be highly expressed in the liver, kidney, and intestine. In contrast, LXR β is more ubiquitously expressed. Both LXRs bind to DNA and regulate transcription as heterodimers with RXRs serving as the common heterodimeric partner with preferred binding to direct repeats of the nuclear receptor half-site AGGTCA separated by 4 nucleotides (DR4). The first link between LXR and lipid metabolism came from the identification of cholesterol derivatives including 22(R)-hydroxycholesterol, 24(S)-hydroxycholesterol, and 24(S),25-epoxycholesterol as ligands that directly bind to both LXR α and LXR β and increase their transcriptional activity. More recent studies using knockout mice that cannot synthesize oxysterols have provided strong support that these cholesterol derivatives function as LXR ligands *in vivo*. The identification of hydroxycholesterols as natural LXR ligands prompted studies to identify the genetic networks controlled by LXR in tissues and cells that play important roles in regulating cholesterol metabolism.

3.1 Regulation of Macrophage Reverse Cholesterol Transport by LXR

Gene expression profiling of LXR agonist-treated mice identified ABCA1 as a direct LXR target gene and this discovery stimulated great interest in the therapeutic potential of LXR agonists given the connections between ABCA1, HDL metabolism and atherosclerosis. ABCA1 is required for the process of reverse cholesterol transport whereby cells efflux internal cholesterol to acceptor proteins on pre- β -HDL particles. Loss of functional ABCA1 results in Tangier disease, a condition in which patients have extremely low levels of

circulating HDL and an increased risk for developing atherosclerosis. Examination of fibroblasts isolated from subjects with Tangier disease reveals that ABCA1 defective cells are unable to efflux cholesterol, suggesting that the low HDL levels and increased risk of atherosclerosis results from a loss of reverse cholesterol transport. Historically Tangier disease patients present with large accumulations of cholesterol-laden macrophages in their lymph tissues, highlighting the role of ABCA1 and reverse cholesterol transport in macrophage cholesterol homeostasis. The accumulation of oxidized LDL cholesterol by macrophages in the arterial wall is an initiating step in the development of atherosclerotic lesions and recent studies with mouse knockouts of ABCA1 further support a link between reverse cholesterol transport and atherosclerosis. In support for the role of LXR as a direct regulator of ABCA1 expression and activity, treatment of primary macrophages or cell lines with LXR agonists results in induction of ABCA1 and an increase in cholesterol efflux. Importantly a binding site for LXR-RXR heterodimers in the ABCA1 promoter has also been described. Subsequent studies identified other proteins involved in the reverse cholesterol transport including ABCG1, ABCG4, and apoE as direct LXR target genes. Interesting genetic deletion of LXR activity in mice (*Lxra*^{-/-}/*Lxrβ*^{-/-}) results in the accumulation of cholesterol-laden macrophages and splenomegaly similar to that observed in Tangier disease patients.

3.2 LXR and atherosclerosis

The accumulation of oxidized LDL cholesterol by macrophages in blood vessel walls is an early event in the pathogenesis of atherosclerosis and it had long been suggested that reversing this process by pumping cholesterol out of macrophage foam cells would have an inhibitory effect on the progression of atherosclerosis. The identification of LXR as a regulator of reverse cholesterol transport in macrophages allowed this hypothesis to be tested. Transplantation of lethally irradiated *apoE*^{-/-} and *Ldlr*^{-/-} mice with bone marrow from wildtype or *Lxra*^{-/-}/*Lxrβ*^{-/-} mice demonstrated that genetic deletion of LXR in hematopoietic cells leads to an increase in atherosclerosis in these well established mouse models. Additionally, treatment of *apoE*^{-/-} and *Ldlr*^{-/-} mice with synthetic LXR agonists leads to a reduction in atherosclerosis. Together the combination of genetic analysis and pharmacology clearly demonstrated the anti-atherogenic activity of LXR. Subsequent studies combining bone marrow transplantation with the administration of synthetic LXR agonists have demonstrated that LXR activity in macrophages is necessary for the anti-atherogenic effect of LXR ligands.

