Impact of continuous and pulsatile insulin delivery on net hepatic glucose uptake

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Grubert, Jaime M., Margaret Lautz, D. Brooks Lacy, Mary C. Moore, Ben Farmer, Angelina Penaloza, Alan D. Cherrington, and Owen P. McGuinness. Impact of continuous and pulsatile insulin delivery on net hepatic glucose uptake. Am J Physiol Endocrinol Metab 289: E232–E240, 2005.—The pancreas releases insulin in a pulsatile manner; however, studies assessing the liver’s response to insulin have used constant infusion rates. Our aims were to determine whether the secretion pattern of insulin in conscious dogs fasted for 42 h received infusions including insulin was not infused. A variable duration, followed by intervals of 11 and 8 min (CON, 1/11, and 4/8, respectively) in which insulin was infused in a pulsatile manner; during a peripheral insulin delivery pattern (1/11; 4/8), respectively. Net hepatic glucose uptake (NHGU) was not augmented by pulsatile insulin delivery, and there is no evidence of entrainment in hepatic glucose metabolism. Thus the loss of insulin pulsatility per se likely has little or no impact on the effectiveness of insulin in regulating glucose metabolism. Therefore, the impact of insulin pulsatility on glucose metabolism has been studied. In 1982, Goodner et al. (5) reported that hepatic glucose production oscillated with fluctuations in insulin and glucagon in the rhesus monkey. The specific roles of insulin and glucagon were not assessed. Studies in humans suggested that pulsatile insulin delivery into a peripheral vein had a greater hypoglycemic effect than more effective than a constant insulin infusion in enhancing glucose disposal (2, 22) during a euglycemic hyperinsulinemic clamp. Other investigators suggested that the efficacy of pulsatile insulin is dependent on the glucose concentration and insulin pulse pattern (17, 19). However, not all studies have observed an augmentation of insulin action with pulsatile insulin delivery (7, 18).

The liver may be able to respond to the pulsatile nature of insulin delivery. Liver glucose production responds rapidly (maximal effect within 15 min) to acute changes in insulin delivery (25, 27). Although evidence suggests that pulsatile insulin delivery may be able to regulate liver glucose production, the impact of pulsatile insulin delivery on liver glucose uptake has not been examined, especially when insulin is delivered via its physiological route. Interestingly, in the presence of hyperglycemia, acute increases in insulin are relatively slow modulators of liver glucose uptake relative to the portal signal (16); yet liver glucose uptake is very responsive to insulin. The presence of hyperglycemia, suppression of hepatic sinusoidal insulin concentrations rapidly shifts the liver from a glucose consumer to a producer (3). Although the time to manifest the peak effect of insulin is relatively slow, its effects on suppression of liver glucose production are rapid and potent. Given that insulin oscillates between concentrations that are both above and below basal levels of insulin, it could switch the liver from a glucose producer to a consumer. Thus it is unclear whether the rapid oscillations in insulin seen with pulsatile insulin delivery will entrain liver glucose uptake.

The aim of the present study was to determine whether liver glucose uptake is influenced by pulsatile nature of insulin secretion. Specifically, this study examined 1) whether pulsatile insulin delivery enhanced liver glucose uptake via its physiological route and 2) whether oscillations in hepatic sinusoidal insulin levels would entrain liver glucose uptake.

METHODS


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5006 (Purina Mills, St. Louis, MO) once daily and had free access to water. The composition of the diet based on dry weight was 52% carbohydrate, 31% protein, 11% fat, and 6% fiber. Dogs were housed in a facility that met American Association for the Accreditation of Laboratory Animal Care International guidelines. The Vanderbilt University Medical Center Animal Care Committee approved the protocols. The health of the animals was determined before surgery as having a good appetite (i.e., consumed ≥75% of the daily ration), normal stools, hematocrit >35%, and leukocyte count <18,000/mm³.

