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Molecular characterization of the mineralocorticoid receptor in pseudohypoaldosteronism

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Pseudohypoaldosteronism (PHA) is characterized by salt-wasting and failure to thrive in the newborn, accompanied by high urinary levels of sodium despite hyponatremia, hyperkalemia and metabolic acidosis, elevation of plasma renin activity, and high plasma aldosterone levels. PHA patients are resistant to mineralocorticoid administration, but their symptoms ameliorate after a period of sodium supplementation, which can be discontinued in older subjects. Binding studies performed on mononuclear leukocytes of the family members affected by the disease have shown the absence of binding of [^{3}H]aldosterone to the mineralocorticoid receptor (MR) in mononuclear leukocytes in two siblings and a marked reduction in another sibling and the father, suggesting either the absence of MR or a defect in the ligand binding domain of the MR in these patients. Molecular analysis of the MR in the members of this family did not reveal any major rearrangement or deletion of the MR gene. In addition, no mutation was found in the entire MR coding sequence by RT-PCR and direct sequencing of MR mRNA, and the semiquantitative RT-PCR analysis of the MR mRNA of one affected patient failed to show any quantitative abnormality in MR expression. These results do not exclude a molecular abnormality present in the MR gene being responsible for PHA. However, they indicate that in this family PHA is not related to a modification of the MR primary structure or to a major abnormality in MR expression. (*Steroids* **60**: 164–167, 1995)

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Introduction

Primary pseudohypoaldosteronism (PHA) is a rare, inherited syndrome of mineralocorticoid resistance. Typically, its first clinical manifestations in the newborn are salt loss, failure to thrive and dehydration of variable severity. The clinical signs are associated with the biochemical picture of hyponatremia, hyperkalemia and metabolic acidosis, elevated plasma renin activity, and high urinary sodium excretion. Patients are resistant to the administration of exogenous mineralocorticoids, but improve quickly after a sodium supplementation, which can be discontinued after a variable period of time. The inheritance of the disease may be autosomal dominant or autosomal recessive; alternatively it may be sporadic.[1,2] The demonstration in 1985 by Armanini and colleagues[3] that patients affected by PHA show absent or strongly reduced [^{3}H]aldosterone binding to the mineralocorticoid receptor (MR) on mononucular leukocytes (MNL) strongly suggested that the pathogenesis of the disease was related to a cellular resistance to mineralocorticoid action.

The first description of hormonal resistance dates back to 1942, when pseudohypoparathyroidism was described by Fuller Albright and coworkers.[4] Since then, the field of hormonal resistance syndromes extended to other hormones, including androgens, vitamin D, cortisol, thyroid hormone, and insulin. In many of these syndromes, molecular analysis performed in order to identify the abnormality underlying the disease was successful in detecting alterations at the level of the genes encoding for the hormone receptor proteins. In particular, syndromes of resistance to steroid/thyroid hormones have been associated with a large variety of molecular abnormalities, ranging from deletions of the entire coding region to single mutations localized

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mainly in the DNA-binding or in the hormone-binding domain of the receptor.[5–8]

The mineralocorticoid receptor (MR; Figure 1) is a member of the steroid/thyroid hormone/retinoic acid receptor superfamily.[9] These are highly specialized transcription factors which, in response to hormone binding, activate *cis*-acting responsive elements located near or within hormonally responsive genes.[10] All the members of the family share the same structural organization in different domains which map to discrete segments of the receptor cDNA. These segments are differentially conserved among the members of the family, the centrally located DNA-binding domain being the most conserved. This domain is separated by the so-called hinge region from the C-terminal hormone binding domain. A constitutive transcription activation function is contained in the non-conserved N-terminal region.

We have previously postulated that the clinical and biochemical picture of PHA was due to an abnormality within the MR gene.[11] In this paper we discuss our results of the molecular analysis of the MR cDNA in a kindred affected by the disease and some major questions to which they give rise.

Experimental

Clinical summary

The family analyzed was already described elsewhere.[11,12] Briefly, the index case was hospitalized for vomiting and dehydration at 6 weeks of age. Serum sodium was 122 mEq/L, potassium 5.9 mEq/L, and urinary sodium was 93 mEq/L. Plasma aldosterone was 2324 nmol/L and PRA 14.8 ng/L/sec. Serum sodium returned to normal values after salt supplementation; however vomiting, serum sodium and potassium, and negative sodium balance were not influenced by the intramuscular administration of 4 mg/day of deoxycorticosterone acetate for 5 days.

The father had a history of vomiting and dehydration during early infancy which necessitated saline infusion. His plasma aldosterone and plasma renin activity were elevated. The mother had no history of salt-wasting or excessive salt appetite, and normal plasma aldosterone values.

One sister had a history of vomiting and dehydration at 3 weeks of age, and was treated with saline infusion. Serum sodium was 130 mEq/L and plasma aldosterone was elevated. The two other sisters both had a history of vomiting until 4 months of age, and showed high plasma aldosterone levels at first consultation.

The biochemical reevaluation of the family members is summarized in Table 1. In all subjects, blood pressure was normal and there was no detectable cardiovascular, respiratory, or renal disease.

As all the offspring are affected by the disease, the most likely mode of inheritance in this family is autosomal dominant. However, the small number of MR detected in the mother does not allow to exclude the possibility of her being an asymptomatic carrier.

Methods

DNA and RNA were extracted from patients' leukocytes by standard techniques.[13,14] For Southern blot analysis, aliquots of DNAs were digested with different restriction enzymes (*Taq*I, *Hin*fI, *Msp*I), subjected to electrophoresis on 1.2% agarose gels and transferred to nylon membranes. Filters were probed with three different ^{32}P-labeled probes obtained by *Eco*RI restriction of the human MR cDNA.[15]

Aliquots of total RNA were subjected to reverse transcription and subsequently amplified by PCR. PCR was carried out with 11 sets of primers covering the whole human MR coding sequence and generating overlapping fragments of approximately 350 bp in size.

