Desensitization of Type 1 Angiotensin II Receptor Subtypes in the Rat Kidney


Differences involving serine residues in the sequence of the carboxyl-terminal tail of type 1 angiotensin II (Ang II) receptor subtypes AT1A and AT1B suggest differences in desensitization ability. We examined the Ang II-induced homologous desensitization patterns of both receptor subtypes in freshly isolated renal structures: glomerulus (Glom), afferent arteriole, and cortical thick ascending limb (CTAL), whose content in each subtype mRNA is different, by measuring variations in intracellular calcium concentration. A preexposure to a maximal dose of Ang II, followed by a second application of the same concentration, induced: 1) a complete desensitization in Glom, where AT1A and AT1B mRNAs were expressed in similar proportions, and 2) no or partial desensitization in afferent arteriole and CTAL, where AT1A mRNA was predominant. In the absence of nephron structure containing only AT1B mRNA, we studied rat anterior pituitary cells that exhibit high content in this subtype and observed that desensitization was not complete. In Glom, CTAL, and pituitary cells, desensitization proceeded in a dose-dependent manner. In Glom and CTAL, desensitization occurred via a PKC-independent mechanism. These results suggest that desensitization does not depend on the nature of Ang II receptor subtype but either on the proportion of each subtype in a given cell and/or on cell specific type. This could allow adaptive biological responses to Ang II appropriate to the specific function of a given cell type. (Endocrinology 142: 4683–4692, 2001)

ANGIOTENSIN II (ANG II) plays an essential role in the renal function. It is mainly involved in the regulation of vascular resistance, filtration rate, and tubular reabsorption. Most of these effects seem to be mediated by type 1 angiotensin II receptor (AT1-R). In rodents, two AT1-R subtypes have been isolated and termed AT1A and AT1B (1–5). In recent studies, Oliverio et al. (6) and Tsuchida et al. (7) show that mice lacking both AT1A and AT1B-R exhibited major alterations in biological functions. These two receptor subtypes belong to the seven-transmembrane domain G protein-coupled receptor family. They are encoded by two different genes localized on different chromosomes. There is limited homology (35%) between the two mRNAs in the 5′- and 3′-untranslated regions. In contrast, the amino acid sequences of these receptors are more than 95% identical. The amino acid differences are distributed throughout the protein but are particularly predominant in the carboxyl-terminal intracellular domain (2). Of particular interest are, on the one hand, differences involving cysteine residues, which may be important for the functional coupling of the receptors to their effector proteins, and on the other hand, difference involving serine residues, which may imply a difference in phosphorylation and subsequent desensitization (1, 5).

Comparison of the pharmacology of rat AT1A-R and AT1B-R in Chinese hamster ovary (CHO) cells, specifically expressing either recombinant receptor, has revealed similar agonist/antagonist potencies with that of the pharmacologically well-defined AT1-R (8–11). It is well documented that AT1A-R and AT1B-R subtypes exhibit disparate tissue-specific expression profiles. AT1A-R mRNA is predominantly expressed in the liver, lung, aorta, and kidney, whereas AT1B-R mRNA is predominantly expressed in the adult anterior pituitary (12–14). In the zona glomerulosa of the adrenal gland and in mesangial cells of the renal glomeruli, both subtypes are found in equal proportions (14, 15). Several reports pointed out the differential regulation of AT1-R subtype mRNA expression, which in addition, seems tissue-specific (4, 14–16).

AT1-R are coupled to at least five different effectors (17). The major signaling pathway involves the activation of G proteins of the Gαi/Gq11 family, which subsequently activates PLC, resulting in [Ca2+]i mobilization and protein kinase C activation (1, 4, 5, 18–20). Rat AT1A-R or AT1B-R expressed in stable transfected cell lines (adrenal Y1 tumor cells) cannot be differentiated by its potency to increase cytosolic free calcium after stimulation by Ang II (21).

