Chapter 6
Involvement of Human Peroxisomes in Biosynthesis and Signaling of Steroid and Peptide Hormones

Isabelle Weinhofer, Markus Kunze, Sonja Forss-Petter, and Johannes Berger

Contents

Abstract Although peroxisomes exert essential biological functions, cell type-specific features of this important organelle are still only superficially characterized. An intriguing new aspect of peroxisomal function was recently uncovered by the observation that the peptide hormones β-lipotropin (β-LPH) and β-endorphin are localized to peroxisomes in various human tissues. This suggests a functional link between peptide hormone metabolism and peroxisomes. In addition, because endocrine manifestations that affect steroid hormones are often found in patients suffering from inherited peroxisomal disorders, the question has been raised whether peroxisomes are also involved in steroidogenesis. With this chapter, we will review several crucial aspects concerning peroxisomes and hormone metabolism.

Keywords Peroxisome • Peptide Hormones • β-lipotropin • β-endorphin • Steroid Hormones

I. Weinhofer (✉) • M. Kunze • S. Forss-Petter • J. Berger
Center for Brain Research, Medical University of Vienna, Spitalgasse 4, 1090 Vienna, Austria
e-mail: isabelle.weinhofer@meduniwien.ac.at

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Abbreviations

ABC-transporter	ATP binding cassette transporter
ACTH	Adrenocorticotropic Hormone
ALDP	Adrenoleukodystrophy protein
DHEA	Dehydroepiandrostenedione
IBA	Indole-3-butyric acid
PC	Prohormone convertase
POMC	Proopiomelanocortin
TGN	*Trans*-Golgi network
VLCFA	Very long-chain fatty acids
X-ALD	X-linked adrenoleukodystrophy
α-MSH	α-Melanocyte-Stimulating Hormone
β-LPH	β-Lipotropin

6.1 Introduction

Peroxisomes are ubiquitous, single membrane-bound organelles that participate in a wide variety of metabolic processes, many of which are related to the metabolism of lipids and reactive oxygen species. In humans and other mammals, these include degradation of pipecolic, phytanic, and very long-chain fatty acids (VLCFA; ≥ 22 carbon atoms), the synthesis of plasmalogens, bile acids and docosahexaenoic acid or the detoxification of hydrogen peroxide (Wanders and Komen 2007). Indeed, one of the most rapidly developing areas of organellar biology is that of peroxisomal function. It was recently demonstrated that peroxisomes are important sites of antiviral signal transduction (Dixit et al. 2010), and that they play a role in invariant natural killer T cell stimulation and maturation (Facciotti et al. 2012), in brain aging and Alzheimer's disease (Kou et al. 2011) as well as in peptide hormone metabolism (Höftberger et al. 2010). The latter work of Höftberger and coworkers demonstrated for the first time an unexpected link between hormone metabolism and peroxisomes.

In general, hormones are defined as chemical substances, which are released by a cell or a gland and are conveyed by the bloodstream to another part of the body to effect physiological activities. In mammals, hormones are derived from amino acids, cholesterol or phospholipids and fall into three chemical classes: peptide hormones, lipid- and phospholipid-derived hormones and monoamines. Intriguingly, a role of peroxisomes in hormone metabolism has already been demonstrated in plants: In the model plant *Arabidopsis thaliana*, indole-3-butyric acid (IBA), a storage precursor of the important morphogenetic plant hormone auxin, is activated in the peroxisome by fatty acid β-oxidation (Zolman et al. 2000, 2007). Here, we will review the current knowledge concerning a role of peroxisomes in mammalian peptide and steroid hormone metabolism.

6.2 The Peptide Hormones β-Lipotropin and β-Endorphin are Localized to Peroxisomes

The peptide hormones β-lipotropin (β-LPH) and β-endorphin are produced by post-translational cleavage from the 31-kDa prohormone precursor protein, proopiomelanocortin (POMC), which is synthesized in the pituitary, in the arcuate nucleus of the hypothalamus, in the adrenal gland and in several other peripheral tissues. The POMC precursor protein is produced in the endoplasmic reticulum and moves to the Golgi complex, where it is sorted for delivery to secretory granules by an N-terminal sequence acting as sorting signal (Fig. 6.1). During the trafficking process, POMC is proteolytically cleaved by the endopeptidases prohormone convertase (PC) 1/3 and 2, resulting in a number of biologically active peptides including β-LPH and β-endorphin. PC1/3 is synthesized as an inactive precursor that does not become

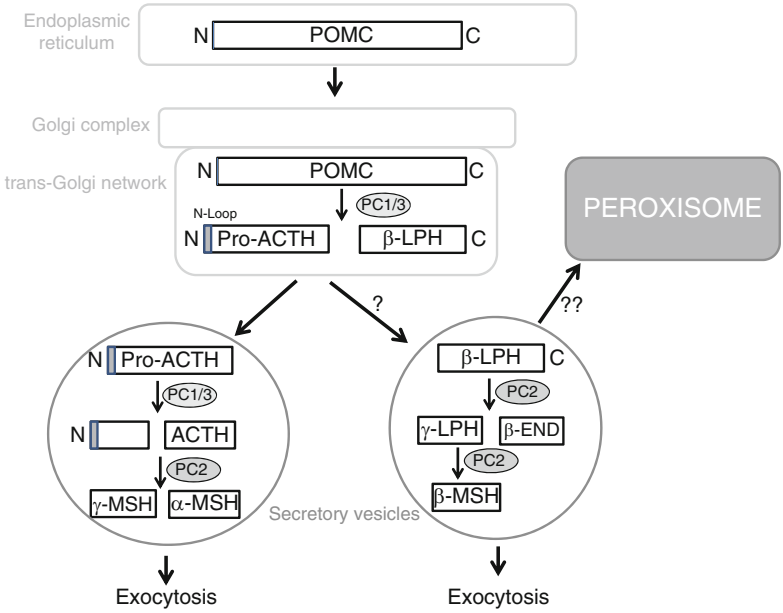

Fig. 6.1 Processing of POMC in the secretory pathway and suggested rerouting of β-LPH and β-endorphin to peroxisomes. In the *trans*-Golgi network, POMC is processed to pro-ACTH and β-LPH by the enzyme prohormone convertase (PC) 1/3. Pro-ACTH and β-LPH are then sorted into secretory granules, possibly into different subpopulations of secretory vesicles, where PC1/3 and PC2 become fully activated and generate ACTH, α-MSH, γ-MSH, β-MSH and β-endorphin. In tissues and cell types that predominantly secrete peptide hormones derived from the N-terminal part of POMC (ACTH, α-MSH or γ-MSH), a localization of the C-terminal peptides β-LPH and β-endorphin to peroxisomes has been observed. Thus, it could be speculated that when predominantly products from the N-terminal part of POMC are secreted, the by-products β-LPH and β-endorphin are prevented from secretion by rerouting them to peroxisomes, possibly resulting in their degradation in this organelle

fully activated until it reaches the immature secretory granules and undergoes an autocatalytic cleavage event (Zhou and Lindberg 1993). However, PC1/3 is partially active before autolysis, resulting in partial processing of POMC to pro-ACTH, the precursor of Adrenocorticotropic Hormone (ACTH) and α-Melanocyte-Stimulating Hormone (MSH), and β-LPH, the precursor of β-MSH and β-endorphin, in the *trans*-Golgi network (TGN) (Jutras et al. 1997). By a mechanism that is not fully understood, pro-ACTH and, presumably, β-LPH are sorted from the TGN into secretory granules. As the N-terminal sorting signal is present on pro-ACTH but not on β-LPH, the sorting of β-LPH apparently occurs through a different mechanism, possibly targeting a different subpopulations of secretory vesicles (Pritchard and White 2007). In the secretory granules, PC1/3 and PC2 become fully activated and generate ACTH, α-MSH, β-MSH γ-MSH and β-endorphin (Benjannet et al. 1991).

There is considerable complexity in this processing pathway with tissue-specific processing of POMC as an important factor conferring selectivity on the production of distinct peptides. In the anterior pituitary, POMC is processed predominantly to ACTH, β-LPH and a 16 kDa N-terminal fragment. Whereas ACTH is critical for the maintenance of adrenocortical function, the biological role of β-LPH is not fully understood, although a role in lipid mobilization such as lipolysis and steroidogenesis was demonstrated (O'Connell et al. 1996; Halabe Bucay 2008). In the hypothalamus and in the intermediate lobe of the pituitary, POMC is more extensively processed: ACTH is further cleaved to produce α-MSH, which influences skin-darkening reactions next to controlling body weight and CNS functions such as memory and learning; β-LPH is processed to γ-LPH and the opioid peptide neurotransmitter β-endorphin, which has a role in modulating pain sensation (Akil et al. 1998). Thus, POMC-expressing neuronal systems cosynthesize, costore and corelease a variety of biologically active peptides of which at least two, α-MSH and β-endorphin, may exert opposing effects upon reaching a common postsynaptic target. The mechanisms involved in regulating these antagonistic activities are unclear. N-terminal acetylation of α-MSH and β-endorphin in the secretory granules, resulting in increased biological activity of α-MSH and reduced binding of β-endorphin to opioid receptors, thus attenuating opiate-mediated analgesia, is proposed as one mechanism for shifting the predominant effects of secreted POMC peptides from endorphinergic to melanocortinergic actions.

