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BRIEF REVIEW

RENOMEDULLARY INTERSTITIAL CELLS: A TARGET FOR ENDOCRINE AND PARACRINE ACTIONS OF VASOACTIVE PEPTIDES IN THE RENAL MEDULLA

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SUMMARY

1. The renal medulla plays an important role in regulating body sodium and fluid balance and blood pressure homeostasis through its unique structural relationships and interactions between renomedullary interstitial cells (RMIC), renal tubules and medullary vasculature.

2. Several endocrine and/or paracrine factors, including angiotensin (Ang)II, endothelin (ET), bradykinin (BK), atrial natriuretic peptide (ANP) and vasopressin (AVP), are implicated in the regulation of renal medullary function and blood pressure by acting on RMIC, tubules and medullary blood vessels.

3. Renomedullary interstitial cells express multiple vasoactive peptide receptors (AT_1, ET_A, ET_B, BK B_2, NPR_A and NPR_B and V_{1a}) in culture and in tissue.

4. In cultured RMIC, AngII, ET, BK, ANP and AVP act on their respective receptors to induce various cellular responses, including contraction, prostaglandin synthesis, cell proliferation and/or extracellular matrix synthesis.

5. Infusion of vasoactive peptides or their antagonists systemically or directly into the medullary interstitium modulates medullary blood flow, sodium excretion and urine osmolarity.

6. Overall, expression of multiple vasoactive peptide receptors in RMIC, which respond to various vasoactive peptides and paracrine factors *in vitro* and *in vivo*, supports the hypothesis that RMIC may be an important paracrine target of various vasoactive peptides in the regulation of renal medullary function and long-term blood pressure homeostasis.

Key words: angiotensin II, bradykinin, endothelin, hypertension, renomedullary interstitial cell, vasopressin.

INTRODUCTION

The roles of the renal medulla in the regulation of arterial blood pressure and body fluid and sodium balance have recently attracted intense investigation. Several reasons account for this growth of interest in the renal medulla and hypertension research. First, there is increasing evidence that abnormalities in the pressure–natriuresis relationship and renal medullary microcirculation may lead to the development and maintenance of hypertension in humans and experimental animals.[1,2] Second, many circulating humoral factors and locally produced paracrine and/or autocrine substances can modulate renal medullary blood flow and urinary sodium and water excretion when administered directly into the medullary interstitium.[1] Third, the receptors and receptor mRNA expression for different vasoactive peptides have been localized in medullary structures, including vasa recta bundles, tubules and renomedullary interstitial cells (RMIC).[3–7] These studies suggest that interactions of different renal medullary structures with vasoactive peptides or paracrine factors may play important roles in long-term blood pressure control and body sodium and fluid balance.

Although the medullary microvasculature and tubules are important in the role of renal medulla in the regulation of blood pressure and body sodium and fluid homeostasis, increasing evidence suggests that RMIC are potential endocrine and paracrine targets of actions of different vasoactive peptides or paracrine substances in the renal medulla. Recent *in vivo* microdialysis experiments have shown that the interstitial fluid contains numerous endocrine and local paracrine substances or autacoids, including angiotensin (Ang)II,[8] bradykinin (BK)[9] and nitric oxide (NO).[10] Renomedullary interstitial cells appear to be capable of synthesizing and releasing a number of autacoids, or vasoactive agents, such as medullipins[11] and prostaglandins,[12] which have potent antihypertensive activity. Furthermore, RMIC express multiple receptors for vasoactive peptide AngII (AT_1),[13] atrial natriuretic peptide (ANP; NPR_A and NPR_B),[5] endothelin (ET)-1 (ET_A and ET_B)[4,14] and BK B_2 receptors.[15] Exposure of RMIC *in vitro* to AngII,[16] ET-1[4,17] or BK elicits a series of biological responses, including increases in intracellular inositol 1,4,5-trisphosphate (IP_3) and calcium concentrations, cell contraction and enhanced DNA synthesis (Table 1). These observations suggest that vasoactive peptides may act on RMIC to exert paracrine influences on sodium and fluid balance, renal medullary microcirculation and urine concentration processes.

The purpose of the present brief review is to provide an overview of the cellular localization and functional roles of several vasoactive peptide receptors in RMIC in the renal medulla. This information may be important for our understanding of how RMIC interact with various vasoactive peptides or paracrine factors in the regulation of long-term blood pressure control and renal medullary function.

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Table 1 Expression and biological actions of vasoactive peptide receptors in cultured renomedullary interstitial cells *in vitro*

Receptors	Responses	Reference
AT_{1A}	↑ PGE_2	12
	↑ $[IP_3]_i$ and $[Ca^{2+}]_i$	16
	↑ DNA synthesis	16
	↑ ECM accumulation	16
ET_A	↑↓ $[Ca^{2+}]_i$, ↑ $[IP_3]_i$	4, 45
	↑ Cell contraction	17
	↑ PGE_2 synthesis	4
	↑ DNA synthesis	45
	↑ ECM accumulation	45
BK_2	↑ $[IP_3]_i$	58
	↑ cAMP & cGMP	58
	↑ DNA synthesis	58
	↑ ECM accumulation	58
	↑ PGE_2 synthesis	12
NPR_A and NPR_B	↑ cGMP	5
	↓ ET-stimulated DNA synthesis	45
AVP (V_{1a})	↑ Cell contraction	17
	↑ PGE_2 synthesis	12

PGE_2, prostaglandin E_2; IP_3, inositol 1,4,5-trisphosphate; ECM, extracellular matrix; ET, endothelin; ↑, increase; ↓, decrease.

