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The Regulation of Glucose-Induced Insulin Secretion by Pre-Stimulus Glucose Level and Tolbutamide in Normal Man

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Summary. The relationship between the pre-stimulus glucose level and immunoreactive insulin responses to a glucose challenge (20-g IV) was studied in normal subjects. When the steady-state pre-stimulus glucose concentration was lowered by a 0.33 mU · $kg^{-1} \cdot min^{-1}$ insulin infusion or raised by a 900 mg/min glucose infusion, no effect on first phase insulin secretion (mean Δ 3–5 min insulin level) was observed. In contrast, the second phase response (10–60 min insulin area after glucose pulse) to intravenous glucose fell during insulin infusion and increased during the glucose infusion. Overall, a linear relationship was found between the change of pre-stimulus glucose level from the control to that during the insulin or glucose infusion and the change in second phase response ($r = 0.65$, $n = 14$, $p < 0.02$). The effect of tolbutamide infusion ($7\ mg \cdot m^{-2} \cdot min^{-1}$) when compared with saline control was to increase both first phase ($+54 \pm 13$ mU/l, $n = 8$, $p < 0.001$, mean ± SEM) and second phase ($+ 972 \pm 256\ mU \cdot min^{-1} \cdot l^{-1}$, $p < 0.01$) insulin secretion. It is concluded that the first phase response to a glucose pulse is independent of the steady-state pre-stimulus glucose concentration and is directly enhanced by tolbutamide; in contrast, second phase is related to both the steady-state pre-stimulus glucose level and tolbutamide. These findings suggest that changes in basal or pre-stimulus plasma glucose during therapy with sulphonylurea drugs may be expected to influence the second phase insulin responses to glucose challenge.

Key words: Intravenous glucose tolerance test, first phase insulin secretion, second phase insulin secretion, glucose potentiation, tolbutamide

The pre-stimulus glucose level is an important determinant of insulin responses to non-glucose stimuli in man [1, 2]; that is, the lower the pre-stimulus glucose level the smaller the insulin response and the higher the level, the greater the response. The present study was designed to determine the relationship between insulin responses to a glucose stimulus and the pre-stimulus glucose level, and to determine whether such a relationship would be important to an analysis of the effect of tolbutamide on glucose-induced insulin release.

Subjects and Methods

Subjects

Fifteen lean male volunteers (percentage ideal body weight = 109 ± 4; mean ± SEM) were studied. Their average age was 31 years (range 21–47 years). None of the subjects was using any medications at the time of study, and none had a history of cardiovascular disease. All subjects had fasting plasma glucose levels of less than 5.83 mmol/l (5.38 ± 0.06 mmol/l) and had no family history of diabetes mellitus. Studies were performed after an overnight fast, and no cigarette smoking was allowed on the days of the studies. Aspirin and aspirin-containing products were not allowed for a period of at least one week before the studies. All subjects were eating ad lib diets prior to the studies. Informed consent was obtained from all subjects.

Study Protocols

All studies were performed in a metabolic ward. A 19-gauge butterfly needle was introduced into each antecubital vein and was kept patent by a slow infusion of 0.154 mol/l sodium chloride. Venous samples were obtained from one IV line for laboratory analyses. The other IV line was used for administration of the drugs during the study. Five-ml samples for measurement of plasma immunoreactive insulin (IRI) and glucose were anticoagulated with EDTA, kept on ice until the plasma was separated by centrifugation, and subsequently frozen for analysis at a later time. Samples of blood (2.5 ml) for measurements of plasma nor-

