European Journal of Endocrinology (2000) **142** 60–65 ISSN 0804-4643

EXPERIMENTAL STUDY

Glucose stimulates and insulin inhibits release of pancreatic TRH *in vitro*

J Benický and V Štrbák

Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic

(Correspondence should be addressed to V Štrbák, Institute of Experimental Endocrinology, Slovak Academy of Sciences, Vlárska 3, Bratislava 833 06, Slovak Republic; Email: ueenstrb@savba.savba.sk)

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.

European Journal of Endocrinology **142** 60–65

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). Although 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 perifused pancreatic islets and this effect is reversed by the TRH analog, pGlu-Phe-Pro-NH_2 (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 $MgSO_4$, 1.2 mmol/l KH_2PO_4, 2.5 mmol/l $CaCl_2$, 25 mmol/l $NaHCO_3$, 20 mmol/l Hepes, 3 mmol/l D-glucose, 0.5% BSA (fraction V, Sigma) and 0.3 mg/ml bacitracin (Sigma), 95% O_2-5% CO_2, 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. Kasafírek, Research Institute of Pharmacology and Biochemistry, Prague) was labeled with $Na^{125}I$ 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 unspecific osmotic influence, the same concentrations of biologically inactive L-glucose were used as controls. The release

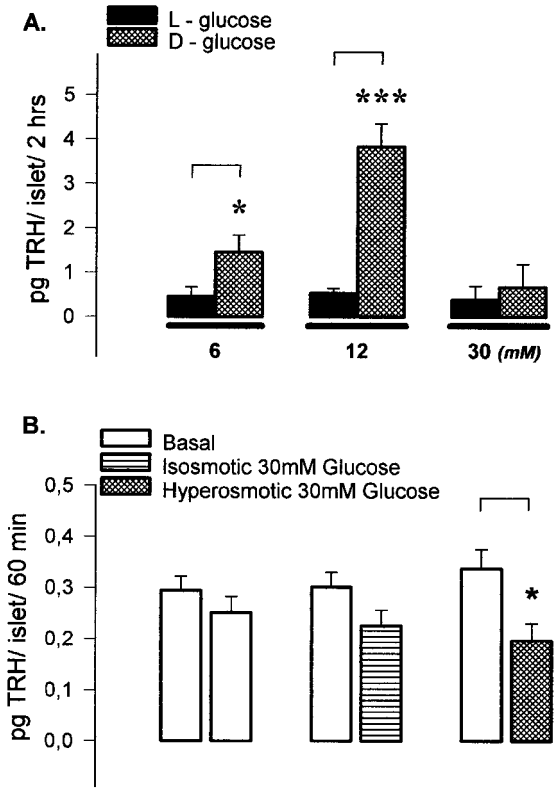

Figure 1 (A) Effect of glucose on the release of TRH. Islets (20 per tube) were incubated for 2 h in medium containing different concentrations of D-glucose (6, 12, and 30 mmol/l respectively). Addition of glucose to medium was not osmotically compensated. Control islets were incubated in the presence of the respective concentrations of biologically inactive L-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.), $*P < 0.05$.

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 hyperosmolarity, we compared the effect of 30 mmol/l D-glucose under either hyperosmotic (330 mosmol/l) or isosmotic (300 mosmol/l; addition of glucose was compensated by reduction of NaCl concentration) conditions (Fig. 1B). Osmotic compensation of 30 mmol/l glucose addition, however, did not restore the stimulatory effect of glucose. Moreover, hyperosmotic medium was inhibitory compared with basal medium. An increase in intracellular cyclic 3′,5′-adenosine monophosphate

