The Interplay Between Bioactive Sphingolipids and Steroid Hormones

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Abstract
Steroid hormones regulate various physiological processes including development, reproduction, and metabolism. These regulatory molecules are synthesized from cholesterol in endocrine organs -such as the adrenal glands and gonads- via a multi-step enzymatic process that is catalyzed by the cytochrome P450 superfamily of monoxygenases and hydroxysteroid dehydrogenases. Steroidogenesis is induced by trophic peptide hormones primarily via the activation of a cAMP/protein kinase A (PKA)-dependent pathway. However, other signaling molecules, including cytokines and growth factors, control the steroid hormone biosynthetic pathway. More recently, sphingolipids, including ceramide, sphingosine-1-phosphate, and sphingosine, have been found to modulate steroid hormone secretion at multiple levels. In this review, we provide a brief overview of the mechanisms by which sphingolipids regulate steroidogenesis. In addition, we discuss how steroid hormones control sphingolipid metabolism. Finally, we outline evidence supporting the emerging role of bioactive sphingolipids in various nuclear processes and discuss a role for nuclear sphingolipid metabolism in the control of gene transcription.

Keywords
Steroidogenesis; sphingolipids; nuclear lipids; ceramide; S1P; sphingosine

1. Introduction
Cortisol, testosterone, progesterone, aldosterone, and estradiol are steroid hormones that regulate multiple physiological processes such as development, metabolism, secondary sex differentiation, and inflammation [1-4]. Members of the cytochrome P450 family of monoxygenases (CYPs) and hydroxysteroid dehydrogenases (HSDs) synthesize these steroid hormones from cholesterol in the adrenal gland, gonads, placenta, intestines, and in the central and peripheral nervous systems [2, 5-10] (Figure 1). Cholesterol metabolism is primarily regulated by trophic peptide hormones, including adrenocorticotropic hormone (ACTH) in the adrenal gland and follicular stimulating hormone (FSH) and leutenizing hormone (LH)
in the gonads. These peptide hormones are released from the anterior pituitary gland and activate a cAMP/PKA-dependent pathway in target tissues via binding to their cognate G-protein coupled receptors (GPCRs). Increased intracellular cAMP leads to a rapid increase in free cholesterol production and its subsequent import into mitochondria as well as the coordinate transcriptional activation of all the genes involved in steroid hormone biosynthesis.

Although the cAMP/PKA pathway is the primary, and most extensively characterized, regulator of steroidogenesis, various other signaling molecules crosstalk with this pathway to modulate hormone production [11]. Growth factors including insulin-like growth factor (IGF)-I and IGF-II, transforming growth factor β 1 (TGFβ1), platelet-derived growth factor (PDGF), and fibroblast growth factor 9 (FGF9) have all been shown to modulate steroid hormone production [12-16]. For example, both IGF-1 and IGF-II regulate steroidogenesis by binding to IGF-I receptors and modulating steroidogenic gene expression [13, 15]. In bovine granulosa cells, TGFβ1 stimulates estradiol production by increasing CYP19 mRNA levels and aromatase activity while inhibiting progesterone production by suppressing StAR, CYP11A1, and 3β-HSD1 transcription [16]. Further, TGFβ1 decreases P450c17α activity in H295R cells by repressing CYP17 gene expression via the activation of activin receptor-like kinase 5 (ALK5) [17]. Finally, mutations in PDGF target genes result in altered hormone production in the testis and ovaries [14] while aberrant FGF9 expression stimulates testosterone production in mouse Leydig cells [12].

Sphingolipids also have multiple established regulatory roles in steroid hormone biosynthesis [18-22]. These signaling lipids belong to a large family of glycolipids and phospholipids that are characterized by a common sphingoid base backbone. A growing number of sphingolipid species, including ceramide (cer), sphingosine-1-phosphate (S1P), sphingosine (SPH), and sphingomyelin (SM), have been reported to modulate various steps of the steroidogenic pathway. They stimulate steroid hormone secretion by regulating steroidogenic gene transcription [23-26], acting as intracellular second messengers [27-32] and/or extracellular paracrine/autocrine regulators [25, 33-35], and by serving as ligands for nuclear receptors [26]. In this review, a summary of studies focused on the various roles of sphingolipids in the regulation of steroidogenesis will be presented. In addition, we highlight emerging data on the novel signaling and regulatory roles for sphingolipids in the nuclei of cells.

