"Loss of high-frequency glucose-induced Ca2+ oscillations in pancreatic islets correlates with impaired glucose tolerance in Trpm5-/- mice"

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Abstract
Glucose homeostasis is critically dependent on insulin release from pancreatic beta-cells, which is strictly regulated by glucose-induced oscillations in membrane potential (V(m)) and the cytosolic calcium level ([Ca(2+)](cyt)). We propose that TRPM5, a Ca(2+)-activated monovalent cation channel, is a positive regulator of glucose-induced insulin release. Immunofluorescence revealed expression of TRPM5 in pancreatic islets. A Ca(2+)-activated nonselective cation current with TRPM5-like properties is significantly reduced in Trpm5(-/-) cells. Ca(2+)-imaging and electrophysiological analysis show that glucose-induced oscillations of V(m) and [Ca(2+)](cyt) have on average a reduced frequency in Trpm5(-/-) islets, specifically due to a lack of fast oscillations. As a consequence, glucose-induced insulin release from Trpm5(-/-) pancreatic islets is significantly reduced, resulting in an impaired glucose tolerance in Trpm5(-/-) mice.

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Loss of high-frequency glucose-induced Ca\(^{2+}\) oscillations in pancreatic islets correlates with impaired glucose tolerance in Trpm5\(^{-/-}\) mice

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Glucose homeostasis is critically dependent on insulin release from pancreatic \(\beta\)-cells, which is strictly regulated by glucose-induced oscillations in membrane potential \((V_m)\) and the cytosolic calcium level \((\left[Ca^{2+}\right]_c)\). We propose that TRPM5, a Ca\(^{2+}\)-activated monovalent cation channel, is a positive regulator of glucose-induced insulin release. Immunofluorescence revealed expression of TRPM5 in pancreatic islets. A Ca\(^{2+}\)-activated nonselective cation current with TRPM5-like properties is significantly reduced in Trpm5\(^{-/-}\) cells. Ca\(^{2+}\)-imaging and electrophysiological analysis show that glucose-induced oscillations of \(V_m\) and \([Ca^{2+}]_c\) have on average a reduced frequency in Trpm5\(^{-/-}\) islets, specifically due to a lack of fast oscillations. As a consequence, glucose-induced insulin release from Trpm5\(^{-/-}\) pancreatic islets is significantly reduced, resulting in an impaired glucose tolerance in Trpm5\(^{-/-}\) mice.

Ca\(^{2+}\) signaling | insulin release | pancreatic \(\beta\)-cells | transient receptor potential ion channels | glucose sensing

The pancreatic \(\beta\)-cell is an electrically excitable cell that secretes insulin when extracellular glucose levels exceed a threshold concentration. Characteristically, upon stimulation with glucose, \(\beta\)-cells display an oscillatory change of the membrane potential \((V_m)\) and, in parallel, of the cytosolic Ca\(^{2+}\) level \((\left[Ca^{2+}\right]_c)\) (1–4). This pattern, consisting of slow waves of depolarized plateaus on which bursts of action potentials are superimposed and separated by electrically silent intervals, plays a critical role in the regulation of insulin secretion. Indeed, in the absence of depolarization, no insulin is released, and the “extent” of electrical activity largely determines the amount of released insulin \((1, 5)\). The bursting pattern of the \(\beta\)-cell is a complex interplay among different ion channels (e.g., ATP-sensitive K\(^{+}\) channels, voltage-gated Ca\(^{2+}\) channels, and Ca\(^{2+}\) and voltage-activated K\(^{+}\) channels), intra-cellular Ca\(^{2+}\) levels \((\left[Ca^{2+}\right]_i)\), and the cellular metabolism of the \(\beta\)-cell (1, 6). The increase in \([Ca^{2+}]_i\) originates from glucose-induced Ca\(^{2+}\) influx through voltage-gated L-type Ca\(^{2+}\) channels and, possibly, Ca\(^{2+}\) mobilization from intracellular stores, the latter promoted by activation of the phospholipase C system and generation ofinositol 1,4,5-trisphosphate \((1, 7, 8)\). Despite intensive investigation, several aspects of the rhythmic electrical activity of \(\beta\)-cells, such as the origin of the variability in oscillation pattern, remain unclear. Indeed, glucose stimulation can result in high-frequency short bursts, low-frequency long bursts, or a combination of these two patterns, also known as compound bursts \((9, 10)\).

