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ORIGINAL ARTICLE

Mechanism of ghrelin-evoked glucagon secretion from the pancreas of diabetic rats

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Abstract

OBJECTIVE: Ghrelin is a newly discovered peptide, which was first demonstrated in the epithelium of rat stomach. The purpose of the study was to examine the effect of ghrelin on glucagon secretion from pancreatic tissue fragments of normal and diabetic rats.

METHODS: Diabetes was induced by streptozotocin (60 mg Kg body weight^{-1}) given intraperitoneally. Four weeks after the onset of diabetes, pancreatic fragments of normal and diabetic rats were incubated with different concentrations (10^{-12}–10^{-6} M) of ghrelin. Glucagon release was measured using radioimmunoassay technique.

RESULTS: Ghrelin failed to stimulate or inhibit glucagon secretion from normal rat pancreas. However, it induced significant increases in glucagon secretion from pancreatic tissue fragments of diabetic rats. Either atropine (muscarinic cholinergic receptor antagonist) or propranolol (β-adrenergic receptor antagonist) or yohimbine (α2-adrenergic receptor antagonist) or diltiazem (calcium channel antagonist) did not affect ghrelin-glucagon interaction. Moreover, a combination of atropine, propranolol and yohimbine had no significant effect on the interaction of ghrelin with glucagon.

CONCLUSION: The ghrelin-induced glucagon secretion in diabetic rats is not controlled via cholinergic or adrenergic pathways. In conclusion, it appears that the main target of ghrelin in the rat endocrine pancreas is not glucagon-producing cells but rather insulin secreting cells which are more involve in weight gain and body growth.

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Introduction

Ghrelin is a newly discovered endogenous ligand for the growth hormone receptor. Ghrelin can stimulate food intake [1] and growth hormone release in lower vertebrates [2] as well as in humans [3]. Ghrelin can stimulate gastric acid secretion [4] as well as adiposity [5] in mammals. Ghrelin has been shown to be present in the pancreas and other peripheral tissues [6]. Ghrelin is present in the arcuate nucleus of the human hypothalamus and pituitary gland [7]. Moreover, ghrelin has also been demonstrated in the anterior medial hypothalamus; nucleus magnocellularis preopticus pars medialis and nucleus chiasmatis of the hypothalamus of the domestic fowl [8]. This indicates that ghrelin may have several physiological actions in the peripheral as well as in the central nervous systems. Since ghrelin has been shown to stimulate insulin secretion [6] and increase food intake and body weight [9] acting in part upon hypothalamic neurons regulating food intake and body composition [10–12] and in part by interaction at the pituitary [13], we hypothesize that ghrelin could have an effect on glucagon, a pancreatic hormone involved in glucose metabolism.

Glucagon-producing cells are the second largest cell type in the endocrine pancreas. Moreover, the number of glucagon-producing cells is increased in experimental diabetes [14]. This increase in the number of glucagon-producing cells is associated with severe hyperglucagonaemia. In addition to this, profound weight loss is seen in rats with streptozotocin-induced diabetes [14].

The purpose of this study was to determine whether ghrelin has a role in the regulation of glucagon secretion from the pancreas of diabetic rats. The study also examine the possible mechanisms by which ghrelin may interact with glucagon in the rat pancreas.

Materials And Methods

Experimental protocol

Male Wistar rats weighing approximately 250g were used in this study. Diabetes was induced by a single dose of streptozotocin (STZ) at 60 mg kg^{-1} according to a previously described method [14]. The rats became diabetic 24 h after the administration of STZ. The control rats were given a similar amount of physiological saline. The rats were divided into two groups, STZ-induced diabetics (n = 8) and age-matched controls (n = 8). Four weeks after the onset of diabetes, all the rats from both groups were killed under chloral hydrate general anesthesia (7% chloral hydrate 6-ml kg^{-1} body weight, injected intraperitoneally). The pancreas was removed and placed in ice-cold Krebs buffer (KB). The experiments were performed according to the recommendations of the Animal Ethics Committee, FMHS, United Arab Emirates University, Al Ain, United Arab Emirates.

