Role of CYP27A1 in progesterone metabolism in vitro and in vivo

Geneviève Escher,1 Isabelle Vögeli,1 Robert Escher,2 Robert C. Tuckey,3 Sandra Erickson,4 Zygmunt Kroowski,5 and Felix J. Frey1

Departments of 1Nephrology and 2General Internal Medicine, University Hospital Berne, Berne, Switzerland; 3Department of Biochemistry and Molecular Biology, School of Biomedical, Biomolecular, and Chemical Sciences, The University of Western Australia, Crawley; 4Baker Heart Research Institute, Melbourne, Australia; and 4Department of Medicine, University of California, San Francisco, San Francisco, California

Submitted 8 May 2009; accepted in final form 8 August 2009

Escher G, Vögeli I, Escher R, Tuckey RC, Erickson S, Kroowski Z, Frey FJ. Role of CYP27A1 in progesterone metabolism in vitro and in vivo. Am J Physiol Endocrinol Metab 297: E949–E955, 2009.—In the kidney, progesterone is inactivated to 20α-dihydro-progesterone (20α-DH-progesterone) to protect the mineralocorticoid receptor from progesterone excess. In an attempt to clone the enzyme with 20α-hydroxysteroid activity using expression cloning in CHO cells and a human kidney expression library, serendipitously cDNA encoding CYP27A1 was isolated. Overexpression of CYP27A1 in CHO cells decreased progesterone conversion to 20α-DH-progesterone in a dose-dependent manner, an effect enhanced by cotransfection with adrenodoxin and adrenodoxin reductase. Incubation of CHO cells with 27-hydroxycholesterol, a product of CYP27A1, increased the ratio of progesterone to 20α-DH-progesterone in a concentration-dependent manner, indicating that the effect of CYP27A1 overexpression was mediated by 27-hydroxycholesterol. To analyze whether these observations are relevant in vivo, progesterone and 20α-DH-progesterone were measured by gas chromatography-mass spectrometry in 24-h urine of CYP27A1 gene knockout (ko) mice and their control wild-type and heterozygote littermates. In CYP27A1 ko mice, urinary progesterone concentrations were decreased, 20α-DH-progesterone increased, and the progesterone-to-20α-DH-progesterone ratio decreased threefold (P < 0.001). Thus CYP27A1 modulates progesterone concentrations. The underlying mechanism is inhibition of 20α-hydroxysteroid dehydrogenase by 27-hydroxycholesterol.

progesterone; steroid 27-hydroxylase; 27-hydroxycholesterol; 20α-steroid dehydrogenase; 20α-dihydro-progesterone

STEROL 27-HYDROXYLASE (CYP27A1) is a mitochondrial enzyme member of the cytochrome P-450 family. It is mainly expressed in the liver and macrophages, but also present in steroidogenic organs. CYP27A1 catalyzes oxidation reactions of cholesterol at C27 to form 27-hydroxycholesterol (27-OH-cholesterol) and cholestenoic acid (2, 4, 7). This reaction requires NADPH, adrenodoxin (ADX), and adrenodoxin reductase (AR) as cofactors. ADX acts as a mobile electron shuttle, with reduced ADX dissociating from AR before binding and delivering its electron to the enzyme (8, 18).

In humans, 50% of the daily net cholesterol is eliminated via bile acid excretion. Therefore, by catalyzing 27-hydroxylation of cholesterol in the initial step of the acidic pathway of bile acid biosynthesis and intermediate steps in the neutral pathway, CYP27A1 plays a major role in bile acids formation (17). Several different mutations have been identified so far in CYP27A1 gene of patients with cerebrotendinous xanthomatosis (CTX), a rare autosomal recessive lipid storage disease, leading to altered expression/function of CYP27A1 (6). Despite normal circulating levels of cholesterol, CTX patients accumulate intracellular cholesterol and develop progressive neurological dysfunction or xanthomatosis, and many have accelerated atherosclerosis, a phenotype best explained by diminished reverse cholesterol transport. CTX patients also have low levels of 25-dihydroxyvitamin D3 in serum, extensive osteoporosis (3), and increased risk of bone fractures, these features being a direct consequence of the reduced hydroxylation at position 25 of vitamin D3 due to CYP27A1 deficiency (15). The complex phenotype observed in these patients reveals the importance of CYP27A1 in bile acid biosynthesis, reverse cholesterol transport, and vitamin D3 biosynthesis.