In this study we identified TRPM5 as a player in the electrical activity of glucose-stimulated pancreatic \(\beta\)-cells. TRPM5 is one of 28 members of the large transient receptor potential (TRP) superfamily \((11–13)\). TRPM5, and its close homologue TRPM4, are Ca\(^{2+}\)-activated cation channels that are permeable for monovalent cations, but not divalent cations, with a conductance of approximately 20 to 25 pS \((14–16)\). Using Trpm5\(^{-/-}\) mice we show here that this channel promotes high-frequency glucose-induced oscillations in \(V_m\) and \([Ca^{2+}]_i\) in pancreatic \(\beta\)-cells. Loss of TRPM5 expression, and high-frequency bursting, is functionally relevant as this leads to reduced glucose-induced insulin release from isolated islets and impaired glucose tolerance.

Results and Discussion
Expression of the Trpm5 Gene in Pancreatic \(\beta\)-Cells. Previously, Trpm5 expression was shown on the mRNA level in several \(\beta\)-cell lines and in human and mouse tissues, including taste buds, intestine, and pancreatic islets \((13, 17–22)\). Here we describe immunostaining of TRPM5 protein in pancreatic islets with a specific antibody (Fig. 1). TRPM5 is colocalized with insulin in WT islets, strongly suggesting expression of TRPM5 in insulin-secreting \(\beta\)-cells. Specific staining with the TRPM5 antibody is absent in Trpm5\(^{-/-}\) islets. Quantitative PCR experiments in a purified \(\beta\)-cell sample confirmed expression of Trpm5 in the \(\beta\)-cells. Expression of Trpm5 could also be detected in purified \(\alpha\)-cells, although to a lower level compared with \(\beta\)-cells (Fig. S1).

Characterization of a Ca\(^{2+}\) Release-Activated Cation Current in Pancreatic Islet Cells. To determine whether TRPM5 is part of the Ca\(^{2+}\)-activated monovalent cation current described earlier in insulin-secreting cell lines and primary \(\beta\)-cells \((23–25)\), we compared whole-cell currents in WT and Trpm5\(^{-/-}\) single pancreatic islet cells. Only cells with a cell capacitance of >5 pF were analyzed, being most likely pancreatic \(\beta\)-cells \((26)\). In one approach, cells were dialyzed with a pipette solution containing 1.5 μM Ca\(^{2+}\). As shown in Fig. 2, a \(\beta\)-dependent current can be readily activated, which is largely reduced in Trpm5\(^{-/-}\) cells (Fig. 2 A–C). Comparable results were obtained when \([Ca^{2+}]_i\) was increased by flash-uncaging during whole-cell experiments \((WT, 6.5 ± 1.4 \mu A/PF; vs. Trpm5\(^{-/-}\), 3.5 ± 0.5 \mu A/PF; \(P = 0.035\) at ±80 mV; WT, 2.53 ± 0.7 \mu A/PF; vs. Trpm5\(^{-/-}\), 1.09 ± 0.2 \mu A/PF; \(P = 0.025\) at ±80 mV; \(n = 21–27\) from five to six mice). Ca\(^{2+}\) uncaging during a step to ±80 mV (Fig. 2D) allows


