Effects of growth hormone-releasing hormone on the secretion of islet hormones and on glucose homeostasis in lean and genetically obese-diabetic (ob/ob) mice and normal rats

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RECEIVED 23 February 1989

ABSTRACT

The effect of synthetic human growth hormone-releasing hormone(1–40) (hGHRH-40) on the function of the endocrine pancreas and on glucose homeostasis in lean and genetically obese-diabetic (ob/ob) mice and normal rats has been examined. The addition of 1 μmol hGHRH-40/l to incubated islets from normal lean mice increased insulin release by 90 and 37% at 5·6 and 16·7 mmol glucose/l respectively. Lower concentrations of hGHRH-40 did not affect insulin release. hGHRH-40 (1 μmol/l) increased pancreatic polypeptide release by 50% at 5·6 mmol glucose/l. A range of concentrations of hGHRH-40 (1 nmol/l–1 μmol/l) reduced glucagon release by 42–73% at 5·6 mmol glucose/l, and by 38–70% at 16·7 mmol glucose/l. Somatostatin release was increased (eightfold) by 1 μmol hGHRH-40/l at 5·6 mmol glucose/l, but at 1 nmol hGHRH-40/l somatostatin release was reduced (by > 50%). At 16·7 mmol glucose/litre 0·01–1 μmol hGHRH-40/l increased somatostatin release (three- to fourfold), but 1 nmol hGHRH-40/l produced a reduction of 50%. In vivo, administration of hGHRH-40 (50 μg/kg body weight i.p.) to fasted lean and ob/ob mice did not alter basal plasma concentrations of glucose and insulin, or the glucose and insulin responses to a concomitant i.p. glucose challenge. Intravenous injection of hGHRH-40 (20 μg/kg body weight) to anaesthetized rats increased plasma concentrations of insulin in the hepatic portal vein. A lower dose of hGHRH-40 (0·2 μg/kg) was ineffective, and neither dose of hGHRH-40 altered plasma glucose. The results indicate that hGHRH-40 exerts dose-dependent effects on the secretion of islet hormones, but this does not appear to be sufficient to produce measurable effects on plasma glucose homeostasis.

Journal of Endocrinology (1989) 123, 19–24

INTRODUCTION

Growth hormone (GH)-releasing hormone (GHRH) has been identified as the hypothalamic peptide with a potent stimulatory effect on the release of GH from the anterior pituitary (Frohman & Jansson, 1986). Several molecular forms have been identified (up to 44 amino acid residues in man) although the full biological activity appears to reside within the 29 amino acid residues at the C-terminus of the molecule (Ling, Laird, Wehrenberg et al. 1984). GHRH bears structural homologies with the glucagon-secretin family of peptides (Rivier, Speiss, Thorner & Vale, 1982), many of which stimulate insulin release (Walsh, 1987). Peripheral circulating concentrations of GHRH are increased by feeding (Penny, Sopwith, Patience et al. 1986; Sopwith, Penny, Grossman et al. 1986). GHRH has been identified in several tissues other than the hypothalamus, including normal human intestine (Christofides, Stephanou, Suzuki et al. 1984; Shibasaki, Kirosawa, Masuda et al. 1984) and pancreas (Bosman, Van Assche, Kruseman et al. 1984; Shibasaki et al. 1984), and human pancreatic islet cell tumours (Guillemin, Brazeau, Bohlem et al. 1982; Rivier et al. 1982).
1982). There is evidence that GHRH may be localized in islet pancreatic polypeptide cells (Bosman et al. 1984).

It has recently been reported that GHRH can enhance glucose-induced insulin secretion, and increase the release of glucagon and somatostatin by the isolated perfused dog pancreas (Hermansen, Kappelgaard, Esmann & Orskov, 1986). The present study investigated the effect of GHRH on insulin, glucagon, somatostatin and pancreatic polypeptide release from mouse islets. The effect of GHRH on glucose homeostasis and concentrations of insulin in normal and genetically obese-diabetic (ob/ob) mice and in normal rats has also been examined.

MATERIALS AND METHODS

Animals

Normal lean (+/+ ) and genetically obese-diabetic (ob/ob) mice from the Aston colony were maintained as described previously (Flatt, Bailey, Kwasowski et al. 1984). The origin and characteristics of these mice have been described elsewhere (Flatt & Bailey, 1981; Bailey, Flatt & Atkins, 1982). Mice were used at 15–20 weeks of age. Adult male Wistar rats were used at about 200 g body weight.

