Can J Diabetes 43 (2019) 115–120

Contents lists available at ScienceDirect

Canadian Journal of Diabetes

journal homepage: www.canadianjournalofdiabetes.com

Original Research

Muscarinic Agonist Ameliorates Insulin Secretion in Wfs1-Deficient Mice

Maarja Toots MSc [a,b,*]; Riin Reimets MSc [a,b]; Mario Plaas PhD [a,b]; Eero Vasar PhD [a,c]

[a] Department of Physiology, Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia
[b] Laboratory Animal Centre, Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia
[c] Centre of Excellence for Genomics and Translational Medicine, University of Tartu, Tartu, Estonia

Key Messages

- Decreased insulin levels in Wfs1 deficiency are at least partially caused by problems in insulin secretion.
- Deficit in decreased insulin secretion in Wfs1 deficiency is improved by stimulation of muscarinic receptors.

ARTICLE INFO

Article history:
Received 1 February 2018
Received in revised form
15 May 2018
Accepted 18 June 2018

Keywords:
diabetes
hyperglycemia
insulin secretion
muscarinic receptor M3
Wolframin1 (Wfs1)

ABSTRACT

Objectives: Similar to patients with Wolfram syndrome and to heterozygous Wolframin1 (Wfs1) mutation carriers, Wfs1-deficient mice exhibit impaired glucose tolerance and lower plasma insulin levels. Muscarinic receptor 3 agonists have previously been shown to potentiate glucose-stimulated insulin secretion. Therefore, the aim of this study was to investigate insulin-secretion dynamics in Wfs1-deficient mice and evaluate carbachol, muscarinic agonist and the ability to ameliorate the insulin secretion deficits caused by the Wfs1 mutation.
Methods: Wild-type Wfs1 heterozygous and Wfs1 mutant mice were used. Blood glucose was measured after glucose and carbachol administration. Insulin secretion was measured from serum using ELISA.
Results: Glucose administration causes hyperglycemia in Wfs1-deficient mice due to decreased insulin secretion. This deficit is abolished by administration of the muscarinic agonist carbachol.
Conclusions: Activation of the muscarinic pathway to potentiate insulin secretion may present a target to manage diabetes resulting from Wfs1 deficiency.

Mots clés :
diabète
hyperglycémie
sécrétion d'insuline
récepteur muscarinique de sous-type M3 (récepteur M3)
wolframine 1 (WFS1)

RÉSUMÉ

Objectifs : Tout comme les patients atteints du syndrome de Wolfram et les porteurs hétérozygotes de la mutation de la wolframine 1 (WFS1), les souris ayant une carence en WFS1 montrent une diminution de la tolérance au glucose et des concentrations plasmatiques plus faibles d'insuline. Il a déjà été démontré que les agonistes des récepteurs muscariniques de sous-type M3 (récepteurs M3) potentialisaient la sécrétion d'insuline induite par le glucose. Par conséquent, l'objectif de la présente étude était d'examiner la dynamique de la sécrétion d'insuline chez les souris ayant une carence en WFS1 (souris WFS1) et d'évaluer l'agoniste des récepteurs muscariniques, le carbachol, et la capacité d'améliorer les déficits de la sécrétion d'insuline dus à la mutation du gène WFS1.
Méthodes : Nous avons utilisé des souris hétérozygotes WFS1 de type sauvage et mutantes WFS1. Nous avons mesuré la glycémie après l'administration de glucose et de carbachol. Nous avons quantifié la sécrétion d'insuline dans le sérum au moyen de l'ELISA.
Résultats : L'administration de glucose provoque une hyperglycémie chez les souris WFS1 en raison de la diminution de la sécrétion d'insuline. L'administration de l'agoniste des récepteurs muscariniques, le carbachol, élimine cette carence.
Conclusions : L'activation des récepteurs muscariniques pour potentialiser la sécrétion d'insuline peut représenter une cible en matière de prise en charge d'un diabète qui résulte de la carence en WFS1.

* Address for correspondence: Maarja Toots, MSc, Institute of Biomedicine and Translational Medicine, University of Tartu, 19 Ravila Street, Tartu 50411, Estonia.
E-mail address: maarja.toots@ut.ee

1499-2671 © 2018 Canadian Diabetes Association.
The Canadian Diabetes Association is the registered owner of the name Diabetes Canada.
https://doi.org/10.1016/j.jcjd.2018.06.007

Introduction

Mutations in the Wolframin1 (Wfs1) gene cause the autosomal recessive disorder Wolfram syndrome (WS). WS is characterized by diabetes insipidus, optic nerve atrophy, deafness and diabetes mellitus resulting from the progressive pancreatic beta-cell loss caused by endoplasmatic reticulum (ER) stress (1,2). Approximately 1% of the general population in the United States are heterozygous Wfs1 gene mutation carriers, and genome-wide association studies have established the increased risk for development of type 2 diabetes caused by single-nucleotide polymorphisms in the Wfs1 gene (3–7). The WFS1 protein is located in the membrane of the ER, where it is responsible for the regulation of Ca^{2+} accumulation. It has been shown that the concentration of free Ca^{2+} in the ER is reduced in Wfs1 knockdown cells and is increased in Wfs1-overexpressing cells (8).

