Advances in endocrinology

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New developments in endocrinology, in common with many specialised branches of medicine, have been dominated by advances in molecular genetics. Although these investigations relate to rare single gene disorders, they have resulted in major breakthroughs in many fields of endocrinology, significantly contributing to our understanding of the cellular mechanisms of hormone action. However it is not just the minute detail of single gene disorders and hormone action that has caught the attention of endocrinologists in recent years. This review will also focus on two broader health issues, one epidemiological and the other therapeutic.

The studies of Barker and colleagues have introduced the concept that disease in adult life has its origin in the fetal environment, and that this process can be attributed to changes in the programming of fetal endocrine axes. On the therapeutic agenda, the most dramatic advance in endocrinology occurred over a decade ago with the introduction of recombinant human growth hormone (hGH). This created wide interest in the use of growth hormone treatment in growth disorders with or without associated hypopituitarism. It is appropriate therefore to summarise our long term experiences with hGH as the first generation of these children achieve final height.

Finally the isolation and characterisation of a new hormone is a rare event in the 1990s. Therefore the discovery of the satiety factor, leptin, initially heralded as a potential cure for obesity, has attracted considerable media attention of endocrinologists in recent years.

Molecular investigation and diagnosis

The tools to treat endocrine disorders have not changed dramatically in recent years, but what has changed is our understanding of the molecular abnormalities that generate these conditions. Endocrinologists have come to rely heavily on molecular diagnostic techniques. For many disorders, it is insufficient to characterise the disease only on clinical and biochemical grounds. A full appraisal requires a molecular diagnosis. There are many individual examples of significant molecular developments in endocrinology (table 1). A few examples will be discussed.

DISORDERS OF G-PROTEINS AND G-PROTEIN COUPLED RECEPTORS

The majority of polypeptide hormones signal to their target cells through G-protein coupled receptors (GPCRs), which are characterised by seven transmembrane helices connected by extracellular and intracellular loops. The N-terminal extracellular domain and one or more extracellular loops are involved in ligand binding while the intracellular loops and the C-terminal tail are coupled to specific G-protein complexes, composed of α, β, and γ subunits. There are many α subunit genes, whose protein products may be associated with stimulation (Gα) or inhibition (Gγ). Activation of a GPCR leads to the dissociation of the G-protein trimer and modulation of the activity of enzymes, such as adenylate cyclase, or ion channels by the α subunit and/or the βγ dimer. Over the last few years, it has been recognised that either gain or loss of function of G-protein intracellular signalling mechanisms due to mutations either in the GPCR or in the G-proteins can generate a wide spectrum of human endocrine disease.

For instance, both loss and gain of function have been described with mutations of genes encoding the receptors of many hormones (for example adenocorticotrophic hormone (ACTH), growth hormone releasing hormone (GHRH), vasopressin, luteinising hormone, follicle stimulating hormone, and thyroid stimulating hormone). Mutations of the ACTH receptor cause resistance to ACTH which is characterised by glucocorticoid but not mineralocorticoid deficiency. Some cases are associated with missense, nonsense or frameshift mutations within the ACTH receptor (ACTHR) gene,51 while others with the features of ACTH resistance (for example the triple A syndrome) have no identified ACTHR mutation,53 implying that postreceptor defects could be responsible. Loss of function in the GHRH receptor is responsible for the “little” mouse,50 but has now been linked to familial isolated growth hormone deficiency, with a homozygous nonsense mutation in the GHRH receptor gene.6 Nephrogenic diabetes insipidus (NDI) is associated with mutations in the V2 vasopressin receptor54 (X linked) or in the aquaporin-2 gene55 (autosomal recessive). Rapid identification of these mutations should lead to the early diagnosis and appropriate management of NDI, hopefully avoiding...
repeated episodes of dehydration, which can cause growth failure and mental retardation.

Mutations within the sixth transmembrane domain of the luteinising hormone receptor gene have recently been linked to male pseudohermaphroditism (female external genitalia, primary amenorrhoea, a short blind ending vagina, and absence of müllerian structures with a 46XY karyotype). It is possible however that other less critical luteinising hormone receptor mutations may account for milder clinical forms of this disorder (for example hypergonadotrophic hypogonadism and microopenis). In contrast activating mutations in the luteinising hormone receptor gene cause familial male precocious puberty which is inherited in an autosomal dominant, male limited pattern. A missense mutation, again in the sixth transmembrane domain of the luteinising hormone receptor, has been found most commonly. Interestingly, no clinical manifestations have been reported in female carriers of the mutant receptor gene.

Endocrine disease is also generated by abnormal G-protein signal transduction. One such example is pseudohypoparathyroidism, a disease with a wide clinical spectrum characterised by insensitivity to parathyroid hormone with or without osteodystrophy and generalised hormone resistance. Defects can occur in the Gα protein or in the association of G-protein subunits with adenylyl cyclase or further down the signalling pathway in the intracellular action of cAMP.

