Glucose stimulates and insulin inhibits release of pancreatic TRH in vitro

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Abstract

Objective: Pancreatic TRH is present in insulin-producing B-cells of the islets of Langerhans. There is fragmentary evidence that it may be involved in glucoregulation. The aim of our present study was to analyze how glucose and insulin affect TRH secretion by the pancreatic islets.

Design: Isolated pancreatic islets were incubated with different concentrations of glucose, insulin and glucagon, and TRH release was measured.

Results: In the present study, 6 and 12 mmol/l D-glucose caused significant TRH release from isolated adult rat pancreatic islets when compared with that in the presence of the same concentrations of biologically ineffective L-glucose. Thirty mmol/l D-glucose was also ineffective, but this was not due to depression of secretion by hyperosmolarity since isosmotic compensation for the high glucose addition did not restore its stimulatory effect. Five μmol/l dibutyryl cyclic 3’,5’-adenosine monophosphate (db-cAMP) increased both basal and glucose-stimulated TRH release, but this effect was not seen with 50 μmol/l db-cAMP. Stimulation of phosphodiesterase by imidazole resulted in decreased basal but not glucose-stimulated release of TRH. Glucagon (10⁻⁷ mol/l) did not affect either basal or glucose-stimulated release of TRH, while insulin (10⁻⁷ and 10⁻⁶ mol/l) inhibited both.

Conclusion: Our present data showing that glucose stimulates and insulin inhibits pancreatic TRH release are compatible with the possibility that this substance may play a role in glucoregulation.

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Introduction

Thyrotropin-releasing hormone (TRH) was originally isolated from mammalian hypothalamus on the basis of its ability to stimulate thyrotropin (TSH) secretion (1, 2). Immunoreactive TRH was later detected in the rat pancreas (3, 4) where its concentration, as well as the levels of its messenger RNA, are highest during the neonatal period (5–8). Within the pancreas, TRH is localized in the insulin-producing B-cells of the islets of Langerhans (9–11). The role of pancreatic TRH is still unclear, its colocalization with insulin in the same cells and possibly even in the same secretory granules (12), indicates that TRH may modulate islet function. In perfused rat pancreas, TRH stimulates basal (13) and potentiates arginine-induced glucagon release (13, 14). Immunoneutralization of exogenous TRH by anti-TRH serum inhibits glucose plus arginine-induced glucagon secretion and somatostatin release (13). TRH may indirectly inhibit glucose-induced insulin release in incubated rat islets by enhancing glucose-induced somatostatin secretion (15, 16). TRH potentiates glucose-induced insulin release by perfused pancreatic islets and this effect is reversed by the TRH analog, pGlu-Phe-Pro-NH₂ (17), thus indicating a direct and specific effect of TRH. TRH gene disruption in experimental mice results in hyperglycemia, accompanied by impaired insulin secretion in response to glucose, providing strong evidence that TRH modulates insulin release (18). It was the aim of our present study to analyze how glucose and insulin affect TRH secretion by the pancreatic islets.

Materials and methods

Isolation and incubation of islets of Langerhans

Males SPF Wistar rats (250–300 g) were anesthetized with pentobarbital (Spofa, Prague, Czech Republic) and islets were isolated by collagenase digestion (19). Briefly, the hepatic part of the common bile duct was cannulated and the distal end was tied near the duodenal exit. The pancreas was then distended by injection of Hank’s balanced salt solution (HBSS, pH 7.4) through a ductal cannula. The distended pancreas was removed, cut into small pieces and incubated at
37°C for 12 min with collagenase (type XI, Sigma, St Louis, MO, USA; 1 mg/ml) in the presence of 0.008% soybean trypsin inhibitor (Sigma). Freed islets were isolated by hand-picking using a dissecting microscope, collected in incubation tubes, and preincubated in basal medium (see below for composition) for 60 min at 37°C. After preincubation, the islets were incubated at 37°C in the indicated medium. Krebs-Ringer-Hepes buffer (118 mmol/l NaCl, 4 mmol/l KCl, 1.2 mmol/l MgSO₄, 1.2 mmol/l KH₂PO₄, 2.5 mmol/l CaCl₂, 25 mmol/l NaHCO₃, 20 mmol/l Hepes, 3 mmol/l d-glucose, 0.5% BSA (fraction V, Sigma) and 0.3 mg/ml bacitracin (Sigma), 95% O₂-5% CO₂, pH 7.4, 300 mosmol/l) was the basal incubation medium. Experimental conditions are indicated in the legends to the particular figures. At the end of each incubation period, the medium was saved and stored at −20°C until TRH radioimmunoassay (RIA) determination.

