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The role of hepatic portal glucose sensing in modulating responses to hypoglycaemia in man

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Abstract

Aims/hypothesis. The role of glucose sensing cells in the human hepatic portal system in the initiation of the neuroendocrine responses to acute hypoglycaemia is not known. We investigated the effect of raising blood glucose concentrations in the hepatic-portal vein on neurohumoral responses during induction of systemic hypoglycaemia in nine healthy male volunteers.
Methods. Each subject received an insulin infusion (3 mU·kg^{-1}·min^{-1}) on two occasions, in random order. Variable rate glucose infusion was used to maintain plasma glucose at 5 mmol/l for 60 min, then 3.2 mmol/l for 60 min. At 20 min prior to hypoglycaemia, subjects drank 20 g of glucose in water or water sweetened with saccharin. In five of the volunteers, the oral glucose was labelled with U-13C6 glucose, which showed peak systemic glucose absorption between 90 and 110 min. Five volunteers also repeated the study with a euglycaemic clamp.
Results. Oral glucose was associated with a reduction in the early adrenaline response to hypoglycaemia, the area under the curve from 90 to 110 min falling from 24.02±20.84 (means ± SD) to 15.26±13.65 nmol/l per 20 min, $p<0.05$. Symptom scores (area under curve) decreased from 99.72±91.86 to 16.39±94.71, $p=0.008$ (total), 51.8±68.61 to 7.78±41.61, $p=0.03$ (autonomic) and 54.17±50.61 to 8.6±57.99 with oral glucose, $p=0.001$ (neuroglycopenic). Oral glucose did not influence symptoms during euglycaemia.
Conclusion/Interpretation. Our data are compatible with the hypothesis that centrally mediated symptomatic and neuroendocrine responses are attenuated by glucose detection in the hepatic portal vein in humans. [Diabetologia (2002) 45:1416–1424]

Keywords Hypoglycaemia unawareness, glucose sensing, counterregulation, hepatic portal venous system, ventromedial nucleus of the hypothalamus.

Perception of a minor decrease in plasma glucose concentration and initiation of a counterregulatory response are essential factors in the defence against severe hypoglycaemia during the pharmacological treatment of diabetes mellitus. The inability to generate or detect the warning symptoms of early hypoglycaemia puts diabetic patients at high risk of episodes of severe hypoglycaemia [1] and often the fear of hypoglycaemia limits the optimisation of glycaemic control [2]. Associated with loss of subjective awareness of hypoglycaemia is a lowering of the glucose concentration required to initiate the counterregulatory response to hypoglycaemia, as well as a reduction in the magnitude and intensity of the counterregulatory response at any given blood glucose concentration [3, 4, 5]. The speculation that these defects are principally the result of defective glucose sensing gains support from data showing similar changes in the neuroendocrine re-

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Abbreviations: VMH, Ventromedial nucleus of the hypothalamus; AUC, area under the curve; G, study with oral glucose drink; S, control study with saccharine.

sponse to experimental hypoglycaemia when non-glucose metabolic fuels are available to replace the glucose [6, 7].

The literature is divided as to the anatomical location of the primary hypoglycaemia sensor of the body. Selective catheterisation studies in dogs showed a reduction in the counterregulatory hormone responses to systemic hypoglycaemia when brain glucose concentrations were maintained artificially, suggesting that the hypoglycaemia sensor is in the brain [8, 9]. More recent studies using microdialysis catheters in rats localised the glucose sensor to the ventromedial nuclei of the hypothalamus (VMH) [10, 11, 12, 13]. Creating cellular glucoprivation in the VMH in euglycaemic animals by local perfusion of 2 de-oxyglucose triggered a counterregulatory response [11], whilst local perfusion of glucose into the VMH during systemic hypoglycaemia suppressed the expected counterregulatory response [12].

In contrast, the authors of other studies using selective catheterisation of the hepatic portal vein in animals suggest that the primary hypoglycaemia sensor is located in the hepatic portal venous system of the liver [14, 15, 16, 17]. Infusion of glucose into the portal vein in both rats and dogs suppressed the catecholamine response to systemic hypoglycaemia [14, 15, 16]. The effect was shown to be dependent on intact innervation of the portal vein [17]. The portal vein infusion of glucose created a portal-arterial glucose gradient in these studies, in order to achieve portal and hepatic normoglycaemia in the face of systemic hypoglycaemia.