Progesterone can bind to the human mineralocorticoid receptor (MR) with the same affinity as aldosterone in vitro; however, it has a low agonist activity in vivo (19). A local inactivation of progesterone to 20α-dihydro-progesterone (20α-DH-progesterone) by the enzyme 20α-steroid dehydrogenase (20α-OHSD) was postulated to explain this observation (21). Thus since progesterone but not 20α-DH-progesterone can bind to the MR, the small anti-MR effect of progesterone in the kidney, despite high tissue progesterone concentrations, was attributed to the conversion of progesterone to 20α-DH-progesterone by 20α-OHSD.

In an attempt to clone the enzyme with 20α-hydroxysteroid activity using expression cloning in CHO cells and a human kidney expression library, cDNA encoding CYP27A1 was isolated. From this, we have uncovered a new role for CYP27A1, that of generating a metabolite, 27-OH-cholesterol, which may protect the mineralocorticoid receptor from progesterone excess. In an attempt to clone the enzyme with 20α-hydroxysteroid activity using expression cloning in CHO cells and a human kidney expression library, cDNA encoding CYP27A1 was isolated. From this, we have uncovered a new role for CYP27A1, that of generating a metabolite, 27-OH-cholesterol, which may protect the mineralocorticoid receptor from progesterone excess.

MATERIALS AND METHODS

Materials. CHOP-C4 cells (16) were grown in RPMI 1640 medium containing 10% FBS, 2 mM l-glutamine, 50 U/ml penicillin/streptomycin, and 0.2 mg/ml G-418. The day before transfection, cells were plated in six-well plates at a density of 1.2 × 106 cells/well. [4,14C]progesterone (0.02 mCi/ml) was obtained from DuPont-New England Nuclear. Progesterone and 4-pregnen-20α-ol-3-one (20α-DH-progesterone) were purchased from Sigma-Aldrich (Switzerland), and 27-OH-cholesterol was from Medical Isotopes.

Library screening. An oriented cDNA library was constructed from a female human kidney poly(A) mRNA in the mammalian expression vector pcDNA1 (Clontech Laboratories). Plasmid DNA was transfected into Escherichia coli MC1062/P3 cells and purified using a Qiagen purification kit. Ninety-six pools of 1,000 clones per pool were transfected in CHO cells using DEAE-dextran, as previously
described (1). After transfection (48 h), 150,000 counts/min of [4-14C]progesterone were added to the culture medium and incubated overnight with the cells. Culture medium was then extracted with 3 volumes of ethyl acetate and evaporated. A screening for the production of 20α/H9251-dihydro-progesterone was performed by thin-layer chromatography (TLC) with the solvent chloroform-ethyl acetate (4:1). A single plasmid was isolated from a positive plasmid pool by sibling selection, sequenced using an ABI prism Big Dye Terminator cycle sequencing kit (Applied Biosystems), and identified using BLAST (Hyperlink http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Fig. 1. Quantification of progesterone (A), 20α-dihydro-progesterone (20α-DH-progesterone; B), and the progesterone-to-20α-DH-progesterone ratio (C) in CHOP cells. CHOP cells were transfected with sterol 27-hydroxylase (CYP27A1) cDNA (hatched bars) or plasmid alone (open bars, mock) for 48 h and incubated for 24 h with the indicated concentrations of unlabeled progesterone before the conversion of [4-14C]progesterone to 20α-[4-14C]DH-progesterone was analyzed. The progesterone-to-20α-DH-progesterone ratio was used to estimate 20α-steroid dehydrogenase (20α-OHSD) activity. RLU, relative light units. Experiments were performed several times in triplicate, and a representative study is depicted. One-way and two-way ANOVA were performed followed by Bonferroni post hoc tests with **P < 0.001 and ***P < 0.0001.

Experiments were performed several times in triplicate, and a representative study is depicted. One-way and two-way ANOVA were performed followed by Bonferroni post hoc tests with **P < 0.001 and ***P < 0.0001.