In a previous report, we have studied, in fresh tissue, the segmental distribution along the rat nephron of AT1A-R and AT1B-R mRNA expression and the calcium signaling of both receptor subtypes. We showed that AT1A-R mRNA is the major subtype in all nephron segments, including the cortical thick ascending limb (CTAL), whereas in the glomerulus (Glom), AT1A-R and AT1B-R mRNA are both expressed (22). Half-maximal increases in cytosolic free calcium were observed at similar Ang II concentration for Glom and CTAL. Despite the fact that there is no nephron structure containing only AT1B-R mRNA, preventing comparative studies, our analysis strongly suggested that both receptor subtypes activate the calcium second-messenger system with the same efficiency (22), as previously shown in recombinant cells.

Abbreviations: Ang II, Angiotensin II; AT1-R, type 1 Ang II receptor; [Ca2+]i, intracellular calcium concentration; CHO, Chinese hamster ovary; CTAL, cortical thick ascending limb; Glom, glomerulus.
However, controversial data are reported concerning the desensitization properties of these two receptor subtypes studied in three distinct cellular models. In stably transfected mouse adrenocortical Y-1 cells, no inhibition of either calcium or inositol phosphate response was observed at high Ang II concentrations for either subtype (21). In contrast, in transfected Xenopus laevis oocytes, the calcium responses to high Ang II concentrations mediated by the rat AT1B-R are reduced, compared with responses dependent on AT1A-R (5). A similar difference in the desensitization pattern of these receptor subtypes was found by Kuroda et al. (23) in CHO cells transfected with human AT1A or AT1B subtypes.

Such discrepancy might be related with the use of overexpressed receptors in stable transfected cells and/or the specificity of the cellular models. Actually, the desensitization process in cells naturally expressing AT1A and AT1B receptors has not been extensively investigated. However, in adrenal glomerulosa cells that naturally equally express AT1A and AT1B receptor mRNA levels (14), Boulay et al. (24) show that short-term desensitization of Ang II receptors is the result of a simple shift of the receptors from a high to a low affinity state, possibly induced by G protein uncoupling. Thus, to gain further insight into this problem, in keeping with physiological relevance, the aim of our work was to look for possible differences in the patterns of desensitization of naturally expressed AT1A-R and AT1B-R subtypes. We have thus chosen Glom, CTAL, and afferent arterioles, three intact structures of the rat kidney, known to express different ratios of AT1-R isoforms, namely 40% AT1A-R and 60% AT1B-R mRNAs in Glom, compared with 90% or 70% AT1A-R mRNA in CTAL and afferent arterioles, respectively (22, 25). In addition, in the

**Figure 1.** Representative recordings of \([Ca^{2+}]_i\) levels, in response to two successive applications of 10^{-7} M Ang II, separated by 5-min washing in Glom (A) and CTAL (B) and by 15-min washing in afferent arteriole (C). In Glom and CTAL, the two applications of Ang II are followed by an application of 10^{-4} M carbachol (A) and 10^{-7} M bradykinin (B), respectively. Mean values of peak responses are indicated in the text.
absence of renal structures predominantly expressing the AT$_{1B}$-R isoform, primary cultures of rat anterior pituitary cells containing predominantly this subtype (80%) were also examined (26). In spite of the absence of specific and selective ligands or antibodies for AT$_{1A}$ or AT$_{1B}$-R subtypes, which did not allow us to evaluate their respective density, clues in the literature strongly suggest that mRNA expression is the reflection of protein abundance. Thus, close correlations were found in the distribution of mRNA (22) and the density of Ang II binding sites (27) along the nephron, and in the variation of AT$_1$ mRNA levels and AT$_1$-R binding site density in afferent arterioles (25) and anterior pituitary (28, 29). Considering these data, homologous desensitization comparative analysis of these various models should allow one to determine whether desensitization properties: 1) are appropriate for discriminating AT$_{1A}$-R from AT$_{1B}$-R; and 2) depend on the proportion of both receptor subtypes in a given cell.

Materials and Methods

Animals

All animal procedures were conducted in agreement with our institutional guidelines for the care and use of laboratory animals. Sprague

![Diagram](image)

**Fig. 2.** Representative recordings of [Ca$^{2+}$]$_i$ levels in response to two successive applications of different doses of Ang II in Glom (A–F). Dose-response curve of desensitization, as a function of the concentration of the first Ang II application ($10^{-11}$–$10^{-7}$ M; bottom panel). On the ordinate, the responses of second applications of $10^{-7}$ M Ang II ($\Delta 2$) are expressed as a percentage of the maximal responses obtained with a first application of $10^{-7}$ M Ang II ($\Delta 1$). Each point represents the mean of four to eight individual determinations.
Dawley rats (Iffa Credo and Charles River Laboratories, Inc., L’Arbresle, France) were used. They were fed normal standard diet (UAR A03, Epinay sur Orge, France) and offered water ad libitum.

