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The Renin-Angiotensin System and the Exocrine Pancreas

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Summary

An accumulating body of evidence strongly indicates a local tissue renin-angiotensin system in the pancreas of a various species. In contrast to the majority of tissues that primarily express the angiotensin type 1 (AT_1) receptor, the pancreas is one of the few tissues that contain a significant proportion of the AT_2 subtype. Moreover, our findings indicate a greater distribution angiotensin II binding sites in the exocrine pancreas. Although the physiological aspects of a local pancreatic renin-angiotensin system remain largely unexplored, recent studies in our laboratory utilizing an acinar cell model demonstrate both functional AT_1 and AT_2 receptors. Indeed, we show that the AR42J cell line expresses all components of an angiotensin system including the mRNA for renin, angiotensinogen, angiotensin converting enzyme (ACE), AT_{1a}, AT_{1b} and AT_2 receptors. Thus, these cells may be of particular value to study the interplay of the AT_1 and AT_2 receptors to regulate cell growth and potentially exocrine function.

Introduction

In 1991, we published the first study demonstrating key components that comprise an intrinsic renin-angiotensin system (RAS) within the canine pancreas [1, 2]. These studies documented the expression of the bioactive peptides angiotensin II, angiotensin III and angiotensin-(1-7), both protein and mRNA levels of the precursor angiotensinogen, as well as the distribution of the AT_2 and AT_1 receptor subtypes. Subsequent studies by other investigators reported comparable findings in the rat, mouse and human pancreas [3, 4, 5]. Indeed, in one of the few reports to study the *in vivo* regulation of pancreatic angiotensin II receptors, Ghiani and Massini [6] demonstrated an increase in angiotensin II binding sites in the pancreas of normotensive rats maintained on a high-salt diet. Although angiotensin II receptors were distributed throughout the pancreas, the highest density of sites, at least in the dog and monkey (see below), comprised the AT_2 receptor subtype and localized to acinar cells and the ductal epithelium [2, 7]. Indeed, the pancreas is one of the few tissues that primarily express the AT_2 receptor subtype. At the time of our initial report, the AT_2 receptor had not been cloned and no functional data had been attributed to this receptor subtype. Only in the last several years has a more complete understanding of the functional role of the AT_2 receptor emerged. In this brief review, we assess the recent data on the AT_2 receptor and the potential influence on the functional aspects of the exocrine pancreas. We also present several novel aspects on the regulation and function of the AT_2 receptor utilizing the AR42J acinar cell line.

Pancreatic Angiotensin II Receptor Characterization

As shown in Figure 1 (top left panel), *in vitro* receptor autoradiography of angiotensin II receptors in the primate pancreas using the non-

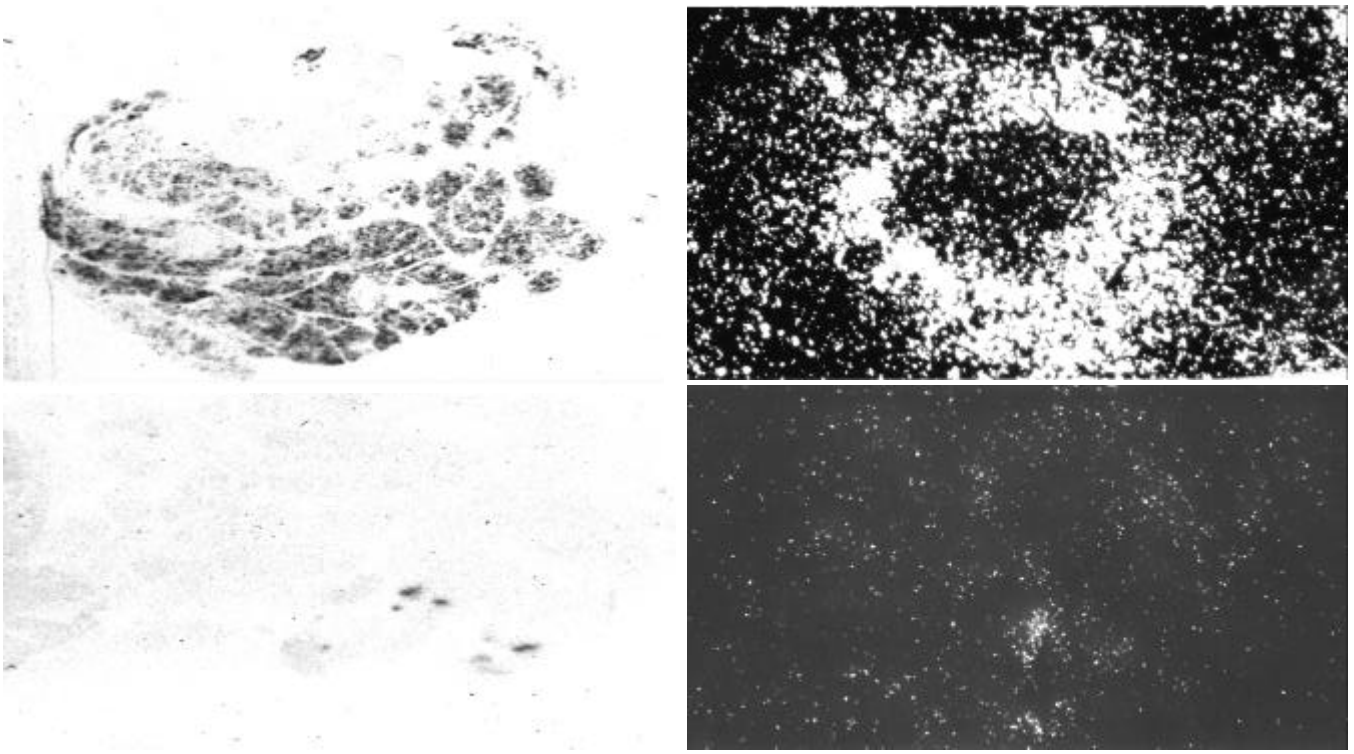

Figure 1. In vitro receptor autoradiography of ^{125}I-[Sar^{1},Thre^{8}]-angiotensin II (Sarthran) binding in the monkey pancreas. Top left panel: Total ^{125}I-Sarthran binding indicates receptor sites throughout the tissue. Bottom left panel: Competition by the AT_2 antagonist PD123319 for ^{125}I-Sarthran binding reveals that the majority of sites are the AT_2 subtype. Top right panel: High-resolution emulsion autoradiograph of ^{125}I-Sarthran binding demonstrates a high density of sites surrounding the islet cells of the monkey pancreas. Bottom right panel: The AT_2 antagonist PD123319 competes for the majority of the ^{125}I-Sarthran binding sites. Adapted from Chappell MC *et al.* [7, 8].

selective angiotensin ligand ^{125}I-[Sar^{1},Thre^{8}]-angiotensin II (Sarthran) revealed the distribution of sites throughout the tissue, but with the highest density on acinar cells [7, 8]. The majority of Sarthran binding (>80%) was attenuated by the AT_2 selective antagonist PD123319 (Figure 1, bottom left panel). High resolution emulsion autoradiography of this tissue revealed a very high expression of Sarthran binding surrounding the islet cells and a lower density of sites within the islet field (Figure 1, top right panel); addition of the PD123319 compound essentially abolished binding (bottom right panel). These findings in the monkey and those in the dog, demonstrating the predominant expression of the AT_2 subtype in exocrine components of the pancreas, prompted further investigation of angiotensin II receptors and other components of the RAS in an acinar cell model. We characterized angiotensin II receptor binding in the AR42J acinar cell line and reported a high density of binding sites (>300 fmol/mg protein) [9]. Similar to monkey and dog tissues, the majority of these receptors were the AT_2 subtype with a minority of sites (<15%) competed for by the AT_1 antagonist losartan. Although the proportion of AT_1 receptors expressed in the AR42J cell line was small, application of angiotensin II to cells loaded with the fluorescent calcium dye Fura-2 resulted in an immediate and significant increase in intracellular calcium. The angiotensin II-dependent rise in calcium was abolished by the AT_1 antagonist, but was not modified by AT_2 antagonists [9]. Consistent with our data, subsequent studies by others also reported AT_1-dependent changes in intracellular calcium by angiotensin II [10]. The biochemical characterization of the AT_2 binding sites

