Chronic glucokinase activation reduces glycaemia and improves glucose tolerance in high-fat diet fed mice

Maria Sörhede Winzell a,⁎, Matthew Coghlan b, Brendan Leighton b, Georgia Frangoudakis c, David M. Smith b, Leonard H. Storlien d, Bo Ahrén a

a Department of Clinical Sciences, Lund University, Biomedical Center, Lund, Sweden
b AstraZeneca, Diabetes & Obesity Drug Discovery, Alderley Park, Macclesfield, Cheshire, UK
c Garvan Institute of Medical Research, Darlinghurst, Australia
d Boden Institute of Obesity, Nutrition & Exercise, University of Sydney, Australia

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A B S T R A C T

Glucokinase (GK) plays a key role in maintaining glucose homeostasis by promoting insulin secretion from pancreatic beta cells and increasing hepatic glucose uptake. Here we investigate the effects of acute and chronic GK activation on glucose tolerance and insulin secretion in mice with diet-induced insulin resistance. In the acute study, a small molecule GK activator (GKA71) was administered to mice fed a high-fat diet for 8 weeks. In the long-term study, GKA71 was provided in the diet for 4 weeks to high-fat diet-fed mice. Glucose tolerance was measured after intravenous glucose administration, and insulin secretion was measured both in vivo and in vitro. Acute GK activation efficiently improved glucose tolerance in association with increased insulin secretion after intravenous glucose both in control and high-fat fed mice. Chronic GK activation significantly reduced basal plasma glucose and insulin, and improved glucose tolerance despite reduced insulin secretion after intravenous glucose, suggesting improved insulin sensitivity. Isolated islets from chronically GKA71-treated mice displayed augmented insulin secretion at 8.3 mmol/l glucose, without affecting glucose oxidation. High-fat diet fed mice had reduced glycogen and increased triglyceride in liver compared to control mice, and these parameters were not altered by long-term GK activation. We conclude that GK activation in high-fat diet-fed mice potently reduces glycaemia and improves glucose tolerance, with combined effect both to stimulate insulin secretion from islets and improve insulin sensitivity.

⁎ Corresponding author at: AstraZeneca R&D, Diabetes and Obesity, SE-431 83 Mölndal, Sweden. Tel.: +46 31 776 1071; fax: +46 31 776 3704.
E-mail address: maria.sorhede-winzell@astrazeneca.com (M.S. Winzell).

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1. Introduction

Type 2 diabetes is characterised by hyperglycaemia resulting from islet dysfunction, manifested as impaired insulin secretion in the presence of insulin resistance (Kahn, 2003). Diabetes is also associated with augmented glucagon secretion resulting in increased hepatic glucose production (Dunning and Gerich, 2007). Glucokinase (GK) is a key enzyme playing an important role in both insulin secretion and hepatic glucose metabolism (Matschinsky, 1996), catalysing phosphorylation of glucose to glucose-6-phosphate, which is the first step in glycolysis. GK has low affinity for glucose with a sigmoidal saturation curve (Matschinsky, 1996; Xu et al., 1995). The enzyme is important both in islet and hepatic function. In islets, GK catalyses the rate-limiting step in glucose-stimulated insulin secretion (Matschinsky, 1996) and in the liver, GK is required for glucose metabolism and glycogen synthesis (Gomis et al., 2000; Seoane et al., 1996; Valera and Bosch, 1994). GK therefore contributes to whole-body glucose disposal and has been suggested to act as the glucose sensor, playing a crucial role in maintaining normoglycaemia (Matschinsky, 2002).