The ability of LXRs to regulate reverse cholesterol transport provides a straight forward explanation for the anti-atherogenic activity of LXR agonists. Experiments using cultured macrophages, however, demonstrate that LXR agonists can also inhibit the expression of several pro-inflammatory genes including iNOS, COX-2, and MMP-9 and these compounds are effective in a murine model of irritant contact dermatitis. Molecular studies indicate that activation of LXR decreases the transcriptional activity of NFκB using many of the same mechanisms described for the PPARs (see section 2.4). Along with anti-inflammatory activity, activation of LXR in macrophages has also recently been shown to reduced endoplasmic reticulum (ER) stress in response to oxidized lipids via up-regulation of fatty acid synthesis (see section 3.3).

Since elevated macrophage cholesterol levels, inflammation, and ER stress have all been shown to contribute to atherosclerosis it is not clear if one or all of these LXR-regulated pathways contribute to the anti-atherogenic activity of LXR ligands (Figure 1). Future studies that combine genetically altered macrophages (e.g. *Abca1*^{-/-}) introduced by bone marrow transplantation along with the administration of LXR agonists can be used to define the individual contributions of these different activities. We believe that such studies will be

critical for development of small molecule LXR ligands for the treatment of cardiovascular disease (see section 4).

3.3 Regulation of Hepatic Lipid Metabolism by LXR

When challenged with a diet rich in cholesterol *Lxra*^{-/-} mice accumulate massive amounts of cholesterol in the liver. Molecular analysis uncovered aberrant regulation of several genes involved in lipid and cholesterol metabolism including Cyp7a1, which encodes cholesterol 7 α hydroxylase, the rate-limiting enzyme in the conversion of cholesterol to bile acids. Subsequently, the ATP binding cassette transporters ABCG5 and ABCG8 which move cholesterol out of the liver and into the lumen of the intestine were identified as LXR target genes. Thus an increase in hepatic cholesterol levels is predicted to lead to an elevation in the concentration of cholesterol-derived LXR ligands resulting in the catabolism of cholesterol to bile acid and the excretion of cholesterol out of the liver (Figure 4). In mice pharmacological activation of LXR has been shown to result in net movement of cholesterol out of the body, a result consistent with the gene expression and genetic knockout studies described above. In concert with the elimination of hepatic cholesterol, activation of LXR also decreases cholesterol uptake by inducing expression of the Inducible Degradator of the LDL receptor (Idol). Over expression of Idol in cell culture models significantly enhances the ubiquitination and proteasome-dependent degradation of the LDL receptor. Similarly, over expression of Idol in the livers of mice lowers the level of LDL receptors and leads to increases in plasma cholesterol. Importantly, at least one structural class of LXR agonists have been shown to elevate LDL cholesterol levels in non-human primates raising the possibility that the LXR-Idol pathway will have important implications for strategies targeting the LXRs for drug development. Additionally, Idol is expressed in macrophages where it may also affect cholesterol uptake. Since the uptake of oxidized forms of LDL cholesterol by macrophages is thought to be primarily mediated by scavenger receptors it is not clear if Idol will directly influence macrophage foam cell formation and atherosclerosis.

Along with effects on cholesterol metabolism activation of LXR agonists also increases expression of genes involved in fatty acid metabolism including the master transcriptional regulator of fatty acid synthesis, the sterol response element binding protein 1c (SREBP1c) (Figure 2). Additionally, several of the genes encoding the enzymes involved in fatty acid metabolism including fatty acid synthase (FAS) and stearoyl CoA desaturase 1 (SCD-1) are regulated directly or indirectly by LXR. The coordinate up-regulation of fatty acid synthesis with reverse cholesterol transport is most likely to provide lipids for the transport and storage of cholesterol. Nevertheless, the elevations in plasma cholesterol and triglyceride levels seen in LXR agonist-treated animals have severely impaired the movement of first generation LXR agonists into the clinic.

