Kisspeptin-13 inhibits insulin secretion without affecting glucagon or somatostatin release: study in the perfused rat pancreas

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Abstract

Kisspeptins are a family of peptides encoded by the KISS1 gene, which binds to G-protein-coupled receptor (GPR54), an orphan GPR54 related to galanin receptors. Endogenous forms composed of 54, 14, and 13 amino acids have been identified. Kisspeptin and GPR54 mRNAs have been detected in pancreatic B and A cells. Furthermore, kisspeptin-54 has been shown to slightly stimulate the last phase of glucose-induced insulin secretion in mouse and human islets and to inhibit insulin release in MIN6 cells. We have investigated the effect of kisspeptin-13 on insulin, glucagon, and somatostatin secretion. The study was performed in the perfused rat pancreas. Glucose, arginine, carbachol, and exendin-4 were used as secretagogues. Hormones were measured by RIA. Kisspeptin-13 reduced glucose-induced insulin secretion in a dose-dependent manner (IC_{50}=1.2 nM) and inhibited the insulin responses to both carbachol and exendin-4. Kisspeptin-13 blocked arginine-induced insulin secretion without affecting the glucagon or somatostatin responses to this amino acid, thus indicating that kisspeptin-13 influences B cells directly, rather than through an A- or D-cell paracrine effect. The reduction of the insulin response to exendin-4 induced by kisspeptin-13 was also observed in pertussis toxin-treated rats, thus suggesting an inhibition independent of Gi proteins. In view of the potent insulinostatic effect of kisspeptin-13, it is tempting to speculate that kisspeptins may be implicated in the regulation of B-cell secretion.

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Introduction

Kisspeptins are a family of peptides encoded by the KISS1 gene (Kotani et al. 2001, Muir et al. 2001, Ohtaki et al. 2001). KISS-1 was originally identified as a putative human metastasis suppressor gene in melanomas and breast cancer cells that acts without affecting tumorigenicity (Lee et al. 1996). Its expression has also been associated with antimetastatic activity in papillary thyroid (Ringel et al. 2002), esophageal (Ikeguchi et al. 2004), and bladder carcinoma (Sánchez-Carbayo et al. 2003).

The predicted KISS1 protein consists of 145 amino acids processed to a 54 amino acid peptide and subsequently amidated at the C-terminus. This peptide has recently been identified by three different groups as the endogenous ligand for an orphan G-protein receptor (GPR54; also known as AXOR-12; Kotani et al. 2001, Muir et al. 2001, Ohtaki et al. 2001) and was termed metastatin (Ohtaki et al. 2001) or kisspeptin (Kotani et al. 2001, Muir et al. 2001). Endogenous forms composed of 14 and 13 amino acids have also been identified (Kotani et al. 2001). Kisspeptin-10, the common C-terminal decapptide shared by these forms, is the minimum sequence necessary for GPR54 receptor activation (Kotani et al. 2001, Muir et al. 2001) and is secreted by cultured human trophoblasts (Bilban et al. 2004).

GPR54 is an orphan G protein-coupled receptor structurally related to galanin receptors, originally cloned from rat brain (Lee et al. 1999). Tissue distribution of GPR54 and its cognate ligand precursor, KISS1, often coincides. The levels of the transcripts of both are the highest in the placenta (Lee et al. 1996, Kotani et al. 2001, Ohtaki et al. 2001). Moreover, both KISS1 and GPR54 are widespread throughout the CNS (higher levels are reported in hypothalamus and pituitary; Lee et al. 1996). The hypothalamic KISS1/kisspeptin/GPR54 system has been shown to play a role as a major gatekeeper of gonadotropin-releasing hormone (GnRH) neurons and the reproductive axis (Mesager 2005, Castellano et al. 2006, Fernández-Fernández et al. 2006, Tena-Sempere 2006).

KISS1 is also expressed in peripheral tissue, testis, pancreas, liver, and small intestine, and kisspeptin receptor is highly expressed in pancreas, with lower expression in spleen, testis, peripheral blood lymphocytes, and adipose tissue (Kotani et al. 2001, Ohtaki et al. 2001, Harms et al. 2003).