STRUCTURAL AND FUNCTIONAL CHARACTERISTICS OF RMIC

Renomedullary interstitial cells, as described in this article, specifically refer to the cells formerly known as type I RMIC[18] or renal medullary fibroblasts, as described recently.[19] These cells are both morphologically and functionally distinct from two other cell types, macrophages and dendritic cells, in the renal medullary interstitium.[19] In the renal medulla, RMIC characteristically form ladder-like structural associations with the long axis of medullary blood vessels and the thin limb of the loop of Henle.[18,19] Through these unique arrangements, these cells have extensive cellular contacts, via their prominent cytoplasmic processes, with basal membranes of vasa recta and the loop of Henle.[18] Thus, RMIC are directly exposed to the interstitial fluid, which contains numerous humoral and paracrine factors. Although these cells are not directly accessible to medullary blood flow, circulating humoral and local vascular and tubular paracrine factors could reach RMIC through the fenestrated endothelium of the ascending vasa recta.[18,20] Whether RMIC are also associated with collecting ducts remains controversial. In a recent imaging study of RMIC *in situ* by confocal fluorescence microscopy, detailed examination of three-dimensional reconstruction of RMIC was unable to demonstrate direct associations between RMIC and collecting ducts.[21]

The ultrastructure of RMIC in the rat kidney is well studied at the electron microscopic level in tissue as well as in cultured cells. Electron micrographs show that these cells have multiple cytoplasmic projections, which contain microtubules and microfilament bundles.[18,19] Through these projections, one RMIC may have extensive contact with several RMIC, renal tubules or vasa recta bundles. Renomedullary interstitial cells are also rich in numerous cellular organelles, including mitochondria, cisternae of rough endoplasmic reticulum, free ribosomes, polysomes and lysosomes. However, the most consistent and characteristic feature of RMIC is numerous heterogeneous lipid droplets in the cytoplasm and, for this reason, these cells are also dubbed 'lipid-laden interstitial cells'.[18] The lipid droplets in RMIC have been suggested to be the source of the production and release of antihypertensive substances, such as prostaglandins,[12] medullipin[11,22] or other unidentified factors.[23]

LOCALIZATION AND ROLE OF AT_1 RECEPTOR EXPRESSION IN RMIC

Cellular localization and *in vivo* regulation of AT_1 receptors in RMIC

Angiotensin AT_1 receptors are the most extensively studied vasoactive peptide receptors in RMIC. The evidence that AngII receptors may occur on RMIC was first provided by Zusman and Keiser, who demonstrated that AngII stimulated prostaglandin biosynthesis in cultured rabbit RMIC.[12] High-affinity AngII receptor-binding sites were subsequently identified in cultured rabbit RMIC.[24] However, whether AngII receptors were present in RMIC in the renal medulla *in vivo* was not investigated by these early studies. To localize AT_1 receptors in the renal medulla at the cellular level, our group developed high-resolution electron microscopic autoradiography.[3] Using this approach, a high density of AT_1 receptor binding was observed in the inner stripe, where silver grains appeared between the renal tubules and the vasa recta bundles. Electron micrographs further showed that AT_1 binding occurred primarily on RMIC situated between the vasa recta and the loop of Henle (Fig. 1b).[3] These AT_1 receptor-bearing interstitial cells display morphological characteristics similar to the well-described RMIC.[18] Angiotensin AT_1 receptors were subsequently confirmed in cultured rat RMIC[25] and are of the AT_{1A} subtype.[16]

Angiotensin AT_1 receptors in RMIC are regulated in a similar manner to those in glomerular mesangial cells during altered activities of the renin–angiotensin system (RAS).[26] High sodium intake and angiotensin-converting enzyme (ACE) inhibitors have been shown to increase AT_1 receptors in RMIC,[27] whereas infusion of exogenous AngII tends to decrease AT_1 receptors in these cells in rats with AngII-dependent hypertension (JL Zhuo, unpubl. obs., 1999). Moreover, *in vivo* occupancy of AT_1 receptors by both circulating and locally formed AngII also contributes to AT_1 receptor regulation in RMIC. For example, AT_1 receptor binding sites in the inner stripe of the outer medulla are poorly labelled by radioligands following systemic delivery *in vivo*.[28] This lack of AT_1 receptor labelling in RMIC of the inner stripe can be reversed by treating rats with a high-sodium diet or ACE inhibitors to reduce endogenous AngII formation.[27] Indeed, AngII levels in the renal medulla have been reported to be approximately four-fold greater than cortical AngII content in the rat kidney.[29] Although the physiological significance of this *in vivo* occupancy of AT_1 receptors in RMIC is not known, these findings suggest that high occupancy of AT_1 receptor binding sites on RMIC by endogenous interstitial AngII may play an important role in the regulation of renal medullary haemodynamics, sodium excretion and urine osmolarity.

