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Steroid and sterol 7-hydroxylation: ancient pathways

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Abstract

B-ring hydroxylation is a major metabolic pathway for cholesterols and some steroids. In liver, 7α-hydroxylation of cholesterols, mediated by CYP7A and CYP39A1, is the rate-limiting step of bile acid synthesis and metabolic elimination. In brain and other tissues, both sterols and some steroids including dehydroepiandrosterone (DHEA) are prominently 7α-hydroxylated by CYP7B. The function of extra-hepatic steroid and sterol 7-hydroxylation is unknown. Nevertheless, 7-oxygenated cholesterols are potent regulators of cell proliferation and apoptosis; 7-oxygenated derivatives of DHEA, pregnenolone, and androstenediol can have major effects in the brain and in the immune system. The receptor targets involved remain obscure. It is argued that B-ring modification predated steroid evolution: non-enzymatic oxidation of membrane sterols primarily results in 7-oxygenation. Such molecules may have provided early growth and stress signals; a relic may be found in hydroxylation at the symmetrical 11-position of glucocorticoids. Early receptor targets probably included intracellular sterol sites, some modern steroids may continue to act at these targets. 7-Hydroxylation of DHEA may reflect conservation of an early signaling pathway. © 2002 Published by Elsevier Science Inc.

Keywords: Cholesterol; Cytochrome P450; CYP7B; DHEA; Evolution; Receptor; Steroid; Hypothesis; Review

1. 7-Oxygenated steroids and sterols

Steroid and sterol oxidoreduction governs biological activity and metabolic fate. Oxidative loss of the cholesterol side-chain generates steroids; oxidoreduction of the steroid nucleus (3-, 11- and 17-positions in particular) dictates activity and specificity. Recent work now points to an important role for B-ring (6- and 7-positions) modification.

7-Oxygenated steroids and sterols are widespread in mammals, birds, fish, and plants. Sterol processing in liver provides the best example of B-ring oxygenation. Hepatic 7α-hydroxylation of cholesterols is the rate-limiting step for bile acid synthesis and elimination (Fig. 1). B-ring hydroxylation of sex steroids in liver may also represent metabolic elimination.

Nevertheless, prominent B-ring hydroxylation is also seen in diverse extra-hepatic tissues. This could argue against simple substrate inactivation. The major 3β-hydroxysteroids including dehydroepiandrosterone (DHEA), pregnenolone, and androstane-3β,17β-diol (A/anediol) are efficiently 7α-hydroxylated in diverse tissues including brain [1–16] (Figs. 2 and 3), with some 6α and 7β modification depending on the substrate conformation. Other steroids are modified: testosterone is 7α-hydroxylated in testis [17,18] while 5α-3α steroids give rise to 6α-hydroxy (OH) derivatives in prostate and lymphocytes [7,13,19].

With the exception of hepatic bile acid formation via 7α-hydroxylation, almost nothing is known of the biological role of B-ring oxygenated sterols and steroids. One insight is provided by studies on the enzymes that catalyze their synthesis.

2. B-ring hydroxylated molecules: enzymes mediating their formation

2.1. Sterol 7-hydroxylation

At least three enzymes mediate sterol B-ring hydroxylation in liver. (1) CYP7A, whose expression is restricted to liver, hydroxylates cholesterols at the 7α-position [20,21]; the enzyme has not been reported to metabolize steroids. (2) Studies on mice lacking CYP7A revealed an alternative pathway for bile acid synthesis via a related enzyme, CYP7B [22,23], expressed in liver and multiple other tissues (below). (3) A hepatic 7α-hydroxylase specific for 24(*S*)-hydroxycholesterol (24(*S*)-OH-cholesterol), CYP39A1, has also been described [24]. In brain, but

Abbreviations: OH, hydroxy; OOH, hydroperoxy; A/anediol, androstane-3β,17β-diol; A/enediol, androstene-3β,17β-diol; DHEA, dehydroepiandrosterone; HSD, hydroxysteroid dehydrogenase; oxo, equivalent to keto

