Oscillatory Membrane Potential Response to Glucose in Islet β-Cells: A Comparison of Islet-Cell Electrical Activity in Mouse and Rat

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In contrast to mouse, rat islet β-cell membrane potential is reported not to oscillate in response to elevated glucose despite demonstrated oscillations in calcium and insulin secretion. We aim to clarify the electrical activity of rat islet β-cells and characterize and compare the electrical activity of both α- and β-cells in rat and mouse islets. We recorded electrical activity from α- and β-cells within intact islets from both mouse and rat using the perforated whole-cell patch clamp technique. Fifty-six percent of both mouse and rat β-cells exhibited an oscillatory response to 11.1 mM glucose. Responses to both 11.1 mM and 2.8 mM glucose were identical in the two species. Rat β-cells exhibited incremental depolarization in a glucose concentration-dependent manner. We also demonstrated electrical activity in human islets recorded under the same conditions. In both mouse and rat α-cells 11 mM glucose caused hyperpolarization of the membrane potential, whereas 2.8 mM glucose produced action potential firing. No species differences were observed in the response of α-cells to glucose. This paper is the first to demonstrate and characterize oscillatory membrane potential fluctuations in the presence of elevated glucose in rat islet β-cells in comparison with mouse. The findings promote the use of rat islets in future electrophysiological studies, enabling consistency between electrophysiological and insulin secretion studies. An inverse response of α-cell membrane potential to glucose furthers our understanding of the mechanisms underlying glucose sensitive glucagon secretion. (Endocrinology 147: 4655–4663, 2006)
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Materials and Methods

Materials

Fura 2-AM was obtained from Invitrogen (Burlington, Ontario, Canada). All other compounds were obtained from Sigma-Aldrich (Oakville, Ontario, Canada).

Islet isolation

Islets were isolated from 2.5-month-old (~250 g) Wistar rats and 2-month-old (~20 g) CD1 mice as previously described (31). Islets were cultured for 1–3 d before use in RPMI 1640 media supplemented with 10% fetal bovine serum, 100 U/ml penicillin/G/sodium, 100 μg/ml streptomycin sulfate, and 11.1 mM glucose at 37 C and 5% CO2. No consistent differences in response were observed between islets cultured for various time periods. Principles of laboratory animal care were followed and protocols approved by the University of Toronto Animal Care Committee. Islets from rat (34 donors) or mouse (14 donors) were generally isolated in duplicate and then pooled. Donor numbers noted for each experiment assume the minimum value possible, e.g. where islets studied from each pool originated from one animal only. Although responses from individual animals could not be compared, no obvious differences between pools were observed. Human islets were obtained from Dr. Jonathan Lakey and the JDRF Human Islet Distribution Program (University of Alberta). Human islets were isolated following the Edmonton Protocol (32) and cultured in RPMI 1640 medium supplemented with 5.5 mM glucose, 10% fetal bovine serum, 25 μM HEPES, 2 mM L-glutamine, 100 μg/ml penicillin, and 100 μg/ml streptomycin at 37 C and 5% CO2.

Electrophysiology

Islets were held in the recording chamber with the assistance of a suction pipette, and current-clamp recordings were performed using the perforated-patch configuration. Pipettes were pulled from 1.5 mm thick-walled borosilicate glass and had a resistance of ~8–12 MΩ when filled with pipette solution. The pipette solution contained (mm) K2SO4 28.4; KCl 63.7; NaCl 11.8; MgCl2 1; HEPES 20.8; EGTA 0.5 (pH 7.2) with KOH plus 0.1 mg/ml amphotericin B. The bath solution contained (mm) NaCl 115; CaCl2 3; MgCl2 2; HEPES 10 (pH 7.2) with NaOH. Islets were superfused at 0.5 ml/min at 34 C. To prevent the influence of leak current and constituted approximately 8% of the cells identified in the glucose response experiments. Independent identification of α-cells by means such as single-cell PCR and immunocytochemistry was not possible in intact islets due to the likelihood of contamination by neighboring cells and the inability to visualize cells on the uppermost surface of the islet respectively. However, a previous study using a fluorescent probe to assess Vm in glucagon-positive mouse islet cells supports hyperpolarization of α-cell Vm in response to elevated glucose (35).

