Lysosomal action of intracrine angiotensin II. Focus on “Intracellular angiotensin II activates rat myometrium”

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FORTY YEARS AGO, Robertson and Khairallah (20) demonstrated that tritiated angiotensin II (ANG II) rapidly localized in myocyte nuclei and mitochondria after intracardiac injection. This finding was confirmed and expanded upon over the subsequent decades with the result that the notion of intracrine ANG II action is now widely accepted. Indeed, there is robust evidence that a variety of hormones, growth factors, cytokines, as well as some enzymes, transcription factors, and DNA-binding proteins, act in an intracrine mode implying that they serve both as intercellular signaling molecules as well as intracellular regulators either in the cells that synthesized them or in target cells. In the case of ANG II, a wide variety of intracellular actions ranging from gene regulation to effects on mitochondrial respiration have been described (15, 16). In this issue, Deliu and coworkers (4) provide additional information regarding intracellular ANG II action by demonstrating that both ANG II and its AT-1 receptor localize in endo-lysosomes. The interaction of ligand and receptor at the lysosome produces changes in calcium release from intracellular stores (an effect with implications for contractility). Thus this work expands our understanding of the action of intracellular ANG II. At the same time this publication prompts the consideration of five areas important to the study and understanding of intracrine ANG II function.

The first is the issue of where in the cell angiotensin acts. In studying intracellular ANG II action it is common to introduce the peptide into the intracellular space. Several methods have been employed including microinjection (as Deliu and colleagues used), microdialysis, or transfection with one or another construct encoding either an intracellular ANG II or a nonsecreted angiotensinogen to serve as a substrate for the synthesis of intracellular ANG II. It is not clear that all these methods introduce ANG II into the same cellular compartments. In general, studies of the effects of ANG II on calcium flux and intercellular conduction have employed microinjection or microdialysis, whereas investigators interested in gene expression and cell growth have employed transfection in cell culture as well as transgenic models, as would be required for the study of longer term effects such as cell growth (1, 5, 8, 15, 16, 18). All methods for introducing ANG II into the cell have resulted in biological effects, but it remains important for future studies to further characterize the site of intracellular ANG II action both in experimental models and free-living organisms. There is evidence for intracellular ANG II trafficking to, and action at, nucleus and mitochondria. Deliu and colleagues provide support for yet another locus of action at lysosomes, apparently following the microautophagy of cytoplasmic ANG II by the organelle. This novel explanation of ANG II trafficking hints at how complex intracellular angiotensin trafficking may be. Delineating other sites of ANG II action both through the use of introduced ANG II as well as the study of unmanipulated cells remains an important goal.

The second is the issue of how the AT-1 receptor, with or without ANG II, trafficks to intracellular sites from the cell membrane. Many receptors and their ligands have been detected in cell nuclei, including G protein-coupled receptors, receptor tyrosine kinases, and their respective ligands (13). In the case of the epidermal growth factor receptor (EGFR) a trafficking pathway has been proposed that involves the passage of this receptor tyrosine kinase to nucleus via endocytic vesicles, the endoplasmic reticulum, the golgi, and the inner nuclear membrane with the receptor then being released into the nucleoplasm (22). This schema is attractive because it explains how a membrane-bound holoreceptor can traffick in the cell to reach nucleus. We have proposed a similar trafficking pathway for the AT-1 receptor (2). The AT-1 trafficking described in the study by Deliu et al. appears to utilize the first portion of this pathway to reach endosomes and lysosomes where the receptor interacts with ANG II, which has possibly been taken up by the organelle through microautophagy. It will be important to clarify and better define all the pathways by which intracrine receptors reach various intracellular target sites from both the cell surface and, possibly in the case of newly synthesized receptor, from the cytoplasm.

A third issue is the mechanism of intracellular ANG II action. ANG II binding sites have been located in association with chromatin, the nuclear membrane, mitochondria, and now lysosomes (6, 7, 12, 14, 18, 20). Both AT-1 and AT-2 receptors have been detected in nuclear membranes, and binding of ligand to these receptors can trigger second messenger generation much as cell surface binding does. Other intracrines also have been reported to act at their intracellular receptors in a manner similar to their interaction with cell surface receptor. However, many intracrines act in unconventional or noncanonical ways, which include direct binding to DNA, transcription factors, and other moieties, actions clearly distinct from their cell surface functionalities. For example, the angiogenic factor angiogenin is an RNase that must traffick to the nucleus to stimulate angiogenesis; the protein’s weak enzymatic activity is similarly required for its function, strongly supporting a noncanonical action (9, 15). ANG II binds to chromatin much as nerve growth factor (NGF), epidermal growth factor (EGF), and midregion parathyroid hormone-related protein (PTHrP) do, implying that it too can function noncanonically (11, 15, 16). In transgenic mice overexpressing an intracellular ANG II fusion protein, hypertension develops associated with trafficking of the construct to mitochondria and its direct binding to electron transport chain proteins, with secondary effects on...
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ATP generation and reactive oxygen species generation (3, 19). This mitochondrial effect also constitutes a noncanonical ANG II action. The angiotensin action described by Deliu et al. is canonical because it is associated with AT-1 receptor, is blocked by losartan, occurs in a membranous compartment, and involves the generation of the second messenger inositol triphosphate. It will thus be important in future studies to define the mechanism of all forms of intracellular ANG II action.

A fourth issue is the role of inhibitors, and, in particular, angiotensin receptor blockers (ARBs), in studying, or therapeutically interfacing with, the action of intracellular ANG II. Deliu and colleagues introduced the AT-1 receptor blocker losartan into cells to demonstrate an AT-1 effect. However, evidence in several systems indicates that losartan, unlike other ARBs, can be actively internalized by, and act in, specific cell types (1). Some investigators have suggested that other ARBs at reasonable concentrations can enter cells but also suggest that intracellular ANG II can have non-AT-1-mediated effects (21). Just as the locus of action of ANG II must be better defined by future research, so too must be the trafficking and compartmentalization of ARBs and other renin-angiotensin system inhibitors in particular, if the intracrine actions of ANG II are to be better understood and if this information is to be rendered therapeutically useful. The observations of Deliu et al. that once inhibitors of ANG II binding (e.g., losartan, saralasin) appear in the cytoplasm they can be taken up by lysosomes via microautophagy adds further complexity to the matter of inhibitor trafficking.

A final issue to be considered, one not touched upon by Deliu et al., is the relationship between intracrine ANG II action and intracrine physiology in general. As mentioned above, there are a wide variety of intracines many of which act in accordance with simple principles. ANG II, while arguably the first intracrine to be described, is one of the hardest to study because of its small molecular weight, its lability, and the fact that its intracellular and extracellular actions are similar, thus making them hard to study individually. Placing ANG II in the wider intracrine context can suggest as yet unappreciated aspects of its biology and thereby point to new directions for experimentation. For too long the field of intracrine biology has been hampered by an epistemological compartmentalization of intracines that has been based solely on their extracelluar physiology (15, 16).

In conclusion, the study by Deliu and colleagues expands our understanding of intracrine angiotensin physiology. Given emerging data that demonstrate a role for intracellular ANG II in sodium reabsorption, hypertension, and diabetes, their findings should also stimulate additional experimentation in this area (3, 10, 19, 21). Finally, the suggestions of Deliu and colleagues related to microautophagy are of interest and bring to mind other atypical modes of intracrine trafficking (17).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES