Liver X receptor opens a new gateway to StAR and to steroid hormones

Colin R. Jefcoate

Commentary

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Liver X receptor opens a new gateway to StAR and to steroid hormones

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Liver X receptors (LXRs) broadly limit cholesterol accumulation by regulating expression of genes involved in cholesterol efflux and storage. In this issue of the *JCI*, Cummins et al. report that LXRα is involved in similar regulation in the adrenal cortex, but it also substantially modulates glucocorticoid synthesis (see the related article beginning on page 1902). LXRα deletion in mice increases the availability of adrenal cholesterol for steroid synthesis by decreasing the expression of cholesterol efflux transporters. Glucocorticoid synthesis requires intramitochondrial cholesterol transport mediated by the steroidogenic acute regulatory protein (StAR). Surprisingly, LXR deletion and stimulation by an agonist each increase glucocorticoid synthesis. This parallels increased expression of StAR and several other steroidogenic genes.

Liver X receptors stimulate expression of genes that lower cholesterol

Liver X receptors (LXRs) belong to a class of ligand-dependent nuclear receptors

that includes the PPAR family and receptors for vitamin D, thyroid hormone, and retinoic acid, which form heterodimers with retinoid X receptors (RXRs). In doing so, LXRs exert transcriptional control on cholesterol and fatty acid homeostasis in a variety of cell types. Two forms of the LXR have been identified: LXRα is expressed at high levels in liver but also at more modest levels in cells that are involved in cholesterol transport and metabolism (e.g., intestines, adipose, macrophages, kidney, and lung) while LXRβ is broadly expressed. LXRs are known to control the expression of genes involved in the transport of excess cholesterol from peripheral tissues to the liver (e.g., the ABC family of membrane transporters, including ABC transporter A1 [ABCA1], ABCG5, ABCG8, and ABCG1), as well as hepatic metabolism of this cholesterol by cytochrome P450 7A1 (CYP7A1) to bile acids (1). The roles of LXRs have recently been expanded through the study of mice deficient in LXRα and LXRβ as well as the use of the LXR agonist T090137 (T1317), which stimulates both receptors (1). Mice deficient in LXRα, LXRβ, or both receptors are fully functional, which indicates that the cholesterol transport processes and fatty acid changes regulated by these receptors play a modulating rather than essential role. The link between the LXRs and cholesterol homeostasis was firmly established by the finding that mice deficient in LXRα lose the ability to convert cholesterol to bile acids in the liver and as a result accumulate cholesterol esters in their liver when challenged with a cholesterol-rich diet (1). LXRs also have extensive extrahepatic functions, including regulation of fatty acid and cholesterol metabolism in macrophages — a critical process in the inflammatory response
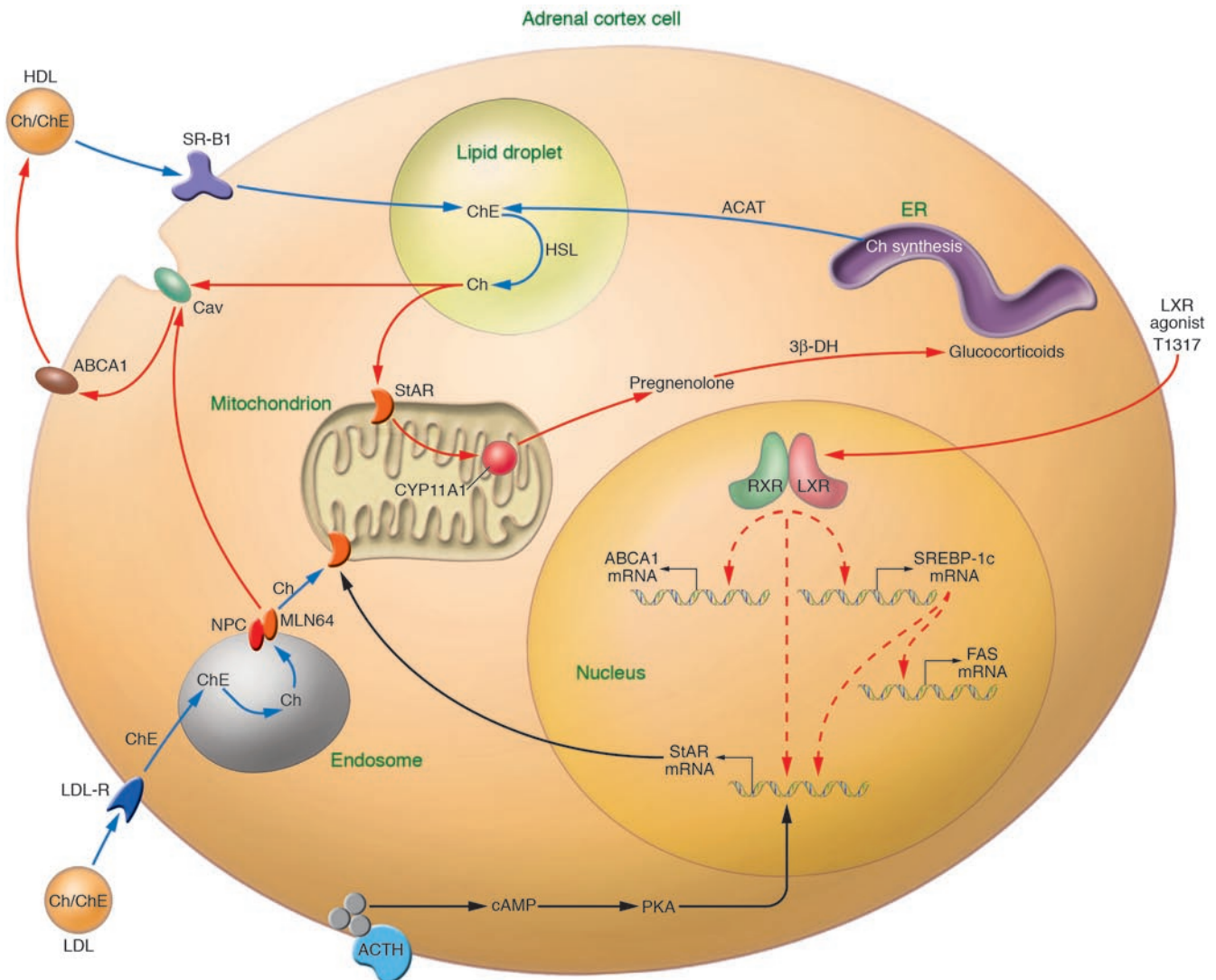
Figure 1
Effect of LXR on cholesterol trafficking in adrenal cortex cells. Cholesterol (Ch) enters adrenal cells through 2 routes that each delivers cholesterol to StAR for steroid synthesis: the LDL/endosome transfer pathway and the HDL/SR-B1/lipid droplet transfer pathway. Cholesterol leaves the cell by transfer from lipid droplets to caveolin (Cav) and then to the ABCA1 pump. LXR stimulates this pathway by increasing the level of ABCA1. Lipid droplets are very extensive in adrenal cells and comprise 95% cholesterol esters (ChE) due to high acyl-CoA:cholesterol acyltransferase (ACAT) activity. Cholesterol is transferred to StAR after activation of HSL, which hydrolyzes ChE to free cholesterol. The endosome transfer of cholesterol to StAR is mediated by the Nieman-Pick type C (NPC) transporter and the StAR-like protein MLN64. StAR mediates transfer of Ch to CYP11A1 inside the mitochondria, which initiates all steroidogenesis. In adrenals, 3β-DH generates progesterone and the activity of 3 cytochrome P450 enzymes (CYP11B1, CYP21, and CYP17) generates cortisol. LXR produces transcriptional changes by combining with RXR in the nucleus. Gene activation is shown as dashed lines. Some effects are mediated by the large increase in a second transcription factor, SREBP-1c, which is stored as an inactive precursor in the ER. StAR is a gene that is activated by both activated SREBP-1c and LXR. LXR-stimulated genes and pathways are highlighted in red. Other cholesterol transfer pathways are shown in blue. For further details, see ref. 3. LDL-R, LDL receptor.

