ADRENAL GLANDS

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ADRENAL FUNCTIONS

• THE ADRENAL GLANDS ACTUALLY HAVE 4 QUITE SEPARATE FUNCTIONS

• 1. MINERALOCORTICOID SECRETION BY THE ZONA GLOMERULOSA OF THE CORTEX

• 2. GLUCOCORTICOID SECRETION BY THE ZONA FASCICULATA OF THE CORTEX

• 3. ANDROGEN SECRETION BY THE ZONA RETICULARIS OF THE CORTEX

• 4. CATECHOLAMINE SECRETION BY THE MEDULLA

• (5) SOME DIFFERENT SECRETIONS IN THE NEWBORN AND BY SOME TUMOURS
CONTROL OF THE CORTEX

• THE SECRETION OF MINERALOCORTICOIDs IS CONTROLLED BY THE RENIN-ANGIOTENSIN SYSTEM AND THE PLASMA K LEVEL

• THE SECRETION OF THE GLUCOCORTICOIDs AND THE SEX STEROIDS IS CONTROLLED BY ACTH FROM THE PITUITARY

• IN THE ABSENCE OF ACTH (SEVERE HYPOPITUITARY STATE) MINERALOCORTICOID SECRETION IS USUALLY PRESERVED
CORTISOL SECRETION

- CRH is released in pulsatile manner by hypothalamus, passes down pituitary stalk and stimulates corticotrophs in pituitary
- Corticotrophs synthesise large molecule proopiomelanocortin which undergoes selective proteolytic cleavage to liberate ACTH
- ACTH is released in pulsatile manner and stimulates the adrenal cortex to produce and secrete cortisol, androgens (androstenedione, DHEA, DHEAS) and to a minor extent aldosterone. ACTH also has a trophic effect on the adrenal cortex, which gradually atrophies in its absence.
- Cortisol has negative feedback effect on pituitary and hypothalamus
- There is a pronounced diurnal variation in CRH, ACTH and cortisol secretion, with the maximum in early to mid morning and minimum round midnight, dependent on the day/night light cycle
A Regulation of cortisol secretion

- Stressors (hypoglycaemia, hypotension, fever, trauma, surgery)
- Diurnal rhythm
- CRH
- ADH
- Cytokines
- Pituitary
- ACTH
- Adrenals
- Metabolism
  - Gluconeogenesis
  - Glycogenolysis
  - Proteolysis
  - Lipolysis
- Cardiovascular system
  - Myocardial contractility
  - Cardiac output
  - Catecholamine pressor effect

B Regulation of aldosterone secretion

- Renal arterial pressure
- β-adrenergic action
- Prostaglandins
- ANP
- Dopamine
- Extracellular volume
- Renal arterial pressure
- Na⁺ (+ water) retention
- K⁺ excretion
- ECF [K⁺]
ASSAYS

• CRH is not usually assayed except in research projects. It is also secreted by the placenta.
• Proteins such as ACTH are assayed by 2-site immunoassays, the two antibodies being specific for the two ends of the molecule. This type of assay is used for all the anterior pituitary hormones.
• Of all the hormones of interest, ACTH is one of the least stable after sample collection and special precautions are needed for assay. Its pulsatile secretion means that results must be interpreted with care.
• Small molecules such as cortisol are assayed by competitive binding methods. The antibody binds either cortisol or a cortisol analogue with a label, and the more cortisol there is, the less the labelled analogue binds. This type of assay is used for all the adrenal cortical hormones, sex steroids and thyroid hormones, vitamins etc.
The first test done to assess adrenal cortical function is usually plasma cortisol. Due to the pronounced diurnal variation, and the variation among individuals, its interpretation is not simple.

Normal range in our laboratory 180-720 nmol/L for morning, 60-500 in afternoon, and at midnight probably about 40-60 but we have no extended series to be certain.

