Desensitisation of somatostatin, TRH and GHRH responses to glucose in the diabetic (Goto-Kakizaki) rat hypothalamus

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Abstract

We have studied the effects of glucose on the release of somatostatin (SS), TRH and GHRH from incubated hypothalami of normal and genetically diabetic, Goto-Kakizaki (GK) rats. The active isomer D-glucose caused a dose-related inhibition of SS, TRH and GHRH from normal rat hypothalami over a 20-min incubation period *in vitro*. In contrast, in GK rats the effects of glucose on TRH and SS were significantly reduced and the effects on GHRH were abolished. These data indicate that the sensitivity of SS-, TRH- and GHRH-producing hypothalamic neurones is reduced in diabetic rats. The effect is most pronounced for GHRH release as there was no change in the release of this peptide with increasing glucose concentrations. In conclusion, it appears that the diabetic state in GK rats causes differential desensitisation (GHRH>TRH and SS) of neuronal responses to subsequent changes in glucose concentrations *in vitro*. This may be due to alterations in the neurotransmitter control and/or a reduction in number, affinity or function of glucose transporters on these peptidergic neurones or other intermediary neuronal pathways.

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Introduction

The role of glucose in mediating the altered patterns of growth hormone (GH) release in human and animal diabetes is unknown. It may be indirect via changes in insulin-like growth factor feedback, or direct via alterations in the release of hypothalamic neuropeptides involved in the neuroregulation of GH. We have shown previously that increasing glucose concentrations inhibit basal hypothalamic somatostatin (SS), thyrotrophin-releasing hormone (TRH) and GH-releasing hormone (GHRH) release from normal rat hypothalami *in vitro* (Lewis *et al.* 1989). The effects are specific, since the release of luteinizing hormone-releasing hormone is unaltered by glucose, and in broad agreement with the work of most (Berelowitz *et al.* 1982, Lengyel *et al.* 1986, Baes & Vale 1990), but not all others (Richardson *et al.* 1983, Yamaguchi *et al.* 1991). Little is known about the influence of glucose on the release of these neuropeptides from the hypothalami of diabetic animals. In this study we have compared the effects of glucose on the release of SS, TRH and GHRH from normal and diabetic rat hypothalami using the diabetic Goto-Kakizaki (GK) rat. The GK rat is a model of non-obese diabetes mellitus, produced by selective inbreeding. These animals show mild basal hyperglycaemia, hyperinsulinaemia, decreased pancreatic insulin stores, carbohydrate intolerance and a reduced insulin response to either oral (Goto *et al.* 1983, Ismail *et al.* 1995) or intravenous (Portha *et al.* 1991) glucose challenge.

Materials and Methods

Animals

Normal male Wistar rats and male diabetic GK rats (also Wistar strain) from the University of Wales College of Medicine breeding colony were maintained in a thermostatically controlled room at 24 °C with a light period of 0700 to 1900 h. The animals were fed on a standard diet (Pilsbury's modified rat and mouse breeding diet). The GK rats were initially gifts from Professor Y Goto of Tohoku University School of Medicine, Sendai, Japan. Six breeding pairs were obtained in 1989 and a colony was established. The diabetic offspring were raised in parallel to the non-diabetic Wistar rats, which were used as controls. Rats were weaned 28 days after birth. To assess the glucose tolerance of the rats, oral glucose (2 g/kg) was given to normal and diabetic animals (n=5) after a 12-h overnight fast and tail vein samples were taken for plasma glucose at 0, 30, 60, 90 and 120 min.

In vitro *methodology*

In vitro experiments were performed on hypothalamic tissue removed from 9-week-old male GK and male control rats. Rats were fasted for 12 h and weighed before tissue removal. The method of *in vitro* hypothalamic incubation has been described in detail previously (Lewis *et al.* 1989). Briefly, the rats were decapitated with

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minimum stress and the brains removed rapidly. Hypothalami, defined by the posterior margin of the optic chiasm and the anterior margin of the mamillary bodies to a depth of 2 mm, were dissected out, weighed, halved longitudinally and the two halves placed together in each well of a multiwell tissue culture plate containing 300 µl Krebs–Ringer bicarbonate solution with 0·1% (w/v) BSA, 5% glucose and bacitracin (300 µg/ml).

In previous studies we have demonstrated that basal release of SS, TRH and GHRH stabilises after incubation for 60 min (authors' unpublished data). The hypothalami were therefore incubated for three successive 20-min periods and the media discarded. The experimental treatment was then applied for the final 20-min period. To control for non-specific and osmolar effects on peptide release, we compared the effects of the active (D)- and the inactive (L)-isomers of glucose at each concentration tested. This methodological approach is important since we have previously demonstrated that even small changes in the osmolarity of the incubation medium can exert significant effects on neuropeptide release (Lewis *et al.* 1989). The medium was then removed, centrifuged and aliquoted for SS, TRH and GHRH assay. In the *in vitro* studies each point represents the mean of at least four replicate wells and is typical of a minimum of three separate experiments.

