Oscillatory Insulin Secretion After Pancreas Transplant

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In vivo studies of β-cell secretory function have demonstrated the existence of rapid insulin oscillations of small amplitude recurring every 8–15 min in normal subjects. This study evaluated the effects of pancreas transplant on rapid insulin oscillations. Samples for glucose, insulin, and C-peptide were drawn during constant glucose infusion at 2-min intervals for 90 min from six successful Px patients with type I diabetes mellitus, from six normal nondiabetic control subjects, and from three Kx subjects. A computerized algorithm (ULTRA) was used for pulse detection. In the Px group, the average insulin pulse period was significantly shorter than in both the control and Kx groups (Px 8.1 ± 0.5, control 12.5 ± 0.7, Kx 12.4 ± 0.5 min, $P < 0.0005$). By contrast, the C-peptide pulse periods (Px 16.8 ± 2.3, control 14.7 ± 1.2, Kx 15.3 ± 1.5 min) were similar in the three groups. Spectral analysis confirmed that the frequency of the insulin pulses was increased in the Px group. The absolute amplitude of the insulin pulses was greater in the Px group ($P < 0.001$) while the amplitude of the C-peptide pulses did not differ between the groups. Cross-correlation analysis demonstrated maximal correlation coefficients at a lag of 0 min between insulin and C-peptide (control $r = 0.33$, $P < 0.0001$; Kx $r = 0.17$, $P = 0.06$) and between insulin and glucose (control $r = 0.21$, $P < 0.001$; Kx $r = 0.20$, $P < 0.02$) in the control and Kx groups, respectively, whereas no significant correlations were observed at any lag in the Px group. Thus, insulin oscillations, which are of larger amplitude and occur with greater frequency than in normal control subjects, may be detected in the peripheral circulation after pancreas transplant. Although their persistence after transplant supports the hypothesis that they reflect the existence of an intrinsic islet cell pacemaker, the increased frequency of the oscillations in the Px group raises the possibility that this intrinsic pacemaker in normal subjects may be modified by extrinsic neural factors.
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In recent years, increasing numbers of patients with type I diabetes mellitus and end-stage renal disease are undergoing successful kidney-pancreas transplants (1–4). Although most subjects are euglycemic while off antidiabetic drugs after this combined transplantation procedure, whether the β-cell secretory responses are similar to those observed in normal subjects is unclear. In normal subjects, many in vivo studies have demonstrated that insulin is released from the β-cell in a pulsatile fashion (5–12). These pulses are characterized by rapid oscillations of small amplitude occurring every 8–15 min that are superimposed on slower (ultradian) oscillations occurring every 1.5–2 h. Whereas the ultradian oscillations appear to be tightly coupled to changes in plasma glucose (11,12), the mechanisms controlling the rapid oscillations have not been clearly defined. The periodicity of these pulses is lengthened in some subjects who have had a truncal vagotomy (8), but experiments using anticholinergic agents have not reproduced these findings (8,13). Furthermore, these pulses have been demonstrated in several in vitro studies using isolated islets (14,15) and the isolated perfused pancreas (16–21). If neural factors were critical for the occurrence of these pulses in vivo, they might not be detectable in subjects who have undergone pancreas transplantation.

The rapid insulin oscillations are of large amplitude in the portal vein (18,22), but because of hepatic extraction

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Type I diabetes, insulin-dependent diabetes mellitus; Px, kidney-pancreas transplant; Kx, kidney transplant; RIA, radioimmunoassay; BUN, blood urea nitrogen; EDTA, ethylenediaminetetraacetic acid; CV, coefficient of variation; ANOVA, analysis of variance.

of insulin and dilution in the systemic circulation, their amplitude in peripheral blood is small, frequently in the range of 7–15 pM, which is very close to the limits of sensitivity of most standard insulin RIAs. This limits their detectability. After a combined kidney-pancreas transplant, where the venous drainage of the transplanted pancreas is into the systemic rather than the portal circulation, the effect from the hepatic extraction of insulin to reduce the amplitude of the pulses is nullified in part. Thus, basal insulin concentrations are higher (1–4) and rapid oscillations, if present, would be expected to be closer in amplitude to those described previously in the portal circulation.

