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Distinct Intracellular Ca^{2+} Response to Extracellular Adenosine Triphosphate in Pancreatic β-Cells in Rats and Mice

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Extracellular adenosine triphosphate (ATP) has distinct effects on insulin secretion from pancreatic β-cells between rats and mice. Using a confocal microscope, we compared changes between rats and mice in cytosolic free calcium concentration ($[Ca^{2+}]_c$) in pancreatic β-cells stimulated by extracellular ATP. Extracellular ATP (50 μ*M*) induced calcium release from intracellular calcium stores by activating P2Y receptors in both rat and mouse β-cells. The intracellular calcium release stimulated by extracellular ATP is significantly smaller in amplitude and longer in duration in rat β-cells than in mouse. In response to extracellular ATP, rat β-cells activate store-operated calcium entry following intracellular calcium release. This response is lacking in mouse β-cells. Rat and mouse β-cells both responded to 9 m*M* glucose by increasing $[Ca^{2+}]_c$. This increase, however, was pronounced only in the rat β-cells. In 9 m*M* glucose, extracellular ATP induced a pro-nounced calcium release above the increased level of $[Ca^{2+}]_c$ in rat β-cells. In mouse β-cells, however, extracellular ATP did not exhibit calcium release on top of the increased level of $[Ca^{2+}]_c$ in 9 m*M* glucose. These results demonstrate distinct responses between rat and mouse β-cells to extracellular ATP under the condition of low and high glucose. Considering that extracellular ATP inhibits insulin secretion from mouse β-cells but stimulates insulin secretion from rat β-cells, we suggest that store-operated Ca^{2+} entry may be related to exocytosis in pancreatic rat β-cells.

Key Words: Islets; insulin; β-cell; $[Ca^{2+}]_i$; store-operated Ca^{2+} currents.

Introduction

Extracellular adenosine triphosphate ($[ATP]_e$) performs many functions through P2 purine receptors in various physiologic systems *(1)*. P2 receptors have been identified in pancreatic β-cells of rats and mice *(2)*. However, β-cells from rats and mice respond differently to $[ATP]_e$. $[ATP]_e$ stimulates insulin secretion in rat pancreatic islets and insulin-secreting cell lines *(2,3)* but inhibits insulin secretion in mouse pancreatic β-cells *(4)*. It is thus suggested that the signaling systems mediating stimulus-secretion coupling are different between rat and mouse β-cells.

Cytosolic free calcium concentration ($[Ca^{2+}]_c$) is a key factor in triggering secretion of hormones, including insulin *(5)*. The increase in $[Ca^{2+}]_c$ is achieved via Ca^{2+} influx through membrane Ca^{2+} channels and/or by Ca^{2+} release from intracellular Ca^{2+} stores (such as endoplasmic reticulum). Glucose increases $[Ca^{2+}]_c$ in pancreatic β-cells through Ca^{2+} influx via voltage-gated Ca^{2+} channels in the plasma membrane *(6)*. $[ATP]_e$ increases $[Ca^{2+}]_c$ by inducing Ca^{2+} release from intracellular Ca^{2+} stores through activating the P2Y receptor or by inducing Ca^{2+} influx through ion channels of P2X receptors *(1)*. Since rat β-cells and mouse β-cells exhibit differences in ATP-induced insulin secretion, changes in the $[Ca^{2+}]_c$ response to ATP should also differ between the two species. Using a confocal microscope system, we investigated the different $[Ca^{2+}]_c$ responses in pancreatic β-cells of rat and mouse in response to $[ATP]_e$ between these two most often used laboratory animals in diabetes research.

Results

Differences in ATP-Induced $[Ca^{2+}]_c$ Increase in β-Cells Between Rat and Mouse

The Fluo-3 intensity in rat pancreatic β-cells was stable under the condition of 2 m*M* glucose. Immediately after inclusion of 50 μ*M* ATP in bath solution, rat β-cells produced a rapid increase in $[Ca^{2+}]_c$, composed of two phases. A transient increase and a sustained increase in $[Ca^{2+}]_c$ under the stimulation of $[ATP]_e$ were recorded (Fig. 1A). The maximum transient increase in Fluo-3 intensity was 2.18 ± 0.23-fold of basal levels. This was deemed significantly higher than basal levels. Five minutes later under ATP stimulation, the Fluo-3 intensity was 1.16 ± 0.023-fold of basal levels, demonstrating sustained higher-than-basal levels (Fig. 1B). Mouse β-cells also responded to $[ATP]_e$ but with a different pattern from rat cells. $[ATP]_e$ induced a transient increase in

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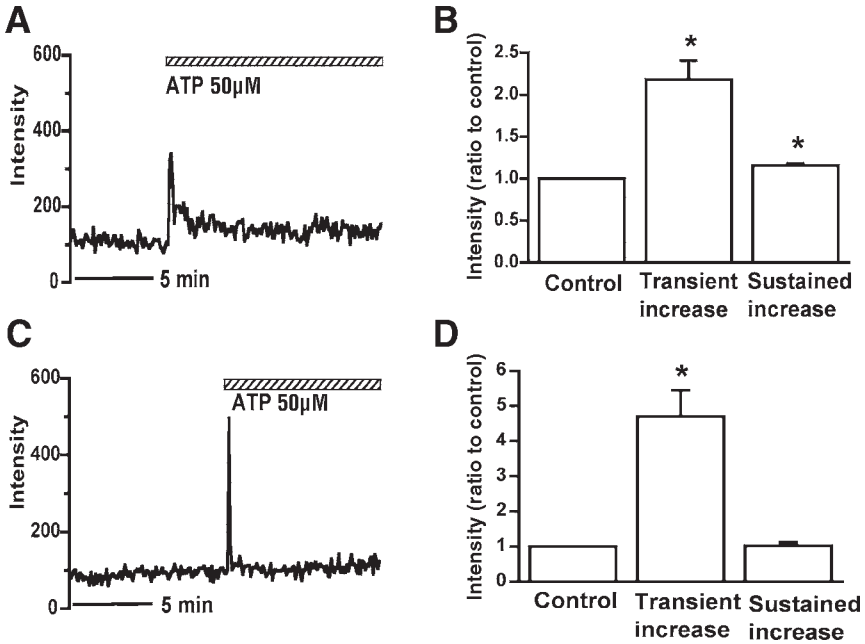

