Differential responses of the growth hormone axis in two rat models of streptozotocin-induced insulinopenic diabetes

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Abstract

The impact of streptozotocin (STZ)-induced, insulinopenic diabetes on the GH axis of rats and mice differs from study to study, where this variation may be related to the induction scheme, severity of the diabetes and/or the genetic background of the animal model used. In order to begin differentiate between these possibilities, we compared the effects of two different STZ induction schemes on the GH axis of male Sprague–Dawley rats: (1) a single high-dose injection of STZ (HI STZ, 80 mg/kg, i.p.), which results in rapid chemical destruction of the pancreatic β -cells, and (2) multiple low-dose injections of STZ (LO STZ, 20 mg/kg for 5 consecutive days, i.p.), which results in a gradual, autoimmune destruction of β -cells. STZ-treated animals were killed after 3 weeks of hyperglycemia (>400 mg/dl), and in both paradigms circulating insulin levels were reduced to $<40\%$ of vehicle-treated controls. HI STZ-treated rats lost weight, while body weights of LO STZ-treated animals gradually increased over time, similar to vehicle-treated controls. As previously reported, HI STZ resulted in a decrease in circulating GH and IGF-I levels which was associated with a rise in hypothalamic neuropeptide Y (NPY) mRNA (355% of vehicle-treated controls) and a fall in GH-releasing hormone (GHRH) mRNA (45% of vehicle-treated controls) levels. Changes in hypothalamic neuropeptide expression were reflected by an increase in immunoreactive NPY within the arcuate and paraventricular nuclei and a decrease in GHRH immunoreactivity in the arcuate nucleus, as assessed by immunohistochemistry. Consistent with the decline in circulating GH

and hypothalamic GHRH, pituitary GH mRNA levels of HI STZ-treated rats were 58% of controls. However, pituitary receptor mRNA levels for GHRH and ghrelin increased and those for somatostatin (sst2, sst3 and sst5) decreased following HI STZ treatment. The impact of LO STZ treatment on the GH axis differed from that observed following HI STZ treatment, despite comparable changes in circulating glucose and insulin. Specifically, LO STZ treatment did suppress circulating IGF-I levels to the same extent as HI STZ treatment; however, the impact on hypothalamic NPY mRNA levels was less dramatic (158% of vehicle-treated controls) where NPY immunoreactivity was increased only within the paraventricular nucleus. Also, there were no changes in circulating GH, hypothalamic GHRH or pituitary receptor expression following LO STZ treatment, with the exception that pituitary sst3 mRNA levels were suppressed compared with vehicle-treated controls. Taken together these results clearly demonstrate that insulinopenia, hyperglycemia and reduced circulating IGF-I levels are not the primary mediators of hypothalamic and pituitary changes in the GH axis of rats following HI STZ treatment. Changes in the GH axis of HI STZ-treated rats were accompanied by weight loss, and these changes are strikingly similar to those observed in the fasted rat, which suggests that factors associated with the catabolic state are critical in modifying the GH axis following STZ-induced diabetes.

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Introduction

A commonly used method to induce insulin-dependent diabetes in rodents is a single high-dose injection (i.p. or i.v.) of streptozotocin (HI STZ). In this paradigm, STZ induces pancreatic β -cell necrosis, which is evident 4 h following STZ injection. Blood glucose levels peak 1–3 days following STZ injection and remain elevated

if the appropriate dose is given (Candela *et al.* 1979, Junod *et al.* 1969, Rossini *et al.* 1977). HI STZ-induced insulinopenia is typically associated with rapid weight loss, due to a decrease in fat stores, leading to a fall in circulating leptin (Havel *et al.* 1998, Sindelar *et al.* 1999) and a compensatory rise in hypothalamic neuropeptide Y (NPY), an orexigenic signal (Ishii *et al.* 2002, Marks *et al.* 1993, White *et al.* 1990). In rats (Sprague–Dawley and

Wistar), these metabolic changes have been associated with suppression of the growth hormone (GH) axis, which includes a decrease in hypothalamic GH-releasing hormone (GHRH) and pituitary GH mRNA levels and a reduction in pulsatile GH release, and mean circulating insulin-like growth factor (IGF)-I levels (Busiguina *et al.* 2000b, Olchovsky *et al.* 1990). Despite these changes, the pituitary of the HI STZ-treated rat is more sensitive to the stimulatory actions of GHRH (Sheppard *et al.* 1989a, 1989b) and GH secretagogue (GHS) receptor (GHS-R) agonists (ipamorelin; Johansen *et al.* 2003) and less sensitive to the inhibitory actions of somatostatin (SRIH; Bruno *et al.* 1994, Sheppard *et al.* 1989b).

