Physiological control of splanchnic blood flow by adrenaline: studies during acute hypoglycaemia in man

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ABSTRACT

Superior mesenteric artery blood flow (SMABF) increases significantly during and after the hypoglycaemia reaction in healthy humans. To investigate the mechanisms controlling this phenomenon, SMABF and plasma catecholamines were measured in healthy human volunteers. In 10 controls, hypoglycaemia was induced by insulin infusion (2.5 m-units min⁻¹ kg⁻¹). In six subjects, β-blockade by propranolol infusion (0.7 μg min⁻¹ kg⁻¹) preceded insulin infusion and was continued throughout the study. Following the hypoglycaemia reaction, the glucose nadir was similar in both groups. In controls, increases in SMABF [42.4 ± 6.1 % (mean ± S.E.M.); P < 0.001], cardiac output (34.3 ± 2.3 %; P < 0.001) and pulse rate (from 63.9 ± 2.7 to 82.5 ± 3.1 beats/min; P < 0.001) occurred. Superior mesenteric artery resistance fell by 32.4 ± 3.3 % (P < 0.001). Under β-blockade, decreases in SMABF (34.8 ± 2.9 %; P < 0.001) and pulse rate (from 59.5 ± 0.2 to 51.8 ± 2.2 beats/min; P < 0.001) occurred. Superior mesenteric artery resistance increased (peak + 30.8 ± 12.3 %; not significant). Subjects showed greater increases in adrenaline (P < 0.001) and noradrenaline (P < 0.022) concentrations than controls. Mesenteric hyperaemia associated with hypoglycaemia in man appears to be mediated by a β-adrenergic mechanism that is activated by increased circulating levels of adrenaline.

INTRODUCTION

Insulin-induced hypoglycaemia in man is associated with adrenergically mediated increases in pulse rate, cardiac output and pulse pressure [1–3]. Using duplex Doppler ultrasound, we have shown previously that hypoglycaemia is associated with a significant increase in superior mesenteric artery blood flow (SMABF) in man, and that this rise has a close temporal relationship with the increase in adrenaline levels [4]. This phenomenon may facilitate the restoration of a normal blood glucose concentration by increasing the delivery of substrates to the liver for gluconeogenesis.

In separate studies we have shown that the peripheral intravenous infusion of adrenaline, producing circulating concentrations within the physiological range and associated with changes in pulse rate, cardiac output and blood pressure of a similar order of magnitude to those seen during hypoglycaemia, induced a dose-dependent increase in SMABF and a selective decrease in superior mesenteric artery blood flow.

Key words: β-adrenoceptor, hypoglycaemia, superior mesenteric artery.

Abbreviations: AUC, area under the curve; R, the time at which the hypoglycaemic reaction occurred; SMA, superior mesenteric artery; SMABF, SMA blood flow.

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mesenteric artery (SMA) resistance within 5 min of commencement [5]. These effects occurred before steady-state plasma levels of adrenaline could have been achieved, and flow continued to increase to give a peak after 15–30 min of infusion. Flow returned to baseline within 5 min of discontinuing adrenaline administration. When propranolol was infused simultaneously with adrenaline, all haemodynamic changes were prevented. These findings suggested that splanchnic vascular resistance is sensitive to circulating adrenaline concentrations and that splanchnic vasodilatation during hypoglycaemia may be mediated via a β-adrenergic mechanism. The present study using β-adrenoceptor blockade was undertaken to examine the possibility that adrenaline secretion is related causally to the mesenteric hyperaemia seen with acute hypoglycaemia.

METHODS

Sixteen healthy, non-smoking volunteers aged between 21 and 30 years with no significant medical or family history were studied. None were taking regular medication. All experiments were carried out the morning after an overnight (12 h) fast, and took place in a temperature-controlled environment (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 for administration of fluids and insulin, and a second cannula was inserted in the 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. The latter samples were centrifuged immediately (600 rev./min for 3 min), and serum was fast-frozen prior to storage at −20 °C after addition of 1 μl of 1 M sodium metabisulphite/ml, as an antioxidant. Other samples were taken to measure insulin, C-peptide, cortisol, growth hormone and C-terminal glucagon (specific for pancreatic glucagon), non-esterified fatty acids, lactate, glycerol and acetoacetate.

Pulse rate, blood pressure, cardiac output, SMABF and blood samples were taken after a 45 min rest (baseline), during insulin infusion, at the time at which the hypoglycaemic reaction occurred (R) and serially up to R + 150 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.

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). 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 SMA was calculated as: (mean arterial pressure × 100)/SMABF. Previous studies have validated this duplex Doppler technique as accurate and reproducible, with coefficients of variability of 3.2–6.3 % [4,5].