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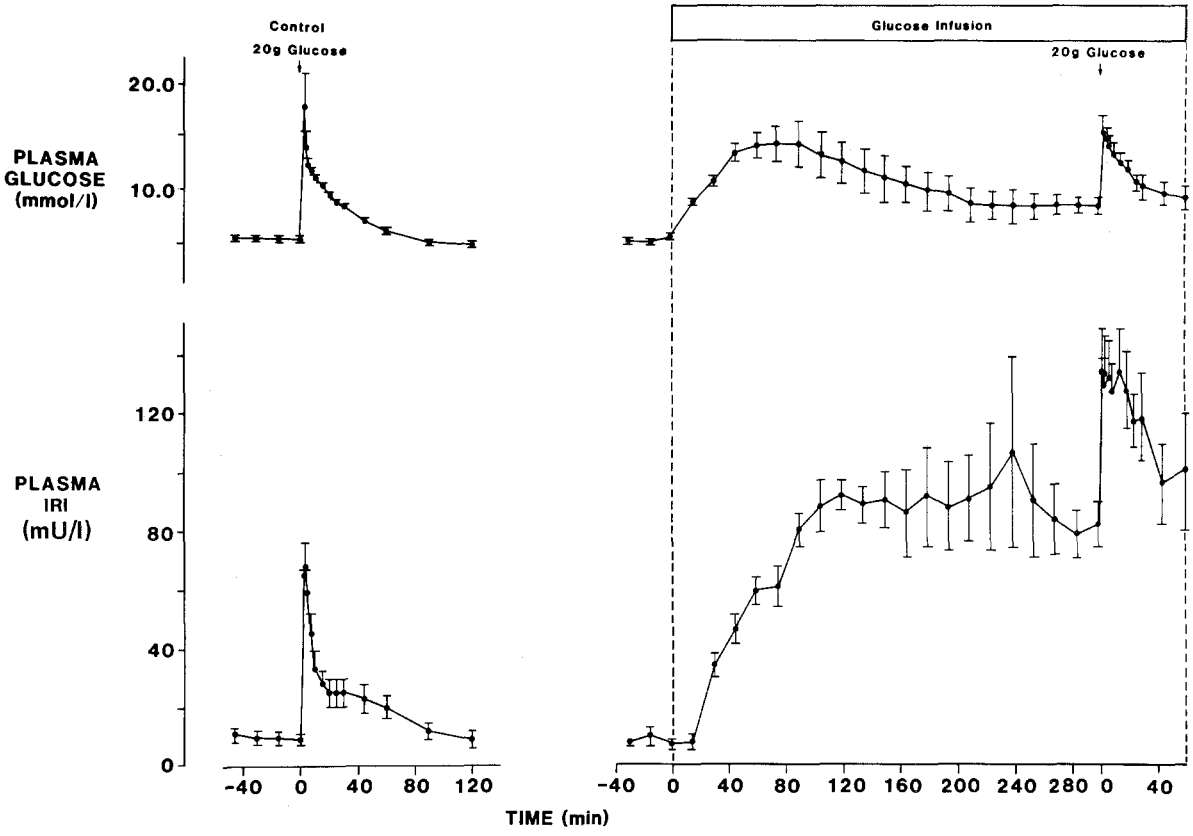

Fig. 1. Effect of an increased pre-stimulus plasma glucose concentration on insulin release to a 20 g IV glucose challenge. Seven normal subjects had the pre-stimulus glucose level elevated and maintained at a new steady-state level by a 5 h infusion of glucose (900 mg/min). The left panel demonstrates a control day for the same subjects. All values are mean ± SEM of seven subjects

adrenaline and adrenaline were collected in pre-chilled tubes containing ethyleneglycoltetraacetic acid (EGTA) for anticoagulation and gluthathione to prevent oxidation. Final blood concentration of EGTA and gluthathione was 4.7 and 3.9 mmol/l, respectively. These samples were kept on ice until the plasma was separated by double centrifugation (within 30 min), and subsequently frozen for analysis at a later time.

In all studies, a 20-g glucose pulse was given rapidly (less than 10 s) intravenously. This dose has been previously determined to be a near maximal stimulus for first phase insulin secretion in normal man [3]. The first phase insulin response to glucose was calculated as the mean increase above the pre-stimulus plasma insulin level at 3, 4, and 5 min after the glucose pulse. The second phase insulin response to glucose was calculated as the area above the pre-stimulus baseline level for the tenth through sixtieth minute period following the glucose pulse. The glucose disappearance rate (K_G) after the glucose pulse is the negative slope of the linear relationship between the natural log of glucose level versus time during the tenth through thirtieth min period following the pulse.

Samples for measurement of plasma catecholamine levels were obtained at 40 and 45 min after placing of the IV lines in the saline control, tolbutamide, and insulin infusion studies. The mean of these two measurements was used as the baseline level and compared with the mean of two samples obtained 5 min before and immediately preceding the administration of each glucose pulse. Catecholamine samples were also obtained every 30 min during the insulin infusion study (described below) to assure that no hypoglycaemia-induced adrenergic response occurred during this period. Serum tolbutamide concentrations were determined after one hour of the tolbutamide infusion (immediately preceding a glucose pulse).

In seven subjects, a 30-min basal period was followed by a 6-h, 900 mg/min glucose infusion. This 6-h infusion was to allow a new steady-state for insulin and glucose to be achieved for at least 90 min before the glucose pulse. The glucose pulse was administered at the initiation of the sixth hour of the concomitant glucose infusion. These results were compared with studies performed on a separate day in the same subjects when the glucose pulse was administered directly after a 30-min basal period.

In seven subjects, a 45-min basal period was followed by a 1 h infusion ($0.33\ mU \cdot kg^{-1} \cdot min^{-1}$) of regular pork-beef insulin (Lilly, Indianapolis, Indiana, USA). Protein was not added to the infusion. The infusion was then discontinued, and a 30-min recovery period was allowed for insulin levels to return to basal values before the administration of the glucose pulse. Samples for measurement of insulin and glucose were obtained during the subsequent 2 h. The results were compared with a similar study done on a separate day when saline was substituted for the insulin infusion.

