The effect of genetically engineered glucagon on glucose recovery after hypoglycaemia in man

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To compare the effect on glucose recovery after insulin-induced hypoglycaemia of intramuscular genetically engineered glucagon, intramuscular glucagon from pancreatic extraction and intravenous glucose, we examined 10 healthy subjects during blockage of glucose counterregulation with somatostatin, propranolol and phentolamine. Each subject was studied on three separate occasions. Thirty min after a bolus injection of 0.075 iu soluble insulin per kilogram body weight the subjects received one of the following treatments: 1 mg glucagon from pancreatic extraction intramuscularly; 1 mg genetically engineered glucagon intramuscularly; and 25 g glucose intravenously, respectively. The two glucagon preparations induced an equally rapid increase in plasma glucose. This was due to an abrupt (within 4 min) and equal increase in glucose appearance rate. The increases in both plasma glucose and in glucose appearance rate were far more protracted after i.m. glucagon than after i.v. glucose. These results suggest that genetically engineered glucagon and glucagon from pancreatic extraction have a similar effect on hepatic glucose production rate. Due to the protracted effect of intramuscular glucagon, a combined treatment consisting of both intravenous glucose and intramuscular glucagon may be more effective in the treatment of hypoglycaemia than any of these given alone.

Keywords hypoglycaemia glucagon glucose counterregulation
glucose appearance rate

Introduction

Hypoglycaemia during insulin treatment is a major hazard, as it accounts for 4% of deaths in diabetics below 50 years of age (Tunbridge, 1981). Furthermore, 10% of all insulin treated diabetics experience at least one severe hypoglycaemic episode each year (Casparie & Elving 1985; Potter *et al.*, 1982). Routine treatment of insulin-induced hypoglycaemia in accident and emergency rooms includes i.v. glucose followed by i.v. saline. At home glucagon intramuscularly is used in hypoglycaemic emergency situations, injected by trained relatives. Today glucagon emergency kits are known by 50% of insulin dependent diabetic patients (Samanta *et al.*, 1986) and the use of glucagon is included in routine diabetes teaching programmes (Muhlhauser *et al.*, 1985).

Parenteral administration of glucagon was established as a treatment of insulin-induced hypoglycaemia by Elrick *et al.* in 1958. The glucose responses to i.v. i.m. and subcutaneous glucagon have been compared with each other and with oral and i.v. glucose (Collier *et al.*, 1987; MacCuish *et al.*, 1970; Muhlhauser *et al.*, 1985 a,b;

Shipp *et al.*, 1964). The glucagon in these studies derived from pancreas collection and extraction. In future the source of glucagon as of insulin may be genetic engineering, since pancreatic extraction subsequently will be abandoned. Therefore the aim of this study was to compare the effect on glucose recovery of i.m. genetically engineered glucagon, i.m. porcine glucagon and i.v. glucose. The experiments were performed during blockade of endogenous secretion of glucagon and insulin with simultaneous inhibition of the cellular actions of adrenaline (Rizza *et al.*, 1979).

Methods

Subjects

Ten healthy male volunteers (24–43 years, 69–98 kg, 169–198 cm) consented to participate in the study after verbal and written information. They were all without a family history of diabetes and received no medication.

The study was approved by the Regional Ethics Committee of Copenhagen.

Experimental procedure

Three different experiments were performed with at least 14 days' interval. On each occasion one of the following three treatments was administered: i.m. genetically engineered glucagon Novo-Nordisk (1 mg), i.m. glucagon from pancreatic extraction Novo-Nordisk (1 mg) or i.v. glucose (25 g). The sequence of the experiments was randomized and the two glucagon preparations were administered in a double-blind fashion.

The subjects arrived at 08.00 h after a 10 h fast having abstained from tobacco in the same period. They rested supine throughout the study. Three i.v. cannulas were inserted, two for infusion in one arm and one for blood sampling in the contralateral arm. A bolus injection of 3-[³H]-glucose (Isotopapoteke, Copenhagen) was administered ($0.2 \mu\text{Ci kg}^{-1}$ body weight) and continuous infusion of 3-[³H]-glucose was started ($0.002 \mu\text{Ci kg}^{-1}$ body weight min^{-1}).

