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L-arginine stimulates insulin secretion from the pancreas of normal and diabetic rats

Short Communication

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Summary. Several reports have shown that nitric oxide (NO) stimulates glucose-induced insulin secretion in the pancreas of normal rat but the effect of L-arginine (a NO donor) on insulin secretion from the pancreas of diabetic pancreas is unknown. Fragments of pancreatic tissue from normal and diabetic rats were incubated for 45 min in Krebs solution containing 100 mM L-arginine. The supernatant was subsequently analyzed for the insulin content using radioimmunoassay technique. L-arginine evoked large increases in insulin secretion from the pancreas of diabetic rat. The insulin secreted from the pancreas of diabetic rat was numerically but not significantly lower compared to that of normal rat pancreas. In conclusion, L-arginine, a nitric oxide donor stimulates insulin secretion from the pancreas of diabetic rats.

Keywords: Amino acids – Insulin secretion – Radioimmunoassay – L-Arginine – NO – Pancreas – Diabetes mellitus – Rat

Introduction

Nitric oxide (NO) is produced by the oxidation of L-arginine. The conversion of L-arginine to NO and L-citrulline is catalyzed by a NADPH and Ca^{2+}/calmodulin dependent enzyme, nitric oxide synthase (NOS) (Anggard, 1994). Neural NOS has been shown to be present in the nerves innervating the pancreas of normal (Adeghate and Parvez, 1998) rat. Neural NOS has been detected specifically in pancreatic islet alpha and delta cells (Spinas et al., 1998) and by Western blotting (Tsuura et al., 1998). The close relationship of NOS-positive cells and nerve fibres with pancreatic acinar and endocrine cells explains why NO plays significant role in the regulation of pancreatic func-

tion. Many reports have shown that L-arginine, through NO stimulates insulin secretion (Schmidt et al., 1992; Spinas et al., 1998). In contrast to this, some other literature reports suggest that NO is a negative modulator of insulin secretion (Akesson et al., 1996). A more recent report by Thams and Capito, 1999) showed that L-arginine potentiation of glucose-induced insulin secretion occurs independently of NO but is mediated via membrane polarization. It was also demonstrated that L-arginine stimulates insulin-mediated glucose uptake in human (Paolisso et al., 1999). The study was designed to examine whether L-arginine, a NO donor can stimulate insulin secretion from the isolated pancreatic tissue fragments of diabetic rats.

Materials and methods

Animals and induction of diabetes mellitus

Twelve-week old male Wistar rats weighing approximately 250 g were used throughout this study. Rats were obtained from the United Arab Emirates University breeding colony and the Animal Research Group's guidelines for the care and use of laboratory animals were followed. The rats were divided into two groups, streptozotocin (STZ)-induced diabetics and age-matched controls. Diabetes was induced by a single intraperitoneal injection of STZ (Sigma, Poole, UK) at 60 mg kg^{-1} body weight prepared in 5 mM citrate buffer pH 4.50 (Adeghate, 1999). The animals were kept in plastic cages and maintained on standard laboratory animal diet with food and water *ad libitum*. The blood glucose estimations were made by One Touch II© Glucometer (LifeScan, Johnson and Johnson, USA) for each individual animal. The animals were considered diabetic if the random blood glucose levels were equal to or more than 300 mg/dl. After six weeks from induction of diabetes all the animals from both groups were sacrificed under chloral hydrate general anesthesia by an intraperitoneal injection of 7% chloral hydrate 6-ml kg^{-1} of body weight. A mid-line abdominal incision was made, and the pancreas was rapidly removed and placed in ice-cold Krebs solution. Representative fragments were taken from the tail end of the pancreas.

Estimation of in vitro pancreatic insulin release

The pancreases of six normal and six diabetic rats were removed and placed in ice-cold Krebs buffer (KB). The pancreases were trimmed free of adherent fat and connective tissue and cut into small fragments (1–2 mm^3). The pancreatic fragments were placed in 2 ml glass vials containing 1 ml of KB and pre-incubated for 30 min in a waterbath at 37°C. In order to wash away any hormones due to cutting of the tissues. After the pre-incubation period KB solution was drained and the fragments were subsequently incubated for 45 min with of 100 mM L-arginine. In the control experiments, the fragments were incubated in KB solution alone for same duration. During the incubation period, each vial was gassed with 95% oxygen and 5% carbon dioxide every 10 min. At the end of experiment the tissues were removed, blotted, weighed and the supernatant stored at −20°C for radioimmunoassay.

Radioimmunoassay

Insulin was determined by using a modified method of Herbert et al. (1965). Insulin measurement was performed using DPC® (Los Angeles, CA, USA) radioimmunoassay kits. All test samples and controls were assayed in duplicates, using tubes coated with

antibodies to insulin. A volume of 200 μl of either calibrators, controls or test samples were pipetted to previously labelled tubes followed by 1 ml of $^{[125]}$ I-Insulin. Each tube was vortexed and incubated for 24 h at room temperature. After the incubation period, the tubes were decanted for 3 min and radioactivity was counted for 1 min using gamma counter (Beckman, Fullerton, CA, USA). Results were analyzed by using a Beckman Immunofit EIA/RIA analysis software, version 2.00. Values were expressed in μIU ml^{-1} (100 mg tissue)$^{-1}$.

