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Steroid hormone synthesis in mitochondria

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ABSTRACT

Mitochondria are essential sites for steroid hormone biosynthesis. Mitochondria in the steroidogenic cells of the adrenal, gonad, placenta and brain contain the cholesterol side-chain cleavage enzyme, P450scc, and its two electron-transfer partners, ferredoxin reductase and ferredoxin. This enzyme system converts cholesterol to pregnenolone and determines net steroidogenic capacity, so that it serves as the chronic regulator of steroidogenesis. Several other steroidogenic enzymes, including 3β-hydroxysteroid dehydrogenase, 11β-hydroxylase and aldosterone synthase also reside in mitochondria. Similarly, the mitochondria of renal tubular cells contain two key enzymes participating in the activation and degradation of vitamin D. The access of cholesterol to the mitochondria is regulated by the steroidogenic acute regulatory protein, StAR, serving as the acute regulator of steroidogenesis. StAR action requires a complex multi-component molecular machine on the outer mitochondrial membrane (OMM). Components of this machine include the 18 kDa translocator protein (TSPO), the voltage-dependent anion chanel (VDAC-1), TSPO-associated protein 7 (PAP7, ACBD3), and protein kinase A regulatory subunit 1α (PKAR1A). The precise fashion in which these proteins interact and move cholesterol from the OMM to P450scc, and the means by which cholesterol is loaded into the OMM, remain unclear. Human deficiency diseases have been described for StAR and for all the mitochondrial steroidogenic enzymes, but not for the electron transfer proteins or for the components of the cholesterol import machine.

1. Introduction

Six classes of steroid hormones, all of which are indispensable for mammalian life, are made from cholesterol via complex biosynthetic pathways that are initiated by specialized, tissue-specific enzymes found in mitochondria. These hormones include glucocorticoids (cortisol, corticosterone) and mineralocorticoids (aldosterone) produced in the adrenal cortex; estrogens (estradiol), progestins (progesterone) and androgens (testosterone, dihydrotestosterone) produced in the gonads; and calciferols (1,25-dihydroxy vitamin D [1,25OH_2D]) produced in the kidney. The biosynthesis of the steroid hormones (Miller and Auchus, 2011) and of 1,25OH_2D (a sterol) (Feldman et al., 2013) from cholesterol have been reviewed recently. There are two specialized aspects to the mitochondria of these steroidogenic tissues – the specialized mechanisms by which cholesterol is delivered to the mitochondria and the specialized intra-mitochondrial enzymes that paricipate in the synthesis of hormonal steroids.

2. Delivery of cholesterol to mitochondria

2.1. Sources of cholesterol

The intracellular transport and distribution of cholesterol prior to its delivery to the mitochondria has been reviewed recently (Miller and Bose, 2011). Cholesterol may be produced *de novo* from acetate via a complex pathway primarily found in the endoplasmic reticulum (ER) (Porter and Herman, 2011), but most steroidogenic

Abbreviations: 1,25$(OH)_2$D, 1,25 dihydroxy vitamin D (calcitriol); 3βHSD, 3β-hydroxysteroid dehydrogenase; ACAT, acyl transferase; ACTH, adrenocorticotropic hormone; ANT, adenine nucleotide transporter; ER, endoplasmic reticulum; CRAC, cholesterol recognition amino acid consensus domain; FAD, flavin adenine dinucleotide; HDL, high density lipoproteis; HSL, hormone-sensitive neutral lipase; HMGCoA, 3-hydroxy-3-methylglutaryl co-enzyme A; IMM, inner mitochondrial membrane; IMS, intramembranous space; Km, Michaelis constant; LAL, lysosomal acid lipase; LDL, low-density lipoproteins; LH, luteinizing hormone; MENTAL, MLN64N-terminal; MENTHO, MLN64N-terminal domain homologue; MLN64, metastatic lymph node clone 64; NADPH, nicotinamide adenine dinucleotide phosphate; NPC, Niemann Pick type C; OMM, outer mitochondrial membrane; PAP7, TSPO-associated protein 7 (ACBD3); PBR, peripheral benzodiazepine receptor; PCP, phosphate carrier protein; PKA, protein kinase A; PKAR1A, protein kinase A regulatory subunit 1α; PRAX1, TSPO-associated protein 1; PTH, parathyroid hormone; P450scc, mitochondrial cytochrome P450 specific for cholesterol side-chain cleavage; SF1, steroidogenic factor 1; SOAT, sterol O-acetyltransferase; SR-B1, scavenger receptor B1; StAR, steroidogenic acute regulatory protein; START, StAR-related lipid transfer domain; SREBPs, sterol regulatory element binding proteins; TSPO, 18 kDa translocator protein; VDAC1, voltage-dependent anion channel.

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cholesterol is derived from circulating lipoproteins. High density lipoproteins (HDLs) may be taken up via scavenger receptor B1 (SR-B1) and low-density lipoproteins (LDLs) are taken up by receptor-mediated endocytosis via LDL receptors. LDL can suppress the rate-limiting enzyme in cholesterol synthesis, 3-hydroxy-3-methylglutaryl co-enzyme A (HMGCoA) reductase. Rodents preferentialy use the HDL/SR-B1 pathway to obtain steroidogenic cholesterol, but the principal human source is receptor-mediated endocytosis of LDL. Nevertheless, patients with congenital abetalipoproteinemia have low LDL cholesterol but have normal basal cortisol concentrations, and only mildly impaired cortisol responses to adrenocorticotropic hormone (ACTH) (Illingworth et al., 1982), and those treated with high doses of statins have no impairment of cortisol secretion (Dobs et al., 2000). Thus endogenously produced cholesterol is sufficient in most situations, and the HDL/SR-B1 system plays a relatively minor role in human steroidogenesis. The regulation of cholesterol uptake, intracellular transport, and utilization is coordinated by a family of basic helix-loop-helix transcription factors called the sterol regulatory element binding proteins (SREBPs). The cell biology of intracellular cholesterol trafficking is summarized in Fig. 1.

