

REVIEW

Tissue renin–angiotensin system: its expression, localization, regulation and potential role in the pancreas

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ABSTRACT

The classical concept of the renin–angiotensin system (RAS) is that of a blood-borne cascade, whose final and bioactive product, angiotensin II, plays an important endocrine role in the maintenance of blood pressure and electrolyte as well as fluid balance. In addition to this circulating RAS, there are an increasing number of studies to suggest the existence of a local angiotensin-generating system in several tissues. The so-called tissue RAS can act locally as a paracrine and/or autocrine factor in meeting specific needs for individual tissues and it can operate, in whole or in part, independently of the circulating counterpart. Recent studies on the expression and localization of key RAS components, particularly angiotensinogen and renin, have provided solid evidence for the existence of an intrinsic, angiotensin-generating system in the pancreas. The

tissue RAS has a potential role in finely regulating exocrine and endocrine functions of the pancreas such as ductal anion secretion and islet hormonal secretion. Some of these effects may be exerted via the markedly vasoconstrictive effects of RAS. Of particular interest in this context are the recent epidemiological data showing that administration of angiotensin-converting enzyme inhibitors appears to be protective against the development of diabetes in hypertensive patients. Moreover, the upregulation of pancreatic RAS has been shown to occur during chronic hypoxia. The significance of changes in pancreatic RAS could have a potential role in acute pancreatitis, islet transplantation and in different shock states, by causing a further decrease of blood perfusion in the pancreas.

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INTRODUCTION

Classically, the renin–angiotensin system (RAS) has been considered a hormonal circulating system. The so-called systemic or circulating RAS plays a crucial role in the maintenance of blood pressure. This is mediated through its constrictive actions on vascular smooth muscle and by its influence on aldosterone secretion from the adrenal cortex, electrolyte transport in kidney tubules (Page & Bumpus 1974, Peach 1977, Reid *et al.* 1978), and on thirst as well as sodium appetite in the brain (Fitzsimons 1998). In addition to its actions on the cardiovascular, renal and nervous systems, RAS has

many other actions on peripheral target tissues, from which it can elicit various specific responses for individual tissue functions (Dzau & Pratt 1986). For example, RAS has been previously demonstrated to play an important role in the regulation of various reproductive functions (Ganong 1995, Leung *et al.* 1999b, Speth *et al.* 1999).

There is a scarcity of information presently available on RAS in the pancreas and its potential roles in the regulation of pancreatic exocrine/endocrine functions are largely undefined. Of interest in this context is the notion of the role of angiotensinogen in essential hypertension, as described recently (Hata 1995). A strong association

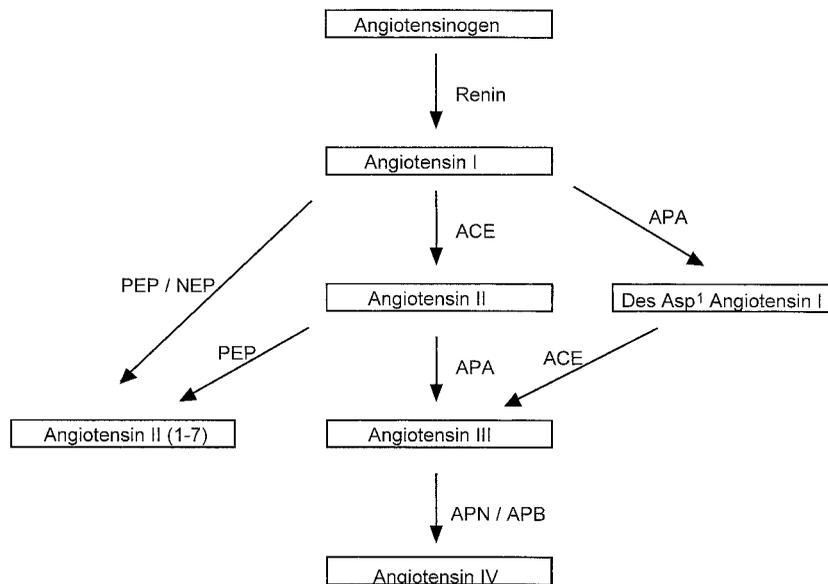


FIGURE 1. Schematic drawing of processing pathways for RAS. APA, aminopeptidase A; APN, aminopeptidase N; APB, aminopeptidase B; PEP, prolylendopeptidase; NEP, neutral endopeptidase.

