Alteration of Cardiac and Renal Functions in Transgenic Mice Overexpressing Human Mineralocorticoid Receptor*

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The mineralocorticoid receptor (MR), a ligand-dependent transcription factor, mediates aldosterone actions in a large variety of tissues. To explore the functional implication of MR in pathophysiology, transgenic mouse models were generated using the proximal human MR (hMR) promoter to drive expression of hMR in aldosterone target tissues. Tissue-specific analysis of transgene expression in two independent transgenic animal (TG) lines by ribonuclease protection assays revealed that hMR is expressed in all mineralocorticoid-sensitive tissues, most notably in the kidney and the heart. TG exhibit both renal and cardiac abnormalities. Enlarged kidneys were histologically associated with renal tubular dilation and cellular vacuolization whose prevalence increased with aging. Renal clearance studies also disclosed a significant decrease in urinary potassium excretion rate in TG. hMR-expressing animals had normal blood pressure but developed mild dilated cardiomyopathy (increased left ventricle diameters and decreased shortening fraction), which was accompanied by a significant increase in heart rate. Differential gene expression analysis revealed a 2- to 5-fold increase in cardiac expression of atrial natriuretic peptide, serum- and glucocorticoid-induced kinase, and early growth response gene 1 as detected by microarrays; renal serum- and glucocorticoid-induced kinase was also induced significantly. Altogether, TG exhibited specific alteration of renal and cardiac functions, thus providing useful pathophysiological models to gain new insights into the tissue-specific mineralocorticoid signaling pathways.

Most of aldosterone actions are mediated by the mineralocorticoid receptor (MR)1 (1), a ligand-activated transcription factor belonging to the steroid receptor superfamily (2). MR is closely related to the glucocorticoid receptor, with which it shares high sequence, structural, and functional homologies. Although glucocorticoid and mineralocorticoid hormones are able to bind MR with the same affinity, several molecular mechanisms intervene to allow specific aldosterone responses despite the large prevalence of plasma glucocorticoid levels (3). MR was found initially to be expressed in sodium-transporting epithelia such as the distal nephron, colon, salivary, and sweat glands. It is now well established that MR is also expressed in a large variety of non-epithelial tissues including the hippocampus (4), the cardiovascular system (5), and brown adipose tissue (6). In tight epithelia, aldosterone is an important regulator of electrolyte and water homeostasis via its binding to MR, inducing sodium reabsorption, and potassium excretion (7). Thus, by acting on volemia, MR has a key role on blood pressure control. Although aldosterone enhances epithelial amiloride-sensitive sodium channels (ENaC), as well as Na+/K+-ATPase expression and activities, it seems unlikely that these sodium channels and pumps represent primary mineralocorticoid-induced genes. Recently, the serum and glucocorticoid-induced kinase (sgk) has been recognized as an early aldosterone-induced protein (8–10). This serine-threonine kinase was also shown to stimulate ENaC activity, consistent with its important role in the early phase of aldosterone-stimulated sodium transport. The aldosterone-MR system has also been shown to play a critical role in the cardiovascular system as aldosterone excess, combined with a high salt diet, has been reported to cause cardiac fibrosis in rats (11). Although the precise mechanisms by which aldosterone modulates cardiovascular function are far from being well understood, it has been suggested that it may potentiate angiotensin II and β-adrenergic agonist effects. In the brain, MR is also involved in neuronal long-term potentiation and in the central regulation of blood pressure, stress, and behavior (4). Finally, specific and pleiotropic effects of mineralocorticoids in other organs such as in the lung (12, 13), eye (14), ear (15), or liver (16) remain to be explored.

