Published in final edited form as:

J Hypertens. 2006 June ; 24(6): 1017–1020.

Intracrine renin and angiotensin II: a novel role in cardiovascular and renal cellular regulation

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Since renin was discovered by Robert Tigerstedt more than a century ago, interest in the renin–angiotensin system (RAS) remains stronger than ever [1]. Tigerstedt injected saline extracts prepared from a rabbit kidney into other rabbits and observed marked increases in arterial blood pressure, which led to the hypothesis that the kidney might release an endocrine substance to control systemic blood pressure [1]. This pressor substance was named renin by Tigerstedt. It was Harry Goldblatt who confirmed and extended Tigerstedt's discovery of the RAS in his legendary studies on two-kidney, one-clip renal hypertension in 1934 [2]. For several decades, angiotensin II was known as an endocrine peptide that is formed by the actions of kidney-derived renin and lung-derived angiotensin-converting enzyme (ACE) and causes systemic hypertension. The RAS is now well recognized as a dual vasoactive system, acting as both a circulating endocrine system and a local tissue paracrine/autocrine system [3,4]. Re further defined intracrine angiotensin II as the effector peptide that is either synthesized within a cell via interactions of renin, angiotensinogen and ACE or internalized from extracellular angiotensin II [5]. Most or all major components of the RAS cascade, including angiotensinogen, renin, ACE and angiotensin II receptors (AT₁ or AT₂), have been demonstrated in the heart, adrenal glands, brain and blood vessels [6,7]. Renin, the rate-limiting enzyme in the cascade, acts on the hepatocyte-synthesized substrate angiotensinogen to form angiotensin I, which is converted to angiotensin II by ACE. Angiotensin II is further metabolized by aminopeptidases into the active fragments angiotensin III and/or angiotensin IV. The principal effector molecule of the RAS cascade is angiotensin II, although other angiotensin fragments such as angiotensin III, angiotensin 1–7 and angiotensin IV have been shown to be biologically active [6]. The presence of renin, angiotensinogen and ACE in many tissues makes local formation of angiotensin II possible, whereas expression of angiotensin II receptors is essential for angiotensin II to induce biological effects at target sites. Two major classes of angiotensin II receptors are expressed in the heart, kidney, brain, adrenals and blood vessels, AT₁ and AT₂ [6,7]. AT₁ is a G protein-coupled receptor belonging to the superfamily of seven transmembrane-spanning proteins. Two subtypes of AT₁, designated AT_{1A} and AT_{1B}, have been identified in rodents, but the former is the predominant isoform in the kidney, blood vessels, and heart [6]. Although the pressor effect of angiotensin II is historically considered its primary action, it also stimulates aldosterone synthesis, promotes body salt and fluid retention, and induces cellular growth and proliferation in cardiovascular and renal tissues [4,6]. Most of the known actions of angiotensin II are mediated by the AT₁ receptor, which is coupled to multiple signaling pathways, including phospholipase C, D, and A₂ signaling, mitogen-activated protein kinase, and tyrosine kinase. Activation of AT₁ leads to phosphoinositide hydrolysis, mobilization of intracellular calcium, and inhibition of adenylate cyclase [6–8].

The classic view of RAS-mediated actions is that extracellular angiotensin II binds its receptors at the plasma membrane, and phosphorylation of the receptor activates downstream signaling and induces intracellular responses [6]. Increasing evidence suggests, however, that binding of angiotensin II to its membrane AT₁ receptors also initiates endocytotic (or internalization) processes that promote trafficking of both the effector and the receptor into intracellular compartments, where interaction of angiotensin II with its receptors may induce intracellular signaling with consequent biological effects [5,6,8,9]. Moreover, there is evidence that renin may be synthesized within or taken up by extrarenal cells, where it may generate angiotensin II intracellularly [10]. Whether internalized or intracellular, renin and/or angiotensin II have important intracrine actions and/or signaling is poorly understood [5]. For example, although the concept of intracellular or intracrine angiotensin II was introduced decades ago, a careful MEDLINE search yielded only dozens of citations specifically on intracellular angiotensin II and its receptors, compared with more than 30 000 citations for extracellular angiotensin II and/or cell surface receptors. There is clearly a huge gap in our understanding of the biological significance of intracrine or intracellular angiotensin II in cardiovascular and renal physiology and disease.