Assays

TRH was measured by specific RIA. The rabbit TRH antibody developed in our laboratory recognizes neither TRH-degradation products (such as TRH-OH, cyclo(His-Pro) or amino acids) nor putative TRH precursor peptides such as TRH-Gly, Gln-His-Pro-Gly-Lys-Arg or Lys-Arg-Gln-His-Pro-Gly-Arg-Arg (cross-reactivity less than 0.1% for TRH-Gly and less than 0.01% for other peptides). Synthetic TRH (a gift from Prof. Kasafiék, Research Institute of Pharmacology and Biochemistry, Prague) was labeled with Na¹²⁵I using the Chloramine-T method and purified on a Sephadex G-15 (Pharmacia, Uppsala, Sweden) column (60 × 1 cm). All assays were performed in a total volume of 400 μl (20). The sensitivity of the assay was 1 pg TRH per tube. TRH standards were prepared in each medium we utilized, and thus correction for recovery was included. All samples from each experiment were analyzed in the same assay to avoid interassay variation. The intra-assay coefficient of variance for the TRH RIA was 4.2%. Medium osmolality was measured cryoscopically using an OSMOMAT 030 (GONOTEC, Berlin, Germany).

Statistics

The results are expressed as means ± s.e. (n = 5–8 parallel samples for each experiment). Statistical analysis was carried out by one-way ANOVA and unpaired Student’s t-test.

Results

The effect of glucose (6, 12, and 30 mmol/l) on the release of TRH by isolated pancreatic islets is shown in Fig. 1A. To avoid possible unspecified osmotic influence, the same concentrations of biologically inactive l-glucose were used as controls. The release of TRH during the 2-h incubation was significantly (P < 0.05 and 0.001) higher in the presence of 6 and 12 mmol/l d-glucose respectively, when compared with the release in the presence of the same concentrations of l-glucose. However, 30 mmol/l d-glucose failed to stimulate TRH secretion. Since a high glucose concentration produces significant medium hyperosmolality, we compared the effect of 30 mmol/l d-glucose under either hyperosmotic (330 mosmol/l) or isosmotic (300 mosmol/l) medium containing 30 mmol/l d-glucose. Results are expressed in pg TRH per islet (mean ± s.e.). *P < 0.05, **P < 0.001. (B) Influence of medium osmolarity on the effect of 30 mmol/l glucose. Islets (30 per tube) were incubated for two subsequent 60-min incubation periods interrupted by medium exchange (as indicated by the pairs of bars). The first set of incubations occurred in basal medium, then it was switched to either isosmotic (300 mosmol/l) or hyperosmotic (330 mosmol/l) medium containing 30 mmol/l d-glucose. Results are expressed in pg TRH per islet (mean ± s.e.), *P < 0.05.
(cAMP) occurs after glucose stimulation of B-cells. Figure 2A shows the effect of dibutyryl cAMP (db-cAMP), a biologically active analog of native cAMP. Like glucose, a 2-h exposure of islets to 5 μmol/l db-cAMP stimulated the release of TRH, while in the presence of 50 μmol/l concentration the stimulatory effect disappeared. A positive role for intracellular cAMP on the release of pancreatic TRH was further confirmed by the negative effect of imidazole, an activator of phosphodiesterase, on the release of TRH (Fig. 2B). The exposure of islets to 5 μmol/l db-cAMP for thirty minutes significantly potentiated the release of TRH induced by 12 mmol/l glucose, whereas imidazole (10 mmol/l) in the same time interval lacked any significant effect on basal or glucose-stimulated TRH release (Fig. 3). Addition of glucagon (10⁻⁷ mol/l) affected neither basal nor glucose-stimulated release of TRH (Fig. 4A). In contrast, 10⁻⁷ and 10⁻⁶ mol/l insulin produced a dose-dependent inhibition of basal TRH release, and 10⁻⁵ mol/l insulin abolished the stimulatory effect of 12 mmol/l glucose (Fig. 4B).