Mice (ICR background) treated with HI STZ (200 mg/kg, i.p. injection) also lose weight and exhibit a dramatic reduction in circulating GH and IGF-I and a decrease in pituitary GH and hypothalamic GHRH expression, which is associated with pituitary GHRH hypersensitivity, *in vitro* (Murao *et al.* 1995). It should be noted that these changes in the GH axis are strikingly similar to those observed in the fasted rat (Park *et al.* 2004, Tannenbaum *et al.* 1979) and therefore might be related to the catabolic condition and not to the absolute circulating levels of insulin and glucose. This hypothesis is consistent with a report showing BALB/c mice, when treated with a single i.v. injection of HI STZ (250–300 mg/kg), have elevated circulating GH levels, which was associated with hypoinsulinemia and hyperglycemia without dramatic weight loss or ketosis (Flyvbjerg *et al.* 1999), a response similar to that reported in poorly controlled type I diabetic humans (Cohen & Abplanalp 1991, Ismail *et al.* 1993, Krassowski *et al.* 1988). However, from these studies it is difficult to say with certainty if the variable effects of STZ on circulating GH levels are due to the severity of catabolic condition or if the differences are more related to species or genetic background of the animal model used. To help clarify this issue we have compared the impact of diabetes on the GH axis of male Sprague–Dawley rats, where diabetes was induced by either HI STZ treatment or by multiple low-dose injections of STZ (LO STZ), where LO STZ results in hypoinsulinemia and hyperglycemia >7 days following the last STZ injection, with the severity of the disease increasing over time due to the gradual autoimmune destruction of pancreatic β -cells (Li *et al.* 2000a, 2000b, Like & Rossini 1976).

Materials and Methods

Animals

Male Sprague–Dawley rats (7–8 weeks; 220–250 g) were housed under controlled environmental conditions (12 h:12 h light/dark). Food and tap water were available *ad libitum*. Experiments were conducted according to the

principles and procedures outlined in the NIH Guide for the Care and Use of Laboratory Animals.

STZ-induced diabetes

HI STZ In order to induce rapid-onset diabetes, rats were treated with vehicle (citrate buffer, pH 4.5; $n=5$) or STZ (80 mg/kg, i.p.; $n=7$) between 14:00 and 16:00 h. Body weights and blood glucose levels were determined on day 0 (time of STZ treatment) and on days 2, 4, 7, 14 and 21. HI STZ-treated animals lost weight (Fig. 1A) and all treated animals displayed hyperglycemia (>22.2 nmol/l or 400 mg/dl) 2 days after STZ injection and remained hyperglycemic until their death (Fig. 1B).

LO STZ To generate a model resembling a more gradual onset of diabetes, rats received vehicle ($n=5$) or STZ (20 mg/kg, i.p.; $n=7$) for 5 consecutive days as previously described (Li *et al.* 2000a, Like & Rossini 1976). Body weight and blood glucose levels were measured at day –4 (time of first STZ treatment), day 0 (time of last STZ treatment) and on days 2, 4, 7, 14, 21 and 28 following the last day of STZ injection. Body weights were not suppressed with treatment (Fig. 1A) and blood glucose levels began to rise to 262 mg/dl at 2 days after the last injection. All LO STZ-treated animals showed hyperglycemia (>22.2 nmol/l or 400 mg/dl) 7 days after last injection and remained hyperglycemic until their death (Fig. 1B).

HI STZ-induced diabetic animals were killed 21 days following bolus STZ treatment, and LO STZ animals were killed 4 weeks after the last STZ injection. Therefore both groups of animals were exposed to hyperglycemia for 3 weeks. Blood, pituitaries and hypothalami were collected and stored at -70°C until further analysis.

Measurement of glucose, GH, IGF-I and insulin concentrations

Glucose levels were measured using blood from the tail vein by GlucoDr Blood Glucose Meter (Allmedicus, Korea; maximal reading 600 mg/dl). Serum GH concentrations were measured by rat GH RIA kit (Amersham Biosciences Co.). Total serum IGF-I levels were assayed using a rat IGF-I RIA kit (Amersham) after acid/ethanol extraction according to the manufacturer's instructions. Serum insulin concentrations were assessed using the rat insulin RIA kit (Amersham).

RNA isolation

Total hypothalamic and pituitary RNA were recovered using standard procedure reported previously (Kamegai *et al.* 1998b, 1998c). RNA was then precipitated with isopropanol, and the pellet was washed with 70% ethanol, air dried, and dissolved in sterile DEPC

Figure 1 Effect of a single high dose of STZ (80 mg/kg, i.p.; $n=5$ vehicle, $n=7$ STZ) and multiple low doses of STZ (20 mg/kg, i.p., for 5 consecutive days; $n=5$ vehicle, $n=7$ STZ) on body weight (A), blood glucose (B) and serum insulin levels (C). Glucose and body weight were measured at the indicated day and insulin concentrations were assessed after animals were killed. Values are the means $\pm$ S.E.M. Values with a different subscript letter are significantly different ($P<0.05$).