The study consisted of 10 control subjects (group 1) made hypoglycaemic, and six study subjects (group 2) in whom β-blockade was induced by intravenous infusion of propranolol (0.7 μg·min⁻¹·kg⁻¹) prior to induction of hypoglycaemia. Propranolol infusion continued in group 2 until the end of the study.

Hypoglycaemia was induced by the intravenous infusion of soluble human insulin (Actrapid) 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) had fallen to 2.5 mmol/l. Plasma glucose subsequently continued to fall until the nadir at which the hypoglycaemic reaction (comprising adrenergic and neuroglycopaenic symptoms and signs) occurred. This method of hypoglycaemia induction has been used previously with safety [4].

The time taken to reach R varied between individuals. Therefore, to ensure comparison of responses over a standardized time scale, R was taken to represent time zero. Statistical comparisons were made between data collected after or before R. It has previously been demonstrated that, unless comparison is made with reference to R in studies into hypoglycaemia, pooling of data from variable points of recovery occurs, and such pooling leads to inaccuracies in interpretation [6].

The research was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association. Ethical approval was granted by the hospital Research Ethics Committee, and informed consent was obtained from the volunteers.

Plasma glucose was assayed using a glucose oxidase autoanalyzer method. Insulin was measured in batches using a double-antibody radioimmunoassay. The plasma concentration of C-peptide was measured using a commercially available radioimmunoassay kit (Amersham); the normal range in fasting healthy subjects is 110–1270 pmol/l. Measurements of plasma cortisol, growth hormone and C-terminal glucagon (specific to pancreatic glucagon) were via radioimmunoassays. Non-esterified fatty acids, lactate, glycerol and acetoacetate concentrations were measured by enzymic colorimetry. Catecholamine assays were performed by liquid chromatography.

All data were tested for normal distribution, and results are expressed as means ± S.E.M. Changes in parameters were analysed by Student’s t-tests; changes with time between groups were compared by area under the curve (AUC). Statistical significance was accepted at P < 0.05.
RESULTS

Results are summarized in Figures 1–4.

The glucose concentration fell significantly in both groups to similar values at $R$, which corresponded to the nadir level (Figure 1). The time taken to reach $R$ was similar in the two groups ($40 \pm 1.5$ min in propranolol-treated subjects (group 2); $38.8 \pm 2$ min in controls (group 1)). The rate of recovery of blood glucose concentration from $R$ to $R + 60$ min was significantly greater in group 1

![Plasma glucose](image1)

![Insulin](image2)

![C-peptide](image3)

Figure 1 Plasma glucose, insulin and C-peptide concentrations in the two groups

Thin line, group 1 (hypoglycaemia); thick line, group 2 (hypoglycaemia + β-blockade). I + 15 represents 15 min after the onset of insulin infusion. Values are means ± S.E.M.; *P < 0.05 and **P < 0.001 compared with baseline values.

![Adrenaline](image4)

![Noradrenaline](image5)

Figure 2 Plasma catecholamine concentrations in the two groups

Thin line, group 1 (hypoglycaemia); thick line, group 2 (hypoglycaemia + β-blockade). I + 15 represents 15 min after the onset of insulin infusion. Values are means ± S.E.M.; *P < 0.05 and **P < 0.001 compared with baseline values.

[3.01 ± 0.11 mmol·l$^{-1}$·h$^{-1}$, compared with $2.55 \pm 0.10$ mmol·l$^{-1}$·h$^{-1}$ in group 2 ($P = 0.012$)]. However, analysis of AUC did not reveal any overall difference between the two groups. The amount of insulin given during the infusion was identical in each group (mean of 10.4 units). Mean insulin concentrations (Figure 1) increased significantly during infusion from a baseline value of $6.4 \pm 0.1$ m-units/litre to a peak of $183.8 \pm 34.3$ m-units/litre in group 1, and from $2.8 \pm 0.9$ to $133.8 \pm 14.1$ m-units/litre in group 2 ($P < 0.001$ within both groups). There was no significant difference between the groups overall. The plasma C-peptide concentration (Figure 1) fell from a baseline of $887 \pm 78.5$ pmol/litre to a trough of $365.2 \pm 30$ pmol/litre at $R + 15$ min in group 1 ($P < 0.001$), and from a baseline of $699.7 \pm 89.1$ to a trough of $312.3 \pm 54.1$ pmol/litre at $R + 60$ min in group 2 ($P < 0.001$). Overall changes in concentrations between the groups were not significantly different.