In vitro effects of AngII on cultured RMIC

Although RMIC have been suggested to exert a variety of actions in the renal medulla, their precise role is far from well understood. In the medulla, RMIC are situated in the medullary interstitium between and anchored closely into the basement membranes of the

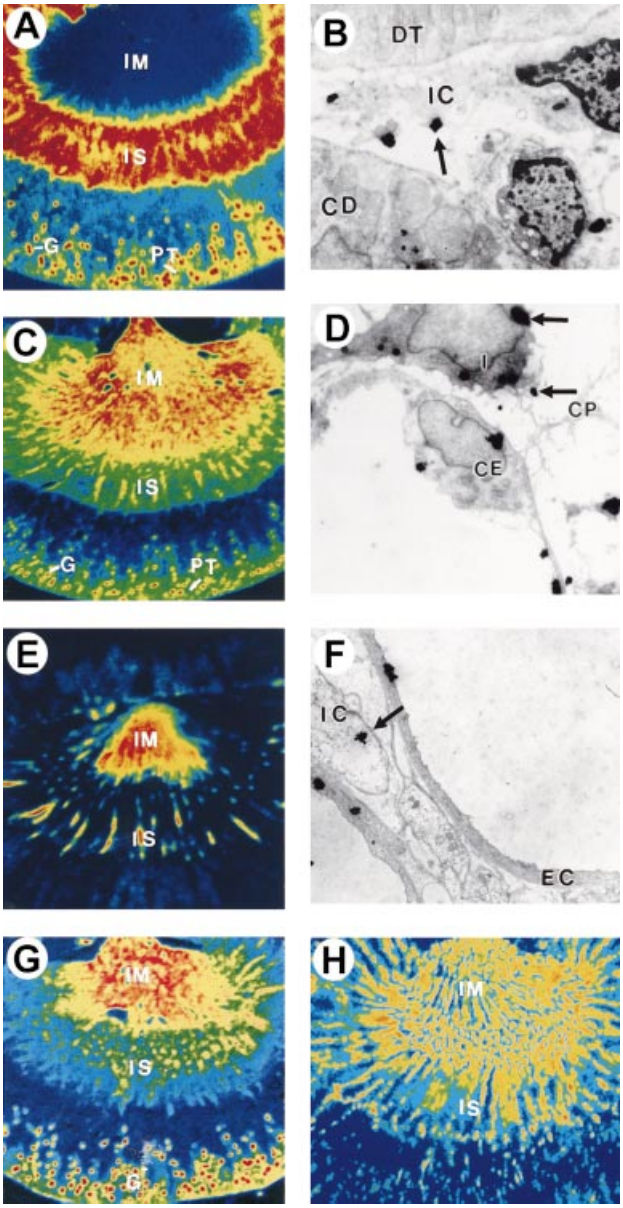

Fig. 1 Autoradiographic mapping of (a,b) angiotensin II AT_1, (c,d) endothelin ET_B, (e,f) bradykinin B_2, (g) atrial natriuretic peptide B type and (h) vasopressin receptors in the rat kidney. Red represents the highest density of receptor binding and blue shows the background level. IM, inner medulla; IS, inner stripe of the outer medulla; PT, proximal tubule; G, glomerulus; DT, distal tubule; IC, interstitial cell; CD, collecting duct; I, interstitial cell; CP, interstitial cell process; CE, capillary endothelial cell; EC, endothelial cell. Data are derived from Zhuo *et al.*[3,13] and Dean *et al.*[14]

loop of Henle and vasa recta blood vessels.[18,20] This unique arrangement renders it difficult to study directly the physiological roles of these cells *in vivo*. Cultured RMIC *in vitro* provide an alternative approach for examining the paracrine and/or autocrine effects of various vasoactive peptides on these cells and the possible mechanisms involved.

Studies on cultured RMIC *in vitro* have suggested that interactions between AngII and RMIC may exert several important influences in the kidney in a similar manner to that between AngII and mesangial cells (Table 1).[30] Like mesangial cells, in which AT_{1A} receptors are expressed and AngII stimulates protein synthesis,[31] AngII also acts on AT_{1A} receptors to increase [^{3}H]-thymidine incorporation and

Table 2 Effects of vasoactive peptides or their inhibitors on renal medullary function *in vivo*

Vasoactive agents	Routes	Effects	Reference
AngII	i.v.	↓ MBF	36
	i.v.	↔ MBF	2
Captopril	i.t.	↑MBF, ↓BP	35
		↓Urine osmolarity	35
Losartan	i.v.	↑ MBF	37
ET-1	i.v.	↑↓MBF	53, 52
BK	i.t.	↑ MBF	60, 61
AVP	i.t.	↓ MBF	71

AngII, angiotensin II; ET-1, endothelin-1; BK, bradykinin; AVP, vasopressin; i.v., intravenous infusion; i.t., medullary interstitial infusion; MBF, medullary blood flow; BP, blood pressure; ↑, increase; ↓, decrease. ↔, unchanged.

induce extracellular matrix (ECM) accumulation.[16] Although AngII has no effect on either basal or forskolin-stimulated adenosine cAMP accumulation, it significantly increases intracellular IP_3 and Ca^{2+} concentrations.[16] These proliferative effects of AngII on RMIC *in vitro* may be both physiologically important in maintaining normal structural arrangements in the renal medulla and in the pathogenesis of progressive renal disease.