To determine the relationship between the fall of plasma glucose during a tolbutamide infusion and the amount of the agent infused, eight normal subjects received different infusion rates of tolbutamide for 1 h on separate days. Plasma glucose levels were determined during these infusions and compared with a 45-min baseline period.

Eight subjects were infused with tolbutamide at a dose of $7\ mg \cdot m^{-2} \cdot min^{-1}$ for 2 h. One hour after starting the tolbutamide infusion, a glucose pulse was given.

In six subjects, a variable rate of glucose infusion was administered during the tolbutamide infusion of $7\ mg \cdot m^{-2} \cdot min^{-1}$ in

Table 1. Glucose and insulin responses to 20 g glucose stimulus under various conditions

	Basal Period				Pre-stimulus period						
	Glucose (mmol/l)	IRI (mU/l)	Noradrenaline (nmol/l)	Adrenaline (nmol/l)	Glucose (mmol/l)	IRI (mU/l)	Noradrenaline (nmol/l)	Adrenaline (nmol/l)	First phase[a] (mU/l)	Second phase[b] mU · min l	K_G (%/min)
Glucose infusion ($n = 7$)											
Control	5.27±0.11	10±2	–	–	5.27±0.11	10±2	–	–	54±7	725±140	1.60±0.28
Glucose infusion (900 mg/min)	5.05±0.11	8±2	–	–	8.60±0.89	80±9	–	–	52±8	1624±320	1.31±0.18
$p<$(glucose infusion versus control)	NS	NS	–	–	0.01	0.001	–	–	NS	0.05	NS
Insulin infusion ($n = 7$)											
Control	5.44±0.11	11±2	1.36±0.22	0.22±0.04	5.33±0.17	8±2	1.52±0.26	0.24±0.05	56±9	663±91	1.90±0.35
Insulin infusion ($0.33\ mU \cdot kg^{-1} \cdot min^{-1}$)	5.44±0.17	10±2	1.61±0.16	0.31±0.09	4.88±0.22	11±2	1.73±0.20	0.23±0.02	60±11	470±148	1.85±0.20
$p<$(insulin infusion versus control)	NS	NS	NS	NS	0.02	NS	NS	NS	NS	0.05	NS
Tolbutamide alone infusion ($n = 8$)											
Control	5.50±0.11	12±2	1.30±0.21	0.22±0.04	5.38±0.11	10±2	1.47±0.23	0.24±0.04	84±32	797±156	2.03±0.33
Tolbutamide infusion ($7\ mg \cdot m^{-2} \cdot min^{-1}$)	5.50±0.11	13±3	1.64±0.16	0.26±0.04	3.94±0.17	22±5	1.74±0.31	0.29±0.08	138±41	1769±371	2.98±0.33
$p<$(tolbutamide infusion versus control)	NS	NS	NS	NS	0.001	0.02	NS	NS	0.001	0.01	0.05
Tolbutamide + glucose infusion ($n = 6$)											
Control	5.50±0.17	10±2	1.41±0.26	0.23±0.05	5.44±0.11	9±2	1.55±0.30	0.26±0.05	61±8	–	2.07±0.33
Tolbutamide infusion alone	5.50±0.17	11±3	1.79±0.17	0.28±0.05	3.89±0.17	18±4	1.86±0.41	0.38±0.14	102±10	–	2.88±0.20
Tolbutamide + glucose infusion	5.33±0.17	11±2	1.72±0.30	0.25±0.07	5.27±0.17	44±6	1.77±0.48	0.29±0.08	133±24	–	3.97±0.70
$p<$(tolbutamide + glucose versus control)	NS	NS	NS	NS	NS	0.001	NS	NS	0.02	–	0.025
$p<$(tolbutamide + glucose versus Tolbutamide infusion)	NS	NS	NS	NS	0.005	0.01	NS	NS	NS	–	NS

All values represent mean ± SEM

[a] First phase = (mean 3, 4 and 5 min IRI value above pre-stimulus IRI) – (pre-stimulus IRI)

[b] Second phase = area above pre-stimulus IRI for the 10–60 min period after the 20 g glucose pulse

IRI = immunoreactive insulin; NS = non-significant ($p > 0.05$)

