Original Article

Individual Mice Can Be Distinguished by the Period of Their Islet Calcium Oscillations

Is There an Intrinsic Islet Period That Is Imprinted In Vivo?

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Pulsatile insulin secretion in vivo is believed to be derived, in part, from the intrinsic glucose-dependent intracellular calcium concentration ($[Ca^{2+}]_i$) pulsatility of individual islets. In isolation, islets display fast, slow, or mixtures of fast and slow $[Ca^{2+}]_i$ oscillations. We show that the period of islet $[Ca^{2+}]_i$ oscillations is unique to each mouse, with the islets from an individual mouse demonstrating similar rhythms to one another. Based on their rhythmic period, mice were broadly classified as being either fast (0.65 ± 0.1 min; n = 6 mice) or slow (4.7 ± 0.2 min; n = 15 mice). To ensure this phenomenon was not an artifact of islet-to-islet communication, we confirmed that islets cultured in isolation (period: 2.9 ± 0.1 min) were not statistically different from islets cultured together from the same mouse (3.1 ± 0.1 min, $P > 0.52$, n = 5 mice). We also compared pulsatile insulin patterns measured in vivo with islet $[Ca^{2+}]_i$ patterns measured in vitro from six mice. Mice with faster insulin pulse periods corresponded to faster islet $[Ca^{2+}]_i$ patterns, whereas slower insulin patterns corresponded to slower $[Ca^{2+}]_i$ patterns, suggesting that the insulin rhythm of each mouse is preserved to some degree by its islets in vitro. We propose that individual mice have characteristic oscillatory $[Ca^{2+}]_i$ patterns, which are imprinted in vivo through an unknown mechanism. *Diabetes* 54:3517–3522, 2005

In humans and animals, insulin secretion is pulsatile, and this pulsatility is advantageous for insulin action on its target tissues (1–3). Furthermore, pulsatile insulin secretion is disrupted in type 2 diabetes, with reduction in the amplitude and possibly frequency of the insulin pulses in diabetic patients (4–6) and their close relatives (7,8).

Because increases in intracellular calcium concentration ($[Ca^{2+}]_i$) are known to be important for insulin exocytosis in pancreatic β-cells (9,10), the well-documented oscillations in $[Ca^{2+}]_i$ observed with >7 mmol/l glucose have been assumed to underlie islet insulin oscillations, as several studies have shown that pulsatile insulin secretion occurs in phase with islet $[Ca^{2+}]_i$ oscillations (11–13). However, the wide range of islet periods observed in mice in vitro is difficult to reconcile with a coherent insulin oscillation in vivo within a single animal. In addition, it is not yet understood how the insulin pulses from different islets are synchronized within the pancreas in vivo, because this would seem to be mandatory to observe distinct oscillations in plasma insulin (14,15).

At least three types of intrinsic rhythms have been identified in isolated islets in vitro. Fast (~10–30 s), slow (~3–5 min), or a mixture of fast and slow islet oscillations have been observed in islet oxygen consumption, $[Ca^{2+}]_i$, glucose, mitochondrial activity, electrical firing patterns, and secretion (12–20). The fast pattern is believed to be electrical in nature, resulting from ion fluxes through calcium and potassium channels (21–23). Slow oscillations, in contrast, may reflect slow underlying oscillations in glucose metabolism (19,24,25), although slow oscillations in $[Ca^{2+}]_i$ may also occur solely because of electrical mechanisms alone (13,25).

By carefully segregating islets from different mice and then systematically studying their $[Ca^{2+}]_i$ oscillations in vitro, we found an intriguing result: islets from a given mouse tended to display strikingly similar $[Ca^{2+}]_i$ oscillations (measured under standard conditions in 11.1 mmol/l glucose). When many mice were compared, we found that some mice were fast and some mice were slow, as assessed by the period of their islet $[Ca^{2+}]_i$ oscillations. These rhythms may be relevant to physiological insulin secretion, because we show for the first time that plasma insulin is pulsatile in response to elevated glucose in conscious mice and that the period of insulin pulses in vivo appears to be related to the $[Ca^{2+}]_i$ oscillations measured in isolated islets in vitro taken from the same mice.