seems to exist between essential hypertension and diabetes mellitus (Stern 1995). The beneficial effects of angiotensin-converting enzyme (ACE) inhibition for pancreatic insulin secretion in many hypertensive patients (Pollare *et al.* 1989, Santoro *et al.* 1992), therefore, underline the importance of our understanding of the physiological and pathophysiological roles of tissue RAS in the pancreas.

Future research on RAS in the pancreas could provide an insight into several pancreatic diseases including inflammatory diseases, diabetes mellitus, cystic fibrosis and cancer. It could also shed new light on our understanding of hypertension and pancreatic islet transplant physiology. This review will focus on the current knowledge of the expression, localization and regulation of a tissue RAS in the pancreas. In addition, the potential physiological and pathophysiological consequences of a tissue RAS in the exocrine and endocrine pancreas will be discussed.

CIRCULATING AND TISSUE RAS

The physiologically active endocrine product of the circulating RAS, angiotensin II, is spliced from its liver-derived precursor angiotensinogen (Menard *et al.* 1993) by a sequential action of two critical enzymes (Fig. 1). The first enzyme, renin, is secreted from juxtaglomerular cells in the kidney (Hackenthal *et al.* 1990). Renin splits a specific

leucine-leucine peptide bond in circulating angiotensinogen, resulting in the formation of the decapeptide angiotensin I. Angiotensin I is then carried via the blood to the lungs, where the second enzyme, ACE, removes two amino acids from the carboxy terminus of angiotensin I to form the active octapeptide angiotensin II. ACE is a membrane-bound enzyme anchored on the highest concentrations found on the vascular epithelium of the lung (Caldwell *et al.* 1976). Other enzymes may act on angiotensin I or angiotensin II to yield various active peptide fragments including angiotensin III, angiotensin II(1-7) and angiotensin IV (Fig. 1). Circulating angiotensin II and these bioactive peptide fragments are dispersed to the target tissues of the body, where they exert a multiplicity of physiological functions via their interactions with specific angiotensin receptors, such as AT_{1a} , AT_{1b} , AT_2 and AT_4 (Matsusaka & Ichikawa 1997, De Gasparo *et al.* 2000).

Recently, the focus has shifted from an endocrine role for RAS to an autocrine/paracrine role in specific tissue functions, such as tissue growth and differentiation. The localization and expression of key RAS components, notably angiotensinogen and renin, which are mandatory for the presence of a tissue RAS, have been reported in a range of tissues (Campbell & Habener 1986, Deschepper *et al.* 1986, Dzau *et al.* 1987). The expression of local RAS components in tissues such as the brain, heart,

kidneys, adrenals and gonads has led to the proposition that these components may either potentiate systemic functions, or have entirely separate activities meeting the specific needs of these individual tissues (Campbell 1987, Dzau 1989, Phillips *et al.* 1993). There is accumulating evidence that changes in tissue/organ-specific RAS may be associated with the pathophysiology of the respective tissue/organ functions. This gives rise to the possibility that drugs acting on tissue RAS might ameliorate some of these disorders. In fact, a recent study has suggested functional consequences of the action of drugs on tissue RAS (Zimmerman & Dunham 1997).

EVIDENCE FOR THE EXISTENCE OF A TISSUE RAS AND ITS EXPRESSION AND LOCALIZATION IN THE PANCREAS

The presence of angiotensin II, angiotensinogen protein and angiotensinogen mRNA have been documented in the dog pancreas (Chappell *et al.* 1991). Moderately high quantities of angiotensin II (524 ± 74 fmol/g tissue), angiotensin III (221 ± 54 fmol/g tissue) and angiotensin II(1–7) levels ($156 \pm$ fmol/g tissue) were found. The concentrations of these peptides were several times higher than those measured in blood, indicating that local generation of angiotensin II may occur in the canine pancreas. The notion for the existence of a tissue RAS in the rat pancreas has been consolidated based on the expression and localization of angiotensinogen, the mandatory component for an intrinsic RAS (Leung *et al.* 1999a). However, the concentration of angiotensinogen in the pancreas of both rats and dogs was low, and constituted only approximately 2–5% of circulating angiotensinogen concentrations (Chappell *et al.* 1991, Leung *et al.* 1999a). Using the Northern blot technique, angiotensinogen mRNA was not detected in the rat pancreas (Campbell & Habener 1986). This discrepancy may be explained by the reduced sensitivity of Northern blots, in combination with the possible degradation of angiotensinogen mRNA during its extraction from the RNAase-rich pancreas. In addition to angiotensinogen, renin mRNA has also been found to be expressed in the rat pancreas (Leung *et al.* 1999a), indicating that, at least in this species, a renin-dependent RAS is operative. However, further investigations are needed to confirm the renin-dependence in the angiotensin II-generating system of the rat pancreas. In the canine pancreas, neither angiotensin I nor renin activity has been detected (Chappell *et al.* 1991), suggesting that this pancreatic RAS may be renin-independent. Multiple

biosynthetic pathways of locally generating renin-independent RAS system have been proposed (Dzau 1989). Kallikrein, an enzyme capable of forming angiotensin II directly from its precursor angiotensinogen, has been reported to be present in the dog pancreas (Hojima *et al.* 1977).

Angiotensin II receptor subtypes have also been localized and characterized autoradiographically and pharmacologically in the pancreas. Preferentially, the AT₂ subtype was found to be expressed in different cell types of the pancreatic tissue in dogs (Chappell *et al.* 1991, 1992, 1995). More recently, specific binding sites for angiotensin II have been detected in both the endocrine and exocrine portions of the rat pancreas (Ghani & Masini 1995). Interestingly, these angiotensin II-binding sites were subject to modulation by sodium loading and depletion. Our recent immunohistochemical studies have shown that both the AT₁ and AT₂ receptor subtypes (Leung *et al.* 1997a) as well as angiotensin II (Leung *et al.* 1998) are localized to various rodent pancreatic cell types, but predominantly to the epithelia of pancreatic ducts and endothelia of blood vessels. Immunoreactivities for the AT₁ and AT₂ receptor subtypes as well as for angiotensin II are also found in the rodent acinar cells, although the immunoreactivity is weaker when compared with that observed in the pancreatic ducts and vasculature. In contrast, a diffuse distribution of both angiotensin II receptor subtypes has been observed throughout the exocrine and endocrine portions of the canine pancreas, i.e. in the acinar cells, duct cells and vascular elements, as well as in the pancreatic islets (Chappell *et al.* 1991, 1992). The discrepancy between the results in the rodent and those obtained in the canine could be due to species difference, or alternatively to the limitations of different techniques employed. A cellular localization of RAS components to the pancreatic islets has recently been reported in the human pancreas. In that study, AT₁ receptors and (pro)renin were localized not only to the exocrine part but also to the beta-cells of the endocrine part of the human pancreas (Tahmasebi *et al.* 1999).

Taken together, all these data support the existence of an intrinsic, angiotensin-generating system in the pancreas, which may be of importance in the regulation of pancreatic exocrine and endocrine functions.

REGULATION OF PANCREATIC TISSUE RAS BY CHRONIC HYPOXIA

The regulation of tissue RAS is subject to a number of factors such as hormones, ions and stress

(Phillips *et al.* 1993). Hypoxic stress is known to be one such factor which may lead to changes of tissue RAS expression in, for example, the kidney (Neylon *et al.* 1996), the lung (Morrell *et al.* 1995), the carotid body (Leung *et al.* 2000b), the epididymis (Leung *et al.* 2001) and the heart (Morrell *et al.* 1997). These data suggest that tissue RAS is differentially affected by hypoxia and is intimately involved in both the physiology and pathophysiology of the respective tissues.