Fig. 2. Time course quantification of progesterone and 20α-DH-progesterone in CHOP cells transfected with CYP27A1 or vector alone. After transfection (48 h), cells were incubated in serum-free medium containing 5 μM progesterone, and 1 ml of medium was extracted and analyzed by gas chromatography-mass spectrometry (GC-MS) at the indicated time points. Progesterone (A) and 20α-DH-progesterone (B) were quantified, and the progesterone/20α-DH-progesterone ratio (C) was calculated. Open bars, CHOP cells transfected with plasmid alone; hatched bars, CHOP cells transfected with CYP27A1 cDNA. All measurements were performed in triplicate, and an independent representative experiment is shown. Two-way ANOVA was performed followed by Bonferroni post hoc tests with *P < 0.01 and ***P < 0.0001 for statistical significance.
Analysis of 20α-OHSD. CHOP cells were transfected with each of the plasmid pools. After transfection (48 h), the monolayer cultures were assessed for their ability to metabolize [4-14C]progesterone to 20α-DH-[4-14C]progesterone. Culture medium was removed and extracted as described above. For quantification of progesterone and 20α-DH-progesterone, TLC plates were exposed to a phosphorimaging plate and analyzed, and the radioactivity in each spot was quantified on the Bioimager BAS-1000 (Fuji) by measuring the relative light units in a constant area. The progesterone-to-20α-DH-progesterone ratio was used to assess 20α-OHSD activity (27).

GC-MS analysis of progesterone metabolites. Progesterone and 20α-DH-progesterone were analyzed in cell culture medium of CHOP cells 48 h following transfection with CYP27A1 or pcDNA3. The monolayer cultures were incubated with 5 μM progesterone, and medium was harvested and analyzed at the indicated time as previously described (9).

Characterization of progesterone metabolism in CHOP cells. CHOP cells were transfected with CYP27A1, vector alone, ADX, and/or AR. After transfection (48 h), the monolayer cultures were assessed for their ability to metabolize [4-14C]progesterone to 20α-DH-[4-14C]progesterone. Culture medium was removed and processed as described above.

Western blot analysis. Cells were transfected with ADX and/or AR cDNA (24, 26) for 48 h and lysed in radiolabeled precipitation buffer. Proteins were separated on 10% SDS polyacrylamide gels for AR or ADX, respectively, followed by immunoblotting using either a rabbit anti-ADX or anti-AR serum (23). Bands were visualized using the Enhanced Chemiluminescence kit (Amersham) and quantified with Image J.

Mouse colonies. CYP27A1 heterozygous (hz) males and females (a gift from Sandra Erickson, University of California, San Francisco, CA) were used for breeding CYP27A1 knockout (ko), wild-type (wt), and hz mice following standard Good Laboratory Practice and local guidelines at the animal facility in Berne, i.e., mice were maintained under a standard 12:12-h light-dark cycle and had free access to chow and water, and the experimental design was approved by the local animal ethics committee. Pups were genotype (for primers and PCR conditions, see Ref. 10) at the age of 3 wk, weaned at the age of 4 wk, and maintained on standard rodent chow and water ad libitum until use. At the age of 4–6 mo, mice were housed separately for 1 wk and placed for preadaptation in a metabolic cage for 3 days. Urine was collected every 24 h for the next 3 days. Steroids were extracted from 1.5 ml urine and analyzed by gas chromatography-mass spectrometry (GC-MS) as previously described (9).

Plasma progesterone concentrations were measured at the hospital laboratory using an electrochemiluminescence immunoassay (Roche Diagnostics).

Statistical analysis. Experiments were performed in triplicate. Means ± SD are presented. To determine statistically significant differences, one-way or two-way ANOVA was used, followed by Bonferroni or Dunnett’s post hoc tests for multiple comparisons.

RESULTS

Expression cloning. A human kidney expression library and CHOP cells were used in an attempt to clone an as yet unidentified 20α-OHSD enzyme. In mock-transfected CHOP cells, progesterone was metabolized with only a small fraction appearing as 20α-DH-progesterone. Following titeration of the library, pools of 1,000 plasmids/pool were transfected, and a total of 96,000 plasmids were screened for their ability to form 20α-DH-[4-14C]progesterone from [4-14C]progesterone. Three plasmid pools increased the ratio of [4-14C]progesterone to a metabolite comigrating with 20α-DH-progesterone on TLC plates. One of these pools was chosen for cloning purification by sibling selection. Isolated after four successive dilutions, the clone was fully sequenced and showed 100% identity with the gene coding for the enzyme 27-sterol hydroxylase CYP27A1.

In CHOP cells transfected with the isolated CYP27A1 cDNA, progesterone and 20α-DH-progesterone represented 15 and 10%, respectively, of the initial amount of [4-14C]progesterone in medium after 12 h of incubation, whereas progesterone was completely metabolized in mock-transfected (pcDNA3, vector alone) CHOP cells (data not shown).