As these levels are dependent on individual variation and more importantly the degree of stress, infection, trauma etc to which the patient has been subjected, a value “within the normal range” cannot be taken as proof of normal adrenal function, and values towards the lower end of the range must be suspect especially if the patient is sick and a stress reaction is expected.

In cases of doubt a stimulation test with a synthetic ACTH analogue is performed and the plasma CORT should increase by not <300 in 60 minutes (some accept 250 at 30 minutes). Others recommend that a level of >550 be achieved post SYNAC, without considering the magnitude of the rise.
NORMAL FUNCTION TESTS - 2

- Normality can be further investigated by measuring ACTH level, but great care in sample collection, transport and handling is essential for the result to be valid due to its instability.
- Aldosterone levels are dependent on the posture and the salt and water balance of the patient, and we usually insist on the patient resting supine for some time before collection. To allow for the fluid volume interaction, it is customary to measure renin at the same time and to calculate an aldosterone/renin ratio which is a more reliable index of the physiological state of the patient.
- Finally, other steroids that are precursors of cortisol or are on branches of the steroid pathway can be measured. Most frequently 17-OH progesterone, androstenedione and DHEA(S), oestradiol or testosterone may be relevant, especially in suspected CAH.
- As cortisol is transported in plasma mostly bound to a protein, the “free cortisol” could be of value (compare FT4) but no assays are commercially available. We can estimate the average free cortisol level over the 24 hours by measuring free cortisol in a 24 hour sample of urine.
- Recently salivary CORT is being used as an index of free cortisol.
HYPOCORTISOL STATES
(including Addison’s disease)

• These can result from hypofunction of the pituitary corticotrophs and lack of ACTH, due to trauma, tumours, ischaemic necrosis, haemorrhage, surgery, cranial irradiation, autoimmune attack etc. In these cases aldosterone secretion is often relatively preserved.

• Hypofunction of the adrenal gland itself – due to infarction, infection (especially tuberculosis and meningococcus), tumours (usually metastatic, commonest from lung), haemorrhage into gland, autoimmune attack (the commonest in Australia) especially in MEN type conditions etc. In these cases the aldosterone secretion is usually also decreased or absent.

• Perhaps the commonest cause at present is suppression of the entire system by administered synthetic corticosteroid for its immunosuppressive and anti-inflammatory effects. When the administration is ceased, even after careful weaning, the pituitary often remains suppressed and the adrenal fails to resume function (compare TSH in treated thyrotoxicosis).

• In pituitary hypofunction or post corticosteroid states, both ACTH and CORT are low or absent and the SYNAC test may fail to stimulate.

• With an adrenal gland cause, ACTH is high and CORT low, SYNAC fails
HYPERCORTISOL STATES (including Cushing’s syndrome)

- In theory, excess CRH from hypothalamus could cause this, but it is normally a short term mechanism by which stress results in high cortisol levels. Excess CRH from placenta probably causes increased ACTH/cortisol in late pregnancy.
- Tumour or hyperplasia of the corticotrophs causes excess ACTH secretion and thus an increase in cortisol. This is called “Cushing’s Disease” as he described this form.
- Tumour or hyperplasia of adrenal cortical cells can result in hypersecretion of the relevant hormones – usually cortisol, more rarely aldosterone, sometimes precursors also.
- Ectopic secretion of ACTH from other tumours (especially small cell carcinoma of lung) can cause high cortisol secretion.
- Administration of high dose corticosteroid causes all the symptoms.
HYPERCORTISOL STATES

Investigations

• The first change is that the diurnal rhythm of CORT is reduced or abolished. The morning CORT may be normal or only a little elevated, but the evening (especially midnight) is much higher than normal. This is an inconvenient test unless the patient is in hospital, so we are experimenting with salivary cortisol which is easier to collect and assayable with sensitive methods. Likewise the urinary free cortisol is elevated.

• If the pituitary is the cause, or an ectopic ACTH source, then the ACTH and the CORT are elevated.

