Intracellular renin-angiotensin system: the tip of the intracrine physiology iceberg

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It is axiomatic that peptide hormones bind to cognate receptors on target cells, with the resultant generation of intracellular second messengers. Not as well known is the fact that a large and growing body of evidence indicates that extracellular signaling peptides, such as hormones and growth factors, also appear, in some cases, to operate in the interiors of cells after internalization or retention in the cells that synthesized them. This type of peptide action has been termed intracrine, and it is particularly relevant in the renin-angiotensin system (13, 15–17). In fact, angiotensin II was one of the first hormones for which such action was proposed.

In this issue of the American Journal of Physiology-Heart and Circulatory Physiology, Singh and colleagues (21) have presented important data indicating the intracellular synthesis and nuclear trafficking of angiotensin II in neonatal cardiac myocytes stimulated by high glucose or isoproterenol. This study is important on several fronts. It represents the most recent fruit of a long progression of research and is best interpreted in that context. As early as 1971, radiolabeled angiotensin II was reported to localize in the nuclei and mitochondria of cardiac myocytes after intracardiac injection in rats (19). In the 1980s, our group demonstrated specific, high-affinity receptors for angiotensin II on cell nuclei in association with chromatin; binding of angiotensin II to nuclei was shown to upregulate transcription (13–17). Later, Booz et al. (2) and Tang et al. (22) reported that nuclear angiotensin II receptors were similar to angiotensin type 1 (AT1) receptors. Still later, studies employing electron microscopy and immunohistochemistry demonstrated angiotensin II in association with euchromatin in normal animals (9). Eggena and colleagues (8) showed that treatment of isolated nuclei with angiotensin II led to the upregulation of renin and angiotensinogen transcription. At the same time, De Mello (7) and then Haller and Luft (11) showed that the introduction of angiotensin II into cells could have important effects on calcium currents; De Mello proposed an important role for intracellular angiotensin II in cardiac myocyte conductance and, by extension, in the pathogenesis of cardiac arrhythmias. Recent studies employing AT1 receptor fluorescent fusion proteins have shown that angiotensin II binding to these receptors is associated with their internalization and trafficking to the nucleus (5, 6, 16). Indeed, our recent studies indicate that, in some cases, AT1 receptor cleavage occurs with the receptor’s cytoplasmic tail trafficking to the nucleus, a phenomenon seen with some tyrosine kinase receptors but not previously associated with G-coupled receptors (6).

One issue raised by the studies described above is the site of synthesis of any angiotensin II that might be active in the intracellular space. That is, does the intracellular generation of angiotensin II occur, or must any intracellular angiotensin be internalized from the extracellular space? The argument was made that angiotensinogen possessed a signal sequence for secretion and was not stored in cells; therefore, it likely would not be available in the intracellular space to serve as substrate for angiotensin I production (3). To be sure, there were reports that strongly suggested intracellular angiotensin II synthesis. Also, Sherrod et al. (20) recently demonstrated that, depending on glycosylation state, angiotensinogen could be retained in astrocytes and, in fact, could be found in association with cell nuclei. Moreover, Peters et al. (12) showed that nonglycosylated renin (which apparently can be found in human circulation) can be internalized by cardiac myocytes, leading to intracellular angiotensin II generation and pathological changes. At the same time, evidence was presented that a second renin transcript lacking the signal sequence for secretion and predicted to be synthesized as an active enzyme, as opposed to a prorenin, was expressed in various tissues (16). Nonetheless, considerable debate surrounded the idea of intracellular angiotensin synthesis (3).

