Increased splanchnic blood flow after hypoglycaemia in diabetic and normal man: evidence against glucagon as a mediator

D. R. PARKER, A. BARGIOTA, G. D. BRAATVEDT, M. HALLIWELL and R. J. M. CORRALL
University of Bristol Department of Medicine, Bristol Royal Infirmary, Bristol BS2 8HW, U.K.

ABSTRACT

Superior mesenteric artery blood flow increases significantly after hypoglycaemia in healthy humans. Glucagon has vasoactive properties but its role in hypoglycaemic hyperaemia is unclear. To assess this role, we studied the superior mesenteric artery blood flow response to hypoglycaemia of patients with uncomplicated Type 1 (insulin-dependent) diabetes mellitus of at least 10 years duration; a group known to have defective glucagon response to hypoglycaemia. Hypoglycaemia was induced using an intravenous infusion of soluble human insulin (2.5 m-units min⁻¹ kg⁻¹) discontinued at a plasma glucose of 2.5 mmol/l. Superior mesenteric artery blood flow was measured using transcutaneous duplex Doppler ultrasound. Plasma samples were assayed for glucose, insulin, glucagon, catecholamines, growth hormone and cortisol. Plasma glucose concentration fell to a nadir of 1.8 (0.3) mmol/l in patients and 1.4 (0.1) mmol/l in controls. Plasma glucagon concentration was unchanged in patients from a baseline level of 111.7 (13.1) ng/l but rose in controls from 105 (8.5) to a peak of 239 (3.1) ng/l (P < 0.001). Superior mesenteric artery blood flow increased in both groups: from 385 (29) to 921 (100) ml/min (140% increase; P < 0.05) in patients and from 517 (50) to 790 (67) (53% increase; P < 0.001) in controls.

This study shows that patients with Type 1 diabetes have a normal splanchnic vascular hyperaemic response to hypoglycaemia despite defective glucagon counter-regulation. These results support our previous work suggesting that glucagon is not a major mediator of this response; it seems likely that circulating adrenaline is the major regulatory mechanism.

INTRODUCTION

Mesenteric hyperaemia occurs in response to insulin-induced hypoglycaemia in healthy young volunteers and a possible role in glucose homoeostasis has been suggested [1,2]. Mesenteric blood flow responses to hypoglycaemia in patients with Type 1 (insulin-dependent) diabetes mellitus have not been studied previously. Patients with more than a 5-year history of Type 1 diabetes have defective glucagon counter-regulation [3] and although glucagon has vasoactive properties, its role in superior mesenteric artery blood flow (SMABF) control in man is uncertain. To assess the role of glucagon in the hyperaemic response of the superior mesenteric artery to hypoglycaemia, we measured SMABF responses to hypoglycaemia in healthy controls and in patients with Type 1 diabetes.

METHOD

Six non-smoking male patients with Type 1 diabetes mellitus, aged 21 to 38 years (age at diagnosis 9–19 years), were examined after an overnight fast having omitted
their morning dose of insulin. All were non-obese (body mass index range 21.2–24.5 kg/m²; median 22.4 kg/m²) and had been diabetic for at least 10 (range 10–20) years. None had any evidence of diabetic complications on clinical examination, although formal autonomic testing was not carried out, and all reported normal hypoglycaemic awareness. All denied any symptomatic hypoglycaemic reactions in the 14 days before each study. Mean fructosamine concentration over the last three visits to the diabetic clinic in the group was 2.9 (0.7) mmol/l (normal range = 1.5–2.4 mmol/l). The diabetic volunteers were advised to continue their regular insulin regimen except for omitting the normal dose on the morning of the study. Five healthy non-obese male volunteers (age 21–30 years) comprised the control group. None of the diabetic volunteers or controls was taking any medication other than insulin.

All studies were carried out in the morning after an overnight (12-h) fast in a temperature-controlled environment of 21–24 °C. All subjects were examined supine; sleep was not permitted. Blood pressure and pulse rate were recorded at 3-min intervals using an automated sphygmomanometer. An intravenous cannula was inserted into the right arm for administration of fluids and insulin and a second was inserted in the right hand in a retrograde direction for blood sampling. This hand was placed into a heated air chamber thermostatically controlled at 55 °C to ensure that arterialized venous blood samples were obtained for measurement of glucose and catecholamines [4]. The latter samples were immediately centrifuged, 1 μl/ml molar sodium metabisulphite was added as an antioxidant and the serum was then fast-frozen and stored at −70 °C. Other samples were taken to measure insulin, cortisol, growth hormone and C-terminal glucagon specific for pancreatic glucagon. SMABF and cardiac output were measured by one operator in all studies using a transcutaneous duplex Doppler ultrasound system (ATL 500, Advanced Technical Laboratories) and signals were processed with a Dopstation (SciMed, U.K.). To avoid operator bias, volume flows were calculated later by taking the mean of three successive sets of five recordings made at each time point. Vascular resistance of the superior mesenteric artery was calculated by:

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\text{Resistance} = \frac{\left[\text{MAP} \times 100\right]}{\text{SMABF}}
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where MAP = mean arterial pressure. The superior mesenteric artery was visualized with the ultrasound scanner head positioned over the upper abdomen. The aortic root was visualized with a pencil probe scanner over the jugular notch to measure cardiac output.

Pulse rate, blood pressure, cardiac output, SMABF and blood samples were taken after 45 min rest (baseline), during insulin infusion, at the hypoglycaemic reaction (R) and serially until R + 120 min. Plasma glucose was measured immediately at the bedside for guidance during the study and plasma was stored for definitive laboratory assay later the same day.

Hypoglycaemia was induced by an intravenous infusion of soluble human insulin at a dose of 2.5 m-units·min⁻¹·kg⁻¹ diluted in 500 ml of normal saline and infused at a rate of 200 ml/h. The infusion was stopped when the plasma glucose concentration measured at the bedside using a Reflolux II meter (Boehringer) fell to 2.5 mmol/l. Plasma glucose subsequently continued to fall until the nadir at which the hypoglycaemic reaction, comprising autonomic and neuroglycopaenic symptoms and signs, occurred. The time at which the hypoglycaemic reaction (R) ensued varied between individuals and the clock was reset at R to ensure that subsequent time points during the recovery phase were comparable between subjects. If resetting had not occurred at this point, data from variable points of recovery from hypoglycaemia would have been pooled leading to inaccuracies in data interpretation [5]. Serial measurements were repeated at intervals for the next 120 min.

At the end of the study, each diabetic patient volunteer was given a light lunch and a hot beverage. Blood glucose was rechecked and when satisfactory, patients were allowed home with advice to restart their insulin regimen as normal with their next meal.

Plasma glucose was assayed using a glucose oxidase auto-analyser method. Insulin was measured in batches by a double-antibody radioimmunoassay [coefficient of variation (COV) 8%]. Measurements of plasma cortisol (COV < 5.3%), growth hormone (COV < 5%) and C-terminal glucagon specific to pancreatic glucagon (COV 5.5%) were via radioimmunoassay. Catecholamine assays were performed by gas liquid chromatography (COV for adrenaline, 4%; COV for noradrenaline, 2%). Ethical approval was granted by the hospital Research Ethics Committee. All patients gave their informed consent.

RESULTS

Results are shown in Figures 1 and 2. Values are expressed as means (S.E.M.) and were compared using Student’s paired t-test; overall changes were measured by comparing areas under the individual curves (AUC). Statistical significance was accepted at P < 0.05.

All control individuals reached hypoglycaemia without complication and recovered from symptoms spontaneously; in two diabetic individuals the blood glucose concentration as measured at the bedside was still under 2.5 mmol/l at 120 min after R and intravenous glucose was administered. All the patients reported that their symptoms of hypoglycaemia were less severe than they normally experienced and occurred at a lower blood glucose concentration. The time taken to reach R in patients (range 60–120 min, median 92 min) was sig-
Hypoglycaemia-induced mesenteric hyperaemia

**Figure 1** Hormonal and metabolic changes after hypoglycaemia

I = insulin infusion; R = hypoglycaemic reaction. Statistical significance: *P < 0.05; **P < 0.01 compared with baseline.

Metabolic and endocrine variables (Figure 1)

Plasma glucose concentration fell from 4.5 (0.2) to 1.4 (0.1) mmol/l in the control group (P < 0.001) and in patients from 12.6 (1.2) to 1.8 (0.3) mmol/l (P < 0.001); concentrations were significantly lower throughout the induction period in controls (P < 0.001). The rate of recovery of glucose in patients was 0.63 (0.11) mmol·h⁻¹·l⁻¹ in the first hour and in the second hour it was 1.47 (0.15) mmol·h⁻¹·l⁻¹. The rate of glucose recovery in controls was significantly higher; 2.9 (0.2) mmol·h⁻¹·l⁻¹ in the first hour post R (P < 0.001) and 3.2 (0.3) mmol·h⁻¹·l⁻¹ (P < 0.001) in the second hour. The amount of insulin administered to patients to induce hypoglycaemia was significantly greater than that administered to controls: 17.3 (1.5) units and 10.4 (0.9) units (P = 0.003) respectively. The concentration of plasma insulin in the control group increased from 7 (0.8) to 196.4 (57.6) m-
Figure 2 Haemodynamic changes after hypoglycaemia

I = insulin infusion; R = hypoglycaemic reaction. Statistical significance: *P < 0.05; **P < 0.01 compared with baseline.