RESEARCH DESIGN AND METHODS

Male Swiss-Webster mice (25–35 g) were used for all experiments using protocols in accordance with the Animal Care and Use Committee of Virginia

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$[Ca^{2+}]_i$, intracellular calcium concentration.

Commonwealth and Vanderbilt Universities. All chemicals were from Sigma-Aldrich (St. Louis, MO) unless otherwise stated.

Islet isolation and culturing. Islets were isolated as previously described (26). Briefly, mice were killed by cervical dislocation, and their pancreata were rapidly removed. Collagenase (Crescent Chemical, Islandia, NY) was then injected at 2 mg/ml into the pancreas via the bile duct or through direct injection, and islets were incubated for 10–20 min to allow them to become free of the exocrine pancreas. Islets were then hand picked twice under a dissecting microscope and placed in RPMI at 37°C in a 95% air/5% CO_2 mixture for ~24 h before experimentation. Islets acquired from Vanderbilt University were isolated in a similar fashion upon completion of the in vivo insulin experiments. Islets were handpicked after collagenase digestion at Vanderbilt University (27), shipped overnight, then rinsed with fresh oxygenated saline, and incubated in RPMI, as above.

In vivo insulin secretion. To measure insulin concentration in conscious mice (28,29), the mice were anesthetized, and the left common carotid artery was catheterized for sampling of arterial blood with a two-part catheter. The right jugular vein was catheterized for infusions with a Silastic catheter. The free ends of the catheters were tunneled under the skin to the back of the neck, where they were attached via stainless steel connectors to lines, which were exteriorized and sealed with stainless steel plugs. Lines were flushed daily with 10–50 μl saline containing 200 units/ml heparin and 5 mg/ml ampicillin. Animals were individually housed after surgery, and body weight was recorded daily. On the day of the study, two blood samples for baseline glucose and insulin were first taken at 10-min intervals. Then a variable infusion of 50% dextrose was infused into the jugular vein to increase the glucose to 235 ± 8 mg/dl, equating to 13.1 ± 0.4 mmol/l. Blood samples (60 μl) were taken every 1 min for 20–30 min. A constant infusion of blood (70 μl/min) from a blood donor animal was infused throughout the rapid-sampling period. In some studies, the rapid-sampling period began 2 min after the onset of the exogenous glucose infusion. Immunoreactive insulin was assayed with a double-antibody method (30) with reagents obtained from Linco Research (St. Louis, MO).

$[Ca^{2+}]_i$ measurements. $[Ca^{2+}]_i$ was measured using the ratiometric dye fura-2/AM, with procedures modified from Zhang et al. (20). All islets were maintained in 11.1 mmol/l glucose to prevent transient states in their oscillatory periods due to shifts in glucose before or during the experiment (25). Islets were fura-loaded (20–40 min), washed, incubated (10–20 min), and then recorded at ~35°C in a standard external solution, containing (in mmol/l): 11.1 glucose, 130.5 NaCl, 3 $CaCl_2$, 5 KCl, 2 $MgCl_2$, and 10 HEPES, pH 7.3. $[Ca^{2+}]_i$ measurements were made by placing mouse islets in a small volume chamber (Warner Instruments, Hamden, CT). The chamber was mounted on the stage of an Olympus IX50 inverted fluorescence microscope equipped with fura-2 optics (Olympus, Tokyo, Japan). Excitation light was supplied to the preparation via a light pipe using a galvanometer-driven mirror and dichroic cube. The light source was a xenon burner, which produced excitation at 340 and 380 nm (HyperSwitch; IonOptix, Milton, MA). Ratios were collected at 510 nm using a photomultiplier (Electron Tubes, Ruislip, Middlesex, U.K. and were analyzed using IonOptix IonWizard software and standard calibration methods (31).

