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Increased AT$_1$ receptor expression and mRNA in kidney glomeruli of AT$_2$ receptor gene-disrupted mice

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Saavedra, Juan M., Walter Häuser, Gladys Ciuffo, Giorgia Egidy, Kwang-Lae Hoe, Olaf Jöhren, Takaaki Sembonmatsu, Tadashi Inagami, and Inés Armando. Increased AT$_1$ receptor expression and mRNA in kidney glomeruli of AT$_2$ receptor gene-disrupted mice. *Am J Physiol Renal Physiol* 280: F71–F78, 2001.—The proposed feedback between angiotensin II AT$_2$ and AT$_1$ receptors prompted us to study AT$_1$ receptor expression in kidneys of male AT$_2$ receptor-gene disrupted mice (*agtr2* −/y). In wild-type (*agtr2* +/y) mice, AT$_1$ receptor binding and mRNA is abundant in glomeruli, and AT$_1$ receptor binding is also high in the inner stripe of the outer medulla. AT$_2$ receptors are scarce, primarily associated to cortical vascular structures. In *agtr2* −/y mice, AT$_1$ receptor binding and mRNA were increased in the kidney glomeruli, and AT$_1$ receptor binding was higher in the rest of the cortex and outer stripe of the outer medulla, but not in its inner stripe, indicating different cellular regulation. Although AT$_2$ receptor expression is very low in male *agtr 2* +/y mice, their gene disruption alters AT$_1$ receptor expression. AT$_1$ upregulation alone may explain the AT$_2$ gene-disrupted mice phenotype such as increased blood pressure, higher sensitivity to angiotensin II, and altered renal function. The indirect AT$_1$/AT$_2$ receptor feedback could have clinical significance because AT$_1$ antagonists are widely used in medical practice.

renin-angiotensin system; angiotensin II receptor types; gene-disrupted models

ANGIOTENSIN II (ANG II), by stimulation of specific, discretely localized ANG II receptors, plays a crucial role in the modulation of renal function in mammals (31). ANG II receptors are classified into AT$_1$ and AT$_2$ types on the basis of their relative affinity for nonpeptidic-selective ligands (46) and molecular cloning (16, 19, 39). Most of the known actions of ANG II on the regulation of water and salt metabolism are dependent on stimulation of AT$_1$ receptors (31, 46). In the kidney, stimulation of AT$_1$ receptors by ANG II modulates both glomerular and tubular function including sodium retention, vasoconstriction of renal vessels, and decreased glomerular filtration rate (2).

AT$_1$ receptors are present in large numbers in the adult mammalian kidney, with a major expression in the glomeruli, and lower levels in the renal cortical tubules, vasculature, medullar interstitial cells, and collecting ducts (1, 4, 6, 22, 23, 30, 36, 38, 40). Of the two AT$_1$ receptor subtypes existing in rodents, AT$_{1A}$ and AT$_{1B}$, the AT$_{1A}$ receptors predominate in the kidney (22). In adult rodents, kidney AT$_2$ receptors were reported to be absent (4, 23, 41) or present at low levels (18, 38). Other studies reported a selective association of AT$_2$ receptors in the adult kidney from different species, including humans, with vascular structures (8, 9, 10, 30, 49). The localized and restricted expression of AT$_2$ receptors in association with renal arteries strongly suggested a function of AT$_2$ receptors different from that of AT$_1$ receptors, perhaps related to inhibition of angiogenesis and vasodilation (3, 34).

The availability of animal models with targeted disruption of specific genes provided an opportunity to further analyze the possible role of AT$_2$ receptors. The targeted disruption of the mouse AT$_2$ receptor gene significantly increased blood pressure and the sensitivity to the pressor action of ANG II, indicating an enhanced response to AT$_1$ receptor stimulation (15, 17). We asked the question whether absence of AT$_2$ receptor transcription could result in alterations in AT$_1$ receptor binding or mRNA expression in selected areas of the kidney in this mouse model.

MATERIALS AND METHODS

Animals. Mice were obtained form the Department of Biochemistry, Nashville University, and were kept under controlled conditions with free access to water and food, according to protocols approved by National Institute of Mental Health (NIMH) Animal Care and Use Committee. We produced the *agtr2* gene-disrupted mice by the injection of *agtr2* disrupted embryonic stem cells (E14−1) from the 129 Ola mouse line into blastocyts derived from C57BL/6 mice, as described previously (17). After the genotype of F$_2$ heterozygous females (*agtr2* −/+) was clearly confirmed, they were backcrossed with C57BL/6 wild-type males for three genera-

tions. Littermate gene-disrupted (*agtr2* −/Y) and control wild-type (*agtr2* +/Y) males were selected from the third backcross progeny to minimize the effect of differences in genetic background.