2. Steroid hormone biosynthesis

As mentioned previously, the biosynthesis of steroid hormones is essential for physiological homeostasis. The vital role that these molecules play in human physiology dictates the need for a complex network of regulatory mechanisms that act concertedly to maintain optimal circulating plasma hormone concentrations. CYPs, HSDs, and other accessory proteins are selectively expressed in different steroidogenic tissues to assure the production of steroid hormones in a tissue-specific manner. For example, zone-specific expression of CYP17 [9] and cytochrome b5 [36-40] allow for glucocorticoid production in the zona fasciculata and androgen synthesis in the zona reticularis, whereas, the absence of CYP17 in the zona glomerulosa allows for mineralocorticoid secretion. In addition, temporal expression of these enzymes controls proper steroid hormone production during development.

Activation of the steroid hormone biosynthetic pathway is initiated when peptide trophic hormones binds to their cognate GPCRs. As mentioned earlier, upon receptor activation, two temporally distinct phases of steroidogenesis occur: a rapid phase and a slower chronic response. In the acute phase, stored cholesterol esters are cleaved and transported to the inner mitochondrial membrane, the site of the first enzymatic step of cholesterol
metabolism. De-esterification of newly imported and stored cholesterol esters facilitates its utilization in steroid hormone production and is catalyzed by hormone sensitive lipase (HSL). The movement of free cholesterol into mitochondria is the rate-limiting step in steroid hormone production and involves several proteins. One key protein is steroidogenic acute regulatory protein (StAR), the founding member of the START (StAR-related lipid transport) family of transport proteins. StAR is rapidly transcribed, translated, and localized to mitochondria upon hormonal stimulation [41-43]. In addition to StAR, a 10-kDa translocator protein (TSPO, formerly known as the peripheral-type benzodiazepine receptor-PBR) [44], PKA regulatory subunit-Iα (PKA-RIα)-associated protein, and voltage-dependent anion channel (VDAC) [41, 45, 46] facilitate the traffic of cholesterol to the inner mitochondrial membrane.

In the chronic phase of steroidogenesis, all the genes responsible for cholesterol metabolism (Figure 1) are transcriptionally activated. The activation of signaling culminates in the interaction of various transcription factors with the promoters of steroidogenic genes [11, 47-55]. In response to cAMP signaling, the nuclear receptor steroidogenic factor-1 (SF-1/Ad4BP/NR5A1) is targeted to most steroidogenic genes [54, 56]. SF-1 is also essential for gonadal and adrenal development [57, 58] as evidenced by the phenotype of targeted disruption of the receptor in mice [59, 60]. The central role of SF-1 in steroidogenesis is also evident in humans where mutations in the receptor result in various clinical pathologies including gonadal dysgenesis, adrenal failure, sex reversal, and underandrogenization [61]. Although SF-1 plays a critical role in conferring coordinate transcription of steroidogenic genes, other transcription factors including GATA4 and GATA6 [62-64], cAMP response element binding protein (CREB) [65, 66], specificity protein (Sp) family members [62, 67], and nerve growth factor 1B (NGF-1B) [68, 69] also participate in steroidogenic transcriptional regulation.

3. The sphingolipid metabolic pathway

Sphingolipids are a large family of glycolipids and phospholipids that share a common sphingoid base backbone. These once called ‘structural’ lipids are now well-established signaling molecules that play multiple roles in a vast number of cellular processes. Some of which include cell growth, differentiation, and migration, apoptosis [22, 26, 70-82], and autophagy [22, 83, 84]. Aberrant sphingolipid metabolism is linked to varied disease states including insulin resistance [85-87], cancer [72, 88, 89], and neurodegeneration [90-92]. The structural diversity of this family of lipids is vast, thus sphingolipid metabolism is a highly regulated process where a series of enzymes work concomitantly to maintain sphingolipid homeostasis.