Against this background, the study by De Mello in the present issue of *Journal of Hypertension* represents a new effort focusing on the potential role of intracellular renin and angiotensinogen in the regulation of cell-to-cell communication in the hamster heart [11]. Historically, it has been difficult to study the role of intracellular renin because it is not easy to differentiate the effects induced by the intracellular enzyme from those mediated by circulating or kidney-derived renin. Likewise, it is hard to separate the effects of intracellular angiotensin II from those mediated by activation of cell surface receptors in cells or animals. To demonstrate whether intracellular renin and angiotensinogen can elicit a biological response in cardiomyocytes, De Mello used in-vitro intracellular dialysis of renin and angiotensinogen and patch clamp techniques to study the changes in inward calcium currents in single cardiomyocytes isolated from hamsters with cardiomyopathy [11]. In this study, when renin and angiotensinogen were added to the extracellular perfusate, the inward calcium current was enhanced. This response was blocked by either extracellular ACE inhibition with enalapril or the AT₁ receptor blocker losartan, suggesting that angiotensin II was formed extracellularly by cardiac ACE [11]. These data are not surprising, and in fact quite expected, since ACE and AT₁ receptors are expressed abundantly in the heart [12]. What is interesting and novel, however, is that simultaneous dialysis of renin and angiotensinogen into cardiomyocytes also increased inward calcium currents in a manner similar to extracellularly applied renin and angiotensinogen. Because intracellular dialysis of losartan suppressed the intracellular renin/angiotensinogen-increased inward calcium current, these findings suggested that the intracellular effects may be due to renin uptake by cardiomyocytes through generation of intracellular angiotensin II [11].

Although De Mello's study clearly shows that direct microdialysis of renin and its substrate angiotensinogen into cardiomyocytes increases the inward calcium current and may therefore have a biological significance on myocyte-to-myocyte communication in the heart, it remains unclear how these findings in single cardiomyocytes *in vitro* are related to normal in-vivo cardiovascular physiology or disease processes [11]. Since the role of intracellular or intracrine renin and angiotensin II remains poorly understood, some important questions arising from De Mello's study deserve further comment. The first question is whether cardiomyocytes synthesize renin and its substrate angiotensinogen intracellularly or take up renin and angiotensinogen from the circulation. This issue has long been controversial. The conventional view holds that cardiac and vascular angiotensin II is synthesized by local renin and/or angiotensinogen. This view has recently been challenged, however, because the heart was found to express very low to undetectable renin mRNA, and cardiac renin activity disappeared completely after bilateral nephrectomy [10]. Moreover, it has been difficult to demonstrate the

presence of renin or angiotensinogen in isolated perfused Langendorff rat hearts [10]. This raises the possibility that cardiac renin is probably not synthesized intracellularly; instead, it may be derived from sequestration or internalization of kidney-released renin through a 'renin receptor' [10]. Indeed, the presence of renin or prorenin receptors or renin-binding proteins has been reported [13,14]. However, although binding of prorenin or renin to the renin receptor (the mannose-6-phosphate receptor) leads to internalization, it does not play an important role in either extracellular or intracellular angiotensin II formation [10,13]. Furthermore, the cloned renin receptor as described by Nguyen *et al.* binds prorenin and renin but does not become internalized after renin binding, although it generates angiotensin II at the cell surface [10, 14]. The precise role of internalized renin/prorenin in generating intracellular angiotensin II therefore remains unknown. This leads to the second question, which is whether cardiac angiotensin II is formed intracellularly or taken up by cardiomyocytes. The study of De Mello as described in this issue of *Journal of Hypertension* shows that dialysis of renin and angiotensinogen into cardiomyocytes increased the inward calcium current, and this effect was blocked by the ACE inhibitor enalapril or the AT₁ receptor blocker losartan [11]. This strongly suggests that angiotensin II is generated inside cardiomyocytes and activates intracellular AT₁ receptors. It may be argued, however, that since only 'crude' renin and angiotensinogen were artificially dialyzed into the cells in this study, we do not know how much renin and angiotensinogen were in fact introduced and whether they were 'physiological' or 'pathophysiological'. Use of a purer or synthesized renin and/or angiotensinogen would have established a clear concentration-dependent response. Moreover, renin or angiotensinogen should have been dialyzed independently into cardiomyocytes rather than administered together to elucidate the role of intracellular renin or angiotensinogen in intracellular angiotensin II production. Clearly, De Mello's study cannot address the question of whether angiotensin II is generated inside the cardiomyocyte. There is increasing evidence that extracellular angiotensin II is accumulated in cardiovascular and renal cells via an AT₁ receptor-mediated mechanism. AT₁ receptor-mediated endocytosis of extracellular angiotensin II has been reported in vascular smooth muscle cells (VSMCs) [8,15], renal proximal tubule cells [16] and the heart [17]. This phenomenon has contributed significantly to high levels of tissue angiotensin II versus the circulating peptide. The idea that extracellular angiotensin II is internalized in cardiovascular cells via receptor-mediated endocytosis is not new. For example, as early as 1971 Robertson and Khairallah reported rapid localization of ¹²⁵I-labeled angiotensin II in the nuclei of VSMCs and cardiac myocytes after intravenous infusion [18]. Anderson and Peach later reported finding internalized angiotensin II and its receptors in endosomes of cultured rat VSMCs [15]. Van Kats *et al.* showed that cardiac ¹²⁵I-angiotensin II was increased to 150% of control after intracardiac infusion in pigs [17]. Despite these efforts, however, whether cardiomyocytes take up extracellular angiotensin II and the mechanisms by which angiotensin II internalization is regulated in cardiomyocytes remain poorly understood.

