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Immunohistochemical localization of renal mineralocorticoid receptor by using an anti-idiotypic antibody that is an internal image of aldosterone

(monoclonal antibody/steroid receptors/kidney)

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ABSTRACT **A monoclonal antibody (H10E), generated by an auto-anti-idiotypic procedure and directed at the aldosterone-binding site of mineralocorticoid receptor (MR), was used in immunohistochemical studies to localize MR in rabbit kidney preparations. In agreement with earlier physiological and biochemical observations, MR was detected in connecting and cortical collecting tubules. Additionally, MR was detected in the distal tubules, the medullary and papillary collecting ducts, and in the epithelial cells lining the papilla. The internal image properties of the antibody were exploited to assess the specificity of MR detection by means of competition studies with hormones and antihormones. Immunostaining was completely abolished by preincubation with aldosterone but not with RU 486, a steroid antagonist that does not bind MR. On the cellular level, immunostaining occurred in the cytoplasm and, in the majority of cells, in the nucleus as well. The nucleocytoplasmic distribution of MR was unaffected by adrenalectomy or by administration of aldosterone. The availability of this specific monoclonal antibody makes it feasible to study MR expression in other target tissues and in pathological disorders.**

Although antibodies have been prepared to most steroid receptors, there is no report of success in generating mineralocorticoid receptor (MR)-specific antibodies by immunization with MR preparations. However, a MR-specific monoclonal antibody (mAb) (H10E) has recently been obtained by an auto-anti-idiotypic approach (1), which, rather than requiring immunization with purified MR, allows immunization with a conjugate of aldosterone, the steroid ligand of MR. The auto-anti-idiotypic antibody H10E is an internal image of aldosterone. This mAb specifically recognizes the aldosterone-binding site of MR and does not crossreact with the glucocorticoid receptor in rabbit kidney cytosol preparations (1). In recent experiments (M.L. and B.F.E., unpublished work), the mAb reacted with human MR expressed transiently in COS cells transfected with pRShMR, a plasmid containing human MR cDNA (2). The availability of this mAb allows the study of MR by specific, sensitive immunologic techniques. Moreover, the reversible and reciprocal competition between the anti-idiotypic antibody and MR-specific ligands for the steroid-binding site of the receptor enables clear demonstration of the specificity of immunodetection.

The kidney is a major target for mineralocorticoid hormones. Although biochemical, autoradiographic, as well as transport studies have indicated that aldosterone acts on the distal nephron, precise localization of aldosterone action along the entire nephron is still controversial. We report on the tissue localization of MR in rabbit kidney and the effect of adrenalectomy and aldosterone treatment on its intracellular distribution through the use of an immunohistochemical technique.

MATERIALS AND METHODS

Reagents. Aldosterone, dexamethasone, and estradiol were from Sigma. Deoxycorticosterone acetate and RU 486 were from Roussel-Uclaf (Romainville, France). SC 9420 was from Searle.

Tissue Preparation. New Zealand White female rabbits weighing 1.5 kg were used in this study. Bilateral adrenalectomy was performed under Fluothan anesthesia. The animals received Syncortyl (1.9 mg/kg; deoxycorticosterone acetate) i.p. at the time of operation and the 2 following days and were given 0.9% saline to drink *ad libitum*. These animals were then used 5 to 7 days after surgery. Aldosterone-treated rabbits received an i.p. injection of 1.5 mg of aldosterone 45 min before the study.

Normal, adrenalectomized, or aldosterone-treated rabbits were anesthetized with 25 to 50 mg of Nembutal and perfused through the abdominal aorta with 500 ml of Zamboni's solution [2% (wt/vol) paraformaldehyde/15% (vol/vol) saturated picric acid solution/300 mM (85%, vol/vol) sodium phosphate buffer, pH 7.4] at 37°C. The perfusion procedure was done as described (3). At the end of perfusion, the kidneys were removed, cut into slices and pyramids (≈2 mm thick), and postfixed for 24 hr in the Zamboni fixative. The kidney slices and pyramids were washed in 70% (vol/vol) ethanol, dehydrated in graded ethanol, cleared in 1-butanol, and embedded in Paraplast. Sections (7 μm) were cut, mounted on histological slides, and processed for immunohistochemistry.

Immunohistochemical Technique. A routine procedure of indirect immunostaining was used (4). Briefly, after deparaffinization and rehydration, sections were incubated with 3% normal horse serum in phosphate-buffered saline (PBS) solution. The anti-idiotypic mAb H10E, a mouse IgG1 immunoglobulin, was used as diluted ascites fluid (1). A nonimmune preparation of mouse IgG (prepared in the laboratory) was used as control. The mAb was used at ≈1–5 μg/ml, followed by a horse biotinylated anti-mouse IgG antibody (Vector Laboratories). The avidin–biotin–peroxidase complex (ABC-Elite from Vector Laboratories) was used as a detection system. After each incubation step, the slides were rinsed in PBS. Peroxidase activity was revealed by diami-

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Abbreviations: MR, mineralocorticoid receptor; mAb, monoclonal antibody.

