Immunohistochemical and Biochemical Evidence for a Cardiovascular Mineralocorticoid Receptor

Marc Lombès, Marie-Edith Oblin, Jean-Marie Gasc, Etienne Emile Baulieu, Nicolette Farman, and Jean-Pierre Bonvalet

The presence of mineralocorticoid receptors (MRs) and their physicochemical characteristics were investigated in the heart and blood vessels of rabbits. Immunohistochemical methods using the monoclonal anti-idiotypic antibody H10E, which interacts with the steroid binding domain of MRs, revealed the presence of immunoreactive material in the heart and large blood vessels. In the heart, a positive staining was observed in myocytes and endothelial cells of atria and ventricles. In vessels, MRs were detected in the aorta and pulmonary artery. They were localized in endothelial and vascular smooth muscle cells. No staining was present in the small vascular bed, arterioles, and capillaries. In all these studies, the mineralocorticoid specificity of the staining was assessed by in situ competition experiments with aldosterone and RU486, a glucocorticoid antagonist. The presence of MRs in the heart and vessels was further demonstrated by specific aldosterone binding to one class of high affinity binding sites in the cytosol of the adrenalectomized rabbit heart (Kd, 0.25 nM; maximum MR concentration, 15–20 fmol/mg protein), whose mineralocorticoid specificity has been clearly established by competition studies. Sedimentation gradient analyses revealed that the cardiovascular MR is an 8.5S hetero-oligomer that includes the heat shock protein 90. The physicochemical characteristics of the cardiovascular MRs are virtually identical to those of the renal MRs. Altogether, our results clearly demonstrate the presence of MRs in the cardiovascular system. This supports the possibility of direct aldosterone actions in the heart and blood vessels. (Circulation Research 1992;71:503–510)

Key Words • aldosterone • monoclonal antibodies • heart • blood vessels • rabbits

The mineralocorticoid hormone aldosterone is involved in the regulation of sodium balance and body fluids and consequently participates in the control of blood pressure (for review, see Reference 1). Besides the action of aldosterone in the kidney, where it regulates electrolyte transport in the distal parts of the nephron, several studies argue in favor of an effect of this hormone on cardiovascular functions through direct actions on the heart and vascular tissues. Aldosterone actions are thought to be mediated through binding to specific intracellular receptors. Although several studies have reported aldosterone binding in the heart,2,3 and arteries,4–8 many uncertainties remain concerning the nature of aldosterone action on the cardiovascular system.

Experimental studies have reported that mineralocorticoids act on arterial smooth muscle cells, leading to an increased vasoreactivity to pressor agents.9,10 They may modulate muscle tone and vessel elasticity and induce variations of electrolyte composition of the arterial wall.11–15 Additional studies have suggested that the heart is a mineralocorticoid-responsive organ since aldosterone stimulates Na⁺,K⁺-ATPase synthesis in rat cardiocytes16 and may directly influence myocardial structure and functions.17,18

Recently, the availability of a monoclonal anti–mineralocorticoid receptor antibody H10E19 renders it possible to directly assess the presence of mineralocorticoid receptors (MRs) in the heart and vessels. This anti-idiotypic antibody H10E, which interacts with the steroid binding domain of MRs, has already been successfully used to immunolocalize the receptor along the rabbit nephron.20,21

The present study was undertaken to localize and characterize MRs in the rabbit heart and vessels by both biochemical and immunohistochemical methods. The biochemical approach allows for the characterization of the cardiovascular MR in terms of affinity and physical structure and the quantification of its abundance among various tissues. In addition, the distribution of MRs among different cell populations can be determined by immunohistochemistry. The present article demonstrates the presence of MRs in the cardiovascular system and provides a basis for further studies of the mechanisms of aldosterone action in these tissues.

Materials and Methods

Buffers

The following buffers were used: TEG buffer, consisting of 20 mM Tris-HCl, 1 mM EDTA, and 10% glycerol;
TEGW buffer, consisting of TEG buffer plus 20 mM sodium tungstate; and TEW buffer, consisting of 20 mM Tris-HCl, 1 mM EDTA, and 20 mM sodium tungstate at pH 7.4. All buffers were adjusted to pH 7.4 (25°C). Zamboni fixative, consisting of 2% paraformaldehyde (wt/vol), 15% saturated picric acid (vol/vol), and 85% 0.15 M sodium phosphate buffer at pH 7.4 (vol/vol), was also used.