We have recently demonstrated the activation of tissue RAS by a rat model of chronic hypoxia in the pancreas (Chan *et al.* 2000). Chronic hypoxia elicited a marked increase in mRNA and protein expression of angiotensinogen (Fig. 2). Moreover, there was a selective upregulation of mRNAs for the AT_{1b} and AT₂ receptor subtypes by chronic hypoxia. Such differential changes of pancreatic RAS components may be responsible for finely regulating the physiology and adaptation of the pancreas during chronic hypoxia, but could also trigger hypoxia-induced pancreatic injury such as acute pancreatitis (*vide infra*).

CURRENT EVIDENCE FOR A PHYSIOLOGICAL ROLE FOR TISSUE RAS IN THE PANCREAS

All these combined data suggest the presence of a pancreatic tissue RAS, which may have physiological effects via a paracrine/autocrine fashion in the exocrine and endocrine pancreas, e.g. in the regulation of pancreatic microcirculation, ductal anion secretion and islet hormonal secretion. In fact, recent results have demonstrated that such local RAS may influence ductal anion secretion in the rat exocrine pancreas (Chan *et al.* 1997, Cheng *et al.* 1999). In those studies, the presence of AT₁ receptors was described in a cystic fibrosis human pancreatic duct cell line, CFPAC-1, and the influence of electrogenic ion transport was demonstrated. The data indicate that angiotensin II may be of importance for normal exocrine function of the pancreas, particularly for the regulation of pancreatic ductal HCO₃⁻ secretion via activation of apical Cl⁻ channels. Similar effects of angiotensin II on electrogenic ion transport have also been observed in other forms of secretory epithelia, including those from the trachea (Norris *et al.* 1991), intestine (Cox *et al.* 1987) and epididymis (Leung *et al.* 1997b).

It has previously been shown that angiotensin II receptors influence prostaglandin synthesis (Jaiswal *et al.* 1990, 1991), which in turn may modulate the secretion of insulin and glucagon from the