In this study, insulin, C-peptide, and glucose concentrations were measured at 2-min intervals for 90 min during constant glucose infusion in six successful Px patients with type I diabetes, and the results were compared with the results from six control and three Kx subjects. The latter group was included to control for the effects of the immunosuppressive regimen and the compromised renal function in the kidney-pancreas recipients. Because measurement error could result in a significant number of false-positive or false-negative pulses, eight replicate measurements of insulin and C-peptide concentrations were performed on each sample to reduce the SE of the mean value at each time point. The data subsequently were analyzed using a previously validated computer algorithm for pulse identification (23). In addition, spectral analysis and cross-correlation analysis were performed.

RESEARCH DESIGN AND METHODS

Studies were performed on six male Px patients, on six male normal control subjects, and on three male Kx subjects. In the Px group, studies were performed ~1–2 yr after the transplant. All pancreas grafts were whole and cadaveric in source. Insulin therapy was discontinued 1 wk after the original operation in all the patients, and at the time of the study none were taking insulin or other medications for diabetes. The immunosuppressive regimens included azathioprine (Px 145 ± 10 mg, Kx 142 ± 8 mg), cyclosporine (Px 268 ± 29 mg, Kx 413 ± 70 mg, $P > 0.05$), and prednisone (Px 10.3 ± 4.4 mg, Kx 11.7 ± 1.7 mg). The individual weights, heights, and ages are given in Table 1. The mean BUN (Px 10.1 ± 1.4 mM, Kx 11.5 ± 2.6 mM) and creatinine (Px 140 ± 10 μM, Kx 206 ± 24 μM, $P > 0.09$) levels were not significantly different between the two transplant groups. In all six Px patients, the venous drainage of the pancreas allograft was into the iliac vessels, thereby providing systemic venous drainage rather than portal drainage of pancreatic endocrine secretions. Exocrine pancreatic secretions were diverted into the bladder in all cases. All Px patients and two of the Kx patients had normal fasting glucose levels at the time of the study. The third Kx patient had slightly elevated fasting glucose levels (6.5 mM). The control subjects did not have personal or family histories of diabetes mellitus. All studies were conducted 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.

TABLE 1
Individual weights, heights, and ages

Subjects	Weight (kg)	Height (cm)	Age (yr)
Px			
1	56.6	166.7	25
2	96.2	190.6	35
3	84.0	177.4	44
4	70.9	171.7	46
5	93.1	179.4	38
6	77.6	180.2	23
Control			
1	86.6	186.7	20
2	78.5	177.4	21
3	79.0	174.8	32
4	68.3	174.5	24
5	95.5	184.8	26
6	79.6	175.2	25
Kx			
1	90.1	176.4	29
2	97.1	185.4	32
3	93.5	180.5	37

Experimental protocol. After a 12-h overnight fast, an intravenous sampling catheter was introduced into a vein on the dorsum of one hand, and an infusion catheter was inserted into the opposite hand. The hand with the sampling catheter was kept in a heating blanket to ensure arterialization of the venous sample.

At 0800, an intravenous glucose infusion (20% dextrose) was started at a rate of 6 $mg \cdot kg^{-1} \cdot min^{-1}$. This was continued for 5.5 h during which time subjects remained recumbent and were not given access to food or water. Commencing at 1200, arterialized venous samples were drawn at 2-min intervals for 90 min for measurement of glucose, insulin, and C-peptide. The 4-h delay between the beginning of the glucose infusion and the beginning of sample collection allowed a steady state to be achieved after the transient glucose rise that is associated with the first few hours of initiating the glucose infusion (10).

Analytical methods. Blood samples for insulin measurements were allowed to clot at room temperature, and the serum was stored at −20°C until assayed. Samples for C-peptide were drawn into tubes at 4°C containing 500 Kallikrein inhibitor U/ml of approtinin (Trasylol) and 1.2 mg/ml EDTA. The plasma was immediately separated and stored frozen until assayed. Serum insulin was assayed by a double-antibody technique (24). Plasma C-peptide immunoreactivity was measured as described previously (25). Plasma glucose was measured with a YSI glucose analyzer (Yellow Springs, OH).

All samples from the same subject were measured in a single assay. To reduce the measurement error inherent in the assays, insulin and C-peptide concentrations were measured on eight aliquots at each time point, whereas glucose levels were measured in quadruplicate.

