Angiotensin II production and distribution in the kidney: I. A kinetic model

MADH Schalekamp[1,2] and AHJ Danser[1,2]

[1]Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands and [2]Department Pharmacology, Erasmus MC, Rotterdam, The Netherlands

Information on the levels of angiotensin II (Ang II) and its receptors in the various renal tissue compartments is still incomplete. A model is presented describing the kinetics of Ang II production, distribution, and disposal in the renal cortex. Basic features are: (1) the model is designed to derive, from Ang II measurements in blood and in whole tissue, estimates of the local densities of the Ang II type 1 (AT_1) and type 2 (AT_2) receptors, and to calculate the concentrations of *endocrine* and *paracrine* Ang II they actually 'see'; (2) glomerular and peritubular tissue are conceived as separate regions (glomerular region (Glom), peritubular region (Pt)); (3) in Glom and in Pt, Ang II is homogeneously distributed in capillary blood and in interstitial fluid; (4) the model allows for local Ang II concentration gradients between interstitium and blood; (5) Ang II from the circulation diffuses into the interstitium of Glom after convective transcapillary transport; (6) Ang II produced in tubules or Pt enters the microcirculation through diffusive overflow from interstitium; (7) the presence of cell-surface-bound Ang II depends on the reaction with AT_1 and AT_2 receptors, and the presence of intracellular Ang II depends on the internalization of Ang II – AT_1 receptor complex; and (8) the model provides for glomerular filtration, vasopeptidase-mediated degradation, and intracellular degradation as mechanisms of elimination. This model can serve as a framework for detailed quantitative studies of the renin–angiotensin system in the kidney.

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Correspondence: *MADH Schalekamp, c/o AHJ Danser, Department of Pharmacology, Room EE1418b, Erasmus MC, Dr Molewaterplein 50, 3015 GE Rotterdam, The Netherlands. E-mail: a.danser@erasmusmc.nl*

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The renin–angiotensin system is part of the complex regulation of blood pressure, renal function, and body sodium and water homeostasis. The most important biologically active end product of this system, angiotensin (Ang) II, is thought to act both as a circulating hormone and as a paracrine factor. The physiological significance of paracrine Ang II, as opposed to circulating Ang II, is still not well understood.

In humans, under normal conditions, the concentration of Ang II in blood plasma is approximately 10 pM at a daily sodium intake typical of Western industrialized countries,[1,2] which is close to the threshold level required to elicit a physiological response. After dietary sodium restriction, either alone or in combination with the use of a diuretic, Ang II can increase 10-fold. Under pathological conditions, it can increase 100-fold or even more. In anesthetized animals, the concentration of circulating Ang II is often higher than the levels normally encountered in humans.

In humans as well as in anesthetized animals, the Ang II concentration in some tissues (per gram of tissue), including the kidney, is higher than in plasma (per ml).[3–7] Some authors measured Ang II levels in the nanomolar range in cardiac and renal tissue fluid,[8–11] which would fit with the value of the equilibrium dissociation constant of the Ang II–Ang II type 1 (AT_1) receptor and Ang II–Ang II type 2 (AT_2) receptor reactions ($K_d = 1$–2 nM). According to others, however, the interstitial fluid levels are much lower.[7,12,13] Rather, it now appears that Ang II in the tissue is cell-associated owing to its binding to cell surface AT_1 and possibly also AT_2 receptors. Studies in rats demonstrated AT_1-receptor-dependent intrarenal accumulation of systemically infused Ang II[14] and provided evidence of AT_1 receptor-mediated renal uptake of endogenous Ang II after dietary salt restriction.[15] The binding to cell surface AT_1 receptors is known to be followed by rapid internalization of Ang II–AT_1 receptor complex.[16]

Experiments in pigs showed that intact ^{125}I-Ang II from the circulation was accumulated in cardiac, renal, and adrenal tissue, and that the uptake of ^{125}I-Ang II was greatly reduced after the animals had been treated with an AT_1 receptor antagonist.[17] The steady-state tissue/blood ^{125}I-Ang II concentration ratio was adrenal > kidney > heart, which is in accordance with the order of AT_1 receptor density in these

tissues as well as with the order of endogenous Ang II concentration.[3,4,7,17]

Published results of Ang II measurements in blood plasma, in tissue extracellular fluid, and in whole tissue raise a number of important questions. How can circulating, endocrine, Ang II act at such a low concentration? What are the local AT_1 and AT_2 receptor concentrations in tissue? What are the Ang II concentrations the AT_1 and AT_2 receptors in tissue actually 'see'? How much of it is of local, paracrine, origin?

This paper focuses on the kidney and, to address the above questions, a quantitative model is presented describing the kinetics of the intrarenal production, distribution, and elimination of Ang II. The model is designed as a tool to calculate the intrarenal levels of extracellular and cell-bound Ang II as well as to estimate the local concentrations of cell surface AT_1 and AT_2 receptors, from Ang II measurements in blood plasma and in whole tissue. The aim of the model also is to provide for separate estimates of endocrine and paracrine Ang II levels.

GENERAL CHARACTERISTICS OF THE MODEL

Tables 1–3 provide a list of abbreviations, symbols, and definitions, as well as the values of the various physical parameters and kinetic constants. An important feature is the distinction between *endocrine* Ang II (IIa) delivered to the tissue via the renal artery and *paracrine* Ang II (IIi) that is produced intrarenally. Ang IIa and Ang IIi in renal venous plasma and in tissue can be measured separately in samples obtained from animals receiving systemic infusions of radiolabeled Ang I or II.[7,17] Similarly, separate measurements of Ang Ia and Ang Ii can be obtained during a systemic infusion of radiolabeled Ang I.

The model considers three cortical tissue regions, the glomerular region (Glom), the tubular region (Tb), and the peritubular region (Pt). The glomerular capillary plasma and interstitial fluid compartments (PcGlom, IsfGlom) are connected in series with the corresponding peritubular compartments (PcPt, IsfPt). The two plasma compartments are connected via the glomerular efferent arterioles, and the two interstitial fluid compartments are connected via the interstitium at the level of the glomerular vascular pole (Figure 1).

Ang I and II from blood reach the glomerular ultrafiltrate via the glomerular capillaries by convection, and it is assumed that some Ang II, after its passage through the capillary endothelium, reaches glomerular (mesangial) cell surface receptors by diffusion into the interstitium (Figure 2). Binding of Ang II to cell surface AT_1 receptors, but not AT_2 receptors, is followed by internalization of the Ang II–AT_1 receptor complex and degradation of Ang II by intracellular (lysosomal) enzymes.[16,18] Free Ang I and II in glomerular interstitial fluid are thought to be homogeneously distributed, under steady-state conditions.