Serine palmitoyltransferase (SPT) catalyzes the first step in sphingolipid de novo biosynthesis. This step involves the condensation of L-serine and palmitoyl-CoA to form the intermediate 3-ketodihydrosphingosine, which is further metabolized into dihydrosphingosine (spinganine) and dihydroceramide (Figure 2). Desaturation of dihydroceramide forms cer (N-acylsphingosine), which constitutes the basic structure of higher order sphingolipids including SM, cerebrosides, and gangliosides (GM). The complex variety of different sphingolipid metabolites is formed by the combination of different head groups such as phosphocholine and carbohydrates O-linked to cer. In addition, the breakdown of cer forms SPH, which can be phosphorylated to generate S1P. Cer can also be phosphorylated to form ceramide-1-phosphate (C1P) (Figure 2). Recently, it was shown that SPT is able to utilize L-alanine [93] and shorter acyl-CoA molecules [94, 95] as alternative substrates, thus generating atypical metabolites and expanding the list of possible physiologically important sphingolipid species.
There are a multitude of physiological and cellular roles for individual sphingolipid species, including GMs, SM, cer, C1P, SPH, and S1P [19, 26, 71, 73, 76, 77, 79, 87, 96-108]. GMs are important constituents of cell membranes and play important roles in cell growth, differentiation, and adhesion [109, 110]. SM is the most abundant sphingolipid in mammalian cells and, in addition to being an important membrane component, is the primary intracellular source of cer. Various stimuli including TNF-α, interleukin 1β (IL-1β), vitamin D3 (1,25-(OH)2D3), and cytotoxic agents can activate SM hydrolysis [31, 103, 108, 111, 112]. Cer participates as a second messenger in numerous cellular events including apoptosis, senescence, and cell cycle arrest [113-115] while its phosphorylated form, C1P, promotes cell differentiation and survival [97, 116, 117]. Similar to cer and C1P, SPH and S1P have opposing roles in cellular processes: the former acts as a pro-apoptotic agent [96, 118, 119] while the latter mediates cell migration, proliferation, and survival [73, 75, 81, 120].

Because different sphingolipids have specific effects on cell function, the intracellular concentrations of each sphingolipid molecular species are tightly controlled by sphingolipid metabolizing enzymes. These include the aforementioned SPT, acid/neutral sphingomyelinase (SMase), SM synthase, acid/neutral/alkaline ceramidase (ASAH), and sphingosine kinase (SK), S1P lyase, and ceramide kinase (CK) (Figure 2). Most of these enzymes are localized to specific sub-cellular locations, where they act maintain sphingolipid homeostasis in distinct microenvironments [121]. Because sphingolipids are mainly hydrophobic and specific mechanisms for sphingolipid transport have not been extensively characterized, the subcellular location where these molecules are generated most likely dictates their site of action.

4. Sphingolipid signaling in steroidogenesis

A growing body of literature has established the integral role that distinct sphingolipid species play in steroid hormone production. As it will be discussed below, cer, SPH, and S1P have all been implicated as secondary modulators of steroidogenesis. These lipids can act at different levels of the steroidogenic signaling pathway including (I) participating in various regulatory signaling cascades as second messengers, (II) acting as paracrine/autocrine regulators, and (III) serving as ligands for nuclear receptors. As previously stated, steroid hormone production is mainly regulated by trophic peptide hormones, which activate multiple signaling cascades at target cells. In addition to the cAMP/PKA pathway, numerous other signaling systems including calcium [122], steroidogenic-inducing protein [123], interleukins (IL-3, IL-6, IL-1β), and TNF-α [23, 124, 125] regulate steroidogenesis. One of the mechanisms by which these extracellular regulators control steroidogenesis is by modulating sphingolipid metabolism. In the adrenal cortex, for example, ACTH rapidly activates sphingolipid metabolism leading to decreased levels of SM, cer, and SPH, with a concomitant increase in S1P secretion [19]. In addition, IL-1β and TNF-α signaling have been shown to regulate cellular functions, including steroid hormone production, through sphingolipid metabolism [126-128].

(I) Sphingolipids as second messengers in steroidogenic regulatory pathways

Cer modulates steroid hormone production primarily by serving as a second messenger in cytokine and growth factor signaling cascades [23, 30, 31, 126, 129-131]. These extracellular mediators, including TNF-α, interferon –γ (INF-γ), and IL-1β, activate SM hydrolysis and promote cer intracellular accumulation [126, 127, 132]. Ultimately, increased cer levels alter cellular steroidogenic output. Given that cer comprises the structural backbone for all sphingolipids, cer can modulate steroid hormone production both directly and indirectly through metabolism into other bioactive sphingolipids (Figure 2). Therefore, it is important to point out that even though cer has been implicated in the regulation of
Steroid hormone biosynthesis, the precise molecular mechanisms involved in its actions are mostly unknown. In addition, data must be carefully interpreted to conclude that the responses observed are indeed a result of cer accumulation and not a bioactive metabolite.