2.2. Lipases and the processing of cholesterol esters

After circulating LDL is internalized by receptor-mediated endocytosis, the resulting endocytic vesicles fuse with lysosomes, where the LDL proteins are degraded by proteolysis, liberating the cholesteryl esters, which are then hydrolyzed to 'free' cholesterol by lysosomal acid lipase (LAL). However, cholesterol is never truly free, as its solubility is only about 20 μmol per liter, so that the term 'free cholesterol' refers to cholesterol that is bound to proteins or membranes, but lacks a covalently linked group. Free cholesterol may be used by the cell or stored in lipid droplets following re-esterification by acyl-coenzyme-A-cholesterol-acyltransferase (ACAT) also known as sterol-O-acetyltransferase (SOAT1). Similarly, HDL cholesteryl esters that enter the cell via SR-B1 are acted on by hormone-sensitive neutral lipase (HSL), following which the free cholesterol may also be used or re-esterified for storage. ACTH and luteinizing hormone (LH) respectively increase intracellular levels of cAMP in the adrenal and gonad, stimulating HSL and inhibiting ACAT, thus increasing the availability of free cholesterol for steroid hormone synthesis. ACTH, LH and other factors that increase cAMP stimulate the activity of HMGCoA reductase and the uptake of LDL cholesterol. When intracellular cholesterol concentrations are high, the genes for the LDL receptor, HMGCoA reductase and LAL are repressed while ACAT is induced, thereby decreasing cholesterol uptake, synthesis and de-esterification. Conversely, when intracellular cholesterol concentrations are low, this process is reversed.

Mutations in the *LIPA* gene encoding LAL cause Wolman disease, characterized by visceral accumulation of cholesteryl esters and triglycerides, with secondary adrenal insufficiency; cholesterol ester storage disease is a milder, adult variant (Lohse et al., 1999). Affected infants quickly develop hepatosplenomegaly, malabsorptive malnutrition and developmental delay; the diagnosis may be suggested by calcification that outlines the adrenal glands, and is established by finding deficient lysosomal acid lipase activity in leukocytes, fibroblasts or prenatal amniocytes. Bone marrow transplantation may ameliorate the disease, but the mechanism is unclear. In contrast to LAL, no known human disease is associated with HSL deficiency.

2.3. Endosomal processing of cholesterol

The entry and exit of cholesterol from lipid droplets involves the NPC proteins, so named because their mutation causes Niemann Pick type C (NPC) disease, which is characterized by endosomal accumulation of LDL-cholesterol and glycosphingolipids. Patients are normal in infancy but develop ataxia, dementia, loss of speech and spasticity at 2–4 years, and usually

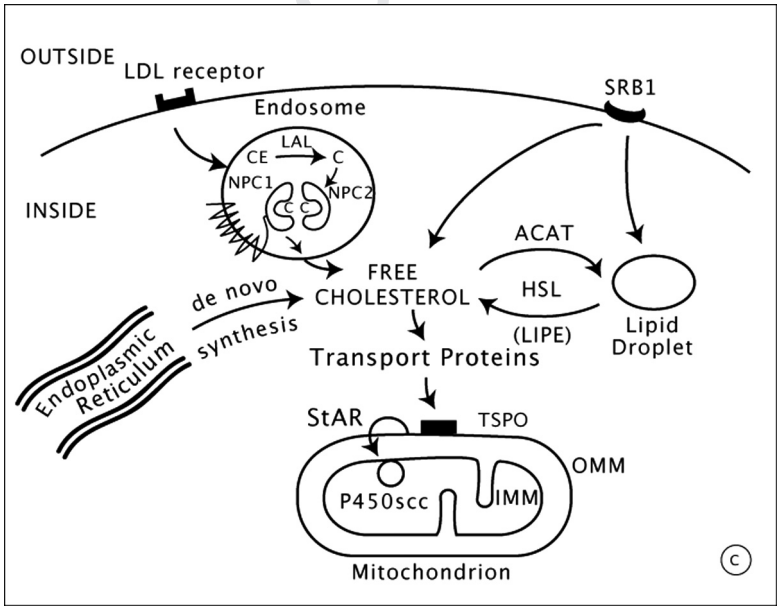

Fig. 1. Intracellular cholesterol trafficing. Human steroidogenic cells take up circulating low-density lipoproteins (LDLs) by receptor-mediated endocytosis, directing the cholesterol to endosomes; rodent cells utilize cholesterol from high-density lipoproteins (HDLs) via scavenger receptor B1 (SRB1). Cholesterol may also be synthesized from acetate in the ER. Cholesteryl esters are cleaved by lysosomal acid lipase (LAL); free cholesterol is then bound by NPC1, transferred to NPC2, and exported. The MLN64/MENTHO system resides in the same endosomes as the NPC system, but its role in cholesterol trafficking remains uncertain. Cholesterol may be re-esterified by acyl-CoA: cholesterol transferase (ACAT) and stored in lipid droplets as cholesteryl esters. Free cholesterol may be produced by hormone-sensitive lipase (HSL). Cholesterol can reach the outer mitochondrial membrane (OMM) by non-vesicular means by utilizing START-domain proteins or other cholesterol transport proteins. Movement of cholesterol from the OMM to the inner mitochondrial membrane (IMM) requires a multi-protein complex on the OMM. In the adrenals and gonads, the steroidogenic acute regulatory protein, StAR, is responsible for the rapid movement of cholesterol from the OMM to the IMM, where it can be converted to pregnenolone by P450scc. (©WL Miller).

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