Calcium imaging

Changes in intracellular Ca2+ concentrations were assessed using fura 2AM. Islets were loaded with 3 μM fura 2AM in bath solution (2.8 mM glucose) at 37 C for 50 min. Islets were then washed and placed in an open chamber on the microscope stage, perfused with bath solution at 1 ml/min at 37 C. Experiments were performed using a BX51WI microscope (Olympus, Tokyo, Japan) with a ×20/0.95 water immersion objective and cooled charge-coupled device camera. Dual excitation at 340/380 nm was used and emission at 510 nm measured (ImageMaster 3 software; Photon Technology International, London, Ontario, Canada).

Statistical analysis

Grouped results were analyzed using the appropriate Student’s t test and a value of P < 0.05 considered statistically significant. N values indicate number of independent observations. Numbers of donors for each data set is indicated in the figure legends.

Results

Characterization of the glucose-dependent oscillatory electrical activity of β-cells in intact islets

We assessed the electrical response of mouse and rat islet β-cells to elevated glucose. Fifty-six percent (22 of 39) of mouse islet β-cells responded to 11.1 mM glucose with an oscillatory electrical pattern (Fig. 1A). In contrast to a previous report (12), 56% (9 of 16) of rat islet β-cells responded to elevated glucose in an oscillatory manner (Fig. 1B). The remaining percentage in both species exhibited a depolarized response to 11.1 mM glucose with small amplitude action potentials, similar to that previously described in rat by Antunes et al. (12) (Fig. 1C).

Although a range of bursting patterns was observed in both mouse and rat islet β-cells (Fig. 1, A and B), quantitative analyses revealed key components of the oscillations and were consistent between species. Mouse and rat oscillations displayed very similar burst plateau (−35.7 ± 2.9 vs. −33.5 ± 2.9 mV; P = NS, n = 9–10) and interburst potentials (−51.8 ± 3.2 vs. −47.3 ± 3.3 mV; P = NS, n = 9–10) (Fig. 1D). The plateau fractions for mouse vs. rat bursting in 11.1 mM glucose were 44.5 ± 2.5 and 51.3 ± 2.0%, respectively (P = NS, n = 9–10) similar to the approximately 50% reported for mouse previously (36). The frequencies of oscillation were also similar between species with more than 75% falling in the range of 1–6 min–1, similar to the range (2–7 min–1) previous classified as rapid oscillations (8).

Our novel report of electrical oscillations in rat islet β-cells is supported by the demonstration of rat islet calcium oscillations in response to 11.1 mM glucose (Fig. 2B), similar to those recorded from mouse islets (Fig. 2A). It is of interest to note that whereas spatially distinct groups of cells within individual mouse islets exhibited synchronous calcium oscillations (Fig. 2Aii vs. 2Aiii), synchrony between groups of cells was not observed in rat islets (Fig. 2Bii vs. 2Biii). One hundred sixty-one paired regions of interest from 17 islets and six donors were assessed and consistently revealed nonsynchronous oscillations. Similarly, human islets have recently been demonstrated to lack the synchronized calcium oscillations observed in mouse (37), suggesting that rat and human islets may function in a similar manner.

Whereas oscillatory mouse and rat β-cells were depolarized in 11.1 mM glucose (mean = −42.2 ± 2.6 and −38.7 ± 2.7 mV, respectively, P = NS, n = 11–16, Fig. 3C), application of 2.8 mM glucose hyperpolarized both mouse and rat β-cells and inhibited action potentials (Fig. 3, A and B), with the mean resting Vm values for each species also being similar (mouse = −62.4 ± 3.4; rat = −64.8 ± 2.8; P = NS, n = 4–6, on 22 June 2018

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In nonoscillating β-cells, the $V_m$ values for mouse vs. rat were: 11.1 mM glucose = $-29.2 \pm 1.0$ mV vs. $-28.8 \pm 1.8$ mV; 2.8 mM glucose = $-65.3 \pm 3.4$ vs. $-65.4 \pm 1.5$ mV ($P$ = NS, $n = 7–11$).