Gα mutations may generate simultaneously loss of function in one tissue (pseudohypoparathyroidism) and gain in another (gonadotrophin independent precocious puberty), as described in two unrelated boys. Expression of the mutant protein showed that it was rapidly degraded at 37°C (that is a loss of function mutation) explaining the pseudohypoparathyroidism phenotype, but at 32°C (compatible with the temperature in the testis) the protein was constitutively activated causing the precocious puberty by stimulating Leydig cell cAMP formation. Another example of disease associated with an activating mutation of the Gα gene is the McCune-Albright syndrome, classically defined by polyostotic fibrous dysplasia, cafe-au-lait spots, sexual precocity, and other hyperfunctional endocrinopathies. Activating mutations of the Gα gene have been found in various tissues, which appear to cause

**Table 1** Examples of a wide range of conditions, relevant to endocrine practice, where the target gene has recently been identified, are shown. References are given for each disorder

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<td>Diastrophic dysplasia sulphate transporter</td>
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<td>Thalassemia</td>
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<td>X linked hypoplasmateric rickets</td>
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<td>Pancreas</td>
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<td>Jansen’s metaphyseal chondrodysplasia</td>
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<td></td>
<td>PTH receptor</td>
<td>Persistent hyperinsulinaemic hypoglycaemia of infancy</td>
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ACTH=adrenocorticotrophic hormone; AMH = anti-müllerian hormone; AW = arginine vasopressin; FGFR = fibroblast growth factor receptor; FSH=follicle stimulating hormone; GH=growth hormone; GHHR=growth hormone releasing hormone; IGF=insulin-like growth factor; LH=luteinising hormone; NP = neurophysin; PTH=parathyroid hormone; TSH=thyroid stimulating hormone.
widespread disease manifestations if they occur early in embryogenesis, but result in focal disease if activated later in development.

ABNORMALITIES IN GROWTH HORMONE SIGNAL TRANSDUCTION
Interest in the wider therapeutic applications of growth hormone has paralleled a rapid advance in our understanding of the cellular mechanisms of growth hormone action. In contrast to the G-protein coupled receptors listed above, the growth hormone receptor (GHR) belongs to the class I cytokine receptor superfamily, whose members possess extracellular, transmembrane and intracellular domains, but lack any intrinsic tyrosine kinase activity. Signalling molecules are recruited from the cytoplasm on activation of the receptor to associate with the intracellular domain. In the case of the GHR, the pivotal signalling molecule is the Janus kinase, Jak 2. Its activation causes a sequence of phosphorylation events down distinct signalling pathways, leading to phosphorylation of mitogen activated protein kinase, signal transducers and activators of transcription, and insulin receptor substrate and eventually changes in nuclear events.

It has been recognised for many years that mutations within the GHR gene, that prevent expression of GHR, can generate growth hormone insensitivity, characterised by extreme short stature and a phenotype similar to that of severe growth hormone deficiency. This condition (Laron’s syndrome) is very rare, usually follows an autosomal recessive inheritance pattern but has been identified throughout the world. An increasing number of mutations within the GHR are being recognised, including instances where the GHR is expressed but dysfunctional or where the GHR appears to be functioning normally but the signal transduction pathway is defective.

Most importantly this rare disorder has led to the recognition that lesser degrees of GHR dysfunction may be relevant to some cases of idiopathic short stature, where clear endocrine deficits have not been identified. Although screening has revealed a low incidence of heterozygous GHR mutations in idiopathic short stature (ISS), the concept of a range of growth hormone sensitivity has been introduced. Such data demonstrate how rapidly the molecular characterisation of a candidate disease, in this case growth hormone insensitivity, can then be used to assess another common disorder, namely ISS.

Overall these developments in our understanding of the molecular basis for defects in hormone receptor signalling have provided: (i) the potential for prenatal diagnosis in affected families, (ii) the opportunity to make an accurate molecular diagnosis where clinical/biochemical uncertainty exists, and most importantly (iii) the recognition of the key receptor sequences or signalling molecules relevant to human disease and therefore the potential to design specific treatments.

