

ANGIOTENSIN II RECEPTOR BLOCKER VALSARTAN ENHANCES GLUCOSE-INDUCED INSULIN SECRETION

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SUMMARY: A number of antihypertensive drugs are known to be diabetogenic. This may contribute to less than expected decrease in the incidence of coronary heart disease with reduction in blood pressure with treatment in hypertensive patients. This study was aimed to determine the effects of a member, Valsartan, of a new class of drugs, angiotensin II receptor blocker, on glucose induced insulin secretion. Male albino rat pancreases were used. The isolated pancreases were perfused with Krebs's solution containing bovine albumin (200 mg/dl) with low glucose (60 mg/dl) followed by high glucose (300 mg/dl) at a rate of 4 ml/min. The dose of Valsartan used was based on the peak plasma level achieved in human at standard single oral dose of 80 mg daily, which was 1.64 mg/L. Five treatment groups were used: Control group, Valsartan at 10%, Valsartan at 100% and Valsartan at 10 times of the 1.64 mg/L, and Diazoxide 10 µg/ml group. Insulin levels in the perfusate were measured by radioimmunoassay. Valsartan at all concentrations significantly increases glucose induced insulin secretion ($p < 0.05$). Valsartan at 10 %, Valsartan at 100% and Valsartan at 10 times of the 16.4 mg/L, increases glucose induced insulin secretion by 226.4 %, 161.7 % and 156.3 %, respectively. Diazoxide, significantly inhibits glucose induced insulin secretion ($p < 0.05$).

Valsartan at all concentrations enhances glucose-induced insulin secretion in isolated rat pancreas technique.

Key Words: Angiotensin II, Valsartan, Pancreas, Hypertension, Insulin.

INTRODUCTION

A number of compounds used in the treatment of hypertension have been shown to impair insulin release (1-3). Disturbances of insulin release caused by antihypertensive drugs may be partly responsible for what has been termed the coronary artery disease paradox. This concept refers to the fact that success in reducing blood pressure

in groups of hypertensive patients who are using antihypertensive drugs fails to reduce mortality and morbidity from coronary artery disease (4). Hypertensive patients receiving pharmacological treatment (diuretics or beta-blockers) had an increased the risk of developing diabetes (5). A study on 12,550 non-diabetic adults who were followed for 6 years, experienced in a significantly increased risk (28%) of developing diabetes after treatment with beta blockers in contrast to diuretics, calcium antagonists and angio-tensin converting enzyme inhibitor (ACEI) (6). Several large clinical trials namely The Captopril Primary Prevention Project (CAPP) (7), Heart Outcomes Prevention Evaluation (HOPE) (8), and Losartan for Interventions for

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Endpoints in Hypertension (LIFE) (9) have demonstrated that blockade of the renin-angiotensin system (RAS) protects against development of diabetes in at risk patients with hypertension. The mechanism underlying this effect is unknown.

The RAS has long been known best for its haemodynamic regulation by means of two major angiotensin II (Ang II) receptor subtypes, AT₁ and AT₂ (10). The two definitive enzymes for this system are renin and angiotensin-converting enzyme (ACE), which determine the generation of the physiologically active peptide, Ang II. The RAS plays an important role in maintenance of blood pressure and electrolytes as well as in fluid balance. In addition to circulation RAS, tissue RAS can act locally as a paracrine and/or autocrine factor for the specific needs of individual tissues independently of the circulating counterpart. In recent years, existence of a local angiotensin-generating system in multiple tissue organs has been shown (11,12). Activity of the local RAS is an important determinant of structure and function in a range of organs, including the heart (13), adrenals (14), gonads (15), and pancreas (16). Recently, the evidence for the presence of a local angiotensin-generating system in the islets of the pancreas has been reported (17). This study demonstrated an inhibitory role for locally produced Ang II on glucose-stimulated insulin secretion, an effect mediated by the AT₁ receptors located on the surface of the islet beta cells. Ang II may also influence islet blood flow regulation in the pancreas, and hence influence insulin release in the isolated rat pancreas study (18). The importance of locally released Ang II in the regulation of both organ structure and function has demonstrated in the islets Zucker diabetic fatty rat (19). This means that blocking AT₁ and/or AT₂ receptor subtypes may influence the local angiotensin-generating system. The blockades of Ang II action by ACEI or angiotensin II receptor blocker (ARB) are commonly the medication of choice. It is however, to date, the effects of direct inhibition on locally produced Ang II particularly by the ARB are largely unknown. The nonpeptide ARB, valsartan, is potent, orally active and is selective for the Ang II receptors subtype AT₁ (20). The direct effect of valsartan on insulin release has not been investigated thus far. We hypothesized that based on previous evidences (17-19) ARB may have a great potential in interrupting the endocrine pancreatic function, probably the ability to inhibit the RAS at receptor levels. For that reasons this study was carried out to study the effect of valsartan, a member of