Data analysis and statistics. The period of $[Ca^{2+}]_i$ oscillations was determined using fast Fourier transform or direct visual inspection. For islets in which fast and slow components were present, the slow component was used for analysis and statistics. Differences between the mean islet periods of individual mice were determined using a one-way ANOVA with a Newman-Keuls multiple comparison post hoc test. A two-tailed t test assuming equal variance was used to compare the mean period of grouped islets and isolated islets (to assess the effects of culturing conditions on islets from the same mouse). To compare $[Ca^{2+}]_i$ oscillations and rhythms in insulin secretion, patterns in insulin concentration were quantified using the Cluster7 pulse detection algorithm (18,32), and the SE for each insulin pattern was calculated from the intervals between each peak.

RESULTS

Individual mice can be distinguished by the period of their islet $[Ca^{2+}]_i$ oscillations. We noticed over the course of our experiments that islets from the same mouse tended to have similar $[Ca^{2+}]_i$ oscillations, whereas the oscillations of islets from different mice appeared to be more distinct. We thus systematically investigated whether the period of $[Ca^{2+}]_i$ oscillations might be related to the individual mouse from which the islets were obtained, and we attempted to rule out the possibility that these similarities were artifactual. In Fig. 1, representative $[Ca^{2+}]_i$ patterns are displayed for five islets from each of three different mice, with the distribution of periods for each full set of sampled islets shown in the *bottom panels*. The $[Ca^{2+}]_i$ oscillations observed among islets from mouse "a" were very slow (~6.5 min; Fig. 1*A*), whereas the oscillations from mouse "o" were faster (~3 min; Fig. 1*B*) and had very rapid patterns superimposed on slower plateaus. For mouse "g," purely fast patterns were observed, with only a suggestion of a slow component (most apparent in the fourth trace of Fig. 1*C*). Note that the letters associated with each mouse refer to Fig. 3*A*, which summarizes the dataset.

To investigate whether the similarity in rhythms seen in islets from the same mouse might be due to islet-to-islet communication or entrainment during overnight culture, some islets were cultured overnight in isolation from one another to eliminate the possibility of islet-to-islet communication, whereas others were cultured in a group. Fig. 2*A* and *B* show representative examples of grouped (*left*) versus isolated (*right*) islets. The $[Ca^{2+}]_i$ patterns from mouse "f" (Fig. 2*A*) displayed a mixed pattern of fast and slow oscillations whether islets were grouped or isolated. In contrast, the rhythms observed in islets from mouse "e" (Fig. 2*B*) displayed a purely fast pattern in both grouped and isolated islets. The period of the $[Ca^{2+}]_i$ patterns did not differ significantly between grouped and isolated islets, presented either as averages of islet periods by mouse ($P > 0.52$, five mice; Fig. 2*C*) or presented as means among all islets ($P > 0.91$; Fig. 2*D*). Because differences in collagenase digestion of pancreatic tissue could also conceivably affect islet oscillations, islets were harvested from pairs of mice in tandem using identical protocols, solutions, and collagenase. Islet rhythms were again found to differ markedly from mouse to mouse even after islets were isolated in parallel, as indicated in pairs d-e, f-g, and k-l in Fig. 3*A*. These findings suggest that the similar $[Ca^{2+}]_i$ patterns observed among islets from the same mouse are not an artifact of islet isolation or culturing.

A summary of the mean period of islet $[Ca^{2+}]_i$ oscillations from 21 different mice is shown in Fig. 3*A* (a–u). Each diamond represents the mean ± SE of three to nine islets for an individual mouse and demonstrates that there was little variance in period in islets from the same mouse but significant diversity in islet periods among different mice. A one-way ANOVA test, used to detect differences among multiple datasets, showed highly significant differences among the 21 sets of islets ($P < 0.0001$). Two subpopulations of islets were apparent, representing fast and slow islets, with a cutoff shown by the dotted line in Fig. 3*A* at ~2 min. In Fig. 3*B*, a histogram of the oscillatory periods of 123 islets is shown. This distribution was bimodal, reflecting the two islet subpopulations. The mean period for fast islets was 0.65 ± 0.1 min, and for slow islets, 4.7 ± 0.2 min. These data thus demonstrate that mice could be differentiated from one another on the basis of their islet $[Ca^{2+}]_i$ periods.

