Adrenals

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Another year has gone by, and having had the privilege of preparing the chapter Adrenals for the *Yearbook of Pediatric Endocrinology* again, it was most impressive to see the continuing developments and ongoing progress that have been achieved in the field of steroidology. A wealth of publications still reflects the constant fascination emanating from these rather old small molecules. In this context may I remind the reader that it is highly remarkable that the steroid structure – speaking in phylogenetic terms – has been preserved throughout evolution over a very long period of time and can be found as early as the appearance of unicellular eukaryotes.

This year's annual meeting of ESPE will take place in France, so another driving force for the huge amount of research and publications in this field might be best explained by a quotation by the well-known French philosopher Jean-Paul Sartre (1905–1980) who, by the way, seems to be – rather calmly – celebrated for the 100th anniversary of his birth this year. One does not necessarily need to be committed to existentialism to realize that his words, 'One of the chief motives of artistic creation is certainly the need of feeling that we are essential in relationship to the world', hold a lot of truth. I hope all of you will unanimously agree that pediatric endocrinology – dealing with growth and development of the human being – without any doubts has, will have, and has to have its firm place in this world as the very own discipline of pediatrics.

This selection of articles is by no means meant as an attempt to rank the research papers that have been published during the last 12 months. In a way, there seem to be too many ranking activities hidden under the concept of quality control. Uncritical use of the impact factor often leads to grotesque arithmetic. Behind all these developments – in the context of decreasing resources – is there not the danger of penetration of an utilitarian view in almost all aspects of life? I leave it up to the reader to imagine what such a trend would mean to disciplines with low shareholder value.

My attempt is to present a broad selection of recent developments in the many aspects related to adrenals and steroids, so the interested reader – basic scientist or clinician – should be able to get a quick update in this field. The reader will find information regarding fetal adrenals, new steroids, steroid metabolism in newborns, the phenomenon of adrenarche, metabolism of steroids, genetic and metabolic aspects on various forms of congenital adrenal hyperplasia (CAH), animal models for CAH, prenatal diagnosis of a new form of CAH, steroid analysis, etc. Due to the limited space, many excellent papers could not be included and I hope that the authors of these papers will understand. I hope that the inclined reader will enjoy reading the following lines after a busy but not too long working day as suggested by Jean Paul Sartre, 'One cannot become a saint when working sixteen hours a day!' – isn't this remarkable for an existentialist?

New mechanisms: the fetal adrenal – still not yet completely understood

Estrogen elicits cortical zone-specific effects on development of the primate fetal adrenal gland

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Endocrinology 2005;146:1737–1744

Background: The levels of endogenous estrogen, which increase with advancing pregnancy, were investigated to discover whether it regulates the growth and development of the fetal adrenal cortex in the baboon.

Methods: Fetal adrenal glands were obtained at mid and late gestation from untreated baboons and at late gestation from animals in which endogenous estrogen production was suppressed by administration of an aromatase inhibitor (CGS 20267) at mid and late gestation. Volumes of the respective cortical zones were determined by zone-specific immunocytochemical staining of steroidogenic enzymes and image analysis.

Results: Fetal adrenal weight and volume rose 3-fold between mid and late gestation and an additional 70% by administration of CGS 20267, which reduced fetal serum estradiol levels by more than 95%. Umbilical artery serum dehydroepiandrosterone sulfate, which is secreted primarily by the fetal zone, was increased almost 3-fold by administration of CGS 20267. Concomitant administration of CGS 20267 and estradiol returned the fetal cortical zone volume and serum dehydroepiandrosterone sulfate levels to normal. In contrast to the effect of estrogen deprivation on the fetal zone, the volumes of the definitive and transitional zones in untreated baboons late in gestation and levels of fetal serum cortisol, a steroid secreted from the transitional zone, were not altered by estrogen suppression.

Conclusion: The authors hypothesize that estrogen acts directly on the fetal adrenal cortex to selectively repress the morphological and functional development of the fetal zone. This may represent a feedback system to maintain physiological secretion of estrogen precursors and thus placental estrogen production to promote normal primate fetal and placental development.