Data analysis. The intra-assay CVs for insulin and C-peptide were determined separately for each subject by calculating the SD of the 8 values at each time point and subsequently obtaining the mean of all 46 values

calculated over the duration of the study. The overall mean intra-assay CVs for C-peptide and insulin were 5.7 ± 0.3 and 9.1 ± 0.5%, respectively, with no significant differences between the three groups. In the subsequent analysis of the insulin and C-peptide pulses, the intra-assay CV for insulin and C-peptide that pertained to the assay for each individual study was used to distinguish true pulses from random assay noise. The inherent measurement error in glucose estimations using the YSI glucose analyzer is lower than that of hormonal assays and averaged 2% across studies and subjects. This value was considered the intra-assay CV for glucose.

To identify statistically significant pulses, each individual 90-min profile of plasma glucose, serum insulin, and plasma C-peptide was submitted to ULTRA, a computer program for pulse detection (23). The general principle of this algorithm is the elimination of all peaks for which 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. For each individual study, the relative SE of the measurements (calculated as the intra-assay CV divided by the square root of the number of replicate estimations) was considered to represent an upper limit for measurement error, and the threshold for pulse detection was set at twice the relative SE. Peaks that did not meet these threshold criteria were eliminated from the data set using an iterative process. The peaks remaining in this series were assumed to represent significant pulses. Absolute pulse amplitude was defined as the difference between the peak and the preceding trough, and relative pulse amplitude was defined as the absolute pulse amplitude divided by the value at the preceding trough.

In addition to the pulse analysis, which identifies statistically significant pulses individually, spectral analysis was performed to investigate whether the pulses in insulin and C-peptide occurred at a preferred periodicity rather than randomly. Each time series was detrended by removal of the first two harmonics. The spectral estimates were calculated with the use of a Tukey window as described by Jenkins and Watts (26). The bandwidth of the window was chosen to be 30 min, yielding a good compromise between stability and fidelity. The insulin and C-peptide spectra were pooled separately for the transplant patients and for each of the two control groups.

The relationships between the rapid oscillations of insulin and C-peptide and the rapid oscillations of insulin and glucose were quantified by calculating, for each experiment, the coefficient of cross-correlation between insulin and C-peptide and between insulin and glucose at time lags of 0, ± 2, ± 4, ± 6, and every 2 min up to ± 20 min. The largest coefficient of cross-correlation was identified for each pair of profiles, providing a global definition of the overall relationship between rapid pulses of insulin and C-peptide/glucose. Because long-term trends can obscure cross-correlation analysis, the data were detrended using a procedure described by Cleveland (27) with a window of 30 min before cross-correlation analysis was performed. Cross-correlation coefficients were pooled and compared using Fisher's z transformation (28).

Statistical analysis. All results are means ± SE. Differences in hormonal concentrations, pulse number, and amplitude between control subjects and transplant recipients were assessed by ANOVA. Significance of peaks in the pooled spectra was evaluated with a paired t test. Differences were regarded as significant if $P < 0.05$.

RESULTS

Profiles of insulin, C-peptide, and glucose. Figure 1 shows typical profiles of glucose, insulin, and C-peptide obtained in two Px patients and in two control subjects. In all subjects, rapid pulses of insulin, C-peptide, and glucose were observed throughout the 90-min study period. The overall mean insulin concentration was significantly higher in the Px group than in the control and Kx groups, whereas the difference between the control and Kx groups did not reach statistical significance (Px 565.5 ± 30.1, control 279.2 ± 44.9, Kx 400.7 ± 20.9 pM, $P < 0.001$). The mean C-peptide concentration was significantly different between the Kx and control groups (Px 2.31 ± 0.22, control 1.56 ± 0.27, Kx 2.79 ± 0.09 nM, $P < 0.02$), whereas the difference between the Px and control group did not quite reach statistical significance ($P > 0.06$). As a result of higher glucose levels during the study in the Kx patient who had elevated fasting glucoses, the mean glucose concentration was higher in the Kx group, but the difference did not reach statistical significance (Px 8.4 ± 0.7, control 7.7 ± 0.4, Kx 11.2 ± 2.2 mM, $P > 0.07$).

