

Quantitative appreciation of steroidogenic gene expression in mouse tissues: new roles for type 2 5 α -reductase, 20 α -hydroxysteroid dehydrogenase and estrogen sulfotransferase

Van Luu-The*, Georges Pelletier, Fernand Labrie

*Molecular Endocrinology and Oncology Research Center, Laval University Hospital Research Center (CRCHUL),
Laval University, Que., Canada G1V 4G2*

Abstract

We have recently developed an improved method for the RealTime PCR quantification of reversed transcribed mRNA (Q-RT-PCR) that allows to obtain absolute mRNA expression levels with high sensitivity and accuracy. Using this Q-RT-PCR method to assess the mRNA expression levels of genes encoding steroidogenic enzymes in male and female mouse tissues allows us to gain quantitative appreciation of the function of these genes. We could thus identify the existence of two types of steroidogenic tissues: those of classical endocrine glands such as the testis, ovary and adrenals which deliver steroids into the circulation, and in which millions of copies/ μ g total RNA are detected, and those of peripheral intracrine tissues where steroids are synthesized locally and exert their action at the site where they are produced (prostate, uterus, etc.), and in which the expression level of steroidogenic enzymes is much lower. We also observed an abnormally high expression levels of type 2 5 α -reductase and 20 α -HSD in the male and female adrenals, respectively, thus indirectly suggesting new roles for these sex-specific enzymes. On the other hand estrogen sulfotransferase, the enzyme that inactivates estrogen, has been found selectively expressed in male tissues, thus suggesting a role for this enzyme to protect male-specific tissues against estrogenic activity.

© 2005 Elsevier Ltd. All rights reserved.

Keywords: Quantitative RealTime PCR; Gene expression; Steroidogenesis; Steroidogenic enzymes; P450c17; P450scc; 3 β -Hydroxysteroid dehydrogenase; 5 α -Reductase; 20 α -Hydroxysteroid dehydrogenase; Estrogen sulfotransferase

1. Introduction

The steroidogenic pathway (Fig. 1) contains 18 main steps catalyzing the transformation of cholesterol into active steroids [1], namely, aldosterone (mineralocorticoid), corticosterone (rodent glucocorticoid), cortisol (human glucocorticoid), progesterone, testosterone (T) and dihydrotestosterone (DHT) (androgens), estradiol (E2) (estrogen), as well as their sulfated- and glucuronosylated-conjugates. Although many of the genes responsible for known human diseases were identified such as pseudohermaphroditism (type 3 17 β -HSD [2] and type 2 5 α -reductase [3]) and congenital adrenal hyperplasia (3 β -hydroxysteroid dehydrogenase [4], and P450c21 [5]), the role and function of many of their isoforms are not well understood. Indeed, because of the existence of clinical syndromes of deficiency in gonadal sex

steroid production and the widespread belief that steroids are freely distributed through blood circulation, it was generally thought that sex steroids are produced exclusively by the gonads. Although this belief is true for laboratory animals, it was proven incomplete in the human, based on studies performed in men who had their testicles removed or their testicular androgen secretion totally blocked by treatment with a luteinizing hormone-releasing hormone (LHRH) agonist ([6] for reviews). It was then found that, while their blood T level was reduced by 90–95% following castration, their intraprostatic concentration of DHT was decreased by only 50%, thus suggesting an intraprostatic biosynthesis of androgens. The development of secondary sexual characteristics in boys deficient in type 2 3 β -HSD [4] and a virilization at adulthood of young men deficient in type 3 17 β -HSD [7] are natural proofs of the conversion of adrenal DHEA (dehydroepiandrosterone) into active sex steroids in target tissues.

The local biosynthesis of sex steroids in peripheral tissues [8] was further confirmed by the cloning of multiple

* Corresponding author. Tel.: +1 418 654 2296; fax: +1 418 654 2741.
E-mail address: van.luu-the@crchul.ulaval.ca (V. Luu-The).

