The Ochsner Journal 14:673–680, 2014

Thirty Years of Intracrinology

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ABSTRACT

Background: Intracrinology is the study of the intracellular actions, regulation, trafficking, and interactions of extracellular signaling peptides/proteins.

Methods: We describe the development of intracrine biology since the term was defined in 1984.

Results: Intracrine biology plays a role in many normal and pathological processes and represents a fertile field for the development of novel therapeutics.

Conclusion: Although 30 years old, the field of intracrinology is only now becoming widely accepted. Intracrine principles can be applied to the investigation of physiological processes and to the development of new therapies.

INTRODUCTION

In 1984, this laboratory introduced the term *intracrine,* meaning the action of a peptide hormone within a cell as opposed to acting at cell surface receptors. Intracrine action could involve the action of a hormone or other extracellular signaling peptide/protein in its cell of synthesis or in target cells after internalization. This notion grew out of studies by our group and others of the intracellular trafficking and actions of the vasoactive peptide angiotensin II. In time, it became clear that peptides/proteins, not usually thought of as hormones, served as extracellular signaling molecules and could also act in the intracellular space: these varied factors displayed intracrine functionality. Surprisingly, included among these proteins were growth factors, cytokines, transcription factors, DNA binding proteins, and enzymes.[1-9]

We developed the basic principles of intracrine action based on observing the functionality of various intracrines.[10-43] For example, many intracrines upregulate either their own synthesis or the synthesis of elements of their signaling cascades. Intracrines can operate in positive feedback loops—that is, in what has been termed a feed-forward mode. For example, angiotensin II action at the nucleus can upregulate the transcription of renin and angiotensinogen. Investigators have reported that high glucose levels stimulate the synthesis of intracellular angiotensin II and establish a feed-forward loop in which angiotensin II upregulates angiotensinogen.[35,38] This feed-forward loop appears to play a role in diabetes-related pathology and in particular diabetic cardiomyopathy. Also, in some cases, intracrines can travel to target cells, be internalized, and act in those cells, including setting up feed-forward loops. This action results in a change of state of the target cells. Indeed, if the secretion of an intracrine ceases, the target cells remain in an altered state by virtue of the intracellular feed-forward loop. This has the result of producing a novel form of differentiation, one based on active feed-forward loops. If target cells in turn secrete intracrines to affect nearby cells, a wave of differentiation can propagate through a tissue. Again, intracellular feed-forward loops can maintain the differentiated state, even after secretion ceases. As an example, one can look to the homeodomain transcription factors that are intracrines. Each of these factors contains a cell penetration sequence that enables it to enter target cells. One such factor, when applied to pancreatic duct cells, enters the cells, upregulates its own synthesis, and converts the ductal cells into islet cells. It appears that intracrines can traffick between cells in a variety of ways including in the fluid phase following secretion, traveling in lipid bodies called exosomes that are frequently released by cells in a variety of circumstances, or potentially between cells in thin nanotubes. Many intracrines traffick to the nucleolus; these

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Keywords: Angiotensin II, cytoplasmic and nuclear receptors, intracellular signaling peptides and proteins, intracrine, intracrinology, renin-angiotensin systems

The author has no financial or proprietary interest in the subject matter of this article.

intracrines almost invariably are either angiogenic or antiangiogenic.[13,14,16,17,22,24]

Although not all intracrines display all the characteristics of intracrine function, these notions form the basis of a coherent view of intracrine functionality, a view that surprisingly has been demonstrated to have a heuristic and predictive value. These ideas serve as the basis of a coherent branch of study, *intracrinology*. Here we discuss some of the findings and implications of this approach. This discussion cannot be comprehensive and, given space constraints, must be somewhat superficial in its treatment of the topic. Although only a few representative examples of the application of intracrinology can be discussed, much of this subject matter has been reviewed in more depth in prior publications. While the intracrine hypothesis originated at Ochsner Clinic Foundation, many scientists around the world have provided the data that support it. It is not possible to credit or reference all these talented people in a short article. References in this manuscript primarily arise from this institution, but these references cite the work of the many others who have made progress possible in this field (Table).[1-54]

INTRACRINOLGY AND THE CARDIOVASCULAR SYSTEM

The first evidence of intracellular peptide action was discovered when tritiated angiotensin II was injected into rats that were immediately killed and subjected to electron microscope autoradiography. Angiotensin II-associated radioactivity was found in the nucleus and mitochondria of the rat cells. Then specific angiotensin II receptors were described on cell nuclei and also in association with euchromatin. The binding of angiotensin II to the nuclei upregulated gene transcription and produced changes in nucleosome/chromatin structure consistent with enhanced transcriptional activity. A variety of laboratories reported angiotensin II AT-1 and AT-2 receptors on isolated nuclei, but it was only years later that electromicroscopic immunohistology confirmed angiotensin II in association with euchromatin.[1-8,10,11] Collectively, these findings indicate that angiotensin II binds to several nuclear sites and regulates transcription.

