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Plasma Insulin Patterns in the Unanesthetized Rat During Intracardial Infusion and Spontaneous Ingestion of Graded Loads of Glucose

J. H. Strubbe and P. R. Bouman

Rats were provided with double permanent heart catheters, allowing simultaneous infusion and rapid blood sampling in the freely moving animals. Intracardial glucose infusion (75, 150, and 300 mg) over 15-min periods induced biphasic plasma insulin responses, their onset and magnitude being correlated with the blood glucose increments. The insulinogenic index decreased at increasing infused loads. After spontaneous oral ingestion of 75, 150, 300, and 750 mg glucose over 2 min or less, plasma insulin increased rapidly during the initial 4 or 8 min. At the highest loads this was followed by a gradual further increase until 15 min. The rapid insulin response increased with the ingested load. About half

of this response had occurred already at 2 min, i.e., prior to the first rise of blood glucose at 3 min. Maximum blood glucose levels (125–135 mg/dl) occurred between 8 and 15 min and did not correlate with the ingested loads. The insulinogenic index increased at higher oral loads. It is suggested that the plasma insulin response to glucose ingestion results from successive and cumulative operation of anticipatory nervously triggered insulin secretion, anticipatory load-dependent potentiation of secretory stimulation by rising blood glucose, and further adjustment of the secretion rate until blood glucose declines. The possible mechanisms are discussed.

STUDIES IN MAN have shown that the pattern and magnitude of glucose-induced insulin secretion depends on the route of glucose administration. Intravenous infusion of glucose elicits a rapid rise of plasma insulin, which may be followed by a slow secondary rise. The magnitude of the early response is closely related to the infused load and the degree and rate of blood glucose elevation.¹⁻⁴ Oral administration of glucose likewise elicits a load-dependent increase of plasma insulin,⁵⁻⁸ but this response develops more gradually and the relations with blood glucose levels appear to be different. Whereas some authors observed a quantitative relationship between the plasma insulin response and blood glucose increment after graded oral loads of glucose,^{6,8} others did not find such a relationship.^{5,7} It is beyond dispute, however, that in terms of blood glucose elevation, oral glucose loading is more effective in stimulating insulin secretion than intravenous glucose administration.^{9,10} This phenomenon is generally attributed to the release of an insulinogenic humoral factor from the gastrointestinal tract.¹⁰⁻¹³

Isolated perfused rat pancreas and perfused rat pancreatic islets have been used for the study of the time and dose kinetics of glucose-induced insulin secretion in the rat.¹⁴⁻¹⁷ However, no data are available on the kinetics of the load-

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dependent plasma insulin response in the intact rat under physiologic conditions. The present study was aimed toward investigating these aspects in the conscious, freely moving rat during spontaneous ingestion of graded loads of glucose. The effect of intracardial infusion of similar loads of glucose was also investigated.

MATERIALS AND METHODS

Animals

Male Wistar rats of about 400 g body weight were kept solitary in Plexiglass cages at a room temperature of about 20°C. Light was on from 6 a.m. to 6 p.m. Food and water were supplied ad libitum. The diet consisted of 20% protein, 53.5% carbohydrate, 4.5% fats, and 22% water and contained appropriate amounts of minerals and vitamins.

Surgery and Blood Sampling

The rats were provided with a double permanent heart catheter in the right atrium, as described by Steffens,¹⁸ using a small swivel joint in the connecting tubes.¹⁹ These catheters allow blood sampling as well as intracardial infusion at the same time. The tip of the infusion catheter extends 3 mm farther downstream into the heart than that of the sample catheter in order to prevent contamination of blood samples with infusion fluid.

Canulated rats were not used for experimentation until they had become adapted to the sampling procedure. Usually, this took about one week.

Blood was sampled in heparinized tubes in volumes of 0.2 ml/sample. After each sample 0.2 ml of citrated blood of a donor rat was transfused through the sample catheter.

Experimental Design and Conditions

Food was removed from the cage 2 hr before the start of the experiment. Each of the various loads of glucose was tested on separate days in randomized order, with an interval of at least one day. Five of the six rats used in this study received the full range of glucose loads both by oral and by intracardial route. The sixth rat was subjected to oral glucose loading only.

Oral glucose loads of 75, 150, and 300 mg were given by allowing the animals to lick volumes of 0.75, 1.5, and 3 ml of a 10% (w/v) glucose solution. A 750-mg oral load was supplied by offering 1.5 ml, of a 50% (w/v) glucose solution. The average time required for the spontaneous ingestion of these amounts was 0.5, 1, 1.8, and 1 min, respectively.

Intracardial glucose loading was performed by infusion of 10% (w/v) glucose in distilled water over a period of 15 min. Loads of 75, 150, and 300 mg were administered by adjusting the infusion rate at 5, 10, or 20 mg/min. Infusion over 15 min was chosen, since the peak levels of blood glucose after oral ingestion of these loads occurred between 8 and 15 min.

Blood sampling times were identical for both routes of glucose administration. Baseline values were established by sampling at -10 and 0 min (start of the glucose administration). The early period of the response to glucose administration was studied by sampling at 1, 2, 3, 4, 5, 6, 8, 10, and 15 min after time zero. Each glucose load was repeated on another day in order to study the later phase of the response in the same animal. In this second test, blood samples were taken at 10, 15, 20, 25, 35, and 45 min after the start of glucose administration, thereby causing an overlap at 10 and 15 min with the corresponding early-phase experiments. The results of the early- and late-phase experiments were pooled for obtaining the time course over 0-45 min, as shown in Figs. 1 and 2. For this reason, the number of observations at 10 and 15 min was twice as high as at any of the other sampling times.

Insulin and Glucose Determinations

Blood samples were immediately chilled and centrifuged at 4°C. Blood glucose was measured with the ferricyanide method of Hoffman in a Technicon Autoanalyser in samples of 50 μ l of whole blood. For insulin determinations the plasma samples were stored at -30°C. Insulin was

measured with double antibody radioimmunoassay kits (Radiochemical Centre, Amersham, England) using a crystalline rat insulin standard. Duplicate assays were performed in 25 μ l of plasma.

Statistical Evaluation

Correlations were calculated with Kendall's rank correlation method. Differences were tested for statistical significance with Student's *t* test, and the level of significance was chosen at $p \leq 0.05$ (double tailed).

RESULTS

Intracardial Glucose Infusion

The results of these experiments are shown in Fig. 1. Blood glucose increased immediately from the start of the infusions, the most rapid rise occurring during the initial 5 min of the infusion period. Blood glucose levels continued to rise until the infusions were discontinued at 15 min. Blood glucose then rapidly decreased, preinfusion baseline values being reached 20 min after the end of the infusion (i.e., 35 min after time zero).

Coincident with the rapid rise of blood glucose, plasma insulin had markedly increased already at 1 min. In fact, the 1-min values marked the initial peak of a typical biphasic pattern of response. After a small additional interphase peak at 5 min, a major secondary rise of plasma insulin occurred from 6 min onward and lasted until the infusion was terminated at 15 min. Plasma insulin then rapidly declined to baseline values. At the 150- and 300-mg loads the levels even fell significantly below the initial values.

The responses of blood glucose and plasma insulin were quantified by a method that could be applied to all glucose loads at both routes of glucose administration. Inspection of Figs. 1 and 2 shows that the plasma insulin and blood glucose values at 8, 10, and 15 min cover a period during which the secondary phase response of these parameters progressed to its highest level. Accordingly, the quantified insulin response in each individual rat was calculated by taking the mean elevation of plasma insulin above the baseline value at 8, 10, and 15 min at each of the administered loads, yielding 15 responses in five infused rats. Similarly, the glycemic stimulation of insulin secretion over the period of maximum blood glucose elevation (8–15 min) was quantified by calculating for each animal at each load the mean elevation of blood glucose above 110 mg/dl at 8, 10, and 15 min. The latter threshold was chosen because pre-stimulatory basal insulin levels coincided with baseline blood glucose values ranging between 100 and 110 mg/dl. The method of quantification is further discussed in the section on oral glucose ingestion.

