The physiological effects of insulin-induced hypoglycaemia in man: responses at differing levels of blood glucose

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Summary

1. The aim of this study was to describe hormonal, cardiovascular and thermoregulatory responses to insulin-induced hypoglycaemia of differing levels of severity.

2. Five normal male volunteers were rendered hypoglycaemic at intervals of 1 week by intravenous infusions of 3, 4 or 6 units of insulin/h, or by intravenous injection of 0.15 unit/kg body weight.

3. Plasma glucose reached nadir values of 2.08 ± 0.10, 1.82 ± 0.21, 1.24 ± 0.08 and 0.92 ± 0.06 mmol/l (means ± SEM) in the four experiments. Non-esterified fatty acid levels fell equally in all experiments but recovery was more rapid with severe hypoglycaemia. In contrast the rate of recovery of plasma glucose was slower with deeper hypoglycaemia and this appeared unrelated to the counter-regulatory response.

4. Plasma glucagon, adrenaline and prolactin levels increased in proportion to the severity of hypoglycaemia, but peak concentrations of cortisol, growth hormone (somatotropin) and noradrenaline did not vary, suggesting that moderate hypoglycaemia had elicited maximal responses. When the areas under the curves were calculated, the cortisol responses were greater for the 6 units infusion and bolus injection than for the other infusions, and the growth hormone responses were similar for all three infusions but significantly greater with the bolus injection.

5. Increases in heart rate and systolic blood pressure were related to the severity of hypoglycaemia, but changes in diastolic blood pressure and peripheral vascular resistance (assessed from calf and from hand blood flow) were not.

6. Central temperature fell by 0.13 ± 0.06°C, 0.30 ± 0.10°C, 0.65 ± 0.14°C and 1.15 ± 0.30°C (means ± SEM) in the four experiments, and the fall in skin temperature had a similar gradation.

7. Many physiological responses to hypoglycaemia are not ‘all-or-none’, but vary according to the intensity of stimulus; some are already maximal at mild degrees of hypoglycaemia. Other changes are more complex, reflecting an interplay between opposing endocrine and neural responses.

Key words: counter-regulatory hormones, glucose, hypoglycaemia, insulin, non-esterified fatty acids, peripheral blood flow, temperature.

Introduction

It has traditionally been believed that physiological responses to hypoglycaemia are triggered only when blood glucose falls below a threshold level in the range 2.0–2.5 mmol/l [1]. Recent studies have thrown doubt on the simple concept of a threshold for response. They show that levels of counter-regulatory hormones rise in response to falls in blood glucose within the normoglycaemic range, and that these increases are inversely proportional to the nadir in blood glucose [2–4].
However, there has been no systematic study of patterns of response at differing levels within the hypoglycaemic range. This omission seems strange, since in the absence of such studies no basis for comparison exists between the many experimental designs which have used differing doses of insulin or methods of administration. Further, endocrine, cardiovascular and thermoregulatory responses have usually been examined in isolation, rather than documented in the same experiment.

Our aim has been to describe the hormonal, cardiovascular and thermoregulatory responses to four different levels of insulin-induced hypoglycaemia in a group of healthy individuals.

Methods

Five lean healthy male volunteers aged 26-35 years took part in the study, which was approved by the Medical School Ethical Committee. Hypoglycaemia was induced on four occasions in each subject at intervals of at least 1 week. Insulin (Actrapid, Novo) was given either as a bolus injection of 0.15 unit/kg body weight, or, in random order, as 1 h infusions of 3, 4 or 6 units/h. Human serum albumin was added to the diluent in the latter experiments. The subjects, who had fasted overnight, wore shorts and rested supine on a mesh bed at a controlled room temperature of 30°C for 1 h before the start of the experiment, which lasted 2 h. Two baseline blood samples were taken 15 min apart before insulin was given, with further samples at 10 min intervals during the first hour and at 20 min intervals during the second hour.

All samples were centrifuged immediately at 4°C and plasma samples were deep-frozen. Samples for glucose determination were taken into tubes with fluoride/oxalate, and plasma glucose was measured with an Autoanalyzer (Technicon AA II) calibrated over the range 0-6.6 mmol/l. Blood for non-esterified fatty acid (NEFA) measurement was taken into tubes containing heparin; assay was by an automated method [5]. Cortisol, growth hormone (somatotropin) and prolactin were measured by radioimmunoassay with standard kits. Insulin was assayed by a double antibody method with radioactively labelled pig insulin. The glucagon assay method has been described [6, 7]. Plasma adrenaline and noradrenaline were measured by high-performance liquid chromatography followed by electrochemical detection; details of this assay have been given elsewhere [8].