Fig. 1. Differences in ATP-induced $[Ca^{2+}]_c$ increase between rat and mouse β-cells. Glucose concentration in the perfusion medium was 2 m*M*, and 50 μ*M* ATP was given as indicated by the horizontal bars. (**A,C**) Recording of ATP-induced increase in $[Ca^{2+}]_c$ in rat and mouse β-cells, respectively; (**B,D**) analytic results of ATP-induced increase in $[Ca^{2+}]_c$ in rat and mouse β-cells, respectively. *$p < 0.05$; $n = 10$ from 12 rats and 14 mice.

$[Ca^{2+}]_c$, but not a sustained increase in $[Ca^{2+}]_c$, under the condition of 2 m*M* glucose (Fig. 1C). Analytic results showed that the maximum transient increase in Fluo-3 intensity was significantly higher (4.69 ± 0.753-fold) than basal levels. Fluo-3 intensity quickly returned to basal levels and was not significantly different from basal levels after 5 min of ATP stimulation (Fig. 1D).

The transient phase of increase in $[Ca^{2+}]_c$ differed between rat and mouse. The maximum amplitude of transient increase in Fluo-3 intensity was 2.18 ± 0.227-fold of basal levels in rat β-cells, but 4.69 ± 0.753-fold of basal levels in mouse β-cells, a result demonstrating a significantly larger transient phase in mouse β-cells ($p < 0.01$; $n = 10$). The duration of the transient phase of increase in $[Ca^{2+}]_c$ was 80 ± 10 s in rat β-cells as compared with only 16 ± 3 s in mouse β-cells ($p < 0.01$; $n = 10$). The area under the curve (AUC) of the transient phase of increase in $[Ca^{2+}]_c$ was significantly lower in mouse β-cells (42.21 ± 6.777) than in rat β-cells (89.38 ± 9.307; $p < 0.01$; $n = 10$) (Table 1).

Table 1
Comparison of the Transient Increase in $[Ca^{2+}]_c$ in Rat and Mouse β-Cells[a]

Species	*n*	Amplitude (ratio to control)	Duration (seconds)	Area under the curve
Rats	10	2.18 ± 0.227	80 ± 10	89.38 ± 9.307
Mice	10	4.69 ± 0.753*	16 ± 3*	42.21 ± 6.777*

[a]Mouse β-cells produced larger amplitudes, shorter duration and smaller area under the curve compared to rat β-cells responding to ATP stimulation. *Means $p < 0.05$ compared to that of rats.

Role of Extracellular Ca^{2+} and Intracellular Ca^{2+} in ATP-Induced $[Ca^{2+}]_c$ Increase in Rat and Mouse β-Cells

We examined the role of extracellular Ca^{2+} and intracellular Ca^{2+} in the $[ATP]_e$-induced increase in $[Ca^{2+}]_c$ in rat and mouse β-cells. In Ca^{2+}-free bath solution (2 m*M* EGTA in 0 m*M* Ca^{2+}), rat β-cells only showed a transient increase in $[Ca^{2+}]_c$ when stimulated by 50 μ*M* $[ATP]_e$; furthermore, the sustained increase in $[Ca^{2+}]_c$ was eliminated (Fig. 2A). The maximum transient increase in Fluo-3 intensity was 1.69 ± 0.099-fold of basal levels, which is significantly higher than the basal levels ($p < 0.01$; $n = 10$). However, Fluo-3 intensity was 0.97 ± 0.04-fold of basal levels 5 min later under ATP stimulation, a value not significantly different from basal levels (Fig. 2B). Mouse β-cells produced a transient phase of increase in $[Ca^{2+}]_c$ when stimulated by 50 μ*M* $[ATP]_e$ in Ca^{2+}-free bath solution similar to the response of β-cells in bath solution with Ca^{2+} (Fig. 2C). The maximum transient increase in Fluo-3 intensity was significantly higher (5.21 ± 0.802-fold) than basal levels ($p < 0.01$; $n = 10$). Fluo-3 intensity quickly returned to basal level and was not significantly different from this 5 min later under ATP stimulation (Fig. 2D).