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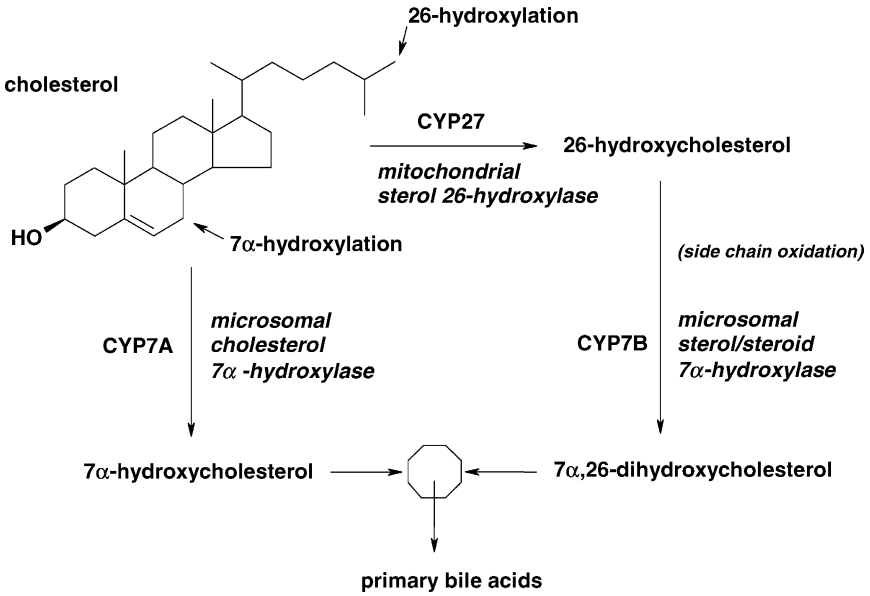

Fig. 1. Co-action of CYP7A and CYP7B on cholesterols to form bile acids. Figure redrawn from Schwarz et al. [30].

not in other tissues, 24(*S*)-hydroxylation of cholesterol is a major export pathway [25–27]; brain-derived 24(*S*)-OH-cholesterol is further metabolized in liver by CYP39A1.

2.2. Steroid 7-hydroxylation

In brain, several different B-ring hydroxylase enzymes were suspected. Although DHEA is primarily 7α-hydroxylated, inhibitor studies pointed to a second enzyme with activity at 7β [12,14–16]. A/anediol is principally 6α-hydroxylated in brain and prostate [6,7,9,28], suggestive of a further enzyme.

We reported molecular cloning of an enzyme from rodent hippocampus, CYP7B, with sequence similarity to CYP7A [29]. The enzyme differs from CYP7A in a number of significant respects. First, in addition to catalyzing the 7α-hydroxylation of sterols (25- and 26-OH-cholesterols; [30–32]), it is robustly active against steroids including

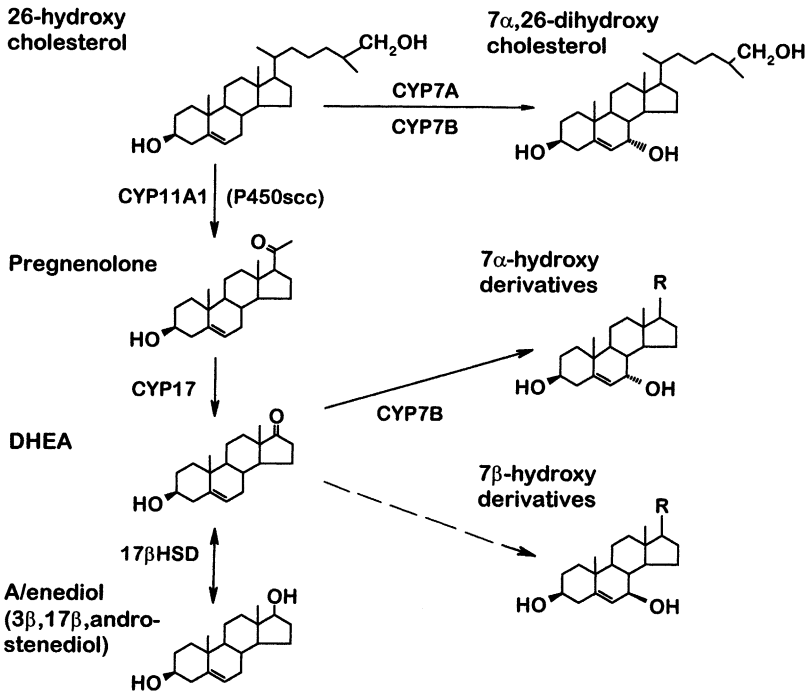

Fig. 2. 7-Hydroxylation of OH-cholesterol and 3β-hydroxysteroids.