It is further assumed that the release of intrarenally produced Ang I and II into the renal interstitial fluid is confined to the peritubular tissue region. The exact sites of Ang I and II production in the kidney are not known, but an extensive series of micropuncture experiments in rats by Navar and co-workers have shown that the levels of Ang I and II in proximal tubular fluid are 10 to 100 times higher than in blood plasma, and that angiotensinogen is also present in high concentrations in proximal tubular fluid.[19,20] These experiments also provided evidence to support that Ang I and II as well as angiotensinogen are secreted by the proximal tubular cells into the tubular fluid.[20,21] The intrarenal localization of angiotensin-converting enzyme (ACE) at the brush border of proximal tubular cells is in accordance with this hypothesis.[22]

In our model, a net transport of Ang I and II is therefore assumed to exist from tubular fluid into the peritubular interstitial fluid compartment and from there into the peritubular capillaries (Figure 3). The capillary–interstitial exchange of Ang I and II in the Pt is thought to depend on diffusion, and here our analysis, while allowing for the existence of an endothelial diffusion barrier, follows the so-called tissue homogeneity model.[23] According to this model, capillary entrances and exits are randomly distributed throughout the tissue, and the interstitial fluid compartment as well as the plasma compartment in capillaries and veins are conceived as being well-mixed.

The model implies that the presence of cell-associated Ang II in the renal cortex depends on the binding of extracellular Ang II to cell surface AT_1 and AT_2 receptors. Experiments using systemic infusions of ^{125}I-Ang I or II in pigs showed more than 90% reduction of the tissue/blood ^{125}I-Ang II concentration ratio in the renal cortex after AT_1 receptor antagonist treatment.[7] This indicates that indeed the bulk of arterially delivered Ang II is cell-associated as a consequence of its reaction with AT_1 receptors. Cell fractionation studies of porcine renal cortical tissue, with the use of differential centrifugation, indicated that not only most of systemically infused ^{125}I-Ang II but also most endogenous Ang II is bound to cell organelles, whereas exogenous and endogenous Ang II were similarly distributed over the various subcellular fractions.[24] Similar data were obtained by others.[25,26]

ARTERIALLY DELIVERED ANG I AND II

Concentrations in interstitial fluid

The concept set out above leads to the following steady-state equations:

$$CIa_{IsfGlom} = CI_{Pa} \quad (1)$$

$$CIIa_{IsfGlom} = CII_{Pa} - RMEIIa_{Glom}/(Q\,FF) \quad (2)$$

$$CIa_{IsfPt} = CIa_{Pv} \quad (3)$$

$$CIIa_{IsfPt} = CIIa_{Pv} - RMEIIa_{Pt}/Cl_{DiffPt} \quad (4)$$

Table 1 | Abbreviations, symbols, and definitions

Symbol or abbreviation	Definition
Renal compartments	
Pa	Renal arterial plasma
Pv	Renal venous plasma
Pc	Capillary plasma
Isf	Interstitial fluid
Tb	Tubular region of renal cortex
T	Renal cortex
Glom	Glomerular region
Pt	Peritubular region of renal cortex
Cs	Cell surface
Ce	Cell interior
Angiotensins from different sources	
I	Ang I
II	Ang II
IIa	Arterially delivered Ang II
IIi	Intrarenally produced Ang II
Angiotensin in different compartments	
II_{Pa}, II_{Pv}	Plasma Ang II in renal artery, vein
II_{PcGlom}, II_{PcPt}	Plasma Ang II in glomerular, peritubular capillaries
$II_{IsfGlom}$, II_{IsfPt}	Ang II in glomerular, peritubular interstitial fluid
II_{C1}	AT_1 receptor-dependent cell-associated (surface-bound and intracellular) Ang II
II_{C1Glom}, II_{C1Pt}	AT_1 receptor-dependent cell-associated Ang II in glomerular, peritubular region
$II_{Cs1Glom}$, II_{Cs1Pt}	Ang II bound to cell surface AT_1 receptors in glomerular, peritubular region
II_{Cs2Pt}	Ang II bound to cell surface AT_2 receptors in peritubular region
II_T	Ang II in renal cortex
II_{Tb}	Ang II in tubular fluid
Receptors	
AT_1R_{CsGlom}, AT_1R_{CsPt}	Cell surface AT_1 receptors in glomerular, peritubular region
AT_2R_{CsPt}	Cell surface AT_2 receptors in peritubular region
Concentrations	
CI, CII	Concentration of Ang I, Ang II
CAT_1R, CAT_2R	Concentration of AT_1-, AT_2 receptors
$(CI)_{1,2}$, $(CII)_{1,2}$	Concentration of Ang I, Ang II in the absence of AT_1- and AT_2 receptor blockade
$(CI)_1$, $(CII)_1$	Concentration of Ang I, Ang II in the presence of AT_2 receptor blockade
$(CI)_2$, $(CII)_2$	Concentration of Ang I, Ang II in the presence of AT_1 receptor blockade
Elimination mechanisms	
VMD	Vasopeptidase-mediated degradation
RME	AT_1 receptor-mediated endocytosis
CI_{RME}	Clearance by RME
Physical parameters	
V_{PcGlom}, V_{PcPt}	Volume of glomerular, peritubular capillary blood plasma
$V_{IsfGlom}$, V_{IsfPt}	Volume of glomerular, peritubular interstitial fluid
Q	Renal cortical plasma flow
FF	Filtration fraction
CI_{DiffPt}	Diffusive clearance across peritubular capillaries
Kinetic constants	
K_d	Equilibrium dissociation constant of the Ang II–AT_1 and Ang II–AT_2 receptor reactions
k_{ass}	Rate constant for Ang II–AT_1 receptor complex formation
k_{diss}	Rate constant for Ang II–AT_1 receptor complex dissociation
k_{int}	Rate constant for AT_1 receptor-mediated Ang II endocytosis
k_{lys}	Rate constant for (lysosomal) intracellular Ang II degradation
k_{tel}	Rate constant for the elimination of cell-associated (surface-bound and intracellular) Ang II