• If the adrenal gland itself or administered steroid is the cause, the ACTH is suppressed. The CORT will be high if it is from adrenal, and low or absent if synthetic steroids are in overdose as these do not react with most of the assays.

• It can be very difficult to exclude mild Cushing’s syndrome in an overweight stressed patient or an alcoholic – perhaps salivary CORT may be best.
• ZONA GLOMERULOSA SECRETES ALDOSTERONE AS A RESULT OF STIMULATION BY ANGIOTENSIN II OR RAISED K⁺

• ANGIOTENSINOGEN (PLASMA PROTEIN MADE IN LIVER) RELEASES ANGIOTENSIN I FROM ACTION OF ENZYME RENIN (FROM JUXTAGLOMERULAR APPARATUS IN KIDNEY)

• ANGIOTENSIN I LOSES TWO AMINOACIDS BY ACE ENZYME IN LUNG TO FORM ANGIOTENSIN II WHICH IS THE ACTIVE FORM
A  Regulation of cortisol secretion

- Stressors (hypoglycaemia, hypotension, fever, trauma, surgery)
- Diurnal rhythm
- CRH
- ACTH
- Adrenals
- Metabolism:
  - Gluconeogenesis
  - Glycogenolysis
  - Proteolysis
  - Lipolysis
- Cardiovascular system:
  - Myocardial contractility
  - Cardiac output
  - Catecholamine pressor effect

B  Regulation of aldosterone secretion

- Renal arterial pressure
- β-adrenergic action
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- Na⁺ (+ water) retention
- K⁺ excretion
- ECF [K⁺]

Liver
Lungs
Angiotensinogen
Renin
Kidneys
Angiotensin I
Angiotensin II
MINERALOCORTICOID - 2

- RENIN IS RELEASED FROM JGA IN KIDNEY IN RESPONSE TO:
- CHEMORECEPTION, PROBABLY OF LOW Cl\(^{-}\) IN TUBULAR FLUID
- PRESSURE MEASUREMENT, BY JG CELLS OR AFFERENT ARTERIOLE STRETCH RECEPTORS
- SYMPATHETIC STIMULATION BY REDUCED BP
- FACTORS SUCH AS K, ANGIOTENSIN II AND ANP
- RELEASE INHIBITED BY HIGH BP AND BY VOLUME EXPANSION (HIGH Na DIET, EXCESS ADH WITHOUT WATER RESTRICTION)
MINERALOCORTICOID - 3

- WE CAN MEASURE ALDOSTERONE IN 24 HOUR URINE BUT PLASMA IS BETTER
- WE MEASURE ALDOSTERONE/RENIN RATIO TO SEE WHETHER RESPONSE IS APPROPRIATE FOR THE TOTAL BODY PHYSIOLOGY
- IMPORTANT TO AVOID DIURETICS AND MANY OTHER DRUGS FOR SOME TIME (UP TO 6/52) AND TO HAVE PATIENT LYING DOWN TO AVOID STANDING STIMULUS TO RENIN SECRETION
- HIGH ALDO/RENIN RATIO SEEN IN TUMOUR OR HYPERPLASIA OF ZONA GLOMERULOSA CELLS
MINERALOCORTICOID - 4

- INADEQUATE SECRETION OF ALDOSTERONE LEADS TO Na LOSS AND K RETENTION IN DISTAL TUBULE AS ALDO RECEPTOR NOT ADEQUATELY STIMULATED
- PROBABLY SIMILAR RESULT IN SWEAT GLANDS, COLONIC MUCOSA AND ? SALIVARY GLANDS
- EXCESS ALDOSTERONE SECRETION LEADS TO Na RETENTION AND K LOSS WITH CONSEQUENT HYPOKALAEMIC ALKALOSIS DEPENDING ON SEVERITY
- Na RETENTION DOES NOT LEAD TO OEDEMA DUE TO SOCALLED “PRESSURE NATRIUREISIS”
TUMOUR LOCALISATION

