Improved blood glucose disposal and altered insulin secretion patterns in adenosine A1 receptor knockout mice

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YANG GK, Fredholm BB, Kieffer TJ, Kwok YN. Improved blood glucose disposal and altered insulin secretion patterns in adenosine A1 receptor knockout mice. Am J Physiol Endocrinol Metab 303: E180–E190, 2012. First published May 1, 2012; doi:10.1152/ajpendo.00050.2012.—Type 2 diabetes mellitus (T2DM) is characterized by the inability of the pancreatic β-cells to secrete enough insulin to meet the demands of the body. Therefore, research of potential therapeutic approaches to treat T2DM has focused on increasing insulin output from β-cells or improving systemic sensitivity to circulating insulin. In this study, we examined the role of the A1 receptor in glucose homeostasis with the use of A1 receptor knockout mice (A1R−/−). A1R−/− mice exhibited superior glucose tolerance compared with wild-type controls. However, glucose-stimulated insulin release, insulin sensitivity, weight gain, and food intake were comparable between the two genotypes. Following a glucose challenge, plasma glucagon levels in wild-type controls decreased, but this was not observed in A1R−/− mice. In addition, pancreas perfusion with oscillatory glucose levels of 10-min intervals produced a regular pattern of pulsatile insulin release with a 10-min cycling period in wild-type controls and 5 min in A1R−/− mice. When the mice were fed a high-fat diet (HFD), both genotypes exhibited impaired glucose tolerance and insulin resistance. Increased insulin release was observed in HFD-fed mice in both genotypes, but increased glucagon release was observed only in HFD-fed A1R−/− mice. In addition, the regular patterns of insulin release following oscillatory glucose perfusion were abolished in HFD-fed mice in both genotypes. In conclusion, A1 receptors in the pancreas are involved in regulating the temporal patterns of insulin release, which could have implications in the development of glucose intolerance seen in T2DM.

Type 2 diabetes mellitus (T2DM) is a debilitating disease characterized by increased blood glucose due to a decreased sensitivity to insulin in the body and the inability of the insulin-secreting pancreatic β-cells to adequately compensate for the higher demand of insulin (19, 43). Therefore, most treatment regimens for T2DM are targeted toward increasing β-cell secretion of insulin and restoring its sensitivity in the body. Even with the current therapies available, individuals with T2DM are typically unable to manage blood glucose excursions as tightly as healthy individuals. Consequently, patients may face excessive periods of hyperglycemia that can contribute to heart and kidney failures, blindness, and other microvascular diseases and reduced life expectancy (9, 53). Currently, an estimated 346 million people worldwide have diabetes (53), and this number is on the constant rise, with 7 million new cases each year (9). Given the growing incidence and reduced quality of life associated with this disease, the demand for new therapeutic approaches to stop or reverse disease progression, rather than delaying the consequences, is more urgent than ever before. Therefore, the integrated control of insulin secretion must be understood better.

Ever since the discovery of purinergic neurotransmission (8), the direct receptor-dependent signaling pathways of adenosine have gained much attention for their therapeutic potential. The ubiquitous distribution of adenosine and metabolic dependency of adenosine levels rationalize the involvement of adenosine signaling in various homeostatic controls (27). In the pancreatic islets, adenosine A1 receptors have been identified on both β-cells and glucagon-releasing α-cells, whereas adenosine A2A receptors have also been identified on α-cells (47). In addition, adenosine and A1 receptor agonists have been shown to inhibit insulin release in the perfused rat pancreas (24) and INS-1 cells (46, 51). Adenosine and its analogs have also been shown to stimulate glucagon release in the perfused rat pancreas in a concentration-dependent manner (12) and to potentiate arginine-induced glucagon secretion (39). These studies illustrate the involvement of adenosine signaling in the endocrine pancreas and specifically the role of A1 receptors in regulating insulin and glucagon release.