Vasopeptidase-mediated degradation

Concerning the elimination of extracellular Ang IIa, the model provides for the following mechanisms: glomerular filtration, vasopeptidase-mediated degradation, and AT_1 receptor-mediated endocytosis. It is known that only a small fraction of systemically infused Ang I is converted into Ang II in the kidney,[27] and studies in pigs, with the use of infusions of either ^{125}I-Ang I or ^{125}I-Ang II, justify the conclusion that,

for the purpose of the present analysis, Ang I–II conversion by renal vasopeptidase can be ignored and that the degradation rate constants are similar for Ang I and II.[17,28,29] The rate of vasopeptidase-mediated degradation of Ang Ia into peptides other than Ang II, at steady state, is therefore given by

$$\mathrm{VMDIa} = \mathrm{CI}_{\mathrm{Pa}}\, Q(1 - \mathrm{FF} - \mathrm{CIa}_{\mathrm{Pv}}/\mathrm{CI}_{\mathrm{Pa}}) \qquad (5)$$

Similarly,

$$\mathrm{VMDIIa} = \mathrm{CII}_{\mathrm{Pa}}\, Q(1 - \mathrm{FF} - \mathrm{CIa}_{\mathrm{Pv}}/\mathrm{CI}_{\mathrm{Pa}}) \qquad (6)$$

Binding to cell surface AT_1 and AT_2 receptors and AT_1 receptor-mediated endocytosis

As mentioned above, experiments using systemic infusions of ^{125}I-Ang I or II in pigs indicate that the bulk of Ang IIa present in tissue is cell-associated as a consequence of its reaction with AT_1 receptors. Ang IIa, after its binding to cell surface AT_1 receptors, reaches the cell interior through AT_1 receptor-mediated endocytosis. For the purpose of the present analysis, it can be stated that

$$\mathrm{CIIa}_{\mathrm{T}} = \mathrm{CIIa}_{\mathrm{C1}} \qquad (7)$$

$$\mathrm{CIIa}_{\mathrm{C1}} = \mathrm{CIIa}_{\mathrm{Cs1}}\, V_{\mathrm{Isf}} + \mathrm{CIIa}_{\mathrm{Ce1}} \qquad (8)$$

Steady-state equations describing the binding of Ang IIa to cell surface AT_1 receptors, its internalization and its intracellular degradation are

$$\mathrm{CIIa}_{\mathrm{Isf}}\mathrm{CAT}_1\mathrm{R}_{\mathrm{Cs}}\, k_{\mathrm{ass}} = \mathrm{CIIa}_{\mathrm{Cs1}}(k_{\mathrm{diss}} + k_{\mathrm{int}}) \qquad (9)$$

$$\mathrm{RMEIIa} = \mathrm{CIIa}_{\mathrm{Cs1}}\, V_{\mathrm{Isf}}\, k_{\mathrm{int}} \qquad (10)$$

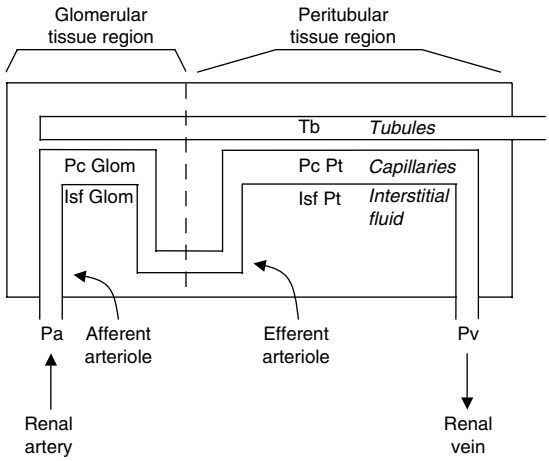

Figure 1 | Schematic representation of the extracellular fluid compartments in the glomerular and peritubular tissue regions

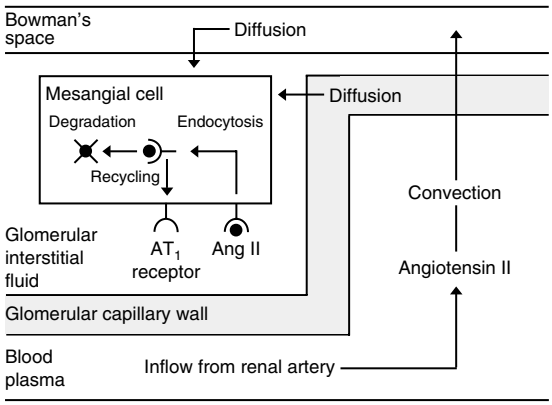

Figure 2 | Schematic representation of extracellular transport and intracellular trafficking of endocrine angiotensin II in the glomerular tissue region.

Table 2 | Units of parameters

Concentrations	
$\mathrm{CII}_{\mathrm{Pa}}$ $\mathrm{CII}_{\mathrm{Pv}}$, $\mathrm{CII}_{\mathrm{Pc}}$	mol/ml of blood plasma
$\mathrm{CII}_{\mathrm{Isf}}$, $\mathrm{CII}_{\mathrm{Cs}}$	mol/ml of interstitial fluid
$\mathrm{CII}_{\mathrm{Tb}}$, $\mathrm{CII}_{\mathrm{T}}$, $\mathrm{CII}_{\mathrm{C1}}$	mol/g of renal cortex
$\mathrm{CAT_1R_{Cs}}$, $\mathrm{CAT_2R_{Cs}}$	mol/ml of interstitial fluid
Physical parameters and kinetic constants	
V_{Pc}, V_{Isf}	ml/g of renal cortex
Q, $\mathrm{CI}_{\mathrm{DiffPt}}$, $\mathrm{CI}_{\mathrm{RME}}$	ml/min per g of renal cortex
K_{d}	mol/ml
k_{ass}	/(mol/ml) per min
k_{diss}, k_{int}, k_{lys}, k_{tel}	min^{-1}
RMEII	mol/min per g of renal cortex
VMDI, VMDII	mol/min per g of renal cortex

Table 3 | Values of physical parameters and kinetic constants of the renal cortex. For an explanation of abbreviations, see Table 1.