Microdissection of nephron segments

Male Sprague Dawley rats, weighing 130–180 g, were anesthetized by ip injection of pentobarbital (6 mg/100 g BW). The left kidney was prepared for nephron microdissection by infusion of 5 ml basal medium, containing 0.3% collagenase (Serva, Heidelberg, Germany), through a catheter placed in aorta just below the left renal artery, as previously described (30). The kidney was then removed and sliced along the corticomedullary axis. Small pyramids were cut and incubated in 0.1% collagenase solution in basal medium bubbled with filtered air, at 30 °C for 15 min. Glom, parietal sheet of Bowman’s capsule, CTAL, and afferent arterioles were isolated by microdissection in basal medium without collagenase, at 4 °C, under stereomicroscopic observation. It was verified in a previous report that similar results were obtained in the presence or absence of collagenase in Glom and CTAL (31).

Mesangial cell culture

Isolation and characterization of rat glomerular mesangial cells were performed as previously described (32). Glom were prepared by mechanical sieving from the cortex of male Sprague Dawley rats weighing 150–200 g. Mesangial cells were cultured on a thin-glass

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**CTAL**

![Graphs showing [Ca²⁺]i levels in response to Ang II](image)

**Fig. 3.** Representative recordings of [Ca²⁺]i levels in response to two successive applications of different doses of Ang II in CTAL (A–E). Dose-response curve of desensitization as a function of the concentration of the first Ang II application (10⁻¹¹–10⁻⁷ M; bottom panel). On the ordinate, the responses of second applications of 10⁻⁷ M Ang II (Δ2) are expressed as a percentage of the maximal responses obtained with a first application of 10⁻⁷ M Ang II (Δ1). Each point represents the mean of four to six individual determinations.

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1 Composition of basal medium, in mM: NaCl, 137; KCl, 5; MgSO₄, 0.8; Na₂HPO₄, 0.33; KH₂PO₄, 0.44; MgCl₂, 1; CaCl₂, 1; HEPES, 20; glucose, 5; lactate, 5; acetate, 10; pyruvate, 1; glutamine, 2; and aspartate, 0.3.
microscope precoated with 0.2% gelatin in RPMI-1640 medium supplemented with glutamine (5 mm), HEPES (15 mm), penicillin (100 U/ml), streptomycin (100 µg/ml), and 10% FCS in an atmosphere of 5% CO₂-95% air. After the cells had reached confluence, they were routinely identified by light microscopy and immunofluorescence staining. They had a stellate appearance, overgrew each other, and showed a network of intracellular fibrils of myosin. They were negative for von Willebrand factor, urokinase, and cytokeratin antibodies, which excluded any contamination by the two other glomerular cell types, endothelial and epithelial cells.

Anterior pituitary cell culture

Anterior pituitaries were collected from female Sprague Dawley rats (175–200 g BW) promptly after decapitation, and cells were enzymatically and mechanically dispersed as previously described (33, 34). In brief, cells from 4–5 pituitaries per experiment were dispersed using trypsin and deoxyribonuclease I (Sigma-Aldrich Corp. Chimie, Saint Quentin, Fallavier, France), suspended in a culture medium consisting in DMEM medium (Sigma-Aldrich Corp.), without phenol red, containing 10% of charcoal-dextran stripped FCS (Roche Molecular Biochemicals, Meylan, France) and antibiotics (penicillin, 50 U/ml; and streptomycin, 50 µg/ml). Dispersed cells were dot-seeded (10⁶ cells in 100 µl) onto glass microscope coverslips placed in 10-cm-diameter culture dishes (Life Technologies, Cergy Pontoise, France). After a 3-h incubation at 37°C in a water-saturated 5% CO₂-95% atmosphere, allowing cell attachment, 8 ml complete culture medium were added per culture dish. Cells were cultured for 6 d before the experiments, with medium renewal on d 3 and d 5. Pituitary and mesangial cells were serum-deprived 2 d before the experiments.