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determination of the time course of activation for this current, in relative isolation from other conductances in β-cells. In WT cells we found a time constant for activation (τ = 24.1 ± 3.5 ms; n = 14), which is similar to what has been shown before for TRPM5 (27). Furthermore, the Ca$^{2+}$-activated current in these conditions in WT cells showed a clear bell-shaped dependency on [Ca$^{2+}$]$_{cyt}$ (Fig. 2F), which corresponds to the Ca$^{2+}$-dependence of TRPM5 (13, 27). Obviously, the current in Trpm5$^{-/-}$ cells is strongly reduced at each [Ca$^{2+}$]$_{cyt}$ level (Fig. 2C and F). Finally, the Ca$^{2+}$-activated current in WT cells displayed a cation conductance sequence Li$^+$>Na$^+$$>$Ca$^{2+}$>NMDG$^-$, which is essentially the same as reported for TRPM5, but different from the background current in Trpm5$^{-/-}$ cells (Na$^+$$>$Li$^+$$>$Ca$^{2+}$>NMDG$^-$). Thus, it is clear that TRPM5 is an important constituent of the calcium-activated cation current in pancreatic β-cells. Other presently known molecular candidates for this class of ion channels include TRPC3 (28, 29), TRPM2 (30), and TRPM4, which may constitute the remaining Ca$^{2+}$-activated cation current in Trpm5$^{-/-}$ β-cells. At least TRPM2 and TRPM4 are also expressed in insulin-secreting cell lines and in mouse pancreatic islets (31, 32). Notably, deletion of the Trpm4 gene has no effect on glucose tolerance or on insulin release from pancreatic islets (33). Finally, note that the current described here is fundamentally different from the “leak” channel, NALCN, proposed recently in β-cells (34, 35).

Slower Intracellular Ca$^{2+}$ Oscillations in Islets Derived from Trpm5$^{-/-}$ Mice. To find clues about the functional role of TRPM5 in pancreatic β-cells, we first turned to glucose-induced Ca$^{2+}$ oscillations. β-Cells display very characteristic [Ca$^{2+}$]$_{cyt}$ oscillations in response to high glucose concentrations, which critically regulate the release of insulin. To analyze glucose-induced signaling in intact islets, we monitored [Ca$^{2+}$]$_{cyt}$ dynamics in intact Fura-2-loaded islets of WT and Trpm5$^{-/-}$ mice. The basal fura-2 fluorescence ratio (F150/F380), in a nonstimulatory glucose concentration (3 mM), was similar in WT and Trpm5$^{-/-}$ islets (WT, 1.16 ± 0.03; vs. Trpm5$^{-/-}$, 1.20 ± 0.02; P = 0.16; n = 27–33 from five to seven mice). Islets from both genotypes had a similar glucose concentration threshold for triggering [Ca$^{2+}$]$_{cyt}$ oscillations (WT, 6.20 ± 0.243; vs. Trpm5$^{-/-}$, 6.21 ± 0.239; P = 0.97; n = 14–15 from four to five mice), indicating that glucose metabolism preceding glucose-induced electrical activity is not changed in Trpm5$^{-/-}$ mice.

Increasing extracellular calcium concentration from 3 to 10 mM elicited an oscillatory increase in [Ca$^{2+}$]$_{cyt}$ in both WT and Trpm5$^{-/-}$
islets (Fig. 3A). During a typical 20-min stimulation with 10 mM glucose, the average increase in [Ca\(^{2+}\)]\(_{\text{cyt}}\) as evaluated from F350/F390, was not different between WT and Trpm5\(^{-/-}\) islets (WT, 0.26±0.03; vs. Trpm5\(^{-/-}\), 0.21±0.02; P = 0.23; n = 28–33 from five to seven mice; Fig. 3B). Strikingly, Trpm5\(^{-/-}\) islets exhibited overall a significantly lower frequency of [Ca\(^{2+}\)]\(_{\text{cyt}}\) oscillations (0.82 ± 0.11 peaks/min in WT; 0.38 ± 0.03 peaks/min in Trpm5\(^{-/-}\) islets; P = 0.00078; n = 28–34 from five to seven mice; Fig. 3C).