Hauge-Evans et al. (2006) have recently demonstrated the presence of kisspeptin and GPR54 mRNAs in both pancreatic B and A cells and GPR54 expression in MIN6
arginine, 10 mM KCl, 2.6 mM CaCl₂, 1.19 mM H₂HPO₄, 1.19 mM MgSO₄ (5.5 mM; Sigma–Aldrich). Kisspeptin-13 was purchased from fraction V bovine albumin (Sigma–Aldrich), and glucose containing 0.1% BSA (Cohn Fraction V, Sigma–Aldrich).

To gain further insight into the effect of kisspeptin on islet cell secretion, we have investigated the effect of kisspeptin-13 on insulin, glucagon, and somatostatin secretion. The study was performed in the isolated perfused rat pancreas.

Animals, materials, and methods

Animals

Male Wistar rats (200–225 g body weight) from our inbred colony were used as donors. Animals were maintained in accordance with the guidelines established by the European Union (86/609). All experiments were performed between 0900 and 1200 h. This is a period of minimal baseline feeding and activity for rodents. Rats had free access to food and water until they were used in the experiments. Feeding activity in rats shows a diurnal rhythmicity. Under ad libitum conditions, rats consume most of their food during the dark phase, in which feeding activity is bimodal, with peaks at the beginning (dusk feeding peak) and toward the end (dawn feeding peak) of the night (Meguid et al. 1992, Varma et al. 1999).

Experimental protocol

Each rat was anesthetized with sodium pentobarbital (50 mg/kg, i.p.), after which the pancreas was dissected and perfused in situ, as previously described (Silvestre et al. 1986). Effluent samples were collected from the portal vein, without recycling, at 1-min intervals (flow rate, 2 ml/min), and frozen at −20 °C until the time of assay. The perfusion medium consisted of a Krebs–Henseleit buffer: 115 mM NaCl, 4.7 mM KCl, 2.6 mM CaCl₂, 1.19 mM H₂HPO₄, 1.19 mM MgSO₄ 7H₂O, and 24.9 mM HNaCO₃ (gas phase 95:5, O₂:CO₂; pH 7.4), supplemented with 4% (w/v) dextran T-70 (Pharmacia LKB Biotechnology, Uppsala, Sweden), 0.5% (w/v) Cohn fraction V bovine albumin (Sigma–Aldrich), and glucose (5.5 mM; Sigma–Aldrich). Kisspeptin-13 was purchased from Bachem AC (Switzerland) and tested at graded concentrations (1 μM, 100 nM, 10 nM, 1 nM, and 0.1 nM).

All substances were dissolved in 0.9% NaCl containing 0.1% BSA (Cohn Fraction V, Sigma–Aldrich). These solutions were prepared daily, immediately before experiments.

After a 35-min equilibration period, baseline samples were collected for 5 min and, at zero time, kisspeptin-13 was infused through a sidearm cannula. Stimulation of hormone secretion was induced by increasing the perfusate glucose concentration from 5.5 to 9 mM and by infusing 10 mM arginine, 10 μM carbachol, or 1 nM exendin-4. The sequence of addition of compounds to the perfusate is indicated in the corresponding figures. In control experiments, an identical volume of buffer was infused.

Pertussis toxin treatment

Treatment with pertussis toxin (PTX) from Bordetella pertussis (Sigma–Aldrich) was performed by injecting a single dose (3 μg/100 g b.w., i.p.) 3 days before perfusions. This PTX treatment has been found to effectively ADP-ryosylate and inactivate Gᵣ-protein coupled to adenylate cyclase (Ui et al. 1984, Komatsu et al. 1994, Silvestre et al. 1994, Egido et al. 2007). Control rats were subjected to a sham treatment consisting of injecting the corresponding buffer (50% glycerol, 0.5 M NaCl, 0.05 M Tris–glycine, pH 7.5).

Hormone determination

Insulin, glucagon, and somatostatin were analyzed by RIA using the charcoal separation method (Herbert et al. 1965, Falloon & Unger 1974, Harris et al. 1978). Experiments were randomized and all samples for each series of experiments were analyzed within the same assay.