and the development of atherosclerosis (1). A further link between LXR and cholesterol homeostasis was provided by the finding that LXRs are activated by binding a number of cholesterol derivatives formed by side-chain hydroxylation (22, 25, and 27 positions), which are formed at low levels in many tissues.

Cholesterol mobilization within adrenal cortex initiates stress-induced glucocorticoid synthesis
Cholesterol is also the precursor to all steroid hormones. The production of glucocorticoids in the adrenal cortex is associated with very high levels of cholesterol transport, lipoprotein receptors

(e.g., LDL receptor [LDL-R] and the HDL receptor, also known as scavenger receptor-B1 [SR-B1]), stored cholesterol esters, and enzymes that metabolize cholesterol (2). Steroidogenic cells and glial cells in the brain possess the distinctive cholesterol transport pathway that supports the conversion of cholesterol to preg-

nenolone (this steroid is the precursor to every steroid hormone) by CYP11A1. The cytochrome is attached to the inner surface of the inner mitochondrial membrane. The transfer of cholesterol to the inner mitochondrial membrane in steroidogenic cells commonly limits pregnenolone formation and the conversion of pregnenolone to progesterone by 3β-sterol dehydrogenase (3β-DH). However, adrenal mitochondria are specialized to manage cholesterol entry, as shown in Figure 1 (2). ACTH enhances the entry of cholesterol across the inner and outer mitochondrial membranes to be metabolized by CYP11A1 to pregnenolone. This unique cholesterol transfer step is mediated by StAR, which is a cAMP-activated cholesterol-binding protein (3).