Heart rate was taken from the electrocardiogram, and blood pressure was measured at 10 min intervals by auscultation of the brachial artery. Hand and calf blood flow were measured by venous-occlusion plethysmography; central body temperature was measured with thermistors introduced into the external auditory meatus and insulated with thick foam pads; skin temperature was taken as the mean reading from eight thermistors taped to the chest, abdomen and thighs [9].

Results

Presentation of results

The data were analysed as group mean results ± SEM at each time point, and as mean maximal responses. In addition, the hormonal responses were calculated as areas under the curves above the baseline. Statistical analysis was by two-way analysis of variance and by Student's paired t-test. Rates of recovery of plasma glucose were analysed by analysis of co-variance. For ease of presentation, results are in all cases given in ascending order of insulin dose (3, 4 and 6 unit infusions, and 0.15 unit/kg body weight bolus). Baseline values did not differ significantly for any of the variables to be described.

![Fig. 1. Effect of insulin administration on plasma insulin levels (logarithmic scale).](image)

Insulin was infused from 0 to 60 min (solid line), or given as a bolus injection at 0 min (dotted line). Values are means ± SEM (n = 5). Each infusion rate produced different steady-state plasma levels of insulin (P < 0.05).
Responses to varying hypoglycaemia

Plasma insulin (Fig. 1)

The total insulin doses given were 3, 4 and 6 and 11.0 (range 9.8-11.6) units respectively. Plateau plasma insulin levels achieved during infusions were 45.2 ± 3.1, 55.0 ± 4.2 and 93.6 ± 3.3 munits/l (means ± SEM). These differences were significant (P < 0.05 for the comparison of the 3 and 4 unit infusion, P < 0.01 for the 4 and 6 unit infusion). Plasma insulin levels fell rapidly at the end of the infusion and were at or below baseline values 20 min later.

With the bolus injection of insulin, the plasma levels reached a peak within the first 10 min and then fell exponentially. As the plasma profile of insulin levels was very different from the three infusions, no statistical comparisons were made.

Plasma glucose (Fig. 2)

Mean baseline values ranged from 4.28 to 4.38 mmol/l; mean nadir values were 2.08 ± 0.10, 1.82 ± 0.21, 1.24 ± 0.08 and 0.92 ± 0.06 mmol/l. These differences were significant (P < 0.05). The rate of fall of plasma glucose was more rapid with the larger doses of insulin, hence nadir values were reached earlier: at 20 min in the bolus experiment, at 40 min with the 6 unit infusion and at 50 min with the 3 unit and 4 unit infusions. The rate of glucose recovery was slower the greater the dose of insulin employed and the deeper the hypoglycaemia (Table 1).

TABLE 1. Rates of recovery of plasma glucose after hypoglycaemia

<table>
<thead>
<tr>
<th>Insulin dose</th>
<th>Rate of recovery of plasma glucose from 60 to 120 min (mmol min⁻¹ l⁻¹)</th>
<th>P (significance of differences from 3 unit infusion)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 units/h</td>
<td>0.045 ± 0.003</td>
<td>N.S.</td>
</tr>
<tr>
<td>4 units/h</td>
<td>0.039 ± 0.004</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>6 units/h</td>
<td>0.036 ± 0.004</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>0.15 unit/kg (bolus)</td>
<td>0.022 ± 0.004</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

Symptoms

All subjects failed to identify the onset of hypoglycaemia during the 3 unit infusion, and three failed to do so during the 4 unit infusion. Sweating was observed to be minimal in those with unawareness of hypoglycaemia. All reported typi-
cal symptoms during the 6 unit infusion and after bolus injection, but the latter was felt to be more unpleasant.

Non-esterified fatty acids (Fig. 3)

Mean baseline values ranged from 0.52 to 0.72 mmol/l; mean nadir values were 0.25 ± 0.03, 0.29 ± 0.02, 0.32 ± 0.05 and 0.35 ± 0.08 mmol/l (not significant). Although recovery of NEFA appeared to be more rapid after the bolus injection, values at 120 min did not differ significantly.