We assessed the rat islet β-cell $V_m$ response to stepwise increases in glucose concentration. Figure 4A depicts representative concentration-dependent effects of glucose during a single recording. Cells were hyperpolarized and electrically silent at 5 mM glucose, with sporadic action potentials generally first being observed at 8 mM glucose. It was not until 11.1 mM glucose was applied that action potential firing became regular and exhibited oscillations. This glucose sensitivity is in contrast to a previous report in which action potentials fired at 5.6 mM glucose but is similar to the mouse data from the same report (12). It can be seen from Fig. 4B that stepwise increases in glucose concentration between 2.8 and 11.1 mM produced a concentration-dependent depolarization ($n = 6–9$).

We also used the perforated-patch technique to record $V_m$ responses to glucose in human islets. Figure 5A shows a $V_m$ recording made from a human islet β-cell. At 11.1 mM glucose individual action potentials are largely observed (Fig. 5Bi and expanded in 5Bi), whereas more complex patterns, similar to rapid $V_m$ oscillations, are observed during membrane repolarization after the application of 2.8 mM glucose (Fig. 5Ci and expanded in 5Cii). The hyperpolarization produced by 2.8 mM glucose is of similar amplitude to that seen in rodent ($-65.6$ mV).
Cells hyperpolarize in response to 11.1 mM glucose in both mouse and rat islets

Glucose-dependent \( V_m \) responses were also assessed in both mouse (Fig. 6A) and rat (Fig. 6B) \( \alpha \)-cells. At 11.1 mM glucose, the \( V_m \) of mouse islet \( \alpha \)-cells was hyperpolarized to \(-67.1 \pm 3.2 \) mV \((n = 5)\), and no difference was observed in the \( V_m \) of rat \( \alpha \)-cells \((-65.2 \pm 2.2 \) mV, \( P = NS, n = 14)\). Similarly, in both species a decrease in glucose concentration to 2.8 mM initiated depolarization of the \( \alpha \)-cell. Recordings were generally switched to voltage clamp mode at this point to confirm that the leak current and access resistance were still acceptable. Typically, upon return to current clamp mode, these cells exhibited action potential firing, as seen on the right-hand side of Fig. 6, A and B (mean \( V_m \): mouse = \(-37.7 \pm 4.9 \) mV; rat = \(-36.6 \pm 4.6 \) mV, \( P = NS, n = 7–9\)). We do not believe this phenomenon is an artifact of switching between voltage and current clamp mode because time-dependent establishment of action potentials is demonstrated in the 20-min continuous current clamp recording made from a mouse \( \alpha \)-cell shown in supplemental Fig. 1, published as supplemental data on The Endocrine Society’s Journals Online Web site at http://endo.endojournals.org. \( V_m \) oscillations were not observed in either mouse or rat \( \alpha \)-cells.

Discussion

Currently many studies compare electrophysiological data recorded from isolated mouse islets to functional studies performed in rat islets. Caution must be used when making such extrapolations because there are numerous reported differences between mouse and rat islet physiology. In fact, one relatively recent report described major differences between the electrical activity of mouse and rat islets (12). However, this report was not in concordance with the rat islet calcium and insulin oscillations that have been widely reported in the literature (13–15, 17–19). We aimed to reassess the electrical activity of both \( \alpha \)- and \( \beta \)-cells within rat islets using a novel approach and to compare our findings with those in mouse islets.

Our principal finding is that a similar percentage of mouse and rat islet \( \beta \)-cells exhibit an oscillatory response to 11.1 mM glucose with no quantitative differences in the mean burst plateau and interburst potentials, plateau fraction, or oscillatory frequency. This is in contrast to others who reported no oscillatory activity in rat islets at glucose concentrations between 5.6 and 16.7 mM (12). However, the response reported by Antunes et al. (12) (depolarization with small amplitude action potentials) was also observed at lower frequency (44%) in both mouse and rat islet \( \beta \)-cells in our study. The range of oscillatory and nonscillatory patterns ob-
served might reflect several factors including variation in normal islet behavior, islet viability, and metabolic status or cell-cell coupling and its modulation by input resistance. It is our understanding that nonoscillatory cells are frequently excluded from studies of β-cell electrical activity in mouse because they may represent δ-cells, although the glucose sensitivity and number of cells in this category would suggest otherwise in our study (33, 34). Antunes et al. (12) also observed no evidence of calcium oscillations in response to glucose in rat islets. However, in our hands, and those of several other groups (13–15, 38), rat islets do exhibit an oscillatory calcium response, supporting our report of oscillations in electrical activity. The lack of synchronicity observed in rat calcium oscillations is in contrast to previous reports (13, 14, 16), and it conceivable that our finding reflects procedural differences. However, islets from both mouse and rat appeared similar in terms of their size range, shape, smooth outline, clear color (islets with a dark center were considered unsuitable for use), and glucose-stimulated insulin secretion capacity (data not shown). It is apparent that further studies are required to clarify the question of calcium oscillation synchronicity in rat.