The results of these calculations are summarized in the upper part of Table 1, together with the insulinogenic index (mean change in insulin/mean change in glucose) at each load of glucose. The individual quantified responses of blood glucose and plasma insulin to glucose infusion ($n = 15$) appear to correlate with the infused glucose loads (loads versus glucose responses: Kendall's $\tau = 0.71$, $p < 0.01$; loads versus insulin responses: $\tau = 0.56$, $p < 0.01$). When the individual insulin responses are matched against the corresponding responses of blood glucose irrespective of the administered glucose load, there

Fig. 1. Plasma insulin and blood glucose patterns induced by intracardial infusion of graded loads of glucose (75, 150, and 300 mg) over 15 min. Blood samples were taken at -10, 0, 1, 2, 3, 4, 5, 6, 8, 10, 15, 20, 25, 35, and 45 min from the start of glucose infusion (time zero). Mean $\pm$ SEM from five rats. For further details see Materials and Methods. Vertical lines mark the time intervals 0-2, 2-8, and 8-15 min.

also appears to be an overall positive correlation between these parameters ($\tau = 0.57$, $p < 0.01$). The insulinogenic index was highest at the 75-mg load and decreased when loads of 150 and 300 mg were given.

Oral Glucose Ingestion

As shown in Fig. 2, the first notable rise of blood glucose levels occurred at 3 min after the start of glucose ingestion. Maximum levels were attained between 8 and 15 min and varied within the relatively narrow range of 125 to 135 mg/dl at all glucose loads employed. As shown in the lower part of Table 1, the quantified mean glucose response (mean glucose increment above 110 mg/dl at 8, 10, and 15 min) seemed to increase slightly with the glucose load, but the

Fig. 2. Plasma insulin and blood glucose patterns following rapid spontaneous ingestion of 75, 150, 300, and 750 mg glucose. Mean $\pm$ SEM from six rats. For times of blood sampling and further details see legend to Fig. 1 and Materials and Methods. Vertical lines mark the time intervals 0-2, 2-8, and 8-15 min.

stepwise increases were not statistically significant, with exception of the difference between the 75 and 750 mg loads ($p = 0.05$). No significant correlation could be demonstrated between the quantified maximum blood glucose elevations ($n = 24$) in individual animals and the size of the ingested loads ($r = 0.28$; $0.1 > p > 0.05$). Correlation with the size of the load was not observed when

Table 1. Quantified Responses of Plasma Insulin and Blood Glucose During Intracardial Infusion and Oral Ingestion of Graded Loads of Glucose

Load (mg)	n	Line	Plasma IR* (μ U/ml)	Blood GR* (mg/dl)	Index IR:GR
Intracardial					
75	5	a	$+32.8 \pm 4.9$	$+32.9 \pm 3.7$	1.0
150	5	b	$+47.2 \pm 5.8$	$+77.3 \pm 3.3$	0.6
300	5	c	$+72.9 \pm 6.9$	$+139.7 \pm 2.4$	0.5
Oral					
75	6	d	$+12.5 \pm 4.5$	$+12.3 \pm 2.4$	1.0
150	6	e	$+28.1 \pm 7.3$	$+16.5 \pm 2.9$	1.7
300	6	f	$+42.0 \pm 7.4$	$+19.8 \pm 1.9$	2.1
750	6	g	$+51.8 \pm 6.1$	$+20.6 \pm 2.1$	2.5

Plasma insulin responses in individual animals were calculated by taking the mean of the elevation above the prestimulatory level at 8, 10, and 15 min. Blood glucose responses were calculated in a similar fashion from the elevations above 110 mg/dl. Values shown denote mean response in n animals $\pm$ S.E.M.