Hormonal responses (Figs. 4 and 5)

Cortisol (Fig. 4). Mean baseline values were 322-347 nmol/l. Maximal responses occurred 20-30 min after the glucose nadir; mean maximal concentrations with the 4 unit and 6 unit infusions and the bolus injection (711 ± 67, 790 ± 48 and 818 ± 73 nmol/l) did not differ significantly but were greater than the response seen with the 3 unit infusion (568 ± 59 nmol/l, \( P < 0.05 \)). When the responses were calculated as areas under the curves, the 6 unit infusion and bolus injection values were similar (28 512 ± 3213, 30 807 ± 5697

![Graphs of Hormonal Responses](image)

Fig. 4. Plasma cortisol, growth hormone, prolactin and glucagon responses to hypoglycaemia induced by insulin administration (●●, 3 units; ○○, 4 units; ■■, 6 units infusions; ▲▲, 0.15 unit/kg body wt., bolus injection). Values are means ± SEM (n = 5). Plasma cortisol rose to an equal extent with the 4 and 6 unit infusions and with the bolus injection. Plasma growth hormone rose equally in all experiments, whereas the prolactin response was proportional to the severity of hypoglycaemia. Plasma glucagon changes were more variable but significant increases were seen with the 6 unit infusion and the bolus injection.
Responses to varying hypoglycaemia

nmol min$^{-1}$ l$^{-1}$) but significantly greater than the values for the other two infusions.

**Growth hormone (Fig. 4).** Mean maximal responses were closely similar, at 20.4 ± 4.6, 23.0 ± 1.4, 22.2 ± 1.0 and 21.6 ± 1.2 munits/l, but one subject showed no response to the 3 unit infusion, although reaching a minimum plasma glucose level of 2.20 mmol/l. When the responses were calculated as areas under the curves, the values for the three infusions were similar but significantly less than for the bolus injection (444 ± 996, 565 ± 79, 619 ± 34, 837 ± 65 munits min$^{-1}$ l$^{-1}$).

**Prolactin (Fig. 4).** Mean baseline values were 131-163 units/l. In contrast to cortisol and growth hormone, mean maximal responses varied with the severity of hypoglycaemia (161 ± 33, 354 ± 107, 996 ± 158 and 1236 ± 190 munits/l). A similar pattern was obtained when the responses were calculated as areas under the curves.

**Glucagon (Fig. 4).** Mean baseline values ranged from 12.4 to 22.2 pmol/l. Mean maximal levels of 30.8 ± 7.9, 39.8 ± 3.5, 39.0 ± 7.8 and 75.3 ± 5.5 pmol/l were reached 20-40 min after the glucose nadir. When the responses were calculated as areas under the curves, there were no significant differences between the 3, 4 and 6 unit infusions. Incomplete data for two subjects during the bolus injection meant that the area under the curve could not be calculated.

**Adrenaline (Fig. 5).** Mean baseline levels were between 0.34 and 0.48 nmol/l. Responses to the 3 and 4 unit infusions did not differ (1.57 ± 0.35 and 1.58 ± 0.45 nmol/l), but peak values during the 6 unit infusion and bolus experiment were significantly greater at 2.77 ± 0.47 and 5.40 ± 0.85 nmol/l ($P < 0.01$). A similar pattern was obtained when the responses were calculated as areas under the curves.

**Noradrenaline (Fig. 5).** Mean baseline values were from 0.72 to 0.83 nmol/l. Plasma noradrenaline rose significantly in all experiments, but the peak levels of 2.35 ± 0.34, 3.16 ± 0.46, 1.91 ± 0.21 and 2.56 ± 0.08 nmol/l did not differ significantly. Similarly, there were no significant differences when the areas under the curves were compared.

**Physical changes**

In the presentation of these results (Figs. 6-8), the maximum or minimum values have been plotted against the nadir values of plasma glucose (a relationship also existed between the rates of fall of plasma glucose and the physical changes, owing to the close association of the former with the nadir glucose values).

**Heart rate and blood pressure (Fig. 6).** Mean baseline heart rates ranged from 60.4 to 63.8 beats/min, with mean maximal increases of 10, 10, 17 and 23 beats/min ($P < 0.05$). Mean baseline systolic blood pressure ranged from 112 to 115 mmHg. Mean maximal increases varied with the severity of hypoglycaemia (11, 13, 20 and 24 mmHg, $P < 0.05$). Diastolic blood pressure fell from mean baseline values of 72-74 mmHg, but nadir values of 63, 67, 63 and 57 mmHg did not differ significantly.