Studies of fluorescently labeled AT-1 receptors demonstrated that angiotensin II binding to cell surface receptors could lead to their internalization and nuclear translocation. Moreover, a variety of models was employed to demonstrate that angiotensin II binding to nuclei in vivo could be associated with cellular proliferation or hypertrophy.[11-18,23,28,54] The discovery of a renin moiety that was not secreted but was predicted to be synthesized in an active form, as opposed to a prohormone form that required activation, supported the notion that intracellular systems could generate physiologically active angiotensin II in the intracellular space.[12,19,54] Indeed, high glucose was later shown to upregulate an intracellular renin-angiotensin system in cardiac myocytes and cardiac fibroblasts, leading to increased intracellular angiotensin II levels. This finding fit nicely with the previously demonstrated increased angiotensin II in cardiac myocytes from patients suffering from diabetic cardiomyopathy.[17,18,28,54] Finally, a transgenic mouse line was developed that overexpressed angiotensin II intracellularly but not extracellularly.[34,40] These animals became hypertensive and developed renal thrombotic microangiopathy. In this model, nuclear localization of internally synthesized angiotensin II was found, but the binding of the hormone to mitochondria was much more prevalent. Indeed, the binding of angiotensin II to 2 specific mitochondrial electron chain proteins was demonstrated, and the binding was associated with altered generations of reactive oxygen species, long assumed to play a role in angiotensin II-induced pathology. This observation pointed out that future studies of intracrine biology in general, and angiotensin II biology in particular, must take into account what we called *noncanonical* intracrine action—that is, an intracrine's action independent of its canonical action at its typical receptors.[38] This in turn implied that total blockade of angiotensin II action could not be achieved with receptor blockers alone. Any therapeutic implications of this observation remain to be determined. Another line of investigation involved the direct physical introduction of angiotensin II into cardiac myocytes. Clear effects on cellular electrical conductance were observed, and these potentially have an important role to play in arrhythmogenesis.[12,15,16,18-43]

Although the study of intracrine angiotensin II led the way toward the study of intracrine processes, especially in the cardiovascular system, other intracrines also pointed the way. Parathyroid hormone-related protein (PTHrP), a hormone associated with the hypercalcemia of malignancy, is an intracrine. One form of the protein is retained in the intracellular space and acts at the nucleus. Another form of the protein is secreted to act at cell-surface receptors. In vascular smooth muscle cells, the binding of the hormone to the nucleus is associated with increased cellular proliferation, while the binding to the cell surface receptor is antiproliferative. This example points out some of the complexity of intracrine biology. Other studies showed the important role of intracrine loops in embryonic cardiac development. In this process, dynorphin B, for example, plays an important role.[14,16,17,19,22,31,49]

A variety of studies revealed that, like angiotensin II, other renin-angiotensin system components are

Table. Intracrines

INTRACRINES

Hormones, Cytokines	Growth Factors	DNA Binding Proteins	Enzymes	Other
Insulin	FGF (1,2,3,10)	Homeoproteins	Phosphoglucose Isomerase/Neuroleukin	Lactoferrin
GLP-1 (28-36)	Midkine	Amphoterin (HMGB1)	Renin/Prorenin (aspartyl-protease)	Endogenous Opioids (Dynorphin)
Angiotensin II	VEGF	IL-33	PD-ECGF/Thymidine Phosphorylase	Galectins
Angiotensin (1-7)	NGF		Granzyme A, B	Tat
Angiotensinogen	PDGF		PLA2-I	Defensins
Prolactin	Pleiotrophin		Urokinase	SHBG
INF beta, gamma	Proenkephalin		Lysyl-tRNA Synthetase	Ribosomal Protein S19
Interleukins	IGF-1		Thioredoxin	Pituitary Adenylate Cyclase Activating Polypeptide
PTHrP	Pigment Epithelium-Derived Factor (a serpin)		Tyrosyl-tRNA Synthetase	Endostatin
Oxytocin	Maspin (a serpin)		Pancreatic Bile Salt-Dependent Lipase	Periostin
Leptin	Schwannoma-Derived Growth Factor		Trp-tRNA Synthetase	Heat Shock Proteins
Growth Hormone	Leukemia Inhibiting Factor		AChE-R	PAI-2 (a serpin)
Somatostatin	Macrophage Colony-Stimulating Factor (CSF-1)		Angiogenin	Reelin
TRH	Hepatopoietin		Angiotensin-Converting Enzyme	PDCD5
LHRH	TGF-alpha			Thrombospondin-1
VIP	Heregulin			C-Peptide
ANP	TGF-beta			STC
Gonadotropin	BMP2			IGF BP-3, 5, 7
Chorionic Gonadotropin	BMP4			TCTP
Endothelin				Wnt 13
Neuropeptide Y				Oncoprotein DEK
Erythropoietin				S100B

