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Multiple Roles for Sphingolipids in Steroid Hormone Biosynthesis

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Abstract

Steroid hormones are essential regulators of a vast number of physiological processes. The biosynthesis of these chemical messengers occurs in specialized steroidogenic tissues via a multi-step process that is catalyzed by members of the cytochrome P450 superfamily of monooxygenases and hydroxysteroid dehydrogenases. Though numerous signaling mediators, including cytokines and growth factors control steroidogenesis, trophic peptide hormones are the primary regulators of steroid hormone production. These peptide hormones activate a cAMP/cAMP-dependent kinase (PKA) signaling pathway, however, studies have shown that crosstalk between multiple signal transduction pathways and signaling molecules modulates optimal steroidogenic capacity. Sphingolipids such as ceramide, sphingosine, sphingosine-1-phosphate, sphingomyelin, and gangliosides have been shown to control the steroid hormone biosynthetic pathway at multiple levels, including regulating steroidogenic gene expression and activity as well as acting as second messengers in signaling cascades. In this review, we provide an overview of recent studies that have investigated the role of sphingolipids in adrenal, gonadal, and neural steroidogenesis.

Keywords

steroidogenesis; sphingolipids; CYP; sphingosine-1-phosphate; ceramide

Introduction

Steroid hormones like testosterone, progesterone, cortisol, aldosterone, and estradiol are important endocrine chemical messengers that are involved in a vast number of physiological processes including metabolism, inflammation, electrolyte and fluid balance, and secondary sex differentiation (Foster, 2004; Ghayee and Auchus, 2007; Newton and Holden, 2007; Williams-Ashman and Reddi, 1971). Steroid hormone synthesis occurs in the gonads, adrenal gland, placenta, intestines (Abdallah *et al.*, 2004; Ghayee and Auchus, 2007; Mueller *et al.*, 2007; Payne and Hales, 2004), and has recently been characterized in brain and peripheral nervous system tissues (Mellon *et al.*, 2004; Mellon, 2007; Tsutsui *et al.*, 2000). Biosynthesis is catalyzed by the sequential activities of cytochrome P450

monooxygenases and hydroxysteroid dehydrogenases that convert cholesterol into the many steroid hormones. Selective expression of these steroidogenic enzymes assures the production of steroid hormones in a tissue-specific manner. Activation of steroidogenesis is initiated by the binding of trophic peptide hormones – adrenocorticotrophin (ACTH), leutenizing hormone (LH), follicle stimulating hormone (FSH) - derived from the anterior pituitary to cognate receptors in target tissues which activates a cAMP/cAMP-dependent protein kinase (PKA) signaling pathway. Activation of cAMP-dependent signaling leads to a rapid increase in cholesterol mobilization and a chronic induction of steroidogenic gene transcription (Figure 1). Although this cAMP-dependent pathway is the main regulator of steroid hormone production, many other signaling systems involving many cytokines and sphingolipids have been reported to modulate steroidogenesis (Sewer *et al.*, 2007).

Sphingolipids, a family of lipids with a common sphingoid base backbone, have recently been identified as important bioactive molecules involved in a variety of cellular processes, including steroidogenesis (Ozbay *et al.*, 2004; Urs *et al.*, 2007; Ledeen and Wu, 2006; Spiegel and Milstein, 2007; Spiegel and Milstein, 2003b; Zheng *et al.*, 2006). Sphingolipids such as ceramide (Cer), sphingosine (SPH), sphingosine-1-phosphate (S1P), sphingomyelin (SM), and gangliosides (GMs) have been shown to modulate the steroidogenic pathway at multiple levels including regulating steroidogenic gene expression and activity as well as acting as secondary messengers in signaling cascades. In this review, we will provide a summary of recent studies that have investigated the role of sphingolipids in steroid hormone biosynthesis, with special emphasis on the role of sphingolipids in adrenal, gonadal, and neural steroidogenesis.

1. Review of Steroidogenesis

Steroidogenesis is a highly regulated biological process that is required for physiological homeostasis. This process takes place in specialized steroidogenic tissues where the biosynthesis of steroid hormones occurs in a highly synchronized manner via the coordinated activity of a series of steroidogenic enzymes. The primary steroidogenic tissues involved in the *de novo* steroid hormone biosynthesis include the gonads (ovaries and testis), the adrenal glands, and the placenta. Cholesterol, the substrate for the synthesis of all steroid hormones, is differentially metabolized into steroid hormones via the concerted action of enzymes localized in these steroidogenic centers (Figure 2).

Activation of steroidogenesis is initiated upon binding of peptide trophic hormones to their cognate G protein-coupled receptors (GPCRs) in the target tissues. As shown in Figure 1, peptide hormones activate two temporally distinct phases of steroid production: a rapid acute response and a slower chronic phase. The acute phase of steroidogenesis involves activation of the steroidogenic acute regulatory protein (StAR) for rapid cholesterol mobilization from the outer to the inner mitochondrial membrane (Miller, 2007; Sewer and Waterman, 2001; Thomson, 1997). Because cholesterol transport to the inner mitochondrial membrane is the rate-limiting step in steroid hormone production, StAR is a key protein necessary for the acute response. In most steroidogenic tissues, StAR expression is mediated by cAMP; in addition, intracellular Ca^{2+} may also play a role in StAR transcription in the adrenal cortex. In addition to StAR, the peripheral benzodiazepine receptor (PBR) and

hormone-sensitive lipase (HSL) are essential proteins for the intracellular transport and production of free cholesterol, respectively (Kraemer *et al.*, 2004; Papadopoulos, 1993). In rodents, the scavenger receptor class B type I (SR-BI) plays a key role in the uptake of cholesterol esters from lipoproteins (Krieger, 1999). The de-esterification of cholesterol esters by HSL is essential for its transport into the mitochondria and therefore its utilization in steroidogenesis (Kraemer *et al.*, 2004). Once cholesterol-laden vesicles are at the outer mitochondrial membrane, a large macromolecular complex containing StAR, PBR, and voltage-dependent anion channel (VDAC) (Hauet T *et al.*, 2002; Liu J *et al.*, 2003; Liu J *et al.*, 2006; Miller, 2007) facilitate import of the substrate into the inner mitochondrial membrane.

The chronic phase of steroidogenesis involves the transcriptional activation of steroidogenic genes that are responsible for cholesterol metabolism. This occurs via activation of adenylyl cyclase and signaling through multiple signaling mechanisms, including a cAMP/PKA pathway, leading to the activation of varied downstream effectors that ultimately promote the binding of transcription factors to the promoters of steroidogenic genes (Arlt and Stewart, 2005; Bassett *et al.*, 2004a; Bornstein *et al.*, 2004; Condon *et al.*, 2002; Jamnongjit and Hammes, 2006; Mendelson *et al.*, 2005; Okamoto *et al.*, 2004; Otis and Gallo-Payet, 2007; Sewer *et al.*, 2007; Sewer and Waterman, 2003; Sirianni *et al.*, 2003). One of the major transcription factors that regulates the expression of most steroidogenic genes in the adrenal gland and gonads of mammals is the nuclear receptor steroidogenic factor-1 (SF1/Ad4BP/NR5A1) (Lala *et al.*, 1992; Morohashi *et al.*, 1992; Parker *et al.*, 2002). The ability of SF-1 to bind to target genes is regulated by post-translational modifications including phosphorylation and acetylation (Chen *et al.*, 2004; Hammer *et al.*, 1999; Ishihara and Morohashi, 2005). More recently, ligand binding has also been implicated in the regulation of SF1 activity (Ishihara and Morohashi, 2005; Krylova *et al.*, 2005; Li *et al.*, 2007; Li *et al.*, 2005; Urs *et al.*, 2006).