A case report of disordered glucoregulation in a patient with a sarcoid deposit in the region of the hypothalamus suggests an important role for this region of the brain in hypoglycaemia sensing in humans [18]. There are no data on the function of possible hepatic portal vein glucose sensors in humans, apart from evidence of a small reduction in adrenaline responses during low dose insulin infusion in patients with recent hepatic transplants [19] and a single study which examined the effect of raising portal glucose concentrations with oral glucose administered after the onset of hypoglycaemia [20]. Our aim was to examine the role of hepatic portal vein glucose sensing during acute hypoglycaemia in humans. We have therefore examined the effects on the magnitude of the counterregulatory response in humans of increasing glucose concentrations in the hepatic portal vein, by oral glucose administration, during the induction of controlled acute hypoglycaemia.

Subjects and methods

Subjects. Nine healthy male volunteers were recruited, between 19 to 39 years of age (mean 27.1±6.86 years). None had any previous relevant medical history or were taking medication. The protocol was approved by the King's College Hospital Ethics Committee and each subject gave written informed consent before participating.

Each subject underwent two hyperinsulinaemic hypoglycaemic clamps, on two occasions, at least 2 weeks apart, in random order. Subjects were kept blinded to the study conditions at all times.

Protocol. Subjects were admitted to the Programmed Investigation Unit in King's College Hospital, in the morning, having fasted from 10:30 pm the previous evening. Two intravenous cannulae were placed in the non-dominant arm using aseptic techniques and 1% intradermal lignocaine to anaesthetize the skin. One cannula was put in the antecubital vein for infusion of insulin, glucose and potassium. The other was placed retrogradely in a distal wrist or hand vein. The hand was rested in a box of heated air (55–60°C) to arterialize venous blood [21]. This cannula was used for sampling arterialized blood.

Not less than 30 min after cannulae insertion, a primed continuous infusion of 3 $mU \cdot kg^{-1} \cdot min^{-1}$ soluble insulin (human Actrapid, Novo Nordisk Pharmaceuticals, Crawley, UK) was started. This dose of insulin was chosen after pilot studies were completed to allow reliable attainment of the required systemic hypoglycaemia in the studies with oral glucose. Arterialized plasma glucose was measured at 5-min intervals by the bedside using a glucose oxidase technique (Yellow Springs glucose analyser, Yellow Springs Instruments, Yellow Springs, Ohio, USA). Plasma glucose was controlled by adjusting a simultaneous variable infusion of 20% glucose (Clintec Nutrition, Slough, UK). Plasma glucose concentrations were maintained at 5 mmol/l for 60 min before being reduced over 10 min to a nadir of 3.2 mmol/l. In three subjects, the nadir was achieved by reducing the rate of infused glucose alone. In six of the subjects however, the initial decrease in plasma glucose concentration was seen to be inadequate and for them the insulin infusion rate was increased transiently (to 9 $mU \cdot kg^{-1} \cdot min^{-1}$ for 3 min then 6 $mU \cdot kg^{-1} \cdot min^{-1}$ for 4 min before being restored to the background rate of 3 $mU \cdot kg^{-1} \cdot min^{-1}$) in addition to reducing the glucose infusion rate. For each of these six subjects, the same increase in insulin infusion was applied on both occasions, so that none of them had a different insulin infusion protocol for his paired study. Once achieved, plasma glucose concentrations were maintained at 3.2 mmol/l for a further 60 min before the plasma glucose concentration was restored to 5 mmol/l and maintained at euglycaemia for the final 30 min [22].

At 50 min into the study, 20 min before the subject achieved the hypoglycaemic plateau, he drank either 20 g oral glucose in 100 ml of water (G) or a similar volume of water sweetened with sodium saccharin (S). The glucose drink was made by dissolving 20 g of glucose monohydrate (Glucose BMS Monohydrate B.P, Bio-Medical Services, Bishopthorpe, York, England), weighed on a laboratory scale, in 100 ml water. The dose was calculated to increase portal glucose by approximately 1 mmol/l, assuming absorption over 2 h, and a portal blood flow of 0.8 l/min. In five of the subjects, the oral glucose drink was labelled with the glucose tracer U-$^{13}C_6$ glucose (98.7 atom % excess, 250 mg in 20 g of glucose) to estimate the rate of systemic absorption of the oral glucose from the gastrointestinal tract. The rate of appearance of the glucose tracer in the systemic circulation was estimated using a one-compartment model [23].

Potassium chloride (80 mmol) (Strong potassium chloride, Antigen Pharmaceuticals, Roscrea, Ireland) was added to a litre of 0.9% isotonic saline and potassium was replaced intravenously at a rate of 4 mmol/h during euglycaemia and at a rate of 8 mmol/h during hypoglycaemia [24]. Serum potassium was checked at 30 min intervals during the study.