Source: Re and Cook,[35] Gressner,[55] Sorci et al,[56] Tsoporis et al,[57] Iosef et al,[58] Saha et al,[59] Tomas et al,[60] Felin et al.[61]
AChE-R, acetylcholinesterase read-through isoform; ANP, atrial natriuretic peptide; BMP, bone morphogenetic protein; BP, binding protein; FGF, fibroblast growth factor; GLP-1 (28-36), glucagon-like peptide 1 fragment 28-36; HMGB1, high-mobility group protein B1; IGF, insulin-like growth factor; IL-33, interleukin-33; INF, interferon; LHRH, luteinizing hormone-releasing hormone; NGF, nerve growth factor; PAI-2, plasminogen activator inhibitor-2; PDCD5, programmed cell death 5; PD-ECGF, platelet-derived endothelial cell growth factor; PDGF, platelet-derived growth factor; PLA2-I, phospholipase A2-I; PTHrP, parathyroid hormone-related protein; SHBG, sex hormone-binding globulin; STC, stanniocalcin; Tat, tyrosine aminotransferase; TCTP, translationally controlled tumor protein; TGF, transforming growth factor; TRH, thyrotropin-releasing hormone; VEGF, vascular endothelial growth factor; VIP, vasoactive intestinal polypeptide.

intracrines. Angiotensinogen, (pro)renin, angiotensin-converting enzyme, and angiotensin (1-7) display intracrine features. Indeed, an intracellular renin-angiotensin system has been described in diabetic cardiomyocytes; this system displays intracrine feed-forward loops and participates in diabetic cardiomyopathy.[18,19,22,27,28,54]

Early on, we predicted that self-sustaining, feed-forward loops played a role in vascular endothelial growth factor (VEGF) biology in angiogenesis. In-

deed, such a feed-forward process was described, albeit in myeloma cells and in hematopoietic stem-cell differentiation. However, even more intricate loops involving fibroblast growth factor 2 (FGF2), angiogenin, and VEGF were later shown to participate in a complex intracrine loop to drive angiogenesis. Angiogenin trafficking to the nucleolus was shown to be critically important in this process, and upregulation of ribosomal RNA synthesis played an important role. Interestingly, the nucleolar protein nucleolin also is located on the cell membrane and trafficks various angiogenic and antiangiogenic factors to the nucleolus. The potent antiangiogenic factor endostatin binds to nucleolin and trafficks to the nucleolus where it blocks nucleolin phosphorylation, thereby blocking angiogenesis. Most of the factors that traffick to the nucleolus are either angiogenic or antiangiogenic. Collectively, these results point to a powerful role of intracrine action at the nucleolus in the process of angiogenesis.[12,22,24,25,29,44-46]

INTRACRINE BIOLOGY AND DEVELOPMENT

Several lines of evidence have been developed that indicate the important role of intracrine biology in development.[31,32,44-49] First, it was discovered that homeodomain protein DNA-binding transcription factors leave cells, traffick to nearby cells, are internalized, traffick to the nucleus, and regulate transcription. This system has been described in the case of retina development. When applied to cultured bile duct cells, the homeodomain transcription factor PDX-1 can be internalized, traffick to the nucleus, upregulate its own synthesis, and produce a transformation of the target cell to an islet cell phenotype complete with the synthesis of insulin. In addition to its potential application to the repopulation of beta cells in patients with diabetes, this finding points to intracrine biology's important role in development. The sequence in the homeodomain transcription factor antennapedia that is responsible for its cell uptake has been employed as a cell-penetrating peptide that when fused to a peptide introduces that peptide into target cells. Intracrine-derived cell-penetrating peptides are currently being used in the study of the intracellular biology of a variety of nonintracrine peptides, and in the future they may provide novel therapeutic opportunities.[31,32]