However, in line with previous work (10, 36, 37), we observed significant variability in the oscillatory pattern among individual islets. Using Fourier analysis, three different oscillatory patterns could be distinguished in WT islets in 10 mM glucose (Fig. S2) (i): slow oscillators display relevant frequencies below 0.015 Hz (ii), fast oscillators display relevant frequencies above 0.015 Hz, and (iii) mixed (compound) oscillators display relevant frequencies in both slow and fast frequency regions (for a detailed description, see Fig. S2). In preparations from WT mice, the proportion of islets exhibiting slow (38.2%), mixed (38.2%), and fast (23.6%) [Ca\(^{2+}\)]\(_{\text{cyt}}\) oscillations (Fig. 3D) was comparable to distributions reported before (10, 37). Remarkably, fast oscillating islets were completely lacking in preparations from Trpm5\(^{-/-}\) mice, in which 89.3% of the islets were classified as slow oscillators and the remaining islets showed mixed oscillations (χ\(^2\) analysis, P = 0.0006; Fig. 3D). An analogous difference is observed when islets were stimulated with a supramaximal concentration of glucose (20 mM). This leads to longer plateaus of increased Ca\(^{2+}\), both in WT and KO islets. In WT islets, a sustained Ca\(^{2+}\) plateau is the most prevalent pattern, whereas Ca\(^{2+}\) oscillations during the stimulation period were observed in only a small subset of the islets. Strikingly, in Trpm5\(^{-/-}\) islets, the oscillating pattern is the more prevalent (Fig. S3; χ\(^2\) analysis, P = 0.025). Finally, it should be noted that increased glucose-induced [Ca\(^{2+}\)]\(_{\text{cyt}}\) oscillation frequency in β-cells lacking the BETA3 subunit of voltage-gated Ca\(^{2+}\) channels can be accounted for by enhanced formation of inositol 1,4,5-trisphosphate and increased Ca\(^{2+}\) mobilization from intracellular stores in Beta3\(^{-/-}\) islets (38). In Trpm5\(^{-/-}\) islets, this pathway is not altered, as release of calcium from the intracellular stores was the same in WT and Trpm5\(^{-/-}\) islets upon stimulation with acetylcholine [an activator of the phospholipase C pathway (39); Fig. S4], revealing no differences in the PI signaling pathway or the amount of Ca\(^{2+}\) in intracellular stores. Taken together, these data demonstrate that TRPM5 is specifically required for the generation of fast [Ca\(^{2+}\)]\(_{\text{cyt}}\) oscillations upon high glucose stimulation.

Membrane Potential Oscillations from WT and Trpm5\(^{-/-}\) Islets. As TRPM5 is a Ca\(^{2+}\)-activated, but Ca\(^{2+}\)-impermeable, cation channel, it can be anticipated that TRPM5 will influence [Ca\(^{2+}\)]\(_{\text{cyt}}\) oscillations through an effect on membrane potential (V\(_{m}\)). Typically, glucose-stimulated pancreatic islets display depolarizing oscillations of membrane potential triggering bursts of action potentials (Fig. 4A). Oscillations in intracellular Ca\(^{2+}\) and V\(_{m}\) in glucose-stimulated islets are strictly coupled (Fig. S5A). Therefore, we performed combined V\(_{m}\) and [Ca\(^{2+}\)]\(_{\text{cyt}}\) measurements, to unravel the role of TRPM5 in this process.

In line with the pattern of [Ca\(^{2+}\)]\(_{\text{cyt}}\) oscillations, glucose-induced V\(_{m}\) oscillations in individual islets can be classified as slow (average frequency <0.015 Hz), fast (average frequency >0.015 Hz; Fig. 4A), or mixed, showing the typical pattern of clusters of V\(_{m}\) oscillations separated by prolonged silent intervals (40). In a group of WT islets (n = 17), the distribution of different oscillation patterns was comparable to the distribution seen in [Ca\(^{2+}\)]\(_{\text{cyt}}\), measurements (χ\(^2\) analysis, P = 0.09): 47% of the islets were oscillating fast (n = 8), whereas 41.2% could be classified as slow oscillators (n = 7) and the remaining 11.8% (n = 2) showed compound oscillations. Likewise, Trpm5\(^{-/-}\) islets (n = 9; χ\(^2\) analysis, P = 0.3), only display slow oscillations. Thus, it is clear that the absence of fast [Ca\(^{2+}\)]\(_{\text{cyt}}\) oscillations is mirrored by the absence of fast V\(_{m}\) oscillations in Trpm5\(^{-/-}\) islets (Fig. 4A; χ\(^2\) analysis, WT vs. Trpm5\(^{-/-}\), P = 0.019).