To examine the role of intracellular Ca^{2+} stores in the $[ATP]_e$-induced increase in $[Ca^{2+}]_c$ in β-cells, rat and mouse β-cells were pretreated with 1 μ*M* thapsigargin for 20 min

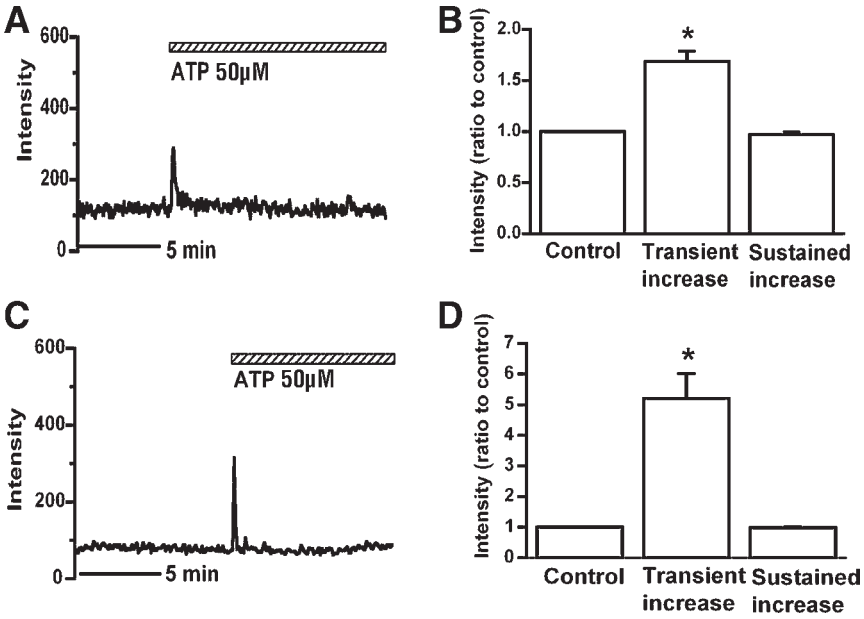

Fig. 2. Role of extracellular Ca^{2+} in ATP-induced $[Ca^{2+}]_c$ increase in rat and mouse β-cells. Rat and mouse β-cells were incubated in Ca^{2+}-free bath solution. (**A,C**) Recording of ATP-induced increase in $[Ca^{2+}]_c$ in rat and mouse β-cells, respectively; (**B,D**) analytic results of ATP-induced increase in $[Ca^{2+}]_c$ in rat and mouse β-cells, respectively. *$p < 0.05$; $n = 10$ from 12 rats and 14 mice.

to deplete the intracellular Ca^{2+} stores. $[Ca^{2+}]_c$ response to $[ATP]_e$ was completely eliminated in both rat and mouse β-cells by this pretreatment (Fig. 3A,C). Analytic results showed that both the transient phase and the sustained phase of increase in $[Ca^{2+}]_c$ in rat β-cells and the transient phase of increase in $[Ca^{2+}]_c$ in mouse β-cells were eliminated. There was no significant difference in Fluo-3 intensity between basal levels and the levels at any time after β-cells were stimulated by $[ATP]_e$ in both rat and mouse (Fig. 3B,D).

The immediate effect of thapsigargin (1 μ*M*) was that it increased $[Ca^{2+}]_c$ in both rat and mouse β-cells. Figure 4A,B shows the thapsigargin-induced increase in $[Ca^{2+}]_c$ in mouse β-cells with and without extracellular Ca^{2+}, respectively. The change in $[Ca^{2+}]_c$ in rat β-cells was identical; the group data are given in Fig. 4C. Fluo-3 intensity was 1.54 ± 0.216-fold of basal levels 5 min after thapsigargin stimulation with the existence of extracellular Ca^{2+} in rat β-cells, which was significantly higher than that of basal levels ($p < 0.01$; $n = 10$) (Fig. 4C). Fluo-3 intensity was decreased to 0.86 ± 0.127-fold of basal levels 5 min after thapsigargin stimulation with the extracellular Ca^{2+} chelated by 2 m*M* EGTA, which was significantly lower than that of basal levels and that with the existence of extracellular Ca^{2+} ($p < 0.01$; $n = 10$) (Fig. 4C). Fluo-3 intensity was 1.51 ± 0.138-fold of basal levels 5 min after thapsigargin stimulation with the existence of extracellular Ca^{2+} in mouse β-cells, which was significantly higher than that of basal levels ($p < 0.01$; $n = 10$) (Fig. 4C). Fluo-3 intensity was decreased to 0.81 ± 0.153-fold of basal levels 5 min after thapsigargin stimulation with the extracellular Ca^{2+} chelated by 2 m*M* EGTA, which was significantly lower than that of basal levels and that with the existence of extracellular Ca^{2+} ($p < 0.01$; $n = 10$) (Fig. 4C).

Signaling Pathway of $[ATP]_e$ in Rat and Mouse β-Cells

To examine the difference of the signaling pathway of $[ATP]_e$ in rat and mouse β-cells, purinergic P2Y receptors were blocked with 30 μ*M* Reactive Blue 2 (RB-2). After inclusion of RB-2 in bath solution for 10 min, the responses of $[Ca^{2+}]_c$ to $[ATP]_e$ were examined. Both the transient increase and sustained increase in $[Ca^{2+}]_c$ induced by $[ATP]_e$ in rat β-cells were eliminated by RB-2 (Fig. 5A,B). The levels of Fluo-3 intensity in the transient phase and the sustained phase after $[ATP]_e$ stimulation were 1.11 ± 0.084-fold and 1.05 ± 0.045-fold of basal levels, respectively. There was no significant difference to basal levels before $[ATP]_e$ stimulation. The transient phase of increase in $[Ca^{2+}]_c$ induced by $[ATP]_e$ in mouse β-cells was also eliminated by RB-2 (Fig. 5C). Analytic results showed that there was no significant difference in Fluo-3 intensity between basal levels and levels after $[ATP]_e$ stimulation (Fig. 5D).