The importance of GK for the control of blood glucose has been demonstrated in several animal models. Liver GK-deficient mice are hyperglycaemic, whilst pancreatic β-cell specific GK-deficient mice or mice with total GK-deficiency die early in life with severe diabetes (Grupe et al., 1995; Postic et al., 1999). Furthermore, mice with heterozygous deletion of GK are mildly hyperglycaemic and develop diabetes when fed a high-fat diet (Gorman et al., 2008; Terauchi et al., 2007). In contrast, adenoviral overexpression of GK restores blood glucose in high-fat diet fed mice (Desai et al., 2001) and in healthy rats GK overexpression results in hypoglycaemia (O’Doherty et al., 1999). Equally, transgenic mice with specific overexpression of GK in the liver have increased liver glycogen levels but reduced plasma glucose levels and improved glucose tolerance (Hariharan et al., 1997), and are protected to high-fat diet induced hyperglycaemia (Hariharan et al., 1997; Shioda et al., 2001). In humans, activating mutations of glucokinase have been described, resulting in hyperinsulinemic hypoglycaemia (Cuesta-Munoz et al., 2004; Dunne et al., 2004). Finally, a number of in vitro and acute studies have shown that small
molecule glucokinase activators (GKA) reducing hepatic glucose output and glycaemia (Grimbsy et al., 2003; Brocklehurst et al., 2004; Coope et al., 2006; Efanov et al., 2005; Johnson et al., 2007).

Due to its significant role in glucose sensing, GK is a potential target for new treatment strategies for the management of type 2 diabetes, as has recently been reviewed (Coghlan and Leighton, 2008; Matschinsky, 2009). It is therefore of importance to examine both short-term and long-term effects of GK activation in glucose metabolism and islet function in models of glucose intolerance. In this study, we therefore examined the effect of both acute and chronic oral administration of a small molecule glucokinase activator, GKA71, on glucose tolerance and islet function in high-fat fed mice, a model which exhibits obesity, insulin resistance, and impaired glucose tolerance due to deficient islet function (Ahrén and Pacini, 2002; Winzell and Ahrén, 2004; Winzell et al., 2007).

2. Materials and methods

2.1. Glucokinase activator compound

A novel small molecule glucokinase activator (GKA71, 3-[(1S)-2-methoxy-1-methyl-ethoxy]-5-(4-methylsulfonylphenoxy)-N-(3-methyl-1,2,4-thiadiazol-5-yl)benzamide) was used in this study (Waring et al., 2011). The compound is selective for glucokinase (in vitro potency for rat GK EC50 0.11 μM, human GK EC50 0.13 μM) with no hexokinase activity (human hexokinase I and II EC50 > 30 μM). In rats, GKA71 has high oral bioavailability (71%). GKAs within the same chemical series as GKA71 have demonstrated good glucose-lowering efficacy (Coope et al., 2006). Related compounds to GKA71 have been tested in toxicity studies and found to have no toxicity at exposure levels in the present study.

For the in vitro studies, the compound was dissolved in 1% DMSO. In the acute in vivo experiments, the compound was formulated in a vehicle consisting of 1% Pluronic F127 (Sigma, St Louis, USA) in water. GKA71 (1 mg/ml) was dissolved in vehicle and stirred overnight prior to oral administration to mice by gavage.

2.2. Animals

Eight-week old female C57BL/6J BomTac mice were purchased from Taconic (Skensved, Denmark). The animals were maintained in a temperature-controlled room (22 °C) on a 12 h-light–dark cycle. One week after arrival to the animal facility at the Biomedical Centre, Lund University, the mice were divided into two groups and fed either a control diet (10% fat by energy; D12450B Research Diets Inc., New Brunswick, NJ) or a high-fat diet (60% fat by energy; D12492, Research Diets). Body weight was measured once a week. In the in vivo experiments, blood samples were taken at indicated time points from the intraorbital, retrobulbar plexus from 4 h-fasted, anaesthetised (20 mg/kg fluanisone/0.8 mg/kg fentanyl [Hypnorm®, Janssen, Beerse, Belgium] and 10 mg/kg midazolam [Dormicum®, Hoffman-LaRoche, Basel, Switzerland]) mice. Principles of laboratory animal care were followed, and the study was approved by the Animal Ethics Committee in Lund/Malmö, Sweden.