Pulsatile insulin measured in vivo and islet $[Ca^{2+}]_i$ in vitro from the same mouse. For such a persistent rhythmic signature to be maintained by a majority of islets from an individual mouse suggests that these rhythms may be physiologically important for pulsatile insulin secretion in vivo. We thus investigated whether the different patterns in islet activity that we observed in different mice were related to their in vivo insulin secretion. Insulin release patterns have not been measured previously in conscious mice because of technical challenges in collect-

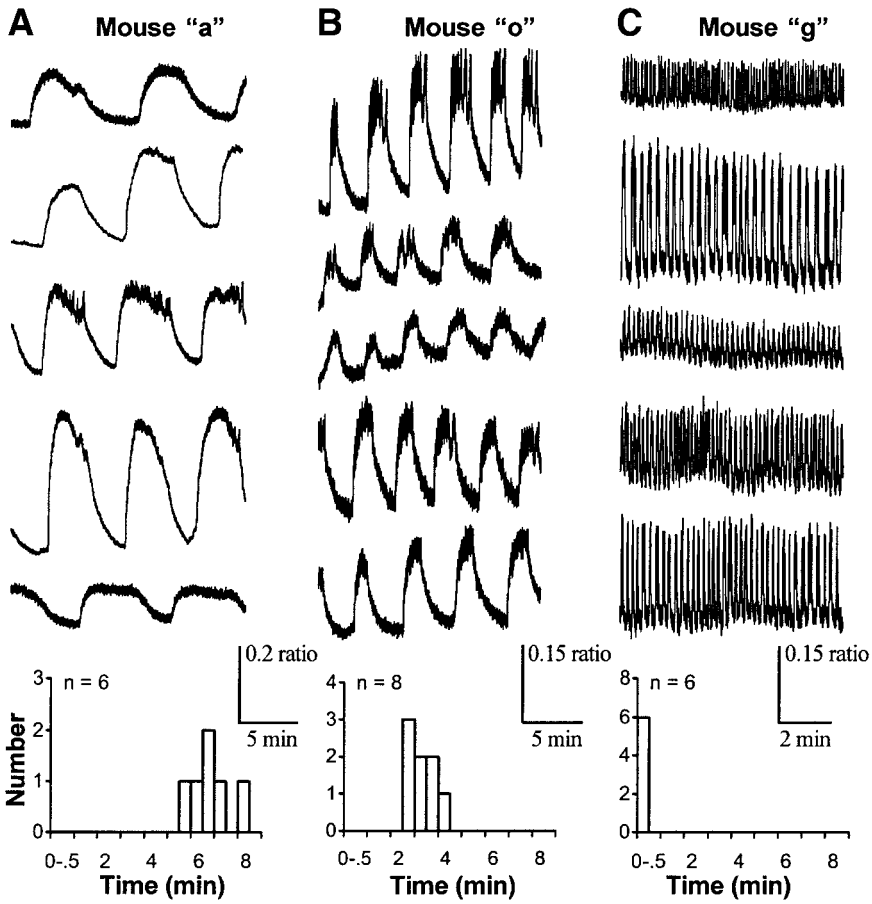

FIG. 1. Islets from the same mouse have similar $[Ca^{2+}]_i$ patterns. *A–C*: Oscillatory $[Ca^{2+}]_i$ patterns made by fura-2 ratiometric imaging are displayed for five islets from each of three mice (*A*, *B*, and *C*). The letters a, o, and g refer to each mouse as represented in the legend to Fig. 3*A*. Scale bars indicate time and fura-2 ratio (340/380 nm) for each set of five $[Ca^{2+}]_i$ traces. The *bottom panels* in *A–C* display histograms of all islets measured from each mouse using 0.5-min bins, with the total number of islets measured for each mouse shown in the *upper left corner* of each histogram. Mean $[Ca^{2+}]_i$ periods differed significantly between mice by two-tailed *t* test ($P < 0.0001$) and by one-way ANOVA ($P < 0.0001$).

ing blood samples from an animal with such a small blood volume. In our approach, blood was sampled using an indwelling carotid arterial catheter implanted 5–7 days before experimentation (28,29). The advantage of this technique is that mice do not need to be handled excessively, and blood samples can be obtained at precise intervals. Glucose clamps were used to raise blood glucose from ~5 to 13 mmol/l and then maintain a constant glucose concentration while blood was collected. Mice were transfused during experiments with blood from a donor mouse to prevent marked decreases in blood volume.