Interactions between AngII and RMIC observed *in vitro* may play an important role in the antihypertensive actions of RMIC. It is well documented that AngII stimulates prostaglandin synthesis in cultured RMIC.[12,24] Prostaglandin is a potent renal vasodilator and causes diuresis and natriuresis in the kidney. Renomedullary interstitial cells are also the major source of the medullipin system. The RMIC secrete medullipin I, which is activated in the liver into medullipin II.[11,22,32] Medullipin II, a vasodilator, exerts various actions that are opposite of the major actions of AngII. Release of prostaglandin and/or medullipins from RMIC by AngII *in vivo* may serve to counter the vasoconstrictive actions of AngII in the renal medulla.

In vivo effects of intrarenally formed interstitial AngII in the renal medulla

The effects of AngII on the medullary microcirculation, sodium excretion and urine concentrating processes have been well studied,[1,2,33,34] but it is not known whether these effects are partially mediated by activation of AT_1 receptors in RMIC *in vivo*. There is indirect evidence that suggests that interactions of AngII and AT_1 receptors in RMIC may be important in the regulation of the renal medullary microcirculation. For example, infusion of the ACE inhibitor captopril directly into renal medullary interstitium selectively increased medullary blood flow, induced hypotension and marked diuresis and natriuresis and reduced urine osmolarity in spontaneously hypertensive rats (Table 2).[35] Systemic infusion of exogenous AngII decreased medullary blood flow in parallel with the cortical blood flow.[36] In contrast, *in vivo* blockade of intrarenal AT_1 receptors by losartan resulted in similar increases in both cortical and medullary blood flows in *ren*-2 transgenic hypertensive rats, in which AT_1 receptors are increased in the cortex and inner stripe of the outer medulla (Fig. 2c,d).[37] Because systemically administered AngII or losartan can reach AT_1 receptor binding sites on RMIC in the inner stripe of the outer medulla (Fig. 2a,b),[27,38] *in vivo* effects of losartan may be partially mediated through actions on RMIC and medullary blood vessels. These *in vivo* observations are also supported by *in vitro* studies in which topically applied AngII caused

potent vasoconstriction and a decrease in vasa recta blood flow in rats.[39,40] These studies suggest that circulating AngII, as well as endogenous renal interstitial AngII, may induce vasoconstriction of medullary blood vessels, at least partly, through paracrine actions on RMIC in addition to direct actions on these vessels. However, caution must be taken to interpret the findings obtained from these *in vivo* studies due to the difficulty in discriminating between the effects of AngII on medullary blood vessels, tubules or RMIC.

LOCALIZATION AND ROLES OF ENDOTHELIN ET_A AND ET_B RECEPTORS IN RMIC

Cellular localization of endothelin receptors in RMIC

Polypeptide ET is one of the most potent vasoconstrictors described.[41] As in other tissues, this peptide acts on two different subtypes of ET-1 receptors, ET_A and ET_B, in the kidney. The ET-1 receptors are widely distributed in the glomeruli, blood vessels and renal tubules.[14,42–44] As visualized by *in vitro* autoradiography, ET-1 receptors are most abundant in the inner medulla or papilla and also in longitudinal bands traversing the inner stripe, with moderate levels of binding throughout the entire inner stripe (Fig. 1c). Both ET_A and ET_B receptors occur in the rat kidney, with ET_B receptors predominating in the cortex and renal medulla.[14,44] At the electron microscopic level, ET_B receptors are localized to endothelial cells of peritubular capillaries and the vasa recta and to RMIC in the inner stripe of the outer medulla and the papilla.[14] The ET-1 receptor binding on RMIC is partially inhibited by both the ET_A receptor antagonist BQ 123 and the ET_B receptor agonist sarafotoxin 6c (S6c), therefore indicating that rat RMIC express both ET_A and ET_B receptors *in vivo* (Fig. 1d).[14] These observations have been confirmed in a similar study using high-resolution electron microscopic autoradiography but with different ET-1 receptor subtype antagonists, namely IRL 1620 (ET_B receptor antagonist) and 97 139 (ET_A receptor antagonist).[44]

Wilkes *et al.* first showed that cultured rat RMIC possess functional ET-1 receptors, which were potently inhibited by sarafotoxin, an ET_B-type receptor agonist, with an IC_{50} of approximately 10 nmol/L.[4] However, this study did not use subtype-specific ET-1 receptor antagonists to discriminate between ET_A and ET_B receptors. A recent reverse transcription–polymerase chain reaction (RT-PCR)