Mice were transported to NIMH, kept for 1 day under controlled conditions as above, and killed by decapitation between 10:00 AM and 11:00 AM. Kidneys were immediately removed, frozen in isopentane at −30°C, and stored at −80°C. For binding studies, sections (16 μm) were cut in a cryostat at −20°C, thaw-mounted on gelatin-coated slides, and dried overnight in a desiccator at 4°C. Sections were stored at −80°C until binding experiments were performed. Consecutive sections were used for ANG II receptor binding studies and in situ hybridization. Every tenth section was stained with hematoxylin and eosin to localize the structures expressing the binding or the receptor mRNA. For in situ hybridization experiments, sections were collected on silanated glass slides (Digene Diagnostics, Beltsville, MD) and stored at −80°C.

ANG II receptor binding. Sar^1-ANG II (Peninsula Laboratories, Belmont, CA) and CGP-42112 (Neosystems Laboratory, Strasbourg, France) were iodinated by New England Nuclear (Boston, MA) to a specific activity of 2,200 Ci/mmol.

Adjacent kidney sections were preincubated for 15 min at 22°C in 10-mM sodium phosphate buffer, pH 7.4, containing 120 mM NaCl, 5 mM EDTA, 0.005% bacitracin (Sigma, St. Louis, MO), and 0.2% protease-free BSA (Sigma), followed by incubation for 2 h at 22°C in fresh buffer, prepared as above with the addition of 50 μM Plummer's inhibitor (Calbiochem, La Jolla, CA), 100 μM phenylmethylsulfonyl fluoride (Sigma), 500 μM phenantrolin (Sigma), and 0.5 nM [^{125}I]Sar^1-ANG II. After an incubation for 120 min at 22°C the sections were washed four times for 1 min each in ice-cold 50 mM Tris-HCl buffer (pH 7.4), followed by a 30 s wash in ice-cold water, and dried under a stream of cold air.

Binding of [^{125}I]Sar^1-ANG II to AT$_1$ receptors was determined in adjacent kidney sections as follows. Some sections were incubated with 0.5 nM [^{125}I]Sar^1-ANG II to determine total binding. Adjacent sections were incubated as above with the addition of 10^{-5} M losartan (DuPont-Merck, Wilmington, DE), to displace binding to AT$_1$ receptors. Binding to AT$_1$ receptors was calculated as the difference between total binding and the binding remaining in adjacent sections incubated in the presence of excess concentration of losartan. Similarly, binding of [^{125}I]Sar^1-ANG II to AT$_2$ receptors was determined as the difference between total binding and binding in adjacent sections incubated in the presence of 10^{-6} M of PD-123319 (Parke-Davis, Ann Arbor, MI) to selectively displace binding to AT$_2$ receptors. The concentrations of the AT$_1$ and AT$_2$ receptor-selective ligands were selected to give maximum-specific displacement (13). Nonspecific or background binding was determined by incubating consecutive sections with 10^{-6} M ANG II (Peninsula). The values remaining were subtracted from all determinations as described above. In the case of areas where we found binding only to AT$_1$ receptors, the values obtained after displacement with excess concentrations of losartan were not significantly different from background values obtained by displacement with excess concentrations of unlabeled ANG II.

In addition, [^{125}I]CGP-42112 binding was performed in another set of adjacent sections to confirm the presence or absence of AT$_2$ receptors. At the concentrations used, [^{125}I]CGP-42112 exclusively labels AT$_2$, and not AT$_1$, receptors (14). Buffers used in this assay had the same composition as those used for the binding with [^{125}I]Sar^1-ANG II. Tissue sections were preincubated for 15 min in incubation buffer followed by incubation for 120 min in fresh buffer containing 0.2 nM [^{125}I]CGP-42112. To determine specific binding to AT$_2$ receptors, consecutive sections were incubated in the presence of 10^{-6} M of PD-123319 to selectively displace binding to AT$_2$ sites. Nonspecific binding was determined by incubating consecutive sections with 5 × 10^{-6} M of ANG II (Peninsula).

To further localize AT$_2$ receptors histologically, [^{125}I]CGP-42112 binding was performed in 6-μm thick kidney sections. Adjacent sections were stained with hematoxylin and eosin. After binding experiments, sections were fixed for 60 min in paraformaldehyde vapors at 80°C and dipped in photo emulsion. After exposure for 2 wk, sections were developed in Kodak D-19 developer for 3 min at 15°C, fixed for 4 min, and counter stained with hematoxylin and eosin.

To determine if the radio-labeled ligands could be significantly metabolized under the conditions of incubation, we analyzed aliquots of buffers obtained before and after incubation of kidney sections by reversed-phase high performance liquid chromatography as described earlier (13). No metabolism of the radio-labeled ligands was observed under the above-mentioned conditions (results not shown).