Recent advances in the understanding of the in vivo function of MR were obtained by genetic ablation in mice (17). The MR knockout mice, which presented with sodium and water wasting, hyperkalemia and hyponatremia, died within the first week of life. This phenotype, closely resembling type I pseudohypoaldosteronism (18), can be rescued by intraperitoneal injections of sodium chloride solution (19). A strong activation of the renin-angiotensin-aldosterone axis was noted hydride-3-phosphate dehydrogenase; ANF, atrial natriuretic factor; m, mouse; b, base; TG, transgenic animals; LV, left ventricle.
Characterization of hMR-overexpressing Transgenic Mice

**MATERIALS AND METHODS**

**Construction of P1-hMR Transgene and Its Functional Characterization**—The P1-hMR plasmid was constructed using the optimized transgenic vector H31 kindly provided by L.-M. Houdebine (Institut National de la Recherche Agronomique, Jouy en Josas, France). A HindIII-AvalII fragment (−965, +216) containing a 1-kilobase pair of hMR P1 proximal promoter sequence and the beginning of exon 1α was blunt-ended by Klenow polymerase and inserted in the unique BstIII cloning site of the H31 plasmid to generate the P1-H31 vector. The AvalII-XmalIII fragment of hMR 3750 plasmid containing 2995 bp of hMR cDNA from position 203 to 3198, including the full coding sequence, was blunt-ended and inserted into the unique SamI cloning site P1-H31 vector (see Fig. 1). The P1.hMR transgene was separated from plasmid vector sequences by NotI digestion and purified after 0.7% low melting agarose gel electrophoresis with Elutip-D columns (Schleicher & Schull) and ethanol precipitation. Transgene DNA resuspended in 10 mM Tris-HCl, pH 7.4, 0.1 mM EDTA was microinjected into fertilized oocytes obtained from B6D2 mice at the Service d'Inbreeding, Ne`se (CNRS, Villejuif, France) (150 μg/ml) injected intraperitoneally. Blood samples were collected from abdominal aorta with lithium-heparinized needle syringes. After centrifugation at 3000 × g for 5 min, plasmas were recovered. Plasma creatinine, urea, and electrolyte concentrations were determined with a Monarch multiparametric autoanalyzer (Instrumentation Laboratory, Paris, France). Plasma aldosterone levels were determined by RIA (Aldoctk-2; Diasorin, Antony, France). Organs were removed and frozen immediately in dry ice for RNA extraction or fixed in 4% paraformaldehyde in phosphate-buffered saline solution for histological examination. Kidneys were immersed in alcoholic Bouin’s solution. Fixed tissues were dehydrated, embedded in paraffin, sectioned at 7-μm thickness, and stained with Mayer’s hemalun, eosin, and saffron.

**RNA Extraction and Analyses**—Total RNA was extracted from various tissues of wild type or transgenic mice. Samples were homogenized with a Polytron homogenizer in Trizol reagent (Life Technologies, Inc.), and total RNA was isolated following the manufacturer’s instructions. Ribonuclease protection assays (RPA) were performed as described previously (23) using generally 50 μg of total RNA. Protected fragments were electrophoresed on denaturing gels. Gels were fixed in 10% acetic acid and dried. Radioactivity was counted overnight with an InstantImager (Packard, Meriden, CT), followed by autoradiography.

Northern blots were performed with 15 μg of total RNA following standard techniques (24). Membranes were then hybridized with [α-32P]dCTP-labeled probes synthesized by random priming (Rediprime II) from specific cDNA fragments. Serial hybridizations with different probes were performed. Signals were quantified and autoradiographed. All results expressed in arbitrary units are normalized to the GAPDH gene control signal.

**Atlas™ Mouse cDNA Expression Array**—The probes were synthesized by reverse transcription of 5 μg of DNAse-treated total RNA from wild type or line 33 transgenic young male hearts using the Atlas mouse cDNA array kit (CLONTECH Laboratories, Palo Alto, CA). The 500 cDNA-containing membranes were hybridized and washed as recommended by the manufacturer and exposed overnight on InstantImager and autoradiographed. Signals were marked by their referenced positions, quantified, and normalized by housekeeping gene spots. Only reproducible differences of at least 5-fold between wild type and transgenic animal values were taken into account.

**Probes**—Some plasmids used to generate DNA or RNA probes were designed in the laboratory. Reverse transcriptions were performed with SuperScript II reverse transcriptase (Life Technology, Inc.) using oligo-dT primers. After 30 cycles of PCR, samples were run on agarose gels, and a specific band was recovered, purified by a Qiagel quick gel extraction kit (Qiagen), and subcloned into pGEM-T-Easy vector (Promega). The insert was sequenced by Genome Express (Paris, France).