**Chemicals**

1,2-[³H]Aldosterone was purchased from Radiochemical Center, Amersham, UK. Unlabeled aldosterone was from Sigma Chemical Co., St. Louis, Mo. RU486 was obtained from Roussel Uclaf Laboratories, Romainville, France. Spironolactone (SC9420) and ZK91587 were from Searle Laboratories, Chicago, Ill., and NEN Research Products, Du Pont de Nemours, France, respectively. To avoid steroid adsorption, the steroid solutions prepared in ethanol were dried and resuspended in 50% polyethylene glycol 300 prepared in TEG buffer to reach a 5% final concentration of polyethylene glycol 300 in the cytosol.

**Antibodies**

The following antibodies were used: H10E4C9F, a mouse immunoglobulin (Ig) G1 monoclonal antibody that interacts with the steroid binding domain of the mineralocorticoid receptor; and 7C10, a mouse IgGl monoclonal antibody directed against the rabbit heat shock protein 90 (hsp90).

**Animals**

Experiments were performed on New Zealand female rabbits weighing 1–2 kg. Some animals were bilaterally adrenalectomized under anesthesia and given 0.9% saline to drink ad libitum for 48 hours. Normal or adrenalectomized animals were perfused in situ with either 500 ml prewarmed Zamboni fixative for immunohistochemical studies or 300 ml cold 0.9% saline followed by 200 ml cold TEGW buffer for biochemical analyses.

**Immunohistochemical Technique**

At the end of the perfusion, small pieces of different tissues were postfixed in Zamboni fixative for 24 hours. They were then washed several times with 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. A routine procedure of indirect immunostaining was used. After deparaffinization and rehydration, sections were incubated with 1% normal horse serum in phosphate-buffered saline. The monoclonal anti-idiotypic antibody H10E, a mouse IgGl immunoglobulin, was incubated as diluted ascites at a concentration of 1–5 μg/ml for 2 hours at room temperature or overnight at 4°C. A mouse IgGl, MOPC 31C (Sigma), was used as control. The mouse antibody was followed by a horse biotinylated anti-mouse antibody (Vector Laboratories, Inc., Burlingame, Calif.), and the avidin-biotin-peroxidase complex (ABC-Elite from Vector Laboratories) was used as a detection system. The slides were rinsed in phosphate-buffered saline after each incubation step. The peroxidase activity was revealed by diaminobenzidine tetrahydrochlo-

ride (0.5 mg/ml) in the presence of 0.01% hydrogen peroxide. Sections were dehydrated and mounted in Canada balsam without counterstaining. In situ competition studies were performed by preincubating tissue sections with 1–5 μM steroid (aldosterone or RU486) in phosphate-buffered saline 30 minutes before incubation with H10E antibody together with the steroid.

**Cytosolic Preparation**

After in situ perfusion, various tissues or organs were cut in small pieces and immediately frozen in liquid nitrogen. Pieces were then ground in a mortar under liquid nitrogen. TEGW buffer was added to the resulting powder (2–3 ml/g). As soon as the mixture had thawed, it was vortexed at 0°C and homogenized with a Teflon-glass Potter apparatus at 4°C. The crude homogenate was centrifuged at 105,000g for 60 minutes at 4°C. The resulting supernatant was immediately used or frozen in liquid nitrogen with no change in the binding activity or physicochemical properties of the MR.

**Binding Studies**

To determine aldosterone binding parameters at equilibrium in the heart, increasing amounts of [³H]aldosterone (0.1–100 nM) were incubated with 50 μl cytosolic aliquots for 4 hours at 4°C. Bound and unbound steroids were separated by dextran-charcoal technique. The evolution of bound steroid as a function of unbound steroid was analyzed by a computerized method. Competition studies were performed by incubating 50 μl cytosolic heart aliquots for 4 hours at 4°C with 5 nM [³H]aldosterone in the absence or presence of unlabeled steroids: aldosterone, SC9420, ZK91587, or RU486. Unbound steroid was removed by dextran-charcoal treatment. The results are expressed as the percentage of [³H]aldosterone binding alone.

