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A Critical Appraisal of the Intrinsic Pancreatic Angiotensin-Generating System

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Summary

The pancreas is a relative newcomer to the stable of tissues with an intrinsic angiotensin-generating system. The involvement of this system in pancreatic activity will be dependent on the angiotensin-generating paths present in the pancreas and their precise cellular location. Thus far, renin, angiotensin-converting enzyme (ACE), angiotensin II and AT_1 and AT_2 receptors have been found. These are components of the "classical" renin-angiotensin system. But there is uncertainty as to their location and site of action. Furthermore, it is not known which, if any, alternative enzymes to renin and ACE are present, which angiotensins in addition to angiotensin II are generated and whether or not there are receptors to angiotensin IV and angiotensin-(1-7). Future research should focus on these aspects in order to provide a mechanistic basis to pancreatic physiological functions and to pathological conditions of clinical relevance.

Introduction

The enzymatic pathway, which leads to the generation of the potent vasoconstrictor octapeptide angiotensin II, is usually understood in terms of the "renin-angiotensin system" (RAS) [1, 2, 3], where the hepatic glycoprotein angiotensinogen is hydrolyzed by the renal enzyme renin in the circulating blood. The decapeptide product, angiotensin I is subsequently converted to angiotensin II by the pulmonary membrane-bound angiotensin converting-enzyme (ACE). This classical model has been incorporated in the illustration in Figure 1. However this pathway to angiotensin II generation is truly skeletal. In the context of contemporary research it is more constructive to view the RAS as consisting of obligatory components (angiotensinogen, angiotensin II and specific receptors) linked by alternative, non-exclusive components (renin, ACE and their functional homologues [4, 5]). It is therefore more accurate to describe the RAS as a major "angiotensin-generating system", rather than the definitive enzymatic cascade of angiotensin II generation. The classical description of the RAS also undermines the reality that angiotensin II formation is not restricted to the circulation but is formed in numerous tissues as diverse in function as the brain [1, 6, 7], pituitary [6, 8], heart [9], kidney [10], gonads [11, 12], and pancreas (*vide infra*). It may even be formed intracellularly [8, 10, 13]. The emphasis on angiotensin II as the biologically relevant product has also undergone extensive revision, as what were once regarded metabolic "fragments" of angiotensin II have become products with specific actions mediated by selective receptors [14]. For example angiotensin III is involved in the central regulation of blood pressure and preferentially binds to the AT_{1b} receptor [15, 16], angiotensin IV has vasodilatory, antiproliferative and cognitive actions [17, 18] and angiotensin-(1-7) is a vasorelaxant [19, 20, 21]. The final level of complexity is added by the variety of angiotensin (AT) receptors. Presently the major recognized AT receptors includes the AT_1 (subtypes a and b)

and AT_2 receptors for angiotensin II and angiotensin III action [1, 2, 3, 9]. Although the gene has yet to be identified, there is strong evidence that angiotensin IV acts via an AT_4 receptor [22], and on the basis of the heterogeneity of ligand binding, via a second AT_{4b} receptor subtype [23]. There is evidence for an AT-(1-7) binding site for angiotensin-(1-7) action, but a definitive identification of this binding site has thus far eluded researchers [19, 20, 21]. This array of enzymatic paths leading to several biologically active angiotensins, targeting tissues with their respective receptors, is a distant and vastly different system from the classical RAS. Its complexity provides the opportunity for functional diversity and for multiple levels of control points. The elucidation and understanding of these angiotensin-generating systems has led to a corresponding expansion of the domain for angiotensin action, far beyond its modest beginning as a vasoconstrictor hormone.