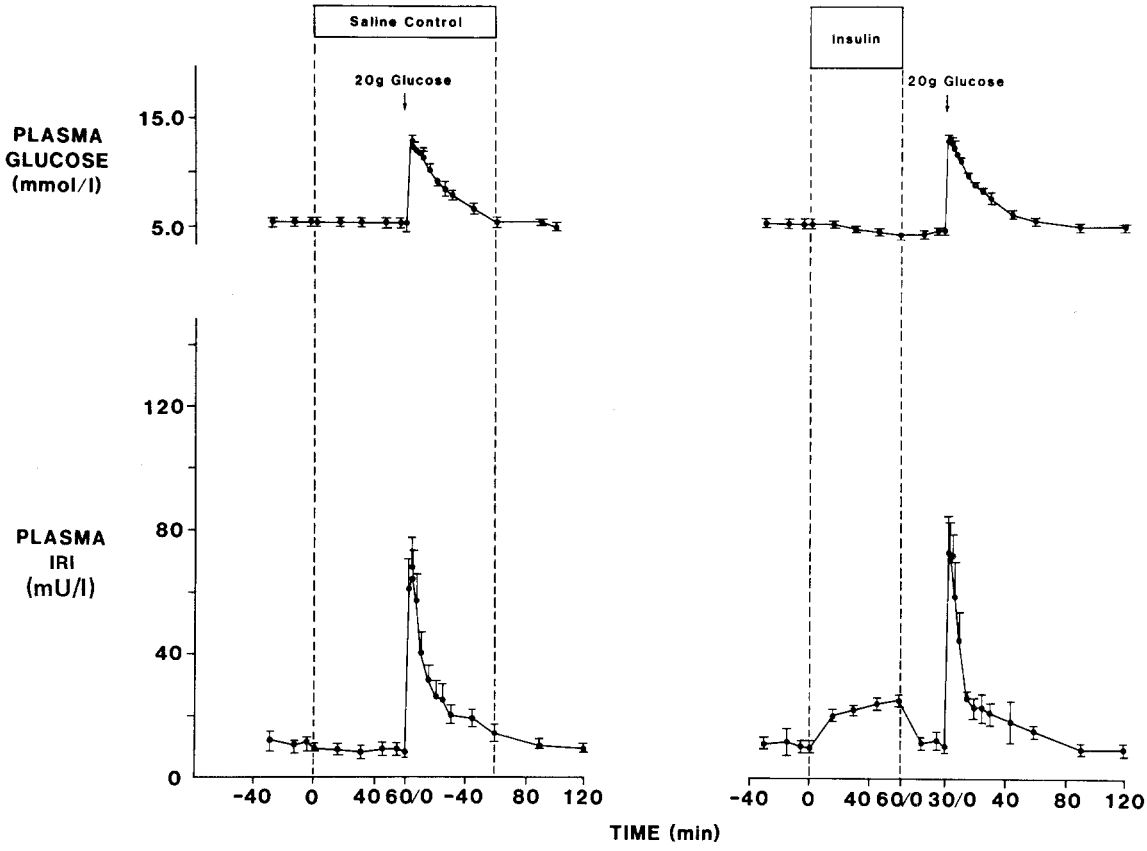

Fig. 2. Effect of a decreased pre-stimulus plasma glucose concentration on insulin release to a 20 g IV glucose challenge. Pre-stimulus plasma glucose concentration was lowered by insulin (0.33 mU · kg^{-1} · min^{-1}) in seven subjects. The left panel shows a saline control for the subjects performed on a different day. All values are mean ± SEM of seven subjects

order to prevent a change in plasma glucose level. The glucose infusion rate was varied every 5 min based on a bedside measurement of plasma glucose using a Beckman glucose analyzer. The variable glucose infusion was begun at a rate of 200 mg/min, 10 min after beginning the tolbutamide infusion. The rate at which the glucose was infused was determined as previously described [2]. The glucose infusion rate at the time the glucose pulse was administered was maintained until the study was terminated (30 min after the glucose pulse).

Analytical Methods

IRI levels were measured by a modification of the double-antibody method of Morgan and Lazarow [4]. Plasma glucose was measured with the autoanalyzer ferricyanide method (Technicon Instruments, Tarrytown, New York). Bedside plasma glucose levels were determined by a glucose oxidase method (Beckman Instruments, Fullerton, California), but these measurements were not used for data analysis. Plasma noradrenaline and adrenaline levels were measured by a single isotope enzymatic assay [5]. The sensitivity of plasma catecholamine assay is 0.11 nmol/l and coefficient of variation is 12% for both noradrenaline and adrenaline measurements. Serum tolbutamide concentrations were determined 1 h after the initiation of the tolbutamide infusion in all subjects. These measurements were performed by the Upjohn Company by a gas-liquid chromatographic method [6]. The sensitivity of the tolbutamide assay is 3.70 μmol/l and the coefficient of variation is 6.4%. Statistical analyses included paired Student's t-test and co-variance analysis with regression.