After 2 h the subjects received an i.v. injection of insulin ($0.075 \text{ i.u. Actrapid Human kg}^{-1}$ body weight). At the same time blockade of neurohumoral glucose counterregulation was started by infusion of: Somatostatin (Dura Scan, Odense, Denmark, i.v. infusion $500 \mu\text{g h}^{-1}$), propranolol (Frekven, Ferrosan, Denmark i.v. bolus injection 5 mg and i.v. infusion $80 \mu\text{g min}^{-1}$) and phentolamine (Regitin, Ciba-Geigy, i.v. bolus 5 mg and i.v. infusion $500 \mu\text{g min}^{-1}$). Thirty min after the i.v. injection of insulin, when the subjects were clinically hypoglycaemic, they received one of the three previously mentioned treatments. Glucagon was injected intramuscularly into the front of the thigh; all injections were performed by the same doctor. The study was terminated 120 min after the insulin bolus.

Blood samples for analysis of plasma glucose, plasma glucagon and activity of 3-[³H]-glucose were drawn every 2 min during the first 10 min after the injection of glucagon or glucose; during the rest of the study blood samples were drawn every 5 min (plasma glucose) and every 10 min (plasma glucagon and 3-[³H]-glucose). Blood samples for analysis of serum insulin and serum-c-peptide were drawn 0, 30, 50 and 100 min after the insulin bolus.

Analytical procedures

Blood samples for analysis of plasma glucagon were drawn into tubes containing EDTA and aprotinin, for analysis of 3-[³H]-glucose into tubes containing heparin. All tubes were icechilled and stored on ice until centrifugation at 4° C. Plasma was stored at -20° C until analysis.

Plasma glucose was measured by the glucose oxidase method (Beckman autoanalyzer, Beckman Instruments Corp, Fullerton, California). Plasma glucagon (Faloona & Unger 1974), serum-insulin (Kuzuya *et al.*, 1977) and serum-c-peptide (Faber *et al.*, 1978) were analyzed as previously described and activity of 3-[³H]-glucose was assayed according to Rizza *et al.* (1979). Glucose appearance rate and glucose disappearance rate were calculated using Steele's equations (Steele, 1959) modified by De Bodo *et al.* (1963). Specific activities

of [³H]-glucose and plasma glucose concentrations during the latter 70 min of the study were smoothed by a moving average of three successive values (Cherrington & Vranic, 1973). The purity of the glucagon preparations was determined by means of reverse phase high performance liquid chromatography (RP-h.p.l.c.). The purity is expressed as the content of native hormone in % of the sum of native glucagon and glucagon-related transformation products.

Statistics

Results are presented as mean $\pm$ s.e. mean. Friedmann test, Wilcoxon test for paired comparisons and Dunns test were applied as appropriate.

P values (two-tailed) less than 0.05 were considered significant.

Results

Concentrations of plasma glucose (Figure 1) just before administering the treatments against hypoglycaemia were not statistically different, and neither was the nadir (1.6 mmol l^{-1} , range $1.2\text{--}2.2 \text{ mmol l}^{-1}$). The time elapsed before fasting glucose was reached (20 min from administration of the treatment, range 15–40 min) was identical for the two glucagon preparations.

Plasma glucose rose to above 10 mmol l^{-1} within 2 min after i.v. glucose. AUC was significantly smaller for the plasma glucose responses to i.v. glucose than for the responses to either of the glucagon preparations ($P = 0.006$).

The basal levels of glucose appearance rate and glucose appearance rate were not statistically different on the three occasions (Figure 1). Both of the glucagon preparations induced an immediate (less than 4 min) increase in glucose appearance rate which increased to maximal values within 10 min. AUC was not statistically significantly different comparing the two glucagon preparations. Glucose appearance rate rose to a peak value of $97.1 \pm 14.4 \text{ mg kg}^{-1} \text{ min}^{-1}$ within 2 min after injection of glucose. The peak value calculated as 25 g glucose divided by 79 kg (mean body weight) divided by 3 min (injection time) was $105 \text{ mg kg}^{-1} \text{ min}^{-1}$.