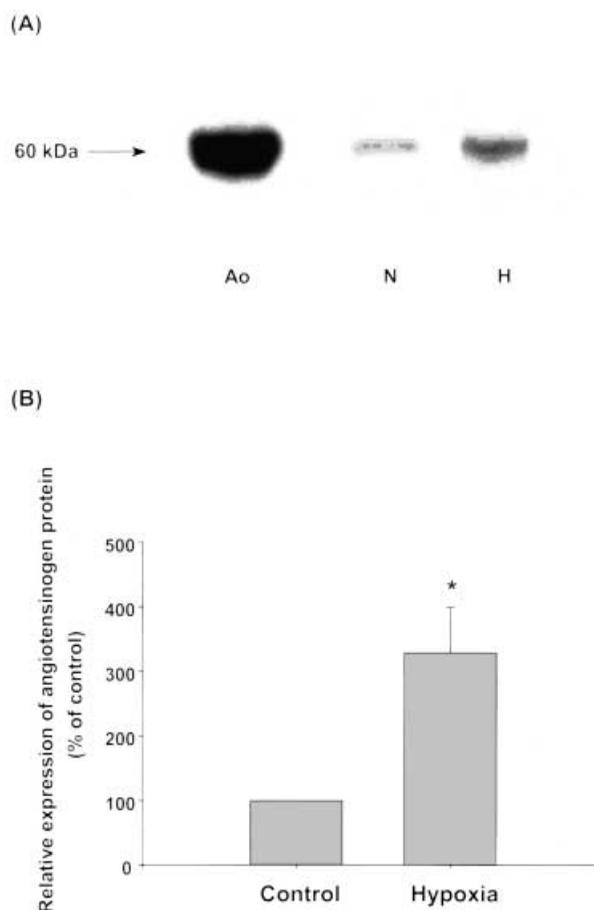


FIGURE 2. (A) Western blot analysis of angiotensinogen protein expression by chronic hypoxia in rat pancreas. Lane Ao shows plasma angiotensinogen purified from nephrectomized rat. A major band of about 60 kDa was detected. Lane N shows the expression by normoxic pancreas (10 µg protein) and lane H shows the expression by chronic hypoxic pancreas (10 µg protein). In this immunoblotting, the primary antibody is a rabbit anti-rat angiotensinogen serum (Thomas & Sernia 1988) at 1:5000 dilution and the secondary antibody is a peroxidase-labeled anti-rabbit IgG at 1:500 dilution. (B) Relative expression of angiotensinogen protein in hypoxic pancreas compared with that of normoxic pancreas. The data are expressed as means \pm S.E.M. ($n=5$ /group). Reproduced from Chan *et al.* (2000) with permission from *Molecular and Cellular Endocrinology*.

endocrine pancreas (Kelly & Laychock 1981). However, no effect of angiotensin II on insulin or glucagon release has been seen in experiments with isolated islets *in vitro* (Dunning *et al.* 1984). In a study where enalaprilate, an inhibitor of ACE, and saralasin, a non-selective angiotensin II receptor antagonist, were administered *in vivo* to rats, an increase in whole pancreatic blood flow, but

preferentially in islet blood flow, was discerned (Carlsson *et al.* 1998a). This finding suggests that islet microvessels produce higher concentrations of angiotensin II than those in the exocrine pancreas, and therefore may be more sensitive to ACE or angiotensin II receptor inhibition. Furthermore, islet blood flow seems to be suppressed by this locally produced angiotensin II during normal conditions. In view of the marked vasoactive effects of RAS, the influence on islet function secondary to angiotensin II-induced vasoconstriction was evaluated by measuring insulin concentrations in the effluents from isolated perfused rat pancreata (Carlsson *et al.* 1998a). In these preparations, addition of angiotensin II to the perfusion medium induced a marked vasoconstriction, which delayed the first phase of insulin release in response to glucose (Fig. 3). This suggests the crucial role of an intact islet blood perfusion for maintenance of an adequate insulin release. Interestingly, i.v. infusion of angiotensin II in a pressor dose (5.0 ng angiotensin II/kg per min) in humans suppressed both basal and pulsatile insulin secretion (Fliser *et al.* 1997). A subpressor dose of angiotensin II (1.0 ng/kg per min) could also suppress insulin secretion. Moreover, the insulinemic response was significantly lower and glucose concentration markedly higher after an oral glucose load when angiotensin II was infused compared with placebo. No study in man has been conducted regarding the influence of angiotensin II on islet blood flow. Vasoconstriction in the islets may nevertheless be regarded as the most likely mechanism for these findings, especially since a number of studies have failed to identify angiotensin II receptors on pancreatic beta-cells.

POTENTIAL PATHOPHYSIOLOGICAL ROLES FOR A TISSUE RAS IN THE PANCREAS

The etiology of acute pancreatitis has been considered to be multifactorial (Whitcomb 1999), although difference in the triggering mechanisms ends up with the same common pathway. This is the autodigestion of pancreatic tissue by the premature activation of proenzymes prior to their release into the duodenum (Wedgewood & Reber 1986). Some of the crucial factors may include activation of proteolytic enzymes, lipase, kinins and other vasoactive peptides such as angiotensin II (Agarwal & Pitchumoni 1993, Lembeck & Griesbacher 1996). In fact, it was previously reported that the plasma renin was significantly activated in acute pancreatitis, suggesting a role for RAS in the development of pancreatitis (Greenstein *et al.* 1987, Pitchumoni *et al.* 1988).

It has previously been reported that hypoxia could result in the decrease of regional blood flow to several tissues including the pancreas, which may enhance tissue inflammation and injury (Kuwahira *et al.* 1993). A vicious cycle of pancreatic microcirculatory changes such as vasoconstriction, capillary stasis, decreased oxygen tension and progressive ischemia has been shown to occur in the course of acute pancreatitis (Knoefel *et al.* 1994). A common etiological factor for pancreatitis is alcohol abuse. Interestingly, alcohol has been suggested to induce hypoxia in the pancreas, which could provide a mechanism for the development of pancreatic injury in this case (Foitzik *et al.* 1995). In view of the upregulation of pancreatic RAS by hypoxic stress and the marked vasoconstrictive effect of this system on pancreatic blood flow (*vide supra*), the role of RAS in acute pancreatitis may be worthy of consideration.