Assays and data analysis

The RIA procedures have been described previously (Lewis *et al.* 1989). SS was measured by standard RIA using a fully characterised antiserum 693 (Patel & Reichlin 1978). Appropriate dilutions of labelled [^{125}I-Tyr^{1}]SS and antiserum were used to obtain 10 000 c.p.m. and 30% binding. Twenty-four hours of preincubation and incubation times were used at 4 °C and non-specific binding was 3–4%. Assay sensitivity was less than 1 pg/tube and SS-14 and SS-28 compete on an equimolar basis for binding of label to this particular antibody (Scanlon *et al.* 1983, Robbins *et al.* 1985). TRH was also measured by a well-characterised RIA (Scanlon *et al.* 1983) which was set up as for SS. Twenty-four hours of preincubation and incubation times were used at 4 °C and non-specific binding was 1–2%. Assay sensitivity was less than 0·5 pg/tube. Rat GHRH was measured using a two-site immunochemiluminometric assay (Page *et al.* 1989). In this assay, a C-terminal antibody was attached to a chemiluminescent probe and the N-terminal was attached to the solid phase. Assay sensitivity was 5 pg/ml and the coefficient of variation was less than 10% at dose levels of 0·5–100 pg. In each assay procedure a standard curve was set up in tubes containing the appropriate amount of solution. Plasma glucose was measured using the glucose oxidase method on a YSI model 2300 glucose analyser (Yellow Springs Instruments Ltd, Yellow Springs, OH, USA).

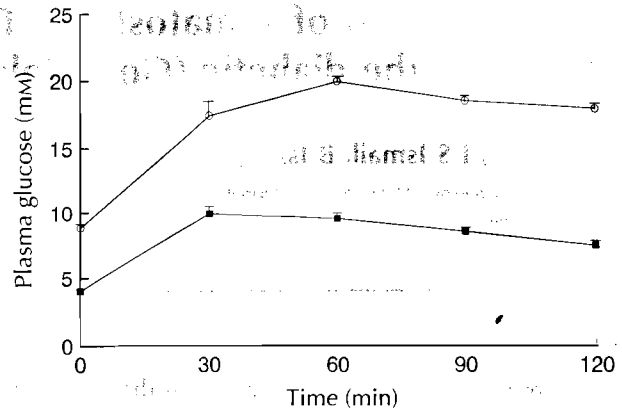

Figure 1 Plasma glucose concentrations in diabetic (○) and normal (■) rats after an oral glucose load (2 g/kg). Results are the mean ± S.E.M. of ten rats.

Data have been calculated as pg peptide per hypothalamus per 20-min incubation period which produces similar results to expression of the data as pg peptide per wet weight or protein content. Parallel incubations were carried out in the presence of the L (inactive)-isomer of glucose and the results are reported as the amount of peptide released in the presence of the D-isomer expressed as a percentage of the amount of peptide released in the presence of the L-isomer. Statistical analysis (diabetic vs normal) was performed by means of the non-paired Student's *t*-test. Where appropriate an ANOVA with post-hoc comparisons by Student–Newman–Keuls multiple comparison test was carried out. The area under the curve (AUC) was calculated by the trapezoidal rule.

All data are expressed as means ± S.E.M. and statistical significance was determined at $P<0·05$.

Results

There was no significant difference between mean body weight (GK vs normal 249 ± 3 vs 254 ± 20 g) or hypothalamic weights (GK vs normal 41·1 ± 2·2 vs 47·3 ± 3·12 mg) in GK and normal rats. Fasting plasma glucose was significantly higher in all GK rats (GK vs normal 8·8 ± 0·3 vs 4·0 ± 0·1 mM, $P<0·001$). After an oral glucose load (2 g/kg), there was an increase in plasma glucose concentrations in both GK (peak 17·5 ± 1·0 mM at 30 min) and normal rats (peak 9·9 ± 0·6 mM at 30 min). The mean glucose AUC was significantly higher in GK rats ($P<0·001$) (Fig. 1).

At physiological concentrations (5 mM) of D-glucose, basal release of all three peptides was similar between normal and diabetic rats (normal vs diabetics 113 ± 11·5 vs 118 ± 11·5 pg SS/hypothalamus per 20 min, not significant (NS); 4 ± 0·8 vs 6·2 ± 0·24 pg TRH/hypothalamus per 20 min, NS; 15·1 ± 3·7 vs 21·9 ± 3·6 pg GHRH/hypothalamus per 20 min, NS).