Estimation of glucagon release from the pancreas of normal and diabetic rats

Pancreatic tissue fragments from normal and diabetic rats were trimmed free of adherent fat and connective tissue and cut into smaller fragments (0.5–1 mm^3). Approximately 100 mg of the pancreatic tissue fragments were placed in 2 ml glass vials containing 1 ml of KB. The pancreatic tissue fragments were pre-incubated for 30 min in a waterbath (37 °C) to wash away any hormone due to cutting of the tissue. After pre-incubation the KB was drained and the specimens were subsequently treated for 1 h with different concentrations (10^{-12}, 10^{-9} and 10^{-6} M) of ghrelin or with ghrelin (10^{-9} M) and atropine (10^{-6} M); ghrelin (10^{-9} M) and propranolol (10^{-6} M); ghrelin (10^{-9} M) and yohimbine (10^{-6} M); ghrelin (10^{-9} M) and diltiazem (10^{-6} M); and ghrelin (10^{-9} M) and atropine, propranolol, yohimbine and diltiazem (10^{-6} M).

Pancreatic tissue fragments of control rats were incubated in KB alone for the same period of time. Some pancreatic tissue fragments were incubated in KB containing atropine (10^{-6} M), propranolol (10^{-6} M), yohimbine (10^{-6} M), and diltiazem (10^{-6} M), either alone or in combination with each other. During the incubation period, each vial was gassed with a mixture of 95% oxygen and 5% carbon dioxide every 3 min. At the end of the incubation, the tissue fragments were removed, blotted and weighed and the effluent stored at –20 °C for glucagon radioimmunoassay.

Measurement of glucagon content in effluent

The glucagon content of the effluent was determined using DPC® (Los Angeles, CA, USA) radioimmunoassay kits. Briefly, all test samples and controls were assayed in duplicates. A volume of 200 μl of either calibrator, control or test sample was placed into previously labelled tubes. After this, 100μl of glucagon antiserum was added to all tubes except NSB (non specific binding) and T (total count) tubes and vortexed. After vortexing the tubes were put in a rack and covered with parafilm before incubation for 24 h at 4 °C. After the first incubation, 100μl of $^{[125]}$I-glucagon was added to every tubes and vortexed. The samples were then incubated for another 24 h at 4 °C and 1 ml of cold precipitating solution was added to all tubes (except the T tubes) and centrifuged for 15 min at 100X g. The tubes were decanted gently onto a blotting paper and radioactivity was counted for 1 min using a gamma counter (Beckman, Fullerton, CA USA). Results were analyzed with Beckman Immunofit EIA/RIA software (Version 2.0). All values were expressed in pg ml^{-1} (100 mg tissue)$^{-1}$.

Chemicals: All other chemicals were purchased from Sigma (Poole, UK) unless otherwise specified.

Statistical analysis: All values were expressed as mean ± standard deviation (SD). Statistical significance was assessed using one-way analysis of variance (ANOVA) and Student's *t*-test where applicable. Values with $p < 0.05$ were accepted as significant.

Results

The effect of ghrelin (10^{-12}, 10^{-9} and 10^{-6} M) on glucagon release from the pancreas of normal rat is shown in Figure 1. Ghrelin at 10^{-12} M caused a small but not significant decrease in glucagon secretion. By contrast, stronger doses of ghrelin (10^{-9} and 10^{-6} M) caused an insignificant increase in glucagon release from the pancreas of normal rats. Thus, ghrelin failed to stimulate or inhibit glucagon release from the pancreas of normal rats when compared to basal.

Ghrelin at 10^{-9} M induced significant (paired Student's *t*-test, $p < 0.03$) increases in glucagon secretion from the pancreas of diabetic rats. Other concentrations (10^{-9} M and 10^{-6} M) of ghrelin caused a slight but not significant increase in glucagon secretion (Fig. 2).

The effect of Atr (a cholinergic muscarinic receptor antagonist), Prop (a β-adrenergic receptor antagonist), Yohim (an α2-adrenergic receptor antagonist), and Dilt (calcium channel antagonist) is depicted in Figure 3. Either Atr, Prop, Yohim, Dilt or a combination of Atr, Prop, Yohim did not have any effect on ghrelin-glucagon interaction in pancreatic tissue fragments of normal rats (Fig. 3).

The effect of a cholinergic muscarinic, β-adrenergic, α2-adrenergic receptor antagonists and calcium channel blocker on ghrelin-glucagon interaction is shown in Figure 4. Either Atr, Prop, Yohim, Dilt or a combination of Atr, Prop, Yohim did not have any significant effect on ghrelin-glucagon interaction in pancreatic tissue fragments of diabetic rats. Atr, Yohim, Dilt, or a combination of Atr, Prop, Yohim induced a slight inhibition in ghrelin-evoked glucagon secretion. This inhibition of glucagon did not reach a significant level.