Plasma glucagon concentration increased after hypoglycaemia in controls from 105 (8.5) to 239 (31) ng/l (P < 0.001). There was no change from baseline [111.7 (13.1) mmol/l] after hypoglycaemia in patients. Between-group comparisons using the AUC revealed a significantly greater increase of glucagon levels in controls (P < 0.05).

Baseline plasma adrenaline levels were significantly greater in patients [60.8 (11) ng/l] than in controls [26.2 (8.2) ng/l; P < 0.05]. After hypoglycaemia adrenaline

units/l (P < 0.05). Baseline plasma insulin concentration was significantly higher in patients (P < 0.05) but there was no significant difference between the peak insulin concentrations of controls and patients. Post-R insulin concentrations were significantly lower in the controls (P < 0.001). A comparison of area under the insulin concentration curves over the period of infusion + 30 min (equivalent to six insulin half-lives) uncorrected for R revealed no significant difference between patients and controls.
concentration increased to 1859.3 (343.8) ng/l (P < 0.05) in diabetic patients and to 531 (152) ng/l (P < 0.05) in controls. The overall adrenaline response to hypoglycaemia was significantly greater in the diabetic patients compared with controls (P < 0.001). Baseline plasma noradrenaline concentrations were similar in controls and patients. An increase was observed after R from 139.6 (14) to 238 (15.4) ng/l (P < 0.05) in the controls and from 177.8 (19.1) ng/l to 634.2 (61.6) ng/l (P < 0.001) in the diabetic patients. Between-group comparisons revealed that diabetic patients showed a greater overall noradrenaline response (P < 0.001).

Baseline cortisol concentration was significantly higher in patients than in controls (P < 0.05). The concentration increased in controls from 280.4 (51.6) to 675.2 (91.8) nmol/l (P < 0.05) and in patients from 528.8 (63.5) to 744.2 (48.5) nmol/l (P < 0.05). There was no significant overall difference between patients and controls. Plasma growth hormone increased from 7 (3.7) to 57 (13) m-units/l in controls (P < 0.05) and from 7.2 (3.6) to 59.3 (6.1) m-units/l (P < 0.05) in patients. The overall response, as measured by the AUC, was not significantly different. The peak growth hormone response to hypoglycaemia tended to occur later in controls than in patients, but this was not significant.

**Haemodynamic variables (Figure 2)**

After hypoglycaemia, SMABF increased in controls from 517.4 (54) to 786.8 (79.6) ml/min (P < 0.001) and in patients from 379.5 (29.5) ml/min to 949.7 (107) ml/min (P < 0.05). The overall response in controls was significantly smaller (P < 0.05), albeit from a higher basal value. Vascular resistance in the superior mesenteric artery (SMAR) fell from baseline by 33 (3) % (P < 0.001) in controls and by −54.3 (1.6) % in patients. The overall fall in superior mesenteric artery resistance (AUC) after hypoglycaemia was significantly greater in diabetic patients (P < 0.05).

Cardiac output increased in controls from 5.8 (0.27) to 7.7 (0.32) litres/min (P < 0.001) and in patients from 6.0 (0.4) to 8.6 (0.7) litres/min (P < 0.05). There was no significant overall difference between controls and patients. Pulse rate increased in controls from a baseline of 59 (3) to a peak of 79 (6) beats/min (P < 0.05) and in patients from a baseline of 68 (4) to a peak of 95 (3) beats/min (P < 0.001). The overall pulse rate response to hypoglycaemia in patients was greater than in healthy controls (P < 0.05). Systolic blood pressure at baseline was significantly lower in controls than in patients (P < 0.05) and increased from 111.6 (3.5) to a peak of 121 (3.7) mmHg (P < 0.05) after hypoglycaemia. In patients, systolic blood pressure increased from 125.7 (3.2) to a peak of 161.7 (9.2) mmHg (P < 0.05). The overall response to hypoglycaemia was significantly greater in patients compared with controls (P < 0.001). Diastolic blood pressure decreased in controls from 63 (4.6) to 57.2 (3.8) mmHg (P < 0.05), but not in patients. There was no significant overall difference between controls and patients. MAP increased in the patients from 85.8 (2.8) to 98.2 (5.6) mmHg (P < 0.05). There was no significant change in MAP in controls. The overall difference in MAP response to hypoglycaemia (AUC) was significantly greater in the diabetic patients (P < 0.05).