**Experimental preparation.** Fourteen to seventeen days before a study, a laparotomy was performed using sterile techniques with general anesthesia (15 mg/kg thiopental sodium iv for induction and 1.0% isoflurane as an inhalant during surgery) on healthy dogs. During the laparotomy, blood sampling catheters (0.04 in. ID) were positioned in the portal and left common hepatic veins. Insuffusion catheters (0.04 in. ID) were placed in the splenic and jejunal veins for portal vein hormone delivery. Flow probes (Transonic Systems, Ithaca, NY) were positioned about the portal vein and hepatic artery. After an incision in the right inguinal region, a sampling catheter (0.04 in. ID) was advanced from the right external iliac artery to the abdominal aorta.

The catheters were filled with 0.9% NaCl (saline) containing heparin (200 U/ml). The free ends of the catheters and flow probes were exteriorized and placed in subcutaneous pockets. The dogs received penicillin G (500,000 U iv) in 1 liter of saline to minimize the possibility of infection. Flunixamine (0.1 mg/kg; Fort Dodge Laboratory, Fort Dodge, IA) was injected intramuscularly immediately after wound closure for acute pain relief. Dogs also received penicillin G (600,000 U im) for 3 days after surgery.

**Experimental protocol.** The studies were performed on 42-h-fasted conscious dogs. Forty-two-hour fasting was chosen because in the dog this fast duration does not cause substantial liver glycogen depletion. It does lead to stable hepatic glycogen levels and basal glucose flux while not altering the ability of the liver to switch to a glucose consumer or respond to insulin. The free ends of all catheters were exteriorized under local anesthesia (2% lidocaine), and their contents were aspirated and flushed with saline. The free ends of the flow probes were also exteriorized and connected to a flowmeter (Transonic Systems). Dogs were placed in a Pavlov harness for the duration of the study.

**Experimental design.** Each experiment consisted of a period to establish a pancreatic clamp (−210 to −150 min), a tracer equilibration period (−150 to −30 min), a basal period (−30 to 0 min), and an experimental period (0–180 min; see Fig. 1). At −210 min, a constant infusion of indocyanine green (0.07 mg/min), somatostatin (SRIF; Bachem, Torrence CA; 0.8 μg·kg⁻¹·min⁻¹), and glucagon (0.9 ng·kg⁻¹·min⁻¹) were given into a peripheral vein. In addition, insulin was infused into the portal vein (0.25 μU·kg⁻¹·min⁻¹) between −120 and 0 min. After 0 min, the time course of the insulin delivery varied, depending on the protocol. At $t = -150$ min a bolus (100 μU), followed by a constant infusion (1 μU/C/min) of $[3-3H]glucose$, was given into a peripheral. Beginning at $t = -150$ min, arterial plasma glucose levels were clamped at 200 mg/dl for the duration of the experiment using a variable glucose infusion containing $[3-3H]glucose$. The addition of tracer to the exogenous glucose helped to minimize excursions in plasma specific activity during the clamp.

After $t = 0$ min, an animal underwent one of three protocols. In **protocol 1** (CON), at the beginning of the experimental period, the intraportal insulin infusion rate was increased to 1 mU·kg⁻¹·min⁻¹. Insulin was delivered continuously at this rate throughout the duration of the study. In **protocol 2** [PULS(1/11)], at the beginning of the experimental period, insulin was infused into the portal vein in a pulsatile manner with an interval of 1 min during which the insulin pump was turned “on,” followed by an interval of 11 min during which the pump was turned “off.” During the “on” period insulin was infused at a rate of 12 mU·kg⁻¹·min⁻¹. Insulin was delivered in this manner throughout the duration of the experiment. In **protocol 3** [PULS(4/8)], at the beginning of the experimental period insulin was infused into the portal vein in a pulsatile manner, with an interval of 4 min, during which the insulin pump was turned “on,” followed by an interval of 8 min, during which the pump was turned “off.” During the “on” period insulin was infused at a rate of 3 mU·kg⁻¹·min⁻¹. Insulin was delivered in this manner throughout the duration of the experiment. In both protocols 2 and 3, during the “off” period insulin was not being infused. Thus the average insulin infusion rate in CON was the same as in PULS(1/11) and PULS(4/8). During the rapid sampling period (120–144 min; see below), the variable glucose infusion was not adjusted.