IR: a vs c, $p < 0.01$; b vs c, $p < 0.01$; d vs f, $p < 0.05$; d vs g, $p < 0.001$; e vs g, $p < 0.05$. GR: a vs b, $p < 0.001$; b vs c, $p < 0.001$; d vs g, $p = 0.05$.

*IR, insulin response; GR, glucose response.

three alternative methods for the quantification of the blood glucose rise were applied (area under the curve over baseline for the period 0–15 min: $\tau = 0.24$; change in glucose 0 $\rightarrow$ 15 min: $\tau = 0.12$; change in glucose 0 min $\rightarrow$ highest level in the period 0–15 min: $\tau = 0.19$). It therefore is evident that the size of the load hardly influenced the magnitude of the maximum blood glucose rise. Increasing the size of the load, however, progressively delayed the decline of blood glucose to baseline values during the period 15–45 min.

At all glucose loads, plasma insulin had increased already at 1 min after the start of glucose ingestion, i.e., 2 min prior to the first notable rise of blood glucose. This blood glucose-independent insulin response seemed to increase at higher oral loads of glucose, in particular at the 750-mg load. In contrast to the prominent biphasic plasma insulin pattern induced by glucose infusion, the insulin responses to oral glucose displayed a multiphasic pattern. Maximum plasma insulin levels were attained between 5 and 15 min and appeared to coincide with the period of maximum blood glucose elevation. Likewise plasma insulin decreased conjointly with blood glucose when their peak levels subsided after 15 min, and like blood glucose the decline of plasma insulin was progressively delayed at higher loads of glucose. At the 75-mg load, plasma insulin fell to levels significantly below the baseline values, but this effect disappeared at higher glucose loads.

Oral ingestion of glucose induced a marked load-dependent increase of the quantified plasma insulin response (Table 1). The 24 individual insulin responses appear to be correlated with the size of the ingested load ($\tau = 0.50$, $p < 0.01$). Although the quantified individual blood glucose responses at 8–15 min do not correlate with the ingested load, there appears to exist an overall positive correlation ($\tau = 0.40$, $p < 0.02$) between the individual insulin responses at 8–15 min and the corresponding responses of blood glucose when these data are matched irrespective of the size of the ingested load.* This apparent relation between plasma insulin and blood glucose following glucose ingestion nevertheless differs from that observed during intracardial glucose infusion. This is shown in Fig. 3, which gives the plasma insulin responses at the various glucose loads as a function of blood glucose increment above 110 mg/dl. The insulin response to oral glucose ingestion increases steeply at minor increments of blood glucose over a narrow range of physiologic concentrations. In the infusion experiments, the slope of the curve is less steep and the curve covers a much wider range of blood glucose increments. The remarkable effectiveness of glucose ingestion in triggering insulin secretion is also demonstrated by the fact that the insulinogenic index increased at higher oral glucose loads, whereas this index decreased at increasing intracardial glucose loads (Table 1).

*This correlation was not detectable when three alternative methods for quantification of the blood glucose and plasma insulin responses were applied [areas under the curves over baselines for the period 0–15 min: $\tau = 0.29$; change in glucose (insulin) 0 $\rightarrow$ 15 min: $\tau = 0.31$; change in glucose (insulin) 0 min $\rightarrow$ highest level in the period 0–15 min: $\tau = 0.23$]. The better performance of our selected method of quantification is probably due to the fact that this quantification expresses the magnitude as well as the duration of the maximum elevation of blood glucose and plasma insulin.