The 280-bp atrial natriuretic factor (ANF) probe was generated by PCR amplification by annealing the primers with forward primer ANF-FP (5'-GAGAGCCCGCGTTCTTGTACCG-3') and reverse primer ANF-RP (5'-CGTGACACACACACGACGTTTGG-3'). A 650-bp ampiclon of the Egr1 gene was also selected by PCR with forward primer Egr1-FP (5'-TTTGGTCTCGTTCACCGTGC-3') and reverse primer Egr1-RP (5'-TGCCAAGTTGTGGCAGCC-3'). The Egr1 plasmid linearized by AvaI was used to synthesize a 287-base riboprobe using T7 RNA polymerase, leading to a protected fragment of 207 bp in the...
The cDNAs of H9251 1N a H11001 K -ATPase (forward primer, 5'-ACGCCC-TCACGCCCCCTCCAA-3'; reverse primer, 5'-CATTTCGAATCACGA-GGGCTT-3') and of H9251 ENaC (forward primer, 5'-CTAATGATGCTGGA-CCACACC-3'; reverse primer, 5'-AAAGCGTCTGTTCCGTGATGC-3'); 54° annealing) were also subcloned in pGEM-T-Easy. The sgk plasmid was kindly provided by Dr. A. Naray-Fejes-Toth (Dartmouth Medical School, Lebanon, NH). The hMR, mMR, and GAPDH plasmids were gifts from Dr. J. Arriza (Salk Institute, San Diego, CA), Dr. G. Schutz (German Cancer Research Center, Heidelberg, Germany), and Dr. B. Escoubet (INSERM U426, Paris, France), respectively. For RPA, the hMR antisense riboprobe is 274 b long, generating a 218-b protected fragment. The mMR antisense riboprobe length is 452 b, hybridizing with 380 b of the target mRNA. The H9251 ENaC antisense riboprobe is 360 b long with a 301-b-long protected fragment. Finally, the 184-b-long GAPDH antisense riboprobe generates a 164-b protected fragment.

Blood Pressure Measurements

Fig. 2. Renal morphological and histological abnormalities in Pi-1MR transgenic mice. A, the two kidneys of a line 42 transgenic mouse are shown, with a marked ballooning of the crypts (magnification × 180). B, renal atrophy in a line 42 transgenic mouse (magnification × 200). C, tubular dilatation (magnification × 200). D, dilated tubules in a line 33 transgenic mouse (magnification × 200). E, dilated tubules in a line 33 transgenic mouse (magnification × 200). F, normal histology of wild type mouse kidney (magnification × 180 and 260, respectively).

Table I
Clinical and biological parameters of 3- to 6-month-old male wild type and line 33 and 42 transgenic mice

| Group        | Transgene copy number | Clinical parameters | Biological parameters |
|--------------|------------------------|---------------------|-----------------------|
|              |                        | Mice BW KW HW       | Mice Na⁺ K⁺ Cl Creatinine Glucose Urea |
|              |                        | n g mg mg %         | n mm mm mm mm mm mm mm |
| Wild type    | 16 29.3 ± 4.4          | 458 ± 76 161 ± 26   | 1.66 ± 0.24 5.3 ± 0.30 |
| Line 33      | 2 29.2 ± 5.5           | 474 ± 96 181 ± 40   | 1.61 ± 0.17 6.23 ± 1.06 |
| Line 42      | 10 27.5 ± 3.2          | 550 ± 101 176 ± 30  | 1.99 ± 0.31 6.38 ± 0.93 |

Values are means ± S.E. *, p < 0.05; **, p < 0.01 vs. wild type group. BW, body weight; KW, kidney weight; HW, heart weight.
rate were measured by the tail-cuff plethysmography method in trained conscious mice placed in a warming restrainer (Marty Technologie, Phymep, Paris, France). Tail-cuff pressure detected by a pressure transducer (SPS844; SensoNorasa, Oslo, Norway) and tail arterial pulsations detected by a piezoelectric pulse sensor were amplified by a signal amplifier Qazap 92204A (ADInstruments). Arterial blood pressure was defined as the tail-cuff inflation pressure at which the waveform was extinguished. For all mice, measurements were repeated for 3 days, between 10 a.m. and 1 p.m. Each day, approximately ten consecutive inflation cycles were performed, and final blood pressure was calculated by averaging successful readings. Heart rate,computerized on-line, was read during stable resting phases preceding inflation cycles.