Quantitative receptor autoradiography. We exposed dry sections to Hyperfilm-^{3}H (Amersham, Arlington Heights, IL) along with 16-μm sections of autoradiographic ^{125}I microscales (Amersham) at 4°C. Films were developed in ice-cold D-19 developer (Eastman Kodak, Rochester, NY) for 4 min, fixed in Kodak rapid fixer for 4 min at 22°C, and rinsed in water for 15 min. We measured optical densities in the autoradiograms by computerized microdensitometry by using the NIH Image 1.6 analysis system (NIMH, Bethesda, MD). For quantitative autoradiography, we measured the optical densities separately in kidney glomeruli, the rest of the cortex, and outer and inner stripes of the outer medulla. The optical densities were related to the concentration of radioactivity present in the sections by comparison with the ^{125}I microscales, and transformed to corresponding values of fmol/mg protein (37). These values should be considered as arbitrary units, because the ligand concentrations used are below saturation, and because the actual protein-tissue-protein concentration varies between the different regions of the kidney (37). We found similar differences between groups when values were calculated as optical densities before comparison to ^{125}I standards.

In situ hybridization histochemistry. In situ hybridization was performed by using ^{35}S-labeled antisense and sense (control) riboprobes (21). A 478-bp *Eco*R I/*Sac*I cDNA fragment of rat AT$_{1A}$ receptor (19, 39) showing 95 and 88% sequence homology to the mouse AT$_{1A}$ and AT$_{1B}$ receptor cDNA, respectively, and 99.4 and 92.5% amino acid homology to mouse AT$_{1A}$ and AT$_{1B}$ receptors, respectively (19, 39), was subcloned into the pBluescript II KS+ vector. Riboprobes were labeled by in vitro transcription by using an RNA labeling kit (Amersham). Because we used a riboprobe to the coding region of the rat AT$_1$ receptor that has a very high homology between AT$_{1A}$ and AT$_{1B}$-rodent subtypes, we did not determine the relative contribution of kidney AT$_{1A}$ or AT$_{1B}$ receptor subtypes in our experiments. However, over 95% of the renal AT$_1$ receptors are of the AT$_{1A}$ subtype (22). Thus the mRNA data presented here predominantly reflects the regulation of the AT$_{1A}$ receptors.

Sections were fixed in 4% paraformaldehyde for 10 min, acetylated for 10 min in 0.1 M triethanolamine HCl, pH 8.0, containing 0.25% acetic anhydride, dehydrated in alcohols, and air dried. Each section was covered with 50-μl hybridization buffer containing 50% formamide, 0.3 M NaCl, 2 mM EDTA, 20 mM Tris, pH 8.0, 1 × Denhardt's solution, 10% dextran sulfate, 100 μg/ml salmon sperm DNA, 250 μg/ml

yeast tRNA, 150 mM DTT, 0.1% SDS, and 40,000-counts per minute/µl sense or antisense probe. Sections were hybridized overnight at 54°C, treated with 40 µg/ml RNase A (Sigma, St. Louis, MO) for 30 min, and washed in sodium chloride/ sodium citrate (SSC) with increasing stringency. After a final wash in 0.1 × SSC at 65°C for 60 min, sections were dehydrated through alcohols and exposed to Hyperfilm-^{3}H (Amersham) along with ^{14}C microscales (Amersham) for 7 days. Films were developed as described above. The intensities of hybridization signals in kidney glomeruli, the rest of the cortex, and outer and inner stripes of the outer medulla, were quantified as nCi/g tissue equivalent by measuring optical film densities by using the NIH Image 1.61 program. Data were calibrated with ^{14}C microscales after subtraction of the values obtained in the same areas of adjacent sections hybridized with sense (control) probes (nonspecific hybridization). The values obtained represent arbitrary units, because the protein content or weight of the different kidney areas may be different (32). We found similar differences between groups when values were calculated as optical densities before comparison to ^{14}C standards.

For cellular localization, slides were dipped in Kodak NTB2 photo emulsion, exposed for 4 wk, developed in Kodak D-19 developer for 3 min at 15°C, fixed for 4 min, and counterstained with hematoxylin and eosin (Fisher Scientific, Fair Lawn, NJ).

Statistical analysis. Results were expressed as means ± SE, calculated and analyzed by using GraphPad Prism (version 2.00) and Microsoft Excel (version 7.0a). Statistical analysis for values obtained from the displacement studies by using single concentrations of the displacers was performed by using a one-way ANOVA followed by post hoc analysis with the Newman-Keuls multiple comparison test. Mean ± SE values of wild-type and AT$_2$ gene-disrupted mice were compared for significance by using unpaired Student's *t*-test.

