Adrenals

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This year we have moved from Reproductive Endocrinology to the Adrenals. As always, we have tried to focus on papers that are clinically relevant or at least herald concepts and mechanisms that might be relevant to pediatric endocrinology in the future. As is often the case, pediatric research – especially in the field of therapeutics – tends to lag behind adult medicine. However, increasing evidence suggests that many aspects of adrenal function can be programmed in early life and many ‘long-term’ consequences of adrenal disease have their roots in childhood or adolescence. Therefore, we are in a prime position to try to relate new concepts and mechanisms to the pediatric population with potentially long-term benefits for individual and population health.

Mechanisms of the year: a new task for the adrenal

TASK channel deletion in mice causes primary hyperaldosteronism

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Background: Inappropriate, autonomous overproduction of aldosterone from the adrenal gland is the most frequent cause of secondary hypertension. However, the etiology of most forms of bilateral idiopathic hyperaldosteronism is unknown. TWIK-related acid-sensitive K (TASK)-1 or TASK-3 channels may play an important role in regulating potassium currents and polarization of the zona glomerulosa cell membrane. Modulation of this system may affect aldosterone release.

Methods: TASK-1- and TASK-3-deleted mice were generated and adrenal function was studied.

Results: Although TASK channel-deleted mice (TASK^{-/-}) were able to adjust urinary sodium excretion and aldosterone production to match sodium intake, these animals produced more aldosterone than control mice across the whole range of Na intake. Aldosterone overproduction was largely independent of renin-angiotensin activity, as renin levels were normal or lower in knockout animals. In addition, TASK^{-/-} deleted mice failed to suppress aldosterone production in response to dietary Na loading and did not normalize aldosterone following candesartan blockade of the angiotensin type 1 receptor.

Conclusion(s): TASK^{-/-} channel knockout mice exhibit a phenotype of primary hyperaldosteronism and represent a novel animal model of nontumorigenic primary hyperaldosteronism. Consequently, TASK channels are potential therapeutic targets for treating primary hyperaldosteronism and for preventing hyperaldosterone-related cardiac and renal damage in the future.

Our traditional view of the hypothalamic-pituitary-adrenal (HPA) axis is of a hierarchical system with integrated feedback loops at different levels. However, it is emerging that local factors such as paracrine influences and ultrashort feedback loops may be important in establishing the ‘set point’ for responsiveness at the different levels of the axis. It is likely that other mechanisms exist that make the adrenal more or less likely to respond to a given stimulus, and inter-individual variability in these mechanisms could be an important factor that contributes to the susceptibility to certain disease states. In this report by Davies et al. (and related findings for the TASK1-deleted mouse by Heitzmann et al. [1], TASK channels were identified as local adrenal regulators of zona glomerulosa function and aldosterone release. TASK channel-deleted animals showed increased aldosterone concentrations across a range of sodium intake states, which were largely independent of renin-angiotensin activity. This model is important as non-tumorigenic hyperaldosteronism is emerging as a significant cause of hypertension (15–20%). Bilateral idiopathic hyperaldosteronism is a major subtype of this, which is usually refractory to standard antihypertensive treatment. The TASK-deleted mouse may provide a

model for this condition and TASK channels or related mechanisms may be important novel therapeutic targets for modulating aldosterone release. It is not yet known whether inter-individual variability in TASK channel activity exists in humans or whether aldosterone hyperresponsiveness might be detected in childhood, which could predict disease later in life. Clearly, long-term exposure to elevated aldosterone is associated with cardiac and renal sequelae, as outlined in the excellent review by Connell et al. [2; see also 3]. Whether this subgroup of the population could be identified and treated at a very early age remains to be seen.

Adrenal GRK2 upregulation mediates sympathetic overdrive in heart failure

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Background: Cardiac overstimulation by the sympathetic nervous system (SNS) is a salient characteristic of heart failure, reflected by elevated circulating levels of catecholamines. This SNS hyperactivity is likely to be pathogenic as β -adrenergic receptor (β AR) antagonists are effective in heart failure treatment. However, sympatholytic agents targeting α 2AR-mediated catecholamine inhibition have been unsuccessful.

Methods: Adrenal adrenergic receptor signaling was investigated in models of heart failure.

Results: During heart failure, there is substantial α 2AR dysregulation in the adrenal gland, triggered by increased expression and activity of G protein-coupled receptor kinase 2 (GRK2). Adrenal gland-specific GRK2 inhibition reversed α 2AR dysregulation in heart failure, resulting in lowered plasma catecholamine levels, improved cardiac β AR signaling and function, and increased sympatholytic efficacy of an α 2AR agonist.

Conclusion(s): Adrenal GRK2 activity is a novel molecular mechanism that may represent a new sympatholytic target to lower SNS activity in the treatment of heart failure.