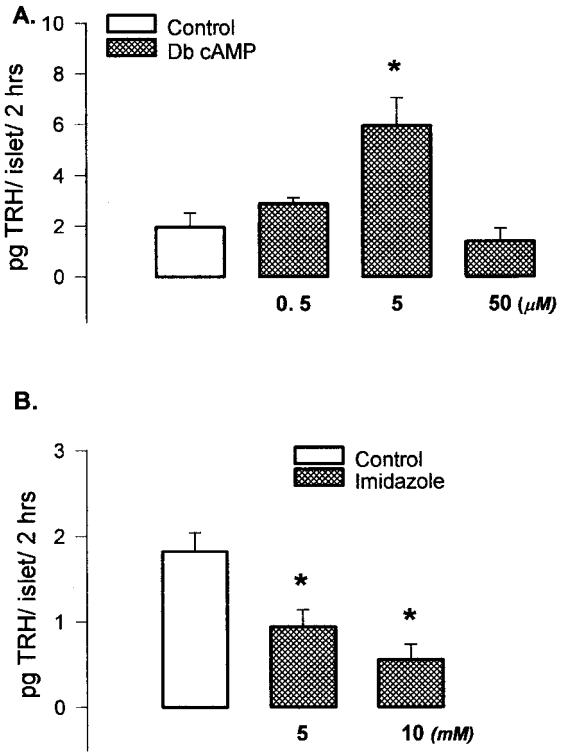

Figure 2 (A) Effect of db-cAMP on the release of TRH. Islets (20 per tube) were incubated for 2 h in the presence of 0.5, 5, and 50 μmol/l db-cAMP (cross-hatched bars). Control islets (open bar) were incubated in basal medium. (B) Effect of imidazole on the release of TRH. Islets were incubated for 2 h in the presence 5 or 10 mmol/l imidazole (cross-hatched bars). Control islets were incubated in basal medium (open bar). Results are expressed in pg TRH per islet (mean ± S.E.), $*P < 0.05$ for individual differences between control and tested group.

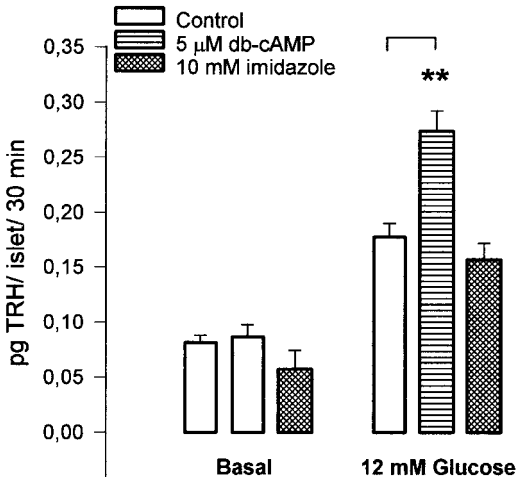

Figure 3 Effect of db-cAMP and imidazole on glucose-stimulated release of TRH. Islets (30 per tube) were incubated for two subsequent 60-min periods interrupted by medium exchange, the first (the left part of the figure) in basal medium and the second (the right part of the figure) in medium containing 12 mmol/l D-glucose and db-cAMP or imidazole, as indicated. Results are expressed in pg TRH per islet (mean ± S.E.), $**P < 0.01$.

(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^{-7} 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 Ca^{2+}-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 Ca^{2+}-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 hyperosmolarity which, on its own, may suppress insulin secretion both *in vitro* (28, 29) and

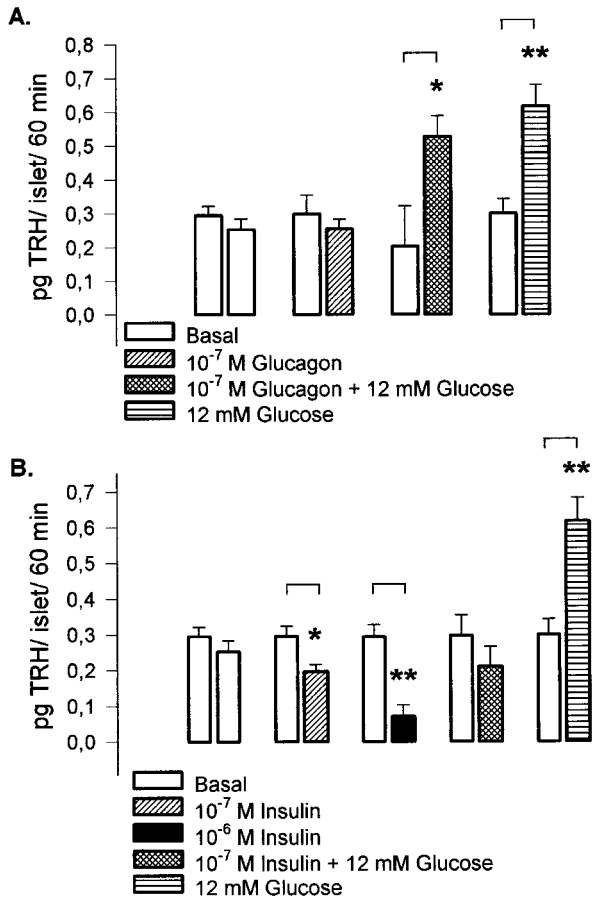

Figure 4 (A) Effect of exogenous glucagon on basal and glucose-stimulated release of TRH. Islets (30 per tube) were incubated for two subsequent 60-min periods, the first in basal medium and the second in medium containing either glucagon alone (10^{-7} mol/l) or 10^{-7} mol/l glucagon plus 12 mmol/l D-glucose, as indicated. (B) Effect of exogenous insulin on the basal and glucose-stimulated release of TRH. Islets were incubated for two subsequent 60-min periods, the first in basal medium and the second in medium containing insulin alone (10^{-7} and 10^{-6} mol/l) or 10^{-7} mol/l insulin plus 12 mmol/l D-glucose. Results are expressed in pg TRH per islet (mean ± S.E.), $*P < 0.05$, $**P < 0.01$.