The fetal adrenal cortex consists of the inner fetal zone, the outer definitive zone and the transitional zone lying between fetal and definitive zones [1]. This unique gland is present in man and the chimpanzee only, but not in earlier evolutionary life forms, just as is the case with human adrenarche. Growth and functional maturation of the human and nonhuman primate fetal adrenal gland are still enigmatic. While ACTH has been shown to play a pivotal role in this process, the potential role of increasing levels of endogenous estrogen on the fetal adrenal cortical zones in vivo in the primate has not been investigated so far. In the baboon, a nonhuman primate model, the authors demonstrate that placental estrogen, synthesized from C19-steroid precursors of the fetal cortical zone, shows a feedback on the latter fetal adrenal zone to restrict its growth and development. Estrogen seems to act selectively on the fetal cortical zone, despite the presence of estrogen receptor- α and β in the definitive and transitional zone. Thus a physiologically normal level of secretion of precursors such as dehydroepiandrosterone and also placental estrogen production might possibly be maintained. It is interesting that despite the presence of estrogen receptors in the outer zones of the baboon fetal adrenal, definitive and transitional zones do not seem to be affected by this mechanism. The physiological relevance of this feedback mechanism needs to be established; however it is likely that it contributes to maintaining the intrauterine hormonal equilibrium required for optimal fetal and placental development.

New hormones: steroids for the heart

Ouabain-like compound changes rapidly on physical exercise in humans and dogs: effects of β -blockade and angiotensin-converting enzyme inhibition

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Hypertension 2005;1024:1024–1028

Background: Ouabain is an inhibitor of the sodium pump. It has been identified in bovine adrenal glands. The authors wanted to investigate whether the release of this cardiotonic steroid is stimulated by physical exercise.

Methods: Athletes and healthy dogs were subjected to ergometry. A ouabain-like compound was determined in venous blood by ELISA as well as by $^{86}\text{Rb}^+$ uptake inhibition (as ouabain equivalents).

Results: The ouabain-like compound increased in venous blood of athletes after 15 min of ergometry from 2.5 ± 0.5 to 86.0 ± 27.2 nmol/l, as did the concentration of a circulating inhibitor of the sodium pump from 7.3 ± 1.7 to 129.8 ± 51 nmol/l (ouabain equivalents). At rest, the ouabain-like compound decreased in humans and dogs with a half life of 3–5 min. In beagles exposed to moderate exercise on a treadmill for 13 min, the levels of ouabain-like compound increased 46-fold. This effect was suppressed when the dogs had been treated for 3 weeks with the β_1 -adrenergic receptor blocker atenolol or the angiotensin-converting enzyme inhibitor benazepril.

Conclusion: The authors conclude that the ouabain-like compound changes rapidly during exercise and that epinephrine and angiotensin II are important for ouabain-like compound release.

This paper was selected to draw the readers' attention to a newly described class of steroid hormones [2]. Ouabain has been isolated from adrenals, blood and hypothalamus, representing one of the endogenous cardiac glycosides circulating in blood plasma. The rapid changes in its plasma concentration suggest that it may play an important role in circulatory regulation. Its main physiological impact might consist in the reduction of heart rate, positive inotropy or increase in blood pressure, but still remains to be elucidated.

Important for clinical practice: the dramatic clinical spectrum of lipid adrenal hyperplasia

A genetic isolate of congenital lipid adrenal hyperplasia with atypical clinical findings

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J Clin Endocrinol Metab 2005;90:835–840

Background: Lipoid congenital adrenal hyperplasia (CAH) represents the most severe form of CAH, capable of destroying all adrenal and gonadal steroidogenesis. Lipoid CAH is caused by mutations in the steroidogenic acute regulatory protein (StAR). This protein facilitates the entry of cholesterol into mitochondria to initiate steroidogenesis. Typically, patients with lipoid CAH present with a salt-losing crisis in the first 2 months of life.

Method: Eight patients from six Saudi Arabian families who were first diagnosed at 1–14 months of age (median 4–7, mean 7 months) are described. Five patients were 46,XY and 3 were 46,XX.

Results: At presentation, all showed hyponatremia, hyperkalemia, elevated ACTH, and low cortisol. Pregnenolone, progesterone, 17-hydroxypregnenolone, 17-hydroxyprogesterone, testosterone, androstenedione, and dehydroepiandrosterone sulfate were all low. DNA sequencing showed that 1 patient was homozygous for the StAR mutation M144R, and the other 7, from 5 apparently unrelated families, were homozygous for the StAR mutation R182H.

Conclusion: The loss of all assayable activity in in vitro expression studies correlated poorly with the later onset of clinical symptoms in these patients. Lipoid CAH may present much later in life than previously thought.