The interaction between the adrenal and kidney in regulating salt (and water) balance is well established, and the discovery of atrial natriuretic peptide generated interest in crosstalk between the adrenal gland and heart. Potential interactions between cardiac function and adrenal regulation are explored further in this study by Lymperopoulos et al., which proposes that adrenal adrenergic receptor signaling is dysregulated in heart failure due to upregulated GRK2 expression, resulting in increased SNS activity. Whilst cardiac failure is far less common in the pediatric population than in adult medicine, the concept of adrenal modulation of SNS activity in relation to cardiac function may have important consequences for certain neonatal and pediatric intensive care settings. This study provides further evidence that the adrenal gland may set the tone for systemic physiology through novel cellular and molecular pathways [4].

New paradigms: nuclear receptors – it's all in your head

Central nervous system-specific knockout of steroidogenic factor 1 results in increased anxiety-like behavior

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Mol Endocrinol 2008;22:1403–1415

Background: Steroidogenic factor 1 (SF1) is a nuclear receptor that plays a key role in many aspects of adrenal and gonadal development, pituitary gonadotropin synthesis, and development of the ventromedial hypothalamic nucleus (VMH). SF1 knockout (KO) mice that are rescued by adrenal transplants show delayed-onset obesity and decreased locomotor activity but it is still unclear whether these effects are central or systemic in origin.

Methods: Using a Cre-loxP system, mice were generated with targeted deletion of SF1 in the central nervous system (CNS) to better define the specific roles of SF1 in the VMH.

Results: CNS-specific SF1 KO mice showed similar VMH structural defects to mice with global SF1 deletion. In multiple behavioral tests, mice with CNS-specific KO of SF1 had significantly more anxiety-like behavior than wild-type littermates. The expression of several stress/anxiety-related genes in the mediobasal hypothalamus was altered (e.g. brain-derived neurotrophic factor, the type 2 receptor for corticotropin-releasing hormone (Crhr2), and urocortin 3). Functional studies showed a direct role for SF1 in regulating Crhr2.

Conclusion(s): SF1 may modulate behavior through the hypothalamic expression of key regulators of anxiety-like behavior.

The nuclear receptor superfamily plays an important role in regulating many aspects of the hypothalamic-pituitary-adrenal axis. For example, glucocorticoid (GR [NR3C1]) and mineralocorticoid (MR [NR3C2]) receptors are essential for mediating the end-organ and feedback actions of adrenal steroids, and several other nuclear receptors (e.g. steroidogenic factor-1 [NR5A1], Nurr1 [NR4A2]) play a key role in HPA biology at different levels and stages of development. In addition to the classic endocrine effects of these nuclear receptors, increasingly literature is focusing on the role of nuclear receptors in behavior and mood. Many studies have reported links between cortisol and memory or depression, and a recent paper has shown, surprisingly, that MR overexpression in the forebrain is associated with decreased anxiety and altered stress response [5, 6]. SF1 is another nuclear receptor that has been shown to play a central role in VMH development and fetal hypothalamic programming as well as adrenal development and steroidogenesis. In this study, Keith Parker's group has shown that a CNS-specific Sf1 knockout mouse model has increased anxiety and alterations in the expression of several key genes thought to have dual HPA axis/stress-mediating effects (e.g. *Bdnf*, *Crhr2*). In humans, behavioral changes associated with adrenal disorders are frequent, and anxiety and depression are common features of adult patients with adrenal disease (see New Treatments). These recent studies on the CNS expression and function of the MR and SF1 suggest that we might need to think beyond just glucocorticoid effects or glucocorticoid:DHEA ratios.

New hope: identifying steroid targets

Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids

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Background: Two key features of asthma are airway inflammation and epithelial remodeling. These processes may be driven by IL-13 and other cytokines produced during T-helper type 2 cell-driven allergic inflammation. These inflammatory processes contribute to airway epithelial goblet cell metaplasia and potentially altered epithelial-mesenchymal signaling, resulting in subepithelial fibrosis or smooth muscle hyperplasia. Corticosteroids may mediate their therapeutic effects in asthma through direct or indirect inhibition of epithelial cell activation by inflammatory cells and cytokines.

Methods: Airway epithelial cells were collected from: (1) asthmatic subjects enrolled in a randomized controlled trial of inhaled corticosteroids; (2) from healthy subjects, and (3) from smokers (disease control). Gene expression microarrays were performed to identify markers of epithelial cell dysfunction and the effects of corticosteroids in asthma treatment.

Results: Three genes upregulated in asthma, but not in smokers, were CLCA1 (chloride channel, calcium-activated, family member 1), periostin, and serpinB2 (serine peptidase inhibitor, clade B (ovalbumin), member 2). Corticosteroid treatment downregulated expression of these three genes. High baseline expression of CLCA1, periostin, and serpinB2 was associated with a good clinical response to

steroid treatment. In contrast, corticosteroid treatment upregulated expression of FKBP51 (FK506-binding protein 51) and high baseline expression of FKBP51 was associated with a poor response. Finally, in cultured airway epithelial cells, IL-13 increased expression of CLCA1, periostin, and serpinB2. This effect was suppressed by corticosteroids. However, corticosteroids induced expression of FKBP51 in this cell system.

Conclusion(s): Airway epithelial cells in asthma have a distinct activation profile. Studying these expression patterns, together with the effects of corticosteroid treatment, may lead to identification of specific mechanisms involved in asthma.