The concentration of adrenaline (Figure 2) rose in group 1 from $25 \pm 4.1$ to $554 \pm 107$ ng/litre at $R$ ($P < 0.001$), and in group 2 from $49.7 \pm 9.8$ to $2220 \pm 566$ ng/litre ($P < 0.05$); the mean peak and overall responses in
group 2 were significantly greater ($P = 0.006$; AUC $P < 0.001$). The noradrenaline concentration (Figure 2) increased in group 1 from $120 \pm 14.8$ to $206 \pm 27.9$ ng/litre at $R$ ($P < 0.001$), and in group 2 from $180 \pm 28$ to $441 \pm 91$ ng/litre ($P = 0.039$); the peak response was significantly greater in group 2 ($P = 0.022$), as was the overall response (AUC: $P < 0.001$).

Vascular SMA resistance (Figure 3) in group 1 had fallen by $32.4\%$ at $R$ ($P < 0.001$); in group 2 it increased to a peak of $+30.8\%$ (not significant) above baseline at $R + 10$ and then fell to a nadir of $-30.2\%$ below baseline ($P = 0.001$) at $R + 60$. Between-group differences (AUC) were significant ($P < 0.001$).

The pulse rate in group 2 prior to hypoglycaemia fell to below 60 beats/min in all volunteers, reflecting adequate β-blockade. At $R$, the pulse rate had increased in group 1 from a baseline of $63.9 \pm 2.7$ to $82.5 \pm 3.1$ beats/min ($P < 0.001$); in group 2 there was a significant decrease at $R$ from a mean baseline value of $59.5 \pm 0.2$ to $51.8 \pm 2.2$ beats/min ($P = 0.021$) (Figure 4). Systolic blood pressure in group 1 increased from $110.7 \pm 1.7$ mmHg to $115.0 \pm 2.4$ mmHg at $R$ ($P = 0.003$), and to a peak of $118.4 \pm 2.1$ mmHg at $R + 10$ min ($P < 0.001$). In the presence of propranolol treatment (group 2), there was no significant change in systolic blood pressure. There was no significant difference between diastolic and mean arterial blood pressure changes in the two groups.
7.6 ± 0.18 litres/min from a baseline value of 5.4 ± 0.21 litres/min ($P < 0.001$); in group 2 there was no significant change. AUC analysis revealed a significantly greater cardiac output following hypoglycaemia in group 1 ($P < 0.05$).

Plasma concentrations of glucagon, cortisol and growth hormone all increased significantly in both groups following hypoglycaemia. Plasma lactate and acetoacetate concentrations increased significantly following hypoglycaemia in both groups, but the plasma glycerol concentration did not change significantly, and non-esterified fatty acid concentrations in plasma fell in both groups. These changes were similar to those in our previous study [4]. The overall responses to hypoglycaemia, as assessed by AUC analysis of these hormones and metabolites, were not significantly different between the two groups.

**DISCUSSION**

Previous studies measuring the effects of adrenaline on hepatic blood flow in man using indirect methods have produced confusing results. Indocyanine clearance studies suggested that supraphysiological doses of adrenaline can produce a sustained increase in total hepatic blood flow and a fall in splanchnic resistance; noradrenaline infusion appears to cause a transient decrease in total hepatic blood flow [7]. However, bromsulphalein extraction studies have suggested that hepatic blood flow does not change significantly during adrenaline infusion [8]. The inconsistency of these results may be a reflection not only of the use of infused catecholamines but also of the relative inaccuracy in measuring blood flow by such methods compared with duplex Doppler ultrasound.

Adrenergic regulation of mesenteric and liver blood flow has been suggested in previous studies by the close temporal relationship between a sustained increase in mesenteric blood flow following insulin-induced hypoglycaemia and catecholamine responses [4]. A significant and selective fall in SMA resistance and splanchnic hyperaemia during the intravenous infusion of physiological amounts of adrenaline has been described, and these changes can be prevented by the concomitant infusion of propranolol [5]. Taken together, these results suggest strongly that splanchnic vasodilatation may be influenced via a $\beta$-adrenergic mechanism, but do not prove physiological regulation by such a mechanism.

