The mass, but not the frequency, of insulin secretory bursts in isolated human islets is entrained by oscillatory glucose exposure

R. A. Ritzel,1,2 J. D. Veldhuis,3 and P. C. Butler1
1Larry Hillblom Islet Research Center, University of California, Los Angeles David Geffen School of Medicine, Los Angeles, California; 2Division of Endocrinology and Diabetes, School of Medicine, University of Heidelberg, Heidelberg, Germany; and 3Endocrine Division, Mayo Medical and Graduate Schools of Medicine, Mayo Clinic, Rochester, Minnesota

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Ritzel, R. A., J. D. Veldhuis, and P. C. Butler. The mass, but not the frequency, of insulin secretory bursts in isolated human islets is entrained by oscillatory glucose exposure. Am J Physiol Endocrinol Metab 290: E750–E756, 2006. First published November 8, 2005; doi:10.1152/ajpendo.00381.2005.—Insulin is secreted in discrete insulin secretory bursts. Regulation of insulin release is accomplished almost exclusively by modulation of insulin pulse mass, whereas the insulin pulse interval remains stable at ~4 min. It has been reported that in vivo insulin pulses can be entrained to a pulse interval of ~10 min by infused glucose oscillations. If oscillations in glucose concentration play an important role in the regulation of pulsatile insulin secretion, abnormal or absent glucose oscillations, which have been described in type 2 diabetes, might contribute to the defective insulin secretion. Using perifused human islets exposed to oscillatory vs. constant glucose, we questioned 1) whether insulin secretory bursts in response to stimulation of insulin secretion by glucose, sulfonylureas, or GLP-1 (10, 23, 26, 29, 30).

Patients with type 2 diabetes have impaired glucose-mediated insulin secretion because they secrete smaller pulses (decreased insulin pulse mass and pulse amplitude) compared with nondiabetic humans (12, 13). Several lines of evidence suggest that this deficit in pulse mass is partly due to the loss of available insulin stores. First, pulse mass is restored in patients with type 2 diabetes by prior overnight inhibition of insulin secretion by somatostatin (12). Second, impaired glucose-mediated insulin secretion due to deficient insulin pulse mass can be reproduced in a porcine model with a loss of β-cell mass comparable with that present in patients with type 2 diabetes (11, 15). Third, isolated human islets that are chronically exposed to glucose concentrations typically present in type 2 diabetes have both decreased insulin content and insulin pulse mass, both of which are avoided if the islets are cultured at the same glucose concentration, with a potassium channel opener to inhibit insulin secretion (31, 35). Although insulin pulse mass is decreased in humans with type 2 diabetes, pigs with a partial loss of β-cell mass, and human islets cultured at high glucose concentrations, pulse frequency is unchanged in all of these circumstances (11–13, 15, 31, 35).

Given the remarkably robust nature of the insulin pulse interval, it was therefore intriguing that studies in nondiabetic humans (25) reported entrainment of the insulin pulse interval from 4 to 10 min by intermittent infusion (every 10 min) of small glucose pulses. This entrainment could not be reproduced in patients with type 2 diabetes (6, 17). Blood glucose concentrations oscillate in healthy humans, with a frequency similar to that of the insulin pulses (14, 17), but these glucose oscillations are impaired in patients with type 2 diabetes (13). Therefore, a change in the glucose concentration profile might contribute to impaired insulin secretion in type 2 diabetes. In the present experiments, we studied perifused isolated human islets at constant vs. oscillatory (10-min) glucose concentrations to address the following questions. 1) Is the frequency of insulin secretory bursts from isolated islets entrained to slower oscillations of glucose, as reported in humans in vivo? 2) Does exposure of
islets to oscillating vs. constant glucose confer an increased signal for insulin secretion?

