Adrenals

Stefan A Wudy and Michaela F Hartmann

Steroid Research and Mass Spectrometry Unit, Division of Pediatric Endocrinology and Diabetology,
Center of Child and Adolescent Medicine, Justus Liebig University, Giessen, Germany

Preface

Recent years have seen a tremendous growth in international communication. The technical means of communication have undergone a major revolution. Scholars and scientists can communicate almost instantly via phone, fax and particularly by e-mail. Some of them have already chosen to post their scientific results on their websites instead of having to wait for months and sometimes years to have them published.

One unspoken or spoken motive of all of these developments is the expectation that the new technical improvements in communication should facilitate research and should bring international discussion of important issues in one's home country. Indeed, such an undertaking as the *Yearbook of Pediatric Endocrinology* would have been hardly possible without the instruments of modern communication. Without the 'net' we authors would have never achieved retrieving and selecting specific literature as well as communicating with each other over large distances in such a small amount of time.

This year's selection directs the readers' attention to new actions of old hormones such as DHEA and its sulfate or cortisol. Furthermore, the response of the adrenals to ACTH will be a major topic. Again, amongst important enzymes to be discussed, the enzyme 11 β -hydroxysteroid dehydrogenase – hope of many fighters of the global epidemic obesity – will be one of the main focuses within our chapter. More than in any other chapter before, we will learn from the steroid metabolism in animals: zebrafish, zebra finches and horses will cross our way. And last but not least, the readers will get a short update on the current role of stem cells in the prospective cure of adrenal disease.

This year's annual meeting of ESPE will be held in Helsinki, Finland's capital. In this context and in the light of the above-mentioned rapid developments of modern communication, it seems to be worthwhile remembering Tekla Hultin (1864–1943), the first female PhD in Finland who on November 11 1889 spoke on the question 'Does the growth of culture bring about happiness for humanity?'. Could she have known of the meanwhile established tradition of the *Yearbook of Pediatric Endocrinology*, I am pretty sure that she would doubtlessly have answered this question with 'yes'.

New concepts: unraveling the mechanisms of septic shock

Dissociation of serum dehydroepiandrosterone and dehydroepiandrosterone sulfate in septic shock

Arlt W, Hammer F, Sanning P, Butcher SK, Lord JM, Allolio B, Annane D, Stewart PM

Division of Medical Sciences, University of Birmingham, Institute of Biomedical Research, Birmingham, UK
w.arlt@bham.ac.uk

J Clin Endocrinol Metab 2006;91:2548–2554

Background: Dehydroepiandrosterone (DHEA) substitution in sepsis has been advocated because DHEA sulfate (DHEAS) decreases in sepsis. Experimental sepsis in rodents leads to downregulation of DHEA sulfotransferase, which inactivates DHEA to DHEAS, thus causing higher DHEA levels. This study was conducted to test whether serum DHEA and DHEAS are dissociated in septic shock.

Method: The study had a cross-sectional design with 181 patients with septic shock, 31 patients with acute trauma, and 60 healthy controls. Serum cortisol, DHEA, and DHEAS were measured before and 60 min after ACTH stimulation.

Results: Serum cortisol significantly increased and DHEAS significantly decreased in both septic shock and trauma patients. Compared with healthy controls, DHEA significantly increased in sepsis but decreased after trauma. In sepsis, neither cortisol nor DHEA increased significantly after ACTH. The cortisol to DHEA ratio was significantly increased in non-survivors of septic shock ($p = 0.026$).

Conclusion: The observed dissociation of DHEA and DHEAS in septic shock is not in accordance with the previous concept of sepsis-associated DHEA deficiency. Increased DHEA levels may maintain the balance between glucocorticoid- and DHEA-mediated immune and vascular effects. Most severe disease and mortality was found to be associated with an increased cortisol to DHEA ratio, which the authors suggest as a novel prognostic marker in septic shock.

Sepsis presents the most common cause of death in non-coronary intensive care unit patients. Adrenal insufficiency might play an important role within this context. The authors examined a large sample of septic shock patients and hypothesized that low circulating DHEAS in septic shock may not indicate true DHEA deficiency. Their results confirmed earlier findings of significantly lower serum DHEAS in septic shock patients. However, the authors found increased DHEA levels in septic patients, thus shattering the previous concept of DHEA deficiency. The upregulation of DHEA might be a sepsis-typical phenomenon which may aim at maintenance of the balance between glucocorticoid- and DHEA-mediated effects on the immune and vascular system. Furthermore, the authors suggest that the ratio cortisol/DHEA is a novel prognostic marker in septic shock, indicating an exhausted counterregulatory mechanism in the most critical patients. More studies are needed, in particular those investigating whether a combined treatment with hydrocortisone and DHEA would be superior to administration of hydrocortisone alone.

