Liver X receptors and cholesterol homoeostasis: spotlight on the adrenal gland

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Abstract

The LXRs (liver X receptors) (LXRα and LXRβ) are nuclear hormone receptors that are activated by oxysterols, endogenous oxidative metabolites of cholesterol. These receptors regulate an integrated network of genes that control whole body cholesterol and lipid homoeostasis. A brief overview of the mechanism of this regulation by LXRs in the liver, macrophage and intestine will be outlined, followed by data from our recent work demonstrating that LXRα is crucial in maintaining adrenal cholesterol homoeostasis. In the adrenal gland, oxysterols are formed as intermediates in the conversion of cholesterol into steroid hormones and can act as endogenous activators of LXR. We have found using both gain- and loss-of-function models that LXR acts to maintain free cholesterol below toxic levels in the adrenal gland, through the co-ordinated regulation of genes involved in cholesterol efflux [ABCA1 (ATP-binding-cassette transporter A1)], storage (sterol-regulatory-element-binding protein-1c and apolipoprotein E) and metabolism to steroid hormones (steroidogenic acute regulatory protein). Furthermore, we show that under chronic dietary stress, the adrenal glands of LXR-null mice (and not wild-type mice) accumulate free cholesterol. These results support the role of LXR as a global regulator of cholesterol homoeostasis, where LXR provides a safety valve to limit free cholesterol in tissues experiencing high cholesterol flux.

Identification and characterization of the LXRs (liver X receptors)

The nuclear hormone receptor superfamily is a large family of transcription factors consisting of 48 members in the human genome [1]. The members of this protein family share a conserved zinc-finger DNA-binding domain, a less well-conserved ligand-binding domain and a C-terminal ligand-dependent activation function domain [2]. The activation function domain is important for the displacement of co-repressors and the recruitment of co-activators to the receptor upon ligand activation [3]. LXRα and LXRβ are members of this superfamily and share approx. 77% sequence identity to one another in both their DNA- and ligand-binding domains [4]. The LXRs were so named because they were cloned from a liver-derived cDNA library, had high liver expression and formed heterodimers with another member of the nuclear receptor superfamily, the RXR (retinoid X receptor) [5]. Both LXRα and LXRβ are broadly expressed, with LXRβ being ubiquitously expressed and LXRα most highly expressed in the liver, intestine, kidney, spleen, adipose, macrophage, muscle and adrenal gland [6].

Key words: adrenal gland, cholesterol, corticosterone, lipid metabolism, liver X receptor (LXR), nuclear receptor.

Abbreviations used: ABC transporter, ATP-binding-cassette transporter; ACAT, acyl-CoA: cholesterol acyltransferase; ApoE, apolipoprotein E; CYP7A1, cholesterol-7α-hydroxylase; HDL, high-density lipoprotein; LXR, liver X receptor; RCT, reverse cholesterol transport; RXR, retinoid X receptor; StAR, steroidogenic acute regulatory protein; SREBP-1c, sterol-regulatory-element-binding protein-1c.

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Although the LXRs were originally termed orphan receptors at the time of cloning because their ligands were unknown, they were considered 'adopted' when oxysterols were discovered to be their physiological ligands [7,8]. The most potent LXR oxysterol ligands are 22(*R*)-hydroxycholesterol, 24(*S*)-hydroxycholesterol and 24(*S*),25-epoxycholesterol [9]. The LXRs form obligate heterodimers with RXR and bind to specific DNA-response elements consisting of two hexanucleotide repeats (ideally AGGTCA) separated by a 4-nt spacer, termed a DR4 (direct repeat-4) [5]. These LXR-response elements appear throughout the genome and are responsible for the physiological responses mediated by the transcriptional activation of LXR by oxysterols.