Inhaled and even systemic steroids remain the mainstay of asthma treatment, but endocrinologists are often called upon to assess the iatrogenic effects of therapy (e.g., HPA axis suppression; metabolic and growth dysregulation; see also Reviews). Identifying the target genes of glucocorticoids in key disease tissues could lead to the development of more specific treatments that would avoid the need for generalized steroid exposure. In this study by Woodruff et al., microarray profiling was undertaken in airway epithelial cells from treated asthmatic subjects compared to controls to identify a subset of factors that are up- and downregulated during glucocorticoid treatment. Several consistent changes in inflammatory targets were observed. This study assumes that the greatest benefits of steroid treatment are via transcriptional regulation of target genes rather than through rapid non-genomic mechanisms. Nevertheless, this approach – with a specific focus on the diseased tissue in question – may ultimately prove important in identifying novel therapeutic targets.

New concerns: short-term dex, long-term effects?

Prenatal dexamethasone exposure induces changes in nonhuman primate offspring cardiometabolic and hypothalamic-pituitary-adrenal axis function

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J Clin Invest 2007;117:1058–1067

Background: Prenatal stress or glucocorticoid administration has been shown to have persisting ‘programming’ effects on offspring in rodents and other model species. However, prenatal glucocorticoids are widely used in several aspects of obstetric practice.

Methods: The aim of this study was to examine glucocorticoid programming in non-human primates (African Vervet) by giving 50, 120, or 200 µg/kg/day of dexamethasone (dex50, dex120, or dex200) orally from mid-term to singleton-bearing pregnant females.

Results: Dexamethasone dose-dependently reduced maternal cortisol levels without affecting maternal blood pressure, glucose, electrolytes, or weight gain. Birth weight was unaffected by any of the dexamethasone doses used, although postnatal growth was attenuated after dex120 and dex200. At 8 months of age, dex120 and dex200 offspring showed impaired glucose tolerance and hyperinsulinemia. At 12 months of age, dex120 and dex200 offspring had reduced (approximately 25%) pancreatic β cell number and increased systolic and diastolic blood pressure. Furthermore, mild stress produced an exaggerated cortisol response in dex200 offspring, which may reflect programming of the hypothalamic-pituitary-adrenal axis.

Conclusion(s): These observations are compatible with the extrapolation of the glucocorticoid programming hypothesis to primates and demonstrate that repeated glucocorticoid therapy and perhaps chronic stress in humans may have long-term effects.

Neonatal dexamethasone treatment for chronic lung disease of prematurity alters the hypothalamus-pituitary-adrenal axis and immune system activity at school age

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Pediatrics 2008;121:e870–e878

Background: Neonatal treatment with dexamethasone or hydrocortisone for chronic lung disease of prematurity may have long-term effects on the hypothalamus-pituitary-adrenal axis and the immune response in children at school age.

Methods: A retrospective, matched cohort study was performed in 156 children born prematurely. Children with chronic lung disease had been treated with dexamethasone ($n = 52$), hydrocortisone ($n = 52$) or nothing. The steroid treated groups were matched for gestational age, birth weight, gender, grade of infant respiratory distress syndrome, grade of periventricular or intraventricular hemorrhage, and year of birth. Plasma ACTH and cortisol in following a social stress task were determined in childhood (7–10 years). Cytokine production was assessed by in vitro stimulation of whole-blood cultures.

Results: The Trier Social Stress Test adapted for children induced an adrenocorticotropic hormone and cortisol response in all of the groups. However, the ACTH response was blunted in the dexamethasone group and overall cortisol concentrations were lower, whereas the hydrocortisone and reference groups showed similar ACTH and cortisol responses. The ratio of T-cell mitogen-induced interferon- γ /interleukin-4 secretion was significantly higher in the dexamethasone group than in the hydrocortisone group. Interferon- γ production and the ratios of interferon- γ /interleukin-4 and interferon- γ /interleukin-10 were significantly higher in the dexamethasone group than the reference group. Production of these cytokines did not differ between the hydrocortisone and the reference groups.

Conclusion(s): Treatment of premature babies with dexamethasone but not with hydrocortisone resulted in long-lasting programming effects on the hypothalamus-pituitary-adrenal axis as well as on T-helper 1/T-helper 2 cytokine balance. Long-term follow-up of these children is needed to investigate the clinical consequences of these effects. Immune and neuroendocrine function should be considered in other studies of long-term outcome following neonatal glucocorticoid treatment.