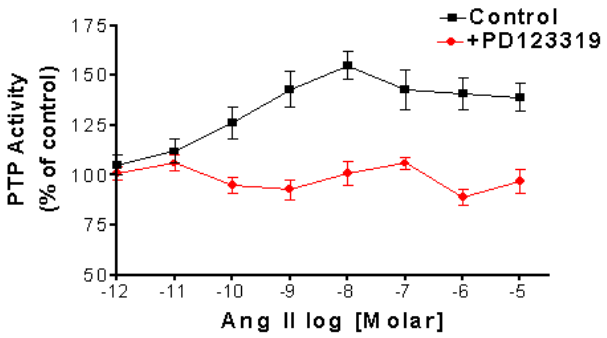

Figure 2. Effect of angiotensin II (Ang II) on tyrosine phosphatase activity (PTP) in the pancreatic acinar AR42J cell line. Cells were treated with varying concentrations of angiotensin II alone or with 1 µM of the AT_2 antagonist PD123319 for 15 minutes. Following the angiotensin II incubation, cells were isolated and phosphatase activity determined with *para*-nitrophenol phosphate (pNPP) as described by Takahasi *et al.* [13]. Data are the mean ± SEM from 3 separate experiments.

utilized cross-linking of radiolabeled Sarthran and SDS/PAGE fractionation. These studies revealed an AT_2 site with a molecular mass of approximately 110 kilodaltons (kDa) that was substantially greater than the predicted mass of 40 kDa based on the protein sequence of the AT_2 receptor. Analysis of the AT_2 sequence indicated a high number of glycosylation sites which likely influences the larger molecular mass observed in these cells, as well that reported in other tissues [11]. Similar to other reports, we could not demonstrate internalization of the AT_2 receptor, another characteristic quite distinct from the rapid down-regulation of the AT_1 receptor subtype following agonist binding [12].

Regarding the functional aspects of the AT_2 receptor, several groups demonstrated a link to activation of tyrosine phosphatase activity [13, 14]. In the AR42J cells, activation of somatotostatin receptors increased tyrosine phosphatase activity and inhibited cell proliferation [15]. We find that in the presence of AT_1 blockade, angiotensin II increased vanadate-inhibitable tyrosine phosphatase activity as measured with *para*-nitrophenol phosphate (Figure 2). In the presence of both AT_2 and AT_1 antagonists, angiotensin II did not change phosphatase activity. Interestingly, these data are consistent with a recent report by Elbaz *et al.* [16] who demonstrated an AT_2-dependent reduction in the phosphorylation of the activated insulin receptor in intact AR42J cells. These authors found that both the AT_2 antagonist PD123319 and a tyrosine phosphatase inhibitor abolished this response [16]. Moreover, others reported that AT_2 activation may contribute to the dephosphorylation of the MAP kinases ERK1 and ERK2 [17, 18] . The activation of tyrosine phosphatase activity may underlie the anti-proliferative actions generally associated with stimulation of the AT_2 receptor [17, 19, 20]. Currently, we do not know whether angiotensin II exerts proliferative or anti-proliferative actions in these cells; the growth effects may likely depend on the relative balance of both receptor subtypes and the overall potency of their cellular signals.

In the AR42J cells, we are investigating the regulation of the AT_2 receptor subtype. As shown in Figure 3, treatment with the glucocorticoid agonist dexamethasone resulted in a significant decline in PD123319-sensitive binding within six hours and a maximal decrease in binding by 24 hours [21]. The addition of cortisol also substantially reduced

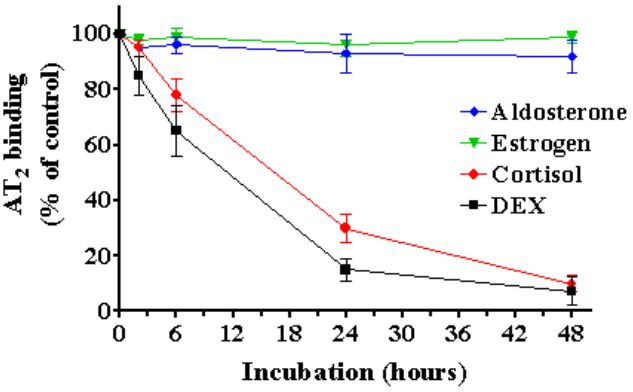

Figure 3. Effect of steroid treatment on AT_2 binding in the pancreatic acinar AR42J cell line. Cells were treated with 1 µM of dexamethasone (DEX), cortisol, aldosterone or 17beta-estradiol (estrogen) for up to 48 hours. AT_2 binding was determined with ^{125}I-[Sar1,Thre^{8}]-angiotensin II in the presence of the AT_1 antagonist losartan in intact cells. Data are the mean±SEM from 3 separate experiments. Adapted from Chappell MC *et al.* [21].