RESEARCH DESIGN AND METHODS

Design. Isolated human islets were studied in an islet perfusion system that has previously been validated for detection and quantification of pulsatile insulin release from human islets (33). After an equilibration period of 40 min without sample collection (40 to 0 min), the islets were studied over a period of 100 min. Superimposed on a baseline glucose concentration of 4 mmol/l, glucose was delivered to the islets in an oscillatory fashion at an interval of 10 min (8 pulses per experiment) and different glucose amplitudes of 0 (control; n = 8 runs), 0.3 ± 0.0 (n = 8 runs), 3.9 ± 0.1 (n = 7 runs), 11.0 ± 0.3 (n = 8 runs), and 22.6 ± 0.9 mmol/l glucose (n = 5 runs). The glucose oscillations were generated by intermittent 2-min infusions of a buffer with higher glucose concentration, alternating with an 8-min period of perfusion at 4 mmol/l glucose. This design was intended to reproduce the previously reported (25) glucose pulse interval (10 min) to entrain insulin pulses in nondiabetic humans in vivo. We reproduced both physiological and supraphysiological glucose amplitudes to reproduce oscillations under conditions of daily living but also to avoid a false-negative outcome. We reasoned that if very large amplitude pulses do not entrain insulin pulses, this negative outcome is safe with smaller pulses present in clinical studies. We define entrainment of pulsatile insulin secretion as a change in pulse frequency so that insulin pulses arise as a consequence of the newly introduced signal (10-min glucose pulse), rather than conforming to the prior intrinsic signal pacemaker generating ~4 min pulses. Islets were recovered from the perfusion chambers for the measurement of islet insulin content. The mean islet insulin content was not significantly different in islets studied at any of the glucose amplitudes: 0, 0.3, 3.9, 11.0, and 22.6 mmol/l (3, 440 ± 390, 2,640 ± 400, 3,080 ± 360, 2,120 ± 200, and 2,840 ± 480 fmol/islet, respectively; P = not significant [NS]). Insulin secretion from the islets is reported as mass units per islet to account for different islet numbers. Islet sizes were measured at the beginning and the end of each perfusion experiment to account for different islet sizes.

The present experiments with oscillatory glucose delivery were compared with islet perfusion experiments that had constant glucose stimulation on the basis of the mean glucose levels of 4, 4.2, 6.3, 9.5, and 14.7 mmol/l (oscillatory) vs. 4, 8, 12, 16, and 24 mmol/l (constant). The comparisons of dose-response relationships are depicted for a glucose range of 4–16 mmol/l. The data of islet insulin secretion profiles from islets perifused at oscillatory glucose was also presented in the perifusion system (33). Deconvolution calculates secretion properties represented by 1) the number, duration, mass (time integral of calculated insulin secretion, but with even subtle (0.3 mmol/l) glucose oscillations, appeared to be present with an interval of 0–25 min. The square of n is proportionate to the variance in the mean insulin concentration, which is explained by a periodic signal that recurs at the corresponding time delay (e.g., 2, 4, 8 min). Spectral analysis was performed to further analyze the periodicity of the data. It decomposes the insulin concentration time series into cyclical components with a few underlying sinusoidal (sine and cosine) functions of particular wavelengths. No detrending procedures were used for data analysis.

Data are presented as means ± SE. ANOVA and the unpaired Student’s t-test were used to contrast insulin concentrations and secretion measures. Nonlinear regression analysis (sigmoidal dose response, variable slope, y = bottom + [top − bottom]/[1 + 10 exp(−log EC50 − x) × Hill slope]) was applied to analyze the relationship between glucose concentration and total insulin secretion, pulse interval, and pulse mass of pulsatile insulin secretion. The assumption of a P value of <0.05 was construed as significant.