Additional arterialized blood samples were collected at predetermined time points for later measurement of catecholamines, glucagon, growth hormone, cortisol and insulin. Samples to measure glucose tracer concentration were taken at 0 min and at 5-min intervals after ingestion of the oral glucose drink. Subjects were asked to complete a standard symptom questionnaire, in which they were asked to grade a standard list of symptoms from 1 (absent) to 7 (severe) on a linear analogue scale [25]. The list included symptoms classified as autonomic (feeling sweaty, shaky, anxious, hot or experiencing palpitations and tingling) and those classified as neuroglycopenic (feeling dizzy, unable to concentrate, confused, drowsy, irritable, or having blurred vision) and each symptom was graded individually by the subject. Thus an asymptomatic person would score 14, 7 and 7 for total, autonomic and neuroglycopenic symptoms respectively and the equivalent maximum scores would be 84, 42 and 42.

To assess the effect of oral glucose alone on the symptoms of hypoglycaemia, five subjects (mean age 28.8±7.3 years) underwent two further clamps on two separate occasions in random order in which plasma glucose was held at 5 mmol/l throughout. They were given oral glucose on one occasion and saccharine on the other and the study protocol was as above except for the absence of hypoglycaemia.

Measurements. Plasma adrenaline and noradrenaline were measured by high performance liquid chromatography [26], glucagon [27], cortisol [28], growth hormone [29] and insulin [30] by a sensitive radioimmunoassay. Inter-assay and intra-assay variabilities were less than 10% for all assays. Paired studies were run in the same assay. The U-$^{13}C_6$ glucose was measured as the trimethylsilyl derivative by gas chromatography mass spectrometry [31].

Statistical analysis. All results are expressed as means ± SD unless otherwise stated. Plasma glucose, serum potassium, hormone responses and symptom scores were calculated as the area under the curve (AUC) using the trapezoidal rule after initiation of hypoglycaemia. For cortisol, where basal levels are high, the AUC of the change in cortisol (delta) during hypoglycaemia was used. Symptom scores were calculated as the change in symptom scores between the period of hypoglycaemia and baseline euglycaemia. Total symptom scores and individual scores for autonomic and neuroglycopenic symptoms were calculated for each individual at each time point by adding the individual incremental scores of all, just the autonomic and just the neuroglycopenic symptoms. Data was analysed by ANOVA with repeated measures design; when there was a statistically significant group-time effect, two-tailed paired *t* tests were used to localize the effects. Glucose infusion rates during studies were analysed by paired Students *t* tests. Results were analysed using SPSS for Windows 10.5 (SPSS, Woking, UK) and differences were regarded as statistically significant if *p* values were less than 0.05.

Results

Insulin, glucose and potassium. Insulin infusion raised circulating insulin concentrations to 180.3±26.4 and 181.4±30.7 mU/l (Fig. 1) in G and S respectively (p=0.94). During hypoglycaemia, the peak levels were 189.7±23.1 and 195.7±11.6 mU/l, (p=0.18). Figure 2 shows the plasma glucose profiles achieved in the two groups which were not significantly different, p=0.31.

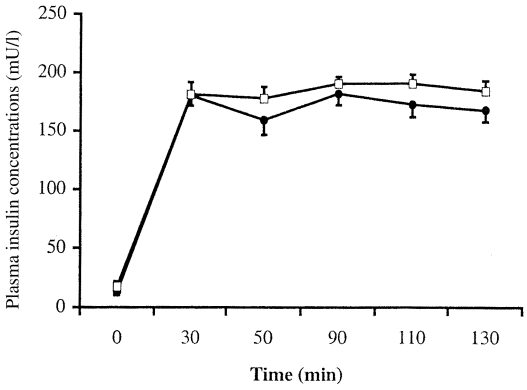

Fig. 1. Plasma insulin concentrations during the hypoglycaemic hyperinsulinaemic clamps in the presence (●) or absence (□) of an oral glucose load. Data expressed as means ± SE

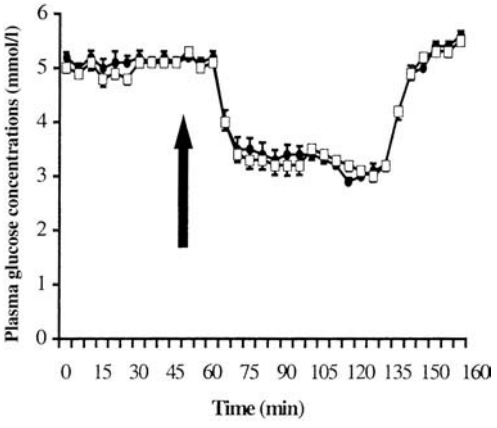

Fig. 2. Arterialised plasma glucose concentrations during euglycaemic-hypoglycaemic clamps, with (●) and without (□) oral glucose administration at onset of hypoglycaemia. The *arrow* represents the time point at which the oral glucose drink was given in the study. Data are expressed as means ± SE

The coefficient of variation in plasma glucose concentration in the first 60 min was 2.91% and 2.03% in G and S respectively. During the hypoglycaemic period the mean plasma glucose concentration in G was 3.28±0.13 mmol/l compared to 3.25±0.17 mmol/l in S, p=0.29.

