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, 2009 and May 31, 2010. Our search yielded more than 5,500 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 2009*, unless progress in the field has been incremental. Emerging themes for this year's chapter include the effect of circadian transcription factors on glucocorticoid receptor action, the implications of ultradian glucocorticoid pulsatility on the expression of target genes, the role of neuropeptide hormone receptors and microRNAs in the diagnosis and treatment of adrenal cancer, and recent advances in the diagnosis and treatment of congenital adrenal hyperplasia.

Mechanism of the year

CLOCK/BMAL1 is a reverse-phase negative regulator of glucocorticoid action

Circadian rhythm transcription factor CLOCK regulates the transcriptional activity of the glucocorticoid receptor by acetylating its hinge region lysine cluster: potential physiological implications

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Background: Glucocorticoids exert their diverse actions through the glucocorticoid receptor (GR). Circulating concentrations of glucocorticoids fluctuate naturally in a circadian fashion and regulate the transcriptional activity of the GR in target tissues. The basic helix-loop-helix protein CLOCK and its heterodimer partner BMAL1 are self-oscillating transcription factors that generate circadian rhythms in both the central nervous system and periphery.

Methods and Results: CLOCK/BMAL1 repressed the GR-induced transcriptional activity in a histone acetyltransferase (HAT) activity-dependent fashion. In serum-shock-synchronized cells, the transactivational activity of GR fluctuated spontaneously in a circadian fashion in reverse phase with CLOCK/BMAL1 mRNA expression. CLOCK and GR interacted with each other physically, and CLOCK suppressed the binding of GR to its DNA recognition sequences by acetylating multiple lysine residues located in its hinge region.

Conclusions: CLOCK/BMAL1 functions as a reverse-phase negative regulator of glucocorticoid action in target tissues, possibly by antagonizing the biological actions of diurnally fluctuating circulating glucocorticoids. In addition, a peripheral target tissue circadian rhythm influences the functions of every organ and tissue indirectly, through modulation of glucocorticoid action.

Interactions of the circadian CLOCK system and the HPA axis

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Organisms have developed concurrent behavioral and physiological adaptations to the strong influence of day/night cycles, as well as to stressful stimuli. These circadian and stress-related responses are achieved by two highly conserved and interrelated regulatory networks, the circadian CLOCK and stress systems, which respectively consist of oscillating molecular pacemakers, the CLOCK/BMAL1 transcription factors, and the hypothalamic-pituitary-adrenal (HPA) axis and its end-effector, the glucocorticoid receptor. These systems communicate with each other at different signaling levels. Uncoupling of or impaired function in either system may result in the development of pathologic conditions. This review summarizes the bidirectional interaction between the circadian CLOCK system and the HPA axis, and discusses their clinical implications.

In humans, circulating cortisol concentrations are tightly regulated by the central components of the HPA axis and fluctuate naturally in a circadian fashion, reaching their zenith in the early morning and their nadir in the late evening [1]. The circadian activity of the HPA axis is generated by the hypothalamic suprachiasmatic nucleus (SCN), the master oscillator and generator of the circadian rhythm of the body. Circadian rhythms of both the central nervous system and peripheral tissues and organs are generated by the coordinated activation/inactivation of self-oscillating transcription factors. Central among them are the circadian locomotor output cycle kaput (CLOCK) and its heterodimer partner brain-muscle-arnt-like protein 1 (BMAL1), which belong to the basic helix-loop-helix (bHLH)-PER-ARNT-SIM (PAS) superfamily of transcription factors [2]. In this study, Kino and colleagues demonstrated that CLOCK/BMAL1 is a reverse-phase negative regulator of glucocorticoid action in target tissues, antagonizing the biological actions of diurnally fluctuating circulating glucocorticoids and providing a local target tissue counter-regulatory feedback loop to the central CLOCK on the HPA axis. The circadian CLOCK system and the HPA axis regulate the activity of one another through multilevel interactions to ultimately coordinate homeostasis against the day/night change and various unforeseen random internal and external stressors (fig. 1). Uncoupling of or dysfunction in either system alters internal homeostasis and causes pathologic changes virtually in all organs and tissues, including those responsible for intermediary metabolism and immunity. Disrupted coupling of cortisol secretion and target tissue sensitivity to glucocorticoids may explain the development of central obesity and the metabolic syndrome in chronically stressed individuals, whose HPA axis circadian rhythm is characterized by blunting of the evening decreases of circulating glucocorticoids, as a result of enhanced input of higher centers upon the hypothalamic paraventricular nucleus secretion of CRH and AVP (fig. 1). Similarly, disrupted coupling of cortisol secretion and target tissue sensitivity to glucocorticoids could explain the increased cardiometabolic risk of subjects exposed to frequent jetlag because of traveling across time zones. At pharmacologic concentrations, the transactivational activity of glucocorticoids is correlated with the side effects of these steroids, while their transrepressive activity is associated mostly with their beneficial anti-inflammatory activity. Since CLOCK may differentially regulate these two major class actions of glucocorticoids, administration of these steroids at a specific period of the circadian cycle might increase their pharmacological efficacy, while at the same time reducing their unwanted side effects.

Fig. 1. A heuristic scheme of **(a)** circadian secretion of cortisol in non-stressed and chronically stressed humans (left panel) and their responses to midnight dexamethasone administration (right panel), **(b)** the corresponding circadian changes of target tissue sensitivity to glucocorticoids and **(c)** mean target tissue sensitivity to glucocorticoids in the human population. Even mild evening cortisol elevations, as those seen in chronically stressed individuals, will exert disproportionately increased glucocorticoid effects because of the natural circadian target tissue sensitivity increase at this time of the day. SS = Chronically stressed individuals, D = midnight dexamethasone administration, HS = hyper-sensitivity, N = normal sensitivity, NS = non-stressed individuals, R = resistance.

Ultradian hormone stimulation induces glucocorticoid receptor-mediated pulses of gene transcription

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Background: Studies on glucocorticoid receptor (GR) action typically assess gene responses by long-term stimulation with synthetic hormones. However, given that glucocorticoids are secreted in a circadian and high-frequency (ultradian) mode, such treatments may not provide an accurate assessment of physiological hormone action.

Methods and Results: Ultradian hormone stimulation induces cyclic GR-mediated transcriptional regulation, or gene pulsing, both in cultured cells and in animal models. Nascent RNA transcripts from GR-regulated genes are released in distinct quanta, demonstrating a profound difference between the transcriptional programs induced by ultradian and constant stimulation. Gene pulsing is driven by rapid GR exchange with response elements and by GR recycling through the chaperone machinery, which promotes GR activation and reactivation in response to the ultradian hormone release.

Conclusions: The GR signaling pathway has been optimized for a prompt and timely response to fluctuations in glucocorticoid concentrations. These findings indicate that biologically accurate regulation of gene targets by GR requires an ultradian mode of hormone stimulation.