Results

The Relationship Between Pre-stimulus Glucose Level and Insulin Responses to IV Glucose

As shown in Figure 1, during the first 5 h of the glucose infusion there was a rise in glucose levels from basal glucose, reaching a zenith at 90 min (Δ glucose: +9.05 ± 1.94 mmol/l, $n = 7$, $p < 0.005$). This was followed by a decline to a plateau at 225 min which was still elevated above the basal level (Δ glucose: +3.61 ± 1.44 mmol/l, $n = 7$, $p < 0.05$; Table 1). This new steady-state glucose level was maintained during the subsequent 135 min until the time of the glucose pulse. Insulin levels reached a plateau by 120 min after the basal period (Δ IRI at 120 min: +84 ± 5 mU/l, $n = 7$, $p < 0.001$) and remained at this level until the glucose pulse. After 6 h, there was a significant difference in steady-state insulin (Δ IRI: +70 ± 7 mU/l, $n = 7$, $p < 0.001$) and glucose levels (Δ glucose: +3.33 ± 0.89 mmol/l, $n = 7$, $p < 0.01$) when compared with the control studies performed on a separate day (Table 1). First phase insulin responses to the 20 g IV glucose challenge were not

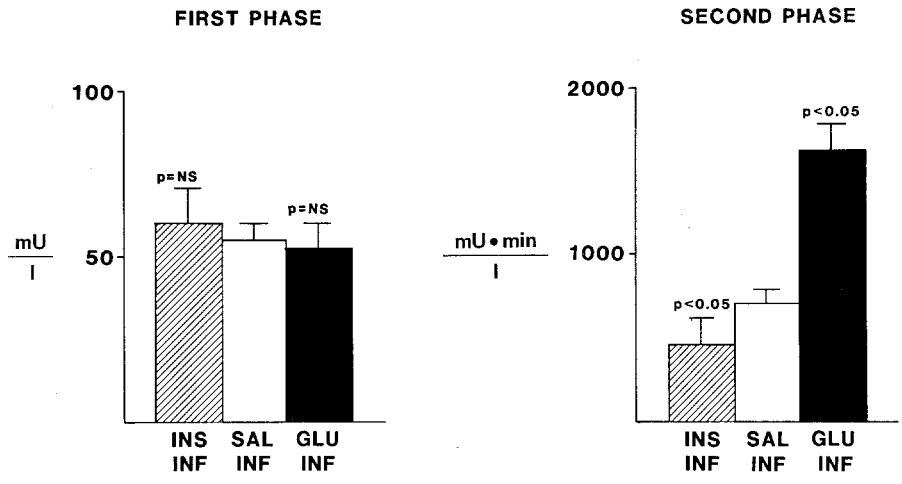

Fig. 3. A summary of the effects of the varying pre-stimulus plasma glucose level on insulin responses to IV glucose. First phase is calculated by the mean change in the 3–5 min insulin value above the pre-stimulus value. Second phase is calculated as the 10–60 min insulin area above the pre-stimulus value. All values are mean ± SEM. ▨ INS INF = insulin infusion protocol; □ SAL INF = saline infusion controls; ■ GLU INF = glucose infusion protocol

significantly different compared to control studies (Δ first phase: -2 ± 8 mU/l, p = NS; Fig. 3). However, second phase was increased during this study (Δ second phase: $+889 \pm 333$ mU·min^{-1} l^{-1}, $p < 0.05$; Fig. 3). Glucose disappearance rate was not changed when compared to the control studies (Δ K_G: $+0.29 \pm 0.27$, p = NS; Table 1).

When plasma glucose levels were decreased by insulin infusion (Fig. 2), there was a significant decrease in the second phase insulin response to the 20 g IV glucose stimulus when compared with the response of the same subjects on a control day (Δ second phase: -193 ± 84 mU min^{-1} l^{-1}, $n = 7$, $p < 0.05$; Fig. 3). However, there was no change in the first phase response (Δ first phase: -5 ± 9 mU/l, $n = 7$, p = NS; Fig. 3). When compared with the control studies carried out on a separate day, there was a significant decrease in pre-stimulus glucose (Δ glucose: -0.44 ± 0.11 mmol/l, $n = 7$, $p < 0.02$) but no significant difference in pre-stimulus insulin (Δ IRI: $+3 \pm 1$ mU/l, $n = 7$, p = NS). Glucose disappearance rate after the glucose pulse was not significantly affected by the insulin infusion (Δ K_G: -0.05 ± 0.24, $n = 7$, p = NS). During and after the insulin infusion, there were no increases in plasma catecholamines compared with the basal period.

Relationship Between Change of Pre-stimulus Glucose Level and Second Phase Insulin Response to IV Glucose: There was a significant linear correlation (Fig. 4) between the change of pre-stimulus glucose from the saline control and the change in second phase insulin secretion from control during either an insulin or glucose infusion ($r = 0.65$, $n = 14$, $p < 0.02$).