RESULTS

ANG II receptor subtype expression and mRNA in wild-type mice. We studied ANG II receptor subtype binding and mRNA in the kidney of male wild-type and AT$_2$ gene-disrupted mice. Binding to AT$_1$ receptors was, as expected, high and selectively localized in the kidney of wild-type mice. In wild-type mice, the highest levels of losartan-sensitive, PD-123319-insensitive, [^{125}I]Sar^1-ANG II binding to AT$_1$ receptors were present on glomeruli (Fig. 1, *A-E*) and lower levels in the inner stripe of the outer medulla (Table 1). Binding to AT$_1$ receptors was also present in the rest of the cortex and the outer stripe of the outer medulla (Table 1).

Emulsion autoradiography in wild-type mice revealed very high losartan-sensitive [^{125}I]Sar^1-ANG II binding in kidney glomeruli and low, diffuse losartan-sensitive binding throughout the whole renal cortex (Fig. 2, *A-C*).

AT$_1$ receptor mRNA was also detected in the kidney of male wild-type mice. As it was the case with AT$_1$ receptor binding, AT$_1$ receptor mRNA was higher in the glomeruli, and lower in the rest of the cortex and in the outer and inner stripes of the outer medulla (Table 1 and Fig. 3, *A* and *B*). Low levels of AT$_1$ mRNA expression were located in the renal vasculature (Fig. 3*C*).

In some cortical structures of wild-type mice, of different shape and size than the glomeruli, we found high-losartan-insensitive, PD-123319-sensitive [^{125}I]Sar^1-ANG II binding, indicative of AT$_2$ re-
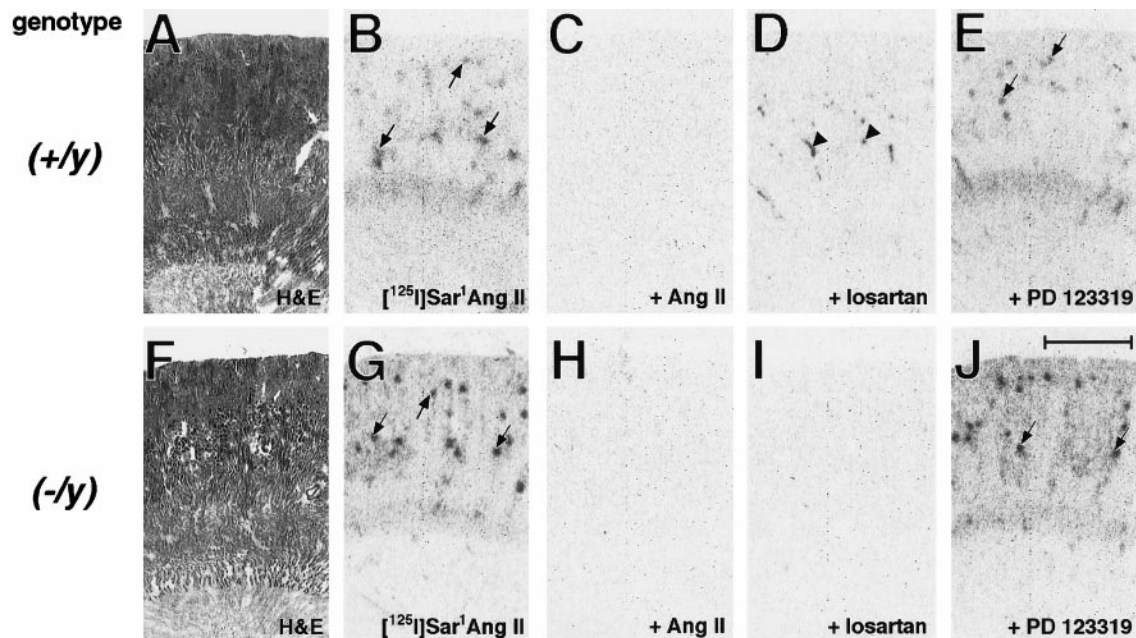

Fig. 1. Autoradiography of ANG II receptor type binding in kidneys from male mice. Hematoxylin-eosin (H & E) staining [wild-type (*A*), AT$_2$ gene deficient (*F*)] and autoradiographs with binding of 0.5 nM [^{125}I]Sar^1-ANG II in kidneys of wild-type (+/y) mice (*B-E*) and AT$_2$ receptor gene-disrupted (-/y) mice (*G-J*) alone (*B*, *G*) or in the presence of 10^{-6} M ANG II (*C*, *H*), 10^{-5} M losartan (*D*, *I*), and 10^{-6} PD-123319 (*E*, *J*). Scale bar, 1 mm. Arrows point to losartan-sensitive [^{125}I]Sar^1-ANG II binding to AT$_1$ receptors located on glomeruli. Arrowheads point to losartan-insensitive [^{125}I]Sar^1-ANG II binding to AT$_2$ receptors that are absent in AT$_2$ receptor gene-disrupted mice (*I*).