Labelled glucose began to appear in the systemic circulation 10 to 15 min after ingestion of the oral glucose with peak systemic absorption of the labelled glucose occurring between 90 to 110 min (Fig. 3).

Serum potassium concentration decreased slightly in both studies from 4.11±0.38 to 3.57±0.23 mmol/l in G compared to 3.94±0.33 to 3.56±0.21 in S, p=0.27.

Glucose infusion rates. Glucose infusion rate was lower in G compared to S during the period of hypoglycaemia (206.78±88.98 mg·kg^{-1}·min^{-1} in G compared to 439.39±130.66 mg·kg^{-1}·min^{-1} in S, p=0.0003, Fig. 4). There was no difference in the rate of glucose infused between the two groups during the preceding period of

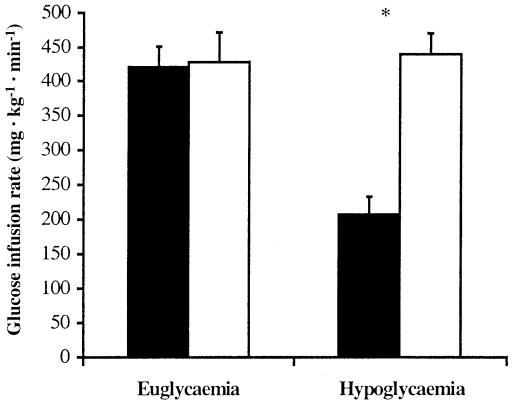

Fig. 3. Glucose infusion rates during euglycaemic-hypoglycaemic clamps, with (*closed bars*) and without (*open bars*) oral glucose administration. *Indicates p value =0.0003. Data are expressed as means ± SE

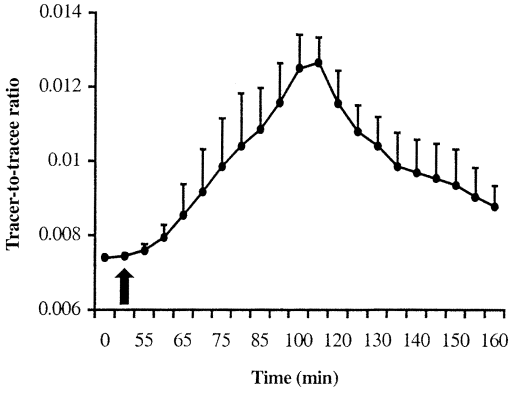

Fig. 4. Rate of appearance of glucose tracer in the systemic circulation over time in five subjects who ingested 20 g labelled glucose at the time indicated by the *arrow*. Data are expressed as means ± SE

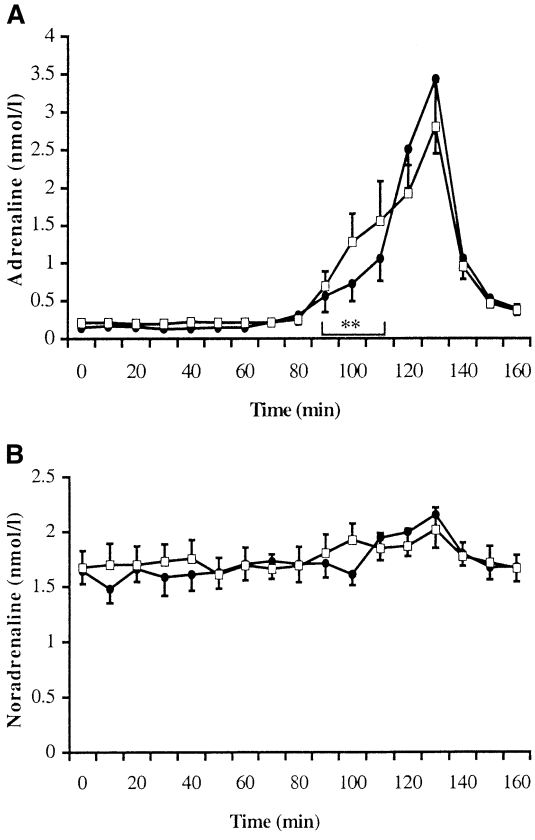

Fig. 5 A, B. Adrenaline (**A**) and noradrenaline (**B**) responses to hypoglycaemia in the presence or absence of oral glucose load. Symbols as in Fig. 1. **Indicates $p<0.05$. Data are expressed as means ± SE

euglycaemia (420.78±78.41 mg·kg^{-1}·min^{-1} in G compared to 427.56±90.83 mg·kg^{-1}·min^{-1} in S, p=0.802).

