

Novel mechanisms for DHEA action

Russell A Prough, Barbara J Clark and Carolyn M Klinge

Department of Biochemistry and Molecular Genetics, Center for Genetics and Molecular Medicine, University of Louisville School of Medicine, Louisville, KY, USA

Correspondence should be addressed to C M Klinge

Email
carolyn.klinge@louisville.edu

Abstract

Dehydroepiandrosterone (3 β -hydroxy-5-androsten-17-one, DHEA), secreted by the adrenal cortex, gastrointestinal tract, gonads, and brain, and its sulfated metabolite DHEA-S are the most abundant endogenous circulating steroid hormones. DHEA actions are classically associated with age-related changes in cardiovascular tissues, female fertility, metabolism, and neuronal/CNS functions. Early work on DHEA action focused on the metabolism to more potent sex hormones, testosterone and estradiol, and the subsequent effect on the activation of the androgen and estrogen steroid receptors. However, it is now clear that DHEA and DHEA-S act directly as ligands for many hepatic nuclear receptors and G-protein-coupled receptors. In addition, it can function to mediate acute cell signaling pathways. This review summarizes the molecular mechanisms by which DHEA acts in cells and animal models with a focus on the 'novel' and physiological modes of DHEA action.

Key Words

- ▶ DHEA
- ▶ DHEA-S
- ▶ nuclear receptors
- ▶ G-protein-coupled receptors

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Introduction

Metabolism of dehydroepiandrosterone (3 β -hydroxy-5-androstene-17-one, DHEA) and its 3 β -sulfated metabolite DHEA-S provides ~50% of androgens in men and ~75% of estrogens in premenopausal women (Maggio *et al.* 2015). Circulating levels of DHEA/DHEA-S decline with age (Labrie 2010), and this age-related decline in DHEA, DHEA-S, and other metabolites is associated with changes in cardiovascular tissues (reviewed in Mannic *et al.* 2015, Ohlsson *et al.* 2015), female fertility (Labrie 2015a, Tartagni *et al.* 2015), and metabolism, and neuronal/CNS functions (reviewed in Maninger *et al.* 2009, Traish *et al.* 2011, Maggio *et al.* 2015). In addition to providing precursors for sex steroids, DHEA binds directly to steroid hormone and nuclear receptors (NRs), activating various membrane receptors and inhibiting voltage-gated T-type Ca²⁺ channels. This review summarizes the established mechanisms by which DHEA activates its biological effects in cells, including receptors and intracellular signaling pathways.

DHEA synthesis and metabolism overview

DHEA synthesis mainly occurs in the adrenal zona reticularis: the inner zone of the adrenal cortex (Parker Jr 1999; Rainey & Nakamura 2008). All steroid hormones are derived from cholesterol with the first enzymatic reaction occurring in the mitochondria. The steroidogenic enzymes and pathway for DHEA synthesis in the adrenal have been well characterized and reviewed in detail and will only be briefly summarized herein (reviewed in Miller & Auchus 2011). Cholesterol transport across the mitochondrial membranes requires the action of the steroidogenic acute regulatory protein (STAR) (Clark & Stocco 1997). The cytochrome P450 (CYP) side-chain cleavage enzyme (CYP11A1 encoded by the *CYP11A1* gene) is located in the inner membrane of the mitochondria and converts cholesterol to pregnenolone. CYP11A1 is part of an electron transport complex that includes the flavin adenine dinucleotide (FAD) FAD-containing adrenodoxin reductase and iron–sulfur protein, adrenodoxin, that transfer reducing equivalents from NADPH to CYP11A1 for two sequential

hydroxylation reactions at C22 and C20 of cholesterol followed by cleavage of the C22–C20 bond to form pregnenolone (Mast *et al.* 2011). In the zona reticularis, pregnenolone is converted to DHEA by the action of CYP 17 α -hydroxylase/17,20-lyase (CYP17A1 encoded by the *CYP17A1* gene) (reviewed in Auchus 2004). CYP17A1 is an integral membrane protein of the endoplasmic reticulum attached via an amino terminal stop anchor sequence. The enzyme is part of a minielectron transport chain with NADPH-CYP oxidoreductase (POR, encoded by the *POR* gene) that donates electron from NADPH to the CYP17A1 (reviewed in Miller 2005). CYP17A1 has two enzymatic reactions: hydroxylation of pregnenolone at C17 to generate 17 α -hydroxypregnenolone, followed by cleavage of the C17–C20 bond of 17 α -hydroxypregnenolone to generate DHEA. The action of CYP17A1 generates a keto group at C17, which is the characteristic feature of the adrenal androgens. DHEA has a double bond between C5 and C6 of the B ring that can be isomerized to the C4–C5 position within the A ring by 3 β -hydroxysteroid dehydrogenase/ Δ 5– Δ 4 isomerase (*HSD3B2*) forming androstenedione (4-androstene-3 β ,17 β -dione, Adione). *HSD3B2* is a member of the short-chain dehydrogenase family that catalyzes both the oxidation of the 3 β -hydroxyl

group to the 3-keto group and the isomerization of the double bond from the C5–C6 to the C4–C5 position in an NAD⁺-dependent reaction (Persson *et al.* 2009). Alternatively, the C17 keto group of DHEA can be reduced by 17 β -hydroxysteroid dehydrogenase type 5 (17BHS5, encoded by the *AKR1C3* gene) to generate 5-androstene-3 β ,17 β -diol (Adiol) (Fig. 1). 17BHS5 is a member of the aldo–keto reductase family that catalyzes reduction–oxidation reactions at C17 for reversible conversion of C17-keto steroids to C17-hydroxysteroids (Jez *et al.* 1997). Both Adione and Adiol have been reported to be activators of NRs as described later. Sulfotransferase enzymes modify steroids through sulfation of free hydroxyl groups, a modification which increases the solubility of the steroid hormone (Mueller *et al.* 2015). DHEA is modified at the 3 β -hydroxyl group to generate DHEA sulfate (DHEA-S). DHEA and DHEA-S synthesis are developmentally and hormonally regulated (reviewed in Rainey & Nakamura 2008). Regeneration of active DHEA occurs in tissues via the action of steroid sulfatase, and this is an important biological function of adipose in postmenopausal women in whom the major source of estradiol is from adrenal DHEA-S conversion to estrogens in fat tissue (Labrie *et al.* 2007).

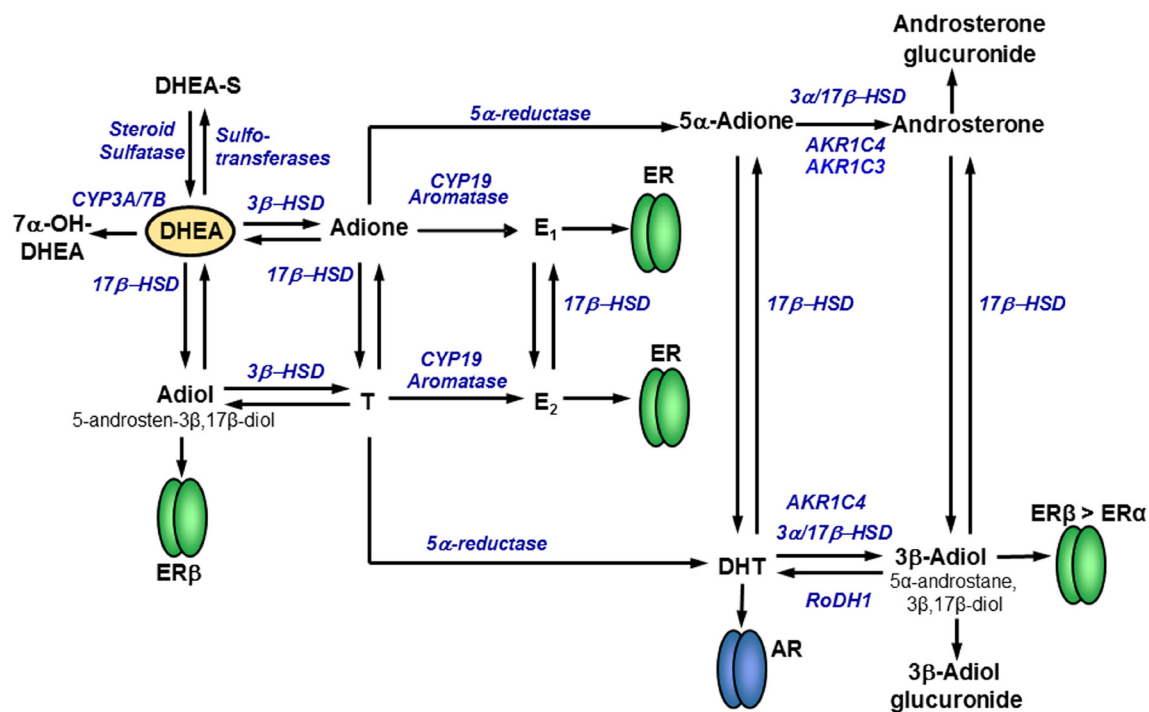


Figure 1

Selected aspects of DHEA metabolism. This model shows some of the pathways and enzymes by which DHEA is metabolized to steroids that bind ER and AR. AKR1C4 is liver specific, whereas AKR1C3 is expressed in many tissues, including the adrenal, brain, kidney, liver, lung mammary gland, placenta, small intestine, colon, spleen, prostate, and testis. This pathway is reviewed in Labrie *et al.* 2001; Labrie *et al.* 2005; Labrie 2015a,b.

Prepubertal elevations in serum DHEA/DHEA-S are coincident with the differentiation of the adrenal zona reticularis in humans, and peak DHEA/DHEA-S levels are observed around age 20 followed by age-dependent decline to prepubertal levels. DHEA levels in plasma in adult men and pre- and postmenopausal women range from 10 to 25 nM, 5 to 30 nM, and 2 to 20 nM, respectively, whereas DHEA-S levels are an order of magnitude higher in the 1–10 μ M range (Mueller *et al.* 2015). These levels decline to the lower nanomolar and micromolar ranges for DHEA and DHEA-S in women and men aged 60–80 years (Labrie *et al.* 1997, Labrie *et al.* 2005, Labrie 2010). In addition, genome-wide association studies indicate that serum levels of DHEA-S are regulated ~60% by genotypes at loci near these genes: *BCL2L11*, *ZKSCAN5*, *ARPC1A*, *TRIM4*, *HHEX*, *CYP2C9*, *BMF*, and *SULT2A1* (Vandenput & Ohlsson 2014).

DHEA synthesis is controlled by the hormonal signaling cascade of the hypothalamic–pituitary–adrenal axis. Corticotropin-releasing hormone released from the hypothalamus stimulates the anterior pituitary to synthesize and secrete adrenocorticotropin hormone (ACTH). ACTH binds to melanocortin-2 plasma membrane (PM) G-protein-coupled receptors (GPCRs) located on adrenocortical cells and activates cAMP-dependent protein kinase A (PKA)-dependent signaling. PKA signaling rapidly increases STAR for cholesterol delivery to the mitochondria and increases *STAR*, *CYP11A1*, and *CYP17A1* gene expression to maintain an increase in steroid output. In adults, DHEA levels peak in the morning, following the circadian pattern of ACTH secretion (Hammer *et al.* 2005).

Metabolism of DHEA to active androgens, including testosterone and 5-dihydrotestosterone (DHT), occurs in the gonads, liver, adrenals, and peripheral tissues (Fig. 1). In men with normal testis function, the contribution of DHEA to circulating testosterone represents a very small fraction, less than 5% of the total testosterone. However, in the absence of testis-derived testosterone, such as castration, adrenal androgens are the important precursors to maintain DHT levels in the prostate (Labrie *et al.* 2005). In premenopausal women, 40–75% of circulating testosterone is derived from peripheral metabolism of DHEA-S, whereas in postmenopausal women, over 90% of

the estrogens are derived from peripheral metabolism of DHEA-S. In both men and women, DHT and testosterone can be metabolized to estradiol (E2) or estrone, respectively, by aromatase (*CYP19A1*).

Declining DHEA and DHEA-S levels are associated with age-related disorders (Lois *et al.* 2000), whereas the overproduction of adrenal androgens contributes to disorders associated with hyperandrogenic states such as in polycystic ovarian syndrome (PCOS) and nonclassical 21-hydroxylase-deficient congenital adrenal hyperplasia (Goodarzi *et al.* 2015). Thus, in PCOS patients with elevated DHEA-S and testosterone compared with age-matched control group (Daan *et al.* 2015, Moran *et al.* 2015, Pinola *et al.* 2015), the novel mechanisms of DHEA action may be more pronounced.