(diethyl pyrocarbonate-treated) water. The concentration and purity of RNA were determined by NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) at wavelengths of 260/280 nm.

RNAse protection assay (RPA) of hypothalamic GHRH, SRIH and NPY mRNA

Hypothalamic GHRH, SRIH and NPY mRNA levels were measured by RPA using the HybSpeed RPA kit

(Ambion, Austin, TX, USA) as previously described (Park *et al.* 2004). Briefly, in a single reaction, probes for GHRH, SRIH, NPY and β -actin were incubated for 20 min at 68 °C in 10 μ l HybSpeed Hybridization Buffer containing 50% total RNA isolated from a single hypothalamus or 50 μ g yeast RNA. Unhybridized probes were removed by treating the reactions with RNase A/T1 mix for 1 h at 37 °C. Protected fragments were separated on a 5% polyacrylamide/8 M urea gel. The gel was dried on chromatography paper and exposed to a phosphorimager screen (Packard Instruments, Fallbrook, CA, USA). Band intensity was evaluated by image-analysis software (Non-linear Dynamics, Newcastle upon Tyne, UK).

Real-time reverse transcriptase (RT)-PCR of pituitary GH, SRIH receptor subtypes, GHRH receptor (GHRH-R) and GHS-R mRNA

Total pituitary RNA (1 μ g) was used as a template to generate cDNA by RT with random hexamer priming. The resultant cDNA was amplified using the LightCycler. Real-time PCR analysis was carried out with SYBR Green I and primers (for GH, sst3, sst4, sst5, GHRH-R and β -actin) or hybridization probes and primers (for sst1, sst2 and GHS-R). The sequences of primers for GH (GenBank accession no. V01237) were as follows: sense, 5'-CTG GCT GCT GAC ACC TAC AAA-3'; antisense, 5'-CAG GAG AGC AGC CCA TAG TTT-3'. Details of the procedure of the real-time PCR for the SRIH receptor subtypes, GHRH-R and GHS-R mRNA levels have been described previously (Park *et al.* 2004).

Statistical analysis

All data are expressed as means $\pm$ S.E.M. Comparisons between groups were made by Student's *t*-test or ANOVA, and $P<0.05$ was considered significant. All comparisons were made between samples electrophoresed on the same gel (for RPAs) or real-time PCR run.

Results

HI STZ treatment resulted in hyperglycemia, hypoinsulinemia and significant weight loss (-27.4%), consistent with previous reports (Busiguina *et al.* 2000b, Marks *et al.* 1993, Olchovsky *et al.* 1990; Fig. 1). Circulating GH and IGF-I levels were also significantly decreased with HI STZ treatment and these changes were associated with a suppression of pituitary GH mRNA and hepatic IGF-I mRNA levels (Fig. 2). LO STZ-treated diabetic animals showed hyperglycemia, hypoinsulinemia (Fig. 1) and decreased circulating IGF-I levels (Fig. 2) similar to that observed in HI STZ-treated rats. In contrast, LO STZ-treated rats did not lose weight (Fig. 1) and circulating GH levels and pituitary GH and

Figure 2 Effect of a single high dose of STZ (80 mg/kg, i.p.; $n=5$ vehicle, $n=7$ STZ) and multiple low doses of STZ (20 mg/kg, i.p., for 5 consecutive days, $n=5$ vehicle, $n=7$ STZ) on circulating GH levels, circulating IGF-I levels, pituitary GH mRNA levels and hepatic IGF-I mRNA levels. GH and IGF-I mRNA levels were measured by real-time RT-PCR and adjusted by β -actin. Circulating GH and IGF-I levels are the means $\pm$ S.E.M. Values with a different subscript letter are significantly different ($P<0.05$). GH and IGF-I mRNA levels are expressed as percentage of respective vehicle-treated controls and are shown as the means $\pm$ S.E.M. ** $P<0.01$.

hepatic IGF-I expression did not differ when compared with vehicle-treated controls (Fig. 2).

In HI STZ-treated diabetic animals, hypothalamic NPY mRNA levels were dramatically increased (355% of vehicle-treated controls; $P<0.01$), while GHRH mRNA levels were decreased to 50% of controls ($P<0.01$; Fig. 3). In contrast, in LO STZ-treated animals, hypothalamic NPY mRNA levels were increased to only 158% of vehicle-treated controls ($P<0.05$; Fig. 3). Under these conditions, STZ failed to alter GHRH mRNA expression. SRIH mRNA levels in HI and LO STZ-treated animals did not differ from controls.