Measurements of intracellular calcium concentration ([Ca²⁺]ₗ)

[Ca²⁺]ₗ was measured as previously described (35). After microdissection, each Glom, parietal sheet of Bowman’s capsule, CTAL, or afferent arteriole (0.2–0.3 mm) was transferred individually onto a thin-glass microscope coverslip in 1 µl basal medium containing 2 mM CaCl₂ and 1% agarose (type IX). Then, the agarose was jellied by cooling the slide for 2 min on ice. We have previously checked that agarose did not modify the calcium response in Glom and CTAL (31). Glom, parietal sheet, CTAL, afferent arterioles, mesangial cells, and anterior pituitary cells were loaded with 5 µM Fura-2 acetylmethoxy ester at room temperature for about 1 h. For fluorescence measurements, each sample was placed on the stage of an inverted microscope and was continuously superfused at a rate of 0.8 ml/min, at 37°C, with basal medium (2 mM CaCl₂), which could be replaced, at any time, by the solutions to be tested. In most experiments, applications of Ang II and/or agonists lasted 5 min and were generally separated by 5-min washings with basal medium, except for some experiments (15 or 30 min) and for afferent arteriole, in which applications of Ang II were always separated by 15-min washings.² The Fura-2-loaded Glom, CTAL, afferent arteriole, mesangial cell, or pituitary cell was alternately excited at wavelengths of 340 (S) and 380 nm (L), every 4 sec. The fluorescence intensity emitted at 510 nm was recorded from a selected area delimited by an adjustable window diaphragm (about 10 cells for Glom, CTAL, and afferent arteriole). The return to the basal calcium level after Ang II application was longer than in the other structures studied.

² A 15-min washing was performed for afferent arterioles, because the return to the basal calcium level after Ang II application was longer than in the other structures studied.
where the dissociation constant \( K_d \) = 224 nM; \( R = S/L \); and \( L_{\text{max}}, L_{\text{min}}, R_{\text{min}}, \) and \( R_{\text{max}} \) are \( L \) and \( R \) values at 0 and saturating concentrations of calcium, respectively. \( L_{\text{max}}, L_{\text{min}}, R_{\text{min}}, \) and \( R_{\text{max}} \) were determined by external calibration, as previously described in (35).

The calcium response was evaluated either by the magnitude of the response \( \Delta[Ca^{2+}] \) equated with the difference between the peak and basal concentration (in nm) or by the integral of the \( Ca^{2+} \) signal (in \( nM \cdot s \)). The integral of the \( Ca^{2+} \) signal was calculated as \( \int_0^t [Ca^{2+}] \cdot dt \), where \( t_0 \) is the time at the start of \( [Ca^{2+}] \) increment, \( t_1 \) is the time when the signal returns to baseline value, and \( dt \) is the interval of time between 2 measurements (4 sec).

All the results are mean values of replicate samples ± se or sd, as indicated. Statistical differences were assessed using \( t \) test. Emax (efficiency concentration giving half of the maximal response) was estimated by fitting the data to a nonlinear regression model using commercially available software (Prism 2.0, GraphPad Software, Inc., San Diego, CA).

### Results

**Homologous desensitization in Glom and CTAL**

Representative patterns of homologous desensitization in Glom and CTAL elicited by two successive applications of \( 10^{-7} \) M Ang II, are shown in Fig. 1, A and B, respectively. This Ang II concentration may be considered close to \( in vivo \) conditions because, although plasma concentration is close to \( 10^{-10} \) M, intrarenal production of Ang II leads to concentrations in glomerular filtrate and tubular lumen that are \( 10^2-10^3 \) fold higher than in plasma (37). Patterns of calcium responses elicited by the first application of Ang II were similar to those reported in our previous study, especially the time required to reach the maximum response (which was significantly longer in the Glom than in CTAL) (22). After a second application of Ang II, the amplitude of the \( [Ca^{2+}] \) response \( \Delta [Ca^{2+}] \) was totally abolished in Glom \( \Delta [Ca^{2+}] = 0 \) vs. \( 79 \pm 11 \) nM, \( n = 12 \); whereas in CTAL, a slight response \( (11 \pm 1\%) \) remained \( \Delta [Ca^{2+}] = 24 \pm 3 \) vs. \( 209 \pm 17 \) nM, \( n = 13 \). Such abolition or attenuation of calcium responses were attributable neither to a loss of cell viability (in Glom and CTAL) nor to a depletion of the calcium stores (in CTAL), because a subsequent application of \( 10^{-4} \) M carbachol (in Glom) or \( 10^{-7} \) M bradykinin (in CTAL) induced calcium responses within the expected range: \( 352 \pm 22 \) nM, \( n = 4 \); and \( 266 \pm 43 \) nM, \( n = 5 \), respectively (38, 39). Calcium responses were of the same order of magnitude when either bradykinin in CTAL or carbachol in Glom was applied in first or third position (data not shown).