Prenatal dexamethasone has been used in the treatment of CAH and preterm labor for several years and postnatal dexamethasone has been used in preterm infants for the prevention and treatment of chronic lung disease. Whilst these treatments are often clinically effective, the debate continues as to whether early dexamethasone exposure can have long-term 'programming' effects on development and function. Two potentially important studies published this year have used somewhat different approaches to try to address these issues. By using a non-human primate model, de Vries et al. have shown that prenatal exposure to repeated high doses of dexamethasone was associated with altered β cell function and blood pressure in offspring between 8 and 12 months of age (see also Hauser et al. [7] for a study in marmoset monkeys). In addition, Karemaker et al. have shown in a retrospective cohort study that postnatal dexamethasone treatment (0.5 mg/kg/day) for lung disease in preterm humans was associated with altered neuroendocrine stress response and immunological parameters in late childhood. These effects were not observed in a 'control' group who had received hydrocortisone treatment (5 mg/kg/day) instead of dexamethasone. Although these cohorts were evenly matched for some important parameters, nonequivalent steroid doses were used, the dexamethasone group were slightly younger and smaller, and the hydrocortisone-treated cohort were all from a single center. Therefore, it is unclear whether selection or treatment variations could have contributed to these differences. These studies highlight some of the many methodological difficulties when dealing with complex diseases and subtle outcome variables. Indeed, with changes in prenatal genetic counseling (e.g. free-fetal DNA analysis in early pregnancy), obstetric practice, and postnatal management of preterm babies (e.g. oscillatory ventilation, nutritional regimen), it is likely that the 'goalposts' will continue to keep moving.

Hydrocortisone therapy for patients with septic shock

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N Engl J Med 2008;358:111–124

Background: Hydrocortisone is widely used in patients with septic shock. However, studies to date have only shown survival benefit in patients who remained hypotensive after fluid and vasopressor resuscitation and whose plasma cortisol response to corticotropin was inadequate.

Methods: A multicenter, randomized, double-blind, placebo-controlled trial was performed in 251 patients (50 mg of intravenous hydrocortisone every 6 h for 5 days) and 248 patient controls (placebo); the dose was then tapered over a 6-day period. All participants had a corticotropin stimulation test performed before treatment/placebo. The primary outcome was death (at 28 days) among patients who did not have a response to a corticotropin test.

Results: Of the 499 patients in the study, 233 (46.7%) had a suboptimal response to corticotropin (125 in the hydrocortisone group and 108 in the placebo group). At 28 days, there was no significant difference in mortality between patients in the two study groups who did not have a response to corticotropin (39.2% in the hydrocortisone group and 36.1% in the placebo group, $p = 0.69$) or between those who had a response to corticotropin (28.8% in the hydrocortisone group and 28.7% in the placebo group, $p = 1.00$). At 28 days, 86 of 251 (34.3%) patients in the hydrocortisone group and 78 of 248 (31.5%) patients in the placebo group had died ($p = 0.51$). Shock was reversed more quickly in the hydrocortisone group compared to the placebo group. However, treatment was associated with more episodes of superinfection, including new sepsis and septic shock.

Conclusion(s): Hydrocortisone did not improve survival or reversal of shock in patients with septic shock, irrespective of the initial cortisol response to corticotropin. However, reversal of shock was quicker with hydrocortisone in patients in whom shock was reversed. (ClinicalTrials.gov number, NCT00147004.)

HPA axis dysregulation frequently occurs in septic shock in children as well as adults, but investigations of adrenal function are often difficult to interpret and the benefits and risks of hydrocortisone replacement/treatment remain very controversial. To address this issue further, a large multicenter, randomized, double-blind, placebo-controlled trial has now been reported involving almost 500 patients undergoing resuscitation for severe sepsis. Hydrocortisone treatment for 5 days did not improve survival at 28 days. Indeed, steroid treatment was associated with more episodes of superinfection and mortality was nonsignificantly higher, even in those patients with an initial poor response to corticotropin stimulation. Although some patients had received prior etomidate, this highlights the challenges of assessing HPA function in the acute setting. It remains unclear whether these findings can be extrapolated to children. Certainly a very small group of children will need steroid treatment in the acute setting due to previously undiagnosed or known adrenal failure or sepsis-related adrenal hemorrhage. And how do you manage those patients refractory to adequate fluid resuscitation and vasopressor support? Recent International Consensus Guidelines from the *Surviving Sepsis Campaign* state that in pediatric severe sepsis ‘steroids (should) only (be used) in children with suspected or proven adrenal insufficiency’ [8]. However, the GRADE classification for evidence strength was low (2C). Until a similarly well-designed study is performed in the pediatric population questions about the risks and benefits of steroid treatment in this age group will remain.

Prevalence of testicular adrenal rest tumors in male children with congenital adrenal hyperplasia due to 21-hydroxylase deficiency

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Eur J Endocrinol 2007;157:339–344

Background: Testicular adrenal rest tumors (TART) are a well-known complication of congenital adrenal hyperplasia (CAH) in adult males, with a reported prevalence of up to 94%. These ‘tumors’ are associated with gonadal dysfunction, which may be secondary to longstanding seminiferous tubule obstruction. The prevalence and consequences of TART in childhood CAH is poorly understood.

Methods: A retrospective analysis was undertaken of scrotal ultrasound findings in 34 boys, aged 2 and 18 years, with CAH due to 21-hydroxylase deficiency, FSH, LH, testosterone and inhibin B concentrations were measured in the serum of 27 of these patients.

Results: TART was detected by ultrasound in 8 of 34 (24%) children (7 with salt-wasting CAH; 1 with simple virilizing CAH). Two of these children had bilateral tumors. Two children were under 10 years old and 6 were older. All lesions were located in the rete testis. Mean tumor size was 4.1 (range 2–8) mm and none were palpable clinically. There were no significant differences in LH, FSH, testosterone and inhibin B levels in boys with or without TART.