At the pituitary level HI STZ treatment resulted in an increase in GHRH-R and GHS-R mRNA levels (Fig. 4) and a decrease in mRNA levels of the SRIH receptor subtypes sst2, sst3 and sst5 (Fig. 5). In contrast, LO STZ treatment only suppressed sst3 mRNA levels compared with vehicle-treated control. In both induction schemes pituitary sst1 and sst4 mRNA levels were not altered.

Discussion

The results of the present study clearly demonstrate that changes in the GH axis following STZ-induced diabetes are dependent on the induction scheme used. HI STZ

treatment led to a significant weight loss which was associated with changes in the GH axis similar to that

Figure 3 Effect of a single high dose of STZ (80 mg/kg, i.p., $n=5$ vehicle, $n=7$ STZ) and multiple low doses of STZ (20 mg/kg, i.p., for 5 consecutive days, $n=5$ vehicle, $n=7$ STZ) on hypothalamic GHRH, NPY and SRIH mRNA levels. Hypothalamic neuropeptide mRNA levels were measured by RPA and NPY and SRIH mRNA levels were adjusted by β -actin. Data are expressed as percentage of respective vehicle-treated controls and are shown as the means $\pm$ S.E.M. * $P<0.05$, ** $P<0.01$.

Figure 4 Effect of a single high dose of STZ (80 mg/kg, i.p., $n=5$ vehicle, $n=7$ STZ) and multiple low doses of STZ (20 mg/kg, i.p., for 5 consecutive days, $n=5$ vehicle, $n=7$ STZ) on pituitary GHRH-R and GHS-R mRNA levels. GHRH-R and GHS-R mRNA levels were measured by real-time RT-PCR and adjusted by β -actin. Data are expressed as percentage of respective vehicle-treated controls and are shown as the means $\pm$ S.E.M. ** $P<0.01$.

observed in the fasted rat (Park *et al.* 2004, Tannenbaum *et al.* 1979), which included suppression of hypothalamic GHRH, pituitary GH and hepatic IGF-I mRNA levels and decreased circulating GH and IGF-I, accompanied by a reciprocal shift in the expression of GH stimulatory and inhibitory receptors. In contrast, LO STZ treatment, which did not alter body weight, had little impact on the GH axis, with the exception that circulating IGF-I levels and pituitary *sst3* mRNA levels were reduced compared with controls. Since both HI STZ and LO STZ treatment

Figure 5 Effect of a single high-dose of STZ (80 mg/kg, i.p., $n=5$ vehicle, $n=7$ STZ) and multiple low doses of STZ (20 mg/kg, i.p., for 5 consecutive days, $n=5$ vehicle, $n=7$ STZ) on pituitary SRIH receptor subtype (*sst1*–*sst5*) mRNA levels. *sst1*–*sst5* mRNA levels were measured by real-time RT-PCR and adjusted by β -actin. Data are expressed as percentage of respective vehicle-treated controls and are shown as the means $\pm$ S.E.M. * $P<0.05$, ** $P<0.01$.

regimens resulted in insulinopenia, hyperglycemia and suppressed circulating IGF-I, we can conclude that these factors are not the primary modulators of the central and pituitary GH axis following STZ treatment. Given the striking similarity between the GH axis of rats treated with HI STZ and those that have been fasted, it is likely that changes associated with the catabolic condition play a key role in precipitating events leading to many of the changes in the GH axis following HI STZ treatment.

One event that may be central to HI STZ-induced changes in the GH axis is the dramatic rise in hypothalamic NPY. In the rat and mouse, food deprivation and HI STZ-induced diabetes lead to increased activity of NPY neurons within the arcuate nucleus (ARC) of the hypothalamus (Marks *et al.* 1993, Mizuno *et al.* 1999, Shimizu-Albergine *et al.* 2001, Vuagnat *et al.* 1998, White *et al.* 1990). In the rat, the fasting- and STZ-induced increases in NPY neuronal activity are associated with a decline in hypothalamic GHRH expression and suppression of pulsatile GH release (Busiguina *et al.* 2000a, Park *et al.* 2004, Tannenbaum 1981, Tannenbaum *et al.* 1979, 1986, 1989, White *et al.* 1990). NPY maybe a key inhibitor of the GH axis in that intracerebroventricular administration of NPY inhibits pulsatile GH release in rats and decreases GHRH mRNA levels in both rats and mice (Pierroz *et al.* 1996, Raposinho *et al.* 2000, 2001, Sainsbury & Herzog 2001, Suzuki *et al.* 1996). The significance of endogenous NPY in regulation of hypothalamic GHRH expression in catabolic states is supported by a recent report from our laboratory demonstrating that NPY-knockout mice do not exhibit fasting-induced suppression of GHRH mRNA (Park *et al.* 2005). In that NPY levels did not rise as dramatically LO STZ-treated rats, compared with HI STZ rats, we might speculate that these changes were not adequate to suppress GHRH expression.