1.1 Adrenal steroidogenesis

The adrenal gland is divided into two regions: cortex and medulla. The medulla, which comprises about 10% of the gland, is made up of neuroendocrine cells that synthesize catecholamines. The adrenal cortex, on the other hand, comprises most of the adrenal gland and is the site of adrenal steroid hormone biosynthesis. This region can be further subdivided into 3 distinct zones, each with a characteristic steroidogenic profile: (1) the zona glomerulosa is the outer cortical zone where the mineralocorticoid aldosterone is produced. (2) The middle zone, zona fasciculata, makes glucocorticoids, (3) while the inner zone, zona reticularis, is the site of androgen biosynthesis. Each of the three cortical zones expresses a unique profile of steroidogenic genes, thereby allowing for zone-specific cholesterol metabolism (Bassett *et al.*, 2004b; Rainey, 1999).

Adrenocortical steroid hormones have a vast array of biological functions. Cortisol, the primary human glucocorticoid, regulates the inflammatory response (Newton and Holden, 2007), carbohydrate and lipid metabolism, and stress response (Kassel and Herrlich, 2007). Aldosterone regulates blood pressure by modulating fluid and electrolyte balance (Brizuela *et al.*, 2006; Foster, 2004). In the adrenal cortex, dehydroepiandrosterone (DHEA),

dehydroepiandrosterone sulfate (DHEA-S), and androstenedione are the androgens produced (Havelock *et al.*, 2004; Rainey *et al.*, 2002).

In the zona fasciculata and reticularis of the adrenal cortex, steroidogenesis is mainly regulated by the binding of ACTH to the melanocortin-2 receptor, whereas in the zona glomerulosa, angiotensin II (AngII) directs aldosterone production by binding to angiotensin receptors. As shown in figure 2, there are two classes of steroidogenic enzymes whose transcription is activated in the chronic phase of steroid hormone production: the cytochrome P450 heme-containing proteins (CYPs) and hydroxysteroid dehydrogenase (HSD) enzymes (Payne and Hales, 2004; Sewer and Waterman, 2003). Additionally, the expression of StAR (Caron *et al.*, 1997; Clark and Combs, 1999; Clem *et al.*, 2005; Reinhart *et al.*, 1999), PBR (Besman *et al.*, 1989), HSL (Kraemer *et al.*, 2004), SR-BI (Azhar *et al.*, 1998), and adrenodoxin (Brentano and Miller, 1992; Chen and Waterman, 1992), the iron-sulfur electron transfer protein, are also induced by trophic hormone stimulation. The P450 side chain cleavage enzyme (encoded by CYP11A1) is an inner mitochondria membrane-bound enzyme that catalyzes the first enzymatic reaction in the synthesis of all steroid hormones: cleavage of free cholesterol into pregnenolone. P450c17 α is encoded by CYP17 and is localized in the endoplasmic reticulum (ER). P450c17 α catalyzes the hydroxylation of progesterone and pregnenolone at the carbon-17 and the conversion of pregnenolone to DHEA in the zona reticularis and the conversion of progesterone into androstenedione in the zona fasciculata. The microsomal 3 β -hydroxysteroid dehydrogenase (3 β HSD) catalyzes the conversion of pregnenolone, 17 α -hydroxypregnenolone, and DHEA into progesterone, 17 α -hydroxyprogesterone, and androstenedione, respectively. P45021 hydroxylase, encoded by CYP21, is also microsomal and catalyzes the conversion of progesterone and 17 α -hydroxyprogesterone into 11-deoxycorticosterone and 11-deoxycortisol, respectively. P45011- β -hydroxylase (CYP11B1) is localized at the inner mitochondrial membrane in the zona fasciculata and converts 11-deoxycorticosterone or 11-deoxycortisol into corticosterone or cortisol. In the zona glomerulosa, aldosterone synthase (encoded by CYP11B2) is expressed in the inner mitochondrial membrane and catalyzes the conversion of 11-deoxycorticosterone into aldosterone. As previously discussed, the zone-specific expression of CYP11B1 and CYP17 in the zona fasciculata and reticularis and CYP11B2 in the zona glomerulosa allow for the differential steroid hormone biosynthesis.

1.2 Gonadal steroidogenesis

The primary sites of androgen and estrogen biosynthesis are the testis and ovaries. Analogous to adrenocortical steroidogenesis, tissue-specific steroidogenic gene expression accounts for differential production of sex hormones. Also, gonadal steroidogenesis occurs via the two above-mentioned temporally distinct phases (acute and chronic) that assures proper and controlled steroid hormone output. In the gonads, LH and FSH regulate acute and chronic steroidogenesis. LH and FSH activate an adenylyl-cyclase/cAMP-dependent pathway in the same manner as ACTH in the adrenal cortex (Jamnongjit and Hammes, 2006; Mendelson *et al.*, 2005; Sewer and Waterman, 2003; Tajima *et al.*, 2005). The steroid hormones produced in the gonads - testosterone, estradiol, and progesterone - function primarily in the control of secondary male and female sex characteristics and in embryogenesis.

As in the adrenal cortex, a specific pattern of steroidogenic enzymes expression allow for the production of the gonadal steroid hormones in a cell-specific manner. Some enzymes expressed in the adrenal are also equally expressed in the gonads: CYP11A1 and 3 β HSD are expressed in the ovaries and testis and catalyzes the same reactions as in the adrenal cortex (Figure 2). CYP17 is also expressed in the Leydig cells of the testis and theca cells of the ovary but, in contrast to the 17 α -hydroxylase reaction that is prevalent in the adrenal cortex, the lyase reaction predominates, resulting in the conversion of pregnenolone into androstenedione. Moreover, additional gonadal-specific enzymes, such as aromatase (CYP19) and 17 α -HSD types 1 and 3 direct the production of gonadal-specific hormones. Testosterone biosynthesis is terminated in the Leydig cells of the testis by the activity of 17 α HSD3, which catalyses the conversion of androstenedione to testosterone. In ovarian granulosa cells, aromatase catalyzes the conversion of androstenedione or testosterone into estrone or estradiol, respectively, while 17 α HSD1 converts estrone to estradiol (Figure 2).

1.3 Neurosteroidogenesis

Adrenal and gonadal steroid hormones have long been known to regulate many important brain functions (Fuxe *et al.*, 1981; McEwen, 1991). In addition, circulating steroids like progesterone, 11-deoxycorticosterone, and testosterone can be converted to neuroactive steroids within the brain (Mellon and Griffin, 2002). More recently, however, many laboratories have reported that nervous tissue is capable of expressing essential steroidogenic enzymes and therefore *de novo* synthesize steroid hormones, that are at least in part independent from classic steroidogenic tissues (Mellon and Griffin, 2002; Tsutsui *et al.*, 2000). Moreover, the expression of StAR has also been detected in neural tissues (Lavaque *et al.*, 2006; Sierra, 2004). Today, the term neurosteroid refers to both *de novo* synthesized steroids by nervous cells and circulating steroids that are subsequently converted to neuroactive forms within nervous tissues.