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nobenzidine tetrahydrochloride (0.5 mg/ml) in the presence of 0.01% H_2O_2. Sections were then dehydrated and mounted in Canada Balsam without counterstaining.

The competition experiments were done by preincubating sections in PBS containing 1 μM steroid (aldosterone, dexamethasone, or estradiol) or anti-hormone (RU 486 or SC 9420) for 30 min before incubation with mAb H10E (1 to 5 μg/ml or ≈30 nM assuming a M_r of 150,000 for an IgG) together with the steroid or the antihormone.

RESULTS AND DISCUSSION

By means of an immunohistochemical technique (4), the anti-idiotypic mAb H10E revealed immunoreactive material in several portions of the rabbit nephron (Fig. 1). Immunostaining was seen in the connecting and cortical collecting tubules (Fig. 1*A*). No staining was found in cells of the glomeruli or in the proximal tubules. This distribution is similar to autoradiographic patterns of aldosterone receptors in rabbit kidney (5, 6) and is in agreement with the preliminary detection of MR mRNA by *in situ* hybridization in rat kidney (7). These observations are also in accord with physiological studies that confine the sites of mineralocorticoid-dependent ionic transport to those parts of the nephron (8).

MR was also detected in the distal tubules and in the medullary and papillary collecting ducts, where autoradiographic and ion-transport studies have also suggested an aldosterone effect. An important observation is the finding of MR in interstitial cells of the papilla and in the epithelium lining the papilla (Fig. 1*B*); the physiological significance of this observation is presently unknown.

Labeling of cells in all kidney structures varied from cell to cell, with some cells of the tubules appearing unstained (Fig. 1 *C* and *E*). This heterogeneity may be related to different cell types. In this regard, principal and intercalated cells (3) have been described with differentiated transport functions and hormonal responsiveness (9, 10).

Fig. 1*C* shows that most positive cells are stained in both cytoplasm and nuclei, whereas some cells are stained only in the cytoplasm. No differences were seen in the immunostaining patterns and intracellular distribution in tissues, whether obtained from intact control rabbits, from aldosterone-treated rabbits, or from animals deprived of aldosterone after adrenalectomy.

The above findings were supported by several control experiments. No staining was observed with an IgG control (data not shown). As a pure MR preparation does not exist, the usual control experiment of specificity by preabsorption of the antibody cannot be done. However, a control experiment that took into account the internal image properties of the antibody (1) was done. Mutual competition between aldosterone and mAb H10E for the steroid-binding site of MR could be shown. Fig. 1*D* shows that when a tissue section was preincubated with aldosterone (1 μM), immunostaining was completely abolished. Other ligands that bind MR had similar effects: extinction was observed with SC 9420 (5 μM), a spirolactone antagonist of aldosterone (11), and with dexamethasone, a synthetic glucocorticoid that binds with high affinity to MR as well as to glucocorticoid receptor (12). In contrast, immunostaining was unaffected by RU 486 (1 μM), a steroid antagonist that does not bind to MR (13), or by estradiol (1 μM), which also does not interact with MR (Fig. 1*E*). Together, these results demonstrate the reliability of the anti-idiotypic mAb H10E for the immunohistochemical detection of MR.

The nucleocytoplasmic distribution of MR detected immunochemically contrasts with an almost exclusive nuclear