It was long thought that patients with lipoid congenital adrenal hyperplasia had a defect in the cholesterol side-chain cleavage enzyme system but analysis of the components of this enzymatic system was negative [3]. The discovery of the StAR protein led to the discovery of StAR mutations in these patients. A two-hit model has been formulated for lipoid hyperplasia: a first hit consists of loss of the acute steroidogenic response secondary to StAR mutations, whereas a second hit consists of loss of StAR-independent steroidogenesis as a consequence of accumulation of cellular cholesterol. This paper reminds us of a rare form of CAH. Furthermore it is impressive to read about the wide spectrum of clinical presentations in the same ethnic group with the same mutation in the same part of the world.

Two prevalent CYP17 mutations and genotype-phenotype correlations in 24 Brazilian patients with 17-hydroxylase deficiency

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J Clin Endocrinol Metab 2004;89:49–60

Background: The authors give us insight into the phenotypic variations in 17-hydroxylase deficiency.

Method: Molecular genetic analyses were performed in 24 Brazilian subjects from 19 families with 17-hydroxylase deficiency.

Results: Seven novel CYP17 mutations were found, of which 2 (W406R and R362C) accounted for 50 and 32% of the mutant alleles, respectively. Expression studies showed that both mutations were completely inactive. However, phenotypic features varied: some 46,XY individuals had partially virilized genitalia while others had unambiguously female external genitalia. Some had normal blood pressure and/or serum potassium levels while others had severe hypermineralocorticoidism. The severity of hypertension, hypokalemia, 17-deoxysteroid excess, and sex steroid deficiency varied, even among patients completely lacking CYP17 enzyme activity. Spontaneous pubertal development occurred only in 46,XX females with partial enzyme deficiency.

Conclusion: The authors conclude that other – hitherto unknown – factors, in addition to the CYP17 genotype, contribute to the phenotype of individual patients with 17-hydroxylase deficiency.

17-Hydroxylase deficiency seems to be a further example for emerging recent evidence that the relationship between genotype and phenotype cannot always be considered linear. Studies like this might be an excellent starting point to shed light on this question because unique cohorts of multiple individuals bearing the same genotype are being investigated and therefore might provide the clue to what extent genotype alone determines phenotype. Much remains to be done!

New mechanisms: back to the roots – origins of 18-hydroxycortisol and 18-oxocortisol identified!

Studies on the origin of circulating 18-hydroxycortisol and 18-oxocortisol in normal human subjects

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J Clin Endocrinol Metab 2004;89:4628–4633

Background: 18-Hydroxycortisol (18-OHF) and 18-oxocortisol (18-oxoF) are derivatives of cortisol found in primary aldosteronism. Their origin and regulation in normal subjects remains unknown. 18-OHF can be synthesized by zona fasciculata 11 β -hydroxylase (CYP11B1); 18-oxoF can only be produced by zona glomerulosa aldosterone synthase (CYP11B2).

Methods: Transfected cell lines expressing either 11 β -hydroxylase or aldosterone synthase were incubated with cortisol and other substrates over a range of concentrations.

Results: Both enzymes could synthesize 18-OHF from cortisol, however only aldosterone synthase could synthesize 18-oxoF. Aldosterone synthase was more efficient than 11 β -hydroxylase at 18-hydroxylation. In patients with adrenal insufficiency maintained on hydrocortisone, urinary free cortisol and cortisone levels were high; 18-oxoF was detectable in all patients and 18-OHF in 3. It is likely that the 18-oxygenated steroids were synthesized from circulating cortisol, either in the zona glomerulosa or at extra-adrenal sites. In male volunteers, dexamethasone treatment decreased urinary excretion rates of free cortisol, cortisone, 18-OHF, and 18-oxoF, confirming dependence of 18-oxygenated steroid levels

on cortisol availability. In both groups, hydrocortisone administration resulted in detectable levels of 18-OHF and raised levels of 18-oxoF. There was close correlation between 18-oxoF and cortisol excretion during hydrocortisone administration in normal subjects.

Conclusion: These data reveal that 18-OHF and 18-oxoF can be synthesized from circulating cortisol. The close correlation between 18-oxoF and cortisol suggests that 18-oxoF is normally produced by the action of aldosterone synthase using circulating cortisol as a substrate. Although 18-OHF can be synthesized using circulating cortisol as substrate, the data suggest that this compound is normally produced in the zona fasciculata by 11 β -hydroxylase from locally available cortisol.