Parameter	Value	Reference
V_{IsfGlom}	$(5) \times 10^{-3}$/ml per g	33
V_{IsfPt}	0.1 ml/g	33
Q	2 ml/min per g	34
FF	0.20	35
$\mathrm{CI}_{\mathrm{DiffPt}}$	0.3 ml/min per g	See text
K_{d} (for AT_1 – and AT_2 receptors)	$(1.5) \times 10^{-12}$ mol/ml	36,37
k_{ass} (for AT_1 receptors)	$(2.4) \times 10^{10}$/(mol/ml) per min	36
k_{diss} (for AT_1 receptors)	$(3.6) \times 10^{-2}\ \mathrm{min}^{-1}$	Derived from K_{d} and k_{ass}
k_{tel}	$(2.7) \times 10^{-2}\ \mathrm{min}^{-1}$	17
k_{int}	$(35) \times 10^{-2}\ \mathrm{min}^{-1}$	See text
k_{lys}	$(2.9) \times 10^{-2}\ \mathrm{min}^{-1}$	Derived from k_{tel} and k_{int} (see Eq. (13))

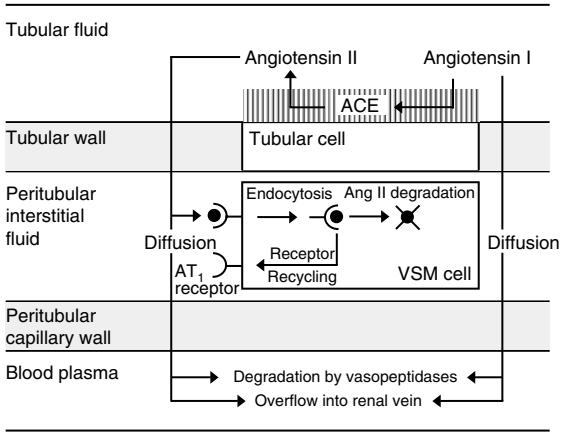

Figure 3 | Schematic representation of extracellular transport and intracellular trafficking of paracrine angiotensin II in the tubular and peritubular cortical tissue regions.

$$\mathrm{CIIa_{Cs1}}\, V_{\mathrm{Isf}}\, k_{\mathrm{int}} = \mathrm{CIIa_{Ce1}}\, k_{\mathrm{lys}} \qquad (11)$$

The experiments in pigs mentioned above showed a quasi-mono-exponential decrease of ^{125}I-Ang II from the renal cortex after the infusion of tracer had been stopped. It can therefore be stated that

$$\mathrm{RMEIIa} = \mathrm{CIIa_T}\, k_{\mathrm{tel}} \qquad (12)$$

From Eqs. (7), (8), and (10)–(12), it follows that

$$k_{\mathrm{tel}} = \frac{k_{\mathrm{int}} k_{\mathrm{lys}}}{(k_{\mathrm{int}} + k_{\mathrm{lys}})} \qquad (13)$$

In accordance with Eqs. (7) and (12), it follows from Eqs. (2) and (4) that

$$\mathrm{CIIa_{IsfGlom}} = \mathrm{CII_{Pa}} - \mathrm{CIIa_{C1Glom}}\, k_{\mathrm{tel}}/(Q\,\mathrm{FF}) \qquad (14)$$

$$\mathrm{CIIa_{IsfPt}} = \mathrm{CIIa_{Pv}} - \mathrm{CIIa_{C1Pt}}\, k_{\mathrm{tel}}/\mathrm{CI_{DiffPt}} \qquad (15)$$

The implicit assumption underlying Eqs. (14) and (15) is that k_{tel} has the same value in the glomerular and peritubular tissue regions.

In Eqs. (5), (6), and (12), the rates of vasopeptidase-mediated Ang Ia and IIa degradation and the rate of AT_1 receptor-mediated Ang IIa endocytosis are expressed as a function of the plasma concentration of Ang I in the renal artery, the concentrations of Ang Ia and Ang IIa in the renal vein, and the total tissue concentration of Ang IIa in the renal cortex. These independent variables can be measured.

INTRARENALLY PRODUCED ANG I AND II

Concentrations in interstitial fluid, spillover into the microcirculation, and vasopeptidase-mediated degradation

The contribution of Ang I that is produced in the renal microcirculation by the action of circulating renin to the total concentration of Ang Ii in the renal vein is very small.[29] It can be ignored for the purpose of the present analysis and, as set out above, the same can be said for the Ang I–II conversion by renal vasopeptidase. It can therefore be stated that the spillover of Ang Ii and IIi into the microcirculation is given by the sum of the vasopeptidase-mediated degradation rate and the outflow via the renal vein. In Eqs. (5) and (6), the rates of vasopeptidase-mediated Ang Ia and IIa degradation are expressed as a fraction of the rate of delivery via the renal artery, the fraction being equal to $(1-\mathrm{FF}-\mathrm{CIa_{Pv}}/\mathrm{CI_{Pa}})$. The rates of vasopeptidase-mediated Ang Ii and IIi degradation can be expressed in a similar way, as a fraction of the spillover rate, so that

$$\text{Spillover Ii} = \mathrm{CIi_{Pv}}\, Q/(\mathrm{FF} + \mathrm{CIa_{Pv}}/\mathrm{CI_{Pa}}) \qquad (16)$$

$$\text{Spillover IIi} = \mathrm{CIIi_{Pv}}\, Q/(\mathrm{FF} + \mathrm{CIa_{Pv}}/\mathrm{CI_{Pa}}) \qquad (17)$$

$$\mathrm{VMDIi} = \mathrm{CIi_{Pv}}\, Q[1/(\mathrm{FF} + \mathrm{CIa_{Pv}}/\mathrm{CI_{Pa}}) - 1] \qquad (18)$$

$$\mathrm{VMDIIi} = \mathrm{CIIi_{Pv}}\, Q[1/(\mathrm{FF} + \mathrm{CIa_{Pv}}/\mathrm{CI_{Pa}}) - 1] \qquad (19)$$

Spillover of Ang Ii and IIi into the microcirculation is related to the concentration gradient across the peritubular capillaries, as follows:

$$\text{Spillover Ii} = (\mathrm{CIi_{IsfPt}} - \mathrm{CIi_{Pv}})\mathrm{Cl_{DiffPt}} \qquad (20)$$

$$\text{Spillover IIi} = (\mathrm{CIIi_{IsfPt}} - \mathrm{CIIi_{Pv}})\mathrm{Cl_{DiffPt}} \qquad (21)$$

From Eqs. (16), (17), (20), and (21), it follows that

$$\mathrm{CIi_{IsfPt}} = \alpha\, \mathrm{CIi_{Pv}} \qquad (22)$$

$$\mathrm{CIIi_{IsfPt}} = \alpha\, \mathrm{CIIi_{Pv}} \qquad (23)$$

where

$$\alpha = 1 + Q/[(\mathrm{FF} + \mathrm{CIa_{Pv}}/\mathrm{CI_{Pa}})\mathrm{Cl_{DiffPt}}] \qquad (24)$$

In Eqs. (16)–(19) and (22)–(24), the spillover of Ang Ii and IIi into the microcirculation, the degradation of Ang Ii and IIi by vasopeptidases, and the concentrations of Ang Ii and IIi in interstitial fluid are expressed as a function of the plasma concentration of Ang I in the renal artery and the concentrations of Ang Ia, Ii, and IIi in the renal vein. These independent variables can be measured.