RESULTS

Inspection of a representative data profile from a perifusion experiment with oscillatory glucose stimulation (Fig. 1) shows that insulin pulses were still evident between glucose pulses and appeared to be present with an interval of ~4–5 min, in contrast to the glucose oscillations of 10-min intervals. The size of the insulin pulses that coincided with glucose pulses were markedly larger than those that coincided with troughs in glucose concentration. Deconvolution analysis confirms that the frequency of insulin pulses did not adapt to the frequency of the oscillatory glucose stimulus (Fig. 1). In Fig. 2, additional insulin secretion profiles from islets perfused at oscillatory glucose vs. constant glucose are shown. When islets were perfused at a constant glucose concentration (Fig. 2A), pulsatile insulin release was apparent, as previously reported (31, 35). In perifusion experiments at a similar glucose concentration, but with even subtle (~0.3 mmol/l) glucose oscillations, a more marked pulsatile pattern of secretion was evident (Fig. 2B).

However, this more marked pulsatile pattern of insulin secretion was not accompanied by entrainment of insulin pulses to the glucose oscillations, even in experiments with higher glucose amplitudes (Fig. 2, C and D). Deconvolution analysis (Fig. 2, E–H) revealed that the reason for the more distinct pulsatile pattern of insulin secretion with oscillating glucose was a twofold increase in the proportion of insulin secreted by the group of islets in each chamber in coordinate pulses (61.5 ± 3.9 vs. 30.6 ± 1.1%, P < 0.0001). This increased proportion of insulin released by groups of islets in coordinate pulses exposed to oscillatory glucose was also
accompanied by a fourfold augmentation of insulin pulse mass \((P < 0.001)\) with oscillatory glucose. However, the frequency of insulin secretory bursts was not different between the islets perfused at oscillating vs. constant glucose across the whole range of glucose concentrations studied (Fig. 3), which affirms the impression from individual insulin concentration profiles that the pacemaker driving insulin pulses was not influenced by oscillations of glucose at 10-min intervals. Cross-correlation analysis indicated that there was a positive relationship between insulin secretion and glucose concentration with zero lag time, affirming that the insulin secretion responded within <1 min to a rise in glucose concentration in the buffer.

A striking finding was the coexistence of large insulin pulses adjacent to small ones, which caused a marked pulse-to-pulse variance in both pulse mass and pulse amplitude in islets exposed to oscillating glucose. In particular, variability in interburst interval was \(\sim 35\%\), whereas that of insulin pulse size was \(\sim 150\%\), and it was greater under intermittent rather than continuous glucose drive \((P < 0.05)\). To examine whether this close proximity of large and small insulin pulses could still be resolved under circumstances that resemble in vivo experiments with sampling from the systemic circulation, we reexamined the raw insulin data from one representative experiment on the basis of the systemic insulin kinetics obtained in humans in vivo (28). In this simulation of systemically sampled data, the insulin concentration profile closely resembled the underlying glucose oscillations (Fig. 4). Therefore, in vivo when sampling from the systemic circulation, small insulin

![Fig. 1. Insulin (solid line, left y-axis) and glucose (dashed line, right y-axis) concentration profiles (A) and insulin secretion rates (derived by deconvolution analysis; B) from 1 representative islet perfusion experiment with oscillatory glucose stimulation at 10-min intervals and amplitudes of \(-0.3\) mmol/l. *Insulin oscillations corresponding to a detected pulse.](image1)

![Fig. 2. Insulin (left y-axes, solid lines) and glucose (right y-axes, dashed lines) concentration profiles (A–D) and insulin secretion rates (derived by deconvolution analysis; E–H) from 4 representative islet perfusion experiments with constant (A and E) or oscillatory glucose stimulation at 10-min intervals and amplitudes of \(-0.3\) (B and F), \(-3.9\) (C and G), and \(-11.8\) mmol/l (D and H). Note the different y-scale for glucose in B.](image2)
pulses might be missed if they were to occur between larger pulses.