In mice, cholesterol reaches the mitochondria after uptake of HDL cholesterol via SR-B1 and storage in lipid droplets, mostly as cholesterol esters. After stimulation of the cells by ACTH, activated hormone-sensitive lipase (HSL) mediates cholesterol ester hydrolysis to free cholesterol, which is then released from lipid droplets to the mitochondria. In humans, predominant is a second pathway in which LDL receptors pass cholesterol and cholesterol esters bound to LDL to the endosomes for hydrolysis (Figure 1). From there, the Nieman-Pick type C (NPC) cholesterol pump and the MLN64 cholesterol-binding protein (related to steroidogenic acute regulatory protein [StAR]) deliver cholesterol to the mitochondria. ACTH acutely increases free cholesterol levels by stimulating expression of the LDL-R, SR-B1 and both the expression and activation of HSL.

The efflux of cholesterol from the cell substantially determines the level of cholesterol and cholesterol esters stored in lipid droplets. Another cholesterol-binding protein, caveolin, organizes localized cholesterol-rich regions of the cell membrane. These caveolae play an important role in LXR-enhanced cholesterol export mediated by ABCA1 (4) (see Figure 1). It is notable that cholesterol entry into caveolae regulates the activity of many plasma membrane receptors, including the insulin receptor, that affect fatty acid and cholesterol homeostasis. To date, there has been no report of LXR effects on caveolae.

LXR modulates conversion of cholesterol to glucocorticoids

In this issue of the *JCI*, Cummins et al. extend the range of LXR activity to the adrenal cortex and steroid synthesis (5). The authors utilized mice with specific LXR deficiencies and the LXR agonist T1317 to show that LXRα limits free cholesterol accumulation in adrenal cortex cells by (a) increasing the expression of ATP-dependent pumps that remove cholesterol (e.g., ABCA1); (b) increasing the expression of apoE, which mediates cholesterol ester/LDL exchange; and (c) increasing the expression of SREBP-1c, a transcription factor that regulates genes contributing to fatty acid synthesis, including fatty acid synthase (FAS). They report that levels of cholesterol esters in adrenal cells of LXRα-deficient mice increased substantially in comparison with those of wild-type mice, consistent with the loss of function of the cholesterol efflux pathway.

Sustained expression of many other genes involved in cholesterol trafficking in the adrenal cells of LXRα-deficient mice shows that most of these genes do not respond to LXR, including those that code for the HDL receptor SR-B1, HMG-CoA reductase (the action of which is the rate-limiting step in cholesterol biosynthesis), and HSL.

This reorganization of cholesterol homeostasis induced by LXRα deficiency doubled basal conversion of cholesterol to corticosterone in vivo and produced an even greater increase in corticosterone synthesis in cultured adrenal cells (5). The increases in corticosterone level in vivo reported by Cummins et al. parallel increases in adrenal free cholesterol levels. Loss of ABC transporters reported here may limit the export response. However, surprisingly, the increase in corticosterone levels in vivo failed to produce the expected suppression of ACTH levels typical of the feedback control exerted by the hypothalamic-pituitary-adrenal axis. An even bigger surprise was that activation of LXR in cultured adrenal cells by T1317 produced large stimulations in corticosterone synthesis, comparable to the elevated synthesis in LXRα-deficient cells. This stimulation occurred even though T1317 might be expected to cause cholesterol depletion from the cells due to elevated contributions from the ABC efflux pumps.

LXRα enhances the expression of StAR and the conversion of cholesterol to steroid hormones

Cummins et al. (5) report several coordinated LXR-responsive changes in key steroidogenic genes that explain this anomaly. These authors find that StAR levels, as well as those of CYP11A1 and 3β-DH, which together generate progesterone, are similarly regulated by LXRα. Remarkably, each is elevated in adrenal cells both after stimulation by the LXR agonist T1317 and in cells of LXRα-deficient mice. These StAR and associated expression changes parallel the increases in glucocorticoid, suggesting that they are more important in this process than the total cell free cholesterol.

StAR is hormonally regulated in all steroidogenic tissues, often in proportion to cholesterol metabolism. The hormonal regulation (6) and mechanism of cholesterol intermembrane transfer (3) are complex. Loss of StAR in either humans or mice leads to dramatic increases in the level of cholesterol esters in adrenal cortex cells and associated adrenal hyperplasia, which arises secondary to a loss of glucocorticoids and a compensatory rise in ACTH (7).