Despite the reduction in GHRH expression following HI STZ treatment, we observed a reciprocal shift in the expression pattern of GH inhibitory and GH stimulatory receptors that would favor GH release and synthesis, similar to that observed following fasting (Park *et al.* 2004). As previously reported by Bruno *et al.* (1994), we also observed that HI STZ treatment resulted in a decline in pituitary *sst2*, *sst3* and *sst5* mRNA levels. In addition, we report for the first time that HI STZ treatment enhances pituitary GHRH-R and GHS-R mRNA levels. It is possible that a reduction in GHRH input to the pituitary is required for some of the changes in pituitary receptor expression following HI STZ treatment in that we have previously reported that GHRH acutely inhibits GHRH-R expression and stimulates *sst2* expression *in vitro* (Kamegai *et al.* 1998a, Park *et al.* 2000).

Changes in pituitary expression of GH regulatory receptors in the HI STZ-treated rats are in line with reports demonstrating changes in pituitary sensitivity to their respective ligands. Specifically, pituitaries of HI

STZ-treated rats are more sensitive to the stimulatory actions of GHRH (Sheppard *et al.* 1989a, 1989b) and less sensitive to the inhibitory actions of SRIH (Bruno *et al.* 1994, Sheppard *et al.* 1989b) *in vitro*. Also, *in vivo* sensitivity to GHRH and a GHS-R ligand, GHRP-6, was negatively correlated with body weight in HI STZ-treated rats (Diz *et al.* 2003). In addition, STZ-treated mice have been reported to display enhanced GH responses to the GHS-R ligand ipamorelin (Johansen *et al.* 2003). Comparable changes in pituitary sensitivity to GH secretagogues are observed in patients with uncontrolled insulin-dependent diabetes (Catalina *et al.* 1998, Krassowski *et al.* 1988). Therefore, it is possible that the enhanced GH output observed in the insulin-dependent diabetic human (Catalina *et al.* 1998, Krassowski *et al.* 1988) may be related, at least in part, to changes in pituitary receptor expression that would favor GH release. It has also been hypothesized that the characteristic reduction in circulating IGF-I and insulin, both known inhibitors of GH synthesis and release (Yamashita & Melmed 1986a, 1986b), could enhance GH output in insulinopenic diabetes (Bereket *et al.* 1999). However, it should be noted that pulsatile GH release is blocked in the HI STZ-induced diabetic rat (Tannenbaum 1981), suggesting that the enhanced sensitivity to GH secretagogues is not sufficient to override metabolic changes in hypothalamic input in this animal model.

In the current study, circulating IGF-I levels were reduced in both HI STZ- and LO STZ-treated rats despite differential effects on pituitary GH synthesis and circulating GH levels, clearly demonstrating that a decrease in GH input is not required for the reduction in IGF-I output observed in the diabetic state. These observations are consistent with a previous report where circulating IGF-I levels are reduced in LO STZ-treated rats, without significant changes in circulating GH levels (Khamaisi *et al.* 2002). The liver is the primary source of IGF-I (Sjogren *et al.* 1999) and in the HI STZ-treated rat the fall in circulating IGF-I was reflected in a decrease in hepatic IGF-I mRNA levels. However, this relationship was not observed in the LO STZ-treated rat, where hepatic IGF-I mRNA levels did not differ from vehicle-treated controls, suggesting that a decrease in IGF-I gene expression is not the only component in modulating circulating IGF-I levels in diabetes. It has been reported that the clearance rate of IGF-I is increased in diabetic rats which may be due to a reduction in circulating IGF-I-binding proteins (IGFBP3 and IGFBP4; Higaki *et al.* 1997, Khamaisi *et al.* 2002).

Studies using STZ, to induce diabetes in rodents, have provided a plethora of valuable information regarding the impact of insulinopenia and hyperglycemia on various physiologic endpoints. However, caution should be exercised when interpreting these results because STZ is a potent toxin that has been shown to damage multiple tissue types, in addition to its experimentally relevant

effect on pancreatic β -cells. These include toxic effects on the neuroendocrine gastrointestinal tract which results in a decrease in gastric motility (Brenna *et al.* 2003), direct toxic effects on hepatocyte function which inhibits biliary excretions (Carnovale & Rodriguez Garay 1984) and toxic effects on the kidneys leading to urinary protein leakage (Palm *et al.* 2004). All of these effects could contribute to the acute weight loss observed in HI STZ-treated rats. Finally, the toxic effects of STZ can extend to the pituitary. Liu *et al.* (2002) have shown HI STZ (100–200 mg/kg) results in the blockade of GH secretory vesicle release and somatotrope rupture in rats suggesting that some of the reduction in pituitary GH output in HI STZ-treated rats could be due to toxic destruction of the somatotropes. Therefore, the time after STZ treatment and STZ dose are critical in differentiating between toxic and metabolic effects of STZ treatment on GH release, and LO STZ-induced diabetes may be a more suitable model.