DHEA binds and activates nuclear receptors

DHEA binds steroid hormone receptors (class I NRs) and selects class II NRs (Table 1) (reviewed in Webb *et al.* 2006, Traish *et al.* 2011) with the following reported affinities: pregnane X receptor/steroid and xenobiotic receptor (PXR/SXR, NR1I2), $K_d \sim 50\text{--}100 \mu\text{M}$ (Webb *et al.* 2006); estrogen receptors α and β (ER α (ESR1) and ER β (ESR2)), $K_d \sim 1.2$ and $0.5 \mu\text{M}$, respectively; androgen receptor (AR), $K_d \sim 1.1 \mu\text{M}$ (Chen *et al.* 2005); peroxisome proliferator-activated receptors (PPAR), $K_m \sim 7 \mu\text{M}$ (Webb *et al.* 2006); and PXR, $K_d \sim 10\text{--}50 \mu\text{M}$ (Ripp *et al.* 2002). Although higher and lower binding affinities have been reported, the binding affinities for the DHEA–NR interactions are much lower compared with cognate ligands for the receptors (Table 1). Therefore, the metabolism of DHEA to the potent androgens (testosterone and DHT) and estrogens, most notably estradiol (E2), is a confounder that must be addressed when assessing DHEA action on steroid NRs, as discussed below.

In addition to direct binding to NRs, DHEA has been shown to modulate the levels of NRs. DHEA (10–100 nM) increased *Er β* > *Era* > *Ar* mRNA levels in mouse osteoblasts (Wang *et al.* 2009), although there was no concentration–response relationship. We reported that the DHEA metabolite Adione bound ER β with an IC_{50} of

Table 1 Relative binding affinities of DHEA to NRs.

AR	ER α	ER β	PXR/SXR	PPAR α
2 μM (Lu <i>et al.</i> 2003)	1.2 μM (Chen <i>et al.</i> 2005) 245 nM (Kuiper <i>et al.</i> 1997)	500 nM (Chen <i>et al.</i> 2005) 163 nM (Kuiper <i>et al.</i> 1997)	50–100 μM (Webb <i>et al.</i> 2006)	No binding affinity determined (Altman <i>et al.</i> 2008)
1 μM (Chen <i>et al.</i> 2005)	5 μM (Adams <i>et al.</i> 1981) (Maggiolini <i>et al.</i> 1999) >500 μM (Michael Miller <i>et al.</i> 2013)	500 μM (Michael Miller <i>et al.</i> 2013)		

~5 nM (Michael Miller *et al.* 2013), whereas Adiol bound both ER α (ESR1) and ER β (ESR2) with nanometer affinities (Kuiper *et al.* 1997).

DHEA activation of steroid receptors

DHEA (1 μ M) activated transfected ER α in an estrogen response element (ERE)-driven luciferase reporter assay in GT1-7 mouse hypothalamic neuronal cells to a similar extent as 10 nM E2, suggesting ~1000-fold lower affinity (Bruder *et al.* 1997). DHEA did not appear to be metabolized to E2 in the GT1-7 cells. In MCF-7 and MCF-7SH (an estrogen nonresponsive subline) breast cancer cells and Ishikawa endometrial cancer cells, DHEA (1 nM–1 μ M) activated the ERE-driven luciferase activity in an ER-dependent manner, ascertained by inhibition by cotreatment with the antiestrogen 4-hydroxytamoxifen (Maggiolini *et al.* 1999). However, in these studies, the authors did not rule out the metabolism of DHEA to estrogens. DHEA also activated ER α in a yeast reporter assay (Bruder *et al.* 1997). Interestingly, DHEA (5 M) was reported to activate the ligand binding domain of ER β , but not ER α , in a mammalian two-hybrid luciferase reporter assay in transiently transfected COS-1 cells (Chen *et al.* 2005). This suggests that DHEA may activate ER α by a mechanism other than direct binding and/or that cell-specific factors mitigate whether DHEA activates ER action. One possibility is that DHEA may activate MAPK that phosphorylates and activates ER α independent of ligand, a possibility that fits with DHEA activation of PM-associated intracellular MAPK signaling in endothelial cells (ECs) (Liu & Dillon 2004, Liu *et al.* 2008).

DHEA binds AR with 1–2 μ M affinity (Table 1). DHEA activation of AR was reported to have antiestrogenic activity in MCF-7 breast cancer cells (Bocuzzi *et al.* 1993). DHEA showed higher transcriptional activation of mutant AR H874Y in prostate cancer cells than wild-type AR (Tan *et al.* 1997). Other studies suggest that DHEA activation of ER β predominates over AR activation in tissues in which both receptors are expressed, including prostate (reviewed in Arnold & Blackman 2005). As DHEA is metabolized to androgens in the prostate, the precise activity of DHEA, or DHEA-S, as ligands for AR is difficult to discern (reviewed in Arnold 2009).

DHEA activation of PPARs

A number of the hepatic NRs, PPAR α , constitutive androstane receptor (CAR), and PXR, that regulate the transcription of *CYP* genes and other foreign compound-metabolizing enzymes are regulated by sterols such as

DHEA, as initially reviewed by Webb and coworkers (Webb *et al.* 2006). Interest in DHEA as a regulator of these NRs came from a series of experimental pathology studies in which rodents were fed diets containing DHEA *ad libitum*. Many beneficial effects of DHEA have been reported in rodent studies, including the maintenance of lean body mass, improved coat color, and decreased incidence of chemically induced cancers, for example, colon cancer caused by 1,2-dimethylhydrazine exposure (Nyce *et al.* 1984), lung cancer caused by installation of 7,12-dimethyl-benzanthracene (Schwartz & Tannen 1981), and skin papilloma caused by painting with 7,12-dimethylbenzanthracene and phorbol esters (Pashko *et al.* 1985). DHEA feeding also decreased the appearance of virally induced, spontaneous breast tumors in female mice (Schwartz 1979). Among the other beneficial effects of DHEA feeding in rodents are decreases in the incidence of genetically disposed obesity (Yen *et al.* 1977), diabetes (Coleman *et al.* 1982), and systemic lupus erythematosus (Lucas *et al.* 1985). Our early work with DHEA led us to study the effect of DHEA feeding in rats with hepatomegaly and induction of the hepatic and renal *Cyp4a* (*Cyp4a10*) genes (Wu *et al.* 1989). After 4 days of feeding rats a diet containing 0.45% DHEA, the levels of total CYP and its flavoprotein oxidoreductase were increased approximately 1.8–2.0-fold at the 50 mg/kg dose given daily for 4 days. Of the enzymatic activities measured in the liver microsomes, the major CYP activity induced was laurate ω -hydroxylase activity, which was induced 16.9-fold. Other marker enzymes for peroxisomal proliferation were also elevated (Wu *et al.* 1989).

In 1993, Sakuma and coworkers reported that Adiol activated peroxisome proliferation in cultured rat hepatocytes (Sakuma *et al.* 1993). Subsequent studies using isolated rat hepatocytes *in vitro* which compared DHEA induction of rat CYP4A *in vivo* with the effect of E₂ and Adiol demonstrated that Adiol was a better inducing agent, that is, required at lower doses for induction of CYP4A and a number of markers of peroxisome proliferation than DHEA itself (Prough *et al.* 1994). E₂ treatment had little or no effect on these markers. These studies also demonstrated that DHEA induction of CYP4A was a transcriptional process.

We also were interested in regulation of these processes by thyroid hormone (T₃). DHEA has been shown to also induce transcription of lipid metabolism genes such as malic enzyme and mitochondrial sn-glycerol-3-phosphate dehydrogenase (Lardy *et al.* 1995, Bobyleva *et al.* 2000), which are also transcriptionally regulated by T₃.

T₃ was shown to be required for induction of malic enzyme by DHEA (Song *et al.* 1989) and Su & Lardy (1991) noted that DHEA induction of these enzymes was suppressed in livers of hypothyroid animals. We subsequently showed that treating rats with T₃ doesn't affect DHEA-dependent peroxisome proliferation as measured by NADPH:CYP oxidoreductase and fatty acyl-CoA oxidase activity (Webb *et al.* 1996). However, supraphysiological T₃ concentrations (50 µg/100 g body weight), suppressed DHEA induction of hepatic CYP4A1, CYP4A2, and CYP4A3 over 70% at the mRNA and protein levels. CYP4A2 transcript in kidney, which only expresses CYP4A2, was also suppressed more than 95% at high T₃ doses. We subsequently demonstrated that this transcriptional regulation was strikingly suppressed in hypothyroid rats treated with 10 µg/100 g body weight doses of T₃ daily for 4 days. This level of T₃ administration results in blood levels of the hormone near the levels of T₃ seen in euthyroid rats. Interestingly, CYP4A2 was most suppressed by the physiological doses of T₃ given to hypothyroid rats.

In light of these studies, we sought to define the receptors involved in DHEA action using transient transfection assays to more easily test which derivatives of DHEA were most potent in regulating receptors. Many of the genes induced by DHEA feeding were noted to have putative peroxisome proliferator responsive elements in their 5'-flanking regions of their genes, but others like NADPH:CYP oxidoreductase do not, suggesting that other receptors may be involved in DHEA induced gene expression.

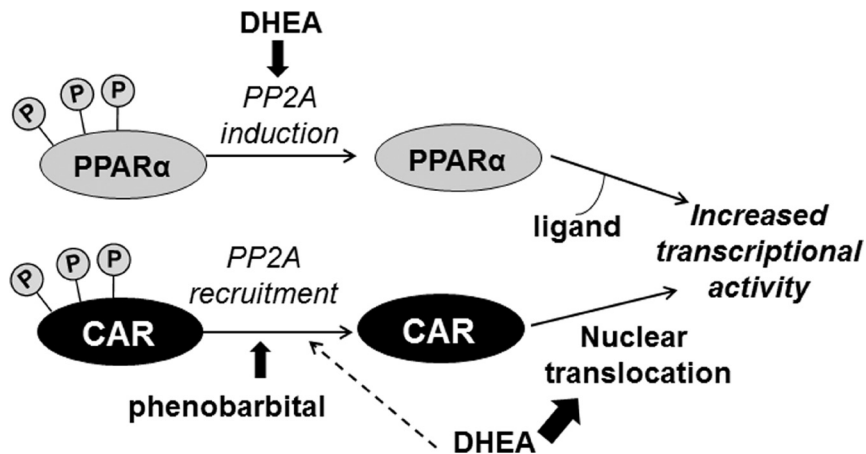
Knockout of *Ppara* in mice *in vivo* blunts the induction of CYP4A expression by Wyeth14643 (WY14643, a synthetic PPAR α agonist (Bernardes *et al.* 2013)) and DHEA-S *in vivo* (Peters *et al.* 1996). Therefore, our first studies were designed to test the ability of various DHEA metabolites to induce the expression of a transfected PPAR α -dependent luciferase reporter gene in cultured human hepatoma HepG2 cells. Much to our surprise, neither DHEA, DHEA-S, nor any of the DHEA metabolites tested increased PPAR α -dependent luciferase reporter activity, whereas nafenopin, a known peroxisome proliferator, increased the activity more than 40-fold (Webb *et al.* 2006). Subsequently, we used primary rat hepatocytes to test the ability of DHEA and its metabolites to induce CYP4A1 expression (Webb *et al.* 2006). DHEA and its metabolites (Adiol, DHEA-S, 7 α -hydroxy-DHEA, 7-oxo-DHEA, and 7-oxo-Adiol) induced the expression of *CYP4A1* mRNA. The apparent K_m for 7-oxo-DHEA for this induction was ~7 µM.

These results demonstrate that induction of CYP4A1 by DHEA and its metabolite in HepG2 cells must require a transcription factor or modulator in addition to PPAR α that is present in the primary rat hepatocytes, but not HepG2 cells.

DHEA and WY14643 induced the levels of PPAR α mRNA and protein by two- to four-fold and concomitantly decreased the phosphorylation of serine 6, 12, and 21, suggesting that the peroxisome-proliferating agents induce PPAR α levels and decrease its overall phosphorylation status (Tamasi *et al.* 2008). We examined if the phosphorylation of these three serine residues (6, 12, 21) in the N-terminal region of PPAR α affects PPAR α transcriptional activity. We demonstrated that the mutation of the serine residues to alanine increased PPAR α transcriptional activity by over 60%, supporting that dephosphorylation of PPAR α stimulates its activity (Tamasi *et al.* 2008). In primary cultured rat hepatocytes, DHEA, nafenopin, and Wy14643 decreased the levels of PPAR α serine 6 and 21 phosphorylation, whereas Okadaic acid, a potent inhibitor of PP2A, a multi-subunit protein phosphatase, causes PPAR α to become fully phosphorylated at those residues. Okadaic acid also blunted PPAR α -luciferase reporter activity stimulated by DHEA treatment of primary hepatocytes. Measurement of *PPP2CA* (the catalytic subunit of PP2A) mRNA levels in primary rat hepatocytes demonstrated that DHEA caused an eighth-fold increase in *PPP2CA* message relative to nafenopin, but mRNA levels decreased rapidly to control levels by 24h. These studies demonstrate that DHEA transcriptionally regulates *PPP2CA* and further protein kinases regulate the transcriptional activity of PPAR α through direct protein phosphorylation in primary rat hepatocytes and HepG2 cells (Fig. 2).