Pulse analysis. Table 2 gives the mean period and amplitude of the insulin, C-peptide, and glucose pulses detected. In all three groups, the C-peptide pulse periods observed were similar. By contrast, a significantly shorter insulin pulse period was observed in the Px group ($P < 0.0005$). Thus, a pulse of insulin was detected, on average, every 8 min in the Px group, whereas insulin pulses were observed approximately every 12.5 and 12.4 min in the control and Kx groups, respectively. The absolute and relative amplitude of the C-peptide pulses in the three groups were similar. Although the absolute amplitude of the insulin pulses was significantly greater in the transplant recipients ($P < 0.001$), the relative amplitudes of the pulses were similar in the three groups. The glucose pulse characteristics were not significantly different between the three groups.

Spectral analysis. Figure 2 shows the individual spectra from the insulin and C-peptide profiles of the two Px patients displayed in Fig. 1 as well as the pooled spectra from all the kidney-pancreas transplant patients. For the entire Px group, a significant peak exists in the pooled insulin spectrum at 5 min ($P < 0.02$), demonstrating that the rapid insulin oscillations occur with that preferred period in that group. There is no similar peak in the pooled C-peptide spectrum, which is dominated by irregular, lower frequency components not removed by the relatively moderate detrending. However, in the individual spectra, small but nonsignificant peaks are visible in the 5- to 7-min range. If the C-peptide data are