**Density Gradient Analyses**

Cytosolic samples were incubated with 5 nM [³H]aldosterone alone or in the presence of either 0.5 μM RU486 or the monoclonal antibody H10E (1/100 diluted ascites) for 4 hours at 4°C. After dextran-charcoal treatment, samples, generally 0.15 ml, were layered onto the top of 15–40% glycerol gradients prepared in TEGW buffer. Gradients were prepared as six discontinuous layers and maintained at least 2 hours at 4°C before use. To determine the subunit composition of the MR, heart cytosol was incubated with either a nonimmune mouse IgGl or the monoclonal anti-hsp90 antibody, 7C10, for 4 hours at 4°C and then layered onto the top of glycerol gradients. Gradients were centrifuged for 18 hours at 4°C at 50,000 rpm in a rotor (model SW60, Beckman Instruments, Gagny, France). Two-drop fractions were collected from the bottom of the tube after puncture, and the radioactivity was counted. Myoglobin (2S region), bovine serum albumin (4.6S region), and aldolase (7.9S region) were used as external standards in parallel tubes. The quantification of MR content in various rabbit tissues was achieved by counting the specific radioactivity recovered in the 8S–9S region of the gradient profile.
Miscellaneous

Protein concentration was determined by Bradford's method, and bovine serum albumin was used as a standard. The position of standard proteins in the gradient fractions was determined by Lowry's method.

Results

Immunolocalization of MRs

Figure 1 exemplifies the immunostaining obtained with the monoclonal antibody H10E in the left atrium of the rabbit heart. A strong labeling is visible in the myocytes and the endothelial cells (Figure 1A). Within the subendothelial connective layer of the left atrium, some cells, presumably fibrocytes, also seem to be labeled. When compared with a serial section stained with Masson's trichrome (Figure 1C), it appears that the immunostaining of myocytes is distributed over both nuclear and cytoplasmic compartments. The specificity of the immunoreactivity for the MR was established by in situ competition studies in which preincubation with aldosterone was performed before incubation with the monoclonal anti-idiotypic antibody H10E. In this condition, the intensity of the staining is strongly reduced in atrial myocytes as well as in the endothelial cells (Figure 1B). A murine monoclonal antibody MOPC31 unrelated to MR, used as a control, did not give any significant staining (data not shown).

The results obtained from rabbit aorta are presented in Figure 2. Both endothelial cells and vascular smooth muscle cells of the arterial wall are positive (Figure 2A). As in the case of the left atrium, preincubation with aldosterone significantly reduced the immunostaining (Figure 2B). In contrast, RU486, a synthetic steroid that does not bind MRs, does not modify the pattern of immunostaining (Figure 2C).

Figure 3 shows immunostaining observed in the left ventricle (Figure 3A) and the pulmonary artery (Figure 3B). These micrographs were taken from the section used for photographs of Figures 1 and 2. This heart section includes the left atrium (Figure 1A), left ventricle (Figure 3A), the initial part of the ascending aorta (Figure 2A), and the pulmonary artery (Figure 3B). The fact that each part of this section was submitted to strictly identical experimental procedures allows a direct comparison of the relative intensity of the immunostaining from one tissue to another. The distribution of immunostaining in the ventricle is very similar to that of the atrium. However, the intensity of labeling is stronger in atrial myocytes than in ventricular myocytes. On the other hand, the immunostaining seems to be weaker in the aorta and the pulmonary artery. Although quantitative interpretation of immunohistochemical data is difficult, our results suggest that MR is relatively more abundant in the heart, in particular the atrium, than in large arteries.

Figure 4 illustrates the immunostaining observed in the smaller vascular bed. In contrast to the aorta (Figure 2A) and the pulmonary artery (Figure 3B), only a weak immunostaining was detected in smaller arteries such as the carotid (Figure 4B), humeral, mesenteric, coronary, and renal arteries (data not shown). No significant staining was apparent in the vena cava and portal vein (data not shown). With respect to the arterioles and capillaries, no MR immunoreactivity was detectable. Examples of the absence of vascular staining are given for an intramyocardial capillary, contrasting with the strong labeling of the myocytes (Figure 4A), and an intrarenal arteriole, contrasting with the heavy immunostaining of a cortical collecting duct (Figure 4C).

To study the effect of the endocrine status on cardiovascular MR expression, we examined heart and blood vessel sections of normal or adrenalectomized rabbits. In these conditions, we could not detect any difference in the intensity of the immunostaining given by H10E or...
one class of specific and high affinity binding sites, with a $K_d$ value of 0.25 nM and a maximum number of sites of 22.1 fmol/mg protein. A nonspecific binding was also detected and corresponded to the $B$ constant reported in the inset of Figure 5. The aldosterone binding sites detected in the cardiac cytosol clearly displayed a mineralocorticoid specificity, as demonstrated by competition assays shown in Figure 6. Unlabeled aldosterone competed with [H]aldosterone for binding to MRs with 50% inhibition occurring for a concentration (IC$_{50}$) of 0.5 nM consistent with the $K_d$ value of 0.25 nM estimated from the Scatchard plot analysis. Two antimineralocorticoid compounds, SC9420 and ZK91587, were potent competitors with IC$_{50}$s of 3 and 10 nM, respectively. In contrast, RU486, a synthetic steroid that does not bind to MRs, was unable to compete for aldosterone binding sites.