ARB class on insulin release *in vitro* preparation. The *in vitro* technique was chosen as it determines a direct effect of valsartan on the isolated rat pancreas in responses to glucose-induced insulin release.

MATERIALS AND METHODS

Drugs and treatment studied

The rats' pancreases were divided into five groups (6 pancreases per group). There were three groups of rats' pancreases treated with valsartan at three different concentrations. Another two control groups were carried out on different set of pancreases. There were a control group with no treatment and a group treated with diazoxide (10 µg/ml). Valsartan (CGP 48933: an active form) was obtained from Novartis Pharma AG Basel, Switzerland. Valsartan concentration was based on the peak plasma/serum concentration achieved in human subject with a single standard oral dose of 80 mg (21). Valsartan was dissolved in 1 molar KOH solution and was then made up to three different concentrations in the perfusate fluid namely, i) peak concentration achieved with a single standard oral dose 80 mg daily in human (1.64 mg/L), ii) at 1/10 of the above concentration (0.164 mg/L), and iii) at 10 times of the above concentration (16.4 mg/L). Diazoxide was purchased from Latoxan Corporation (rue Leon Blum, Valence France). Diazoxide (10 µg/ml) was used as a positive control and is a known potent inhibitor of insulin release. Diazoxide's molecular weight was 230.7. It is dissolved easily in methanol and slightly diluted in aqueous base. A concentration of 10 µg/ml of diazoxide was prepared in NaOH (10%) solution. The diazoxide solution was kept from light throughout the study by protecting the conicals with aluminium foil.

Surgical technique

Male Albino rats weighing approximately 250-350 gram were used. The rats were fed a standard laboratory diet (BARAS-TOC). The twelve hours fasted rats (*water ad libitum*) were anesthetized with pentobarbitone (60 mg/kg body weight) intraperitoneally. The pancreas was dissected according to the technique of Loubatieres *et al.* (22).

Perfusion technique

Bovine serum albumin (RIA grade) was obtained from Sigma Chemicals Company (St. Louis, Mo). The bovine albumin (200 mg/dl) was added to Krebs's solution. The isolated pancreas was perfused with Krebs's solution at 37°C containing bovine albumin (200 mg/dl) with low glucose (60 mg/dl) concentration for 5 to 10 minutes to stabilize the pancreas, and followed by taking a total of 5 samples (one sample each minute). This was switched to Krebs's solution containing high glucose (300 mg/dl) for a further 20 minutes. Samples were collected for 15 seconds at the beginning of each minute and were then frozen and stored at -20°C for future assay. The perfusate was bubbled with a mixture of 95% oxygen and 5% carbon dioxide. The system was non-recirculating and the perfusion was carried out using a roller pump at a con-

stant rate of 4.0 ml/minute. Insulin secretion was assayed as integrated insulin release in the first (1-5 minute) phase with low glucose and the second phase (6-25 minute) with high glucose.