Table 1. *ANG II receptor AT_1 binding and mRNA in mouse kidney*

	Wild-Type	AT_2 Gene-Disrupted	%Change
Glomeruli			
Binding	72 ± 19	164 ± 5†	$+127$
mRNA	456 ± 45	695 ± 63†	$+52$
Rest of the cortex			
Binding	9 ± 4	27 ± 5*	$+200$
mRNA	93 ± 31	134 ± 32	$+44$
Outer stripe of the outer medulla			
Binding	8 ± 1	15 ± 2*	$+87$
mRNA	106 ± 12	166 ± 27	$+56$
Inner stripe of the outer medulla			
Binding	29 ± 6	28 ± 3	-4
mRNA			

Values are means $\pm$ SE. Binding to ANGII type 1 (AT_1) receptors, as determined by quantitative autoradiography, is expressed in fmol/mg protein. AT_1 receptor mRNA, determined by in situ hybridization, is expressed in nCi/g. $*P < 0.05$; †$P < 0.001$, statistically significant differences between wild-type and AT_2 gene-disrupted mice.

ceptors (Fig. 1*D*). To confirm the presence of AT_2 receptors in the cortical structures distinct from glomeruli, we used AT_2 receptor-selective [^{125}I]CGP-42112 binding (Fig. 4). All [^{125}I]CGP-42112 binding was displaced by ANG II (Fig. 4*B*) and by the AT_2 receptor-specific ligand PD-123319 (Fig. 4*D*) but not by the AT_1 receptor-specific ligand losartan (Fig. 4*C*).
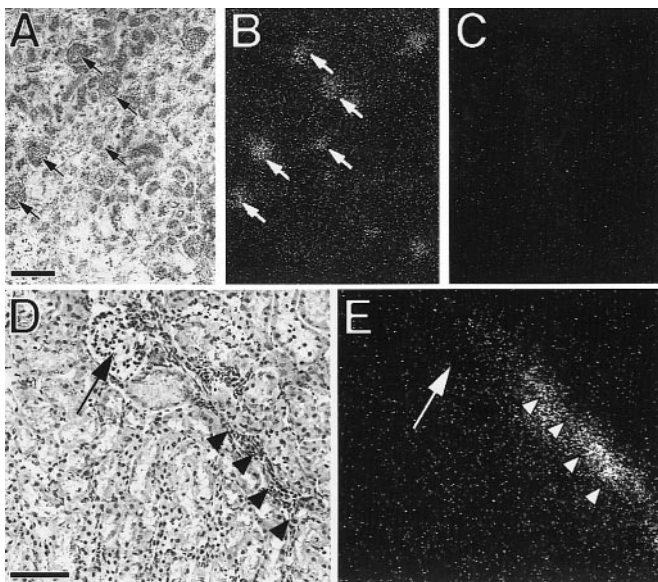

Fig. 2. Emulsion autoradiography of binding to AT_1 and AT_2 receptors in kidneys from male mice. Emulsion autoradiographs of binding of 0.5 nM [^{125}I]Sar^1-ANG II to glomeruli of kidneys from AT_2 receptor gene-disrupted (-/y) mice in the absence (*B*) and presence of 10^{-5} M losartan (*C*). In addition, [^{125}I]CGP-42112 binding is shown in vascular structures of kidneys from wild-type (+/y) mice (*E*). *A* and *D*: histology of *B* and *E*, respectively. *A* and *D*: arrows point to glomeruli. *D* and *E*: arrowheads point to a blood vessel. Scale bars, 60 μm. Scale bar in *A* applies to *A*-*C*. Scale bar in *D* applies to *D* and *E*.
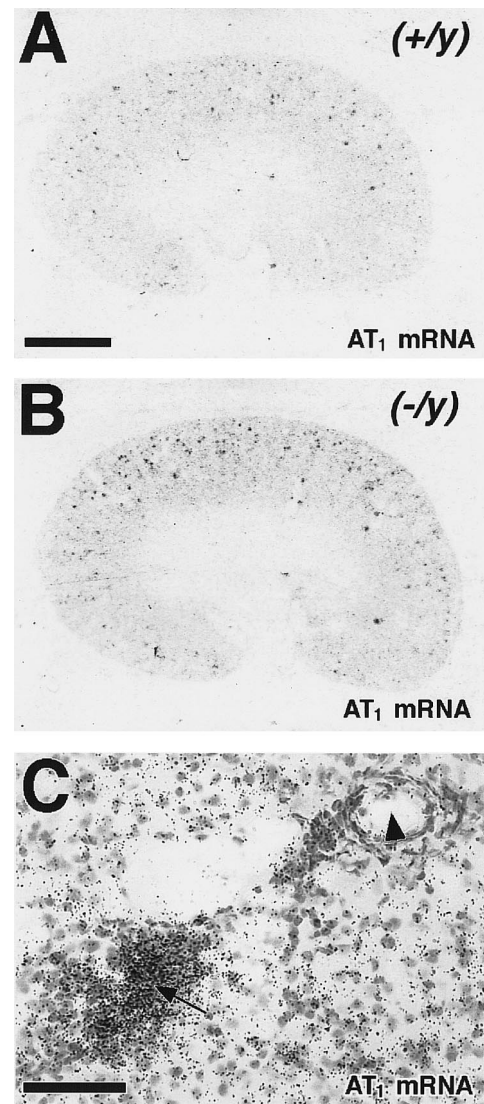