Counterregulatory responses. Catecholamine, growth hormone and cortisol levels increased in response to the hypoglycaemia in all studies. The ingestion of oral glucose altered the adrenaline responses, which were significantly different during the increase of labelled glucose in the systemic circulation. Although the overall area under the adrenaline response curve across the whole time course of the hypoglycaemia was not different between the two studies (Fig. 5), the rise in adrenaline was slower in study G, where there was a reduction in the early adrenaline response compared to S, during the time of peak appearance of labelled glucose in the systemic circulation, between 90 to 110 min. The area under the adrenaline curve from 90 to 110 min was 15.3±13.7 nmol/l per 20 min in G, compared to 24.0±20.8 nmol/l per 20 min in S ($p<0.05$). After 110 minutes, the adrenaline responses came together (43.8±29.8 nmol/l per 40 min in G compared to 42.7±23.2 nmol/l per 40 min in S, $p>0.05$).

A reduction in the magnitude of the norepinephrine response (Fig. 5) between the 90 to the 110 min period of the study in G was not significant (34.5±6.8 nmol/l per 20 min in G compared to 37.5±8.6 nmol/l per 20 min in S, p=0.22). Similarly, although the cortisol responses showed an apparent dimunition in G compared to S (Fig. 6), the difference did not reach statistical significance ($AUC_{\Delta 90-110\ min}$=3962.8±3041.0 nmol/20 min in G compared to 7113.3±3787.2 nmol/20 min in S, p=0.099).

The growth hormone responses to hypoglycaemia were not different between the two groups (Fig. 6), peak growth hormone response 23.7±23.9 mU/l in G compared to 22.6±16.2 mU/l in S, p=0.57. There was no glucagon response in either group, 80.4±7.8 to 83.3±15.9 pg/ml in G compared to 92.8±20.7 to 91.5±18.5 pg/ml in S, p=0.56 (Fig. 6).

Symptom scores. There was a rise in symptom scores from baseline values in both groups during hypoglycaemia. However, the rise in symptom scores was less in G at the 85 and 110 min assessments (Fig. 7). Total symptom scores (AUC) were only 16.4±94.7 in

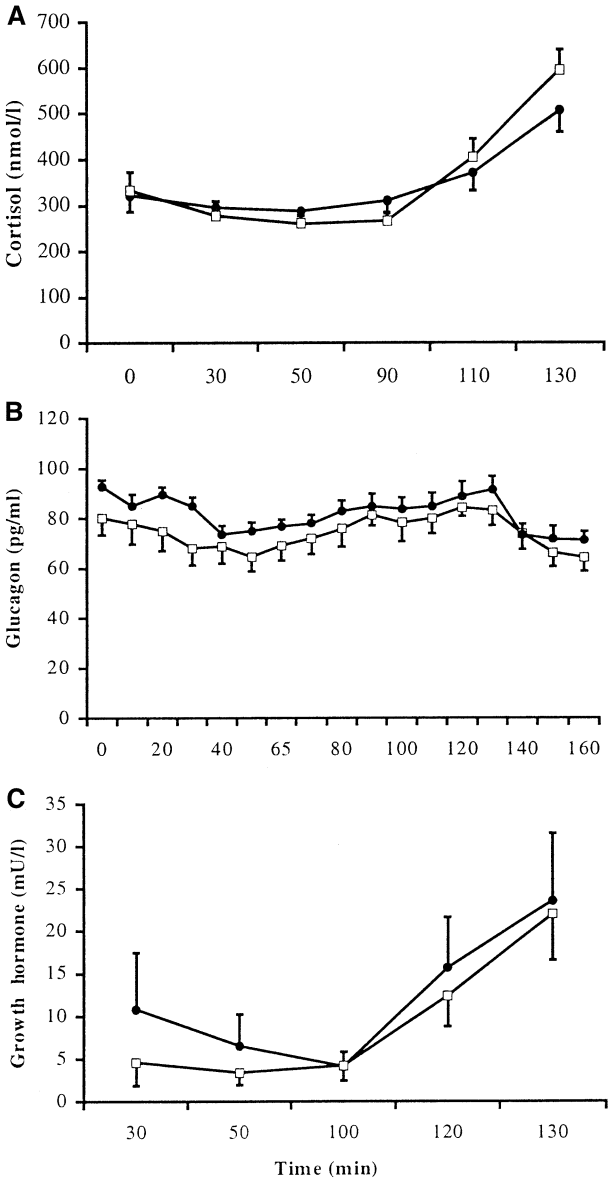

Fig. 6 A–C. Cortisol (**A**), glucagon (**B**) and growth hormone (**C**) responses to acute hypoglycaemia in the presence and absence or oral glucose. Symbols as in Fig. 1. Data are expressed as means ± SE