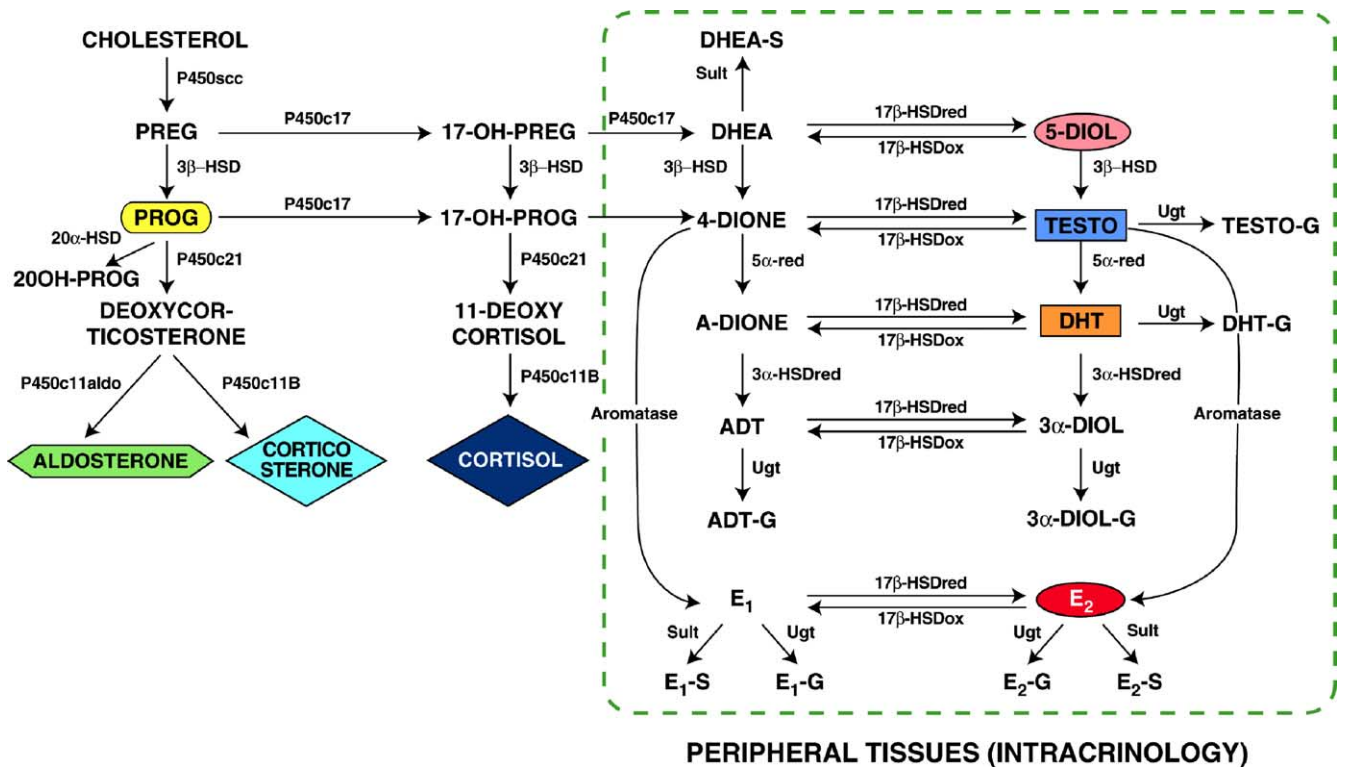


Fig. 1. Schematic representation of the main steroidogenic pathways, active steroids, namely glucocorticoids, mineralocorticoids, progesterone, androgens and estrogens are boxed.

types of steroidogenic enzymes, namely type 1 and 2 3 β -HSD [9–12], types 1 to 12 17 β -HSD [2,13–26], types 1 and 2 5 α -reductases [3,27–29], as well as types 1 and 2 11 β -hydroxysteroid dehydrogenase. The expression of these enzymes is tissue-specific, many of them being expressed only in peripheral tissues.

In the study reported here, we used double-correction RealTime PCR measurements [30] to quantify, by estimating the number of copies/ μ g total RNA, the expression levels of P450_{scc}, P450_{c21}, P450_{c17}, 3 β -HSDs, 5 α -reductases, 20 α -HSD and estrogen sulfotransferase (EST) in male and female mice in order to improve knowledge about their tissue distribution and thus their functional role and also the function of their human orthologs. It is noteworthy that, in a single liver cell, the amount of total RNA is estimated to 50 pg [31] and thus an expression level of 20,000 copies/ μ g total RNA could correspond to approximately 1 copy/cell in a homogenous cell population.

2. Double correction quantitative RealTime PCR

Quantitative RealTime PCR (Q-RT-PCR), a recently developed fluorescent method of mRNA quantification [32–34] has improved greatly the mRNA quantification performed with PCR. Indeed, Q-RT-PCR permits to control PCR conditions most rigorously and the quantification takes place within an exponential phase of the amplification curve [35,36] thus

minimizing errors due to the PCR process. The efficiency coefficient is closed to the theoretical value of 2, thus indicating that errors due to the PCR amplification process is negligible. The most important cause of errors is the RNA preparation and handling [36] because they will be amplified exponentially in the amplification process. The important improvement of Q-RT-PCR compared to classical PCR is that Q-RT-PCR permits to follow the kinetics of DNA production in real-time and to quantify the initial amount of mRNA using a standard curve resulting in much lower variability [37]. On the other hand, classical PCR quantification in which end product signal included exponentially amplified errors [38], shows very high variability.

Two modes of detection are generally used, one using a gene-specific fluorescent hybridization probe in which a fluorescent signal is increased [39,40] or decreased [41] by energy transfer from one fluorescent dye to another, and a second one using a common SYBR Green I fluorescent dye that binds to a minor groove of DNA [42]. With a proper choice of primers and amplification conditions assisted by informatics, it has been shown that Q-RT-PCR using SYBR Green I is a rapid, sensitive and accurate method to quantify mRNAs [35,42].

There are also two methods of determination of a crossing point (C_p) value, a cycle number in a log-linear region (Fig. 2) which will be used to calculate the quantitative value of Q-RT-PCR. One method, namely second derivative, calculates a second derivative [35,37] value of the real-time fluorescence intensity curve (Fig. 2A), only one value is obtained.