Previous studies demonstrated that adenosine A1 receptor knockout mice (A1R−/−) exhibit greater insulin and glucagon release following an intraperitoneal (ip) glucose challenge compared with wild-type controls (29). Furthermore, in the perfused pancreas model, A1R−/− mice appear to have greater insulin release upon high glucose perfusion than wild-type controls (29). In addition, a temporal analysis revealed that A1R−/− mouse pancreata exhibited regular pulses of insulin release following a steady infusion of high glucose conditions, and these pulses were not observed in wild-type controls (42). Taken together, these recent studies support the notion that A1 receptors are involved in the regulation of insulin release. However, it remains unclear whether or not these changes in the levels and temporal patterns of insulin release will have any phenotypic consequences in A1R−/− mice.

To assess the role of adenosine in pancreatic endocrine secretions, we examined the glucose homeostatic controls of A1R−/− mice, with specific focus on the release of the hormones insulin and glucagon. Furthermore, A1R−/− mice were placed on a high-fat diet (HFD) to determine whether or not alterations in glucose homeostasis in these mice would predispose or protect them from the development of glucose intolerance in the presence of a metabolic stressor. In addition, the pulsatile insulin release patterns were investigated using an external oscillatory glucose entrainment technique. The results obtained highlight the involvement of A1 receptors in regulating temporal insulin release and in determining systemic glucose tolerance.
MATERIALS AND METHODS

Animals. Mice were treated in accordance with guidelines of the University of British Columbia Committee on Animal Care. Generation of A1R−/− mice was described previously (28), and the mice were back-crossed for more than 10 generations onto a C57BL/6 background. Male mice were housed in temperature-controlled rooms with 12:12-h light-dark cycles and received, in the first set of studies, a standard diet (25.3% dietary fat; LabDiet, St. Louis, MO) and, in the second set of studies, a control diet (CD; 10% fat; Research Diets, New Brunswick, NJ) or a HFD (45% fat; Research Diets). Daily monitoring and maintenance of animal health and living conditions were carried out by the staff of the animal facilities.

Food and water intake tracking. Mice were caged individually in an enriched environment with a measured amount of food and water at the start of the experiment. Food intake was tracked every 2 days by weighing the amount of food left on the cage tops. Food crumbs that had fallen into the cages were also weighed to minimize error in the measurements. Water intake was recorded by tracking the changes in the mass of the water bottle every 2 days. To account for the potential difference in the rate of water dispensing or leakage from the bottle, the water bottles between experimental animals were interchanged every week.

Nutrient and hormone challenge tests. Oral (OGTT) and ip glucose tolerance tests (IPGTT) were performed following a 6-h fast with 2 g/kg of glucose, as reported previously to be the optimal conditions at differentiating between mice with normal and abnormal glucose tolerance (1). Insulin tolerance tests were also carried out following a 6-h fast with an ip injection of 0.75 U/kg synthetic human insulin (Novo Nordisk, Toronto, ON, Canada). For an assessment of the glucose-induced changes in plasma glucagon levels, 2 g/kg ip glucose was administered following an overnight fast. Blood samples were collected from the saphenous vein. Glucose levels were determined using the OneTouch Ultra glucometer (LifeScan Canada, Burnaby, BC, Canada). Insulin levels were determined by a specific enzyme-linked immunosorbent assay kit (80-INSMSU-E01; Alpco, Salem, NH).

Pancreas perfusion. The surgical isolation of the mouse pancreas has been described previously (18). Mice were anesthetized with ip injections of xylazine (15 mg/kg; Bayer, Toronto, ON, Canada) and ketamine (120 mg/kg; Bioniche Animal Health Canada, Belleville, ON, Canada). The preparation of perfusate has also been described elsewhere (56). Perfusion containing different glucose and arginine concentrations was used to selectively stimulate either β-cell insulin secretion or α-cell glucagon secretion, as demonstrated previously (20). Specifically, perfusate containing 2 mM glucose and 10 mM arginine was used to stimulate glucagon secretion, whereas perfusate containing 20 mM glucose and 10 mM arginine was used to stimulate insulin secretion. Prior to the start of sample collection, the pancreas preparations were perfused with perfusate containing 5 mM glucose for 30 min. The peristaltic pump was set to perfuse at a rate of 1 ml/min, and 1-min samples were collected. Specific radioimmunoassays were used to determine quantities of insulin (RI-13K; Millipore, Billerica, MA) and glucagon (GL-32K; Millipore).