Neurosteroids appear to mainly function as neurotransmitters in a paracrine and autocrine fashion in the modulation of many brain functions including myelination, inhibition of neuronal toxicity and ischemia, behavioral aspects, and neuronal survival, growth, and differentiation (Griffin *et al.*, 2004; Mellon *et al.*, 2004; Mellon, 2007; Mukai *et al.*, 2006). Such neurosteroids include progesterone, pregnenolone, allopregnanolone, DHEA, their sulfate esters, and 5 α /5 β -tetrahydroprogesterone, some of which were previously viewed as inactive metabolites or steroid precursors (Plassart-Schiess and Baulieu, 2001; Sakamoto *et al.*, 2007).

Glial cells, oligodendrocytes and type I astrocytes, are considered the major neuronal steroidogenic cells (Tsutsui and Ukena, 1999). However, other cell types including Schwann cells, cerebellar Purkinje cells, and neurons have also been reported as capable of steroid hormone production (Mellon and Griffin, 2002; Plassart-Schiess and Baulieu, 2001). Purkinje cells, for example, are one of the major site of *de novo* progesterone and pregnenolone sulfate production (Tsutsui and Ukena, 1999).

The neurosteroidogenic pathway involves the same group of cytochrome P450 and HSDs enzymes as in classical steroidogenic tissues. Tissue-specific expression of a unique panel of steroidogenic enzymes also occurs in different brain regions and nerve cells (Mellon and

Deschepper, 1993). However, some enzymes are expressed at higher levels in the brain than in other steroidogenic tissues and vary during development (Mellon *et al.*, 2004). CYP11A1, 3 β -HSD, and CYP17 are expressed in many brain regions including the cortex, cerebellum, and hypothalamus (Mellon and Griffin, 2002). These three brain regions also express CYP11B1 and CYP11B2 as well as StAR (reviewed in ref. (Mellon and Griffin, 2002)).

A significant amount of data relating the function of neurosteroids to nervous system development comes from the Niemann-Pick Type C-1 (NPC-1) knockout mouse model (Griffin *et al.*, 2004; Mellon *et al.*, 2004). This mouse has a mutation in the *npc1* gene, which codes for a late endosomal membrane protein that is involved in the trafficking of cholesterol out of late endosomes for steroidogenesis (Blanchette-Mackie, 2000). Mutation of this gene leads to the accumulation of cholesterol and GMs in lysosomes and impaired neurosteroidogenesis (Mellon *et al.*, 2004).

1.4 Other steroidogenic tissues

The liver, placenta, and small intestines have also been reported as steroidogenic centers where a selective set of cytochrome P450 and HSD enzymes are expressed. CYP17 is expressed in the rat liver and this expression fluctuates during development, indicating a pattern of expression exclusively tuned for the needs of this tissue (Vianello *et al.*, 1997). Mueller *et al.*, (Mueller *et al.*, 2007) reported that murine intestinal epithelial cells are capable of producing glucocorticoids utilizing a differently regulated steroidogenic pathway than in adrenocortical cells. cAMP accumulation abrogates glucocorticoid production in this intestinal cells, illustrating a diversification of the classical steroidogenic pathway, which has likely evolved as a result of the different requirements of their environment. The placenta is an important steroidogenic tissue during pregnancy, which secretes human chorionic gonadotropin (hCG) hormone that stimulates progesterone production by the corpus luteum (Pepe and Albrecht, 1995). It has been reported that in addition to the corpus luteum, other nongonadal tissues including the kidney, lungs, pancreas and liver express hCG receptors (Abdallah *et al.*, 2004). Although the role of these receptors is unknown, the expression of steroidogenic genes in these tissues suggests that placental hCG may mediate additional pleiotropic effects in the developing fetus.

2. Review of sphingolipids

Sphingolipids comprise a family of phospholipids and glycolipids that are characterized by the presence of a common sphingoid base (such as SPH) backbone. This family of lipids has a large structural diversity that allows for the existence of many structurally similar moieties yet with crucial differences in biochemical and biophysical properties. The role of this class of lipids in membrane structure is well-established (Goni and Alonso, 2006). Although the precise amount may vary considerably, as high as 30% of lipids that make up plasma membranes are sphingolipids, especially complex sphingolipids such as SM and glycosphingolipids (GSL) (Smith and Merrill, 2002). Furthermore, certain sphingolipids like SM, Cer, and glucosylceramides may aggregate to form higher order domains termed “lipid rafts”, which are believed to be important in cell signaling (Huwiler *et al.*, 2000; Smith and Merrill, 2002; Tani *et al.*, 2007). Significantly, a large body of data has established a role for sphingolipids as key bioactive molecules in a variety of biological processes (Brizuela *et al.*,

2006; Budnik *et al.*, 1999; Cuvillier *et al.*, 1996; Degnan *et al.*, 1996; Gomez-Munoz, 2006; Hannun, 1996; Kihara *et al.*, 2007; Meroni *et al.*, 2000; Rabano *et al.*, 2003; Thon *et al.*, 2005) (Table 1). These processes include cell differentiation, growth, apoptosis, cell-cell interaction, and mediation of signaling pathways and gene expression (Merrill *et al.*, 1999; Zeidan and Hannun, 2007; Zheng *et al.*, 2006). Roles for sphingolipids in vascular function (Lorenz *et al.*, 2007), neurodegeneration (Tamboli *et al.*, 2005), cancer [reviewed in ref. (Ogretmen, 2006)], autophagy (Lavieu *et al.*, 2006; Lavieu *et al.*, 2007), and insulin resistance (Adams *et al.*, 2004; Turinsky *et al.*, 1990) have also been reported, which illustrate the broad spectrum of cellular processes that can be directed or indirectly mediated by these bioactive lipid molecules. In addition, a series of recent studies examining the role of sphingolipids, primarily Cer and S1P, in adrenal and gonadal steroidogenesis (Brizuela *et al.*, 2007; Brizuela *et al.*, 2006; Budnik *et al.*, 1999; Li *et al.*, 2001; Meroni *et al.*, 2000; Ozbay *et al.*, 2006; Rabano *et al.*, 2003) add to a rapidly expanding list of roles for sphingolipids in cellular processes.

The simplest sphingolipids, SPH ((2S, 3R, 4E)-2-aminooctadec-4-ene-1,3-diol -Ser) and Cer (N-acylsphingosine) shown in Figure 3, constitute the basic structure of higher order sphingolipids like SM, cerebroside, GMs, and other GSLs (Goni and Alonso, 2006). Complex sphingolipids are synthesized by Cer metabolism. Cer can be produced by the degradation of SM or by *de novo* biosynthesis. *De novo* Cer biosynthesis occurs from the condensation of serine and palmitoyl-CoA by the sequential action of the enzymes serine palmitoyltransferase, ketodihydro-sphingosine reductase, and ceramide synthase (Hanada *et al.*, 2003) (Figure 3). SM hydrolysis is the major cellular source of Cer and it can be activated in response to a variety of extracellular signals, e.g. vitamin D3, cytokines, growth factors, and cytotoxic agents (Andrieu-Abadie and Levade, 2002; Huwiler *et al.*, 2000; Okazaki *et al.*, 1989). Alternatively, SPH formed as a product of complex sphingolipid metabolism can be recycled back into Cer via the scavenger pathway (Zeidan and Hannun, 2007).

