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Research Article

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Entrainment of Pulsatile Insulin Secretion by Oscillatory Glucose Infusion

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Abstract

Ultradian "oscillations" or "pulses" of insulin secretion with periods around 120 min occur in man. It is not known whether glucose plays an active role in generating these oscillations, or if an intrapancreatic pacemaker generates oscillations in insulin secretion that entrain glucose passively. To determine if the frequency of pulses of insulin secretion could be modified by oscillatory glucose infusion, seven normal men were studied on three separate occasions. The first study involved a constant glucose infusion administered at a rate of 6 mg/kg per min for 28 h. During the two subsequent studies, the subjects received an oscillatory glucose infusion for 28 h with the same mean rate, an amplitude of 33% above and below the mean infusion rate, a sinusoidal waveshape and a period either 20% longer ("slow oscillatory infusion") or 20% shorter ("rapid oscillatory infusion") than the periodicity observed during constant glucose infusion. Samples for insulin, C-peptide, and glucose were drawn at 10-min intervals during the last 24 h of each study. Insulin secretion rates were calculated by deconvolution of C-peptide levels. During constant glucose infusion, the respective periods of oscillation of glucose and insulin secretion averaged 126±5 min and 118±3 min (mean±SEM). During the slow oscillatory infusion, the period of infusion was 155±7 min and the periods of insulin secretion and glucose were, respectively, 155±7 min and 150±5 min. During rapid oscillatory infusion, the period of infusion was 103±5 min and the period of both insulin secretion and glucose was 105±5 min. Thus the periodicity of both insulin secretion and plasma glucose changed in parallel with the exogenous periodicity, indicating complete entrainment of the secretory oscillations. These results suggest that the ultradian oscillations of insulin secretion are caused by the feedback loop linking glucose and insulin. (*J. Clin. Invest.* 1991. 87:439–445.) Key words: ultradian oscillations • feedback • generating mechanism • pancreatic function • human insulin secretion

Introduction

A complex temporal organization underlies human insulin secretion with "oscillations" or "pulses" of secretion occurring at two discrete periodicities. A series of clinical studies have reported the existence of rapid, small amplitude oscillations recurring approximately every 10–15 min (1–3). In addition to these rapid pulses, slower, larger amplitude, ultradian oscillations of secretion with periods ranging from 80–150 min have been observed in response to meals (4, 5), during continuous enteral nutrition (6), and during constant glucose infusion (7, 8). These oscillations are closely associated with similar changes in glucose levels. They are irregular and reduced in amplitude in patients with non-insulin dependent diabetes mellitus (9), suggesting that their presence may be of physiological significance. In experiments in which samples were drawn at 2-min intervals for a total of 8 h, Simon and co-workers (6) clearly showed that the rapid and slower oscillations coexist in the same individual and that the latter do not represent an artifact of infrequent sampling. The temporal organization of beta cell secretion is therefore similar to that of luteinizing hormone and growth hormone for which rapid, low amplitude, and slow, large amplitude, secretory pulses have been demonstrated (10, 11).

The persistence of the rapid oscillations of secretion in the isolated perfused pancreas (12) and in isolated islets (13) is consistent with the hypothesis that they originate from the activity of an intrapancreatic pacemaker. The origin of the slower ultradian oscillations remains to be elucidated. In particular, it is not known whether glucose plays an active role in generating the oscillations, or if a glucose-independent intrapancreatic pacemaker generates oscillations of insulin secretion that force glucose to oscillate passively and in synchrony with insulin. These studies were designed to determine whether glucose is involved in the generation of the oscillations in insulin secretion, which would imply that they are a by-product of the insulin–glucose feedback system. If these ultradian oscillations are due to the nonlinear feedback mechanisms involved in glucose regulation and insulin secretion, the theory of nonlinear systems (14, 15) predicts that it should be possible to entrain them by an oscillatory infusion of exogenous glucose, if the period of infusion is within the limits of entrainment of the system. According to this hypothesis, during exogenous oscillatory glucose infusion, the oscillations of insulin secretion should have the same period as the exogenous infusion, without residual pulsatility reflecting glucose-independent oscillatory activity. On the other hand, if the insulin oscillations were due to the activity of a glucose-independent intrapancreatic pacemaker, it should not be possible to entrain them with oscillatory glucose infusions and complex pulsatile patterns, reflecting the response to the entraining period and the persistence of the endogenous period should be observed. To distinguish between these alternative mechanisms, we examined the temporal patterns of insulin secretion in normal men receiving oscillatory glucose infusions with periods either longer or shorter than their endogenous period of oscillation.

