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ORIGINAL ARTICLE

Mechanism of ipamorelin-evoked insulin release from the pancreas of normal and diabetic rats

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Abstract

OBJECTIVE: To examine the effect of ipamorelin (IPA), a novel pentapeptide with a strong growth hormone releasing potency, on insulin secretion from pancreatic tissue fragments of normal and diabetic rats.

MATERIALS AND METHODS: Diabetes mellitus was induced by streptozotocin (60 mg kg^{-1}). Four weeks after the induction of diabetes, pancreatic tissue fragments of normal and diabetic rats were removed and incubated with different concentrations (10^{-12}–10^{-6} M) of IPA. Insulin release from the pancreas was measured by radioimmunoassay.

RESULTS: Ipamorelin evoked significant ($p<0.04$) increases in insulin secretion from the pancreas of normal and diabetic rats. Either diltiazem or yohimbine or propranolol or a combination of atropine, propranolol and yohimbine inhibited IPA-evoked insulin secretion significantly ($p<0.03$) from the pancreas of normal and diabetic rats. Atropine caused a significant ($p<0.007$) reduction in the IPA-induced insulin secretion in diabetic but not in normal rats.

CONCLUSION: IPA stimulates insulin release through the calcium channel and the adrenergic receptor pathways. This is the first study to examine the effect of ipamorelin on insulin secretion in the pancreas.

Introduction

Ipamorelin (IPA) is a newly discovered pentapeptide with a strong potency for growth hormone release in vivo and in vitro [1]. The administration of IPA significantly increases body weight in rats [2, 3]. IPA can also cause an increase in bone mineral content in rats [4] and induce an increased serum leptin level and food intake in growth hormone-intact mice [5]. For example IPA, when given at 100 microg/kg three times daily, can counteract glucocorticoid-induced decrease in muscle strength and bone formation in rat [6]. The physiological role of IPA is similar to those described for ghrelin [7–8]. In fact, ghrelin has been shown to regulate insulin release from the pancreas of normal and diabetic rats [7]. IPA increases the percentage of somatotroph cells,

without modifying the ratio of strongly/weakly immunostained GH cells. Moreover, IPA can increase intracellular GH content suggesting that IPA is able to exert a dynamic control on the somatotroph population and on GH hormone content of rat [9].

All of the above properties of IPA indicate that IPA may have a potential role for the treatment of growth retardation and associated illnesses. Since insulin is important in the control of body weight and normal growth, it is pertinent to examine the role of IPA on insulin secretion. The aim of this study was to examine whether IPA has a role in the regulation of insulin secretion from the pancreas of normal and diabetic rats. The study was also designed to examine the mechanisms by which IPA influences insulin secretion.

Materials and Methods

Experimental animals and induction of diabetes mellitus

Twelve-week old male Wistar rats weighing approximately 250 grams were used in this study. The rats were divided into two groups, streptozotocin (STZ)-induced diabetics (n = 8) and age-matched controls (n = 8). Diabetes mellitus was induced by a single dose of STZ at 60 mg kg^{-1} according to a previously described method [10]. Four weeks from the date of induction of diabetes, all rats from both groups were sacrificed under chloral hydrate general anesthesia (7% chloral hydrate 6-ml kg^{-1} of body weight, injected intraperitoneally). The abdomen was opened and the pancreas was expeditiously removed and placed in ice-cold Krebs buffer (KB). The study was in line with the guidelines of the Animal Ethics Committee, FMHS, UAE University, Al Ain, United Arab Emirates.

Determination of insulin release from pancreatic fragments of normal and diabetic rats

The pancreata of normal and diabetic rats were cut into small pieces and trimmed free of adherent fat and connective tissue and minced into small fragments (0.5–1 mm^3). The pancreatic tissue 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 hormone due to cutting of the tissues. Immediately after the pre-incubation step the KB solution was carefully drained and the specimens were subsequently incubated for 1 h with different concentrations (10^{-12} – 10^{-6} M) of ipamorelin (IPA) or with IPA (10^{-9} M) and atropine (10^{-6} M); IPA (10^{-9} M) and yohimbine (10^{-6} M); IPA (10^{-9}M) and propranolol (10^{-6} M), IPA (10^{-9} M) and diltiazem (10^{-6} M) and IPA (10^{-9} M) and atropine and yohimbine and propranolol (10^{-6} M). In the control experiments, the isolated pancreatic fragments were incubated in KB solution alone for the same period. The vials were gassed with a mixture of 95% oxygen and 5% carbon dioxide every 3 min. At the end of the incubation period the pancreatic tissue fragments were removed, blotted, weighed and the effluent stored at –20°C for insulin radioimmunoassay.