Distribution over the interstitial and tubular fluid compartments

In our model, it is assumed that Ang I in tissue is confined to the extracellular fluid compartments so that, in the absence of Ang I production in the glomerular tissue region, under steady-state conditions

$$\mathrm{CIi_T} = \mathrm{CIi_{PcPt}}\, V_{\mathrm{PcPt}} + \mathrm{CIi_{IsfPt}}\, V_{\mathrm{IsfPt}} + \mathrm{CIi_{Tb}} \qquad (25)$$

We further define the distribution of Ang Ii by

$$\beta = \mathrm{CIi_{Tb}}/(\mathrm{CIi_{IsfPt}}\, V_{\mathrm{IsfPt}}) \qquad (26)$$

With respect to the distribution of Ang IIi in the presence of AT_1 receptor blockade and in the absence of AT_1 and AT_2 receptor blockade, the notations β_2 and $\beta_{1,2}$, respectively, are used. This distinction is necessary, because the clearance of Ang IIi from peritubular interstitial fluid, in contrast with the clearance of Ang Ii, does not only depend on diffusion into blood but also on AT_1 receptor-mediated endocytosis.

Anatomically, the tubular tissue region comprises the tubular fluid compartment as well as its cell lining. It seems, however, reasonable to assume that the basolateral tubular cell receptors are exposed to a concentration of Ang II not too different from the concentration in peritubular interstitial fluid, whereas apical receptors are indeed exposed to an Ang II concentration equal to that in the tubular fluid compartment. The apical receptors are bathed in a relatively large volume of tubular fluid and it is therefore assumed that only a small fraction of the total amount of Ang IIi in tubular fluid is bound to these receptors and that this fraction can be ignored. The term $CIi_{PcPt}\ V_{PcPt}$ in Eq. (25) is very small as compared with CIi_T[7] and, for the practical purpose of our analysis, it is also ignored.

Thus, in accordance with Eq. (22), it follows that

$$\beta_2 = \beta = [(CIi_T/CIi_{Pv})/(\alpha V_{IsfPt})] - 1 \qquad (27)$$

$$\beta_{1,2} = \beta[(ClIIi_{PtRME})_{1,2} + Cl_{DiffPt}]/Cl_{DiffPt} \qquad (28)$$

In Eq. (27), the factor β is expressed as a function of the plasma concentration of Ang Ii in the renal vein, the total tissue concentration of Ang Ii in the renal cortex, the plasma concentration of Ang I in the renal artery, and the concentration of arterially delivered Ang I in the renal vein. These independent variables can be measured.

Binding to cell surface AT_1 and AT_2 receptors and AT_1 receptor-mediated endocytosis

Part of Ang IIi in the renal cortex is localized in the tissue extracellular fluid compartments, and part of it is cell-bound as a consequence of the binding to cell surface AT_1 and AT_2 receptors and AT_1 receptor-mediated endocytosis. It is further assumed that the bulk of cell-associated Ang IIi is localized in the peritubular tissue region, possibly including the basolateral part of tubular cells, so that under steady-state conditions

$$\begin{aligned} CIIi_T =& CIIi_{PcPt}\ V_{PcPt} + CIIi_{IsfPt}\ V_{IsfPt} + CIIi_{Tb} \\ &+ CIIi_{C1Pt} + CIIi_{Cs2Pt}\ V_{IsfPt} \end{aligned} \qquad (29)$$

The tissue concentration of Ang IIi that is confined to the fluid-phase extracellular milieu is represented by the first three terms of the right part of this equation, and the tissue concentration of cell-associated Ang IIi is represented by the last two terms. The term $CIIi_{PcPt}V_{PcPt}$ is very small as compared with $CIIi_T$,[7] and can be ignored, so that Eq. (29) can be simplified into

$$\begin{aligned} CIIi_T =& CIIi_{IsfPt}\ V_{IsfPt} + CIIi_{Tb} + CIIi_{C1Pt} \\ &+ CIIi_{Cs2Pt}\ V_{IsfPt} \end{aligned} \qquad (30)$$

In order to distinguish the experimental data obtained under AT_1 receptor blockade from data obtained in the absence of AT_1 and AT_2 receptor blockade, the notations $(CIIi)_2$ and $(CIIi)_{1,2}$, respectively, will be used. Thus,

$$\begin{aligned} (CIIi_T)_{1,2} =& (CIIi_{IsfPt})_{1,2} V_{IsfPt} + (CIIi_{Tb})_{1,2} \\ &+ (CIIi_{C1Pt})_{1,2} + (CIIi_{Cs2Pt})_{1,2} V_{IsfPt} \end{aligned} \qquad (31)$$

$$\begin{aligned} (CIIi_T)_2 =& (CIIi_{IsfPt})_2 V_{IsfPt} + (CIIi_{Tb})_2 \\ &+ (CIIi_{Cs2Pt})_2\ V_{IsfPt} \end{aligned} \qquad (32)$$

In accordance with Eq. (26), it follows from Eqs. (31) and (32) that

$$\begin{aligned} (CIIi_{C1Pt})_{1,2} =& (CIIi_T)_{1,2} - (\beta_{1,2} + 1)(CIIi_{IsfPt})_{1,2} V_{IsfPt} \\ &- (CIIi_{Cs2Pt})_{1,2} V_{IsfPt} \end{aligned} \qquad (33)$$

$$(CIIi_{Cs2Pt})_2 = (CIIi_T)_2/V_{IsfPt} - (\beta_2 + 1)(CIIi_{IsfPt})_2 \qquad (34)$$

Assuming the concentration of cell surface AT_2 receptors (free plus occupied) not being altered by AT_1 receptor blockade, it can be stated that

$$(CIIi_{Cs2Pt})_{1,2}/(CIIi_{IsfPt})_{1,2} = (CIIi_{Cs2Pt})_2/(CIIi_{IsfPt})_2 \qquad (35)$$

In accordance with Eq. (23), it follows from Eqs. (33) to (35) that

$$(CIIi_{C1Pt})_{1,2} = \gamma(CIIi_{Pv})_{1,2} - \alpha(\beta_{1,2} - \beta_2)(CIIi_{Pv})_{1,2} V_{IsfPt} \qquad (36)$$

where

$$\gamma = (CIIi_T)_{1,2}/(CIIi_{Pv})_{1,2} - (CIIi_T)_2/(CIIi_{Pv})_2 \qquad (37)$$

In accordance with Eq. (12), it can be stated that

$$(RMEIIi_{Pt})_{1,2} = (CIIi_{C1Pt})_{1,2} k_{tel} \qquad (38)$$

so that

$$(ClIIi_{PtRME})_{1,2} = (CIIi_{C1Pt})_{1,2} k_{tel}/(CIIi_{IsfPt})_{1,2} \qquad (39)$$

In accordance with Eq. (23), it follows from Eqs. (27), (28), and (36)–(39) that

$$\beta_{1,2} - \beta_2 = (\gamma/\alpha)\beta k_{tel}/(Cl_{DiffPt} + \beta V_{IsfPt} k_{tel}) \qquad (40)$$

where α is given by Eq. (24), β by Eq. (27), and γ by Eq. (37).

From Eqs. (27), (28), and (38)–(40), it follows that

$$(CIIi_{C1Pt})_{1,2} = \frac{\gamma(CIIi_{Pv})_{1,2}\, Cl_{DiffPt}}{(Cl_{DiffPt} + \beta V_{IsfPt} k_{tel})} \qquad (41)$$

$$(RMEIIi_{Pt})_{1,2} = \frac{\gamma(CIIi_{Pv})_{1,2}\, Cl_{DiffPt} k_{tel}}{(Cl_{DiffPt} + \beta V_{IsfPt} k_{tel})} \qquad (42)$$

In accordance with Eq. (23), it follows from Eqs. (27) and (34) that

$$(CIIi_{Cs2Pt})_2 = (CIIi_T)_2/V_{IsfPt} - \alpha \cdot (\beta + 1)(CIIi_{Pv})_2 \qquad (43)$$

In Eqs. (41) and (42), the AT_1 receptor-dependent concentration of cell-associated Ang IIi and the rate of AT_1 receptor-mediated endocytosis are expressed as a function of the plasma concentrations of Ang Ii and Ang IIi in the renal vein and the total tissue concentrations of Ang Ii and Ang IIi in the renal cortex. These independent variables can be measured. In Eq. (43), the AT_2 receptor-dependent concentration of cell-associated Ang IIi is expressed as a function of the same variables in combination with the factor α, as given by Eq. (24). This factor depends on the plasma concentration of Ang I in the renal artery and the concentration of arterially delivered Ang I in the renal vein; both variables can be measured.

Regional AT_1 and AT_2 receptor concentrations

On the basis of Eqs. (7), (9), (10), and (12), the concentrations of cell surface AT_1 receptors in glomerular and peritubular interstitial fluid can be expressed as a function of the $CIIa_{C1Glom}/CIIa_{IsfGlom}$ and $CIIa_{C1Pt}/CIIa_{IsfPt}$ ratios, as follows:

$$(CAT_1R_{CsGlom})_{1,2} = \frac{(\lambda/V_{IsfGlom})(CIIa_{C1Glom})_{1,2}}{(CIIa_{IsfGlom})_{1,2}} \quad (44)$$

$$(CAT_1R_{CsPt})_{1,2} = \frac{(\lambda/V_{IsfPt})(CIIa_{C1Pt})_{1,2}}{(CIIa_{IsfPt})_{1,2}} \quad (45)$$

where

$$\lambda = (k_{diss} + k_{int})k_{tel}/(k_{ass}k_{int}) \quad (46)$$

The mechanisms of binding to cell surface AT_1 receptors, AT_1 receptor-mediated endocytosis, and intracellular degradation are the same for Ang IIi and Ang IIa, so that

$$(CIIi_{C1Pt})_{1,2}/(CIIi_{IsfPt})_{1,2} = (CIIa_{C1Pt})_{1,2}/(CIIa_{IsfPt})_{1,2} \quad (47)$$

In accordance with Eq. (23), it follows from Eqs. (41) and (45)–(47) that

$$(CAT_1R_{CsPt})_{1,2} = \frac{(\gamma/\alpha)(\lambda/V_{IsfPt})Cl_{DiffPt}}{(Cl_{DiffPt} + \beta V_{IsfPt}k_{tel})} \quad (48)$$

In accordance with Eq. (15), it follows from Eqs. (45) and (48) that

$$(CIIa_{C1Pt})_{1,2} = \frac{\gamma(CIIa_{Pv})_{1,2}Cl_{DiffPt}}{(\alpha\, Cl_{DiffPt} + \alpha\beta\, V_{IsfPt}\, k_{tel} + \gamma\, k_{tel})} \quad (49)$$

In Eqs. (48) and (49), α is given by Eq. (24), β by Eq. (27), and γ by Eq. (37). In accordance with Eq. (14), it follows from Eq. (44) that

$$(CAT_1R_{CsGlom})_{1,2} = \frac{(\lambda/V_{IsfGlom})(CIIa_{C1Glom})_{1,2}Q\,FF}{[(CII_{Pa})_{1,2}Q\,FF - (CIIa_{C1Glom})_{1,2}k_{tel}]} \quad (50)$$

According to Eq. (7), and allowing for our statements on the distribution of Ang II over the interstitial and tubular fluid compartments, it can be concluded that

$$(CIIa_{C1Glom})_{1,2} = (CIIa_T)_{1,2} - (CIIa_{C1Pt})_{1,2} \quad (51)$$

CAT_2R_{CsPt} can be expressed as a function of the $CIIi_{Cs2Pt}/CIIi_{IsfPt}$ ratio, as follows

$$CAT_2R_{CsPt} = K_d\, CIIi_{Cs2Pt}/CIIi_{IsfPt} \quad (52)$$

In accordance with Eq. (23), it follows from Eqs. (43) and (52) that

$$CAT_2R_{CsPt} = \frac{[K_d/(\alpha V_{IsfPt})](CIIi_T)_2}{(CIIi_{Pv})_2} - (\beta + 1)K_d \quad (53)$$

where α is given by Eq. (24), and β by Eq. (27).