Methods

Subjects

Studies were performed on seven normal men. Age, weight, and body mass index (mean±SEM) were 26.9±1.2 yr, 69.4±3.7 kg, and 22.9±1.2

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kg/m^2, respectively. All were within 10% of ideal body weight and none had a personal or family history of diabetes. The studies were carried out in the Clinical Research Center of the University of Chicago after written informed consent had been obtained. The experimental protocol was approved by the Institutional Review Board.

Experimental protocol

The subjects were studied in the recumbent position after a 10–12-h overnight fast for a period of 28 h beginning at 0800. An intravenous sampling catheter was inserted in a retrograde direction in a dorsal vein of the left hand with its tip in place as distally as possible. The hand was kept in a heating blanket to ensure arterialization of the venous sample. A second catheter for glucose administration was inserted into the antecubital vein of the right arm.

Each subject was studied on three separate occasions. Each study consisted of an initial 4-h period (0800–1200 hours) for equilibration of the glucose infusion followed by a subsequent period of 24 h (1200–1200 hours) during which samples were drawn at 10-min intervals for measurement of glucose, insulin, and C-peptide. Lights were dimmed between 2300 and 0700 hours, to allow the subjects to sleep. Potassium (40 meq) was given orally every 12 h and subjects were allowed free access to water but not given any food for the duration of the experiment. During the three studies, glucose was administered as a 20% solution via a computer-controlled pump (Flo-gard 8000 volumetric infusion pump; Travenol Laboratories, Deerfield, IL) following three different patterns. First, each subject received a constant infusion at a rate of 6 mg/kg per min and the data derived enabled the individual period of endogenous oscillation to be estimated. The subsequent two studies involved the administration of oscillatory glucose infusions with periods 20% shorter (hereafter referred to as the rapid oscillatory infusion) or 20% longer (hereafter referred to as the slow oscillatory infusion) than the period observed during the constant glucose infusion. The software controlling the glucose infusion was custom written to allow the actual infusion rate to be recorded so that possible interruptions could be detected. Each oscillation was shaped as a sine wave and the amplitude was 33% above and below the mean infusion rate. Subjects received identical volumes of glucose during each 28-h infusion period. In one subject, an additional oscillatory glucose infusion was administered in which the exogenous period was double the period estimated during the constant glucose infusion.

In addition to the studies described above, each subject also received a bolus intravenous injection of biosynthetic human C-peptide as described elsewhere (16). This allowed the parameters describing C-peptide kinetics to be defined in each individual based on a two-compartment model of C-peptide distribution and metabolism. The average parameter values were 3,371±164 ml for the volume of distribution, 0.0497±0.0045 min^{-1} for K_1, 0.0513±0.0046 min^{-1} for K_2, and 0.0680±0.0040 min^{-1} for K_3. These corresponded to a short half-life of 4.84±0.024 min, a long half-life of 30.01±1.78 min, and a fraction associated with the short half life of 0.78±0.02. These kinetic parameters were used to derive, in each time interval between successive blood samplings, the insulin secretion rate from the peripheral C-peptide concentrations by deconvolution (16, 17).

Glucose, insulin and C-peptide assays

Glucose concentrations were measured by a YSI analyzer (Model 23A; Yellow Springs Instrument Co., Yellow Springs, OH). The coefficient of variation of this method is < 3%. Serum insulin was assayed by a double antibody technique (18) with a lower limit of sensitivity of 20 pmol/liter and an average intraassay coefficient of variation of 6%. Plasma C-peptide was measured as previously described (19). The lower limit of sensitivity of the assay is 0.02 pmol/ml and the intraassay coefficient of variation averaged 6%. All samples were measured in duplicate. For each hormone, samples from individual subjects were measured in a single assay.

Data analysis

Smoothing and estimation of insulin secretion rates. The individual glucose, insulin, and C-peptide profiles were smoothed using a three-point moving average as in previous studies of oscillations of insulin secretion (1, 6). This procedure consists of replacing the value observed at time t by the arithmetic mean of the values observed at time $t - \Delta$, t and $t + \Delta$, where Δ is the sampling interval, i.e., 10 min. This procedure strongly dampens all fluctuations shorter than 30 min allowing a better visualization of slower oscillations at the expense of a modest reduction in their amplitude. It also reduces measurement error by a factor of $\sqrt{3}$. All further calculations were performed on the smoothed profiles. The smoothed C-peptide curve was used to derive insulin secretion rates (ISR)[1] by deconvolution.