Discussion

TRH is present in insulin-producing B-cells of the islets of Langerhans (9, 11). Although its role in this location remains to be fully explored, several studies indicate that it modulates insulin and glucagon release (13–15, 17, 18, 21), and thereby participates in glucoregulation. In the present study, d-glucose stimulated TRH release by isolated pancreatic islets. Exposure of pancreatic B-cells to glucose induces sustained cell-swelling and depolarization, leading to insulin secretion (22). Cell-swelling induced by extracellular hyposmolarity or by isosmolar addition of permeant molecules induces a rapid and short-lasting insulin secretory response in vitro (23, 24), and increased serum insulin levels were observed in patients with plasma hyposmolarity (25). Isosmolar addition of d-glucose and its poorly metabolized transport analog, 3-O-methyl d-glucose, stimulate insulin release even under Ca²⁺-free conditions, possibly due to a cell-swelling-mediated mechanism, since the same concentrations of both in a hyperosmolar addition suppressed secretion (26). We have previously reported that cell-swelling induced in vitro by exposure of islets to 30% hypotonicity or isotonic permeant solution results in the immediate Ca²⁺-independent release of TRH (27). Therefore, we suggest that glucose-induced cell-swelling may play an important role in mediating the effect of glucose on the release of TRH. Surprisingly, increasing glucose concentration to 30 mmol/l resulted in the disappearance of the stimulatory effect. Addition of high glucose concentration produces medium hyposmolarity which, on its own, may suppress insulin secretion both in vitro (28, 29) and
in vivo (26, 30). This hyperosmotic influence, however, was not crucial in our study, since the same concentration of glucose in isosmotic medium was also ineffective. Moreover, a similar effect was observed for stimulation of TRH release by db-cAMP, a biologically active analog of cAMP, the intracellular content of which increases after glucose stimulation (31–34). Db-cAMP was used in concentrations (0.5–50 μmol/l) too low to affect the osmotic properties of the medium. The involvement of paracrine- or autocrine-like effects after glucose stimulation is, therefore, suggested.

Yamaguchi et al. (35) found that in vivo hypoglycemia and low glucose in the medium decreased in vitro release of TRH from the rat hypothalamus, while Lewis et al. (36) reported that increasing concentrations of glucose inhibited the release of TRH from hypothalamic fragments in vitro. Both these studies used hypothalamic areas comprising different TRH systems. When we studied, specifically, the effect of glucose on hypophysiotropic TRH in the hypothalamic paraventricular nucleus and median eminence, no effect of glucose on either basal or depolarization-induced TRH release was found (M Nikodemová & V Strbák, unpublished observations). It seems, therefore, that the effect of glucose is specific and may vary in different TRH systems.

The islets of Langerhans produce two main glucoregulatory hormones, insulin and glucagon, involvement of which may contribute to the observed effects on TRH release after glucose load. Addition of glucagon to the medium had no effects on either basal or glucose-stimulated TRH release. Insulin, on the other hand, dose-dependently inhibited both. Although we used relatively high doses of insulin in this study (10⁻⁷ and 10⁻⁶ mol/l), comparable concentrations probably occur in the proximity of the B-cell surface after glucose stimulation. Insulin inhibits its own secretion under both in vivo (37, 38) and in vitro conditions using isolated pancreatic islets (39, 40). However, it is unclear if this inhibition is mediated through a direct autocrine influence of insulin on the B-cell or if it is a consequence of indirect paracrine effects on surrounding islet cells. There are insulin-binding sites on the surface of cultured B-cells (41, 42) and Harbeck et al. (43) demonstrated insulin receptor mRNA expression in B-cells. It is, therefore, highly probable that exogenous insulin acts directly through its own receptors on the B-cell surface and that the effect on TRH release observed in our study is, at least in part, mediated through this direct influence. Moreover, insulin in the medium prevented stimulation of TRH release by 12 mmol/l D-glucose. These data indicate an autocrine role of insulin in the control of pancreatic TRH secretion. Additional involvement of other islet hormones and/or bioactive substances cannot be excluded. We have shown that basal TRH release is inhibited by addition of imidazole, an activator of phosphodiesterase which lowers the intracellular content of cAMP. Pancreatic somatostatin inhibits intracellular cAMP production (44–46) and is stimulated by glucose (47–49). Since somatostatin inhibits the release of TRH (50), its involvement in the absence of the stimulatory effect of 30 mmol/l glucose is probable.

Permanent hyperglycemia in mice with the targeted disruption of the TRH gene (18) and impaired insulin response to glucose demonstrated the importance of TRH or pro-TRH-derived peptides in glucoregulation. Our present data, by showing that glucose stimulates and insulin inhibits TRH secretion from pancreatic islets, suggest that in this location the neurohormone might be involved in a delicate mechanism of glucoregulation. The possible mechanism could involve direct local autocrine or paracrine effects. Recent data showing that orally administered TRH suppresses...
glucose absorption, and insulin, C-peptide and pro-insulin secretion (51) suggest that the mechanism might be more complex.

In conclusion, we demonstrated that TRH secretion from pancreatic islets is affected by glucose and insulin in opposite directions.

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