Glycerol gradient centrifugation analyses of heart cytosol labeled with tritiated aldosterone in the absence or presence of RU486 or of the monoclonal antibody H10E were performed to determine the sedimentation characteristics of the heart MR. As observed in Figure 7A, aldosterone-MR complexes sedimented as an 8.5S species ($8.78 \pm 0.26S$, $n=5$). H10E completely abolished the [H]aldosterone binding to this 8S–9S form of MR (Figure 7A), confirming the immunological recognition of cardiac MRs by the monoclonal anti-idiotypic antibody H10E. As expected, RU486 did not modify the sedimentation profile of these receptors (data not shown). Like other steroid receptors, MR under its large untransformed state is associated with a non-DNA binding protein identified as the heat shock protein of $M_r \approx 90,000$ (hsp90). Therefore, the subunit composition of the heart MR was studied using 7C10, a monoclonal antibody that specifically recognized rabbit hsp90. When aldosterone-labeled receptors were incubated in the presence of 7C10, the peak of radioactivity was clearly shifted to the 11S region of the gradient as illustrated in Figure 7B. This shift is ac-

**FIGURE 2.** Immunodetection of mineralocorticoid receptors in the rabbit aorta. The sections include the endothelium (arrows), the arterial wall with the vascular smooth muscle cells, and the tunica adventitia (bottom of each panel). Panel A: Monoclonal antibody H10E reveals immunoreactive material in the endothelial cells (indicated by the arrows) as well as in the vascular smooth muscle cells of the aortic wall. Panel B: When aldosterone is applied to the tissue section together with the monoclonal antibody H10E, the immunostaining is clearly reduced. Panel C: In contrast, excess RU486 does not impair the immunoreactivity. Magnification, ×344.

**FIGURE 3.** Immunostaining of the left ventricle and pulmonary artery with monoclonal antibody H10E. Panel A: In the left ventricle, a strong positive staining is observed over the myocytes (m). Magnification, ×202.5. Panel B: In the pulmonary artery, mineralocorticoid receptors are detected in the endothelial cells (e) and within the entire arterial wall. Magnification, ×127.5.

Biochemical Characterization of MRs
Since a specific immunostaining was observed in the heart and the large blood vessels, we characterized [H]aldosterone binding in the cardiovascular system and studied the physicochemical properties of MRs in these tissues. Biochemical analyses were performed in the cytosolic fractions of several tissues of adrenalectomized rabbits. We first measured aldosterone binding in the heart cytosol. Scatchard plot representation of [H]aldosterone binding is presented in Figure 5. Computerized analysis of the experimental data revealed in the relative cellular distribution of the labeling among various tissues examined.
counted for by the increase of the molecular weight resulting from the binding of the antibody to the untransformed MR complex, indicating that the cardiac MR is clearly a hetero-oligomer that includes hsp90.

The quantification of MR can be easily achieved by counting the radioactivity recovered in the 8S–9S region of the gradient. Using this method, MR concentration in the heart cytosol was estimated to be 16.9 fmol/mg protein, a figure close to that calculated from the Scatchard plot analysis. We have also investigated the presence of MRs in the aorta and the vena cava by aldosterone binding assays. As a reference, the binding of tritiated aldosterone to the cytosol of kidney and salivary glands was also examined in the same animal. The binding of [3H]aldosterone to MRs was measured by means of glycerol density gradient analyses, and the specificity was confirmed by competition with either RU486 or H10E, as described above. Under these conditions, all cytosolic fractions were found to contain MR that sedimented in the 8S–9S region (data not shown). The quantification of MR content in these various tissues is given in Figure 8. The concentration of aortic MR was slightly lower than the one estimated in the heart. In accordance with immunohistochemical data, specific aldosterone binding in the cytosol of the vena cava was almost undetectable.