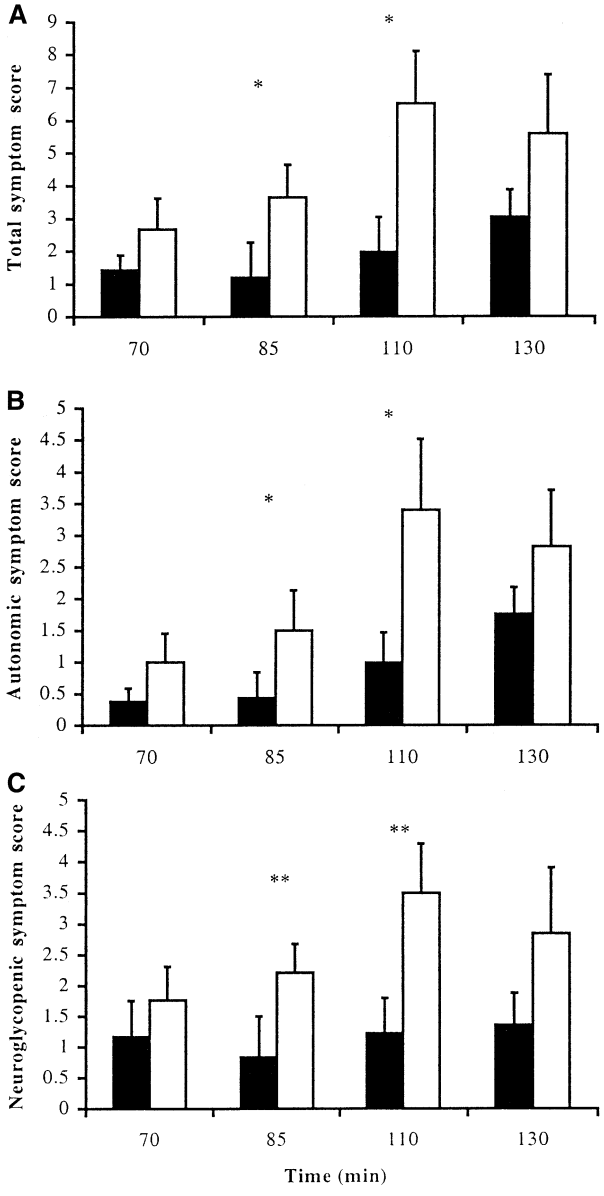

Fig. 7 A–C. The change from baseline in total (**A**), autonomic (**B**) and neuroglycopenic (**C**) symptom scores during 60 min of hypoglycaemia only, with (*closed bars*) or without (*open bars*) oral glucose. Data are expressed as means ± SE. *Indicates $p<0.05$; **$p<0.005$

G, compared to 99.7±91.9 in S, $p=0.008$. Autonomic symptom scores were 7.8±41.6 in G, compared to 51.8±68.6 in S, $p=0.03$, and neuroglycopenic symptoms were 8.6±58.0 in G, compared to 54.2±50.6 in S, $p=0.001$.

In the additional euglycaemic study, the equivalent numbers for the AUC for total symptoms, autonomic and neuroglycopenic symptoms were 10.2±39.59 in G, compared to 7.25±16.83 in S, $p=0.86$; 2.25±5.03 in G, compared to 2.5±5.59 in S, $p=0.95$ and 12.5±40.70 in G, compared to in S, 9.75±21.37, $p=0.89$ respectively.

Discussion

Understanding the mechanisms of hypoglycaemia sensing is becoming critical for the better treatment of people with diabetes. Elucidating the mechanisms of hypoglycaemia detection and the initiation of symptomatic and neuro-humoral responses to early hypoglycaemia in humans should help us devise ways of restoring hypoglycaemia awareness to those that have lost it. Our data, showing attenuation of symptomatic, and to some extent adrenergic, responses to induced systemic hypoglycaemia during absorption of an oral glucose load, elucidates these mechanisms.