Cer has been shown to regulate progesterone and testosterone production (Figure 3). In ovarian granulosa cells, activation of SMase by IL-1β suppresses progesterone production in a cer-dependent manner [31]. Further, Budnik et al. reported that SM hydrolysis also resulted in decreased progesterone secretion in MA-10 cells by suppressing StAR protein expression [23]. Importantly, the authors discussed that although SM hydrolysis also led to S1P accumulation, the inhibitory action of this cytokine was not reversed by SK inhibition. Similarly, cer-dependent StAR protein suppression was also reported in rat Leydig cells where it resulted in decreased testosterone synthesis [24]. Significantly, other chemically similar sphingolipids, such as SPH or S1P, did not mirror the effect of cer. Notably, cer accumulation observed in these reports was triggered by TNF-α signaling, which, similar to IL-1β, activates SMase activity [133]. Santana et al. reported that TNF-α signaling represses P450 aromatase activity in granulosa cells through a mechanism involving cer production [131]. Cells treated with bacterial SMase or cell-permeable cer displayed equivalent inhibition of aromatase activity, suggesting that TNF-α mediated activation of SM hydrolysis and subsequent cer production are involved in the regulation of this P450 enzyme. In rat Leydig and luteal cells, cer suppresses human chorionic gonadotropin (hCG)-stimulated testosterone and progesterone production, respectively, in a dose-dependent manner [28, 30]. Finally, cer was shown to modulate the mRNA expression of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), the glucocorticoid reactivation enzyme, in preadipocytes [129]. Cell-permeable C2-cer (N-acetyoyl-D-erythro-sphingosine) induced the expression and recruitment of CCAAT/enhancer binding protein β (C/EBPβ) to the 11β-HSD1 gene. In addition, cer treatment upregulated 11β-HSD1 activity in these cells [129], thus suggesting a role for cer in regulating active circulating steroid hormones.

Although most studies demonstrate a role for cer as an inhibitor of steroidogenesis, other reports present contradicting findings. Soboloff et al. reported that different acyl-chain length cer have opposite effects on LH-induced progesterone production in hen granulosa cells [32]. C6-cer (N-hexanoyl-D-erythro-sphingosine) and C8-cer (N-octanoyl-D-erythro-sphingosine) increased intracellular [Ca2+] and progesterone secretion whereas C2-cer had no effect on intracellular Ca2+ and suppressed steroid hormone production. Further, Kwon et al. demonstrated that C2-cer increases basal and hCG-stimulated progesterone production in MA-10 Leydig cells through a mechanism not linked to the induction of apoptosis [27]. The reason for these discrepancies is unknown, but the use of cer with different acyl-chain lengths may underlie the inconsistencies observed. Of note, Soboloff et al. [32] and others [134-139] have found that different molecular species of cer exert different cellular effects. Also, because sphingolipid metabolism is a highly dynamic process, the cellular responses reported may stem from the conversion of cer into another bioactive metabolite. Nonetheless, the majority of reports collectively suggest that the role of cer in steroidogenesis is likely independent from its role in cell growth and/or apoptosis because cell death was not reported as a reason for the decrease in the net steroid hormone output [27, 140].

(II) Sphingolipids as paracrine/autocrine regulators

S1P is well-established to induce cellular responses in a paracrine and/or autocrine manner through its binding to a family of GPCRs (S1PRs). In fact, the most important functions of S1P are mediated through the activation of these cell surface receptors [141, 142]. There are 5 S1PRs with each receptor coupling to multiple heterotrimeric G proteins [143-145]. Significant work has been done to characterize the downstream signaling cascades and cellular responses associated with the activation of the different S1PRs. S1PR1 couples to Gi,

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and activates the phospholipase C (PLC), phosphatidylinositol 3-kinase (PI3K), and extracellular-signal regulated kinase (ERK) pathways. S1PR$_2$ and S1PR$_3$ couple to G$_i$, G$_{12/13}$, and G$_q$ and active multiple downstream cascades including PLC, PI3K, ERK, and Rho GTPase. S1PR$_4$ activates PLC and ERK whereas S1PR$_5$ inhibits adenylyl cyclase and ERK (reviewed in [146]).