New hopes: obesity – a treatable disorder of steroid metabolism? Part I

Depot-specific modulation of rat intra-abdominal adipose tissue lipid metabolism by pharmacologic inhibition of 11 β -hydroxysteroid dehydrogenase type 1

Berthiaume M, Laplante M, Festuccia W, Gelinas Y, Poulin S, Lalonde J, Joanisse DR, Thieringer R, Deshaies Y
Laval Hospital Research Center and Department of Anatomy and Physiology, Faculty of Medicine, Laval University, Quebec, QC, Canada; Division of Kinesiology, Department of Social and Preventive Medicine, Faculty of Medicine, Laval University, Quebec, QC, Canada, and Department of External Scientific Affairs, Merck Research Laboratories, Rahway, N.J., USA

Endocrinology 2007;148:2391–2397 [Epub ahead of print]

Background: The metabolic consequences of visceral obesity have been correlated with amplification of glucocorticoid action by 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) in adipose tissue. The authors assessed in a rat model of diet-induced obesity the effects of pharmacologic 11 β -HSD1 inhibition on the morphology and expression of key genes of lipid metabolism in intra-abdominal adipose depots.

Method: Rats fed a high-sucrose, high-fat diet were treated or not with a specific 11 β -HSD1 inhibitor (Compound A) for 3 weeks.

Results: Compound A did not alter food intake or body weight gain, but specifically reduced mesenteric adipose weight and adipocyte size. In mesenteric fat, the inhibitor decreased mRNA levels of genes involved in lipid synthesis and fatty acid cycling, and increased the activity of the fatty acid oxidation-promoting enzyme CPT1. In striking contrast, in the epididymal depot, 11 β -HSD1 inhibition increased mRNA levels of those genes related to lipid synthesis/cycling and slightly decreased CPT1 activity, whereas gene expression remained unaffected in the retroperitoneal depot.

Conclusion: The authors found that pharmacologic inhibition of 11 β -HSD1, at a dose that does not alter food intake, fat accretion specifically in the mesenteric adipose depot was reduced and that divergent intra-abdominal depot-specific effects on genes of lipid metabolism were exerted reducing steatosis and lipemia.

The concept of 'a mini-Cushing's syndrome' has been suggested in the genesis of visceral obesity and metabolic syndrome [1]. Within this context the enzyme 11 β -HSD1 in adipose tissue seems to play a major role amplifying glucocorticoid action. Therefore, pharmacologic inhibition of 11 β -HSD1 seems to be a highly attractive therapeutic approach for the metabolic effects of visceral obesity. This study assesses the effects of a 3-week course of pharmacological inhibition of this key enzyme of cortisol metabolism on intra-abdominal adipose depot distribution as well as on the metabolic profile of rats. The authors found reduced fat accretion, decreased lipogenic gene expression, and increased oxidative enzyme activity in the mesenteric fat depot. This study in rats suggests pharmacologic 11 β -HSD1 inhibition an attractive approach in metabolic syndrome. However, we need more studies with respect to correct dosage as well as duration of such therapeutic agents. In particular, proper caution should be exerted because these drugs might also affect the brain and the central regulation of energy balance!

New paradigms: ACTH testing – a horse's perspective

The adrenocorticotropin stimulation test: contribution of a physiologically based model developed in horse for its interpretation in different pathophysiological situations encountered in man

Bousquet-Melou A, Formentini E, Picard-Hagen N, Delage L, Laroute V, Toutain PL

Unité Mixte de Recherche 181 de Physiopathologie et Toxicologie Expérimentales, Institut National de la Recherche Agronomique et Ecole Nationale Vétérinaire de Toulouse, Ecole Nationale Vétérinaire de Toulouse, Toulouse, France
a.bousquet-melou@envt.fr

Endocrinology 2006;147:4281–4291

Background: The authors characterized the adrenal response to ACTH in horses. They developed a model coupling the non-linear disposition of cortisol with a physiologically based model for cortisol secretion by the adrenals. They assumed that the response to ACTH resulted from two mechanisms: a stimulation of the cortisol secretion rate and control of the duration of the secretion.

Method: Seven doses of ACTH were tested in horses, a species similar to man with respect to adrenal function.