Glucose entrainment. Introduction of an oscillating glucose concentration was achieved with a Standard Infuse/Withdraw PHD Ultra Syringe Pump (Harvard Apparatus, Holliston, MA). Oscillating glucose levels between 6.3 and 7.7 mM were perfused into the isolated pancreas at 1 ml/min with intervals of 10 min. These glucose concentrations have been demonstrated previously to generate oscillatory insulin release in the perfused rat pancreas (44). To ensure that the perfusion pressure remained steady in the setup during the experiment the pump speed was optimized for each preparation, with the pressure monitored by a pressure transducer/signal amplifier that transmitted the signals to a computer via LabView 8.0 (National Instruments, Austin, TX). The pressure readings were monitored only prior to the experiment for optimization of the pump speed.

Data analysis. Data analysis was carried out using Prism 5.0 (GraphPad, La Jolla, CA). Results are expressed as means ± SE for all of the hormones measured. For comparison of glucose, insulin, and glucagon levels, two-way analysis of variance was performed with Bonferroni posttest. Comparisons of summarized data between two groups were performed by two-tailed Student t-tests. For glucose entrainment studies examining correlation coefficients, MATLAB 7.8 (MathWorks, Natick, MA) was used. For all statistical analyses, P ≤ 0.05 is considered to be significant.

RESULTS

Long-term tracking of a cohort of A1R−/− and wild-type control mice revealed comparable body weight gain and food intake between the two genotypes (Fig. 1, A and B). However, water intake was higher in A1R−/− mice than in wild-type controls (Fig. 1C). To examine glucose homeostasis in the A1R−/− compared with the wild-type controls, OGTTs were performed on 7- and 13-wk-old mice. At both ages tested, A1R−/− mice had better glucose tolerance compared with the wild-type controls (Fig. 2, A and C). This was especially apparent at the 15-min time point. No differences were observed in the 2-h postprandial glucose between the two groups. An assessment of plasma insulin levels following the oral

Fig. 1. Body weight, food intake, and water intake of A1 receptor knockout (A1R−/−) mice. Tracking data for body weight (A), food intake (B), and water intake (C) for A1R−/− mice (○) and the wild-type controls (A1R+/+; ●) starting at 5 wk of age; n ≥ 5. *P ≤ 0.05 when the A1R−/− are compared with the wild-type controls.
Fig. 2. Oral glucose tolerance test in A1R<sup>-/-</sup> and wild-type control mice. Oral glucose gavages were performed when A1R<sup>-/-</sup> (○) and wild-type control (A1R<sup>+/+</sup>; ●) mice were at 7 (A and B) and 13 wk (C and D) of age. Blood glucose measurements were taken at 0, 7, 15, 30, 60, 90, and 120 min following glucose administration (A and C). Plasma samples were collected at 0, 7, 15, and 60 min following glucose administration and later assayed for insulin (B and D). Insets show area under the curve (AUC) with arbitrary units; n = 5. **P ≤ 0.01 and ***P ≤ 0.001 when A1R<sup>-/-</sup> mice are compared with the wild-type controls.

Glucose gavage did not reveal any significant differences between the two groups (Fig. 2, B and D).

To determine whether the better glucose tolerance in A1R<sup>-/-</sup> mice is related to changes in the rate of gastric emptying and nutrient absorption, IPGTTs were carried out to bypass the gastrointestinal tract. At the ages of 9 and 12 wk, A1R<sup>-/-</sup> mice had better glucose tolerance compared with wild-type controls (Fig. 3, A and C). However, similar to the plasma insulin levels observed following an oral glucose gavage, no differences in plasma insulin levels were observed between the two groups following ip glucose administration (Fig. 3, B and D).