Conclusion(s): TART can be found in CAH children before the age of 10 years and is increasingly prevalent in adolescence. The absence of gonadal dysfunction in this group of children suggests that TART-associated gonadal dysfunction develops after childhood.

Testicular adrenal rest tumors and Leydig and Sertoli cell function in boys with classical congenital adrenal hyperplasia

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J Clin Endocrinol Metab 2007;92:4583–4589

Background: Infertility in adult males with congenital adrenal hyperplasia (CAH) is associated with testicular adrenal rest tumors (TART). These tumors may originate during childhood.

Methods: The prevalence of TART and Sertoli and Leydig cell function was assessed in a group of 19 boys with classic CAH (aged 2–10 years) and 13 normal controls matched for age (age 5.9 vs. 5.6 years; $p = 0.67$) and bone age:chronological age ratio. A complete physical examination was performed and high-resolution ultrasound was undertaken to determine the prevalence of TART. Inhibin B and anti-müllerian hormone were used as markers of Sertoli cell function. The ratio of basal:hCG-stimulated testosterone (72 h; 5,000 U/m² hCG) was used to evaluate Leydig cell response.

Results: TART prevalence was 4 of 19 (21%) in the CAH group. Lower values for inhibin B (49.2. vs. 65.2 pg/ml; $p = 0.018$), anti-müllerian hormone (70.1 vs. 94.2 ng/ml; $p = 0.002$), and (T72–T0)/T0 (5.6 vs. 13.6; $p < 0.01$) were observed in the CAH group.

Conclusion(s): TART can be found during childhood in prepubertal males with classic CAH. Differences in markers of gonadal function may exist in a subgroup of patients, especially in those with inadequate control.

The long-term consequences of CAH in adulthood are a major concern. Many excellent review articles have been published recently that address some of these issues [for example, see 9, 10], and several important papers have appeared this year to provide additional long-term follow-up data on bone mineral density [11], fracture risk [12], and the consequences of genital surgery [13, 14]. Whilst much focus has quite rightly been on females with CAH, attention is now turning to the long-term effects of CAH in males. As pediatric endocrinologists our main focus for the male CAH patient is on adrenal insufficiency (salt-loss, hypoglycemia, glucocorticoid replacement) and growth. However, infertility is emerging as a significant problem in adulthood, which may be related to the presence of TART. A series of recent publications from the Nijmegen group have described the adrenal-like characteristics of TART [15], the frequent location of this tissue around the rete testis [16] and no significant improvement in

fertility following testis-sparing surgical resection of 'tumor' tissue in a small group of adults [17]. Given these findings, it has been hypothesized that TART originates in childhood or adolescence and anatomical obstruction of the rete testis may subsequently damage testicular function and spermatogenic capacity. Two cross-sectional studies have now been published which show that subclinical TART can be detected by ultrasound in around 20–25% of CAH boys in adolescence and even in mid to late childhood. The obvious questions that arise, therefore, are: (1) whether those boys with poor control or late diagnosis are more likely to be affected; (2) whether childhood TART correlates with adult TART and impaired fertility in a longitudinal study; (3) whether improving control in late childhood and adolescence would be associated with regression (or a failure of progression) of disease and improved fertility potential, and (4) whether testis-sparing surgery might be more effective in childhood or early adolescence. Only the first question can be addressed in these cross-sectional studies, and the answer is unclear. Claahsen-van der Grinten et al. found no differences in basal LH, FSH, testosterone and inhibin B in those boys with or without TART. Most of the affected group (7/8) were salt-wasters (17/26, non-TART group), but there was no obviously greater incidence of poor control or advanced bone age in the tumor-affected group. Martinez-Aguayo et al. reported potentially worse control in 3 of the 4 boys diagnosed with TART and lower anti-müllerian hormone, but these numbers are very small to draw firm conclusions. Obviously larger, longitudinal studies are needed to address these issues properly. However, this may represent yet another area in which the pediatric endocrinologist can influence long-term outcome.

Diagnostic tests for children who are referred for the investigation of Cushing syndrome

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Pediatrics 2007;120:e575–e586

Background: Endogenous Cushing syndrome (CS) in children is a rare disorder that is most frequently caused by pituitary or adrenocortical tumors. Diagnostic criteria for CS have largely been based on studies in adult patients. However, the physiology of the hypothalamic-pituitary-adrenal axis differs in childhood and the epidemiology of CS in children is not the same as in adults. This study aimed to assess the reliability and efficiency of diagnostic tests for pituitary or adrenal tumors in a large cohort of pediatric patients with CS.

Methods: A retrospective review of clinical data was conducted in 125 children who were referred to a tertiary care center for evaluation of CS between 1997–2005. Within this cohort, 105 children were found to have CS (confirmed histologically) whereas 20 children who did not transpire to have CS or any other endocrinopathy served as the control group. All children had the following tests undertaken: midnight and morning cortisol; ACTH; urinary free cortisol and 17-hydroxycorticosteroid; a corticotropin-releasing hormone (ovine) stimulation test, and overnight high-dosage dexamethasone suppression test. Pituitary and adrenal imaging was also performed. The main outcome measure was the sensitivity of these tests for the diagnosis and differential diagnosis of CS at 100% specificity.