The stimulation of StAR expression by LXR activation is explained by the identification by Cummins et al. of a new LXR response element in the StAR promoter (5). This DNA sequence binds the LXR/RXR heterodimer and mediates transcriptional activation. The authors note that the combination of suppression by basal LXRα and stimulation by T1317-activated LXR is not uncommon for LXR-responsive genes. However, the parallel responses of StAR, CYP11A1, and 3β-DH to LXRα depletion and activation suggest a shared transcriptional regulation by LXRα.

The large increase in SREBP-1c produced by LXR stimulation triggers further transcriptional responses that coordinate the crosstalk between cholesterol and fatty acids in cells. As shown in Figure 1, LXR and SREBP-1c can function in combination or separately. StAR, like several other genes that respond to LXRs, is also stimulated by direct promoter effects of SREBP-1c (8). The LXR/SREBP combination also integrates with the effects of ACTH/cAMP-dependent factors (CREB, SF1, GATA). This stimulation of StAR by LXR/SREBP1c counteracts the removal of cholesterol favored by LXR stimulation of ABCA1. This dual LXR/SREBP stimulation enhances the expression of several genes involved in fatty acid synthesis, notably stearoyl desaturase type 1 (SCD1), a contributor to cholesterol ester formation

(9). Other genes are regulated selectively by either LXR (e.g., ABCA1) or SREBP-1c (e.g., FAS). Dietary polyunsaturated fatty acids suppress both LXR and SREBP-1c but are also crucial to StAR expression (6). It is notable that the proteolytic activation of SREBP-1c, unlike that of other SREBPs, is regulated by insulin and probably not suppressed by cholesterol (10).

Little is known about the physiological ligands for LXR. However, there is evidence that mitochondrial cholesterol metabolism may be a source of these activators. StAR may therefore also participate as an activator of LXR activity. The steroid intermediate 22R-hydroxycholesterol, which is formed by CYP11A1, is a potent LXR agonist but is unlikely to be released from the mitochondrial cholesterol-cleavage process. However, StAR can also mediate cholesterol transfer to mitochondrial cholesterol hydroxylases that may generate LXR agonists. Other members of the StAR family — StARD4 and StARD5 — exhibit cholesterol transfer activity in steroidogenic cells and macrophages, respectively (11). Interestingly, elevation of these StAR relatives (and perhaps also of StAR) causes LXR activation, possibly by forming a hydroxysterol agonist. Hydroxysterols also stimulate StAR expression in steroidogenic cells (12), possibly through this new LXR mechanism.

This connection between LXR and StAR, introduced in this issue of the *JCI* by Cummins et al. (5), provides a new avenue for regulation of steroid synthesis. This may extend to other steroidogenic processes, including testosterone synthesis in the Leydig cells of the testis, estrogens in the ovary, and even neurosteroids produced in glial cells of the brain, each of which utilize StAR. It should be noted that cholesterol homeostasis in human adrenals is primarily mediated by LDL rather than HDL (13). It remains to be determined whether the LXR gateway to StAR and steroid synthesis remains open in tissues where cholesterol fluxes are less than in the adrenal or when the LDL pathway partially bypasses the lipid droplets (see Figure 1).

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New insights into the regulation of inflammation by adenosine

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Adenosine, long known as a regulator of cardiovascular function, has recently been identified as a significant paracrine inhibitor of inflammation that acts primarily by activation of A_{2A} adenosine receptors (A_{2A}ARs) on lymphoid or myeloid cells. In this issue of the *JCI*, Yang et al. describe a proinflammatory phenotype resulting from deletion of the gene encoding the A_{2B} adenosine receptor (A_{2B}AR) in the mouse, suggesting that activation of the A_{2B}AR can also have antiinflammatory effects (see the related article beginning on page 1913). Nevertheless, the role of the A_{2B}AR remains enigmatic since its activation can either stimulate or inhibit the release of proinflammatory cytokines in different cells and tissues.

There is growing interest in elucidating the mechanisms by which adenosine inhibits the immune system, since these inhibitory adenosine receptors and their downstream signaling pathways are promising targets for new antiinflammatory therapies. By signaling through the A_{2A} adenosine receptor (A_{2A}AR), adenosine suppresses the immune system, primarily by inhibiting lymphoid or myeloid cells including neutrophils (1), macrophages (2), lymphocytes (3, 4), and platelets (5). These responses are amplified by rapid induction of A_{2A}AR mRNA in macrophages and T lymphocytes in response to inflammatory or ischemic stimuli (2, 3, 6, 7). The A_{2B} adenosine receptor (A_{2B}AR) also appears to mediate antiinflammatory effects in macrophages by inhibiting the production of TNF-α and IL-1β, stimulating IL-10 and inhibiting macrophage proliferation (8–11) (Figure 1A). Macrophage A_{2B}AR signaling increases during