DHEA activation of CAR

The suspicion that other NRs are activated by DHEA came from the observations of DHEA stimulation of NADPH:CYP oxidoreductase (POR) and CYP3A23 mRNA and protein levels in rat liver, genes with no observable PPREs in their 5'-flanking regions (data not shown). POR is regulated through CAR activation, being a member of the gene battery that is upregulated by phenobarbital as well as by other compounds that possibly act as direct CAR ligands (Kobayashi *et al.* 2015). Other studies showed that mice fed DHEA- or DHEA-S-containing food (0.4% w/v for 15 days) had hepatomegaly, peroxisome proliferation, and activation of liver CAR β as assessed by DNA-binding activity *in vitro* in an electrophoretic mobility shift assay (EMS) and increased *Cyp2b10* transcript levels *in vivo*

**Figure 2**

Possible mechanism by which DHEA activates PPAR α independent of direct binding. Phenobarbital was reported to activate CAR by stimulating PP2A recruitment to the CAR:HSP90 complex to dephosphorylate and increase CAR nuclear translocation (Yoshinari *et al.* 2003; Mutoh *et al.* 2013; Kobayashi *et al.* 2015). DHEA induces PP2A that dephosphorylates PPAR α , which may enhance ligand-activated transcription (Tamasi *et al.* 2008). DHEA stimulates CAR nuclear translocation (Kohalmy *et al.* 2007); hence, we suggest a role for PP2A that appears to be common for both PPAR α and CAR activation. (Solid arrows, established facts; dashed arrow, speculation based on experimental evidence).

(Fujita *et al.* 2002). However, this study showed only increased nuclear localization of RXR and not CAR β after DHEA feeding, leaving open the question of how DHEA activates CAR β . Therefore, we collaborated with Urs Meyer and Katalin Monostory to document that CAR is activated to increase its transcriptional activity (Kohalmy *et al.* 2007). In primary human hepatocytes, *CYP2B6* mRNA was induced upon treatment with DHEA alone, but not with 7 α -hydroxy-DHEA or 7-oxo-DHEA. This induction was blunted by the addition of the CAR antagonist, androstanol. Finally, like phenobarbital or 3,3',5,5'-tetrachloro-1,4-bis(pyridyloxy)benzene (a direct ligand for CAR), DHEA caused the change in the subcellular localization of CAR from the cytoplasm to the nuclei, accompanied by dephosphorylation of CAR at threonine 38 (Kohalmy *et al.* 2007).

In a manner similar to our work on PPAR α and PP2A, Negishi and coworkers have shown that protein phosphatase 2A (PP2A) is required for CAR activation by mediating the translocation of CAR from the cytosol to the nucleus (Kobayashi *et al.* 2015). Phosphorylated CAR is localized in a cytoplasmic protein complex that is disrupted by PP2A-dependent dephosphorylation of CAR, resulting in exposure of CAR's nuclear localization signal and CAR translocation to the nucleus to activate the gene expression of its targets (Sueyoshi *et al.* 2008, Mutoh *et al.* 2009). Recently, Mutoh and coworkers described the activation of CAR through a CAR ligand-independent mechanism, in which phenobarbital blocks the binding of epidermal growth factor (EGF) to EGF receptor (EGFR) in the cell membrane (Mutoh *et al.* 2013). EGFR modulates the activation of SRC kinase and ERK that keep threonine 38 of CAR phosphorylated and, therefore, CAR is sequestered in a cytoplasmic protein complex (Kobayashi *et al.* 2015). Only when PP2A is present through *de novo* synthesis does that complex

dissociate (after CAR dephosphorylation) allowing nuclear transport (Fig. 2). In addition to PP2A activation of CAR, direct ligand binding to CAR also disrupts the cytoplasmic protein complex releasing CAR for nuclear localization. We suggest a role for PP2A that appears to be common for both PPAR α and CAR activation, which we speculate based on our research cited above may also be stimulated by DHEA to activate CAR (Fig. 2).

DHEA activation of PXR

PXR binds a number of pregnane-related steroids (Chai *et al.* 2013). We observed the induction of P4503A23 in the livers of rats fed DHEA and, because DHEA is a pregnane derivative, it was likely that DHEA and its metabolites may act as a ligand of PXR (Ripp *et al.* 2002). Using *Ppar α* -null mice fed DHEA, we were unable to detect the induction of *Cyp4a1*, but there was a two-fold increase in the enzymatic activity and mRNA specific for *Cyp3a11* in both wild-type and PPAR α -null mice (Ripp *et al.* 2002). In *Pxr*-null mice, we did not observe P4503A11 induction upon DHEA feeding (S J Webb and R A Prough, unpublished observations). Other experiments demonstrated that DHEA, Adiol, and Adione activate PXR in luciferase assays in HepG2 cells, using a vector containing two copies of the PXR responsive element (PXRE) from the rat *Cyp3a23* gene (Ripp *et al.* 2002). However, other oxidized metabolites of DHEA have no effect in stimulating PXRE-luciferase activity at low micromolar concentrations (Ripp *et al.* 2002). Human and murine PXR have been shown to have differences, in which the ligand activates each receptor; the human receptor is activated by rifampicin but not the murine receptor. Pregnenolone 16 α -carbonitrile is a good ligand for the mouse receptor, but not for the human receptor (Kliwer *et al.* 1998).

For DHEA metabolites, we observed similar species differences between the human and mouse receptors. For human PXR, Adiol is not a good ligand activator as DHEA or Adione in activating human PXR, based upon their concentration dependence of activation (Ripp *et al.* 2002). By contrast, Adiol is a better ligand activator than DHEA and Adione for murine PXR. These results further demonstrate the differences in the active sites of the human and mouse PXR (Jones *et al.* 2000).

DHEA activation of mineralocorticoid receptor

One study has indicated that the mineralocorticoid receptor (MR) is required for DHEA-mediated signaling in human umbilical vein endothelial cells (HUVECs), human skin fibroblasts, and either human or rat aortic vascular smooth muscle cells (VSMCs) (Lindschau *et al.* 2011). DHEA (100 nM) rapidly (10–20 min) increased the activation of ERK1/2 and increased FOXO1 phosphorylation and nuclear localization in rat VSMCs. Both ERK1/2 and FOXO1 activation were blocked by transfection with siRNA directed against or by the MR antagonist eplerenone (10 μ M). However, DHEA did not activate the MR-luciferase reporter activity in transiently transfected CHO-K1 cells, leading the authors to conclude that DHEA is not a direct ligand of MR (Lindschau *et al.* 2011). Although further studies are needed on DHEA activation of MR, DHEA-MR cross talk mediated by other membrane receptors, for example, EGFR, may be a possibility (Meinel *et al.* 2014).

Conclusion about DHEA activation of NRs that are activated by foreign chemicals

Although the effective concentrations for 50% activation are higher (5–10 μ M vs 0.3 μ M) for PPAR α , CAR, and PXR than for ERs (Table 1), the concentrations required for the activation approached that observed for the concentration for circulating DHEA-S. We suggest that under adverse pathological states in which these sterols may be accumulated in the liver, one might expect the activation of genes regulated by the steroid hormone NRs. In addition, the hydroxylated metabolites of DHEA are rapidly interconverted by the enzyme 11 β -hydroxysteroid dehydrogenase (Robinson *et al.* 2003). There may be other receptors activated by these further oxidation products of DHEA as will be discussed in the following section.

DHEA activation of GPCRs

DHEA has been reported to bind and activate PM receptors in a cell-specific manner (Table 2, Fig. 3). To our knowledge, the first report of DHEA's activation of a GPCR was from Dillon's laboratory (Liu & Dillon 2002). This group has demonstrated that DHEA binds and activates a DHEA-specific GPCR in caveolae in the PM of vascular ECs with a K_d ~49 pM leading to the activation of MAPK and endothelial nitric oxide synthase (eNOS) (Liu & Dillon 2002, 2004, Simoncini *et al.* 2003, Liu *et al.* 2008, Liu *et al.* 2010, Olivo *et al.* 2010). These authors demonstrated that a membrane-impermeable DHEA-BSA conjugate (1 nM) activated MAPK and induced EC proliferation and cellular DHEA activities. DHEA and DHEA-BSA conjugate (1 nM, each) induced endothelial H₂O₂ production in a Gi/o protein-dependent manner, and the increase in H₂O₂ was critical for DHEA-simulated cell proliferation (Iruthayanathan *et al.* 2011). A biotinylated DHEA conjugate identified DHEA binding proteins of 55, 80, and 150 kDa in the PM of bovine aortic ECs (BAECs) (Liu *et al.* 2010, Olivo *et al.* 2010). Data from inhibitor studies by other investigators indicate that neither ER subtype (ER α or ER β) nor AR is involved in DHEA (10 nM) activation of MAPK and downstream effects in BAECs (Williams *et al.* 2004).

DHEA-S (1 μ M) induced the release of granule-associated β -HEX from RBL-2H3 cells by activating a GPCR (Mizota *et al.* 2005). Progesterone-BSA and E₂ (each at 10 μ M) inhibited the rapid (5–15 min) activity of DHEA-S in these cells, suggesting a common GPCR. Antiprogestin RU486, Fulvestrant (an ER antagonist), Wortmannin (a PI3K inhibitor), and pertussis toxin (PTX, a G-protein inhibitor) did not block this effect, whereas inhibitors of phospholipase C and inositol trisphosphate (InsP₃) receptor and pretreatment with an antisense oligomer for G $\alpha_{q/11}$ blocked the DHEA-S activity. The authors concluded that DHEA-S induced degranulation by interacting with a Gq/11 protein-coupled membrane receptor (Mizota *et al.* 2005).

In mouse spermatogenic GC-2 cells that lack steroid sulfatase, DHEA-S (1 nM) activated phosphorylation of ERK1/2, c-SRC, ATF1, and CREB (CREB1) after 30–180 min, suggesting a direct rapid effect of DHEA-S rather than DHEA or a metabolite thereof (Shihan *et al.* 2013). Knockdown of *Ar* did not block DHEA-S-stimulated ERK_{1/2} activation, but silencing of *Gna11* (the equivalent of *Gaq/11*) inhibited DHEA-S-induced phosphorylation of ERK1/2, c-SRC, ATF1, and CREB (Shihan *et al.* 2013). This group of investigators recently reported that testosterone binds ZIP9, a Zn²⁺ transporter from the family of the

Table 2 DHEA activation of PM receptors.

PM receptor	Cell/tissue	Affinity/concentration	Activity	References
G-protein coupled Galpha i2, 3-coupled	BAECs; HUVEC	$K_d = 48.7 \text{ pM}$ $B_{max} = 500 \text{ fmol/mg protein}$; lower binding in the heart and liver, very low in the kidney; [^3H]DHEA binding sites were not competed with testosterone, androstenedione, or E_2	10 nM DHEA activated eNOS in 15 min No effect on intracellular Ca^{2+} fluxes Activated MAPK	Liu & Dillon (2002, 2004)
G-protein coupled $\text{G}\alpha_{q/11}$ receptor	RBL-2H3 mast cells	Activity blocked by a PLC inhibitor, an antagonist to IP3R receptor	DHEA-5 (1 μM)-induced the release of granule-associated β -HEX from RBL-2H3 cells	Mizota <i>et al.</i> (2005)
G-protein coupled $\text{G}\alpha_{q/11}$ receptor	Mouse spermatogenic GC-2 cells		DHEA-5 (1 nM) activated phosphorylation of ERK1/2, c-SRC, ATF1, and CREB	Shihan <i>et al.</i> (2013)
G-protein coupled	hCASMC		Inhibited activation of the AKT/GSK-3 β axis	Bonnet <i>et al.</i> (2009)
GPER/GPR30		7 β -Hydroxy-epiandrosterone	Stimulated SKBR3 and MCF-7 cell proliferation	Niro <i>et al.</i> (2012)
GPER	HepG2 cells	10 nM	Stimulated EGFR, SRC, ER1/2, PI3K signaling; miR-21 transcription	Teng <i>et al.</i> (2015)
NMDA receptor	Rat hippocampal slices Mouse embryonic neocortical neurons Primary human neural stem cells	50 nM–1 μM 1 pM	Increased intracellular Ca^{2+}	Monnet <i>et al.</i> (1995), Compagnone & Mellon (1998), Suzuki <i>et al.</i> (2004)
Voltage-gated T-type Ca^{2+} channels: CaV3.1, CaV3.2, or CaV3.3	Transfected NG108-15 cells-somatic mouse neuroblastoma/rat glioblastoma cell line	10 μM	Inhibited peak current	Chevalier <i>et al.</i> (2012)
Sigma 1 (σ 1) receptor	C6 rat glioma cells PC12 cells	$K_d > 50 \mu\text{M}$	PI3K/AKT, SRC, PKA, PKC, and MAPK pathways	Rybczynska <i>et al.</i> (2009), Moriguchi <i>et al.</i> (2013)
TRKA and p75 ^{NTR} membrane receptors of neurotrophin NGF family	PC12 rat adrenal pheochromocytoma cells	K_d : $7.4 \pm 1.75 \text{ nM}$ and $5.6 \pm 0.55 \text{ nM}$ for TRKA and p75 ^{NTR} receptors, respectively, in HEK-293 cells	Induced TRKA phosphorylation and NGF receptor-mediated signaling; SHC, AKT, and ERK1/2 kinases downstream to TRKA receptors and TRAF6, RIP2, and RhoGDI interactors of p75 ^{NTR} receptors	Lazaridis <i>et al.</i> (2011), Gravanis <i>et al.</i> (2012), Anagnostopoulou <i>et al.</i> (2013), Padiaditakis <i>et al.</i> (2015)
GABA _A Rs	HEK-293 cells transfected with GABA _A R		DHEA blocked	Svob Strac <i>et al.</i> (2012)
IGF-1 R	Human mesenchymal stem cells	10 nM	IGF1 receptor, PI3K, P38 MAPK, and ERK1/2 MAPK, but not PKA or CREB	Liang <i>et al.</i> (2015)
Dendritic brain microtubule-associated protein MAP2C	<i>In vitro</i> experiments with MAP2C expressed in <i>Escherichia coli</i> and purified; methods used: circular dichroism and isothermal titration calorimetry	$K_d = 27 \mu\text{M}$	Promotes tubulin polymerization and stabilizes microtubules	Laurine <i>et al.</i> (2003)

ZRT- and IRT-like proteins (ZRT, zinc-regulated transporter; IRT, iron-regulated transporter), which directly interacts with the G-protein $G\alpha_{11}$ and acts as a membrane-bound *Ar* in GC-2 cells leading to ERK1/2, CREB, or ATF-1 activation (Shihan *et al.* 2015). Whether DHEA or DHEA-S activates ZIP9 in GC-2 cells has not yet been examined.