2.3. Experimental design for acute in vivo effect of GKA71

To evaluate the glucose lowering effect of GKA71, 10 mg/kg was given by gavage (0.5 ml in 1% Pluronic F127) to anaesthetised high-fat diet fed, female mice as described above. Control high-fat diet fed mice received only vehicle. Blood samples (50 μl) were taken immediately before GKA71 administration and either after 0.5, 1, 2, 4 and 6 h or after 30, 60, 90 and 120 min. Following immediate centrifugation at 4 °C, plasma was separated and analysed for glucose (in the 6 h experiment) or for glucose and insulin. To evaluate the acute effect of GK activation on glucose tolerance and insulin secretion, intravenous glucose tolerance test (IVGTT) was undertaken in mice fed high-fat diet for 9 weeks. Mice were fasted 4 h prior to the IVGTT. GKA71 (10 mg/kg), dissolved in 0.5 ml vehicle, was given by gavage 1.5 h prior to the IVGTT, whilst high-fat diet fed and control diet fed mice were given vehicle only. Mice were anaesthetized 30 min prior to the IVGTT, as described above, and a blood sample (50 μl) was taken from the retrobulbar, intraorbital, capillary plexus. Thereafter, p-glucose (0.75 g/kg, Sigma, St. Louis, MO) was injected intravenously in a tail vein (volume load 10 ml/kg). Additional blood samples were taken in heparinized tubes at 1, 5, 10, 20, 50 and 75 min after injection. Following immediate centrifugation at 4 °C, plasma was separated and stored at −20 °C until analysis of glucose and insulin.

2.4. Experimental design for chronic effect of GKA71

After an initial 8-week of high-fat diet feeding, mice were divided into two groups. One group was provided with high-fat diet supplemented with 150 mg/g GKA71. Control mice continued on the high-fat diet and the control diet, respectively. Mice were fed ad libitum and had free access to tap water throughout the study. Food intake and body weight was recorded daily during the first week and thereafter once a week. After one and four weeks with GKA71 in the diet, mice were subjected to intravenous glucose tolerance tests as described above. Two or three days later, islets were isolated and insulin secretion and glucose oxidation were measured.

2.5. Islet isolation and insulin secretion

Mouse islets were isolated from the pancreas by collagenase digestion and handpicked under the microscope. Batches of freshly isolated islets were preincubated in HEPES balanced salt solution containing 125 mmol/l NaCl, 5.9 mmol/l KCl, 1.28 mmol/l CaCl2, 1.2 mmol/l MgCl2, 25 mmol/l HEPES (pH 7.4), 5.6 mmol/l glucose and 0.1% fatty acid free BSA (Boehringer Mannheim, GmbH, Germany) for 60 min. Thereafter, islets in groups of three were incubated in 200 μl of the above described buffer with varying concentrations of glucose. In the experiments where the direct effect of GKA71 on insulin secretion was studied, isolated islets from mice fed the control diet were incubated with or without 1 μmol/l GKA71 and glucose in different concentrations. Islets were incubated for 60 min at 37 °C, where after aliquots of 25 μl in duplicates were collected and stored at −20 °C until analysis of insulin.

2.6. Islet glucose oxidation

Batches of 30 islets in quadruplicates were incubated in a reaction mixture containing 0.1 μCi or 0.7 μCi [14C]-glucose (NEN, Boston, MA, specific activity 310 mCi/mmol) as tracer, at final concentrations of 2.8, 8.3 or 16.7 mmol/l glucose. To study the acute effect on glucose oxidation, 1 μmol/l GKA71 was included in the reaction mixture. The reaction was terminated by addition of trichloroacetic acid after incubation of the samples for 2 h at 37 °C, and the amount of released 14CO2, trapped with benzotrion hydroxide, was determined by liquid scintillation counting.

2.7. Glucose and insulin measurements

Glucose was measured with the glucose oxidase method using 2,2′-azino-bis-[3-ethyl-benzothiazoline-6-sulfonate] as substrate and the absorbance was measured at 420 nm on a microtiter plate reader (Fluostar/Polarstar Galaxy, BMG Labtechnologies, Offenburg, Germany). Insulin was determined radioimmunochemically (Linco Res., St Charles, MO). Radioactivity was measured on a gamma counter (Wallac Wizard 1470, Perkin Elmer, Turku, Finland).
2.8. Western blotting