Echoangiographic Assessments—Transesophageal echocardiography was performed in 6- to 12-week-old wild type and transgenic mice using an Acuson 128XP/10 cardiac ultrasound machine with an Acuson L10 transducer (6–11 MHz) (Mountain View, CA). Mice were slightly anesthetized by ventilation with 0.5–1% isoflurane (Foreno®, Abbott) in O₂. The heart was first imaged in the two-dimensional mode in the parasternal long-axis and/or parasternal short-axis views with mice in the supine position, to position the M-mode cursor perpendicular to the interventricular septum and the left ventricle (LV) posterior wall. Then, M-mode images were obtained at a 100-mm/s speed. Echocardiographic measurements were performed on-line from images captured on cine loops and by using the software of the ultrasound machine. Measurements were made from at least three beats of at least three separate acquisitions and were averaged by one observer blinded to prior results; interventricular septum thickness, posterior wall thickness, and LV end-diastolic and end-systolic diameters were measured by the use of the leading edge convention of the American Society of Echocardiography. LV shortening fraction was calculated as (LV end-diastolic diameter – LV end-systolic diameter/LV end-diastolic diameter) (25). The intraobserver reproducibility assessed by the percentage of the standard deviation to the mean value ratio was 12 ± 1% (α = 31) for the LV posterior wall thickness and 4.5 ± 0.5% (α = 31) for LV end-diastolic diameter measurements.

Renal Function Study—Male wild type and transgenic mice (25 to 28 g) were anesthetized with thioth rubarbitral (inactin; 100 mg/kg, intraperitoneal) and ketamine (75 mg/kg, intramuscular), and their rectal temperature was maintained at 37 °C on a servo-controlled heated surgical table. Tracheostomy was performed, and a PE-50 catheter hand-drawn to the appropriate size was inserted into the right femoral artery for blood pressure measurements (Gould transducer) and blood sampling. The right external jugular vein was cannulated with a hand-drawn PE-50 catheter for continuous infusion (0.9 ml/h/100 g of body weight) of a solution containing 105 mM NaCl, 5 mM KCl, 25 mM NaHCO₃, 4 mM Na₂HPO₄, 1 mM MgSO₄, 1.8 mM CaCl₂, and 5 mM glucose. This solution also contained [methoxy-³H]inulin (priming dose of 12.5 μCi followed by 30 μCi/h). After a 45–60-min equilibration period, a timed urine collection was obtained through a bladder catheter under water-equilibrated paraffin oil. The urine volume was measured gravimetrically. Blood samples (~10 μl) were taken from the femoral artery in heparinized glass capillaries before and after the clearance period. At the end of the experiment, a larger blood sample was obtained for blood gas measurements, and the kidneys were excised, decapsulated, blotted, and weighed. The radioactivity of [methoxy-³H]inulin in plasma and urine samples (5 μl) was measured by liquid scintillation counting (1209 rackbeta counter; LKB) for determination of the glomerular filtration rate.

Statistical Analysis—Comparisons between groups were performed by analysis of variance, Student’s t tests, or non-parametric tests, as appropriate by using the software Instat, Version 2.01 (GraphPad Software, San Diego, CA), or Statview (GraphPad Prism program). For tail-cuff blood pressure and heart rate measurements, grouping factors were taken into account by using multiway variance analysis. p values of 0.05 or less were considered significant.