Pulsatile insulin patterns were detected using the Cluster7 algorithm (18,32) in six of seven mice, for which $[Ca^{2+}]_i$ measurements were also made in vitro for each corresponding mouse. We confirmed by a two-tailed *t* test that there were no differences in terms of period (3.96 vs. 3.30 min, $P > 0.46$), SE (16.7 vs. 24.4, $P > 0.12$), or coefficient of variation (23.4 vs. 43.3, $P > 0.16$) between islets prepared on site and shipped islets, respectively. In Fig. 4, *left panels*, representative examples of $[Ca^{2+}]_i$ oscillations are shown for four mice. Corresponding insulin secretory patterns are shown in the *right panel* for each mouse. In these examples, the periods of both the mean $[Ca^{2+}]_i$ and insulin oscillations are progressively shorter in viewing from Fig. 4*A–D*. The insulin pattern in Fig. 4*A* was one of the slowest at 4.5 min, with corresponding $[Ca^{2+}]_i$ oscillations averaging 4.3 ± 0.2 min among nine islets. Figure 4*B* was similar, except the mean period of $[Ca^{2+}]_i$ oscillations was 0.8 min faster than the corresponding insulin pulse interval. In Fig. 4*C*, the mean period of $[Ca^{2+}]_i$ oscillations was 1.6 min, which was briefer than the 2-min limit of detection for insulin pulses. Although an up-down cycle of 2 min was maintained for the first one-half of the corresponding insulin pattern in Fig. 4*C*, the pattern appeared to change in the latter one-half of the record, resulting in a 3-min period overall. In Fig. 4*D*, the even faster $[Ca^{2+}]_i$ oscillations that we observed corresponded to a record lacking clear insulin pulses, suggesting that the islet secretory pulses may have been too rapid to produce detectable pulses in the circulating blood. Alternatively, we cannot rule out that in this particular mouse, islet activity was not well synchronized or that the islets were not oscillating in vivo.

Although these examples suggest that islet $[Ca^{2+}]_i$ patterns are related to the in vivo insulin secretory patterns of the same mouse, many factors could affect the relationship between these rhythms, including in vivo neural and hormonal input and feedback that are lacking in vitro, the glucose concentration being slightly higher for the in vivo studies (~13 vs. 11.1 mmol/l), and slight variations in the pulse period measured across a fairly short sampling duration. Despite these sources of variability, however, these data suggest a direct relationship between the insulin secretory patterns of individual mice measured in vivo and the corresponding $[Ca^{2+}]_i$ oscillations of their islets measured in vitro. Further study will be needed, however, to more fully elucidate the quantitative nature of this relationship and in vivo factors, such as neural inputs and interactions with the liver, that can modify the relationship.

DISCUSSION

Oscillatory islet activity and pulsatile insulin secretion are key features of the endocrine regulation of blood glucose.