Representative recordings of \( [Ca^{2+}] \) levels, in response to two successive applications of different doses of Ang II in Glom and CTAL, are depicted on Figs. 2 (A–F) and 3 (A–E). The first application varied from \( 10^{-7} \) to \( 10^{-11} \) M Ang II, the second one being always \( 10^{-7} \) M Ang II. Mean dose-response curves (Figs. 2 and 3, bottom panels) indicated that desensitization is a function of the concentration of the first Ang II application in the two structures. Half-maximal desensitization occurred at \( 7.1 \pm 2.2 \) \( 10^{-9} \) and \( 4.4 \pm 2.1 \) \( 10^{-9} \) M (mean ± sd) for Glom and CTAL, respectively, \( P < 0.01 \).

Regarding Glom, it was important to discriminate the response of the different cell populations. This was done by comparing cultures of pure mesangial cells and microdissected parietal sheet of Bowman’s capsule. The calcium response elicited by Ang II \( (10^{-7} \) M) was observed in mesangial cells (32, 40) \( \Delta [Ca^{2+}] = 282 \pm 48 \) nM, \( n = 5 \) (Fig. 4A) and not in epithelial cells of Bowman’s capsule (41) (Fig. 4B). The absence of calcium response in epithelial cells is consistent with recent data indicating that their AT1-R are coupled to the adenylylcyclase pathway (42). To check that this absence of calcium response in parietal sheet was not caused by a cellular death, good cell viability in our experimental conditions was attested by their response to carbachol (38) (Fig. 4B).

In mesangial cells, as in Glom, total desensitization was found after a second application of \( 10^{-7} \) M Ang II (Fig. 4A). In these preparations, cellular integrity and absence of depletion of the calcium stores were checked by a subsequent application of \( 10^{-7} \) M bradykinin, which induced the expected increase in \( [Ca^{2+}] \).

**Homologous desensitization in afferent arterioles**

Because a difference in desensitization properties was found between Glom and CTAL when using \( 10^{-7} \) M Ang II, we extended our studies to another renal structure, afferent arterioles, which contained proportions of the two subtypes similar to CTAL.

Fig. 1C shows representative recordings of \( [Ca^{2+}] \) levels in response to two successive applications of \( 10^{-7} \) M Ang II, separated by 15-min washing. We observed no desensitization in afferent arterioles \( \Delta [Ca^{2+}] = 229 \pm 31 \) vs. \( 207 \pm 25 \) nM, \( n = 4 \). We showed also that no desensitization occurred.

**Fig. 5.** Dose-response curve, response to a single application of Ang II in anterior pituitary cells. Half-maximal effective response was \( 4.5 \pm 1.8 \) \( 10^{-9} \) M (mean ± sd). \( \Delta [Ca^{2+}] \), variation in \( [Ca^{2+}] \), expressed as a peak increase in \( [Ca^{2+}] \), above basal values. Each point represents the mean calculated from three to nine individual determinations.
with two successive applications of $10^{-8}$ or $10^{-9}$ m Ang II (data not shown).

**Homologous desensitization in anterior pituitary cells**

Dose-response characteristics of the calcium mobilization induced by Ang II in cultured pituitary cells is shown in Fig. 5. Maximal stimulation and half-maximal response were obtained at $10^{-7}$ and $4.5 \pm 1.8 \times 10^{-9}$ m Ang II, respectively (mean ± sd).

