Adrenals

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For this year's chapter on 'Adrenals', we have searched the PubMed for articles on 'adrenal' and 'steroidogenesis' published in English between June 1, 2010 and May 31, 2011. Our search yielded more than 5,000 citations. We have examined all citations individually and selected the following collection of basic research and clinical articles. Whenever possible, we have avoided topics that have been discussed in the *Yearbook 2010*, unless progress in the field has been incremental. Emerging themes for this year's chapter include the role of K⁺ channel mutations in aldosterone production and hereditary hypertension, novel mechanisms that determine tissue sensitivity to glucocorticoids, the implications of the glucocorticoid receptor- β expression for metabolism, the role of microRNAs in the diagnosis and treatment of adrenal cancer and recent guidelines for the management of congenital adrenal hyperplasia.

Mechanism of the year

K⁺ channel mutations are a cause of hereditary hypertension

K⁺ channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension

Choi M, Scholl UI, Yue P, Björklund P, Zhao B, Nelson-Williams C, Ji W, Cho Y, Patel A, Men CJ, Lolis E, Wisgerhof MV, Geller DS, Mane S, Hellman P, Westin G, Åkerström G, Wang W, Carling T, Lifton RP
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Science 2011;331:768–72.

Background: Aldosterone-producing adrenal adenomas (APAs) are a cause of severe hypertension and feature constitutive hormone production and unrestrained cell proliferation.

Methods and Results: 22 patients with APAs were studied and two recurrent somatic mutations in and near the selectivity filter of the potassium (K⁺) channel KCNJ5 were identified in 8 of them. Functional studies demonstrated that both mutations resulted in increased sodium (Na⁺) conductance and cell depolarization, which in adrenal glomerulosa cells produces calcium (Ca²⁺) entry, the signal for aldosterone production and cell proliferation. A third KCNJ5 mutation that produces increased Na⁺ conductance was identified in a mendelian form of severe aldosteronism and massive bilateral adrenal hyperplasia.

Conclusions: These findings delineate the pathogenesis in a subset of patients with severe hypertension and implicate loss of K⁺ channel selectivity in constitutive cell proliferation and hormone production.

Aldosterone is synthesized by the adrenal glomerulosa in response to intravascular volume depletion and hyperkalemia. Volume depletion activates the renin-angiotensin system, leading to the secretion of angiotensin II (All), which signals via its G-protein-coupled receptor (GPCR) in glomerulosa cells. The resting membrane potential is set by K⁺ channel activity [1]. Both All signaling and hyperkalemia cause membrane depolarization and activation of voltage-gated Ca²⁺ channels. Increased intracellular Ca²⁺ provides the normal signal for aldosterone production, and sustained increases lead to glomerulosa cell proliferation. In primary hyperaldosteronism, the adrenal gland constitutively produces aldosterone in the absence of All or hyperkalemia, resulting in hypertension and variable hypokalemia. In this article, Choi et al. report two different mutations in the gene encoding the potassium channel KCNJ5 in 8 out of 22 patients with an APA. A third mutation in the same gene was also identified in

a father and his two daughters with florid adrenal hyperplasia, a hereditary condition that is treated by adrenalectomy in early childhood. These findings provide a better understanding of adrenal physiology and pathology, and implicate inherited and acquired mutations in *KCNJ5* in primary aldosteronism associated with autonomous cell proliferation. They also demonstrate a role for ion channel mutations in neoplasia. Primary hyperaldosteronism accounts for ~10% of patients referred for evaluation of hypertension, while APA accounts for approximately one third of these cases. Patients with primary hyperaldosteronism have a considerably higher incidence of cardiovascular disease than age-, sex- and blood pressure-matched patients with 'essential' hypertension [2]. The identification of mutations in the *KCNJ5* gene elucidates further the etiology of primary hyperaldosteronism and contributes significantly towards its detection and management.

New paradigms

Implications of the expression of GR β and hypomethylation of POMC and GR genes in metabolism

Discovery of glucocorticoid receptor- β in mice with a role in metabolism

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Mol Endocrinol 2010;24:1715–27.

Background: Glucocorticoids regulate several physiologic functions essential for life and exert their actions via the glucocorticoid receptor (GR). In humans (h), alternative splicing of the GR gene in exon 9 generates two highly homologous receptor isoforms, termed α and β . The hGR α represents the classic hGR that mediates the actions of glucocorticoids, while hGR β acts as a dominant negative inhibitor of hGR α , leading to glucocorticoid resistance. This study investigated the expression of GR β mRNA and protein in the mouse (m).

Methods and Results: The mGR β isoform arises from a distinct alternative splicing mechanism utilizing intron 8, rather than exon 9, as in humans. The splicing event produces a β isoform that is similar in structure and functionality to hGR β . The mGR β isoform cannot bind the glucocorticoid agonist, dexamethasone, inhibits the transcriptional activity of mGR α , and is upregulated by inflammatory signals. These properties are the same as those reported for the hGR β . In addition, novel data are presented that indicate that mGR β plays a role in metabolism.

Conclusions: This study provides the rodent model of GR β for delineating the role of this GR isoform in health and disease.

The human glucocorticoid receptor (hGR) is expressed as two major isoforms, hGR α and hGR β [3]. The hGR α represents the classic hGR that functions as a ligand-dependent transcription factor and mediates the actions of glucocorticoids. On the other hand, hGR β does not bind to glucocorticoids and inhibits the transcriptional activity of hGR α in a dose-dependent manner, leading to glucocorticoid resistance [4]. Indeed, increased expression of hGR β has been documented in severe asthma, leukemia, ulcerative colitis and systemic lupus erythematosus, and has been associated with increased activation of proinflammatory transcription factors and cytokines, and poor response to glucocorticoid treatment [5]. These observations suggest an important role for GR β as a homeostatic mechanism in the normal attenuation of glucocorticoid responses, and as a possible culprit in hormone-resistant disease states. In this study, Hinds et al. report the expression of GR β mRNA and protein in the mouse. They demonstrate that the mGR β shares several structural and functional similarities to the hGR β . In addition, the mGR β is involved in metabolic processes. Glucocorticoid induction of the gluconeogenic enzymes PDK4 and G6Pase was inhibited by mGR β , suggesting that one function of the GR β isoform, like insulin, is to block glucose production. In cells treated with insulin, mGR α expression was unchanged, but mGR β increased substantially. In cells treated with dexamethasone, mGR β was also increased, suggesting that both glucocorticoids and insulin share upregulation of mGR β as a common mechanism for antagonism of mGR α . Similarly, in mice subjected to fasting refeeding (which is known to produce a robust stimulation of hepatic metabolism by insulin), a large

increase of GR β was seen in the liver, whereas mGR α was once again unchanged. These data provide the first evidence that GR β upregulation may be an important mechanism for maintaining organ sensitivity to insulin. In addition, this work provides the much-needed rodent model of GR β for delineating the role of this GR isoform in health and disease.

Epigenetic changes in the hypothalamic proopiomelanocortin and glucocorticoid receptor genes in the ovine fetus after periconceptual undernutrition

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Endocrinology 2010;151:3652–64.

Background: Maternal undernutrition impacts on fetal development and leads to obesity in the offspring. This study examined how maternal undernutrition in sheep affects the fetal hypothalamic glucocorticoid receptor (GR) and the appetite-regulating neuropeptides, proopiomelanocortin (POMC) and neuropeptide Y (NPY).