In steroidogenesis, the regulatory functions of S1P are primarily mediated through S1PRs. We have demonstrated that the S1P secreted from cAMP-stimulated human H295R adrenocortical cells induces CYP17 transcription via a paracrine mechanism that requires S1PR activation and nuclear translocation of sterol regulatory element binding protein 1 (SREBP1) [25]. In the zona fasciculata of bovine adrenal cells, S1P stimulates cortisol biosynthesis by activating protein kinase C (PKC) and phospholipase D (PLD) through a pertussis toxin-sensitive receptor-mediated mechanism [35]. S1P-induced cortisol production, unlike ACTH stimulation, did not stimulate cAMP formation but rather was mediated through intracellular [Ca$^{2+}$]. Similar S1PR-dependent PLD activation by S1P was also reported in adrenal glomerulosa cells [34]. Brizuela et al. characterized S1P as an inducer of aldosterone production through an S1PR$_{1/3}$-mediated mechanism involving the activation of PI3K/Akt and ERK pathways [33]. In addition, S1P activates prostaglandin-E2 (PGE$_2$) production [147], which induces CYP19 gene expression [148, 149]. PGE$_2$ also regulates 11β-HSD1 activity in luteinizing granulosa cells [150] (Figure 3). Interestingly, we have demonstrated that S1P induces the expression of liver receptor homologue-1 (LRH-1, NR5A2) in MCF-7 breast cancer cell [151], which regulates CYP19 transcription and estrogen production [152]. Given that aberrant estrogen production is a feature of breast cancer development and S1P induces tumorgenesis, it is likely that increased S1P concentrations in breast cells controls local estrogen production. Recently, an S1P-specific humanized monoclonal antibody called LT1009 (sonepcizumab) was developed and found to inhibit S1P-induced cell growth, migration, and angiogenesis [153-155]. This antibody is currently under Phase I trials for cancer treatment. Therefore, given the role of S1P in LRH-1 transcription and tumorgenesis, it is tempting to speculate that some of its chemopreventive effects are mediated by lowering estrogen production.

(III) Sphingolipids as ligands for nuclear receptors

Our laboratory has contributed to expanding the role of sphingolipids in steroidogenesis by demonstrating that SPH is an antagonist for SF-1 [26] (Figure 3). SPH is bound to the receptor under basal conditions and exchanged for PA, an SF-1-agonist [156], upon cAMP stimulation. SPH antagonizes cAMP-stimulated CYP17 reporter gene activity and antagonizes coactivator recruitment. Interestingly, silencing ASAH1 (acid ceramidase) expression mimicked cAMP-induced CYP17 transcription, further supporting the role of SPH as a repressor of SF-1 activity [26]. We have found that lysoSM (sphingosylphosphorylcholine) is also able to bind SF-1 in H295R cells under basal conditions and that cAMP treatment promotes its dissociation from the receptor [26]. The implications of this binding are unknown but it suggests that multiple sphingolipids can potentially regulate receptor activity. Although the mechanisms controlling ligand availability are yet to be uncovered, the recently described nuclear localization of sphingolipid metabolizing enzymes (discussed bellow) suggests that nuclear sphingolipid concentrations are locally controlled. In addition, we have found that cAMP stimulation leads to an increase in nuclear ceramidase activity (Lucki and Sewer, unpublished observations), thus demonstrating a potential link between ACTH/cAMP signaling and nuclear SPH production.
(IV) Regulation of sphingolipid enzymes by trophic hormones

As discussed above, the sphingolipid metabolic pathway is comprised of a series of enzymes that dynamically regulate sphingolipid concentrations (Figure 2). Because different sphingolipid species have unique cellular functions, these enzymes play a central role in modulating the bioactive activity of these molecules. A growing list of factors modulates the activity of sphingolipid enzymes including steroidogenic regulatory agents such as TNF-α, IL-1β, ACTH, and growth factors [19, 25, 127, 130, 157, 158].

