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Review

The post-translational regulation of 17,20 lyase activity

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ABSTRACT

A single enzyme, microsomal P450c17, catalyzes the 17α-hydroxylase activity needed to make cortisol and the subsequent 17,20 lyase activity needed to produce the 19-carbon precursors of sex steroids. The biochemical decision concerning whether P450c17 stops after 17α-hydroxylation or proceeds to 17,20 lyase activity is largely dependent on three post-translational factors. First, 17,20 lyase activity is especially sensitive to the molar abundance of the electron-transfer protein P450 oxidoreductase (POR). Second, cytochrome b_5 strongly promotes 17,20 lyase activity, principally by acting as an allosteric factor promoting the interaction of P450c17 with POR, although a minor role as an alternative electron-transfer protein has not been wholly excluded. Third, the serine/threonine phosphorylation of P450c17 itself promotes 17,20 lyase activity, again apparently by promoting the interaction of P450c17 with POR. The principal kinase that phosphorylates P450c17 to confer 17,20 lyase activity appears to be p38α (MAPK14), which increases the maximum velocity of the 17,20 lyase reaction, while having no effect on the Michaelis constant for 17,20 lyase or any detectable effect on the 17α-hydroxylase reaction. Other kinases can also phosphorylate P450c17, but only p38α has been shown to affect its enzymology. Understanding the mechanisms regulating 17,20 lyase activity is essential for the understanding of hyperandrogenic disorders such as premature, exaggerated adrenarche and the polycystic ovary syndrome, and also for the design of selective 17,20 lyase inhibitors for use in hyperandrogenic states and in sex-steroid dependent cancers.

Contents

1. Introduction

The principal pathways of adrenal and gonadal steroidogenesis have been known for over 50 years (reviewed by Miller and Auchus, 2011). Cholesterol is first converted to pregnenolone via three reactions, 20-hydroxylation, 22-hydroxylation and scission of the 20,22 carbon–carbon bond, all catalyzed by mitochondrial P450scc. Pregnenolone may then be converted to other Δ5 steroids: 17α-OH-pregnenolone (17-Preg) and DHEA by P450c17, and thence to androstenediol by several forms of HSD17B. These Δ5 steroids may be converted to the corresponding Δ4 steroids, progesterone, 17α-OH-progesterone (17OHP), androstenedione and testosterone, by adrenal or gonadal 3β-hydroxysteroid dehydrogenase, type 2 (3βHSD2). P450c17 readily catalyzes the 17α-hydroxylation of

Abbreviations: 3βHSD2, 3β-hydroxysteroid dehydrogenase, type 2; 17-Preg, 17α-OH-pregnenolone; 17OHP, 17α-OH-progesterone; ACTH, adrenocorticotropic hormone; C_{19}, steroids having 19 carbon atoms, typically androgens and their precursors; C_{21}, steroids having 21 carbon atoms, including mineralocorticoids and glucocorticoids; DHEA, dehydroepiandrosterone; DHEAS, DHEA sulfate; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; Km, Michaelis constant; MAPK, mitogen-activated protein kinase; NADPH, reduced nicotinamide adenine dinucleotide phosphate; Vmax, maximum velocities; POR, P450 oxidoreductase; PCOS, polycystic ovary syndrome; ROCK1, rho-associated, coiled-coil containing protein kinase 1.

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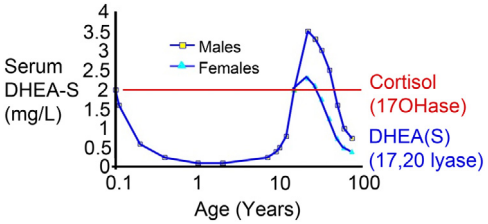

Fig. 1. Concentrations of DHEAS, a marker of 17,20 lyase activity change dramatically as a function of age, while cortisol concentrations, a marker of 17α-hydroxylase activity, remain essentially constant (as adjusted for the diurnal rhythm). Note that the x-axis is on a log scale. DHEAS values in males reach higher values than in females because the gene for steroid sulfatase is X-linked, hence males have only one gene copy and have less sulfatase activity; cortisol values in males and females are essentially the same. The line for cortisol is only illustrative; the DHEAS data are redrawn based on the data of Orentreich et al., 1984.

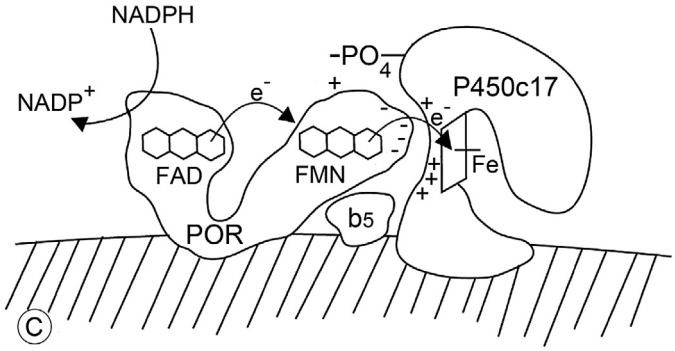

Fig. 2. Cell biology of P450c17. P450 oxidoreductase (POR), bound to the endoplasmic reticulum, receives two electrons (e-) from NADPH and transfers them to its FAD moiety. Receipt of the electrons elicits a conformational change in POR, permitting the isoalloxazine rings of the FAD and FMN moieties to come close together, so that the electrons pass from the FAD to the FMN. The POR then returns to its original orientation, permitting POR's negatively charged FMN domain to interact with the positively charged redox-partner binding site of P450c17. The electrons then reach the heme group and mediate catalysis. The interaction of P450c17 and POR is facilitated by the allosteric action of cytochrome b_5, and by the serine phosphorylation of P450c17. Copyright W.L. Miller.

