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

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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$^{-/-}$). In wild-type (agtr2$^{+/+}$) 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$^{-/-}$ mice, AT$_1$ receptor binding and mRNA were increased in the kidney glomeruli, and AT$_2$ 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 agtr2$^{+/-}$ 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, discreetly 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 reten tion, 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$_1$ 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 from 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 blastocysts 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-

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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 (Digenic Diagnostics, Beltsville, MD) and stored at −80°C.

ANG II receptor binding. Sar1-ANG II (Peninsula Laboratories, Belmont, MA) 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 phenanthroline (Sigma), and 0.5 mM [125I]Sar1-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 [125I]Sar1-ANG II to AT1 receptors was determined in adjacent kidney sections as follows. Some sections were incubated with 0.5 nM [125I]Sar1-ANG II to determine total binding. Adjacent sections were incubated as above with the addition of 10−5 μM losartan (DuPont-Merck, Wilmington, DE), to displace binding to AT1 receptors. Binding to AT1 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 [125I]Sar1-ANG II to AT2 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 AT2 receptors. The concentrations of the AT1 and AT2 receptor-selective ligands were selected to give maximum-specific displacement (13). Nonspecific or background binding was determined as the difference between total binding and binding in adjacent kidney sections as follows. Some sections were incubated with 0.5 nM [125I]Sar1-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.

In addition, [125I]CGP-42112 binding was performed in another set of adjacent sections to confirm the presence or absence of AT2 receptors. At the concentrations used, [125I]CGP-42112 exclusively labels AT2, and not AT1, receptors (14). Buffers used in this assay had the same composition as those used for the binding with [125I]Sar1-ANG II. Tissue sections were preincubated for 15 min in incubation buffer followed by incubation for 120 min in fresh buffer containing 0.2 mM [125I]CGP-42112. To determine specific binding to AT2 receptors, consecutive sections were incubated in the presence of 10−6 M of PD-123319 to selectively displace binding to AT2 sites. Nonspecific binding was determined by incubating consecutive sections with 5 × 10−6 M of ANG II (Peninsula).

To further localize AT2 receptors histologically, [125I]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-125I (Amersham, Arlington Heights, IL) along with 16-μm sections of autoradiographic 125I 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 125I microscales, and transformed to corresponding values of fmol/mg protein (37). These values should be considered as arbitrary units, because the ligand concentrations used were below saturation, and because the actual protein-tissue-protein concentration varies between the different regions of the kidney (37). We found similar differences between sections when values were calculated as optical densities before comparison to 125I standards.

In situ hybridization histochemistry. In situ hybridization was performed by using 35S-labeled antisense and sense (control) riboprobes (21). A 478-bp EcoR I/SalI cDNA fragment of rat AT1A receptor (19, 39) showing 95 and 88% sequence homology to the mouse AT1A and AT1B receptor cDNA, respectively, and 99.4 and 92.5% amino acid homology to mouse AT1A and AT1B 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 AT1 receptor that has a very high homology between AT1A and AT1B rodent subtypes, we did not determine the relative contribution of kidney AT1A or AT1B receptor subtypes in our experiments. However, over 95% of the renal AT1 receptors are of the AT1A subtype (22). Thus the mRNA data presented here predominantly reflects the regulation of the AT1A 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 dehy-
drated through alcohols and exposed to Hyperfilm-3H (Am-
ersham) along with 14C 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 14C microscales after subtraction of the
values obtained in the same areas of adjacent sections hy-
bridized with sense (control) probes (nonspecific hybridiza-
tion). 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 be-
fore comparison to 14C 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 Scien-
tific, Fair Lawn, NJ).

Statistical analysis. Results were expressed as means ±
SE, calculated and analyzed by using GraphPad Prism (ver-
sion 2.00) and Microsoft Excel (version 7.0a). Statistical anal-
ysis 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 AT2 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
AT2 gene-disrupted mice. Binding to AT1 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,
[125I]Sar1-ANG II binding to AT1 receptors were
present on glomeruli (Fig. 1, A-E) and lower levels in
the inner stripe of the outer medulla (Table 1). Binding
to AT1 receptors was also present in the rest of the
cortex and the outer stripe of the outer medulla (Ta-
ble 1).