**DISCUSSION**

SMABF in this group of patients with Type 1 diabetes of up to 20 years’ duration increased significantly after insulin-induced hypoglycaemia. This observation is in agreement with the findings of Braatvedt et al. [1] in normal volunteers and suggests that patients with Type 1 diabetes have a normal splanchnic vascular hyperaemic response to hypoglycaemia. The absence of a plasma glucagon response to hypoglycaemia in the patients examined in this study was in accordance with previous studies, including that of Bolli et al. [3], but was accompanied in all cases by significant increments in SMABF. We have previously demonstrated that suppression of endogenous glucagon secretion with octreotide does not prevent SMABF hyperaemia after insulin-induced hypoglycaemia in healthy volunteers [6], and the present observations support our suggestion that glucagon is not a major mediator of the post-hypoglycaemia SMABF increase. Previous data suggest that a β- adrenergic mechanism governs the SMABF response to hypoglycaemia [2]; the current observation that an increase in SMABF occurs in diabetic patients who have intact catecholamine responses but impaired glucagon responses to hypoglycaemia is consistent with this conclusion.

The diabetic patients began this study with higher glucose and insulin concentrations than controls. This led to the diabetic patients requiring a more prolonged insulin infusion to induce hypoglycaemia; as a result the hypoglycaemic stimulus was not identical in patients and controls. In retrospect, the ideal design would have employed a modified glucose clamp technique to control glucose and insulin levels overnight before the induction of hypoglycaemia. This would have resulted in the hypoglycaemic stimulus being more comparable in patients and controls as well as avoiding the possibility of antecedent hypoglycaemia.

The unequal hypoglycaemic stimulus in the two groups explains several of the observed differences in response to hypoglycaemia in this study. The greater adrenaline and noradrenaline responses in diabetic patients reflect greater autonomic stimulation in this group. The bigger catecholamine response explains the greater increase in MAP in diabetic individuals, and if, as we believe, hypoglycaemia-induced mesenteric hyperaemia is adrenergically mediated, this would also explain
the greater overall SMABF increase and the greater fall in superior mesenteric artery resistance. The lower rate of blood glucose restoration in the diabetic patients is also explicable by their greater hypoglycaemic stimulus. The observed differences in baseline SMABF, pulse rate and systolic blood pressure are difficult to explain but may possibly be related to the elevated baseline plasma adrenaline levels in the diabetic patients. This last observation has been made previously [7], but further studies are needed to clarify these results.

The lower rate of blood glucose restoration in this small group of diabetic patients is explicable in part by the greater hypoglycaemic stimulus but the absent glucagon response may also play a role. The principal counter-regulatory hormones are glucagon and adrenaline [8]. Counter-regulatory failure with an inability to mount a normal endocrine secretory response to a hypoglycaemic stimulus may either represent a reduced capacity to secrete the hormone or an alteration in the glycaemic threshold at which hormone secretion is initiated. Both types of abnormality are observed in patients with Type 1 diabetes and deficient responses to hypoglycaemia have been described for all the principal counter-regulatory hormones [9]. The true prevalence of hormonal deficiencies in insulin-treated patients is unknown but the frequency of deficient counter-regulatory responses is known to increase with the duration of diabetes. Bolli et al. [3] investigated the hormonal response to a standard insulin infusion test and found that patients with diabetes of recent onset had a normal response but patients with diabetes of 1 to 5 years’ duration had impaired glucagon responses. Patients with a longer duration of diabetes (most of whom had autonomic neuropathy) had deficient glucagon and adrenaline responses and concomitant attenuation of blood glucose recovery. An impaired glucagon response thus delays recovery from hypoglycaemia and this has been confirmed in other reports [10–12]. The present study is in keeping with the work of Bolli et al. [3] and confirms that the glucagon response in a group of patients with Type 1 diabetes of relatively short duration is attenuated and is associated with a significant delay in blood glucose recovery.

In summary, hypoglycaemia-induced mesenteric vascular hyperaemia is preserved in diabetic patients exhibiting counter-regulatory failure of glucagon. This suggests that glucagon is not a significant mediator of the hyperaemic response.

REFERENCES


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