RESULTS

Generation and Characterization of hMR-overexpressing Transgenic Mice—P1-hMR transgenic mice with a B6D2 genetic background were generated, and lines 33 and 42 were studied extensively. These animals were viable and fertile, and no overmorbidity was noted. Table I summarizes the major clinical and biological parameters of these animals compared with their wild type littermates. No major abnormality was detected except that kidney weights, as well as kidney to body weight ratios, were significantly increased in line 42 transgenic animals. This was in accordance with the surprising macroscopic observation of renal abnormalities in ~25% of 6-month-to 1-year-old line 42 animals (Fig. 2A). In some cases, a renal atrophy occurred with a compensatory hypertrophy of the op-
posite kidney (Fig. 2B). Histologically, there was a pyelocalyceal dilatation with renal cortex atrophy (Fig. 2C). Systematic histological examination performed in the two transgenic lines revealed glomeruli with dilatation of the Bowman’s spaces (Fig. 2D), whereas the renal tubules displayed either dilation (Fig. 2E) or sometimes epithelial necrosis with vacuolization of some tubular cells (Fig. 2F). These histological alterations, not found in wild type animals (Fig. 2, G and H), were more pronounced in older animals and were compatible with morphological modifications observed during potassium depletion (26).

Tissue-specific Expression of Recombinant hMR—hMR expression was examined in testis, kidney, heart, lung, brain, and colon of line 42 and 33 P1.hMR animals. To differentiate expression of hMR transgene from that of the endogenous mMR, the species specificity of hMR and mMR riboprobes designed for RNase protection assay protocol was assessed first. As shown in Fig. 3A, the hMR riboprobe does not cross-hybridize with mMR mRNA, whereas the mMR probe does not hybridize with hMR transcripts isolated from a renal rabbit cell line stably transfected with hMR (27). As expected from our previous targeted oncogenesis studies using the same P1 proximal promoter (23), transgene expression was detected in all MR-expressing tissues, even in liver, spleen, and salivary glands (not shown). Quantification and normalization by GAPDH signals showed a higher hMR expression in line 33 animals, with no correlation with the integrated transgene copy number as estimated by Southern blot (2 in line 33, 10 in line 42; data not shown). A mean relative transgene expression pattern was established from animals for each line (data not shown). The hMR expression in the testis was always higher than that detected in other organs for the two lines, consistent with previous in vivo P1 promoter analysis (23). In line 33, high transgene expression was also found in the lung, heart, kidney, and colon, whereas hMR mRNA levels were lower in the brain. Line 42 animals exhibited a high transgene expression in lung and kidney, moderate in heart and brain, and very low if not undetectable in colon.

Renal Investigation—Examination of tubular function was performed by measuring renal clearances in young adult line 42 and wild type males. Results are reported in Table II. Under our experimental conditions, arterial blood pressure measured through intra-arterial catheter in anesthetized animals remained in the normal range and was identical in the two groups. Arterial pH (7.31 ± 0.02 versus 7.28 ± 0.04) and plasma bicarbonate (19.4 ± 0.6 versus 19.9 ± 0.9 mM) levels were not significantly different between wild type and TG mice. The glomerular filtration rate, as well as the urinary flow rate, both expressed as μl/min or μl/min/g of kidney weight, were also similar between transgenic and wild type animals (Table

![Figure 4. P1.hMR mice exhibit mild dilated cardiomyopathy. Upper panel illustrates M-mode echocardiography of wild type (WT) and TG mice. SEP, septum; PW, posterior wall; EDD, left ventricle end-diastolic dimension; ESD, left ventricle end-systolic diameter. Lower panel, measurements of left ventricle dimensions in wild type, line 42 and 33 transgenic mice. Results are means ± S.E. of nine to fourteen determinations. *, p < 0.05; ***, p < 0.001.](image-url)

### Table III

| Group (n) | Age (wk) mean value (range) | Number of values (total number of measurements) | Blood pressure | Heart rate |
|-----------|-----------------------------|-----------------------------------------------|----------------|-----------|
| Wild type (15) | 24.4 (14–30) | 42 (419) | 119 ± 16 | 597 ± 92 |
| Line 33 (8) | 16.5 (11–22) | 24 (239) | 119 ± 18 | 698 ± 78*** |
| Line 42 (12) | 24.2 (21–32) | 34 (351) | 120 ± 13 | 668 ± 69*** |