In this context of regulating antagonistic hormone activities, the findings of Höftberger and coworkers are of particular interest: Using confocal laser microscopy and immunoelectron microscopy, a peroxisomal localization of β-LPH and β-endorphin could be demonstrated in the anterior pituitary gland (Höftberger et al. 2010). The presence of β-LPH and β-endorphin in peroxisomes was confirmed by colocalization using antibodies against several different peroxisomal proteins, such as catalase and adrenoleukodystrophy protein (ALDP). In the study, peroxisomes were shown to be distinctly separated from secretory vesicles, as ALDP did not colocalize with secretogranin, a typical marker for secretory versicles. Interestingly, the peroxisomal localization of β-LPH and β-endorphin was not restricted to the anterior pituitary gland but could be observed in several specific

cell types and tissues throughout the human body including dorsal root ganglia, adrenal cortex, distal tubules of kidney, and skin.

Intriguingly, an association of β-LPH and β-endorphin with peroxisomes was found only in tissues and cell types that predominantly secrete peptide hormones derived from the N-terminal part of POMC, such as ACTH in the pituitary, γ-MSH in the adrenal cortex or kidney and α-MSH in the skin (Höftberger et al. 2010). In contrast, in tissues like the adrenal medulla that secrete peptide hormones derived from the C-terminal part of POMC, such as β-endorphin, no localization of the peptide hormone in peroxisomes could be detected (Höftberger et al. 2010). Thus, it appears that when predominantly products from the N-terminal part of POMC are to be secreted, the arising by-products β-LPH and β-endorphin are retained by rerouting to peroxisomes, possibly resulting in their degradation in this organelle (Fig. 6.1). In contrast, cell types and tissues destined to secrete C-terminal POMC products, namely β-LPH and β-endorphin, do not show a peroxisomal localization of these peptide hormones.

Alternatively, it could be argued that β-LPH and β-endorphin are addressed to peroxisomes to serve as intracellular messengers to modify peroxisomal functions in response to specific cellular conditions. Several other neuropeptide and hormone products encoded by a single gene, e.g. growth hormone, proenkephalin or insulin, have previously been demonstrated to locate to more than one intracellular compartment including nucleus and mitochondria (Morel 1994; Mertani et al. 1996). The physiological implications of these findings remain unclear.

Interestingly, the peroxisomal localization of β-LPH and β-endorphin is restricted to cells that express the peroxisomal ATP-binding cassette (ABC) transporter ALDP, suggesting a link between peroxisomal β-LPH and β-endorphin and the function of ALDP. Defects in ALDP due to mutations in the *ABCD1* gene lead to X-linked adrenoleukodystrophy (X-ALD), the most common peroxisomal disorder, primarily affecting the adrenal cortex, testes and the central nervous system (Berger and Gärtner 2006). The biochemical hallmark of X-ALD is an abnormal accumulation of VLCFA in tissues, plasma and body fluids due to alterations in peroxisomal β-oxidation as well as in fatty acid chain elongation (Berger and Gärtner 2006). However, since β-LPH and β-endorphin are also found in peroxisomes of tissues from X-ALD patients lacking functional ALDP (Höftberger et al. 2010), ALDP does not seem to be involved in the transport of these peptide hormones into peroxisomes. Thus, further experiments are necessary to establish whether there is a yet unknown, direct link between ALDP and the peroxisomal localization of the peptide hormones or whether the restricted colocalisaztion of β-LPH and β-endorphin to peroxisomes of cell types expressing ALDP is a metabolic coincidence of ALDP function and peptide hormone secretion.

In summary, the peptide hormones β-LPH and β-endorphin, which were classically thought to be destined for secretion to act on distant target cells via specific receptors can also be translocated to another cellular site, namely the peroxisome, within the cells in which they are synthesized. The physiological relevance of these findings which possibly expand the physiological actions of these peptide hormones to peroxisomal function, remains to be elucidated.