The amounts of different sphingolipid moieties within a cell is controlled by multiple enzymes including sphingomyelinases (SMases) that hydrolyze SM into Cer; ceramidases that convert Cer into SPH and a free fatty acid; ceramide synthase which catalyzes the *de novo* synthesis of Cer from SPH; sphingomyelinase synthase that catalyzes the production of SM; and sphingosine kinases (SKs) and ceramide kinases (Cerk) that phosphorylate SPH and Cer, respectively (Figure 3) (Huwiler *et al.*, 2000; Maceyka *et al.*, 2002; Maceyka *et al.*, 2005; Pettus *et al.*, 2003; Spiegel and Milstien, 2007). All of the above-mentioned enzymes play a central role in regulating the many bioactive sphingolipid types. Because the activity of these enzymes can be regulated by multiple signaling factors (Huwiler *et al.*, 2000; Maceyka *et al.*, 2002; Maceyka *et al.*, 2005; Pettus *et al.*, 2003; Spiegel and Milstien, 2007), the sphingolipid metabolic profile in any given cell at any given point in time is comprised of a unique set of bioactive sphingolipids. Therefore, the amounts and molecular species of the different sphingolipids are in a constant dynamic flux.

Of all known sphingolipid moieties, a large body of research has established physiological roles for SPH, Cer, S1P, and C1P (Adams *et al.*, 2004; Andrieu-Abadie and Levade, 2002; Brizuela *et al.*, 2007; Brizuela *et al.*, 2006; Budnik *et al.*, 1999; Hadizadeh *et al.*, 2007;

Kihara *et al.*, 2007; Li *et al.*, 2001; Meroni *et al.*, 2000; Ozbay *et al.*, 2006; Pettus *et al.*, 2003; Rabano *et al.*, 2003). Although these molecules are structurally similar and can be interconverted by one or two-step reactions, they have unique, and sometimes opposing, biological functions (reviewed in ref. (Kihara *et al.*, 2007)). As shown in Figure 3, the dynamic balance of these sphingolipid metabolites is maintained by enzymes such as ceramidases, SKs, and S1P phosphatases whose activity is regulated by a variety of intra- and extracellular signals including TNF- α , Fas ligand, and cytokines (Cai *et al.*, 1997; Cai *et al.*, 2007; Hannun, 1996; Ruvolo *et al.*, 2002; Sawai *et al.*, 1997; Spiegel and Milstien, 2003a).

SM, the most abundant sphingolipid in mammalian cells, has also been implicated as a bioactive sphingolipid (Degnan *et al.*, 1996; Ding *et al.*, 2007; Porn *et al.*, 1991). SMase activity is triggered by diverse stimuli, which results in the cleavage of membrane SM into free Cer. This process, now called the “sphingomyelin cycle”, is a receptor-mediated signaling system that plays an important role in regulating sphingolipids homeostasis (Andrieu-Abadie and Levade, 2002; Hannun, 1996; Ziulkoski *et al.*, 2001). Cytokines such as TNF- α and interleukin 1 β (IL-1 β) have been found to activate SMase activity (Santana *et al.*, 1996; Zeidan *et al.*, 2006).

Cer, aside from the precursor of complex sphingolipids, is a second messenger molecule involved in a series of cellular events including differentiation, senescence, proliferation, mediation of stress response, cell cycle arrest, and apoptosis (Gomez-Munoz, 2006; Kolesnick, 2002; Pettus *et al.*, 2002). Cytokines and fatty acids have been reported as mediators of intracellular ceramide production and subsequent activity (Lu *et al.*, 2003; Osawa *et al.*, 2005; Zeidan *et al.*, 2006). Conversely, C1P, a major metabolite of Cer produced by Cerk-catalytic phosphorylation of Cer, has been identified as having antagonistic effects than Cer by being an inhibitor of apoptosis and a cell survival inducer (Gomez-Munoz *et al.*, 2004). C1P has also been reported to play a role in inflammation and phagocytosis (Gomez-Munoz, 2006) and in arachidonic acid release (AA) and prostanoid production (Pettus *et al.*, 2003). Since a role for AA in steroid hormone production stimulation has been found (Castilla *et al.*, 2004), C1P may be a regulator of steroidogenesis.

Like Cer, SPH acts as a pro-apoptotic signal (Hung *et al.*, 1999; Sakakura *et al.*, 1998; Sweeney *et al.*, 1998) as well as an inhibitor of protein kinase C (PKC) (Hannun *et al.*, 1986), phospholipase D (PLD) (Natarajan *et al.*, 1994), and calmodulin-dependent kinase (Jefferson and Schulman, 1988) in diverse cell types. SPH has also been shown to activate diacylglycerol (DAG) kinase (Yamada and Sakane, 1993). S1P is involved in cell survival and proliferation (Olivera *et al.*, 1999; Olivera and Spiegel, 1993; Spiegel and Milstien, 2002). In contrast to Cer, which is membrane-bound, S1P can diffuse into the cytosol and be secreted into the extracellular space, where it can exert its signaling properties by binding to cell surface receptors (reviewed in ref. (Kihara *et al.*, 2007)).

S1P is formed by phosphorylation of SPH by SKs. There are two isoforms of SK (1 and 2) whose function and subcellular location are distinct (Liu *et al.*, 2003; Maceyka *et al.*, 2005; Okada *et al.*, 2005). SK2 prevents apoptosis and leads to increase in S1P metabolism back to

ceramide (Spiegel and Milstien, 2007). SK1, however, appears to be a critical regulator in the intracellular amounts of S1P and its precursors SPH and Cer. Overexpression of SK1 leads to accumulation of S1P and tumorigenesis (Le Stunff *et al.*, 2007; Spiegel and Milstien, 2007). Therefore, there is evidence to suggest that these two SK isoforms may have distinct physiological functions and effect on sphingolipid metabolism. External ligands including acetylcholine, prosaposin, lysophosphatidic acid (LPA), formylmethionine peptide, and even S1P itself have been reported as agonists of SK activity via GPCR signaling (Maceyka *et al.*, 2002). Downstream targets of GPCR signaling are still under investigation, but PKC and ERK2 activation have been shown to regulate SK1 activity (Spiegel and Milstien, 2007) and may be important in regulating the dynamic balance of intracellular S1P, SPH, and Cer.

Given the opposite functions of Cer/SPH and S1P in cell viability, it has been proposed that the balance of these lipid mediators is a key factor in determining cell fate toward proliferation or apoptosis (Cuvillier *et al.*, 1996). As a result of such important role in cell viability, these sphingolipid metabolites are under extensive investigation as potential anti-cancer targets (Mimeault, 2002; Pettus *et al.*, 2002; Thon *et al.*, 2005). Nonetheless, the dynamic balance of these sphingolipid molecules will also affect other biological processes including steroidogenesis.