**Blood pressure and heart rate in 3- to 6-month-old male wild type and transgenic mice**

Blood pressure and heart rate were measured by tail-cuff method as described under “Materials and Methods.” Values are mean ± S.D. for the number of values. ***, p < 0.001 vs. wild type group.**
II). In contrast, a significant decrease in urinary potassium concentration was observed in transgenic mice leading to a 30% reduction in the urinary potassium excretion rate (398 ± 49 versus 592 ± 86 nmol/min/g in control; p < 0.04). The urinary chloride excretion rate, like that of potassium, was reduced (849 ± 139 versus 1224 ± 133 nmol/min/g in control; p < 0.04) whereas sodium urinary concentration and excretion rate remained unchanged. Given the lack of hypokalemia observed in transgenic mice, these results were suggestive of an increased potassium reabsorption by the renal tubule possibly consequent to a chronic potassium depletion. Of note, plasma aldosterone levels were slightly higher in transgenic male animals than in wild type animals (873.7 ± 85.9; n = 20 versus 604.9 ± 79.5 pg/ml; n = 15, p < 0.04 with Mann Whitney test).

**Echocardiographic Assessment of Cardiac Function**—LV function was assessed in 12- to 18-week-old transgenic and wild type males using transthoracic echocardiography (Fig. 4). Importantly, this was performed on slightly anesthetized animals as that allows the maintenance of heart rate close to the physiological values (450–500 beats/min), a condition necessary for accurate measurements of LV diameters. As shown in Fig. 4B, whereas no significant difference was noted in interventricular septal thickness (0.59 ± 0.02 mm; n = 15 and 0.61 ± 0.02; n = 8, for TG42 and TG33, respectively, versus 0.57 ± 0.03; n = 16, in wild type mice), left ventricular posterior wall thickness was reduced significantly in the two transgenic mouse lines. Interestingly enough, both left ventricle end-diastolic and end-systolic dimensions were significantly
higher in lines 42 and 33 animals with a significant decrease of the shortening fraction in TG42 (46.2 ± 1.3%; n = 14, p < 0.05) and TG33 mice (45.5 ± 2.3%; n = 8, p < 0.05) as compared with wild type mice (51.2 ± 1.3%; n = 15). These results were consistent with a mild dilated hypokinetic cardiomyopathy. It is noteworthy that similar responses to dobutamine were observed in both transgenic and wild type animals (data not shown), excluding major modifications in cardiac β-adrenergic sensitivity. Finally, no cardiac fibrosis development was found by systematic histological examination (data not shown).

**Blood Pressure Assessments**—The tail-cuff method was used to measure blood pressure and heart rate on transgenic and wild type animals. Results are summarized in Table III. Mean arterial blood pressure values were not different between transgenic and wild type animals, but importantly a highly significant increase in heart rate was observed in transgenic animals. Furthermore, we also noted that 3 of 20 transgenic animals presented with typical cardiac rhythmic abnormalities as depicted in Fig. 5. They consist in arrhythmia and/or bursts of tachycardia that were systematically noted during five independent pulsation measurements.

**Study of Mineralocorticoid-related Gene Expression**—To examine the molecular consequences of the observed phenotypes, expression of several genes in heart and kidney was compared in young wild type and TG male mice by Northern blot analyses or RPA. At least three different RNA samples from animals of each line were used to minimize individual differences. First of all, as shown in Fig. 6, expression of endogenous mMR was reduced in kidneys of the two transgenic lines compared with wild type mice, but no change was observed in heart and brain (not shown). We next examined the steady state mRNA levels of two well recognized aldosterone-regulated genes, Na+/K+-ATPase and sgk. As shown in Fig. 7, expression of the α1 subunit of the Na+/K+-ATPase was not modified significantly.  

**FIG. 7. Increased expression of sgk but not of α1 Na+/K+-ATPase in transgenic mice. Upper panels, Northern blot analyses were performed with heart and kidney RNA samples from wild type (WT) and transgenic mice. Lower panels, results normalized by GAPDH are expressed as means ± S.E. in arbitrary units. *, p < 0.05.**

**FIG. 8. Cardiac ANF expression is enhanced in transgenic animals. Levels of ANF and GAPDH mRNA were determined by Northern blot in line 33 (TG 33), line 42 (TG 42), and wild type control littermates (WT) using 15 μg of total RNA. Results normalized by GAPDH are expressed in arbitrary units and represent means ± S.E. *, p < 0.05.**
In contrast, expression of sgk was enhanced significantly by 
~5-fold in both the kidneys and hearts of transgenic mice. 
Although sgk has been clearly implicated in ENaC activation in 
tight epithelia, its role in the heart remains unclear.