To determine the contribution of TRPM5 to the bursting pattern in glucose-stimulated islets, we performed a detailed comparison of V\(_{m}\) changes in islets from both mouse strains. Detailed analysis of the individual action potentials on top of the depolarized plateau, revealed no significant differences between WT and Trpm5\(^{-/-}\) islets concerning parameters like duration and maximal slope of the upstroke, duration of repolarization, and width at half-maximal amplitude. A tendency, although not statistically significant, toward reduced action potential amplitude was apparent in Trpm5\(^{-/-}\) islets (Table S1 and Fig. S6). Slow burst oscillations were indistinguishable between WT and Trpm5\(^{-/-}\) islets, with respect to the duration of the interburst interval, the slope of depolarization during the interburst interval, the threshold potential for burst initiation (Fig. 4B), the average burst duration, plateau fraction, resting potential, interburst potential, and the plateau potential (Table 1). As fast bursting is missing from Trpm5\(^{-/-}\) mice, it seems evident that TRPM5 activity must be hidden in the difference between fast and slow bursting in WT islets. The most obvious differences in that comparison are burst

**Fig. 3.** Overall reduced frequency of intracellular Ca\(^{2+}\) oscillations in isolated islets from Trpm5\(^{-/-}\) mice as a result of a lack of fast oscillations. (A) Effect of glucose (10 mM) on the [Ca\(^{2+}\)]\(_{\text{cyt}}\) in islets from WT and Trpm5\(^{-/-}\) mice. Initially, the islets were bathed in a solution containing 3 mM glucose. Arrows indicate application of 10 mM glucose. (B) Average increase in ratio (F350/F390) after stimulation with 10 mM glucose in islets from WT and Trpm5\(^{-/-}\) mice (n = 28–34 from five to seven mice; P = 0.23). (C) Frequency of oscillations in individual experiments from WT and Trpm5\(^{-/-}\) islets, counted as the number of peaks per min (n = 28–34 from five to seven mice). [Ca\(^{2+}\)]\(_{\text{cyt}}\) increase of 15% was considered to be an oscillation, when 100% is the amplitude between the baseline and the highest level reached in 10 mM glucose. ***P < 0.001. (D) Proportion of islets showing slow, mixed, and fast oscillation patterns according to Fourier analysis in WT (38.2%, 38.2%, and 23.6%, respectively) and Trpm5\(^{-/-}\) islets (89.3%, 10.7%, and 0%, respectively); n = 28 to 34 from five to seven mice: χ\(^2\) analysis WT vs. Trpm5\(^{-/-}\); P = 0.0006.
is clear from combined [Ca\(^{2+}\)]\(_{\text{cyt}}\) and V\(_m\) measurements that the Ca\(^{2+}\)-transient overlaps with the interburst interval (Fig. S5A), so it is likely that TRPM5 is active during the interburst interval. As the increased slope is lacking in Trpm5\(^{-/-}\) islets, our data strongly suggest that TRPM5 is contributing to the depolarizing current during the interburst interval to drive the V\(_m\) toward the threshold for a new burst of activity.

Interestingly, a mathematical model of Ca\(^{2+}\) and V\(_m\) changes in a glucose-stimulated pancreatic β-cell reproduces this phenotype (Fig. S5B). In this model, removing a Ca\(^{2+}\)-activated monovalent cation conductance significantly reduces the oscillation frequency, as we observe in our KO mouse model. This is a result of the lack of a depolarizing current in the interburst interval, which shapes the slow depolarization of the membrane potential to reach the threshold for a new burst of activity (Fig. S5). Note that the increased Ca\(^{2+}\) during the burst of activity overlaps to a large part with the interburst interval, as we also observe in simultaneous Ca\(^{2+}\) and V\(_m\) measurements (Fig. S5 A and B), explaining why TRPM5 would be active in this period.