Methods and Results: In fetuses from ewes undernourished from –60 to +30 days around conception, there was increased histone H3K9 acetylation (1.63-fold) and marked hypomethylation (62% decrease) of the POMC gene promoter but no change in POMC expression. In addition, there was increased acetylation of histone H3K9 associated with the hypothalamic GR gene (1.60-fold), marked hypomethylation of the GR gene promoter region (53% decrease) and an increase in hypothalamic GR expression (4.7-fold). Hypomethylation of both the GR and POMC promoter markers in fetal hypothalami was also identified after maternal undernutrition from –60 to 0 days and –2 to +30 days.

Conclusions: Periconceptual undernutrition is associated with marked epigenetic changes in hypothalamic GR and POMC genes. The increased GR expression in the undernourished group may contribute to fetal programming towards a predisposition to obesity, via altered GR regulation of POMC and NPY. These epigenetic changes may also predispose the offspring to altered regulation of food intake, energy expenditure and glucose homeostasis later in life.

In the last decades there is a marked rise in the incidence of metabolic syndrome, which affects up to 25% of the population in the United States [6]. At the core of the problem is the association between obesity and insulin resistance, which may lead to atherosclerotic cardiovascular disease and diabetes [7]. Maternal undernutrition is known to have a significant impact on fetal development, leading to obesity in adulthood [8]. In this study, Stevens et al. examined whether epigenetic changes in POMC and GR were present in the hypothalami of late gestation fetuses from undernourished mothers in sheep, a paradigm chosen because of the similarity with the human fetal hypothalamic-pituitary-adrenal axis and placental function. The authors identified important epigenetic changes in POMC and GR genes in the fetal hypothalamus after periconceptual maternal undernutrition. These findings suggest that epigenetic changes could act as a programming mechanism for the hypothalamic POMC and GR genes and predispose hypothalamic feeding centers to abnormal regulation later in life. Indeed, this was confirmed by further studies that demonstrated that the 10-month-old offspring from ewes undernourished using the same protocol had abnormal glucose tolerance and increased body weight [9]. It appears that an early nutritional insult alters the development of fetal hypothalamic appetite regulation centers to increase postnatal survival, provided that undernutrition continues postnatally. However, if such changes persist postnatally, when food is abundant, a defect in normal appetite regulation may subsequently lead to hyperphagia and obesity. This may explain several findings that animals undernourished prenatally are hyperphagic when given hypercaloric or high-fat diet postnatally, compared with control animals. Therefore, early life epigenetic regulation of these neuropeptides by maternal nutritional status may have implications for the glucose homeostasis, food intake and energy balance of the adult offspring.

Differential expression of microRNA-675, microRNA-139-3p and microRNA-335 in benign and malignant adrenocortical tumors

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J Clin Pathol 2011;64:529–35.

Background: The differential diagnosis between adrenocortical adenomas (ACAs) and adrenocortical carcinomas (ACCs) is crucial for the clinical management of patients with these lesions. Histomorphologically based scoring systems do not allow precise separation between ACAs and ACCs, and are not sufficient to prove malignant behavior. This study investigated whether novel markers, such as microRNAs (miRNAs), allow a more reliable separation of benign from malignant adrenocortical lesions.

Methods: In order to elucidate the diagnostic impact of miRNA expression in adrenocortical neoplasms, a cohort of 20 adrenocortical specimens, including normal adrenal tissue (n = 4), ACAs (n = 9), ACCs (n = 4) and metastases (n = 3), was analyzed using TaqMan low-density arrays to identify specific miRNA profiles that might distinguish between benign and malignant adrenocortical lesions. Results were validated in a validation cohort (n = 16).

Results: Concerning the differential diagnosis of ACAs and ACCs, 159 out of 667 miRNAs were up- and 89 were downregulated in ACAs. Real-time PCR analysis of three of the most significantly expressed single key miRNAs allowed separation of ACAs from ACCs. ACCs exhibited significantly lower levels of miR-139-3p (up to 8.49-fold, $p < 0.001$), miR-675 (up to 23.25-fold, $p < 0.001$) and miR-335 (up to 5.25-fold, $p < 0.001$). A validation cohort of 16 specimens with known Weiss score showed upregulation of miR-335 and miR-675 in the majority of cases with probable malignant course, although overlapping values were noted.

Conclusions: miRNA profiling of miR-675 and miR-335 helps discriminate ACCs from ACAs, and may indicate malignant behavior in cases with indeterminate malignant potential.

MicroRNAs (miRNAs) are a class of recently discovered small RNA molecules that regulate the expression of genes at the translational level [10]. MiRNAs are endogenously expressed short non-coding RNAs with 18–25 nucleotides in length capable of repressing protein translation through binding to target messenger RNA (mRNA). Recent studies indicate that miRNA-mediated gene regulation is likely to play a key role in human development, cellular differentiation and oncogenesis, and that evaluation of miRNA expression levels may substantially contribute to the understanding of cancer development, progression and treatment [11]. In order to investigate the miRNA expression profiles in adrenal tissue, the authors performed miRNA analyses on a series of normal, benign and malignant adrenocortical tissues to analyze 667 human miRNAs, followed by real-time PCR-based validation of miRNA expression of single key miRNAs with potential diagnostic value in discriminating ACC from ACA. In a third step, they compared miRNA expression levels of key miRNAs with the Weiss score in a validation cohort. They showed that up to 146 miRNAs were differentially expressed at statistically significant level in the various subgroups analyzed. Furthermore, up to 159 miRNAs were upregulated and up to 89 were downregulated in ACCs compared to ACAs. Of those, miR-335 and miR-675 showed a potential diagnostic value in predicting the biological behavior of adrenocortical tumors. This study confirms the power of molecular profiling as a novel diagnostic tool for the diagnosis of adrenocortical tumors. Using proposed cut-off values, the expression analysis of miR-675 and miR-335 seems to offer highly valuable information about the potential subsequent malignant behavior of lesions with indeterminate malignancy.

Poor sleep and altered hypothalamic-pituitary-adrenocortical and sympatho-adrenal-medullary system activity in children

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J Clin Endocrinol Metab 2010;95:2254–61.

Background: Poor sleep is associated with poor health, but the underlying mechanism is not clear. The aim of this study was to determine whether an actigraphy-based sleep pattern is associated with hypothalamic-pituitary-adrenocortical axis and sympatho-adrenal-medullary system activity in children.

Methods: In a cross-sectional study in a birth cohort in Helsinki, Finland, 282 8-year-old children were studied. Diurnal salivary cortisol concentrations and α -amylase (a sympatho-adrenal-medullary system marker) responses to the Trier Social Stress Test for Children (TSST-C) were determined in all subjects.

Results: Children with short (<7.7 h) vs. average sleep duration (7.8–9.3 h) displayed higher cortisol awakening response and nadir ($p < 0.042$). Children with low ($<77.4\%$) vs. average-high sleep efficiency ($>77.4\%$) displayed higher diurnal cortisol concentrations across the entire day ($p < 0.03$), higher cortisol concentrations after the TSST-C stressor ($p < 0.04$), and higher overall α -amylase concentrations across the entire TSST-C protocol ($p < 0.05$).

Conclusions: Poor sleep may indicate altered neuroendocrine functioning in children. These findings may offer insight into the pathways linking poor sleep with poor health.