Insulin

Anti-pig insulin serum (I8510, Sigma–Aldrich) and rat insulin standards (Novo Nordisk, Bagsvaerd, Copenhagen, Denmark) were employed. The inter- and intra-assay variations were 5-3 and 4% respectively.

Glucagon

Anti-glucagon serum (30 K) was kindly donated by R.H. Unger (University of Texas, Health Sciences Center, Dallas, TX, USA). The inter- and intra-assay variations were 6-4 and 4-5% respectively.

Somatostatin

Anti-somatostatin serum (180C) was also donated by R.H. Unger (University of Texas, Health Sciences Center). The inter- and intra-assay variations were 5-4 and 4-6% respectively.

Statistical analysis

Results are expressed as the mean±S.E.M. Insulin response was calculated as the integrated area of the curve above the mean preinfusion level (average of all the baseline levels for each perfusion) using the trapezoidal method. The normal distribution of our data was demonstrated by the Kolmogorov–Smirnov test (Siegel 1978). Differences between values were tested for significance by repeated-measures ANOVA in each group and by the unpaired Student’s t-test.

Results

Figure 1 shows the effect of 1 μM kisspeptin-13 on basal and on arginine-induced insulin (Fig. 1A), glucagon (Fig. 1B),...
and somatostatin (Fig. 1C) secretion. Infusion of 1 μM kisspeptin-13 did not significantly alter basal insulin, glucagon, or somatostatin secretion at a constant glucose concentration (5.5 mM). At 1 μM, kisspeptin-13 blocked both phases of the arginine-induced insulin response (incremental areas: first phase: 14 ± 7 ng/5 min versus 144 ± 80 ng/5 min in controls; \( P < 0.01 \); second phase: 9.4 ± 11 ng/15 min versus 298 ± 58 ng/15 min in controls; \( P < 0.01 \)) and did not significantly alter the glucagon or somatostatin responses elicited by this amino acid (incremental areas: 14 ± 4 ng/20 min versus 15 ± 1.3 ng/20 min in control perfusions, \( P = 0.757 \); and 397 ± 119 pg/20 min versus 479 ± 106 pg/20 min in control perfusions, \( P = 0.71 \) respectively). Furthermore, ANOVA has demonstrated that basal insulin secretion was not affected by a prolonged (20 min) infusion of 1 μM kisspeptin-13 (\( F = 1.53; \) NS; data not shown).

Figure 2 shows the effect of graded concentrations of kisspeptin-13 on glucose-stimulated insulin secretion. As shown in Fig. 2A, infusion of kisspeptin-13 at 1 μM did not significantly alter basal insulin secretion at a constant glucose concentration (5.5 mM), but suppressed both phases of the insulin response induced by increasing the perfusate glucose concentration from 5.5 to 9 mM (incremental areas: first phase: \(-1.7 ± 1.7 \) ng/5 min versus 41.6 ± 5.1 ng/5 min in controls; \( P < 0.01 \); second phase: 0.1 ± 5.8 ng/15 min versus 103 ± 27 ng/15 min in controls; \( P < 0.01 \)). The insulin response to glucose observed in control perfusions (incremental area: 145 ± 32 ng/20 min) was significantly inhibited by kisspeptin-13 at 100 nM (incremental areas: 4.2 ± 8 ng/20 min; \( P < 0.01 \); Fig. 2B) and at 10 nM (incremental areas: 38 ± 16 ng/20 min; \( P < 0.025 \); Fig. 2C). At 1 nM, kisspeptin-13 reduced glucose-induced insulin secretion (incremental area: 77 ± 28 ng/20 min), although the difference was not statistically significant (Fig. 2D). At 0.1 nM, kisspeptin-13 did not significantly affect glucose-induced insulin secretion (incremental areas: 151 ± 63 ng/20 min versus 145 ± 33 ng/20 min in controls; \( P = 0.918 \); Fig. 2E). The dose–response curve was fitted to a sigmoidal curve (\( R^2 = 0.99 \)) with an IC_{50} of 1.2 nM (Fig. 3).