The study was done to investigate the role of portal vein glucose sensing in human responses to hypoglycaemia. For a long time, the major mammalian hypoglycaemia sensor has been considered to be within the brain, particularly including the hypothalamus. The evidence for this includes the inhibition of neuroendocrine counterregulatory responses to systemic hypoglycaemia when brain glucose concentrations are maintained by selective glucose infusion [8, 9]; the development of hyperglycaemia after hypothalamic lesions [10]; the induction of a counterregulatory response in euglycaemic animals by localised glucoprivation in the ventromedial hypothalamus [11] and the amelioration of counterregulatory responses to acute hypoglycaemia in animals when hypothalamic glucose concentrations are sustained by microdialysis of glucose solutions into the area [12]. However, the primacy of the cranial hypoglycaemia sensor has been challenged by studies showing similar suppression of counterregulatory responses to acute hypoglycaemia when portal vein and hepatic glucose concentrations are sustained by local infusion in rats and dogs [14, 15, 16]. Certainly, glucose sensing cells and the beta-cell transporter GLUT2 are found both in the brain [32] and in the hepatic portal vein [33]. Which of the glucose sensing regions acts as the primary hypoglycaemia sensor in humans remains controversial. There are almost no data on a possible portal vein glucose sensing mechanism in humans, except observations of slightly impaired hormonal responses to systemic hypoglycaemia in patients with denervated liver in the months after therapeutic orthoptic liver transplantation [19] and a conflicting study in which a small increase in hormonal responses to hypoglycaemia was noted when portal vein glucose sensors were stimulated by oral glucose after the onset of hypoglycaemia [20].

To investigate the role of portal vein glucose sensing in the responses to acute hypoglycaemia in humans, we gave an oral glucose load immediately before inducing controlled mild systemic hypoglycaemia to diminish portal vein hypoglycaemia during the onset of acute systemic hypoglycaemia. The time course of the appearance of labelled glucose from the oral load into the peripheral circulation allowed us to define a time span in which we could be confident that portal vein hypoglycaemia was being reduced by the absorption of the oral glucose. It was during this time that the effects of oral glucose on counterregulatory responses was observed. In order to do this while achieving a similar degree of peripheral hypoglycaemia in the presence or absence of oral glucose, a high dose of insulin had to be used, which could have influenced counterregulation. However, the insulin infusion regimens were matched for each subject in the study.

Our data show the presence of active glucose sensors in the liver or hepatic portal vein in humans. They are consistent with the hypothesis that these portal vein glucose sensors can act to modulate centrally mediated counterregulatory response to acute hypoglycaemia as portal vein glucose concentrations increase after glucose ingestion, with a major effect on symptom generation and a smaller but significant effect on adrenaline responses. There was no change in symptom scores during euglycaemia as a result of oral glucose ingestion; therefore, the oral glucose effect was specific to the hypoglycaemic state.

The ingested glucose creates a transient glucose concentration gradient between the portal vein and the systemic arterial circulation, as occurs in feeding. A reduction in sympathetic tone in response to feeding has been described [34]. Such a phenomenon would explain the findings of the animal studies in which portal vein glucose infusion resulted in reduced catecholamine responses to systemic hypoglycaemia [14, 15, 16, 17]. Our data might also be consistent with the hypothesis that the portal vein glucose sensors in humans are the primary hypoglycaemia sensors, driving the central responses. In this interpretation, the diminished early adrenaline and symptomatic responses are due to a diminished hypoglycaemic challenge to the portal hypoglycaemia sensor. The relatively small suppression of adrenaline responses might then be due to incomplete amelioration of the portal hypoglycaemia by our oral glucose load, although we can calculate that 20 g oral glucose will raise portal venous glucose by at least 1.1 mmol/l, as the tracer data show that the glucose was absorbed in parabolic manner over about 2 h, and we know that portal blood flow (2/3 total liver blood flow which itself at rest is 25% of the total cardiac output of 5 l/min) is 0.8 l/min or 96 l in total. We therefore would have expected a more substantial loss of counterregulatory responses if the portal vein glucose sensors were the primary hypoglycaemia sensor, although we cannot rule the possibility out altogether because we cannot measure portal glucose directly. Also against this interpretation is the failure of studies to show a substantial loss of hypoglycaemia responses in human patients with denervated livers, early after orthoptic liver transplantation [19]. Although a small reduction in adrenaline responses was noted during hypoglycaemia in response to low dose insulin infusion in recent recipients of liver transplant recipients (in whom the liver and portal vein are denervated), interpretation of these data is difficult because the hypoglycaemic challenge was also slower in those patients than in the control subjects. Teleologically, the portal glucose sensors are ideally placed to monitor glucose absorption from the gastrointestinal tract, while a hypothalamic glucose sensor is well placed to monitor glucose supplies to the brain.