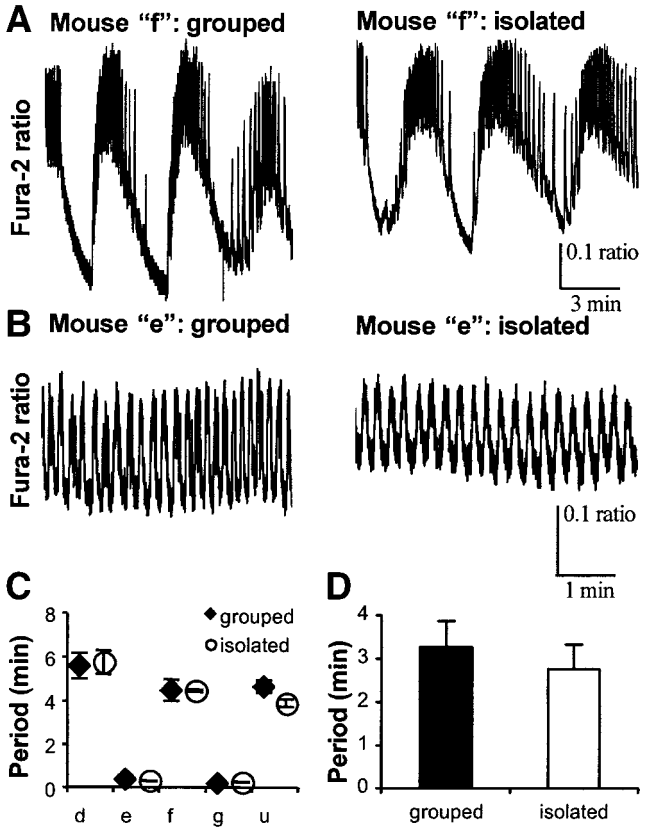

FIG. 2. $[Ca^{2+}]_i$ oscillations from the same mouse were similar whether islets were cultured together or in isolation. *A* and *B*: Two representative examples (*A*, mouse f; *B*, mouse e) of oscillatory $[Ca^{2+}]_i$ patterns measured from islets that were either cultured overnight together (grouped) or cultured in isolation with only one islet per culture dish (isolated). The letters f and e refer to each mouse as represented in summary Fig. 3*A*. *C*: Mean periods measured among grouped (filled) and isolated (open) islets are displayed in pairs by individual mouse (a–e). *D*: Mean periods of all grouped (n = 15) islets compared with all isolated (n = 15) islets. Periods were not significantly different between grouped and isolated islets as presented in either *C* or *D*.

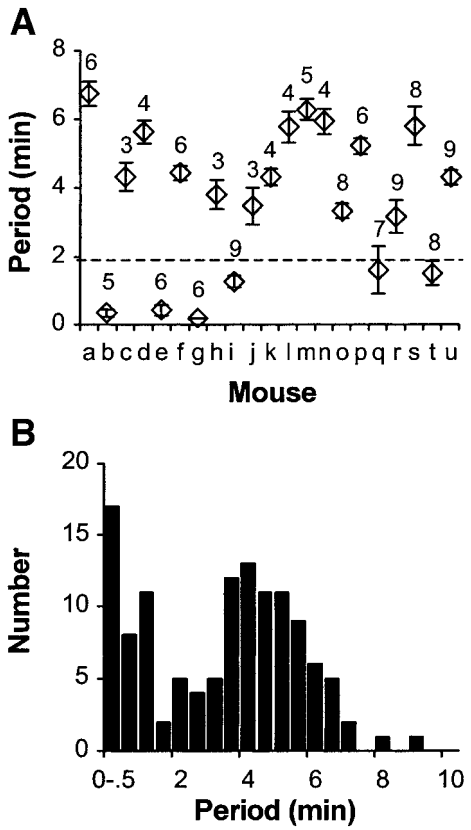

FIG. 3. Summary of islet $[Ca^{2+}]_i$ oscillations from 21 different mice. *A*: Mean periods ± SE of $[Ca^{2+}]_i$ oscillations measured from three to nine islets (numbers indicated above each data point) for each of the 21 mice (a–u). *B*: Oscillatory $[Ca^{2+}]_i$ periods binned at 0.5-min intervals exhibit a bimodal distribution for all islets (n = 123).