Effects of $[ATP]_e$ on $[Ca^{2+}]_c$ Increase in Rat and Mouse β-Cells Under High Glucose

The levels of $[Ca^{2+}]_c$ in rat β-cells were low and stable under the condition of 2 m*M* glucose. A concentration of 9 m*M* glucose induced an increase in $[Ca^{2+}]_c$ in these β-cells. While rat β-cells were exposed to 9 m*M* glucose, the addi-

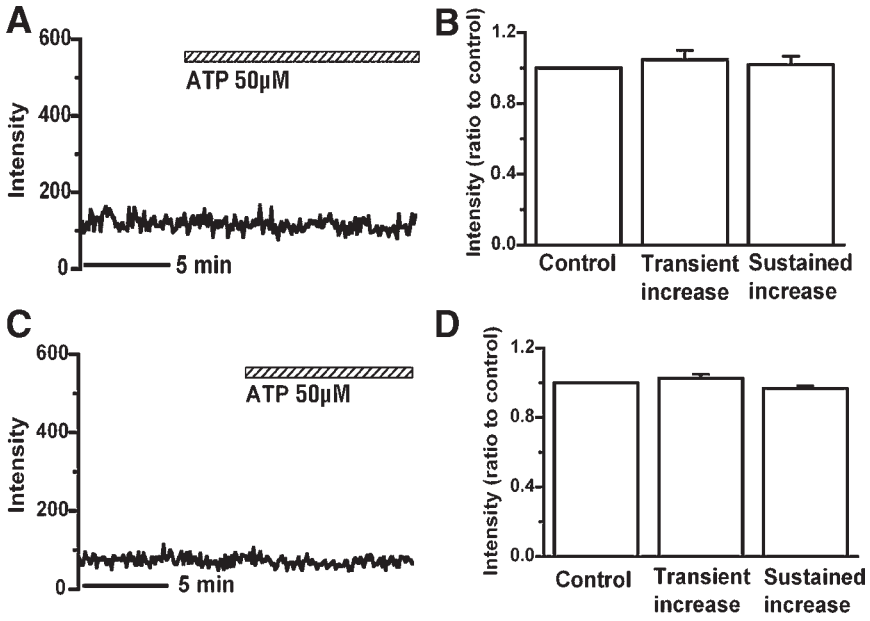

Fig. 3. Role of intracellular Ca^{2+} stores in ATP-induced $[Ca^{2+}]_c$ increase in rat and mouse β-cells. Rat and mouse β-cells were pretreated with 1 μ*M* thapsigargin for 20 min to deplete intracellular Ca^{2+} stores. (**A,C**) Recording of ATP-induced increase in $[Ca^{2+}]_c$ in rat and mouse β-cells, respectively; (**B,D**) analytic results of ATP-induced increase in $[Ca^{2+}]_c$ in rat and mouse β-cells, respectively ($n = 10$ from 12 rats and 14 mice).

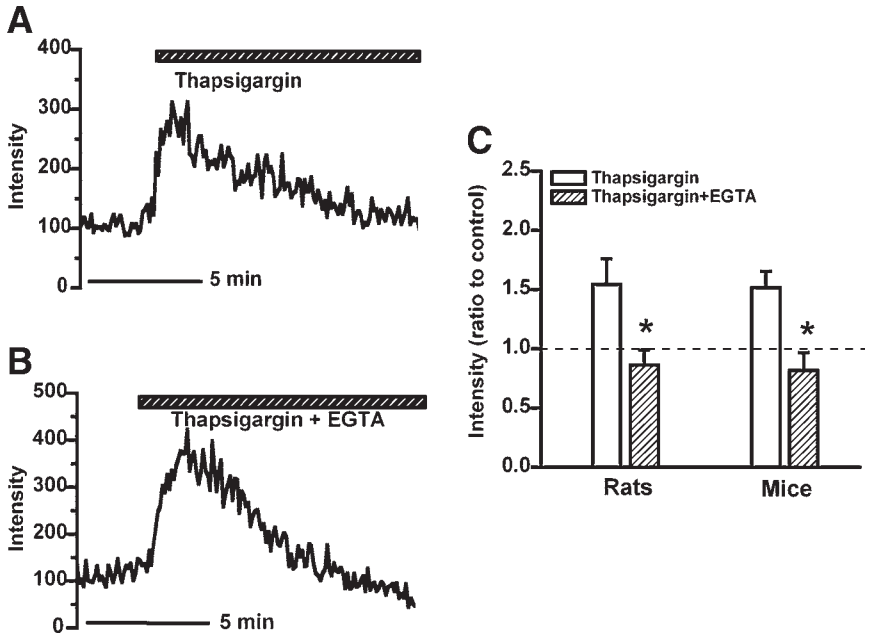

Fig. 4. Activation of Ca^{2+} influx by releasing intracellular Ca^{2+} stores with 1 μ*M* thapsigargin. (**A**) Recordings of thapsigargin-induced increase in $[Ca^{2+}]_c$ with extracellular Ca^{2+} (2.6 m*M*) in mouse β-cells. (**B**) Recording of thapsigargin-induced increase in $[Ca^{2+}]_c$ with extracellular Ca^{2+} completely chelated by 2 m*M* EGTA in mouse β-cells. Rat β-cells had the identical thapsigargin-induced $[Ca^{2+}]_c$ change. (**C**) Analytic results showing the levels of $[Ca^{2+}]_c$ 5 min after thapsigargin administration with or without extracellular Ca^{2+} in rat and mouse β-cells. *$p < 0.05$; $n = 10$ from 12 rats and 14 mice.