Although we could not measure portal glucose concentration, the systemic appearance of radiolabelled glucose from the glucose drink in our study implies

the presence of a portal arterial glucose gradient early after glucose ingestion. Such gradients have been measured directly in animals in response to an intragastric glucose load. Intragastric administration of glucose to animals causes an immediate increase in portal vein glucose concentration, greater than that shown in the systemic arterial circulation, creating a positive portal arterial glucose concentration gradient [35, 36, 37] which can last for up to 60 min and is then lost as the glucose concentration in the portal vein falls [38]. In our study we can assume that the $U^{13}C_6$ glucose appears in the systemic circulation slightly later than any appearance of glucose in the portal system, indicating a positive portal arterial glucose concentration gradient at the time of inducing hypoglycaemia as the tracer levels in the circulation are increasing. Portal-arterial glucose gradients have been implicated in the control of net hepatic glucose uptake [38, 39, 40], food intake [41], and recently tissue glucose utilisation [42]. Alternatively, any effects we observed could have been a direct result of changes in stimulus to the portal vein glucose sensors themselves.

It is likely that any portal signal is conveyed to the central sympathetic pathways via nerves. A recent paper [17] found that denervation of the portal vein reduced the symapatho-adrenal response to whole body hypoglycaemia. However, other animal studies have found no effect of either hepatic denervation [43] or blocking the function of the vagus nerve [44, 45] on counterregulation, although very recent data in which capsacin was used to block sympathetic transmission have suggested it is the sympathetic nerves, rather than the vagus, that could carry the signal to the brain [46]. The effects of total severance of the neuronal connections from the portal vein glucose sensors is thus still controversial.

The major effect of the oral glucose in our study was the reduction in autonomic and neuroglycopenic symptoms, also at time points compatible with the presence of the portal signal. This could relate to the reduced adrenaline responses but the size of the effect on neuroglycopenic as well as autonomic symptoms suggests additional mechanisms are involved. The portal signal can control food intake independent of catecholamines [41]. The lateral hypothalamus contains glucose-sensitive neurones that are stimulated by hypoglycaemia indirectly [47] via signals transmitted from the nucleus tractus solitarius [48]. The nucleus tractus solitarius receives input from vagal nerve afferents supplying the liver [49] and contains neurones that are stimulated by falling glucose [48] and inhibited by rising glucose in the portal circulation [50]. These pathways form a route through which the portal signal can influence hypothalamic and cortical function during hypoglycaemia [51] and could explain the reduction in both autonomic and neuroglycopenic symptoms.

Our study design forced continuation of the hypoglycaemia despite the symptomatic and adrenaline response and this could have minimized the effect of oral glucose on a less artificial hypoglycaemic episode. This could explain why we did not find a significant reduction in the noradrenaline, cortisol and growth hormone responses to hypoglycaemia. The study was powered on the basis of the adrenaline responses, which are the fastest and most robust of the hormonal responses to hypoglycaemia in this setting [4, 22] and so it is possible we were underpowered to detect changes in the other hormones. In the case of growth hormone, the release of ghrelin from the stomach might have acted as a direct stimulus of growth hormone release independent of hypoglycaemia [52]. The absent glucagon response is probably related to the high dose insulin infusion used to achieve consistent hypoglycaemia during the study [53].

A recently published study found a late enhancement of adrenaline, growth hormone and glucagon responses after oral glucose ingestion to induced hypoglycaemia between 1 to 2 h after the onset of hypoglycaemia [20]. In that study, the oral glucose was given after the achievement of the hypoglycaemic nadir and so the effect of portal vein glucose sensing on the initiation of the counterregulatory hormone responses could not be examined. Furthermore, the glucose drink could have contained caffeine as well as glucose, which would be expected to enhance counterregulatory hormone responses to hypoglycaemia [54].

We must be cautious in applying our data on our healthy volunteers to diabetic patients, but an effective portal signal giving early warning of glucose ingestion and able to attenuate a central stress response to systemic hypoglycaemia could be valuable in limiting post-hypoglycaemic hyperglycaemia in diabetic patients [55]. Currently however, these data are of most importance by indicating the interactions between the different glucose sensing areas of the human body in coordinating the neuroendocrine responses to acute hypoglycaemia. In conclusion, the portal vein glucose sensors do have a role in determining the physiological responses to acute hypoglycaemia in humans, primarily by influencing symptom generation and, to some extent, by modulating the centrally mediated adrenergic responses.

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