The protein expression levels of GK in islets and GK and fatty acid synthase in liver were analysed using Western blot. The tissues were homogenised in a buffer containing 150 mmol/l NaCl, 2 mmol/l EDTA, 20 mmol/l Tris–HCl pH 7.5, 1% Triton X-100 and 0.2% protease inhibitor cocktail (Sigma). The total amount of proteins in each sample was measured using a BCA Protein assay reagent kit (Pierce, Rockford, IL). Aliquots of tissue homogenates containing equal amounts of protein (80 μg) were separated on SDS-PAGE, and electroblotted onto nitrocellulose membranes (Hybond-c extra, Amersham Pharmacia Biotech, Uppsala, Sweden). The membranes were probed with primary antibodies against GK, fatty acid synthase and actin (Santa Cruz Biotechnology, Santa Cruz, CA). The secondary antibody was a horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody (Amersham Pharmacia Biotech, Sweden). The blots were developed by enhanced chemiluminescence (SuperSignal, Pierce, Rockford, IL) and the proteins were detected and quantified using a CD camera (LAS 1000, Fuji, Tokyo, Japan).

2.9. Liver triglyceride content

Liver biopsies (50 mg) were homogenised in ice-cold 20 mmol/l Tris–HCl pH 7.5, 150 mmol/l NaCl, 2 mmol/l EDTA and 1% Triton X-100. Triglycerides were extracted from the tissue homogenates with chloroform:methanol (2:1). The amount of extracted triglycerides was measured using a commercially available kit (Infinity Triglycerides Liquid Stable Reagent, Thermo Electron, Melbourne, Australia), using triolein (Sigma) as standard. The triglyceride content was corrected for the total protein content in the liver homogenates, determined with the BCA Protein Assay kit (Pierce, Rockford, IL).

2.10. Liver glycogen content

Liver glycogen content was measured after incubation of the homogenates (as above) with 20 μg/ml amyloglucosidase (Sigma) for 45 min in 50 °C. The samples were put on ice for 5 min and then centrifuged before the released glucose was measured in the supernatant with the glucose oxidase method. Rabbit glycogen (Sigma) was used as the standard.

2.11. Islet insulin content

Batches of ten islets were first frozen, thawed and then sonicated in acidic ethanol (0.2 mol/l HCl in 87.5% ethanol). The procedure was repeated twice. The samples were then centrifuged and total insulin content was measured in the supernatant.

2.12. Statistical analysis

All data are presented as mean ± S.E.M.. The acute insulin response (AIR) to intravenous glucose was calculated as the mean of suprabasal 1 and 5 min values, and the glucose elimination was quantified using the glucose elimination constant (Ke) calculated as the slope of the logarithmic transformation of circulating glucose between 1 and 20 min after the glucose bolus. Insulin sensitivity was estimated as the ratio between Ke and the suprabasal area under the curve for insulin during the 50 min study period (Pacini et al., 2009). Multiple comparisons between the different groups were performed by one-way ANOVA. Tukey’s or Bonferroni’s post hoc test were used to calculate statistical differences between the groups. Significant statistical difference was considered at P<0.05.

3. Results

3.1. In vitro insulin secretion after GK activation

At non-stimulatory glucose concentration (2.8 mmol/l), basal insulin secretion from freshly isolated islets was slightly increased after addition of 1 μmol/l GKA71 (Fig. 1A) and this correlated with elevated glucose oxidation (Fig. 1B). At physiological glucose level (8.3 mmol/l) there was a pronounced increase in both insulin secretion (0.63 ± 0.06 vs. 0.13 ± 0.02 ng/h per islet, P<0.001) and glucose oxidation (17.1 ± 1.5 vs. 9.6 ± 0.5 pmol/h per islet, P<0.001), whilst at a supra-physiological glucose level (16.7 mmol/l), GKA71 had no additional effect on either insulin secretion or glucose oxidation.