tion of 50 μ*M* $[ATP]_e$ induced a pronounced $[Ca^{2+}]_c$ peak on the elevated level of $[Ca^{2+}]_c$ (Fig. 6A). By contrast, mouse β-cells produced an evident increase in $[Ca^{2+}]_c$ when they were exposed to 9 m*M* glucose from 2 m*M* glucose. At the elevated level of $[Ca^{2+}]_c$ induced by 9 m*M* glucose, 50 μ*M* $[ATP]_e$ did not further induce an increase in $[Ca^{2+}]_c$ in mouse β-cells (Fig. 6B). The maximum level of Fluo-3 intensity after stimulation of 50 μ*M* $[ATP]_e$ was significantly higher (2.49 ± 0.244-fold) than the levels just before the addition of $[ATP]_e$ in rat β-cells ($p < 0.01$; $n = 8$). By contrast, the maximum level of Fluo-3 intensity after stimulation of 50 μ*M* $[ATP]_e$ was not significantly different (1.19 ± 0.180-

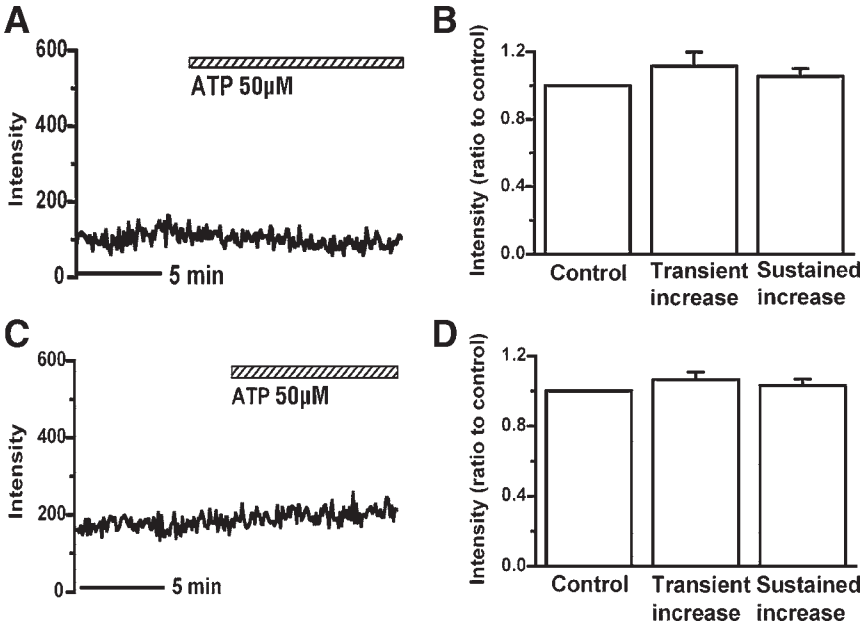

Fig. 5. Effects of RB-2 on ATP-induced $[Ca^{2+}]_c$ increase in rat and mouse β-cells. Rat and mouse β-cells were pretreated with 30 μ*M* RB-2 for 10 min before recording. (**A,C**) Recording of ATP-induced increase in $[Ca^{2+}]_c$ in rat and mouse β-cells, respectively; (**B,D**) analytic results of ATP-induced increase in $[Ca^{2+}]_c$ in rat and mouse β-cells, respectively (n = 10 from 12 rats and 14 mice).

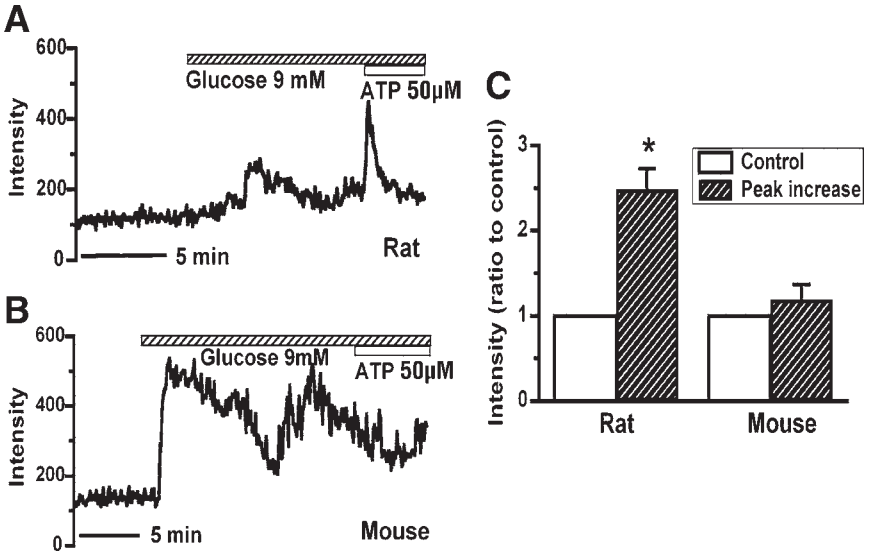

Fig. 6. Differences in ATP-induced $[Ca^{2+}]_c$ increase in rat and mouse β-cells under high glucose levels. Glucose concentration in the perfusion medium was raised from 2 to 9 m*M*, and ATP was given following the addition of 9 m*M* glucose, as indicated by the horizontal bars. (**A,B**) Recording of glucose and ATP-induced increase in $[Ca^{2+}]_c$ in rat and mouse β-cells, respectively. (**C**) analytic results of ATP-induced increase in $[Ca^{2+}]_c$ following addition of 9 m*M* glucose in rat and mouse β-cells. *$p < 0.05$; n = 10 from 12 rats and 14 mice.

fold) from the levels just before the addition of $[ATP]_e$ in mouse β-cells (Fig. 6C).