The dilated cardiomyopathy phenotype observed in trans-
genomic animals prompted us to study expression of ANF, which 
is known to be up-regulated during cardiac hypertrophy and 
involved in cardiomyocyte differentiation and growth (28). As 
illustrated in Fig. 8, cardiac ANF expression was increased 
significantly \((p < 0.05)\) in the two TG lines, providing molecu-
lar support for cardiac remodeling.

Finally, we used a mouse atlas cDNA array to search for 
altered expression of some unexpected induced or repressed 
genes in transgenic animals. Hybridization was performed 
with probes generated using heart total RNA samples from 
three line 33 transgenic and wild type males. Among the 500 
cDNAs blotted on the membranes, only 15% of the genes were 
sufficiently expressed to ensure accurate quantification. Thus, 
expression of egr1, a three-zinc-finger transcription factor re-
lated to c-Jun and c-Fos (29), was increased reproducibly by at 
least 5-fold (Fig. 9A). The increase in egr1 expression in the 
heart of transgenic animals was confirmed by RPA (Fig. 9B), 
cardiac egr1 transcript levels being 2- to 3-fold higher in the 
two transgenic line animals than in controls. Egr1 expression 
was also strongly enhanced in the kidneys of transgenic 33 
and 42 animals (data not shown); however, because of the large 
individual variations found in transgenic mice, no significant 
increase could be reached. Egr1 has also been shown to be 
overexpressed in the presence of high urea concentration or 
under hypo-osmotic stress in kidney, as well as in cell cultures 
of renal origin (30). It could be hypothesized that renal expres-
sion of egr1, which greatly varies among transgenic animals, 
amay correlate with the degree of alteration in kidney function.

**DISCUSSION**

In the present study, we have generated and characterized 
transgenic mouse models in which hMR is expressed under the 
control of one of its own alternative promoters. The main find-
ing is the development of both cardiac and renal phenotypes in 
hMR-overexpressing transgenic animals. These phenotypes re-
sult from the superimposition of functional and molecular con-
sequences of targeted overexpression of hMR and their adapt-
ive compensatory mechanisms.

Although the degree of overexpression of recombinant hMR 
compared with that of the endogenous receptor could not be 
assessed directly at the protein level, most notably because of 
the lack of species-specific antibodies suitable for quantifica-
tion purposes, RPA experiments indicated that the amounts of 
hMR transcripts found in aldosterone target tissues were in the 
same range than those detected for mouse MR. Moreover, the 
tissue-specific pattern of transgene expression was superim-
posable to that of endogenous mMR in particular in the kidney 
and the heart, both of which are now recognized as mineralo-
corticoid target tissues. As reported already and confirmed in 
this study the P1 promoter directs a strong transgene expres-
sion in the testis (23), which raises the question of the exact 
role played by the receptor in reproductive processes.

Transgenic mice developed mild dilated hypokinetic car-
diomyopathy without induction of cardiac fibrosis. Contrary to 
our expectations, there was no evidence for an increase in 
arterial blood pressure in hMR-overexpressing animals. This 
result was a strong indication that cardiomyopathy was not a 
consequence of hemodynamic alterations responsible for car-
diac overloading but rather because of a direct effect of targeted 
transgene expression presumably in the heart. The cardiac 
phenotype of these mice differs significantly from that of aldos-
terone/salt-induced cardiac hypertrophy and fibrosis (11, 31, 
32). The lack of cardiac fibrosis in our models questions on the
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exact contribution of MR in cardiac remodeling and seems to indicate that the aldosterone and sodium status per se may play a predominant role in cardiac hypertrophy and fibrosis (33). Along with this hypothesis, cardiac aldosterone production (34) was shown to be increased by high sodium intake (35), thus contributing to synergistically induce cardiac fibrosis.