AUC for glucose appearance rate did not differ statistically between the glucagon experiments. Basal values of plasma glucagon were not statistically different (Figure 1). Plasma glucagon rose to a maximum of $1.8 \pm 0.6 \text{ ng ml}^{-1}$ (genetically engineered glucagon) and $1.6 \pm 1.1 \text{ ng ml}^{-1}$ (glucagon from pancreatic extraction). Neither maximum values nor AUC for plasma glucagon responses differed significantly in the experiments with glucagon treatment. Values of plasma glucagon were at the end of the study period for genetically engineered glucagon and for glucagon from pancreatic extraction, $0.56 \pm 0.34 \text{ ng ml}^{-1}$ and $0.58 \pm 0.24 \text{ ng ml}^{-1}$, respectively. In the experiments with glucose injection plasma glucagon remained completely suppressed throughout the study as plasma glucagon was $0.03 \pm 0.03 \text{ ng ml}^{-1}$ just before start of counterregulatory blockade and $0.01 \pm 0.02 \text{ ng ml}^{-1}$ at the end of the study period (NS). Values of serum insulin were not statistically different prior to

Figure 1 Plasma glucose, glucose appearance rate (Ra), glucose disappearance rate (Rd) and plasma glucagon before and after insulin-induced hypoglycaemia treated with i.v. glucose (▲), genetically engineered glucagon (■) and glucagon from pancreatic extraction (●) in healthy subjects.

i.m. glucagon or i.v. glucose on the three study days and neither was the fall in plasma-C-peptide (data not shown). The purity of the glucagon preparations sampled at random was 98.3% (genetically engineered glucagon) and 91.9% (glucagon from pancreatic extraction).

One subject (treated with glucagon from pancreatic extraction) experienced a short episode of nausea,

vomiting and diarrhoea 15 min after termination of one of the experiments. No other side effects were observed.

Discussion

The present study confirms i.m. injection of genetically engineered glucagon 1 mg to be as effective in the treatment of hypoglycaemia as i.m. injection of glucagon 1 mg from pancreatic extraction. The rapid rise in plasma glucose was due to an abrupt and equal rise in glucose appearance rate (Figure 1), indicating an equal effect on the liver glucose production rate of the two glucagon preparations.

Comparing the plasma glucose responses (Figure 1), there was a more protracted rise in plasma glucose when using i.m. glucagon than when using i.v. glucose. This confirmed the results of Shipp *et al.* (1964) who compared the hyperglycaemic effect during hypoglycaemia of subcutaneous glucagon and oral glucose in diabetic teenagers, and who found a more protracted effect on plasma glucose of glucagon than of glucose.

Collier *et al.* (1987) compared the hyperglycaemic effect of i.v. glucagon and of i.v. glucose. At the end of their study period which was only 30 min after treatment of hypoglycaemia, the plasma glucose had risen to a value of approximately 4.5 mmol l⁻¹ (i.v. glucagon) and 7.3 mmol l⁻¹ (i.v. glucose). The study of Collier *et al.* (1987) was performed with patients in an accident and emergency department, and therefore the insulin effect might be more protracted than in our study. Furthermore, the effect on glucose recovery of i.v. glucagon is of shorter duration than the effect of i.m. glucagon (insert ref.), thus explaining a comparatively small effect of glucagon in their study. The lesser hyperglycaemic effect of i.v. glucose in our study could as previously mentioned be due to blockade of the counterregulatory system, preventing the subjects from augmenting plasma glucose by means of endogenous glucagon and catecholamine secretion. Supporting this hypothesis plasma glucagon levels in the experiments with i.v. glucose remained low.

The maximum value of glucose appearance rate (97.1 mg kg⁻¹ min⁻¹) in the glucose experiments estimated from Steele's equations (Steele, 1959) was only 8% below the calculated value (105 mg kg⁻¹ min⁻¹), validating the accuracy of Steele's equations during non steady-state hyperglycaemia.

In conclusion, the effects of i.m. glucagon from pancreatic extraction and of i.m. genetically engineered glucagon on glucose production rate are identical after hypoglycaemia. Furthermore, in this experimental system allowing expression of the isolated effect of the drugs, i.m. glucagon had a much more protracted hyperglycaemic effect than i.v. glucose, as previously suggested for oral glucose (Shipp *et al.*, 1964). Thus combined treatment of insulin-induced hypoglycaemia consisting of both i.v. glucose and i.m. glucagon could be more effective than any of these given alone.

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