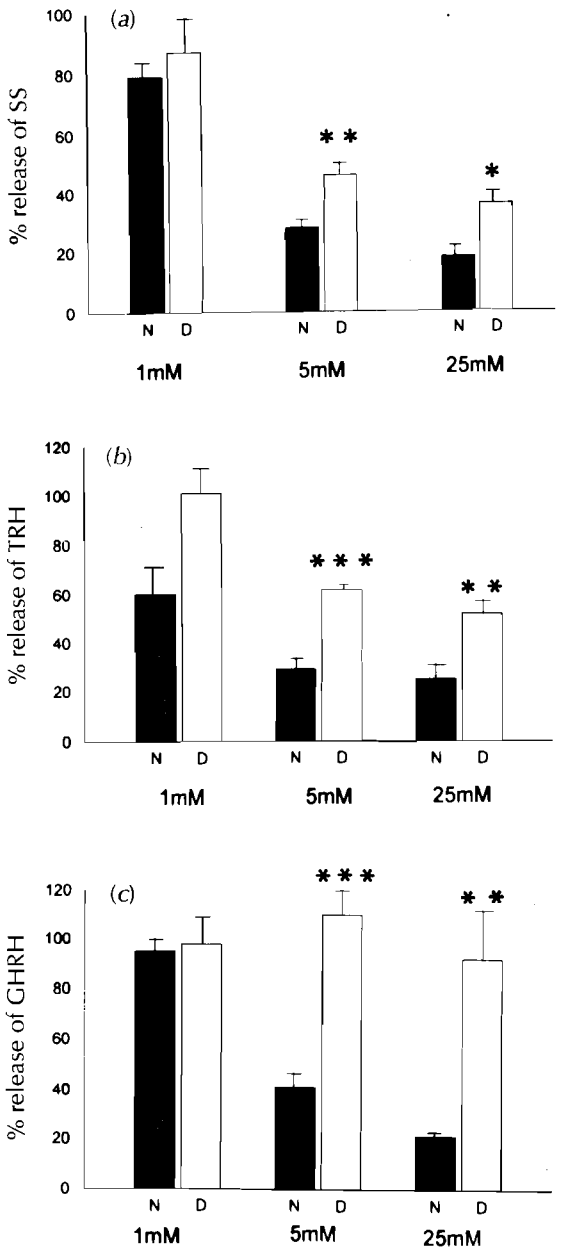

Figure 2 (a) SS, (b) TRH and (c) GHRH responses to D-glucose as a percentage of the amount of peptide released in the presence of L-glucose. Each point represents the mean ± S.E.M. of four replicates and the results are taken from at least three separate experiments. The statistical significance of differences across the doses in normal (N) and diabetic (D) rats were calculated by Student–Newman–Keuls multiple range test and the data are indicated in the text. *P<0·02, **P<0·01, ***P<0·001 compared with normal rats (Student's t-test).

Figure 2 shows the effect of 1, 5 and 25 mM concentrations of glucose on SS, TRH and GHRH release from normal rat hypothalami compared with GK rat hypothalami. The data are controlled for osmolar changes by expression of peptide release in the presence of D-glucose as a percentage of the amount released in the presence of L-glucose. The results demonstrated that when the osmolar effects of glucose are taken into account by using L-glucose as a control, significant differences emerge between normal and diabetic rats for each neuropeptide studied. A dose-related inhibitory action of D-glucose on SS release was present in both normal and diabetic hypothalami (Fig. 2*a*). The data were analysed using one-way ANOVA followed by Student–Newman–Keuls multiple comparison test (normal and diabetic hypothalami 1 vs 5 mM, P<0·001; 1 vs 25 mM, P<0·001; 5 vs 25 mM, NS). However, the inhibitory action of D-glucose on SS release was significantly less in diabetic than in normal hypothalami at 5 and 25 mM concentration (% release: 1 mM 87·8 ± 9·0%, NS vs normal; 5 mM 45·6 ± 4·5%, P<0·01 vs normal; 25 mM 35·7 ± 6·2%, P<0·02 vs normal; data were analysed using a non-paired Student's t-test).

A dose-related inhibitory action of D-glucose on TRH release was also present (Fig. 2*b*). Again, when the data were analysed by Student–Newman–Keuls multiple comparison test significant differences were observed (normal and diabetic hypothalami 1 mM vs 5 mM, P<0·01; 1 mM vs 25 mM, P<0·01; 5 mM vs 25 mM, NS). When the data were analysed using Student's t-test comparing diabetic with normal rats the inhibitory action of D-glucose on TRH was significantly less in diabetic than in normal hypothalami at 5 and 25 mM concentrations (% release: 1 mM 100·7 ± 13·6%, NS vs normal; 5 mM 62·3 ± 2·3%, P<0·001 vs normal; 25 mM 51·6 ± 5·1%, P<0·01 vs normal).