Pulse analysis. To identify significant pulses in insulin secretion and glucose, each profile was analyzed with Ultra, a computer program for pulse detection and quantification (20). The general principle of this algorithm is the elimination of all peaks for which either the increment (difference between the peak and the preceding trough) or the decrement (difference between the peak and the next trough) does not exceed a certain threshold related to measurement error. Extensive simulation studies (20) have indicated that a threshold of twice the intraassay coefficient of variation generally minimizes both false-positive and false-negative errors. However, because deconvolution involves an amplification of measurement error, a more conservative threshold of three times the intraassay coefficient of variation of C-peptide has been previously used to quantify pulses of ISR (5, 7, 9). In this study, pulse analysis was performed on the profiles smoothed by the three-point moving average so that the measurement errors were divided by $\sqrt{3}$. Thus, peaks of insulin secretion and glucose were considered significant if their respective increments and decrements exceeded 10.39% (i.e., $3 \times 6\% \div \sqrt{3}$) and 3.46% (i.e., $2 \times 3\% \div \sqrt{3}$), respectively. For each significant pulse, the relative increment was defined as the difference between the level at the peak and the level at the preceding trough, divided by the level at the preceding trough. Group statistics on relative pulse increments were based on medians, rather than means, because of the non-Gaussian nature of pulse distribution.

Because glucose has a relatively long half-life, changes in production and/or utilization may not be reflected as significant peaks in the glucose concentration curve. We therefore identified all the "shoulders" in the glucose profiles. To do this, instantaneous derivatives of each individual glucose curve were estimated as the slope of the glucose changes during each 10-min sampling interval, and the shoulders were identified as slopes with an absolute value of zero, preceded and followed by slopes of the same sign.

Analysis of the temporal association between oscillations of glucose and oscillations in ISR. Temporal associations between oscillations in glucose and ISR were quantified by pulse-by-pulse analysis of the concomitancy as well as by estimations of overall cross-correlation. Significant pulses of glucose and insulin secretion were considered concomitant if their peak values occurred within 10 min of each other. The concomitance ratio of glucose pulses with ISR pulses was calculated as the number of concomitant glucose and ISR pulses divided by the total number of glucose pulses. Conversely, the concomitance ratio of ISR pulses with glucose pulses was calculated as the number of concomitant glucose and ISR pulses divided by the total number of ISR pulses.

For each pair of individual profiles, the coefficient of cross-correlation at time lags of 0 min (i.e., simultaneous glucose and ISR values), ±10 min (i.e., glucose leading ISR by 10 min or vice versa), and at increasing 10-min intervals up to±240 min was computed. These calculations provide a global definition of the temporal relationship between glucose and ISR oscillations and indicate whether increases and decreases in plasma levels tend to occur simultaneously or whether changes in one of the variables tend to precede or follow the other.

Results

Mean levels of plasma glucose, serum insulin, and ISR. Overall mean glucose levels over the 24-h study period were 7.78±0.17 mmol/liter during the constant glucose infusion, and

1. *Abbreviation used in this paper:* ISR, insulin secretion rates.