Results: A midnight cortisol value of $\geq 4.4 \mu\text{g/dl}$ (121 nmol/l) confirmed the diagnosis of CS with a sensitivity of 99% and a specificity of 100%. Suppression of morning cortisol levels $>20\%$ in response to an overnight, high-dosage dexamethasone test excluded all patients with adrenal tumors and identified most patients with pituitary tumors (sensitivity 97.5%; specificity 100%).

Conclusion(s): In this cohort of children referred for the evaluation of possible CS, a single cortisol value at midnight followed by overnight high-dosage dexamethasone test led to rapid and accurate confirmation and diagnostic differentiation of hypercortisolemia caused by pituitary and adrenal tumors, respectively.

Non-iatrogenic CS is a rare but extremely important diagnosis to make in childhood. Clinical features can be subtle and it is often difficult to know how intensively to investigate the obese child with potential CS features unless there is obvious growth failure. Although consensus guidelines and useful overviews of the investigation and management of CS have been published this year [18–20], it is clear that the criteria used for diagnosis in adults cannot be automatically applied to the pediatric or adolescent population. This retrospective study of a large cohort of children referred to a specialist center with potential CS provides an important contribution to the literature. A midnight cortisol

measurement and response to overnight, high-dose dexamethasone test were the most useful investigations. Although the strength of this study is clearly the large number of children identified with significant pathology, a little caution is needed as this cohort represents a highly pre-selected group; only 20/105 children turned out *not* to have significant adrenal pathology, and formed the control group. It would be useful to know if these cutoffs have the same specificity and positive predictive value for diagnosing Cushing disease or adrenal tumors in a less rigid clinical setting, where the proportion of children with obesity, hirsutism and/or depression is higher and the diagnostic yield is likely to be lower.

Clinical trials, new treatments: getting the delivery right

Impaired subjective health status in 256 patients with adrenal insufficiency on standard therapy based on cross-sectional analysis

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Background: Increasing evidence suggests that the current replacement regimen fails to fully restore health-related subjective health status in patients with adrenal insufficiency (AI). Here, the subjective health status in primary and secondary AI and the effect of concomitant disease was evaluated.

Methods: A cross-sectional study of subjective health status was performed in primary and secondary AI and the effect of concomitant disease was evaluated. All AI patients registered with the University Hospital Würzburg ($n = 148$) or with the German Self-Help Network ($n = 200$) were contacted by mail and medical records were reviewed to verify underlying diagnoses and comorbidities. Patients completed three validated self-assessment questionnaires [Short Form 36 (SF-36), Giessen Complaint List (GBB-24), Hospital Anxiety and Depression Scale (HADS)]. Age- and gender-matched controls were obtained from the questionnaire-specific reference cohorts.

Results: Of 348 patients identified, 256 agreed to participate and 210 completed the questionnaires [primary adrenal insufficiency ($n = 132$), secondary adrenal insufficiency ($n = 78$)]. A significant impairment of subjective health status was seen in both AI cohorts for seven of eight SF-36 dimensions, all five GBB-24 scales, and the HADS anxiety score (all $p < 0.001$). Even after exclusion of all patients with any concomitant disease, subjective health status remained significantly impaired in five SF-36 subscales and four GBB-24 subscales. Individuals with secondary AI were slightly more compromised than those with primary AI, significant with regard to two SF-36 scales ($p < 0.05$) and the HADS depression score ($p < 0.001$). Furthermore, a total of 18.3% of the AI patients were out of work compared to 4.1% in the general population.

Conclusion(s): Patients with AI on current standard replacement suffer from significantly impaired health-related subjective health status, irrespective of whether the adrenal disease is primary or secondary or whether concomitant disease is present. Future studies will have to assess whether these features might be improved by using more physiological glucocorticoid replacement strategies.

Modified-release hydrocortisone for circadian therapy: a proof-of-principle study in dexamethasone-suppressed normal volunteers

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Clin Endocrinol (Oxf) 2008;68:130–135

Background: Existing long-term glucocorticoid replacement therapy is suboptimal and does not reproduce the normal nocturnal rise in cortisol and waking morning peak. A more physiological pattern could be produced using oral delayed and sustained release preparations of hydrocortisone [Cortisol(ds)].

Methods: The study group consisted of 6 healthy male volunteers who attended on two occasions, in a single-dose, open-label, nonrandomized study. Endogenous cortisol secretion was suppressed by

administration of dexamethasone. Cortisol(ds) (formulation A or B) was administered at 22.00 h on day 1. Blood samples for measurement of cortisol were taken from 22.00 h every 30 min until 07.00 h, then hourly until 22.00 h on day 2. The control group comprised 15 body mass index (BMI)-matched subjects who had serum cortisol levels measured at 20-min intervals for 24 h. Serum cortisol profiles and pharmacokinetics after Cortisol(ds) in the study group were compared with the spontaneous cortisol profiles of the controls.