Homologous desensitization studies were performed. Representative recordings of $[\text{Ca}^{2+}]_i$ levels in response to two successive applications of different doses of Ang II are depicted on Fig. 6, A–D. Half-maximal desensitization occurred at $1.7 \pm 1.3 \times 10^{-9}$ m (mean ± sd, Fig. 6, bottom panel). Comparison of this value with those calculated in Glom ($7.1 \pm 2.2 \times 10^{-9}$ m) and CTAL ($4.4 \pm 2.1 \times 10^{-9}$ m), showed significant differences ($P < 0.001$ and $P < 0.01$, respectively). By using two successive applications of $10^{-7}$ m Ang II, desensitization occurred but was not complete, because $[\text{Ca}^{2+}]_i$ increase reached $26 \pm 5\%$ ($n = 6$) of the response elicited by the first application ($\Delta [\text{Ca}^{2+}] = 58 \pm 9$ vs. $236 \pm 34$ nm, $n = 6$) (Fig. 6A). After two applications of $10^{-7}$ m Ang II, TRH ($10^{-7}$ m), known to induce an increase in $[\text{Ca}^{2+}]_i$ in lactotropes and thyrotropes (43), was as effective ($\Delta [\text{Ca}^{2+}] = 246 \pm 76$ nm, $n = 6$) as a first application of Ang II. In keeping with the fact that measurements were performed on single cells and that thyrotropes are devoid of AT$_1$-R (44), the response to TRH indicates that lactotropes were undamaged and their calcium stores not depleted.

After these results of homologous desensitization, obtained in different cell types, additional data and results were presented in Table 1: 1) To validate calcium results expressed as the difference between the peak and basal concentration ($[\text{Ca}^{2+}]_i$), we measured the area under curve (integral of the calcium signal). Data showed that the percent of desensitization was similar to that obtained with the first calculation.
method. 2) By using $10^{-8}$ M Ang II in CTAL, to equal the amount of calcium released by $10^{-7}$ M Ang II in Glom, we checked that the percent of desensitization does not depend on the amount of calcium released. 3) Whatever the washing time (5, 15, or 30 min), the desensitization occurring in Glom is systematically more important than in CTAL. Indeed, after 30-min washing, CTAL exhibits normal sensitivity in response to a second application of $10^{-7}$ M Ang II, whereas Glom remains partially unresponsive. These data were in good agreement with the notion that desensitization and resensitization are generally linked (45, 46). They also showed that both these phenomena are dependent upon the cell type studied (47, 48).

Possible involvement of PKC in AT1-R desensitization processes

In view of the ability of PKC to phosphorylate (and thereby down-regulate) AT1-R, we looked for a possible involvement of PKC in the homologous desensitization process by using bisindolylmaleimide, a potent PKC inhibitor. Glom and CTAL were superfused with bisindolylmaleimide (1 μM) or DMSO alone (control) for 20 min before the first application of Ang II and during all the experiments. As depicted in Table 2, the presence of the PKC inhibitor increased the calcium responses elicited by the first application of $10^{-7}$ M Ang II in both structures and did not alter the homologous desensitization level that remained total in Glom and partial in CTAL.

Discussion

The cloning of AT1a-R and AT1b-R cDNAs in rodents has allowed exploration of the properties of these two receptor subtypes in stable cell lines specifically expressing either recombinant receptor. However, no study has attempted to examine the desensitization process of these receptors in intact tissue naturally expressing both isoforms in various proportions. In the present work, we report, for the first time in three different structures of the kidney, differences in the pattern of homologous desensitization of Ang II receptor subtypes, AT1a-R and AT1b-R. In CTAL and afferent arterioles, both predominantly expressing AT1a-R mRNA, we obtained an unexpected finding, i.e. a partial desensitization and no desensitization, respectively. In Glom, which contains a relatively similar proportion of the two receptor subtype mRNAs, total desensitization was observed (Table 3). This latter result may be attributed to mesangial cells, because after two successive applications of $10^{-7}$ M Ang II, a complete desensitization was observed in these cells containing 42% AT1a-R and 58% AT1b-R mRNA (15). Comparison of results obtained at a maximal dose of Ang II in CTAL and afferent arterioles, and those obtained in Glom, might suggest that the AT1b-R subtype is more sensitive to homologous desensitization. This hypothesis is in good agreement with the prominent desensitization of AT1b-R observed previously in Xenopus Laevis oocytes expressing rat AT1b-R (5).