Many children suffer from insufficient sleep quantity and poor sleep quality, and the prevalence of sleep problems in children ranges from 20 to 30%. Both human and animal studies have demonstrated that there is a bidirectional interaction between sleep and the hypothalamic-pituitary-adrenal (HPA) axis: experimental alterations in the HPA axis lead to altered sleep and alterations in sleep lead to altered HPA axis function [12]. Given that the HPA axis activity impacts on health, psychological functioning and psychopathology, altered HPA axis function may, at least in part, account for the effects of poor sleep on health [13]. However, studies testing the associations between sleep and HPA axis functioning in children are limited. In this study, Räikkönen et al. investigated the association of actigraphy-based sleep pattern with diurnal salivary cortisol concentrations, as well as with salivary cortisol responses to a standardized psychosocial stress test, the Trier Social Stress Test for Children (TSST-C), in 282 8-year-old children. They also investigated the association between sleep pattern and salivary α -amylase responses to stress, a marker of sympatho-adrenal-medullary system (SAMS) activity. They demonstrated that short sleep duration is associated with higher salivary cortisol concentrations in response to awakening and towards bedtime, and that low sleep efficiency is associated with higher diurnal salivary cortisol concentrations throughout the entire day and after stress. For children with low sleep efficiency, salivary α -amylase concentrations were also higher across the entire stress protocol. These findings indicate that poor sleep is associated with altered neuroendocrine functioning in healthy children. The cross-sectional design of this study does not allow inferences of causality. However, in adult humans, sleep and the HPA axis activity may be causally and reciprocally related. Sleep deprivation in adults leads to HPA axis activation by increased cortisol concentrations, while corticosteroid treatment may induce sleep problems. Given that we live in an era where the total number of hours of sleep is gradually decreasing, these findings may offer insight into why poor sleep is associated with poor health.

Maternal prenatal anxiety and stress predict infant illnesses and health complaints

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Pediatrics 2010;126:e401–9.

Background: Evidence from both animal and human studies suggests that maternal prenatal anxiety and stress can have adverse consequences on the offspring's development. However, in humans, studies on the effects of acquiring illnesses are scarce. The aim of this study was to examine whether maternal prenatal anxiety and stress, measured both by self-report and by cortisol physiology, are related to more infant illnesses and antibiotic use during the first year of life.

Methods: 174 mothers with normal pregnancies and term deliveries participated in the study. The mothers filled out a third-trimester questionnaires on general and pregnancy-specific anxiety and stress, and provided saliva samples for determination of circadian cortisol concentrations. Information on infant illnesses and antibiotic use was obtained through monthly maternal interviews throughout the infant's first year of life.

Results: Hierarchical multiple regressions showed that, even after controlling for many relevant confounders, prenatal anxiety and stress predicted a considerable amount of variance in infant illnesses and antibiotic use: 9.3% for respiratory, 10.7% for general, 8.9% for skin, and 7.6% for antibiotic use. Digestive illnesses were not related to prenatal anxiety and stress.

Conclusions: These findings are the first to demonstrate that maternal prenatal anxiety and stress are linked to infant illnesses and antibiotic use early in life.

In this study, Beijers et al. demonstrated that both higher evening cortisol concentrations and flattened diurnal cortisol rhythms during late pregnancy were associated with a greater number of illnesses in infancy. Although previous studies have linked these markers to psychopathology, in the present study both psychological predictors and cortisol concentrations independently predicted infant illnesses during the first year of life. This indicates that determining prenatal stress on the psychological and physiologic levels can be complementary and advantageous. The mechanism underlying the association between infant illnesses and prenatal anxiety and stress is not clear. Increased maternal cortisol concentrations normally do not lead to increased cortisol concentrations in the fetus. However, animal studies have shown that prenatal stress can affect the placental function, including the regulation of the placental barrier enzyme 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2), which converts cortisol to cortisone [14]. In humans, maternal anxiety may also increase the permeability of the placenta to cortisol by downregulating the placental 11 β -HSD2 activity, which may allow more cortisol to cross from maternal to fetal blood [15]. Since cortisol plays a key role in the regulation of the immune system, where it regulates the magnitude and duration of the inflammatory responses and the maturation of lymphocytes, exposure to abnormal cortisol concentrations in utero may program the immune function of the fetus towards an increased susceptibility to illnesses during infancy. Follow-up studies are necessary to determine whether the effects of prenatal anxiety and stress on infant susceptibility to illnesses are transient, persistent or even progressive.

Sexually dimorphic actions of glucocorticoids provide a link to inflammatory diseases with gender differences in prevalence

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Background: Males and females show differences in the prevalence of many major diseases, including autoimmune diseases, hepatocellular carcinoma, diabetes and osteoporosis, which are largely considered to reflect the actions of sex hormones on the susceptibility to inflammatory stimuli. Inflammation reflects a balance between pro- and anti-inflammatory signals. Glucocorticoids are the primary physiological anti-inflammatory hormones in mammals and synthetic derivatives of these hormones are prescribed as anti-inflammatory agents irrespective of patient gender.

Methods and Results: This study explored the possibility that sexually dimorphic actions of glucocorticoid regulation of gene expression may contribute to the dimorphic basis of inflammatory disease by evaluating the rat liver, a classic glucocorticoid-responsive organ. Eight distinct patterns of glucocorticoid-regulated gene expression were identified, which included sex-specific genes. The experiments also defined specific genes with altered expression in response to glucocorticoid treatment in both sexes, but in opposite directions. Pathway analysis identified sex-specific glucocorticoid-regulated gene expression in several canonical pathways involved in susceptibility to and progression of diseases with gender differences in prevalence. Furthermore, a comparison of the number of genes involved in inflammatory disorders between sexes revealed 84 additional glucocorticoid-responsive genes in the male, suggesting that the anti-inflammatory actions of glucocorticoids are more effective in males. These gender-specific actions of glucocorticoids in liver were substantiated in vivo with a sepsis model of systemic inflammation.

Conclusions: Gender-specific actions of glucocorticoids might contribute to the susceptibility to, development or progression of inflammatory diseases with differences in gender prevalence.

Stress and sex versus immunity and inflammation

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Glucocorticoids influence almost all aspects of mammalian physiology. These steroids exert their effects on a large network of primary, secondary and tertiary target genes, encompassing up to 20% of the expressed genome in a tissue. New evidence shows quantitative and qualitative gender-specific differences in the actions of glucocorticoids on the rat liver transcriptome, suggesting that the pervasive actions of these hormones are modulated by gender, both as an inherent property of the target tissues and as a result of exposure of these tissues to estrogens and possibly androgens. Generally, female mammals have more robust behavioral and somatic responses to stress and more potent immune and inflammatory reactions than males, differences that are inherent, sex steroid-mediated or both and possibly the evolutionary products of natural selection of female and male roles.

Gender-based biology has identified physiological and pharmacological differences between women and men at the subcellular, cellular, tissue, organ and system levels, as well as differences in the propensity for specific diseases [16]. Indeed, gender emerges as an important epidemiological risk factor for the incidence, presentation, and progression of several diseases with known inflammatory components. Glucocorticoids regulate intermediary metabolism, vascular tone, central nervous system function, development and programmed cell death [3]. They also function as anti-inflammatory and immunosuppressive effectors, and synthetic glucocorticoids are a mainstay in the treatment of many inflammatory and autoimmune diseases [17]. Given that inflammation appears to be a common feature underlying many diseases that exhibit gender-based differences in prevalence, the authors con-

sidered the possibility that gender may influence the anti-inflammatory actions of glucocorticoids. With the rat liver as a model, genome-wide complementary DNA (cDNA) microarray studies revealed that glucocorticoid treatment expands the set of liver genes that exhibit sexually dimorphic expression. This study was limited to the gender specificity of the rat liver transcriptome, first in the basal state, which showed major overlaps but also substantial differences, and subsequently in the glucocorticoid-stimulated state, which demonstrated a quantitative expansion of the gender-specific transcriptome, with substantial qualitative changes between the sexes. These findings, supported by in silico analyses and in vivo experiments, suggest that glucocorticoids selectively modulate the inflammatory response in male and female livers through distinct signaling pathways. Therefore, these gender-specific actions of glucocorticoids might contribute to the susceptibility to development or progression of inflammatory diseases with differences in gender prevalence.