Radioimmunoassay

The insulin content of the effluent was determined by using the protocol provided by Linco® (St. Charles, MO, USA), the vendor of the insulin radioimmunoassay kit used in this study. All test samples and controls were assayed in duplicates. A volume of 100 μl of either calibrators, controls or test samples were pipetted into previously labelled tubes followed by 100 μl of $^{[125]}$I-Insulin. Each tube was vortexed and incubated for 24 h at room temperature. After the incubation period, the tubes were centrifuged and 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 RIA analysis software version 2.00 and values were expressed in ng ml^{-1} (100 mg tissue)$^{-1}$. Pancreatic tissue fragments were homogenized in KB solution to determine pancreatic insulin content of normal and diabetic rats.

Chemicals and Statistical analysis

Ipamorelin (Catalogue # SC1329) was obtained from Neosystem® (Strassbourg, France). All other chemicals were purchased from Sigma (Poole, UK) unless otherwise specified. All values were expressed as mean ± standard deviation (SD). Statistical significance was assessed using paired Student's *t*-test. Values with $p < 0.05$ were accepted as significant.

Results

The effect of different concentrations (10^{-12}–10^{-6} M) of ipamorelin (IPA) on insulin secretion from pancreatic fragments of normal and diabetic rats is shown in figure 1. IPA evoked large and significant ($p< 0.04$) increases in insulin secretion from the pancreas of normal rats when compared to basal. In normal rat the stimulatory effect of IPA on insulin secretion from the pancreas was maximal at 10^{-9} M and weakest at 10^{-12} M. IPA also induced a large and significant ($p<0.004$) increases in insulin secretion from the pancreas of diabetic rats at 10^{-9} M. The pancreatic insulin content in normal and diabetic rats was 21.87 ± 1.3 and 3.2 ± 0.2 ng ml^{-1} (100 mg tissue)$^{-1}$, respectively. If the pancreatic insulin contents are compared to the insulin released from the pancreas of both normal [1.06 ± 0.1 ng ml^{-1} (100 mg tissue)$^{-1}$] and diabetic [0.13 ± 0.01 ng ml^{-1} (100 mg tissue)$^{-1}$] rats after incubation with 10^{-9} M of IPA, it becomes clear that the potency of IPA in stimulating insulin release from the pancreas of normal rat (4.8%) is comparable to that on diabetic rats (4.0%).

The effect of cholinergic, α- and β-adrenergic receptor antagonists on IPA-evoked insulin release is depicted in figure 2. Either yohimbine (α2-adrenergic receptor antagonist or propranolol (β-adrenergic antagonist) or diltiazem (calcium channel blocker) or a combination of atropine and yohimbine and propranolol caused a significant ($p<0.03$) reduction in IPA-induced insulin secretion from the pancreas of normal rats. Atropine induced a small but not significant reduction in IPA-induced insulin secretion from the pancreas of normal rats. It was interesting to observe that,

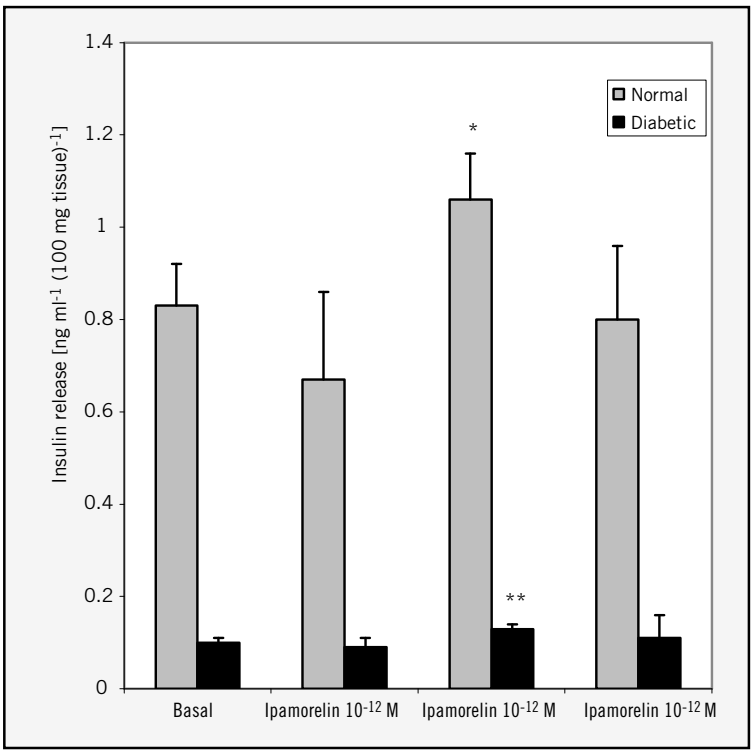

Figure 1 shows the effect of ipamorelin on insulin secretion from pancreatic tissue fragments of normal and diabetic rats. Basal insulin output is also shown for comparison. (Data are mean ± SD, n=8). Note that ipamorelin at 10^{-9} M evoked a large and significant ($p<0.04$)* increase in insulin release when compared with basal. In diabetic rat pancreas ipamorelin strongly ($p<0.0004$)** stimulates insulin secretion when compared with basal.