It’s important to mention here, however, that it is unclear what the exact mechanism behind the variability of the oscillation pattern of a pancreatic islet is (9), and why TRPM5 is apparently only functionally relevant in a (fast-oscillating) subpopulation of the islets. Several models for glucose-induced Ca\(^{2+}\) oscillations exist, of which the most successful propose a complex interplay among [Ca\(^{2+}\)]\(_{\text{cyt}}\) levels, ion channel activity, glycolytic rate, and mitochondrial respiration (6). Bertram et al. (6) propose that slow oscillations (period between 2 and 7 min) represent oscillations in glycolytic activity and that fast oscillations (period as long as tens of seconds) are controlled by ion channel activity during persistent high glycolytic activity. Compound bursting would represent a complex interplay among several of the aforementioned factors. Our data fit well into this model, in a sense that we can show that TRPM5 is essential for the occurrence of fast but not slow oscillations and that also compound bursting is largely lacking in Trpm5\(^{-/-}\) islets. An interesting hypothesis might be that the weight of TRPM5-mediated depolarization is coupled to the glycolytic rate in the cell. Thus, at a constantly high glycolytic rate, which is a necessity for fast oscillations according to Bertram et al. (6), TRPM5 activity would be able to depolarize V\(_m\) in the interburst interval, as the hyperpolarizing K\(_{\text{ATP}}\) current is largely inactive at that point. Conversely, during an oscillating glycolytic rate, TRPM5 would be inadequate to depolarize V\(_m\) during the low point of glycolytic activity, corresponding to the interburst interval between slow oscillations (6) as a result of high activity of K\(_{\text{ATP}}\).

![Figure 4](image_url)

**Table 1. Characteristics of V\(_m\) measurements in WT and Trpm5\(^{-/-}\) islets during stimulation with 10 mM glucose**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>WT Slow (n = 7 from 5 mice)</th>
<th>WT Fast (n = 8 from 6 mice)</th>
<th>Trpm5(^{-/-}) Slow (n = 9 from 5 mice)</th>
<th>WT: Slow vs. fast</th>
<th>Slow: WT vs. Trpm5(^{-/-})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of interburst interval, s</td>
<td>180.8 ± 55.4</td>
<td>118.1 ± 1.4</td>
<td>153.5 ± 34.0</td>
<td>0.013*</td>
<td>0.78</td>
</tr>
<tr>
<td>Slope of depolarization in the interburst interval, mV</td>
<td>0.023 ± 0.004</td>
<td>0.13 ± 0.02</td>
<td>0.034 ± 0.01</td>
<td>0.0001*</td>
<td>0.18</td>
</tr>
<tr>
<td>Threshold potential for burst initiation, mV</td>
<td>−56.9 ± 1.1</td>
<td>−54.3 ± 2.1</td>
<td>−59.1 ± 2.4</td>
<td>0.27</td>
<td>0.64</td>
</tr>
<tr>
<td>Average duration of burst plateau, s</td>
<td>120.5 ± 17.6</td>
<td>12.7 ± 3.5</td>
<td>114.7 ± 17.4</td>
<td>&lt;0.0001*</td>
<td>0.82</td>
</tr>
<tr>
<td>Plateau fraction, %</td>
<td>0.47 ± 0.04</td>
<td>0.55 ± 0.06</td>
<td>0.6 ± 0.05</td>
<td>0.22</td>
<td>0.56</td>
</tr>
<tr>
<td>Plateau potential, mV</td>
<td>−42.2 ± 2.5</td>
<td>−49.2 ± 3.0</td>
<td>−46.2 ± 4.4</td>
<td>0.091</td>
<td>0.41</td>
</tr>
<tr>
<td>Interburst potential, mV</td>
<td>−61.2 ± 2.3</td>
<td>−59.4 ± 2.72</td>
<td>−66.7 ± 3.1</td>
<td>0.61</td>
<td>0.14</td>
</tr>
<tr>
<td>Resting potential, mV</td>
<td>−70.4 ± 0.8</td>
<td>−71.5 ± 1.0</td>
<td>−73.0 ± 2.1</td>
<td>0.12</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Comparison of different parameters (in mean ± SEM) of V\(_m\) in WT slow (n = 7 from 5 mice) vs. WT fast (n = 8 from 6 mice) oscillating islets and WT slow vs. Trpm5\(^{-/-}\) slow oscillating islets (n = 9 from 5 mice). The plateau fraction is calculated as the sum of burst lengths divided by the total period of glucose stimulation.