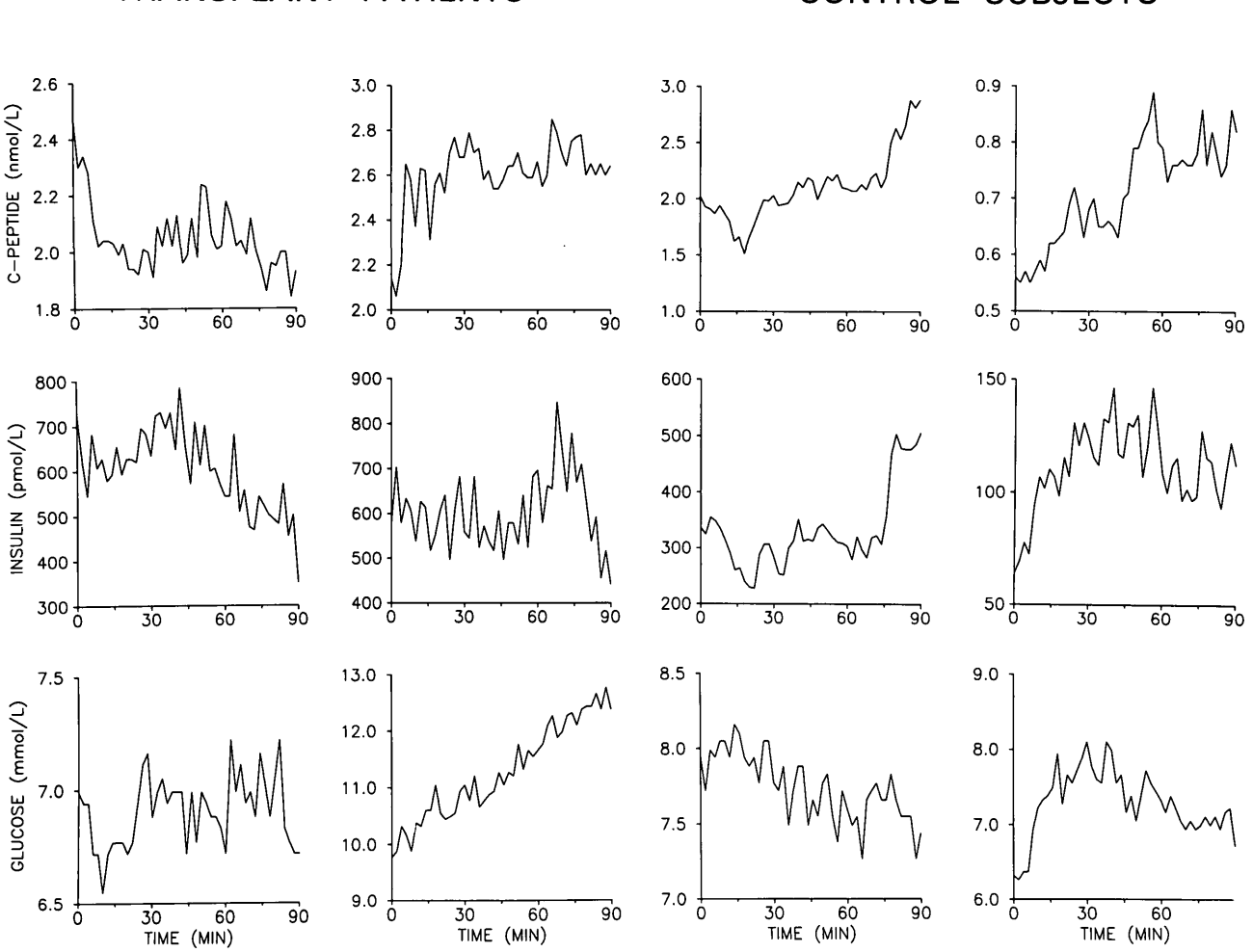

FIG. 1. Examples of glucose, insulin, and C-peptide profiles obtained in two Px patients and two control subjects over 90 min. Except for mean glucose concentration in one Px patient being higher than in the other five patients, examples are representative.

detrended by use of the first difference filter that strongly dampens all low-frequency components, a peak is visible in the pooled C-peptide spectrum at 5–7 min (data not shown). The pooled spectra for the control and Kx groups revealed no increased frequency of the insulin or C-peptide pulses.

Cross-correlation analysis. Cross-correlation between insulin and C-peptide showed a maximal positive asso-

TABLE 2
Pulse characteristics for insulin, C-peptide, and glucose for three study groups

	n	Pulse period (min)	Amplitude	
			Absolute	Relative
Insulin				
Px recipients	6	8.1 ± 0.5*	115 ± 10 pM†	0.24 ± 0.03
Control	6	12.5 ± 0.7	54 ± 8 pM	0.24 ± 0.03
Kx recipients	3	12.4 ± 0.5	67 ± 6 pM	0.19 ± 0.01
C-peptide				
Px recipients	6	16.8 ± 2.3	330 ± 58 pM	0.18 ± 0.03
Control	6	14.7 ± 1.2	237 ± 47 pM	0.18 ± 0.02
Kx recipients	3	15.3 ± 1.5	263 ± 10 pM	0.10 ± 0.01
Glucose				
Px recipients	6	15.4 ± 1.7	0.62 ± 0.13 mM	0.07 ± 0.01
Control	6	13.2 ± 1.0	0.51 ± 0.04 mM	0.07 ± 0.01
Kx recipients	3	18.5 ± 2.2	0.72 ± 0.14 mM	0.07 ± 0.01

Data are means ± SE.
*$P < 0.0005$, †$P < 0.001$, vs. other two groups (ANOVA).

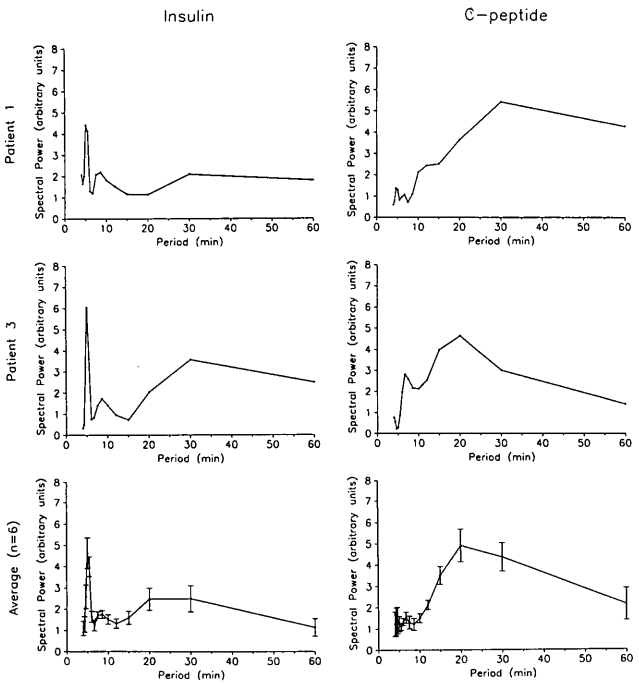

FIG. 2. Spectral analysis of insulin and C-peptide profiles from two Px patients illustrated in Fig. 1 as well as average spectra of all six Px patients. A strong peak exists at 5 min in all insulin spectra.