6.3 A Possible Role of Peroxisomes in Steroid Hormone Metabolism

In mammalian species, there are seven families of steroid hormones that are classified on both structural and biological (hormonal) basis: estrogens (female sex steroids), androgens (male sex steroids), progestins, mineralcorticoids, glucocorticoids, vitamin D and bile acids. In contrast to peptide hormones, steroid hormones are synthesized in the mitochondria and rough ER and require the presence of specific enzymes that convert cholesterol into the appropriate steroid. The "classical" steroid-producing endocrine glands are the adrenal cortex and the gonads. In addition, the kidneys produce the active steroid metabolite of vitamin D.

In vertebrates and other higher organisms, cholesterol, which is an important component of many cellular membranes, is also the obligatory precursor for synthesis of steroid hormones and bile acids. Cholesterol is either obtained from the diet or synthesized de novo from acetate in a multi-step process involving nearly 30 enzymes. It has been debated for a long time, whether the pre-squalene segment of the cholesterol biosynthetic pathway is, at least under certain conditions, localized to peroxisomes and whether acetyl-CoA derived from peroxisomal β-oxidation of VLCFA and dicarboxylic acids is channeled preferentially to cholesterol synthesis inside the peroxisomes (Kovacs et al. 2007; Hogenboom et al. 2004a, b). As the outcome of these studies was inconclusive, a role of peroxisomes in synthesizing the steroid hormone precursor cholesterol has not yet been clarified.

6.3.1 Peroxisomes and Bile Acid Synthesis

Bile acids have long been known to facilitate digestion and absorption of lipids. Only recently, bile acids were demonstrated to function also as hormones that bind to specific nuclear receptor transcription factors to modulate expression of genes involved in cholesterol homeostasis (Chiang 2004). The conversion of cholesterol into the principal mammalian bile acids cholic acid and chenodeoxycholic acid takes place largely in the liver by a sequence of enzymatic modifications involving several enzymes and multiple subcellular compartments. One step involves side chain shortening by β-oxidation and subsequent conjugation with the amino acids taurine or glycine; both reactions are accepted to take place in peroxisomes. Accordingly, patients with generalized peroxisome deficiency disorders accumulate bile acid intermediates; and the peroxisomal enzymes involved in bile acid biosynthesis have all been identified, for a review see (Ferdinandusse et al. 2009). Only the transporters required for transfer of the C27-bile acid intermediates into the peroxisome and for the conjugated C24-bile acids out of the peroxisome remain unidentified, although a role of the *Abcd3*-encoded protein PMP70 was discussed in this context (Ferdinandusse et al. 2009).

6.3.2 *Peroxisomes and Steroid Hormone Synthesis in the Adrenal Cortex*

The cortex of the adrenal glands is, next to the gonads, the major steroid hormone producing site. The main secretory products of the adrenal cortex are the glucocorticoid cortisol; the mineralcorticoid aldosterone; and the androgens, androstenedione and dehydroepiandrostenedione (DHEA). Whereas cortisol is an important metabolic hormone and aldosterone has a role in salt and water homeostasis, the androgens produced by the adrenal cortex are regarded to have little physiological significance when gonadal function is normal. As discussed above, synthesis of steroid hormones begins with cholesterol. Circulating plasma lipoproteins derived from liver cholesterol synthesis are the major source of adrenal cholesterol, although de novo synthesis from acetate also occurs within the adrenal gland. In addition, cholesterol stored as cholesterol-esters in lipid droplets within adrenocortical cells is also used for steroidogenesis.