Insulin measurement

The insulin in the perfusate was determined by a radioimmunoassay technique by using Linco Rat Insulin RIA Kit (LINCO Research, Inc. St. Charles, Missouri USA). This method utilizes ¹²⁵I-labeled insulin and a rat insulin antiserum for the quantitative determination of rat insulin in serum, plasma, perfusion fluid or other tissue culture media. In this procedure, a fixed concentration of labeled tracer antigen was incubated with a constant dilution of antiserum such that the concentration of antigen binding sites on the antibody was limited. If unlabeled antigen was added to this system, there was competition between labeled tracer and unlabeled antigen for the limited and constant number of binding sites on the antibody. Thus, the amounts of tracer bound to antibody were decreased as the concentration of unlabeled antigen increases. This can be measured after separating antibody-bound from free tracer and counting one or the other, or both fractions. A standard curve was set up with increasing concentrations of standard unlabeled antigen and from this curve the amount of antigen in unknown samples can be calculated by using a gamma counter. The sensitivity of the assay was 0.1 ng/ml. The insulin secretion at each time interval (minute) was expressed as ng insulin per minute per pancreas.

Ethics

The ethic committee of the Faculty of Medicine of IUM approved the protocol of this study. All animals received humane care according to the 'Guide for the Care and Use of laboratory animals' by the National Academy of Sciences.

Statistical analysis

The data were given as means ± SEM from the six different groups consisting of 6 pancreases per group. Statistical significance was assessed with Mann-Whitney U statistical test and the significance of the difference was taken at p < 0.05 by comparing the results of valsartan with that of the control. All analyses were performed with SPSS 11.5 statistical software for Windows.

RESULTS

Normal response of insulin release to low and high glucose concentrations

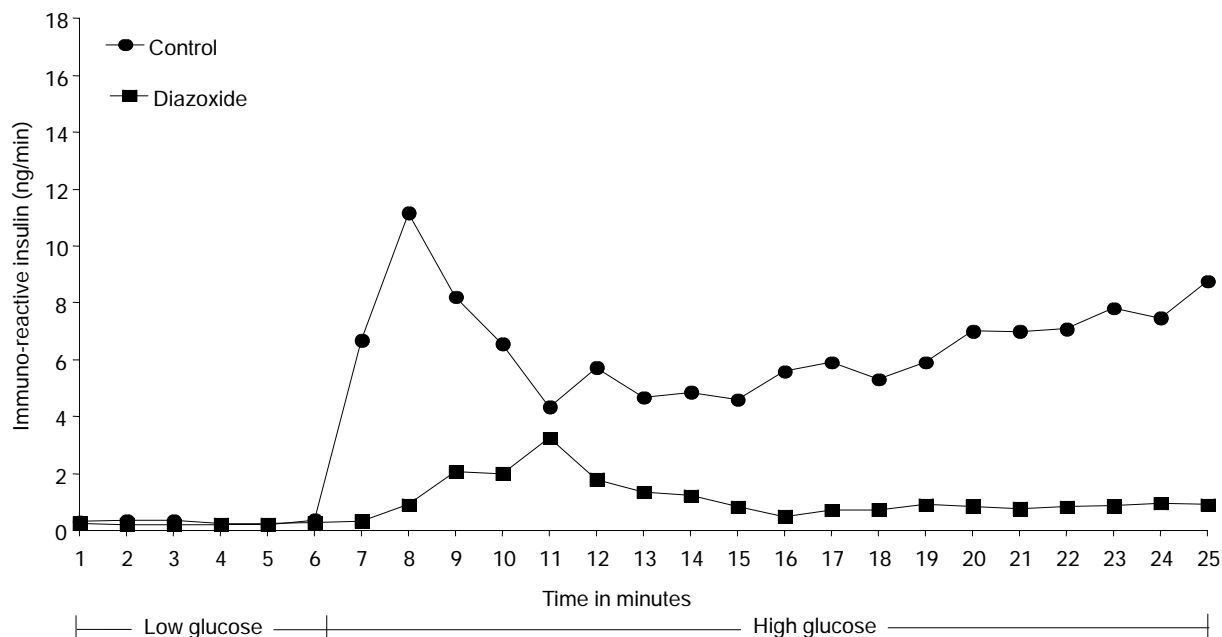
Six pancreases were used in the control group. A total of 6 samples were collected at low glucose concentrations, whereas 19 samples at high glucose concentrations. The details immuno-reactive insulin secretions are as shown in Table 1. At low glucose concentration, the release of insulin from the pancreas was approximately 0.31ng/minute. At high glucose concentration, insulin

Table 1: Effects of valsartan (0.164 mg/L, 1.64 mg/L and 16.4 mg/L) and positive control (diazoxide 10 µg/ml) on average insulin secretion/minute/pancreas in low and high glucose perfusion.