3. The role of sphingolipids in steroid hormone production

As previously summarized, steroidogenesis involves the binding of the trophic hormones ACTH, LH, and FSH to their cognate receptors which lead to the subsequent activation of a series of cascade pathways (Figure 1), primarily the cAMP-dependent/PKA pathway leading to the activation of many downstream targets. Calcium is also important for maximal steroidogenesis (Gallo-Payet and Payet, 1989). Additionally, cAMP-independent signaling systems play an integral role in the regulation of steroidogenesis (Bornstein *et al.*, 2004; Okamoto *et al.*, 2004; Otis and Gallo-Payet, 2007). Interleukins (IL-3, IL-6, IL-1 β , TNF- α) (Budnik *et al.*, 1999; Hedger, 1997; Weber *et al.*, 1997), calmidazolium (Choi and Cooke, 1992), steroidogenic-inducing protein (SIP) (Stocco and Khan, 1992), and chloride ions (Choi and Cooke, 1990; Gallo-Payet *et al.*, 1999; Ramnath *et al.*, 1997) are a few of the many regulators of steroid hormone production.

In addition, recent data suggests that sphingolipids can also act as secondary modulators of steroid hormone production. In the adrenal cortex, for example, ACTH/cAMP rapidly activates sphingolipid metabolism by decreasing intracellular amounts of SM, Cer, and SPH while increasing S1P production via SK activation (Ozbay *et al.*, 2004; Ozbay *et al.*, 2006). Bioactive sphingolipids have been identified as modulators of steroidogenesis by acting at different levels of the steroidogenic signaling pathway (Table 1). Some points of regulation include: (1) regulating cytochrome P450 gene expression, (2) serving as ligands for the major steroidogenic transcription factor SF-1, and (3) participating in secondary signaling cascades and second messenger systems. Some of these key findings are described below.

3.1 Ceramide

Current data suggests that the primary role of Cer in steroidogenesis is as a mediator in cytokine and growth factors signaling pathways, which ultimately lead to a change in basal

steroid hormone production (Arai *et al.*, 2007; Budnik *et al.*, 1999; Cai *et al.*, 1997; Degnan *et al.*, 1996; Meroni *et al.*, 2000; Santana *et al.*, 1995; Santana *et al.*, 1996). TNF- α , Fas ligand, interferon- γ (INF- γ), and IL-1 β modulate intracellular Cer concentrations via the activation of SMases (Cai *et al.*, 1997; Hannun, 1996; Sawai *et al.*, 1997). Of note, although some of the end point results of Cer accumulation in steroid production have been reported (Budnik *et al.*, 1999; Cai *et al.*, 1997; Degnan *et al.*, 1996; Meroni *et al.*, 2000; Santana *et al.*, 1995; Santana *et al.*, 1996), the precise molecular mechanism of action of Cer is still mostly unknown.

Cer has been shown to negatively regulate progesterone production in granulosa cells (Santana *et al.*, 1996). The accumulation of intracellular Cer is a result of activation of SM hydrolysis by IL-1 β . Similarly, TNF- α , which has many overlapping cellular effects with IL-1 β , was shown to activate SMases and generate intracellular Cer in both MA-10 murine Leydig cells (Degnan *et al.*, 1996) and Jeg-3 human choriocarcinoma cells (McClellan *et al.*, 1997). Cer was also reported to suppress human choriogonadotropin (hCG)-stimulated testosterone production in rat Leydig cells (Meroni *et al.*, 2000) and progesterone production in rat luteal cells in a dose-dependent manner (Li *et al.*, 2001). Budnik *et al.* (Budnik *et al.*, 1999) reported that regulation of progesterone biosynthesis by TNF- α /Cer signaling occurs via inhibition of StAR expression in MA-10 cells. Attenuation of StAR expression and reduced testosterone secretion by TNF- α and Cer was also seen in rat Leydig cells (Morales *et al.*, 2003). It has been proposed that the apoptotic effect of TNF- α signaling is mediated via SM degradation into Cer (Mimeault, 2002; Thon *et al.*, 2005). It is important to point out that the role of Cer in steroid hormone production is likely to be independent from the role of Cer in cell proliferation and/or apoptosis because in the majority of these studies, apoptosis was not a reported reason for the decrease in steroid hormone production (Son *et al.*, 2004).

Cer can also modulate the activity and/or expression of steroidogenic enzymes. Meroni and colleagues have shown that Cer can regulate P450c17 α enzymatic activity as well as inhibit cAMP production (Meroni *et al.*, 2000). In another report, Cer was identified as a novel regulator of 11 β -HSD1 in preadipocytes (Arai *et al.*, 2007). Cer was observed to cause an increase in CCAAT/enhancer binding protein- β (C/EBP β) recruitment to the 11 β -HSD1 promoter, and in this way activate gene expression (Arai *et al.*, 2007). Furthermore, Cer can inhibit hCG-induced aromatase activity and estradiol production in granulosa cells (Santana *et al.*, 1995; Son *et al.*, 2004).

Enzymes involved in Cer metabolism may also play a key role in steroidogenic regulation. Cortisol can activate the expression of the acid ceramidase gene (ASAH1) and promote Cer degradation (Lucki and Sewer, unpublished observations), thereby increasing the cellular concentrations of SPH, the antagonist for SF-1 (Urs *et al.*, 2006). The regulation of ASAH1 by glucocorticoids may represent an intra-adrenal feedback mechanism that controls optimal steroid hormone output by repressing the ability of SF-1 to induce the transcription of steroidogenic genes.

3.2 Sphingosine

SF-1 was once classified as an orphan nuclear receptor because the identity of the endogenous ligand for the receptor was unknown. However, investigations of the endogenous receptor have identified SPH as a *bonafide* ligand (Urs *et al.*, 2006) and recent crystallographic studies in bacterially expressed SF-1 confirmed that the ligand binding domain (LBD) of SF-1 is bound by phospholipids (Krylova *et al.*, 2005; Li *et al.*, 2005; Wang *et al.*, 2005). SPH is an antagonist for SF-1 and its binding decreases CYP17 expression (Urs *et al.*, 2006). cAMP stimulation reverses the antagonistic action of SPH possibly by displacing SPH from the SF-1 ligand binding pocket and promoting PA binding (Figure 4). Notably, we have also demonstrated that phosphatidic acid (PA) is an endogenous activating ligand for SF1 in H295R adrenocortical cells (Li *et al.*, 2007). The identification of SPH as a ligand for SF-1 adds yet another level of regulation for sphingolipids in controlling steroid hormone production. Moreover, since SF-1 also regulates the expression of genes involved in endocrine development and sex differentiation, it is possible that SPH may also control these processes by antagonizing SF-1.

3.3 Sphingosine-1-Phosphate

As previously mentioned, S1P is an amphiphilic molecule that can exert its signaling functions both within the cell and extracellularly by binding to specific G protein-coupled receptors (S1PRs) (Kihara *et al.*, 2007). There are 5 S1PRs and each couples to multiple heterotrimeric G protein leading to the activation of specific intracellular targets (An *et al.*, 1997; Im *et al.*, 2000; Lee *et al.*, 1998; Van Brocklyn *et al.*, 2000; Yamazaki *et al.*, 2000). S1PR₁ couples to G_i and activates the phospholipase C (PLC), phosphatidylinositol 3-kinase (PI3K), and extracellular regulated kinase (ERK) pathways. S1PR₂ and S1PR₃ couple to G_i, G_{12/13}, and G_q and activate pathways such as PLC, PI3K, ERK, and Rho. S1PR₄ activates the PLC and ERK pathways, while S1P₅ has been shown to inhibit adenylyl cyclase and ERK (reviewed in ref. (Kihara *et al.*, 2007). Binding of S1P to its cognate receptor seems to be the major mode of signaling and the PI3K/Akt and ERK pathways have been identified as downstream targets (An *et al.*, 1997; Payne *et al.*, 2004; Spiegel and Milstien, 2002; Spiegel and Milstien, 2003b). Previous reports have found a link between PI3K and ERK activation and steroidogenesis, which supports a role for S1P in steroid hormone production (Meroni *et al.*, 2002; Shah *et al.*, 2005).