To examine whether the differences in glucose tolerance observed between the A1R<sup>-/-</sup> and wild-type mice were attributed to a difference in insulin sensitivity, insulin tolerance tests were performed. At the two ages examined, insulin sensitivity was comparable between the two genotypes (Fig. 4). The absence of a difference in the insulin sensitivity and glucose-stimulated insulin secretion between the two genotypes suggests that the difference in glucose tolerance could arise from...
differences in the release of other hormones, such as glucagon. To assess glucose-induced changes in glucagon release, a single ip injection of glucose was carried out, and glucagon levels were measured 10 min following injection. Whereas blood glucose levels increased in both the A1R−/− and the wild-type controls, a decrease in plasma glucagon following glucagon administration was observed only in the wild-type controls (Fig. 5).

The improved glucose tolerance in A1R−/− mice warranted the determination of whether these mice would be protected from metabolic stressors. Therefore, a separate cohort of A1R−/− mice and wild-type control mice was divided into two groups each, with one receiving a HFD and the other receiving the CD. Prior to the splitting of the diet, these cohorts of animals with the same genotype exhibited similar glucose tolerance and insulin sensitivity (Fig. 6), as observed previously; the A1R−/− mice were still able to clear glucose more efficiently than the wild-type control mice, as in the previous cohort (Fig. 6, A and C). However, no differences in glucose-stimulated insulin secretion (Fig. 6, B and D) and insulin sensitivity (Fig. 6E) were detected between the two genotypes. Glucose tolerance and insulin sensitivity were again examined following 3–5 wk on the HFD (Fig. 7). A1R−/− mice were not protected from HFD-induced impaired glucose tolerance. Interestingly, glucose disposal over time following an ip glucose injection exhibited a different pattern in CD-fed A1R−/− mice compared with CD-fed wild-type controls (Fig. 7C). Specifically, the peak in blood glucose levels was lower in CD-fed A1R−/− compared with CD-fed wild-type controls, but blood glucose levels 2 h after injection were higher in CD-fed A1R−/− than in the CD-fed wild-type controls. However, insulin release was not significantly different between the genotypes (Fig. 7, B and D). Furthermore, this difference in ip glucose tolerance was not attributed to a difference in insulin sensitivity since an insulin challenge did not produce different glucose-lowering effects between the genotypes fed with the same diet (Fig. 7E).

We did not observe a difference in food intake observed between genotypes (Fig. 8A). An examination of the body weight changes and fasting blood glucose levels over the course of the experimental periods showed that mice on HFD exhibited faster weight gain and had elevated fasting glucose levels compared with mice on CD (Fig. 8, B and C). However, no significant differences were observed between the genotypes.

The isolated vascularly perfused pancreas was used to better assess the patterns of release of glucagon and insulin. Glucagon release was stimulated with 2 mM glucose and 10 mM L-arginine, whereas insulin release was stimulated with 20 mM glucose and 10 mM L-arginine. L-Arginine was used because it has been demonstrated previously to potentiate glucagon release under low extracellular glucose levels and insulin release under high extracellular glucose levels (20). In both genotypes, insulin release was significantly higher in the HFD group compared with the CD group (Fig. 9, A–C). However, no significant differences were observed between the genotypes. Whereas glucagon release did not vary significantly between the different diet treatments in the wild-type mice (Fig. 9D), A1R−/− mice on the HFD exhibited significantly elevated release of glucagon compared with their CD counterparts (Fig. 9, E and F). Upon the administration of high glucose (20 mM), the A1R−/− HFD pancreases also exhibited a delayed reduction in glucagon release compared with their CD counterparts (time points 30–50; Fig. 9E).