Fig. 3. Autoradiography of ANG II receptor subtype mRNA in kidneys from male mice. Film (*A* and *B*) and emulsion (*C*) autoradiograms after in situ hybridization by using an AT_1 receptor-specific riboprobe. *A*: wild-type mice. *B*: AT_2 gene-disrupted mice. Note the presence of AT_1 receptor mRNA in glomeruli from wild-type mice (arrow in *C*). *C*: arrowhead points to a blood vessel. Scale bar in *A* is 2 mm (also applies to *B*). Scale bar in *C* is 250 μm.

Specific [^{125}I]CGP-42112 binding sites were detected in the kidney cortex and, as determined by emulsion autoradiography, were associated only with vascular structures, in particular the arcuate arteries (Fig. 2, *D* and *E*). Emulsion autoradiographic analysis of other structures in the kidney cortex, and in particular the kidney glomeruli, did not reveal accumulation of silver grains above background levels.

ANG II AT_1 receptor expression and mRNA in AT_2 gene-disrupted mice. As expected, the AT_2 receptor gene-disrupted male did not express losartan-insensitive, PD-123319-sensitive (AT_2) [^{125}I]Sar^1-ANG II binding sites (Fig. 1, *F-J*) or AT_2 receptor-selective [^{125}I]CGP-42112 binding (Fig. 4, *E-H*) in the renal cortex or in other areas of the kidney.
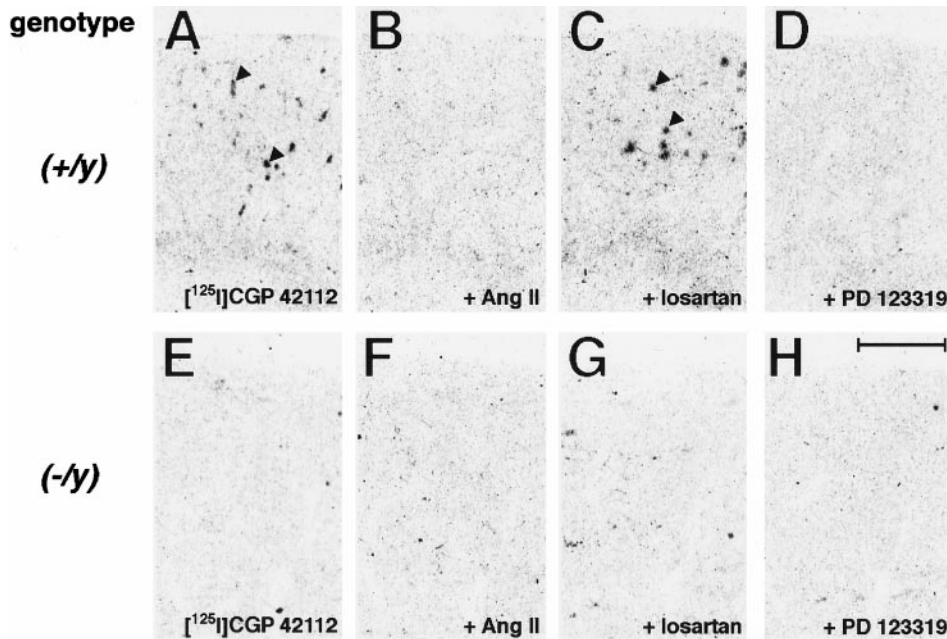
Fig. 4. Autoradiography of binding to AT$_2$ receptors in kidneys from male mice. Binding of 0.2 nM [^{125}I]CGP-42112 in kidneys of wild-type (+/y) mice (*A-D*) and AT$_2$ receptor gene-disrupted (-/y) mice (*E-H*) alone (*A, E*) and in the presence of 5×10^{-6} ANG II (*B, F*), 10^{-5} M losartan (*C, G*), and 10^{-6} PD-123319 (*D, H*). Arrowheads point to specific binding of [^{125}I]CGP-42112 to AT$_2$ receptors. Scale bar, 1 mm (*A-H*).