• INACTIVE TUMOURS OF THE ADRENAL ARE RELATIVELY COMMON AND ARE FOUND ON CT EXAMINATION OF ABDOMEN - “INCIDENTALOMA”

• IT IS THEREFORE IMPORTANT THAT WHEN A HORMONALLY ACTIVE TUMOUR IS SUSPECTED, MUST SAMPLE BLOOD FROM EACH ADRENAL SEPARATELY TO SHOW EXCESS HORMONE (ALDO OR CORT) COMES FROM ONLY ONE SIDE

• FINDING A TUMOUR ON CT DOES NOT PROVE IT IS THE SOURCE – IT COULD BE A BILATERAL HYPERPLASIA + “INCIDENTALOMA”
EFFECT OF STRESS

• ESPECIALLY IN THE INTENSIVE CARE UNIT, OR OTHER SITE OF STRESS, THE HYPOTHALAMUS IS STIMULATED TO SECRETE MORE CRH WHICH LEADS TO INCREASED ACTH AND CORT

• SEVERAL OTHER ADJUSTMENTS TO HYPOTHALAMIC FUNCTION FOLLOW INCLUDING:
  • PARTIAL OR COMPLETE SUPPRESSION OF TSH
  • PARTIAL OR COMPLETE SUPPRESSION OF FSH/LH
  • CORT ITSELF AFFECTS OTHER ENDOCRINES:
    • INHIBITS T4 $\rightarrow$ T3 (NON THYROIDAL ILLNESS)
Carbohydrate/lipid metabolism:
- Hepatic glycogen deposition
- Peripheral insulin resistance
- Gluconeogenesis
- Free fatty acid production
- Overall diabetogenic effect

Adipose tissue distribution:
- Promotes visceral obesity

Bone and calcium metabolism:
- Bone formation
- Bone mass and osteoporosis

Skin/muscle/connective tissue:
- Protein catabolism/collagen breakdown
- Skin thinning
- Muscular atrophy

Endocrine system:
- LH, FSH release
- TSH release
- GH secretion

Eye:
- Glaucoma

GI tract:
- Peptic ulcerations

Cardiovascular/renal:
- Salt and water retention
- Hypertension

Growth and development:
- Linear growth

Immune system:
- Anti-inflammatory action
- Immunosuppression
CONGENITAL ADRENAL HYPERPLASIA (CAH)

- There are 5 enzymatic steps necessary to convert cholesterol to cortisol, and some of these steps are shared by the aldosterone or the sex hormone synthetic pathways.
- Failure to synthesise adequate cortisol in the fetus due to a defect in one of these enzymes means that the feedback on ACTH is inadequate, and the excess ACTH drives the adrenal to make excessive quantities of precursors before the blocked reaction.
- Depending on the enzyme that is defective, the excess cortisol precursors may overload alternative pathways and lead to synthesis of excess sex steroids. This occurs with 21 hydroxylase deficiency (commonest, because of recombination with adjacent pseudogene) and 11 hydroxylase deficiency. May not manifest till puberty in females and be a cause for hirsuitism and/or infertility and be confused with conditions such as polycystic ovary syndrome.
- Excess androgens can result in masculinisation of the female fetus, with ambiguous genitalia and incorrect sex allocation. Androgen lack can cause feminisation of a male, and often aldosterone is also deficient and severe or even fatal neonatal salt losing syndromes occur.
- See diagrams on next slides for discussion.
Cholesterol → CYP11A1 → Pregnenolone → CYP 17 → 17-OH-Pregnenolone → CYP 17 → DHEA