A Wfs1-deficient mouse line, generated at Tartu University (Estonia), exhibits impaired glucose tolerance, increased urine glucose concentration and growth retardation (9,10). In 6-month-old male Wfs1-deficient mice, these traits are accompanied by significantly reduced levels of insulin compared to wild-type (WT) littermates (10).

Insulin secretion in response to glucose is divided into 2 phases. In the transient first phase, high amounts of insulin are secreted during a very short period; in the slowly developing, sustained second phase, insulin is secreted more slowly during a longer period (11). For glucose-stimulated insulin secretion, glucose enters beta cells through GLUT2 transporters (12). Glucose is then metabolized, increasing the adenosine triphosphate/adenosine diphosphate ratio. The increased adenosine triphosphate/adenosine diphosphate ratio leads to closure of adenosine triphosphate-sensitive K^+ channels and membrane depolarization (13,14). Membrane depolarization leads to the opening of voltage-operated Ca^{2+} channels, induces Ca^{2+} influx, increases the intracellular Ca^{2+} concentration and activates the exocytosis machinery. The exocytosis process begins a few minutes after glucose enters the pancreatic beta cells (15,16).

In addition to glucose concentration, insulin secretion from the pancreatic beta cells is also influenced by various neurotransmitters and hormones, which can amplify glucose-stimulated insulin secretion. This amplification is impaired in patients suffering from type 2 diabetes (17). Acetylcholine belongs to the neurotransmitters affecting glucose-stimulated insulin secretion. Acetylcholine interacts with 5 types of muscarinic receptors (M1 through M5), of which M3 has a critical role in regulating insulin release (18,19). Acetylcholine, released from the parasympathetic nerve endings or pancreatic alpha cells, binds to the M3 muscarinic receptors and, through a signaling cascade, leads to generation of inositol-1,4,5-triphosphate (IP3) and diacylglycerol. IP3 binds to IP3 receptors on the surface of the ER, mobilizing intracellular Ca^{2+} stores, which leads to an increase in cytoplasmic Ca^{2+} levels, thus potentiating glucose-stimulated insulin secretion (20–22). Previous reports have shown that the muscarinic agonist carbachol can restore insulin secretion in mice fed a high-fat diet (23) and in spontaneously diabetic Goto-Kakizaki rats (24).

The aim of the present study was to investigate insulin-release dynamics in Wfs1-deficient mice and to determine whether stimulating muscarinic receptors diminishes deficiency in insulin secretion caused by the Wfs1 defect.

Methods

Animals

The animal experiments described in this study were performed according to permission from the Estonian National Board of Animal Experiments (# 37, 14.08.2014 and #55, 14.05.2015) and in accordance with the European Communities Directive of September 2010 (2010/63/EU). Generation of Wfs1 exon 8 knockout mice has been described by Luuk et al (25). All studies were performed in 2-month-old male F2 (129S6/SvEvTac×129S6/SvEvTac)×(129S6/SvEvTac×129S6/SvEvTac) mice. The average weight of animals was 22.9±0.3 g for WT mice, 23.2±0.3 g for heterozygous (HET) mice and 19.2±0.3 g for homozygous (KO) Wfs1-deficient mice. Altogether, 180 mice were used. Breeding and genotyping were conducted in the Department of Biomedicine and Translational Medicine, University of Tartu. Mice were housed in groups of 7 to 8 at 20±2°C under a 12/12-h light/dark cycle (lights on at 7:00 AM) with free access to standard food pellets and water. All animal experiments were performed between 10:00 AM and 3:00 PM. Wfs1-deficient homozygous and HET mice were always used in parallel with their WT littermates, and the animals were randomly divided between the experimental groups.

Intraperitoneal glucose tolerance test

For the intraperitoneal glucose tolerance test, food was removed 90 min prior to the beginning of the experiment, and 90-min fasting was used because it was sufficient to allow blood glucose levels to equalize while having smaller effects on metabolic parameters, effects that have been observed with longer fasting (26–28). Basal levels of blood glucose were determined from the tail vein by needle puncture using a commercial glucose meter (Accu-Chek Performa, Roche, Basel, Switzerland). After that, mice were given intraperitoneal injections of: 1) 2 g/kg glucose; 2) 0.2 mg/kg carbachol (carbamoylcholine chloride, muscarinic receptor agonist); or 3) 2 g/kg glucose + 0.2 mg/kg carbachol. Then, 5, 10 or 30 min later, animals were euthanized, blood samples were collected for insulin measurement and blood glucose levels were determined. Carbachol (Sigma-Aldrich, St. Louis, Missouri, United States) and D-(+)-glucose (Sigma-Aldrich) were dissolved in saline (0.9% NaCl; B. Braun, Melsungen, Germany). A carbachol concentration of 0.2 mg/kg was chosen because it corresponds to a 1 µmol/kg dose and approximately a 10 µM concentration in blood, showing strong effects in previously reported experiments (29–31).