PCR products were gel-purified and submitted to direct cycle sequencing as described elsewhere.[16]

Semiquantitative analysis of the amount of mRNA from lymphocytes was performed by RT-PCR with human β-actin mRNA as an internal control. Band intensities at 30 cycles of PCR were analyzed by computer-assisted densitometry and the results expressed as the ratio of MR:β-actin.

Results and discussion

Over the past few years, the study of hormonal resistance syndromes has provided much insight into the molecular mechanisms underlying hormone action. Different receptor abnormalities detected at the biochemical level were found to correspond to definite alterations of the primary structure of the receptor molecule. In particular, the absence of hormone-receptor binding was associated with modifications in the DNA region coding for the hormone binding domain of the receptor.[7,17] With regard to these results, and based on the analogy of the clinical picture, one would have expected to detect similar molecular abnormalities in the mineralocorticoid receptor of patients affected by PHA. However, this turned out not to be the case. Indeed, in the family studied, we failed to detect any major rearrangement of the MR, as demonstrated by the normal Southern blot analysis, and no mutation was present in the entire MR coding region. In addition, quantitation of MR mRNA revealed that in our patient MR are expressed in apparently normal amounts, at least in leukocytes.[16] Analogous results were obtained in another patient investigated by Komesaroff and coworkers.[18] The fact that there is no quantitative abnormality in MR mRNA expression suggests that PHA is not due to mutations which alter the mRNA stability and seems to eliminate the possibility of alterations in gene regulatory regions being responsible for the disease.

However, on the basis of these results, there remain major questions to address. First is whether what we detect in leukocytes reflects what happens in the kidney. MRs are expressed in so-called "classical" target tissues, which are sodium transporting epithelia (kidney, colon, salivary, and sweat glands) and in a variety of non-epithelial target tissues, such as hippocampus, (MNL), large blood vessels,

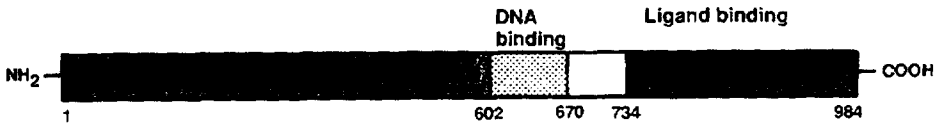

Figure 1 The human mineralocorticoid receptor protein.

Table 1 Biochemical data of the index case and the family members

	I1	I2	II1	II2	II3	II4
Serum Na (136–143 mmol/L)	142	136	141	140	N.D.	142
Serum K (3.5–4.5 mmol/L)	4	4.3	3.7	4.1	N.D.	3.8
Angiotensinogen (841–1287 ng AI/mL)	1295	1276	1840	1095	1100	1313
Total renin (113–299 pg/mL)	315	475	N.D.	468	298	453
Active renin (15–50 pg/mL)	75	41	53	35	35	90
Prorenin (101–297 pg/mL)	240	434	N.D.	433	263	363
Angiotensin converting enzyme (23.3–38.3 mU/mL)	36	30	27	32	N.D.	28
Plasma aldosterone (210–1008 nmol/L)	7672	588	3472	700	882	5250
Urinary aldosterone (0.8–4.4 mmol/mmol creatinine)	15.8	7.8	35.5	11.7	N.D.	36.1
Deoxycorticosterone (DOC) (0.18–0.7 nmol/L)	0.58	0.25	N.D.	0.41	0.28	0.72
18-OH-DOC (0.20–0.52 nmol/L)	0.46	0.32	N.D.	0.31	0.50	0.41
Corticosterone (B) (37–66 nmol/L)	24	30	N.D.	26	28	28
18-OH-B (2.4–4.7 ng/mL)	8.6	3.8	N.D.	5.8	3.6	6.4
MR (150–400 per cell)	104	96	0	119	N.D.	0

Normal values are shown in parentheses.
N.D., not determined.

and the heart. However, mineralocorticoid resistance seems to affect mainly two groups of organs: either the kidney alone (most frequent form) or the kidney, the colon, and the salivary and sweat glands.[2,19] In no case has abnormal mental or neurological development been observed in subjects affected by PHA, despite the fact that MR expression in the hippocampus is even higher than in the kidney. This fact may suggest differential mechanisms of aldosterone action at the level of its target tissues. In this context, the normal expression of MR mRNA in leukocytes would not exclude abnormal MR expression in other organs such as the kidney. Even if this is the case, the question remains of the meaning of the strongly reduced or absent aldosterone binding on MNL. If this is a matter of receptor down-regulation in a state of sustained hyperaldosteronism,[20,21] the mechanism(s) whereby aldosterone is regulating the number of receptor molecules are unclear. In rat hippocampus the expression of MR mRNA is increased after adrenalectomy,[22] whereas rat distal colonic MRs are incapable of homologous down-regulation of their own protein and mRNA levels in response to an MR agonist.[23] Indeed, recent studies suggest the presence of at least two different promoters for the rat MR,[22] which might be responsible for differential expression and regulation of distinct MR mRNA's. Although a more detailed analysis of these regulatory mechanisms is beyond the scope of this paper, they underline the complexity of mineralocorticoid effector mechanisms.

In this context, further studies of PHA might include genetic analysis, using polymorphic markers located in the MR gene, or positional cloning of the disease locus in large affected pedigree. Both approaches may provide an important step forward in the understanding of hormonal and in particular mineralocorticoid action.

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