Group	Glucose Concentration	Sample	Mean immuno-reactive of insulin (ng/min) ^a ± SEM
Control n = 6	Low (60 mg/dl)	1 - 6	0.31 ± 0.03
	High (300 mg/dl)	7 - 25	6.56 ± 0.36
Valsartan 0.164 mg/L n = 6	Low (60 mg/dl)	1 - 6	1.20 ± 0.04
	High (300 mg/dl)	7 - 25	14.85 ± 1.70*
Valsartan 1.64 mg/L n = 6	Low (60 mg/dl)	1 - 6	0.69 ± 0.04
	High (300 mg/dl)	7 - 25	10.61 ± 1.25*
Valsartan 16.4 mg/L n = 6	Low (60 mg/dl)	1 - 6	1.02 ± 0.05
	High (300 mg/dl)	7 - 25	10.25 ± 1.26*
Diazoxide 10 µg/ml n = 6	Low (60 mg/dl)	1 - 6	0.23 ± 0.01
	High (300 mg/dl)	7 - 25	1.15 ± 0.16*

* p < 0.05 Significant

^aImmuno-reactive insulin in nanogram/minute

Figure 1: Effects of diazoxide (10 $\mu\text{g/ml}$) on insulin release (ng/min) in low and high glucose perfusion in isolated rat pancreas (n=6).

secretion was approximately 6.56 ng/minute. The biphasic response of insulin secretion to glucose was demonstrated as shown in Figure 1.

Effects of diazoxide on insulin release

Diazoxide is a potent inhibitor of insulin release. In this preparation, diazoxide 10 $\mu\text{g/ml}$ reduced the basal insulin secretion to approximately 0.23 ng/minute with low glucose, and with high glucose concentration, the release of insulin was significantly reduced (1.15 ng/minute, $p < 0.05$) compared to the control. The biphasic response was abolished (Figure 1).

Effects of valsartan on insulin release

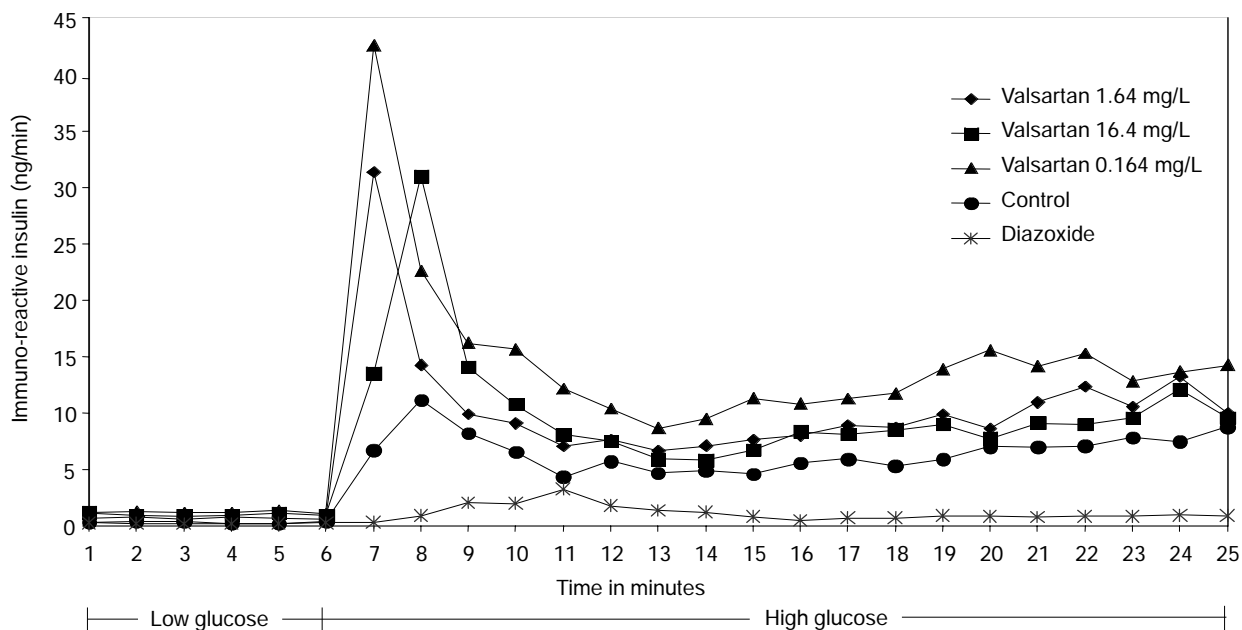
The 3 different concentrations of valsartan compared to the control value resulted in a significant increase of insulin secretion ($p < 0.05$) (Table 1). The patterns of insulin release to low and high glucose perfusion are as shown in Figure 2. Valsartan at 1/10 of the peak concentrations have been shown to stimulate insulin secretion greater than at peak and high concentrations. The amount of immunoreactive insulin (ng/pancreas/19 minutes) for valsartan at 0.164 mg/L, 1.64 mg/L and 16.4 mg/L were 14.85 ng/min, 10.61 ng/min and 10.25 ng/min, respectively, and the percentage observed were 226.4%, 161.7% and 156.3% compared to the control value, respectively (Figure 3).