Statistical analysis

All values were expressed as mean ± standard error of the mean (SEM). Statistical significance was assessed using Students' *t*-test. Values with $p < 0.05$ were accepted as significant.

Results

Figure 1 shows a family of histograms depicting the effect of L-arginine (100 mM L^{-1}) on insulin secretion from the isolated pancreatic tissues of nor-

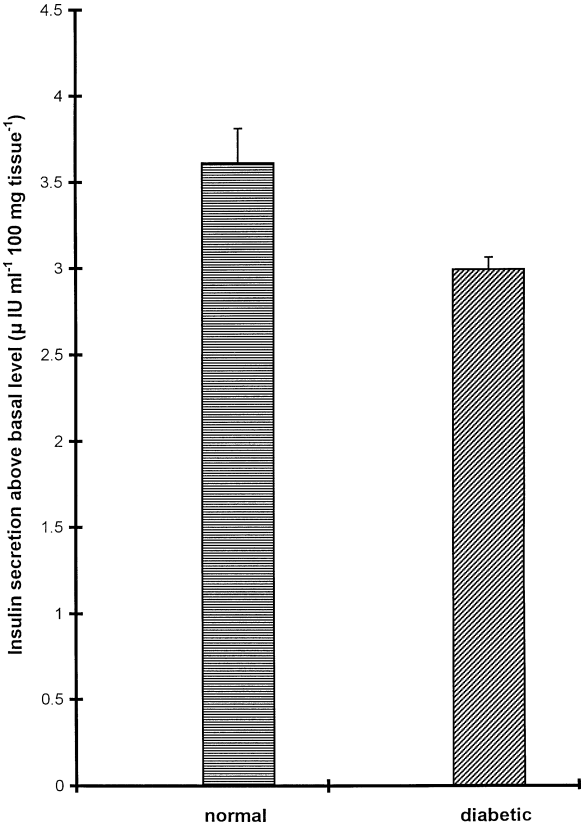

Fig. 1. Family of histograms showing insulin secretion from pancreatic tissue fragments of normal (n = 6), and diabetic (n = 6) rats after incubation with 100 mM L-arginine for 45 min. Note that there is no statistically significant difference between insulin secretion from normal and diabetic rat pancreatic fragments

mal and diabetic rats. The value of insulin secreted (above basal level) from the pancreas of normal (n = 6) and diabetic (n = 6) rats were 3.61 ± 0.2 and 2.99 ± 0.07 μIU ml^{-1} 100 mg tissue^{-1}, respectively. Basal insulin secretion from the pancreas of normal and diabetic rats were 8.46 ± 1.04 and 5.27 ± 1.05 μIU ml^{-1}100 mg tissue^{-1}, respectively. L-arginine stimulated insulin secretion from the pancreatic tissue fragments of both normal and diabetic rats. The insulin secreted from the pancreas of diabetic rat was numerically but not significantly lower compared to that of normal rat pancreas.

Discussion

The present study shows that L-arginine, a nitric oxide (NO), donor can stimulate insulin secretion from the isolated pancreatic tissue fragments of normal and diabetic rats. It is of great interest that L-arginine was able to stimulate insulin secretion from diabetic rats which has a relatively lower pool of insulin reserve. It is well known that other secretagogues like vasoactive intestinal polypeptide or acetylcholine can stimulate insulin secretion from diabetic rat pancreas (Adeghate et al., 2000a, b). This shows that the insulin reserve in diabetic rats is not completely depleted. A complete depletion of insulin would probably be incompatible with life. What is the possible mechanism of action of L-arginine on stimulating insulin secretion from the rat pancreas? It is possible that L-arginine stimulates insulin secretion directly since it is known that a number of amino acids, including arginine can stimulate insulin secretion (Guyton, 1981). In fact many reports have shown that L-arginine stimulates insulin secretion via membrane depolarization and not through the mediation of NO. It was shown that L-arginine causes membrane depolarization, which stimulates insulin secretion through protein kinase A- and C-sensitive mechanisms (Thams and Capito, 1999). On the other, it is also possible that L-arginine stimulates insulin secretion via NO. It has been demonstrated that NO mediates the stimulatory action of L-arginine on insulin secretion in normal human (Coiro et al., 1997) and animal subjects (Schmidt et al., 1992). NO can also influence insulin secretion independently of membrane depolarization events (Salehi et al., 1998). The result of this study did not agree with those investigations, which reports that islet NO system is a negative modulator of insulin secretion (Akesson et al., 1996).

In conclusion, L-arginine, a NO donor, is capable of stimulating insulin secretion from pancreatic tissue fragments of diabetic rat. This indicates that L-arginine is a strong secretagogue of insulin secretion bearing in mind that the insulin content of diabetic rat pancreas is significantly lower than that of normal pancreas.

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