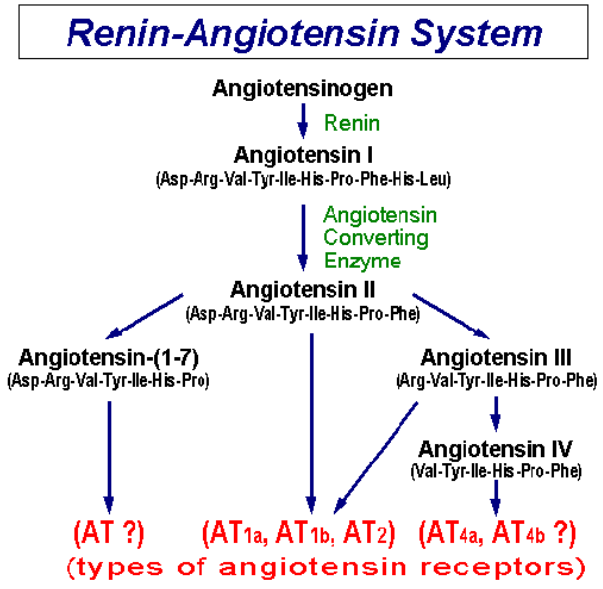

Figure 1. Diagram of the classical renin-angiotensin II pathway with additional biologically active angiotensins and their receptors.

Angiotensin-Generating Systems in the Pancreas

Evidence for intrinsic angiotensin-generating systems has been found in pancreatic tissues and cell lines of the mouse, rat, dog and man [24, 25, 26, 27, 28, 29]. The evidence has been summarized in Table 1. The protein for the obligatory component angiotensinogen has been reported for the rat [24] and dog [25] pancreas and its mRNA identified by reverse transcriptase polymerase chain reaction (RT-PCR). Immunocytochemistry has shown it to be localized in the epithelium of pancreatic ducts and in the vascular endothelium [24]. The islet cells do not show angiotensinogen immunocytochemically, yet islet cell lines do express angiotensinogen [30]. Thus a low or intermittent expression of angiotensinogen in islet cells in particular stimulatory circumstances, such as hypoxia [31], pancreatitis [32, 33] or other inflammatory conditions, should not be overlooked. The tissue content of angiotensinogen is low compared to blood [25], indicating the presence of highly localized angiotensin generation in the vasculature and ducts, and possibly the islets. Renin, (more precisely, prorenin), has been identified in the human pancreas [26]. While renin was localized by immunocytochemistry to the vascular endothelium and beta-cells, renin mRNA was found in vascular fibroblasts and reticular fibres of the islets. This cellular separation of prorenin from its mRNA suggests that prorenin may be used in the intracellular generation of angiotensin II by endothelium and beta-cells, in addition to any extracellular role. There is partial support for an intracellular angiotensin II system from the findings of Leung *et al* [29], which localized angiotensin II immunocytochemically to endothelial cells and ductal epithelia. However they found no angiotensin II in islet cells, implying that the renin in beta-cells [24] is not used to generate angiotensin II or more likely, that angiotensin II is formed and immediately secreted. This secreted angiotensin II would then act in a paracrine fashion on beta-cells via AT_1 receptors and on adjacent islet cells which, at least in the

rodent, express higher densities of AT receptors than beta-cells [34]. The direct function of angiotensin II on islets cells may well be the regulation of cell growth rather than the regulation of hormone secretion [35, 36]. ACE is predictably found in the vascular endothelium, preferentially in the islet vasculature [37], as well as in islet cells [36]. One of the striking features of the research on the angiotensin-generating systems in the pancreas is the coincidence in the localization of renin, angiotensinogen and angiotensin II. Based on these current observations the predominant pathway to angiotensin II production in the pancreas strongly favours the classical angiotensinogen-renin-ACE axis. The failure to detect angiotensin I by Chappell *et al* [25] seems to undermine this view, until one considers that these authors also failed to detect renin activity as well, in contrast to later studies using renin-specific techniques [24, 26].

Table 1. Summary of the presence or absence and the location in the pancreas of major components of the renin-angiotensin system.