TNF-α and IL-1β have both been shown to activate SMase in multiple cell types [29, 31, 127, 130, 158]. In Jeg-3 choriocarcinoma cells, SMase activity mediates TNF-α-dependent hormone production [29]. In addition, SM degradation was linked to cholesterol mitochondrial movement and increase in steroid hormone biosynthesis in mouse Leydig cells [159]. Degnan et al. demonstrated that Leydig cells incubated with exogenous SMase produced lower levels of testosterone in both basal and hCG-stimulated cells [130]. As discussed above, cer generated by SM hydrolysis can participate in various signaling cascades to regulate steroidogenesis. Interestingly, TNF-α was also shown to activate SK and increase endogenous S1P in human umbilical vein endothelial cells [160]. In addition, S1P was also reported to accumulate in response to TNF-α treatment in MA-10 cells [23], suggesting that this cytokine not only regulates SMase function, but also stimulates SK activity in steroidogenic cells.

In H295R cells, ACTH rapidly activates sphingolipid metabolism by decreasing the intracellular amounts of SM, cer, and SPH, while increasing S1P production via SK activation [19, 25]. These findings point to a role for ACTH signaling in controlling the activity of multiple sphingolipid enzymes. We have recently reported that cAMP increases the transcription, translation, and catalytic activity of ASAH1 in H295R cells [161]. Interestingly, we have also found that SK1 is rapidly translocated into the nucleus of H295R cells in response to ACTH/cAMP stimulation (Li et al., unpublished observations), thus indicating that S1P is potentially being produced in the nucleus of these cells. Because SPH suppresses SF-1-dependent steroidogenic gene transcription [25], it is possible that SK1 nuclear import facilitates SPH phosphorylation into S1P and consequently modulates the transcription of steroidogenic genes. Further studies are required to define the physiological significance of SK1 in modulating nuclear SPH concentrations.

4. Sphingolipid-mediated actions of steroid hormones

An equally important concept in the relationship between sphingolipid metabolism and steroidogenesis is the regulation of sphingolipid metabolism by steroid hormones. Sphingolipids have been reported to mediate multiple actions of estrogens, glucocorticoids, neurosteroids, and vitamin D₃ [6, 162-170]. SK has been reported to bridge crosstalk between estrogens and growth factor signaling in breast cancer cells. 17β-estradiol (E2)-dependent SK activity is essential for E2-dependent cell growth and SK overexpression mimics the mitogenic effects of ER [170]. In addition, SK mediates E2-stimulated endothelial growth factor receptor (EGFR) transactivation, Ca²⁺ mobilization, and ERK1/2 activation [169, 170]. The observed E2-induced SK activation appear to be at least in part, dependent on G-protein coupled receptor 30 (GPR30) because when this receptor is downregulated [169] or absent [170], E2-dependent SK activity is inhibited. In addition, SK1 has been reported to have anti-apoptotic effects in breast cancer cells in an E2-dependent manner through inhibition of caspase-7 activation and poly(ADP-ribose) polymerase (PARP) cleavage [167]. Recently, Sukocheva et al. further strengthened the link between SK and ER-dependent cancer progression by demonstrating that SK1 confers tamoxifen resistance in MCF-7 cells [171]. Further, a recent clinical study reported that ASAH1 expression strongly correlates with ER-positive tumor cells and is a significant
prognostic marker [168]. Thus, it is plausible to speculate that ER-induced breast cancer cell growth is, at least in part, dependent on ASAH1 and SK.

Glucocorticoids have also been shown to act through sphingolipid metabolism. Dexamethasone (dex) was reported to protect human fibroblast from apoptosis by inducing S1P formation [162]. Nieuwenhuis et al. recently demonstrated that this process involves S1P export through the ATP binding cassette (ABC)-transporter ABCC1 and activation of S1PR3 [163]. In addition, dex-induced intracellular S1P formation was shown to occur via upregulation of SK1, but not SK2, transcription and protein expression [163]. Similarly, dex-induced thymocyte apoptosis was shown to involve acid SMase activation and subsequent increase in cer levels [172]. In fact, cer is becoming appreciated as an important player in glucocorticoid-induced myopathy [173] and glucocorticoids have been shown to induce cer formation in various cell types [85, 101, 174]. Cer is known to induce mitochondrial dysfunction, oxidative stress, and insulin resistance, which are characteristics of glucocorticoid-mediated myopathy [173]. However the precise molecular mechanisms through which cer mediates this process are not entirely understood.