Results: The main finding was that the secretion rate of the adrenal gland could be described by a zero-order process that was maximal for a relatively low dose of ACTH (0.1 μ g/kg). At higher doses, the increasing adrenal gland response was only due to the prolongation of the time of its secretion.

Conclusion: The authors were able to reproduce and explain many adrenal gland responses that were dimmed by the different non-linearities of the system.

Did you know that horses are similar to humans? At least when it comes to comparing adrenal function this seems to be true. I do not know whether horses like maths, but the inclined reader of this paper should not be repelled by mathematical models. For all those not indulging in mathematical formulas since school any more, the essentials of this remarkable paper are that ACTH tests seem to be only able to explore concentration-dependent mechanisms of the adrenal response at very low ACTH doses. As soon as the dose exceeds 0.1 μ g/kg, the response reflects different time-dependent mechanisms. These results are in agreement with the fact that the adrenal glands do not accumulate a pool of releasable cortisol but instead only increase cortisol synthesis. I still suggest to carry out experiments transferring these findings to the human being and then to draw consequences for reasonable ACTH testing.

Mechanisms of disease: the adrenocorticotropin receptor and disease

Clark AJ, Metherell LA

Centre for Endocrinology, William Harvey Research Institute at Barts, London, UK

a.j.clark@qmul.ac.uk

Nat Clin Pract Endocrinol Metab 2006;2:282–290

Background: The action of ACTH to stimulate glucocorticoid production by the adrenals is an essential physiologic process. It depends on a single unique genetic component – the ACTH receptor or melanocortin-2 receptor. Genetic defects causing abnormalities in this receptor or in a protein required for its expression at the cell surface result in a potentially fatal disease (familial glucocorticoid deficiency). Overexpression of this receptor or inability to desensitize it can be seen in adrenal adenomas or hyperplasia associated with glucocorticoid overproduction (Cushing syndrome). Regarding depressive illness and septic shock, the origin of these latter disturbances is undoubtedly complex and multifactorial, but there is strong evidence that a component of this phenomenon is an altered responsiveness of the ACTH receptor to ACTH.

Please consider that (1) ACTH plays a key role in mediating the stress response, (2) inactivating mutations in the ACTH receptor cause the inherited syndrome of ACTH insensitivity, (3) benign and malignant adrenal neoplasms often bear disturbances of ACTH receptor expression, and (4) in common disorders such as depression or septic shock an altered responsiveness to ACTH is often present. This excellent review does not deserve to be further commented, no – to read this article is a must for every serious endocrinologist.

New concepts: cortisol – a growth hormone for the fetal heart?

Cortisol stimulates cell cycle activity in the cardiomyocyte of the sheep fetus

Giraud GD, Louey S, Jonker S, Schultz J, Thornburg KL

Heart Research Center, Oregon Health and Science University, Portland, Oreg., USA

Endocrinology 2006;147:3643–3649

Background: The authors hypothesized that cortisol would suppress cardiomyocyte proliferation and stimulate cardiomyocyte binucleation and enlargement, which are signs of terminal differentiation.

Method: Cardiomyocyte dimensions and percent binucleation were determined in isolated cardiac myocytes from 7 cortisol-treated and 7 control fetuses. Cortisol was infused into the circumflex coronary artery at subpressor rates.

Results: Cortisol infusion had no hemodynamic effects. It increased heart weight significantly. Heart to body weight ratio was greater in treated hearts. Ventricular myocyte length, width, and percent binucleation were not different between groups.

Conclusion: Increases in fetal heart mass associated with subpressor doses of cortisol are due to cardiomyocyte proliferation and not hypertrophic growth.

The authors present a somewhat unexpected and exciting finding that cortisol acts as a growth hormone in the ovine fetal heart, stimulating cardiac myocyte hyperplasia and not maturation. On the one hand, this paper presents a valuable contribution to understand the role of corticosteroids in cardiomyocyte maturation. On the other hand, this observation raises at least two major issues. First, it is still unknown by which receptor these effects are mediated. Second, we have to investigate more intensively possible implications for premature human neonates receiving exogenous glucocorticoids before birth! [2]

In humans, early cortisol biosynthesis provides a mechanism to safeguard female sexual development

Goto M, Piper Hanley K, Marcos J, Wood PJ, Wright S, Postle AD, Cameron IT, Mason JI, Wilson DI, Hanley NA
Human Genetics Division and Early Human Development and Stem Cells Group, University of Southampton,
Southampton, UK

J Clin Invest 2006;116:953–960

Background: Differentiation of the human external genitalia is established at 7–12 weeks post-conception. During this period, maintaining the appropriate intrauterine hormone environment is critical. This regulation extends to the human fetal adrenal cortex, as evidenced by the virilization that is associated with various forms of congenital adrenal hyperplasia. The mechanism underlying these clinical findings has remained unknown.