Discussion

Our study provides evidence for the presence of MRs in the rabbit heart and large blood vessels. These findings are supported by the results obtained by two complementary methodological approaches. Biochemical methods have been used to characterize binding and structural properties of the cardiovascular MR. Immunohistochemistry has been performed to determine the

Figure 4. Immunodetection of reactivity in the smaller arteries, arterioles, and capillaries. Panel A: A capillary (c) in the left atrium is devoid of any immunoreactivity with H10E antibody. This contrasts with the strong surrounding positive staining of the atrial myocytes (m). Panel B: A significant although weak immunostaining is detected in the carotid artery. The arrow e indicates the position of the endothelial cells. Panel C: In the kidney, an interlobular artery (a) is negative as compared to the positivity of a cortical collecting duct (ccd). Magnification, ×344.

Figure 5. Scatchard plot of aldosterone binding to rabbit heart cytosol. Nmax, maximum number of binding sites. Cytosolic aliquots were incubated with increasing concentrations of [3H]aldosterone (0.1–100 nM) for 4 hours at 4°C. Bound (B) and unbound (U) hormone were separated by dextran-coated charcoal treatment. All points are means of triplicate determinations.

Figure 6. Graph showing specificity of tritiated aldosterone binding in rabbit heart cytosol. Cytosol was incubated for 4 hours at 4°C with 1 nM [3H]aldosterone either alone or in the presence of increasing concentrations of unlabeled competitors: aldosterone (ALDO, •), spironolactone (SC9420, □), ZK91587 (▲), and RU486 (○). Results are expressed as the percentage of binding measured with tritiated aldosterone alone (16.3 fmol/mg protein).
cellular distribution of MRs within the cardiovascular system. Aldosterone binding has been identified previously in the rat heart; in rabbit, bovine, and rat arteries; and in human vascular smooth muscle cells in culture. In the present study, we have shown that cardiovascular MRs possess characteristics virtually identical to those of the rabbit kidney. In the cytosolic fraction of the rabbit heart and aorta, we found a specific binding of aldosterone to one class of high affinity binding sites whose mineralocorticoid specificity was clearly demonstrated by competition experiments. The amount of MR present in the heart is relatively high, only two times lower than in the kidney, which is the main site of action of aldosterone. In addition, ultracentrifugation analysis together with immunological probes identified the cardiac MR as a hetero-oligomeric entity that includes hsp90, which is considered to play an important role in the activation process of MRs. The structural similarity between cardiac MRs and MRs from other classic aldosterone target tissues, such as the kidney or the colon, suggests that in the cardiovascular system MRs might function through the same general transduction pathway.

The present immunohistochemical study allows us to describe the tissue and cellular distribution of MRs. By using the monoclonal antibody H10E, a positive immunostaining was detected in the atria, ventricles, aorta, and pulmonary artery. The antibody H10E is an anti-idiotypic antibody that possesses internal image properties of aldosterone; i.e., its binding to the MR could be easily inhibited by an excess of MR ligands. This property is unique in that one can clearly attribute immunoreactivity to MRs by in situ competition experiments. Extinction of the immunostaining by preincubation with mineralocorticoid ligands, such as aldosterone, associated with the absence of extinction by synthetic ligands with no affinity for MRs, such as RU486, allows us to assess mineralocorticoid specificity of the immunostaining. Beyond the direct demonstration of the presence of MRs in the heart and large vessels, immunohistochemistry brings important information, since it identifies the cell type possessing the MR. It appears clearly that the MR is not restricted to smooth muscle cells and myocytes. It is also present in endothelial cells of the heart and vessels and possibly in the fibrocytes.

Quantitative interpretation of immunohistochemical experiments has to be considered with caution. However, comparison of the intensity of staining within cell populations of the same slide, which reduces the variability originating from different experimental processes, allows valuable observations. It appears that the range of intensity of staining was as follows: atrium > ventricle > aorta > pulmonary artery. This apparent regional variation could actually reflect differences in the abundance of MRs among these tissues. Such an interpretation is in good agreement with our observation of a specific aldosterone binding higher in the heart than in the aorta.

When immunoreactivity in vessels of different size is compared, it appears that the intensity of staining decreases with the size of the arteries. The immunostaining is higher in the aorta and pulmonary artery than in the carotid, renal, and mesenteric arteries and is virtually undetectable in small arterioles and capillaries. The absence of staining probably reflects an actual absence or very low level of MRs in small intramycocardial and intrarenal arterioles, for which direct comparisons are possible with the strong staining of the sur-
rounding tissues (from the same slide) such as myocytes and the renal cortical collecting duct, respectively. By contrast, for the reasons explained above, the fact that different histological sections were used to examine large and medium-sized arteries precludes a firm conclusion concerning the relative abundance of MRs in these vessels.