A remarkable difference in ANG II AT$_1$ receptor binding studied with quantitative autoradiography, and mRNA, as determined by in situ hybridization, was noted in AT$_2$ gene-disrupted mice compared with wild-type controls. In AT$_2$ receptor gene-disrupted mice, both [^{125}I]Sar^1-ANG II binding to AT$_1$ receptors and AT$_1$ receptor mRNA were higher compared with values in wild-type mice. Significant increases in AT$_1$ receptor binding (~125%) (Table 1, Fig. 1, *G* and *J*) and AT$_1$ mRNA (~50%) (Table 1, Fig. 3, *B* and *C*) were found in glomeruli.

Binding was significantly increased in the rest of the renal cortex (3-fold) and in the outer stripe of the outer medulla (~90%). In these areas there was a tendency toward increased expression of AT$_1$ mRNA, a ~45% increase in the rest of the renal cortex, and about a ~55% increase in the outer stripe of the outer medulla (Table 1).

In the rest of the renal cortex and the outer stripe of the outer medulla of AT$_2$ gene-disrupted mice, AT$_1$ receptor expression was also significantly higher (Table 1). In these areas there was increased expression of AT$_1$ mRNA, but the results did not achieve statistical significance (Table 1). Conversely, no significant differences were found in AT$_1$ receptor binding or mRNA in the inner stripe of the outer medulla between wild-type animals and AT$_2$ gene-deficient male mice (Table 1).

DISCUSSION

We report that gene disruption of the ANG II AT$_2$ receptor results in marked and selective alterations on the protein and mRNA expression of the renal AT$_1$ receptors in the male mouse.

First, we analyzed the distribution of AT$_1$ receptor binding and mRNA in the kidney of wild-type male mice. The highest numbers of AT$_1$ receptors and highest AT$_1$ mRNA expression occur in the glomeruli, significant AT$_1$ receptor binding, and mRNA in the inner stripe of the outer medulla, and lower binding and receptor mRNA in the rest of the kidney cortex. These results are in agreement with previous data from other mammalian species and with the demonstration of AT$_1$ receptors, not only in glomeruli but also in proximal and distal tubules, medullar interstitial cells, and the vasculature (1, 4, 6–10, 22, 23, 30, 36, 38, 40, 48). In addition, we detected low levels of AT$_1$ receptor binding, and significant levels of AT$_1$ receptor mRNA, in the outer stripe of the outer medulla of wild-type mice. This indicates that some AT$_1$ receptors may be located, in the mouse, in medullar structures that do not express AT$_1$ receptors in the rat (47).

We found a clear expression of AT$_2$ receptor binding in the kidney of the wild-type mice, a finding that again contrasts with the reported absence of AT$_2$ receptors in adult rats (1, 4, 5). The number of AT$_2$ receptors in the adult mouse kidney was much lower than that of AT$_1$ receptors, and followed a different pattern of localization. AT$_2$ receptor binding was restricted to very selective cortical areas associated with renal vessels. This is in agreement with the reported localization of AT$_2$ receptors in renal vessels of other species, including humans (8, 9, 10, 30, 48). Under the conditions of our assay, we could not find AT$_2$ receptor binding in kidney glomeruli and interstitial cells, as proposed with immunocytochemical techniques (38). If present, these receptors might be expressed in low amounts below the sensitivity of our assay.

Thus our analysis of ANG II receptor subtypes in the adult mice indicated that both receptors were clearly expressed, that the AT$_1$ receptor predominated, and that the localization of the receptor subtypes occurred, at least for the most part, with exception of the vasculature, in separate renal structures.

Next, we studied ANG II receptor subtype expression and mRNA in AT$_2$ gene-disrupted mice. In the kidney of adult AT$_2$ gene-disrupted mice there was no PD-123319-sensitive, losartan-insensitive binding and no [^{125}I]CGP 42112, AT$_2$-selective binding. The total absence of AT$_2$ receptor binding in the AT$_2$ gene-disrupted mouse was expected, because AT$_2$ receptors are encoded by a single gene (16, 19, 39), and confirmed the identity of AT$_2$ receptor binding sites with the cloned AT$_2$ receptor.