3β-HSDII → Progesterone → CYP 17 → 17-OH-Progesterone → Androstenedione

CYP 21A2 → DOC → CYP11B1 → Corticosterone → CYP11B2 → Aldosterone

MINERALOCORTICOID

CYP 21A2 → 11-Deoxycortisol → CYP11B1 → Cortisol

GLUCOCORTICOID

ANDROGENS
**E15. Analyse the following sets of results in patients A - J**

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ADRENAL MEDULLA - 1

• THE ADRENAL MEDULLA IS REALLY PART OF THE SYMPATHETIC NERVOUS SYSTEM, AND IS THE BIGGEST COLLECTION OF CELLS WHICH SECRETE NORADRENALINE AND ADRENALINE WHEN STIMULATED BY THESE NERVES

• THERE ARE OTHER SMALL COLLECTIONS OF SUCH CELLS ON OR NEAR THE SYMPATHETIC CHAIN THAT CAN FORM TUMOURS (PHAEOCHROMOCYTOMAS) BUT MOST OF THESE TUMOURS ARE IN THE ADRENAL ITSELF

• NEUROBLASTOMA CELLS OFTEN DO NOT CONTAIN ALL THE ENZYMES NEEDED TO MAKE NORADRENALINE AND RELEASE PRECURSORS
Catechol → Tyrosine → Dopa → Dopamine → Norepinephrine → Epinephrine
ADRENAL MEDULLA - 2

• ADRENALINE AND NORADRENALINE ARE SYNTHESISED FROM TYROSINE BY WAY OF DOPA (DIHYDROXYPHENYLALANINE)
• THEY ARE RELEASED IN SHORT TERM EMERGENCIES
• THEY ARE METABOLISED FIRST BY ADDITION OF A METHYL (-CH₃) GROUP TO ONE OF THE –OH GROUPS ON THE BENZENE RING, TO GIVE “METANEPHRINES” – ACTUALLY METANEPHRINE AND NORMETANEPHRINE
• THEY ARE THEN FURTHER METABOLISED TO MANDELCIC ACID DERIVATIVES (VMA)
ADRENAL MEDULLA - 3

• WE CAN TEST FOR THE PRODUCTION OF THESE “CATECHOLAMINES” EITHER:

• BY ANALYSING A 24-HOUR URINE FOR THE PARENT SUBSTANCES AND THE METANEPHRINES, IN WHICH CASE THE URINE MUST BE COLLECTED IN HCl WITH METABISULPHITE TO INHIBIT OXIDATIVE DESTRUCTION (pH NOT >3)

• OR BY ANALYSING PLASMA FOR METANEPHRINE (LONG HALF LIFE) OR CATECHOLAMINE (SHORT HALF LIFE) CONTENT; IN THE LATTER CASE AVOID STRESSFUL COLLECTION!
ADRENAL MEDULLA - 4

• STRESSED PATIENTS OFTEN HAVE A MINOR ELEVATION OF DOPAMINE, NORADRENALINE OR ADRENALINE IN THE 24 HOUR URINE
• PLASMA CATECHOLAMINES AND METANEPHRINES ARE BECOMING MORE ACCEPTABLE, BUT THE ANALYSIS IS NOT EASY
• PHAEOCHROMOCYTOMAS OFTEN HAVE EPISODIC RELEASE OF HORMONE SO BEST TO TAKE BLOOD WHEN THE BLOOD PRESSURE IS HIGH – IF POSSIBLE – IF MEASURING CATECHOLAMINES
• SO THERE ARE PROS AND CONS FOR EACH TEST
NEUROBLASTOMA

- THESE MALIGNANT TUMOURS IN YOUNG CHILDREN OFTEN SECRETE PRECURSORS SUCH AS DOPAMINE, WHICH IS METABOLISED BY OXIDATION AND METHYLATION TO A DIOXYPHENYLACETIC ACID DERIVATIVE {HOMOVANILLIC ACID (HVA)}
- WE DO NOT OFTEN SEE THIS PRODUCT IN HIGH AMOUNTS IN PHAEOCHROMOCYTOMAS IN ADULTS