Finally, regardless of any merits or drawbacks of using intracellular microdialysis as a tool to introduce renin and angiotensinogen into cells, De Mello's finding that intracellular administration of renin and angiotensinogen increases the inward calcium current in cardiomyocytes may add valuable information to the current debate on whether intracellular angiotensin II has a biological role in cardiovascular and renal cellular regulation [11]. There is no doubt that extracellular angiotensin II plays a central role in normal blood pressure, cardiovascular and renal regulation, nor is there any dispute regarding the important role of extracellular angiotensin II in the pathogenesis of hypertension and cardiovascular and renal diseases by acting on cell surface G-protein-coupled receptors. The classical acute effects (as well as desensitization) of angiotensin II on VSMC contraction, and therefore arterial blood pressure, aldosterone synthesis and release, cardiac performance, or renal hemodynamics and tubular transport, are clearly mediated by activation of cell membrane AT₁ receptors [6,7]. However, binding of extracellular angiotensin II to the membrane receptor also induces

phosphorylation of the AT₁ receptor carboxyl terminus and initiates endocytosis/internalization of the agonist (angiotensin II)/AT₁ receptor complex in many cardiovascular and renal cells [8,9,15–19]. After endocytosis, the complex is trafficked to endosomes, where the internalized AT₁ receptor is dissociated from angiotensin II and recycles back to the cell membrane. Internalized angiotensin II is either degraded after trafficking to lysosomes or remains an active peptide inside the cell. Although the precise role of internalized angiotensin II is not known, it has long been speculated that intracellular angiotensin II may play a pivotal role in promoting cellular growth, differentiation and proliferation [4,5], which may contribute to target organ damage in hypertension, cardiac and vascular remodeling and chronic renal diseases. Although it remains difficult to define the precise role of intracellular/intracrine angiotensin II, attempts have been made to study the role of intracellular angiotensin II in recent years. Haller *et al.* showed that microinjection of angiotensin II directly into VSMCs induced rapid increases in intracellular calcium through activation of intracellular AT₁ receptors [20]. We recently reported that extracellular angiotensin II accumulates in proximal tubule cells via AT₁ receptor-mediated endocytosis [16] and that microinjection of angiotensin II into single proximal tubule cells stimulates intracellular calcium mobilization by acting on intracellular AT₁ receptors [21]. Eto *et al.* found that, in arterial myocytes, intracellular dialysis of angiotensin II also affects voltage-activated calcium currents [22]. In hepatocytes, Cook *et al.* expressed a nonsecreted form of angiotensinogen and found a mitogenic effect that could be blocked by losartan [23]. Finally, in isolated hepatocyte nuclei, angiotensin II was reported to stimulate transcription of renin and angiotensinogen mRNAs [24]. Taken together, these studies provide evidence that intracellular angiotensin II, whether synthesized intracellularly or internalized via receptor-mediated processes, may indeed play an important role in cardiovascular and renal cellular function beyond what was previously recognized. The long-term beneficial effects of ACE inhibitors and AT₁ receptor blockers in treating hypertension and cardiovascular and kidney diseases may be explained in part by their effects on blocking angiotensin II formation and/or reducing receptor-mediated angiotensin II endocytosis, thereby inhibiting the actions of intracellular angiotensin II. The studies of De Mello [11] and others [20–24] on the role of intracrine renin and/or angiotensin II in different target cells may therefore provide a new impetus to drive further investigations in this emerging and exciting field.

Acknowledgements

This article is dedicated in part to my PhD mentor and teacher, Sanford L. Skinner, MD, Department of Physiology, University of Melbourne, Australia, who was one of the pioneers in renin and prorenin research and introduced me to the RAS research.

Sponsorship: This work is supported by a grant from the National Institute of Diabetes, Digestive and Kidney Diseases (1RO1DK067299), National Institutes of Health; and a Henry Ford Health System institutional grant (A10217).

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