3.4 LXR and Diabetes

Interestingly, along with up-regulating fatty acid synthesis, activation of LXR also represses expression of the genes encoding the enzymes of gluconeogenesis in the liver including phosphoenolpyruvate carboxy kinase (PEPCK) and glucose 6-phosphatase and induces expression of GLUT4 in adipose tissue. Thus in many ways activation of LXR mimics treatment with insulin. Perhaps not surprisingly in light of this “insulin-like” activity, LXR ligands decrease hepatic glucose output and lower blood glucose levels in animal models of type II diabetes. The observation that LXR ligands can behave as insulin sensitizers even in face of relatively large increases in plasma triglyceride levels suggests the possibility of a broader role for LXR in regulating metabolism beyond the control of lipid levels. Interestingly a number of studies have demonstrated that LXRs, particularly LXR β , are expressed in pancreatic beta cells. In beta cells ABCA1 and ABCG1 have been shown to facilitate insulin secretion and LXR activation has been shown to indirectly lead to increases

in expression of the insulin gene. Thus in beta cells activation of LXR should promote insulin secretion. The observation that LXRs are also active in skeletal muscle and that *Lxra*^{-/-}/*Lxrβ*^{-/-} mice are resistant to diet-induced obesity further supports a role for the LXRs as important coordinators of energy metabolism. Despite these suggestive observations of LXR activity in adipose, pancreas and muscle a hyperinsulinemic-euglycemic clamp study in high-fat-fed insulin-resistant rats indicated, at least in this model, that the primary anti-diabetic effect of LXR ligands is the suppression of hepatic gluconeogenesis.

4. Therapeutic Potential of the Metabolic Nuclear Receptors

The anti-atherogenic, anti-inflammatory, and anti-diabetic activities of the PPARs and LXRs highlight the therapeutic potential of small molecules that regulate the activity of the metabolic nuclear receptors. Nevertheless, the experience with these two subclasses of the nuclear receptor family also illustrates the challenges in targeting receptors that regulate metabolism. The fibrates and TZDs, agonists of PPARα and PPARγ respectively, have for the most part proven to be safe and effective in humans. The cardiac toxicity observed with the TZDs and with the PPARα/γ dual agonist muraglitazar, however, has called into question the long term safety of these drugs. Importantly, numerous more potent and efficacious PPARα, PPARγ, dual PPARα/γ, and PPAR pan agonists have failed in pre-clinical studies or clinical trials for safety issues. In particular drug-related increases in tumors have been observed in mice and rats treated with this new generation of PPAR agonists and the FDA now requires 2 year rodent carcinogenicity studies prior to the initiation of clinical trials longer than 6 months. In retrospect the fibrates and TZDs are relatively weak agonists. Returning to the idea that proper metabolic control requires that the activity of opposing pathways to be in balance, it may be that these more potent and efficacious PPAR ligands shift this balance back too far in the one direction, uncovering unwanted and unexpected side effects. In contrast relatively weaker agonists may provide a more subtle effect that effectively rebalances pathways that are perturbed by metabolic disease.

The ability of LXR agonists to reduce atherosclerosis even in the face of significant ligand-induced increases in plasma lipids has stimulated great interest in the LXRs as new drug targets for cardiovascular disease. Treatment of patients with a drug that raises lipids, however, is not a viable option for the treatment of metabolic diseases and approaches to separate the beneficial activities of LXR ligands from unwanted side effects need to be explored. Furthermore, studies in human cells have shown that LXR agonists also increase expression of the gene encoding the cholesterol ester transfer protein (CETP). CETP functions to transfer cholesterol esters from HDL to apolipoprotein B containing lipoprotein particles and CETP activity has been shown to inversely correlate with atherosclerosis. Indeed CETP inhibitors are currently being explored for the treatment of atherosclerosis.