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References

- Bereket A, Lang CH & Wilson TA 1999 Alterations in the growth hormone–insulin-like growth factor axis in insulin dependent diabetes mellitus. *Hormone and Metabolic Research* **31** 172–181.
- Brenna O, Qvigstad G, Brenna E & Waldum HL 2003 Cytotoxicity of streptozotocin on neuroendocrine cells of the pancreas and the gut. *Digestive Diseases and Sciences* **48** 906–910.
- Bruno JF, Xu Y, Song J & Berelowitz M 1994 Pituitary and hypothalamic somatostatin receptor subtype messenger ribonucleic acid expression in the food-deprived and diabetic rat. *Endocrinology* **135** 1787–1792.
- Busiguina S, Argente J, Garcia-Segura LM & Chowen JA 2000a Anatomically specific changes in the expression of somatostatin, growth hormone-releasing hormone and growth hormone receptor mRNA in diabetic rats. *Journal of Neuroendocrinology* **12** 29–39.
- Busiguina S, Argente J, Garcia-Segura LM & Chowen JA 2000b Anatomically specific changes in the expression of somatostatin, growth hormone-releasing hormone and growth hormone receptor mRNA in diabetic rats. *Journal of Neuroendocrinology* **12** 29–39.
- Candela S, Hernandez RE & Gagliardino JJ 1979 Circadian variation of the streptozotocin-diabetogenic effect in mice. *Experientia* **35** 1256–1257.
- Carnovale CE & Rodriguez Garay EA 1984 Reversible impairment of hepatobiliary function induced by streptozotocin in the rat. *Experientia* **40** 248–250.
- Catalina PF, Mallo F, Andrade MA, Garcia-Mayor RV & Dieguez C 1998 Growth hormone (GH) response to GH-releasing peptide-6