At times of molecular biology, it is a pleasure to read a metabolic paper. There are still so many unsolved questions regarding classical steroidology and this paper is an elegant example of how these problems can successfully be addressed. This paper reminds us of the pioneering work of Chu and Ulick [4] who in the early 1980s of the last century isolated the hybrid corticosteroids 18-OHF and 18-oxoF. A basic paper worth reading for all enjoying basic steroid metabolism. Furthermore, the final significance of these interesting steroids on pathophysiology and their potential role as diagnostic parameters of disorders of mineralocorticoid metabolism has yet to be established.

Concepts revised: the DHEA-DHEAS shuttle revisited

No evidence for hepatic conversion of dehydroepiandrosterone (DHEA) sulfate to DHEA: in vivo and in vitro studies

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J Clin Endocrinol Metab 2005;90:3600–3605

Background: Dehydroepiandrosterone sulfate (DHEAS) is the most abundant steroid in the human circulation. It is believed to represent the circulating hydrophilic storage form of dehydroepiandrosterone (DHEA). The authors state that there is general agreement that DHEA and DHEAS inter-convert freely and continuously via hydroxysteroid sulfotransferases and steroid sulfatase and that only desulfated DHEA can be converted downstream to sex steroids.

Method: To study DHEA/DHEAS interconversion in vivo and in vitro, the authors administered oral DHEA (100 mg) and i.v. DHEAS (25 mg) to 8 healthy young men and carried out incubation experiments with liver cells.

Results: Oral administration of DHEA and i.v. administration of DHEAS resulted in similar increases in serum DHEAS compared with baseline. However, DHEA administration significantly increased serum DHEA, while no such increase was observed after DHEAS. It was DHEA but not DHEAS which led to a rise in androstenedione, estrone, and androstenediol glucuronide thus suggesting further downstream metabolism. The striking absence of conversion of DHEAS to DHEA was reflected by their in vitro findings in HepG2 cells, revealing dose-dependent conversion of DHEA to DHEAS but no conversion of DHEAS.

Conclusion: The results show a lack of hepatic conversion of DHEAS to DHEA which, according to the authors, challenges the concept of free interconversion of DHEA and DHEAS. The authors conclude that DHEAS does not seem to represent a circulating storage pool for DHEA regeneration, and therefore serum DHEAS is unlikely to reflect bioavailable DHEA.

This is an interesting observation shedding a bit more light on the still enigmatic steroids DHEA and DHEAS. In particular it shatters the widely believed endocrine dogma that DHEA and DHEAS inter-convert freely and continuously via hydroxysteroid sulfotransferases and steroid sulfatase and that DHEAS acts as a storage pool for bioavailable DHEA. But who formulated this dogma and who knows the respective reference? On the other hand, many of us will have noted from clinical practice that when assessing female hirsutism, DHEA and DHEAS do not always behave in parallel. It is the great merit of the authors to have led the endocrine community's attention to a weak point in today's

concept of steroid metabolism. We learn that serum DHEAS does not appropriately reflect corresponding levels of biologically active DHEA and that it may be useful to determine both steroids especially in pathological conditions. More work is needed to reassess the role of DHEA measurements in pediatric endocrinology.

Food for thought: from nappies to dynamic endocrinology

Assessing cortisol production in preterm infants: do not dispose of the nappies

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Pediatr Res 2005;57:412–418

Background: The authors aimed to develop a practical procedure for reliable and noninvasive assessment of cortisol production rates in premature infants.

Methods: Daily urinary excretion rates of glucocorticoids were measured using a hydraulic compression method to collect urine from cellulose nappies. Glucocorticoid metabolites were then profiled by quantitative gas chromatography-mass spectrometry (GC-MS).

Results: Recovery of steroids after hydraulic extraction from nappies was excellent and approximated 100%. The median urinary excretion rate of glucocorticoids in 9 healthy preterm infants increased significantly during the first 5 days of life and remained between 566 $\mu\text{g/kg/day}$ at day 5 and 302 $\mu\text{g/kg/day}$ at 4 weeks of age. However, this increase in urinary excretion rates of glucocorticoids in the first days of life was no longer significant when corrected for urinary creatinine. When calculated per square meter body surface area, the median urinary excretion rates of glucocorticoids were 5.1, 4.2, 4.1, and 3.7 $\text{mg/m}^2/\text{day}$ on day 5, and at weeks 2, 3, and 4, respectively, thus revealing that the urinary excretion rates of glucocorticoids in premature babies constitute approximately 70% of the natural cortisol production rate as determined by stable isotope dilution technique in older children. In 3 of 5 preterm infants with arterial hypotension requiring treatment with catecholamines, low cortisol production was detected.