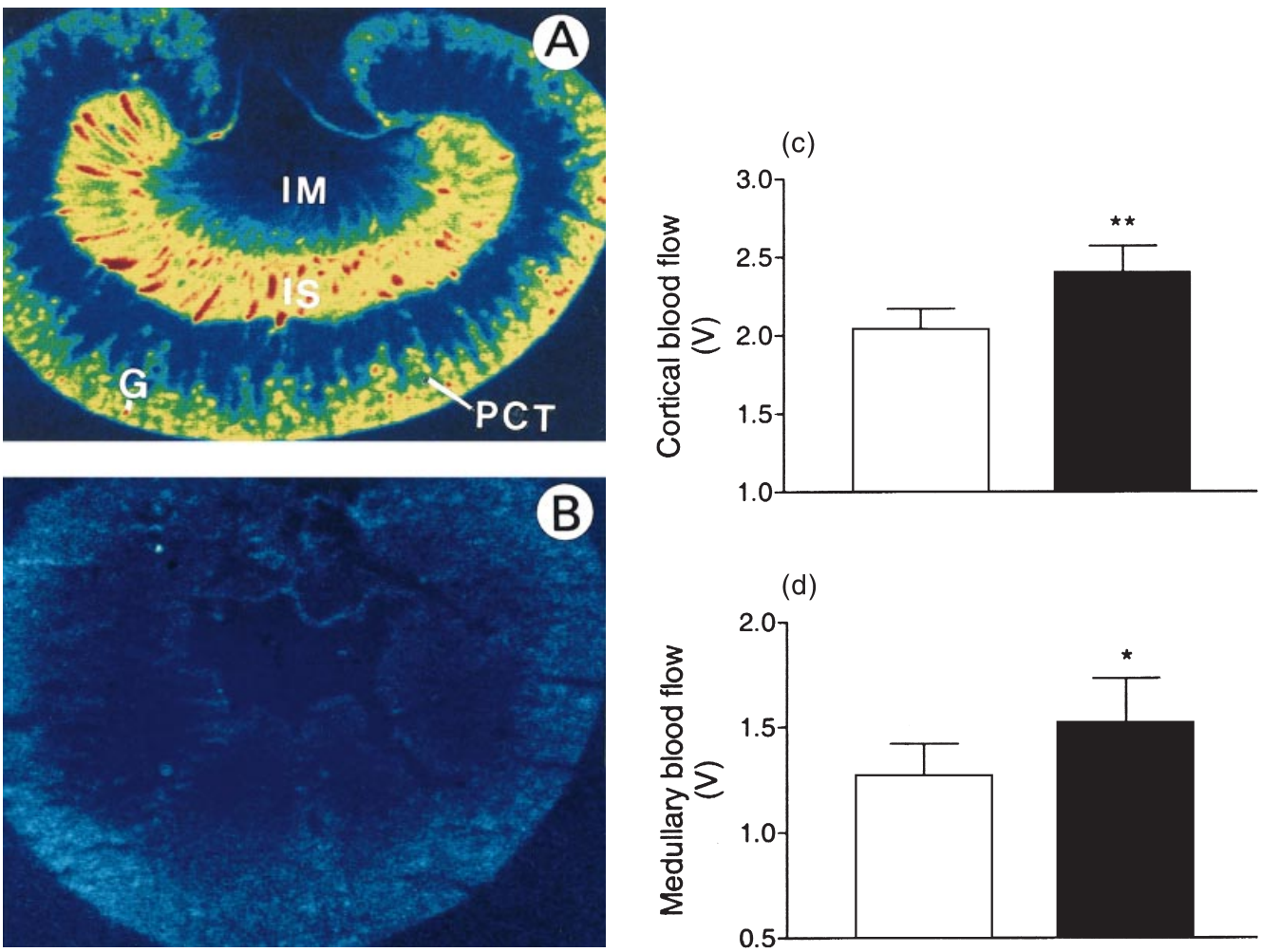

Fig. 2 (a,b) *In vivo* effects of the AT_1 receptor antagonist losartan on AT_1 receptor binding ((a) control; (b) losartan treated) in the cortex and inner stripe of the outer medulla and on renal (c) cortical and (d) medullary blood flows (□, control; ■, losartan treated) in *ren*-2 transgenic hypertensive rat kidney. Data are derived from Zhuo *et al.*[27,37,38] Red represents the highest density of receptor binding and blue shows the background level. $*P < 0.05$, $**P < 0.01$ compared with control. IM, inner medulla; IS, inner stripe of the outer medulla; G, glomerulus; PCT, proximal convoluted tubule.

and Southern blot analysis on cultured rat RMIC detected a 478 b.p. band corresponding to the expected size of the ET_A receptor PCR product.[45] Interestingly, an ET_B receptor PCR product was not detected in cultured rat RMIC.[45] The finding observed in cultured rat RMIC *in vitro* is in contrast with autoradiographic studies in tissues, in which ET_A and ET_B receptors were colocalized in RMIC of the renal medulla.[14,44] The differences between these studies raise the possibility that ET_B receptor expression may have been suppressed under culture conditions *in vitro*.