The main finding in our study was the demonstration of a significant increase in AT$_1$ receptor expression and mRNA in the kidney of adult AT$_2$ gene-disrupted mice. Ours is a clear example of how the gene disruption of one receptor can alter the expression of another receptor. This is particularly remarkable considering that the expression of renal AT$_2$ receptors in wild-type mice is very low, compared with that of AT$_1$ receptors. Our results further support the hypothesis that the level of expression of the AT$_2$ receptors inversely correlates with that of AT$_1$ receptors. Earlier studies demonstrated that the cardiac specific over-expression of AT$_2$ receptors attenuates the AT$_1$-mediated pressor and chronotropic effects of ANG II (29). In addition, AT$_2$ receptors are highly expressed in the fetal kidney, and their numbers decrease during gestation in parallel with a concomitant increase in AT$_1$ receptor expression (4).

Activation of AT$_1$ and AT$_2$ receptors in wild-type animals results in effects different, and in most cases opposite; and stimulation of AT$_2$ receptors was postulated to limit the response of AT$_1$ receptors to ANG II (17). Stimulation of kidney AT$_1$ receptors decreases cortical and medullary blood flow and decreases urine flow and natriuresis, the principal renal effects of ANG II, shifting pressure natriuresis to the right (11). Conversely, in wild-type animals, stimulation of AT$_2$ receptors was reported to increase pressure natriuresis (26) and their stimulation results in renal vasodilatation, probably as a consequence of production of cGMP, (42), nitric oxide (43), and prostaglandin F2 alpha (44), and increased arachidonic acid release (20). AT$_1$ receptor blockade produces vasodilatation, diuresis and natriuresis, and this was proposed to be due to unopposed AT$_2$ receptor stimulation (3, 33, 35, 42). The evidence of a physiological role of renal AT$_2$ receptors, however, is controversial. AT$_2$ receptor blockade was reported to potentiate the ANG II-induced PGE$_2$ production after AT$_1$ receptor stimulation (42) and prevents the effect of ANG II on the vasculature (24). However, other studies indicate that chronic blockade of AT$_2$ receptors in wild-type adult animals does not alter the response to AT$_1$ receptor stimulation by ANG II (33).

In mice lacking AT$_2$ receptor expression there is increased sodium retention, rightward shift in pressure natriuresis and diuresis, reduction in cortical and medullary blood flow, increased blood pressure, and enhanced hypertensive response to ANG II administration (12, 15, 17). The phenotype of the AT$_2$ receptor gene-disrupted mouse may be related to the upregulation of the AT$_1$ receptor, rather than negatively reflecting the actions of the AT$_2$ receptor (12, 45). It is also possible that the AT$_2$ receptor gene-disrupted phenotype could be the result of a lost balance between AT$_1$ and AT$_2$ receptors, with a shift toward predominance of AT$_1$-related effects. In addition, higher AT$_1$ receptor transcription and expression could explain the enhanced fibrosis after ureteral occlusion in AT$_2$ gene-deficient mice (25, 28), because kidney fibrosis induced by ANG II is AT$_1$ receptor dependent (27).

There is a recent report (12) of increased AT$_1$ receptor mRNA in the whole kidney of AT$_2$ gene-disrupted mice. Our findings of increased AT$_1$ receptor binding indicate that an actual increase in AT$_1$ receptor expression occurs in AT$_2$ receptor gene-disrupted mice. In addition we demonstrate here that upregulation of renal AT$_1$ receptors predominantly occurs in cortical structures, the kidney glomeruli, and probably in the tubular epithelium, and in the outer stripe of the outer medulla. In AT$_2$ receptor gene-disrupted mice, there are no changes in AT$_1$ receptor expression in the inner stripe of the outer medulla. Thus the AT$_1$ upregulation and the AT$_2$-AT$_1$ feedback in this model are structure and cell specific. Although the expression of glomerular and cortical interstitial AT$_1$ receptors may depend on AT$_2$ receptor expression, the AT$_1$ receptor expression in the inner stripe of the outer medulla does not. We conclude from our results that AT$_1$ receptors in renal glomeruli, interstitial, and medullary cells are differentially regulated.

Last, our findings further support the hypothesis of a crosstalk or feedback between AT$_1$ and AT$_2$ receptor subtypes. It appears that AT$_1$ receptors for the most part do not coexist with AT$_2$ receptors in the same renal cells. For this reason, the mechanism of feedback is probably indirect, through modifications in kidney function related to the absence of AT$_2$ receptors, and the upregulation of renal AT$_1$ receptors.

In our model, when alterations in the transcription and expression of AT$_2$ receptors are present from birth, renal AT$_1$ receptor expression and consequently renal function, are profoundly affected in the adult. Because AT$_1$ receptor antagonists are widely used in the treatment of cardiovascular disease, the crosstalk/feedback between AT$_1$ and AT$_2$ receptors has significant pathophysiological and clinical relevance.

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