Isolated islets

Islets of Langerhans were prepared by collagenase digestion (Lacy & Kostianovsky, 1967) from lean mice fasted for 18 h. The pancreas was destended by a subcapsular injection of Krebs–Ringer bicarbonate buffer (KRB; pH 7.4), excised, minced and shaken for about 30 min at 37 °C in KRB containing glucose (5·6 mmol/l), collagenase (5 mg/ml) (Worthington type IV; Lorn Diagnostics, Bury St Edmunds, Suffolk, U.K.), Hepes (3·8 g/l; Sigma Chemical Co., Poole, Dorset, U.K.) and bovine serum albumin (BSA) fraction V (0·2 mg/ml; Sigma) pregassed with 95% O2: 5% CO2. The digest was washed three times in collagenase-free buffer and islets were harvested with the addition of DNase (1 μg/ml; Sigma). After preincubation for 15 min at 37 °C in pregassed KRB with glucose, Hepes and BSA as above, individual islets of visually the same size were test-incubated in multiwell plates (Costar, Cambridge, MA, U.S.A.) for 30 min at 37 °C in 1 ml pregassed buffer. The buffer comprised KRB, Hepes and BSA as above, supplemented with glucose at either 5·6 or 16·7 mmol/l plus synthetic human GHRH(1–40) (hGHRH-40; Cambridge Research Biochemicals, Harston, Cambs, U.K.) at 1–1000 nmol/l. After test incubations, samples of the medium were stored at −20 °C with the addition of aprotinin (1000 KIU/ml; Trasylol; Bayer, Haywards Heath, West Sussex, U.K.).

In-vivo studies

Norman lean (+/+ ) and obese-diabetic (ob/ob) mice which had previously been familiarized with the handling procedure were fasted for 24 h before and throughout the experiments. The following substances were administered by i.p. injection: 0·9% (w/v) NaCl (5 ml/kg body weight), glucose (2 g/kg body weight in a 40%, w/v, solution) and hGHRH-40 (50 μg/kg body weight). Blood samples (60 μl) were taken from the tail tip immediately before and at 5, 15 and 30 min after administration of test substances.

Fed rats were anaesthetized with sodium pentobarbitone (50 mg/kg body weight, i.p.). A fine polythene cannula (pp 10; Portex, Hythe, Kent, U.K.) was introduced into the right jugular vein and the following substances were administered by a bolus i.v. injection: 0·9% (w/v) NaCl (0·2 ml/kg body weight), glucose (200 mg/kg body weight in a 20%, w/v, solution) and synthetic hGHRH-40 (20 and 0·2 μg/kg body weight). Blood samples (80 μl) were withdrawn from a fine heparinized needle introduced into the hepatic portal vein just proximal to the liver. The samples were taken immediately before and at 2, 5, 10 and 15 min after administration of test substances.

Analyses

Plasma was separated and stored at −20 °C. Plasma glucose was measured by an automated glucose oxidase procedure (Stevens, 1971). Insulin was measured by double-antibody radioimmunoassay using rat insulin as standard (Bailey & Ahmed-Sorour, 1980). Glucagon, somatostatin and pancreatic polypeptide were determined by dextran-charcoal radioimmunoassays. Glucagon was measured using glucagon antiserum YY89 (mid to C-terminal specific), and the MRC International Reference Preparation of porcine glucagon was used as standard (Stout, Henry & Buchanan, 1976). Somatostatin was measured using an antiserum directed towards the mid-region of somatostatin-14, which recognizes both somatostatin-14 and somatostatin-28 on an approximately equimolar basis (Ballman & Conlon, 1985). Synthetic cyclic somatostatin was used as a standard. Pancreatic polypeptide was measured using antibody 221/9 which was raised against the synthetic C-terminal hexapeptide of pancreatic polypeptide. This antibody recognizes rat pancreatic polypeptide. Synthetic rat pancreatic polypeptide (Eli Lilly, Indianapolis, IN, U.S.A.) was used as standard (O’Hare, Daly & Buchanan, 1983). At concentrations up to 10 nmol/l, the synthetic hGHRH-40 did not cross-react with any
of the antisera. Glucagon (10 nmol/l) showed 0.5% cross-reactivity in the pancreatic polypeptide assay.

**Statistics**

Data are presented as means ± S.E.M. and were compared by one-way analysis of variance. Differences between individual groups were confirmed using Student's unpaired t-test, with confidence limits set at \( P<0.05 \) and \( P<0.01 \).