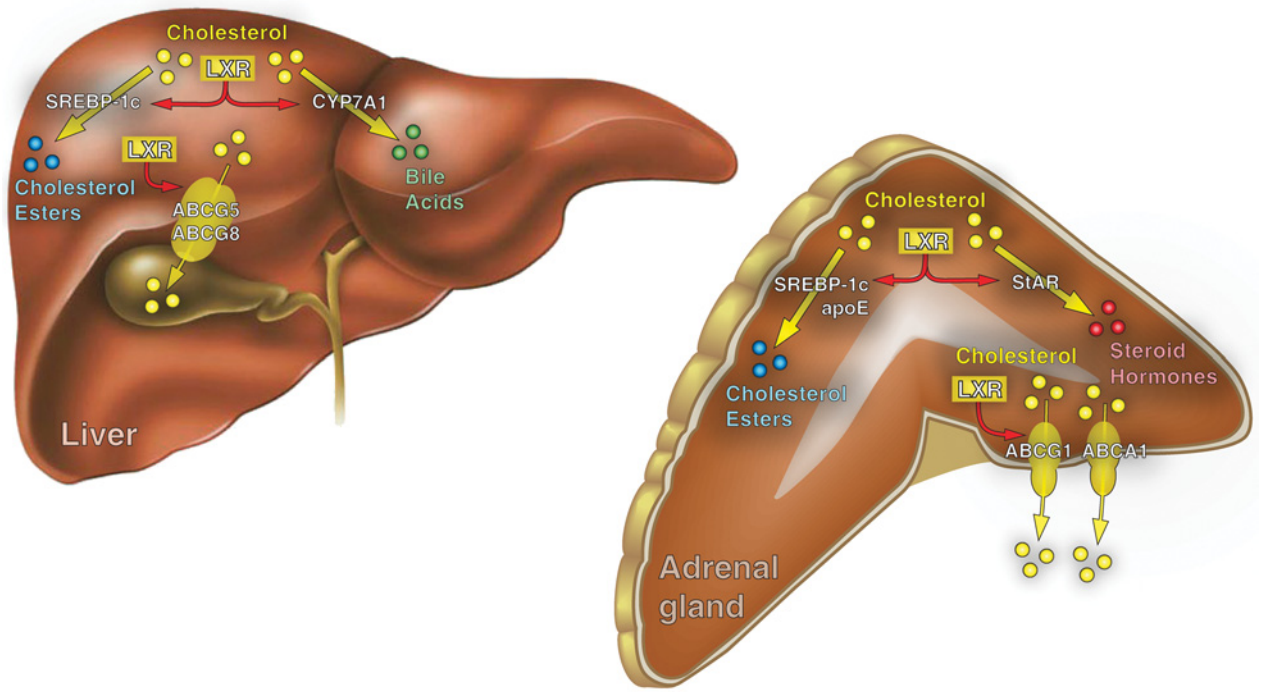
Discovery of a role for LXR in cholesterol homoeostasis

LXR in the liver

The creation of the LXRα-null mouse was critical for understanding the role of LXR in cholesterol homoeostasis. These mice, when fed a cholesterol-enriched diet, developed severe hepatomegaly and a light-coloured liver due to an increased build-up of liver cholesterol [10]. Furthermore, these mice had impaired bile acid metabolism, suggesting that LXR was important for maintaining the intracellular levels of cholesterol in the liver. An important target gene identified as part of this phenotype was CYP7A1 (cholesterol-7α-hydroxylase) [10]. CYP7A1 carries out the rate-limiting step in the conversion of cholesterol into bile acids (the major

Figure 1 | Complementary roles of LXR in the liver and the adrenal gland

In the liver, storage, efflux and metabolism to bile acids are all regulated by LXR. In the adrenal gland, similar pathways are regulated by LXR through a complementary set of genes: storage (SREBP-1c and ApoE), efflux (ABCA1 and ABCG1), and metabolism to steroid hormones (StAR).



metabolite of cholesterol in the liver). Bile acids are then actively transported across the bile canalicular membrane, providing a means of metabolizing and removing excess cholesterol from the liver [11]. Intriguingly, this effect is LXRα-specific because LXRβ was unable to compensate for the loss of LXRα in the single-knockout animal, and LXR$\beta^{-/-}$ mice did not exhibit the same phenotype upon dietary cholesterol challenge [12].

It was later discovered that both LXRα and LXRβ can regulate the key transcription factor responsible for the control of fatty acid synthesis, SREBP-1c (sterol-regulatory-element-binding protein-1c) [13]. This provided the first link between dietary cholesterol intake and triacylglycerol synthesis. It was postulated that this mechanism of increased fatty acid synthesis could allow for more co-substrate to be available for cholesterol esterification, providing a storage form of cholesterol in the liver.