Blood insulin measurements

For baseline insulin measurements, blood was collected without prior manipulations. The same baseline group was used as the control group for study of the effects of glucose and carbachol. Blood was allowed to clot at room temperature for 30 min, followed by centrifugation for 10 min, 2000×*g* in at 2°C to 4°C. Serum was collected and stored in –80°C until further analysis. An ELISA test was performed on blood serum using the Ultra Sensitive Mouse Insulin ELISA Kit (Crystal Chem, Elk Grove Village, Illinois, United States) according to the manufacturer's protocol.

Statistical analyses

Mean values and SEMs are presented in the figures. All data were analyzed using Statistica for Windows v. 8.0 (StatSoft, Dell, Round Rock, Texas, United States). The Shapiro-Wilks normality test was used to verify normal distribution of data, followed by 1-way ANOVA or factorial ANOVA and the Tukey HSD post hoc test for identification of statistical significance. For area-under-the-curve calculations, results from the same group of animals at different time points were combined.

Results

Wfs1 KO mice had impaired insulin secretion in response to glucose administration.

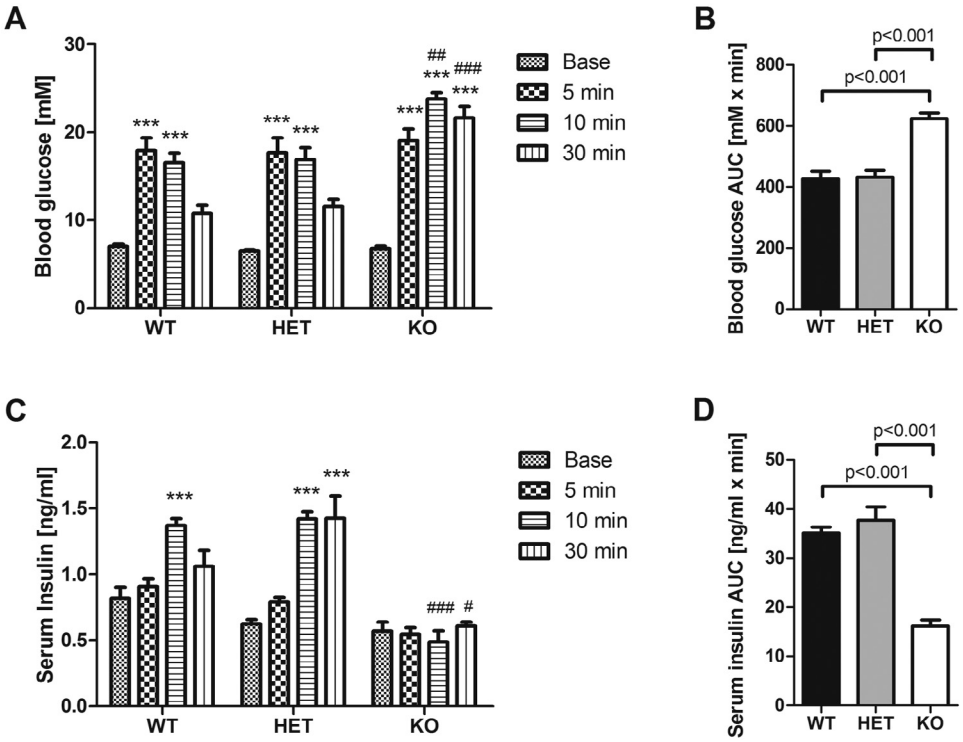

Figure 1. Response to 2 g/kg glucose injection. Blood glucose (A,B) and insulin (C,D) levels 5, 10 or 30 min after administration and corresponding AUC. ***p<0.001 compared to the baseline level of the same genotype. #p<0.05. ##p<0.01. ###p<0.001 compared to WT at the same time-point. *AUC,* area under the curve; *HET,* heterozygous Wfs1 mutation carrier; *KO,* homozygous Wfs1 mutation carrier; *WT,* wild-type mice. n=5 to 7.

There were no differences between genotypes in the basal blood glucose level (Figure 1A). A glucose injection increased the blood glucose level 5 and 10 min after injection in all mice ($F[3,59]=86.97$; $p<0.001$). Blood glucose levels in KO mice were significantly higher than in WT mice and remained elevated for 30 min ($p<0.001$) ($F[2.59]=28.42$; $p<0.001$ [genotype]; $F[6.59]=7.90$; $p<0.001$ [genotype×time]) (Figure 1A,B).