Vitamin D3 has long been reported as an inducer of SM turnover [103] and 1,25-(OH)2D3-dependent cer formation plays a role in cell differentiation in HL-60 cells [175, 176]. In addition, the anti-apoptotic properties of S1P were also shown to mediate the cytoprotective actions of 1,25-(OH)2D3 [164, 165, 177, 178]. Vitamin D3 activates SK and inhibits cer-induced apoptosis in a time- and dose-dependent manner [164]. Further, S1P formation was shown to protect keratinocytes from apoptosis despite acute SMase activation and cer formation in these cells [165]. More recently, Sauer et al. reported that Vitamin D3-induced S1P formation protected human fibroblast from apoptosis by increasing Bcl-2 expression [177].

Finally, neurosteroids have joined the list of sphingolipid modulators. Griffin et al. [179] have demonstrated that the neurosteroid allopregnanolone stimulates the degradation of complex sphingolipids and improves neurodegeneration in the Niemann-Pick Type C-1 (NPC-1) mouse model [6]. Further, Mellon et al. reported that the neuroprotective actions of allopregnanolone are partially mediated through GABA_A and pregnane-X receptors [180].

5. Nuclear Sphingolipid Metabolism

Nuclear lipid metabolism is an important mechanism through which bioactive lipids modulate cell function. Recent studies have uncovered the extensive metabolism of lipids that occurs in the nuclei of various cell types [181, 182]. These studies have pointed towards important signaling and regulatory roles for nuclear lipids, including sphingolipids [18, 183-185]. For example, nuclear cer has been shown to participate in Fas-induced apoptosis in Jurkat T-cells as a result of caspase-3-dependent activation of SMase [184]. Also, glycosphingolipids have been shown to promote cytoprotection through regulation of nuclear Ca2+[186]. The ganglioside GM1 forms a complex with a sodium-calcium exchanger in the nuclear envelope (NE) and facilitates the transfer of Ca2+ from the nucleoplasm to the endoplasmic reticulum (ER) [187]. Finally, Hait et al. linked nuclear SK2/S1P to epigenetic regulation of gene expression by demonstrating that SK2 is associated with histone deacetylase 1 and 2 (HDAC1/2) in repressor complexes. S1P inhibits HDAC1/2 activity and SK2 induces p21 and c-fos gene transcription by enhancing histone H3 acetylation [183].

To date, multiple sphingolipid enzymes have been detected in the nuclei of various cell types. SMase was reported in the nuclear matrix [188-190], NE [191], and chromatin [188] whereas SM synthase was detected in chromatin and NE [192]. In addition, nuclear ceramidase and SK activities were demonstrated in rat hepatocytes and Swiss 3T3 cells,
respectively [193-195]. However, SK2 is the predominantly nuclear isoform of SK in many cells [196]. Due to the hydrophobic nature of most sphingolipids, the nuclear expression of sphingolipid enzymes suggest that these bioactive lipids may have unique roles in nuclear function that are independent of the cytoplasmic functions of these molecules.

In steroidogenesis, recent data supports a role for these lipids in the regulation of gene expression. The characterization of SPH as an antagonist for SF-1 [26] was the first clue to the role of nuclear sphingolipids in the regulation of steroidogenesis. Further, due to the predominantly nuclear localization of SK2 [196] and cAMP-induced nuclear translocation of SK1 (Li et al., unpublished observations), S1P is likely being formed in the nucleus, perhaps as a way to control SPH nuclear levels and/or activate another uncharacterized nuclear process. In addition, we have found that ASAH1 is localized to the nucleus of H295R adrenocortical cells and ASAH1 represses SF-1-dependent CYP17 reporter gene activity by a direct enzyme-receptor (Lucki et al., unpublished observations). Thus, although further experimental evidence is necessary to dissect the mechanisms through which nuclear sphingolipids modulate steroidogenic gene transcription, it is evident that these molecules are likely to play key regulatory roles in the nucleus. Mass spectrometry, coupled with cell fraction, biophysical approaches, and microscopy are likely to provide more insight into the functional role(s) of bioactive sphingolipids in regulating gene transcription and other nuclear processes.

6. Conclusion

This brief review presents a summary of the established roles for sphingolipids in steroid hormone production. These bioactive molecules modulate steroidogenesis by regulating the expression of steroidogenic genes and enzymes, functioning as second messengers in various signaling pathways, acting as paracrine/autocrine regulators, and serving as ligands for nuclear receptors. Cer, SPH, and S1P play unique modulatory roles in steroidogenesis and trophic hormones, including ACTH, TNF-α, and IL-1β, regulate the activity of sphingolipid enzymes. In addition, sphingolipids have been shown to mediate the actions of various steroid hormones.