It was recently discovered that two members of the ABC transporter (ATP-binding cassette transporter) superfamily are up-regulated in the liver by LXR ligands. ABCG5 and ABCG8 are co-ordinately induced upon LXR activation and form a functional heterodimer unit that actively transports cholesterol into the bile [14]. LXR is considered a cholesterol 'sensor' because oxysterols [for example, 24(*S*), 25-epoxycholesterol] are thought to accumulate in the liver with increasing concentrations of cholesterol, thereby initiating LXR activation. This mechanism provides a direct molecular method of eliminating excess cholesterol from the liver. The multiple transcriptional pathways regulated by LXR for maintaining cholesterol balance in the liver include (i) metabolism, (ii) storage and (iii) efflux (illustrated in Figure 1).

LXR in the intestine

Cholesterol enters the intestine from two sources: dietary cholesterol and cholesterol secreted from the bile into the upper duodenum. Activation by an LXR ligand causes the up-regulation of the transporters ABCG5, ABCG8 and ABCA1 in the intestine [14–16]. These transporters have been shown to play a role in limiting the extent of cholesterol (and plant sterol) absorption after activation. These findings further extend the role of LXR in the enterohepatic circulation, highlighting the co-ordinated effort between the liver and intestine to prevent excess cholesterol accumulation.

LXR in the macrophage

Cholesterol accumulation in peripheral tissues can be limited through a process termed RCT (reverse cholesterol transport). RCT is the process by which peripheral cholesterol is transferred to HDL (high-density lipoprotein)-cholesterol and shuttled back to the liver for further processing. RCT is particularly important in circulating macrophages to prevent the formation of foam cells (a pro-atherogenic step), and LXR is critical in mediating this response. The genes that have been shown to be involved in this process include the transporters

ABCA1 and ABCG1, both found to enhance cholesterol and phospholipid transport to lipoproteins [16–18]. Furthermore, ApoE (apolipoprotein E) has also been shown to be strongly up-regulated by LXR agonists in the macrophage, and the secretion of this protein helps to promote incorporation of cholesterol into the lipid-poor HDL-particles [19]. Treatment of atherogenic mouse models [such as the LDLR (low-density lipoprotein receptor)-knockout and ApoE$^{-/-}$] with LXR agonists significantly decreased the lesion area for atherosclerotic plaques, suggesting that LXR is a promising target for the treatment or prevention of atherosclerosis [20,21].

LXRs in adrenal cholesterol homoeostasis

Parallel pathways between the liver and adrenal gland

The liver is responsible for critical metabolic functions required for the processing and elimination of cholesterol. Similar to the liver, the adrenal gland contains many parallel pathways for cholesterol utilization. For example, both organs require cholesterol as the obligate precursor for the formation of their primary metabolites: in the liver, this is conversion into bile acids, and in the adrenal gland it is the formation of steroid hormones. Furthermore, both organs undergo high cholesterol flux, whether for maintaining RCT (liver), or during a stress response (adrenal gland). Because of these striking parallels, we undertook the investigation of the role of LXR in the adrenal gland.

Adrenal gland cholesterol flux

Cholesterol is primarily taken up by the murine adrenal gland through the interaction of ApoA1 in circulating HDL particles, with the scavenger receptor B1 localized on the plasma membrane [22,23]. Under basal or resting conditions, cholesterol delivered to the adrenal gland is primarily stored {by conversion into cholesteryl esters by the ACAT (acyl-CoA:cholesterol acyltransferase) enzyme [24]} or effluxed back to the circulation. Under conditions of acute stress, intracellular cholesteryl ester stores are rapidly mobilized and the selective uptake pathway is up-regulated to allow an abundant supply of cholesterol to meet the demands of steroid hormone production. Similarly, chronic stress (environmental or dietary) can also result in the sustained import of cholesterol to the adrenal gland. At the end of the stress response, the system must be reset to allow for basal conditions to return (in which cholesterol efflux and storage predominate).