To verify this hypothesis, in the absence of a renal structure containing only AT1b-R mRNA (22), we studied calcium response in primary cultures of rat anterior pituitary cells where AT1b-R mRNA predominates (80% of total AT1-R mRNA) (26). In these cells, identified as lactotropes, surprisingly, desensitization was not complete after two successive applications of $10^{-7}$ M Ang II.

Taken together, the data obtained in Glom, CTAL, afferent arterioles, and anterior pituitary cells suggest that when one of both AT1-R subtype is predominantly expressed in intact tissue or cells, the desensitization process is partially or not at all observed; whereas when both receptors are expressed together in similar proportions, total desensitization occurred (Table 3). One hypothesis to explain this differential pattern of desensitization would be a possible molecular interaction between both subtypes. Interconversion between monomeric and dimeric forms between AT1-R subtypes has been evoked to play an important role in modulating receptor function (49, 50). This was also supported by findings on the dimerization of a number of seven-transmembrane domain G protein-coupled receptors such as β2-adrenergic re-

### TABLE 1. Percent of desensitization calculated from measurements of areas under curve

<table>
<thead>
<tr>
<th>Washing time (min)</th>
<th>Areas under curve (nM × sec)</th>
<th>Desensitization (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First response</td>
<td>Second response</td>
</tr>
<tr>
<td>Glom (12)</td>
<td>5</td>
<td>11,766 ± 1,410</td>
</tr>
<tr>
<td>Glom (6)</td>
<td>15</td>
<td>12,142 ± 1,297</td>
</tr>
<tr>
<td>Glom (5)</td>
<td>30</td>
<td>12,275 ± 4,029</td>
</tr>
<tr>
<td>CTAL (6)</td>
<td>5</td>
<td>14,937 ± 4,424</td>
</tr>
<tr>
<td>CTAL (13)</td>
<td>5</td>
<td>17,819 ± 1,752</td>
</tr>
<tr>
<td>CTAL (7)</td>
<td>15</td>
<td>17,307 ± 2,647</td>
</tr>
<tr>
<td>CTAL (4)</td>
<td>30</td>
<td>20,931 ± 3,427</td>
</tr>
<tr>
<td>Anterior pituitary cells (6)</td>
<td>5</td>
<td>7,991 ± 1,714</td>
</tr>
<tr>
<td>Afferent arterioles (4)</td>
<td>15</td>
<td>198,307 ± 13,944</td>
</tr>
</tbody>
</table>

Washing time, Time between two applications of Ang II; nd, nondetectable. Number of determinations are in parentheses.

### TABLE 2. Involvement of PKC in the desensitization of AT1-R subtypes to Ang II in Glom and CTAL

<table>
<thead>
<tr>
<th>PKC inhibitor</th>
<th>n</th>
<th>First calcium response (in nM)</th>
<th>Second calcium response (in nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glom</td>
<td>−</td>
<td>7</td>
<td>63 ± 6</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>6</td>
<td>91 ± 8</td>
</tr>
<tr>
<td>CTAL</td>
<td>−</td>
<td>13</td>
<td>209 ± 17</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>7</td>
<td>410 ± 45&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Both calcium responses were induced by $10^{-7}$ M Ang II, separated by 5-min washing. Values are means ± se; nd, Nondetectable; n, number of determinations. Comparison of calcium response in the absence (−) and in the presence (+) of 1 μM of the PKC inhibitor, bisindolylmaleimide.

<sup>a</sup> P < 0.05.
<sup>b</sup> P = 0.001.
TABLE 3. Proportion of AT1-R subtype mRNA and amplitude of desensitization in different structures studied