If extrapolated and generalized, these data suggest that stress potentiates gender differences in the responsiveness of tissues to glucocorticoids, and that these differences are both quantitative and qualitative. These data also provide us with a better understanding of differences in the ability of males and females to deal with stress and immune and inflammatory reactions. A more potent stress response and immune and inflammatory reaction in women than men may translate into the higher prevalence of stress-related behavioral syndromes, such as anxiety, depression, psychosomatic and eating disorders, and autoimmune, inflammatory or allergic disorders, such as rheumatoid arthritis, systemic lupus erythematosus and multiple sclerosis or asthma, respectively [18, 19]. The presence of androgenic steroids might also contribute to the overall lower prevalence of behavioral stress-related syndromes and autoimmune disorders in men as compared with women because of their ability to inhibit the HPA axis and the immune and inflammatory reaction. The usual superiority of women in dealing with stress and infections is complemented by the protective effects of estrogen on the cardiovascular system and appears to be balanced by their increased vulnerability to stress-related behavioral syndromes and autoimmune inflammatory and allergic disorders. On the other hand, men have increased vulnerability to brain developmental disorders, such as attention deficit hyperactivity disorder, autism, and schizophrenia, conditions possibly related to the male brain sexual differentiation process.

Important for clinical practice

Guidelines for the management of congenital adrenal hyperplasia

Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an Endocrine Society clinical practice guideline

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J Clin Endocrinol Metab 2010;95:4133–60.

Background: Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders characterized by impaired cortisol synthesis. The objective of this study was to develop clinical practice guidelines for the management of CAH due to 21-hydroxylase deficiency.

Methods: The Task Force included a chair, selected by The Endocrine Society Clinical Guidelines Subcommittee (CGS), ten additional clinicians experienced in treating CAH, a methodologist and a medical writer. Consensus was guided by systematic reviews of evidence and discussions. The guidelines were reviewed and approved sequentially by The Endocrine Society's CGS and Clinical Affairs Core Committee, members responding to a web posting, and The Endocrine Society Council. At each stage, the Task Force incorporated changes in response to written comments.

Results and Conclusions: The authors recommend: (i) universal newborn screening for severe steroid 21-hydroxylase deficiency, which should be followed by confirmatory tests; (ii) prenatal treatment of CAH should continue to be regarded as experimental; (iii) the diagnosis of CAH should be made according to clinical and endocrine data, while genotyping should be reserved for equivocal cases and

genetic counseling; (iv) glucocorticoid dosage should be minimized to avoid iatrogenic Cushing's syndrome; (v) mineralocorticoids and, in infants, supplemental sodium should be given in patients with the classic form of the disease; (vi) the routine use of experimental therapies to promote growth and delay puberty, and adrenalectomy should be avoided; (vii) the early single-stage genital repair for severely virilized girls should be performed by experienced surgeons; (viii) clinicians should consider patients' quality of life; (ix) at the transition to adulthood, patients should be monitored for potential complications of CAH, and (x) judicious use of medication during pregnancy and in symptomatic patients with non-classic CAH.

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders characterized by impaired cortisol synthesis [20]. The incidence of CAH ranges from 1:10,000 to 1:20,000 births and is more prevalent in certain ethnic groups. The most common form of the disease is due to 21-hydroxylase deficiency, which accounts for approximately 95% of cases of CAH. Classic CAH is characterized by impaired cortisol and often aldosterone secretion, impaired development and function of the adrenal medulla, and adrenal hyperandrogenism. Non-classic CAH is more prevalent, occurring in approximately 0.1–0.2% in the general Caucasian population but in up to 1–2% among inbred populations, such as Eastern European (Ashkenazi) Jews. Non-classic CAH may be asymptomatic or present with variable degrees of postnatal androgen excess. The mild subclinical impairment of cortisol synthesis in non-classic CAH generally does not lead to addisonian crises [20]. The present clinical practice guidelines provide evidence-based recommendations on newborn screening, prenatal treatment, diagnosis, medical treatment, complications, feminizing surgery, experimental therapies and transition to adulthood in patients with classic and non-classic CAH.

Guidelines for the development of comprehensive care centers for congenital adrenal hyperplasia: guidance from the CARES Foundation Initiative

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Int J Pediatr Endocrinol 2010;2010:275213.

Patients with rare and complex diseases, such as congenital adrenal hyperplasia (CAH), often do not receive optimal care unless efforts are coordinated among providers. The aim of this study was to translate the concepts of the medical home and comprehensive healthcare for individuals with CAH in order to offer many benefits for the affected individuals and their families. This paper represents the recommendations of a 1.5-day meeting held to discuss the ideal goals for comprehensive care centers (CCC) for newborns, infants, children, adolescents and adults with CAH. Participants included pediatric endocrinologists, internal medicine and reproductive endocrinologists, pediatric urologists, pediatric surgeons, psychologists, pediatric endocrine nurse educators, as well as patients with CAH. Representatives of Health Research and Services Administration (HRSA), New York-Mid-Atlantic Consortium for Genetics and Newborn Screening Services (NYMAC), and National Newborn Screening and Genetics Resource Center (NNSGRC) also participated. This paper should serve as a 'roadmap' for the development phases of CCC for individuals and families affected by CAH.

Patients with rare and complex diseases often receive suboptimal care unless efforts are coordinated among providers. For some diseases, such as cancer and cystic fibrosis, the development of comprehensive care centers (CCCs) has dramatically improved outcomes and quality of life [21]. To establish guidelines for the development of CCCs for the treatment of CAH, the Congenital Adrenal hyperplasia Research, Education, and Support (CARES) Foundation, Inc., organized a conference attended by pediatric endocrinologists, medical endocrinologists, reproductive endocrinologists, pediatric urologists/surgeons, endocrine nurses, psychologists, as well as subjects affected with the condition. The

diverse backgrounds of the participants were fundamental to the planning process and influenced the idealized design of these centers. The essence of a CCC is patient-centered efficient coordination of care among multiple healthcare providers, extending from diagnosis through all stages of growth and development. Personalized, family-centered, holistic, culturally sensitive and effective care should create the foundation for a CCC for patients and families affected with CAH. Comprehensive care should consider the medical, emotional, psychosocial and financial needs of patients and their families throughout the lifespan. Internal medicine and pediatric physicians, nurses, behavioral/psychosocial health providers, nutritionists, genetic counselors, care coordinators and others should collaborate as a team to create a comprehensive plan for patients with CAH. The individual and his or her long-term health outcomes and quality of life should be the central focus of the CAH CCC. Important components of a CCC must be collaboration, cooperation, teamwork, clinical investigation and training.

Adult height in patients with congenital adrenal hyperplasia: a systematic review and meta-analysis

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J Clin Endocrinol Metab 2010;95:4161–72.

Background: Treatment for patients with congenital adrenal hyperplasia (CAH) may affect their final height. The aim of this study was to determine the distribution of final height in patients with classic CAH diagnosed in infancy or early childhood and treated with glucocorticoids.