These reports show that DHEA and DHEA-S activate $G\alpha_q/11$ signaling in EC, BAEC, and GC2 cells, suggesting a common GPCR-activated signaling pathway, even if the GPCR may be different between cells.

DHEA was reported to inhibit human carotid artery smooth muscle cell (hCASMC) proliferation by blocking the activation of the AKT/GSK3 β axis independently of both ER α and AR, but through a PM GPCR (Bonnet *et al.* 2009). This conclusion was based on the inhibition of AKT and GSK-3 β phosphorylation in hCASMC treated with BSA-conjugated DHEA. In pulmonary artery smooth muscle cells isolated from patients with idiopathic pulmonary arterial hypertension, DHEA (100 μ M for 48h) inhibited SRC/STAT3 activation and decreased STAT3 target genes, that is, *PIM1*, *NFATC2*, *BMPR2*, and Survivin (Paulin *et al.* 2011). However, no mechanism was identified for DHEA's inhibition of STAT3 activation. More recently, DHEA (100 μ M) has suppressed the inflammatory response of rat aortic VSMCs to Angiotensin 2 (ANG2, 1 μ M) by inhibiting ROS production and p38 MAPK and MAPK (pERK1/2) activation, preventing degradation of

cytoplasmic 1 κ B and thereby reducing NF κ B activation; however, the mechanism by which DHEA achieved these effects was not identified (Chen *et al.* 2014).

DHEA's rapid activation of miR-21 expression via G-protein ER

G-protein ER (GPER; originally called GPR30) is an integral PM receptor coupled to $G\alpha_s$ in its inactive state and, when activated, forms heterotrimeric G proteins that stimulate adenylate cyclase, SRC, and EGFR signaling (Gaudet *et al.* 2015, Prossnitz & Barton 2014). It was suggested that the DHEA metabolite, 7 β -hydroxy-epiandrosterone, binds to GPR30/GPER in SKBR3 and MCF-7 breast cancer cells based on its ability to inhibit GPER-specific agonist G-1-induced cell proliferation (Niro *et al.* 2012). We have recently reported that DHEA (10nM) increased the transcription of miR-21 in HepG2 cells in part via GPER signaling. DHEA had a biphasic effect on miR-21 transcription with an initial peak at 1h followed by a decrease and then an increase again after 3h which is sustained to 12h (Teng *et al.* 2014, Teng *et al.* 2015). The DHEA-induced increase in pri-miR-21 and miR-21 was inhibited by the transcriptional inhibitor actinomycin D, but not cycloheximide, suggesting a primary transcriptional effect of DHEA. The initial rapid DHEA-induced increase in *MIR21* (miR-21) transcription that peaked at 1h involved

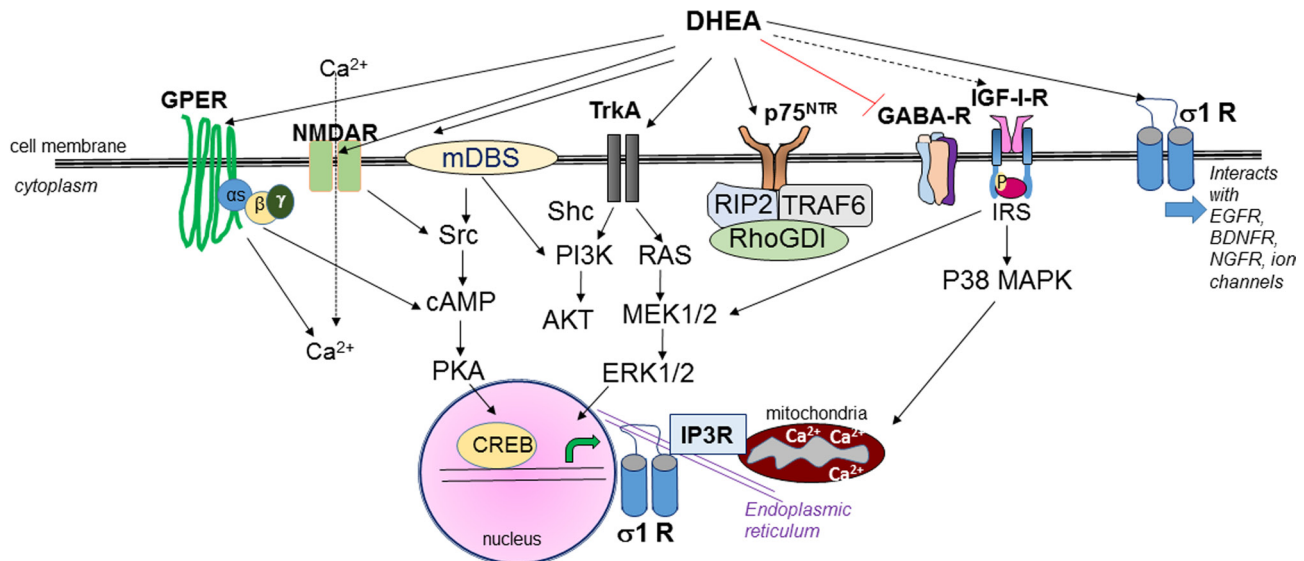


Figure 3

DHEA activation or inhibition of PM receptors. This model summarizes the interaction of DHEA with PM receptors as described in the text and in Table 2 with references to each receptor included therein. The sigma-1 receptor plays a key role interface between the endoplasmic reticulum and the mitochondria (referred to as mitochondrial-associated endoplasmic reticulum membrane (MAM) (Cheng *et al.* 2008; Bernard-Marissal *et al.* 2015; Lewis *et al.* 2016)). IP3R modulates Ca²⁺ homeostasis (Ruscher & Wieloch 2015).

GPER, ER α 36, EGFR signaling, and G protein activation of c-SRC, ERK1/2, and PI3K. We showed that the GPER-specific antagonist G-15 attenuated DHEA- and BSA-conjugated DHEA-stimulated pri-miR-21 transcription (Teng *et al.* 2015). DHEA, such as GPER agonist G-1, increased GPER and ER α 36 mRNA and protein levels. DHEA also increased ERK1/2 and c-SRC phosphorylation in a GPER-responsive manner in HepG2 cells. The pERK/ERK ratio remained elevated (>1) up to 6 h after DHEA treatment. DHEA also increased ER α ser 118 phosphorylation, an established target of MAPK (Joel *et al.* 1998). MAPK activation by GPER has been reported to increase the expression of downstream transcription factors (Prossnitz & Maggiolini 2009). For example, GPER activation by 1 μ M E₂ increased c-FOS protein in SKBR3 (ER α -) breast cancer cells (Maggiolini *et al.* 2004) and G-1 increased c-JUN and c-FOS expression in an MAPK-dependent manner in PC-3 prostate cancer cells (Chan *et al.* 2010). We reported that DHEA increased c-JUN but had no effect on c-FOS protein levels in HepG2 cells.

Overexpression of GPER induced ER α 36 transcription in HEK-293 and COS-7 cells (Chaudhri *et al.* 2012). ER α 36 is a PM-associated splice variant of ER α , which has been proposed to mediate the membrane-initiated effects of E₂. ER α 36 is a direct transcriptional target of GPER activation of the SRC/MEK1/2/AP1 pathway (Kang *et al.* 2010, Wang & Yin 2015); as stated previously, we observed that DHEA increased ER α 36 expression. Specifically, 10 nM DHEA or G-1, serving as a positive control (Kang *et al.* 2010), increased ER α 36 mRNA expression after only 1 h, and this increase was abrogated by siGPER (Teng *et al.* 2015). However, the induction of ER α 36 was transient as levels returned to baseline control after 6 h of DHEA or G-1 treatment. This result helped us to distinguish the mechanisms responsible for the rapid activation of GPER with \leq 1 h of DHEA versus the longer term effect of DHEA that involved its metabolites after 6 h of treatment (Teng *et al.* 2014).

Efficient signal transduction requires signaling molecules to be preorganized, sequestered, and compartmentalized into microdomains at the PM (Martinez-Outschoorn *et al.* 2015). Therefore, we tested a role for caveolae in DHEA signaling. Using methyl- β -cyclodextrin, an agent that removes cholesterol from the PM (Rodal *et al.* 1999), we observed that DHEA-induced pri-miR-21 and mature miR-21 transcript expression was inhibited (Teng *et al.* 2015). Thus, our data suggested a need for intact lipid raft structure for rapid DHEA signaling.

Altogether, our data suggest that the initial rapid increase in pri-miR-21 transcription in DHEA-treated HepG2 cells was

mediated by activation of GPER and ER α 36 and subsequent SRC, EGFR, and MAPK signaling resulting in increased c-JUN protein expression and ER α phosphorylation. The second phase of pri-miR-21 transcription, examined after 6 h of DHEA treatment, was mediated by DHEA metabolites produced in these cells that activate AR and ER β recruitment to the miR-21 promoter. Importantly, these results are from cells treated with 10 nM DHEA, that is, a physiologically relevant level in human serum (Labrie 2010).

DHEA increases IGF-I receptor

DHEA increases the bone mineral density in men and women (Traish *et al.* 2011, Weiss *et al.* 2009). DHEA inhibited secretion of IL6, an osteolytic cytokine, and stimulated osteoblast differentiation of human mesenchymal stem cells by increasing IGF1 gene transcription *in vitro* through a mechanism involving IGF 1 receptor, PI3K, P38 MAPK, and ERK1/2 MAPK, but not PKA or CREB (Liang *et al.* 2015). However, whether DHEA binds IGF1R was not examined.

DHEA action in neuronal/CNS cells

DHEA and DHEA-S are well-established allosteric modulators of the neurotransmitter receptors N-methyl-D-aspartate (NMDA) receptor, γ -aminobutyric-acid type A (GABA_A), and sigma-1 receptors (Table 1). The activation of these receptors provided some of the first evidence for rapid, nongenomic action of DHEA/DHEA-S and supported the concept for potential beneficial effects of DHEA in alleviating a spectrum of neurological disorders associated with activation or suppression of these receptors (Monnet & Maurice 2006, Yadid *et al.* 2010, Hashimoto 2013). The NMDA receptor is a PM receptor composed of a large family of glutamate receptors, which are large heterotetrameric complexes that bind neurotransmitters and allosteric effectors to regulate transmembrane ion channels involved in learning and memory (Regan *et al.* 2015). Mutation and altered activity of NMDA receptors have been associated with autism and epilepsy (Burnashev & Szepietowski 2015) and with major depressive and bipolar disorders (Ghasemi *et al.* 2014). The GABA_A receptors (GABA_A Rs) are heteropentameric ligand-gated chloride and bicarbonate channels that promote hyperpolarizing postsynaptic responses, that is, the inhibitory postsynaptic potential when activated (Braat & Kooy 2015, Farrant & Nusser 2005). The sigma-1 receptor is a 25 kDa protein that has been associated with the endoplasmic reticulum,

nuclear membrane, mitochondrial membrane, and PM in neurons, astrocytes, oligodendrocytes, and microglia. In the endoplasmic reticulum, the receptor has dual roles: it binds to the InsP3 receptor (IP3R) and modulates cellular calcium homeostasis and functions as a chaperone protein in response to endoplasmic reticulum stress (Maurice 2004, Hashimoto 2013, Ruscher & Wieloch 2015). Sigma-1 also interacts with PM-bound receptors and ion channels to regulate their function and is found at ER-mitochondrial membrane junctions and mitochondrial function, including ROS generation. Early studies showed that DHEA-S (50 nM–1 μ M) activated NMDA receptors in rat hippocampal slices (Monnet *et al.* 1995) and mouse embryonic neocortical neurons (Compagnone & Mellon 1998). Use of pharmacological inhibitors showed specificity for DHEA (1 pM), and not DHEA-S (up to 1 μ M), for rapid NMDA activation and subsequent increase in intracellular calcium in mouse embryonic neocortical neurons (Compagnone & Mellon 1998). By contrast, DHEA-S acted as an allosteric antagonist of a recombinant GABA_A R and was shown to block GABA_A R currents in transfected HEK-293 cells (Svob Strac *et al.* 2012, Sachidanandan & Bera 2015).