3.2. Acute effect of GK activation on glucose tolerance and insulin secretion

After establishing a significant effect of GKA71 on insulin secretion from isolated islets, the glucose lowering effect of GKA71 was examined in vivo in mice rendered insulin resistant and glucose intolerant by feeding a high-fat diet. Body weight was increased compared to the control mice (21.3 ± 0.2 g and 26.0 ± 0.6 g, respectively, P<0.001) and basal blood glucose levels was elevated (6.8 ± 0.3 mmol/l vs. 8.6 mmol/l in high-fat diet fed mice, P<0.001), which is in agreement with earlier studies (Winzell and Ahrén, 2004). Acute administration of GKA71 (10 mg/kg) to high-fat diet fed mice reduced blood glucose maximally after 1 h (5.8 ± 0.3 vs. 8.3 ± 0.3 mmol/l in high-fat diet control, P<0.001), and the effect was stable after 2 h, declining after 4 h. Six hours after oral administration of the compound, glucose levels were not different from the vehicle treated group. Acute oral GKA71 administration to control mice also reduced glucose, as in high-fat diet-fed mice (Fig. 2A), whilst insulin levels remained unchanged (Fig. 2B).

The acute effect of GKA71 on glucose tolerance in high-fat diet fed mice was next examined after intravenous glucose administration. Since the effect of oral GKA71 was apparent 1 h after administration of the compound and maintained for up to 4 h, GKA71 was administered orally 90 min prior to the intravenous glucose tolerance test to ensure greater than enzyme EC50 coverage throughout the experiment. Glucose levels were significantly reduced 90 min after GKA71 administration (5.8 ± 0.2 vs. 9.2 ± 0.4 mmol/l in high-fat diet vehicle group, P<0.001; Fig. 3A). After intravenous glucose, glucose elimination was significantly improved in GKA71 administered mice, and completely normalised, with no difference in glucose elimination (Kg) compared to control diet fed mice (Fig. 3B). The improved glucose tolerance was accompanied by increased insulin secretion compared to high-fat diet fed control mice (Fig. 3C), which could be demonstrated
as increased acute insulin response (Fig. 3D). However, insulin secretion was not restored to the same levels as observed in control diet fed mice.

3.3. Glucose tolerance in high-fat diet fed mice after chronic GK activation

Chronic GKA71 administration in high-fat diet fed mice, significantly decreased glucose from 8.8 ± 0.4 to 5.4 ± 0.2 mmol/l (P < 0.001). The effect was apparent after 1 week and remained throughout the 4-week treatment period (Fig. 4A). In the high-fat diet fed group, basal insulin levels increased significantly at weeks 2 and 4, whilst GKA71 treated mice remained on the same basal insulin level as control diet fed mice (Fig. 4B). Body weight was similar in the high-fat diet fed groups, with or without GKA71, during the first week with a tendency of increased body weight gain in the GKA treated mice, whilst GKA71 treated mice remained on the same basal insulin level as control diet fed mice (Fig. 4C). In the subsequent weeks, the GKA71 treated mice gained less weight than the high-fat diet fed control mice (3.3 ± 0.5 in high-fat diet control mice vs. 0.8 ± 0.3 g after 4 weeks with GKA71, P < 0.05). There was no significant difference in food intake between the high-fat diet control and the GKA71 groups, whilst the expected difference in caloric intake between control diet and high-fat diet groups was observed (Fig. 4D).

Intravenous glucose tolerance tests were performed after 1 week (data not shown) and after 4 weeks (Fig. 5), with almost identical results. In mice receiving GKA71, glucose tolerance was significantly improved and normalised (Fig. 5A); being similar to control diet fed mice (Kg: 3.8 ± 0.2%/min vs. 4.0 ± 0.5%/min in HFD-GKA71; Fig. 5B), whilst in the high-fat diet fed control mice, Kg was significantly reduced (2.5 ± 0.2%/min, P = 0.004) compared to GKA71. The improved glucose elimination could not be explained by increased insulin secretion (Fig. 5C). The acute insulin response in GKA71 treated mice was not significantly different compared to the high-fat diet fed control mice (Fig. 5D), but reduced compared to control diet fed mice (890 ± 73 vs. 458 ± 117 pmol/l, P = 0.005). Plotting the individual Kg values against the acute insulin response following intravenous glucose after 1 and 4 weeks GKA71 administration, demonstrated that in the mice receiving GKA71 treatment, most of the individuals displayed improved glucose tolerance despite similar insulin levels, as observed in high-fat diet fed control mice and lower levels than in control diet fed mice (Fig. 5E). However, increased insulin secretion in the GKA71 treated mice resulted in significantly increased glucose elimination, whilst in high-fat diet fed control mice, increased insulin levels did not result in improved glucose tolerance. Insulin sensitivity, as estimated from the intravenous glucose after 1 and 4 weeks GKA71 administration, improved and normalised (Fig. 5A); being similar to control diet fed mice (Fig. 5D). In contrast, in control diet fed mice, insulin sensitivity was reversed by GKA treatment (Fig. 5F).