Insulin secretion (Figure 1C,D) significantly differed between genotypes ($F[2.58]=52.08$; $p<0.001$). Blood insulin levels in WT mice were slightly elevated after 10 min and returned to the baseline level at 30 min. In HET mice, the elevation of insulin levels lasted longer but was otherwise similar to that in WT mice. KO mice insulin levels remained at the baseline level and were significantly lower compared to WT mice throughout the experiment ($F[3.58]=19.07$; $p<0.001$ [time]; $F[6.58]=8.03$; $p<0.001$ [genotype×time]).

Wfs1-deficient mice displayed defects in the first phase of insulin secretion

The muscarinic agonist carbachol caused no significant decrease in blood glucose levels in WT and HET animals and caused a minor increase in KO animals at 10 min (Figure 2A,B), as also seen previously; they were caused by an increased stress response (32) ($F[2.60]=5.33$; $p<0.01$ [genotype]; $F[3.60]=4.52$; $p<0.01$ [time]; $F[6.60]=3.22$; $p<0.01$ [genotype×time]).

Initiating only the first phase of insulin secretion with carbachol revealed the deficits in insulin secretion in both HET and KO animals ($F[2.60]=12.84$; $p<0.001$). In WT animals, insulin levels were significantly increased compared to baseline levels at both 5 and 10 min after carbachol injection ($p<0.001$), but there was only a modest increase in HET mice and no statistical increase in KO animals (Figure 2C,D); the early response (5 min after injection) of their blood insulin was significantly lower than in WT animals ($F[3.60]=33.99$; $p<0.001$ [time]; $F[6.60]=4.34$; $p<0.01$ [genotype×time]).

Carbachol and glucose coadministration releases insulin in Wfs1 KO mice and diminishes hyperglycemia

Coadministration of carbachol and glucose increased blood glucose levels (Figure 3A,B) in all genotypes. The glucose level in KO mice was somewhat higher at 10 min compared to other genotypes. However, hyperglycemia was absent in all genotypes at 30 min after injection ($F[2.61]=8.82$; $p<0.001$ [genotype]; $F[3.61]=77.38$; $p<0.001$ [time]; $F[6.61]=5.63$; $p<0.001$ [genotype×time]).

Insulin levels in WT and HET mice 5 and 10 min after injection were 10 times higher than baseline levels (Figure 3C). KO mice displayed significantly lower insulin secretion than WT mice (Figure 3D) ($F[2.61]=142.82$; $p<0.001$ [genotype]; $F[3.61]=233.09$; $p<0.001$ [time]; $F[6.61]=53.36$; $p<0.001$ [genotype×time]).

However, coinjected KO mice blood glucose levels were similar to those of WT mice receiving glucose (Figure 4A) ($F[1.41]=1.07$; $p=0.31$), and their insulin secretion was as strong as in glucose-treated WT mice (Figure 4B) ($F[1.40]=10.94$; $p<0.01$ [genotype]; $F[3.40]=18.53$; $p<0.001$ [time]; [(3.40)=15.62; $p<0.001$ [genotype×time]).

Discussion

Our findings confirm the significant impairment in insulin secretion in Wfs1-deficient mice. Glucose injection caused strong hyperglycemia in Wfs1 KO mice, which is consistent with previous reports in mice, rats and humans (Figure 1) (1,2,10,33). Hyperglycemia was explained by the lack of insulin secretion in response to glucose, evident from blood serum insulin levels.

Carbachol and glucose coreceiving Wfs1 KO mice were able to secrete insulin at levels similar to those of WT mice receiving only glucose and, thus, preventing the long-term hyperglycemia that was present in KO animals receiving only glucose (Figure 1, Figure 3,