Arterial blood samples were taken every 10 min throughout the study, except during the rapid sampling period (120–144 min), when they were taken every minute. Blood samples from the hepatic vein and portal vein were collected at 10-min intervals during the basal period (−30 to 0 min) and at the end of the experimental period (150–180 min), and at 1 min intervals during the rapid sampling period (120–144 min) within the experimental period (0–180 min).

**Sample processing and analysis.** Blood samples were collected and placed in EDTA-containing tubes centrifuged on the day of the study. Plasma glucose was measured using a Beckman glucose analyzer. Plasma glucose specific activity (SP) was measured in triplicate using the Somogyi-Nelson method (29). Immunoreactive insulin and glucagon were assayed using double-antibody techniques with intra-assay coefficients of variation of 11 and 10%, respectively (14).

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**Fig. 1.** Experimental protocol for protocols 1 (equal amounts continuously at 4X basal; CON), 2 [pulsatile for 1 min on/11 min off; PULS(1/11)], and 3 [pulsatile for 4 min on/8 min off; PULS(4/8)].
Hepatic artery and portal vein blood flows were assessed using Transonic flow probes. Indocyanine green dye infusion was used to assess liver blood flow in two cases where flow probes were not functioning (9). In those two cases, the contribution of hepatic artery and portal vein blood flow to total hepatic blood flow was assumed to equal the mean contribution in the other studies in which the Transonic flow probes functioned.

**Calculations.** The glucose and hormone (insulin) loads entering the liver were calculated as the sum of the loads in the hepatic artery and portal vein, \( \frac{A_s}{H_{ABF}} + \frac{P_s}{H_{PBF}} \), where \( A_s \) and \( P_s \) represent substrate or hormone concentrations in the artery and portal vein, and \( H_{ABF} \) and \( H_{PBF} \) represent blood flows in the hepatic artery and portal vein. Plasma flow, rather than blood flow, was used when hormone loads were calculated. Similarly, the substrate load leaving the liver equaled \( H_e \times H_{THBF} \), in which \( H_e \) and \( H_{THBF} \) represent the hepatic vein substrate concentration and total hepatic blood flow (HABF + PBF). Sinusoidal insulin levels were calculated as the ratio of the entering insulin load to the liver and the hepatic plasma flow. Net hepatic glucose uptake (NHGU) rate was calculated as the difference between the entering and exiting glucose loads. Net hepatic fractional extraction of glucose was calculated as the ratio of NHGU and hepatic glucose load.

Tracer-determined hepatic glucose uptake (HGU) was calculated as the ratio of \([\text{H}]\)glucose uptake by the liver and inflowing \([\text{H}]\)glucose SA; assumptions of this method have been previously described (12, 13). Hepatic glucose production was the difference between HGU and NHGU.

**Spectral analysis.** Spectral analysis was used to test the possibility that the pulsatile input (insulin concentration) produced an HGU response with a pulsatile component at the input frequency (Matlab, Natick, MA). This analysis revealed the nature of the insulin input pulses. NHGU was examined by taking advantage of special properties of deconvolution, with filtering of high frequency components resulting from system noise. The power spectrum was then examined to determine the estimated magnitude of the glucose uptake response. The power spectrum of each signal was determined by first fitting detrended data with a 7\( q \)-order autoregression model with the structure \( A(q) \times y(t) = e(t) \) where \( A(q) \) is the matrix of the parameters of...
the autoregression model, \( y(t) \) is the time series data, and \( e(t) \) is the loss function. The power spectral density is the frequency response calculated for this matrix. This gives the frequency components of the input and output signals (sinusoidal insulin levels and NHGU, respectively) without the significant noise components seen with fast Fourier transform analysis of the raw data.

Cross-correlation analysis. Cross-correlation analysis was used to estimate the degree to which sinusoidal plasma insulin levels and NHGU were correlated. The cross-correlation at delay, \( d \), was defined as

\[
 r = \frac{\sum_i [(x(i) - m(x)) \cdot (y(i - d) - m(y))] / \sqrt{\sum_i [x(i) - m(x)]^2 \cdot \sum_i [y(i - d) - m(y)]^2}}{
}

for two series \( x(i) \) (sinusoidal insulin concentrations) and \( y(i) \) (NHGU), where \( m(x) \) and \( m(y) \) were the means of the corresponding series and \( i = 0, 1, 2 \ldots n - 1 \). Correlations between signals were evaluated up to a maximum delay of 4 min. In other words, the output signal (NHGU) was shifted across the input signal (sinusoidal insulin). For each shift, the sum of the product of the newly lined-up terms in the series was computed. Large correlation coefficients \( (r^2 > 0.5 \text{ or } 50\%) \) would suggest that the variation in NHGU may have been attributed to the corresponding differences in plasma insulin concentrations.