Glucocorticoids are released from the adrenal glands in a daily, circadian cycle as a result of the activity of the highly dynamic HPA axis. The pattern of glucocorticoid secretion is highly pulsatile (ultradian), with a periodicity of approximately 1 h. Temporal HPA axis activity drives a pattern of GR action that leads to gene stimulation that is reflective of the HPA axis profile. Physical and psychological stressors induce a rise in plasma glucocorticoid concentrations, superimposed on the ultradian and circadian rhythm. Due to its plasticity, the HPA axis integrates many internal and external stimuli, which in turn have a direct impact on GR-regulated transcriptional programs. The GR has been shown to exchange rapidly with response elements in living cells and its residence time on regulatory elements is measured in seconds. In this article, Hager and colleagues evaluated the implications of these complex aspects of glucocorticoid action (the rapid fluctuation of serum glucocorticoid concentrations and the fast dynamics of GR interactions with chromatin) on the GR-mediated transcriptional program. They demonstrated that, in contrast to treatment with long-acting glucocorticoid formulations, the ultradian mode of hormone secretion induces cyclic GR-mediated transcriptional regulation, or gene pulsing. The dynamics of GR-template interaction, as well as RNA Pol II loading and exchange, fluctuate together with the changes in extracellular hormone concentration. As a result, nascent RNA transcripts from a number of GR-regulated genes are released in distinct quanta during ultradian treatment, demonstrating profound differences in the transcriptional program induced by pulsatile ligand stimulation compared with that induced by constant stimulation. These findings suggest that gene pulsing in the GR system is necessary for correct transcriptional programming. As a result, even low doses of synthetic glucocorticoids, such as dexamethasone, are expected to alter the transcription program set by ultradian hormone release significantly. Furthermore, the inability to simulate ultradian hormone pulsatility may account for the fatigue, a common and debilitating manifestation often reported by patients with adrenal insufficiency, despite optimal glucocorticoid substitution therapy. These data provide a basis for a more complex view of gene regulation by GR and open new approaches for the development of synthetic glucocorticoids.

Genomic and non-genomic cross talk between the gonadotropin-releasing hormone receptor and glucocorticoid receptor signaling pathways

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Background: The GnRH receptor (GnRHR) is a central regulator of reproductive function in all vertebrates. GnRH exerts its effects by binding to the GnRHR in pituitary gonadotropes. This study investigated the mechanisms of regulation of transcription of the mGnRHR gene.

Methods and Results: Reporter assays with transfected mGnRHR promoter showed that both dexamethasone and GnRH increased the transcription of the mGnRHR gene via an activating protein-1 (AP-1) site. Small interfering RNA experiments revealed a requirement for the glucocorticoid receptor (GR) for both the dexamethasone and GnRH response. Chromatin immunoprecipitation (ChIP) and immunofluorescence assays showed that both GnRH and dexamethasone up-regulate the GnRHR gene via nuclear translocation and interaction of the GR with the AP-1 region on the mGnRHR promoter. GnRH activated the unliganded GR by rapid phosphorylation of the GR at Ser-234 in a GnRHR-dependent fashion. Also, a direct link between GnRH-induced protein kinase C and MAPK activation was established, leading to unliganded GR phosphorylation at Ser-234 and transactivation of the glucocorticoid response element. Finally, GnRH and dexamethasone synergistically activated the endogenous GnRHR promoter via a mechanism involving steroid receptor coactivator-1 recruitment to the GnRHR AP-1 region.

Conclusions: These findings demonstrate a novel mechanism of rapid non-genomic cross-talk between the hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary-adrenal (HPA) axes via GnRHR-dependent phosphorylation and activation of the unliganded GR in response to GnRH.

Ligand-independent activation of steroid receptors by phosphorylation is a common mechanism of receptor activation in several different cells via several different pathways. This article provides the first evidence for a novel mechanism of rapid non-genomic cross-talk between the HPG and HPA axes via GnRHR-dependent phosphorylation and activation of the unliganded GR in response to GnRH. Given that GnRH regulates several genes in pituitary gonadotropes, this non-genomic cross-talk represents a mechanism whereby the GnRHR-activated, unliganded GR may modulate the expression of several GnRH and GR target genes [3]. Furthermore, this GnRHR-GR cross-talk may have important physiologic implications, since it participates in the interplay between reproductive, stress and immune responses. It is well established that the neuroendocrine, immune, inflammatory and stress-response systems are integrated functionally and regulated bidirectionally [4]. For example, stress or chronic activation of the HPA axis suppresses reproduction via the elevated glucocorticoid concentrations that exert their effects at all levels of the HPG axis [5]. This study shows a direct transcriptional effect of glucocorticoids on GnRHR gene expression mediated by the GR, which represents another mechanism whereby the HPA axis could modulate the HPG axis.

New hope A big role for small molecules

Expression of neuropeptide hormone receptors in human adrenal tumors and cell lines: antiproliferative effects of peptide analogues

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Proc Natl Acad Sci USA 2009;106:15879–15884

Background: Peptide analogues targeting neuropeptide receptors have been used effectively in cancer therapy. Adrenocortical tumor formation is characterized by the aberrant expression of peptide receptors relating to uncontrolled cell proliferation and excess hormone secretion.

Methods: A comprehensive analysis of relevant receptors in human adrenomedullary and adrenocortical tumors was performed and the antiproliferative effects of peptide analogues targeting these receptors were tested. Specifically, the receptor expression of somatostatin-type-2 (sst2) receptor, growth hormone-releasing hormone (GHRH) receptor or GHRH receptor splice variant-1 (SV-1) and luteinizing hormone-releasing hormone (LHRH) receptor were examined at the mRNA and protein levels in normal human adrenal tissues, adrenocortical and adrenomedullary tumors, and cell lines.

Results: The cytotoxic derivatives of somatostatin AN-238 and AN-162 reduced the cell numbers of adrenomedullary pheochromocytoma cells and adrenocortical cancer cells. Both the SV-1 and the LHRH receptor were expressed in adrenocortical cancer but not in pheochromocytoma cell lines. The GHRH receptor antagonist MZ-4-71 and LHRH antagonist Cetrorelix significantly reduced cell growth in the adrenocortical cancer cell lines.

Conclusions: The expression of receptors for somatostatin, GHRH and LHRH in the normal human adrenal and in adrenal tumors, in association with the growth-inhibitory effects of antitumor peptide analogues, may improve current treatment of adrenal tumors.