Effect of Tolbutamide Studies

Figure 5 depicts the relationship between the dose of tolbutamide infused and the decrease of plasma glucose level from basal glucose levels 60 min after the start of the infusion in eight normal subjects. A dose of 7 mg·m^{-2}·min^{-1} was chosen for the subsequent tolbutamide infusion studies because it caused a modest decline in plasma glucose levels (Δ glucose: -1.61 ± 0.17 mmol/l, $n = 8$, $p < 0.001$) without an increase in plasma catecholamines (Δ noradrenaline: $+0.09 \pm 0.24$ nmol/l, $n = 8$, p = NS; Δ adrenaline: $+0.09 \pm 0.10$ nmol/l, $n = 8$, p = NS).

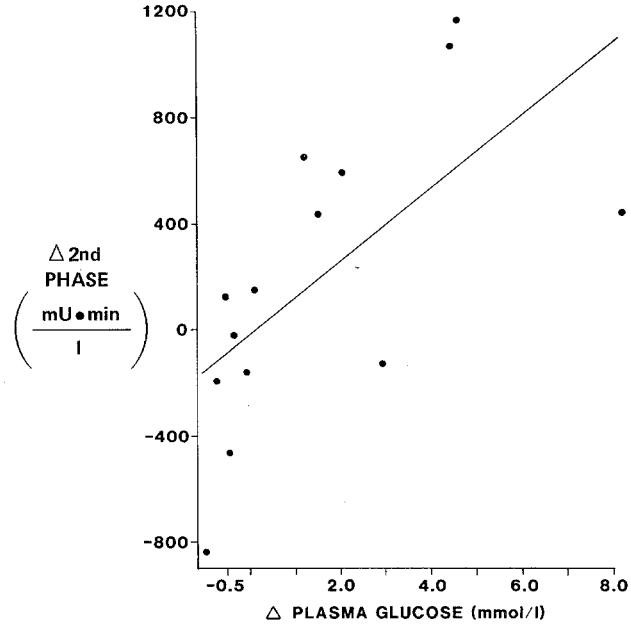

Fig. 4. Relationship of the change in plasma glucose concentration to the change of second phase insulin release in normal man. The change in plasma glucose concentration was calculated as the pre-stimulus plasma glucose level during either the insulin infusion or glucose infusion protocol minus the pre-stimulus plasma glucose during saline control day. The change of the second phase insulin response was calculated in a similar manner. Second phase = 10–60 min IRI area after glucose pulse. There was a significant linear relationship ($y = 8.03x - 2.15$, $r = 0.65$, $p < 0.02$)

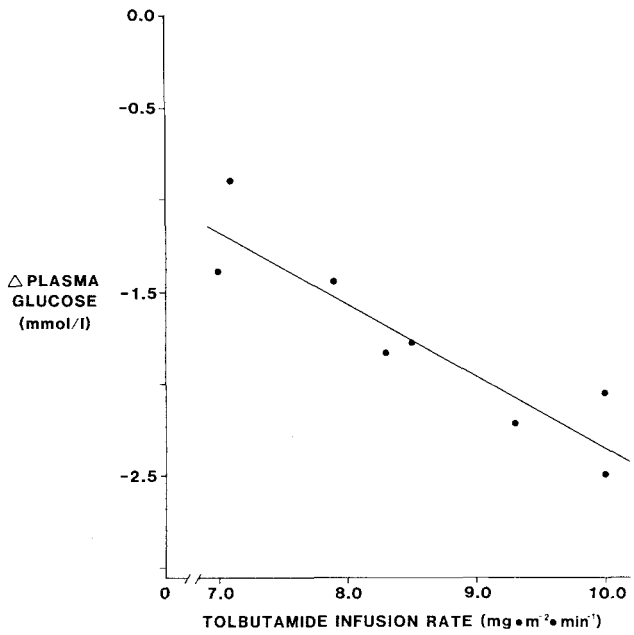

Fig. 5. The relationship between infusion rates of tolbutamide and changes of plasma glucose from basal levels. The change in plasma glucose was determined by subtracting the basal glucose level from that after a 60-min infusion of tolbutamide. There was a significant linear relationship ($y = 7.15x + 29.00$, $r = 0.93$, $p < 0.001$)

A 20-g glucose pulse was given 1 h after the initiation of a 7 $mg \cdot m^{-2} \cdot min^{-1}$ tolbutamide infusion in eight subjects (Fig. 6). A presistent increase in plasma insulin from basal levels was achieved by 30 min (Δ IRI at 30 min: $+13 \pm 4$ mU/l, $p < 0.01$). This was associated with a significant decrease in glucose levels during the first hour of the tolbutamide infusion (Δ glucose: -1.67 ± 0.17 mmol/l, $p < 0.001$). When compared to saline control studies (Table 1), there was a significant increase in both the first phase (Δ 1st phase: $+54 \pm 13$ mU/l, $p < 0.001$) and second phase (Δ 2nd phase: $+972 \pm 256$ $mU \cdot min^{-1} \cdot l^{-1}$, $p < 0.01$) responses to IV glucose. In addition, an increase in K_G was observed (Δ K_G: $+0.95 \pm 0.38$, $p < 0.05$). Table 1 demonstrates that there was no elevation of plasma catecholamines from basal levels (Δ noradrenaline: $+0.11 \pm 0.25$ nmol/l, p = NS; Δ adrenaline: $+0.09 \pm 0.09$ nmol/l, p = NS) during this modest fall of plasma glucose.