Statistics. For the majority of data, results are expressed as means \( \pm \) SE. Statistical comparisons were made with a two-way ANOVA for repeated measures and post hoc analysis by a Tukey test (SigmaStat). The level of significance was set at \( P < 0.05 \).

RESULTS

In each group, the peripheral glucose infusion doubled the plasma glucose level. During the basal period, arterial glucose levels were \( 217 \pm 8, 212 \pm 2, \text{ and } 217 \pm 4 \text{ mg/dl for CON, PULS(1/11), and PULS(4/8) respectively} \). They remained relatively stable for the experimental period at \( 202 \pm 4, 189 \pm 3, \text{ and } 209 \pm 2 \text{ mg/dl, respectively} \) (Fig. 2).

Hepatic blood flow was \( 25 \pm 1, 31 \pm 3, \text{ and } 29 \pm 3 \text{ ml kg}^{-1} \text{ min}^{-1} \) during the basal period and did not change.
significantly during the experimental period (26 ± 1, 32 ± 2, and 29 ± 2 ml·kg⁻¹·min⁻¹). Hepatic glucose loads were similar between the basal (38 ± 3, 47 ± 4, and 45 ± 6 mg·kg⁻¹·min⁻¹) and experimental periods (37 ± 3, 43 ± 2, and 44 ± 3 mg·kg⁻¹·min⁻¹) for each group.

Mean arterial plasma insulin levels were not different among groups throughout the study. In CON, the arterial insulin levels increased from 6 ± 1 μU/ml in the basal period to 22 ± 4 μU/ml in the experimental period. In PULS(1/11) and PULS(4/8), the arterial insulin levels oscillated between 5 ± 1 and 71 ± 21 μU/ml and between 5 ± 1 and 45 ± 5 μU/ml, respectively, during the experimental period (Fig. 3). Sinusoidal insulin levels during the basal period were 18 ± 4, 12 ± 3, and 14 ± 2 μU/ml in CON, PULS(4/8), and PULS(1/11), respectively. In CON, sinusoidal levels were increased to 50 ± 6 μU/ml during the experimental period and remained stable for the duration of the study (Fig. 4). In PULS(4/8) and PULS(1/11), arterial insulin levels oscillated during the experimental period from a nadir of 6 ± 1 and 5 ± 1 μU/ml to a peak of 123 ± 43 and 416 ± 79 μU/ml, respectively. Arterial plasma glucagon levels remained basal and did not differ between groups [47 ± 5 to 43 ± 5, 44 ± 5 to 39 ± 5, and 44 ± 3 to 39 ± 3 pg/ml; basal to experimental period in CON, PULS(1/11), and PULS(4/8), respectively]. In all groups, arterial C-peptide levels were very low (~0.2 ng/ml) and did not change during the study, indicating that endogenous insulin secretion was suppressed.

During the basal period, NHGU was −0.8 ± 0.3, 0.4 ± 0.2, and −0.9 ± 0.4 mg·kg⁻¹·min⁻¹ in CON, PULS(1/11), and PULS(4/8), respectively. Pulsatile and continuous insulin infusion resulted in similar rates of NHGU (Fig. 5) during the experimental period [1.3 ± 0.2, 1.7 ± 0.7, and 2.4 ± 0.4 mg·kg⁻¹·min⁻¹ in CON, PULS(1/11), and PULS(4/8), respectively]. Tracer-determined HGU was also similar during the experimental period (1.6 ± 0.2, 2.1 ± 0.8, and 2.6 ± 0.4 mg·kg⁻¹·min⁻¹). NHGU and tracer-determined HGU were slightly lower in the control group than in the other two groups, but this resulted from the lower hepatic blood flow and, consequently, a reduced hepatic glucose load. Net fractional hepatic glucose extraction avoids this difficulty and indicates clearly that the pattern of insulin infusion had no effect on the liver (0.04 ± 0.01, 0.04 ± 0.01, and 0.05 ± 0.01, respectively). Hepatic glucose production was also not differ-

Fig. 4. Sinusoidal plasma insulin levels for 42-h-fasted, conscious dogs during the rapid sampling interval for insulin infused in CON and PULS(1/11) or PULS(4/8); n = 5/group. Studies were performed in the presence of SRIF, basal glucagon, peripheral glucose infusion (2× basal). Data are means ± SE.