Adrenal gland phenotype of LXR-null mice: chronic dietary stress

The adrenal glands of LXR$\alpha^{-/-}$ and LXR$\alpha/\beta^{-/-}$ animals are 30–40% heavier than those of wild-type mice [25]. The observed adrenomegaly is due to a build-up of cholesteryl esters in the LXR-null animals. Because this observation was made under normal chow-fed conditions, we tested the effect of chronic dietary stress on the adrenal phenotype by treating the animals with a Western-style (high-fat and high-cholesterol) diet. After 7 weeks of high-fat feeding, the LXR$\alpha/\beta^{-/-}$ adrenals were severely blanched and contained significantly more cholesteryl esters and free cholesterol compared with wild-type mice [25]. These results suggest that LXRα protects the adrenal gland locally against a build-up of excessive free and esterified cholesterol. Under basal conditions, LXR-α- and LXRα/β-null mice also had 2-fold higher circulating basal corticosterone levels compared with wild-type mice [25,26]. The increased glucocorticoid secretion was independent of dysregulation in the hypothalamic–pituitary–adrenal axis, since no difference in ACTH was observed between LXR genotypes and excessive glucocorticoid secretion was observed even from primary adrenal cell culture of LXR$\alpha/\beta^{-/-}$ mice [25]. Addition of an LXR agonist also significantly increased circulating levels of corticosterone [25,26]. The molecular basis for the observed adrenal phenotype of LXR-null mice is described below and summarized in Figure 1.

Cholesterol storage and efflux in the adrenal gland

Under basal conditions, LXR$\alpha/\beta^{-/-}$ animals expressed considerably less adrenal ABCA1 protein relative to wild-type mice. When treated with an LXR agonist, the wild-type mice could increase the expression of ABCA1 and ABCG1, but this effect was absent from LXR$\alpha/\beta^{-/-}$ mice [25]. These results suggest that the adrenomegaly caused by increased cholesteryl esters in LXR$\alpha/\beta^{-/-}$ mice was due to a deficiency of ABCA1 and the inability of the null mice to up-regulate the expression of ABCA1/ABCG1. In the absence of the efflux pathway, esterification by ACAT can compensate and convert the excess cholesterol into cholesteryl esters. Furthermore, we found that SREBP-1c and ApoE were both up-regulated in the adrenal gland of wild-type mice, but not LXR-null mice, when treated with LXR agonist. In a pathway analogous to that seen in the liver, the regulation of SREBP-1c may play a role in ensuring enough fatty acid co-substrate for the formation of the cholesteryl ester [13]. However, in contrast with the role of ApoE in the macrophage, adrenal gland ApoE is present in intracellular compartments and helps to sequester cholesteryl esters away from the steroidogenic pathway [27–29]. This function is thought to be important for maintaining cholesteryl ester stores under conditions of chronic stress [30].

Adrenal gland steroid hormone synthesis

A novel LXR target gene in the adrenal gland was identified during these studies. The StAR (steroidogenic acute regulatory protein) is positively regulated by LXR [25]. StAR transfers cholesterol from the outer to the inner mitochondrial membrane and this process represents the rate-limiting step in the conversion of cholesterol into steroid hormones [31,32]. Furthermore, the expression of StAR is basally derepressed in the LXR$\alpha/\beta^{-/-}$ mice, explaining the enhanced basal level of corticosterone in the LXR$\alpha/\beta^{-/-}$ animals

and the inducibility of corticosterone with LXR agonist. In summary, these findings tie together the paradoxical phenotype of the LXR-null mice in which both cholesterol storage and steroidogenesis were increased (loss of efflux pathway and de-repression of the steroidogenic pathway). These results suggest that LXR provides a safety mechanism to help maintain the appropriate compartmentalization of cholesterol under the dynamic range of conditions that exist in the adrenal gland, including the basal state and under chronic dietary stress. The parallel pathways of LXR function in the adrenal gland and liver are highlighted in Figure 1.

Perspectives

The nuclear hormone receptors represent attractive candidates for drug therapy because they are ligand-activated and easily targeted for small molecule discovery. The LXRs in particular have been proposed as molecular targets for the treatment of atherosclerosis, dyslipidaemia and diabetes [21,33,34]. However, as our understanding of the effects of LXR in various tissues expands, the requirement for the small molecule activator to have selectivity becomes increasingly important. For example, because LXR targets the regulation of SREBP-1c and results in the elevation of circulating triacylglycerols (an undesirable side effect), activation of this pathway must be avoided [13]. Furthermore, our recent studies in the adrenal gland suggest that treatment with LXR agonists may increase glucocorticoid levels through the activation of StAR [25]. Nevertheless, there continues to be optimism that selective LXR agonists can be obtained because of the successful development of other selective nuclear receptor ligands (such as the selective oestrogen receptor modulators).

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