3.4. Liver effects after chronic GK activation

High-fat feeding reduced liver glycogen and increased triglyceride levels in both control and in GKA71 treated mice compared to control diet fed mice. There was no significant difference in either glycogen or triglyceride levels in liver between the two high-fat diet fed groups.
Glucose oxidation was similar in all three groups with no difference at either low or stimulatory glucose concentration (Fig. 7B). Islet insulin content was significantly increased in GKA71 treated mice compared to control mice (Fig. 7C). Islet GK protein expression was examined and found to be similar in the three treatment groups (Fig. 7D).

3.5. *In vitro* islets effect of chronic GK activation

After the 4-week treatment period, islets were isolated and examined for glucose-stimulated insulin secretion (GSIS). Normally, in islets from high-fat diet fed mice, basal insulin secretion is elevated, which was observed also in this study (Fig. 7A). However, in islets from mice treated with high-fat diet and GKA71, basal insulin secretion was not increased compared to control diet. At physiological glucose levels (8.3 mmol/l), GSIS was impaired in islets from high-fat diet control mice (1.8 ± 0.3 fold increase over basal glucose insulin secretion) compared to control diet fed control mice (3.1 ± 0.6 fold). GKA71 treatment restored GSIS to a 4.4 ± 0.9 fold increase over basal (P = 0.016 compared to islets from high-fat diet fed mice) At high glucose (16.7 mmol/l) there was no significant difference between the treatment groups, although the fold increase in glucose-stimulated insulin secretion was impaired in control islets (4.4 ± 0.6 vs. 8.3 ± 1.4 in control diet and 7.7 ± 1.0 fold increase in HFD-GKA71, P = 0.033).
4. Discussion

The present study investigated the chronic effect of a small molecule GK activator in a murine model of dietary-induced insulin resistance and obesity. The results demonstrate improved glycaemia, reduced basal insulin levels, improved glucose tolerance and reduced body weight gain by GKA treatment. Of equal importance, pancreatic islet function was assessed after chronic GKA71 treatment and the results demonstrate an islet protective effect, manifested as improved glucose-stimulated insulin secretion in isolated islets. The results therefore render further support for the idea of glucokinase as a target for treatment of type 2 diabetes (Coghlan and Leighton, 2008; Matschinsky, 2009).

In healthy individuals, blood glucose is tightly regulated via multiple mechanisms. Important regulating factors are insulin and glucagon, which together balance blood glucose under fasting conditions as well as after a meal. GK is the major enzyme that senses plasma glucose levels and it is expressed in several tissues including liver, gut, islet and brain (Matschinsky, 1996; Schuit et al., 2001). In patients with type 2 diabetes, insulin secretion is not sufficient to reduce plasma glucose and glucose sensitivity is reduced in both pancreas and in the liver (Home and Pacini, 2008; Kahn, 2003). In this study we examined the chronic effect of GK activation in mice fed a high-fat diet. These mice develop insulin resistance and impaired glucose tolerance (Ahrén and Pacini, 2002; Winzell and Ahrén, 2004; Winzell et al., 2007). Basal plasma glucose is slightly but significantly increased by approximately 1 mM whilst basal insulin is continuously increased over time, demonstrating decreasing insulin sensitivity in association with adapted islet function, to counteract the aggravating insulin resistance (Pacini et al., 2001). The mechanisms behind these perturbations are not established but may involve reduced insulin sensitivity in peripheral tissues (Zierath and Kawano, 2003) and islet adaptation mediated by fatty acids, adipokines, incretins and the autonomic nervous system (Ahrén and Pacini, 2005).