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Spectral estimates of sinusoidal insulin concentrations and NHGU taken during the rapid sampling interval indicated that there was no pulsatile component for the input in CON (Figs. 6 and 7). The system output, or NHGU signal, did not reveal pulsatile components with periods of 12 min in any of the groups. Furthermore, spectral analysis did not reveal any pulsatile components across the spectrum that were distinguishable from random noise. Cross-correlation analysis between the sinusoidal insulin concentrations and NHGU supported the results from the spectral analysis. For all conditions, \( r^2 \) values were <0.05 (Table 1). These data reveal that there was no significant correlation between oscillations in NHGU and the oscillations in sinusoidal insulin levels.

**DISCUSSION**

It is controversial whether the pulsatile nature of insulin secretion enhances the effectiveness of insulin on whole body glucose metabolism. Many of the studies, however, did not examine specific effects on the liver and did not deliver insulin via its physiological route. In addition, no studies have examined the effect of the pulsatile nature of insulin on HGU. The present study demonstrated that, in the presence of hyperglycemia, hyperinsulinemia and basal glucagon, NHGU was not differentially augmented by the pulsatile nature of insulin that was delivered into the portal vein.

This study investigated whether pulsatile vs. continuous insulin delivery influenced the liver’s ability to consume glucose. Our results suggest that, regardless of the delivery pattern, liver glucose uptake responds to the amount of insulin delivered rather than the infusion pattern itself. The amount of insulin delivered was the same in each group throughout the study, and the magnitude of the NHGU response was similar in each group. NHGU ranged from 1.3 ± 0.2 to 2.4 ± 0.4 mg·kg\(^{-1}\)·min\(^{-1}\) and was stable for the duration of the study. This is consistent with previous data obtained in the conscious dog under similar hyperglycemic hyperinsulinemic conditions (1, 15). Nevertheless, NHGU tended to be slightly less during the experimental period in the control group than in the other two groups. This difference was due to the differing hepatic glucose load in CON, which was a consequence of a lower hepatic blood flow in CON. Tracer-determined HGU revealed a similar trend. Measurements that account for differences in

[Fig. 5. Net hepatic glucose uptake (NHGU) and tracer-determined hepatic glucose uptake (HGU; top), and net hepatic fractional extraction (NHFE) and tracer-determined fractional extraction (bottom) of glucose in 42-h-fasted, conscious dogs during the rapid sampling interval for CON, PULS(1/11), or PULS(4/8); \( n = 5 \) group. Studies were performed in the presence of SRIF, basal glucagon, and peripheral glucose infusion (2× basal). Data are means ± SE.]

[Fig. 6. NHGU in 42-h-fasted, conscious dogs during the rapid sampling interval for CON or PULS(1/11) and PULS(4/8); \( n = 5 \) group. Studies were performed in presence of SRIF, basal glucagon, peripheral glucose infusion (2× basal). Data are means ± SE.]
glucose load, i.e., net hepatic fractional extraction and tracer-determined fractional extraction of glucose, were identical in all three groups, clearly attesting to the inability of pulsatile insulin delivery to modify the overall response of the liver to insulin.