ciation occurring at a lag of 0 min in the control and Kx groups (control $r = 0.33$, $P < 0.0001$; Kx $r = 0.17$, $P = 0.06$). By contrast, no significant coefficient of cross-correlation was observed at any lag in the Px group. Similarly, a significant coefficient of cross-correlation between insulin and glucose occurred at a lag of 0 min in the control and Kx groups (control $r = 0.21$, $P < 0.001$; Kx $r = 0.20$, $P < 0.02$), whereas in the Px group, no significant coefficient of cross-correlation was observed.

DISCUSSION

Several studies have reported the changes in insulin and C-peptide concentrations that occur in diabetic patients who have undergone a combined kidney-pancreas transplant (1–4). Although these reports have demonstrated hyperinsulinemia in transplant recipients, only one published study previously has addressed whether the rapid pulses of insulin secretion persist in the transplanted pancreas (29). That study, which did not demonstrate any difference in the rapid pulses after transplant, evaluated rapid insulin secretory pulses in the fasting state through deconvolution of peripheral C-peptide levels but did not address the issue of period or amplitude of the insulin pulses in the study subjects.

The data in this study demonstrate that rapid oscillations in peripheral insulin levels occur with greater frequency after a pancreas transplant. This observation was made using two contrasting analytical approaches for characterizing oscillatory behavior: *1*) through the use of the pulse detection program ULTRA that analyzes each oscillation in the time series separately and *2*) through the use of spectral analysis that assesses the periodicity of oscillatory behavior in the time series. Although these pulses of insulin were of larger amplitude in absolute terms than in the control subjects (a finding that probably reflects the reduced impact of hepatic extraction on peripheral insulin levels in the Px group), the relative amplitude of the pulses in both groups was similar. Because the relative, rather than the absolute, amplitude of the pulses was used to identify significant pulses in this study, the greater number of insulin pulses in the Px group cannot be attributed to the differences in absolute pulse amplitude between the groups. Furthermore, the difference in pulse period between the Px and control group cannot be attributed to the effects of the immunosuppression because the Kx subjects received similar immunosuppressive therapy but nevertheless had pulse periods similar to those observed in the control group. Renal impairment is also unlikely to be a major contributing factor because the Kx patients on average had slightly higher creatinine levels than the Px patients yet had insulin and C-peptide pulse periods similar to the control subjects.

Although rapid oscillations in serum insulin levels may reflect the altered secretion of insulin, other factors including variations in clearance of insulin and measurement error (leading to random fluctuations in serum insulin concentrations) may also be involved in producing the overall temporal pattern of peripheral concentrations. This study attempted to reduce the impact of measurement error on the mean concentration by performing eight replicate measurements of insulin at each time point. In an attempt to establish which insulin pulses might reflect true pulses in insulin secretion, C-peptide concentrations were also measured at each time point and the results were submitted to formal pulse analysis. Although the number of insulin pulses in the control and Kx subjects was close to the number of C-peptide pulses, large discrepancies were observed between the number of insulin and C-peptide pulses in the Px patients. The long half-life of C-peptide (30 min) in contrast to insulin (4 min) will dampen the relative amplitude of many of the C-peptide oscillations in the peripheral circulation, and this militates against their detectability. Computer simulations performed in our laboratory (data not shown) have demonstrated that the difficulty in detecting pulses with a half-life comparable to that of C-peptide becomes greater as the interpulse interval decreases. This may explain why the Px group with the shortest interpulse interval has the greatest discrepancy between insulin and C-peptide pulse period. The ease of detectability of rapid insulin pulses in contrast to C-peptide pulses has been noted previously by Lang et al. (7), who also attributed this observation to the shorter half-life of insulin.