As depicted in Figure 4, our laboratory has demonstrated that cAMP-mediated S1P accumulation lead to an increase in CYP17 expression by activating cleavage of the sterol regulatory element binding protein 1 (SREBP1) in H295R human adrenocortical cells (Ozbay *et al.*, 2006). In the zona fasciculata of bovine adrenal cells, S1P was demonstrated to illicit cortisol secretion via binding to a S1PR and subsequent activation of PKC and PLD (Rabano *et al.*, 2003). In addition, Brizuela *et al.* (Brizuela *et al.*, 2006) reported that S1P stimulate aldosterone secretion in bovine glomerulosa adrenal cells. This aldosterone modulation is mediated by S1P binding to its cognate S1PR and activating the PLD/ phosphatidate phosphohydrolase pathway with PKC and extracellular Ca²⁺ as possible components of the signaling cascade.

Interestingly, we have also found that SK1 is rapidly translocated to the nucleus of H295R human adrenocortical cells in response to ACTH/cAMP stimulation (Li *et al.*, unpublished observations). Since SPH inhibits steroidogenesis by antagonizing SF-1, it is likely that nuclear import of SK1 facilitates conversion of SPH to S1P and acts to promote activated gene steroidogenic gene transcription. Given the identification of sphingolipids in the nucleus (Ledeen and Wu, 2006), future investigation into the significance of these molecules in nuclear function is likely to reveal novel roles for these bioactive lipids in controlling diverse cellular processes.

Akt and ERK 1/2 have also been identified as direct S1P targets (Brizuela *et al.*, 2007). The finding that inhibiting PI3K and MEK prevents S1P-mediated aldosterone secretion led Brizuela *et al.* to propose a model for S1P in the regulation of aldosterone production in which stimulation of PI3K/Akt and MEK/ERK pathways activate PLD and ultimately result in aldosterone production. S1P was identified as an inducer of CYP19 expression and estrogen production in granulosa cells by mediating the production of prostaglandin-2 (PGE₂), which is an established activator of CYP19 (Cai *et al.*, 1997). Given that S1P induces the expression of liver receptor homologue-1 (LRH-1) (Hadizadeh *et al.*, 2007) and LRH-1 regulates CYP19 expression in breast cancer cells (Clyne *et al.*, 2002), it is likely that estradiol production in both physiological and pathophysiological conditions is controlled by the amounts of S1P.

3.4 Sphingomyelin

SM is present in the plasma membrane and can be hydrolyzed into Cer via the action of SMases. SM hydrolysis is mediated by a series of stimuli including TNF- α , IL- β 1, and Fas ligands (Degnan *et al.*, 1996; Hannun, 1996), which, as described above, activate ceramide production. Therefore, SM seems to be a target for a series of external signals that modulate, among other cellular processes, steroidogenesis. S1P and C1P have been reported to repress acid SMase and may perhaps be part of a negative feedback loop that regulates flux through the sphingolipid metabolic pathway (Gomez-Munoz *et al.*, 2003; Gomez-Munoz *et al.*, 2004).

In mouse Leydig testicular cells, SM degradation was shown to be correlated with cholesterol movement from the plasma membrane to the mitochondria and subsequent increase in steroid hormone secretion (Porn *et al.*, 1991). Conversely, SMase activity was shown to inhibit Leydig cell function via degradation of SM and accumulation of the proapoptotic sphingolipid Cer (Degnan *et al.*, 1996). In addition, our laboratory has found that lysoSM (sphingosylphosphorylcholine) is able to bind to SF-1 in H295R adrenocortical cells under basal conditions, and that cAMP treatment promotes dissociation of the sphingolipid from SF1 (Urs *et al.*, 2006). Given that different sphingolipid species are in a dynamic balance at any given point within a cell, SM can play an important role in steroidogenesis regulation by serving as a precursor for Cer, lysoSM, and S1P.

3.5 Glycosphingolipids (gangliosides)

Thus far, the roles of bioactive sphingolipids in the modulation of steroidogenesis have been discussed. However, an equally important aspect of the relationship between sphingolipids

and steroid hormones involves the regulation of sphingolipid metabolism by steroid hormones. This concept is exemplified in neurosteroidogenesis between the complex sphingolipids, GMs, and the steroid hormone allopregnanolone (Griffin *et al.*, 2004; Mellon *et al.*, 2004). Mellon *et al.* have demonstrated that allopregnanolone treatment reduces GM accumulation and ameliorates neurodegeneration in the NPC-1 mouse model (Mellon, 2007). Even though the precise molecular mechanism by which these molecules regulate NPC progression is not unclear, these findings indicate the intimate relationship between steroid hormone biosynthesis and sphingolipid metabolism and emphasize the complexity of the regulatory mechanisms that control steroidogenesis. Complex GMs have also been found to be essential for optimal testosterone production and spermatogenesis in mice (Takamiya *et al.*, 1998). Testosterone has also been found to regulate GM levels in rat kidney (Anic and Mesaric, 1998).

Summary and Future Outlook

A significant amount of recent studies have pointed toward an important role for sphingolipids in the regulation of steroidogenesis. These bioactive lipids are key mediators that act at different levels of steroid hormone production including regulation of steroidogenic enzymes expression and activity as well as participating in regulatory signaling cascades. Although the studies discussed in this review significantly increased our understanding of the multiple mechanisms by which sphingolipids control steroidogenesis, it is evident that future studies are necessary to fully elucidate the roles of sphingolipids in steroid hormone biosynthesis. Technologies such as mass spectroscopy, metabolomic profiling, and proteomics are likely to be valuable tools in continued study of the relationship between these two classes of lipids. The identification and characterization of novel bioactive sphingolipids, quantification of flux through both the sphingolipid and steroidogenic metabolic pathways in response to various factors, and the examination of the role of steroid hormones as regulators of sphingolipid production are just a few research avenues that will provide more insight into the roles of sphingolipids in steroid hormone biosynthesis and endocrine function.