Conclusion: The authors found that 24-hour urine collection using disposable nappies in combination with GC-MS steroid profiling proved to be a reliable, noninvasive, non-stressful procedure to assess cortisol production and metabolism in premature infants.

Concerning the important question whether early adrenal insufficiency – possibly associated with increased pulmonary and circulatory morbidity – might be present in premature babies, no major breakthrough has been achieved. Most studies have been based on plasma steroid concentrations. However, we must not forget that a plasma concentration does not represent a time-integrated parameter and therefore does not necessarily allow assessment of the hormonal production rate! Furthermore no commercially available cortisol immunoassay has been developed that is appropriate for use in the unique hormonal milieu of premature infants or neonates [5]. The authors therefore tried to develop a suitable alternative research tool consisting of 24-hour urine collection using disposable nappies in combination with GC-MS steroid profiling. This procedure proved to reliably assess cortisol production rates and to be as noninvasive and gentle as possible with respect to the fragile premature infant. In contrast to previous studies using static plasma concentrations, the authors believe that assessing production rates provides a more appropriate dynamic and time-integrated approach.

LBP proteins modulate SF1-independent expression of P450scc in human placental JEG-3 cells

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Mol Endocrinol 2005;19:409–420

Background: The cholesterol side-chain cleavage enzyme, P450scc, is the first, rate-limiting and hormonally regulated enzymatic step in the biosynthesis of all steroid hormones. Steroidogenic factor-1 (SF1) is essential for the expression of all steroidogenic genes in the adrenals and gonads. Its expression is undetectable in human placenta. P450scc expression in human placental JEG-3 cells utilizes an SF1-independent cis-acting element at –155/–131 that is inactive in adrenals and gonads. The authors have previously cloned two transcription factors, the long terminal repeat binding protein (LBP)-1b and LBP-9, from human placental JEG-3 cells.

Methods and Results: To determine the roles of these factors on the intact P450scc gene, the authors expressed LBP-1b or LBP-9 in JEG-3 cells. They found that all cell lines stably expressing a fusion protein of LBP-1b and enhanced green fluorescent protein increased P450scc expression, but cell lines stably expressing LBP-9 fused to enhanced green fluorescent protein either increased or decreased P450scc expression.

Conclusion: It was concluded that LBP-1b is an important SF1-independent transcriptional activator stimulating P450scc expression in human placental JEG-3 cells, whereas LBP-9 modulates the action of LBP-1b, exerting both positive and negative effects.

The human placenta produces large amounts of progesterone, which are required to suppress uterine contractility so that pregnancy can proceed. During human pregnancy, progesterone is initially provided by the mother's ovarian corpus luteum, but from the 8th–10th week onwards practically all progesterone is produced by the fetal tissue placenta requiring very high levels of placental P450scc transcription. It is important to understand the SF1-independent mechanism regulating human placental P450scc transcription and steroid biosynthesis because, without placental progesterone or interference with the action of progesterone, spontaneous abortion would occur. The LBP-1 family of mammalian transcription factors was initially described in HeLa cells. Their name refers to long terminal repeat binding protein [6]. The authors nicely demonstrate that the widely expressed LBP-1b and LBP-9 appear to be involved in placental steroidogenesis.

Important for clinical practice: measuring hormones in pediatric endocrinology – method development is still taking place!

Ketosteroid profiling using Girard T derivatives and electrospray ionization tandem mass spectrometry: direct plasma analysis of androstenedione, 17-hydroxyprogesterone and cortisol

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Rapid Commun Mass Spectrom 2005;19:193–200

Background: The ratio between the three ketosteroids, [(androstenedione + 17 α -hydroxyprogesterone)/cortisol], discriminates better than the concentration of 17 α -hydroxyprogesterone alone in the diagnosis of the inherited disease congenital adrenal hyperplasia (CAH).

Methods: The authors succeeded in developing a rapid, direct infusion electrospray ionization tandem mass spectrometry (ESI-MS/MS) method to measure this ratio in plasma (100 μ l). Steroids were derivatized using Girard T reagent. Androstenedione, 17 α -hydroxyprogesterone and cortisol were individually quantitated by isotope dilution.

Results: An approximately tenfold increase in sensitivity could be achieved by using Girard T derivatives. The value of the ratio function of the three ketosteroids was 0.01–0.16 for normal controls (n = 26) and 1.83–18.7 for patients with severe CAH (n = 4).

Conclusions: A simple ESI-MS/MS method has been developed for the simultaneous isotope dilution quantitation of the three ketosteroids androstenedione, 17 α -hydroxyprogesterone and cortisol in plasma of neonates.