The overexpression or aberrant expression of G-protein-coupled receptors for neuropeptides in human adrenal tissue has been associated with adrenal tumor formation and excessive hormone production [6, 7]. In addition, peptide hormone receptors have been detected in adrenocortical cancers, as well as in malignant pheochromocytomas. The identification of these receptors may allow the development of pharmacologic interventions as an alternative approach to current therapy. The present study evaluated specific receptor-targeted chemotherapeutic peptide antagonists and agonists for their potential future use in the antineoplastic therapy of adrenal tumors. It demonstrated that both adrenal tumor tissue and two adrenal tumor cell lines expressed receptors for somatostatin, GHRH or the SV-1 splice variant, as well as for LHRH. The immunohistochemical staining of adrenal tissue showed strong staining for sst2 in normal adrenal cortex, adrenocortical adenoma and carcinoma, as well as benign and malignant pheochromocytomas. The cytotoxic derivatives of somatostatin AN-238 and AN-162 reduced cell numbers of adrenomedullary pheochromocytoma cells and adrenocortical cancer cells, while the GHRH receptor antagonist MZ-4-71 and LHRH antagonist Cetrorelix significantly reduced cell growth in the adrenocortical cancer cell line. These results demonstrate a promising approach for delivering therapeutic compounds selectively to tumor cells, and raise hope for improved targeted treatment strategies for adrenal diseases.

MicroRNA signature of primary pigmented nodular adrenocortical disease: clinical correlations and regulation of Wnt signaling

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Cancer Res 2009;69:3278–3282

Background: MicroRNAs comprise a novel group of gene regulators implicated in the development of different types of cancer. However, their role in primary pigmented nodular adrenocortical disease (PPNAD), a rare form of bilateral adrenal hyperplasia caused by protein kinase A (PKA) regulatory subunit type 1A (PRKARIA)-inactivating mutations, has not been investigated.

Methods and Results: A 44-microRNA gene signature of PPNAD was identified following comparison of PPNAD with normal adrenal samples: 33 microRNAs were up-regulated and 11 were down-regulated in PPNAD compared with normal adrenal tissues. Comparison of microRNA microarray data with hormonal measurements showed a negative correlation between let-7b expression and cortisol concentrations in patients with PPNAD. Also, nine microRNA-gene target pairs were identified as playing a potential role in adrenal pathogenesis. MiR-449 was up-regulated and WNT1-inducible signaling pathway protein 2 (WISP2) was identified as its direct target. Finally, pharmacologic inhibition of PKA resulted in the up-regulation of miR-449 leading to the suppression of WISP2.

Conclusions: The microRNA profile and its clinical significance in PPNAD were investigated for the first time. The above findings suggest that PKA, via microRNA regulation, affects the Wnt signaling pathway, which is thought to be a primary mediator of PRKAR1A-related tumorigenesis.

An aberrant expression signature of microRNAs (miRNAs), small non-coding RNAs, is a hallmark of several diseases, including cancer. MicroRNA expression profiling by microarray techniques has pro-

vided a powerful tool to reveal the involvement of these molecules in tumor development and progression, showing that they are differentially expressed in tumors compared with normal tissues. Moreover, specific miRNA signatures have been associated with histopathological and clinical features, suggesting a potential role of these molecules as prognostic and predictive markers [8, 9]. Focusing then on their biological effects and role in cancer, it has been shown that miRNAs can function as potential oncogenes or oncosuppressor genes, depending on the cellular context and on the target genes they regulate. This is the first study of miRNAs in PPNAD, and indeed, in any form of adrenal hyperplasia. In this study, the authors identified a miRNA gene signature for PPNAD [10] and demonstrated that let-7b expression is highly associated with midnight cortisol concentrations, an index of clinical severity of the Cushing syndrome caused by PPNAD tumors. The let-7 miRNA family appears to play a tumor suppressor role in cancer. In addition, using a PPNAD cell line, the authors identified the inhibition of miR-449 and up-regulation of its target gene WISP2. These results suggest that PKA, through miRNA regulation, affects the Wnt signaling pathway, which is important in the regulation of PRKAR1A-related tumorigenesis and in adrenocortical oncogenesis in general. The possibility to modulate miRNA expression either in vitro or in vivo by developing synthetic pre-miRNA molecules or antisense oligonucleotides provides a powerful tool towards a better understanding of the molecular mechanisms regulated by these molecules, and suggests the intriguing and promising perspective of their possible use in therapy.

New concerns

Long-term effects of early-life stress

Childhood maltreatment and telomere shortening: preliminary support for an effect of early stress on cellular aging

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Biol Psychiatry 2010;676:531–534

Background: Advanced cellular aging has been proposed as a potential mechanism for the association between psychological stress and several medical and psychiatric illnesses. Previous studies linked chronic psychosocial stress and activation of the hypothalamus-pituitary-adrenal axis to shorter telomere length. Telomeres are DNA repeats that cap the ends of chromosomes and promote stability. They shorten progressively with each cell division and their length is a marker of biological aging. This study was designed to investigate the effect of childhood adversity on telomere length.

Methods: 31 adults with no current or past major axis I psychiatric disorder were recruited to participate in this cross-sectional study. Subjects reported on their history of childhood maltreatment with a retrospective self-report questionnaire (CTQ). Telomere length was measured following DNA extraction from whole blood.

Results: Participants reporting a history of childhood maltreatment had significantly shorter telomeres than those who did not report a history of maltreatment. This finding was not due to the effects of age, sex, smoking, body mass index or other demographic factors. Both physical neglect and emotional neglect were significantly linked to telomere length.

Conclusions: Childhood maltreatment could influence cellular aging.

Stressful life experiences have been associated with an increased risk for psychiatric disorders, as well as cardiovascular and immune diseases [11]. The results of the present study extend previous reports linking shortened leukocyte telomere length and caregiver stress to more remote stressful experiences in childhood. In this study, reported maltreatment was moderate to severe, and a variety of types of abuse and neglect were represented. Analysis of subtypes of maltreatment suggested that both emotional and physical neglect may have the most robust effects. Therefore, in addition to the psychological effects of stress, it is possible that physical stressors might have contributed to these findings. Although the mechanism underlying the above association remains to be elucidated, gluco-

corticoid-associated oxidative stress damage is likely to play an important role. Glucocorticoids increase neuronal oxidative stress damage [12] and oxidative stress reduces telomere length in vitro [13]. Limitations of this study that should be taken into consideration are the modest sample size, the cross-sectional nature of the study, and the fact that the CTQ is a brief, retrospective self-report questionnaire that may be subject to recall and other biases. Longitudinal, prospective, larger studies are needed to elucidate the effect of psychosocial stress on telomere length over time.

Quality of life in children and adolescents one year after cure of Cushing syndrome: a prospective study

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Background: Cushing syndrome (CS) in children is associated with symptoms that may impair health-related quality of life (HRQL). Prospective studies investigating the HRQL in children with CS are lacking.

Methods: 40 children (age range 5–18 years) with CS were studied prospectively prior to and 1 year post-treatment for CS. The Child Health Questionnaire (CHQ) was used to assess HRQL, the Wechsler Intelligence Scale for Children (WASI) was used to assess cognitive function, and a CS symptom checklist was used to assess patient-reported symptoms.

Results: Active CS was associated with low physical and psychosocial summary scores compared to US population data. Although these scores improved 1 year post-cure, residual impairment remained in physical summary and function. Incomplete recovery of adrenal function at 1 year post-treatment was associated with impaired scores. WASI IQ scores declined and a correlation was noted between age at first evaluation and IQ score changes. Most self-reported CS symptoms improved following treatment, with the exception of forgetfulness, unclear thinking and decreased attention span.