Figure 4 shows that infusion of kisspeptin-13 at 10 nM inhibited the insulin response to 10 μM carbachol (incremental areas: 124 ± 24 ng/20 min versus 465 ± 85 ng/20 min; \( P < 0.01 \)).

Finally, we examined the effect of kisspeptin-13 on the insulin response to exendin-4 both in PTX-treated and untreated rats (Fig. 5). As shown in Fig. 5A, in pancreases isolated from untreated rats, infusion of 10 nM kisspeptin-13 blocked the insulin response to exendin-4 (incremental areas: 19 ± 6.7 ng/15 min versus 80 ± 19 ng/15 min in controls; \( P < 0.05 \)). The inhibitory effect of kisspeptin-13 on the insulin response to exendin-4 was also observed in pancreases from PTX-treated rats (incremental areas: 15 ± 8.7 ng/15 min versus 88 ± 13 ng/15 min in controls; \( P < 0.05 \); Fig. 5B).
Discussion

The foregoing results demonstrate that, in pancreases isolated from normal rats, infusion of kisspeptin-13 dose dependently inhibits glucose-induced insulin secretion. This inhibitory effect of kisspeptin-13 was observed when insulin secretion was stimulated by an increase in the perfusate glucose concentration (from 5.5 to 9 mM), resembling that occurring in normal subjects upon consuming a carbohydrate-rich meal; however, no effect of kisspeptin-13 on basal insulin secretion was observed (with constant infusion of 5.5 mM glucose).

Hauge-Evans et al. (2006) have recently shown that 1 μM kisspeptin induced a slight, but significant, inhibitory effect on insulin secretion from MIN6 cells, while it potentiated the late phase of glucose-induced insulin secretion from mouse and human perfused islets. The discrepancy between our findings and those of the above-mentioned authors may be due to the
different experimental protocols employed in these investigations. The isolated rat pancreas may be more sensitive to several secretagogues and inhibitors of insulin secretion than islets obtained by collagenase digestion of the pancreas. On the other hand, in the present study, we have employed kisspeptin-13, the C-terminal fragment of kisspeptin, a fact that might also explain the discrepancies observed with regard to the reported effect of kisspeptin on glucose-induced insulin secretion. Concerning this point, it should be pointed out that, in cells overexpressing GPR54, all kisspeptin fragments have been found to have a similar affinity and efficacy for this receptor, thus indicating that the C-terminal portion of the kisspeptin molecule is responsible for the activation of GPR54 (Kotani et al. 2001). However, other studies have found that kisspeptin-54 shows lower agonistic potency toward AXOR-12/GPR54 than kisspeptin-13 (Muir et al. 2001).

In our experimental model, the insulinostatic effect of kisspeptin-13 on glucose-induced insulin secretion is dose dependent, and the IC$_{50}$ (1.2 nM) is comparable with the EC$_{50}$ value (1.38 nM) reported for binding of kisspeptin-13.

Figure 3 Dose–response curve corresponding to the inhibitory effect of kisspeptin-13 on the glucose-induced insulin response in the perfused rat pancreas. Hormone responses were calculated as the incremental areas under the curve. Means ± S.E.M.

Figure 4 Effect of 10 nM kisspeptin-13 on the insulin response to 10 μM carbachol at 5.5 mM glucose. Solid line (n=4): control, carbachol alone. Broken line (n=5): carbachol + kisspeptin-13. Means ± S.E.M.

Figure 5 Effect of 10 nM kisspeptin-13 on the insulin response to 1 nM exendin-4 at 5.5 mM glucose. (A) Control rats. Solid line (n=7): control, exendin-4 alone. Broken line (n=5): exendin-4 + kisspeptin-13. (B) PTX-treated rats. Solid line (n=11): control, exendin-4 alone. Broken line (n=8): exendin-4 + kisspeptin-13. Means ± S.E.M.
to rat GPR54 (Kotani et al. 2001). The inhibition of insulin secretion by kisspeptin-13 in the nanomolar range resembles the effect induced by other insulinostatic peptides – galanin (Sharp et al. 1989, Drews et al. 1994), somatostatin (Schuit et al. 1989), and 26RFa (Egido et al. 2007).