Electrospray ionization tandem mass spectrometry (ESI-MS/MS) has become a powerful means of metabolic profiling used in the delineation of many inherited human diseases. This development demonstrates that significant progress has not only been made in the field of molecular genetics but also in the field of instrumental (mass spectrometric) analysis of metabolites and particularly in the field of steroid analysis [7]. In last year's chapter I selected a paper on LC-MS/MS steroid profiling in blood spots from neonates. The current paper will undoubtedly mean a further step forward in the development of highly specific plasma methods needed for the hormonal diagnosis of congenital adrenal hyperplasia. The use of Girard T derivatives presents an elegant means of improving sensitivity and enabling rapid profiling of these ketosteroids.

**New mechanisms: What remains straightforward in this world?
Not even activities of human 17 β -hydroxysteroid dehydrogenases are
unidirectional any more**

Human 17 β -hydroxysteroid dehydrogenase types 1, 2, and 3 catalyze bi-directional equilibrium reactions, rather than unidirectional metabolism, in HEK-293 cells

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Arch Biochem Biophys 2004;429:50–59

Background: Human 17 β -hydroxysteroid dehydrogenases catalyze the interconversion of weak and potent androgen and estrogen pairs. Although the reactions using purified enzymes can be driven in either direction, these enzymes appear to function unidirectionally in intact cells: only reductive reactions for 17 β HSD1 (ovarian granulosa cells) and 17 β HSD3 (testicular Leydig cells) and only oxidative reactions for 17 β HSD2 (peripheral tissues).

Methods and Results: In exhaustive incubation experiments the authors were able to demonstrate with either 17 β -hydroxy- or 17-ketosteroid that the medium for HEK-293 cells expressing 17 β HSD1 or 17 β HSD3 contained a 92:8 ratio of reduced:oxidized steroid. Likewise, 17 β HSD2 yielded a >95:5 ratio of oxidized:reduced steroids for both androgens and estrogens. Dual-isotope kinetic measurements showed that the rates of the forward and reverse reactions were identical at these functional equilibrium states in intact cells for all three 17 β HSD isoforms, and that these rates were much faster than those estimated from single-isotope flux studies.

Conclusion: 17 β HSD types 1, 2, and 3 catalyze both oxidative and reductive reactions in HEK-293 cells at intrinsic rates that are much faster than those estimated from single-isotope studies. Activities of these 17 β HSD isoforms do not drive steroid flux in one direction but rather may achieve functional equilibrium in intact cells.

The human 17 β -hydroxysteroid dehydrogenases are decisive enzymes in the regulation of androgen and estrogen potency. Due to the reductive activities of 17 β HSD1 and 17 β HSD3 the weak (17-ketosteroid) and potent (17-hydroxysteroid) steroid pairs, such as estrone/estradiol and androstenedione/testosterone, are interconverted in ovarian granulosa cells and testicular Leydig cells, respectively. Paradoxically, purified enzymes in reconstituted assays possess either activities dependent on the concentrations of reduced and oxidized cofactors (enzymes, such as 11 β HSD, which preferentially use NADPH/NADP⁺ act as reductases), whereas utilization of NADH/NAD⁺ leads to

oxidative activity. However, bi-directional steroid metabolism by 17 β HSDs has not yet been convincingly demonstrated in intact cells. In a series of cell culture, transfection, and incubation experiments as well as using dual-isotope kinetic measurements the authors were able to demonstrate that the 17 β HSD isoforms achieve functional equilibrium in intact cells, reflecting thermodynamically driven steroid distributions. The authors' findings have a couple of noteworthy implications for steroid hormone biology: e.g. oxidative 17 β HSD cannot absolutely protect peripheral tissues against high concentrations of either active or inactive 17-oxo steroids, or the extent of the steroid distribution ratio might not be strictly fixed by the properties of the enzyme alone but might be influenced by various intracellular factors.

Important for clinical practice: more on associations between low birth weight and adrenal androgen secretion

Opposing influences of prenatal and postnatal weight gain on adrenarche in normal boys and girls

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J Clin Endocrinol Biochem 2004;89:2647–2651

Background: So far, associations between low birth weight and higher adrenal androgen secretion before puberty have only been reported in case-control studies in girls.

Methods: The authors examined the influence of birth weight and early postnatal weight gain on overnight-fasting adrenal androgen and cortisol levels in a large normal United Kingdom birth cohort at age 8 years.