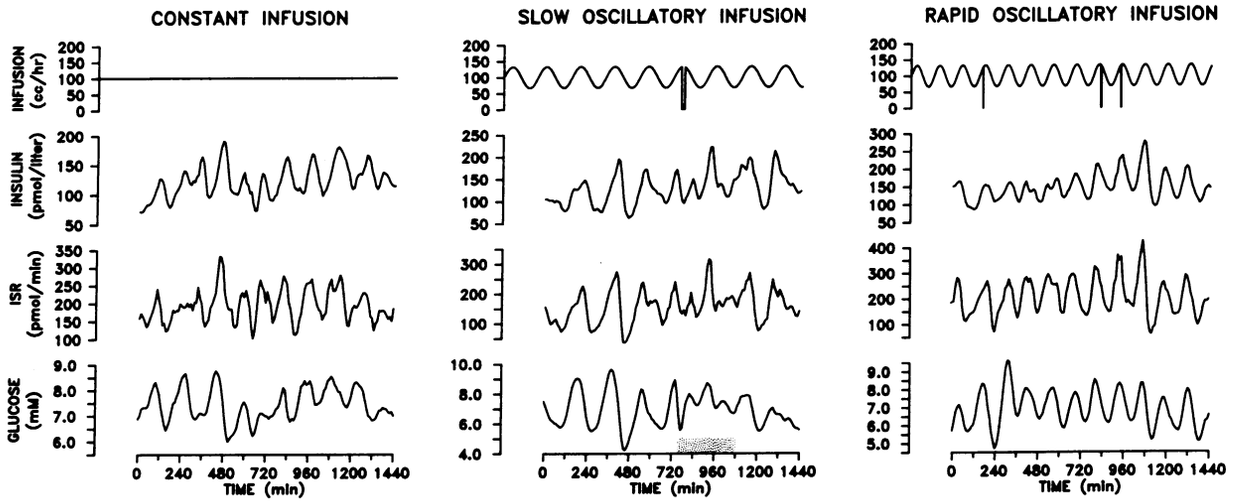

Figure 1. 24-h profiles of glucose, plasma insulin, and insulin secretory rates in subject 1 during constant glucose infusion (*left*), oscillatory glucose infusion with a period of 192 min (*center*), and oscillatory glucose infusion with a period of 128 min. During the slow infusion (*center*), a technical problem in the pump caused an interruption of infusion of 18 min. Entrainment was lost after this interruption but was restored after a transient period of about 300 min (*shaded bar*).

7.72±0.16 and 7.67±0.19 mmol/liter during the rapid and slow oscillatory glucose infusions, respectively. Similarly, overall mean levels of serum insulin were 223±32 pmol/liter during constant glucose infusion, and 215±18 and 220±33 pmol/liter during the rapid and slow oscillatory infusions. Finally, overall mean levels of ISR were 314±47, 304±41, and 294±38 pmol/min during the constant glucose and the rapid and slow oscillatory glucose infusions. There were no significant differences between the three study conditions for either glucose, insulin, or ISR.

Pulses of glucose and ISR during constant glucose infusion. All subjects exhibited significant pulses of glucose, insulin, and ISR during constant glucose infusion with representative examples being shown in the left panels of Figs. 1 and 2. The number of pulses identified from the profiles of insulin concentrations was similar to the number of pulses derived from the profiles of ISR in all subjects. Since the insulin secretion rates provide a more quantitatively accurate measure of beta cell secretory activity, subsequent analyses use the insulin secretion rates rather than the insulin concentrations.

As shown in Table I, during constant glucose infusion, more pulses of ISR than glucose were identified in all subjects. Careful inspection of the simultaneous changes in glucose levels and insulin secretory rates indicated that in each pair of individual profiles several pulses of insulin secretion were concomitant with a significant shoulder on an ascending or declining limb of a glucose pulse. In view of the prolonged half life of plasma glucose, both significant pulses and shoulders concomitant with a significant ISR pulse were considered in estimating the oscillatory period of glucose. The oscillatory period for ISR was calculated as the total duration of sampling divided by the number of significant pulses.

Selection of the period for the oscillatory glucose infusion. For subjects 1 and 2, the determination of the period of oscillation during constant glucose was based on pulse analysis of the glucose profiles which were readily available at the completion

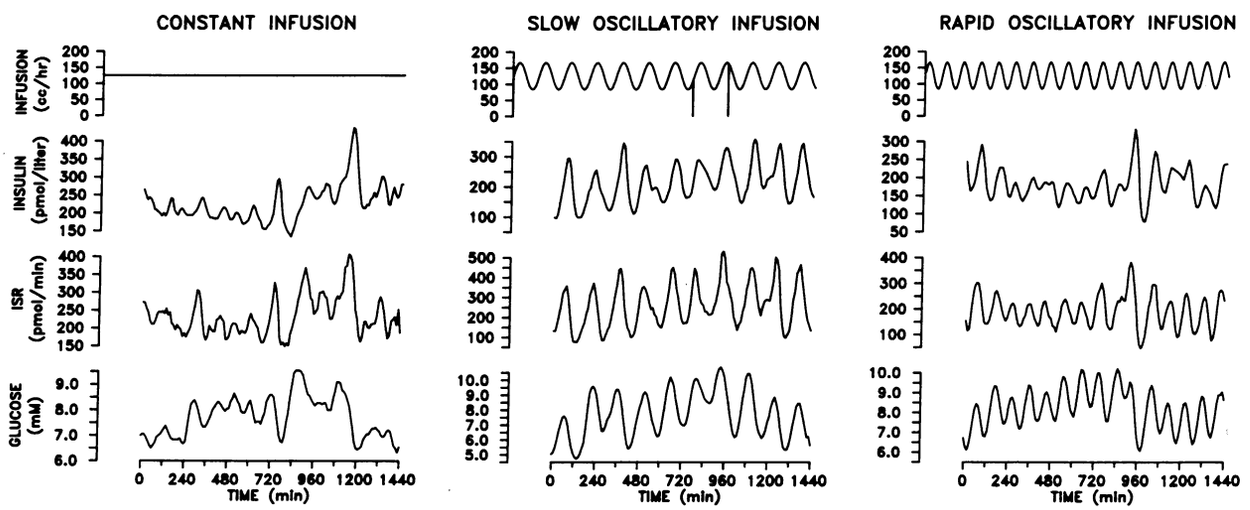

Figure 2. 24-h profiles of glucose, plasma insulin, and insulin secretory rates in subject 4 during constant glucose infusion (*left*), oscillatory glucose infusion with a period of 144 min (*center*), and oscillatory glucose infusion with a period of 96 min (*right*).

Table I. Pulse Analysis of the Profiles of Plasma Glucose and Insulin Secretory Rates during Constant and Oscillatory Glucose Infusions

		Glucose			ISR			
Subject	Period of infusion	Nb of pulses	Median relative increment	Nb of shoulders	Mean period	Nb of pulses	Median relative increment	Mean period
	min		%		*min*		%	*min*
Constant glucose infusion								
1	—	8	24	2	144	12	78	120
2	—	8	12	4	120	12	29	120
3	—	8	16	4	120	14	42	103
4	—	10	14	1	131	11	48	131
5	—	8	16	2	144	12	47	120
6	—	8	17	5	111	13	41	111
7	—	9	14	4	111	12	41	120
Mean	—	8.4	16	3.1	126	12.3	47	118
SEM	—	0.3	2	0.6	5	0.4	6	3
Slow oscillatory infusion								
1	192	7.7*	67	0	188	9.6*	210	150
2	173	8	42	0	180	8	89	180
3	144	10	47	0	144	10	175	144
4	144	10	54	0	144	10	219	144
5	144	10	38	0	144	10	61	144
6	144	10	54	0	144	10	204	144
7	144	10	46	0	144	10	140	144
Mean	155	9.4	50	0	155	9.7	157	150
SEM	7	0.4	4	0	7	0.3	24	5
Rapid oscillatory infusion								
1	128	11	36	0	131	11	141	131
2	115	13	41	0	111	13	146	111
3	96	15	41	0	96	15	231	96
4	96	14	36	0	103	14	130	103
5	96	15	24	0	96	15	85	96
6	96	14	43	0	103	14	123	103
7	96	14	29	1	96	15	72	96
Mean	103	13.7	36	0.1	105	13.9	133	105
SEM	5	0.5	3	0.0	5	0.6	19	5

* Estimated by extrapolation after an accidental interruption of the infusion.

of the experiment. This preliminary approach estimated the oscillatory period during constant glucose infusion to be 160 and 144 min in these two subjects, respectively. Subsequent detailed analysis of simultaneous variations of glucose and ISR indicated that, on average, the period of oscillation during constant glucose infusion was close to 120 min in all subjects and this was the oscillatory period selected for the glucose infusions in subjects 3–7. The respective periods for the slow and rapid oscillatory glucose infusion were 20% slower or faster than the period during constant glucose infusion (Table I).

Oscillatory glucose infusions. During the oscillatory glucose infusions, the oscillations of plasma glucose, insulin, and ISR entrained to match the exogenous infusion pattern with remarkable accuracy when the period of the exogenous infusion was both longer and shorter than the period observed during constant glucose infusion. Data from two representative subjects depicted in Figs. 1 and 2 illustrate these findings. In all subjects, the period of ISR oscillations closely matched the period of the exogenous glucose infusion (Table I and Fig. 3).

The effects of oscillatory glucose infusion on the distribution of the interpulse intervals of ISR are illustrated in Fig. 4. During constant glucose infusion, there was a relatively wide distribution of interpulse intervals centered around a mean of 126 min (median 110 min). 75% of the total number of pulses had interpulse intervals between 63 min and 154 min. Slow oscillatory infusion resulted in a concentration of interpulse intervals above 110 min with essentially no residual endogenous pulsatility below 110 min. Indeed, during slow oscillatory infusion, only 2.2% of the interpulse intervals were shorter than 110 min vs. 48.1% during constant infusion (chi-squared = 28.6, $P < 0.001$). Conversely, rapid oscillatory infusion had the effect of concentrating interpulse intervals in the range below 110 min and of markedly suppressing pulsatility in the range above 110 min. Specifically, during rapid oscillatory glu-

cose infusion only 9.0% of interpulse intervals were longer than 110 min, vs. 40.5% during constant infusion. (Chi-squared = 10.1, $P < 0.01$.)

Amplitude of oscillations. The amplitude of oscillation was estimated for both glucose and ISR as the median relative increment of significant pulses. Slow oscillatory glucose infusion resulted in an approximately threefold increase in the amplitude of both glucose and ISR oscillations observed during constant infusion (Table I, $P < 0.001$). Rapid oscillatory infusion also magnified the glucose and ISR oscillations (Table I, $P < 0.001$). However, entrained glucose oscillations were larger during slow oscillatory infusion than during rapid oscillatory infusion ($P < 0.01$). The amplitude of entrained ISR oscillations was similar during both oscillatory regimens of delivery. Under the three experimental conditions, the amplitude of the ISR oscillations was strongly correlated with the amplitude of the glucose oscillations (Fig. 5).

Temporal association between oscillations in glucose and ISR. During constant glucose infusion, 90.7±4.3% of glucose pulses were concomitant with an ISR pulse, and 85.3±4.6% of ISR pulses were concomitant with a glucose pulse. During slow and rapid oscillatory infusions, the concomitancy ratios were higher than during constant glucose infusion, and increased to 100±0% and 96.1±1.4% for glucose, and 95.8±2.9%, and 96.1±1.4%, respectively, for ISR. Differences in concomitancy rate between glucose and ISR were nonsignificant. Similarly, differences in concomitancy rates across study conditions were nonsignificant for both glucose and ISR. Cross-correlation analysis confirmed the significance of the temporal association between oscillations in glucose levels and oscillations in ISR both during constant and oscillatory glucose infusions. During constant glucose infusion, the maximum cross-correlation was observed at lag 0 min and averaged 0.62±0.05 ($P < 0.01$). Because the ISR is calculated at the midpoint of each 10-min sampling interval, this result suggests that the glucose oscillation precedes the oscillation in ISR by 5 min on average. Oscillatory infusion enhanced the temporal association (mean maximum cross-correlation was 0.81±0.04 for slow and 0.77±0.05 for rapid oscillatory infusions, respectively; $P < 0.02$ as compared with constant glucose infusion.) During both modes of oscillatory infusion, the maximum cross-correlation occurred at a lag of +10 min, revealing that, in entrained conditions, on average, the oscillations in glucose occur 15 min in advance of the oscillations in ISR.

Oscillatory infusion with an ultra slow period. Subject 1 was studied on an additional occasion, using a period of infusion of 320 min, thus more than twice as long as the endogenous period (Table I). The profiles for glucose and C-peptide are shown in Fig. 6. Two large amplitude oscillations of both glucose and ISR were observed for every single oscillation in the exogenous infusion. This pattern of entrainment, commonly referred to as "2:1 entrainment," is typical of oscillatory systems "forced" or "entrained" with periods that are outside of their limits of en-

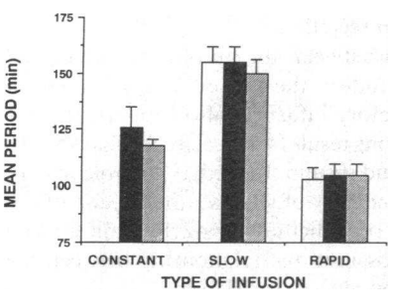

Figure 3. Mean (and SEM) period of oscillation during constant glucose infusion, slow oscillatory glucose infusion, and rapid oscillatory glucose infusion for glucose (■) and ISR (▨). During both slow and rapid oscillatory infusion, the mean period of both glucose and insulin oscillations did not differ significantly from the period of the exogenous infusion (□).

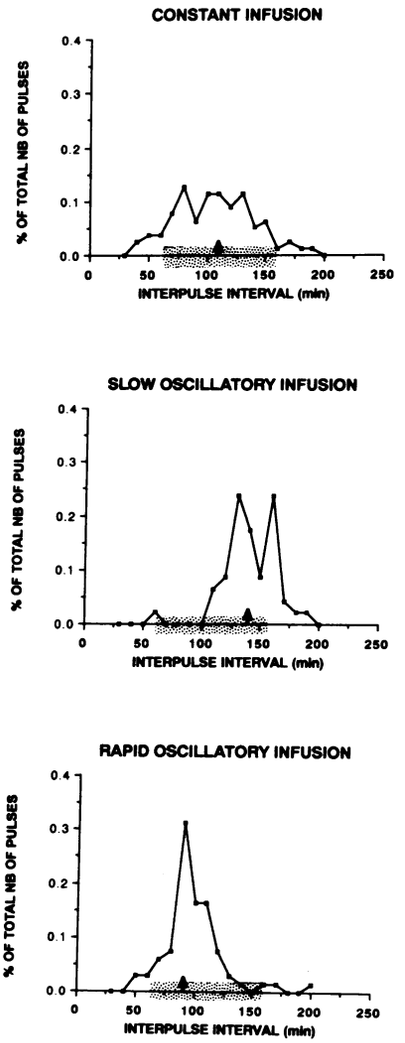

Figure 4. Histograms of interpulse intervals during constant glucose infusion (*top*), oscillatory glucose infusion with a period shorter than the endogenous period (*bottom*). The median of the distribution (*arrow*) was 110 min during constant glucose infusion, 140 min during slow oscillatory infusion, and 90 min during rapid oscillatory infusion. The shaded area represents the range of interpulse intervals including 75% of the pulses observed during constant glucose infusion. Note that during slow oscillatory infusion (*center*), rapid pulsatile activity in the range below 110 min was essentially totally suppressed. Conversely, during rapid oscillatory infusion, slow pulsatile activity in the range above 110 min was strongly inhibited.

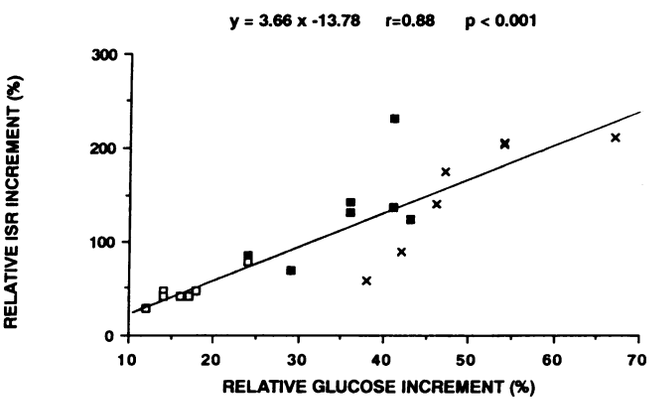

Figure 5. Relationship between the relative amplitude of ISR oscillations and the relative amplitude of glucose oscillations during constant glucose infusion (□) and during slow (×) and rapid (■) oscillatory glucose infusions. The amplitude was estimated as the relative increment over the preceding trough.

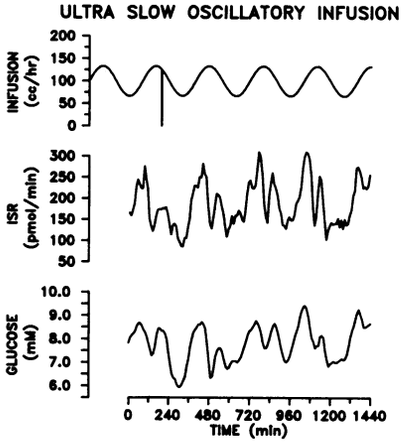

Figure 6. 24-h profiles of glucose and insulin secretory rates in subject 1 during oscillatory glucose infusion with an ultra slow period of 320 min. This period of exogenous infusion was outside the range of entrainment of the mechanism generating the endogenous oscillations and the coexistence of oscillations around 160 min (i.e., the first harmonic of the exogenous period) and of slow oscillations matching the period of infusion was observed.

trainment but that are approximately twice as long as periods within the range of entrainment (15).

Discussion

A number of recent studies have demonstrated that human insulin secretion occurs in an oscillatory fashion, with pulses recurring with a periodicity of ~ 120 min both under basal conditions and in response to a variety of secretory stimuli (4–8). Glucose changes closely parallel these ultradian variations in insulin secretion. Oscillations of glucose and insulin in the same frequency range had been previously observed in dogs receiving constant glucose infusions (21) and shown to be associated with periodic variations in glucose uptake (22). A further study by the same group (23) indicated that these oscillations are of functional importance because they enhance the efficiency and stability of glucose disposal. Our finding that the pulsatile patterns of postmeal insulin secretion are abnormal in patients with non-insulin dependent diabetes mellitus (9) suggests that these ultradian oscillations may also be of physiological significance in man.

Although the existence of these pulses or oscillations has been well documented, the factors responsible for their origin are not known. However, two mechanisms that are possible from a theoretical standpoint can be ruled out on the basis of recent experimental observations. First, the oscillations are not dependent on the central neurological connections of the pancreas, since they persist in insulin-deficient patients with type I diabetes after successful whole pancreas transplantation (24). Second, an analysis of simultaneous variations of cortisol, growth hormone, and glucagon levels has failed to identify correlations with oscillations of glucose and insulin secretion, indicating that they are not caused by temporal variations in these counterregulatory hormones (7).