Fetal origin of adult disease
Restricted fetal and early infant growth has been associated with the later development of significant risk factors for cardiovascular disease, in particular hypertension. These relationships were initially demonstrated using retrospective data on cohorts born earlier this century in the UK, on whom detailed obstetric and perinatal growth data were available (reviewed in Barker4). The association between low birth weight and postnatal development of hypertension has been demonstrated prospectively in rat models. The relationship has not been confirmed in UK adolescents.68 The timing of the growth restriction and hence the body phenotype generated are proposed to influence the risk of other conditions in addition to raised blood pressure. Thus syndrome X (hypertension, impaired glucose tolerance, non-insulin-dependent diabetes mellitus (NIDDM), hypertriglyceridaemia, low high density lipoprotein-cholesterol, and central obesity) is more likely to occur in those with mid-trimester growth retardation, who are thin at birth but regain normal weight by one year, while abnormal lipid and haemostatic profiles occur in those with poor growth in late pregnancy, who have reduced abdominal circumference, are short at birth and remain so postnatally.64 Although many studies support the fetal origin hypothesis, it has been suggested that selection bias, confounding variables, and inconsistency between studies have not been fully eliminated.67 68 It is important therefore to continue to rigorously test this hypothesis, and to unravel the mechanisms that generate these associations.

It is postulated that alterations in fetal nutrition lead to permanent changes in hormone sensitivity. Evidence that programming within the insulin and growth hormone-insulin-like growth factor (IGF) axes to generate a multihormone resistant syndrome is accumulating both from studies in animals and in children who have suffered intrauterine growth retardation (IUGR).72 73 Such studies have demonstrated that growth retarded neonates can develop altered insulin:glucose ratios, indicative of insulin resistance, raised growth hormone and IGF-I concentrations, suggesting insensitivity within the growth axis, and raised IGF binding protein-1 levels, that may reduce IGF-I bioavailability. Persistence of these abnormalities may permanently reset homoeostatic control of hormonal axes, leading to a clinically significant insulin resistant syndrome in adulthood. In both UK and Indian children, serum IGF-I concentrations in early to mid-childhood were higher in taller and heavier children, but also inversely related to birth weight. Thus children with low birth weight had higher IGF-I levels than would be expected for their size. The development of NIDDM in subjects, who had disproportionate IUGR but became obese adults, provides further evidence for a link between prenatal and postnatal growth. Additionally there is some evidence that the adrenal axis may be programmed: adrenal androgen and glucocorticoid excretion, assessed in mid-childhood,
was raised in those with low birth weights.76 One caveat to these data is that all these common health problems will usually occur in those without a history of IUGR; fetal environment is but one of many other factors (lifestyle, activity, diet, and smoking) that contribute.

This is an area with considerable implications not only to obstetricians and neonatologists but to all paediatricians. An understanding of the key events involved in reprogramming an endocrine axis offers the possibility that therapeutic manipulation of the prenatal or postnatal environment could modify the incidence of some of the most common disorders of Western society.

**Efficacy of long term growth hormone treatment**

Although molecular investigation has dominated most specialised areas of paediatrics, accumulating knowledge on practical therapeutic issues should not be overlooked. This is of particular relevance to the field of growth hormone treatment. It is just over a decade since the first recombinant (r)hGH was issued a license in the UK for the treatment of growth hormone deficiency. Since that time, many other conditions associated with growth failure have been treated with growth hormone. As many of these children are now approaching final height, it is pertinent to review our experiences with growth hormone and decide just how effective such treatment has been.

The final height prognosis of those with isolated growth hormone deficiency or multiple pituitary hormone deficiency has improved over the last decade, in part related to the optimisation of growth hormone administration schedules, including adjustment of growth hormone dose according to body size. Most will achieve a height within the normal range, but still below that predicted from parental target range.77–79 In a study of children who received rhGH from the prepubertal years to near adult height, factors that influenced the adult height achieved included the duration of growth hormone treatment, age at starting treatment (the younger the child at the start, the greater the adult height), height at the start and growth rate in the first year.77 These data clearly indicate that prompt diagnosis and initiation of growth hormone treatment are the principal factors that can be modified in order to improve further the efficacy of growth hormone in growth hormone deficiency.

Growth hormone has been licensed to promote growth in Turner’s syndrome since 1989; this indication accounts for up to 25% of the caseload in growth clinics. Studies from the USA, Canada, and Europe are now reporting final heights after four to 10 years of growth hormone treatment80–84: the parameter used to judge success has been the gain in height over that projected from disease specific growth charts at the start of treatment, a technique that can be compromised by secular trends and application of national standards to a different population. Nevertheless growth hormone treatment would appear to promote an average gain in height above that predicted of 3–8 cm, with individuals gaining at best >15 cm of extra height. Factors influencing this gain include the timing of pubertal induction and the length of growth hormone treatment. The adjuvant use of oxandrolone would appear to provide only a small additional benefit to final height. Despite these studies, the optimal times for starting growth hormone treatment and for the induction of puberty have not been defined, nor has the wide range of individual response to growth hormone been adequately explained. Coordinated treatment policies across the growth centres in the UK may help to address these issues over the next decade.