<table>
<thead>
<tr>
<th>Structures</th>
<th>% of AT1A-R mRNA</th>
<th>% of AT1B-R mRNA</th>
<th>Ref.</th>
<th>Desensitization amplitude of the Ca2+ response after two applications of a maximal dose of Ang II</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTAL</td>
<td>90</td>
<td>10</td>
<td>22</td>
<td>Partial desensitization</td>
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<tr>
<td>Afferent arterioles</td>
<td>70</td>
<td>30</td>
<td>25</td>
<td>No desensitization</td>
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<td>Glomeruli</td>
<td>40</td>
<td>60</td>
<td>22</td>
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</tr>
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<td>Mesangial cells</td>
<td>42</td>
<td>58</td>
<td>15</td>
<td>Total desensitization</td>
</tr>
<tr>
<td>Anterior pituitary cells</td>
<td>20</td>
<td>80</td>
<td>26</td>
<td>Partial desensitization</td>
</tr>
</tbody>
</table>

* References where the proportions of the two mRNA receptor subtypes were evaluated.

ceptors (51), δ opioid receptors (52), and AT1-R/bradykinin type 2 receptor (53). Another variable that may affect homologous desensitization was receptor number expressed by an individual cell. Indeed, a total desensitization was observed in mesangial cells, which expressed the highest density of AT1-R binding sites (15) [~3-fold that obtained in CTAL (27, 54) and afferent arterioles (25)]. However, either partial or no desensitization to Ang II was observed in CTAL and afferent arterioles, respectively, although they exhibit a similar number of AT1-R binding sites. Thus, the comparison of AT1-R binding site density values and the degree of desensitization to Ang II obtained in different renal structures does not allow evidence of a clear correlation between these parameters. Finally, another hypothesis would be related to the nature of the cell expressing these receptors, as suggested by studies performed in two types of eucaryote cells. Indeed, no inhibition of the maximal Ca2+ response was observed at high concentrations of Ang II in transfected mouse adrenal Y-1 carcinoma cells expressing AT1A or AT1B receptor subtypes (21). In contrast, a change in maximal Ca2+ response with high concentrations of Ang II was observed in transfected CHO cells expressing the AT1B receptor subtype but not in those expressing the AT1A receptor subtype (23). Whatever the considered hypothesis (heterodimerization and/or importance of the cell type), it could allow an adaptive response to Ang II according to the specific function of a given cell type. As an example, in afferent arterioles, where AT1A is predominantly expressed, the absence of desensitization should correspond to a permanent requirement for limiting the renal blood flow through vasoconstrictor effects of Ang II. Besides, the importance of AT1A receptor, in the brain, on blood pressure regulation was recently described by Davison et al. (55). In contrast, in mesangial cells, the important desensitization observed would limit the Ang II induced-cell contraction and thus would avoid an important fall in glomerular filtration. These differential desensitization patterns could be considered as a protective device, in regard to physiological regulation of glomerular filtration. This extends the importance of AT1-R subtypes in regulating physiological functions mediated by the renin angiotensin system. Indeed, the absence of either of the AT1-R isoforms alone can be compensated, in varying degrees, by the other isoform (6).

AT1A-R and AT1B-R are coupled to the phospholipid hydrolysis/[Ca2+]i mobilization pathway (22) that leads to the activation of protein kinase C (PKC). In addition, the carboxyl-terminal region of the AT1-R contains several serine and threonine residues, which constitute potential consensus sites for PKC phosphorylation (1, 5). The involvement of PKC in the desensitization of these two subtypes has been investigated, but controversial data were reported (24, 56–58). So, we examined, in our experimental conditions, the involvement of PKC in the desensitization process on Glom and CTAL naturally expressing AT1A-R and AT1B-R subtypes. Our results indicated that desensitization phenomenon was caused by a PKC-independent mechanism in both studied structures. However, note that the development of the calcium response induced by the first application of 10–7 M Ang II was PKC-dependent, because the amplitude of responses was significantly increased in the presence of PKC inhibitor in Glom and CTAL, as recently shown by Meszaros et al. (48).

In conclusion, this study, performed on different tissues, naturally and constitutively expressing both AT1-R isoforms in various proportions, shows that AT1-R subtypes can be desensitized, in response to two successive doses of Ang II, in a dose-dependent manner. Homologous desensitization phenomenon does not depend on the nature of the expressed subtype but, more probably, on the proportion of each subtype in a given cell without excluding the importance of the cell type. Homologous desensitization occurs via a PKC-independent process. Taken together, these observations suggest that the differential desensitization pattern of AT1A-R and AT1B-R constitutes a way to adapt the Ang II response to a given cell type requirement, according to the physiological conditions.

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