We could not detect any significant difference in the immunostaining patterns between normal and adrenalectomized rabbits. Nevertheless, limitation in quantitative interpretation of immunohistochemical studies does not rule out the possibility of a hormonal regulation of cardiovascular MR expression. However, it is noticeable that such an absence of steroid effects on renal MR expression has already been reported with immunohistochemical techniques by us and others.

In contrast with corroborating evidence of the presence of MRs in the cardiovascular system, the nature of specific aldosterone actions in the heart and blood vessels is still under debate. Studies on direct effects of mineralocorticoids on cardiac activity remain rather limited. In the heart, aldosterone has been proposed to exert direct inotropic effects, but the mechanisms of these actions remain to be identified. In addition, in vivo studies have reported that aldosterone might influence myocardial structure by participating in the development of cardiac fibrosis during ventricular hypertrophy. Along this line, our observation of MR-containing cells in the subendothelial connective layer of the heart might be of interest and requires further studies. Recently, aldosterone has been shown to modulate gene expression of the Na⁺,K⁺-ATPase α₁-subunit and intracellular electrolyte content in rat cardiomyocytes. These results, together with the present demonstration of MRs in the heart, support the proposal of direct actions of aldosterone on heart functions.

In blood vessels, several studies have reported an influence of aldosterone on sodium movements across the cell plasma membrane. However, at the present time, no general agreement has been reached concerning the nature and the mechanism of these mineralocorticoid actions. Both membrane permeability to sodium and active Na⁺,K⁺-ATPase-dependent cell sodium extrusion have been proposed to participate in the aldosterone-dependent cellular ion modifications. Free intracellular sodium content in smooth muscle cells has been shown to be decreased, unchanged, or increased by aldosterone. Whereas aldosterone seems to increase membrane permeability to sodium, it results in either a rise in sodium influx into the cellular compartment or a rise in sodium efflux from the cell to the extracellular compartment. Most of these studies reported that aldosterone increases the active Na⁺,K⁺-ATPase-dependent sodium extrusion, except one. Thus, although all these studies point out an important impact of aldosterone on sodium movements in arterial walls, the nature of these effects is still debatable. Several factors may contribute to the variability of the results, such as differences in the species studied, the experimental approach, and the nature and the dose of the mineralocorticoid hormone used. Moreover, a supplementary degree of complexity has to be taken into consideration for the interpretation of these physiological studies. Indeed, interactions of sodium movements with other ions, such as calcium or potassium, as well as interactions of aldosterone with other hormonal systems, such as vasopressin, angiotensin, and catecholamines, have been repeatedly reported in vessels. On the whole, whereas one can expect an effect of mineralocorticoid hormones on sodium movements in cardiovascular cells possessing the MR, further studies are required to determine its precise nature and mechanism.

Recently, it has been shown that the expression of selective mineralocorticoid action in MR-containing cells depends on the presence of an enzyme (11β-hydroxysteroid dehydrogenase) degrading natural glucocorticoids. Consequently, aldosterone effects can be clearly evidenced in tissues possessing this enzyme, e.g., the renal tubule, colon, and toad bladder. By contrast, the presence of 11β-hydroxysteroid dehydrogenase activity in the heart is still controversial: some authors did not detect it and others reported it. Concerning the vascular system, the presence of 11β-hydroxysteroid dehydrogenase activity was reported in the rat aorta, caudal artery, and the mesenteric artery. This enzyme activity that permits aldosterone access to MRs varies in relation to the availability of the cofactor NADP in the preparation and also varies from one cardiovascular tissue to another. Such variations could be at the origin of some discrepancies concerning the regulatory effect of aldosterone on ion movements.

Finally, the possibility exists that some aldosterone actions might be mediated independent of the classical receptor pathway. This could be suggested for the heart and vessels as it has already been reported for other steroid hormones, in particular, the central nervous system. Along this line, the finding that aldosterone very rapidly stimulates the sodium–proton exchanger might be of critical importance in determining the action of aldosterone on the cardiovascular system.

In conclusion, we have demonstrated the presence of MRs in the rabbit heart and vessels. Immunohistochemical methods localize this receptor in the endothelial cells, myocytes, and vascular smooth muscle cells of the arterial walls. The precise actions and mechanisms of action of aldosterone in these cells remain to be clarified.

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M Lombès, M E Oblin, J M Gasc, E E Baulieu, N Farman and J P Bonvalet

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