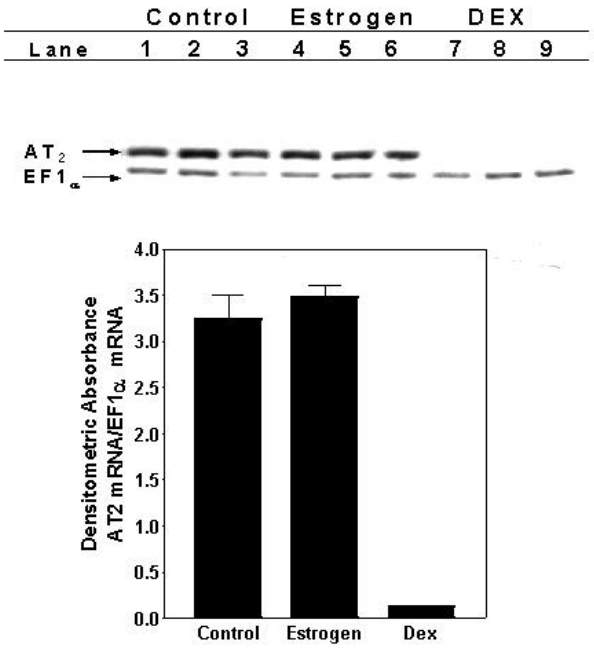

Figure 4. Effect of dexamethasone (DEX) and 17beta-estradiol (estrogen) on AT_2 mRNA concentration in the pancreatic acinar AR42J cell line. Top panel: Autoradiogram of the amplified products using primers specific for the rat AT_2 receptor and the elongation factor-1alpha ($EF1_\alpha$). Data are from 3 sets of cells untreated (Control, lanes 1-3), treated for 24 hrs with 1 µM estrogen (lanes 4-6) or 1 µM dexamethasone (lanes 7-9). Bottom panel: Densitometric quantification of the $AT_2/EF1_\alpha$ mRNA; mean+SEM.

binding, but other steroid agents including estrogen, and aldosterone had little or no effect (Figure 3). Although not shown, saturation analysis of the dexamethasone-induced inhibition of the AT_2 binding reflected a decrease in the number of receptor sites (B_{max}) and no change in the relative affinity (K_D) of the receptor to the Sarthran ligand. Consistent with the decrease in receptor number, the assessment of AT_2 mRNA levels by RT-PCR revealed an almost complete inhibition of mRNA expression by dexamethasone in these cells (Figure 4). In contrast, estrogen treatment had no effect on angiotensin II binding or AT_2 mRNA expression. Further studies are necessary to determine whether this reduction in AT_2 mRNA results from an attenuation in transcriptional activity or decreased mRNA stability. However, our results are quite consistent with those of Kijima *et al.* [22] who reported that dexamethasone treatment reduced AT_2 mRNA levels in the adrenomedullary PC12 cell line. In their study, dexamethasone treatment primarily reduced the message stability to attenuate AT_2 mRNA half-life [22]. In view of the contrasting actions of AT_1 and AT_2 receptors, glucocorticoids are known to increase AT_1 binding and AT_1 mRNA, as well as ACE activity [23, 24]. Thus, glucocorticoid-induced hypertension may comprise a shift in the balance of effects between the AT_1 and AT_2 receptors in the presence of elevated levels of angiotensin II. Glucocorticoid down-regulation of the AT_2 receptors may also be relevant to the recent findings that endogenous glucocorticoids suppress apoptosis in an induced- pancreatitis model [25]. Leung and colleagues [26, 27] demonstrated up-regulation of the pancreatic RAS including increased expression of AT_2 mRNA in a chronic model of hypoxia, as well as augmented angiotensinogen in induced pancreatitis. In this regard, perhaps the activation of a pancreatic RAS, particularly the AT_2 receptor, may promote cellular apoptosis and influence pancreatitis. Transient up-regulation of the AT_2 receptor has been reported in other tissues such as brain and kidney [12].