**Fig. 3.** Representative traces showing hyperpolarization of islet β-cell membrane potential after a change of glucose concentration from 11.1 to 2.8 mM in mouse (A) and rat (B) at approximately 34°C. C, Grouped data showing mean membrane potential at 11.1 or 2.8 mM glucose in mouse (white bars) and rat (black bars) islet β-cells (n = 4–16, donors > 4–5).

**Fig. 4.** A, Representative trace showing change in \( V_m \) in a single rat islet β-cell in response to step increases in glucose concentration at approximately 34°C. B, Grouped data showing glucose concentration-dependent depolarization of rat islet β-cell \( V_m \) (n = 6–9, donors > 3 each group).
Second, we report that the resting \( V_m \) of \( \beta \)-cells is similar in rat and mouse, in contrast to the more depolarized \( V_m \) previously reported in rat (12). In addition, we find the glucose sensitivity of rat islets to be more similar to mouse than previously reported (12). There are several possible explanations for the discrepancies observed between laboratories, including slightly different methodologies used. Unlike Antunes et al. (12), we chose to culture both mouse and rat islets in 11.1 mM glucose to enable direct comparison between species. These conditions are standard and widely used in the field for recording \( V_m \) from mouse islets (39–43). Another difference between the two studies is the recording technique chosen; perforated patch-clamp in our study vs. sharp electrode in that of Antunes et al. Although reports of \( V_m \) oscillations in mouse islets using either technique indicates that this difference may not explain the reported discrepancies in rat electrical activity, direct comparison of the two techniques has suggested that perforated patch clamp is more accurate than sharp electrode for the measurement of \( V_m \) (44). Indeed, it has been reported that researchers may draw radically different conclusions if the recordings were made with patch electrodes; for example, microelectrode recordings suggest *Xenopus* embryo neurons to be poorly excitable (45), whereas patch-clamp recordings demonstrate the ability of these cells to rhythmically fire (46). Although the differences observed in neuronal cells may not be applicable to \( \beta \)-cells, such technical issues might explain the more hyperpolarized rat \( \beta \)-cell resting membrane potential and observation of \( V_m \) oscillations described in our study. Our use of the perforated patch-clamp technique necessitated the slight

![Fig. 5. A, Human islet \( \beta \)-cell \( V_m \) response after a change of glucose concentration from 11.1 to 2.8 mM at approximately 34 C. At 11.1 mM glucose, individual action potentials are largely observed (Bi and expanded in Bii), whereas more complex patterns, similar to rapid \( V_m \) oscillations, are observed during membrane repolarization after the application of 2.8 mM glucose (Ci and expanded in Cii).](https://academic.oup.com/endo/article-abstract/147/10/4655/2500206)
lowering of temperature in these studies to 32–34°C to enable giga-ohm seal formation, in comparison with the 37°C used in our calcium imaging experiments and the studies of Antunes et al. (12). We do not believe that this 3°C drop in temperature underlies our novel observation of V_m oscillations in rat islets in which previous studies failed because in general it is cooling that inhibits robust bursting. Mouse V_m oscillations have been previously recorded at both 37 and 32–34°C, and we similarly observe calcium oscillations in both mouse and rat islets at 37°C.

Human islet β-cells have been previously reported to exhibit a greater complexity and wider variety of secretogogue-induced patterns of electrical activity than rodent β-cells (47, 48). Our recording of human β-cell electrical activity in response to changes in glucose concentration is in concordance with this literature. These data also indicate that the perforated patch-clamp technique is suitable for recording membrane potential from intact islets in several species. Whereas a single recording from a human islet cannot provide definitive characterization of the species, the lack of data currently available means that individual reports have significant value in advancing our understanding of human islet physiology.