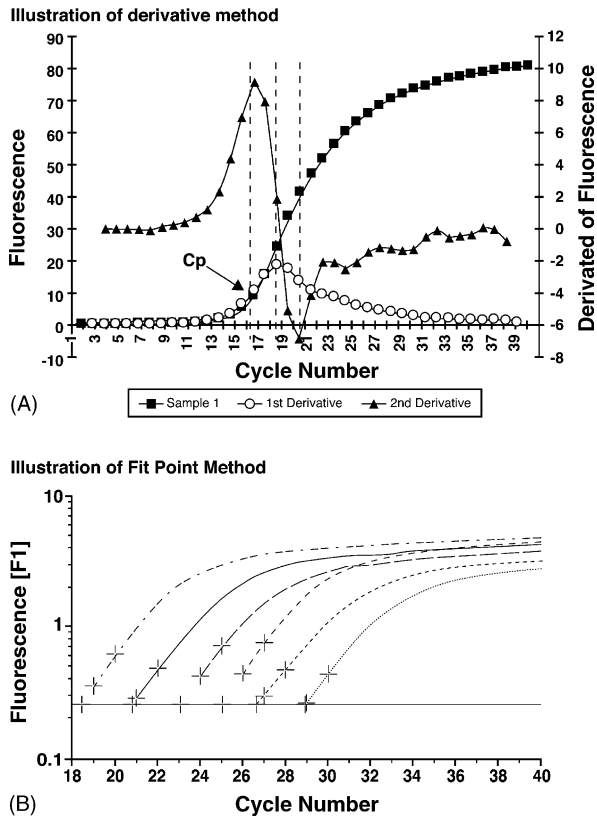


Fig. 2. Illustration of the second derivative (A) and Fit point methods (B). Illustrations taken from a LightCycler manual (Roche Inc.), showing the variation of fluorescence signal vs. number of cycle and the method to determine crossing point using second derivative peak (A) and Fit point (B). In the second derivative method, a crossing point (Cp) corresponds to the first peak of a second derivative curve. This peak corresponds to a beginning of a log-linear phase (A). In the Fit point method (B), a Cp is determined by the intersection of a parallel to the threshold line in the log-linear region.

The second, namely Fit point [35] (Fig. 2B) is performed by drawing a line parallel to the X-axis in the log-linear region of the real-time fluorescence intensity curve: a somewhat variable user-dependent value can be obtained by this method. The Fit point method is the most currently used method and the calculation is user-dependent. The second derivative calculation, on the other hand, does not involve any decision by the user since a positive peak corresponds to the beginning of the log-linear phase of the original data.

In this report, we use our improved method of quantification to determine Cp value based on the second derivative method and a double correction. The first correction serves to minimize errors due to handling and RNA preparation using a house keeping gene of the assay as internal standard. The second correction using a reference expression level of the same house keeping gene in the tissue or cells allows to express as microgram of total RNA and thus to avoid the variation of the results with the variability of housekeeping in each tissue, especially in experiments using various treatments. Quantification of absolute value (copies/ μg total RNA) is performed using standard curve of the reference house keeping gene subunit O of ATPase (ATP5o) that have been shown to be a gene having stable expression levels from embryonic life through adulthood in various tissues [43].

3. Marked difference of mRNA expression levels in classical endocrine tissues and peripheral tissues

As illustrated in Fig. 3, quantification of mRNA expression levels of steroidogenic enzymes shows that there is a good agreement between mRNA expression levels and enzymatic activities in a specific tissue. The data permit to clearly

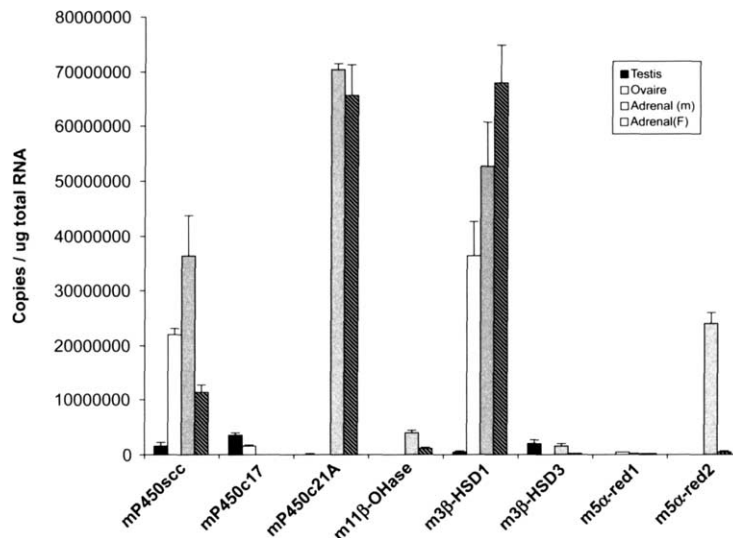


Fig. 3. Quantification of mRNA expression levels of P450scc, P450c17, P450c21, 3 β -HSDs and 5 α -reductases in classical steroidogenic mouse tissues. Twenty micrograms of total RNA extracted from mouse testis, ovary and adrenals were used for quantification by Q-RT-PCR. The experimental procedure was as described in part 2, using the second derivative calculation method and double correction. The LightCycler RealTime PCR apparatus as well as reagents were from Hoffman-La Roche Inc. (Nutley, NJ). The expression level is indicated as the number of copies/ μg of RNA. The data are expressed as mean \pm S.E.M. of triplicate assays.

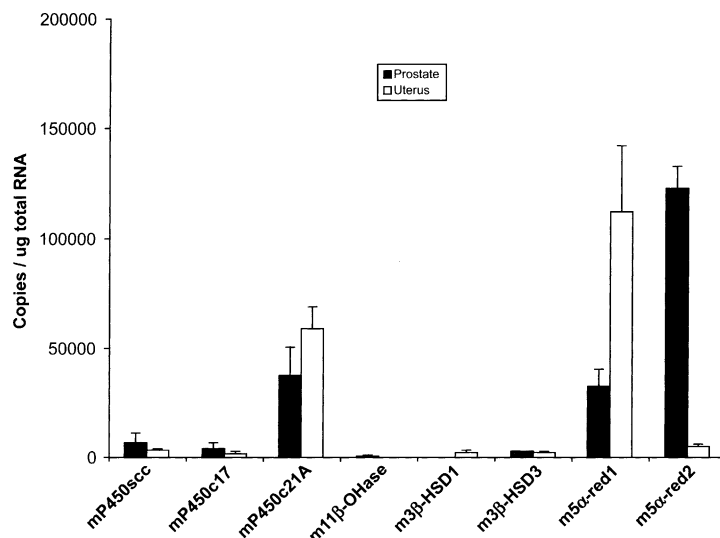


Fig. 4. Quantification of mRNA expression levels of P450scc, P450c17, P450c21, 3 β -HSDs 20 α -HSD and 5 α -reductases in the mouse prostate and uterus. Experimental procedures were as described in Fig. 3.

identify the existence of two types of steroidogenic tissues: those of classical endocrine glands such as the testis, ovary and adrenals which deliver steroids into the circulation (very important dilution), and in which millions of copies/ μ g total RNA are detected, and those of peripheral intracrine tissues where steroids are synthesized locally and exert their action at the site where they are produced (prostate, uterus, etc.), and in which the expression level of steroidogenic enzymes is much lower.