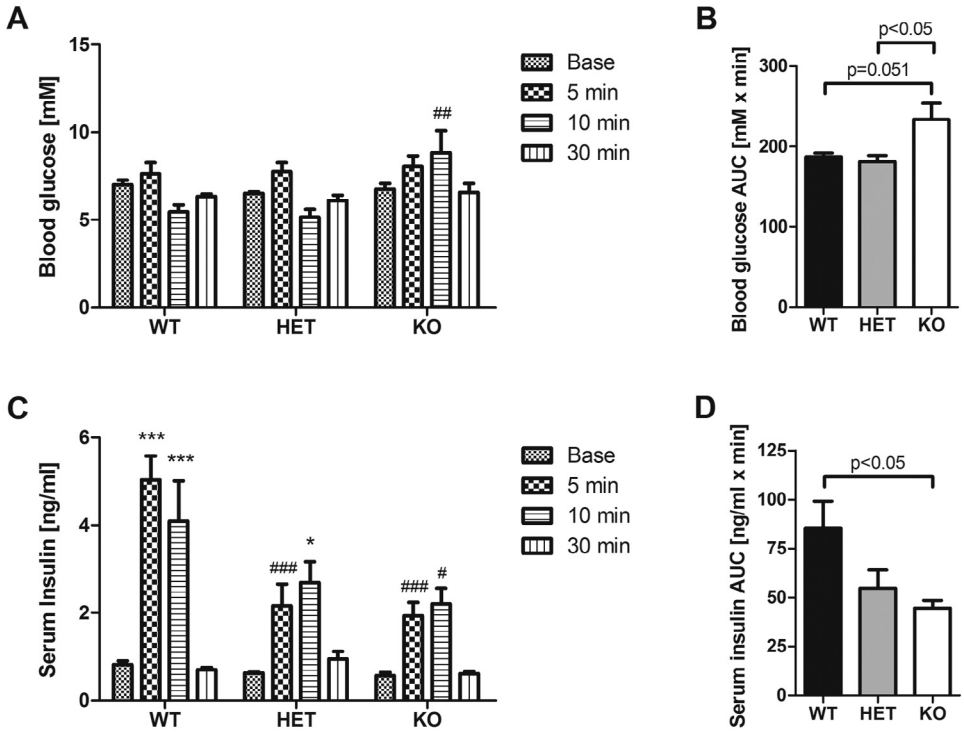

Figure 2. Response to 0.02 mg/kg carbachol injection. Blood glucose (A,B) and insulin (C,D) levels 5, 10 or 30 min after administration and corresponding AUC. *p<0.05. ***p<0.001 compared to the baseline level of the same genotype. #p<0.05. ##p<0.01. ###p<0.001 compared to WT at the same timepoint. *AUC*, area under the curve; *HET*, heterozygous Wfs1 mutation carrier; *KO*, homozygous Wfs1 mutation carrier; *WT*, wild-type mice. n=5 to 7.

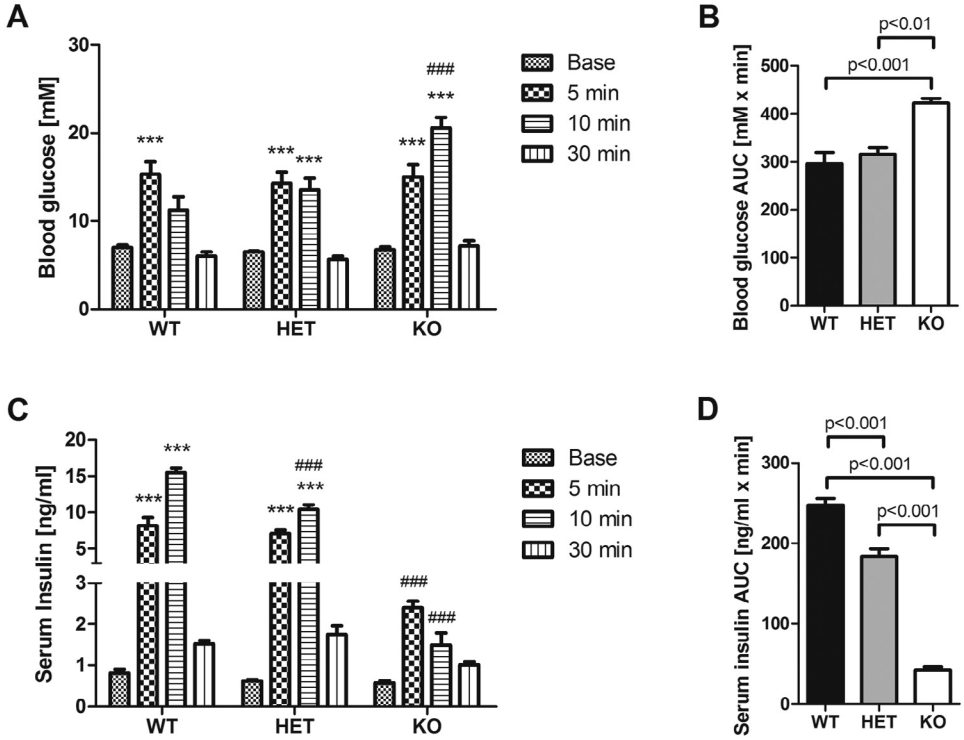

Figure 3. Response to 0.02 mg/kg carbachol and 2 g/kg glucose coinjection. Blood glucose (A,B) and insulin (C,D) levels 5, 10 or 30 min after administration and corresponding AUC. **p<0.001 compared to the baseline level of the same genotype. ### p<0.001 compared to WT at the same timepoint. *AUC*, area under the curve; *HET*, heterozygous Wfs1 mutation carrier; *KO*, homozygous Wfs1 mutation carrier; *WT*, wild-type mice. n=5 to 7.