Results: Formulations A and B were associated with delayed drug release (by 2 and 4 h, respectively), with median peak cortisol concentrations at 4.5 h (02.45 h) and 10 h (08.00 h), respectively. Total cortisol exposure was not different from controls.

Conclusion(s): It is possible to mimic the normal circadian rhythm of circulating cortisol with an oral modified-release formulation of hydrocortisone. This approach could provide the basis for the development of physiological circadian replacement therapy in patients with adrenal insufficiency.

Continuous subcutaneous hydrocortisone infusion in Addison's disease

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Background: Conventional approaches to steroid replacement therapy in Addison's disease (AD) do not restore the normal diurnal cortisol rhythm. Continuous subcutaneous hydrocortisone infusion (CSHI) could be an alternative mode of glucocorticoid replacement therapy.

Methods: Seven patients with Addison's disease were treated with CSHI in an open-labelled clinical study for up to 3 months. The adequacy of glucocorticoid replacement was assessed by 24-hour blood and saliva sampling in 1 patient and by outpatient salivary cortisol day curves in 6 others. Subjective health status was monitored using the Short Form-36 questionnaire.

Results: CSHI reestablished the circadian variation and normal levels of cortisol in the patients, with minor day-to-day variation. Most patients were able to reduce their glucocorticoid dose considerably without adverse reactions. The treatment was well tolerated by the patients and evaluated positively.

Conclusion(s): CSHI is technically feasible and safe in patients with Addison's disease. A daily dose of approximately 10 mg/m² body surface area/day restores the circadian variation and normal levels of salivary cortisol in most patients, which is close to the estimated daily requirement.

The introduction of steroid replacement therapy was a major advance in saving the lives of patients with Addison's disease and adrenal insufficiency. However, with the exception of the DHEA replacement debate, our approach to steroid replacement therapy has shown little progress in recent years. As most glucocorticoids are nonproprietary and the number of patients affected is relatively small there has been little commercial incentive for the pharmaceutical industry to invest into research and drug development in this area. However, several studies have now been published that show that adults on adrenal replacement therapy have significant psychological morbidity (see Hahner et al., above). Whilst some of these studies may be subject to recruitment bias or patients' wellbeing may have been affected by confounding factors such as panhypopituitarism and autoimmune thyroid disease, it is well-established that current adrenal steroid replacement strategies are unphysiological, especially as the early morning rise in cortisol is delayed. Proof-of-principle studies using controlled release hydrocortisone or subcutaneous hydrocortisone infusions have now been published that may allow more physiological circadian delivery of adrenal steroid replacement in patients with adrenal disease [see also, 21, 22]. Will these replacement strategies improve wellbeing in adults or in children with adrenal disease? Will they be useful in improving control in CAH by suppressing an early morning ACTH drive? What will happen during sickness or with glycemic control in younger children? Many questions remain to be answered. It will clearly be impossible to undertake placebo-controlled studies to address these points, so there is risk of selection bias especially when psychological parameters are assessed. Nevertheless, adrenal steroid replacement may soon be catapulted into the 21st century.

Mutation in PDE8B, a cyclic AMP-specific phosphodiesterase in adrenal hyperplasia

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This year has seen relatively few new genetic discoveries related to adrenal disease in humans. One potential defect is a mutation in PDE8B in a child with bilateral micronodular adrenocortical hyperplasia leading to Cushing syndrome. The cyclic AMP-specific phosphodiesterase system is now well established as a mediator of certain aspects of the Carney Complex/adrenal hyperplasia, and this correspondence by Stratakis and colleagues describes a PDE8B change in a girl with adrenal overactivity. Notably her father carried the same change but had only mild symptoms (e.g. obesity, hypertension, abnormal midnight cortisol). Other interesting reports of adrenal disease this year include adrenal hypoplasia as part of SERKAL syndrome (female sex reversal and dysgenesis of kidneys, adrenals and lungs) in a child with a homozygous mutation in WNT4 [23].

New hormones: oranges and lemons ...

Human adrenal glands secrete vitamin C in response to adrenocorticotrophic hormone

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Background: Fasting plasma concentrations of vitamin C do not exceed 80 $\mu\text{mol/l}$, irrespective of dietary intake. Therefore, it is possible that local or paracrine variability in vitamin C may have biological effects.

Methods: To assess whether paracrine secretion of vitamin C occurs from the adrenal glands, adrenal and peripheral vein vitamin C and cortisol measurements were taken following intravenous administration of ACTH in 26 patients with hyperaldosteronism.

Results: Adrenal vein vitamin C concentrations increased in all cases and reached a peak of $176 \pm 71 \mu\text{mol/l}$ at 1–4 min, whereas the corresponding peripheral vein vitamin C concentrations were $35 \pm 15 \mu\text{mol/l}$ ($p < 0.0001$). Mean adrenal vein vitamin C increased from $39 \pm 15 \mu\text{mol/l}$ at 0 min, rose to $162 \pm 101 \mu\text{mol/l}$ at 2 min, and returned to $55 \pm 16 \mu\text{mol/l}$ at 15 min. Adrenal vein vitamin C release preceded the release of adrenal vein cortisol (and peripheral cortisol), which peaked at 15 min.