DISCUSSION

Intracardial Infusion

Intracardial infusion of glucose in the conscious rat elicits a biphasic pattern of insulin secretion essentially similar to that seen in the isolated perfused rat pancreas.^{14,15} The initiation, termination, and overall magnitude of the plasma insulin response were closely related to the time of onset and the magnitude of blood glucose changes, indicating that insulin secretion was triggered and governed by blood glucose elevation. Nervous and humoral factors may play an additional role in shaping the secretory response by modulating the sensitivity of the B-cell to glucose stimulation.²⁰ The latter does not apply to the biphasic pattern of the secretory response, which is known to reflect an intrinsic property of the pancreatic islets.¹⁷

The load-dependent increase of the overall insulin response to glucose infusion was mainly accounted for by increased secondary phase secretion. The rapid initial phase of insulin secretion did not further increase when the infused load was increased from 150 to 300 mg. The reduction of the insulinogenic index when the infused load was raised from 75 to 150 mg was somewhat unexpected, since the 150-mg load caused blood glucose to proceed to the range of 150 to 200 mg/dl, which is most effective in stimulating insulin secretion from isolated perfused rat pancreas.^{15,16} In the intact animal, rapid elevation of blood glucose beyond 150 mg/dl may therefore activate an inhibitory mechanism that decreases islet glucose sensitivity. There is evidence to suggest that such a mechanism might involve adrenergic activation.²¹

Termination of glucose infusion resulted in rapidly declining plasma insulin levels. After infusion of 150 and 300 mg of glucose, plasma insulin even fell to levels significantly below the initial preinfusion values. This effect cannot be explained in terms of blood glucose, since blood glucose did not fall below the preinfusion level at the 150-mg load. Alternative explanations of this phenomenon might be the active inhibition of basal insulin secretion by adrenergic activation induced by the rapid decline of blood glucose, or the inability to establish previous basal rates of insulin secretion due to exhaustion of readily available pools of stored hormone.

Oral Ingestion

For obvious reasons the insulin response to spontaneous oral ingestion of glucose should be considered as the true physiologic response. It exhibits a number of striking and fundamental differences with the secretory response to direct infusion of glucose into the circulation. These differences pertain to the initiation of the insulin response, its pattern, and the quantitative relations with the degree of blood glucose elevation.

Spontaneous ingestion of glucose caused plasma insulin to rise 2 min prior to the first notable elevation of blood glucose, confirming previous observations of a similar nature in dogs and rats.^{22,23} This blood glucose-independent early insulin response (EIR) also occurs during ingestion of indigestible dummy meals, which may cause plasma insulin elevations of the order of $+30 \mu\text{U/ml}$ lasting for 5 min at least.²³ The EIR is probably triggered by afferent nervous signals arising from the oropharyngeal cavity.^{24,25} The efferent pathways have

Fig. 3. Mean plasma insulin increment at 8–15 min after rapid ingestion of glucose loads of 75, 150, 300, and 750 mg (six rats) and at intracardial glucose infusion of 75, 150, and 300 mg (five rats) plotted against the corresponding mean blood glucose increment above 110 mg/dl at each of these loads. Details on the method of calculation are given in the section Results.

not yet been identified. Since the 750-mg load caused the highest insulin response at 1 min, the EIR may be load dependent. However, the higher concentration of the glucose solution used for administering this load might also be involved.

Oral ingestion of glucose failed to elicit the prominent biphasic pattern of insulin secretion seen during glucose infusion. This may be due to the lower rate of blood glucose elevation following glucose ingestion. Studies in isolated perfused rat pancreas and in man have demonstrated the importance of the rate of glucose elevation in this respect.^{4,15,26} After the blood glucose-independent EIR (0–2 min) the further secretory pattern was characterized by successive bursts of secretory activity resulting in a multiphasic secretory response. The apparent half-life of acutely expelled insulin was only 1–2 min, as evidenced by the rapid decline of the prominent initial plasma insulin peak in the infusion experiments. The plasma insulin patterns therefore reasonably reflect rapidly changing rates of insulin secretion. During the period of rising blood glucose levels (2–8 min), there usually occurred two separate spikes of secretory activity that appeared to be load dependent. These spikes were superposed on the plasma insulin elevation induced by the preceding EIR (0–2 min). They seem to be glucose-triggered and as such they may be analogous to the initial phase and interphase peaks seen during glucose infusion. The subsequent rise of plasma insulin (8–15 min) to its final load-dependent level was more gradual and coincided with the plateau of blood glucose elevation. This rise resembles secondary phase secretion from isolated perfused rat pancreas during constant stimulation with glucose.^{15,16} It should be emphasized again, however, that the load-dependent increase of the overall plasma insulin response was mainly due to secretory activity during the initial 8 min following glucose ingestion (Fig. 2).