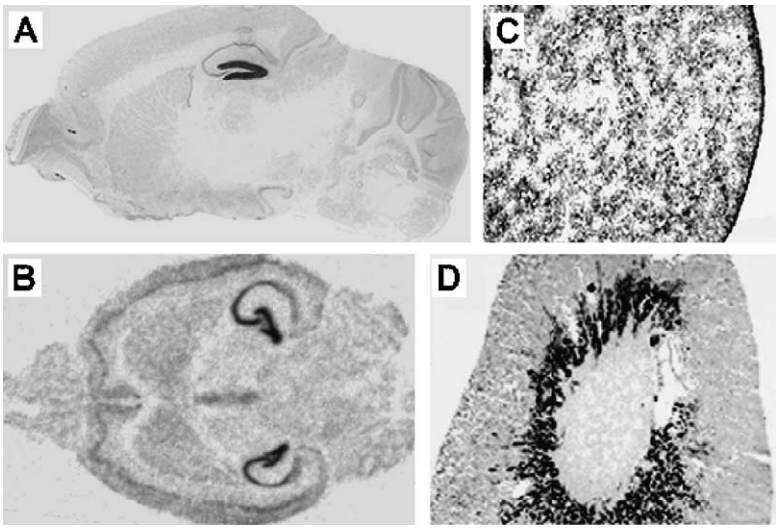

Fig. 3. CYP7B expression in brain and liver and kidney. Panels A, C, and D depict reporter gene expression (dark coloration), in transgenic mice, under control of CYP7B regulatory elements (A, brain, sagittal section; C, liver; D, kidney). Panel B shows in situ hybridization (dark/grey coloration) using a CYP7B probe (brain, horizonal section) [33]. Expression in neonates is very much more widespread and abundant [138].

DHEA, pregnenolone, and androstene-3β,17β-diol (A/enediol); 17β-estradiol was also modified at a lower rate [31]. Second, CYP7B is not restricted to liver, and is expressed widely in the brain and other tissues [29,33], Fig. 3. In addition to affecting sterol 7α-hydroxylation in liver [34], CYP7B gene disruption abolished steroid and sterol hydroxylation in diverse tissues including brain, spleen, thymus, heart, lung (in male), prostate, uterus, and mammary gland [33]. Third, CYP7B may modify other positions including 6α and 7β: knockout mice fail to modify A/anediol (that is normally 6α-hydroxylated) while recombinant CYP7B enzyme expressed in vaccinia or in yeast generates minor secondary metabolites including 7β-hydroxysteroids ([33]; Vico et al., Yeast, in press); 7β metabolites are also abolished in CYP7B knockout mice [33] though onwards metabolism from 7α was not excluded (see Lardy, this volume).

2.3. Other B-ring hydroxylases

Hepatic steroid metabolism includes hydroxylation at the 6β-position by members of the CYP3A family [35,36]. Outwith liver, CYP7B appears to be the primary steroid and sterol hydroxylase, though a distinct testosterone 7α-hydroxylase has been described in testis (CYP2A9/15 [17,18]) and in human (but not rodent) prostate, 6α-hydroxylation of 5α-3α steroids is performed by a non-P450 enzyme [7,13]. Other enzymes may exist.

3. Signaling by B-ring oxygenated sterols

Sterol hydroxylation at the 7α-position is central to bile acid synthesis in liver; the role of steroid and sterol B-ring hydroxylation in other tissues is unknown. There is evidence that they may play regulatory and/or signaling roles, exemplified (below) by effects on cholesterol regulation and apoptosis; other diverse effects of B-ring modified sterols have been noted (not reviewed).

3.1. Cholesterol homeostasis

Excess cholesterol represses its own synthesis and upregulates hepatic CYP7A expression to promote elimination. Conversely, bile acid excess can repress expression of CYP7A. Complex transcriptional regulation acts via a series of nuclear hormone receptors including LXRα, LXRβ, FXR and LRH-1 [37–39] that mediate responses to oxygenated sterols and bile acids. B-ring modification does not appear to be essential for this regulation. While 6α-OH-cholesterol may activate LXR [40] and 6α-epoxycholesterols and 7-oxocholesterol can inhibit [41], LXR is most efficiently activated by 22(*R*)- and 24(*S*)-OH-cholesterols [42,43]. Though 6- or 7-hydroxylated sterols could contribute to this regulation, B-ring hydroxylated sterols may exert effects via other pathways.