In light of the above findings, several hypotheses regarding the mechanism responsible for the ultradian oscillations in insulin secretion and glucose levels can be formulated. The ultradian insulin oscillations could result from the activity of an intrinsic pancreatic pacemaker, with glucose being passively forced to oscillate following the changes in insulin levels. Manipulating the period of glucose oscillation by rhythmic infusions of exogenous glucose would not suppress the activity of this glucose-independent pancreatic pacemaker and the resulting secretory profiles would reflect the coexistence of both the endogenous oscillation and the response to the exogenous periodicity. Alternatively, these oscillations could be an inherent feature of the insulin–glucose feedback mechanism, and thus glucose would have an active role in their generation. Nonlinear negative feedback systems that contain delays are often unstable or oscillatory (14, 15). In the insulin–glucose feedback loop, there is a delay between increases in insulin secretion and the resulting reduction of glucose production (25). Furthermore, there is evidence to indicate that the biological activity of insulin occurs from a distant peripheral compartment (26). These dynamic characteristics of the system are compatible with the concept that oscillatory or pulsatile insulin secretion is an intrinsic feature of the insulin–glucose feedback loop. If this were the case, glucose would play an active role in driving the pulses of insulin secretion, glucose-independent secretory activity should not be present, and it should be possible to control the oscillatory period of beta cell secretion with exogenous glucose. Finally, there is also the theoretical possibility that there is an intrapancreatic pacemaker whose period can be altered by glucose. The observation that during overnight fasting the number of ISR oscillations is greater than the number of glucose oscillations (5) can be considered as evidence in favor of this hypothesis. However, as shown in previous studies (8) and as can be seen in Fig. 5, the relative amplitude of the ISR oscillation is approximately three times the relative amplitude of the glucose oscillation. Therefore, the apparent lack of concomitancy between pulses of ISR and pulses of glucose during fasting, when pulse amplitudes are reduced, could reflect the limitations on pulse detection.

While the results of the entrainment experiments described here cannot exclude the existence of an intrapancreatic pacemaker, they do define an important role for glucose in determining the oscillatory period of ISR. Indeed, during both slow and rapid oscillatory glucose infusion, oscillations in insulin secretion clearly entrained to the exogenous period and glucose-independent insulin secretory activity was essentially absent, with a concomitancy ratio of ISR pulses with glucose pulses exceeding 95%. Furthermore, during these oscillatory infusions, pulsatile activity at periods observed during constant glucose infusion was suppressed. Slow oscillatory infusion almost completely eliminated short interpulse intervals with only 2.2% of interpulse intervals being shorter than 110 min. Conversely, rapid oscillatory infusion markedly suppressed pulsatility in the range above 110 min. In view of the limitations on the precision of such biological estimates, these results strongly suggest that pulsatile insulin secretion was completely entrained by the periodic infusion of exogenous glucose and indicate that plasma glucose is an active part of the mechanism causing oscillatory insulin secretion.

The high correlation between the amplitude of the ISR oscillation and the amplitude of the glucose oscillation during both constant and oscillatory infusions also supports the hypothesis that the oscillations result from a close feedback interaction between glucose and insulin. Indeed, if pulsatile insulin activity occurred independently of glucose, the mixing of endogenous and exogenous periodicities during rhythmic glucose infusions should have obscured the relationship between the amplitudes of the ISR and glucose oscillation.

The results obtained in subject 1 during ultra slow oscillatory infusion further demonstrate that there is a limit to the range of entrainment. Thus, when the period of the oscillatory

glucose infusion was twice the period during constant glucose infusion, the exogenous glucose oscillation was no longer able to entrain the endogenous period of insulin secretion in a simple fashion (i.e., one oscillation in plasma glucose and one oscillation in ISR for every oscillation in the exogenous glucose infusion). Instead, a more complex type of entrainment (2:1 entrainment) occurred and two pulses in both glucose and ISR were observed for every oscillation in exogenous glucose, indicating that the endogenous oscillation was entrained to the first harmonic of the exogenous oscillation. It is a universal feature of nonlinear systems that such modes of entrainment will occur, depending on the period of the exogenous stimulus (14).

In summary, these results suggest that the ultradian oscillations of the ISR are an inherent feature of the insulin–glucose feedback mechanism and define an active role for glucose in driving the oscillatory period. Our findings are consistent with the observation that ultradian oscillations of ISR occur under all circumstances where the insulin–glucose feedback is operative, such as following a variety of stimuli to insulin secretion and after successful pancreas transplantation, and although present, are abnormal, in situations where the insulin–glucose feedback control is abnormal, as in non-insulin dependent diabetes.

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