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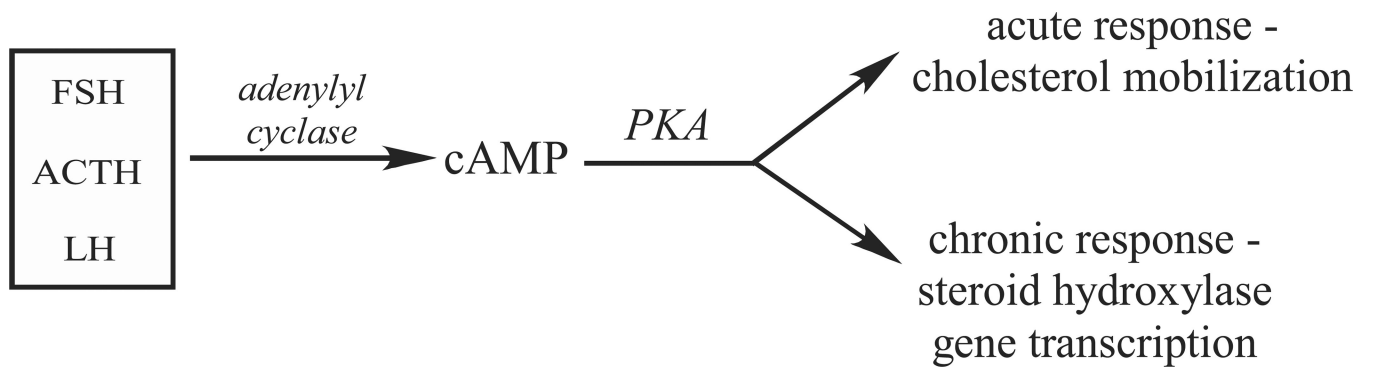


Figure 1.
Temporal regulation of steroidogenesis by peptide hormones.

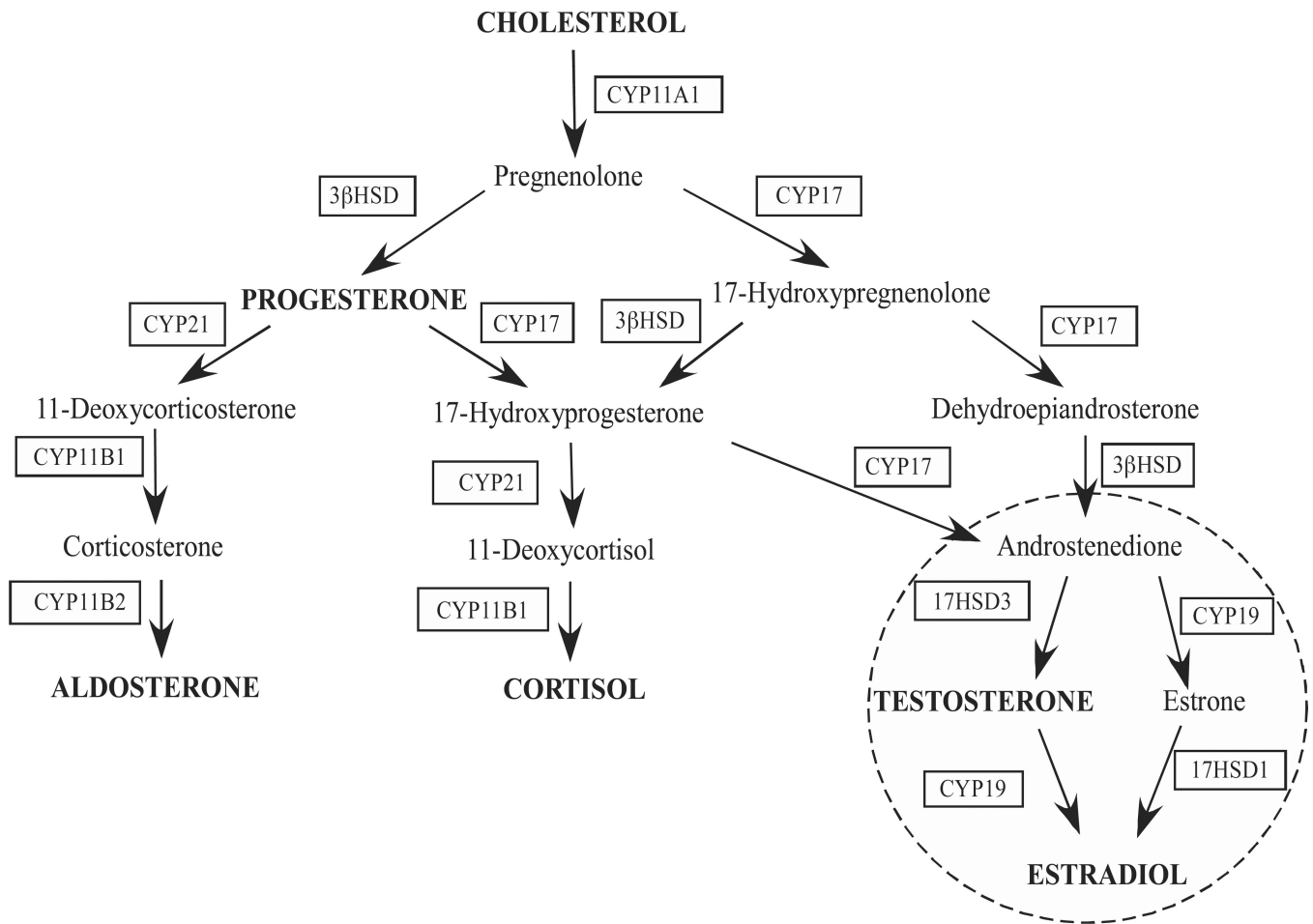


Figure 2.
Steroid hormone biosynthetic pathways.