When the fall of plasma glucose during tolbutamide was prevented by a concomitant variable rate glucose infusion, there was no significant change in the first phase insulin response to glucose or the K_G when compared to the study of tolbutamide alone ($+31 \pm 16$ mU/l, $n = 6$, p = NS; Δ K_G: $+1.08 \pm 0.70$, $n = 6$, p = NS; Table 1). The mean increments observed were all due to one subject who had a marked increase in first phase response to glucose. However, there was little or no change in the first phase response in the other five subjects. Serum tolbutamide levels were comparable in these studies to those performed without the glucose infusion (tolbutamide infusion alone: 292.58 ± 31.44 μmol/l, $n = 6$; tolbutamide plus glucose infusion: 291.10 ± 11.10 μmol/l, $n = 6$, p = NS).

When the tolbutamide plus glucose infusion protocol was compared to saline control studies, both first phase (Δ first phase: $+72 \pm 20$ mU/l, $p < 0.02$) and K_G (Δ K_G: $+1.90 \pm 0.59$, $p < 0.025$) were increased. When this study was allowed to proceed for 60 min rather than 30 min, there was such a marked increase in insulin levels that glucose levels fell below 1.67 mmol/l. Second phase insulin release could not be evaluated with this protocol because of the counter regulatory adrenergic responses that occurred.

Discussion

We have found that the first phase insulin response to a 20 g glucose challenge is not dependent upon the pre-stimulus glucose level. However, the second phase insulin response is dependent upon the level of glucose before the challenge, in that the higher the pre-stimulus glucose level the greater the second phase response and the lower the pre-stimulus glucose the smaller the second phase. This is consistent with a previous study [7], in which the first phase response to a 5 g glucose pulse was unchanged during a 20 h glucose infusion. In apparent contrast, some studies found a decrease in first phase insulin response during a short glucose infusion [7] or in first phase after stopping a glucose infusion [8–10]. The differences between these studies and our study is the lack of a steady-state in the previous studies. It appears that first phase insulin secretion is pre-stimulus glucose level independent only when a steady-state is achieved.

In contrast, we have found that second phase insulin response to a 20-g glucose challenge is pre-stimulus glucose level dependent. Thus, the change of second phase responses to varying pre-stimulus glucose are similar to the changes seen with non-glucose stimuli [1, 2]. Therefore, any evaluation in second phase response to glucose would necessitate consideration of the pre-stimulus glucose level.

The infusion of tolbutamide was associated with an increase of *both* first and second phase insulin responses to a 20-g glucose pulse in normal subjects. These effects occurred in spite of a decrease in pre-stimulus glucose level. When the plasma glucose level was held constant during the tolbutamide infusion by a variable glucose infusion, the first phase insulin