Impaired function of the adrenal gland to produce steroid hormones is known as Addison disease and is characterized by the loss of more than 90 % of both adrenal cortices. Intriguingly, many peroxisomal disorders are accompanied by adrenocortical dysfunction, with X-ALD being the most frequent genetic disorder leading to adrenal insufficiency. Histopathological examinations of the adrenal cortex from patients with X-ALD revealed ballooned adrenocortical cells with lamellar inclusions rich in VLCFA esterified with cholesterol (Powers 1980). In addition to entrapping cholesterol in VLCFA-esters, and thus limiting intracellular cholesterol for steroid synthesis, VLCFA accumulation is thought to have a direct toxic effect on intracellular membranes and enzymes, resulting in gradual adrenocortical destruction (Powers 1980). In the initial phase, basal steroid secretion is still within a normal range but cortisol secretion fails to increase in response to stress (ACTH challenge). With further loss of cortical tissue, also basal steroid secretion becomes deficient with the consequence of increased ACTH plasma levels because of decreased negative feedback inhibition due to the lack of cortisol. Accordingly, lowering of VLFCA levels by administration of Lorenzo's oil, a combination of erucic acid and oleic acid, to X-ALD patients with subclinical adrenal failure normalized elevated ACTH levels within 6 months, thus rendering steroid replacement therapy unnecessary (Cappa et al. 2011). An issue that has not been adequately explored, is why X-ALD mice, which also accumulate high levels of VLCFA in the adrenal gland, do not display signs of adrenal atrophy or clinical hypocortisolism (Lu et al. 2007). Also intriguing, and not yet understood, is the finding that very low plasma levels of DHEA(S), the sulfate ester of DHEA, which is the major secretory steroidal product of the adrenal glands, are also found in patients with no apparent adrenal dysfunction and normal plasma cortisol and ACTH levels (Wichers-Rother et al. 2005; Assies et al. 2003). Together, these observations point to a role of peroxisomes in adrenocortical steroidogenesis and forms the foundation for an interesting but still speculative hypothesis.

6.3.3 *Peroxisomes and Steroid Hormone Synthesis in the Gonads*

The ovary and the testis, like the adrenal gland, secrete cholesterol-derived steroid hormones under the control of the stimulatory releasing hormones of the hypothalamo-pituitary axis, mainly through actions of the gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH), produced by the pituitary gland. Just like the action of ACTH on adrenal cortical cells, LH and FSH increase intracellular concentrations of free cholesterol and its intracellular transport. The biosynthetic pathways to sex hormones in both male and female gonads include the production of the androgens androstenedione and DHEA. Testes and ovaries contain an additional enzyme, 17-β-hydroxysteroid-dehydrogenase (17βHSD), which enables the conversion of androgens to testosterone.

A role of peroxisomes in androgen metabolism has been discussed because patients suffering from the X-ALD phenotypic variant, adrenomyeloneuropathy, present hypogonadism, impairment of Leydig cells and testosterone levels that are in the lower part of the normal range (Stradomska et al. 2012; Brennemann et al. 1997). Additional evidence for a potential involvement of ALDP and peroxisomes in androgen metabolism was also provided by the observation that skin fibroblasts from X-ALD patients less actively convert testosterone to the more potent androgen dihydrotestosterone (DHT) than do fibroblasts derived from healthy control subjects (Petroni et al. 2000). The metabolic reaction of testosterone conversion to DHT is catalyzed by 5alpha-reductase isoform 2 (5α-R2), a key enzyme of steroid hormone metabolism. Intriguingly, 5α-R2 expression in X-ALD fibroblasts dissociates from the observed reduced enzymatic activity as increased 5α-R2 mRNA levels were reported (Petroni et al. 2000).

6.4 Conclusion

The POMC-derived peptide hormones β-LPH and β-endorphin are localized in peroxisomes of various cell types of the human body. Intriguingly, this association of β-LPH and β-endorphin with peroxisomes was found only in tissues and cell types that predominantly secrete peptide hormones derived from the N-terminal part of POMC, such as ACTH, α-MSH or γ-MSH and not from the C-terminal part, such as β-LPH or β-endorphin. As some of these products like α-MSH and β-endorphin have antagonistic activities in target tissues, it could be speculated that whenever predominantly products from the N-terminal part of POMC are to be secreted, the arising by-products β-LPH and β-endorphin are retained by rerouting to peroxisomes, possibly resulting in their degradation in this organelle. Thus, peroxisomes seem to play a role in regulating peptide hormone signaling by preventing the action of β-LPH and β-endorphin in specific situations.

Concerning steroid hormone metabolism, peroxisomal disorders are generally associated with alterations in steroidogenic tisssues, however, a role for peroxisomes in steroidogenesis and steroid signaling is still speculative. Taken together, a role of peroxisomes in hormone metabolism is implied by several findings but is a largely unexplored field that needs to be pursued. No effective treatment is available for most patients with peroxisomal disorders and the development of novel therapeutic strategies requires a detailed knowledge about peroxisomal function. Therefore, it is of major significance to address the cell biological questions about the role of peroxisomes in hormone metabolism.

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