Method: The authors investigated the expression of the orphan nuclear receptor nerve growth factor IB-like (NGFI-B) and its regulatory target, the steroidogenic enzyme 3 β -hydroxysteroid dehydrogenase type 2 (HSD3B2).

Results: Cortisol biosynthesis was maximal at 8–9 weeks post-conception under the regulation of ACTH. Negative feedback was apparent at the anterior pituitary corticotrophs. ACTH also stimulated the adrenal gland to secrete androstenedione and testosterone.

Conclusion: These data show a distinctive mechanism for normal human development whereby cortisol production, determined by transient NGFI-B and HSD3B2 expression, provides feedback at the anterior pituitary to modulate androgen biosynthesis and safeguard normal female sexual differentiation.

This is a very interesting contribution to understand the role of the fetal adrenal cortex and its capacity of cortisol and androgen biosynthesis during the first trimester of human development. The authors suggest a delicate balance during early female differentiation which is vulnerable to androgen before the protective appearance of placental aromatase. A transient expression of adrenocortical nerve growth factor IB-like inducing 3 β -hydroxysteroid dehydrogenase type 2 would cause early cortisol biosynthesis and thus inhibit ACTH production by the anterior pituitary. This would minimize ACTH-induced androgen secretion and thus cause a transient mechanism safeguarding the most sensitive period of female sexual development. To conclude, becoming a female seems by no means to be as passive a process than has previously been thought.

New concerns: your daily dose of DHEA – a critical reflection

Dehydroepiandrosterone is an anabolic steroid like dihydrotestosterone, the most potent natural androgen, and tetrahydrogestrinone

Labrie F, Luu-The V, Martel C, Chernomoretz A, Calvo E, Morissette J, Labrie C
Molecular Endocrinology and Oncology Laboratory, Laval University Hospital Research Center (CRCHUL) and
Laval University, Quebec City, Que., Canada
fernand.labrie@crchul.ulaval.ca

J Steroid Biochem Mol Biol 2006;100:52–58

Background: In 2004, the US Controlled Substances Act was modified to include androstenedione (4-dione) as an anabolic steroid. However, despite the common knowledge that dehydroepiandrosterone (DHEA) is the precursor of testosterone, DHEA was excluded from the list of anabolic steroids.

Method: The authors used DNA microarray technology to analyze the expression profile of practically all the 30,000 genes of the mouse genome modulated by DHEA and dihydrotestosterone (DHT) in classical androgen-sensitive tissues.

Results: Daily subcutaneous injections of DHT or DHEA for 1 month in gonadectomized mice increased ventral prostate, dorsal prostate, seminal vesicle and preputial gland weight. As early as 24 h after a single

injection of the two steroids, 878, 2,681 and 14 probe sets were commonly stimulated or inhibited in the prostate, seminal vesicles and preputial glands, respectively, compared to tissues from gonadectomized control animals.

Conclusion: The present microarray data show proof of the androgenic/anabolic activity of DHEA. The data reveal that DHEA is transformed into androgens in the human peripheral tissues as well as in laboratory animal species exerting potent androgenic/anabolic activity.

DHEA [3] has so far not been listed by the World Anti-Doping Agency as an anabolic steroid. In many countries the substance is regarded as an anti-aging drug and is available over the counter. Using gene expression profiling, the authors have convincingly shown that DHEA is an anabolic steroid like DHT. Another interesting aspect of this paper is that of 'intracrinology'. While ovaries and testes are the exclusive sources of androgens and estrogens in lower mammals, in man and higher primates, sex steroids are largely synthesized locally in peripheral tissues. DHEA, at physiological concentrations, induces high levels of intraprostatic DHT resulting in marked stimulation of ventral prostate weight and increased expression of androgen-sensitive genes. Therefore the question arises whether the measurement of serum androgens reliably reflects biological and clinical phenomena!

New mechanisms: singing – a function of steroids? Music lessons by the zebra finch

Widespread capacity for steroid synthesis in the avian brain and song system

London SE, Monks DA, Wade J, Schlinger BA
University of California, Los Angeles, Los Angeles, Calif., USA
slondon@igb.uiuc.edu
Endocrinology 2006;147:5975–5987

Background: The zebra finch song system presents a sensorimotor neural circuit sensitive to steroids throughout life. It organizes and functions largely independent of gonadally derived steroids.