We have demonstrated here that islets from an individual mouse, long after being removed from their normal in vivo environment, exhibit $[Ca^{2+}]_i$ oscillations in vitro that have a signature period that appears to be unique to that mouse. Furthermore, we observed that islets isolated from mice having faster rhythms in plasma insulin had correspondingly faster islet $[Ca^{2+}]_i$ oscillations, whereas slower insulin rhythms corresponded to slower $[Ca^{2+}]_i$ oscillations. This suggests that the in vivo insulin pulsatility of each mouse is preserved to some degree by its islets in vitro. Although this rhythm might change from day to day or over the life of the mouse, after islet isolation, the islets of a given mouse appear to reflect the in vivo insulin pulsatility of the mouse. We propose that the islets of individual mice have their own characteristic oscillatory $[Ca^{2+}]_i$ patterns, which are imprinted in vivo through an as-yet-unknown mechanism. The data support the hypothesis that the $[Ca^{2+}]_i$ pulsatility, which is intrinsic to mouse islets, strongly contributes to the pulsatility of insulin secretion that we observed in vivo in mouse. We also note that, to the best of our knowledge, this study is the first demonstration that insulin levels measured under glucose clamp in mouse in vivo show the pulsatility that is characteristic of other species.

What might be the benefit of having islets with similar $[Ca^{2+}]_i$ patterns? One possibility is that this may facilitate the coordination of insulin pulses between islets of the whole pancreas. The synchronization of these islet rhythms is believed to be a prerequisite for producing regular pulses of insulin in the peripheral plasma (14,15). By maintaining intrinsically similar rhythms, the islets of a given mouse would be expected to be more easily entrained to a single pancreatic secretory pattern via a number of putative synchronizing mechanisms, such as an intrapancreatic ganglion pacemaker (33–35), circulating interislet factors (36), or feedback interactions with the liver (37,38). We note that in vivo experiments to date have only reported fast oscillations of membrane potential and $[Ca^{2+}]_i$ (39,40), which do not necessarily appear to be synchronized (41). We believe that slow islet oscillations in $[Ca^{2+}]_i$ likely occur in vivo, as suggested by slow islet oscillations in oxygen tension measured in vivo (42), and that these oscillations synchronize to drive the in vivo insulin pulses. Modeling predictions done by our group indicate that islets exhibiting mixed patterns of fast oscillations superimposed on slower oscillations can synchronize on the slow time scale while remaining asynchronous on the fast time scale and that islets having similar intrinsic periods are more readily synchronized than islets with heterogeneous periods (R.B., unpublished data). Further study is therefore necessary to fully understand the significance of these imprinted $[Ca^{2+}]_i$ patterns for islet-to-islet synchronization of insulin secretion within the pancreas.