3.2. Apoptosis control

One pathway links cholesterol supply to cell proliferation and/or programmed cell death. Oxysterols are inhibitors of cell activation and proliferation, and can induce cell death, particularly in lymphocytes [44–47]. 7β-OH and 7-oxocholesterols are neurotoxic [48]. The most potent apoptosis-inducing activity found in oxidized LDL was identified as 7β-hydroperoxycholesterol (7β-OOH-cholesterol) [49,50]. Death in these models can be via classical apoptotic pathways [51,52].

Cell death may be a consequence of cholesterol biosynthesis inhibition; however, the specific pathways by which sterols can induce apoptosis remain to be elucidated.

4. Signaling by B-ring hydroxylated steroids

4.1. Brain function

The major metabolic route for DHEA in extra-hepatic tissues is via 7α-hydroxylation [31–33]. The metabolism of DHEA is of some interest. DHEA (and pregnenolone) promote synaptic plasticity and memory function in experimental animals [53–59]. Further, blood DHEA levels fall markedly with age in primates [60–65]. Cognitive decline in old age could be causally linked to DHEA decline [66–69]. However, oral DHEA replacement has not brought the hoped-for improvements in cognitive function [66,70,71] although beneficial effects are reported in adrenal dysfunction [72].

DHEA may require metabolism in target tissues. It is of note that the DHEA metabolizing enzyme CYP7B is particularly well-expressed in the hippocampus [29,33], a brain region centrally involved in memory formation. Lardy et al. [73] suggested that 7α-hydroxylation of DHEA is on a metabolic pathway to more potent derivatives and recently reported that 7-oxoDHEA (that may interconvert with 7-OH derivatives) is more active in promoting brain function than DHEA [74]. We have observed that 7α-OH-DHEA is more active than DHEA in preventing hypoxic cell death of neurones in vitro (Sundström, Martin, Lathe, Seckl, and Wulfert, unpublished data). In the brain, therefore, 7-oxygenation seems to be associated with activation of DHEA.

4.2. The immune system

DHEA and its metabolites promote the immune response in experimental animals [75–84]; however, attempts to boost immune-responsiveness in the elderly by DHEA replacement have not been entirely promising [85].

As in brain, DHEA may require metabolism for bioactivity. CYP7B is expressed in thymus and in lymphocytes ([33]; our unpublished data). There is debate about the stereoconfiguration of the active metabolite. 7α-OH-DHEA is a major immunity-promoting derivative of DHEA [86,87] others have argued that 7β-OH derivatives of A/enediol are most effective ([78,82,88,89]; Loria, this volume).

4.3. Origins of 7β-hydroxylated molecules

Both 7α- and 7β-modified molecules have biological activity, particularly in the immune system, but the origin of 7β-OH molecules is enigmatic (see Lardy, this volume). Several routes are possible. (1) Enzymatic hydroxylation: trace 7β-modified molecules are seen in CYP7B reactions [31]; allosteric modulation could favor 7β modification [12]. (2) Epimerization: 7α-hydroperoxycholesterol (7α-OOH-cholesterol) and 7α-OH-cholesterol may spontaneously epimerize to their 7β counterparts [88,89]; a 7-epimerase similar to the 3-epimerase enzyme [90] could contribute. (3) Dehydrogenation and reduction: 11β-HSD activity against 7α-OH-cholesterol [91] generates 7-oxo molecules that could in turn generate 7β-OH derivatives. All three are consistent with abolition of both 7α and 7β derivatives by disruption of the CYP7B [33].

5. Did signaling by B-ring hydroxylated molecules predate conventional steroid signaling?

5.1. Dearth of conventional receptor targets

No dedicated conventional (nuclear) receptor has been identified for 7-OH steroids. These could then act through via gating (ligand inactivation) of typical nuclear receptors, through the modulation of cell-surface ion channels (particularly in brain), or at atypical receptors.

A/anediol and DHEA are modest agonists of the estrogen and androgen receptors (ER and AR) [92,93]. 7-Oxygenation reduces activity of both molecules [1,94]. Clearly hydroxylation can gate nuclear receptor access, but the significance in vivo is unclear.

Hydroxylation of steroids (and possibly sterols) may modulate activity at cell-surface ion channels. Diverse channels respond to steroids [95], but the $GABA_A$ receptor has received most attention. DHEA and related steroids are antagonists of $GABA_A$, promoting neuronal activity (while 3α-5α steroids are agonists with potent anaesthetic properties). B-ring hydroxylation of DHEA and related steroids could gate access to these receptors.