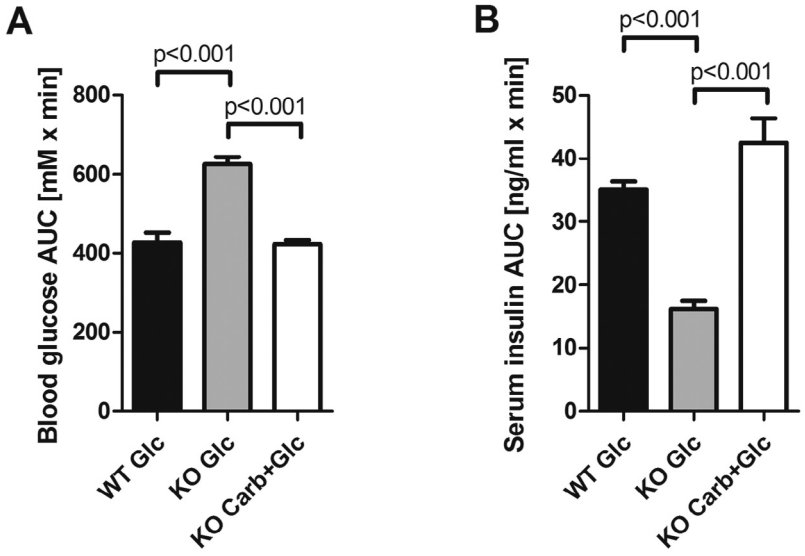

Figure 4. Improvement of blood glucose and serum insulin regulation in Wfs1 KO mice. Blood glucose (A) and insulin (B) AUC in response to 2 g/kg glucose or 0.02 mg/kg carbachol and 2 g/kg glucose coinjection. *AUC,* area under the curve; *KO,* homozygous Wfs1 mutation carrier; *WT,* wild-type mice. n=5 to 6.

Figure 4). Therefore, Wfs1 KO mice have deficits in insulin release but not in insulin sensitivity, coinciding with a previous report demonstrating no changes in insulin sensitivity in Wfs1 KO mice (10).

Muscarinic agonists such as carbachol have previously been shown to initiate insulin secretion in models with diabetes, an effect that is abolished by the muscarinic antagonist atropine (23,24). Also, stimulation of downstream targets of carbachol was able to provide similar effects in spontaneously diabetic Goto-Kakizaki rats (24). Carbachol was able to initiate insulin secretion in both heterozygous and homozygous Wfs1-deficient mice, but this effect was much weaker than in WT mice (Figure 2C,D). Even though heterozygous Wfs1 mice did not display glucose intolerance, these changes indicate alterations in beta cells; as in humans, defective Wfs1 gene carriers have higher risk for the development of type 2 diabetes (4–7).

Muscarinic receptors are widely expressed in various tissues, so there is also the possibility of some indirect influence on insulin secretion. Glucagon-like peptide-1 (GLP-1) has the ability to potentiate insulin secretion from pancreatic beta cells. GLP-1 is secreted in response to meal ingestion by ileal L cells, with a measurable increase about 10 min after meal intake. These cells also respond to muscarinic-receptor stimulation. However, for the secretion of GLP-1 from L cells, nutrients must reach the intestine, making the carbachol effect via the GLP-1 mechanism in fasted animals futile (34,35).

Studies investigating acetylcholine effects on pancreatic beta cells and insulin secretion have revealed the central role of the M3 muscarinic receptor; M3-deficient mice do not exhibit increased glucose-stimulated insulin secretion after treatment with the muscarinic agonist oxotremorine (18). However, it should be taken into account that a small proportion of the activity of carbachol may be related to other muscarinic receptor subtypes and even to nicotine receptors, but the direct effect and even the expression of receptors other than M3 is still debated (18,36).

Many psychiatric drugs inhibit the M3 receptor as a side effect (e.g. amitriptyline, desipramine, imipramine, nortriptyline, doxepin, dosulepin, maprotiline, paroxetine). These drugs increase disturbances of insulin secretion and are associated with the development of type 2 diabetes (37). It has been shown that amitriptyline and desipramine aggravate glucose-induced hyperglycemia in Wfs1 KO mice and induce stronger increases in blood glucose levels in mice with the heterozygous Wfs1 mutation, compared to WT mice (32). Both Wfs1 HET and KO mice present changes in insulin secretion in response to carbachol (Figure 2C,D), so the use of drugs with M3 antagonistic properties might be contraindicated in people with mutations in the Wfs1 gene.

Muscarinic receptors are widely expressed in various tissues. At this point, no drugs are able to activate the M3 receptor selectively without activating other muscarinic receptors (M1 through M5). Acetylcholine, released from the parasympathetic nerve endings or pancreatic alpha cells, binds to M3 muscarinic receptors and, through a signaling cascade, leads to the generation of IP3 and diacylglycerol. IP3 binds to IP3 receptors on the surface of the ER, mobilizing intracellular Ca^{2+} stores, which leads to an increase in cytoplasmatic Ca^{2+} levels, thus potentiating glucose-stimulated insulin secretion (20–22). However, the pathway by which carbachol exerts its effects is similar for many different G-protein-coupled receptors (GPCRs). GPCRs have emerged as potential therapeutic targets in type 2 diabetes, exhibiting glucose-dependent potentiating effects on insulin secretion (38,39). Similar to the M3 receptor, GPR40 and GPR120 (free fatty acid receptors 1 and 4, respectively) expressed in Langerhans islets are also coupled with $G_{\alpha q}$ protein, and there are currently several ongoing clinical trials promising medications with this novel mechanism of action (40).