In a rat model of experimental pancreatitis, markedly increased expression of mRNAs for angiotensinogen was observed (Fig. 4). The expression of the AT₂ receptor was also markedly augmented during this condition (Leung *et al.* 2000a). The functional role for AT₂ receptors in many tissues, e.g. in the pancreas, remains unsettled. In addition, a significant increase in the mRNA expression for the AT_{1a} subtype, but not for the AT_{1b} subtype, was seen during pancreatitis. It appears that subtype-specific activation of AT₁ receptor could play a role in the pathophysiology of acute pancreatitis. However, species differences should be taken into consideration, particularly the fact that such subtypes have not been described in humans. On the other hand, the reasons for and the implications of an increased expression of subtype AT_{1a} during acute pancreatitis (Leung *et al.* 2000a) and of subtype AT_{1b} during chronic hypoxia (Chan *et al.* 2000) remain to be elucidated.

There are indications that activation of the pancreatic RAS occurs during exposure to other forms of stress, such as cardiogenic (Reilly *et al.* 1997), septic (Oldner *et al.* 1999) or trauma (Kincaid *et al.* 1998) shocks. In cardiogenic shock, a selective angiotensin II-mediated pancreatic vasoconstriction is seen that causes severe pancreatic ischemia/hypoxia (Reilly *et al.* 1997). In the two latter conditions, splanchnic blood perfusion is markedly improved following the treatment of angiotensin II receptor antagonism (Kincaid *et al.* 1998, Oldner *et al.* 1999).

Little information exists on pancreatic RAS in pancreatic cancer. An indication of a potential role is that ACE inhibitors decrease mitosis frequency and influence gene expression in pancreatic cancer cells (Reddy *et al.* 1995). Angiotensinogen gene expression has also been demonstrated in a cell line