Effects of ET-1 on RMIC *in vitro*

Intrarenal blood vessels and renal tubules have been shown to express mRNA for endothelin peptides[46] and endothelin-converting enzyme.[47] Therefore, endothelin peptides can be synthesized in, or released from, renal vessels and tubules and, subsequently, exert a paracrine influence on adjacent RMIC. Exposure of cultured RMIC to ET-1 produces a biphasic increase in cytosolic calcium concentration (Table 1).[4] This cellular response is associated with activation of phosphatidylinositol-specific phospholipase C (PI-PLC).[4] Endothelin-1 also markedly stimulates prostaglandin (PG)E_2 synthesis, which parallels activation of PI-PLC and intracellular calcium mobilization (Table 1). Furthermore, activation of phospholipase A_2[48] and phospholipase D[49] forms another important action of ET-1 in RMIC. These results suggest that ET-1 acts on ET-1 receptors in cultured rat RMIC and activates a series of signal transduction pathways to mediate cellular responses.

One of the major cellular actions of ET-1 on RMIC is to promote cell proliferation and ECM synthesis through interaction with the NO system (Table 1).[45] To define which ET-1 receptor subtype mediates cellular responses to ET-1 in cultured rat RMIC, Maric *et al*. examined the effects of ET-1 on RMIC cell proliferation and ECM synthesis.[45] In that study, ET-1 significantly increased intracellular IP_3 and calcium concentrations. Endothelin-1 stimulated intracellular cAMP and cGMP concentrations by interacting with the NO system. Moreover, ET-1 both directly, or indirectly through interaction with NO, stimulated [^{3}H]-thymidine incorporation and ECM synthesis.[45] These cell-proliferative effects of ET-1 were effectively abolished by the ET_A receptor antagonist BQ 123, while the ET_B receptor agonist S6c, or the ET_B receptor antagonist IRL 1038, was without effect.[45] These findings are consistent with the presence of functional ET_A receptors in cultured RMIC.

The other important biological action of ET-1 on cultured RMIC is that this vasoactive peptide induces a potent contractile response *in vitro* (Table 1).[17] Hughes *et al*. showed that ET-1 induced a dose-dependent reduction of cell surface area in RMIC accompanied by a marked increase in the number and intensity of F-actin microfilament staining. This contractile response was mediated by protein kinase C, but did not involve NO, cAMP or cGMP.[17] Thus, the intracellular signal transduction pathways associated with the contractile response are different from that thought to mediate ET-induced cell proliferation and ECM synthesis in cultured rat RMIC.[45] The physiological significance of the contractile response of RMIC to ET-1 *in vitro* remains to be elucidated. However, contraction of RMIC *in vivo* may be of importance in the regulation of urinary concentration and medullary blood flow.[17]

Effects of ET-1 in the renal medulla *in vivo*

Although the levels of endothelin peptides are higher in the renal medulla than in the cortex, the precise physiological levels of these peptides are uncertain.[46] Recent evidence suggests that the renal endothelin system may be implicated in normal development. For example, mice with ET-1 gene disruption show prominent craniofacial developmental abnormalities and have paradoxical elevation of blood pressure without other morphological abnormalities in the heart, lung, blood vessels and kidney.[50] An elevated basal blood pressure in the ET knock-out mice is highly unexpected, because ET-1 is considered the most potent vasoconstrictor.[41]

Animal studies on renal vascular and tubular responses to ET-1 or its antagonists *in vitro* and *in vivo* suggest that ET-1 may play a physiological role in the renal medulla. *In vitro*, ET-1 caused a potent vasoconstrictor response in the isolated outer medullary descending vasa recta of the rat and this action of ET-1 was markedly inhibited by PGE_2, a major paracrine factor synthesized in renal tubules and RMIC.[51] However, *in vivo* systemic administration of ET-1 in rats either transiently increased, or did not alter, medullary blood flow, while consistently reducing cortical blood flow (Table 2).[52,53] This medullary vasodilator response appears to be mediated by ET_B receptors, because a specific ET_B-receptor agonist (IRL 1620) caused similar vasodilatation in the renal medulla. The increase in medullary blood flow in response to ET-1 may also depend on activation of the renal NO system in the medulla, because inhibition of NO synthase (NOS) abolished this ET_B receptor-mediated effect.[52] In contrast, the ET_A-receptor antagonist BQ 123 was without any effect, although it effectively blocked the ET-1-induced cortical vasoconstriction.[52] The results of this study are consistent with results of receptor mapping studies that show that ET_B receptors predominate in both the cortex and medulla of the rat kidney.[13,14,44] Because ET-1 production may be increased in the kidney in acute and chronic renal diseases, the interactions between renal endothelin peptides and the NO/PGE_2 system, which is mediated by ET_B receptors, may provide a protective effect in the medulla against ischaemia caused by ET_A receptor-mediated vasoconstriction.