Conclusions: CS in children and adolescents is associated with impaired HRQL, with residual impairment 1 year after cure.

Chronic exposure to excess endogenous glucocorticoids in adults, children and adolescents with CS is associated with detrimental health effects including truncal obesity, hypertension, insulin resistance, hyperglycemia, impaired wound healing, hypercoagulability, osteoporosis and gonadal dysfunction. Adult studies have also demonstrated that glucocorticoid excess is associated with impaired cognition. This study showed that children and adolescents with active CS had low physical and psychosocial summary scores compared to their peers in the US population. One year after successful cure of CS, children and adolescents showed residual impairments in HRQL scores for physical summary scores, physical function, global health perception, role-physical and emotional impact on parent. These findings are consistent with previous studies demonstrating that children with CS experienced a decline in cognitive function after cure of CS, despite reversal of cerebral atrophy [14]. This is in contrast to studies of adults with CS, which reported that cognitive impairment and loss of brain volume is partially reversible after successful treatment [15]. In addition, younger children were more likely to experience negative changes in cognitive function from pre- to post-treatment, although all post-treatment IQ scores remained within a normal range. Long-term follow-up of children and adolescents with CS, with particular reference to HRQL, is essential.

Dehydroepiandrosterone sulfate directly activates protein kinase C- β to increase human neutrophil superoxide generation

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Background: Dehydroepiandrosterone (DHEA) sulfate (DHEAS) is the most abundant steroid in human circulation, and is secreted in an age-dependent fashion. DHEAS is considered an inactive metabolite that can be activated following cleavage of the sulfate group, thus generating DHEA, an important sex steroid precursor.

Methods: Human neutrophils were isolated from healthy male donors. Neutrophil superoxide production, OATP, STS and SULT2A1 mRNA expression analysis, DHEAS uptake assays, protein kinase C (PKC) activation assays and phosphorylation of p47phox were studied.

Results: DHEAS, but not DHEA, increased superoxide generation in primed human neutrophils in a dose-dependent fashion. This effect was not prevented by coincubation with androgen and estrogen receptor antagonists but was reversed by inhibition of PKC activity. Neutrophils were found to be unique among leukocytes in expressing an organic anion-transporting polypeptide D, able to mediate active DHEAS influx transport whereas they did not express steroid sulfatase that activates DHEAS to DHEA. DHEAS directly activated recombinant PKC- β in a cell-free assay. Enhanced PKC- β activation by DHEAS resulted in increased phosphorylation of p47phox, a crucial component of the active reduced nicotinamide adenine dinucleotide phosphate complex responsible for neutrophil superoxide generation.

Conclusions: PKC- β acts as an intracellular receptor for DHEAS in human neutrophils, a signaling mechanism entirely distinct from the role of DHEA as sex steroid precursor and with important implications for immune senescence, which includes reduced neutrophil superoxide generation in response to pathogens, thereby impacting on a key bactericidal mechanism.

DHEA and its sulfate ester, DHEAS, are the most abundant steroids in human circulation, representing the major products of the adrenal zona reticularis. In humans and higher primates, DHEAS secretion shows a characteristic, age-associated pattern with very high concentrations in the neonatal period, a decline to very low concentrations during the first few months of life, and a continuous increase starting between the sixth and tenth year of age, also termed 'adrenarche' [16]. Peak DHEAS concentrations are achieved during the third decade of life followed by a steady decline starting in the fifth decade (adrenopause) with concentrations decreasing to 10–20% of maximal levels around 70 years of age [16]. This age-related decline in DHEAS does not reflect a general loss of adrenocortical output because cortisol concentrations are maintained and are even slightly raised with age [17]. This study challenges the previously held views that DHEAS is an inactive metabolite, and provides evidence for a novel signaling mechanism of DHEAS, which is distinct from its role as a precursor to DHEA and downstream sex steroid synthesis. In human neutrophils, PKC- β acts as an intracellular receptor for DHEAS, mediating its stimulatory effects on neutrophil superoxide generation via activation of the NADPH oxidase complex. This is the first report of the direct activation of a major serine/threonine protein kinase by DHEAS. These effects of DHEAS may have important clinical implications, with particular reference to immunologic conditions and/or old age.

Microarray gene expression and immunohistochemistry analyses of adrenocortical tumors identify IGF2 and Ki-67 as useful in differentiating carcinomas from adenomas

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Endocr Relat Cancer 2009;16:573–583

Background: The Weiss score is the most widely used system for the diagnosis of adrenocortical tumors (ACTs). An ACT is scored from 0 to 9, with a higher score correlating with increased malignancy. However, ACTs with a score of 3 can be phenotypically benign or malignant.

Methods: Microarray profiling of a cohort of adrenocortical carcinomas (ACCs) and adrenocortical adenomas (ACAs) was used to identify discriminatory genes that could be used as an adjunct to the Weiss score. Genes with high-discriminatory power were identified by univariate and multivariate analyses and confirmed by quantitative real-time reverse transcription PCR and immunohistochemistry (IHC).

Results: Compared with ACAs, ACCs demonstrated significantly higher expression of IGF2, MAD2L1 and CCNB1 but lower expression of ABLIM1, NAV3, SEPT4 and RPRM. Several proteins, including IGF2, MAD2L1, CCNB1 and Ki-67 had high-diagnostic accuracy in differentiating ACCs from ACAs. A combination of IGF2 and Ki-67 diagnosed ACCs with 96% sensitivity and 100% specificity.

Conclusions: Microarray gene expression profiling accurately differentiates ACCs from ACAs. The combination of IGF2 and Ki-67 IHC is highly accurate in distinguishing between the two groups and is particularly helpful in ACTs with a Weiss score of 3.

With the advent of improved imaging techniques, adrenal tumors have been detected with increasing frequency. Distinguishing between adrenocortical adenomas (ACAs) and adrenocortical carcinomas (ACCs) can be difficult. The Weiss score, a 9-point histopathological scoring system, is presently the most widely used system for classifying adrenocortical tumors (ACTs) as benign or malignant [18]. However, whilst the Weiss score reliably classifies ACTs with a score of 0–2 as ACAs and those with a score of 4–9 as ACCs, the biological behavior of ACTs with a score of 3 can be difficult to predict accurately. Microarray gene expression profiling is a powerful tool that is able to characterize the transcription profile of a large number of genes in a tumor sample. This study has demonstrated that microarray gene expression profiling can accurately categorize ACTs into ACCs and ACAs. Immunohistochemistry (IHC) using IGF2 and a marker of proliferation can also distinguish ACCs from ACAs. Given that microarray gene expression profiling is expensive and its use in the clinical setting is limited, for the group of ACTs with a Weiss score of 3, the authors recommend the addition of IHC with IGF2 and a marker of proliferation (Ki-67 because of its wide availability and easy interpretation), in an attempt to classify and predict the biological behavior of these tumors accurately. This would lead to expeditious treatment for patients with 'true' ACCs, and ultimately lead to better prognosis and improved quality of life.