Discussion

$[ATP]_e$ has different effects on insulin secretion between rat and mouse. The present study is the first to characterize and compare $[Ca^{2+}]_c$ responses to $[ATP]_e$ in rat and mouse pancreatic β-cells. Our results show that a clear kinetic difference in $[Ca^{2+}]_c$ responses to $[ATP]_e$ exists between rat and mouse β-cells. This finding warrants consideration when comparing the functional data obtained from these two, often used animal models in diabetes research. The

major differences in $[Ca^{2+}]_c$ in β-cells responding to $[ATP]_e$ include bigger but shorter transient increases in $[Ca^{2+}]_c$, lack of sustained increase in $[Ca^{2+}]_c$, and lack of increase in $[Ca^{2+}]_c$ on the addition of high glucose in mouse β-cells.

$[ATP]_e$ stimulates a transient increase in $[Ca^{2+}]_c$ in both rat β-cells and mouse β-cells; however, this increase differs between rats and mice. While the amplitude of transient increase in $[Ca^{2+}]_c$ is smaller in rat β-cells compared with that in mouse β-cells, the duration of transient increase in $[Ca^{2+}]_c$ is much longer. Therefore, the AUC of transient increase is larger in rats than in mice. This transient increase in $[Ca^{2+}]_c$ could not be blocked by chelating extracellular Ca^{2+}, whereas it was completely eliminated in both rats and mice by depletion of intracellular Ca^{2+} with thapsigargin, the inhibitor of Ca^{2+}-ATPase on the endoplasmic reticulum. It is therefore clear that the transient increase in $[Ca^{2+}]_c$, in both rat and mouse β-cells, is due to Ca^{2+} release from intracellular Ca^{2+} stores. Further results showed that RB-2, a blocker of P2Y receptors, eliminated the action of $[ATP]_e$ to induce intracellular Ca^{2+} release in both rat and mouse β-cells. This demonstrates that $[ATP]_e$ induces Ca^{2+} release from intracellular Ca^{2+} stores through P2Y receptors in β-cells of both species. Tokuyama et al. *(7)* cloned P2Y receptors from both rat and mouse insulin cells and showed that the subtypes of P2Y receptors in these cells are the same and belong to type 1 P2Y receptors. These receptors are coupled to G-proteins and activate phospholipase C to stimulate production of IP3 *(7)*. As such, it appears that the observed differences in the transient increase in $[Ca^{2+}]_c$ between rat and mouse β-cells could not have resulted from either differences in receptors of $[ATP]_e$ or sources of Ca^{2+}. We postulate that the differences may be caused by a differing ability of β-cells to clear elevated $[Ca^{2+}]_c$ between rat and mouse. One obvious candidate for this process is the storage of intracellular Ca^{2+}. As demonstrated, the transient $[Ca^{2+}]_c$ response to $[ATP]_e$ in mouse β-cells is large and short-lasting, suggesting well-synchronized Ca^{2+} stores with a developed ability to clear Ca^{2+}. The Ca^{2+} stores in rat β-cells may be less synchronized. The difference between rat and mouse β-cells in handling $[Ca^{2+}]_c$ has also been confirmed in myocytes. Results of a recent report suggest that intracellular stores of Ca^{2+} are larger in rat than in mouse myocytes and that rat, compared with mouse, myocytes have a less efficient ability to clear intracellular Ca^{2+} after calcium elevation *(8)*. Further studies are warranted to examine the differential expression and function of Ca^{2+}-ATPase and Na^+/Ca^{2+} exchangers on endoplasmic reticulum and plasma membrane between these species.

Another possible explanation for the difference in $[ATP]_e$-induced increase in $[Ca^{2+}]_c$ between rats and mice is that mouse β-cells lack a store-operated Ca^{2+} entry following $[ATP]_e$-induced Ca^{2+} release from intracellular stores. Rat β-cells exhibited a sustained increase in $[Ca^{2+}]_c$ after the transient increase following stimulation with $[ATP]_e$. However, mouse β-cells lack this sustained increase. Given that this sustained increase in $[Ca^{2+}]_c$ in rat β-cells was abolished in a Ca^{2+}-free solution, it is concluded that this sustained increase was a result of an influx of extracellular Ca^{2+}. The pathways of influx of extracellular Ca^{2+} into cells include voltage-gated Ca^{2+} channels, ligand-gated Ca^{2+} channels, and store-operated Ca^{2+} entry (also known as Ca^{2+} release–activated Ca^{2+} [CRAC] influx, capacitative Ca^{2+} entry). All these types of channels are represented in pancreatic β-cells *(9–11)*. Voltage-gated Ca^{2+} channels play a dominant role in glucose-induced, but not $[ATP]_e$-induced, increase in $[Ca^{2+}]_c$. First, $[ATP]_e$ inhibits action potentials and voltage-dependent Ca^{2+} currents in rat pancreatic β-cells by inducing an increase in $[Ca^{2+}]_c$ released from intracellular Ca^{2+} stores *(12)*. The inhibitory effect of $[ATP]_e$ on voltage-dependent Ca^{2+} currents has also been observed in rat dorsal root ganglion neurons *(13)*. Second, when intracellular Ca^{2+} stores were depleted by thapsigargin, it is in the presence of extracellular Ca^{2+} that the sustained increase in $[Ca^{2+}]_c$ stimulated by $[ATP]_e$ is eliminated in rat β-cells. Therefore, voltage-dependent Ca^{2+} channels are excluded from involvement in the influx of extracellular Ca^{2+} stimulated by $[ATP]_e$. Some reports show that P2X receptors are expressed in rat islets *(14,15)*, raising the possibility that P2X receptors are involved in the action of $[ATP]_e$ to increase $[Ca^{2+}]_c$ in rat β-cells. However, although P2X receptors are expressed in islets, we cannot conclude that P2X receptors are expressed in pancreatic β-cells because there are many cell types in pancreatic islets *(16,17)*. A concentration of 30 μ*M* RB-2, much lower than the concentration used to inhibit P2X receptors *(18)*, completely blocked the sustained increase in $[Ca^{2+}]_c$ stimulated by $[ATP]_e$ in rat β-cells. This result suggests that P2X receptors are not involved in the sustained increase in $[Ca^{2+}]_c$. Furthermore, even in the presence of extracellular Ca^{2+}, the sustained increase in $[Ca^{2+}]_c$ stimulated by $[ATP]_e$ was eliminated following depletion of intracellular Ca^{2+} stores. As such, the involvement of P2X receptors in the sustained increase in $[Ca^{2+}]_c$ stimulated by $[ATP]_e$ can be excluded. Observations in rat osteoclasts also support the notion that P2X receptors are not involved in rises in $[Ca^{2+}]_c$ induced by $[ATP]_e$ *(19)*.