Method and Results: The authors demonstrated that the steroidogenic acute regulatory protein (StAR), cytochrome P₄₅₀ side-chain cleavage (CYP11A1), and 3 β -hydroxysteroid dehydrogenase/ Δ 5- Δ 4 isomerase are expressed in both developing and adult zebra finch brain.

Conclusion: The data suggest neurosteroids may modulate multiple brain functions, including sensory and motor systems. Notably, whereas expression of other steroidogenic genes such as aromatase has been essentially absent from the song system, each of the major song nuclei express at least a subset of steroidogenic genes described here, establishing the song system as a potential steroidogenic circuit.

The zebra finch song system is steroid-sensitive at all ages, but its incomplete dependence on gonadally derived steroids suggests that the brain may provide steroids essential to its organization and function. Thus, neurosteroidogenesis may broaden the role of steroids in the brain [4]. By uncoupling the synthesis of sex steroids from gonads, the role of sex steroids may be expanded in the brains beyond those primarily useful for reproductive behaviors. From the example of the zebra finch, we learn that neurosteroidogenesis may be a crucial factor by which the brain regulates sensory and motor functions.

Important for clinical practice: the correlation between body and psyche in CAH

Gender development in women with congenital adrenal hyperplasia as a function of disorder severity

Meyer-Bahlburg HF, Dolezal C, Baker SW, Ehrhardt AA, New MI
NYS Psychiatric Institute/Department of Psychiatry, Columbia University, NYSPI Unit 15, New York, N.Y., USA
meyerb@childpsych.columbia.edu
Arch Sex Behav 2006;35:667–684

Background: Prenatal-onset CAH in 46,XX individuals is associated with variable masculinization/defeminization of the genitalia and of behavior. This is presumably both due to excess prenatal androgen production. The purpose of this study was (1) to extend the gender-behavioral investigation to the mildest subtype of 46,XX CAH, the non-classical variant, (2) to replicate previous findings on moderate and severe variants of 46,XX CAH using a battery of diversely constructed assessment instruments, and (3) to evaluate the utility of the chosen assessment instruments for this area of work.

Method: 63 women with classical CAH were studied (42 had salt wasting and 21 were simple virilizers), 82 women had the non-classical type, and 24 related non-CAH sisters and female cousins served as controls.

Results: Non-classical women showed a few signs of gender shifts in the expected direction, simple virilizing women were intermediate, and salt wasting women were found to be most severely affected. Regarding gender identity, two salt wasting women were gender-dysphoric, and a third had changed to male in adulthood. All others identified as women.

Conclusion: Behavioral masculinization/defeminization was pronounced in salt wasting-CAH women, slight but still clearly present in simple virilizing women, and probable, but still in need of replication in non-classical women.

The clinical spectrum of patients with 21-hydroxylase deficiency extends from the mildest 'non-classical' forms to the severer classical forms (simple virilizers, salt wasters). This study for the first time investigates gender outcome in a relatively large sample of women with non-classical 21-hydroxylase deficiency in comparison to the classical forms of 21-hydroxylase deficiency and normal controls. Behavioral masculinization/defeminization correlated with the severity of 21-hydroxylase deficiency: it was pronounced in women with a salt wasting form, slight but clearly demonstrable in simple virilizing women, but questionable in non-classical women. This study is a valuable contribution to a neglected field of research. However, the assessment instruments in this study clearly had their limitations: they were not well suited as measures of discrete behaviors that could be compared to those behavioral units used by animal researches in the investigation of hormone-, dose-, and timing-specific hormone behavior relationships. The authors furthermore point out further hindrances of such a study such as a relatively small sample size, high rates of non-participation and a cross-sectional design. This reviewer wonders whether a further study could be conducted on an ESPE-wide basis?

Important for clinical practice: StAR's mechanism, a lesson in endocrine astronomy

Mechanism of StAR's regulation of mitochondrial cholesterol import

Miller WL
Department of Pediatrics, University of California, San Francisco, Calif., USA
Mol Cell Endocrinol 2007;265–266:46–50

The steroidogenic acute regulatory protein (StAR) regulates the acute steroidogenic response by moving cholesterol from the outer to inner mitochondrial membrane. However, the mechanism of StAR's action has remained enigmatic. The authors showed that StAR acts on the outer membrane, needs cholesterol binding, and requires the structural change previously described as a pH-dependent molten globule.