Methods: The authors searched the MEDLINE, EMBASE, Cochrane Library, ISI Web of Science and Scopus through September 2008, the reference sections of included studies and expert files. Eligible studies included patients diagnosed with CAH before the age of 5 years and followed to final height. The SD score (SDS) for final height and corrected height (defined as final height SDS – midparental height SDS) were estimated from each study and pooled using random-effects meta-analysis. The I^2 statistic was used to assess inconsistency in results across studies.

Results: 35 eligible studies were identified, most of which were retrospective single-cohort studies. The final height SDS achieved by CAH patients was -1.38 (-1.56 to -1.20 ; $I^2 = 90.2\%$), and the corrected height SDS was -1.03 (-1.20 to -0.86 ; $I^2 = 63.1\%$). There was no significant association between final height SDS and age at diagnosis, gender, type and dose of steroid, and age of onset of puberty. Mineralocorticoid users had a better height outcome compared with non-users ($p = 0.02$).

Conclusions: The final height of patients with CAH treated with glucocorticoids is lower than the population norm and is lower than expected given parental height.

In classic 21-hydroxylase deficiency, glucocorticoid treatment aims to reduce the excess secretion of CRH and ACTH from the hypothalamus and anterior pituitary, respectively, and to suppress the increased production of adrenal sex steroids. However, patients on treatment often fluctuate between periods of hyperandrogenism and hypercortisolism, which both contribute to poor final height outcome in this population. In addition, the course of CAH is often complicated by central precocious puberty, which further contributes to compromised final height [20]. The authors conducted a systematic review and meta-analyses to summarize the available evidence about height outcomes in treated CAH patients, who were diagnosed in early childhood and followed until final height. Evidence derived from observational studies suggests that the final height of CAH patients treated with glucocorticoids is lower than the population norm and is lower than what would be predicted based on parental height. The authors found that the final height of these patients on average was 1.38 SDS lower than the population norm. This effect was also present when height was referenced to the genetic height potential. However, heterogeneity limits the reliability of meta-analytic estimates. The quality of evidence was considered very low due to the serious methodological limitations of included studies, large inconsistency of results across studies and unclear importance of the outcome. Most of the studies were limited by lack of universal newborn screening with delay in diagnosis and some also by higher doses of glucocorticoid use than in current practice. They also found that patients who required mineralocorticoids had a better outcome compared with the non-salt-wasting patients. This might be in part due to the delayed diagnosis of the non-salt wasters

contributing to increased height velocity in the early years of life and hence a less favorable final height outcome. Finally, meta-regression suggests that treatment in recent years may be more effective in improving patients' height. This can reflect improved patients' care in recent years, better nutrition or overall healthcare, variability in treatment dosing and preparation or many other confounding variables. Inference from this regression model is quite limited considering that the overall methodology of these studies is limited and the numerous potential alternative explanations.

Health status of adults with congenital adrenal hyperplasia: a cohort study of 203 patients

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Background: Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders characterized by impaired cortisol synthesis. The objective of this study was to establish the health status of adults with CAH.

Methods: A prospective cross-sectional study of adults with CAH attending specialized endocrine centers across the United Kingdom was performed. Participants included 203 patients with CAH: 138 women, 65 men, median age 34 (range 18–69) years. Anthropometric, metabolic and subjective health status was evaluated.

Results: Glucocorticoid treatment consisted of hydrocortisone (26%), prednisolone (43%), dexamethasone (19%), or a combination (10%), with reverse circadian administration in 41% of patients. Control of androgens was highly variable with a normal serum androstenedione found in only 36% of patients, whereas 38% had suppressed levels suggesting glucocorticoid overtreatment. In comparison with Health Survey for England participants, patients with CAH were significantly shorter and had a higher body mass index, and women with classic CAH had increased diastolic blood pressure. Metabolic abnormalities were common, including obesity (41%), hypercholesterolemia (46%), insulin resistance (29%), osteopenia (40%) and osteoporosis (7%). Subjective health status was significantly impaired and fertility was compromised.

Conclusions: Currently, a minority of adult patients with CAH in the United Kingdom appear to be under endocrine specialist care. In the patients studied, glucocorticoid replacement was generally non-physiological and androgen levels were poorly controlled. This was associated with an adverse metabolic profile and impaired fertility and quality of life. Improvements in the clinical management of adults with CAH are required.

CAH is a life-long chronic disorder. Management of CAH focuses on gender assignment, genital surgery, and optimization of growth and pubertal development during childhood and adolescence, on fertility in early adult life and on the prevention of metabolic syndrome and osteoporosis in middle and older age [20]. Given the relative paucity of data on the health status of adults with CAH, the authors performed a large, multi-center cross-sectional study assessing the subjective and objective health status of adults with CAH in the United Kingdom. They showed that both the objective and subjective health status in these subjects is poor. This was observed for both sexes and for patients with both classic and non-classic forms of the disease. Females with classic CAH were the most affected. All patients, but in particular women with classic CAH, suffered from a high prevalence of obesity, hypercholesterolemia, insulin resistance, osteopenia and osteoporosis, and women with classic CAH also had increased diastolic blood pressure. Glucocorticoid overtreatment might be responsible for the observed adverse metabolic profile, given that women with classic CAH most frequently receive long-acting synthetic glucocorticoids, combined treatment with several glucocorticoid preparations, and reverse circadian glucocorticoid administration. It is recommended that all adult patients with CAH should be offered care in endocrine centers with appropriate expertise, involving a multidisciplinary team, including endocrinologists, gynecologists, geneticists, psychologists, urologists and specialist nurses. It is also suggested that long-acting synthetic glucocorticoids and in particular reverse circadian glucocorticoid administration should be avoided, while improved options for glucocorticoid treatment should be explored.

Prenatal dexamethasone use for the prevention of virilization in pregnancies at risk for classical congenital adrenal hyperplasia because of 21-hydroxylase (CYP21A2) deficiency: a systematic review and meta-analyses

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Clin Endocrinol (Oxf) 2010;73:436–44.

Background: Prenatal treatment with dexamethasone to prevent virilization in pregnancies at risk for classic congenital adrenal hyperplasia (CAH) remains controversial. The aim of this study was to conduct a systematic review and meta-analyses of studies that evaluated the effects of dexamethasone administration during pregnancies at risk for classical CAH due to 21-hydroxylase deficiency.

Methods: MEDLINE, EMBASE and Cochrane central were searched from inception through August 2009. Review of reference lists and contact with CAH experts further identified candidate studies. Eligible studies reported the effects on either fetal or maternal outcomes of dexamethasone administered during pregnancy compared to a control group that did not receive any treatment.

Results: Only four eligible observational studies (325 pregnancies treated with dexamethasone) were identified. The methodological quality of the included studies was overall low. Meta-analysis demonstrated a reduction in fetus virilization measured by Prader score in female fetuses treated with dexamethasone initiated early during pregnancy (weighted mean difference -2.33 , 95% CI -3.38 , -1.27). No deleterious effects of dexamethasone on stillbirths, spontaneous abortions, fetal malformations, neuropsychological or developmental outcomes were detected. There was increased edema and striae in the mothers treated with dexamethasone. There were no data on long-term follow-up of physical and metabolic outcomes in children exposed to dexamethasone.

Conclusions: Dexamethasone seems to be associated with reduction in fetus virilization without significant maternal or fetal adverse effects. However, the observational nature of the available evidence and the overall small sample size of the body of the literature significantly weaken inferences about the benefits and harms of dexamethasone in this setting. The decision about initiating treatment should be based on patients' values and preferences, and requires fully informed and consenting parents.