We have shown that by measuring just a few islets from

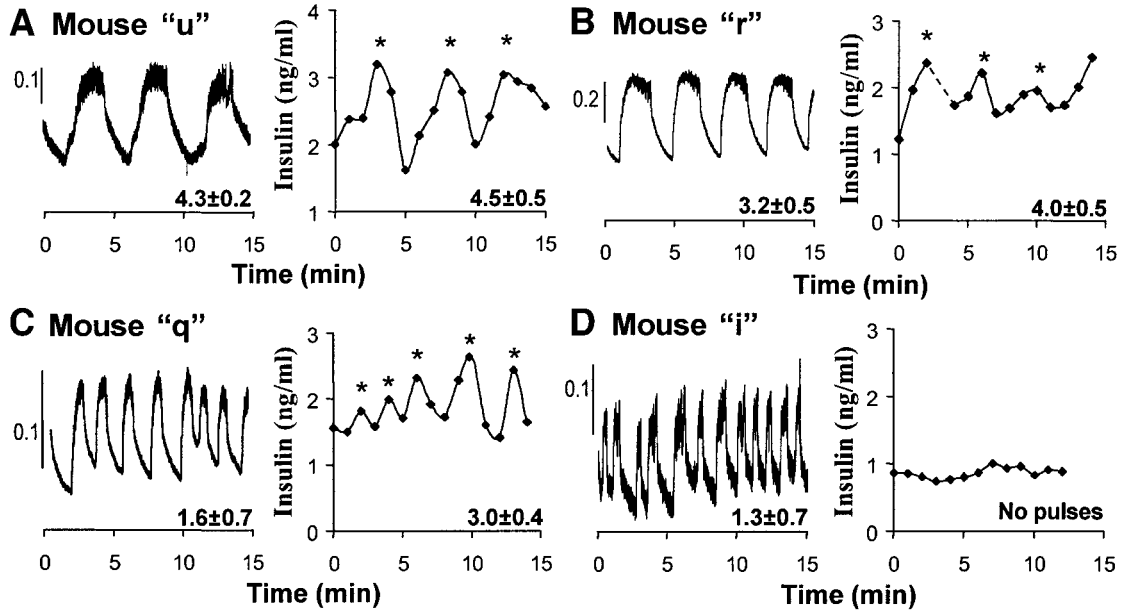

FIG. 4. Pulsatile insulin measured in vivo and islet $[Ca^{2+}]_i$ measured in vitro from the same mouse. *A–D*: $[Ca^{2+}]_i$ recordings (*left traces*) and insulin secretion (*right traces*) from each of four mice are displayed from longest to shortest period in *A–D*. Each $[Ca^{2+}]_i$ recording is representative of the mean among seven to nine islet recordings from each mouse. Mean periods ± SE for $[Ca^{2+}]_i$ or insulin is displayed in the corner of each panel. Lowercase letters in quotes refer to mouse labels from Fig. 3. Note that technical difficulties with sampling prevented a longer insulin record for *D*.

a given mouse, one can distinguish between mice having either fast (~30%) or slow rhythms (70%). Of interest, similar fractions of fast versus slow/mixed patterns have been reported for $[Ca^{2+}]_i$ oscillations (43) as well as oxygen and glucose consumption in mouse islets (19). This is the first report, however, to demonstrate that these different patterns may be linked, at least in part, to the individual mouse from which the islets were obtained.

Although we do not know at this time what causes islets from some mice to be fast and others to be slow, we recently proposed that both metabolic and ionic oscillations may quantitatively interact to mediate the overall oscillatory behavior of islets (25). According to this hypothesis, the slower patterns observed reflect glycolytic oscillations, whereas the faster ones depend primarily on ionic processes (25). Differences in key cellular parameters such as glucokinase activity or the pattern of expression of ATP-sensitive K^+ channel or K_{Ca} channels are plausible candidates to account for these diverse patterns. Alternatively, because it is well known that intracellular cAMP levels can markedly alter the period of islet oscillations, differences in the activity of the PKA/cAMP pathway in given islets could also account for the differences we observed between mice. Additional studies will be necessary to identify the key characteristics responsible for differences in islet pulsatility and to determine how these characteristics might be imprinted in vivo.

Differences in imprinted rhythms at the level of the whole animal could also possibly reflect the natural heterogeneity of the outbred strain of mice used in these studies. To determine whether this is the case, inbred mouse strains could be used as controls for potential genetic variability, whereas different transgenic models could be used to investigate the potential role of specific candidate genes on the imprinted islet rhythms. Genetic mutations in enzymes of the metabolic pathway and receptors for hormones that regulate energy metabolism are known to alter the rhythmic activity and insulin secretion of islets. For example, irregular insulin secretory patterns have been reported in humans with mutations in the muscle isoform phosphofructokinase (6), nonoscillatory insulin secretion has been reported in humans with a glucokinase-activating mutation (44), and mutations in leptin signaling produce slow and irregular oscillations in the islets of *ob/ob* mice (43) and also much slower insulin oscillations in Zucker rats compared with controls (45). Likewise, nongenetic factors are also known to influence insulin patterns and could contribute to the imprinting process. For example, it is known that insulin pulse frequency is reduced in people after weight loss (46). An unbiased approach, such as the use of gene arrays, may be appropriate in future studies to determine the underlying causes and significance of these apparent imprinted characteristics for mouse physiology.

In summary, our findings suggest the possibility that a powerful in vivo drive maintains similar intrinsic $[Ca^{2+}]_i$ oscillations among the islets of an individual mouse. This imprint of in vivo activity and the corresponding in vivo patterns of insulin secretion appear to differ from one mouse to the next, suggesting that there is measurable heterogeneity among otherwise normal wild-type mice. We do not yet know how this imprinting occurs or whether it could potentially influence the susceptibility of individuals to the development of metabolic diseases such as diabetes.

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