To assess the substrate specificity of CYP27A1 in this cell system, 10 different putative substrates were added to mock- or CYP27A1 cDNA-transfected CHOP cells. There was no difference between vector alone and CYP27A1 cDNA-transfected CHOP cells when corticosterone, androsterone, aldosterone, estradiol, androstane, retinol, and methylentrienolone were used as substrates. However, when CHOP cells were incubated with the precursor of progesterone, pregnenolone, the formation of two different metabolites was observed in cells transfected with CYP27A1 cDNA compared with mock-transfected cells, one migrating to the same position as progesterone and the other comigrating with 20α-DH-progesterone (data not shown).

Characterization of progesterone metabolism in CHOP cells transfected with CYP27A1 cDNA. Progesterone metabolism was studied in CHOP cells transfected with vector alone or vector containing CYP27A1 cDNA, in the presence of increasing concentrations of cold progesterone in cell culture medium (Fig. 1), and [4-14C]progesterone and 20α-DH-[4-14C]proges-

Fig. 3. Expression of adrenodoxin (ADX) and adrenodoxin reductase (AR) in CHOP cells. Six-well plates were transfected with plasmids containing the cDNA of ADX or AR or both, combined with or without the cDNA of CYP27A1. The amount of plasmid cDNA was kept constant (3 μg) and completed with pcDNA3 when required. Cells were harvested 48 h after transfection, and 10 μg protein were loaded on a polyacrylamide gel. A: Western blot with anti-ADX antibody. Lane 1, purified ADX (1 nmol); lane 2, mock-transfected CHOP cells; lane 3, CHOP cells transfected with ADX cDNA; lane 4, CHOP cells transfected with CYP27A1 + ADX cDNA; lane 5, CHOP cells transfected with CYP27A1 + ADX + AR. B: Western blot with anti-AR antibody. Lane 1, purified AR (1 nmol); lane 2, mock-transfected CHOP; lane 3, CHOP cells transfected with AR; lane 4, CHOP cells transfected with CYP27A1 + AR; lane 5, CHOP cells transfected with CYP27A1 + ADX + AR.
terone were quantified. Whereas all the progesterone was metabolized when only \[^{[4-14}C\]progesterone was added, increasing concentrations of cold progesterone up to \(10^{-5}\) M resulted in increased progesterone in mock-transfected cells; these concentrations remained lower than those measured in cells transfected with the CYP27A1 construct \((P < 0.0001)\) (Fig. 1A). The amount of 20\(\alpha\)-DH-\[^{[4-14}C\]progesterone formation increased with increasing concentrations of progesterone in mock-transfected cells, whereas it remained almost constant in cells transfected with CYP27A1 cDNA (Fig. 1B). The progesterone-to-20\(\alpha\)-OHSD ratio was used to assess 20\(\alpha\)-OHSD, with a high ratio indicating low enzyme activity. This ratio was 25-fold higher in CYP27A1 cDNA-transfected than in mock-transfected CHOP cells when \[^{[4-14}C\]progesterone alone was added to the cells (5.25 \pm 0.17 vs. 0.21 \pm 0.05, \(P < 0.0001\)), and this difference decreased but was still significant \((P < 0.0001)\) (Fig. 1C) when increasing concentrations of cold progesterone were added.

Progesterone was also quantified by GC-MS in the medium of CHOP cells transfected with CYP27A1 or vector alone and incubated for the indicated time with 5 \(\mu\)M nonlabeled progesterone (Fig. 2). Progesterone concentrations remained always higher in CHOP cells transfected with CYP27A1 than in those transfected with pcDNA3 \((P < 0.0001;\) Fig. 2A), and 20\(\alpha\)-DH-progesterone increased both in CHOP cells transfected with CYP27A1 or pcDNA3 (Fig. 2B). Compared with progesterone concentrations, 20\(\alpha\)-DH-progesterone only represents a small fraction of steroids, and the progesterone-to-20\(\alpha\)-DH-progesterone ratio was always higher in CHOP cells transfected with CYP27A1 cDNA than in those transfected with the vector alone \((P < 0.0001);\) Fig. 2C).