Indeed, P450scc and 3 β -HSD – which are responsible for the formation of all steroid hormones – are expressed extremely abundantly in the adrenals and gonads, at more than 10 millions (M) copies/ μ g total RNA. P450c21, which catalyzes the hydroxylation at position C21 of the steroid nucleus and is involved exclusively in the formation of glucocorticoids and mineralocorticoids, is highly expressed in the adrenals.

In addition, it is noteworthy that in the mouse prostate and uterus, two well recognized intracrine organs, the expression levels of steroidogenic enzyme mRNAs are rather low, around hundreds of thousands of copies/ μ g total RNA or less, as compared to more than 10 million copies/ μ g total RNA in classical endocrine glands. As illustrated in Fig. 4 type 2 5 α -reductase mRNA is predominantly expressed in the prostate at 123,000 copies/ μ g total RNA, while type 1 5 α -reductase is predominantly expressed in the mouse uterus at 112,000 copies/ μ g total RNA.

4. Absence of mouse P450c17 mRNA expression in the mouse adrenals

In rodents, P450c17 is involved exclusively in the formation of sex steroids. It is thus highly expressed in the testis and ovary (2.2 and 1.6 million copies/ μ g total RNA,

respectively) and weakly in the adrenals (10,000 copies/ μ g total RNA) (Fig. 3). The relatively low expression levels of P450c17 in the mouse and rat adrenals [44,45] could explain the absence of cortisol (the main human glucocorticoid) in the mouse. The main glucocorticoid in the mouse is indeed corticosterone. Fig. 5 illustrates the importance of P450c17 in the human adrenals. While the expression levels of human and mouse P450c21 are both very high in the adrenals, only human P450c17 is highly observed in the adrenals. Our results confirm that the lack of formation of cortisol and the low levels of circulating adrenal steroid precursors (DHEA and

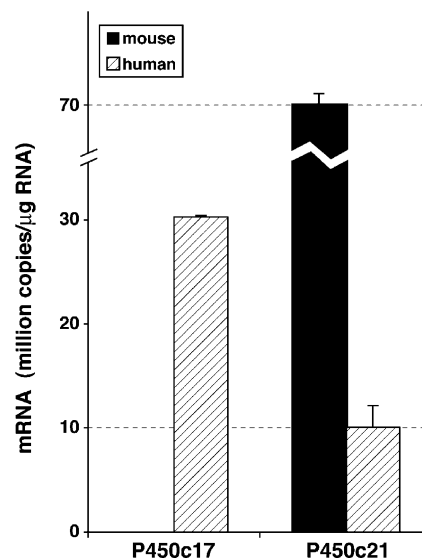


Fig. 5. Comparison of P450c17 and P450c21 mRNA expression levels in human and mouse adrenals. mRNA expression levels of P450c17 and P450c21 in human and mouse adrenals were quantified using RealTime PCR. Expression of P450c17 is extremely high in human but very low in the mouse. Expression of P450c21 is high in both human and mouse adrenals. Experimental procedures were performed as described in Fig. 3.