DHEA activation of sigma-1 receptor was shown to reverse memory deficits induced in mouse models by either prenatal exposure to cocaine (Meunier & Maurice 2004) or transient bilateral common carotid artery ligation to simulate stroke (Yabuki *et al.* 2015). Studies have shown that DHEA-S competes with a sigma-1 agonist in C6 glioma cells, although the apparent K_d was >50 μ M (Rybczynska *et al.* 2009). DHEA activated both NMDA and sigma-1 receptors to stimulate the proliferation of primary human neural stem cells derived from the fetal cortex (ItNSC^{ctx} cells) (Suzuki *et al.* 2004).

Other potential DHEA receptors have been reported in PC12 rat sympathoadrenal cells derived from a pheochromocytoma of the rat adrenal medulla. DHEA activated a membrane DHEA binding site, which was described as a PM receptor leading to sequential activation of pSRC, MAPK, PICK, PKC, AKT, cAMP, PKA, and increased NF- κ B and CREB (Charalampopoulos *et al.* 2008). This group later reported that DHEA and DHEA-S bind to PM receptors TRKA and p75NTR in PC12 cells (Lazaridis *et al.* 2011) and that transfection of HEK-293 cells with cDNAs encoding TRKA or p75NTR revealed [³H]DHEA binding with K_{ds} of 7.4 \pm 1.7 nM and 5.6 \pm 0.5 nM, respectively (Gravanis *et al.* 2012). TRKA and p75NTR are neurotrophin nerve growth factor (NGF)-binding transmembrane tyrosine kinase receptors (K_d for NGF=0.01 and 1 nM, respectively) (Lazaridis *et al.* 2011).

DHEA (100 nM for 20 min) induced TRKA and p75NTR signaling in transfected HEK-293 cells and PC12 cells, including increased phospho-ERK1/2 and AKT (Lazaridis *et al.* 2011). More recent studies have shown that [³H] DHEA binds Ltrk, ApTrk, or AmphiTrk invertebrate neurotrophin receptors expressed in HEK-293 cells with K_d : 3.6 \pm 0.4 nM, 1.14 \pm 0.11 nM, and 0.47 \pm 0.18 nM, respectively (Pediaditakis *et al.* 2015). DHEA upregulated the expression of the TRKA receptor in PC12 cells as well as in DU145 prostate cancer cells and increased TRKA phosphorylation and the interaction of p75NTR with its effectors RhoGDI and RIP2 (Anagnostopoulou *et al.* 2013). DHEA (100 nM for 24 h) inhibited serum deprivation-induced apoptosis in DU145 prostate and Caco2 colorectal carcinoma cells in a TRKA- and p75NTR-specific manner (Anagnostopoulou *et al.* 2013). Another group synthesized a photoreactive DHEA analog, showed that it is specifically bound to the PM of PC12 cells, and activated adenylyl cyclase to increase cAMP in HepG2 human hepatoma cells (Waschatko *et al.* 2011).

DHEA binds MAP2C

DHEA was reported to bind to the dendritic brain microtubule-associated protein MAP2C *in vitro* by interaction with a hydrophobic pocket with a K_d =27 μ M (Laurine *et al.* 2003). The microtubule-associated proteins (MAPs) promote tubulin polymerization and stabilize microtubules, and MAP2 is highly expressed in the brain (Sánchez *et al.* 2000). MAP2 promotes tubulin polymerization and stabilizes microtubules, and loss of MAP2 is involved in schizophrenia (Shelton *et al.* 2015) and aging-related deficits in rats (Ma *et al.* 2014). MAP2C interacts with SRC, BRB2, and PKA (Lim *et al.* 2013).

DHEA action in the heart

DHEA inhibits voltage-gated T-type Ca²⁺ channels: CaV3.1, CaV3.2, or CaV3.3

Whether DHEA/DHEA-S is beneficial on the outcome of cardiovascular disease is highly controversial (reviewed in Savineau *et al.* 2013, Mannic *et al.* 2015). DHEA (10 μ M) inhibited voltage-gated T-type Ca²⁺ channels: CaV3.1, CaV3.2, or CaV3.3 in transfected NG108-15 cells-somatic mouse neuroblastoma/rat glioblastoma cell line (Chevalier *et al.* 2012). The authors concluded that this accounts for DHEA inhibition of pulmonary artery contraction and relates to the therapeutic action and/or physiological effects in cardiovascular and

neuronal diseases. However, DHEA has additional activities involving PPAR α , sigma-1R, and unknown mechanisms in cardiovascular tissues (reviewed in [Mannic *et al.* 2015](#)). The authors report an inverse association with DHEA-S and cardiovascular risk, morbidity, and mortality, even after correcting for usual confounding factors in multiple epidemiological studies ([Mannic *et al.* 2015](#)). They review the possible mechanisms involved from cell-based studies, for example, NOS activation in HUVECs ([Liu & Dillon 2002, 2004](#)), although most are not determined.

DHEA regulation of mitochondrial function

DHEA (1 μ M) preserved the isolated rat brain mitochondrial function in response to stresses, including anoxia-reoxygenation, uncoupling, and apoptosis by a direct effect on mitochondrial membranes ([Morin *et al.* 2002](#)). DHEA (166 μ M) was reported to act like rotenone and inhibit respiratory complex I in isolated rat kidney mitochondria, inhibiting NADH oxidation, ATP generation, and mitochondrial membrane potential, and increasing ROS generation, calcium release, and permeability transition ([Correa *et al.* 2003](#)). DHEA (10 and 100 nM for 24h) increased ATP synthesis, ROS production, mitochondrial membrane potential, and oxygen consumption rate (OCR) in human SH-SY5Y neuroblastoma cells and primary mouse cortical neurons ([Grimm *et al.* 2014](#)), but no mechanism was elucidated.

Conclusions

Reviewing the literature makes it clear that despite numerous studies of DHEA's effects in a variety of human, animal, and cell-based studies, there remain many questions about its possible molecular mechanisms of action to be resolved. The physiological role of circulation DHEA and DHEA-S as sources of bulk androgen cannot be overlooked ([Rainey *et al.* 2004](#), [Rainey & Nakamura 2008](#), [Rege & Rainey 2012](#)), because a source of androgen is required to rapidly produce the sex-related hormones during adrenarche and parturition. Our studies of DHEA metabolism and the biological action of DHEA and its metabolites on hepatic metabolism have provided evidence that induction of the enzymes normally involved in foreign compound metabolism by DHEA is common to those induced by drugs and chemical toxicants ([Webb *et al.* 2006](#)). The NRs involved appear to serve as sensors of excessive accumulation of sterols, and their activation leads to the induction of the enzymes needed to clear the liver of high levels of sterols, including DHEA.

These results possibly account for the enhanced fatty acid metabolism (through PPARs) and drug detoxification (through CAR and PXR), leading to the beneficial effects of DHEA observed in some models of obesity and cancer ([Traish *et al.* 2011](#)). Based on our recent studies as well as the review of the literature herein, we speculate that in the liver, there may be biphasic activities of DHEA mediated by different receptors, for example, PM-bound receptors and NRs, that are differentially responsive to low (nM) versus supraphysiological (μ M) DHEA in a time-dependent manner. For example, low DHEA may activate membrane receptors leading to EGFR activation and downstream to MAPK or other pathways, whereas high concentrations for longer times activate PP2A to dephosphorylate and activate PPAR α and CAR.

With the identification of a G-coupled receptor for DHEA by Dillon and coworkers ([Liu & Dillon 2002](#)), the possible receptor families available for DHEA activation were greatly expanded. In addition, this and other observations suggest the possibility that DHEA-S itself may be a signaling molecule. A series of different signaling systems have since been defined, providing an appreciation that DHEA, which is the most abundant sterol in the circulation, may have many functions heretofore not realized. Our recent observation that DHEA potently upregulates miR-21 opens a new mechanism for DHEA action that is unique to its role in transcriptional activation of gene expression by receptors. Systemic RNA seq (RNA sequencing of the entire transcriptome of a cell or tissue) studies are needed to define the miRNA transcriptome, which would then theoretically lead to regulation of hundreds of downstream genes.

Although there is great interest in DHEA as a neurosteroid and its effects in cognitive function ([Maggio *et al.* 2015](#)), some studies on the sigma-1 receptor have used concentrations of DHEA (50 μ M) that are higher than DHEA concentrations reported in the brain ([Hill *et al.* 2015](#)). The biological effects of DHEA in the brain on mood and cognition have been recently reviewed ([Pluchino *et al.* 2015](#)). [Tables 1 and 2](#) summarize what is known about the direct interaction of DHEA with NRs and PM-associated receptors. However, future studies addressing the affinity and time/tissue-dependent downstream effects of DHEA are warranted to fully understand the role of this sterol and its sulfated form in neurobiological function.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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References

- Adams J, Garcia M & Rochefort H 1981 Estrogenic effects of physiological concentrations of 5-androstene-3 β ,17 β -diol and its metabolism in MCF7 human breast cancer cells. *Cancer Research* **41** 4720–4726.
- Altman R, Motton DD, Kota RS & Rutledge JC 2008 Inhibition of vascular inflammation by dehydroepiandrosterone sulfate in human aortic endothelial cells: Roles of PPAR α and NF- κ B. *Vascular Pharmacology* **48** 76–84. (doi:10.1016/j.vph.2007.12.002)
- Anagnostopoulou V, Peditakis I, Alkahtani S, Alarifi SA, Schmidt E-M, Lang F, Gravanis A, Charalampopoulos I & Stourmaras C 2013 Differential effects of dehydroepiandrosterone and testosterone in prostate and colon cancer cell apoptosis: the role of Nerve Growth Factor (NGF) receptors. *Endocrinology* **154** 2446–2456. (doi:10.1210/en.2012-2249)
- Arnold JT 2009 DHEA metabolism in prostate: For better or worse? *Molecular and Cellular Endocrinology* **301** 83–88. (doi:10.1016/j.mce.2008.10.019)
- Arnold JT & Blackman MR 2005 Does DHEA exert direct effects on androgen and estrogen receptors, and does it promote or prevent prostate cancer? *Endocrinology* **146** 4565–4567.
- Auchus RJ 2004 Overview of dehydroepiandrosterone biosynthesis. *Seminars in Reproductive Medicine* **22** 281–288. (doi:10.1055/s-2004-861545)
- Bernard-Marissal N, Medard JJ, Azzedine H & Chrast R 2015 Dysfunction in endoplasmic reticulum-mitochondria crosstalk underlies SIGMAR1 loss of function mediated motor neuron degeneration. *Brain* **138** 875–890. (doi:10.1093/brain/aww008)
- Bernardes A, Souza PCT, Muniz JRC, Ricci CG, Ayers SD, Parekh NM, Godoy AS, Trivella DBB, Reinach P, Webb P, et al. 2013 Molecular mechanism of peroxisome proliferator-activated receptor α activation by WY14643: A new mode of ligand recognition and receptor stabilization. *Journal of Molecular Biology* **425** 2878–2893. (doi:10.1016/j.jmb.2013.05.010)
- Bobyleva V, Paziienza L, Muscatello U, Kneer N & Lardy H 2000 Short-term hypothermia activates hepatic mitochondrial sn-glycerol-3-phosphate dehydrogenase and thermogenic systems. *Archives of Biochemistry and Biophysics* **380** 367–372. (doi:10.1006/abbi.2000.1942)
- Bocuzzi G, Di Monaco M, Brignardello E, Leonardi L, Gatto V, Pizzini A & Gallo M 1993 Dehydroepiandrosterone antiestrogenic action through androgen receptor in MCF-7 human breast cancer cell line. *Anticancer Research* **13** 2267–2272.
- Bonnet S, Paulin R, Sutendra G, Dromparis P, Roy M, Watson KO, Nagendran J, Haromy A, Dyck JRB & Michelakis ED 2009 Dehydroepiandrosterone reverses systemic vascular remodeling through the inhibition of the Akt/GSK3- β /NFAT Axis. *Circulation* **120** 1231–1240. (doi:10.1161/CIRCULATIONAHA.109.848911)
- Braat S & Kooy RF 2015 The GABAA receptor as a therapeutic target for neurodevelopmental disorders. *Neuron* **86** 1119–1130. (doi:10.1016/j.neuron.2015.03.042)
- Bruder JM, Sobek L & Oettel M 1997 Dehydroepiandrosterone stimulates the estrogen response element. *Journal of Steroid Biochemistry and Molecular Biology* **62** 461–466. (doi:10.1016/S0960-0760(97)00056-3)
- Burnashev N & Szepietowski P 2015 NMDA receptor subunit mutations in neurodevelopmental disorders. *Current Opinion in Pharmacology* **20** 73–82. (doi:10.1016/j.coph.2014.11.008)
- Chai X, Zeng S & Xie W 2013 Nuclear receptors PXR and CAR: implications for drug metabolism regulation, pharmacogenomics and beyond. *Expert Opinion on Drug Metabolism Toxicology* **9** 253–266. (doi:10.1517/17425255.2013.754010)
- Chan QKY, Lam HM, Ng CF, Lee AYY, Chan ESY, Ng HK, Ho SM & Lau KM 2010 Activation of GPR30 inhibits the growth of prostate cancer cells through sustained activation of Erk1/2, c-jun/c-fos-dependent upregulation of p21, and induction of G2 cell-cycle arrest. *Cell Death and Differentiation* **17** 1511–1523. (doi:10.1038/cdd.2010.20)
- Charalampopoulos I, Margioris AN & Gravanis A 2008 Neurosteroid dehydroepiandrosterone exerts anti-apoptotic effects by membrane-mediated, integrated genomic and non-genomic pro-survival signaling pathways. *Journal of Neurochemistry* **107** 1457–1469. (doi:10.1111/j.1471-4159.2008.05732.x)
- Chaudhri RA, Olivares-Navarrete R, Cuenca N, Hadadi A, Boyan BD & Schwartz Z 2012 Membrane estrogen signaling enhances tumorigenesis and metastatic potential of breast cancer cells via estrogen receptor- α 36 (ER α 36). *Journal of Biological Chemistry* **287** 7169–7181. (doi:10.1074/jbc.M111.292946)
- Chen F, Knecht K, Birzin E, Fisher J, Wilkinson H, Mojena M, Moreno CT, Schmidt A, Harada S-i, Freedman LP, et al. 2005 Direct agonist/antagonist functions of dehydroepiandrosterone. *Endocrinology* **146** 4568–4576. (doi:10.1210/en.2005-0368)
- Chen J, Xu L & Huang C 2014 DHEA inhibits vascular remodeling following arterial injury: A possible role in suppression of inflammation and oxidative stress derived from vascular smooth muscle cells. *Molecular and Cellular Biochemistry* **388** 75–84. (doi:10.1007/s11010-013-1900-7)
- Cheng Z-X, Lan D-M, Wu P-Y, Zhu Y-H, Dong Y, Ma L & Zheng P 2008 Neurosteroid dehydroepiandrosterone sulphate inhibits persistent sodium currents in rat medial prefrontal cortex via activation of sigma-1 receptors. *Experimental Neurology* **210** 128–136. (doi:10.1016/j.expneurol.2007.10.004)
- Chevalier M, Gilbert G, Lory P, Marthan R, Quignard JF & Savineau JP 2012 Dehydroepiandrosterone (DHEA) inhibits voltage-gated T-type calcium channels. *Biochemical Pharmacology* **83** 1530–1539. (doi:10.1016/j.bcp.2012.02.025)
- Clark BJ & Stocco DM 1997 Steroidogenic acute regulatory protein: the StAR still shines brightly. *Molecular and Cellular Endocrinology* **134** 1–8. (doi:10.1016/S0303-7207(97)00166-4)
- Coleman DL, Leiter EH & Schwizer RW 1982 Therapeutic effects of dehydroepiandrosterone (DHEA) in diabetic mice. *Diabetes* **31** 830–833. (doi:10.2337/diab.31.9.830)
- Compagnone NA & Mellon SH 1998 Dehydroepiandrosterone: a potential signalling molecule for neocortical organization during development. *PNAS* **95** 4678–4683. (doi:10.1073/pnas.95.8.4678)
- Correa F, García N, García G & Chávez E 2003 Dehydroepiandrosterone as an inducer of mitochondrial permeability transition. *Journal of Steroid Biochemistry and Molecular Biology* **87** 279–284. (doi:10.1016/j.jsbmb.2003.09.002)
- Daan NM, Jaspers L, Koster MP, Broekmans FJ, de Rijke YB, Franco OH, Laven JS, Kavousi M & Fauser BC 2015 Androgen levels in women with various forms of ovarian dysfunction: Associations with cardiometabolic features. *Human Reproduction* **30** 2376–2386. (doi:10.1093/humrep/dev195)
- Farrant M & Nusser Z 2005 Variations on an inhibitory theme: phasic and tonic activation of GABAA receptors. *Nature Reviews Neuroscience* **6** 215–229. (doi:10.1038/nrn1625)
- Fujita A, Furutama D, Tanaka T, Sakai R, Koyama A, Hanafusa T, Mitsuhashi T & Ohsawa N 2002 In vivo activation of the constitutive androstane receptor beta (CARbeta) by treatment with dehydroepiandrosterone (DHEA) or DHEA sulfate (DHEA-S). *FEBS Letters* **532** 373–378. (doi:10.1016/S0014-5793(02)03712-2)
- Gaudet HM, Cheng SB, Christensen EM & Filardo EJ 2015 The G-protein-coupled estrogen receptor, GPER: the inside and inside-out story. *Molecular and Cellular Endocrinology* **418** 207–219. (doi:10.1016/j.mce.2015.07.016)