Kisspeptin-13 has been detected in peripheral plasma (Horikoshi et al. 2003, Panidis et al. 2006), where its levels correlate negatively with fasting plasma insulin levels (Panidis et al. 2006). Plasma kisspeptin concentrations are in the picomolar range, much lower than the concentrations employed in this study. However, kisspeptin has also been found in the pancreas (Kotani et al. 2001, Harms et al. 2003, Masui et al. 2004), and it can be assumed that in addition to the kisspeptin reaching the B cell through the systemic circulation, the local production of this peptide would augment its intra-islet concentration. Interestingly, plasma kisspeptin levels are dramatically elevated during pregnancy (up to 7000-fold higher than the normal value). This increase is in accord with the progression of pregnancy and seems to be related to the placental production of this family of peptides (Horikoshi et al. 2003).

The precise mechanism of action by which kisspeptin-13 could reduce B-cell secretory activity is not known. We have found that, besides reducing glucose-induced insulin secretion, kisspeptin-13 inhibits the insulin response to secretagogues that act on the B cell via different mechanisms, i.e. arginine, which depolarizes the B cell (Flatt 2003), secretagogues that act on the B cell via different mechanisms, found that, besides reducing glucose-induced insulin could reduce B-cell secretory activity is not known. We have observed that the inhibitory effect of kisspeptin-13 on the insulin response to exendin-4 is maintained in PTX-treated rats, thus indicating that the insulinostatic effect of kisspeptin-13 is independent of PTX-sensitive G proteins. It should be mentioned that, in our perfused pancreas system, the same batch of PTX has been shown to be effective in preventing the inhibition of exendin-4-induced insulin secretion elicited by other insulinostatic peptides, such as 26RFa (Egido et al. 2007) or ghrelin (unpublished data).

We have found that kisspeptin-13, at a concentration that totally blocked insulin release (1 μM), failed to significantly modify either basal or arginine-stimulated glucagon output. In agreement with this, Hauge-Evans et al. (2006) have shown that, at the same concentration, kisspeptin failed to significantly modify glucagon secretion in the presence of either 2 or 20 mM glucose. The present study provides the first data on the effect of kisspeptin-13 on somatostatin release. The lack of effect of kisspeptin-13 on glucagon and somatostatin secretion would rule out the possibility that the insulinostatic effect of kisspeptin-13 be due to a paracrine mechanism mediated by A- or D-cell secretion.

Kisspeptins belong to a peptide family with a common Arg-Phe-NH2 motif at their C-terminal end, and collectively termed RFamide-related peptides (RFPs; Kotani et al. 2001). Interestingly, other RFamide peptides have been shown to reduce insulin secretion at micromolar concentrations (Sorensen et al. 1984, Fehmann et al. 1990). Recently, using our perfused pancreas model, we have found that 26RFa, another RFamide peptide, behaves as a potent inhibitor of insulin secretion (Egido et al. 2007). The insulinostatic effect of 26RFa resembles that of kisspeptin-13. Both peptides dose dependently inhibited glucose-induced insulin secretion (with IC50 at the nanomolar level) and reduced the insulin responses to arginine and exendin-4, thus suggesting that RFamide peptides may play a role in the control of B-cell secretion.

Conclusion

In view of the potent insulinostatic effect of kisspeptin-13, it is tempting to speculate that the kisspeptin family may be implicated in the regulation of B-cell secretion. The lack of effect of kisspeptin-13 on both glucagon and somatostatin secretion would indicate that it influences the B cell directly, rather than through an A- or D-cell paracrine effect.

As for the pathological relevance of our findings, it can be hypothesized that, by impairing insulin secretion, elevation of plasma kisspeptins could constitute a diabetogenic condition. Given the massive elevation of circulating kisspeptins during pregnancy, gestational diabetes might represent one of these conditions, a point worthy of investigation.
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