Emulsion autoradiography in wild-type mice re-
vealed very high losartan-sensitive [125I]Sar1-ANG II
binding in kidney glomeruli and low, diffuse losartan-
sensitive binding throughout the whole renal cortex
(Fig. 2, A-C).

AT1 receptor mRNA was also detected in the kidney
of male wild-type mice. As it was the case with AT1
receptor binding, AT1 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 AT1 mRNA
expression were located in the renal vasculature (Fig.
3C).

In some cortical structures of wild-type mice, of
different shape and size than the glomeruli, we
found high-losartan-insensitive, PD-123319-sensi-
tive [125I]Sar1-ANG II binding, indicative of AT2 re-

Fig. 1. Autoradiography of ANG II receptor type binding in kidneys from male mice. Hematoxylin-eosin (H & E)
staining (wild-type (A), AT2 gene deficient (F)) and autoradiographs with binding of 0.5 nM [125I]Sar1-ANG II in
kidneys of wild-type (+/y) mice (B-E) and AT2 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 [125I]Sar1-ANG II binding to AT1 receptors located on glomeruli. Arrowheads point to
losartan-insensitive [125I]Sar1-ANG II binding to AT2 receptors that are absent in AT2 receptor gene-disrupted
mice (I).
ceptors (Fig. 1D). To confirm the presence of AT₂ receptors in the cortical structures distinct from glomeruli, we used AT₂ receptor-selective [¹²⁵I]CGP-42112 binding (Fig. 4). All [¹²⁵I]CGP-42112 binding was displaced by ANG II (Fig. 4B) and by the AT₂ receptor-specific ligand PD-123319 (Fig. 4D) but not by the AT₁ receptor-specific ligand losartan (Fig. 4C).

Table 1. ANG II receptor AT₁ binding and mRNA in mouse kidney

|                      | Wild-Type | AT₂ 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 ± SE. Binding to ANGII type 1 (AT₁) receptors, as determined by quantitative autoradiography, is expressed in fmol/mg protein. AT₁ 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 AT₂ gene-disrupted mice.

Specific [¹²⁵I]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 AT₁ receptor expression and mRNA in AT₂ gene-disrupted mice. As expected, the AT₂ receptor gene-disrupted male did not express losartan-insensitive, PD-123319-sensitive (AT₂) [¹²⁵I]Sar¹-ANG II binding sites (Fig. 1, F-J) or AT₂ receptor-selective [¹²⁵I]CGP-42112 binding (Fig. 4, E-H) in the renal cortex or in other areas of the kidney.
A remarkable difference in ANG II AT₁ receptor binding studied with quantitative autoradiography, and mRNA, as determined by in situ hybridization, was noted in AT₂ gene-disrupted mice compared with wild-type controls. In AT₂ receptor gene-disrupted mice, both [¹²⁵I]Sar¹-ANG II binding to AT₁ receptors and AT₁ receptor mRNA were higher compared with values in wild-type mice. Significant increases in AT₁ receptor binding (~125%; Table 1, Fig. 1, G and J) and AT₁ 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 AT₁ 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 AT₂ gene-disrupted mice, AT₁ receptor expression was also significantly higher (Table 1). In these areas there was increased expression of AT₁ mRNA, but the results did not achieve statistical significance (Table 1). Conversely, no significant differences were found in AT₁ receptor binding or mRNA in the inner stripe of the outer medulla between wild-type animals and AT₂ gene-deficient male mice (Table 1).

**DISCUSSION**

We report that gene disruption of the ANG II AT₂ receptor results in marked and selective alterations on the protein and mRNA expression of the renal AT₁ receptors in the male mouse.

First, we analyzed the distribution of AT₁ receptor binding and mRNA in the kidney of wild-type male mice. The highest numbers of AT₁ receptors and highest AT₁ mRNA expression occur in the glomeruli, significant AT₁ 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 AT₁ 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 AT₁ receptor binding, and significant levels of AT₁ receptor mRNA, in the outer stripe of the outer medulla of wild-type mice. This indicates that some AT₁ receptors may be located, in the mouse, in medullar structures that do not express AT₁ receptors in the rat (47).