*Significant at P < 0.05.
Metabolic Phenotype of Trpm5−/− Mice. To determine whether the lack of fast oscillations has consequences for pancreatic islet function, glucose-induced insulin release from freshly isolated islets was measured. Insulin release was significantly reduced in Trpm5−/− islets when stimulated with 10 or 20 mM glucose (Fig. S4 and Fig. S5). Importantly, the insulin content of individual islets (WT, 65.9 ± 5.5 ng insulin/islet; vs. Trpm5−/−, 57.2 ± 5.2 ng insulin/islet; P = 0.27; n = 8) and the pancreatic insulin content in Trpm5−/− islets (WT, 45.9 ± 6.6 μg insulin per pancreas; vs. Trpm5−/−, 51.6 ± 6.8 μg insulin per pancreas; P = 0.58; and WT, 90.8 ± 12.7 μg insulin per g of pancreas; vs. Trpm5−/−, 105.1 ± 1.3 μg insulin per g of pancreas; P = 0.38; n = 3) was unchanged, indicating that Trpm5 is not required for insulin synthesis or storage. β-Cells release insulin through Ca2+-dependent exocytosis of membrane vesicles (41). Cell capacitance measurements from isolated β-cells reveal a similar exocytotic response to the Ca2+-ionophore ionomycin (ΔCm ± 0.70 ± 0.26 pF in WT cells vs. 0.74 ± 0.18 pF in Trpm5−/−cells; P = 0.77; n = 6 per group), excluding a defect in Ca2+-dependent exocytosis as a result of Trpm5 gene deletion. Considering that the time-averaged [Ca2+]cyt signal (and the plateau fraction in Vm measurements) upon glucose stimulation was not significantly different from WT, it may seem difficult to explain a significant reduction in Ca2+-dependent insulin release. However, in this aspect, our results are fully consistent with previous work in β-cells (38) as well as in other secretory cell types such as pulmonary alveolar cells (42) and somatotropes (43), showing that fast Ca2+-dependent oscillations are more efficient than slow oscillations in triggering exocytosis of secretory vesicles. It is clear that Ca2+ exhibits a strong cooperativity in triggering exocytosis from β-cells, suggesting that four to five Ca2+ ions bind to the exocytotic machinery to induce vesicle fusion (44). This nonlinearity may, at least partially, underlie the more efficient insulin release in rapidly oscillating islets. Moreover, oscillatory changes in [Ca2+]cyt in β-cells were also shown to be much more efficient than sustained changes in mobilizing and/or priming vesicles for release (38).

Finally, in conscious mice, overnight fasting blood glucose levels were significantly higher in Trpm5−/− mice (95 ± 3 mg/dL) versus WT mice (72 ± 2 mg/dL; n = 25–28; P = 0.0009). No changes were observed between both mouse lines in an insulin tolerance test (Fig. S7). However, in agreement with the reduced insulin release measured from isolated islets, plasma insulin levels after i.p. glucose injection in overnight fasted male mice were significantly reduced in Trpm5−/− mice compared with WT mice (Fig. 5B). As a consequence, results of oral glucose tolerance test (OGTT) and i.p. glucose tolerance test (IPGTT) show a reduced glucose tolerance in Trpm5−/− mice (Fig. 5C). These data convincingly show that reduced glucose-induced insulin release from β-cells leads to a moderate but significant glucose intolerance in Trpm5−/− mice.

Conclusion

We identified Trpm5 as an important gene for the function of mouse pancreatic β-cells. Deletion of Trpm5 results in an impaired glucose tolerance caused by a reduced glucose-induced insulin release from pancreatic islets. In this way, insight in pancreatic TRPM5 function could consolidate our understanding of the pathogenesis of type II diabetes and might provide a unique target for the treatment of this disease.