Serum steroid profiling for congenital adrenal hyperplasia using liquid chromatography-tandem mass spectrometry

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Background: The diagnosis of congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency is based on the quantification of 17-hydroxyprogesterone (17-OHP) by an immunoassay. However, during the neonatal period the specificity of screening for CAH is low, leading to high false-positive rates.

A robust, specific and selective method using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC/MS/MS) has been developed for the measurement of serum concentrations of cortisol, 21-deoxycortisol, 11-deoxycortisol, 4-androstene-3,17-dione (A4) and 17-OHP.

Methods: The steroids were extracted from 50 µl of serum using methyl-tert-butyl-ether and the analysis was performed on a UPLC tandem quadrupole mass spectrometer system.

Results: The assay was linear over each analyte concentration range. Inter- and intra-assay CVs were ≤10% across the analytical range. Simultaneous measurement of the full range of steroids in the pathway of cortisol biosynthesis allowed confirmation of the affected steroidogenic enzyme.

Conclusions: A second-tier test for the diagnosis of CAH has been developed. The method allows for detection and quantification of 5 steroids with good linearity, sensitivity and precision.

Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency accounts for 95% of all cases of CAH [19]. Early diagnosis and treatment is associated with a significant reduction in morbidity and mortality, and newborn screening has been advocated in many countries. The introduction of an immunoassay for 17-hydroxyprogesterone (17-OHP) in the late 1970s has contributed substantially to the uptake of CAH newborn screening programs. However, despite the wide availability and usage of this test, its diagnostic value is often limited, owing to low analytical antibody specificity and the limited diagnostic specificity of an isolated raised 17-OHP concentration. Transient elevations of 17-OHP concentrations are often seen in premature or severely ill newborns in the screening period and contribute to a high false-positive rate [20]. Analytical methods based on mass spectrometry present the most specific quantitative methods for determination of steroid concentrations [21–23]. A new method for diagnosing CAH accurately has been developed. This method is based on LC/MS/MS. It is fast, robust and reproducible, allows quantification of 5 steroids related to CAH in 5 min, and demonstrates excellent resolution between 21-deoxycortisol and 11-deoxycortisol, thereby indicating the enzymatic defect in the vast majority of cases. The application of steroid profiling by LC/MS/MS to the newborn screening for CAH is expected to reduce the false-positive results through its high analytical specificity and the potential to quantify several compounds in the same analytical run.

Clinical trials – new treatments

A delayed- and extended-release formulation of hydrocortisone for the treatment of congenital adrenal hyperplasia

A pharmacokinetic and pharmacodynamic study of delayed- and extended-release hydrocortisone (Chronocort™) vs. conventional hydrocortisone (Cortef™) in the treatment of congenital adrenal hyperplasia

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Background: Current glucocorticoid therapy for congenital adrenal hyperplasia (CAH) is suboptimal and non-physiologic. This study investigated whether a new modified-release hydrocortisone (MR-HC) formulation (Chronocort™) is able to simulate physiologic cortisol secretion more closely compared with the conventional hydrocortisone (HC) formulation (Cortef™) in patients with CAH.

Methods: A phase 2, open-label, crossover pharmacokinetic and pharmacodynamic study was conducted in 14 patients (age range 17–55 years) with classic 21-hydroxylase deficiency. One week of thrice-daily HC (10, 5 and 15 mg) was followed by 1 month of once-daily MR-HC (30 mg at 22:00 h). Cortisol, 17-hydroxyprogesterone (17-OHP), androstenedione and ACTH concentrations were determined over a 24-hour period.

Results: Hydrocortisone therapy resulted in three cortisol peaks, while MR-HC resulted in a single cortisol peak at approximately 06:00 h. Treatment with MR-HC resulted in significantly lower afternoon (12:00–20:00 h) and night-time (20:00–04:00 h), but higher morning (04:00–12:00 h) cortisol concen-

trations compared with HC. Patients on MR-HC had significantly higher afternoon (12:00–20:00 h) 17-OHP, androstenedione and ACTH, but significantly lower 08:00 h 17-OHP concentrations. No serious adverse events occurred.

Conclusions: Modified-release hydrocortisone represents a promising new treatment for CAH. Overnight adrenal androgens were well controlled, but rose in the afternoon with once-daily dosing, suggesting that a morning dose of glucocorticoid is needed.

Novel strategies for hydrocortisone replacement

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Current therapy with immediate-release hydrocortisone is the most commonly used regimen for replacement in patients with primary and secondary adrenal insufficiency. However, conventional hydrocortisone treatment cannot simulate physiologic cortisol production. Twice- or thrice-daily doses of hydrocortisone substitution inevitably result in temporary over- or under-replacement. Therefore, patients with adrenal insufficiency have a poor quality of life and an increased mortality despite optimal doses and adherence to treatment. Recent studies have investigated circadian hydrocortisone therapy imitating the physiologic circadian cortisol rhythm. Proof-of-concept studies using hydrocortisone infusions predict an improvement in both the biochemical control and the quality of life in treated patients. Delayed- and sustained-release oral formulations of hydrocortisone are being developed, which are expected to offer a more practical and effective solution for patients with adrenal insufficiency and congenital adrenal hyperplasia.

Classic CAH is characterized by a defect in cortisol and aldosterone secretion, impaired development and function of the adrenal medulla, and adrenal hyperandrogenism [19]. Current treatment aims to provide adequate glucocorticoid and, when necessary, mineralocorticoid substitution to prevent adrenal crises, and to suppress the excess secretion of androgens and steroid precursors from the adrenal cortex. However, the currently available formulations of hydrocortisone are not able to simulate the normal cortisol circadian rhythm, and patients are often at risk for developing in tandem iatrogenic Cushing's syndrome and hyperandrogenism [24, 25]. The use of a delayed- and extended-release formulation of hydrocortisone represents a new treatment approach to CAH that offers the prospect of a more physiologic cortisol replacement. Chronocort™ is a novel modified-release formulation of hydrocortisone designed to approximate physiologic cortisol secretion. In a proof-of-principle study, Chronocort™ was shown to mimic the circadian rhythm of serum cortisol secretion in healthy volunteers [26]. In patients with classic CAH, Chronocort™ achieved good overnight and early morning (but not afternoon or evening) control of ACTH and adrenal androgen secretion. Physiologic cortisol replacement in patients with CAH or adrenal insufficiency is expected to offer improved biochemical control and quality of life, as well as to decrease the risk for the development of co-morbidities owing to under- and/or over-treatment with glucocorticoids in these patients. Further studies are needed to determine the optimal dosing regimen and long-term clinical outcome.

Non-coding RNA gas5 is a growth arrest- and starvation-associated repressor of the glucocorticoid receptor

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Background: The availability of nutrients influences cellular growth and survival by affecting gene transcription. Glucocorticoids also influence gene transcription and have diverse activities on cell growth, energy expenditure and survival.