We found a clear expression of AT₂ receptor binding in the kidney of the wild-type mice, a finding that again contrasts with the reported absence of AT₂ receptors in adult rats (1, 4, 5). The number of AT₂ receptors in the adult mouse kidney was much lower than that of AT₁ receptors, and followed a different pattern of localization. AT₂ receptor binding was restricted to very selective cortical areas associated with renal vessels. This is in agreement with the reported localization of AT₂ receptors in renal vessels of other species, including humans (8, 9, 10, 30, 48). Under the conditions of our assay, we could not find AT₂ 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 AT₁ 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 AT₂ gene-disrupted mice. In the kidney of adult AT₂ gene-disrupted mice there was no PD-123319-sensitive, losartan-insensitive binding and no [¹²⁵I]CGP 42112, AT₂-selective binding. The total absence of AT₂ receptor binding in the AT₂ gene-disrupted mouse was expected, because AT₂ receptors are encoded by a single gene (16, 19, 39), and confirmed the identity of AT₂ receptor binding sites with the cloned AT₂ receptor.

The main finding in our study was the demonstration of a significant increase in AT₁ receptor expression and mRNA in the kidney of adult AT₂ 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 AT₂ receptors in wild-type mice is very low, compared with that of AT₁ receptors. Our results further support the hypothesis that the level of expression of the AT₂ receptors inversely correlates with that of AT₁ receptors. Earlier studies demonstrated that the cardiac specific over-expression of AT₂ receptors attenuates the AT₁-mediated pressor response (4). AT₁ receptor antagonists are widely used in the treatment of cardiovascular disease, the crosstalk/feedback between AT₁ and AT₂ receptors has significant pathological and clinical relevance.

Activation of AT₁ and AT₂ receptors in wild-type animals results in effects different, and in most cases opposite; and stimulation of AT₂ receptors was postulated to limit the response of AT₁ receptors to ANG II (17). Stimulation of kidney AT₁ receptors decreases cortical and medullar blood flow and decreases urine flow and natriuresis, the principal renal effects of ANG II (29). In addition, AT₂ receptors are highly expressed in the fetal kidney, and their numbers decrease during gestation in parallel with a concomitant increase in AT₁ receptor expression (4).

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In mice lacking AT₂ receptor expression there is increased sodium retention, rightward shift in pressure natriuresis and diuresis, reduction in cortical and medullar blood flow, increased blood pressure, and enhanced hypertensive response to ANG II administration (12, 15, 17). The phenotype of the AT₂ receptor gene-disrupted mouse may be related to the upregulation of the AT₁ receptor, rather than negatively reflecting the actions of the AT₂ receptor (12, 45). It is also possible that the AT₂ receptor gene-disrupted phenotype could be the result of a lost balance between AT₁ and AT₂ receptors, with a shift toward predominance of AT₂-related effects. In addition, higher AT₁ receptor transcription and expression could explain the enhanced fibrosis after ureteral occlusion in AT₂ gene-deficient mice (25, 28), because kidney fibrosis induced by ANG II is AT₁ receptor dependent (27).

There is a recent report (12) of increased AT₁ receptor mRNA in the whole kidney of AT₂ gene-disrupted mice. Our findings of increased AT₁ receptor binding indicate that an actual increase in AT₁ receptor expression occurs in AT₂ receptor gene-disrupted mice. In addition we demonstrate here that upregulation of renal AT₁ 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 AT₂ receptor gene-disrupted mice, there are no changes in AT₁ receptor expression in the inner stripe of the outer medulla. Thus the AT₁ upregulation and the AT₂-AT₁ feedback in this model are structure and cell specific. Although the expression of glomerular and cortical interstitial AT₁ receptors may depend on AT₂ receptor expression, the AT₁ receptor expression in the inner stripe of the outer medulla does not. We conclude from our results that AT₁ receptors in renal glomeruli, interstitial, and medullar cells are differentially regulated.

Last, our findings further support the hypothesis of a crosstalk or feedback between AT₁ and AT₂ receptor subtypes. It appears that AT₁ receptors for the most part do not coexist with AT₂ 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 AT₂ receptors, and the upregulation of renal AT₁ receptors.

In our model, when alterations in the transcription and expression of AT₂ receptors are present from birth, renal AT₁ receptor expression and consequently renal function are profoundly affected in the adult. Because AT₁ receptor antagonists are widely used in the treatment of cardiovascular disease, the crosstalk/feedback between AT₁ and AT₂ receptors has significant pathophysiological and clinical relevance.

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