Conclusion(s): ACTH stimulation increased adrenal vein vitamin C concentrations, but not peripheral vein concentrations. Adrenal vitamin C paracrine secretion may be part of a stress response. Tight control of peripheral vitamin C concentration is permissive of higher local concentrations that may have paracrine functions.

The medical benefits of vitamin C and consequences of vitamin C deficiency have been known for hundreds of years since reports of the treatment of scurvy on long distance sailing expeditions. Whilst the importance of dietary vitamin C is clear, much less attention has been paid to the local or paracrine actions of this vitamin. This challenging paper from this NIDDKD group shows a rapid rise in adrenal vein vitamin C concentrations following ACTH stimulation. These changes precede a rise in cortisol. Although this study has limitations as it was performed in patients undergoing investigation for hyperaldosteronism, it is probably unlikely that the underlying disease process had any affect on the phenomenon observed. Vitamin C is a water-soluble vitamin that is well known for its antioxidant

effects. Intra-adrenal concentrations of vitamin C reach levels within the *millimolar* range, as opposed to *micromolar* concentrations in the circulation. Within the adrenal gland, vitamin C may play a role in regulating oxidants generated during steroidogenesis as well as being a cofactor for norepinephrine synthesis in the medulla. Whether release of adrenal vitamin C into the local circulation in response to stress has any specific additional effects, or whether this observation is simply a 'washout' of vitamin C secondary to increased local blood flow is unclear. Nevertheless, this study hypothesizes that vitamin C may be a 'new' stress hormone, which certainly warrants further investigation.

Reviews: an inflammatory hypothesis

The hypothalamic-pituitary-adrenal axis in asthmatic children

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Overview: The hypothalamic-pituitary-adrenal (HPA) axis may be suppressed in patients with various chronic allergic inflammatory disorders and a blunted HPA axis response has been reported in poorly controlled asthmatics *before* long-term inhaled corticosteroids (ICS) treatment. Pro- and anti-inflammatory cytokines might be involved in the attenuation of cortisol and adrenocorticotrophic hormone (ACTH) responses to stress in these patients. Although long-term ICS treatment might produce mild adrenal suppression in some asthmatic children, improvement of adrenal function has been detected in the majority of cases.

Conclusion(s): This article postulates that the anti-inflammatory effects of ICS can result both in asthma remission and an improvement in HPA axis activity. Adrenal suppression of some asthmatic patients on maintenance ICS seems to be a separate phenomenon, possibly constitutionally or genetically determined.

Asthma is a major disease burden in children. Inhaled and sometimes systemic steroids are a mainstay of treatment. Certain inhaled steroids are reported to have quite potent dose-dependent adrenal suppression especially when higher than conventional doses are used. Whilst these side-effects are undoubtedly seen in some individuals, this challenging review claims that – in many cases – HPA activity is actually *improved* following the control of asthma by steroids once the repressive effects of circulating inflammatory agents are reduced. Larger systematic studies will be needed to prove this. However, this is a clear example of how individual variability in response to treatment agents and their potential side effects might be an important consideration as we enter the era of personalized pharmacogenomics.

Food for thought: Is your pet your next paper?

Adrenal gland disease in ferrets

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Overview: Adrenal gland disease in ferrets has unique characteristics, with clinical signs and pathophysiological differences from those common adrenal disorders seen in the dog. The prevalence of adrenal disease in ferrets appears to be increasing; 70% of pet ferrets in the United States were affected in 2003. The exact etiology of the adrenal gland changes that lead to the disease are not known. Possible contributing factors include early oophorohysterectomy and neutering, combined with the artificially prolonged photoperiod experienced by indoor pet ferrets, and a possible genetic component. Signs of

adrenal gland disease include progressive hair loss, pruritis, lethargy, wasting, and, in female ferrets, vulvar swelling.

It is well known that many aspects of adrenal development are different in different species. For example, the large fetal adrenal zone is found only in higher primates whereas mice have an X zone that regresses in adolescence or after pregnancy. Similarly, steroidogenic pathways and metabolites are very different between different species and humans (e.g. production of cortisol versus corticosterone; 17,20-lyase affinity for Δ^4 precursors). Whilst this biological variability provides some challenges for animal-based research, there are a number of animal models that are providing novel insights into adrenal and steroidogenic function that might be relevant to human disease states (e.g. the 'backdoor pathway' of testosterone synthesis in the Tamar wallaby and its relevance for understanding P450 oxidoreductase deficiency). One intriguing naturally occurring phenomenon widely known amongst veterinarians is the extremely high incidence of adrenal tumors in pet ferrets. This pathology often presents following castration with progressive hair loss on the side of the body, wasting, and large adrenal masses. Whether studying these animals could uncover novel mechanisms that might be involved in human adrenal development and tumorigenesis remains to be seen. Looking beyond mouse models into other animal systems may well have benefits in the future: the recent publication of the dog genome and rapid progress in understanding speciation and species specific canine disease is a prime example of the potential use of other animal systems [24, 25]. So, is your pet your next paper?

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