**Hand and calf blood flow (Fig. 7).** Mean baseline hand blood flow ranged from 13.1 to 14.8 ml min$^{-1}$ 100 ml$^{-1}$ of tissue. Hand blood flow fell in all subjects during the 3 unit infusion, and hand vascular resistance increased from 6.5 ± 1.0 to 16.9 ± 3.3 arbitrary units. The lowest hand blood flow in each individual occurred 50-60 min after the infusion was started, and coincided with peak plasma adrenaline levels. The fall in hand blood flow was less marked in the 4 and 6 unit infusions, with increases in mean vascular resistance to 9.4 ± 1.9 and 11.3 ± 1.3 arbitrary units respec-
FIG. 6. Heart rates (●) and systolic (○) and diastolic (□) blood pressures at four levels of hypoglycaemia; values are means ± 1 SEM (n = 5). The value on the right of each plot is the value before insulin administration; the other values are those recorded within 5 min of the glucose nadir.

FIG. 7. Calf and hand blood flows at four levels of hypoglycaemia; values are means ± 1 SEM (n = 5). The value on the right of each plot is the value before insulin administration; the other values are those recorded within 5 min of the glucose nadir.
Growth hormone rose equally in all experiments, suggesting that the weakest hypoglycaemic stimulus had elicited a maximal response. Peak cortisol levels were significantly greater with the 4 unit than with the 3 unit infusion, but showed no further increase with deeper levels of hypoglycaemia. When the cortisol responses were calculated as areas under the curves, the 4 unit infusion was less effective than the 6 unit infusion or bolus injection. We did not measure adrenocorticotropic hormone (corticotropin), but it has been shown that adrenocorticotropic hormone and cortisol levels become dissociated during hypoglycaemia [10], presumably because the maximal secretory capacity of the adrenal cortex has been exceeded. Our results suggest that the standard bolus injection of insulin (0.15 unit/kg body weight) used to assess anterior pituitary function produces peak levels of cortisol and growth hormone no greater than those seen after infusion of 4 units of insulin/h in non-obese subjects, and the symptoms are notably more unpleasant.

In contrast, prolactin release required a more powerful stimulus, as shown by a previous study [11], with no significant response to the 3 unit infusion but large increments with more severe hypoglycaemia. This is the only hormone that we measured which had a clear threshold for response within the hypoglycaemic range. Glucagon levels doubled with the 3 and 4 unit infusions, and quadrupled in the bolus experiment. Adrenaline showed similar but more dramatic increases, rising three- to four-fold with the 3 unit infusion and 11-fold in the bolus experiment. A similar relationship between the depth of hypoglycaemia and the magnitude of the adrenaline response was found by Christensen et al. [12]. However, noradrenaline rose by about three-fold in all experiments, suggesting that, as with growth hormone, the threshold for a maximal response had been exceeded.

Non-esterified fatty acid levels fell to an equal depth in all experiments, but recovery was more rapid with severe hypoglycaemia, consistent with the greater magnitude of adrenaline and glucagon responses. In contrast, the rate of recovery of plasma glucose was slower with deeper hypoglycaemia, and thus appeared to be unrelated to the counter-regulatory response. Since differing methods of insulin administration were used, comparison of plasma insulin levels during the recovery period would be difficult, and potentially misleading because the effects of an infusion or bolus injection of insulin persist long after the plasma levels return to basal [13].

The effects of hypoglycaemia upon systolic and diastolic blood pressure resemble those of adrenaline infusion [14], and are abolished by adrenalectomy [15, 16], adrenal denervation [17] or ganglion blockade [18]. The increase in heart rate, however, is diminished but not abolished by these procedures. In our experiment heart rate increased by 10 beats/min during the 3 unit infusion and by 23 beats/min in response to the bolus injection. Maximum increases coincided with peak adrenaline levels, an observation that conflicts with that of Christensen et al. [12], although we confirmed that the heart rate returned to normal after hypoglycaemia at a time when adrenaline levels were still greatly increased.