Gating of either sex steroid receptors or ion channels such as $GABA_A$ does not easily explain the apopotic regulatory action and brain/immune system effects of these molecules. For instance, GABA agonists can inhibit apoptosis, but steroids are orders of magnitude more effective than the classic GABA agonist, muscimol [96]. This implies that they are binding to other receptors. This could make sense if these targets predated both the development of ion-channel sensitivity to steroids and the radiation of the steroid hormone receptor superfamily.

5.2. Late emergence of steroid signaling

Traditional wisdom depicts the evolution of intercellular steroid signaling from intracellular sterol signaling by an evolutionary breakthrough—the oxidative removal of the long hydrophobic side-chain of cholesterol via the action of the P450scc (side-chain cleavage; scc) enzyme, CYP11B. This interpretation may be incomplete.

Steroid signaling proper emerged late in eukaryotic evolution. The genome of the yeast, *Saccharomyces cerevisiae*, contains no homolog to the vertebrate steroid hormone receptor family. Steroid signaling proper has been placed with

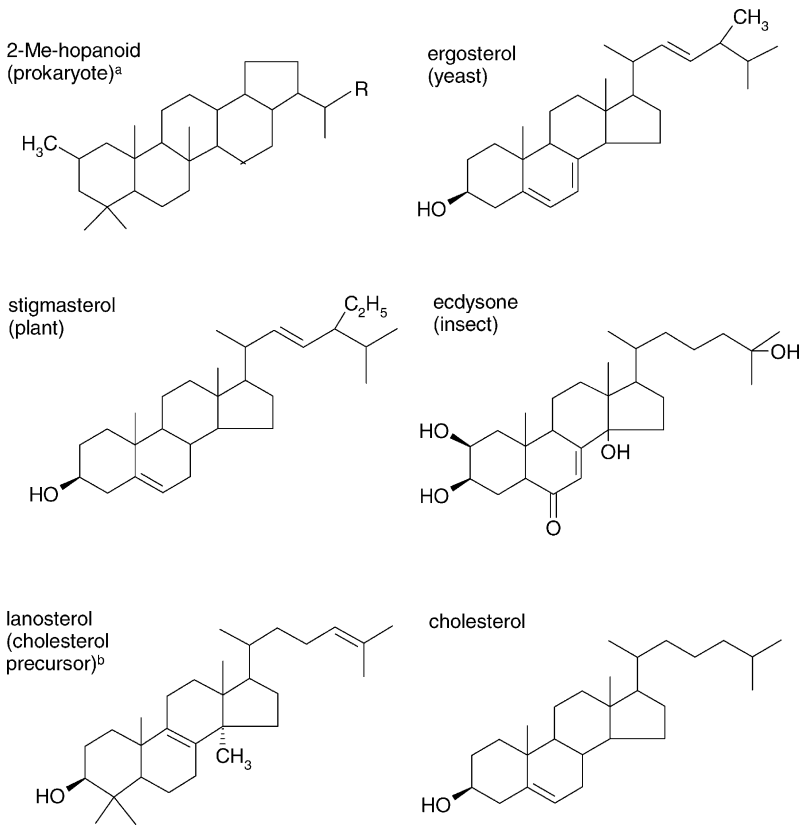

Fig. 4. Sterols/steroids from different organisms: (a) 2- (and 3-) methyl-bacteriohopanoids are commonly substituted (R in the figure) with a long side-chain (C8 outwith the hopanoid nucleus) bearing clustered OH groups (e.g. bacteriohopantetrol [103]); (b) cycloartenol rather than lanosterol is the sterol precursor in plants.

primitive fishes during the massive radiations taking place in the Cambrian period [97–100]. Ion-channel sensitivity to steroids only appears late in chordate evolution [101]. Therefore, the full spectrum of growth, differentiation, and reproduction was achieved, in precursors to the vertebrate lineage, without conventional steroid signaling at either nuclear or ion-channel receptors. These processes might have been subserved by sterols (rather than steroids) acting at atypical receptors.