The endogenous opiate dynorphin drives positive intracrine feedback loops that regulate cardiac stem cell differentiation and thus acts in an intracrine fashion to direct cardiac embryogenesis.[31,32,49] Neurons secrete the read-through isoform of acetylcholinesterase (AChE-R) that is the precursor of a fragment generated in the extracellular space. This fragment is then internalized by neurons and has the effect of enhancing fear-conditioned memory. The same sequence of events occurs in hematopoietic stem cells. In this case, there is clear evidence that the internalized fragment upregulates AChE-R synthesis by target cells, resulting in a feed-forward positive loop, and antisense to AChE-R blocks differentiation of the stem cells.[31,32,47,48]

It is also possible to suggest future applications of intracrine biology to regenerative medicine. Retroviral-mediated transduction of fibroblasts with 4 genes (Oct3/4, Sox2, c-Myc, and Klf4) can reprogram the cells to an embryonic stem cell–like state.[31,32] This is of interest because Oct3/4 encodes a homeodomain transcription factor and can likely be internalized by target cells. Sox2 encodes a protein containing a high mobility group motif, and because there is evidence that the high-mobility group box 1 protein functions in an intracrine fashion, Sox2 protein may also be internalized by cells. Thus, 2 of the 4 transducing factors potentially could be delivered without the use of retroviruses. Moreover, the transcription factor Nanog can also be involved in reprogramming. Because it is a homeodomain protein, it likely can be applied extracellularly and function. Because retroviral transduction can be associated with viral integration in the genome and subsequent tumor formation, the direct application of proteins would appear to be considerably safer. The additional transducing factors could potentially be coupled with cell-penetrating peptides to avoid the use of viral transduction.[31,32] The first steps of the process have already been reported.[50] While speculative, these ideas suggest the potential of the intracrine paradigm.

INTRACRINE BIOLOGY AND CANCER

Given the important role played by intracrine biology in growth and development and given the fact that growth factors such as FGF2, VEGF, and insulin-like growth factor 1 are intracrines, it is not surprising that intracrines play a role in the development and progression of cancer. For example, angiogenesis is an important component of cancer growth, and the important role played by FGF2, VEGF, and angiogenin acting in concert has already been discussed in the cardiovascular system section. Angiogenin has been detected in the nuclei of breast cancer cells, and pharmacologic inhibition of angiogenin trafficking to the nucleus blunts cancer cell proliferation. The presence of angiogenin in the nuclei of breast cancer cells strongly suggests a role for angiogenin in cancer physiology, just as the intracrine VEGF loop in myeloma cells previously described indicates a role for VEGF in that disorder. Additionally, the existence of the same feed-forward loops in cancer cells and vascular cells led us to suggest the notion of intracrine reciprocity, whereby

intracrines in the vasculature or other tissues can spill over and strengthen intracrine loops in cancer cells and vice versa. Similarly, intracrine biology can predict the response of some cancers to therapy. For example, the epidermal growth factor receptor 1 (HER1) is a receptor for a variety of intracrine growth factors such as the epidermal growth factor (EGF) and neuregulin. HER1 is a therapeutic target in breast cancer, and antibodies targeting the receptor as well as tyrosine-kinase inhibitors blocking its signaling have been successfully used in the clinic. However, not infrequently drug resistance develops, and this resistance was initially ascribed solely to receptor mutation. Given the intracrine nature of the growth factors involved, we predicted that upregulation of the intracrine HER1 ligands could produce resistance by allowing the intracellular system to signal, unaffected by antibodies working solely at the cell surface. Similarly, upregulation of the intracrine system could bypass the HER1 tyrosine-kinase inhibitor. Soon thereafter, this kind of resistance was demonstrated, pointing to the need for an expanded approach to interrupting the EGF growth network.[14,29,32,38,44-46,51]

Additionally, the intracrine view suggests novel approaches to cancer therapy. For example, the nucleolar protein nucleolin is intimately involved in the regulation of ribosomes, but it also serves as a cell surface protein that shuttles intracrines from the cell surface to the nucleolus. Nucleolin is upregulated on the surface of a variety of cancer cell types. The interruption of this shuttle pathway in endothelial cells inhibits angiogenesis, and the interruption of the nucleolin shuttle or other trafficking pathways to the nucleus inhibits the growth of various cancer cells**.** For example, the drug neamine inhibits the trafficking of angiogenin to the nuclei of breast and prostate cancer cells and inhibits their proliferation.[24,25,29,52,53]