- Ghasemi M, Phillips C, Trillo L, De Miguel Z, Das D & Salehi A 2014 The role of NMDA receptors in the pathophysiology and treatment of mood disorders. *Neuroscience & Biobehavioral Reviews* **47** 336–358.
- Goodarzi MO, Carmina E & Azziz R 2015 DHEA, DHEAS and PCOS. *Journal of Steroid Biochemistry and Molecular Biology* **145** 213–225. (doi:10.1016/j.jsbmb.2014.06.003)
- Gravanis A, Calogeropoulou T, Panoutsakopoulou V, Thermos K, Neophytou C & Charalampopoulos I 2012 Neurosteroids and microneurotrophins signal through NGF receptors to induce prosurvival signaling in neuronal cells. *Science Signaling* **5** pt8.
- Grimm A, Schmitt K, Lang UE, Mensah-Nyagan AG & Eckert A 2014 Improvement of neuronal bioenergetics by neurosteroids: Implications for age-related neurodegenerative disorders. *Biochimica et Biophysica Acta (BBA) – Molecular Basis of Disease* **1842** 2427–2438. (doi:10.1016/j.bbadis.2014.09.013)
- Hammer F, Subtil S, Lux P, Maser-Gluth C, Stewart PM, Allolio B & Arlt W 2005 No evidence for hepatic conversion of dehydroepiandrosterone (DHEA) sulfate to DHEA: *in vivo* and *in vitro* studies. *Journal of Clinical Endocrinology and Metabolism* **90** 3600–3605. (doi:10.1210/jc.2004.2386)
- Hashimoto K 2013 Sigma-1 receptor chaperone and brain-derived neurotrophic factor: emerging links between cardiovascular disease and depression. *Progress in Neurobiology* **100** 15–29. (doi:10.1016/j.pneurobio.2012.09.001)
- Hill M, Dušková M & Stárka L 2015 Dehydroepiandrosterone, its metabolites and ion channels. *Journal of Steroid Biochemistry and Molecular Biology* **145** 293–314. (doi:10.1016/j.jsbmb.2014.05.006)
- Iruthayanathan M, O'Leary B, Paul G & Dillon JS 2011 Hydrogen peroxide signaling mediates DHEA-induced vascular endothelial cell proliferation. *Steroids* **76** 1483–1490. (doi:10.1016/j.steroids.2011.08.002)
- Jez JM, Flynn TG & Penning TM 1997 A new nomenclature for the aldo-keto reductase superfamily. *Biochemical Pharmacology* **54** 639–647. (doi:10.1016/S0006-2952(97)84253-0)
- Joel PB, Traish AM & Lannigan DA 1998 Estradiol-induced phosphorylation of Serine 118 in the estrogen receptor is independent of p42/p44 mitogen-activated protein kinase. *Journal of Biological Chemistry* **273** 13317–13323. (doi:10.1074/jbc.273.21.13317)
- Jones SA, Moore LB, Shenk JL, Wisely GB, Hamilton GA, McKee DD, Tomkinson NC, LeCluyse EL, Lambert MH, Willson TM *et al.* 2000 The pregnane X receptor: a promiscuous xenobiotic receptor that has diverged during evolution. *Molecular Endocrinology* **14** 27–39. (doi:10.1210/mend.14.1.0409)
- Kang L, Zhang X, Xie Y, Tu Y, Wang D, Liu Z & Wang Z-Y 2010 Involvement of estrogen receptor variant ER- α 36, not GPR30, in nongenomic estrogen signaling. *Molecular Endocrinology* **24** 709–721. (doi:10.1210/me.2009-0317)
- Kliwer SA, Moore JT, Wade L, Staudinger JL, Watson MA, Jones SA, McKee DD, Oliver BB, Willson TM, Zetterstrom RH *et al.* 1998 An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. *Cell* **92** 73–82. (doi:10.1016/S0092-8674(00)80900-9)
- Kobayashi K, Hashimoto M, Honkakoski P & Negishi M 2015 Regulation of gene expression by CAR: an update. *Archives of Toxicology* **89** 1045–1055. (doi:10.1007/s00204-015-1522-9)
- Kohalmly K, Tamasi V, Kobori L, Sarvary E, Pascucci J-M, Porrogi P, Rozman D, Prough RA, Meyer UA & Monostory K 2007 Dehydroepiandrosterone induces human CYP2B6 through the constitutive androstane receptor. *Drug Metabolism and Disposition* **35** 1495–1501. (doi:10.1124/dmd.107.016303)
- Kuiper GG, Carlsson B, Grandien J, Enmark E, Haggblad J, Nilsson S & Gustafsson J-A 1997 Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β . *Endocrinology* **138** 863–870.
- Labrie F 2010 DHEA, Important source of sex steroids in men and even more in women. In *Progress in Brain Research*, pp 97–148. Ed M Luciano. Cambridge, MA, USA: Elsevier. (doi:10.1016/S0079-6123(10)82004-7)
- Labrie F 2015a All sex steroids are made intracellularly in peripheral tissues by the mechanisms of intracrinology after menopause. *Journal of Steroid Biochemistry and Molecular Biology* **145** 133–138. (doi:10.1016/j.jsbmb.2014.06.001)
- Labrie F 2015b Intracrinology in action: Importance of extragonadal sex steroid biosynthesis and inactivation in peripheral tissues in both women and men. *Journal of Steroid Biochemistry and Molecular Biology* **145** 131–132. (doi:10.1016/j.jsbmb.2014.09.012)
- Labrie F, Bélanger A, Cusan L, Gomez J-L & Candas B 1997 Marked decline in serum concentrations of adrenal C19 sex steroid precursors and conjugated androgen metabolites during aging. *Journal of Clinical Endocrinology and Metabolism* **82** 2396–2402. (doi:10.1210/jcem.82.8.4160)
- Labrie F, Luu-The V, Labrie C & Simard J 2001 DHEA and its transformation into androgens and estrogens in peripheral target tissues: intracrinology. *Frontiers in Neuroendocrinology* **22** 185–212. (doi:10.1006/frne.2001.0216)
- Labrie F, Luu-The V, Bélanger A, Lin S-X, Simard J, Pelletier G & Labrie C 2005 Is dehydroepiandrosterone a hormone? *Journal of Endocrinology* **187** 169–196. (doi:10.1677/joe.1.06264)
- Labrie F, Bélanger A, Labrie C, Candas B, Cusan L & Gomez JL 2007 Bioavailability and metabolism of oral and percutaneous dehydroepiandrosterone in postmenopausal women. *Journal of Steroid Biochemistry and Molecular Biology* **107** 57–69. (doi:10.1016/j.jsbmb.2007.02.007)
- Lardy H, Partridge B, Kneer N & Wei Y 1995 Ergosteroids: Induction of thermogenic enzymes in liver of rats treated with steroids derived from dehydroepiandrosterone. *PNAS* **92** 6617–6619. (doi:10.1073/pnas.92.14.6617)
- Laurine E, Lafitte D, Gregoire C, Seree E, Loret E, Douillard S, Michel B, Briand C & Verdier J-M 2003 Specific binding of dehydroepiandrosterone to the N terminus of the microtubule-associated protein MAP2. *Journal of Biological Chemistry* **278** 29979–29986. (doi:10.1074/jbc.M303242200)
- Lazaridis I, Charalampopoulos I, Alexaki V-I, Avlonitis N, Padiaditakis I, Efsthathopoulos P, Calogeropoulou T, Castanas E & Gravanis A 2011 Neurosteroid dehydroepiandrosterone interacts with Nerve Growth Factor (NGF) receptors, preventing neuronal apoptosis. *PLoS Biology* **9** e1001051.
- Lewis A, Tsai SY & Su TP 2016 Detection of isolated mitochondria-associated ER membranes using the sigma-1 receptor. *Methods in Molecular Biology* **1376** 133–140. (doi:10.1007/978-1-4939-3170-5_11)
- Liang X, Glowacki J, Hahne J, Xie L, LeBoff MS & Zhou S 2016 Dehydroepiandrosterone stimulation of osteoblastogenesis in human MSCs requires IGF-I signaling. *Journal of Cellular Biochemistry* [in press]. (doi:10.1002/jcb.25475)
- Lim W, Jeong W, Kim J, Yoshimura Y, Bazer FW, Han JY & Song G 2013 Expression and regulation of beta-defensin 11 in the oviduct in response to estrogen and in ovarian tumors of chickens. *Molecular and Cellular Endocrinology* **366** 1–8. (doi:10.1016/j.mce.2012.10.031)
- Lindschau C, Kirsch T, Klinge U, Kolkhof P, Peters I & Fiebeler A 2011 Dehydroepiandrosterone-induced phosphorylation and translocation of FoxO1 depend on the mineralocorticoid receptor. *Hypertension* **58** 471–478. (doi:10.1161/HYPERTENSIONAHA.111.171280)
- Liu D & Dillon JS 2002 Dehydroepiandrosterone activates endothelial cell nitric-oxide synthase by a specific plasma membrane receptor coupled to Galpha i2,3. *Journal of Biological Chemistry* **277** 21379–21388. (doi:10.1074/jbc.M200491200)
- Liu D & Dillon JS 2004 Dehydroepiandrosterone stimulates nitric oxide release in vascular endothelial cells: Evidence for a cell surface receptor. *Steroids* **69** 279–289. (doi:10.1016/j.steroids.2004.02.004)
- Liu D, Iruthayanathan M, Homan LL, Wang Y, Yang L, Wang Y & Dillon JS 2008 Dehydroepiandrosterone stimulates endothelial