In our hMR transgenic mice, we showed that cardiac expression of ANF, which is a well recognized and premature molecular marker of developing heart failure, was greatly induced. Unexpectedly, this was accompanied by an increase in sgh expression. Although this serine-threonine kinase has been reported to increase ENaC activity in sodium-transporting epithelial cells (7), its role in cardiomyocytes remains elusive. Identification of regulated genes represents an important step in understanding the physiopathological function of hMR in the heart. Interestingly, using cDNA microarrays and RPA experiments, we have identified egr1 as a predominant up-regulated gene in the heart of transgenic animals. Egr1, an immediate response gene (29) acting as a transcription factor modulating cellular growth and differentiation, was shown to be overexpressed in hypertrophied hearts of transgenic mice or after infusion of adrenoreceptor agonists (36). Furthermore, the major role played by egr1 in cardiac remodeling was confirmed further by the demonstration of a blunted catecholamine-induced cardiac hypertrophy response in egr1-deficient mice (37). Given the overexpression of egr1 in our hMR transgenic mice, egr1 may represent an early aldosterone gene similarly to that described in the kidney for K-Ras2 (38) or for the proto-oncogene Fos-related antigen 2 (7). Further experiments are needed to elucidate this hypothesis. Our findings raise the question concerning the mechanism by which overexpression of hMR causes dilated cardiomyopathy and what are the primary target genes dysregulated.

Another interesting cardiac phenotype observed in hMR transgenic mice is the increased heart rate and the frequency of dysrhythmia. This could suggest a higher sensitivity to adrenergic agonists. However, because no difference in dobutamine-induced increase in shortening fraction was noted, it is likely that activation of cardiac mineralocorticoid signaling pathway contributes to the incidence of dysrhythmia. This is in accordance with the beneficial effects of aldosterone receptor blockade on mortality and presumably arrhythmic sudden death of patients with congestive heart failure (39) and with the recent report that indicates that spironolactone treatment reduces significantly the frequency of ventricular premature complexes and episodes of non-sustained ventricular tachycardia (40). In vivo, telemetric electrocardiography recordings from transgenic animals might be necessary to identify precisely the arrhythmic effect of MR.

With respect to the renal phenotype, the enhanced transcriptional activity of hMR in transgenic mice, as revealed by a strong increase in renal sgh, has important functional consequences. It is conceivable that acute activation of MR signaling cascade leads to sodium retention, increased extracellular fluid volume, and potassium depletion. These modifications might not be necessarily associated with either hypertension or modification of serum sodium and potassium concentrations but could eventually induce chronic intracellular changes in ion concentrations such as chronic potassium depletion (hypokaliemia). In response to such chronic alterations, transgenic mice need to adjust their physiological parameters by compensatory adaptive mechanisms. We propose that in response to potassium depletion, hMR transgenic mice might increase their renal potassium reabsorption as detected during the acute renal function analysis. These findings provide strong evidence for an unbalanced sodium/potassium ratio pointing to a distal tubular dysfunction. Concerning morphological modifications observed in the kidneys of transgenic mice despite the slight increase in plasma aldosterone levels, it is worth noting that patients affected by aldosterone-producing adenoma develop renal cysts (41). Renal histological alterations of transgenic animals were also compatible with the diagnosis of hypokalemic nephropathy (42).

In conclusion, the hMR-expressing mice not only provide us with important basic information concerning the role of hMR in regulating renal and cardiac functions but also allow us to address several important pathophysiologic questions. Among them, the nature of adaptive and compensatory mechanisms of activation of the mineralocorticoid signaling pathway. It would be interesting to test the effects of diet and/or pharmacological compounds on these transgenic animals. The complexity of the observed phenotypes reflects functional and molecular consequences of targeted hMR overexpression but also its transcriptional control directed by its own regulatory sequences i.e. hMR P1 proximal promotor. It will be of great interest to compare these phenotypes with those induced by tissue-specific targeted overexpression of hMR in the heart or in the distal nephron. Our prediction is that they might not be totally superimposable.

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