Methods and Results: The growth arrest-specific 5 (Gas5) non-coding RNA, which is abundant in cells whose growth has been arrested because of lack of nutrients or growth factors, induced apoptosis by suppressing glucocorticoid-mediated induction of several responsive genes, including the cellular inhibitor of apoptosis 2. Gas5 bound to the DNA-binding domain of the glucocorticoid receptor (GR) and competed with DNA glucocorticoid-response elements for binding to the GR.

Conclusions: Gas5 modulates the transcriptional activity of the GR by acting as a 'riborepressor', thereby influencing cell survival and metabolic activities during starvation.

Non-coding RNAs (ncRNAs) have diverse regulatory functions and may affect every aspect of organismal biology by influencing messenger RNA (mRNA) transcription, degradation and translation, the nuclear translocation of proteins, or both protein abundance and localization [27]. One such single-strand ncRNA is the growth arrest-specific 5 (Gas5) [28]. Gas5 is expressed in growth-arrested cells as a result of nutrient deprivation or growth factor withdrawal. The present study has demonstrated that Gas5 is a strong interactant of the DNA-binding domain (DBD) of the GR, another ubiquitous molecule with major functions in behavioral, cardiovascular, metabolic and immune homeostasis. More specifically, Gas5 ncRNA interacts with the DBD of the ligand-activated GR through a decoy RNA 'GRE' and suppresses the GR-induced transcriptional activity of endogenous glucocorticoid-responsive genes by inhibiting binding of the GRs to target genes' GREs. These findings indicate that Gas5 ncRNA functions as a starvation- or growth arrest-linked riborepressor of the GR and possibly other steroid hormone receptors. Given that relative starvation produces a favorable metabolic profile and prolongs life in several organisms, while increased glucocorticoid secretion or activity is associated with an unfavorable metabolic profile and decreased life expectancy, the observed Gas5-GR interaction might be of physiologic and/or pathologic importance.

Aldo-keto reductase 1B7 and prostaglandin F_{2α} are regulators of adrenal endocrine functions

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Background: Prostaglandin F_{2α} (PGF_{2α}) represses ovarian steroidogenesis and initiates parturition in mammals but its impact on adrenal gland is not known. Prostaglandin biosynthesis depends on the sequential action of upstream cyclooxygenases (COX) and terminal synthases but no PGF_{2α} synthases (PGFS) were functionally identified in mammalian cells. In vitro, the most efficient mammalian PGFS belong to aldo-keto reductase 1B (AKR1B) family. The adrenal gland is a major site of AKR1B expression in both human (AKR1B1) and mouse (AKR1B3, AKR1B7).

Methods and Results: This study examined the PGF_{2α} biosynthetic pathway and its functional impact on both cortical and medullary zones using in vivo approaches and murine cell culture models. Both cortical and medullary compartments were shown to produce PGF_{2α} but expressed different biosynthetic isozymes. In chromaffin cells, PGF_{2α} secretion appeared constitutive and correlated to continuous

expression of COX1 and AKR1B3. In steroidogenic cells, PGF_{2α} secretion was stimulated by ACTH and correlated to ACTH responsiveness of both COX2 and AKR1B7/B1. The pivotal role of AKR1B7 in ACTH-induced PGF_{2α} release and functional coupling with COX2 was demonstrated using over- and down-expression in cell lines. PGF_{2α} receptor was only detected in chromaffin cells, making medulla the primary target of PGF_{2α} action.

Conclusions: PGF_{2α} repressed glucocorticoid secretion by a decrease in catecholamine release, which in turn decreased adrenal steroidogenesis. PGF_{2α} may be regarded as a negative autocrine/paracrine regulator within a novel intra-adrenal feedback loop.

PGF_{2α} is an essential autocrine/paracrine regulator of ovarian and testicular steroidogenesis. However, little was known about its possible impact on the adrenal gland. The present study provides a new insight in the understanding of PGF_{2α} biosynthesis and its role in the function of the adrenal gland. Martinez and colleagues established for the first time that PGF_{2α} acts as a negative regulator of both adrenocortical and adrenomedullary functions, and that the coordinate cell-specific regulation of both cyclooxygenases (COX1 and COX2) and aldo-keto reductases of the AKR1B subfamily (AKR1B7, AKR1B1, AKR1B3) could play a pivotal role in the generation of this signal.

The role of TASK1 in aldosterone production and its expression in normal adrenal and aldosterone-producing adenomas

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Background: Aldosterone production in the adrenal glomerulosa is mainly regulated by angiotensin II and K⁺. In mice, genetic deletion of subunits of K⁺-selective leak-channels (KCNK), TASK1 and/or TASK3, leads to hyperaldosteronism and histological changes in the adrenal cortex. This study investigated the expression of TASK1 in human adrenocortical cells and its role in aldosterone production in H295R cells.

Methods: TASK1 expression was investigated by comparative microarray analysis of aldosterone-producing adenomas (APA) and normal adrenals (NA). The effects of TASK1 knockdown by siRNA transfection were investigated in H295R cells. Fluo-4 fluorescent measurements of intracellular Ca²⁺ and pharmacological inhibition of Ca²⁺-dependent calmodulin kinases (CaMK) were performed to better define the effects of TASK1 on Ca²⁺ signaling pathways.

Results: The expression of TASK1 did not differ between APA and NA. However, in APA, NA and H295R cells the expression of TASK1 was predominant when compared to other KCNK family members. Knockdown of TASK1 induced the expression of steroidogenic acute regulatory (StAR) protein and aldosterone synthase (CYP11B2), and stimulated pregnenolone and aldosterone production. Cells transfected with siTASK1 had increased intracellular Ca²⁺, leading to activation of CaMK and increased expression of CYP11B2.

Conclusions: Human adrenal cortex displays predominant expression of TASK1 over other KCNK family genes. In addition, TASK1 plays an important role in the regulation of human aldosterone production through regulation of intracellular Ca²⁺ and CaMK signaling pathways.

Primary hyperaldosteronism (PA) is the most common cause of secondary hypertension. Aldosterone biosynthesis in the zona glomerulosa of the adrenal cortex is physiologically regulated by angiotensin II (Ang II), K⁺ and ACTH. Binding of Ang II to its type 1 receptor (AT1R) stimulates a variety of signaling cascades, leading to the release of Ca²⁺ from the endoplasmic reticulum and subsequent cell membrane depolarization with additional flow of extracellular Ca²⁺ into the cytoplasm. These events stimulate the early and the late regulatory steps in aldosterone production, StAR protein and CYP11B2 respectively, culminating with elevated aldosterone production. Adrenal glomerulosa cells are sensitive to small increases in extracellular K⁺, a characteristic that is associated with the expression of high levels of KCNK channels, including the TWIK-related acid sensitive K⁺ 1 and 3 (TASK1 and TASK3). Recent studies have demonstrated that deletion of TASK1 and TASK3 in animal models leads to PA [29, 30]. Furthermore, a loss-of-function TASK3 mutation has been described in patients with Birk Barel syndrome [31]. The present study sought to define the role of TASK1 in adrenal cell aldosterone production, as well as its potential role in PA. The authors demonstrated for the first time that

TASK1 is the predominant KCNK family member expressed in the human adrenal cortex, and that TASK1 knockdown stimulated aldosterone production through augmentation of Ca^{2+} flux and activation of CaMK in human adrenocortical cells. These intracellular signaling events culminated with the activation of early (StAR) and late (CYP11B2) rate-limiting steps in aldosterone production. Further studies are necessary to evaluate functional changes in TASK1 that could potentially contribute to conditions associated with increased aldosterone production. A TASK1-related clinical syndrome has not been described yet.