**RESULTS**

*Islets in vitro*

Islets isolated from the pancreas of normal lean mice showed an increased release of insulin \( (P<0.01) \) and somatostatin \( (P<0.01) \) during incubations for 30 min in the presence of high (16.7 mmol/l) compared with low (5.6 mmol/l) concentrations of glucose (Fig. 1). The addition of hGHRH-40 to the incubation medium at a concentration of 1 μmol/l increased insulin release by 90% at the low concentration of glucose and by 37% at the high concentration of glucose, but lower concentrations of hGHRH-40 (1–100 nmol/l) were not effective. Glucagon release was reduced by 42–73% at the low concentration of glucose and by 38–70% at the high concentration of glucose with all concentrations of hGHRH-40 tested (1–1000 nmol/l). Somatostatin release was increased (eightfold) with 1 μmol hGHRH-40/l at low glucose, but reduced below the level of accurate detection of the assay \(< 10 \text{ pmol/islet per 30 min} \) with 1 nmol hGHRH-40/l. At a high concentration of glucose, 10–1000 nmol hGHRH-40/l increased somatostatin release (three- to fourfold), while 1 nmol hGHRH-40/l reduced somatostatin release by 50%. The highest concentration of hGHRH-40 (1 μmol/l) increased pancreatic polypeptide release by 50% at the low concentration of glucose, but hGHRH-40 did not exert a significant effect at lower concentrations, or in the presence of a high concentration of glucose.

*Glucose homeostasis in vivo*

Compared with normal lean mice (Fig. 2a), genetically obese-diabetic \((ob/ob)\) mice characteristically exhibited basal hyperglycaemia and hyperinsulinaemia, impaired glucose tolerance and a defective plasma insulin response to an i.p. glucose challenge (Fig. 2b). Administration of hGHRH-40 (50 μg/kg body weight, i.p.) did not significantly alter basal plasma concentrations of glucose and insulin, or plasma glucose and insulin responses to an i.p. glucose challenge in either fasted normal lean or obese-diabetic \((ob/ob)\) mice.

**FIGURE 1.** Effect of synthetic human growth hormone-releasing hormone \((1–40)\) (hGHRH-40; 1–1000 nmol/l) on the release of insulin, glucagon, somatostatin and pancreatic polypeptide by isolated mouse islets during incubations for 30 min in the presence of 5.6 (left panels) and 16.7 (right panels) mmol glucose/l. Values are means ± S.E.M.; \( n = 12–14 \). *\( P<0.05 \), **\( P<0.01 \) compared with control \( (\text{no hGHRH-40}) \) (Student's unpaired t-test); †Value below 10 pmol/islet per 30 min.

Intravenous injection of glucose (200 mg/kg body weight) rapidly increased plasma concentrations of glucose and insulin in the hepatic portal vein of fed anaesthetized rats (Fig. 3). Compared with an i.v. injection of saline alone, injection of hGHRH-40 at a dose of 20 μg/kg body weight increased plasma insulin in the hepatic portal vein without altering plasma glucose. Injection of a lower dose of hGHRH-40 (0.2 μg/kg body weight) did not affect either plasma glucose or insulin.

**DISCUSSION**

The stimulatory effect of hGHRH-40 on insulin release by mouse islets and rat pancreas at physiological concentrations of glucose is consistent with previous
studies using the perfused dog pancreas (Hermansen et al. 1986; Hermansen & Kappelgaard, 1987). These results contrast with in-vivo observations in other species including man (Thorner, Rivier, Speiss et al. 1983; Foltzer-Jourdainne, Harvey, Karmann & Mialhe, 1987) which failed to demonstrate an effect of hGHRH-40 on insulin release. The observation that hGHRH-44 stimulates insulin release in the rat (Green, Lo, Crowther et al. 1986) but not in the dog (Hermansen et al. 1986) further raises the question of species differences. The fact that hGHRH-40 enhanced insulin release in the mouse in vitro but not in vivo in the present study indicates, however, that peptide concentration, site of delivery and extrapancreatic effects each contribute to the overall effectiveness of hGHRH-40 as a stimulus for insulin secretion.

Although GHRH is present at low concentrations in plasma (typically less than 50 pg/ml, approximately 10 pmol/l; Thorner, Frohman, Leong et al. 1984; Kashio, Chihara, Kita et al. 1987), its occurrence within the islets of Langerhans (Bosman et al. 1984; Shibasaki et al. 1984) suggests a more important role in the local modulation of islet hormone secretions. This view is supported by the present study which demonstrated that hGHRH-40 exerts dose-dependent effects on the secretion of insulin, glucagon, somatostatin and pancreatic polypeptide. Since these hormones will influence the secretory activity of neighbouring endocrine cells through paracrine interactions, it is difficult to distinguish the effects of GHRH on individual A, B, D and pancreatic polypeptide cell types from the effects on the overall islet unit. It is apparent from the present study, however, that the insulin-releasing effect of hGHRH-40 is not attributable to stimulation of glucagon, since glucagon release was reduced. This contrasts with a glucagon-releasing effect of hGHRH-40 in the dog pancreas (Hermansen et al. 1986), although it is notable that this study examined only a small concentration range (5–30 nmol/l).