Another potential area of interest is the notion of conditional stem cells. As we have discussed, intracrines play an important role in stem cell regulation. The existence of cancer stem cells has been proposed. These cancer stem cells are proposed to be slow growing but immortal cells that can repopulate tumors after chemotherapy. However, very few cancer cells display traditional stem cell intracrines, and few if any express all needed factors. Thus, we suggested that a reciprocity could occur in which cancer stem cells lacking 1 or another intracrine factor could take up an intracrine factor from a neighboring cell and possibly return a different factor to be internalized by the neighboring cell.[24,25,31,32] Thus, 2 conditional cancer stem cells would become fully functional. The interaction of these conditional cancer stem cells is analogous to intracrine reciprocity, and although this kind of intracrine interaction has not yet been demonstrated, it potentially could provide new targets for cancer therapy.[21,24,25,31,32,52,53]

INTRACRINE BIOLOGY IN OTHER DISORDERS

Intracrine functionality can be found in multiple physiologic processes, leading us to establish the principles of what might be called intracrine pharmacology.[29] The interruption of intracrine synthesis, extracellular trafficking, intracellular trafficking, canonical signaling, and noncanonical signaling are targets for the development of therapies for a variety of disorders. Indeed, some currently available drugs such as endostatin have been shown to act through intracrine pathways in target cells. Endostatin colocalizes with nucleolin on the surface of endothelial cells and trafficks with it to the nucleolus. There it inhibits the phosphorylation of nucleolin that is necessary for cell proliferation. Endostatin is, therefore, antiangiogenic, but it must traffick to the nucleolus to block angiogenesis.[24,25,29] The principles of intracrine pharmacology can be applied to the development of therapies for the conditions already discussed and to other processes that involve intracrine action.[35,37-39] For example, the process of cellular senescence is complex and involves a senescence secretory response on the part of senescent cells.[32] This response involves the intercellular trafficking of intracrines, including trafficking via exosomes. Understanding this process and developing agents to modulate it could provide the opportunity to influence senescence and apoptosis in a variety of circumstances.

Transmissible spongiform encephalopathies, diseases such as mad cow disease and Creutzfeld-Jakob disease, involve the spread of abnormally folded prion proteins from cell to cell, with the abnormal protein subsequently causing the misfolding of normal proteins in target cells. The disease is thus propagated by intercellular trafficking proteins. This process can be viewed as a primitive intracrine system. Similar propagation of misfolded proteins occurs in Alzheimer disease, Parkinson disease, amyotrophic lateral sclerosis, and chronic traumatic encephalopathy, among other diseases. One could ask if a more robust intracrine action is involved in these disorders. For example, intracellular trafficking occurs not only by secretion of abnormal proteins or the release of the proteins from dying cells, but rather the evidence shows that intercellular trafficking occurs via exosomes and nanotubes, modalities also associated with intracrine trafficking.[43] A more important issue is whether intracrine-like protein feed-forward

amplification occurs in these neurodegenerative disorders. If so, interruption of these loops could provide a framework for the development of novel therapies for these disorders.[31,32,43]

CONCLUSION

Intracrinology is 30 years old and yet is only now becoming widely accepted. However, the evidence to support its validity is overwhelming, suggesting that in the near future intracrine principles will be widely applied to the investigation of physiological processes and to the development of novel therapies. Although this cursory review cannot fully explore all aspects of intracrine biology, it can serve as a guide for interested readers.

Finally, it is appropriate to recognize the researchers at Ochsner Clinic Foundation who have contributed to this effort. Initially, I had the opportunity to work with a talented technician, Meera Parab, without whom the project would have never gotten off the ground. After several years, I had the good fortune of establishing a collaboration with the late Dr Sara Bryan, professor of biology at the University of New Orleans. Along with Jean Brown, Dale Seth, Michael Saucier (who now is a physician on the Ochsner staff), and others, we made great progress in understanding the genomic actions of intracrine angiotensin II. This collaboration only ended with Dr Bryan's death. After several more years, Dr Julia Cook, co-director of the Ochsner Molecular Genetics Laboratory, agreed to collaborate on the study of intracrine angiotensin II. This marked a turning point in the work on intracrine action here and nationwide. Dr Cook's participation led to a near-explosive increase in understanding the operative mechanisms of intracrine angiotensin II action. Dr Cook is an enormously talented and creative scientist whose application of molecular genetic techniques led the way to understanding much of the basic biology of intracrine angiotensin II. Thanks to Dr Cook, during this period Ochsner remained the leader in the emerging field of intracrinology. Collectively, this team has accomplished a great deal.

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