By using Eqs. (48)–(53), the local concentrations of AT_1 and AT_2 receptors can be calculated from measurements of Ang I and Ang II in blood and in whole tissue. In Figures 4 and 5, the parameters $CIIa_{IsfPt}/CII_{Pa}$ and CAT_1R_{CsPt} are plotted against the $CIIa_{C1Pt}/CIIa_T$ ratio. These graphs are based on Eqs. (15), (45), and (46), and refer to $Cl_{DiffPt} = 0.1$, 0.3, and 0.5 ml/min. Experimental data are from van Kats *et al.*[7] It appears that the $CIIa_{IsfPt}/CII_{Pa}$ ratio decreases as CAT_1R_{CsPt} increases, and that CAT_1R_{CsPt} is minimally 10–30K_d at a $CIIa_{C1Pt}/CIIa_T$ ratio > 0.05. Thus, the calculated concentration of cell surface AT_1 receptors in peritubular interstitial fluid is high, even when only a very small fraction of cell-associated Ang IIa is localized in this region.

In Figure 6, the parameters $CIIa_{IsfGlom}/CII_{Pa}$ and CAT_1R_{CsGlom} are plotted against the $CIIa_{C1Pt}/CIIa_T$ ratio by using Eqs. (14), (44), and (49)–(51). Comparison with Figure 5 shows that the calculated concentration of cell surface AT_1 receptors in the glomerular interstitial fluid decreases from about 850K_d to 700K_d, as the calculated AT_1 receptor concentration in peritubular tissue increases from zero to more than 50K_d. The model-based calculations further demonstrate that the glomerular AT_1 receptor concentration

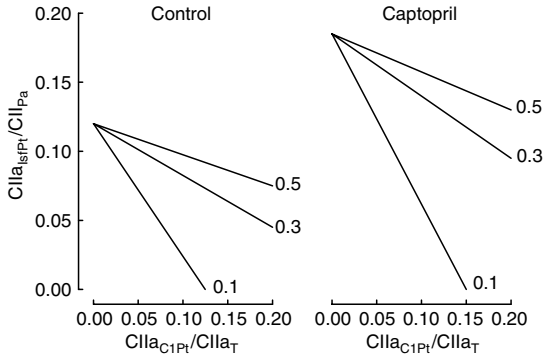

Figure 4 | Line graphs showing the concentration of arterially delivered angiotensin II in the peritubular interstitial fluid compartment ($CIIa_{IsfPt}$), as a function of the concentration of cell-associated arterially delivered angiotensin II in peritubular tissue ($CIIa_{C1Pt}$). $CIIa_{IsfPt}$ is expressed relative to the plasma concentration of angiotensin II in the renal artery (CII_{Pa}). $CIIa_{C1Pt}$ is expressed relative to the total concentration of arterially delivered angiotensin II in the renal cortex ($CIIa_T$). Graphs refer to peritubular transcapillary diffusive angiotensin II clearance rates (Cl_{DiffPt}) of 0.1, 0.3 and 0.5 ml/min per g of renal cortex.

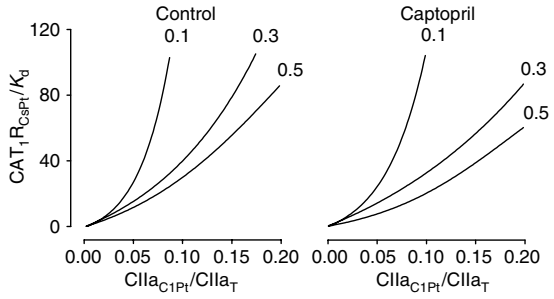

Figure 5 | Line graphs showing the concentration of cell surface AT_1 receptors in the peritubular interstitial fluid compartment (CAT_1R_{CsPt}), as a function of the concentration of cell-associated arterially delivered angiotensin II in peritubular tissue ($CIIa_{C1Pt}$). CAT_1R_{CsPt} is expressed relative to the equilibrium dissociation constant (K_d) of the angiotensin II–AT_1 receptor reaction. $CIIa_{C1Pt}$ is expressed relative to the total concentration of arterially delivered angiotensin II in the renal cortex ($CIIa_T$). Graphs refer to peritubular transcapillary diffusive angiotensin II clearance rates (Cl_{DiffPt}) of 0.1, 0.3, and 0.5 ml/min per g of renal cortex.

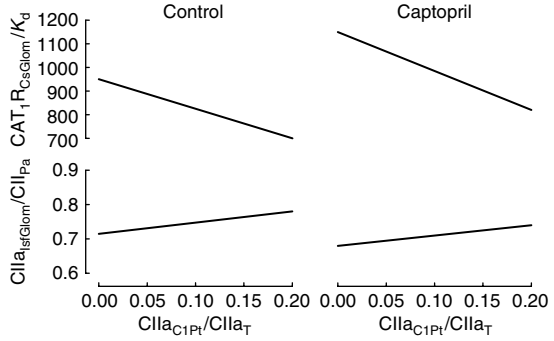

Figure 6 | Line graphs showing the concentration of arterially delivered angiotensin II in the glomerular interstitial fluid compartment ($CIIa_{IsfGlom}$) and the concentration of cell surface AT_1 receptors in glomerular interstitial fluid (CAT_1R_{CsGlom}), as a function of the concentration of cell-associated arterially delivered angiotensin II in peritubular tissue ($CIIa_{C1Pt}$). $CIIa_{IsfGlom}$ is expressed relative to the plasma concentration of angiotensin II in the renal artery (CII_{Pa}). CAT_1R_{CsGlom} is expressed relative to the equilibrium dissociation constant (K_d) of the angiotensin II–AT_1 receptor reaction. $CIIa_{C1Pt}$ is expressed relative to the total concentration of arterially delivered angiotensin II in the renal cortex ($CIIa_T$).

is still above $200K_d$, even in the hypothetical case where the peritubular AT_1 receptor concentration is close to infinitely high.