The activity of CYP27A1 is known to be enhanced by its electron transfer partners ADX and AR \((8, 18)\). Therefore, these two proteins were overexpressed in CHOP cells transfected with CYP27A1 cDNA. The expression of endogenous and overexpressed ADX and AR was monitored by Western blotting using specific antibodies against ADX or AR (Fig. 3). Low endogenous levels of ADX and AR were detected in CHOP cells, and, following transfection with ADX and AR constructs, expression was equally increased in CHOP cells transfected with CYP27A1 and ADX or AR or both. The increase in protein expression was 6-fold for ADX and \(>10\)-fold for AR (Fig. 3). ADX and AR constructs alone or combined had no effect on the metabolism of progesterone (Fig. 4A). When equal amounts of ADX and AR cDNA were cotransfected alone or combined with CYP27A1 cDNA, the amount of progesterone was increased significantly (Fig. 4A). 20\(\alpha\)-DH-progesterone increased significantly only when ADX and AR were expressed simultaneously (Fig. 4B). The progesterone-to-20\(\alpha\)-DH-progesterone ratio was increased significantly when one or the other or both constructs encoding ADX and AR were cotransfected with CYP27A1 (Fig. 4C). These observations indicate that the increase of electron transfer via ADX and AR increases CYP27A1 activity and further reduces the metabolism of progesterone in CHOP cells.

---

**Fig. 4. Effect of ADX and AR on progesterone metabolism in CHOP cells.** CHOP cells were cotransfected as described in Fig. 3, and 20\(\alpha\)-OHSD activity assay was performed by incubating the cells for 12 h with \[^{[4-14}C\]progesterone. \[^{[4-14}C\]progesterone \((A)\) and formation of 20\(\alpha\)-DH-\[^{[4-14}C\]progesterone \((B)\) were measured, and the progesterone-to-20\(\alpha\)-DH-progesterone ratio \((C)\) was calculated. CYP, CYP27A1 cDNA. One-way ANOVA followed by Dunnett’s post hoc test for multiple comparisons using CYP as control was used, with **\(P < 0.001\) and ***\(P < 0.0001\).
CYP27A1 catalyzes the conversion of cholesterol to 27-OH-cholesterol. Therefore, we hypothesized that 27-OH-cholesterol might inhibit the 20α-OHSD. CHOP cells transfected with CYP27A1 construct or vector alone were preincubated for 24 h with increasing concentrations of 27-OH-cholesterol, and 20α-OHSD activity was measured. Preincubation of CHOP cells with 27-OH-cholesterol increased the progesterone-to-20α-DH-progesterone ratio (P < 0.001) in mock-transfected cells in a concentration-dependent manner, an effect less pronounced in cells transfected with CYP27A1 cDNA (Fig. 5). The progesterone-to-20α-DH-progesterone ratio always remained higher in CHOP cells transfected with CYP27A1 (P < 0.0001).

Effect of CYP27A1 activity on progesterone metabolism in CYP27A1 ko mice. To prove the relevance of this oxysterol-mediated regulation on 20α-OHSD in vivo, we studied progesterone metabolism in CYP27A1 ko mice. If 27-OH-cholesterol is a key regulator of 20α-OHSD in vivo, the absence of sterol 27-hydroxylase activity in CYP27A1 ko mice should result in increased 20α-OHSD activity and decrease the progesterone-to-20α-DH-progesterone ratio. To study this, urine from CYP27A1 ko mice and their wt and littermate controls was collected for 24 h, and progesterone and 20α-DH-progesterone were quantified by GC-MS. Progesterone concentrations were decreased in ko mice compared with wt mice [26.24 ± 4.02 vs. 40.1 ± 1.64 (SE) ng/24 h, P < 0.0008] (Fig. 6A). 20α-DH-progesterone was increased about threefold in CYP27A1 ko mice [35.7 ± 1.35 vs. 113.7 ± 12.14 (SE) ng/24 h, P < 0.0001] (Fig. 6B). The net result was that the progesterone-to-20α-DH-progesterone ratio was decreased in CYP27A1 ko mice [0.32 ± 0.05 vs. 1.12 ± 0.06 (SE), P < 0.0003] (Fig. 6C). The highest progesterone concentrations were seen in CYP27A1 hz mice [46.51 ± 7.24 (SE)] in which CYP27A1 mRNA expression is known to be reduced by 50% (10). In CYP27A1 hz mice, the concentration of 20α-DH-progesterone was higher than in CYP27A1 wt mice and lower than in CYP27A1 ko mice [49.49 ± 8.37 (SE)] so that, when the progesterone-to-20α-DH-progesterone ratio was calculated, it remained unchanged when compared with wt mice.