Results: In univariate analyses, adrenal androgen levels (dehydroepiandrosterone sulfate, androstenedione) were inversely related to birth weight SD score in each sex. In multivariate analyses, both lower birth weight and larger current body weight were predictive of higher adrenal androgen levels. Children who showed rapid postnatal weight gain between 0 and 3 years had higher dehydroepiandrosterone sulfate and androstenedione levels at 8 years. Cortisol levels were found to be unrelated to birth weight or current body size.

Conclusion: A continuous relationship between lower birth weight and higher childhood adrenal androgen levels was found throughout the range of normal birth weights, and was similar in boys and girls. Adrenal androgen levels were highest in small infants who gained weight rapidly during early childhood.

This paper contains data from the Avon Longitudinal Study of Parents and Children (ALSPAC), which is a prospectively designed ongoing study in England. The data stem from a cohort of 770 children. One of the most important findings of this study is that adrenal androgen levels were highest in those small infants who became heavier than average during early childhood. Such a growth pattern is usually seen after in utero growth retardation. The findings are of clinical importance since higher adrenal androgen secretion is likely to contribute to links between early growth and adult disease risks, possibly by enhancing insulin resistance and central fat deposition. Long-term follow-up of this cohort will provide results regarding development of body mass index and puberty in relation to adrenal maturation. In this context the reader's attention should also be drawn to a recent study on urinary markers of adrenarche in 400 healthy children and adolescents of the DONALD study (Dortmund Nutritional and Anthropometric Longitudinally Designed study) showing that adrenarche is a gradual process starting much earlier than hitherto believed [8].

Congenital adrenal hyperplasia: the molecular basis of 21-hydroxylase deficiency in H-2(aw18) mice

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J Clin Endocrinol Metab 2005;146:2563–2574

Background: The only available animal model of 21-hydroxylase deficiency is the mouse strain H-2(aw18). A deletion of the active Cyp21a1 gene has been postulated, the exact changes at the nucleotide level are still unknown, however.

Methods: The authors have performed a detailed analysis of the Cyp21 locus in H-2(aw18) mice.

Results: They could demonstrate that 21-hydroxylase deficiency is caused by unequal crossing over between the active Cyp21a1 gene and the pseudogene resulting in a hybrid Cyp21a1-Cyp21a2-p gene including a partial deletion of Cyp21a1. Next to several pseudogene-specific point mutations, various novel missense mutations and a nonsense mutation were found to be present. The mutations were classified in three classes: I (no or minor decrease in enzyme activity), R238Q, P465L, R361K, A362V, P458L; II (loss of enzyme activity caused by inefficient electron flux), R346H, R400C; and III (loss of activity due to deficient substrate binding), I462F, L464F.

Conclusion: Interestingly, the underlying genetic mechanisms are also known to be responsible for 21-OHD in humans. Consecutively rodent 21-OHD turns out to be an excellent genetic model for studying the human disease.

This paper provides a detailed description of an animal model for 21-hydroxylase deficiency. Nevertheless, there are important differences between mice and men: in the absence of 17-hydroxylase, corticosterone is the major glucocorticoid in rodents. Furthermore, shunting of steroid precursors in the androgen pathway cannot occur and sexual differentiation is therefore normal. Enzyme activity for each point mutation was determined in vitro, and the structure-function relationship was studied by sequence conservation analysis and by a three-dimensional murine 21-hydroxylase protein (Cyp21) structure model. These approaches provide a valuable tool to understand the role of the different mutations and polymorphisms on enzyme activity. Metabolic studies could further characterize the steroid metabolome of CAH mice and might prove useful to address unsolved questions in human CAH.

Important for clinical practice: the unique potential of GC-MS urinary steroid profiling enables prenatal diagnosis of a new form of CAH

Prenatal diagnosis of P450 oxidoreductase deficiency (ORD): a disorder causing low pregnancy estriol, maternal and fetal virilization, and the Antley-Bixler syndrome phenotype

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Am J Med Genet 2004;129:105–112

Background: This is a case report on the second pregnancy of a woman who had previously given birth to a virilized female infant. Then, the cause of the virilization could not be detected; common forms of congenital adrenal hyperplasia (CAH) were excluded.

Methods: Urinary steroid profiling by gas chromatography-mass spectrometry (GC-MS) was used for longitudinal monitoring of the second pregnancy.