In Figure 7, the $CIIa_{C1Pt}/CIIa_T$ ratio is plotted as a function of the factor γ, as defined by Eq. (37). This factor is a measure of the AT_1 receptor-dependent tissue concentration of cell-associated paracrine Ang II. The graphs are based on Eqs. (24), (27), (37), and (49). The values of CIa_{Pv}/CI_{Pa} in Eq. (24) and $CIi_T/(CIi_{Pv}\,V_{IsfPt})$ in Eq. (27) are taken to be 0.15 and 180, respectively, as derived from measurements in control animals.[7]

DISCUSSION

The model presented in this paper is designed to calculate, from measurements in blood and in whole tissue, the

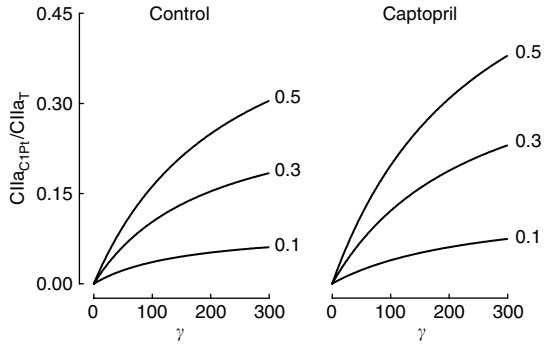

Figure 7 | Line graphs showing the concentration of cell-associated arterially delivered angiotensin II in peritubular tissue ($CIIa_{C1Pt}$), as a function of the factor γ. $CIIa_{C1Pt}$ is expressed relative to the total concentration of arterially delivered angiotensin II in the renal cortex ($CIIa_T$). The factor γ, as defined by Eq. (37), is a measure of the AT_1 receptor-dependent tissue concentration of cell-associated paracrine Ang II. Graphs refer to peritubular transcapillary diffusive angiotensin II clearance rates (Cl_{DiffPt}) of 0.1, 0.3, and 0.5 ml/min per g of renal cortex.

concentrations of Ang I and II in various glomerular and peritubular tissue compartments. The model-based calculations of the local intrarenal concentrations of Ang II and its receptors also require quantitative information about a number of kinetic constants and physical parameters. Fortunately, such information is at hand for most variables, but we are not aware of any direct information about the value of Cl_{DiffPt}, the diffusive Ang II clearance rate across the peritubular capillaries. Our estimate of 0.3 ml/min per g of renal cortical tissue is based on published measurements of the distribution half-time of intravenously administered vasopressin (mol. wt. similar to that of Ang II) in humans, which is 4–8 min.[30] Here $t_{1/2} = 5$ min was chosen, corresponding with a whole-body fractional clearance from plasma of $0.14\,min^{-1}$. Assuming the cardiac output to be equal to blood volume/min and the regional plasma clearance during the distribution phase to be proportional to the regional plasma flow, a fractional clearance of $0.14\,min^{-1}$ corresponds to a peritubular transcapillary clearance rate of 0.3 ml/min per g of renal cortex. In view of the uncertainty about the exact value of Cl_{DiffPt}, the calculations were extended to values of Cl_{DiffPt} ranging from 0.1 to 0.5 ml/min.

Convection may contribute to transport from interstitium to blood. This tends to decrease the transcapillary concentrations gradients of locally produced Ang I and II and to increase the gradient of arterially delivered Ang I and II, resulting in lower calculated levels of $CIIi_{IsfPt}$ and $CIIa_{IsfPt}$ and therefore higher values of CAT_1R_{CsPt} and CAT_2R_{CsPt}.

Values of k_{tel} in cardiac and adrenal tissue where similar to k_{tel} of the renal cortex.[17] As reviewed by Thomas *et al.*,[16] studies of a wide variety of cells, including transfected Chinese hamster cells, showed a half-time value for AT_1 receptor-mediated Ang II endocytosis ranging from 2 to 10 min, corresponding with k_{int} ranging from 0.35 to $0.07\,min^{-1}$. We chose $0.35\,min^{-1}$; lower values will lead to higher calculated values of λ, and therefore to higher

calculated AT_1 receptor concentrations in the Glom and Tb (see Eqs. (44)–(46)).

Calculations presented in Figures 4–7 made use of data obtained in animals treated with an ACE inhibitor or AT_1 receptor antagonist as well as in control animals. Renal plasma flow (Q) and filtration fraction (FF) are dealt with in our model as being constant, whereas in reality ACE inhibitors and AT_1 receptor antagonists can affect these parameters. The effects on Q and FF that are to be expected, however, are small as compared with the effects on Ang I and II levels, so that our approach appears justified.

Our calculations were made on the assumption that paracrine Ang II (Ang IIi) in the kidney is mainly confined to the tubular and interstitial tissue compartments. Evidence to support the presence of renin and Ang II in juxtaglomerular cells has been reported,[31] but it is not known whether this Ang II is derived from intrarenal production or from blood plasma.

In this context, it is important to note that the practical usefulness of our model does not critically depend on *a priori* quantitative information about plasma and tissue concentrations of paracrine Ang II. Without such information, the model can still be used for calculating the local receptor concentrations and the levels of endocrine and paracrine Ang II they actually 'see', namely when the quantitative distribution of arterially delivered Ang II (AngIIa) over the glomerular and peritubular structures is known. Such information can be provided by more detailed *in vivo* studies using systemically or locally (intra-arterially) administered radiolabeled Ang II, supplemented by *in vitro* studies of the kinetics of binding of Ang II to cell surface AT_1 and AT_2 receptors and its intracellular trafficking. The model predicts that arterially delivered Ang II mainly binds to cell surface AT_1 receptors in the glomerular tissue region and that the concentration of these receptors in glomerular interstitial fluid is more than two orders of magnitude higher than K_d. This is in accordance with autoradiographic studies demonstrating that, in rat renal cortex, arterially delivered ^{125}I-Ang II is mainly confined to the Glom.[32] This finding, combined with the high concentration of glomerular AT_1 receptors predicted by the model, may well explain why glomerular hemodynamics and, as a consequence, renal sodium handling, are so responsive to low-dose Ang II infusions. The model further predicts that AT_1 receptors in the peritubular tissue region are exposed to paracrine rather than endocrine Ang II, and that the concentration of cell surface AT_1 receptors in peritubular interstitial fluid is probably high, that is $>10K_d$.

It is often believed that paracrine secretion serves to establish a high local hormone concentration. However, a high rate of hormone–receptor complex formation can be achieved when the concentration of free hormone is low, provided the local receptor concentration is high. In fact, binding of paracrine hormone to functional receptors and subsequent internalization of the receptor–hormone complex can function as a trapping mechanism to prevent leakage of hormone into the circulation. Under such conditions, there is no need for postulating other mechanisms, such as local extracellular enzymatic degradation, to prevent the escape of hormone from its local environment.

Experimental data to validate the model are as yet incomplete, but the model can serve as a framework for further studies to obtain detailed information on the local concentrations of AT_1 and AT_2 receptors and on the concentrations of endocrine and paracrine Ang II they actually 'see'.

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