Preliminary evidence has linked the stimulatory effect of hGHRH-44 on insulin secretion in the rat to activation of adenylate cyclase and increases in intracellular cyclic AMP (Green et al. 1986). The release of

![Figure 2: Effect of synthetic human growth hormone-releasing hormone (1–40) (hGHRH-40; 50 µg/kg body weight, i.p.) on plasma concentrations of glucose and insulin of fasted lean (upper panels) and genetically obese diabetic (ob/ob) (lower panels) mice. Animals were injected with saline (5 ml/kg body weight, i.p.; ○), hGHRH-40 (50 µg/kg per 5 ml body weight i.p.; ●), glucose (2 g/5 ml per kg body weight, i.p.; □) or hGHRH-40 (50 µg/kg body weight dissolved in glucose 2 g/5 ml per kg body weight, i.p.; ■). Values are means ± S.E.M.; n = 6.](image)
somatostatin by hGHRH-40 in the present study may, however, have been sufficient to suppress the secretion of glucagon and possibly insulin (Gerich, 1983). Interestingly, stimulation of somatostatin release by hGHRH-40 in mouse islets was more sensitive at high concentrations of glucose (10–1000 nmol hGHRH-40/l was effective) than at low concentrations of glucose (only 1 μmol hGHRH-40/l was effective). Stimulation of somatostatin release by hGHRH-40 (15–30 nmol/l) was also noted in dog pancreas (Hermansen et al. 1986), but the present study has further shown that a lower concentration of hGHRH-40 (1 nmol/l) reduced somatostatin release by mouse islets at both low and high concentrations of glucose. An ability of hGHRH-40 to suppress plasma concentrations of somatostatin has also been noted in young ducks (Foltzer-Jourdainne et al. 1987).

Peripheral concentrations of GHRH have been shown to rise after a meal (Penny et al. 1986; Sopwith et al. 1986; Kashio et al. 1987), raising the possibility that extrahypothalamic GHRH might exert an effect on islet function and influence normal glucose homeostasis. An i.p. injection of hGHRH-40 (50 μg/kg body weight) in normal lean and ob/ob mice failed, however, to produce any significant alterations of plasma concentrations of glucose and insulin in the basal state or after an i.p. glucose challenge. The dose of 50 μg hGHRH-40/kg is expected to raise peripheral circulating concentrations by several orders of magnitude. The need for high peripheral circulating concentrations of GHRH to stimulate insulin release in vivo was demonstrated by the bolus i.v. injection of hGHRH-40 to anaesthetized rats. A dose of 0.2 μg/kg body weight, which is likely to produce a transient circulating concentration approaching 1 nmol/l, did not alter the concentration of insulin in the hepatic portal vein close to the liver. A dose of 20 μg/kg body weight, likely to produce a transient circulating concentration approaching 100 nmol/l, produced only a transient increase of insulin in the portal vein.

The results suggest that hGHRH-40 affects endocrine pancreatic function by reducing somatostatin release at low concentrations (1 nmol/l) and increasing somatostatin release at higher concentrations. Despite increased somatostatin release, high concentrations of hGHRH-40 (for example 1 μmol/l in mouse islets) enhanced insulin release, but plasma concentrations of glucose were not acutely affected.

**REFERENCES**


**FIGURE 3.** Effect of synthetic human growth hormone-releasing hormone (1–40) (hGHRH-40; 20 and 0.2 μg/ml per kg body weight, i.v.) on plasma concentrations of glucose and insulin in the hepatic portal vein of anaesthetized rats. Animals were injected with saline (1 ml/kg body weight, i.v.; ○), glucose (0.5 g/ml per kg body weight, i.v.; □), hGHRH-40 (20 μg/ml per kg body weight, i.v. dissolved in saline; ●) or hGHRH-40 (0.2 μg/ml per kg body weight, i.v. dissolved in saline; ▲). Values are means ± S.E.M.; n = 5–6. *P < 0.05, **P < 0.01 compared with saline only (Student’s unpaired t-test).


