

The expression of the ACTH receptor

L.L.K. Elias^{1,2} and
A.J.L. Clark¹

¹Molecular Endocrinology Laboratory, Department of Chemical Endocrinology, St. Bartholomew's and the Royal London School of Medicine and Dentistry, London, England

²Divisão de Endocrinologia, Departamento de Clínica Médica, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brasil

Abstract

Adrenal glucocorticoid secretion is regulated by adrenocorticotrophic hormone (ACTH) acting through a specific cell membrane receptor (ACTH-R). The ACTH-R is a member of the G protein superfamily-coupled receptors and belongs to the subfamily of melanocortin receptors. The ACTH-R is mainly expressed in the adrenocortical cells showing a restricted tissue specificity, although ACTH is recognized by the other four melanocortin receptors. The cloning of the ACTH-R was followed by the study of this gene in human diseases such as familial glucocorticoid deficiency (FGD) and adrenocortical tumors. FGD is a rare autosomal recessive disease characterized by glucocorticoid deficiency, elevated plasma ACTH levels and preserved renin/aldosterone secretion. This disorder has been ascribed to an impaired adrenal responsiveness to ACTH due to a defective ACTH-R, a defect in intracellular signal transduction or an abnormality in adrenal cortical development. Mutations of the ACTH-R have been described in patients with FGD in segregation with the disease. The functional characterization of these mutations has been prevented by difficulties in expressing human ACTH-R in cells that lack endogenous melanocortin receptor activity. To overcome these difficulties we used Y6 cells, a mutant variant of the Y1 cell line, which possesses a non-expressed ACTH-R gene allowing the functional study without any background activity. Our results demonstrated that the several mutations of the ACTH-R found in FGD result in an impaired cAMP response or loss of sensitivity to ACTH stimulation. An ACTH-binding study showed an impairment of ligand binding with loss of the high affinity site in most of the mutations studied.

Key words

- ACTH receptor
- Resistance
- Mutation
- Transfection

Correspondence

L.L.K. Elias
Departamento de Clínica Médica
FMRP, USP
14049-900 Ribeirão Preto, SP
Brasil
Fax: + 55-16-633-1144
E-mail: llelias@hotmail.com

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Introduction

Glucocorticoid secretion by the adrenal gland is under the control of pituitary adrenocorticotropin hormone (ACTH), which acts through a specific cell membrane receptor. The ACTH receptor (ACTH-R) is a member of the superfamily of G protein (guanine

nucleotide binding regulatory proteins)-coupled receptors and belongs to the melanocortin receptor family (1). The first two receptors of this family cloned were the MSH-R, also named MC1-R, and the ACTH-R, also named MC2-R (2). Further investigations have identified three other receptors named MC3-R, MC4-R and MC5-R, which

are involved in the melanocortin system in the brain and play a role in the control of weight homeostasis and in the autonomic control of the cardiovascular system (3,4).

The ACTH-R is one of the smallest G protein-coupled receptors identified, having a molecular weight of 33 kDa and containing 297 amino acids. Two potential sites for N-linked glycosylation in the extracellular N-terminal region may result in a molecule of 43 kDa. The ACTH-R is mainly expressed in the adrenal gland (2) and shows low expression in skin and adipose tissue (5,6). The major specific expression of the ACTH-R in the adrenal is determined by the presence of specific regulatory elements in the promoter region of the ACTH-R gene. The orphan nuclear receptor steroidogenic factor 1, a key regulator of adrenal development and function (7), has been shown to be involved in the regulation of the steroidogenic specific activity of the ACTH-R (8,9). The induction of ACTH-R promoter activity is also sensitive to cAMP, which could explain the ACTH-R up-regulation through its own ligand (10,11).

The ACTH-R shows an exclusive affinity for ACTH, although this hormone is recognized by the other four melanocortin receptors (12,13). The ACTH-R requires for ligand binding not only the conserved His-Phe-Arg-Trp sequence but also a highly basic motif present solely in the ACTH molecule.

Following binding to the receptor, ACTH induces a conformational change that allows the G protein to interact with the receptor, eliciting the exchange of GDP for GTP and dissociation of the heterotrimeric G protein into an α subunit and a $\beta\gamma$ subunit. This activation ultimately results in an increase of cAMP, a second messenger that activates protein kinase A (PKA) which phosphorylates cAMP response element binding protein increasing the transcription of the genes involved in steroidogenesis such as steroidogenic regulatory protein (14). PKA also phosphorylates proteins on threonine and

serine residues, a fact which is likely to be important for steroidogenesis (15). Cyclic AMP also acts by inhibiting a potassium channel resulting in an increase of intracellular calcium concentration (16).

ACTH insensitivity

Resistance to ACTH action has been described in a group of inherited disorders which includes familial glucocorticoid deficiency (FGD) and the triple A syndrome (adrenal failure, achalasia, alacrima) (17). Linkage analysis excluded the ACTH-R locus as the cause of triple A syndrome, leading to the search for another candidate gene which has mapped this disease to a locus on chromosome 12q13 (18,19).

FGD is a rare autosomal recessive disease characterized by ACTH insensitivity with glucocorticoid deficiency, high plasma ACTH levels and normal renin-aldosterone axis. The pathogenesis of FGD has been ascribed to the following hypotheses: defect in adrenocortical development, degeneration of the adrenal gland, defect in the intracellular signalling response to ACTH, and defect of the ACTH-R.

The cloning of the human ACTH-R gene in 1992 allowed the study of this gene in FGD and several mutations have been found in segregation with this disease (20-25). Mutations are distributed throughout the ACTH-R molecule and should result in different effects on receptor function.

In order to characterize the functional effects of the mutations found in FGD, expression studies were carried out using mutant ACTH-R. Previous studies used cells with some endogenous melanocortin receptor activity and their results did not permit a distinction between endogenous and transfected melanocortin receptor functions (26,27). To overcome this difficulty we used Y6 cells which are a mutant variant of Y1 mouse adrenocortical tumor cells that possess a non-expressed ACTH-R gene and

therefore show no background of melanocortin receptor activity. Using this cell line we could successfully express the human ACTH-R and characterized mutations found in FGD with an impaired cAMP response to ACTH or loss of sensitivity showing a right shift of the dose-response curve (28). Most of the mutations result in a loss of the high affinity ACTH binding site. As a group, we may consider the mutations as quantitative or qualitative defects according to the possibility of overcoming the defect with higher

doses of ACTH. Moreover we found a variable degree of resistance to homologous desensitization by these ACTH-R mutations suggesting an impairment of G protein coupling (29). The resistance to ACTH-induced desensitization observed in ACTH-R mutations found in FGD could be a mechanism for preserving some cortisol production to overcome the receptor resistance to ACTH. Taken as a whole, the data obtained confirm the ACTH-R mutations as a cause of FGD.

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