Our results show that the Ca^{2+} influx, responsible for the sustained increase in $[Ca^{2+}]_c$, is dependent on intracellular Ca^{2+} release stimulated by $[ATP]_e$. After elimination of intracellular Ca^{2+} release, the Ca^{2+} influx is concomitantly eliminated in the presence of extracellular Ca^{2+}. Thus, it appears possible that the release of intracellular calcium activates Ca^{2+} influx stimulated by $[ATP]_e$, and as such, the sustained increase in $[Ca^{2+}]_c$ is by means of store-operated Ca^{2+} entry. From our results we conclude that $[ATP]_e$ induces store-operated Ca^{2+} entry following the release of intracellular Ca^{2+} rat β-cells. Although $[ATP]_e$ stimulates the release of intracellular Ca^{2+} in mouse β-cells, it does not induce store-operated Ca^{2+} entry in these cells.

Store-operated Ca^{2+} entry, mediated by CRAC channels, is a common characteristic of cells *(20)*. However, we observed, surprisingly, that there was no store-operated Ca^{2+} entry in mouse β-cells responding to $[ATP]_e$. We observed store-operated Ca^{2+} entry in both rat and mouse β-cells in response to immediate administration of thapsigargin, which demonstrates convincingly the existence of store-operated Ca^{2+} entry or CRAC channels. In the literature, carbachol is able to induce intracellular Ca^{2+} release and store-operated Ca^{2+} entry in mouse β-cells *(10,21)*. We therefore conclude that the lack of store-operated Ca^{2+} entry in mouse β-cells responding to $[ATP]_e$ we observed in this report is not owing to a lack of expression of CRAC channels. Rather, the lack of store-operated Ca^{2+} entry in mouse β-cells responding to $[ATP]_e$ may be related to inhibitory effects of $[ATP]_e$ on insulin secretion. Recent studies have provided evidence demonstrating that activation of store-operated Ca^{2+} entry is coupled to the process of exocytosis. Depletion of intracellular Ca^{2+} stores causes fusion of vesicles containing CRAC channels with the plasma membrane, allowing an influx of extracellular Ca^{2+} *(22,23)*. In mouse β-cells $[ATP]_e$ blocks the exocytosis of vesicles containing insulin at a level downstream to an elevation of $[Ca^{2+}]_c$ *(24)*, and thus we proposed that $[ATP]_e$ also blocks the exocytosis of vesicles containing CRAC and as such restrains store-operated Ca^{2+} entry in mouse β-cells. $[ATP]_e$ stimulates insulin secretion and concomitantly induces store-operated Ca^{2+} entry in rat β-cells, which further supports the coupling of store-operated Ca^{2+} entry and exocytosis in pancreatic β-cells. Further study is warranted to clarify the relationship between vesicles containing insulin and vesicles containing CRAC.

The effect of $[ATP]_e$ on $[Ca^{2+}]_c$ of pancreatic β-cells exposed to high glucose levels (9 m*M*) is different between rats and mice. In mouse β-cells, this level of glucose induced a quickly started and long-lasting increase in $[Ca^{2+}]_c$ with oscillations. In rat β-cells, however, this level of glucose induced a slowly started and short-lasting $[Ca^{2+}]_c$. The difference observed is consistent with previous reports *(28)*. With the addition of 9 m*M* glucose, $[ATP]_e$ induced a further increase in $[Ca^{2+}]_c$ in rat but not in mouse β-cells. It is well known that $[Ca^{2+}]_c$ regulates Ca^{2+} release from endoplasmic reticulum. Cytosolic Ca^{2+} potentiates intracellular Ca^{2+} release at lower concentrations and inhibits intracellular Ca^{2+} release at higher concentrations *(25–27)*. In rat β-cells, we observed with the addition of 9 m*M* glucose a less potent increase in $[Ca^{2+}]_c$ compared with that in mouse β-cells. However, following this in rat β-cells, $[ATP]_e$ induces a potent Ca^{2+} release and may augment insulin secretion. By contrast, glucose induces a potent increase in $[Ca^{2+}]_c$ in mouse β-cells, and this high concentration of $[Ca^{2+}]_c$ produces inhibitory effects on intracellular Ca^{2+} release. Therefore, in mouse β-cells, $[ATP]_e$ is unable to induce the release of Ca^{2+} following the addition of 9 m*M* glucose. It thus appears that the signaling pathway of $[ATP]_e$ to inhibit exocytosis of mouse β-cells is not blocked by the addition of 9 m*M* glucose, and the $[ATP]_e$ may inhibit insulin secretion even when $[Ca^{2+}]_c$ is high. These results sustain the notion that inhibition of $[ATP]_e$ on insulin secretion is downstream of an increase in $[Ca^{2+}]_c$ in mouse β-cells.

