

I Special Lecture

Hypothalamic control of energy homeostasis by *Agrp* neurons

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Control of energy balance by the central nervous system depends on the ability of first-order hypothalamic neurons to sense and respond to changes leptin, insulin, and other circulating indicators of peripheral energy stores. One group of these neurons in the medial portion of the arcuate nucleus can be recognized by expression of the neuropeptide *Agrp*, whose transcription is upregulated 10–20 fold in response to food deprivation or hypoleptinemia. To better understand the molecular mechanisms that couple changes in peripheral energy stores to alterations in hypothalamic gene expression, we have employed two parallel approaches, transgenic reporter analysis in mice and comparative sequence analysis between mouse and human, to define a region of DNA necessary and sufficient to regulate the spatial expression and fasting response of *Agrp*. Proper regulation of *Agrp* is dependent on DNA sequences located in a 6 kb region approximately 35 kb upstream of the *Agrp* transcriptional initiation site. Using homologous recombination in *E. coli* and BAC transgenesis, we have made precise alterations of the conserved regions to determine whether they mediate the effects of leptin on *Agrp* transcription. As a complementary approach we have created animals that express Cre recombinase specifically in *Agrp* neurons, and we are using these animals to remove individual signal transduction molecules from *Agrp* neurons to assess their role in *Agrp* transcription. The Cre transgenic mice have also been injected with adenoviral vectors that encode Cre-activatable reporters for intracellular signaling molecules. Using multiphoton microscopy of living brain slices, changes in fluorescence resonance energy transfer or membrane localization can be used to measure alterations of intracellular calcium or phosphatidylinositol-3 kinase (PI-3K) activity, respectively. This approach allows changes in intracellular signaling to be measured in individual *Agrp* neurons, and provides the opportunity to understand how different signaling events are integrated into a neuronal circuit.

2 RECENT DEVELOPMENT OF CONGENITAL ADRENAL HYPERPLASIA

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Congenital adrenal hyperplasia (CAH) describes a group of inherited autosomal recessive disorders characterized by an enzymatic defect in cortisol biosynthesis, compensatory increases in corticotrophin secretion. 21-OH deficiency is responsible for more than 95% of cases. 21-OHD shows a wide range of phenotypic expression due to genetic variation, however genotype-phenotype is not always correlated. Reproductive, metabolic, and other comorbid conditions, including risk for tumors, are further investigated. Lipoid CAH is a severe inborn error of steroid hormone synthesis that disrupts the synthesis of all adrenal and gonadal steroids. This disease is proved to have two genetic causes; namely StAR and P450scc defects.

Now we are investigating whether a human disease caused by NADPH-cytochrome P450 oxidoreductase (CYPOR) gene defect exists or not. Because CYPOR is an essential component of the microsomal P450 mixed-function oxidase system, several P450s including P450_{arom}, P450C21, P450C17, P450RA, and P450C51 etc would be impaired, resulting in embryonic lethal.

The 21-OHD mouse has provided a useful model with which to examine disease mechanisms. Using this mouse, abnormality of HPA axis was investigated. We are also testing gene therapy. In addition, we perform adrenal capsule transplantation and observe regeneration of adrenal cortex.

1 Molecular genetics, diagnosis and management of adrenal diseases in childhood

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Currently advance of molecular genetics expands our understanding of pathogenesis of adrenal diseases in childhood. Hence, this minireview will summarize newer clinical observation and molecular findings of these diseases and approaches to clinical diagnosis and management.

1. Impaired steroidogenesis

Congenital adrenal hyperplasia (CAH) is a group of diseases whose common feature is an enzymatic defect in the steroidogenic pathway to cortisol. The mutations of genes encoding steroidogenic enzymes that cause all form of CAH have been elucidated. Abnormalities in both the structure and function of the adrenal medulla have been shown in patients with CAH, and the degree of adrenomedullary impairment may be a biomarker of disease severity.

The common treatment in all forms of CAH is cortisol replacement therapy, which corrects the cortisol deficiency and reverses the abnormal hormonal patterns resulting from excessive ACTH secretion. Prenatal diagnosis and prenatal therapy would be available in female patients with 21OHD to prevent prenatal masculinization.

2. Adrenal development and congenital hypoplasia

Development of adrenal glands depends on complex process multiple interacting factors. The mutations of DAX-1, SF-1, which are important transcription factor for normal development of adrenal cortex, have been clarified as a cause of congenital adrenal hypoplasia.

3. Impaired metabolism of adrenocortical hormones

Two isozymes of 11 β -hydroxysteroid dehydrogenase (11 β HSD) interconvert active cortisol and inactive cortisone. Mutations of 11 β HSD type 2 gene expressed in aldosterone target tissues are responsible for the human syndrome of apparent mineralocorticoid excess. While, increased 11 β HSD type 1 activity has been reported to be associated with metabolic syndrome.

3 Molecular and Clinical Aspects of Adrenal Hypoplasia Congenita

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Adrenal Hypoplasia Congenita (AHC) is a rare disorder presenting with primary adrenal insufficiency, usually in early infancy. AHC occurs in two distinct forms: autosomal recessive and X-linked. Hypogonadotropic hypogonadism (HHG) is also commonly associated with X-linked AHC. Mutations in *DAX1/AHC (NR0B1)* located in the Xp21 DSS region, are responsible for both AHC and HHG. On the other hand, it has been reported that *SF-1/Ad4BP (NR5A1)* mutations cause autosomal AHC.

DAX-1 gene encodes an unusual orphan member of the nuclear hormone receptor superfamily of transcription factors, that lacks the typical zinc finger DNA-binding motif. *DAX-1* is expressed in the fetal gonad, testis, ovary, adrenal cortex, anterior pituitary and hypothalamus, overlapping the expression of the another orphan nuclear receptor, *Ad4BP/SF-1 (NR5A1)*, which plays a key role in the development and function of these tissues. In the fetal gonad, there is a sexually dimorphic expression pattern of these two factors.

Dax-1 represses *Ad4BP/SF-1* mediated transcription, and is up-regulated by *Ad4BP/SF-1*. While both *DAX-1* and *SF-1* interact with each other and affect the development of adrenal glands, no obvious factors which are influenced by these two factors have been not identified yet. The molecular mechanisms how mutated *DAX-1* or *SF-1* cause AHC remains unclear. Furthermore, in IMAGe association, which manifests intrauterine growth retardation, metaphyseal dysplasia, AHC, and genital anomalies, mutations of *SF-1* or *DAX-1* are not detected, and also pathogenesis remains unclear.