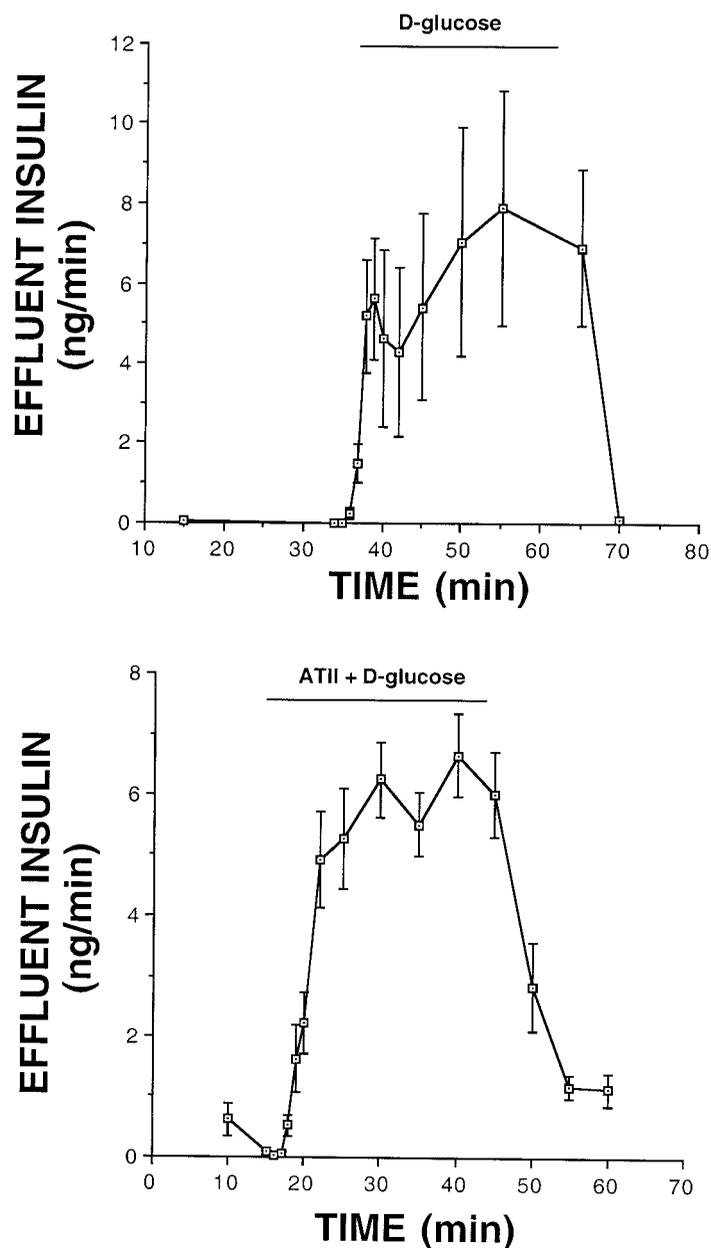


FIGURE 3. Insulin concentrations in effluent medium collected from perfused pancreata of male Sprague-Dawley rats. The upper panel shows insulin secretion in response to a 30 min period with 16.7 mmol/l D-glucose (bar) added to the perfusion medium. The lower panel shows insulin secretion in response to a 30 min period with 16.7 mmol/l D-glucose + 10 ng/ml angiotensin II (bar). Values represent means \pm s.e.m. for six or seven experiments. Reproduced from Carlsson *et al.* (1998a) with permission from *Diabetologia*.

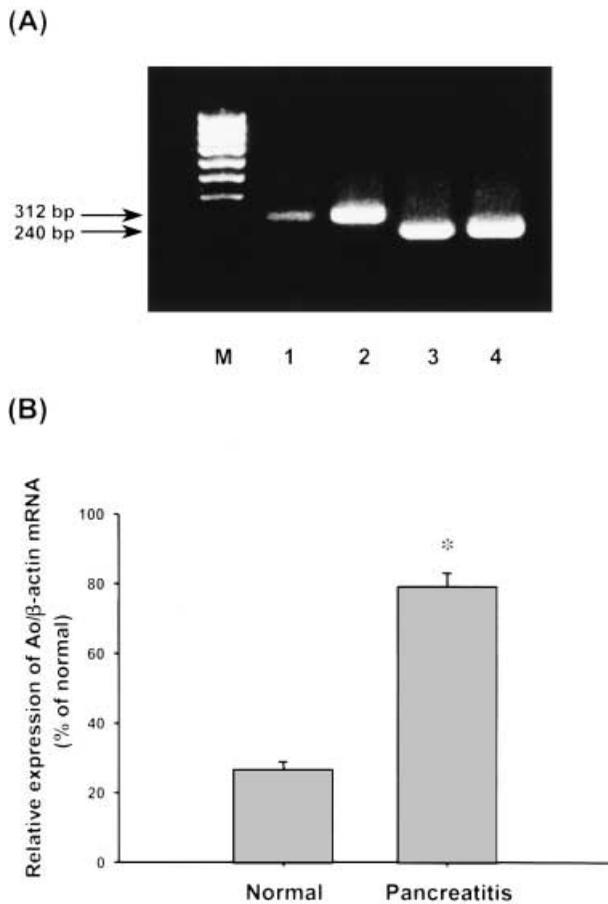


FIGURE 4. (A) RT-PCR analysis of angiotensinogen mRNA in rat pancreatitis. Lane M, DNA marker (X174 RF/HaeIII fragments); lane 1, angiotensinogen expression in normal rats; lane 2, angiotensinogen expression in pancreatitis rats; lane 3, β -actin expression in normal rats; lane 4, β -actin expression in pancreatitis rats. The arrows indicate the expected size of amplified products from angiotensinogen (312 bp) and β -actin (240 bp). (B) The relative expression of angiotensinogen/ β -actin mRNA. The data are expressed as means \pm S.E.M. ($n=5$ /group). Reproduced from Leung *et al.* (2000a) with permission from *Molecular and Cellular Endocrinology*.

derived from a pancreatic endocrine tumor in the rat (Brasier *et al.* 1986). The significance of this latter finding remains to be determined.