Final height data after growth hormone are also being reported in those children, variably classified as short normal, normal variant, or idiopathic short stature.85–87 These cohorts are not growth hormone deficient by classical criteria. Their early response to growth hormone was encouraging, but in some studies this has not been maintained to final height, with insignificant increments in height above that predicted (for example mean 2.5 cm in girls and 2.8 cm in boys79). Those with chronic renal failure88 and with IUGR89 have also been targeted for growth hormone treatment, but as yet final height data are not available in significant numbers. Certainly growth rate can be improved by supraphysiological doses of growth hormone, but experience with short normal children might suggest that we should be cautious in our predictions for final height.

Biosynthetic hGH would appear to be a safe agent with undisputed benefit in congenital and acquired growth hormone deficiency. In fact the lifelong metabolic consequences of growth hormone deficiency have led to its introduction as replacement therapy in the adult growth hormone deficient population. In all non-growth hormone deficient growth disorders, there would appear to be a range of sensitivity to growth hormone with potential benefits to many of the recipients. It remains our task to work out which individuals with which conditions are the most appropriate targets for treatment.

**Regulation of leptin**

In 1994 a new hormone, exclusively expressed in adipose tissue, was characterised and cloned from the genetically obese (ob/ob) mouse.90 The hormone, named leptin (leptos = thin), was rapidly identified as a potent modulator of appetite and thermogenesis. Its administration to the ob/ob mouse resulted in a marked reduction in food intake and hence weight loss.91–93 The leptin receptor was soon cloned,93 and mutations in this gene result in the obese phenotype of the db/db mouse.94–97 It had been postulated for many years that a factor produced in fat may exist, which would be capable of signalling to central mechanisms governing appetite. Leptin appears to be a candidate for such a role, but its actions may be even more significant in that it also has an additional direct effect on the reproductive system.95 It is also present in significant amounts in amniotic fluid and cord blood,96 the
level in the latter correlating with birth and placental weight, suggesting that it may have a role in fetal growth and metabolism.

Leptin mRNA is expressed exclusively in adipose tissue, while the leptin receptor (a member of class I cytokine family) is found in a range of tissues, which include the hypothalamus, choroid plexus but also lung and gonadal tissues. Circulating concentrations of leptin in humans are most closely correlated to body fat mass and are acutely regulated by starvation, insulin, and glucocorticoids (reviewed in Saladin et al.115). It is postulated that leptin exerts its central effects on appetite through a long feedback loop from the periphery to the central nervous system to decrease the expression of neuropeptide-Y and hence appetite (reviewed in Campfield et al.105 and Rohner-Jeanrenaud et al.106). Its potential therapeutic role as an anorectic factor, however, is confounded by the fact that the majority of cases of human obesity appear to be associated with a leptin resistant state. Nevertheless it is thought that 5% of those with obesity may have a leptin concentration lower than expected for their fat mass.107 Of particular interest to paediatricians is the recent discovery of the first cases of the Ob gene mutation, analogous to that found in the ob/ob mouse, in two massively obese children from a consanguineous family.108 Although very rare, this diagnosis should be considered in children, who develop progressive severe obesity in infancy.

The ob/ob mouse is recognised to be sterile, but it was found that parenteral administration of leptin not only induced weight loss but also stimulated the reproductive system109 and restored fertility.99 110 A series of experiments in normal rats and mice have demonstrated conclusively that leptin administration is also able to bring forward the age at which reproductive ability is achieved, despite lowering body weight.111–115 It will also partially reverse the relative hypopituitarism induced by starvation of mice.116 These effects are likely to be mediated through actions on the gonadotrophin releasing hormone pulse generator, but could also involve stimulation directly of the gonads. In humans, hypothalamic hypogonadism can be induced by weight loss and anorexia, while the obese child can proceed to body mass index and to increasing age.115 Leptin concentrations peak in early puberty in both sexes, then decline to adulthood in boys, but show a late rise in girls. It is possible therefore that leptin may play a part in facilitating progress into puberty. In a more general context, it may act as a marker of the interaction between nutrition and maturation.

Summary

Molecular genetics will continue to help us to make precise diagnoses. At present, the expertise to achieve this for a specific disease is often exclusive to one unit with a research interest. It will be important to establish a coordinated approach at a suprarregional level to provide molecular diagnosis for rare disorders as a fast reliable clinical service. In addition understanding the molecular mechanisms of disease is likely to direct a search for new treatments. For instance, calcium channel blockers have been used in nesidioblastosis to reduce the hypersecretion of insulin, as a result of the recognition of the role that calcium has in the function of the β-cell ATP sensitive K+ channel.116

Although the potential benefits of hGH are now being clearly defined in a range of growth disorders, the treatment is invasive and expensive. It is likely that future endocrine therapeutic developments could include slow release growth hormone preparations, orally active growth hormone mimetics, or even hormone production from an ectopic viral cDNA vector. The next “advances in endocrinology” will also reveal whether leptin will have a therapeutic role in appetite control or even the modulation of pubertal development.

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Advances in endocrinology