To test whether the difference in glucose tolerance could be attributed to a different pulsatile pattern of insulin release, the
vascularly perfused isolated pancreas model was again used with exogenous application of oscillating glucose to induce pulses of insulin release. When glucose was infused in an oscillatory manner between 7 \pm 0.7 \text{mM}, clear pulses of insulin release were observed and revealed with autocorrelation analyses (Fig. 10). The wild-type mice exhibited an oscillatory pattern at intervals of 10 min, which coincides with glucose oscillations (Fig. 10, A and B), whereas A1R\textsuperscript{-/-} mice exhibited an oscillatory interval of 5 min (Fig. 10, C and D). To determine the effect of HFD on the pulsatile release patterns of insulin release, pancreas perfusions were performed on mice following 14 wk of HFD feeding. The pulses of insulin release were disrupted in HFD-fed A1R\textsuperscript{-/-} and wild-type mice, and the autocorrelation analysis no longer revealed an apparent pattern (Fig. 10, E–H).

**DISCUSSION**

In this study, we determined that A1R\textsuperscript{-/-} mice were able to clear blood glucose more efficiently than wild-type mice following both oral and ip glucose challenges. Furthermore, this phenomenon was not due to a difference in insulin sensitivity between the two genotypes. Following the HFD challenge it was determined that both wild-type and A1R\textsuperscript{-/-} mice developed glucose intolerance and insulin resistance, demonstrating that the better glucose tolerance initially observed in A1R\textsuperscript{-/-} mice did not protect them from such metabolic stress. It was also shown that HFD-fed A1R\textsuperscript{-/-} mice exhibited elevated glucagon secretion in the perfused pancreas. In addition, exogenous infusion of oscillating glucose into the perfused pancreas revealed that A1R\textsuperscript{-/-} mice release insulin in a pulsatile manner with a greater frequency than the wild-type controls, although this was equally disrupted in both groups of mice following a HFD. Our results point to an important role of A1R signaling in islet function.

Over 50 days of tracking, no difference in food intake was observed between A1R\textsuperscript{-/-} mice and wild-type controls when fed the same diet. This is in contrast with a recent study that also tracked food intake for 10 consecutive days and observed slightly elevated food intake in A1R\textsuperscript{-/-} mice (16). This discrepancy could be due to the different sexes of mice used. In the present study only male mice were used, whereas the previous study examined food intake from a cohort of mixed sexes (16). Consistent with the previous assessment (16), we detected an elevated water intake in A1R\textsuperscript{-/-} mice relative to

![Fig. 6. Group matching for the high-fat diet study in A1R\textsuperscript{-/-} mice prior to diet change. Glucose was administered orally (A and B) or via intraperitoneal route (C and D) to A1R\textsuperscript{-/-} and wild-type control (A1R\textsuperscript{+/+}) mice. B and D: plasma samples were collected for measurement of insulin levels. Insets show AUC with arbitrary units. Left 2 bars, mice fed a control diet (CD); right 2 bars, mice fed a high-fat diet (HFD). E: insulin was injected via intraperitoneal route, and plasma glucose levels were monitored; n = 6. †P ≤ 0.05, ††P ≤ 0.01, and †††P ≤ 0.001 when A1R\textsuperscript{-/-} mice are compared with A1R\textsuperscript{+/+}. ‡‡‡P ≤ 0.001 for the comparison as indicated by the bar. Mice were 5–7 wk of age.](http://ajpendo.physiology.org/doi/10.1152/ajpendo.00050.2012)
the controls. This could be attributed to the role of A1 receptors in the kidneys for regulating tubuloglomerular feedback (5). The net increase in filtration rate in A1R-/- mice appears to result in a higher demand for rehydration.