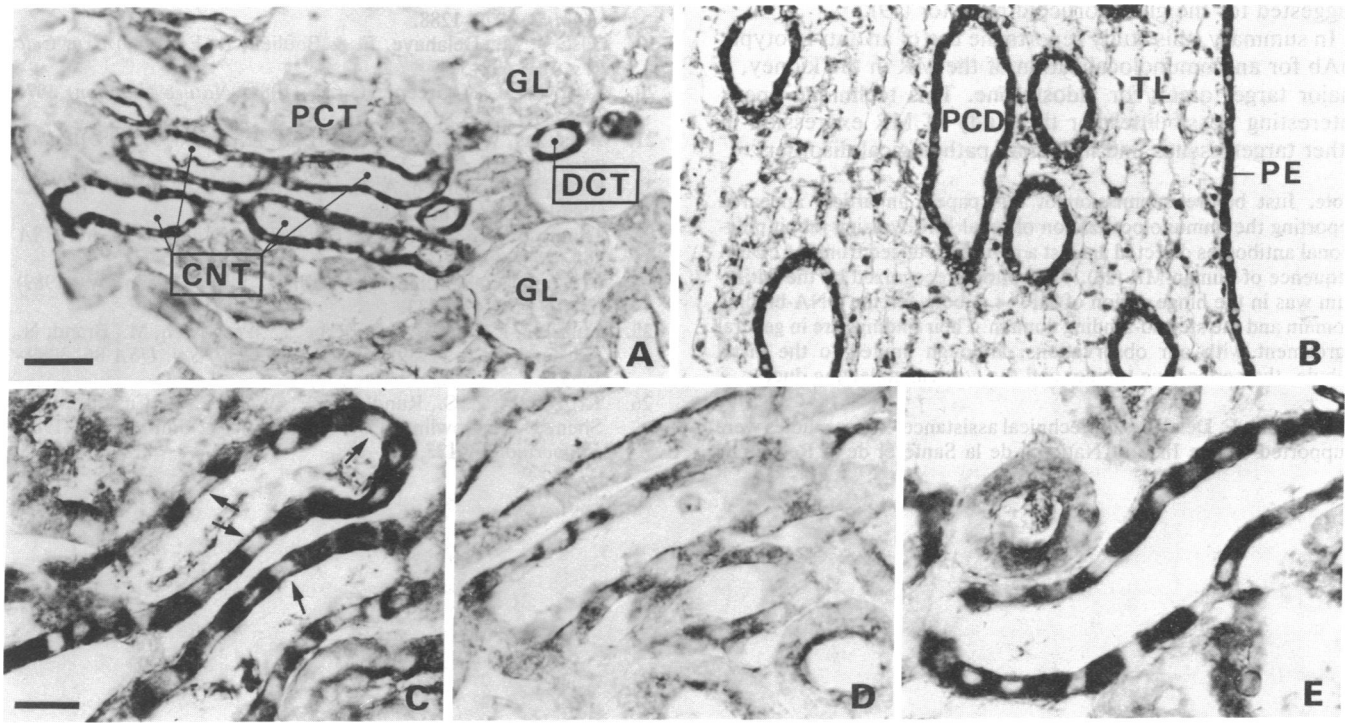

FIG. 1. Immunodetection of MR in rabbit kidney with the anti-idiotypic mAb H10E. (*A*) In kidney cortex, an intense immunostaining is seen in the connecting (CNT) and collecting and distal tubules (DCT). The glomeruli (GL) and the proximal tubules (PCT) are not reactive. (*B*) In the medulla and papilla, MR is revealed in the collecting tubules (PCD), Henle's loops (TL), and papillary epithelium (PE). (*C*) Along the cortical collecting tubules, some cells appear devoid of staining (arrows). Most positive cells are stained in both the cytoplasm and the nucleus. A few cells display only cytoplasmic staining. These cells are seen whether or not the animal has circulating aldosterone. This picture is from an intact animal; staining is the same for an animal deprived of aldosterone after adrenalectomy as in *B*. (*D*) When excess aldosterone (1 μM) is applied to the section together with mAb H10E, immunostaining is abolished. (*E*) Excess RU 486 (1 μM), a steroid antagonist that does not bind to MR, does not impair immunostaining. No counterstaining was done. (Bar = 40 μm in *A* and *B*; comparable length = 10 μm for *C*, *D*, and *E*).

localization of hormone–receptor complexes shown by autohistoradiography after exposure of intact isolated rabbit tubules to [^{3}H]aldosterone (5, 6). The conditions required by the respective methods may explain this difference. Autoradiography, which is performed after incubation of tissues with tritiated ligand, would reveal only receptors still complexed with [^{3}H]aldosterone. In contrast, the antigen–antibody interaction occurs in a fixed tissue preparation and detects receptors irrespective of the presence of bound hormone.

Within the limits of the sensitivity of the immunohistochemical technique, we found no difference in the nucleocytoplasmic distribution of MR, regardless of the endocrine status of the animal. The absence of a clear nuclear translocation of MR after hormone exposure and the apparent distribution in both the nucleus and the cytoplasm are in agreement with results reported for the glucocorticoid receptor (14–16), although others have reported hormone-dependent nuclear translocation of glucocorticoid receptor (17, 18). Distribution of MR differs from the solely nuclear distribution described for the estrogen and progesterone receptors (19–21). Because the mAb mimics aldosterone, we cannot absolutely exclude the possibility that part of the cytoplasmic staining is due to cross-reactivity with a nonreceptor aldosterone-binding protein analogous to transcortin (22) or an enzyme of aldosterone metabolism. The receptor-like specificity found in the competition experiments, however, strongly supports the specific detection of MR. It is also possible that, as with thyroid (23) and retinoic acid receptors (24, 25), multiple forms of MR exist, which have different physiological functions requiring diverse partitioning in cell compartments. Two forms of receptors differing by their intracellular distribution and functions have already been suggested for the glucocorticoid receptor (19).

In summary, this study reports the use of an anti-idiotypic mAb for an immunolocalization of the MR in the kidney, a major target organ for aldosterone. This technique opens interesting possibilities for the study of MR expression in other target tissues and in human pathological disorders.

Note. Just before submission of this paper, an article appeared reporting the immunolocalization of renal MR by using rabbit polyclonal antibodies directed against a peptide deduced from the cDNA sequence of human MR (26). The epitope recognized by the antiserum was in the hinge region of MR—i.e, between the DNA-binding domain and the steroid-binding domain. Their findings are in general agreement with our observations, although limited to the distal tubule, the connecting tubule, and the cortical collecting duct.

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