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 Nikodémová & V Štrbák, 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.

Acknowledgements

We are indebted to A Krupková for technical assistance. This work was supported by grant 2/4133/97 of the Slovak Academy of Sciences (VEGA).

References

1 Burgus R, Dunn TF, Desiderio D & Guillemin R. Molecular structure of the hypothalamic hypophysiotropic TRF factor of ovine origin: mass spectrometry demonstration of the PCA-His-Pro-NH_2 sequence. *Comptes Rendus Hebdomadaires des Seances de l'Academie des Sciences. D: Sciences Naturelles* 1969 **269** 1870–1873.

2 Boler J, Enzmann F, Folkers K, Bowers CY & Schally AV. The identity of chemical and hormonal properties of the thyrotropin releasing hormone and pyroglutamyl-histidyl-proline amide. *Biochemical and Biophysical Research Communications* 1969 **37** 705–710.

3 Morley JE, Garvin TJ, Pekary AE & Hershman JM. Thyrotropin-releasing hormone in the gastrointestinal tract. *Biochemical and Biophysical Research Communications* 1977 **79** 314–318.

4 Martino E, Lernmark A, Seo H, Steiner DF & Refetoff S. High concentration of thyrotropin-releasing hormone in pancreatic islets. *Proceedings of the National Academy of Sciences of the USA* 1978 **75** 4265–4267.

5 Martino E, Seo H, Lernmark A & Refetoff S. Ontogenetic patterns of thyrotropin-releasing hormone-like material in rat hypothalamus, pancreas, and retina: selective effect of light deprivation. *Proceedings of the National Academy of Sciences of the USA* 1980 **77** 4345–4348.

6 Dutour A, Giraud P, Kowalski C, Ouafik L, Salers P, Štrbák V *et al.* Ontogenesis of TRH mRNA in the rat pancreas. *Biochemical and Biophysical Research Communications* 1987 **146** 354–360.

7 Engler D, Scanlon MF & Jackson IM. Thyrotropin-releasing hormone in the systemic circulation of the neonatal rat is derived from the pancreas and other extraneural tissues. *Journal of Clinical Investigation* 1981 **67** 800–808.

8 Aratan-Spire S, Wolf B & Czernichow P. Developmental pattern of TRH-degrading activity and TRH content in rat pancreas. *Acta Endocrinologica* 1984 **106** 102–108.

9 Aratan-Spire S, Wolf B, Portha B, Bailbe D & Czernichow P. Streptozotocin treatment at birth induces a parallel depletion of thyrotropin-releasing hormone and insulin in the rat pancreas during development. *Endocrinology* 1984 **114** 2369–2373.

10 Kawano H, Daikoku S & Saito S. Location of thyrotropin-releasing hormone-like immunoreactivity in rat pancreas. *Endocrinology* 1983 **112** 951–955.

11 Leduque P, Aratan-Spire S, Wolf B, Dubois PM & Czernichow P. Localization of thyrotropin-releasing hormone and insulin immunoreactivity in the pancreas of neonatal rats after injection of streptozotocin at birth. *Cell and Tissue Research* 1987 **248** 89–94.

12 Leduque P, Aratan-Spire S, Scharfmann R, Basmaciogullari A, Czernichow P & Dubois PM. Coexistence of thyrotropin-releasing hormone and insulin in cultured fetal rat islets: a light and electron microscopic immunocytochemical study during islet neoformation. *Biology of the Cell* 1989 **66** 291–296.

13 Ebiou JC, Grouselle D & Aratan-Spire S. Antithyrotropin-releasing hormone serum inhibits secretion of glucagon from isolated perfused rat pancreas: an experimental model for positive feedback regulation of glucagon secretion. *Endocrinology* 1992 **131** 765–771.

14 Morley JE, Levin SR, Pehlevanian M, Adachi R, Pekary AE & Hershman JM. The effects of thyrotropin-releasing hormone on the endocrine pancreas. *Endocrinology* 1979 **104** 137–139.