Although A1R-/- mice were able to clear exogenously administered glucose more efficiently than wild-type controls, no significant differences were observed in systemic insulin levels or insulin sensitivity between the two genotypes. In wild-type control mice, ip glucose induced a greater increase in plasma glucose levels than an oral glucose challenge. This is expected because ip glucose bypasses the regulatory effects of gastric emptying and incretin hormones. However, in A1R-/- mice, the plasma glucose differences between IPGTT and OGGT are less pronounced, suggesting that, despite the faster delivery of ip glucose to the A1R-/- pancreas, blood glucose levels were brought back to basal levels rapidly, and thus these mice exhibit a greater capacity clearly at blood glucose. The absence of a significant difference in insulin release between the genotypes suggest that the changes in glucose homeostasis between A1R-/- mice and wild-type controls may be due to the different pattern of temporal release of insulin. This notion is supported by previous studies showing that a pulsatile administration of insulin is more effective at lower hepatic glucose output than a continuous insulin infusion (4).

Although we did not observe a significant difference in plasma insulin levels following glucose challenges, it was reported previously by Johansson et al. (29) that glucose tolerance following ip glucose injections was comparable between A1R-/- and wild-type mice but that plasma insulin levels were elevated in A1R-/- mice. The variation between observations could be due to a different glucose dose (1 g/kg), different method of blood collection (retrobulbar), different age of mice (3–4 mo), and variation in feeding state (freely fed) used in the previous study (29). The present study used a glucose challenge regimen that has been found to be the most useful protocol for differentiating glucose intolerance under various conditions (1). Fasting of mice prior to an assessment of glucose tolerance minimizes the variable feeding states among individual mice that could alter the initial blood glucose and plasma insulin levels. Furthermore, because A1R-/- mice have increased sensitivity to pain (28), getting the mice accustomed to a less invasive blood collection procedure of saphenous bleeding performed in the present study could minimize the stress-induced differences in glucose and insulin responses between the two genotypes. Yet a recent study by Faulhaber-Walter et al. (16) showed that A1R-/- mice have worse glucose tolerance than wild-type controls following ip glucose administration, contrary to the present study. These authors
used a longer fasting period (10–12 h). A prolonged fasting period could have affected the release of counterregulatory hormones to insulin such as glucagon. A paradoxical elevation in glucagon level was observed previously in A1R−/− mice following an ip glucose infusion (29), suggesting that the normal regulation of glucagon secretion may be affected in the absence of A1 receptor signaling. This notion is bolstered by our finding of elevated glucagon secretion from A1R−/− mice compared with wild-type controls. In the present study, it is possible that, with a prolonged fasting period, elevated plasma glucagon levels in A1R−/− mice compared with wild-type controls may have contributed to a worsened glucose tolerance observed in the previous study (16). Changes in the regulation of glucagon release over time could affect the hepatic responses to glucagon and therefore glucose homeostasis under both fasting and feeding states. Hyperglucagonemia has been linked to the initiation and maintenance of hyperglycemia seen in people with diabetes (48–50). In addition, inhibition of glucagon secretion or activity in diabetic mice and rats can alleviate hyperglycemia (30, 52, 57). Therefore, we cannot rule out the potential contribution of altered glucagon signaling in A1R−/− mice to the observed changes in glucose homeostasis. To clarify this point, experiments utilizing α-cell-specific A1 receptor knockout animals would be warranted.

A key point to note is that the A1R−/− mice used by Faulhaber-Walter et al. (16) were of a different strain than those used by Johansson et al. (29) and in the present study. The significant improvement in glucose tolerance in A1R−/− mice in this study warranted examination to determine whether or not these mice would be protected from metabolic stressors. However, both the present study and the recent report by Faulhaber-Walter et al. (16) demonstrated that A1R−/− mice were not protected from HFD-induced impaired glucose tolerance and insulin resistance.

