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Pseudohypoaldosteronism: Evaluation of type I receptors by radioreceptor assay and by antireceptor antibodies

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We have previously demonstrated a deficiency of mineralocorticoid receptors in pseudohypoaldosteronism, by radioreceptorassay. We now report findings with an antireceptor antibody derived from the immunogenic region of the receptor. Lymphocytes from normal controls and from two cases of pseudohypoaldosteronism previously shown to lack receptor binding were tested. After the plasma membrane of lymphocytes was permeabilized with methanol the cells were incubated with a 1:200 dilution of antibody followed by fluorescent antirabbit immunoglobulin mouse serum. After washing fluorescence was detected by microscopy and cytofluorimetry in both controls and patients with pseudohypoaldosteronism. Recent studies on mineralocorticoid receptor cDNA in pseudohypoaldosteronism have not established a mutation in the sequence. We thus suggest that the pathogenesis of pseudohypoaldosteronism is not related to an abnormality of the receptor but rather due to intracellular factor(s) which can block the binding of aldosterone to its receptor. (*Steroids* **60**:161–163, 1995)

Keywords: pseudohypoaldosteronism; aldosterone receptors; immunofluorescence; radioreceptorassay; cytofluorimetry

Introduction

Congenital pseudohypoaldosteronism (PHA) is a rare disease which is characterized by renal sodium wasting, hyperreninemia, hyperaldosteronism, increased serum potassium, and insensitivity to mineralocorticoids in infancy. Therapy with prolonged sodium supplementation normalizes sodium balance, with aldosterone remaining elevated.[1] In 1985 we established a radioreceptorassay (RRA) for measuring the number of mineralocorticoid receptors (MR) in human mononuclear leukocytes (MNL),[2] and found an absence or marked reduction in aldosterone receptor levels in PHA. Accordingly, we postulated that the pathogenesis of the disease may reflect a congenital reduction or absence of mineralocorticoid receptors.[3,4] Subsequent studies have analyzed the molecular structure of the human MR gene in pseudohypoaldosteronism. Southern blot analysis of the DNA obtained from the lymphocytes of patients with pseudohypoaldosteronism showed no major rearrangement of the MR gene. In addition point mutations in the cDNA of the MR were also excluded by reverse transcription-polymerase chain reaction of mRNA extracted from leukocytes of PHA patients.[5]

In the present study we have evaluated MR in the syndrome of pseudohypoaldosteronism by immunofluorescence, using an antireceptor antibody, and compared the results with those obtained by RRA in the same cells.

Experimental

We used a previously evaluated rabbit polyclonal antibody against a peptide sequence deduced from the immunogenic region of the cloned MR. In sections of human kidney, cells of segments corresponding to distal tubule, connecting piece, and initial cortical collecting duct were stained, consistent with the known sites of mineralocorticoid action.[6]

Heparinized blood was layered onto a gradient of Percoll and, after centrifugation, the mononuclear layer was used. The cells were then washed and viability (99%) checked. An aliquot of cells was then incubated for 20 min in pure methanol to permeabilize the plasma membranes. After washing out methanol the cells were incubated at 4°C with 1:200 diluted antibody. After overnight incubation the cells were washed and a fluorescent anti-rabbit immunoglobulin polyclonal mouse serum was added, and after one hour the cells were washed again. An aliquot of the cells was used for fluorescence microscopy and for cytofluorimetric evaluation (Cytoron Ortho).

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Measurement of the number of receptors by RRA has already been described in detail.[2] In brief, an aliquot of cells was incubated with increasing amounts of [^{3}H]aldosterone alone or with addition of excess of nonradioactive aldosterone. In all tubes 100-fold excess RU 28362 was added to confine binding to MR. After incubation the cells were washed, their radioactivity counted, and the number of receptors per cell calculated by Scatchard analysis. Normal values of MR in MNL of children are 100–400 and in adults 175–400 receptors per cell. Measurement of glucocorticoid receptors (GR) was done in parallel using [^{3}H]dexamethasone as tracer and excess nonradioactive dexamethasone to determine nonspecific binding. Normal values are 1800–5000 receptors per cell.

Results

In normal subjects specific fluorescence was found in almost all the cells (Fig. 1), while no fluorescence was evident in control cells preincubated with normal rabbit serum and treated with secondary fluorescent antibody.

In two families with pseudohypoaldosteronism similar results were seen both in the affected patients and in their parents. Similar results were also obtained by cytofluorimetry: 93–97% of the cells were positive for fluorescence, while the same cells incubated with the secondary antibody after preincubation with normal rabbit serum were less than 0.5% positive.