Although spectral analysis identified a regular insulin peak at 5 min in the Px patients, no consistent insulin peak was observed in the spectra of the control and Kx subjects. Despite this observation, an average insulin pulse period of 12–13 min was identified by ULTRA in both control and Kx subjects. That these pulses were false-positives is unlikely, because the false-positive yield from ULTRA using the criteria used to identify

significant pulses in this study is extremely low (23). The failure to detect a preferred period using spectral analysis is the result of the irregularity of the oscillatory pattern in the control subjects. The absence of regular oscillations in a proportion of normal subjects studied under basal conditions has recently been emphasized (30), and in a preliminary communication, Flax and Matthews (31) showed that the infusion of dextrose disrupts the distinctly regular period that is present in some subjects under basal conditions. The use of intravenous glucose in this study may explain in part why the control subjects did not exhibit regular oscillatory behavior that could be detected by spectral analysis.

All subjects were studied within 2 yr of transplant, and the transplanted pancreas is unlikely to have been reinnervated to any significant degree in this period as supported by Diem et al. (32). The persistence of rapid oscillations after transplant therefore suggests that neural factors may not be primarily responsible for the rapid oscillations in insulin levels seen in normal subjects. This is consistent with previous in vitro studies demonstrating rapid oscillations of insulin in the isolated perfused pancreas (16–21) and in isolated islets (14,15) and supports the hypothesis that these rapid oscillations are generated through intrinsic intra-islet mechanisms (14,15,33). Many of these in vitro studies have demonstrated that the periodicity of the oscillations is different from the periodicity seen in vivo, suggesting that, in the intact animal or human, extrinsic factors may modify the activity of this intrinsic pacemaker. Indeed, several studies that use the isolated perfused pancreas extracted from dogs, rats, monkeys, and baboons have demonstrated shorter oscillatory periods ranging from 5.5 to 7.5 min (17,19,20), and recently published studies that use the perfused human pancreas have also indicated a pulse period of 6 min (21). These periods are very close to the 5- to 8-min oscillatory period observed in the transplant recipients in this study, but they are markedly different from the prolonged oscillatory periods of 16–17.6 min observed in isolated rat islets (14,15). In this regard, the in vitro model of the isolated pancreas may resemble more closely the model of the transplanted pancreas than do isolated islets because, in the latter, the islets are also isolated from the intrapancreatic nervous system and experimental evidence implicates these intrapancreatic neural pathways in the regulation of the oscillatory period of the β-cell (34,35). Studies have also been performed evaluating rapid insulin secretory pulses in patients who have had a truncal vagotomy (8). The prolonged oscillatory period observed in these vagotomized patients may have resulted from an underdetection of pulses, in part the result of the reduced amplitude of the oscillatory period in this setting (18). Also, potential differences in neural innervation between the control subjects and transplant recipients might not be the only factor accounting for the increased frequency of the insulin oscillations in the transplant group. The long history of diabetes antedating the pancreas transplant might also be a contributory factor.

This study does not address whether these rapid pulses are of any physiological significance in the periphery. The lower amplitude of these pulses in the peripheral circulation in the control subjects suggests that, after further dilution in the interstitium, they will not be transmitted at the cellular level. In the Px patients, one might expect that the larger absolute amplitude of these pulses would have some effect on glucose disposal rates, but consistent decreases in plasma glucose levels even after the largest insulin oscillations were not observed in this study. In view of the findings of Luzi et al. (36), it is possible that the group receiving prednisone were insulin resistant, but direct measures to quantify glucose disposal were not used here. Cross-correlation analysis in the control and Kx subjects demonstrated a significant correlation between the rapid insulin and glucose pulses that was not evident in the Px patients. In the control and Kx groups, insulin is released from the β-cell into the portal circulation where the amplitude of the rapid oscillations is much higher than in the peripheral circulation. Consequently, any potential physiological effect of rapid insulin pulses is likely to be manifested at the level of the hepatocyte. Conversely, the lack of a relationship between the insulin and glucose pulses in the Px patients might result partially from initial insulin release into the systemic rather than the portal circulation after transplantation and, as a result of the ensuing dilution, portal levels of insulin are unlikely to be as high as in control and Kx subjects.

In summary, this study confirms the presence of rapid oscillations of insulin in subjects who have undergone pancreas transplantation. These insulin oscillations occur more frequently and are of larger amplitude in absolute terms compared with those observed in control subjects. These findings support the hypothesis that the oscillations are generated by intrinsic intra-islet mechanisms but that their frequency may be influenced by the central neurological connections of the pancreas.

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