Since the mice used were global knockouts of A1 receptor, a multitude of pathways may have been altered to result in the differences in glucose tolerance compared with wild-type mice. With regard to glucose metabolism and insulin sensitivity, previous studies have demonstrated that adenosine antagonism increases insulin sensitivity in muscle following diet-induced insulin resistance (6) and in Zucker fatty rats (10). Conversely, decreased insulin sensitivity in muscle has been observed with adenosine agonists (7), and this effect has been attributed to A1 receptor activation (11, 33). In adipocytes, A1 receptor activation leads to improved insulin sensitivity (31), whereas prolonged exposure leads to receptor downregulation and insulin resistance (23). Desensitization of A1 receptor on adipocytes has also been correlated with diabetes, suggesting an etiological link (2). In addition, overexpression of the A1 receptor in adipocytes of transgenic mice protected them from HFD-induced insulin resistance (13). These studies illustrate the diverse and tissue-dependent effects of A1 receptor signaling on insulin sensitivity. Whereas Johansson et al. (29) demonstrated that muscle insulin sensitivity in A1R−/− mice was unaffected, Faulhaber-Walter et al. (16) have shown that A1R−/− mice have increased fat mass and decreased lean mass compared with wild-type controls. In addition, the effects of adenosine signaling in the central nervous system have been well characterized with various potential therapeutic applications (3). Specifically, A1R−/− mice show signs of increased anxiety and sensitivity to pain (21, 28). Changes in neuronal activity in response to tissue hypoxia are also decreased in A1R−/− mice compared with wild-type controls (5, 54). Furthermore, adenosine A1 receptors may play an important role in maintaining tonic activity in the synaptic junctions (35), and A1R−/− mice have been associated with decreased neuronal plasticity (22).

In the present study, it is possible that the lower stress tolerance in A1R−/− mice may have contributed to the development of glucose intolerance following HFD-induced oxidative stress. The protective effect of the higher frequency pulsatile insulin release in A1R−/− mice may be overridden by the poor hypoxic response and decreased adipocyte insulin sensitivity following a HFD. Therefore, to better illustrate the involvement of A1 receptors in energy homeostasis, tissue-specific knockouts or isolated organ perfusion models should be employed.

The more efficient glucose tolerance observed in A1R−/− in the present study could be due to more pronounced pulsatile release patterns of insulin with higher amplitudes. The impor-
The significant improvement in glucose tolerance in A1R−/− mice has been drawn from correlative studies with the development of diabetes (4, 32, 34, 37, 40). Exogenous infusion of minute pulses of glucose can amplify oscillations in insulin secretion in healthy individuals, but this property is disturbed in people with diabetes or prediabetes (14, 25, 36). This method relies on the glucose entrainment properties of insulin secretion such that the pulses of insulin release are synchronized to the oscillatory patterns of exogenously administered glucose (45). With the same concept, pancreas perfusions with oscillating glucose levels generated synchronized insulin pulses in Wistar and Zucker control rats but not in the Zucker diabetic fatty rats (44). Therefore, glucose entrainment presents a viable method to examine the insulin secretory health in A1R−/− mice. The present study demonstrated that, by inducing pulsatile insulin release with an oscillatory glucose infusion, A1R−/− mice exhibited a higher frequency of regular pulsatile insulin release than wild-type controls. Pulses of insulin release from β-cells are dependent on intracellular signals that regulate the priming of reserve insulin granules and the exocytotic machinery (38). Examples of these signals are the cAMP-activated factors PKA and Epac2 (15, 41). Since A1 receptor signaling has been demonstrated to inhibit adenylate cyclase activity and decrease intracellular cAMP levels (17), results from the present and previous studies may suggest that A1 receptor signaling could be involved in the priming and exocytosis of insulin granules and thereby govern the temporal release patterns of insulin from β-cells.

The significant improvement in glucose tolerance in A1R−/− mice in this study warranted examination to determine whether or not these mice would be protected from metabolic stressors. However, both the present study and the recent report by Faulhaber-Walter et al. (16) demonstrated that A1R−/− were not protected from HFD-induced impaired glucose tolerance and insulin resistance. Thus the higher frequency of pulsatile insulin secretion in A1R−/− may have contributed to improved glucose metabolism in vivo due to improved hepatic actions (4). However, the pulses of insulin release were disrupted in HFD-fed A1R−/− and wild-type mice, suggesting that HFD-induced impaired glucose tolerance also results in disrupted pulsatile insulin release patterns. In addition, the HFD-induced disruption of the higher frequency of insulin pulses in A1R−/− may have led to the loss of the superior glucose tolerance observed under CD feeding. Further examination on the sequence of events of disruption in pulsatile insulin release and impaired glucose tolerance may provide insight into the exact cause and effects of HFD-induced defects.