Results: During pregnancy estriol excretion failed to increase normally. The mother showed signs of virilization by the 23rd week of gestation. Urinary steroid analysis was not indicative of aromatase deficiency. However, excretion of the androgen metabolite androsterone increased rapidly at the beginning of pregnancy and peaked around the 20th week, suggesting increased production of testosterone and 5 α DHT. Urinary steroid analysis by GC-MS showed gradually increasing excretion of the normally minor metabolite 5 α -pregnane-3 β ,20 α -diol (epiallopregnanediol), an epimer of the dominant progesterone metabolite pregnanediol (5 β -pregnane-3 α ,20 α -diol). The male baby born of this pregnancy had normal genitalia but showed a urinary steroid profile indicating partial deficiencies of P450c17 and P450c21. However, no mutations in the corresponding CYP17 and CYP21 genes were identified. This disorder was caused by mutations in P450 oxidoreductase (OR), the essential redox partner for CYP17 and CYP21 hydroxylases.

Conclusion: The authors speculated that epiallopregnanediol was largely the maternal urinary excretion product of fetal 5-pregnene-3 β ,20 α -diol, the principal metabolite of pregnenolone, implying a build-up of the latter steroid in the fetal adrenal. These findings suggested that the 'block' in the estriol biosynthetic pathway occurred at an early stage with 17-hydroxylation of pregnenolone being affected.

The exciting story on P450 oxidoreductase deficiency – a new form of congenital adrenal hyperplasia – is moving on. This paper impressively demonstrates on the one hand that this disorder can already be detected non-invasively by steroid profiling of maternal urinary steroids. On the other hand the paper again confronts us with the unique diagnostic potential of GC-MS urinary steroid profiling [9]. So please add another item to the list of diseases which can be diagnosed antenatally by this method.

New mechanisms: in the search for missing links between phenotype and genotype

Decreased expression of ABCD4 and BG1 genes early in the pathogenesis of X-linked adrenoleukodystrophy

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Background: Childhood cerebral adrenoleukodystrophy, adrenomyeloneuropathy and adrenomyeloneuropathy with cerebral demyelination are the main phenotypic variants of X-linked adrenoleukodystrophy. The disease is caused by mutations in the ABCD1 gene which encodes a half-size peroxisomal transporter that has to dimerize to become functional. The leading biochemical characteristic of adrenoleukodystrophy is the accumulation of very-long chain fatty acids in plasma and tissues. However, there is no correlation between the adrenoleukodystrophy phenotype and the ABCD1 gene mutations or the accumulation of very long-chain fatty acids in plasma and fibroblasts from adrenoleukodystrophy patients.

Methods: To investigate the mechanisms underlying the phenotypic variability of adrenoleukodystrophy, the expression of ABCD1, three other peroxisomal transporter genes of the same family (ABCD2, ABCD3 and ABCD4) and two very long-chain fatty acids synthetase genes (VLCS and BG1) involved in very long-chain fatty acid metabolism, as well as the very long-chain fatty acid concentrations in the normal white matter from adrenoleukodystrophy patients with childhood cerebral adrenoleukodystrophy, adrenomyeloneuropathy with cerebral demyelination and adrenomyeloneuropathy phenotypes were studied.

Results and conclusions: The main findings of this study can be summarized as follows: (1) ABCD1 gene mutations leading to truncated adrenoleukodystrophy protein are unlikely to cause variation in the adrenoleukodystrophy phenotype; (2) accumulation of saturated very long-chain fatty acids in normal-appearing white matter correlates with adrenoleukodystrophy phenotype, and (3) expression of the ABCD4 and BG1, but not of the ABCD2, ABCD3 and VLCS genes, tends to be correlated with the severity of the disease, acting early in the pathogenesis of adrenoleukodystrophy.

Remember that X-linked adrenoleukodystrophy is a severe peroxisomal disorder. Its phenotypic presentation of identical mutations in the ABCD1 gene is highly variable, even within the same family. In the absence of a mouse model of adrenoleukodystrophy reproducing the phenotypic variability, the search for modifying genes remains challenging. The authors focused their investigations on two sets of genes: the family of peroxisomal ABC transporters and two very long-chain fatty acids synthetases. The authors found that the expression of ABCD4 and BG1 correlated with the severity of the disease. However, it was not clear whether the modifications in the expression of these genes result from metabolic changes or are primary determinants of the adrenoleukodystrophy phenotype. A further important finding was that the accumulation of saturated very long-chain fatty acids correlated with the adrenoleukodystrophy phenotype. The results suggest that the threshold of very long-chain fatty acids accumulation is probably essential for the initial demyelinating process.

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