The current concept is that StAR's interaction with protonated phospholipid head groups on the outer mitochondrial membrane leads to a molten globule transition needed for StAR to take up cholesterol. A functional interaction between StAR and the peripheral benzodiazepine receptor is suggested. Whereas many models have revealed that StAR delivers cholesterol to peripheral benzodiazepine receptor, the authors suggest that StAR removes cholesterol from the cholesterol-binding domain of peripheral benzodiazepine receptor and delivers it to the inner mitochondrial membrane.

This excellent review is not only for all of those indulging in endocrine mechanisms! It should be a pleasure for all real endocrinologists to read this excellent update on the molecular mechanisms concerning steroidogenic acute regulatory protein (StAR). May the authors remind our readers of last year's *Yearbook of Pediatric Endocrinology*, where we have already dwelled on this highly interesting 'two-hit' disease [5]?

Important for clinical practice: pheochromocytoma update

Pheochromocytoma: recommendations for clinical practice from the First International Symposium

Pacak K, Eisenhofer G, Ahlman H, Bornstein SR, Gimenez-Roqueplo AP, Grossman AB, Kimura N, Mannelli M, McNicol AM, Tischler AS

National Institute of Child Health and Development, NIH, Bethesda, Md., USA

Nat Clin Pract Endocrinol Metab 2007;3:92–102

The First International Symposium on Pheochromocytoma was held in October 2005. Recommendations were made concerning biochemical diagnosis, localization, genetics, and treatment. Measurement of plasma or urinary fractionated metanephrines, the most accurate screening approach, was recommended as the first-line test for diagnosis. Localization studies should only follow reasonable clinical evidence of a tumor. Preoperative pharmacologic blockade of circulatory responses to catecholamines is mandatory. Mutation testing should be considered; however, it is not currently cost-effective to test every gene in every patient. Inadequate methods to distinguish malignant from benign tumors and a lack of effective treatments for malignancy are important problems requiring further studies.

This article reminds us of the fact that the adrenals are not exclusively composed of steroidogenic tissue. The paper is devoted to the adrenal medulla and summarizes the latest recommendations of the First International Symposium on Pheochromocytoma held in 2005. It is an excellent clinical help regarding the latest updates on diagnosis, localization, genetics and treatment of pheochromocytomas – a 'must' for the clinical endocrinologist's library!

Food for thought: diabetes – a disease not only affecting insulin!

Exaggerated adrenarche and altered cortisol metabolism in type 1 diabetic children

Remer T, Maser-Gluth C, Boye KR, Hartmann MF, Heinze E, Wudy SA

Department of Nutrition and Health, Research Institute of Child Nutrition, Dortmund, Germany

remer@fke-do.de

Steroids 2006;71:591–598

Background: A few data from the literature suggest altered steroid metabolism in type 1 diabetes. The aim of this study was to test the hypothesis that adrenarche is affected under conventional intensive insulin therapy.

Method: In 24-hour urine samples of 109 patients aged 4–18 years with type 1 diabetes of more than 1 year, steroids were profiled using gas chromatography-mass spectrometry. Additionally, urinary free cortisol and cortisone were quantified by RIA after extraction and chromatographic purification. Data on urinary steroids from 400 healthy controls served as reference values. Enzyme activities were assessed by established steroid metabolite ratios, e.g. 5 α -reductase and 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2) by 5 α -tetrahydrocortisol/tetrahydrocortisol and urinary free cortisol/cortisone, respectively. Urinary markers of adrenarche, especially dehydroepiandrosterone and its direct metabolites, were elevated in patients, as were urinary 6 β -hydroxycortisol, urinary free cortisol, and 11 β -HSD2 activity.

Results: Overall cortisol secretion, reflected by the sum of major urinary cortisol metabolites, was mostly normal and activity of 5 α -reductase clearly reduced.

Conclusion: The authors found evidence for an exaggerated adrenarche in type 1 diabetes children, which may account for hyperandrogenic symptoms in diabetic females. Furthermore, a reduced cortisol inactivation via 5 α -reductase that was not compensated by a fall in cortisol secretion was found.

This paper draws our attention on the consequences of chronic hyperglycemia on steroid metabolism, a topic rarely touched upon. The authors provide evidence for a potentially clinically relevant alteration in cortisol metabolism with a markedly increased 11 β -HSD2 activity and an exaggerated adrenarche [6] in type 1 diabetic children and adolescents. Obviously, insulin can increase the metabolic clearance of DHEA and its sulfate while DHEA-S secretion is likely to be increased. Elevated excretion rates of 6 β -hydroxycortisol and cortisone might point to a mild form of hypercortisolism and to a potential role of steroids in the induction of insulin resistance.