New genes

Germline mutations in TMEM127 confer susceptibility to pheochromocytoma

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Background: Pheochromocytomas are catecholamine-secreting tumors of neural crest origin that are often hereditary. The molecular basis of the majority of these tumors is not known.

Methods and Results: The transmembrane-encoding gene TMEM127 was identified on chromosome 2q11 as a new pheochromocytoma susceptibility gene. In a cohort of 103 samples, truncating germline TMEM127 mutations were detected in approximately 30% of familial tumors and 3% of sporadic pheochromocytomas without a known genetic cause. In tumor DNA, the wild-type allele was consistently deleted, indicating a classic mechanism of tumor suppressor gene inactivation. Pheochromocytomas with mutations in TMEM127 showed hyperphosphorylation of mammalian target of rapamycin (mTOR) effector proteins. Accordingly, in vitro gain-of-function and loss-of-function analyses indicated that TMEM127 is a negative regulator of mTOR.

Conclusions: This study identified TMEM127 as a tumor suppressor gene and validated the power of hereditary tumors to elucidate cancer pathogenesis.

The present study identified TMEM127 as a new gene conferring susceptibility to pheochromocytoma. TMEM127 has features of a classic tumor suppressor gene and TMEM127 mutations are associated with predisposition to pheochromocytoma development. Clinically, subjects with TMEM127 mutations developed pheochromocytomas in the fourth decade of life, which is similar to the mean age at diagnosis of sporadic pheochromocytomas, but notably older than the mean age at diagnosis of syndromic cases. All tumors arose from the adrenal medulla and were bilateral in approximately half of the cases. No malignancies or recurrences were detected during follow-up of the affected subjects. TMEM127 is predicted to encode a protein with three transmembrane regions and no clearly recognizable functional domains. Its sequence is highly conserved throughout evolution and putative orthologs can be identified in many species, from mammals to fish. Human TMEM127 is broadly expressed both in normal tissue and in a diverse group of cancer cell lines, with variable transcription levels that may reflect tissue of origin. The results of this study support the notion that disruptions of TMEM127 function might underlie tumors with an aberrant mTOR pathway and validate the relevance of hereditary tumor models to shed light on cell growth-related signals.

Isolated 17,20-lyase deficiency due to the cytochrome b5 mutation W27X

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Background: Cytochrome P450c17 (P450c17) is a bifunctional enzyme necessary for the production of glucocorticoids (17-hydroxylase activity) and sex steroids (17,20-lyase activity). Isolated 17,20-lyase deficiency is a rare condition characterized by deficient production of androgens, leading to 46,XY disorders of sex development (DSD), while the production of glucocorticoids is intact. Cytochrome b5 (CytB5) is an important factor in 17,20-lyase activity, probably by acting as an allosteric factor. This study investigated the role of CytB5 in a patient with 17,20-lyase deficiency.

Methods: A 46,XY DSD patient with 17,20-lyase deficiency was studied. No mutation in the CYP17A1 gene had been detected in the patient and his parents. Steroid hormone concentrations were determined and sequencing the CYB5 gene was performed.

Results: A homozygous W27X mutation was detected, leading to the formation of a premature stop codon. The parents of the index case were heterozygous carriers of this mutation. This mutation results in the absence of residues E48 and E49 of CytB5, which are necessary for an intact 17,20-lyase activity.

Conclusion: In addition to CYP17A1 gene mutations, CytB5 mutations can result in 17,20-lyase deficiency.

Cytochrome P450c17 (P450c17) catalyzes the 17-hydroxylase and 17,20-lyase reactions required for the production of glucocorticoids and sex steroids. To exert its activities, P450c17 requires electron donation from reduced nicotinamide adenine dinucleotide phosphate through its redox partner protein cytochrome P450 oxidoreductase (POR). POR mutations have been discussed at length in previous *Yearbooks*. The cofactor cytochrome b5 (CytB5) is also required for optimal 17,20-lyase activity. This protein has electron-donating capacity, can accept electrons from POR and appears to participate in electron transfer for some cytochrome P450 reactions. CytB5 is not an effective electron donor to P450c17, but rather acts as an allosteric factor that fosters the interactions of POR with P450c17, enhancing 17,20-lyase activity without influencing 17-hydroxylase activity [32]. The absence of CytB5 results in low, but not absent, 17,20-lyase activity. In the patient described in this article, the clinical manifestations (bifid scrotum, penoscrotal hypospadias, bilaterally palpable gonads), endocrine evaluation and increased methemoglobin concentrations indicated impaired CytB5 function. Sequencing of the CytB5 gene revealed a mutation that led to a premature stop codon and a truncated protein lacking the E48 and E49 residues that are necessary for intact 17,20-lyase activity [33]. A case of CytB5 deficiency has been reported previously in a patient with ambiguous genitalia and methemoglobinemia, however no steroid hormone concentrations were determined [34]. In patients with 46XY DSD and evidence of 17,20-lyase deficiency, screening for mutations in the CytB5 gene, as well as for mutations in CYP17A1 and POR genes, should be considered.

Reviews

Stress and disorders of the stress system

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All living organisms maintain a complex dynamic equilibrium, or homeostasis, which is constantly challenged by internal or external adverse forces termed stressors. Stress occurs when homeostasis is threatened or perceived to be so; homeostasis is re-established by various physiologic and behavioral adaptive responses. Neuroendocrine hormones have major roles in the regulation of both basal homeostasis and responses to threats, and are involved in the pathogenesis of diseases characterized by dyshomeostasis or cacostasis. The stress response is mediated by the stress system, partly located in the central nervous system

Fig. 2. Homeostatic systems exert their effects in an inverse, U-type dose response. Eustasis is in the middle, optimal range of the curve. Suboptimal effects may be on either side of the curve and can lead to suboptimal adaptation, termed 'allostasis' or, more correctly, 'cacostasis', which may be harmful for the organism in the short or long term.

and partly in peripheral organs. Optimal basal activity and responsiveness of the stress system is essential for a sense of well-being, successful performance of tasks, and appropriate social interactions. By contrast, excessive or inadequate basal activity and responsiveness of this system might impair development, growth and body composition, and lead to a host of behavioral and somatic pathologic conditions.