- in type 1 diabetic patients with exaggerated GH-releasing hormone-stimulated GH secretion. *Journal of Clinical Endocrinology and Metabolism* **83** 3663–3667.
- Cohen RM & Abplanalp WA 1991 Resistance of growth hormone secretion to somatostatin in men with type I diabetes mellitus. *Diabetes* **40** 1251–1258.
- Diz CY, Spuch CC, Perez TD & Mallo FF 2003 GH responses to GHRH and GHRP-6 in Streptozotocin (STZ)-diabetic rats. *Life Sciences* **73** 3375–3385.
- Flyvbjerg A, Bennett WF, Rasch R, Kopchick JJ & Scarlett JA 1999 Inhibitory effect of a growth hormone receptor antagonist (G120K-PEG) on renal enlargement, glomerular hypertrophy, and urinary albumin excretion in experimental diabetes in mice. *Diabetes* **48** 377–382.
- Havel PJ, Uriu-Hare JY, Liu T, Stanhope KL, Stern JS, Keen CL & Ahren B 1998 Marked and rapid decreases of circulating leptin in streptozotocin diabetic rats: reversal by insulin. *American Journal of Physiology Regulatory, Integrative and Comparative Physiology* **274** R1482–R1491.
- Higaki K, Matsumoto Y, Fujimoto R, Kurosaki Y & Kimura T 1997 Pharmacokinetics of recombinant human insulin-like growth factor-I in diabetic rats. *Drug Metabolism and Disposition* **25** 1324–1327.
- Ishii S, Kamegai J, Tamura H, Shimizu T, Sugihara H & Oikawa S 2002 Role of ghrelin in streptozotocin-induced diabetic hyperphagia. *Endocrinology* **143** 4934–4937.
- Ismail IS, Scanlon MF & Peters JR 1993 Cholinergic control of growth hormone (GH) responses to GH-releasing hormone in insulin dependent diabetics: evidence for attenuated hypothalamic somatostatinergic tone and decreased GH autorefeedback. *Clinical Endocrinology* **38** 149–157.
- Johansen PB, Segev Y, Landau D, Phillip M & Flyvbjerg A 2003 Growth hormone (GH) hypersecretion and GH receptor resistance in streptozotocin diabetic mice in response to a GH secretagogue. *Experimental Diabetes Research* **4** 73–81.
- Junod A, Lambert AE, Stauffacher W & Renold AE 1969 Diabetogenic action of streptozotocin: relationship of dose to metabolic response. *Journal of Clinical Investigation* **48** 2129–2139.
- Kamegai J, Aleppo G, Frohman LA, & Kineman RD 1998a Homologous down-regulation of growth hormone-releasing hormone receptor (GHRH-R) mRNA is associated with an increase in inducible cAMP early repressor (ICER) mRNA *in vitro*. *Program and Abstracts of 80th Annual Meeting of Endocrine Society, New Orleans* **63**.
- Kamegai J, Unterman TG, Frohman LA & Kineman RD 1998b Hypothalamic/pituitary-axis of the spontaneous dwarf rat: autorefeedback regulation of growth hormone (GH) includes suppression of GH releasing-hormone receptor messenger ribonucleic acid. *Endocrinology* **139** 3554–3560.
- Kamegai J, Wakabayashi I, Miyamoto K, Unterman TG, Kineman RD & Frohman LA 1998c Growth hormone (GH)-dependent regulation of pituitary GH secretagogue receptor (GHS-R) mRNA levels in the spontaneous dwarf rat. *Neuroendocrinology* **68** 312–318.
- Khamaisi M, Flyvbjerg A, Haramati Z, Raz G, Wexler ID & Raz I 2002 Effect of mild hypoinsulinemia on renal hypertrophy: growth hormone/insulin-like growth factor I system in mild streptozotocin diabetes. *International Journal of Experimental Diabetes Research* **3** 257–264.
- Krassowski J, Felber JP, Rogala H, Jeske W & Zygliszynski S 1988 Exaggerated growth hormone response to growth hormone-releasing hormone in type 1 diabetes mellitus. *Acta Endocrinologica* **117** 225–229.
- Li Z, Karlsson FA & Sandler S 2000a Islet loss and alpha cell expansion in type 1 diabetes induced by multiple low-dose streptozotocin administration in mice. *Journal of Endocrinology* **165** 93–99.
- Li Z, Zhao L, Sandler S & Karlsson FA 2000b Expression of pancreatic islet MHC class I, insulin, and ICA 512 tyrosine phosphatase in low-dose streptozotocin-induced diabetes in mice. *Journal of Histochemistry and Cytochemistry* **48** 761–767.
- Like AA & Rossini AA 1976 Streptozotocin-induced pancreatic insulinitis: new model of diabetes mellitus. *Science* **193** 415–417.
- Liu K, Paterson AJ, Konrad RJ, Parlow AF, Jimi S, Roh M, Chin E Jr & Kudlow JE 2002 Streptozotocin, an O-GlcNAcase inhibitor, blunts insulin and growth hormone secretion. *Molecular and Cellular Endocrinology* **194** 135–146.
- Marks JL, Waite K & Li M 1993 Effects of streptozotocin-induced diabetes mellitus and insulin treatment on neuropeptide Y mRNA in the rat hypothalamus. *Diabetologia* **36** 497–502.
- Mizuno TM, Makimura H, Silverstein J, Roberts JL, Lopingco T & Mobbs CV 1999 Fasting regulates hypothalamic neuropeptide Y, agouti-related peptide, and proopiomelanocortin in diabetic mice independent of changes in leptin or insulin. *Endocrinology* **140** 4557.
- Murao S, Sato M, Tamaki M, Niimi M, Ishida T & Takahara J 1995 Suppression of episodic growth hormone secretion streptozotocin-induced diabetic mice: time-course studies on the hypothalamic pituitary axis. *Endocrinology* **136** 4498–4504.
- Olchovsky D, Bruno JF, Wood TL, Gelato MC, Leidy JW Jr, Gilbert JM Jr & Berelowitz M 1990 Altered pituitary growth hormone (GH) regulation in streptozotocin-diabetic rats: a combined defect of hypothalamic somatostatin and GH-releasing factor. *Endocrinology* **126** 53–61.
- Palm F, Ortsater H, Hansell P, Liss P & Carlsson PO 2004 Differentiating between effects of streptozotocin per se and subsequent hyperglycemia on renal function and metabolism in the streptozotocin-diabetic rat model. *Diabetes Metabolism and Research Review* **20** 452–459.
- Park S, Kamegai J, Johnson TA, Frohman LA & Kineman RD 2000 Modulation of pituitary somatostatin receptor subtype (sst1–5) messenger ribonucleic acid levels by changes in the growth hormone axis. *Endocrinology* **141** 3556–3560.
- Park S, Sohn S & Kineman RD 2004 Fasting-induced changes in the hypothalamic-pituitary-GH axis in the absence of GH expression: lessons from the spontaneous dwarf rat. *Journal of Endocrinology* **180** 369–378.
- Park S, Peng X-D, Frohman LA & Kineman RD 2005 Expression analysis of hypothalamic and pituitary components of the growth hormone-axis in fasted and streptozotocin-treated NPY intact and NPY knockout mice. *Neuroendocrinology* (in press).
- Pierroz DD, Catzeffis C, Aebi AC, Rivier JE & Aubert ML 1996 Chronic administration of neuropeptide Y into the lateral ventricle inhibits both the pituitary-testicular axis and growth hormone and insulin-like growth factor I secretion in intact adult male rats. *Endocrinology* **137** 3–12.
- Raposo PD, Castillo E, d'Alleva V, Broqua P, Pralong FP & Aubert ML 2000 Chronic blockade of the melanocortin 4 receptor subtype leads to obesity independently of neuropeptide Y action, with no adverse effects on the gonadotropic and somatotrophic axis. *Endocrinology* **141** 4419–4427.
- Raposo PD, Pierroz DD, Broqua P, White RB, Pedrazzini T & Aubert ML 2001 Chronic administration of neuropeptide Y into the lateral ventricle of C57BL/6J male mice produces an obesity syndrome including hyperphagia, hyperleptinemia, insulin resistance, and hypogonadism. *Molecular and Cellular Endocrinology* **185** 195–204.
- Rossini AA, Appel MC, Williams RM & Like AA 1977 Genetic influence of the streptozotocin-induced insulinitis and hyperglycemia. *Diabetes* **26** 916–920.
- Sainsbury A & Herzog H 2001 Inhibitory effects of central neuropeptide Y on the somatotrophic and gonadotropic axes in male rats are independent of adrenal hormones. *Peptides* **22** 467–471.
- Sheppard MS, Eatock BA & Bala RM 1989a Altered release of growth hormone from dispersed adenohypophyseal cells of