- proliferation and angiogenesis through extracellular signal-regulated kinase 1/2-mediated mechanisms. *Endocrinology* **149** 889–898. (doi:10.1210/en.2007-1125)
- Liu D, O'Leary B, Iruthayanathan M, Love-Homan L, Perez-Hernandez N, Olivo HF & Dillon JS 2010 Evaluation of a novel photoactive and biotinylated dehydroepiandrosterone analog. *Molecular and Cellular Endocrinology* **328** 56–62. (doi:10.1016/j.mce.2010.07.002)
- Lois K, Kassi E, Prokopiou M & Chrousos GP 2000 Adrenal androgens and aging. In *Endotext*. Eds LJ De Groot, P Beck-Peccoz, G Chrousos, K Dungan, A Grossman, JM Hershman, C Koch, R McLachlan, M New, R Rebar *et al.* South Dartmouth, MA, USA: MDText.com, Inc. (available at: <http://www.ncbi.nlm.nih.gov/books/NBK279006/>)
- Lu S-F, Mo Q, Hu S, Garippa C & Simon NG 2003 Dehydroepiandrosterone upregulates neural androgen receptor level and transcriptional activity. *Journal of Neurobiology* **57** 163–171. (doi:10.1016/S0074-7742(06)73003-3)
- Lucas JA, Ahmed SA, Casey ML & MacDonald PC 1985 Prevention of autoantibody formation and prolonged survival in New Zealand black/New Zealand white F1 mice fed dehydroisoandrosterone. *Journal of Clinical Investigation* **75** 2091–2093. (doi:10.1172/JCI111929)
- Ma QL, Zuo X, Yang F, Ubuda OJ, Gant DJ, Alaverdyan M, Kiose NC, Nazari S, Chen PP, Nothias F, *et al.* 2014 Loss of MAP function leads to hippocampal synapse loss and deficits in the Morris Water Maze with aging. *Journal of Neuroscience* **34** 7124–7136. (doi:10.1523/JNEUROSCI.3439-13.2014)
- Maggio M, De Vita F, Fischella A, Colizzi E, Provenzano S, Lauretani F, Luci M, Ceresini G, Dall'Aglia E, Caffarra P, *et al.* 2015 DHEA and cognitive function in the elderly. *Journal of Steroid Biochemistry and Molecular Biology* **145** 281–292. (doi:10.1016/j.jsbmb.2014.03.014)
- Maggiolini M, Donze O, Jeannin E, Ando S & Picard D 1999 Adrenal androgens stimulate the proliferation of breast cancer cells as direct activators of estrogen receptor alpha. *Cancer Research* **59** 4864–4869.
- Maggiolini M, Vivacqua A, Fasanella G, Recchia AG, Sisci D, Pezzi V, Montanaro D, Musti AM, Picard D & Ando S 2004 The G protein-coupled receptor GPR30 mediates c-fos Up-regulation by 17[beta]-estradiol and phytoestrogens in breast cancer cells. *Journal of Biological Chemistry* **279** 27008–27016. (doi:10.1074/jbc.M403588200)
- Maninger N, Wolkowitz OM, Reus VI, Epel ES & Mellon SH 2009 Neurobiological and neuropsychiatric effects of dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS). *Frontiers in Neuroendocrinology* **30** 65–91. (doi:10.1016/j.yfrne.2008.11.002)
- Mannic T, Viguie J & Rossier MF 2015 In vivo and in vitro evidences of dehydroepiandrosterone protective role on the cardiovascular system. *International Journal of Endocrinology Metabolism* **13** e24660.
- Martinez-Outschoorn UE, Sotgia F & Lisanti MP 2015 Caveolae and signalling in cancer. *Nature Reviews Cancer* **15** 225–237. (doi:10.1038/nrc3915)
- Mast N, Annalora AJ, Lodowski DT, Palczewski K, Stout CD & Pikuleva IA 2011 Structural basis for three-step sequential catalysis by the cholesterol side chain cleavage enzyme CYP11A1. *Journal of Biological Chemistry* **286** 5607–5613. (doi:10.1074/jbc.M110.188433)
- Maurice T 2004 Neurosteroids and sigma1 receptors, biochemical and behavioral relevance. *Pharmacopsychiatry* **37** Suppl 3 S171–S182. (doi:10.1055/s-2004-832675)
- Meinel S, Gekle M & Grossmann C 2014 Mineralocorticoid receptor signaling: Crosstalk with membrane receptors and other modulators. *Steroids* **91** 3–10. (doi:10.1016/j.steroids.2014.05.017)
- Meunier J & Maurice T 2004 Beneficial effects of the sigma1 receptor agonists igmesine and dehydroepiandrosterone against learning impairments in rats prenatally exposed to cocaine. *Neurotoxicology and Teratology* **26** 783–797. (doi:10.1016/j.ntt.2004.07.003)
- Michael Miller KK, Al-Rayyan N, Ivanova MM, Mattingly KA, Ripp SL, Klinge CM & Prough RA 2013 DHEA metabolites activate estrogen receptors alpha and beta. *Steroids* **78** 15–25. (doi:10.1016/j.steroids.2012.10.002)
- Miller WL 2005 Disorders of androgen synthesis – From cholesterol to dehydroepiandrosterone. *Medical Principles and Practice* **14** (Supplement 1) 58–68. (doi:10.1159/000086185)
- Miller WL & Auchus RJ 2011 The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. *Endocrine Reviews* **32** 81–151. (doi:10.1210/er.2010-0013)
- Mizota K, Yoshida A, Uchida H, Fujita R & Ueda H 2005 Novel type of Gq/11 protein-coupled neurosteroid receptor sensitive to endocrine disrupting chemicals in mast cell line (RBL-2H3). *British Journal of Pharmacology* **145** 545–550.
- Monnet FP, Mahe V, Robel P & Baulieu EE 1995 Neurosteroids, via sigma receptors, modulate the [3H]norepinephrine release evoked by N-methyl-D-aspartate in the rat hippocampus. *PNAS* **92** 3774–3778. (doi:10.1073/pnas.92.9.3774)
- Monnet FP & Maurice T 2006 The sigma1 protein as a target for the non-genomic effects of neuro(active)steroids: molecular, physiological, and behavioral aspects. *Journal of Pharmacological Science* **100** 93–118. (doi:10.1016/j.jcsc.2011.05.008)
- Moran C, Arriaga M, Archavaleta-Velasco F & Moran S 2015 Adrenal androgen excess and body mass index in polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* **100** 942–950. (doi:10.1210/jc.2014-2569)
- Moriguchi S, Shinoda Y, Yamamoto Y, Sasaki Y, Miyajima K, Tagashira H & Fukunaga K 2013 Stimulation of the sigma-1 receptor by DHEA enhances synaptic efficacy and neurogenesis in the hippocampal dentate gyrus of olfactory bulbectomized mice. *PLoS ONE* **8** e60863. (doi:10.1371/journal.pone.0060863)
- Morin C, Zini R, Simon N & Tillement JP 2002 Dehydroepiandrosterone and [alpha]-estradiol limit the functional alterations of rat brain mitochondria submitted to different experimental stresses. *Neuroscience* **115** 415–424. (doi:10.1016/S0304-4522(02)00416-5)
- Mueller JW, Gilligan LC, Idkowiak J, Arlt W & Foster PA 2015 The regulation of steroid action by sulfation and desulfation. *Endocrine Reviews* **36** 526–563. (doi:10.1210/er.2015-1036)
- Mutoh S, Osabe M, Inoue K, Moore R, Pedersen L, Perera L, Rebollosa Y, Sueyoshi T & Negishi M 2009 Dephosphorylation of threonine 38 is required for nuclear translocation and activation of human xenobiotic receptor CAR (NR1H3). *Journal of Biological Chemistry* **284** 34785–34792. (doi:10.1074/jbc.M109.048108)
- Mutoh S, Sobhany M, Moore R, Perera L, Pedersen L, Sueyoshi T & Negishi M 2013 Phenobarbital indirectly activates the constitutive active androstane receptor (CAR) by inhibition of epidermal growth factor receptor signaling. *Science Signaling* **6** ra31. (doi:10.1126/scisignal.2003705)
- Niro S, Pereira E, Pélissier M-A, Morfin R & Hennebert O 2012 The DHEA metabolite 7β-hydroxy-epiandrosterone exerts anti-estrogenic effects on breast cancer cell lines. *Steroids* **77** 542–551. (doi:10.1016/j.steroids.2012.01.019)
- Nyce JW, Magee PN, Hard GC & Schwartz AG 1984 Inhibition of 1,2-dimethylhydrazine-induced colon tumorigenesis in Balb/c mice by dehydroepiandrosterone. *Carcinogenesis* **5** 57–62. (doi:10.1093/carcin/5.1.57)
- Ohlsson C, Vandenput L & Tivesten Å 2015 DHEA and mortality: What is the nature of the association? *Journal of Steroid Biochemistry and Molecular Biology* **145** 248–253. (doi:10.1016/j.jsbmb.2014.03.006)
- Olivo HF, Perez-Hernandez N, Liu D, Iruthayanathan M, O'Leary B, Homan LL & Dillon JS 2010 Synthesis and application of a photoaffinity analog of dehydroepiandrosterone (DHEA). *Bioorganic & Medicinal Chemistry Letters* **20** 1153–1155. (doi:10.1016/j.bmcl.2009.12.019)
- Parker Jr CR 1999 Dehydroepiandrosterone and dehydroepiandrosterone sulfate production in the human adrenal during development and aging. *Steroids* **64** 640–647. (doi:10.1016/S0039-128X(99)00046-X)
- Pashko LL, Hard GC, Rovito RJ, Williams JR, Sobel EL & Schwartz AG 1985 Inhibition of 7,12-dimethylbenz(a)anthracene-induced skin