We also assessed the electrical activity of mouse and rat α-cells in response to glucose and report an inverse response to that of β-cells. Understanding α-cell function is of consequence to our understanding of diabetes because whereas β-cell dysfunction often leads to insulin levels that are relatively low, given the prevailing hyperglycemia in patients with this disease, glucagon levels are elevated, exacerbating the hyperglycemia (49, 50). In the healthy islet α-cell, glucagon secretion is active at low glucose and inhibited by elevated glucose concentrations (24, 51). The mechanism of glucose-dependent inhibition of glucagon secretion in α-cells is poorly understood and their electrical activity little studied. Although there is currently no agreed upon model of

![Fig. 6. Representative traces showing depolarization and action potential firing of islet α-cells after a change of glucose concentration from 11.1 to 2.8 mM and hyperpolarization after return to 11.1 mM glucose in mouse (A) and rat (B) at approximately 34°C. Grouped data showing mean V_m at 11.1 or 2.8 mM glucose in mouse (white bars) and rat (black bars) islet α-cells (n = 5–14, donors > 4–5).](https://academic.oup.com/endo/article-abstract/147/10/4655/2500206)
stimulus-secretion coupling in islet α-cells, one proposed mechanism suggests that, in a manner similar to that described in the β-cell, glucose metabolism leads to ATP-sensitive potassium channel (K<sub>ATP</sub>) channel inhibition in high glucose, which depolarizes the α-cell, paradoxically inhibiting action potential firing due to voltage-dependent inactivation of T-type Ca<sup>2+</sup> and Na<sup>+</sup> channels (22). This mechanism is not without contest, and debate exists over the expression level, ATP-sensitivity, and role of K<sub>ATP</sub> channels in α-cells (25–27, 33, 52).

Our findings are also in conflict with the described depolarization of the α-cell by glucose because we observed a distinct hyperpolarization of the membrane potential in the presence of 11.1 mM glucose, which is supported by previous reports of glucose-induced hyperpolarization of mouse α-cells (21, 25, 26, 35). The reasons underlying this discrepancy may lie in a second group of glucagon secretion regulators: negative paracrine factors released from the β-cell in response to glucose. It has been reported that in fluorescence-activated cell sorter-isolated rat α-cells, elevated glucose in fact stimulates electrical activity and glucagon secretion, whereas in intact islets, this is suppressed, demonstrating the importance of paracrine signaling in the regulation of glucagon secretion (28, 29).

Indeed, the earlier study in which increased depolarization was observed in response to glucose was performed in dispersed α-cells (21), a preparation in which paracrine signaling is likely minimal. Several factors have been demonstrated to mediate this negative regulation including: zinc, which acts via K<sub>ATP</sub> channels (53); γ-aminobutyric acid, which increases chloride permeability (54); insulin, which activates both K<sub>ATP</sub> channels and γ-aminobutyric acid receptors (30, 55); and somatostatin, whose mechanism of action remains less well characterized (56, 57).

Each of these factors is proposed to hyperpolarize α-cells in intact islets in response to glucose, in concordance with our findings in this study.

Species differences in the regulation of glucagon secretion in rat and mouse have been reported (28–30;52) and may also underlie the conflicting V<sub>m</sub> responses of α-cells in our study and that of Gobel et al. (21). However, we observe similar V<sub>m</sub> hyperpolarization in response to glucose in both rat and mouse α-cells in intact islets. We believe our novel characterization of rat α-cell electrical activity is of importance in understanding α-cell function and acknowledging that additional studies are required to more fully determine the mechanisms involved in glucagon secretion.

In conclusion, these data are the first to systematically characterize the electrical responses of rat α- and β-cells to high and low glucose in intact islets and to compare them with mouse. The finding that rat islet β-cells display similar V<sub>m</sub> oscillations to mouse suggests that the electrical activity is functionally similar in the two species and clarifies the previous anomaly between the reported nonscillatory V<sub>m</sub> and the well-documented oscillations in cellular calcium and insulin secretion in rat. This finding is important for the understanding of rat electrical activity and promotes the use of the rat in electrophysiological studies as well as insulin secretion studies in which it is already the model of choice.

The inverse response to glucose recorded from α-cells contests the notion that these cells depolarize in response to elevated glucose and again provides strong evidence that mouse and rat islet cells function in a similar manner.

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