The present study demonstrates that adequate $\beta$-adrenoceptor blockade during insulin-induced hypoglycaemia transforms increased flow and decreased resistance into a significant fall in SMABF and a significant increase in SMA resistance. In addition, the increase in cardiac output usually seen at the hypoglycaemic reaction was abolished by propranolol, and there was a fall in the pulse rate. The fall in SMABF at the hypoglycaemic reaction probably reflects unopposed $\alpha$-adrenoceptor stimulation enhanced by raised circulating catecholamine levels, and suggests that the mesenteric hyperaemia associated with hypoglycaemia in man is normally mediated by $\beta$-adrenoceptor stimulation. The significant fall in SMABF post-hypoglycaemia during $\beta$-blockade and the unchanged cardiac output suggest that changes in SMABF were due to *bona fide* alterations in mesenteric vascular resistance rather than a reflex response to changes in cardiac output. The initial increase in SMA resistance was followed by a reversal late in hypoglycaemic recovery, and there was a small increase in SMABF at $R + 60$. Catecholamine levels at this time were still elevated, suggesting $\beta$-adrenoceptor mediation, but an additional late non-adrenoceptor mechanism may also be involved in control of SMABF, possibly involving neurotensin [9].

Following the commencement of propranolol infusion and before hypoglycaemia had been induced, SMABF fell slightly (by 9%; not significant) in this study (Figure 3), and we have demonstrated a similar phenomenon previously [5]. Others have shown a similar decrease in portal venous flow 2 h after the onset of $\beta$-blockade [10]. We believe that this small reduction in splanchnic blood flow following $\beta$-adrenergic blockade in the absence of hypoglycaemia is not the explanation for the significant fall in SMABF observed following induction of hypoglycaemia in the present study. Ideally, a second control group undergoing $\beta$-blockade for 120 min without hypoglycaemia would have addressed this issue.

The mesenteric artery and arterioles are densely innervated by sympathetic post-ganglionic fibres, electrical stimulation of which produces a triphasic response: initial vasoconstriction is followed by a recovery of flow if stimulation continues (termed autoregulatory escape), and finally a hyperaemic phase occurs in which flow increases above baseline once stimulation ceases [11]. Adrenaline has been reported to have variable effects on the mesenteric vasculature [12], and these inconsistencies reflect the use of different supraphysiological or pharmacological doses, different routes of administration, different species studied and the fact that work *in vitro* is complicated by the effects of denervation. The evidence suggests, however, that adrenaline is a splanchnic vasodilator in cats, an effect that is increased by $\alpha$-adrenoceptor blockade [13,14]. Similarly, in dogs, adrenaline under conditions of $\beta$-blockade produces vasoconstriction [15]. In the primate circulation, adrenaline appears to be a vasodilator at low doses and acts as a vasoconstrictor only at higher doses. The dilator response to adrenaline is potentiated by $\alpha$-blockade, but can be blocked by $\beta$-adrenergic receptor blockade [16]. $\alpha$-Adrenoceptor agonists, such as phenylephrine and noradrenaline, cause mesenteric and hepatic artery vas-
constriction that can be blocked by $\alpha$-adrenergic blockade in rats, cats and dogs [12,17]. The non-selective $\beta$-agonist isoprenaline increases mesenteric and hepatic artery blood flow in dogs, cats, man and rats [12,18–22]. $\beta_2$-Adrenoceptor agonism by dobutamine relaxes mesenteric resistance vessels in rats [19], and $\beta_2$-adrenoceptor stimulation with salbutamol increases flow in rats and dogs [22,23]. Non-selective $\beta$-blockade with propranolol antagonizes the intestinal vasodilator response to isoprenaline in cats, dogs and rats [12,19]. Selective $\beta_1$-blockers such as atenolol have a less noticeable effect. These data suggest that hepatic and mesenteric arterial resistance sites have substantial $\beta$-receptor complements mediating vasodilatation via $\beta_2$- and, to a lesser degree, $\beta_1$-adrenoceptors. These previous studies suggest that there are functionally opposite roles for adrenoceptors in the mesenteric vascular bed, with the $\alpha$-receptor mediating vasoconstriction and the $\beta$-receptor mediating vasodilatation. The results of the present study are consistent with such a system of physiological control operating in vivo in man. There is evidence to suggest that endothelial nitric oxide release [19,24] and peripheral capsaicin-sensitive afferent nerves [21,25] are also involved in the control of intestinal blood flow.

Restoration of euglycaemia following insulin-induced hypoglycaemia is impaired during non-selective $\beta$-adrenoceptor blockade with propranolol [26,27]. It has been suggested that $\beta$-adrenergic receptor activation is involved in initiating gluconeogenesis [28,29], and this may explain, at least in part, the impairment of blood glucose recovery by propranolol.

The evidence presented here is in agreement with our previous studies into the mechanism of splanchnic hyperaemia during hypoglycaemia, and suggests that, in the SMA, $\alpha$-receptors are vasoconstrictory and $\beta$-receptors stimulated by circulating adrenaline are vasodilatory.

REFERENCES


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