We first demonstrated that GKA efficiently increased glucose-stimulated insulin secretion in isolated mouse islets. The effect was significant at 2.8 mM and 8.3 mM glucose, whilst at higher concentration, GKA had no additional effect, demonstrating a left-shift of the glucose dose–response curve. This finding correlated with elevated glucose oxidation, demonstrating that the GKA71 efficiently increases the flux of glucose through glycolysis and the TCA cycle, thereby increasing insulin secretion. This is in agreement with other studies which have shown that GK activation results in increased insulin secretion at physiological glucose concentrations (Efanov et al., 2005; Johnson et al., 2007).

Acute oral administration of GKA71 reduced basal glucose and glucose tolerance during an intravenous glucose tolerance test. In fact, glucose elimination was similar in GKA71 treated mice as in control diet fed mice. This effect could partly be explained by increased insulin secretion, since the acute insulin response was significantly elevated by GKA71, although not restored to the same levels as observed in the control mice. This demonstrates that GKA71 also reduces glycaemia via some other mechanism(s), probably through increased hepatic glucose uptake, which has been demonstrated in other studies with other GKAs (Brocklehurst et al., 2004; Efanov et al., 2005).

To study the long-term effect of GK activation, a second cohort of mice were provided with high-fat diet supplemented with GKA71 for up to four weeks. This resulted in reduced glycaemia compared to control mice, together with reduced basal levels of insulin, suggesting that GKA71 treatment may improve insulin sensitivity. This was further supported by the results obtained from the intravenous glucose tolerance test, where improved glucose elimination was observed despite lower insulin levels. The exact role of GK activation for insulin sensitivity is not established. Further studies using euglycaemic hyperglycaemic clamps with tracer administration are needed to define the contributions from the different tissues involved in this apparent improvement in insulin action after GK activation.

Another interesting finding in this study is that the mice treated with GKA71 gained significantly less body weight during the 4-week treatment period compared to the high-fat diet-fed control mice. This effect was seen despite no differences in food intake. The mechanism behind this effect is, however, not known. It was recently demonstrated that enhanced hepatic glycolysis by overexpression of GK resulted in reduced obesity in obese KK/H1J mice (Wu et al., 2005). Overexpression of GK resulted in increased glycolysis in the liver and reduced hepatic glucose production, with reduced whole body glucose disposal, suggesting a switch from glucose to fatty acids as fuel in the peripheral tissues (e.g. skeletal muscle). This notion was supported by the fact that these mice demonstrated increased energy expenditure. Furthermore, transgenic or adenoviral overexpression of GK in the liver resulted in a similar phenotype as observed in this study after GKA71 administration, with reduced glycaemia and insulin levels and also reduced body weight (Desai et al., 2001; Hariharan et al., 1997). It seems therefore possible that lowering of plasma glucose has secondary effects in that it stimulates fat oxidation and preserves glucose sensitivity in the liver, skeletal muscle and in the islets. Bessesen et al. (2008) have recently provided a thoughtful analysis of the relationship between increased fat trafficking and resistance to obesity.

To evaluate the long term effect of GKA71 treatment on islet function, islets were isolated and examined after the 4-week treatment period. High-fat diet normally results in increased basal insulin secretion and this was not noted in islets after GKA71 treatment. Furthermore, the glucose-stimulated insulin secretion was potentiated with similar fold increase compared to normal islets. The reason for this improvement of islet function is not known. One possibility would be increased GK expression and enhanced glucose oxidation, but both glucose oxidation and GK expression were similar in all three groups. There was a slight increase in the total insulin content in islets from GKA71 treated mice, suggesting that less insulin is needed to control plasma glucose levels and therefore more insulin is retained within the islets. Another possibility is that glycaemia is efficiently reduced by GKA71 treatment, and the demand for elevated insulin secretion is relieved and therefore, beta cell function is improved.

In conclusion, this study demonstrates that activation of GK in mice with high-fat diet-induced insulin resistance improves islet function and normalises glucose tolerance similar to healthy, lean mice. The study further supports GK as a target for ameliorating hyperglycaemia and that the glucokinase activators may have potential for the treatment of type 2 diabetes.

Duality of interest

M. Sörhede Winzell, M. Coghlan, B. Leighton, G. Frangioudakis, D. M. Smith and L. Storlien, are or have been, employed by AstraZeneca. B. Ahrén has received fees for consultancy work from AstraZeneca.

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