Our group transfected H4-II-E-C3 rat hepatoma cells that expressed the nonsecreted renin transcript with an angiotensinogen construct lacking the signal sequence for secretion. Intracellular angiotensin synthesis occurred, accompanied by increased cellular proliferation (4). In a second series of studies, transfection of vascular smooth muscle cells with a construct encoding an exclusively intracellular angiotensin II fluorescent fusion protein led to the nuclear trafficking of the fluorescent protein and enhanced cellular proliferation (5). Next, Baker and colleagues (1) made a similar nonsecreted angiotensin II and demonstrated that its expression resulted in rapid (96 h) hypertrophy of neonatal cardiac myocytes; moreover, when a plasmid consisting of the angiotensin II construct driven by an α-myosin heavy chain promoter was injected into mice, marked ventricular hypertrophy occurred within 96 h. This work is a stunning demonstration of the physiological potential of intracrine angiotensin II. The present study by Singh et al. (21) from this same group advances the field by shedding light on several of the questions raised above.

First, the previously reported studies of the effects of intracellular angiotensin II were criticized, because they employed transfected cells (3). Singh et al. (21) extend the field by demonstrating physiological effects (upregulation of renin and angiotensinogen) in nontransfected cells, putting to rest the unlikely possibility that the prior cell models were not representative of unengineered cells. Second, Singh et al. used angiotensin receptor blockade to prevent angiotensin II uptake by the cells. The upregulation of intracellular angiotensin II in this circumstance is strong evidence in favor of intracellular angiotensin II synthesis. The study of Singh et al. also opens new avenues of investigation. The upregulation of local angiotensinogen and renin synthesis by high-glucose media is ex-
intracrine physiology in biology and medicine. This finding is consistent with earlier reports from Frustaci et al. (10) showing increased cellular angiotensin II in the cardiac myocytes in diabetic cardiomyopathy patients. The study of Singh et al. clearly suggests that hyperglycemia contributes to diabetic cardiomyopathy by upregulating the intracellular angiotensin system. The implications of this suggestion are potentially very important, because they support the idea that the intracellular space is the locus for effective therapeutic intervention (16, 17). This point is made even more forcefully through the demonstration by Singh et al. that the renin inhibitor aliskiren can enter the myocytes and inhibit the intracellular generation of angiotensin II. The potential clinical implications of this observation speak for themselves, although much investigation is required to determine whether this analysis is correct. Singh et al. also report the pathways involved in angiotensin II synthesis after glucose or isoproterenol stimulation. They conclude, on the basis of inhibitor studies, that isoproterenol-induced angiotensin II production is driven by angiotensin-converting enzyme (ACE), whereas glucose-mediated upregulation is driven by chymase. These inhibitor studies are not clear-cut and depend on the uptake and trafficking of the various inhibitors introduced into the extracellular space. At the same time, these studies point out the need for detailed studies of the intracellular trafficking of ACE inhibitors, angiotensin receptor blockers, and, now, renin inhibitors in various cell types.

Arguably, the work of Singh et al. (21), similar to the other studies discussed above, represents only the tip of what can be called the “intracrine iceberg.” Not only is angiotensin II an intracrine signaling peptide, but ACE, angiotensinogen, angiotensin-(1–7), and renin appear to be as well (16, 17). Moreover, many other peptide hormones (e.g., parathyroid hormone-related peptide), growth factors (e.g., fibroblast growth factor-2), enzymes (e.g., angiogenin), DNA binding proteins (e.g., high-mobility group box type 1), and other factors (e.g., lactoferrin) are, in fact, extracellular signaling proteins that are found, and likely act, within cells. Although these factors are structurally diverse, we have identified principles of intracellular action and have suggested a role for this intracrine physiology in cellular/tissue memory, differentiation, hormonal responsiveness, and, in particular, angiogenesis (16–18). One aspect of this proposal for intracellular differentiation involves the establishment of long-lived, finite-gain positive intracellular intracrine feedback loops. In this regard, the upregulation of renin and angiotensinogen by activation of the intracellular renin system reported by Singh et al. (21) is consistent with these principles.

In summary, the work of Singh et al. (21) is an exciting next step in understanding the renin-angiotensin system. It is also representative of the growing appreciation of the importance of intracrine physiology in biology and medicine.

REFERENCES