RAS component	Absence/Presence	Location	Reference
Angiotensinogen	Present	Epithelium of ducts, Vascular endothelium	[24, 25]
(Pro)renin	Present	Beta-cells (protein) Fibroblasts (mRNA)	[24, 26]
Angiotensin-converting enzyme (ACE)	Present	Vasculature, Pancreatic cell line	[36, 37]
Angiotensin II	Present	Ductal epithelium, Vascular endothelium	[29]
Angiotensin III	Present	?	[25]
Angiotensin IV	?	?	
Angiotensin-(1-7)	Present	?	[25]
AT_1 receptor	AT_{1a}, AT_{1b} present	Throughout pancreas	[24, 26, 27, 28, 31, 34]
AT_2 receptor	Present	Throughout pancreas	[24, 27, 28, 34]
AT_4 receptor	?	?	
AT-(1-7) site	?	?	

Receptors, which mediate angiotensin II action, have a widespread distribution in the pancreas [26, 27, 28, 31, 33, 34, 38, 39]. The abundance of AT_2 receptors appears to be greater than AT_1 receptors [27, 34], nevertheless their distribution covers islets cells, acinar cells, duct cells and vascular tissue. Such a wide distribution is an indication of the variety of roles in which angiotensin II is likely involved in addition to the already established regulation of pancreatic blood [37] and ducal anion secretion [40]. Further support for this view is provided by the presence of both AT_{1a} and AT_{1b} subtypes and the predominance of AT_2 receptors [24, 26, 27, 28, 34], which are known to mediate growth and differentiation in direct opposition to AT_1-mediated actions [9, 41]. It is thus appropriate that the pancreatic RAS be considered of importance in conditions like pancreatitis, hypoxia and in any inflammatory pancreatic event.

While there is an impressive amount still to be uncovered about the "classical" pancreatic RAS, the most deficient area is in the investigation of alternate pathways to the production of angiotensins [1, 2, 4, 5, 14, 15], and in the expression of non-angiotensin II AT receptors [3, 18, 20, 22]. Kallikreins capable of generating angiotensin II independently of renin are present in the pancreas [5], thus implying the potential for alternative angiotensin II generating pathways. In addition, angiotensin III and angiotensin-(1-7) have been identified, albeit at lower concentrations than angiotensin II, in the canine pancreas [25]. Their presence implies the existence of aminopeptidases capable of cleaving these peptides directly from angiotensinogen or from angiotensin II.

However, the peptidases or proteases responsible for angiotensin II and angiotensin-(1-7) production have not been identified in the pancreas. It is interesting that insulin has the ability to change the activity of aminopeptidases capable of angiotensin hydrolysis [42]. Hence there may be a reciprocal role between insulin regulation of local angiotensins production and the RAS regulation of pancreatic activity. Angiotensin-(1-7), having actions which oppose those of angiotensin II [19, 20, 21], raises the possibility of differential blood flow in the pancreas due to a balance between angiotensin II and angiotensin-(1-7). The present limited data do not allow more than speculation on this possibility. Finally, the hexapeptide angiotensin IV has in recent times attracted a great deal of attention because of its vasodilatory, antihypertrophic and cognitive-enhancing actions [3, 17, 18]. Furthermore, there is at least one AT_4 receptor site selective for angiotensin IV that has been partially characterized [22]. Based on ligand-binding heterogeneity, there may be a second AT_{4b} site [23]. There is no information on any aspect of angiotensin IV physiology in the pancreas. The peptide has not been identified or localized nor has its function been tested. Similarly, AT_4 receptors have not been identified and localized in the pancreas.

Future Directions

The current evidence for an intrinsic RAS in the pancreas is persuasive and indicates that it is of importance in normal exocrine and endocrine functions, as well as in pathological conditions. There is still uncertainty as to the precise location of the components, their relative abundance and which intracellular or extracellular pathways are operative in the generation of angiotensin II. However, the least understood aspects are found in our understanding of the "non-classical" RAS which contains enzymes alternative to ACE and renin, the production of biologically active angiotensins in addition to angiotensin II and the presence of receptors for these additional angiotensin peptides. These areas of research should lead to a fuller understanding of the mechanisms which operate in the normal pancreas and how their derangement leads to pathological manifestations, and *vice versa*.

Key words Angiotensins; Receptors, Angiotensin; Renin-Angiotensin System; Pancreas; Tissues

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