5.3. Emergence of sterol-derived messengers: B-ring modified derivatives can be generated non-enzymatically

Membrane sterols probably arose from terpenoids including the hopanoids of bacteria [102,103] with which they share axial and longitudinal dimensions required for membrane stabilization (but not the 3β-OH group of steroids/sterols). Sterols of modern eukaryotes generally contain the 3β-OH group, including ergosterol, and lanosterol of fission and budding yeasts (*S. cerevisiae*, *Schizosaccharomyces pombe*), plant phytosterols such as stigmasterol, and the insect (and crab) hormone ecdysone (Fig. 4).

Signaling molecules can arise from abundant cell components. Membrane sterols are relatively insoluble; chemical oxidation of cholesterol primarily generates the more soluble 7α-OH, 7β-OH and 7-oxo derivatives (Fig. 5). Lower amounts of 6α-OH molecules, 5α-6α epoxides, and 7α- and β-hydroperoxides are also produced, as are side-chain oxidized cholesterols. Oxygenation at the 5-6 unsaturated bond (perhaps facilitiated by the 3β-OH group) may produce 5-6 epoxides that convert to 7-hydroperoxides, followed by thermal degradation to produce 7α-OH, 7β-OH, and 7-oxocholesterols [88,89,104–107]. Products of other membrane sterols may be similar [108]. B-ring oxidation is promoted by horseradish peroxidase, lipoxygenases, gamma irradiation, and metal ions (most particularly copper ion) and reduced in the presence of metal chelating agents (reviewed by Schroepfer [109]).

5.4. What do we know about the earliest sterol messengers?

Through increased solubility, and non-enzymatic production, 7-oxygenated sterols have considerable signaling potential. First, oxidized cholesterols are toxic, can bind to DNA and have mutagenic activity [110–112] possibly providing an early driving force for inducible elimination (a relic of which may be found in the CYP7A export pathway). Second, they have the potential to signal both sterol abundance (growth) or sterol oxygenation (oxidative stress), suggestive of early growth and stress signals.

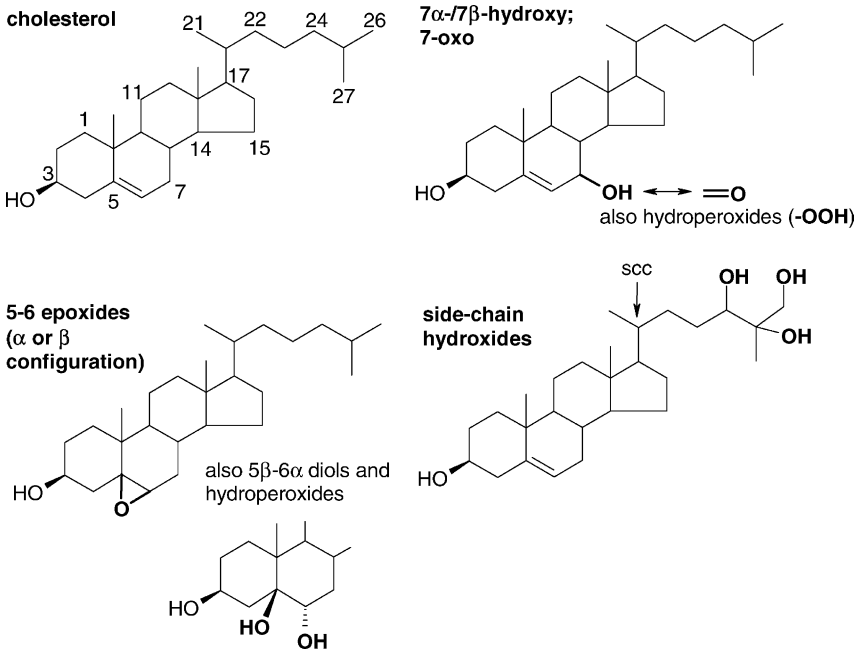

Fig. 5. Non-enzymatic oxidation products of cholesterol.

This latter idea finds some support in the structure of modern steroid receptors. The earliest steroid-type nuclear receptor most resembled the present estrogen receptor; this primordial receptor subsequently diverged to generate the estradiol/growth (ERα, ERβ, ERR) and glucocorticoid/stress families (GR, MR, PR, and later AR) [113-115].