D-glucose also showed a dose-related inhibition of GHRH from normal hypothalami over a 20-min period (Fig. 2*c*). Data were analysed by Student–Newman–Keuls test (1 vs 5 mM, P<0·001; 1 vs 25 mM, P<0·001; 5 vs 25 mM, P<0·01). However, in contrast to the results obtained for SS and TRH, D-glucose did not inhibit GHRH release from diabetic hypothalami. When the results were analysed using Student's t-test there was a significant difference in release from normal hypothalami compared with diabetic hypothalami (% release: 1 mM 91·5 ± 22·3%, NS vs normal; 5 mM 109·8 ± 10·7%, P<0·001 vs normal; 25 mM 91·5 ± 22·4, P<0·01 vs normal).

Discussion

The GK rats used in this study showed fasting hyperglycaemia and impaired glucose tolerance although body weight was normal. Basal release of TRH, SS and GHRH was unchanged in GK rats compared with normal rats at physiological glucose concentrations (5 mM D-glucose) which contrasts with some previous data. Bestetti *et al.* (1989) reported a decrease in basal TRH release from streptozotocin (STZ)-treated diabetic rats. Richardson &

Twente (1987) reported that SS release from dispersed adult hypothalamic cells of STZ-treated rats was diminished both basally and after stimulation by membrane depolarisation. In contrast, Joanny *et al.* (1992) showed an increase in SS release from incubated hypothalamic slices from STZ-treated diabetic rats. Some workers have suggested that this increase in SS release, associated with a decrease in hypothalamic GHRH mRNA content reported elsewhere (Olchovsky *et al.* 1990), may contribute to the decreased GH secretion found in diabetic rats (Harrison & Robinson 1980, Tannenbaum 1981, Gonzalez & Jolin 1985, Joanny *et al.* 1992). Possible explanations for these observed differences include variations in body weight, severity of diabetes and type of animal model used and, with regard to the latter, it should be remembered that the actions of STZ are not confined to the β-cell (Patel *et al.* 1978). However, these data should be treated cautiously since basal neuropeptide release from isolated hypothalami may not be a reliable reflection of *in vivo* events.

Despite these conflicting data regarding basal neuropeptide release we have confirmed the potent effects of glucose on hypothalamic peptide release (Berelowitz *et al.* 1982, Lengyel *et al.* 1986, 1988, Lewis *et al.* 1989, Baes & Vale 1990). Active D-glucose can be viewed as exerting a biphasic action around the physiological concentration of 5 mM with release of SS, TRH and GHRH being stimulated at concentrations below 5 mM and inhibited at concentrations above 5 mM. It is possible that different mechanisms mediate these actions and preliminary data indicate that the peptide-releasing effects of glucose concentrations less than 5 mM are mediated by glutamate pathways (Lewis *et al.* 1992, Sato & Frohman 1993) as are other neurological responses to hypoglycaemia (Tasker *et al.* 1992). Whether the inhibitory action of glucose concentrations above 5 mM reflects a direct action of glucose on peptidergic neurones is unknown at present.

The effect of low glucose on GHRH release in normal rats is controversial. While the results of Baes & Vale (1990) are in agreement with ours, Frohman *et al.* (1990) did not show parallel increases in GHRH and SS release in hypophysial portal blood of sheep in response to insulin-induced hypoglycaemia. Furthermore, Murao *et al.* (1994) reported that rat hypothalamic SS mRNA levels were increased dramatically by hypoglycaemia whereas GHRH mRNA levels were unaffected. These discrepancies may be due to the different methods involved in GHRH detection, species variation and the difference in experimental techniques or a combination of all these.

In GK, in contrast to normal hypothalami, the effects of D-glucose on neuropeptide release were significantly different: with regard to SS and TRH, the effects of glucose concentrations below and above 5 mM were significantly reduced whilst the effects of glucose on GHRH were totally abolished in the presence of isosmolar concentrations of L-glucose. It appears therefore that these peptidergic neurones become differentially desensitised to the effects of glucose (GHRH>TRH and SS) in the presence of diabetes mellitus. Consequent changes in the *in vivo* pattern of neuropeptide release could contribute to the altered GH secretion which occurs in GK rats (Ismail *et al.* 1995) and in other hyperglycaemic rat models (Tannenbaum 1981, Tannenbaum *et al.* 1981, Locatelli *et al.* 1985, Ndon *et al.* 1992), although the precise mechanisms are unknown at present.

In conclusion, we have shown that the sensitivity of GHRH-, SS- and TRH-producing hypothalamic neurones to glucose is markedly reduced in diabetic GK rats with a particularly pronounced loss of sensitivity by GHRH peptidergic neurones. The mechanisms underlying these effects are unknown but it appears that the diabetic state reduces the sensitivity of hypothalamic neurones to subsequent changes in glucose concentrations. This could be via alterations in neurotransmitter control of peptidergic neurones and/or reduction in hypothalamic glucose transporter number or function. Our subsequent studies will address these issues.

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