LOCALIZATION AND ROLES OF BK B_2 RECEPTORS IN RMIC

Cellular localization of B_2 receptors

Bradykinin is the major effector molecule of the kallikrein–kinin system. The kallikrein–kinin system is considered an important intrarenal paracrine and autocrine system in the regulation of renal medullary function.[2,54] Although many renal structures express mRNA for the kallikrein–kinin system, BK is mainly produced in the tubular epithelium, where it may play a dual role: autocrine influence in the tubules and a paracrine role in the adjacent renal vasculature and interstitial cells.[55] High levels of BK have been reported in the renal interstitial fluid to which the interstitial cells are directly exposed.[9]

Bradykinin elicits biological actions by acting on two subtypes of BK receptors, namely B_1 and B_2. The B_1 receptors are involved in tissue inflammatory processes and play little role in physiological control of renal function. The B_2 receptors are G-protein-coupled receptors and appear to mediate the known renal haemodynamic and tubular effects of BK *in vivo* and *in vitro*.[56]

Cellular localization of B_2 receptors in the renal medulla is important for understanding the roles of BK in the renal medulla. Manning and Snyder, in their autoradiographic studies, suggested that BK receptors occur in the renal interstitium between the collecting ducts.[57] The presence of BK receptors in RMIC was initially sug-

gested by early studies on cultured rabbit RMIC.[12] In cultured rabbit RMIC, BK potently stimulated prostaglandin synthesis along with AngII and AVP.[12] Subsequently, specific B_2 receptor-binding sites were identified in cultured rat RMIC by radioreceptor assays.[6] However, whether B_2 receptors occur in RMIC of the renal medulla was not determined in those early studies.

Using the radioligand [^{125}I]-HPP-HOE 140, a specific B_2 receptor antagonist, our group have identified B_2 receptor-binding sites in the rat kidney using both *in vitro* and *in vivo* labelling techniques.[15,58] In the medulla, B_2 receptor binding occurs in high density throughout the inner medulla and in longitudinal bands traversing the inner stripe of the outer medulla (Fig. 1e). Bradykinin B_2 receptor binding is not readily detectable in the outer stripe of the outer medulla or in the entire cortex.[15] High levels of B_2 receptor binding occur widely over the renal tubules, vasa recta bundles and interstitium in the inner stripe of the outer medulla and the papilla. At higher magnification, silver grains are localized to tubular cells of thin limbs of the loop of Henle, distal tubules and collecting ducts and endothelial cells of peritubular capillaries. Both B_2 receptor binding sites and mRNA are also present in RMIC (Fig. 1f).[15,58] Taken together, these studies provide evidence that RMIC in the renal medulla express B_2 receptors.

In vitro effects of bradykinin in RMIC

Although the haemodynamic and tubular effects of BK in the kidney have been studied extensively in animals, the biological actions of BK on cultured RMIC *in vitro* and its receptor subtypes involved are not fully understood. Bradykinin is considered an important intrarenal paracrine and/or autocrine factor, which causes vasodilatation, diuresis and natriuresis and lowers urine osmolarity.[2,58,59] In cultured RMIC, BK significantly increased cAMP and cGMP levels, but the latter response appears to be dependent on the production of NO.[58] Interestingly, BK also stimulated [^{3}H]-thymidine incorporation and ECM synthesis, which was mediated by the B_2 receptors (Table 1).[58] This proliferative effect of BK in cultured RMIC *in vitro* is unexpected, because actions of the vasodilator BK usually contrast with those of vasoconstrictors, including AngII and ET-1, *in vivo*. Furthermore, RMIC appear to respond to BK to increase prostaglandin synthesis *in vitro* (Table 1).[12] The presence and expression of B_2 receptor in RMIC and demonstration of biological responses to BK in RMIC in culture supports an important paracrine role for BK in the renal medulla through B_2 receptors.

In vivo effects of BK in the renal medulla

It is generally agreed that BK exerts important physiological influences on medullary haemodynamics and tubular function. In rats with volume expansion, papillary blood flow was significantly increased and B_2 receptor blockade effectively prevented the rise of papillary blood flow following volume expansion with saline.[60] Bradykinin, acting via B_2 receptors, also appears to modulate renal medullary haemodynamic responses to systemic ACE inhibition or AngII infusion in anaesthetized rats.[2,61] When BK was infused systemically or directly into the renal medullary interstitium, it increased renal papillary blood flow, as measured by laser-Doppler flowmetry and enhanced sodium excretion without altering total renal blood flow and glomerular filtration rate (Table 2).[60,61] Because the increase in papillary blood flow caused by interstitial infusion of BK can be blocked by pretreatment of the rats with an NOS inhibitor, BK may regulate the renal medullary function through a NO-dependent mechanism.[58] Interactions between BK with AngII and NO may influence renal medullary microcirculation and tubular function *in vivo*.

OTHER VASOACTIVE PEPTIDE RECEPTORS IN RMIC

Atrial natriuretic peptide

In addition to the above-described vasoactive peptide receptors, there is evidence that RMIC also contain other vasoactive peptide receptors. Atrial natriuretic peptide is a cardiac hormone initially isolated from the atrial tissue, but it is also found in different structures of the kidney.[62] In the kidney, ANP induces marked glomerular hyperfiltration and pronounced natriuresis through its haemodynamic and tubular actions.[62,63] Two distinct classes of ANP receptors have been cloned, B-ANP and C-ANP.[64,65] The B-ANP receptors are subclassified further into two subtypes, namely NPR_A and NPR_B; both are coupled to cGMP and mediate biological responses to ANP in tissues.[62] The C-ANP (NPR_C) receptors, which are dubbed 'clearance receptors', do not mediate the known biological responses to ANP in tissues.[66]