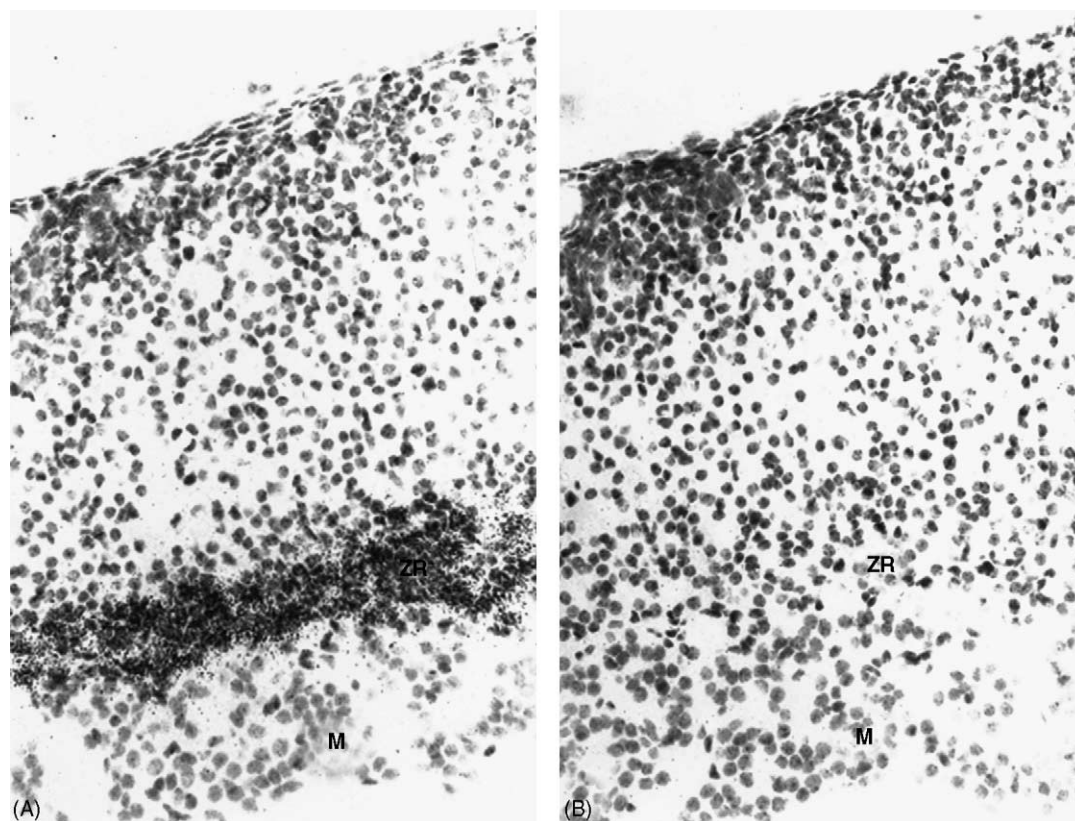


Fig. 6. Localization of 20α -HSD in the adrenals by in situ hybridization. (A) Micrograph showing the strong labeling obtained in the zona reticularis of the adrenal cortex of a female mouse after 1-day exposure time. (B) In the male, only weak labeling could be obtained in the reticularis zone after 45-day exposure time. ZR, zona reticularis; M, medulla.

or 4-androstenedione) in the mouse are not only due to mutations that altered the ability of mouse P450c17 to convert pregnenolone into DHEA, but it is mainly due to the alteration of the expression of mouse P450c17 in the adrenals. Since this enzyme catalyzes efficiently the conversion of progesterone into 4-androstenedione in the mouse testis and ovary, the presence of the enzyme will lead to a high production of circulating adrenal sex steroid precursor, 4-androstenedione in the mouse, as has been observed for DHEA production in human. This is indeed not observed in mice.

5. High expression levels of type 2 5α -reductase in mouse adrenals

From the study of knock-out mice [46] it has been suggested that type 1 5α -red is more important for the female mice physiology while type 2 5α -reductase is more important in the male. Our data agree with these observations since we observed a low expression level of type 2 5α -reductase in female-specific tissues (uterus, vagina and ovary), where type 1 5α -reductase is expressed at higher levels. On the other hand, type 2 5α -reductase is expressed significantly in male-specific tissues such as the prostate (Fig. 4). The unusually very high expression levels of type 2 5α -reductase in the adrenals strongly suggest a new role for 5α -reductase

in the rapid inactivation of progesterone, mineralocorticoids and glucocorticoids.

6. Specific expression of mouse 20α -HSD in the reticularis layer

As shown in Fig. 6, 20α -HSD, the enzyme that catalyzes the transformation of progesterone into 20α -hydroxyprogesterone [47] is mostly located in the zone surrounding the female reticularis layer, the layer which produces DHEA and 4-dione in the human. Since P450c17 is absent in mice, progesterone, which is not used in the glomerulosa and fasciculata layers to produce mineralocorticoid and glucocorticoid precursors, cannot be converted into androgen precursors (4-dione) in the reticularis. It is thus likely that the adrenal layer has a limited tolerance for progesterone, and the excess of progesterone not totally degraded by low levels of type 2 5α -reductase in female adrenals could be inactivated by 20α -HSD. In the male, the high expression of type 2 5α -reductase in the adrenals – 100-fold higher than in the female – strongly suggests that the preferred inactivation pathway of progesterone is through the reduction of progesterone into 5α -pregnane-3,20-dione by type 2 5α -reductase followed by the transformation of 5α -pregnenedione into 5α -pregnane-3 α -ol-20-one by type 2 3α -HSD. It is worth noting that mouse

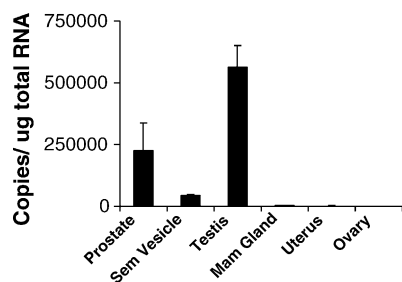


Fig. 7. mRNA expression levels of estrogen sulfotransferase in the mouse prostate, seminal vesicles, testis, mammary gland, uterus and ovary. Experimental procedures were performed as described in Fig. 3.

adrenals is the site for the formation of important amounts of progesterone, which is the precursor of glucocorticoids and mineralocorticoids.

7. Selective expression of estrogen sulfotransferase in male-specific tissues

As illustrated in Fig. 7, *SULT1E1*, the enzyme that catalyzes the sulfonation of estrone and estradiol [48,49] and is thus involved in estrogen inactivation shows a highly male-specific expression profile. It is expressed mainly in male-specific organs (prostate, testis, seminal vesicles) and is almost absent in the mammary gland uterus and ovary. The role of this enzyme is most probably to protect male tissues against estrogenic action. It is, indeed, generally observed that estrogens antagonize androgenic action.