Table 1 shows levels of MR and GR measured by RRA and detected by immunofluorescence.

Another study was also performed on immortalized lymphocytes from 5 members of a family described originally by Roy.[6] On RRA no MR were seen, with 2000–9400 GR per cell and with MR detected by immunofluorescence (95–99% fluorescence-positive cells).

Discussion

The pathogenesis of pseudohypoaldosteronism is still debated. After the observation of absent or reduced number of MR by RRA it seemed clear that the abnormality was at the level of the receptor,[3,4] but subsequent studies carried out on the receptor sequence have not demonstrated any abnormality.[5] The present study confirmed that MR are expressed

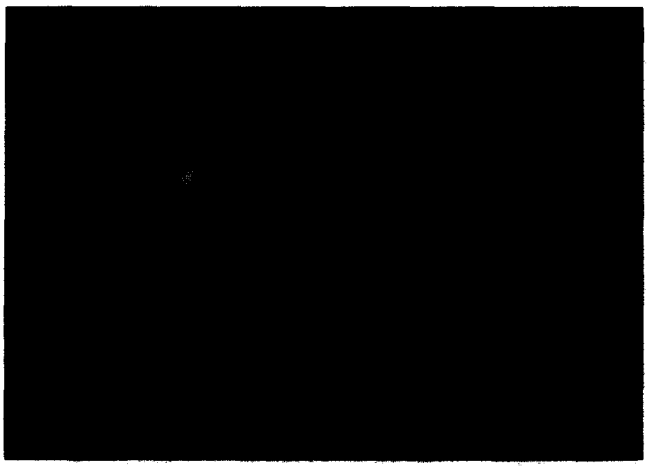

Figure 1 Specific fluorescence of mononuclear leucocytes of a normal control by fluorescence microscopy.

Table 1 Mineralocorticoid and glucocorticoid receptor evaluation by radioreceptorassay and by anti-receptor antibody in the members of a family with pseudohypoaldosteronism

	MR RRA n × cell	GR RRA n × cell	MR:antireceptor antibody (% fluorescent cells)
Father	125	1714	89
Mother	580	1347	88
Affected child	0	4870	94

MR = mineralocorticoid receptors.
GR = glucocorticoid receptors.
RRA = radioreceptorassay.

in MNL of affected patients, since normal immunofluorescence is detectable with an anti-receptor antibody. The only possible explanation of the data obtained with the three different approaches is that in pseudohypoaldosteronism, aldosterone cannot bind to the receptor.

Our recent data on patients with acquired pseudohypoaldosteronism due to obstructive uropathy and infection are interesting at this point. These patients have greatly reduced MR numbers during the period of obstruction and/or infection, while after surgical correction or relief of obstruction the receptor status normalizes.[7] It thus seems that a factor present in the aldosterone target cell in both primary and secondary pseudohypoaldosteronism limits or excludes the binding of aldosterone to its receptor. This factor appears to be intracellular, since after washing out the plasma the receptors are not measurable. In acquired pseudohypoaldosteronism such a factor may be a bacterial toxin which acts at the intracellular level by blocking the binding of aldosterone to its receptor. In primary pseudohypoaldosteronism the putative factor is apparently congenital, and persists in immortalized cells which do not have MR binding, though GR binding is normal or even slightly increased.

In the present study we have evaluated two families with no consanguinity and probable autosomal dominant transmission. The possibility cannot be excluded that autosomal recessive cases, which are usually present in the offspring of consanguineous parents, might give a different result.

Our knowledge of the regulation of the effector mechanism of aldosterone is incomplete. From recent findings it is clear that factors independent of the receptor can regulate the effects and concentration of the steroid. One of these factors is 11β-hydroxysteroid dehydrogenase, which has been involved in the apparent mineralocorticoid excess syndrome[8] and in licorice-induced hypertension.[9] We have also found evidence for other factors: for example, during pregnancy[10] plasma aldosterone is high though aldosterone receptor number in mononuclear leukocytes, and aldosterone effector mechanisms, are normal.

The last point of interest is how these patients are able to compensate for the abnormality in electrolyte and water balance without the classical effector mechanism of aldosterone. The most obvious interpretation is that the osmoreceptors can reset and directly regulate electrolyte balance. It remains to be clarified why the normalization of electrolyte balance is not followed by normalization of the renin-aldosterone system, given that the regulation of renin se-

cretion is mediated by volume and sodium changes at the level of the juxtaglomerular apparatus.

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