Early ligands could have included 7-modified molecules. (1) Estrogen receptors: these respond to diverse 3β-hydroxylated steroids [116,117]; ligand binding to modern ERα is promoted by small 7α-substitutions that fit into an unoccupied cavity in the receptor [117]. (2) Glucocorticoid receptors: these are activated by 11β-hydroxylated steroids. Crucially, the 11β- and 7α-positions are rotationally symmetrical (Fig. 6): emphasized by an 11β-hydroxysteroid dehydrogenase (HSD) with dual 11β- and 7α-dehydrogenase activity [91] and promotion of ligand binding to ERα by 11β-substitutions [117]. Early 11β modifications may have exploited receptor targets binding 7α molecules. Thus, existing 7-modified molecules (produced non-enzymatically) could have been early ligands for the joint precursor to ER and GR.

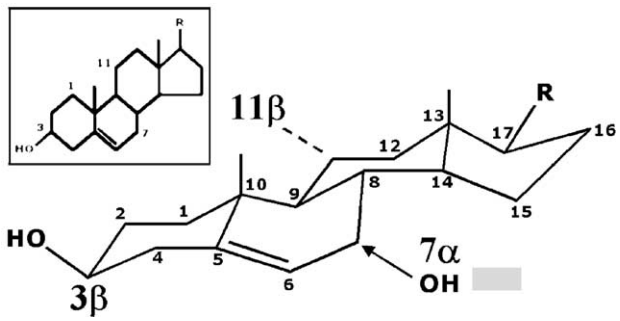

Fig. 6. Rotational symmetry between the 7α- and 11β-positions of the steroid nucleus. Some binding sites for 7α-modified molecules may accept 11β-modified equivalents.

6. Early receptors may have included intracellular sterol sites

If signaling by sterols, possibly 7-modified sterols, predated steroid signaling proper, what were the earliest targets for regulatory sterols? Molecular cloning experiments have begun to reveal a class of intracellular sterol-responsive targets (see Moebius, this volume; reviewed in [118]). These include the emopamil binding protein (EBP), the sigma site, and the peripheral benzodiazepine receptor (PBR). (1) EBP encodes a sterol C8-C7 isomerase that catalyzes the penultimate step in the synthesis of cholesterol [119–123]. (2) Sigma-1 shares significant homology with yeast ERG2 (ergosterol synthesis; C8-C7 sterol isomerase) enzyme [122,124,125] but its catalytic activity has not yet been elucidated; related sigma-2 and -3 receptors have been discussed. (3) The PBR participates in translocating cholesterol from the outer to the inner mitochondrial membrane [126–128]. These sites emerged early in evolution. Sigma finds a strict equivalent in the *S. cerevisiae* ERG2 gene product. PBR has only distant relatives in *S. cerevisiae* but a close counterpart in the fission yeast *S. pombe* (SPBC725.10); EBP has no obvious match in either yeast but the EBP-related protein EBRP is highly homologous to a *S. cerevisiae* gene product, YDL222C, of unknown function.

These and other enzymes and transporters accompanied the evolutionary switch to sterol-rich membranes, and are contenders for the early regulatory targets for oxygenated sterols. However, it is not known which, if any, were modulated by B-ring oxygenated molecules.

Some major drugs target intracellular sterol sites. Ligands have marked effects on apoptosis and the immune system. An anti-estrogen (tamoxifen) used in hormone-responsive breast cancer may act via sterol sites; important brain-active drugs, including anti-epileptics (diazepam), anti-ischemics (emopamil), and neuroleptics (haloperidol) are ligands for sterol sites. Sterols modulate the risk of Alzheimer's disease. An understanding of these primitive pathways is vital.

6.1. Did the first steroids act at sterol sites?—the oxysterol hypothesis

The first steroids, sterols lacking the hydrophobic side-chain of cholesterol, may have targeted existing sterol sites. In support, steroid action at oxysterol targets has been demonstrated. Some sterol sites have significant affinity for natural steroids including glucocorticoids, estrogens (and anti-estrogens) and DHEA [129–137]. Different steroids can have markedly different downstream effects at the same sterol site. Functional overlap between sterols and steroids is emphasized by present-day enzymes (CYP7B, 11β-HSD) that can modify both types of molecule.

Modern systemic steroids (including DHEA, estradiol, and glucocorticoids and their metabolites) continue to target sterol sites, acting in concert or in competition with endogenous sterols. By this means steroids could, and can, control cell life and death at a systemic level. 7-Oxygenation of 3β-hydroxysteroids including DHEA may reflect conservation of early signaling pathways. In the search for targets for B-ring modified steroids, intracellular sterol sites may deserve some attention.

Acknowledgments

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