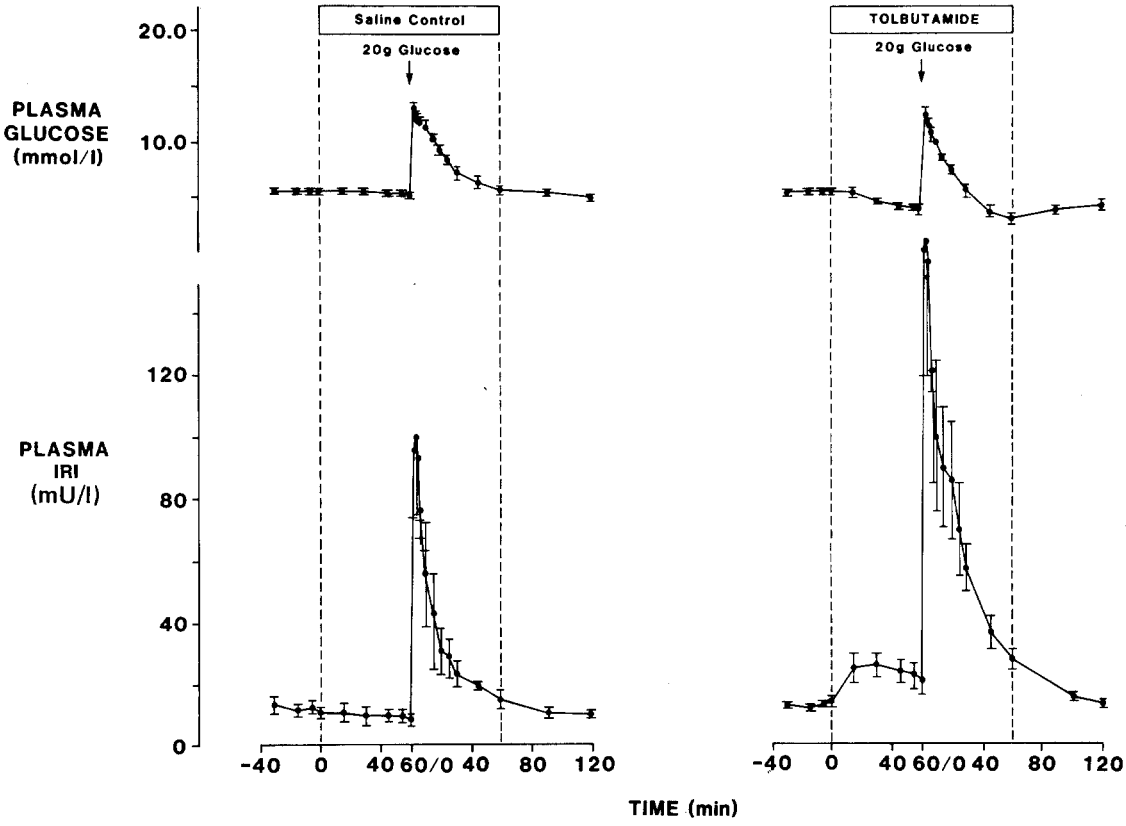

Fig. 6. The effect of a tolbutamide infusion (7 mg · m^{-2} · min^{-1}) on the insulin response to a 20-g glucose induced insulin secretion. The control (saline) day is included in the left panel for comparison. All values represent a mean ± SEM of eight normal subjects

response was the same as that during tolbutamide alone (Table 1). Thus, enhancement of first phase insulin secretion during tolbutamide is due to the drug and is not related to the decrease in glucose level. This lack of effect of pre-stimulus glucose level on first phase insulin secretion during a tolbutamide infusion is consistent with our findings that first phase insulin secretion is not dependent upon the pre-stimulus plasma glucose levels in the absence of tolbutamide.

The second phase insulin response was also increased by tolbutamide in spite of the decrease in plasma glucose level during the drug infusion (Fig. 6). During the tolbutamide plus variable glucose infusion, there was such a greatly increased insulin response during the initial portion of the second phase time period that hypoglycaemic glucose levels were reached and a counter-regulatory adrenergic response occurred in the latter portion of the second phase time period. Thus, the studies were terminated by stopping the infusions and giving subjects additional glucose before the entire second phase period had been completed. We would speculate that if these subjects had not become hypoglycaemic and the second phase could have been completed, there would have been an even larger increase in second phase. This would have been consistent with our previous findings of glucose level dependency of second phase insulin responses.

The physiological significance of the change in first phase responses in these studies is indicated by K_G. When the first phase was augmented (the tolbutamide alone and the tolbutamide plus glucose protocols), K_G was increased. In contrast, K_G did not change in studies in which first phase was not changed (insulin and glucose infusions). These results are consistent with the previously observed relationship of K_G to first phase response [11].

These studies of the control of first and second phase insulin secretion by pre-stimulus glucose level may help clarify some questions raised by studies of the chronic effects of sulphonylurea therapy. Hecht et al. [12] observed an increase in first phase insulin secretion to glucose challenge during the course of treatment of diabetics with chlorpropamide. However, second phase insulin secretion was not statistically different from untreated levels. These changes were consistent after one week, one month, and three months of therapy. However, the fasting glucose levels were already decreased at one week and were essentially unchanged thereafter. Our data suggest that this unchanged second phase response in the

presence of a lower pre-stimulus glucose is still compatible with an islet potentiating action of the drug. This concept was also suggested by Johansen and Ørskov [13] when they observed a decrease in urinary insulin excretion 2–3 days after stopping maintenance sulphonylurea therapy implying that some chronic insulinotrophic effect had been removed.

In summary, the present study shows that second phase, but not first phase, insulin secretion during an IV glucose tolerance test is dependent on the pre-stimulus glucose level in normal man and that tolbutamide increases both first and second phase insulin secretion. These findings may explain some of the apparent discrepancies concerning the ability of the sulphonylureas to influence insulin secretion in diabetic patients. It seems likely that sulphonylurea therapy always enhances insulin secretion but that the effect on second phase insulin secretion may be obscured by a fall in plasma glucose. Since there is a slow fall of plasma glucose after treatment with an oral agent, we hypothesize that studies performed during the early phase of treatment or which specifically test first phase insulin release to glucose will show an increase of insulin release [12, 14]. In contrast, studies which are performed later after a new lower steady-state glucose level has been achieved and which examine oral glucose tolerance or second phase insulin secretion, would find no apparent change in insulin secretion [15–19]. We suggest that these apparent discrepancies can be resolved by recognising that the pre-stimulus glucose level is an important modulator of second phase insulin responses to glucose.

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