The present review identified very few studies that evaluated the effects of prenatal dexamethasone administration on pregnancy outcomes in pregnancies at risk of 21-hydroxylase deficiency. Early administration of dexamethasone ameliorated virilization in female fetuses affected by classic CAH without increasing significantly the rate of stillbirths, spontaneous abortions or malformations. When reported, fetal and postnatal growth showed no statistical significant differences between the exposed and non-exposed to dexamethasone groups. On the other hand, mothers treated with dexamethasone had significant increase in typical adverse effects of hypercortisolism, such as striae and edema, but not in gestational diabetes or hypertension. There were no studies reporting on long-term metabolic and cardiovascular outcomes in children exposed to dexamethasone during pregnancy, while inconsistent and sparse results were found in terms of possible long-term outcomes of dexamethasone treatment on cognitive and neuropsychological effects in early childhood and puberty. The observational nature of the available evidence and the overall small sample size of the studies examined significantly weaken inferences about the benefits and harms of dexamethasone in this setting. Further studies are clearly indicated to assess the short- and long-term effects of dexamethasone in this setting. The decision about initiating treatment requires fully informed and consenting parents.

Cellular processing of the glucocorticoid receptor gene and protein: new mechanisms for generating tissue-specific actions of glucocorticoids

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Glucocorticoids regulate numerous physiological processes essential for life and are the mainstay in the treatment of many inflammatory, autoimmune and lymphoproliferative disorders. The traditional view that glucocorticoids act through a single glucocorticoid receptor (GR) protein has changed in recent years with the discovery of a large cohort of receptor isoforms that arise from alternative processing of the GR gene. These isoforms differ in their expression, gene regulatory and functional profiles. Posttranslational modification of these proteins further expands GR diversity. This paper discusses the origin and molecular properties of the GR isoforms and their contribution to the sensitivity and specificity of the glucocorticoid response.

Glucocorticoid receptor- α isoform-selective regulation of antiapoptotic genes in osteosarcoma cells: a new mechanism for glucocorticoid resistance

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Background: Glucocorticoids regulate a variety of physiological processes and are commonly used to treat several inflammatory, autoimmune and lymphoproliferative disorders. The actions of glucocorticoids are mediated by the classic glucocorticoid receptor (GR) α isoform. Recent data suggest that the mature GR α mRNA is translated into multiple N-terminal isoforms that have distinct biochemical properties and gene regulatory profiles. Interestingly, osteosarcoma cells stably expressing the GR α -D translational isoform are unique in that they are resistant to glucocorticoid-induced apoptosis.

Methods and Results: This study investigated whether GR α isoform-specific differences in the regulation of antiapoptotic genes contribute to this resistant phenotype. Unlike the other receptor isoforms, GR α -D does not inhibit the activity of a nuclear factor κ B (NF- κ B)-responsive reporter gene and does not efficiently repress either the transcription or protein production of the antiapoptotic genes Bcl-xL, cellular inhibitor of apoptosis protein 1 (cIAP1) and survivin. The inability of GR α -D to downregulate the expression of these genes is associated with a decreased interaction between GR α -D and NF- κ B, most likely due to sequestration of GR α -D in the nucleus. Deletion of the GR α N-terminal amino acids 98–335 also results in a nuclear localization of the GR, which fails to interact with NF- κ B and to promote apoptosis in response to glucocorticoids.

Conclusions: These data suggest that the N-terminal translational isoforms of GR α selectively regulate antiapoptotic genes and that the GR α -D isoform may contribute to the resistance of certain cancer cells to glucocorticoid-induced apoptosis.

Glucocorticoids regulate several physiologic functions essential for life, including the immune function, skeletal growth, reproduction, cognition, behavior, cell proliferation and survival, and play an important role in maintaining basal and stress-related homeostasis. Because of their powerful anti-inflammatory and immunosuppressive actions, synthetic glucocorticoids are widely prescribed for the treatment of acute and chronic inflammatory diseases, autoimmune diseases, organ transplant rejection and malignancies of the lymphoid system [3, 17]. Both the physiological and pharmacological actions of glucocorticoids are mediated by the glucocorticoid receptor (GR; NR3C1), a member of the nuclear receptor superfamily of ligand-dependent transcription factors [3] (fig. 1). Consistent with the pleiotropic effects of glucocorticoids, the receptor is ubiquitously expressed and necessary for life

Fig. 1. GR signaling pathway. Upon binding to glucocorticoids, cytoplasmic GR undergoes a change in conformation, becomes hyperphosphorylated (P), dissociates from accessory proteins, and translocates into the nucleus, where it regulates gene expression. GR enhances or represses the transcription of target genes by direct GRE binding **(a)**, by tethering itself to other transcription factors apart from DNA binding **(b)**, or in a composite manner by both direct GRE binding and interactions with transcription factors bound to neighboring sites **(c)**. *Inset:* GR is composed of an NTD, a BD, a hinge region (H), and an LBD. Regions involved in transcriptional activation (AF1 and AF2), dimerization, nuclear localization and chaperone hsp90 binding are indicated. Position numbers are for the human GR. NPC = Nuclear pore complex; BTM = basal transcription machinery; TBP = TATA-binding protein; nGRE = negative GRE; RE = response element.

Fig. 2. a GR isoforms generated by alternative splicing. The human GR primary transcript is composed of nine exons, with exon 2 encoding most of the NTD, exons 3 and 4 encoding the DBD, and exons 5–9 encoding the hinge region (H) and LBD. The classic GRα protein results from splicing of exon 8 to the beginning of exon 9. GRβ is produced from an alternative splice acceptor site that links the end of exon 8 to downstream sequences in exon 9, encoding a variant with a unique 15-amino-acid C-terminus (positions 728–742). GRγ is generated by an alternative splice donor site in the intronic sequence separating exons 3 and 4, resulting in a protein with an arginine insertion (Arg-452) between the two zinc fingers of the DBD. GR-Α is produced from alternative splicing that joins exon 4 to exon 8, deleting the proximal 185 amino acids of the LBD (Ala-490–Ser-674) encoded by exons 5–7. GR-P is formed by a failure to splice exon 7 to exon 8. The retained intronic sequence introduces a stop codon, resulting in a truncated receptor mutant missing the distal half of the LBD. **b** GRα isoforms generated by alternative translation initiation and sites of posttranslational modification. Initiation of translation from eight different AUG start codons in a single GRα mRNA generates receptor isoforms with progressively shorter NTDs. Approximate locations of the AUG start codons in the exon 2 sequences of the GRα mRNA are designated by asterisks. The initiator methionines, AF1 region (amino acids 77–262), and sites of posttranslational modifications (phosphorylation (P), sumoylation (S), ubiquitination (U), and acetylation (A)) are indicated for the human GRα isoforms. H = Hinge region.

after birth. The sensitivity to glucocorticoids differs not only among individuals but also within tissues of the same individual and even within the same cell during the cell cycle. Moreover, tissue-specific glucocorticoid resistance frequently develops in patients on chronic glucocorticoid therapy. The therapeutic benefit of glucocorticoids is also limited by severe side effects such as osteoporosis, abdominal obesity, glaucoma, growth retardation in children, and hypertension. Elucidating the molecular mechanisms governing the diversity in the cellular response to glucocorticoids should facilitate the development of new glucocorticoids with improved therapeutic indices.