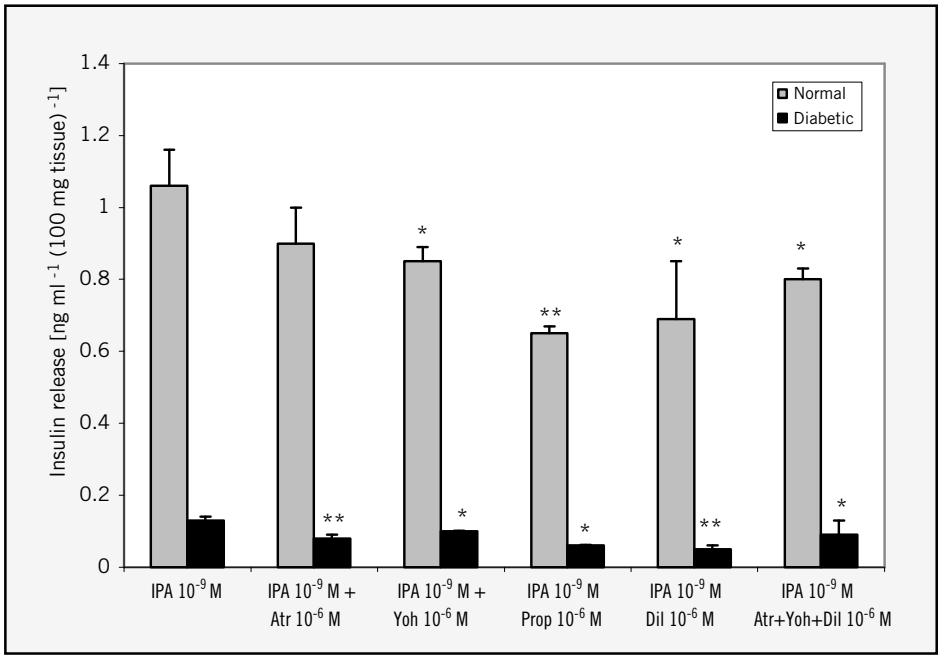

Figure 2 shows the effect of cholinergic, adrenergic and calcium channel antagonists on ipamorelin (IPA)-evoked insulin release from the pancreas of normal and diabetic rats. (Data are mean ± SD, n = 8). Note that yohimbine (Yoh) or propranolol (Prop) or diltiazem (Dil) or a combination of atropine (Atr) and yohimbine and propranolol caused a significant ($p<0.03$) reduction in IPA-induced insulin secretion from the pancreas of normal and diabetic rats. Atropine induced a small but not significant reduction in IPA-induced insulin secretion from the pancreas of normal rats, but it induced a significant ($p < 0.007$) reduction in IPA-evoked insulin release in diabetic rat pancreas.
* = $p< 0.03$ (IPA + antagonist versus IPA alone);
** = $p< 0.007$ (IPA + antagonist versus IPA alone).

either atropine, yohimbine or propranolol or diltiazem or a combination of atropine and yohimbine and propranolol significantly ($p<0.05$) inhibited IPA-induced insulin release from the pancreas of diabetic rat.

Discussion

The ability of ipamorelin (IPA) to equally stimulate insulin release from the pancreas of both normal and diabetic rats shows that IPA could be of use in the treatment of type II diabetes. IPA may also help in the stimulation of insulin release from diabetic patients with some viable pancreatic beta cells. Since this is the first study to examine the effect of IPA on insulin secretion, it is not possible to compare the results obtained in this study with other reports. However, previous reports have shown that IPA can significantly increase body weight [2, 3] and bone mineral content [4]. The ability of IPA to increase food intake and subsequent weight gain may be exerted through the stimulation of insulin release. The insulin that is released through the action of IPA may in turn stimulate glucose uptake and thus increase the body weight of the subject. The ability of the muscarinic receptor antagonist, atropine, and alpha (yohimbine) and beta (propranolol) adrenergic receptor antagonist to inhibit IPA-induced insulin secretion is interesting. It thus indicates that IPA may act on pancreatic beta cells through muscarinic cholinergic and adrenergic receptors on the cell membrane of pancreatic beta cells. In conclusion, IPA stimulates insulin secretion through a number of pathways including cholinergic and $\alpha 2$ and β-adrenergic receptor pathways and calcium channels.

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