progesterone and pregnenolone to 17-Preg and 17OHP, respectively. When progesterone is combined with P450c17, about 30% is 16α-hydroxylated (Swart et al., 1993, 2010) and about 1% is 21-hydroxylated (Yoshimoto et al., 2012), but physiologic relevance has not been established for these reactions. P450c17 then may also catalyze the 17,20 lyase activity that converts 21-carbon (C_{21}) 17-hydroxy steroids such as 17-Preg or 17OHP to 19-carbon (C_{19}) precursors of sex steroids. Much effort has centered on the inhibition of 17,20 lyase activity for therapy of sex steroid dependent malignancies, such as breast and prostate cancer (DeVore and Scott, 2012; O'Donnell et al., 2004; Salvador et al., 2013).

P450c17 is the microsomal P450 enzyme that catalyzes both 17α-hydroxylase and 17,20 lyase activities (reviewed by Miller and Auchus, 2011). These two activities were once thought to be catalyzed by separate enzymes that differed in the adrenals and gonads; however, the two activities co-purify (Nakajin et al., 1981), expression of bovine P450c17 in nonsteroidogenic COS-1 cells conferred both 17α-hydroxylase and 17,20 lyase activities (Zuber et al., 1986), and the cloning of identical cDNAs from adrenal and testis (Chung et al., 1987) showed that both came from the same gene (Picado-Leonard and Miller, 1987). Thus both activities had to be catalyzed by a single enzyme, and the distinction between 17α-hydroxylase and 17,20 lyase had to be functional and not genetic or structural. Nevertheless, clinical observations showed that adrenal 17α-hydroxylase activity (reflected by serum cortisol concentrations) was fairly constant throughout life, whereas adrenal 17,20 lyase activity (reflected by serum DHEA and DHEAS concentrations) was low in early childhood but rose abruptly during adrenarche beginning at ages 8–10 years and reaching maximal values in the mid 20s (Apter et al., 1979; Orentreich et al., 1984) (Fig. 1). As P450c17 has both 17α-hydroxylase and 17,20 lyase activities, it is the key branch point in steroidogenesis: P450c17 is not expressed in the zona glomerulosa, hence neither activity is present and pregnenolone is converted to mineralocorticoids; the zona fasciculata has 17α-hydroxylase activity but not 17,20 lyase activity, hence pregnenolone is converted to cortisol; in the zona reticularis, both activities are present, so that pregnenolone is converted to precursors of sex steroids. Research on the 17,20 lyase activity of P450c17 has been confounded by differences among various species: the predominant pathway to C_{19} steroids for human (Auchus et al., 1998; Lin et al., 1991, 1993) and bovine (Zuber et al., 1986) P450c17 is via the Δ5 pathway, but pig (Nakajin et al., 1981; Yanagibashi and Hall, 1986), frog (Lutz et al., 2001), and trout (Sakai et al., 1992) P450c17 have readily measured Δ4 17, 20-lyase activity, and the Δ4 pathway predominates with the rat (Fevold et al., 1989), hamster (Mathieu et al., 2002) and guinea pig (Kominami et al., 1992; Tremblay et al., 1994).

2. Cell biology and enzymology of P450c17

The principal factor regulating the 17,20 lyase activity of P450c17 is electron transfer (reviewed by Miller, 2005). Like all microsomal P450 enzymes, P450c17 receives electrons from reduced nicotinamide adenine dinucleotide phosphate (NADPH) via the membrane-bound flavoprotein, P450 oxidoreductase (POR) and mediates catalysis via the iron atom in the P450 heme ring. POR receives two electrons from NADPH and transfers them one at a time to the P450 (Wang et al., 1997). The crystal structures of N-terminally truncated forms of rat POR (Hubbard et al., 2001; Wang et al., 1997), dynamic modeling based on nuclear magnetic resonance and small-angle X-ray scattering (Ellis et al., 2009), crystallography of disulfide cross-linked mutants (Xia et al., 2011), and computational modeling (Pandey and Flück, 2013) show that POR is a butterfly-shaped protein: one 'wing' contains a flavin adenine dinucleotide (FAD) moiety, and the other contains a flavin mononucleotide (FMN) moiety; these two domains are separated by a flexible hinge region (Fig. 2). When the FAD moiety of POR receives two electrons from NADPH there is a conformational change in the hinge region, bringing the two 'wings' together and aligning the isoalloxazine rings of the FAD and the FMN, permitting the electrons to flow from the FAD to the FMN. The POR then returns to its initial, more open conformation, allowing the FMN domain to associate with the redox-partner binding site of the P450 by electrostatic interactions: the surface of the electron-donating FMN domain has numerous acidic residues, whereas the redox-partner binding site of P450 enzymes contain numerous basic residues (Auchus and Miller, 1999). The electrons from the FMN domain then flow to the heme iron atom of the P450 (where catalysis is mediated) and the POR dissociates from the P450 (reviewed by Miller, 2005).

The initial observations indicating a crucial role for electron transfer in 17,20 lyase activity were that the molar ratio of POR to P450c17 is three- to four-fold higher in porcine testes than in porcine adrenals, and that adding purified POR to porcine P450c17 in vitro increased 17,20 lyase more than 17-hydroxylase (Yanagibashi and Hall, 1986). This POR-dependency of the 17,20 lyase reaction was then confirmed in cell transfection experiments where an increased POR/P450c17 ratio led to more 17,20 lyase activity (Lin et al., 1993). Work with rodent, bovine and porcine P450c17 indicated that both 17-Preg and 17OHP were equally good substrates for the 17,20 lyase