- papillomas and carcinomas by dehydroepiandrosterone and 3-beta-methylandrosterone-5-en-17-one in mice. *Cancer Research* **45** 164–166.
- Paulin R, Meloche J, Jacob MH, Bissierier M, Courboulain A & Bonnet S 2011 Dehydroepiandrosterone inhibits the SRC/STAT3 constitutive activation in pulmonary arterial hypertension. *American Journal of Physiology: Heart and Circulatory Physiology* **301** H1798–H1809. (doi:10.1186/1465-9921-12-128)
- Pediaditakis I, Iliopoulos I, Theologidis I, Delivanoglou N, Margioris AN, Charalampopoulos I & Gravanis A 2015 Dehydroepiandrosterone: an ancestral ligand of neurotrophin receptors. *Endocrinology* **156** 16–23. (doi:10.1210/en.2014-1596)
- Persson B, Kallberg Y, Bray JE, Bruford E, Dellaporta SL, Favia AD, Duarte RG, Jornvall H, Kavanagh KL, Kedishvili N, et al. 2009 The SDR (short-chain dehydrogenase/reductase and related enzymes) nomenclature initiative. *Chemico-Biological Interactions* **178** 94–98. (doi:10.1016/j.cbi.2008.10.040)
- Peters JM, Zhou YC, Ram PA, Lee SS, Gonzalez FJ & Waxman DJ 1996 Peroxisome proliferator-activated receptor alpha required for gene induction by dehydroepiandrosterone-3 beta-sulfate. *Molecular Pharmacology* **50** 67–74.
- Pinola P, Piltonen TT, Puurunen J, Vanky E, Sundstrom-Poromaa I, Stener-Victorin E, Ruokonen A, Puukka K, Tapanainen JS & Morin-Papunen LC 2015 Androgen profile through life in women with polycystic ovary syndrome: a nordic multicenter collaboration study. *Journal of Clinical Endocrinology and Metabolism* **100** 3400–3407. (doi:10.1210/jc.2015-2123)
- Pluchino N, Drakopoulos P, Bianchi-Demicheli F, Wenger JM, Petignat P & Genazzani AR 2015 Neurobiology of DHEA and effects on sexuality, mood and cognition. *Journal of Steroid Biochemistry and Molecular Biology* **145** 273–280. (doi:10.1016/j.jsbmb.2014.04.012)
- Prossnitz ER & Maggiolini M 2009 Mechanisms of estrogen signaling and gene expression via GPR30. *Molecular and Cellular Endocrinology* **308** 32–38. (doi:10.1016/j.mce.2009.03.026)
- Prossnitz ER & Barton M 2014 Estrogen biology: New insights into GPER function and clinical opportunities. *Molecular and Cellular Endocrinology* **389** 71–83. (doi:10.1016/j.mce.2014.02.002)
- Prough RA, Webb SJ, Wu HQ, Lapenson DP & Waxman DJ 1994 Induction of microsomal and peroxisomal enzymes by dehydroepiandrosterone and its reduced metabolite in rats. *Cancer Research* **54** 2878–2886.
- Rainey WE & Nakamura Y 2008 Regulation of the adrenal androgen biosynthesis. *Journal of Steroid Biochemistry and Molecular Biology* **108** 281–286. (doi:10.1210/jc.2015-3239)
- Rainey WE, Rehman KS & Carr BR 2004 The human fetal adrenal: making adrenal androgens for placental estrogens. *Seminars in Reproductive Medicine* **22** 327–336. (doi:10.1055/s-2004-861549)
- Regan MC, Romero-Hernandez A & Furukawa H 2015 A structural biology perspective on NMDA receptor pharmacology and function. *Current Opinion in Structural Biology* **33** 68–75. (doi:10.1016/j.sbi.2015.07.012)
- Rege J & Rainey WE 2012 The steroid metabolome of adrenarche. *Journal of Endocrinology* **214** 133–143. (doi:10.1530/JOE-12-0183)
- Ripp SL, Fitzpatrick JL, Peters JM & Prough RA 2002 Induction of CYP3A expression by dehydroepiandrosterone: Involvement of the pregnane X receptor. *Drug Metabolism and Disposition* **30** 570–575. (doi:10.1124/dmd.30.5.570)
- Robinson B, Michael KK, Ripp SL, Winters SJ & Prough RA 2003 Glucocorticoids inhibit interconversion of 7-hydroxy and 7-oxo metabolites of dehydroepiandrosterone: a role for 11beta-hydroxysteroid dehydrogenases? *Archives of Biochemistry and Biophysics* **412** 251–258. (doi:10.1016/S0003-9861(03)00056-0)
- Rodal SK, Skretting G, Garred O, Vilhardt F, van Deurs B & Sandvig K 1999 Extraction of cholesterol with methyl-beta-cyclodextrin perturbs formation of clathrin-coated endocytic vesicles. *Molecular Biology of the Cell* **10** 961–974. (doi:10.1091/mbc.10.4.961)
- Ruscher K & Wieloch T 2015 The involvement of the sigma-1 receptor in neurodegeneration and neurorestoration. *Journal of Pharmacological Sciences* **127** 30–35. (doi:10.1016/j.jphs.2014.11.011)
- Rybczynska AA, Elsinga PH, Sijbesma JW, Ishiwata K, de Jong JR, de Vries EF, Dierckx RA & van Waarde A 2009 Steroid hormones affect binding of the sigma ligand 11C-SA4503 in tumour cells and tumour-bearing rats. *European Journal of Nuclear Medicine and Molecular Imaging* **36** 1167–1175. (doi:10.1007/s00259-009-1076-2)
- Sachidanandan D & Bera AK 2015 Inhibition of the GABAA receptor by sulfated neurosteroids: a mechanistic comparison study between pregnenolone sulfate and dehydroepiandrosterone sulfate. *Journal of Molecular Neuroscience* **56** 868–877. (doi:10.1007/s12031-015-0527-4)
- Sakuma M, Yamada J & Suga T 1993 Induction of peroxisomal beta-oxidation by structural analogues of dehydroepiandrosterone in cultured rat hepatocytes: structure-activity relationships. *Biochimica et Biophysica Acta* **1169** 66–72. (doi:10.1016/0005-2760(93)90083-L)
- Sánchez C, Díaz-Nido J & Avila J 2000 Phosphorylation of microtubule-associated protein 2 (MAP2) and its relevance for the regulation of the neuronal cytoskeleton function. *Progress in Neurobiology* **61** 133–168. (doi:10.1186/s12879-015-0972-2)
- Savineau JP, Marthan R & Dumas de la Roque E 2013 Role of DHEA in cardiovascular diseases. *Biochemical Pharmacology* **85** 718–726. (doi:10.1016/j.bcp.2012.12.004)
- Schwartz AG 1979 Inhibition of spontaneous breast cancer formation in female C3H(Avy/a) mice by long-term treatment with dehydroepiandrosterone. *Cancer Research* **39** 1129–1132.
- Schwartz AG & Tannen RH 1981 Inhibition of 7,12-dimethylbenz[a]anthracene- and urethan-induced lung tumor formation in A/J mice by long-term treatment with dehydroepiandrosterone. *Carcinogenesis* **2** 1335–1337. (doi:10.1093/carcin/2.12.1335)
- Shelton MA, Newman JT, Gu H, Sampson AR, Fish KN, MacDonald ML, Moyer CE, DiBitetto JV, Dorph-Petersen K-A, Penzes P, et al. 2015 Loss of microtubule-associated protein 2 immunoreactivity linked to dendritic spine loss in schizophrenia. *Biological Psychiatry* **78** 374–385. (doi:10.1016/j.biopsych.2014.12.029)
- Shihan M, Kirch U & Scheiner-Bobis G 2013 Dehydroepiandrosterone sulfate mediates activation of transcription factors CREB and ATF-1 via a Gα11-coupled receptor in the spermatogenic cell line GC-2. *Biochimica et Biophysica Acta (BBA) – Molecular Cell Research* **1833** 3064–3075. (doi:10.1016/j.bbamcr.2013.08.015)
- Shihan M, Chan K-H, Konrad L & Scheiner-Bobis G 2015 Non-classical testosterone signaling in spermatogenic GC-2 cells is mediated through ZIP9 interacting with Gα11. *Cellular Signalling* **27** 2077–2086. (doi:10.1016/j.cellsig.2015.07.013)
- Simoncini T, Mannella P, Fornari L, Varone G, Caruso A & Genazzani AR 2003 Dehydroepiandrosterone modulates endothelial nitric oxide synthesis via direct genomic and nongenomic mechanisms. *Endocrinology* **144** 3449–3455. (doi:10.1210/en.2003-0044)
- Song MK, Grieco D, Rall JE & Nikodem VM 1989 Thyroid hormone-mediated transcriptional activation of the rat liver malic enzyme gene by dehydroepiandrosterone. *Journal of Biological Chemistry* **264** 18981–18985.
- Su CY & Lardy H 1991 Induction of hepatic mitochondrial glycerophosphate dehydrogenase in rats by dehydroepiandrosterone. *Journal of Biochemistry* **110** 207–213.
- Sueyoshi T, Moore R, Sugatani J, Matsumura Y & Negishi M 2008 PPP1R16A, the membrane subunit of protein phosphatase 1[beta], signals nuclear translocation of the nuclear receptor constitutive active/androstane receptor. *Molecular Pharmacology* **73** 1113–1121. (doi:10.1124/mol.107.042960)
- Suzuki M, Wright LS, Marwah P, Lardy HA & Svendsen CN 2004 Mitotic and neurogenic effects of dehydroepiandrosterone (DHEA) on human neural stem cell cultures derived from the fetal cortex. *PNAS* **101** 3202–3207. (doi:10.1073/pnas.0307325101)

- Svob Strac D, Jazvinscak Jembrek M, Erhardt J, Mirkovic Kos K & Peric D 2012 Modulation of recombinant GABA(A) receptors by neurosteroid dehydroepiandrosterone sulfate. *Pharmacology* **89** 163–171. (doi:10.1159/000336058)
- Tamasi V, Miller KKM, Ripp SL, Vila E, Geoghegan TE & Prough RA 2008 Modulation of receptor phosphorylation contributes to activation of peroxisome proliferator activated receptor α by dehydroepiandrosterone and other peroxisome proliferators. *Molecular Pharmacology* **73** 968–976.
- Tan J-a, Sharief Y, Hamil KG, Gregory CW, Zang D-Y, Sar M, Gumerlock PH, deVere White RW, Pretlow TG, Harris SE, et al. 1997 Dehydroepiandrosterone activates mutant androgen receptors expressed in the androgen-dependent human prostate cancer xenograft CWR22 and LNCaP cells. *Molecular Endocrinology* **11** 450–459. (doi:10.1210/mend.11.4.9906)
- Tartagni M, Cicinelli MV, Baldini D, Tartagni MV, Alrasheed H, DeSalvia MA, Loverro G & Montagnani M 2015 Dehydroepiandrosterone decreases the age-related decline of the *in vitro* fertilization outcome in women younger than 40 years old. *Reproductive Biology and Endocrinology* **13** 18. (doi:10.1186/s12958-015-0014-3)
- Teng Y, Litchfield LM, Ivanova MM, Prough RA, Clark BJ & Klinge CM 2014 Dehydroepiandrosterone induces miR-21 transcription in HepG2 cells through estrogen receptor β and androgen receptor. *Molecular and Cellular Endocrinology* **392** 23–36. (doi:10.1016/j.mce.2014.05.007)
- Teng Y, Radde BN, Litchfield LM, Ivanova MM, Prough RA, Clark BJ, Doll MA, Hein DW & Klinge CM 2015 Dehydroepiandrosterone activation of G-protein-coupled estrogen receptor rapidly stimulates microRNA-21 transcription in human hepatocellular carcinoma cells. *Journal of Biological Chemistry* **290** 15799–15811. (doi:10.1074/jbc.M115.641167)
- Traish AM, Kang HP, Saad F & Guay AT 2011 Dehydroepiandrosterone (DHEA) – a precursor steroid or an active hormone in human physiology (CME). *Journal of Sexual Medicine* **8** 2960–2982. (doi:10.1111/j.1743-6109.2011.02523.x)
- Vandenput L & Ohlsson C 2014 Genome-wide association studies on serum sex steroid levels. *Molecular and Cellular Endocrinology* **382** 758–766. (doi:10.1016/j.mce.2013.03.009)
- Wang L, Wang YD, Wang WJ & Li DJ 2009 Differential regulation of dehydroepiandrosterone and estrogen on bone and uterus in ovariectomized mice. *Osteoporosis International* **20** 79–92. (doi:10.1007/s00198-008-0631-1)
- Wang Z-Y & Yin L 2015 Estrogen receptor alpha-36 (ER- α 36): a new player in human breast cancer. *Molecular and Cellular Endocrinology* **418** 193–206. (doi:10.1016/j.mce.2015.04.017)
- Waschatko G, Kojro E, Zahnow M & Gehrig-Burger K 2011 Photo-DHEA – A functional photoreactive dehydroepiandrosterone (DHEA) analog. *Steroids* **76** 502–507. (doi:10.1016/j.steroids.2011.01.009)
- Webb SJ, Xiao GH, Geoghegan TE & Prough RA 1996 Regulation of CYP4A expression in rat by dehydroepiandrosterone and thyroid hormone. *Molecular Pharmacology* **49** 276–287.
- Webb SJ, Geoghegan TE, Prough RA & Michael Miller KK 2006 The biological actions of dehydroepiandrosterone involves multiple receptors. *Drug Metabolism Reviews* **38** 89–116. (doi:10.1080/03602530600569877)
- Weiss EP, Shah K, Fontana L, Lambert CP, Holloszy JO & Villareal DT 2009 Dehydroepiandrosterone replacement therapy in older adults: 1- and 2-y effects on bone. *American Journal of Clinical Nutrition* **89** 1459–1467. (doi:10.3945/ajcn.2008.27265)
- Williams MR, Dawood T, Ling S, Dai A, Lew R, Myles K, Funder JW, Sudhir K & Komesaroff PA 2004 Dehydroepiandrosterone increases endothelial cell proliferation *in vitro* and improves endothelial function *in vivo* by mechanisms independent of androgen and estrogen receptors. *Journal of Clinical Endocrinology and Metabolism* **89** 4708–4715. (doi:10.1210/jc.2003-031560)
- Wu HQ, Masset-Brown J, Tweedie DJ, Milewich L, Frenkel RA, Martin-Wixtrom C, Estabrook RW & Prough RA 1989 Induction of microsomal NADPH-cytochrome P-450 reductase and cytochrome P-450IVA1 (P-450LA omega) by dehydroepiandrosterone in rats: a possible peroxisomal proliferator. *Cancer Research* **49** 2337–2343.
- Yabuki Y, Shinoda Y, Izumi H, Ikuno T, Shioda N & Fukunaga K 2015 Dehydroepiandrosterone administration improves memory deficits following transient brain ischemia through sigma-1 receptor stimulation. *Brain Research* **1622** 102–113. (doi:10.1016/j.brainres.2015.05.006)
- Yadid G, Sudai E, Maayan R, Gispan I & Weizman A 2010 The role of dehydroepiandrosterone (DHEA) in drug-seeking behavior. *Neuroscience & Biobehavioral Reviews* **35** 303–314. (doi:10.1016/j.neubiorev.2010.03.003)
- Yen TT, Allan JA, Pearson DV, Acton JM & Greenberg MM 1977 Prevention of obesity in Avy/a mice by dehydroepiandrosterone. *Lipids* **12** 409–413. (doi:10.1007/BF02533624)
- Yoshinari K, Kobayashi K, Moore R, Kawamoto T & Negishi M 2003 Identification of the nuclear receptor CAR:HSP90 complex in mouse liver and recruitment of protein phosphatase 2A in response to phenobarbital. *FEBS Letters* **548** 17–20. (doi:10.1016/S0014-5793(03)00720-8)

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