8. Conclusion

The quantitative appreciation of steroidogenic gene expression in the mouse tissues using Q-RT-PCR shows a good agreement between mRNA expression levels of genes of in the steroidogenic pathway and their physiological function. There is, indeed, a notable difference in mRNA expression levels of genes encoding steroidogenic enzymes between classical endocrine tissues such as the testis ovary and adrenals which deliver steroid into the circulation and peripheral intracrine tissues such as the uterus and the prostate which synthesize steroid hormones locally. Evidence showing that the mouse prostate and uterus can behave as intracrine organs is given by the well accepted fact that, while in gonadectomized mice the size of the prostate and uterus is reduced drastically, the administration of inactive steroid precursors (DHEA and 4-androstenedione) to these gonadectomized animals allows the return of these organs to a normal size due to local intracrine transformation of these precursors into active androgens and estrogens. This model is widely used to study androgen and estrogen actions in the mouse. In the mouse, sex steroids are produced almost exclusively by the gonads. Thus, upon gonadectomy, the size of androgen- and estrogen-sensitive organs such as the prostate and uterus,

respectively, is reduced drastically. However, administration of an inactive steroid precursor to gonadectomized mice allows the return of the androgen- and estrogen-sensitive organs to a normal size.

This animal model constitutes a useful and widely used tool for the study of sex steroids formation in peripheral target tissues, a system that accounts for 40–50% of the production of androgens in the human prostate supplied with the steroid precursor DHEA found in high levels in the circulation [8]. Furthermore, the data reveal many unpredicted tissue distributions suggesting new roles and functions for these enzymes. These data will improve considerably our understanding of the role of steroidogenesis in target tissues.

The unusually high level of type 2 5α -reductase mRNAs in the adrenals, strongly suggests its involvement in the reduction of excess 3keto- Δ^4 -steroids in the adrenal, namely, progesterone, glucocorticoids and mineralocorticoids into their 5α -reduced steroids

Since in the mouse adrenal, P450c17 is absent (Fig. 5) [50], progesterone which is not used in the glomerulosa and fasciculata layers to produce mineralocorticoid and glucocorticoid precursors, cannot be converted into androgen precursors (DHEA, 4-dione) in the reticularis. It is thus likely that the adrenal layer has a limited tolerance for progesterone, and the excess of progesterone not totally degraded by low levels of type 2 5α -reductase in female adrenals could be inactivated by 20α -HSD.

Our finding that mouse *SULT1E1*, an estrogen inactivating enzyme, is expressed almost exclusively in male-specific tissues strongly suggests that this enzyme is involved in the protection of male-specific tissues against estrogen action. Indeed, it is generally observed that androgens and estrogens possess antagonist effects on estrogen- and androgen-sensitive cells, respectively [51,52].

Acknowledgements

This study is part of the ATLAS project supported by Genome Quebec and Genome Canada. Nathalie Paquet and Mélanie Robitaille are acknowledged for their skillful technical assistance.

References

- [1] W.L. Miller, Molecular biology of steroid hormone synthesis, *Endocrinol. Rev.* 9 (1988) 295–318.
- [2] W.M. Geissler, D.L. Davis, L. Wu, K.D. Bradshaw, S. Patel, B.B. Mendonca, K.O. Elliston, J.D. Wilson, D.W. Russell, S. Andersson, Male pseudohermaphroditism caused by mutations of testicular 17 beta-hydroxysteroid dehydrogenase 3, *Nat. Genet.* 7 (1994) 34–39.
- [3] S. Andersson, D.M. Berman, E.P. Jenkins, D.W. Russell, Deletion of steroid 5 alpha-reductase 2 gene in male pseudohermaphroditism, *Nature* 354 (1991) 159–161.
- [4] E. Rheume, J. Simard, Y. Morel, F. Mebarki, M. Zachmann, M.G. Forest, M.I. New, F. Labrie, Congenital adrenal hyperplasia due to