Autoradiographic studies show that the distribution of ANP receptors overlaps with those of AT_1 receptors and ET-1 receptors in the glomeruli and the inner stripe of the outer medulla (Fig. 1g).[67] However, like ET-1 receptors, high levels of ANP receptors also occur in the inner medulla, although the precise cellular distribution of ANP receptors in the medulla remains inconclusive. The B-type ANP receptors have been identified in cultured rat RMIC with high density (approximately 23 000 sites/cell) and high affinity (K_d = 50 pmol/L).[5] Exposure of these cells to ANP *in vitro* resulted in a marked increase in cGMP in the culture medium. Co-administration of sodium nitroprusside, a NO donor, with ANP *in vitro* further increased cGMP, suggesting an interaction between ANP and NO in RMIC (Table 1).[5]

Roles of ANP in the regulation of renal medullary function are not fully studied. Atrial natriuretic peptide has been shown to increase medullary and/or vasa recta blood flow in rats,[68] but it is not clear whether RMIC are involved. Interestingly, mice with a disrupted ANP gene[69] or NPR_A receptor gene[70] develop salt-sensitive hypertension. These studies suggest that the vasorelaxant and natriuretic effects of ANP may be important in maintaining normal body salt and fluid balance and arterial blood pressure.

Vasopressin

The neurohypophyseal hormone AVP is another vasoactive peptide known to play an important physiological role in the regulation of renal medullary haemodynamics and urinary concentrating processes.[71] Vasopressin interacts with three specific receptors, V_{1a}, V_{1b} and V_2, to elicit a variety of biological actions in tissues. There is evidence that AVP may be produced intrarenally and, subsequently, exerts a paracrine role in the kidney.[72] In the kidney, AVP receptors occur predominantly in longitudinal bands traversing the inner stripe of the outer medulla and in the papilla (Fig. 1h). The V_2 receptors are mainly localized in collecting duct tubules, whereas V_{1a} recep-

tors are expressed predominantly in medullary blood vessels.[7] The V_2 receptors mediate the classic antidiuretic effect of AVP, whereas V_{1a} receptors modulate vascular effects of this peptide. Although AVP receptors have not been characterized in RMIC *in vivo* and *in vitro*, autoradiographic studies using [^{3}H]-AVP or [^{3}H]-SR 49059, a specific V_{1a} receptor antagonist, show that V_{1a} receptors are localized in the outer part of the inner medulla and the inner stripe of the outer medulla.[73] In the inner stripe of the outer medulla, V_{1a} receptors occur in the vasa recta bundles as well as in RMIC, a pattern of distribution overlapping with those of AT_1, ET_B and B_2 receptors.[13] In cultured rabbit RMIC, AVP stimulates PGE_2 synthesis and this effect can be inhibited by indomethacin, a prostaglandin synthesis inhibitor.[12] Recently, AVP was reported to induce cell contraction in cultured rat RMIC, characterized by a reduction of cell surface area and an increase in F-actin microfilment staining (Table 1).[17] These responses are mediated by V_{1a} receptors in RMIC. The V_{1a} receptors also mediate AVP-induced vasoconstriction of outer medullary descending vasa recta in the rat kidney[74] or reduction of medullary blood flow *in vivo* after medullary interstitial administration (Table 2).

FUTURE PERSPECTIVES

There is increasing evidence that supports the hypothesis that RMIC are an important endocrine and paracrine target of actions of vasoactive peptides in the renal medulla. Renomedullary interstitial cells are strategically situated in such a unique position in the renal medulla that allows these cells to be in close contact with adjacent tubules and vasa recta bundles. Renomedullary interstitial cells are also exposed to the interstitial fluid, which contains a variety of circulating hormones and locally produced vasoactive paracrine or autocrine substances derived from blood vessels, tubules and interstitium, such as AngII, ET-1, BK, prostaglandins and NO. Expression of multiple vasoactive peptide receptors in RMIC enables these cells to respond to various vasoactive peptides *in vitro* and *in vivo*. Although the consequences of biological interactions between vasoactive peptides and RMIC *in vivo* remain to be elucidated, attractive possibilities may include cell contraction, proliferation, ECM synthesis and release of paracrine or autocrine factors, such as prostaglandins, medullipins and NO. These vasoactive peptide-induced cellular responses may directly or indirectly influence the renal medullary microcirculation and urinary concentrating processes or tubular function. New experimental approaches and molecular biology techniques are now available to study the roles of RMIC. For example, chronically implanted optic fibres and laser-Doppler flowmetry can be used to monitor physiological responses of renal medullary blood flow to interstitial administration of vasoactive peptides or their receptor antagonists in conscious animals with or without genetic disruption of specific vasoactive peptide receptors. Renomedullary interstitial cells can be cultured from mice with overexpression or deletion of specific vasoactive peptide receptors for studies of their cellular responses to various stimulations or inhibitions *in vitro*. These new studies will provide important information on how RMIC interact with different circulating vasoactive peptides, paracrine or autocrine factors to regulate renal medullary microcirculation, urine concentrating processes and long-term blood pressure homeostasis.

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