No correlation could be demonstrated between the size of the ingested glucose load and the subsequent maximum blood glucose increments, which varied within a remarkably narrow range. Nevertheless, the individual maximum responses of blood glucose and plasma insulin were correlated, suggesting that blood glucose increment was involved in determining the load-dependent secretory response to glucose ingestion. However, this relation differed markedly from that during glucose infusion (Fig. 3). In terms of blood glucose increment, oral glucose was more effective in stimulating insulin secretion, which agrees with similar observations in man.^{9,10} The insulinogenic index, furthermore,

increased at increasing oral loads, in contrast to the decreasing index when these loads were infused. These findings indicate that the load-dependent increase of the insulin response to glucose ingestion was determined by factors other than blood glucose increment alone.

Two essential features characterize the secretory response to glucose ingestion, the anticipatory nature of its early phases with respect to the ingested load and the subsequent adjustment of the secretion rate to prevailing blood glucose levels. The combination of these characteristics confines the course of blood glucose within narrow limits even after ingestion of widely diverging glucose loads. The possible mechanisms involved are summarized in the following conceptual outline.

The anticipatory response (0-8 min) ensures the immediate and load-dependent release of insulin both prior to and during the rise of blood glucose. In view of experiences with the artificial beta-cell,²⁷⁻²⁹ the short latency of this response will minimize the subsequent insulin requirement for maintaining normal glucose tolerance. The load-dependent anticipatory response may be caused by cumulative operation of the neurally triggered EIR,²²⁻²⁵ and load-dependent release of intestinal hormones known to augment the stimulating action of glucose on insulin secretion.¹⁰⁻¹³ This would provide load-dependent augmentation of the insulinogenic effect of the virtually equal blood glucose increments between 2 and 8 min. The same potentiating mechanism may continue to operate during the further adjustment of the secretion rate between 8 and 15 min, when high glucose loads cause blood glucose elevation to persist until 15 min. The resultant glucose-dependent "secondary phase" secretion is halted immediately when blood glucose starts to fall progressively.

The active principle of the enteroinsular axis has not yet been identified. Secretin, pancreaticozym, and other intestinal peptides have been advocated as likely candidates for this role,¹¹⁻¹³ but relevant studies in the intact rat are relatively scarce. Nevertheless, pancreaticozym and certain peptide fractions of intestinal mucosa induce rapid insulin secretion or potentiate the early phases of glucose-induced insulin secretion on intravenous administration to intact rats.³⁰⁻³⁴ Oral administration of glucose has furthermore been observed to induce a rapid increase of the insulinogenic activity of rat duodenal mucosa extract.³⁵ Various insulinogenic intestinal hormone preparations activate adenylate cyclase activity in mouse and rat pancreatic islets.³⁶⁻³⁹ The active principle may therefore enhance the glucose-induced elevation of islet cAMP,⁴⁰⁻⁴² which is known to be a determinant factor in the regulation of insulin secretion during glucose stimulation *in vitro*.⁴¹⁻⁴³ This mechanism of action, in combination with rapid load-dependent release of the active principle following glucose ingestion, would furnish an attractive model to explain anticipatory load-dependent potentiation of the insulinogenic effect of alimentary hyperglycemia.

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