New mechanisms: development of pituitary-interrenal interaction – lessons from the zebrafish

Pituitary-interrenal interaction in zebrafish interrenal organ development

To TT, Hahner S, Nica G, Rohr KB, Hammerschmidt M, Winkler C, Allolio B
Endocrinology and Diabetes Unit, Department of Medicine, University of Wuerzburg, Wuerzburg, Germany
Allolio_b@medizin.uni-wuerzburg.de
Mol Endocrinol 2007;21:472–485

Background: The authors elucidated pituitary adrenal interactions during development by studying the organogenesis of the interrenal organ, which is the teleost homolog of the mammalian adrenal gland in zebrafish.

Method: Wild-type zebrafish interrenal development was compared with that of mutants lacking pituitary cell types including corticotrophs.

Results: Until 2 days post-fertilization (2 dpf), interrenal development assessed by transcripts of key steroidogenic genes (*cyp11a1*, *mc2r*, *star*) was found to be independent of proopiomelanocortin. However, at 5 dpf, lack of pituitary cells leads to reduced expression of steroidogenic genes at both the transcriptional and the protein level.

Conclusion: The authors demonstrated a gradual transition from early pituitary-independent interrenal organogenesis to developmental control by the anterior domain of pituitary corticotrophs acting via Mc2 receptors.

The inclined reader will again note the importance of animal models in steroid research. It actually seems that this year's selection of papers is more or less devoted to animals helping to understand steroid metabolism and its regulation. While the zebra finch has nicely demonstrated the importance of – gonadally indifferent – neurosteroids, the zebrafish interrenal glands seem to be a suitable model for studying adrenal development. Indeed the authors demonstrate that interrenal development in the zebrafish shares many conserved molecular and developmental mechanisms with higher vertebrates, in particular when it comes to studying transcription factors involved in adrenal development. In this paper we learn that it is the Mc2 receptor which after an early phase of pituitary independent development and steroidogenic enzyme activity is needed for further functional differentiation of the interrenal organ.

Inhibition of 11 β -HSD1 activity in vivo limits glucocorticoid exposure to human adipose tissue and decreases lipolysis

Tomlinson JW, Sherlock M, Hughes B, Hughes SV, Kilvington F, Bartlett W, Courtney R, Rejto P, Carley W, Stewart PM
Division of Medical Sciences, Institute of Biomedical Research, University of Birmingham, Queen Elizabeth Hospital, Edgbaston, Birmingham, UK; Department of Clinical Chemistry and Immunology, Heart of England NHS Foundation Trust, Birmingham, UK, and Pfizer Global R&D, La Jolla Laboratories, San Diego, Calif., USA

J Clin Endocrinol Metab 2007;92:857–864

Background: The authors aimed at comparing markers of 11 β -HSD1 activity and demonstrating that inhibition of 11 β -HSD1 activity limits glucocorticoid availability to adipose tissue.

Methods: Seven healthy male volunteers participated in this clinical study. Carbenoxolone was used in a single dose (100 mg) and the probands received 72 h of continuous treatment (300 mg/day). Inhibition of 11 β -HSD1 was examined using five different mechanistic biomarkers (serum cortisol and prednisolone generation, urinary corticosteroid metabolite analysis by gas chromatography/mass spectrometry, and adipose tissue microdialysis examining cortisol generation and glucocorticoid-mediated glycerol release).

Results: Each biomarker demonstrated reduced 11 β -HSD1 activity after CBX administration.

Conclusion: Carbenoxolone is able to inhibit rapidly the generation of active GC in human adipose tissue. Limiting glucocorticoid availability in vivo has functional consequences including decreased glycerol release.

Carbenoxolone – a derivative of glycyrrhetic acid, the active principal of licorice – is an inhibitor of 11 β -hydroxysteroid dehydrogenase type 1 and type 2. Clinical studies in patients with type 2 diabetes have shown improvement of insulin sensitivity and decreased glucose production rates. In this paper the authors show in healthy adult males that carbenoxolone is able to access adipose tissue and to inhibit the generation of bioactive cortisol and prednisolone through inhibition of 11 β -hydroxysteroid dehydrogenase type 1. This observation has important implications for selective inhibition of 11 β -hydroxysteroid dehydrogenase type 1 as a therapeutic strategy in humans. However, carbenoxolone is not a selective inhibitor of both type 1 and type 2 11 β -hydroxysteroid dehydrogenases and therefore bears the risk of inducing apparent mineralocorticoid excess, as all chronic users of licorice will know. Consequently, this substance will not be suited as a future therapeutic drug.