Conclusions

Wfs1 deficiency causes glucose intolerance as a result of decreased insulin secretion. Selective M3 agonists and, possibly, other ligands able to activate G-protein-coupled receptors on beta cells are a promising way to release insulin and reverse glucose intolerance resulting from Wfs1 deficiency.

Funding

This research was supported by the European Union through the European Regional Development Fund (Project # 2014–2020.4.01.15–0012), by grants PUT784 (AT), IUT14064T (MP) and IUT20-41 (EV) from the Estonian Research Council.

Author Disclosures

Conflicts of interest: None.

Author Contributions

MP and MT designed the experiments; MP and EV directed the study; MT and RR performed the animal experiments; MT and RR performed biochemical analysis; MT wrote the manuscript. All authors have read and approved the final version of the manuscript.

References

1. Wolfram DJ, Wagener HP. Diabetes mellitus and simple optic atrophy among siblings: Report of four cases. Mayo Clinic Proc 1938;13:715–8.
2. Inoue H, Tanizawa Y, Wasson J, et al. A gene encoding a transmembrane protein is mutated in patients with diabetes mellitus and optic atrophy (Wolfram syndrome). Nat Genet 1998;20:143–8.
3. Swift RG, Perkins DO, Chase CL, Sadler DB, Swift M. Psychiatric disorders in 36 families with Wolfram syndrome. Am J Psychiatry 1991;148:775–9.
4. Han X, Luo Y, Ren Q, et al. Implication of genetic variants near SLC30A8, HHEX, CDKAL1, CDKN2A/B, IGF2BP2, FTO, TCF2, KCNQ1, and WFS1 in type 2 diabetes in a Chinese population. BMC Med Genet 2010;11:81.
5. Sandhu MS, Weedon MN, Fawcett KA, et al. Common variants in WFS1 confer risk of type 2 diabetes. Nat Genet 2007;39:951–3.
6. Franks PW, Rolandsson O, Debenham SL, et al. Replication of the association between variants in WFS1 and risk of type 2 diabetes in European populations. Diabetologia 2008;51:458–63.
7. Cheurfa N, Brenner GM, Reis AF, et al. Decreased insulin secretion and increased risk of type 2 diabetes associated with allelic variations of the WFS1 gene: The Data from Epidemiological Study on the Insulin Resistance Syndrome (DESIR) prospective study. Diabetologia 2011;54:554–62.
8. Takei D, Ishihara H, Yamaguchi S, et al. WFS1 protein modulates the free Ca(2+) concentration in the endoplasmic reticulum. FEBS Lett 2006;580:5635–40.
9. Koks S, Soomets U, Paya-Cano JL, et al. Wfs1 gene deletion causes growth retardation in mice and interferes with the growth hormone pathway. Physiol Genom 2009;37:249–59.
10. Terasmaa A, Soomets U, Oflijan J, et al. Wfs1 mutation makes mice sensitive to insulin-like effect of acute valproic acid and resistant to streptozocin. J Physiol Biochem 2011;67:381–90.
11. Rorsman P. Insulin secretion: Function and therapy of pancreatic beta-cells in diabetes. Br J Diabetes Vasc Dis 2005;5:187–91.
12. Coore HG, Randle PJ. Regulation of insulin secretion studied with pieces of rabbit pancreas incubated in vitro. Biochem J 1964;93:66–78.
13. Cook DL, Hales CN. Intracellular ATP directly blocks K+ channels in pancreatic B-cells. Nature 1984;311:271–3.
14. Ashcroft FM, Harrison DE, Ashcroft SJ. Glucose induces closure of single potassium channels in isolated rat pancreatic beta-cells. Nature 1984;312:446–8.
15. Milner RD, Hales CN. The role of calcium and magnesium in insulin secretion from rabbit pancreas studied in vitro. Diabetologia 1967;3:47–9.
16. Henquin JC. Triggering and amplifying pathways of regulation of insulin secretion by glucose. Diabetes 2000;49:1751–60.
17. Del Guerra S, Lupi R, Marselli L, et al. Functional and molecular defects of pancreatic islets in human type 2 diabetes. Diabetes 2005;54:727–35.
18. Duttaroy A, Zimliki CL, Gautam D, Cui Y, Mears D, Wess J. Muscarinic stimulation of pancreatic insulin and glucagon release is abolished in m3 muscarinic acetylcholine receptor-deficient mice. Diabetes 2004;53:1714–20.
19. Gautam D, Han SJ, Hamdan FF, et al. A critical role for beta cell M3 muscarinic acetylcholine receptors in regulating insulin release and blood glucose homeostasis in vivo. Cell Metab 2006;3:449–61.