The steady-state insulin and glucose levels that we chose in the constant infusion group were designed to suppress hepatic glucose production, allowing us to see the full effects of pulsatile insulin on glucose uptake by the liver. Yet at the same time, the trough of insulin in the pulsatile groups fell below basal levels, allowing changes in glucose production, which is known to entrain to a pulsatile insulin signal in the absence of hyperglycemia, to contribute to any oscillatory behavior of NHGU. In our studies, mean arterial plasma insulin levels were four times the basal level regardless of whether the insulin was infused continuously or as a continuous or pulsatile infusion. In addition, glucose was infused peripherally to raise the glucose load to the liver to twice basal. Under these conditions, tracer determined hepatic glucose release was suppressed to <0.4 mg·kg⁻¹·min⁻¹ in all groups. This observation is consistent with previous studies in which plasma glucose concentrations >190 mg/dl in the presence of elevated insulin levels (4× basal) were shown to effectively suppress glucose production by the liver (23, 26). It is surprising in the pulsatile groups that, at the trough of insulin levels, hepatic glucose production did not increase.

Previous studies have demonstrated that the effects of pulsatile insulin are particularly sensitive to the pulse frequency and plasma glucagon levels (18, 19). When infusing insulin in the present study, we selected a pulse pattern that reflected a physiological period and amplitude. Our pulse period (12 min) was chosen based on physiological data (ranging from 9 to 12 min/cycle) reported in dogs (6, 10, 30). Pulse amplitudes for plasma insulin in the artery [200–500% of the mean arterial plasma insulin concentration for PULS(1/11) and PULS(4/8), respectively], were also consistent with previous studies in dogs and humans in which the amplitude of insulin levels in the artery varied from 50 to 800% of the mean. Insulin was delivered intraportally so that the infusion route would mimic the physiological release of insulin from the pancreas. This way, the liver sinusoid could experience the full effect of oscillations in the insulin levels. This approach was in contrast to the peripheral infusion sites used in earlier studies, which may have resulted in degradation or attenuation of the insulin pulse by the time the signal reached the liver sinusoid. In conducting these studies, arterial plasma glucagon were kept at basal levels, thereby permitting the enhanced effects of pulsatile insulin to be observed should they have occurred (19).

Arterial plasma insulin concentrations clearly depicted the hormone delivery pattern, with levels remaining in the physiological range and oscillating between 5 ± 1 and 71 ± 21 μU/ml in PULS(1/11) and between 6 ± 1 and 45 ± 5 μU/ml in PULS(4/8). In PULS(1/11), arterial insulin abruptly rose at the onset of the insulin infusion and rapidly declined upon cessation of the pump. In PULS(4/8), arterial plasma insulin levels gradually rose, peaking at 4 min and rapidly declining upon discontinuation of the pump. Estimated sinusoidal insulin levels did not depict the pulse pattern and increments over time with the same fidelity as in the artery. This is because portal vein insulin levels were prone to random noise due to incomplete mixing arising from portal vein infusion into the laminar flow of the portal vein. The estimated sinusoidal concentrations

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Table 1. Cross-correlation coefficients (r²) for comparison of sinusoidal plasma insulin levels and NHGU for time shifts of 0–4 min in CON, PULS(1/11), and PULS(4/8)

<table>
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<th>PULS(4/8)</th>
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Comparisons were performed during the rapid sampling interval (120–144 min) in 42 h fasted conscious dogs (n = 5/group) CON, equal amounts continuously at 4× basal; PULS(1/11), pulsatile for 1 min on/11 min off; PULS(4/8), pulsatile for 4 min on/8 min off; NHGU, net hepatic glucose uptake.

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Fig. 7. Spectral estimates of plasma insulin in the hepatic sinusoid and NHGU in 42-h-fasted, conscious dogs during the rapid sampling interval for CON (top), PULS(1/11) (middle), and PULS(4/8) (bottom). Insulin was infused with pulses having a period of 12 min. Studies were performed in the presence of SRIF, basal glucagon, and peripheral glucose infusion (2× basal). Estimates are representative data of one dog in each group.
were in the physiological range for all groups. In CON, sinusoidal insulin levels increased from 18 ± 4 μU/ml in the basal period to 62 ± 8 μU/ml in the experimental period. In PULS(4/8), sinusoidal insulin levels oscillated between 6 ± 1 and 123 ± 23 μU/ml and reached a steady state within 2 min after the pulse began (Fig. 5). On the basis of previous studies (15) establishing the dose-response relationship between sinusoidal insulin levels and NHGU in the conscious dog, insulin concentrations remained consistently below the saturating threshold (Fig. 5). In PULS(1/11), sinusoidal insulin levels peaked (416 ± 79 μU/ml) above saturation threshold (~200 μU/ml (15)), so that for a brief period the liver was presented with more insulin than was required for maximal activation of NHGU. With such a substantial amount of insulin being infused so quickly, it is likely that some insulin was degraded without imparting a biological effect. Because of this, the functional amount of insulin to which the liver was exposed could be argued to be less in PULS(1/11) than in CON and in PULS(4/8). Hypothetically, one might expect that the area under the curve (AUC) for NHGU in PULS(1/11) would also be less. In the present study this was not the case; AUCs for all groups were similar.