Materials and Methods

Experimental procedures are described in more detail in SI Materials and Methods.

Mice. Trpm5−/− mice (45) were backcrossed eight generations in the C57BL/6J background and WT C57Bl/6J mice were used as controls. Only male mice were used for experiments. All animal experiments were carried out in accordance with the European Union Community Council guidelines and approved by the local ethics committee.

Preparation of Islets. Islets were isolated from male mice at 10 to 14 weeks of age via collagenase digestion as described previously (46).

Solutions. For whole-cell current measurements, pipette solution contained (in mM) 20 NaCl, 120 NaAsp, 10 Heps, MgCl2, CaCl2, and the appropriate Ca2+ buffer were added according to the CaBuf program (ftp://ftp.cc.kuleuven.ac.be/pub/rodgmans/cabuf.zip) to obtain 1 mM free Mg2+ and the desired free Ca2+-concentration. Bath solution contained (in mM) 150 NaCl, 5 MgCl2, 10 Heps, and 10 glucose. Pipette solution for flush-uncaging contained (in mM) 20 CsCl, 120 CsAsp, 2 Heps, 2 CaCl2, 5 DMNP-EDTA (1-(4.5-dimethoxy-2-nitrophenyl)-EDTA, Molecular Probes), 1 Fura-2FF (Teflabs); and bath solution contained (in mM) 140 CsAsp, 5 MgCl2, 10 Heps, 1 glucose, 1 μM nifedipine (Sigma-Aldrich) and 100 mM tetrodotoxin (Sankyo). Standard extracellular solution for calcium imaging measurements contained (in mM): 120 NaCl, 4.8 KCL, 2.5 CaCl2, 1.2 MgCl2, 10 Heps, pH 7.4, with NaOH, with different concentrations of glucose added as indicated. For Vcyt and combined measurements, bath solution contained (in mM): 138 NaCl, 5.6 KCl, 1.2 MgCl2, 2.6 CaCl2, 10 Heps, pH 7.4, with NaOH; and pipette solution contained (in mM): 10 KCl, 10 NaCl, 70 K2SO4, 7 MgCl2, 5 Heps, pH 7.35, with KOH, 300 μg/mL nystatin (Sigma-Aldrich) (47).

Electrophysiology and Calcium Measurements. Whole cell currents were measured at 31 °C to 33 °C from cells with a capacitance >5 pF (WT, 6.6 ± 0.3 pF; vs. Trpm5−/−, 6.9 ± 0.3 pF; P = 0.42; n = 29–37), being most likely β-cells (26). [Ca2+]cyt was measured by monitoring the Fura-2FF fluorescence signal (F/F0/F0). Calibration of the fura signal was done as previously described (27). Ramp protocol consisted of a 400-ms ramp from −100 mV toward +100 mV (whole cell current measurements) applied at 1 Hz or from +100 mV toward −100 mV (calcium
uncaging, applied at 0.5 Hz (holding potential, 0 mV). Flash photolysis was performed after 60 ms during a 300-ms depolarizing step at +80 mV. \([\text{Ca}^{2+}\text{free}]_{\text{j}}\) from Fura-2–loaded islets was measured monitoring fluorescence ratio \((F_{340}/F_{380})\) every second (after correction for background fluorescence) at 37 °C. \(V_{\text{m}}\) measurements were performed at 31 °C to 33 °C in the perforated patch configuration under current-clamp conditions. In combined measurements, \(V_{\text{m}}\), of a single cell within the islet and \([\text{Ca}^{2+}\text{free}]_{\text{i}}\), of a region centered around the patch pipette were simultaneously monitored.

**Glucose and Insulin Tolerance Tests.** Glucose and insulin tolerance were analyzed in overnight-fasted and 6 h–fasted mice of 10–14 weeks age, respectively.

**Insulin Release.** Insulin release was measured from statically incubated, size-matched, freshly isolated islets using a commercially available ELISA kit (Merodia).