DISCUSSION

The study was designed to measure the effects of antihypertensive drugs on insulin release. The technique in this study was adopted from that of Loubatieres *et al.* (22). This *in vitro* experiment was performed to identify the direct effects of the tested drugs on insulin release, an important determinant for glucose metabolism. Although, the study of insulin secretion can be performed *in vivo* (i.e. oral glucose tolerance test) instead of *in vitro*, the effects on insulin release observed *in vivo* are not necessarily due to the direct action on the beta-cells of the islet Langerhans. Other factors such as the effects of other endogenous mediators may be involved. In the present study, all concentrations of valsartan stimulated insulin secretion in isolated rat pancreas. The stimulation of insulin secretion by valsartan *in vitro* may partly suggest that the effect was exerted directly on the beta-islets cells of pancreas and/or possibly on the microvessels of pancreatic islets.

Valsartan has no agonist effect on the AT_1 receptors (21), however the present study has demonstrated that it seems to induce insulin secretion. A possible explanation for this is as follows. Firstly, Valsartan displaces Ang II from AT_1 subtype receptors, thus antagonizing the effects of Ang II (23). The antagonistic effect of valsartan may subsequently cause less inhibitory effect of Ang II on AT_1 receptors on the endocrine, exocrine and vascular cells of

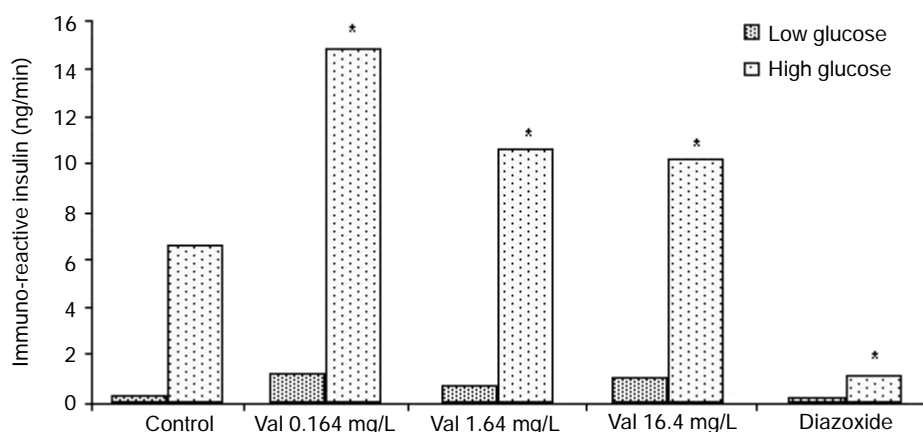
Figure 2: Effects of valsartan (0.164 mg/L, 1.64 mg/L and 16.4 mg/L) on insulin release (ng/min) in low and high glucose perfusion in isolated rat pancreas (n=6).