Discussion

The present observations showed that ghrelin has no significant effect on ghrelin-glucagon interaction in the pancreas of normal rats. However, it induced significant increases in glucagon secretion from the pancreas of diabetic rats. The failure of ghrelin to stimulate glucagon release in the pancreas of normal rats is not surprising. Previous reports from our laboratory have demonstrated that ghrelin is a potent secretagogue of insulin secretion from the pancreas of normal and diabetic rats [6]. Since insulin and glucagon have opposite effects in the endocrine pancreas, it is logical that if ghrelin stimulates insulin, it should not stimulate glucagon release. However, it was a surprise to observe that ghrelin stimulated glucagon secretion from the pancreas of diabetic rats. Why should ghrelin stimulate glucagon release from the pancreas of diabetic rats when it cannot do so in the pancreas of normal rats? A possible reason for this difference is that signal transduction involving the calcium pathway is impaired in diabetic rats [15]. This defect in calcium signaling pathway in diabetic pancreas may contribute to the differences in the response of normal and diabetic rat pancreas to ghrelin.

Another possible reason why ghrelin failed to induce glucagon secretion in normal rat is that glucagon is not associated with increased weight gain. In fact lean diabetic rats have high blood levels of glucagon [14]. It thus appears that ghrelin stimulates the secretion of hormones, such as insulin [6] and growth hormones [3] that increases weight and growth and have no effect on those hormones that have a negative effect on growth or body weight gain.

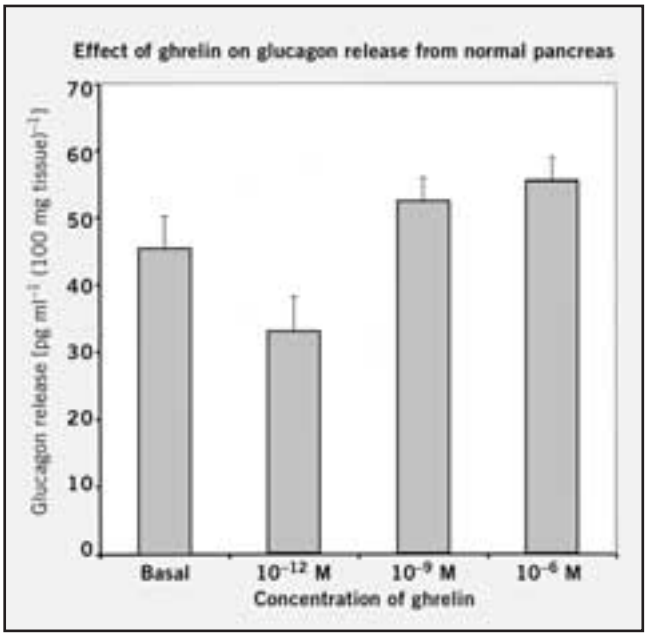

Figure 1 shows the effect of ghrelin on glucagon release from the pancreas of normal rats (n =8). Basal glucagon release is also depicted for comparison. Ghrelin, failed to evoke significant increases or decreases in glucagon release. Ghrelin at 10^{-12} M caused a small but not significant decrease in glucagon secretion. By contrast, stronger doses of ghrelin (10^{-9} and 10^{-6} M) caused an insignificant increase in glucagon release from the pancreas of normal rats.

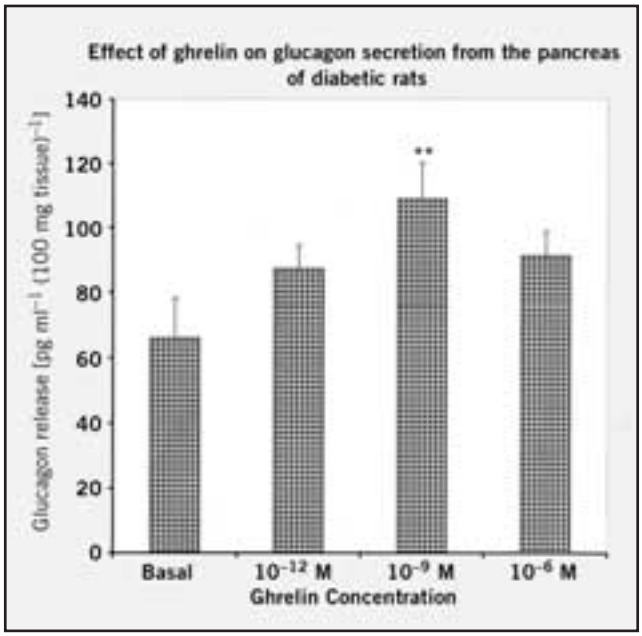

Figure 2. Histograms showing the effect of ghrelin on glucagon secretion from the pancreas of diabetic rats (n =8). Basal glucagon output is also shown for comparison. Ghrelin (10^{-9} M) caused a large and significant increase in glucagon release. $^{**}p < 0.03$ (Ghrelin versus basal).