A strong linkage seems to exist between type 2 diabetes and essential hypertension (National High Blood Pressure Education Program Working Group 1994, Stern 1995). It has therefore been hypothesized that some factor(s) common to hypertension and diabetes may underlie the strong association between these diseases. Peripheral insulin resistance is commonly found in patients with essential hypertension and type 2 diabetes (Ferrannini *et al.*

1987). However, it seems that type 2 diabetes does not develop as long as the pancreatic beta-cells can secrete sufficient quantities of insulin to maintain a normal glucose homeostasis (Hellerstrom 1984). Interestingly, several studies in hypertensive patients receiving long-term treatment with ACE inhibitors have described an increased first phase insulin peak in response to i.v. glucose administration (Pollare *et al.* 1989, Haenni *et al.* 1994) or oral glucose (Santoro *et al.* 1992). The mechanism for this is unknown, since angiotensin II receptors have not been described on beta-cells. In view of the profound effects of angiotensin II on islet blood flow, it may be speculated that hyperactivity of the angiotensin system in islet vasculature can impair insulin release (cf. Carlsson *et al.* 1998a). Indeed, increased ACE concentrations occur in the mesenteric vasculature under the diabetic state, at least in animals (Jandeleit *et al.* 1992). An increased vasopressor responsiveness to angiotensin II in diabetic patients has also been observed (Christlieb *et al.* 1976, Drury *et al.* 1984). In addition, changes in vascular ACE seem to occur in various models of hypertension (Jandeleit *et al.* 1991). The influence of ACE inhibition on diabetes incidence has been evaluated in clinical trials. When patients at high risk of cardiovascular events, e.g. due to hypertension, were treated with the ACE inhibitor ramipril, a marked reduction in the incidence of diabetes was observed (Heart Outcomes Prevention Evaluation Study 2000). Similar findings were obtained in the Captopril Prevention Project randomized trial (Hansson *et al.* 1999). In view of these combined findings, it may be of importance to investigate more closely the role of the islet RAS in human diabetes and hypertension, especially with regard to tentative circulatory effects.

In a recent study, infusion of angiotensin II, in a dose that caused no changes in islet blood flow or vascular conductance in endogenous pancreatic islets, caused a marked decrease in both blood flow and vascular conductance in transplanted rat islets (Olsson *et al.* 2000). These data therefore suggest that the vascular response to angiotensin II in islet transplants is augmented compared with endogenous islets. Interestingly, a chronically marked decrease in graft tissue oxygen tension is seen after transplantation of pancreatic islets (Carlsson *et al.* 1998c, 2000, 2001). Whether upregulation of RAS occurs secondarily to low oxygen tension levels in the grafted islets remains to be determined. In either case, an increased sensitivity to RAS components may be deleterious for islet graft function. This could be by further reducing islet transplant blood perfusion (cf. Carlsson *et al.* 1998b,c, 2000, 2001), or by inducing the generation of free radicals (Griendling

et al. 1994, Ushio-Fukai *et al.* 1998, Warnholtz *et al.* 1999) harmful to the scavenger-deficient islets.

CONCLUSIONS

Recent studies on the expression and localization of key RAS components, particularly angiotensinogen and renin, have provided solid evidence for the existence of an intrinsic, angiotensin-generating system in the pancreas. This tissue RAS may play a potential role in regulating exocrine and endocrine functions of the pancreas such as ductal anion secretion and islet hormonal secretion. Such pancreatic RAS is subjected to the regulation of, for example, chronic hypoxia, and is affected during several other conditions, e.g. acute pancreatitis and different forms of shock. The significance of tissue RAS in the pancreas and its regulation by chronic hypoxia and other factors could be of potential importance in the physiology and pathophysiology of the pancreas. The target for the pancreatic RAS could provide an insight into several pancreatic diseases, including acute pancreatitis, pancreatic cancer, diabetes mellitus and cystic fibrosis. The pancreatic RAS could also have implications for pancreatic islet transplantation.

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