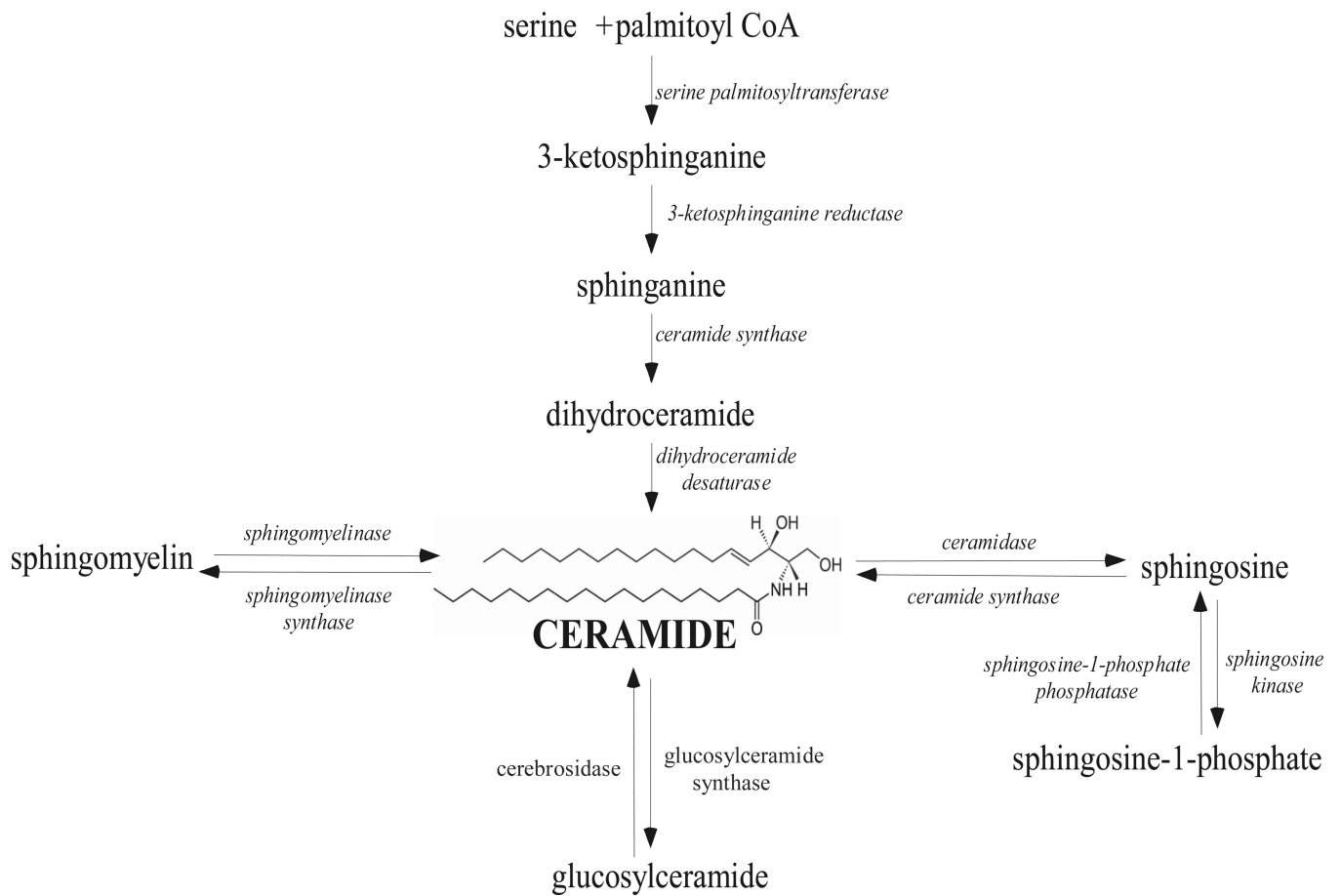


Figure 3.
Overview of sphingolipid metabolic pathways.

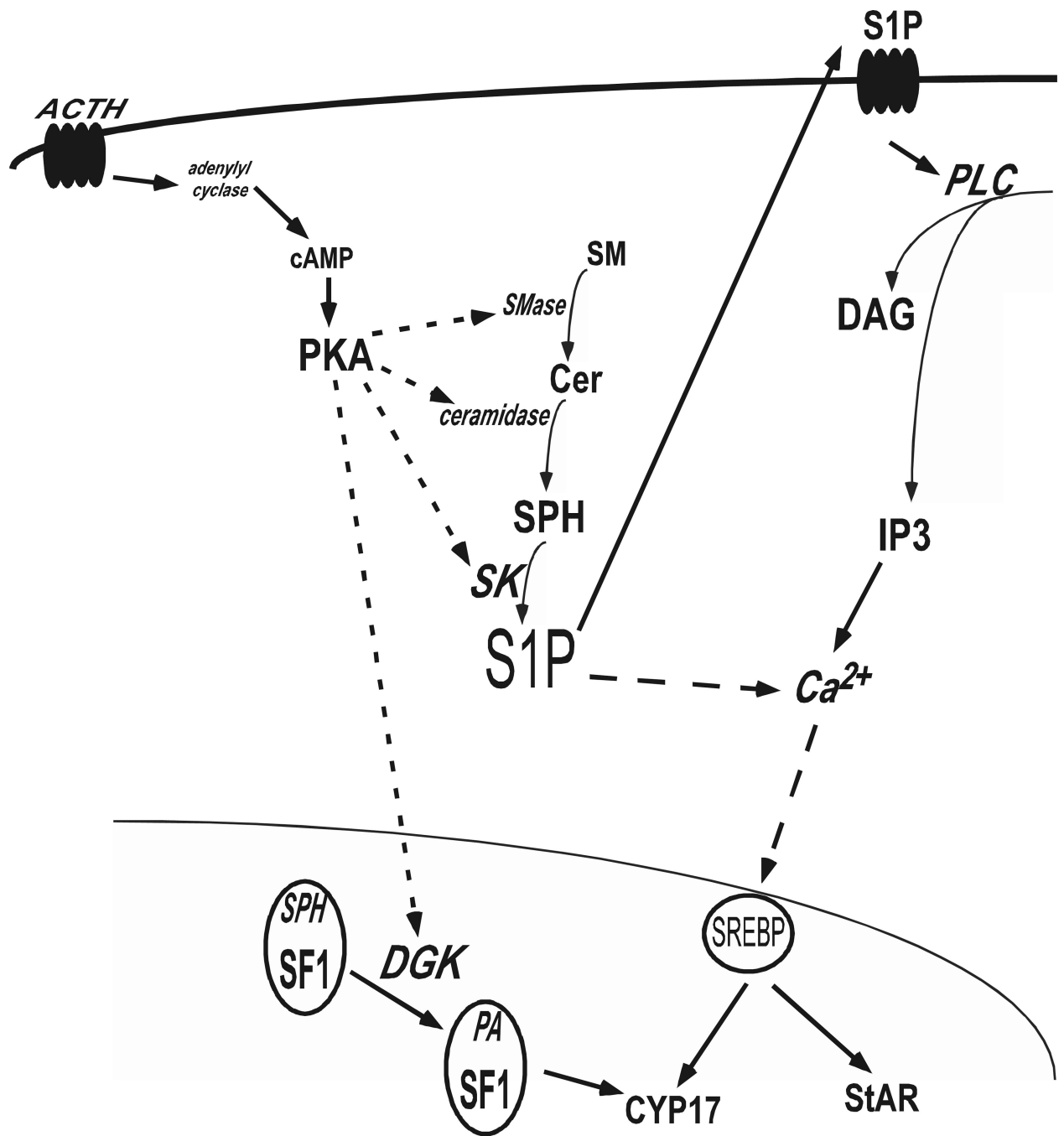


Figure 4. Relationship between sphingolipid metabolism and steroid hormone biosynthesis in the human adrenal cortex.

Table 1

Summary of recent data obtained for selected bioactive sphingolipids in steroidogenesis as well as their multiple downstream molecular targets and upstream molecular inducers.

Sphingolipid	Role in steroidogenesis	Upstream inducers	Downstream targets
Cer	Suppress progesterone and testosterone production; attenuate StAR expression; regulate CYP17 α activity; inhibit cAMP production; regulate 11 β -HSD1 and CYP19arom activity.	TNF- α , Fas ligand, INF- γ , IL-1 β , SMase	ERK1/2, SAPK, p38, c-Jun, caspases, CAPK, CAPP
SIP	Upregulate CYP17 and CYP19 expression; increase cortisol, estrogen, and aldosterone secretion;	SK1/2 activity. PKC, ERK2, and external S1PR ligands regulate SK activity.	ERK and PI3K/Akt pathways, PKC, PLD, PLC, S1PR ligand
SPH	Antagonist ligand		SF1
SM	Precursor of ceramide	Acid/neutral/alkaline ceramidase enzymatic activity	
GMs	Neurosteroidogenesis: degradation by allopregnanolone	allopregnanolone	

¹ Abbreviations used: tumor necrosis factor- α (TNF- α), interferon- γ (INF- γ), interleukin-1 β (IL-1 β), sphingosine kinase (SK), protein kinase C (PKC), sphingomyelinase (SMase), extracellular-regulated mitogen-activated protein kinase (ERK), stress-activated protein kinase (SAPK), ceramide activated protein kinase (CAPK), ceramide activated protein phosphatase (CAPP), mitogen activated kinase (MEK), phosphatidylinositol-3-kinase (PI3K), phospholipase C (PLC), phospholipase D (PLD), S1PR (S1P receptor).