In summary, we have shown distinct $[Ca^{2+}]_c$ responses to $[ATP]_e$ in pancreatic β-cells between rats and mice. $[ATP]_e$ induces different patterns of intracellular Ca^{2+} release through P2Y receptors in rat and mouse β-cells. Mouse β-cells lack store-operated Ca^{2+} entry in response to $[ATP]_e$ and also are unable to induce further elevation of $[Ca^{2+}]_c$ following the addition of high levels of glucose. This study suggests that store-operated Ca^{2+} entry may be related to exocytosis in pancreatic β-cells and warrants further study with a focus on investigating the manner by which the signaling pathway of $[ATP]_e$ inhibits the process of exocytosis.

Materials and Methods

Preparation of Pancreatic Islet Cell Culture

Pancreatic islets were isolated from 8- to 10-wk-old Spraque-Dawley male rats and 8- to 10-wk-old C57BL/6 male mice, respectively. The rats and mice were killed by decapitation as approved by the Monash Medical Centre Animal Ethics Committee. Each pancreas was inflated by injecting Hank's solution containing 1 mg/mL of collagenase (type V, Sigma, St. Louis, MO) through the bile duct into the pancreas. The pancreas was then isolated and digested at 37°C for 20 min. The digested tissue was disassembled, then filtered through a mesh. Islets were separated from the digested pancreas by Histopaque-1077 (Sigma) centrifugation and selected by hand under a stereomicroscope *(29)*. The islets were dispersed into single cells by digestion with Dispase (1 mg/mL; Sigma) *(30)*. The cells were then plated onto 22-mm-diameter glass slips coated with 0.01% poly-L-lysine and cultured in RPMI-1640 medium containing 11 m*M* glucose for mouse islet cells and 7 m*M* glucose for rat islet cells *(28)*. The culture medium was supplemented with 10% heat-inactivated fetal calf serum, 100 IU/mL of penicillin, and 100 μg/mL of streptomycin and replenished every 2 d. The experiments were performed during d 2–5 in culture.

Single-Cell $[Ca^{2+}]_c$ Measurement

Cultured islet cells were loaded with 1 μ*M* Fluo-3/AM for 30 min at 37°C in RPMI-1640 medium. Before the recording, the cells were rinsed with and kept for at least 20 min in bath solution to allow for full deetherification of the dye and to equilibrate the cells. The bath solution used for $[Ca^{2+}]_c$ measurement was composed of 140 m*M* NaCl, 4.7 m*M* KCl, 2.6 m*M* $CaCl_2$, 1.2 m*M* $MgCl_2$, 2 m*M* $NaHCO_3$, 1.2 m*M* KH_2PO_4, 2 m*M* glucose, and 5 m*M* HEPES (pH = 7.4 with NaOH and osmolarity = 300 mosM). $[Ca^{2+}]_c$ levels

were reflected by the fluorescent intensity of Fluo-3. The cells were constantly perfused with bath solution at a speed of 2 mL/min. Experimental reagents (such as high glucose, ATP) were dissolved in the bath solution and given through the perfusion system. Fluo-3 fluorescence was excited with a laser at 480 nm, and emitted light was detected by a confocal detector at wavelengths of >515 nm. Images were acquired every 6 s under 500 × 500 pixel with X-Y scan mode. Obtainment and analysis of Fluo-3 intensity were performed using the software Fluoview-300 supplied by Olympus. Fluorescent intensity was artificially assigned to approx 100 in basal condition. To rule out the influence of different cell batches and Fluo-3 loadings, the ratio was compared to basal levels between different groups. The alternation of $[Ca^{2+}]_c$ in each cell during the whole process was reflected by the fluorescent intensity of the cell. When AUC was used, it was calculated by the increase in fluorescent intensity multiplied by time (seconds).

Identification of Cell Types by Immunocytochemical Methods

Dishes containing recorded cells were fixed onto a microscope stage without movement following $[Ca^{2+}]_c$ recording. The cells were washed with phosphate-buffered saline (PBS) and fixed with 4% paraformaldehyde for 20 min. Fluo-3 in cells was bleached by strong ultraviolet light to an undetectable level before the immunocytochemical process was started. Nonspecific binding sites were blocked by incubation with 5% bovine serum albumin in PBS for 30 min. The cells were first incubated with insulin antibodies (guinea pig antiswine, 1:200; Dako) for 1 h. After washing, fluorescin isothiocyanate–conjugated secondary antibodies (rabbit antiguinea pig, 1:100; Dako) were added to stain the β-cells. Fluorescent signal was visualized using the same system for $[Ca^{2+}]_c$ measurement. In this way, recorded $[Ca^{2+}]_c$ signaling could be corresponded to the identified β-cells.

Data Analysis

The initial level of Fluo-3 intensity in each cell before the addition of $[ATP]_e$ was taken as the control level. The rate of $[Ca^{2+}]_c$ increase was estimated by the ratio of intensity level after stimulation of $[ATP]_e$ to the control level. Experimental cells were recorded independently from 10 batches of rat cells and 10 batches of mouse cells. Each batch included cells obtained from one to two rats or one to two mice. Twelve rats and 14 mice were used in total in these experiments. Data quoted are the mean values ± SEM of the indicated number of experimental cells. Statistical significance was evaluated using student's *t*-test for unpaired data. Results were considered significant at $p < 0.05$.

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