Nonetheless, there are still gaps in our understanding of the precise molecular mechanisms involved in sphingolipid-mediated steroidogenesis. Undoubtedly, future studies will uncover novel roles for these multi-faceted molecules. In addition, as novel bioactive sphingolipid species are discovered, the list of sphingolipid steroidogenic regulators is likely to expand. Finally, the recent discovery of nuclear sphingolipid metabolism and novel functions for these molecules in nuclear processes illustrates expanding functional diversity and hint to additional cellular functions that are yet to be discovered.

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Figure 1.
Steroid hormone biosynthetic pathways. Diagram representing the sequential metabolism of cholesterol into the major steroid hormones in the adrenal cortex (square) and gonads (circle). The steroidogenic gene responsible for each enzymatic reaction is indicated above the arrow. CYP11A1 (encodes P450scc); CYP17 (encodes P450c17α); 3β-HSD (encodes 3β hydroxysteroid dehydrogenase); CYP21 (encodes P450c21); CYP11B1 (encodes P45011β); CYP11B2 (encodes aldosterone synthase); CYP19 (encodes aromatase); 17α-HSD (encodes 17α hydroxysteroid dehydrogenase).
Figure 2.
Overview of the sphingolipid metabolic pathway. Ceramide (cer) is central to sphingolipid metabolism and can be generated via de novo biosynthesis, through the degradation of complex sphingolipids, or by the recycling of sphingosine. Degradation of ceramide leads to the formation of sphingosine and sphingosine-1-phosphate (S1P). Abbreviations: serine palmitoyl transferase (SPT), ceramide (cer), ceramide kinase (CK), sphingomyelin (SM), sphingomyelinase (SMase), sphingomyelin synthase (SM synthase), sphingosine kinase (SK), sphingosine-1-phosphate (S1P).
Figure 3.
The structural similarities of ceramide (CER), sphingosine (SPH), and sphingosine-1-phosphate (S1P) and their established roles in steroid hormone production. Although these lipids are structurally similar, they have unique roles in cellular function. CER functions as an intracellular second messenger to suppress progesterone and testosterone biosynthesis while S1P acts primarily via binding to cell-membrane S1P receptors to induce cortisol and aldosterone production. SPH binds to and antagonizes the function of steroidogenic factor 1 (SF-1). Abbreviations: Steroidogenic acute regulatory protein (StAR); cytochrome P450 17α-hydroxylase (P450c17α); cytochrome P450 aromatase (P450arom); 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1); prostaglandin E2 (PGE2); liver receptor homologue-1 (LRH-1).
Summary of recent data obtained for the regulation of sphingolipid enzymes by steroid hormones, their sphingolipid mediators, and cellular responses that result from this regulation. Abbreviations: sphingosine kinase (SK), sphingomyelinase (SMase), acid ceramidase (ASAH1), sphingosine-1-phosphate (S1P), sphingosine (SPH), ceramide (cer), endothelial growth factor receptor (EGFR), extracellular regulated kinase (Erk), poly(ADP-ribose) polymerase (PARP). ? Indicates sphingolipid mediator has not yet been identified.

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<td>SK</td>
<td>↑ Activity</td>
<td>S1P</td>
<td>EGFR activation, Ca(^{2+}) mobilization, Erk1/2 activation, caspase 7 inhibition, PARP cleavage, Tamoxifen resistance.</td>
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<tr>
<td></td>
<td>ASAH1</td>
<td>↑ Gene transcription</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td><strong>Glucocorticoids</strong></td>
<td>SK1</td>
<td>↑ Gene transcription and activity</td>
<td>S1P</td>
<td>Cell survival</td>
</tr>
<tr>
<td></td>
<td>Acid SMase</td>
<td>↑ Activity</td>
<td>cer</td>
<td>Apoptosis</td>
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<tr>
<td><strong>Allopregnanolone</strong></td>
<td></td>
<td>↑ Activity</td>
<td>?</td>
<td>↓Neurodegeneration</td>
</tr>
<tr>
<td><strong>Vitamin D(_1)</strong></td>
<td>SMase</td>
<td>↑ Activity</td>
<td>cer</td>
<td>Cell differentiation</td>
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<tr>
<td></td>
<td>SK</td>
<td>↑ Activity</td>
<td>S1P</td>
<td>Cell survival/ cytoprotective</td>
</tr>
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