When progesterone was measured in plasma (Fig. 6D), no significant changes were seen between the genotypes.

DISCUSSION

A clone encoding CYP27A1 was isolated serendipitously by screening a human kidney cDNA library for the conversion of progesterone to 20α-DH-progesterone in an attempt to clone a specific 20α-OHSD enzyme responsible for the local inactivation of progesterone in the human kidney. To the best of our knowledge, this enzyme has not been cloned previously. The expression of CYP27A1 markedly modulates the metabolism of progesterone in CHOP cells mostly by inhibiting progesterone degradation, as demonstrated with the two different analytical methods used (Figs. 1 and 2). Interestingly, the concentration of 20α-DH-progesterone also increased. This might be explained as follows: the enzyme 20α-OHSD not only converts progesterone to 20α-DH-progesterone but also metabolizes progesterone at three other levels (conversion of 5α-DH-progesterone to 20α-DH, 5α-DH-progesterone, 3β,5α-TH-progesterone to 20α-DH, 3β, 5α-TH-progesterone, and 17α-OH-progesterone to 17α-OH, 20α-DH-progesterone) (20). It is reasonable to assume that the inhibition of the 20α-OHSD by 27-OH-cholesterol diminishes these three other reactions. As a corollary, the progesterone concentrations increase tremendously; therefore, the reduced activity of the 20α-OHSD still generates more 20α-DH-progesterone in CYP27A1 than in mock-transfected cells. In the present investigation, we focused on the mechanism accounting for the decline in the enzymatic activity accounting for the increased progesterone-to-20α-DH-progesterone ratio. A model for this is proposed in Fig. 7.

We showed, first, that factors known to enhance CYP27A1 activity, i.e., ADX and/or AR, enhanced the progesterone-to-20α-DH-progesterone ratio; second, that an important metabolite of CYP27A1, 27-OH-cholesterol, decreases the apparent progesterone-to-20α-DH-progesterone conversion activity in a concentration-dependent manner; and, third, that the ratio of progesterone to 20α-DH-progesterone is decreased when the urinary steroid metabolites are measured by GC-MS in CYP27A1 ko mice. Thus it is reasonable to conclude that CYP27A1 is responsible, at least in part, for the inhibition of the conversion of progesterone to 20α-DH-progesterone and that this effect is mediated by 27-OH-cholesterol. Surprisingly, the progesterone concentrations tended to be slightly higher, rather than lower as expected, in urines of CYP27A1 hz mice than in those of their wt littermates. Considering the fact that the progesterone-to-20α-DH-progesterone ratio remained unchanged in the urine of CYP27A1 hz mice in which CYP27A1 transcription was reduced by 50% but not abolished, the reduced tissue concentration of 27-OH-cholesterol in CYP27A1 hz mice was too low to completely inhibit the 20α-OHSD enzyme accounting for progesterone metabolism. In line with this hypothesis is the observation that a relatively high concentration of 27-OH-cholesterol is required to increase the progres-
The concentration of progesterone in plasma of the gene knockout animals did not change (Fig. 6D). This indicates that the regulation of progesterone metabolism described in the present study occurs only intracellularly at the prereceptor level. This prereceptor level modulation of steroid hormone availability is reminiscent of mechanisms found in cortisol metabolism. For this hormone, it was clearly shown that 11β-hydroxysteroid dehydrogenase (11β-OHSD) enzymes regulate the availability of steroid hormones without changing the serum concentrations (11–13, 25). Bumke-Vogt et al. (5) have recently shown a high rate of conversion of progesterone to 20α-DH-progesterone in kidney tissue and considered this to be a protective mechanism for avoiding MR activation by progesterone. This mechanism might be especially important during states of high progesterone concentrations such as pregnancy. An impaired activity of 20α-OHSD could be deleterious in the rare carriers of the MR bearing S810L mutation (MRL810) who develop early onset hypertension (14). When compared with MR, the MRL810 mutation exhibits 100-fold higher affinity for progesterone. Therefore, during pregnancy, women with MRL810 are at risk to develop life-threatening hypertension.

The biological relevance of this finding is unknown at the present time. However, the known multitude of effects of progesterone on pregnancy, preeclampsia, menstrual cycle, and possibly renal sodium retention by means of aldosterone re-
CYP27A1 MODULATES PROGESTERONE METABOLISM

E955