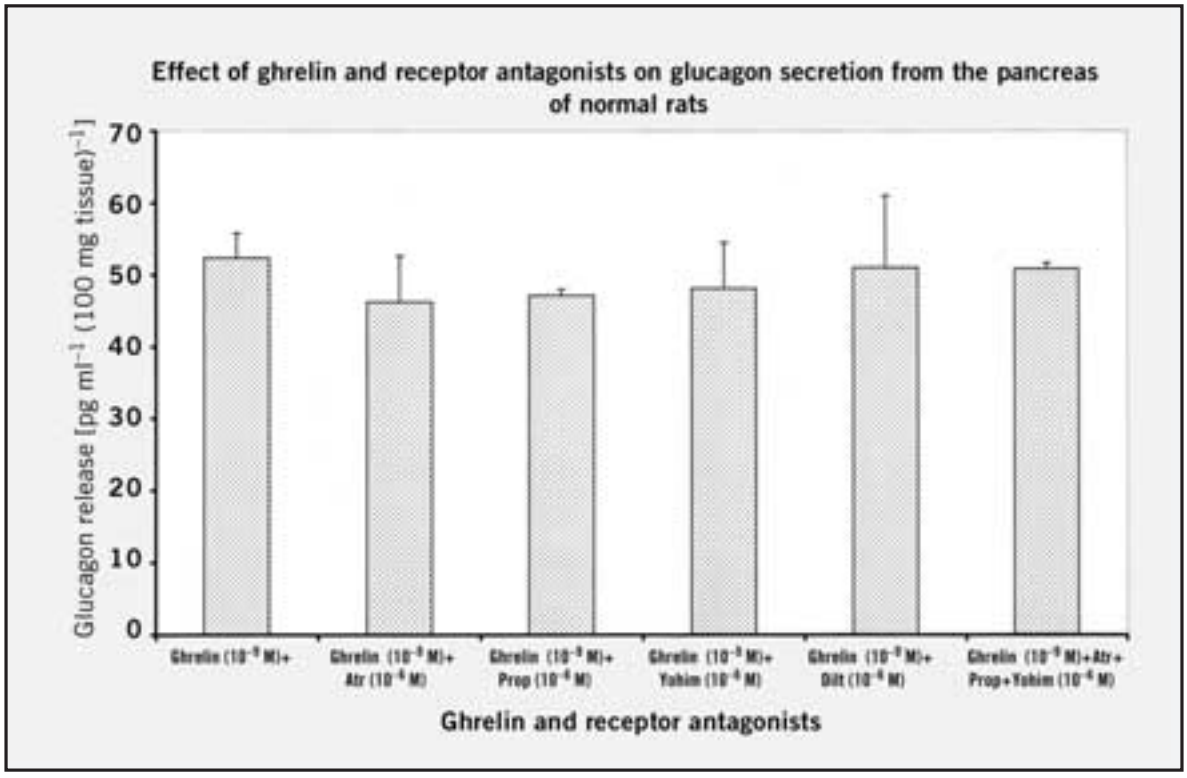

Figure 3 shows the effect of cholinergic, adrenergic receptor antagonists and calcium channel blocker on ghrelin-glucagon interaction in the pancreas of normal rats (n =8). Atropine (Atr), propranolol (Prop), yohimbine (Yohim) and diltiazem (Dilt) have no effect of ghrelin interaction. Although Atr, Prop, Yohim caused a slight decrease in the glucagon release from the pancreas of diabetic rats, this decrease did not reach a significant level.