Stress occurs when homeostasis is threatened or perceived to be threatened. The stressors are potentially adverse forces, which can be emotional or physical. Both the magnitude and the chronicity of stressors are important. When any stressor exceeds a certain severity or temporal threshold, the adaptive homeostatic mechanisms of the organism activate compensatory responses that functionally correspond to the stressor. The homeostatic mechanisms exert their effects in an inverted U-shaped dose-response curve. Basal, healthy homeostasis (eustasis) is achieved in the central, optimal range of the curve, whereas suboptimal effects may occur on either side of the curve and can lead to insufficient adaptation (allostasis or cacostasis) (fig. 2). The latter might be harmful for the organism, and might account for many acute or chronic disorders. Prenatal development, infancy, childhood and adolescence are times of increased vulnerability to stressors.

Narrative review: the emerging clinical implications of the role of aldosterone in the metabolic syndrome and resistant hypertension

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The prevalence of obesity, diabetes, hypertension, cardiovascular and chronic kidney disease is increasing in developed countries. Obesity, insulin resistance and hypertension commonly cluster with other risk factors for cardiovascular and chronic kidney disease to form the metabolic syndrome. Emerging evidence suggests that excess circulating aldosterone concentrations impair insulin metabolic signaling and endothelial function, which in turn leads to insulin resistance and cardiovascular and renal structural and functional abnormalities. Furthermore, the cardiovascular and renal abnormalities associated with insulin resistance are mediated in part by aldosterone acting on the mineralocorticoid receptor. Mineralocorticoid receptor blockade improves pancreatic insulin release, insulin-mediated glucose utilization, and endothelium-dependent vasorelaxation. Aldosterone excess has detrimental metabolic effects that contribute to the metabolic syndrome and endothelial dysfunction, which in turn lead to the development of resistant hypertension, as well as cardiovascular disease and chronic kidney disease.

Obesity, insulin resistance, and hypertension commonly cluster with other risk factors for cardiovascular or chronic kidney disease to form the metabolic syndrome, which is associated with increased cardiovascular morbidity and mortality [35]. A new paradigm indicates that elevated concentrations of plasma aldosterone mediate several maladaptive changes that contribute to the pathogenesis of the metabolic syndrome, resistant hypertension, and associated cardiovascular and renal structural and functional abnormalities [36, 37]. Many of these adverse effects are mediated through rapid, membrane-initiated non-genomic actions of aldosterone. Emerging evidence suggests that mineralocorticoid receptor blockade is useful in treating resistant hypertension and in preventing cardiovascular and chronic kidney disease in patients with the metabolic syndrome and diabetes. Future research studies should further delineate the role of mineralocorticoid receptor blockade in the management of the metabolic syndrome and resistant hypertension.

Food for thought

Social disadvantage and overcommitment are associated with impaired HPA function

Higher overcommitment to work is associated with higher plasma cortisol but not ACTH responses in the combined dexamethasone/CRH test in apparently healthy men and women

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Background: Overcommitment (OC) is a pattern of excessive striving that has been associated with alterations in the hypothalamic-pituitary-adrenal (HPA) axis. This study investigated whether OC is associated with alterations in the function of HPA axis.

Methods: 92 men and 108 women of a wide range of OC scores were recruited to participate in this cross-sectional study. OC, depressive symptoms (Beck Depression Inventory, BDI) and work stress (effort-reward-imbalance, ERI) were assessed by questionnaires. Cortisol and ACTH concentrations were determined following a combined dexamethasone/CRH test.

Results: Independent of age and gender, higher OC was associated with higher repeated cortisol but not ACTH secretion. Similarly, higher cortisol but not ACTH increase following CRH stimulation was predicted by higher OC. Depressive symptoms (BDI score) and work stress scores (effort-reward-ratio) did not relate to neuroendocrine responses to the dexamethasone/CRH test. OC was not associated with ACTH or cortisol pre-test concentrations.

Conclusions: With increasing OC scores, a higher reactivity of the adrenal cortex together with a normal reactivity of the pituitary is observed following CRH stimulation.

Overcommitment (OC) is considered to be an enduring cognitive-motivational pattern of maladaptive coping with demands characterized by the inability to withdraw from obligations combined with a high need for control and approval [38]. OC individuals are extremely ambitious and tend to exaggerate their efforts while at the same time they overestimate their resources. Prospective studies have demonstrated that OC independently increases the risk for coronary heart disease (CHD) [39–41]. Although the underlying mechanisms have not been fully delineated, emerging evidence suggests that OC is associated with alterations in the HPA axis function. In this study, Zobel and colleagues demonstrated that OC is independently associated with higher increases in cortisol but not ACTH concentrations following a combined dexamethasone/CRH test, suggesting an association between OC and HPA axis dysregulation. From a clinical perspective, OC is associated with an increased risk of depression. OC and depression are known to increase the risk of CHD and might share a common biological mechanism of HPA axis dysregulation. Further studies are required to determine the clinical implications of these findings (including insulin resistance, obesity, hyperten-

sion and atherosclerotic cardiovascular disease), as well as the mechanisms underlying the link between OC and CHD.

Follow-up on Yearbook 2009

Fertility in non-classic congenital adrenal hyperplasia

Fertility in women with non-classical congenital adrenal hyperplasia due to 21-hydroxylase deficiency

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Background: Although fertility has been evaluated in women with the classic form of congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency, little is known about fertility in women with the non-classic form of the disease (NC-CAH).

Methods: 161 women with NC-CAH (age range 13–52 years), who presented with hirsutism (78%), menstrual irregularities (61%) or infertility (12%), and 29 affected female relatives were studied. The diagnosis had been confirmed by an ACTH stimulation test and sequencing of the CYP21A2 gene.

Results: 95 of the 190 women (age 26.7 ± 8.9 years) wanted to become pregnant; 187 pregnancies occurred in 85 women, which resulted in 141 births in 82 of them; 83% of pregnancies were obtained within 1 year. The rate of miscarriages was 6.5% for pregnancies obtained with glucocorticoid treatment and 26.3% for pregnancies obtained without treatment.

Conclusions: Subfertility is mild in NC-CAH. However, the rate of miscarriages is lower in pregnancies occurring with glucocorticoid treatment, suggesting that glucocorticoid treatment should be instituted in patients who want to conceive.

Although fertility problems are addressed in adulthood, the issue is often raised by the pediatric endocrinologists and is discussed with patients and their parents during adolescence. Last year's *Yearbook* addressed fertility in classic CAH. The present study provides a follow-up, investigating fertility in a large group ($n = 190$) of women with non-classic CAH. The study showed that fertility problems in NC-CAH are mild compared to the classic forms of the disease [42, 43]. The cumulated pregnancy rate for the 90 women who wanted to become pregnant was 76% at 1 year, which is slightly less than in the general French population (92%). Interestingly, the group of patients not receiving glucocorticoid treatment reported a higher number of miscarriages (26%) compared with the group receiving glucocorticoid treatment (6.5%) or the general population (10–15%). These findings suggest that introducing glucocorticoid treatment in women with NC-CAH who want to conceive may improve pregnancy rates.

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