The traditional view that glucocorticoids exert their diverse effects through one receptor protein has changed dramatically over the last two decades with the discovery of multiple GR isoforms arising

from the single GR gene. GR subtypes with unique expression and gene regulatory profiles are generated by alternative splicing of the nascent transcript, alternative translation initiation of the mature mRNA, and posttranslational modifications of the receptor protein (fig. 2). The capacity of a cell to generate dozens of GR isoforms that control specific sets of genes and/or differentially regulate common sets provides enormous potential for signaling diversity. Further contributing to the tissue- and cell-specific effects of glucocorticoids is the potential for these isoforms to heterodimerize with each other and cross-talk with other signaling molecules [3]. A greater understanding of the role that GR heterogeneity plays in the cellular response to glucocorticoids should aid in the development of safer and more effective glucocorticoid therapies.

The data presented here by Dr. Cidlowski's group suggest that the N-terminal GR α translational isoforms have selective effects on the expression of antiapoptotic genes and cell survival. In contrast to GR α -wt, GR α -A, GR α -B and GR α -C, the GR α -D isoform does not mediate glucocorticoid-induced cell death in osteosarcoma cells. In addition, GR α -D does not efficiently inhibit basal NF- κ B reporter gene activity nor repress expression of the antiapoptotic genes Bcl-xL, cIAP1, and surviving. Furthermore, the ability of GR α -D to interact with the p65 subunit of NF- κ B and to be recruited to the promoters of the three antiapoptotic genes was impaired. The unique nuclear compartmentalization of GR α -D that results in its physical separation from the majority of p65 in the cytoplasm likely contributes to the reduced association between GR α -D and p65. Mutational studies confirm the importance of the N-terminal amino acids 98–335, which are absent in GR α -D, for receptor subcellular localization, interaction with p65, and efficient induction of apoptosis by glucocorticoids. Taken together, these data highlight a novel role for the GR α N-terminus in modulating NF- κ B signaling and cell death and suggests that GR α -D expression may be a contributing factor to glucocorticoid resistance.

New genes

Genetics of cortisol secretion and depressive symptoms: a candidate gene and genome-wide association approach

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Background: Depressive patients are known to have altered cortisol secretion, but few genetic variants have been linked to both cortisol secretion and depression. The aim of this study was to identify genes related to both conditions. The authors: (i) tested the association of single nucleotide polymorphisms (SNPs) in hypothalamic-pituitary-adrenal (HPA) axis candidate genes with a summary measure of total cortisol secretion during the day (cortisol AUC), (ii) performed a genome-wide association study (GWAS) of cortisol AUC, and (iii) tested the association of identified cortisol-related SNPs with depressive symptoms.

Methods: This study analyzed data on SNPs for the HPA axis, genome-wide scans of cortisol secretion ($n = 1,711$) and depressive symptoms (Centre for Epidemiology Studies Depression Scale, CES-D) ($n = 2,928$) in elderly subjects of the Rotterdam Study. Data from the Whitehall II study ($n = 2,836$) were used to replicate the GWAS.

Results: Of the 1,456 SNPs in 33 candidate genes, minor alleles of 4 SNPs (rs9470080, rs9394309, rs7748266 and rs1360780) in the FKBP5 gene were associated with a decreased cortisol AUC ($p < 1 \times 10^{-4}$) after correction for multiple testing using permutations. These SNPs were also associated with an increased risk of depressive symptoms (rs9470080: OR 1.19 (95% CI 1.0, 1.4)). The GWAS for cortisol yielded 2 SNPs with p values of 1×10^{-6} (rs8062512, rs2252459), but these associations could not be replicated.

Conclusions: Variation in the FKBP5 gene is associated with both cortisol AUC and the likelihood of depressive symptoms.

Several studies have shown that depression is associated with hyperactivity of the HPA axis, resulting in high cortisol concentrations and abnormal cortisol response to standard stimuli, such as the DEX/CRH suppression test. It has been suggested that these alterations in cortisol secretion are causally related to the development of depression and that the identification of genes related to cortisol secretion may enhance the discovery of genes associated with depression. SNPs within the glucocorticoid receptor (GR) gene that lead to glucocorticoid hypersensitivity have been associated with depression and the response to antidepressant treatment. Similarly, the FK506 binding protein 5 (FKBP5), which acts as co-chaperone of GR, is associated with glucocorticoid hypersensitivity and SNPs within the FKBP5 gene have been associated with depression, treatment response and recurrence of depressive episodes. However, most of these studies examined cortisol secretion under stressful circumstances without an assessment of basal cortisol secretion. This study from two well-characterized European cohorts of middle-aged or elderly adults used candidate gene and genome-wide approaches to identify genes associated with diurnal cortisol secretion and their association with depressive symptoms. The findings showed an association of FKBP5 SNPs with a decrease in cortisol AUC in a population-based cohort. Carriers of FKBP5 minor alleles were also at increased risk of clinically relevant depressive symptoms. However, the finding of an association between FKBP5 SNP and cortisol AUC in the Rotterdam Study ($n = 2,928$) could not be replicated in the Whitehall II study ($n = 2,836$), nor was it found in two earlier GWA studies. Although these findings represent consistent evidence for the physiological and clinical relevance of variants in this HPA-axis regulating gene in an epidemiological study, future laboratory studies are needed to establish the causal mechanism behind these associations.

Reviews

The molecular biology, biochemistry and physiology of human steroidogenesis, and its disorders

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Steroidogenesis entails processes by which cholesterol is converted to biologically active steroid hormones. Although most endocrine reviews discuss adrenal, ovarian, testicular, placental, and other steroidogenic processes in a gland-specific fashion, steroidogenesis is better understood as a single process that is repeated in each gland with cell-type-specific variations on a single theme. Therefore, understanding steroidogenesis is rooted in an understanding of the biochemistry of the various steroidogenic enzymes and cofactors and the genes that encode them. The first and rate-limiting step in steroidogenesis is the conversion of cholesterol to pregnenolone by a single enzyme, P450_{scc} (CYP11A1), but this enzymatically complex step is subject to multiple regulatory mechanisms. Qualitative regulation determining the type of steroid to be produced is mediated by many enzymes and cofactors. Steroidogenic enzymes fall into two groups: cytochrome P450 enzymes and hydroxysteroid dehydrogenases. The activities of these enzymes are modulated by posttranslational modifications and by cofactors, especially electron-donating redox partners. The elucidation of the precise roles of these various enzymes and cofactors has been greatly facilitated by identifying the genetic bases of rare disorders of steroidogenesis. Understanding steroidogenesis is of fundamental importance to understanding physiological homeostasis, as well as disorders of sexual differentiation, reproduction, fertility, hypertension and obesity.

Steroid hormones regulate a wide variety of developmental and physiological processes from fetal life to adulthood. They are all synthesized from cholesterol and hence have closely related structures based on the classic cyclopentanophenanthrene 4-ring structure. Isolation of some key steroidogenic enzymes from animal sources and the cloning of many of their cDNAs and genes in the 1980s showed that there were fewer steroidogenic enzymes than there were steroidogenic reactions and that, in most cases, a particular steroidogenic reaction was catalyzed by the same enzyme in all tissues. The last 23 years have witnessed major developments in several areas of steroidogenesis, including the

cloning of the steroidogenic acute regulatory protein (StAR), the expanding array of hydroxysteroid dehydrogenases (HSDs), the expanding roles of electron transfer proteins and other cofactors in disease and the elucidation of additional pathways of steroidogenesis in classic and extraglandular tissues. This review discusses the molecular biology, biochemistry and physiology of human steroidogenesis and its disorders. It emphasizes the importance of understanding steroidogenesis to understanding physiology, as well as disorders of sexual differentiation, reproduction, fertility, hypertension and obesity.