15 Vara E & Tamarit-Rodriguez J. Islet secretion of immunoreactive thyrotropin-releasing hormone and the 'paracrine-like' effects of its exogenous administration. *Acta Endocrinologica* 1988 **118** 429–436.

16 Schusdziarra V. Somatostatin – a regulatory modulator connecting nutrient entry and metabolism. *Hormone and Metabolic Research* 1980 **12** 563–577.

17 Kulkarni RN, Wang ZL, Akinsanya KO, Bennet WM, Wang RM, Smith DM *et al.* Pyroglutamyl-phenylalanyl-proline amide attenuates thyrotropin-releasing hormone-stimulated insulin secretion in perifused rat islets and insulin-secreting clonal beta-cell lines. *Endocrinology* 1995 **136** 5155–5164.

18 Yamada M, Saga Y, Shibusawa N, Hirato J, Murakami M, Iwasaki T *et al.* Tertiary hypothyroidism and hyperglycemia in mice with targeted disruption of the thyrotropin-releasing hormone gene. *Proceedings of the National Academy of Sciences of the USA* 1997 **94** 10862–10867.

19 Lacy PE & Kostianovsky M. Method for the isolation of intact islets of Langerhans from the rat pancreas. *Diabetes* 1967 **16** 35–39.

20 Oliver C, Eskay RL, Ben-Jonathan N & Porter JC. Distribution and concentration of TRH in the rat brain. *Endocrinology* 1974 **95** 540–546.

21 Fedotov VP, Sadovnikova NV, Pluzhnikova GN, Scholz R & Shvachkin IP. Effect of thyroliberin on pancreatic islet cell function in rats. *Problemy Endokrinologii* 1985 **31** 63–65.

22 Miley HE, Sheader EA, Brown PD & Best L. Glucose-induced swelling in rat pancreatic beta-cells. *Journal of Physiology* 1997 **504** 191–198.

23 Blackard WG, Kikuchi M, Rabinovitch A & Renold AE. An effect of hyposmolarity on insulin release *in vitro*. *American Journal of Physiology* 1975 **228** 706–713.

24 Lund PE, Berts A & Hellman B. Stimulation of insulin release by isosmolar addition of permeant molecules. *Molecular and Cellular Biochemistry* 1992 **109** 77–81.

25 Oshimoto K, Shimizu H, Sato N & Mori M. A case of Addison's disease which became worse during interferon therapy: insulin secretion under hyposmolarity. *Nippon Naibunpi Gakkai Zasshi* 1994 **70** 511–516.

26 Berts A, Lund PE, Dryselius S & Hellman B. Glucose has regulatory effects on insulin release also in the virtual absence of extracellular Ca^{2+}. *Biochemistry International* 1991 **24** 1085–1091.

27 Benický J, Greer MA & Štrbák V. Hyposmolar medium and ethanol in isosmotic solution induce the release of thyrotropin-releasing hormone (TRH) by isolated rat pancreatic islets. *Life Sciences* 1997 **60** 865–872.

28 Hermans MP & Henquin JC. Is there a role for osmotic events in the exocytotic release of insulin? *Endocrinology* 1986 **119** 105–111.

29 Sato N, Kashima K, Shimizu H, Shimomura Y & Mori M. Hypertonic glucose impairs glucose-induced increases in cytosol Ca^{2+} concentration and insulin secretion by HIT-T 15 cells. *Cell Calcium* 1996 **20** 273–278.

30 Tasaka Y, Matsumoto H, Inoue Y, Mochizuki N & Hirata Y. Plasma insulin and glucagon and their release from pancreatic islets in hyperosmolar diabetic rat. *Endocrinologia Japonica* 1991 **38** 53–59.

31 Sakuma N, Ishikawa S, Okada K, Miyazaki J & Saito T. Glucose induces calcium-dependent and calcium-independent insulin secretion from the pancreatic beta cell line MIN6. *European Journal of Endocrinology* 1995 **133** 227–234.

32 Zhang HJ, Walseth TF & Robertson RP. Insulin secretion and cAMP metabolism in HIT cells. Reciprocal and serial passage-dependent relationships. *Diabetes* 1989 **38** 44–48.