pancreas. This is based on the fact that the presence of an intrinsic RAS in the rat pancreas may play a role in the regulation of pancreatic functions (24). Therefore, the less inhibitory effect of Ang II would subsequently increase insulin release particularly in the presence of valsartan in the high glucose concentration. Secondly, there is a possi-

bility that valsartan has an effect on microvessels of the pancreas, in which it may play an important role in regulating insulin release. Carlsson *et al.* (18) found that the first phase of insulin release was delayed in response to glucose with the presence of Ang II (exogenous) in the medium. This was presumably due to the vasoconstrictive

Figure 3: Effects of valsartan (0.164 mg/L, 1.64 mg/L and 16.4 mg/L) on average insulin/min/pancreas (Mean ± SEM) in low and high glucose perfusion in isolated rat pancreas (n=6, *p<0.05).



action of Ang II. With the presence of valsartan, it may inhibit the vasoconstrictive effect of the basal intra-pancreatic Ang II. This would lead to vasodilatation of the intra-pancreatic microvessels. Even though, the evaluations of Ang II effects were beyond the scope of the present study, the influence of Ang II on insulin release is still significant (17). Thirdly, some of the hormones, neurotransmitters, and neuropeptides were indirectly involved in the regulation of insulin release. It is possible that valsartan may inhibit the intra-pancreatic Ang II or neurotransmitters. Thus modulates the insulin release. It has been demonstrated that Ang II receptors influence prostaglandin synthesis (25,26), which in turn may modulate secretion of insulin and glucagons (27). On the other hand, Ang II may also modulate the secretion of other regulatory pancreatic hormones such as cholecystokinin, pancreatic polypeptide or somatostatin, which then influence alpha-cell or beta-cell functions. However, this needs to be shown by further research.

Carlsson *et al.* (18) reported that the RAS is important in regulation of islet blood flow and points to a pivotal role of islet blood perfusion for an adequate insulin release. In isolated perfused rat pancreas study (18), enalaprilate affected neither basal nor glucose-stimulated insulin release, whereas Ang II delayed the first phase of insulin release in response to glucose. The effect of Ang II was shown to be due to vasoconstriction, and suggests a crucial role of intact islet blood perfusion for maintenance of an adequate insulin release (18). These findings were further supported by the fact that an intrinsic RAS was demonstrated in the rat pancreas which may play a role in the regulation of pancreatic functions (24). Despite the putative presence of angiotensin receptors in the pancreas (28), Ang II has been reported not to have marked effects on the release of insulin or glucagon from isolated rat islets (29). However, the islets used in these *in vitro* studies lack both blood supply and innervation, which is likely to affect their response to Ang II. In a human study, Ang II has demonstrated a potent vasoconstrictor function, which reduce the total, basal, and pulsatile insulin secretion at pressor dose and tended to suppress insulin secretion at subpressor dose in humans (30). The amount of insulin released that was measured in this preparation may be partly influenced by the inhibition effect of valsartan on Ang II. Therefore, this may partly suggest that the presence of the RAS component in the pancreas would be also

involved in the regulation of insulin release. A direct interaction of signaling of the AT₁ receptor and insulin receptor in the pathogenesis of insulin resistance was recently suggested (31). The authors found that valsartan increased insulin sensitivity and glucose uptake in skeletal muscle of KK-Ay mice via stimulating the insulin signaling cascade and consequent enhancement of GLUT4 translocation to the plasma membrane (31). This finding might suggest the involvement of ARB, valsartan in particular on insulin sensitivity and insulin receptors.

A potential effect of valsartan on insulin secretion is intriguing as this agent may or may not provide clinically meaningful progress toward lowering the development of coronary artery disease in hypertensive subjects. The fact that some of the antihypertensive drugs were known to inhibit insulin release, means that the patients are predisposed to other risk factors of CHD. Our results showed that valsartan produced no diabetogenic effect and is suitable for the management of hypertensive and hypertensive diabetic patients. In conclusion, valsartan at all concentrations based on the peak plasma concentration achieved with a single standard oral dose of 80 mg daily stimulates insulin secretion in isolated rat pancreas. These observations may be of relevance in the management of hypertension, especially in hypertensive subjects with diabetes.

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