- streptozotocin diabetic rats. II. Effects of a phorbol ester and secretagogues which increase cyclic AMP. *Canadian Journal of Physiology and Pharmacology* **67** 1321–1325.
- Sheppard MS, Eatock BA & Bala RM 1989b Altered release of growth hormone from dispersed adenohypophysial cells of streptozotocin diabetic rats. I. Effects of growth hormone releasing factor and somatostatin. *Canadian Journal of Physiology and Pharmacology* **67** 1315–1320.
- Shimizu-Albergine M, Ippolito D & Beavo JA 2001 Down regulation of fasting-induced cAMP response element-mediated gene induction by leptin in neuropeptide Y neurons of the arcuate nucleus. *Journal of Neuroscience* **23** 1238–1246.
- Sindelar DK, Havel PJ, Seeley RJ, Wilkinson CW, Woods SC & Schwartz MW 1999 Low plasma leptin levels contribute to diabetic hyperphagia in rats. *Diabetes* **48** 1275–1280.
- Sjogren K, Liu J-L, Blad K, Skrtic S, Vidal O, Wallenius V, LeRoith D, Tornell J, Isaksson O, Jansson J *et al.* 1999 Liver-derived insulin-like growth factor I (IGF-I) is the principal source of IGF-I in blood but is not required for postnatal body growth in mice. *PNAS* **96** 7088–7092.
- Suzuki N, Okada K, Minami S & Wakabayashi I 1996 Inhibitory effect of neuropeptide Y on growth hormone secretion in rats is mediated by both Y1- and Y2-receptor subtypes and abolished after anterolateral deafferentation of the medial basal hypothalamus. *Regulatory Peptides* **65** 145–151.
- Tannenbaum GS 1981 Growth hormone secretory dynamics in streptozotocin diabetes: evidence of a role for endogenous circulating somatostatin. *Endocrinology* **108** 76–82.
- Tannenbaum GS, Epelbaum J, Colle E, Brazeau P & Martin JB 1978 Antiserum to somatostatin reverses starvation-induced inhibition of growth hormone but not insulin secretion. *Endocrinology* **102** 1909–1914.
- Tannenbaum GS, Rorstad O & Brazeau P 1979 Effects of prolonged food deprivation on the ultradian growth hormone rhythm and immunoreactive somatostatin tissue levels in the rat. *Endocrinology* **104** 1733–1738.
- Tannenbaum GS, Painson JC, Lengyel AMJ & Brazeau P 1989 Paradoxical enhancement of pituitary growth hormone (GH) responsiveness to GH-releasing factor in the face of high somatostatin tone. *Endocrinology* **124** 1380–1388.
- Vuagnat BA, Pierroz DD, Lalaoui M, Englaro P, Pralong FP, Blum WF & Aubert ML 1998 Evidence for a leptin-neuropeptide Y axis for the regulation of growth hormone secretion in the rat. *Neuroendocrinology* **67** 291–300.
- White JD, Olchovsky D, Kershaw M & Berelowitz M 1990 Increased hypothalamic content of preproneuropeptide-Y messenger ribonucleic acid in streptozotocin-diabetic rats. *Endocrinology* **126** 765–772.
- Yamashita S & Melmed S 1986a Effects of insulin on rat anterior pituitary cells. Inhibition of growth hormone secretion and mRNA levels. *Diabetes* **35** 440–447.
- Yamashita S & Melmed S 1986b Insulin-like growth factor I action on rat anterior pituitary cells: suppression of growth hormone secretion and messenger ribonucleic acid levels. *Endocrinology* **118** 176–182.

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