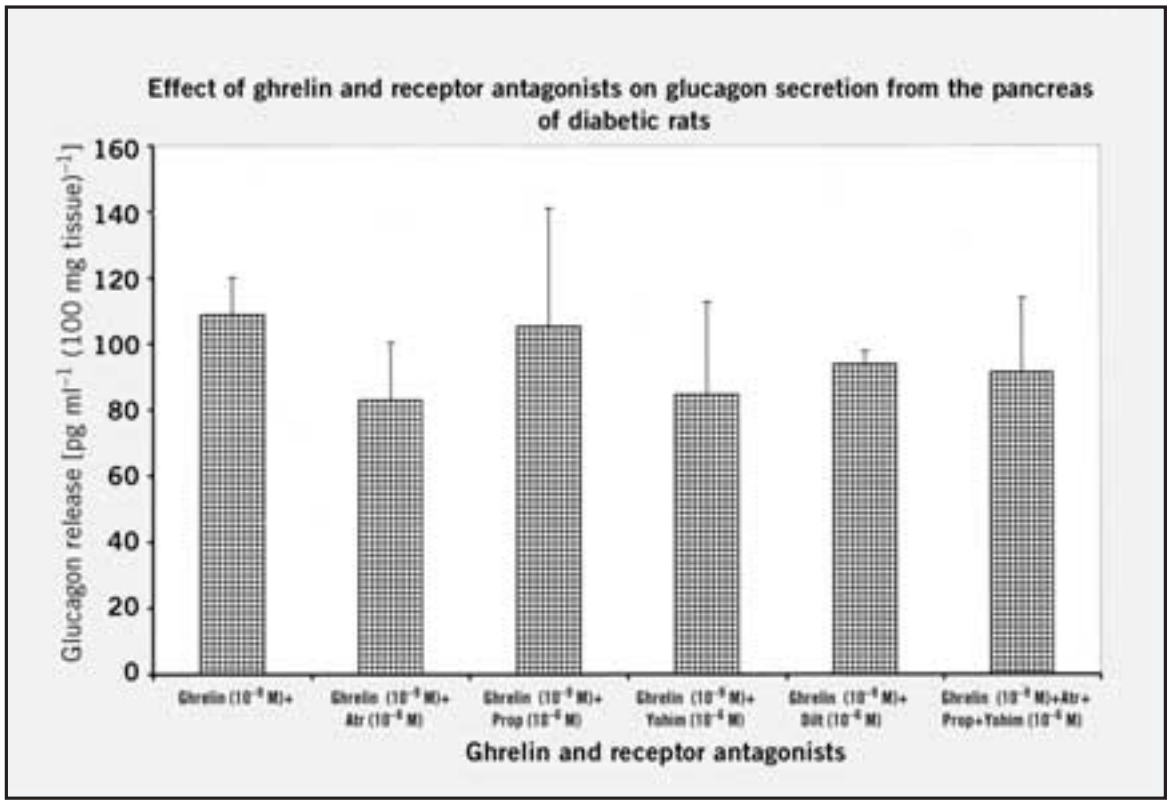

Figure 4. Histograms showing the effect of cholinergic, adrenergic receptor antagonists and calcium channel blocker on ghrelin-evoked glucagon release from the pancreas of diabetic rats (n = 8). Atropine (Atr), propranolol (Prop), yohimbine (Yohim) and diltiazem (Dilt) did not significantly affect ghrelin-evoked glucagon release from the pancreas of diabetic rats. However, Atr (10^{-6} M), Yohim (10^{-6} M) , Dilt and a combination of Atr, Prop and Yohim were able to induce various degree of slight but no significant reduction in ghrelin-evoked glucagon secretion. The degree of this reduction did not reach a significant level.

The results of this study corroborates that of Egido et al [16] who reported that ghrelin has no effect on arginine-induced glucagon release from the pancreas of normal rats.

Neither Dilt nor a robust combination of Atr, Prop and Yohim significantly affected ghrelin-evoked glucagon release from the pancreas of normal and diabetic rats. Although, Dilt (a calcium channel antagonist) did not significantly affect ghrelin-evoked glucagon secretion, it produced a slight inhibition in ghrelin-induced glucagon release. This observation supports previous reports that calcium is involved in the control of ghrelin-induced insulin release [6]. Ghrelin-glucagon interaction was not affected by either atropine, propranolol nor yohimbine when applied alone or in combination with each other. This shows that ghrelin-glucagon interaction is not controlled via cholinergic and or adrenergic pathways.

In conclusion, ghrelin does not have any effect on glucagon secretion from the pancreas of normal rat. However, it induced significant increases in glucagon secretion from the pancreas of diabetic rat. Ghrelin-glucagon interaction is not regulated by either cholinergic or adrenergic pathway.

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