Changes in hand blood flow during hypoglycaemia probably represent a balance between α-adrenoceptor-mediated vasoconstriction due to circulating adrenaline, opposed by a release of sympathetic vasoconstrictor tone [15, 17, 19, 20]. Uniform vasoconstriction was observed with the 3 unit infusion; the bolus injection produced initial vasoconstriction in all five subjects, but was rapidly succeeded by marked vasodilatation in three. The effect of hypoglycaemia on subcutaneous blood flow has recently been studied. The reduction of serum glucose to 2.5 mmol/l in normal subjects was associated with a 50% reduction in subcutaneous blood flow [21], a result which is consistent with the effects on hand blood flow of reducing plasma glucose to 2.2 mmol/l in the present study.

Forearm and calf blood flow also increase during hypoglycaemia (the latter was measured in our experiment, since both arms were cannulated). The increase appears to be due to β-adrenoceptor-mediated vasodilatation in muscle, and an atropine-sensitive vasodilator pathway to skin. These effects over-ride α-adrenoceptor-mediated vasoconstriction due to circulating adrenaline [8, 19, 20]. Changes in both calf and hand vascular resistance and diastolic blood pressure did not show a graded response to hypoglycaemia of increasing severity.

Hypothermia has been recognized as a complication of hypoglycaemia for many years [22]. We have shown that central temperature falls despite an increase in resting heat production [9]. This increase can be blocked with propranolol, and is thus likely to be a thermogenic response to high circulating catecholamine levels [8]. The net fall in temperature results from rapid heat loss at the body surface due to peripheral vasodilatation and sweating.

The present experiments were performed in a thermoneutral environment (30°C). Skin temperature fell in all experiments as sweating developed. The mean fall in central temperature ranged from 0.13°C with the 3 unit infusion to 1.15°C in the bolus experiment. This fall in central temperature was proportional to the fall in skin temperature,
Responses to varying hypoglycaemia

We have examined a number of hormonal, cardiovascular and thermal responses to different degrees of insulin-induced hypoglycaemia. Three rates of insulin infusion were used, as well as the conventional 0.15 unit/kg body weight bolus injection of insulin. This approach allowed the responses to four different levels of biochemical hypoglycaemia to be compared. Since the rate and magnitude of the fall in plasma glucose were closely related, we cannot judge the relative importance of these two possible stimuli.

It has long been believed that hormonal responses are triggered only as blood glucose falls below a threshold value of 2.0-2.5 mmol/l [1]. This concept has been challenged by recent studies which have shown that levels of growth hormone, cortisol, glucagon, adrenaline and noradrenaline all rise when blood glucose is lowered rapidly, but within the physiological range, by infusion of insulin [2, 3]. Significant increases in levels of all these hormones were found when blood glucose was lowered, more slowly, to mean levels of 3.5 and 3.0 mmol/l by infusion rates of 0.025 and 0.05 unit of insulin h⁻¹ kg⁻¹ [4]. These studies suggest that there is no absolute glucose threshold for hormonal responses, but rather a progressive release of regulatory hormones as blood glucose falls below fasting levels in response to exogenous insulin.

Our study has extended these observations by examining patterns of hormonal response at differing blood glucose levels within the hypoglycaemic range. Hormonal responses were calculated in terms of both peak levels and areas under the curves, but care must be taken in interpreting the latter because of the varied time courses of the responses.
Responses to varying hypoglycaemia

and both changes were related to the severity of hypoglycaemia. The greatest falls in central temperature occurred in those in whom sweating was combined with cutaneous vasodilatation. Thus, in the bolus experiment, central temperature fell by 0.32°C and 0.64°C in the two subjects with persistent vasoconstriction, and by 1.10°C, 1.68°C and 1.92°C in those with vasodilatation. This implies that cutaneous vasodilatation plays an important role in the hypothermia of hypoglycaemia.

In conclusion, we have shown that some endocrine, cardiovascular and thermoregulatory changes observed during insulin-induced hypoglycaemia are in fact already maximal as plasma glucose falls to around 2 mmol/l. The growth hormone response is an example of this pattern. Other responses, for example the rise of plasma adrenaline, increase in direct proportion to the severity of hypoglycaemia. Yet other variables, such as hand blood flow, show a more complex pattern of change, reflecting an interplay between opposing endocrine and neural responses. Thus the concept of a hypoglycaemic threshold, which, once reached, results in a unified set of responses, appears to be invalid. In contrast, each element of the response may have its own threshold, and many responses appear to be activated at plasma glucose levels above 2.5 mmol/l.

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References