Analysis from the present study revealed that J) frequency components of the input signal (insulin pulse) were distinct from the frequency components of the output signal (NHGU), and 2) the oscillations in sinusoidal insulin levels were not correlated to oscillations in NHGU (Fig. 7). To explain why NHGU was not entrained by oscillations in insulin involves examining the time dependency of the liver to changes in sinusoidal insulin levels. Past studies have demonstrated that net hepatic glucose output or production responds quickly to changes in insulin. Within 15 min, the liver responds to increases and decreases in insulin (26, 31). By contrast, in the presence of hyperglycemia, a fourfold rise in insulin delivered intraportally had little effect in 15 min and was not maximal until 90 min (16). Yet, if sinusoidal insulin was decreased to below basal, as was the case in both pulsatile groups, hepatic glucose production rapidly increased despite development of hyperglycemia (3). The time course in which the liver responds to a decrease in insulin in the presence of sustained hyperglycemia had not been examined previously. The excursions in hepatic sinusoidal insulin levels were large; peak values were more than 20-fold increased from the nadir, and the nadir was one-third of that seen in the basal insulin infusion period. Thus the liver should have switched from a modest producer of glucose at the nadir to a significant consumer of glucose at the peak insulin levels (25). On the basis of the present study, no such excursion was observed. Thus NHGU is too slow to respond to the rapid changes in insulin in the presence of hyperglycemia and/or the magnitude of the change in NHGU during a 12-min oscillatory period was too small to be evident. Taken in context, although the pancreas secretes insulin in a pulsatile manner, the pulsatile nature of the secretory process has little beneficial effect on insulin action for durations typically required to absorb a meal. Previous reports observed only modest beneficial effects of pulsatile insulin delivery generally, and when present it was evident only after a few hours (11, 17). Moreover, in most of the studies the effect on the liver was not examined. In addition, the delivery of pulsatile insulin into a peripheral vein may not mimic the response seen with portal delivery. We chose to expose the liver to a pulsatile signal for 3.5 h because liver glucose uptake occurs physiologically only when a meal is being absorbed, which typically lasts no more than 3 h. It is possible, however, that increasing the duration of the pulsatile insulin signal to a nonphysiological duration may have unmasked a pulsatile-dependent effect in liver glucose uptake. However, we feel that this is unlikely, since we found no evidence that liver glucose uptake would even entrain to a pulsatile insulin signal. This contrasts with the regulation of glucose production, which can be entrained to a pulsatile insulin signal. The peak arterial insulin concentration is much higher when insulin is given peripherally. One possibility is that peripheral pulsatile insulin delivery may have suppressed nonesterified fatty acids (NEFA), which in turn would indirectly amplify insulin suppression of liver glucose production (20, 28). This could explain the variable response seen when investigators examined the regulation of glucose production by pulsatile insulin secretion. However, recent work suggests that, at least with doses of insulin that induce mild hypoglycemia, fatty acids were equally suppressed by both constant and pulsatile insulin delivery (4). We did not measure NEFA concentrations; however, the combined hyperglycemia and hyperinsulinemia should suppress NEFA concentrations, and thus eliminate this indirect mediator of insulin action (21, 24).

In summary, the presence of pulsatile insulin secretion for a physiological duration does not amplify the effects of insulin in stimulating liver glucose uptake. Moreover, the pulsatile nature of insulin secretion is unable to entrain liver glucose uptake. Thus, although defects in pulsatile insulin secretion may presage degradation of pancreatic function, the loss of pulsatility per se likely has little or no impact on the effectiveness of insulin in regulating liver glucose uptake.

GRANTS

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