The canonical pathway of glucose-induced insulin release has been well described. In addition, the cAMP signaling pathway is critical in the supplemental pathway of insulin secretion from β-cells. It has long been suggested that adenosine may be an important regulator of insulin release via its effects on adenylate cyclase (26). The A1 receptor is coupled to the Gα protein; therefore, receptor activation and G protein activation lead to downstream inhibition of cAMP activity (17). It is conceivable that, in A1R−/− mice, expression of adenylate cyclase and the downstream effectors PKA and Epac2 could be altered. Since PKA and Epac2 have profound effects on the canonical pathway of insulin secretion acting on glucose transporters and ion channels, it is possible that these are also altered in the A1R−/− mouse β-cells. It has been demonstrated that the A1 receptor antagonist DPCPX can suppress the actions of adenosine without affecting intracellular Ca2+ transients (17). These results suggest that there are
molecular differences in β-cell physiology following acute and chronic A1 receptor blockade. It is possible that chronic alterations in A1 receptor signaling can then lead to changes in β-cell architecture, leading to pathological states. Interestingly, our recent study demonstrated that the adenosine level in the isolated mouse pancreatic islets is 5.6 ± 0.6 μM (55), which is within the concentration that has been shown previously to affect insulin secretion (24, 46, 51). Furthermore, adenosine levels in the islets were inversely correlated with exogenous glucose concentrations, suggesting a potential link in glucose-mediated changes in islet hormone secretion (55). More attention is needed in future investigations to draw more evidence in this relationship. Future studies should examine changes in the intracellular signaling molecules in isolated pancreatic islets or β-cells from A1R−/− mice compared with wild-type controls.

In conclusion, we find that A1R−/− mice have better glucose tolerance than wild-type controls, and one of the factors contributing to this phenomenon could be a higher frequency of pulsatile insulin release from the A1R−/− pancreas. In addition, a correlation was also noted between impaired glucose toler-

Fig. 10. Insulin secretion induced by oscillatory glucose infusion in vascularly perfused pancreas from wild-type controls and A1R−/− mice. Oscillatory infusion of 7 ± 0.7 mM was achieved with a periodicity of 10 min. Insulin release was measured in samples collected at 1-min intervals. One representative A1R+/+ mouse (A) and 1 A1R−/− mouse (C) fed a CD are shown here with their corresponding autocorrelation analysis (B and D); n = 3. One representative A1R+/+ mouse (F) and 1 A1R−/− mice (G) fed a HFD are shown here with their corresponding autocorrelation analysis (F and H); n = 3.
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GRANTS

This study was supported by the Wah Sheung Fund. G. K. Yang is a recipient of the Alexander Graham Bell Canada Graduate Scholarship granted by the Natural Sciences and Engineering Research Council of Canada. T. J. Kieffer is a Senior Scholar of the Michael Smith Foundation for Health Research and received grant funding from the Canadian Institutes of Health Research and the Canadian Diabetes Association.

DISCLOSURES

The authors have no conflicts to disclose, financial or otherwise.

AUTHOR CONTRIBUTIONS

G.K.Y., T.J.K., and Y.N.K. did the conception and design of the research; G.K.Y. performed the experiments; G.K.Y. analyzed the data; G.K.Y., B.B.F., T.J.K., and Y.N.K. interpreted the results of the experiments; G.K.Y. prepared the figures; G.K.Y. drafted the manuscript; G.K.Y., B.B.F., T.J.K., and Y.N.K. edited and revised the manuscript; G.K.Y., B.B.F., T.J.K., and Y.N.K. approved the final version of the manuscript.

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