New hope: a further step to eternal life? Stem cell research in steroidology

Differentiation of adult stem cells derived from bone marrow stroma into Leydig or adrenocortical cells

Yazawa T, Mizutani T, Yamada K, Kawata H, Sekiguchi T, Yoshino M, Kajitani T, Shou Z, Umezawa A, Miyamoto K
Department of Biochemistry, Faculty of Medical Sciences, University of Fukui, Fukui, Japan

Endocrinology 2006;147:4104–4111

Background: The authors investigated whether MSCs from rat, mouse, and human are able to differentiate into steroidogenic cells.

Method and Results: It was found that when transplanted into immature rat testes, adherent marrow-derived cells were found to be engrafted and differentiated into steroidogenic cells that were indistinguishable from Leydig cells. Isolated murine marrow-derived cells transfected with green fluorescence protein driven by the promoter of P₄₅₀ side-chain cleaving enzyme gene (CYP11A), a steroidogenic cell-specific gene, were used to examine steroidogenic cell production in vitro. During in vitro differentiation, green fluorescence protein-positive cells similar to Leydig cells, were found. Stable transfection of murine marrow-derived cells with a transcription factor, steroidogenic factor-1, followed by treatment with cAMP almost recapitulated the properties of Leydig cells. Transfection of human marrow-derived cells

with steroidogenic factor-1 also led to their conversion to steroidogenic cells, but they appeared to be glucocorticoid rather than testosterone-producing cells.

Conclusion: These results indicate that marrow-derived cells represent a useful source of stem cells for producing steroidogenic cells that may provide basis for their use in cell and gene therapy.

Stem cells are self-renewing elements. They have the capacity to generate multiple distinct cell lineages. Even in adults they exist in various tissues and have been isolated from a variety of differentiated tissues such as bone marrow, umbilical blood, brain and fat, while bone marrow-derived mesenchymal stem cells have been shown to differentiate into adipocytes chondrocytes and osteoblasts both in vivo and ex vivo. The authors in this paper show that rodent mesenchymal stem cells have the potential to differentiate into steroidogenic cells in vivo and in vitro. Thus, mesenchymal stem cells represent a powerful tool not only for studying the differentiation of the steroidogenic lineage but also for future therapeutic approaches concerning steroidogenic organs. Let's look forward to the future!

Closing remark

Of course, phone, fax and e-mail have tremendously helped in writing another yearbook chapter. But do we only profit from these developments in communication or is there a slight danger that one day there will be no more 'real' ESPE meetings but only 'virtual' ESPE conferences? Indeed the unresolved question seems to be whether technical means can serve the same function as face-to-face personal interaction? Therefore we will have to show – more than ever before – that personal relationships and personal contacts within ESPE are of continued importance. Let's all look forward to meeting personally in Helsinki again!

It has now been for 4 years that these 'first-generation' authors (S.A.W. and M.H.) have had the privilege of compiling the Adrenals' chapter in the *Yearbook of Pediatric Endocrinology*. Despite this huge workload, the authors have always enjoyed this task because it permitted them the opportunity to get an excellent overview over current research and developments in the field of steroidology. We hope that our readers have enjoyed and profited from the selections as well. To maintain a balance in selecting and commenting the literature it is now time for a change, and we wish our successors all the best with this highly challenging and interesting task.

References

1. Wolf G: Glucocorticoids in adipocytes stimulate visceral obesity. *Nutr Rev* 2002;60:148–151.
2. Newnham JP: Is prenatal glucocorticoid administration another origin of adult disease? *Clin Exp Pharmacol Physiol* 2001;28:957–961.
3. Labrie F, Luu-The V, Belanger A, Lin SX, Simard J, Labrie C: Is DHEA a hormone? Starling review. *J Endocrinol* 2005;187:169–196.
4. Mellon SH, Griffin LD: Neurosteroids: biochemistry and clinical significance. *Trends Endocrinol Metab* 2002;13:35–43.
5. Wudy SA, Hartmann MF: Adrenals; in Carel JC, Hochberg Z (eds): *Yearbook of Pediatric Endocrinology* 2006. Basel, Karger, 2006, pp 103–115.
6. Remer T, Boye KR, Hartmann MF, Wudy SA: Urinary markers of adrenarche: reference values in healthy subjects aged 3–18 years. *J Clin Endocrinol Metab* 2005;90:2015–2021.