Autocorrelation analysis, which was used for analysis of insulin concentration time series in vivo (6, 25), showed a dominant periodicity of 10 min in experiments with oscillatory glucose delivery, although it did not detect regular insulin pulses in experiments with constant glucose perfusion (Fig. 5C). Also, the autocorrelograms of individual data sets and the corresponding modeled systemic insulin concentration (by re-convolution of insulin secretion as described above) together revealed oscillations in insulin concentration at the periodicity of the applied glucose oscillations (Fig. 5D). Spectral analysis has also been used for analysis of insulin time series in vivo (6, 25). It detects dominant periodicities at the frequency of infused glucose oscillations but does not distinguish underlying sinusoidal functions of particular wavelengths in experiments with constant glucose (Fig. 5A). In the present studies, when spectral analysis was applied to individual insulin concentration time series, an additional cyclical component with a period of ~5 min was uncovered. However, the ~5-min component was not recognized using the corresponding modeled systemic insulin concentration (Fig. 5B).

The dose-response curves for total insulin secretion in response to oscillating or constant glucose were remarkably similar (Fig. 6). Analysis of the time course of insulin secretion revealed that over a period of 1 h, there was no time-dependent difference of insulin secretion in experiments with constant vs. oscillatory glucose stimulation (Fig. 7).

DISCUSSION

In the present studies, we report that the pacemaker activity for insulin secretion in isolated human islets is insensitive to oscillatory glucose stimulation. Also, an oscillatory pattern of glucose concentrations does not provide a quantitative advantage with respect to total insulin secretion over stimulation with a comparable, but constant, glucose concentration. Therefore, we postulate that entrainment of pulsatile insulin to infused glucose oscillations in vivo likely reflects preferential detection of intermittently enhanced insulin pulses, which occasionally coincide with the momentary glucose elevation.

![Fig. 4. Dampening of insulin concentration profile as it occurs in systemic circulation in vivo. A: original insulin concentration profile (solid line) from 1 representative islet perfusion experiment with oscillatory glucose stimulation (10-min interval, amplitude ~0.3 mmol/l). B: calculated systemic concentration data derived by convolution of insulin secretion time series corresponding to A. Computations are based on insulin kinetics previously established in humans (see Ref. 28).](http://ajpendo.physiology.org/)

![Fig. 5. Mean spectral density (A) and mean autocorrelograms (C) of insulin concentration profiles obtained during islet perfusion experiments with oscillatory (interval of 10 min) or constant glucose. Individual spectral analysis (B) and autocorrelograms (D) correspond to insulin concentration profiles shown in Fig. 4B.](http://ajpendo.physiology.org/)
The concept of entrainment of insulin oscillations was originally introduced to describe the frequency shift of ultradian insulin oscillations (occurring at an \( \sim 100–120 \) min interval) by pulsed glucose infusions (19, 36). The present study is focusing on high-frequency insulin oscillations (\( \sim 4–6 \) min interval) that arise as a consequence of discrete secretory bursts. Although the term entrainment was used in several studies of high-frequency insulin oscillations in response to administration of glucose pulses in vivo (17, 25) and in vitro (2), the precise meaning of entrainment has not been clearly defined. In the present analyses, entrainment denotes that the intermittent stimulus (i.e., glucose) would change the frequency of the underlying pacemaker to correspond consistently to that of the experimental stimulus. It has been suggested that entrainment of high-frequency insulin pulses is of potential use as a discriminator between insulin secretion in health and type 2 diabetes and has been examined in perifused rat islets (2) and humans in vivo (7, 17, 25). In rat islets, perifusion with a glucose pulse every 40 min increased insulin pulse periodicity from \( \sim 25 \) min to \( \sim 40 \) min (2). However, in that study, insulin concentrations were only evaluated at \( \sim 3 \) min intervals, a sampling interval that is inadequate to reliably detect high-frequency insulin oscillations that occur every 4–6 min (27). In technical terminology, insufficiently frequent sampling induces aliasing, an artifact that is due to intermittent false-negative estimates. In an in vivo study, glucose boluses were given to diabetic and nondiabetic subjects at an interval of \( \sim 29 \) min. Spectral analysis of smoothed insulin concentration data (3-point moving average) showed a shift of insulin pulse frequency from \( \sim 10 \) to \( \sim 15 \) min in nondiabetic humans (17), which was interpreted as entrainment, although the insulin pulse interval did not adapt to the \( \sim 29 \)-min input periodicity that is required by the present definition of entrainment. On the other hand, entrainment of insulin oscillations to a train of 10-min glucose pulses was reported in nondiabetic volunteers (25). The present study illustrates that the latter outcome is consistent with the expected loss of discrimination in systemic blood of underlying high-frequency pulses during intermittent glucose pulsing (18). In the same study, the pulse interval detected with autocorrelation was \( \sim 10 \) min in both type 2 diabetes and controls with or without glucose pulses, but the autocorrelation coefficient was reduced in type 2 diabetes compared with controls during glucose pulses. This important observation could reflect the technical limitation of autocorrelation analysis to identify only those periodicities that approximate multiples of twice the sampling interval and recur with suitably high consistency. Interestingly, spectral analysis of insulin concentration profiles from islets perifused with oscillatory glucose was able to reveal the intrinsic \( \sim 4-\text{to-}5\-\text{min} \) pulse interval. However, when the modeled insulin concentration profile anticipated in the systemic circulation was examined by spectral analysis, like autocorrelation, it failed to reliably recognize the \( \sim 4-\text{to-}5\-\text{min} \) pulse interval next to the dominant \( \sim 10\-\text{min} \) insulin oscillations, thus incorrectly implying entrainment.

The present data indicate that variability in insulin burst mass reflects occasional concordance between a stable islet pulse frequency and exogenously fixed glucose stimuli. Detection of discrete insulin bursts in the systemic circulation is often a consequence of overlap between small and large insulin pulses, the latter arising as a consequence of the coincident increase in glucose concentration. Stability of the frequency of pulsatile insulin secretion to time-invariant stimulation with various secretagogues has been inferred by others (8, 9, 28–30). The present study extends this notion by direct assessment of pulsatile insulin secretion by isolated human islets with oscillatory glucose. Although the mechanism for generation of insulin pulses is independent of prevailing glucose concentrations, in the present conditions, insulin-secretory burst mass rose severalfold whenever glucose maxima coincided with the expected time of insulin pulses. Cross-correlation analysis using concomitant 1-min glucose and insulin data disclosed that the response lag was \( <1 \) min. The secretion pattern in response to oscillatory glucose was therefore characterized by the coexistence of large insulin pulses next to small ones. Given the waveform damping and delay of the insulin secretion profile reaching the systemic circulation in vivo (27, 34), the simplified model prediction (Fig. 4) is that the smaller insulin pulses between the amplified pulses would not be resolved at this sampling site, wherein diffusion and turbulent blood flow introduce further nonuniformities. Although we cannot be certain that the same circumstances prevail in the intact pancreas as observed here in isolated islets, given the robust nature of the pacemaker dictating pulsatile insulin secretion the ac-
companying data allow the unifying postulate that the apparent entrainment of insulin pulses in vivo reflects the intermittent concordance of pacemaker activity with glucose elevations, undersampling, and false-negative errors in systemic pulse identification in that circumstance. An important question is, why was entrainment not present in patients with type 2 diabetes?

The most likely explanation is that in type 2 diabetes there is an attenuated response of insulin secretion to glucose stimulation (12, 21, 22, 38). Typically, β-cell function is assessed clinically by supraphysiological glucose stimulation followed by measurement of first-phase insulin secretion (4, 20, 21). The elegant studies of Pørksen et al. (25) established a much more physiological protocol by intermittent infusion of small glucose pulses (amplitude \( \sim 0.3 \text{ mmol/l} \)), which correspond to glucose oscillations that are present in humans (17). Given the high sensitivity of physiological vs. supraphysiological stimulation tests in endocrinology, the use of physiological glucose pulses might be a more sensitive discriminator, although this has not been formally verified to date. The present data allow the hypothesis that failure to detect entrainment of insulin pulses in patients with type 2 diabetes, given small glucose pulses, reflects lesser stimulation by glucose of concordant insulin pulses compared with nondiabetic subjects.

In the present islet perfusion studies, oscillating vs. constant glucose delivery also had a prominent effect to synchronize pulsatile insulin release by the 10–30 islets in each perfusion chamber. It is well documented that each independent islet has the capacity to serve as a pacemaker (2, 16, 18) and secrete insulin in a pulsatile manner at an interval of \( \sim 4 \text{ min} \) (32, 33). Single perfused islets secrete \( \sim 60\% \) of insulin in pulses (16, 33), a comparable proportion with that present in vivo. However, groups of perfused islets release a much lower proportion of their net insulin release in measurable insulin pulses (30–40%) (32, 33). The likely explanation for this difference is that groups of islets in the perfusion system are only partially synchronized, so although each individual islet is likely releasing most of its insulin in pulses, only those islets that are synchronized secrete insulin in pulses that are detectable in the perfusate. Interestingly, intermittent glucose pulses, even at a pulse interval that exceeded the inherent pulse interval for pulsatile insulin secretion, led to a marked increase (twofold) in the proportion of insulin secreted as detectable insulin pulses from the groups of islets. It is unlikely that this coordinating action is responsible for the reported capacity of infused glucose pulses to discriminate between patients with type 2 diabetes and control subjects (6, 17), because the proportion of insulin secreted in pulses is comparable in these two groups.

In the neuroendocrine system, pulsatile hormone secretion is important for eliciting a sustained and appropriate target tissue response. For example, pulsatile luteinizing hormone-releasing hormone (LHRH) release elicits greater secretion response of luteinizing hormone (LH) than does constant LHRH delivery (3). In healthy humans, blood glucose concentrations oscillate with a frequency (\( \sim 4 \text{ min} \)) that is similar to high-frequency insulin pulses (6, 17). This raises the possibility that one of the contributory factors toward impaired insulin secretion in response to a glucose challenge seen in type 2 diabetes is downregulation of this system due to constant hyperglycemia in type 2 diabetes. However, the present data argue against this notion. We report that there are no time-dependent differences in the insulin secretion rate with oscillatory or constant glucose stimulation (Fig. 7). Therefore, we reject the second hypothesis, that insulin secretion might be enhanced by oscillating vs. constant glucose stimulation. However, one limitation of the present experiments is that the perfusion experiments were performed for a period of only 100 min to avoid functional deterioration of the islets. It is possible that exposure of islets to oscillating vs. constant glucose over much longer periods would result in enhanced insulin release in response to oscillating glucose.

In conclusion, the present experiments demonstrate that the burst interval of pulsatile insulin secretion from isolated human islets is not entrained to glucose oscillations. The entrainment of pulsatile insulin to infused glucose oscillations in nondiabetic humans in vivo likely reflects entrainment of pulse mass without changes in underlying pacemaker activity. Oscillations of stimulatory glucose concentrations provide no quantitative advantage over constant glucose stimulation of insulin secretion but selectively enhance the pulsatile component of insulin secretion. Therefore, it is unlikely that the loss of glucose oscillations contributes to deficient insulin secretion in type 2 diabetes. Given the limitations of high-frequency pulse detection during intermittent glucose oscillations while sampling from the systemic circulation, any future efforts to prove the concept of insulin pulse entrainment in vivo should include portal vein sampling at 1-min intervals and a validated approach for pulse detection. Because entrainment of insulin pulses is absent in the present studies, perhaps it might be more appropriate to consider the phenomenon as the induction of amplified insulin pulses by coincident intermittent glucose pulses.

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