- point mutations in the type II 3 beta-hydroxysteroid dehydrogenase gene, *Nat. Genet.* 1 (1992) 239–245.
- [5] M. Amor, K.L. Parker, H. Globerman, M.I. New, P.C. White, Mutation in the CYP21B gene (Ile-172 → Asn) causes steroid 21-hydroxylase deficiency, *Proc. Natl. Acad. Sci. U.S.A.* 85 (1988) 1600–1604.
- [6] F. Labrie, A. Dupont, A. Belanger, M. Giguere, Y. Lacoursiere, J. Emond, G. Monfette, V. Bergeron, Combination therapy with flutamide and castration (LHRH agonist or orchiectomy) in advanced prostate cancer: a marked improvement in response and survival, *J. Steroid Biochem.* 23 (1985) 833–841.
- [7] S. Andersson, W.M. Geissler, L. Wu, D.L. Davis, M.M. Grumbach, M.I. New, H.P. Schwarz, S.L. Blethen, B.B. Mendonca, W. Bloise, S.F. Witchel, G.B. Cutler Jr., J.E. Griffin, J.D. Wilson, D.W. Russell, Molecular genetics and pathophysiology of 17 beta-hydroxysteroid dehydrogenase 3 deficiency, *J. Clin. Endocrinol. Metab.* 81 (1996) 130–136.
- [8] F. Labrie, V. Luu-The, C. Labrie, A. Belanger, J. Simard, S.X. Lin, G. Pelletier, Endocrine and intracrine sources of androgens in women: inhibition of breast cancer and other roles of androgens and their precursor dehydroepiandrosterone, *Endocrinol. Rev.* 24 (2003) 152–182.
- [9] Y. Lachance, V. Luu-The, C. Labrie, J. Simard, M. Dumont, Y. de Launoit, S. Guérin, G. Leblanc, F. Labrie, Characterization of human 3b-hydroxysteroid dehydrogenase/D5-D4 isomerase gene and its expression in mammalian cells, *J. Biol. Chem.* 265 (1990) 20469–20475.
- [10] Y. Lachance, V. Luu-The, H. Verreault, M. Dumont, É. Rhéaume, G. Leblanc, F. Labrie, Structure of the human type II 3b-hydroxysteroid dehydrogenase/D5-D4 isomerase (3b-HSD) gene: adrenal and gonadal specificity, *DNA Cell Biol.* 10 (1991) 701–711.
- [11] V. Luu-The, Y. Lachance, C. Labrie, G. Leblanc, J.L. Thomas, R.C. Strickler, F. Labrie, Full length cDNA structure and deduced amino acid sequence of human 3b-hydroxy-5-ene steroid dehydrogenase, *Mol. Endocrinol.* 3 (1989) 1310–1312.
- [12] M.C. Lorence, C.J. Corbin, N. Kamimura, M.S. Mahendroo, J.I. Mason, Structural analysis of the gene encoding human 3 beta-hydroxysteroid dehydrogenase/delta 5 → 4-isomerase, *Mol. Endocrinol.* 4 (1990) 1850–1855.
- [13] V. Luu The, C. Labrie, H.F. Zhao, J. Couet, Y. Lachance, J. Simard, G. Leblanc, J. Cote, D. Berube, R. Gagne, et al., Characterization of cDNAs for human estradiol 17 beta-dehydrogenase and assignment of the gene to chromosome 17: evidence of two mRNA species with distinct 5'-termini in human placenta, *Mol. Endocrinol.* 3 (1989) 1301–1309.
- [14] V. Luu-The, C. Labrie, J. Simard, Y. Lachance, H.-F. Zhao, J. Couët, G. Leblanc, F. Labrie, Structure of two in tandem human 17b-hydroxysteroid dehydrogenase genes, *Mol. Endocrinol.* 4 (1990) 268–275.
- [15] H. Peltoketo, V. Isomaa, O. Maentausta, R. Vihko, Complete amino acid sequence of human placental 17 beta-hydroxysteroid dehydrogenase deduced from cDNA, *FEBS Lett.* 239 (1988) 73–77.
- [16] L. Wu, M. Einstein, W.M. Geissler, H.K. Chan, K.O. Elliston, S. Andersson, Expression cloning and characterization of human 17 beta-hydroxysteroid dehydrogenase type 2, a microsomal enzyme possessing 20 alpha-hydroxysteroid dehydrogenase activity, *J. Biol. Chem.* 268 (1993) 12964–12969.
- [17] J. Adamski, T. Normand, F. Leenders, D. Monte, A. Begue, D. Stehelin, P.W. Jungblut, Y. de Launoit, Molecular cloning of a novel widely expressed human 80 kDa 17 beta-hydroxysteroid dehydrogenase IV, *Biochem. J.* 311 (1995) 437–443.
- [18] F. Leenders, B. Husen, H.H. Thole, J. Adamski, The sequence of porcine 80 kDa 17 beta-estradiol dehydrogenase reveals similarities to the short chain alcohol dehydrogenase family, to actin binding motifs and to sterol carrier protein 2, *Mol. Cell Endocrinol.* 104 (1994) 127–131.
- [19] Y. Labrie, F. Durocher, Y. Lachance, C. Turgeon, J. Simard, C. Labrie, F. Labrie, The human type II 17 beta-hydroxysteroid dehydrogenase gene encodes two alternatively spliced mRNA species, *DNA Cell Biol.* 14 (1995) 849–861.
- [20] I. Dufort, P. Rheault, X.F. Huang, P. Soucy, V. Luu-The, Characteristics of a highly labile human type 5 17beta-hydroxysteroid dehydrogenase, *Endocrinology* 140 (1999) 568–574.
- [21] A. Krazeisen, R. Breitling, K. Imai, S. Fritz, G. Moller, J. Adamski, Determination of cDNA, gene structure and chromosomal localization of the novel human 17beta-hydroxysteroid dehydrogenase type 7(1), *FEBS Lett.* 460 (1999) 373–379.
- [22] W. Qiu, M. Zhou, F. Labrie, S.X. Lin, Crystal structures of the multispecific 17beta-hydroxysteroid dehydrogenase type 5: critical androgen regulation in human peripheral tissues, *Mol. Endocrinol.* 18 (2004) 1798–1807.
- [23] J. Fomitcheva, M.E. Baker, E. Anderson, G.Y. Lee, N. Aziz, Characterization of Ke 6, a new 17beta-hydroxysteroid dehydrogenase, and its expression in gonadal tissues, *J. Biol. Chem.* 273 (1998) 22664–22671.
- [24] V. Luu-The, Analysis and characteristics of multiple types of human 17b-hydroxysteroid dehydrogenases, *J. Steroid Biochem. Mol. Biol.* 76 (2001) 143–151.
- [25] M.G. Biswas, D.W. Russell, Expression cloning and characterization of oxidative 17beta- and 3alpha-hydroxysteroid dehydrogenases from rat and human prostate, *J. Biol. Chem.* 272 (1997) 15959–15966.
- [26] J.L. Napoli, 17beta-Hydroxysteroid dehydrogenase type 9 and other short-chain dehydrogenases/reductases that catalyze retinoid, 17beta- and 3alpha-hydroxysteroid metabolism, *Mol. Cell Endocrinol.* 171 (2001) 103–109.
- [27] S. Andersson, D.W. Russell, Structural and biochemical properties of cloned and expressed human and rat steroid 5 alpha-reductases, *Proc. Natl. Acad. Sci. U.S.A.* 87 (1990) 3640–3644.
- [28] E.P. Jenkins, C.L. Hsieh, A. Milatovich, K. Normington, D.M. Berman, U. Francke, D.W. Russell, Characterization and chromosomal mapping of a human steroid 5 alpha-reductase gene and pseudogene and mapping of the mouse homologue, *Genomics* 11 (1991) 1102–1112.
- [29] F. Labrie, Y. Sugimoto, V. Luu-The, J. Simard, Y. Lachance, D. Bachvarov, G. Leblanc, F. Durocher, N. Paquet, Structure of human type II 5a-reductase gene, *Endocrinology* 131 (1992) 1571–1573.
- [30] V. Luu The, N. Paquet, E. Calvo, J. Cumps, Quantitative RealTime RT-PCR, Improved method for high throughput measurements using second derivative calculation and double correction, *BioTechniques* 38 (2005) 287–293.
- [31] L.C.T. Manual, LightCycler Presentation, Roche Molecular Biochemicals, Quebec, 2002, p. 32.
- [32] R. Higuchi, C. Fockler, G. Dollinger, R. Watson, Kinetic PCR analysis: real-time monitoring of DNA amplification reactions, *Biotechnology (NY)* 11 (1993) 1026–1030.
- [33] C.T. Wittwer, K.M. Ririe, R.V. Andrew, D.A. David, R.A. Gundry, U.J. Balis, The LightCycler: a microvolume multisample fluorimeter with rapid temperature control, *Biotechniques* 22 (1997) 176–181.
- [34] T.D. Schmittgen, Real-time quantitative PCR, *Methods* 25 (2001) 383–385.
- [35] R. Rasmussen, in: S. Meucr, C. Wittwer, K. Nakagawara (Eds.), Quantification on the LightCycler Instrument in Rapid Cycle Real-time PCR: Methods and Application, Springer, Heidelberg, 2001, pp. 21–34.
- [36] F. Souaze, A. Ntodou-Thome, C.Y. Tran, W. Rostene, P. Forgez, Quantitative RT-PCR: limits and accuracy, *Biotechniques* 21 (1996) 280–285.
- [37] C.T. Wittwer, M. Gutekunst, S. Lohmann, Method for quantification of an analyte in United States Patent No. US 6,303,305 B1 United State (1999).
- [38] F. Ferre, Quantitative or semi-quantitative PCR: reality versus myth, *PCR Meth. Appl.* 2 (1992) 1–9.
- [39] V. Lyamichev, M.A. Brow, V.E. Varvel, J.E. Dahlberg, Comparison of the 5' nuclease activities of taq DNA polymerase and its