Finally, in view of our incomplete understanding of the generation of angiotensin II in the pancreas and other tissues such as the kidney, heart and brain, we have begun to investigate the expression of additional components of the pancreatic RAS in the AR42J cells. As shown in Figure 5, molecular analysis using RT-PCR revealed that the AR42J cells express mRNA for both AT_{1a} and AT_{1b} isotypes, as well as that for renin, angiotensinogen and ACE. Indeed, to our knowledge this is the first demonstration that this pancreatic cell line exhibits all components of the RAS. Although the expression of these components may result from the transformed phenotype, the AR42J cells constitute a unique cell model to explore the processing of angiotensin II and angiotensin I. Indeed, these cells may more closely model an autocrine

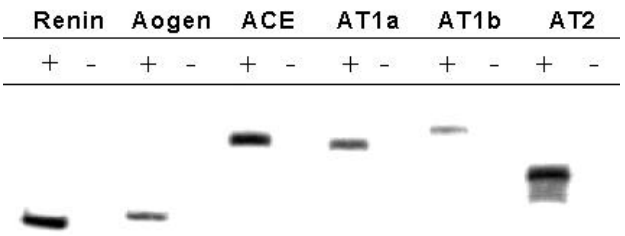

Figure 5. RT-PCR analysis of the mRNA components of the renin-angiotensin system in the pancreatic acinar AR42J cell line. DNase-treated total RNA from AR42J cells was transcribed with (+) or without (-) reverse transcriptase (RT) and amplified with primers specific for rat renin, angiotensinogen (Aogen), angiotensin converting enzyme (ACE), and the angiotensin II receptor subtypes AT_{1a}, AT_{1b}, and AT_2.

system in which the local production of angiotensin II or other active metabolites acting through different receptor subtypes may feedback to influence its tissue of origin. This may be of particular relevance in hypertensive patients as AT_1 receptor blockers (ARBs) may supplant ACE inhibitors and other anti-hypertensive treatments. ARB treatment not only blocks AT_1 receptors, but significantly increases angiotensin II levels (due to the disinhibition of renin release) that may result in greater activation of the AT_2 and other receptor subtypes. Furthermore, the acinar cell model may be of relevance to study more novel components of the RAS such as the AT_4 receptor and the biologically active ligands, angiotensin-(3-8) and angiotensin-(3-7); these endogenous peptides exhibit high affinity for the AT_4 binding site [12, 28]. Although a high density of AT_4 sites are found in a number of tissues including the heart, adrenal gland, and the vascular endothelium, whether this site is expressed on the exocrine or endocrine elements of the pancreas is not known at this time. In addition, numerous studies demonstrate a functional role for angiotensin-(1-7) in the vasculature, brain and kidney that is mediated by a non-AT_1,-AT_2 receptor [29, 30]. Indeed, elevated levels of angiotensin-(1-7) contribute to the anti-hypertensive actions of ACE inhibitors and AT_1 receptor antagonists [31, 32]. Although we originally measured significant angiotensin-(1-7) levels in the dog pancreas, whether this peptide influences pancreatic function is also unknown.

Future Perspectives

The RAS cascade has historically been viewed as a key factor in the role of cardiovascular regulation primarily to maintain arterial blood pressure and water and sodium balance, with its regulation and expression predominantly controlled through the kidney. Although relatively few studies addressed the role of angiotensin II or other active fragments in the regulation of endocrine or exocrine aspects of the pancreas, substantial evidence indicates local systems in other tissues that may exhibit paracrine or autocrine actions. Indeed, the data from other tissues provide a new understanding that this important hormonal system is actually a pleiotropic system encompassing both vasopressor/depressor and proliferative/anti-proliferative actions. Moreover, the regulation of individual components of the system is tissue specific and may be under the control of local factors. Thus, the mechanisms of the angiotensin system now include multiple receptors, different ligands and a diverse number of target tissues including the pancreas.

Key words Angiotensin II; Angiotensinogen; Glucocorticoids; Pancreas; Peptidyl-Dipeptidase A; Protein-Tyrosine-Phosphatase; Receptors, Angiotensin

Abbreviations ARB: AT_1 receptor blocker; Sarthran: [Sar^1,$Thre^8$]-angiotensin II

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