20. Sharp R, Culbert S, Cook J, Jennings A, Burr IM. Cholinergic modification of glucose-induced biphasic insulin release in vitro. J Clin Invest 1974;53:710–6.
21. Gilon P, Henquin JC. Mechanisms and physiological significance of the cholinergic control of pancreatic beta-cell function. Endocr Rev 2001;22:565–604.
22. Molina J, Rodriguez-Diaz R, Fachado A, Jacques-Silva MC, Berggren PO, Caicedo A. Control of insulin secretion by cholinergic signaling in the human pancreatic islet. Diabetes 2014;63:2714–26.
23. Ahren B, Simonsson E, Scheurink AJ, Mulder H, Myrsen U, Sundler F. Dissociated insulinotropic sensitivity to glucose and carbachol in high-fat diet-induced insulin resistance in C57BL/6J mice. Metabolism 1997;46:97–106.
24. Guenifi A, Simonsson E, Karlsson S, Ahren B, Abdel-Halim SM. Carbachol restores insulin release in diabetic GK rat islets by mechanisms largely involving hydrolysis of diacylglycerol and direct interaction with the exocytotic machinery. Pancreas 2001;22:164–71.
25. Luuk H, Koks S, Plaas M, Hannibal J, Rehfeld JF, Vasar E. Distribution of Wfs1 protein in the central nervous system of the mouse and its relation to clinical symptoms of the Wolfram syndrome. J Comp Neurol 2008;509:642–60.
26. Andrikopoulos S, Blair AR, Deluca N, Fam BC, Proietto J. Evaluating the glucose tolerance test in mice. Am J Physiol Endocrinol Metab 2008;295:E1323–32.
27. Palou M, Priego T, Sanchez J, et al. Sequential changes in the expression of genes involved in lipid metabolism in adipose tissue and liver in response to fasting. Pflugers Arch 2008;456:825–36.
28. Agouni A, Owen C, Czopek A, Mody N, Delibegovic M. In vivo differential effects of fasting, re-feeding, insulin and insulin stimulation time course on insulin signaling pathway components in peripheral tissues. Biochem Biophys Res Commun 2010;401:104–11.
29. Renuka TR, Robinson R, Paulose CS. Increased insulin secretion by muscarinic M1 and M3 receptor function from rat pancreatic islets in vitro. Neurochem Res 2006;31:313–20.
30. Gautam D, Han SJ, Duttaroy A, et al. Role of the M3 muscarinic acetylcholine receptor in beta-cell function and glucose homeostasis. Diabetes Obes Metab 2007;9(Suppl. 2):158–69.
31. Johnson DE, Yamazaki H, Ward KM, et al. Inhibitory effects of antipsychotics on carbachol-enhanced insulin secretion from perifused rat islets: Role of muscarinic antagonism in antipsychotic-induced diabetes and hyperglycemia. Diabetes 2005;54:1552–8.
32. Reimets R, Raud S, Loomets M, et al. Variability in the effect of antidepressants upon Wfs1-deficient mice is dependent on the drugs' mechanism of actions. Behav Brain Res 2016;308:53–63.
33. Plaas M, Seppa K, Reimets R, et al. Wfs1-deficient rats develop primary symptoms of Wolfram syndrome: Insulin-dependent diabetes, optic nerve atrophy and medullary degeneration. Sci Rep 2017;7:10220.
34. Rocca AS, Brubaker PL. Role of the vagus nerve in mediating proximal nutrient-induced glucagon-like peptide-1 secretion. Endocrinology 1999;140:1687–94.
35. Anini Y, Brubaker PL. Muscarinic receptors control glucagon-like peptide 1 secretion by human endocrine L cells. Endocrinology 2003;144:3244–50.
36. Yoshikawa H, Hellstrom-Lindahl E, Grill V. Evidence for functional nicotinic receptors on pancreatic beta cells. Metabolism 2005;54:247–54.
37. Tran YH, Schuiling-Veninga CCM, Bergman JEH, Groen H, Wilffert B. Impact of muscarinic M3 receptor antagonism on the risk of type 2 diabetes in antidepressant-treated patients: A case-controlled study. CNS Drugs 2017;31:483–93.
38. Reimann F, Gribble FM. G protein-coupled receptors as new therapeutic targets for type 2 diabetes. Diabetologia 2016;59:229–33.
39. Tan T, Bloom S. Gut hormones as therapeutic agents in treatment of diabetes and obesity. Curr Opin Pharmacol 2013;13:996–1001.
40. Peng XV, Marcinak JF, Raanan MG, Cao C. Combining the G-protein-coupled receptor 40 agonist fasiglifam with sitagliptin improves glycaemic control in patients with type 2 diabetes with or without metformin: A randomized, 12-week trial. Diabetes Obes Metab 2017;19:1127–34.