- isolated nuclease domain, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 6143–6148.
- [40] S. Tyagi, F.R. Kramer, Molecular beacons: probes that fluoresce upon hybridization, Nat. Biotechnol. 14 (1996) 303–308.
- [41] K.J. Livak, S.J. Flood, J. Marmaro, W. Giusti, K. Deetz, Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization, PCR Meth. Appl. 4 (1995) 357–362.
- [42] T.B. Morrison, J.J. Weis, C.T. Wittwer, Quantification of low-copy transcripts by continuous SYBR Green I monitoring during amplification, Biotechniques 24 (1998) 954–958, 960 and 962.
- [43] J.A. Warrington, A. Nair, M. Mahadevappa, M. Tsyganskaya, Comparison of human adult and fetal expression and identification of 535 housekeeping/maintenance genes, Physiol. Genomics 2 (2000) 143–147.
- [44] B.J. Brock, M.R. Waterman, Biochemical differences between rat and human cytochrome P450c17 support the different steroidogenic needs of these two species, Biochemistry 38 (1999) 1598–1606.
- [45] L.M. Perkins, A.H. Payne, Quantification of P450sc, P450(17) alpha, and iron sulfur protein reductase in Leydig cells and adrenals of inbred strains of mice, Endocrinology 123 (1988) 2675–2682.
- [46] M.S. Mahendroo, A. Porter, D.W. Russell, R.A. Word, The parturition defect in steroid 5alpha-reductase type 1 knockout mice is due to impaired cervical ripening, Mol. Endocrinol. 13 (1999) 981–992.
- [47] Y. Zhang, I. Dufort, P. Rheault, V. Luu-The, Characterization of a human 20alpha-hydroxysteroid dehydrogenase, J. Mol. Endocrinol. 25 (2000) 221–228.
- [48] W.F. Demyan, C.S. Song, D.S. Kim, S. Her, W. Gallwitz, T.R. Rao, M. Slomczynska, B. Chatterjee, A.K. Roy, Estrogen sulfotransferase of the rat liver: complementary DNA cloning and age- and sex-specific regulation of messenger RNA, Mol. Endocrinol. 6 (1992) 589–597.
- [49] C. Her, I.A. Aksoy, S. Kimura, B.F. Brandriff, J.J. Wasmuth, R.M. Weinshilboum, Human estrogen sulfotransferase gene (STE): cloning, structure, and chromosomal localization, Genomics 29 (1995) 16–23.
- [50] A.H. Payne, I.G. Abbaszade, T.R. Clarke, P.A. Bain, C.H. Park, The multiple murine 3 beta-hydroxysteroid dehydrogenase isoforms: structure, function, and tissue- and developmentally specific expression, Steroids 62 (1997) 169–175.
- [51] F. Labrie, J. Simard, R. Poulin, A.C. Hatton, C. Labrie, S. Dauvois, H.F. Zhao, L. Petitclerc, J. Couet, M. Dumont, et al., Potent antagonism between estrogens and androgens on GCDFP-15 expression and cell growth in the ZR-75-1 human breast cancer cells, Ann. NY Acad. Sci. 586 (1990) 174–187.
- [52] R. Poulin, D. Baker, F. Labrie, Androgens inhibit basal and estrogen-induced cell proliferation in the ZR-75-1 human breast cancer cell line, Breast Cancer Res. Treat. 12 (1988) 213–225.