Adrenocortical carcinoma: a clinician's update

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Adrenocortical carcinoma (ACC) is a rare heterogeneous neoplasm with an incompletely understood pathogenesis and a poor prognosis. Previous studies have demonstrated that overexpression of insulin-like growth factor 2 (IGF-2) and constitutive activation of β -catenin play an important role in the development of ACC. Although most patients present with steroid hormone excess or abdominal mass effects, a growing proportion of patients with ACC is initially diagnosed incidentally. In patients with suspected ACC, a thorough endocrine and imaging work-up is recommended to guide the surgical approach aimed at complete resection of the tumor. To establish an adequate basis for treatment decisions, pathology reports include the Weiss score to assess malignancy, the resection status and the Ki67 index. As recurrence is frequent, close follow-up initially every 3 months is mandatory. Most patients benefit from adjuvant mitotane treatment. In metastatic disease, mitotane is the cornerstone of initial treatment and cytotoxic drugs should be added in case of progression. New targeted therapies, such as IGF-1 receptor (IGF-1R) inhibitors, are under investigation and may soon improve current treatment options.

Within the past decade, collaborative international efforts have paved the way for unprecedented progress in the care of patients with ACCs. Insights into the molecular pathogenesis of ACCs have led to the first clinical trials using targeted strategies, such as IGF-1R inhibitors. The European network ENSAT has established a validated ACC staging system and has outlined a detailed work-up for patients with suspected ACC. This review provides a detailed update on the pathophysiology, molecular biology, clinical presentation, diagnostic work-up, treatment, prognosis and predictors of clinical outcome of patients with ACC. In addition, the first ever phase III trial in ACC will hopefully lead to a first-line treatment in advanced ACC that fulfills the standards of high-level, evidence-based medicine. Given these international efforts, it is expected that within the next decade the combination of basic science, translational research and clinical trials will substantially improve the clinical outcome of patients with this rare disorder.

Steroidogenic factor-1 (SF-1, NR5A1) and human disease

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Steroidogenic factor-1 (SF-1) is a key regulator of adrenal and reproductive development and function. Recent evidence suggests that variations in SF-1 can be found not only in association with adrenal failure but also with a wide range of human reproductive phenotypes, such as 46,XY disorders of sex development (DSD), hypospadias, anorchia, male factor infertility or primary ovarian insufficiency in women. Overexpression or overactivity of SF-1 is reported in some adrenal tumors or endometriosis. Therefore, the clinical spectrum of phenotypes associated with variations in SF-1 is expanding and the importance of this nuclear receptor in human endocrine disease is now firmly established.

Steroidogenic factor-1 (SF-1) was originally identified as a master-regulator of steroidogenic enzymes in the early 1990s and has since been shown to control many aspects of adrenal and gonadal devel-

opment and function. Although initially thought that variations in SF-1 in humans would be rare events associated with specific phenotypes, more recent studies suggest that SF-1 changes may be associated with a wide range of human conditions. Some of these conditions are rare, sporadic events due to 'private' de novo changes in the coding sequence of NR5A1, while others are more common, such as male factor infertility or primary ovarian insufficiency. In this paper, the authors review the range of human phenotypes that are emerging in association with SF-1/NR5A1 variants. Understanding the role of SF-1 in human disease is of fundamental importance to understanding and dealing with disorders of sexual differentiation, reproduction, fertility and adrenal function.

Steroid assays in pediatric endocrinology

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Most steroid disorders of the adrenal cortex come to clinical attention in infancy and childhood. The investigation of these disorders may be challenging to the laboratory. The analysis of sex steroids in biological fluids of neonates, children and adolescents may present challenges of specificities and concentrations often in small sample sizes. Different reference ranges are also needed for interpretations. For approximately 40 years, quantitative assays for the steroids and their regulatory peptide hormones have been possible using immunoassay techniques. This review summarizes the benefits and failings of immunoassays and introduces the indications for which tandem mass spectrometry is anticipated to meet the clinical needs for steroid analysis in pediatric endocrine investigations. A close collaboration between clinicians and biochemists is crucial, particularly when presented with difficult to diagnose steroid disorders or when laboratory results do not make sense in the context of clinical and endocrine findings.

The analysis of steroids for investigations in pediatric endocrinology is often challenging for the laboratory. The diagnosis of several steroid disorders may be difficult and requires an in-depth understanding of physiology, as well as of adrenal and gonadal development and function. Biochemists must also address abnormal results and keep abreast of new analytical developments that may improve the service. In the present review, Dr. J. Honour summarizes the advantages and disadvantages of immunoassays, and introduces the indications for which tandem mass spectrometry is expected to meet the clinical needs for steroid analysis in pediatric endocrine practice. Immunoassays have replaced chemical tests of steroids based on colorimetry and will remain important in the field of steroid testing. Chromatography can be used before immunoassays to improve specificity, however, it is too labor intensive for routine use. Gas-liquid chromatography with flame ionization and electrochemical detection and high-performance liquid chromatography (HPLC) with UV detection is useful for measuring steroids at the higher ranges of concentrations in biological fluids. Gas chromatography-mass spectrometry (GC-MS) of urinary steroids is an extremely powerful diagnostic tool for defects in adrenocortical function and will remain important in revealing the nature of steroids in a sample without selection. MS is a powerful analytical tool for qualitative and quantitative analysis when coupled with GC and LC. Tandem mass spectrometry (MS-MS) will bring benefits of new technology to broaden applications. LC-MS-MS is often regarded as reference technology. Accuracy and specificity are better than immunoassay, but LC-MS-MS does not yet meet the higher precision standards achieved with GC-MS. Bioassays are unlikely to compete routinely with immunoassay or MS methods. Clinicians should work closely with biochemists, particularly when presented with unusual results, since there may be alternative investigations that can be analyzed by a tandem mass spectrometric approach.

Glucocorticoids enhance extinction-based psychotherapy

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Background: Anxiety disorders are generally treated with behavioral exposure therapy, which is thought to rely on fear extinction. Preclinical studies have shown that glucocorticoids can promote extinction processes. This study investigated whether the administration of glucocorticoids might be useful in enhancing exposure therapy.

Methods: In a randomized, double-blind, placebo-controlled study, 40 patients with specific phobia for heights were treated with three sessions of exposure therapy using virtual reality exposure to heights. Cortisol (20 mg) or placebo was administered orally 1 h before each of the treatment sessions. Subjects returned for a posttreatment assessment 3–5 days after the last treatment session and for a follow-up assessment after 1 month.

Results: Adding cortisol to exposure therapy resulted in a significantly greater reduction in fear of heights, as measured by the acrophobia questionnaire (AQ) both at posttreatment and at follow-up, compared with placebo. Furthermore, subjects receiving cortisol showed a significantly greater reduction in acute anxiety during virtual exposure to a phobic situation at posttreatment and a significantly smaller exposure-induced increase in skin conductance level at follow-up.

Conclusions: Adding cortisol to exposure therapy can enhance extinction-based psychotherapy.

Glucocorticoids can enhance memory consolidation of new information, but impair memory retrieval of already stored information. Especially emotionally arousing information, including traumatic memory, is sensitive to the retrieval-reducing effects of glucocorticoids. This could be the reason for successfully reducing anxiety when cortisol was added to exposure therapy in patients with phobia for height. The authors showed the same effect in patients with spider phobia. The finding that glucocorticoids enhance extinction-based psychotherapy can contribute to the development of novel therapeutic strategies to treat anxiety disorders. Although corticosteroids are one of the most widely used medications for the treatment of many diverse conditions, new disorders are being added to the list of therapeutic indications for these compounds.

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