33 Loubatieres-Mariani MM. Current data on insulin secretion and its regulation. *Journal de Pharmacologie* 1986 **17** (Suppl 2) 83–103.
34 Grill V. Cyclic AMP and insulin release. *Acta Paediatrica* (Suppl) 1977 41–47.
35 Yamaguchi M, Iriuchijima T, Michimata T & Mori M. Glucose affects the release of thyrotropin-releasing hormone from rat hypothalamus. *Neuroendocrinology* 1991 **53** 423–427.
36 Lewis BM, Dieguez C, Ham J, Page MD, Creagh FM, Peters JR *et al.* Effects of glucose on thyrotropin-releasing hormone, growth hormone-releasing hormone, somatostatin and luteinizing hormone-releasing hormone release from rat hypothalamus *in vitro*. *Journal of Neuroendocrinology* 1989 **1** 437–441.
37 Argoud GM, Schade DS & Eaton RP. Insulin suppresses its own secretion *in vivo*. *Diabetes* 1987 **36** 959–962.
38 DeFronzo RA, Binder C, Wahren J, Felig P, Ferrannini E & Faber OK. Sensitivity of insulin secretion to feedback inhibition by hyperinsulinaemia. *Acta Endocrinologica* 1981 **98** 81–86.
39 Verspohl EJ, Handel M, Hagenloh I & Ammon HP. *In vitro* effect of exogenous insulin on insulin secretion. Studies with glucose, leucine, arginine, aminophylline and tolbutamide. *Acta Diabetologica Latina* 1982 **19** 303–317.
40 Draznin B, Goodman M, Leitner JW & Sussman KE. Feedback inhibition of insulin on insulin secretion in isolated pancreatic islets. *Endocrinology* 1986 **118** 1054–1058.
41 Patel YC, Amherdt M & Orci L. Quantitative electron microscopic autoradiography of insulin, glucagon, and somatostatin binding sites on islets. *Science* 1982 **217** 1155–1156.
42 Gazzano H, Halban P, Prentki M, Ballotti R, Brandenburg D, Fehlmann M *et al.* Identification of functional insulin receptors on membranes from an insulin-producing cell line (RINm5F). *Biochemical Journal* 1985 **226** 867–872.
43 Harbeck MC, Louie DC, Howland J, Wolf BA & Rothenberg PL. Expression of insulin receptor mRNA and insulin receptor substrate 1 in pancreatic islet beta-cells. *Diabetes* 1996 **45** 711–717.
44 Bent-Hansen L, Capito K & Hedeskov CJ. The effect of calcium on somatostatin inhibition of insulin release and cyclic AMP production in mouse pancreatic islets. *Biochimica et Biophysica Acta* 1979 **585** 240–249.
45 Claro A, Grill V, Efendic S & Luft R. Studies on the mechanisms of somatostatin action on insulin release. IV. Effect of somatostatin on cyclic AMP levels and phosphodiesterase activity in isolated rat pancreatic islets. *Acta Endocrinologica* 1977 **85** 379–388.
46 Dachicourt N, Serradas P, Giroix MH, Gangnerau MN & Portha B. Decreased glucose-induced cAMP and insulin release in islets of diabetic rats: reversal by IBMX, glucagon, GIP. *American Journal of Physiology* 1996 **271** E725–E732.
47 Gerber PP, Trimble ER, Wollheim CB, Renold AE & Miller RE. Glucose and cyclic AMP as stimulators of somatostatin and insulin secretion from the isolated, perfused rat pancreas: a quantitative study. *Diabetes* 1981 **30** 40–44.
48 Schauder P, McIntosh C, Herberg L, Arends J, Koop H, Frerichs H *et al.* Increased somatostatin secretion from pancreatic islets of streptozotocin-diabetic rats in response to glucose. *Molecular and Cellular Endocrinology* 1980 **20** 243–250.
49 Schauder P, McIntosh C, Arends J, Arnold R, Frerichs H & Creutzfeldt W. Somatostatin and insulin release from isolated rat pancreatic islets in response to D-glucose, L-leucine, alpha-ketoisocaproic acid or D-glyceraldehyde: evidence for a regulatory role of adenosine-3′,5′-cyclic monophosphate. *Biochemical and Biophysical Research Communications* 1977 **75** 630–635.
50 Dutour A, Giraud P, Maltese JY, Becquet D, Pesce G, Salers P *et al.* Regulation of TRH release by the cultured neonate rat pancreas. *Peptides* 1990 **11** 1081–1085.
51 Duntas LH, Papanastasiou L, Mantzou E, Jehle P, Mantzos I & Koutras DA. Inhibitory action of oral thyrotropin-releasing hormone on the regulatory response of the oral glucose tolerance test. *Thyroid* 1998 **8** 923–933.

Received 27 May 1999
Accepted 9 September 1999