Interestingly, defects in hepatic cholesterol metabolism are detected in *Lxra*^{-/-} single knockout mice indicating that LXRβ is not functionally redundant with LXRα. In contrast, cholesterol and triglyceride levels appear normal in *Lxrβ*^{-/-} mice suggesting that LXRα mediates most, if not all, of the effects of LXR ligands on triglyceride metabolism. The relatively low level of LXRβ in the liver most likely accounts for lack of functional redundancy in this tissue. Nevertheless, in macrophages either LXRα or LXRβ alone appears to be sufficient to mediate the effects of LXR ligands on reverse cholesterol transport and inflammatory gene expression. Additionally a recent study by Bradley et al. indicates that the anti-atherogenic activity of LXR agonists are maintained in *apoE*^{-/-}/*Lxra*^{-/-} double knockout mice, suggesting that LXRβ alone is sufficient to limit atherosclerosis. Taken together these observations have led several investigators to suggest

that LXR β -selective ligands may provide a mechanistic basis for identification of LXR ligands with improved therapeutic profiles. The enthusiasm for LXR β -selective ligands must be countered with the realization that the spectrum of activities measured in the complete absence of LXR α activity may differ when a sub-type selective synthetic ligand is used. Additionally, the observation that the ligand binding pockets of LXR α and LXR β defined by crystallography differ by only one amino acid suggests that identification of selective ligands may not be simple.

Importantly, the function of the LXRs in the liver and in macrophages differs. The liver is essentially always exposed to cholesterol (and LXR ligands) so in this tissue LXRs function as a rheostat to fine tune cholesterol uptake and excretion in response to small changes in cholesterol levels. Macrophages, in contrast, respond to cholesterol levels acutely. In this case LXRs function as on-off switches repressing gene expression when cholesterol is absent and activating when cholesterol is elevated; for instance when a macrophage engulfs an infected cell. Given these tissue-specific differences one might predict that once again a relatively weak agonist would provide a beneficial therapeutic profile. Such a compound would most likely have a significant effect in macrophages (going from no activity to some activity) while having little or no biological effect in the liver. Indeed the Wyeth group has described such a partial agonist, WAY-252623, that retains anti-atherogenic activity in animal models but limits lipid elevations in animals and in humans. Unfortunately the clinical development of this compound has been discontinued due to unexpected neurological activity in the clinic.

Clearly the experience with the PPARs and LXRs indicate that small molecules targeting the metabolic nuclear receptors will need to exhibit a restricted set of activities that allow the separation of beneficial therapeutic activities from unwanted side effects. Perhaps the best examples of such compounds are the selective estrogen receptor modulators such as roloxifene that function as estrogen receptor agonists in some tissues and estrogen receptor antagonists in others. A common feature of all these selective receptor modulators is that they appear to function as partial or weak agonists when characterized *in vitro*. When bound to receptors selective modulators produce unique conformational changes that cannot be achieved by more typical agonists. The outcome of these unique conformations is an alteration in interactions between receptors and the down-stream coregulator proteins that mediate the transcriptional response leading to ligand-specific effects on gene expression. Future studies that define the biologically relevant receptor-coregulator interactions should pave the way for the rational identification of the next generation of nuclear receptor ligands.

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Figure 1. LXR activity in Macrophages

Activation of LXR in macrophages promotes reverse cholesterol transport via induction of ABCA1 and ABCG1, inhibits ER stress via activation of SREBP1c and SCD-1 and inhibits inflammation by repression of NFκβ.

Figure 2. LXR activity in the liver

Activation of LXR in the liver results in the up-regulation of ABCG5, ABCG8, and Cyp7A1 promoting the excretion and catabolism of cholesterol. Induction of Idol leads to enhanced degradation of the LDL receptor and decreased cholesterol uptake. Finally, induction of SREBP1c leads to increased fatty acid and triglyceride production.