ARA290 Improves Insulin Release and Glucose Tolerance in Type 2 Diabetic Goto-Kakizaki Rats

Carole Muller,1* Kamal Yassin,1* Luo-Sheng Li,1,2* Magnus Palmblad,3 Suad Efendic,1 Per-Olof Berggren,1,2 Anthony Cerami,4 Michael Brines,4 and Claes-Göran Östenson1,2

1Dept of Molecular Medicine and Surgery, Karolinska Institutet (KI), Stockholm, Sweden; 2The Rolf Luft Research Centre for Diabetes and Endocrinology, KI, Stockholm, Sweden; 3Dept Vascular Surgery, Leiden University Medical Center, The Netherlands; 4Araim Pharmaceuticals, Tarrytown, New York, United States of America

Effects of ARA290 on glucose homeostasis were studied in type 2 diabetic Goto-Kakizaki (GK) rats. In GK rats receiving ARA290 daily for up to 4 wks, plasma glucose concentrations were lower after 3 and 4 wks, and hemoglobin A1c (Hb A1c) was reduced by ~20% without changes in whole body and hepatic insulin sensitivity. Glucose-stimulated insulin secretion was increased in islets from ARA290-treated rats. Additionally, in response to glucose, carbachol and KCl, islet cytoplasmic free Ca2+ concentrations, [Ca2+]i, were higher and the frequency of [Ca2+]i oscillations enhanced compared with placebo. ARA290 also improved stimulus-secretion coupling for glucose in GK rat islets, as shown by an improved glucose oxidation rate, ATP production and acutely enhanced glucose-stimulated insulin secretion. ARA290 also exerted an effect distal to the ATP-sensitive potassium (KATP) channel on the insulin exocytotic pathway, since the insulin response was improved following islet depolarization by KCl when KATP channels were kept open by diazoxide. Finally, inhibition of protein kinase A completely abolished effects of ARA290 on insulin secretion. In conclusion, ARA290 improved glucose tolerance without affecting hematocrit in diabetic GK rats. This effect appears to be due to improved β-cell glucose metabolism and (Ca2+)i handling, and thereby enhanced glucose-induced insulin release.

Online address: http://www.molmed.org
doi: 10.2119/molmed.2015.00267

INTRODUCTION

Impaired β-cell function and insulin secretion play a primary role in type 2 diabetes (1,2). Although the mechanisms behind impaired insulin secretion may reside in inherited defects related to β-cell development and metabolism, immunological events such as low-grade inflammation and apoptosis may also contribute to β-cell dysfunction. Indeed, increased expression of cytokines and chemokines has been demonstrated in pancreatic islets of patients with type 2 diabetes as well as in animal models of the disease (3,4).

Erythropoietin (EPO) is a cytokine that regulates hematopoiesis mediated by its binding to the erythropoietin receptor (EPOR), that is present also in nonerythroid tissues, including pancreatic islets (5). In addition to its hematopoietic action, EPO has been shown to exert antiinflammatory, antiapoptotic and cytoprotective effects in a wide variety of cell types by binding to the innate repair receptor (IRR) which is a heteromer of EPOR and CD131, the β common receptor (6). EPO treatment has been shown to protect against diabetes development in streptozotocin-induced and db/db mouse models of type 1 and type 2 diabetes, respectively, while exerting antiapoptotic, antiinflammatory, proliferative and angiogenic effects within the islets (7).

Since prolonged treatment with EPO can increase the hematocrit and provoke thrombosis, we have studied an EPO analogue, ARA290 (8). This 11 amino acid peptide lacks hematopoietic action, binds to the IRR and protects a number of tissues in response to injury (9). A recent phase 2 clinical trial evaluating ARA290 in patients with type 2 diabetes and painful neuropathy showed that ARA290 significantly reduced hemoglobin A1c (Hb A1c) levels as well as neuropathic symptoms (10). In an effort to explore the mechanism of action of ARA290 in diabetes, we now report effects of ARA290 on different aspects of glucose homeostasis in spontaneously diabetic Goto-Kakizaki (GK) rats (2) compared with nondiabetic controls.
MATERIALS AND METHODS

ARA290

The nonhematopoietic erythropoietin analogue ARA290 consists of 11 amino acids (MW 1258 daltons) (8), and was supplied by Araim Pharmaceuticals. It was dissolved in phosphate buffered saline (PBS) at a concentration of 2 mg/mL and kept at 4°C for up to 4 wks.

Animals

Diabetic Goto-Kakizaki (GK) rats, originating from Wistar rats, were bred in our department (2). Normal Wistar (W) rats were purchased from a commercial breeder (B&K Universal) and used as nondiabetic controls. All animals were about six weeks old and with body weights 100 to 150 g when treatment was initiated. They were kept at 22°C on a reversed 12-h light–dark cycle with free access to food, except when fasted overnight as noted below. The study was approved by the Laboratory Animal Ethics Committee of Karolinska Institutet (N333/09). All experiments were performed in overnight fasted rats by collagenase digestion of the exocrine pancreas as previously described (12). Islets were separated using a Histopaque gradient and picked up under a stereomicroscope and cultured overnight in RPMI 1640 medium supplemented with 11 mmol/L glucose, 2 mmol/L glutamine, 10% heat inactivated FCS, 100 IU/mL penicillin, and 0.1 mg/mL streptomycin at 37°C, under a 95% O2, 5% CO2 atmosphere.

Islet Experiments

Islets were isolated from W and GK rats by collagenase digestion of the exocrine pancreas as previously described (12). Islets were separated using a Histopaque gradient and picked up under a stereomicroscope and cultured overnight in RPMI 1640 medium supplemented with 11 mmol/L glucose, 2 mmol/L glutamine, 10% heat inactivated FCS, 100 IU/mL penicillin, and 0.1 mg/mL streptomycin at 37°C, under a 95% O2, 5% CO2 atmosphere.

Tolerance Testing

Prior to treatment and after 2 and 4 wks, intraperitoneal (IP) glucose tolerance tests (IPGTT; 2 mg glucose/g BW) were performed in overnight fasted rats. Additionally, plasma samples were collected at 0 and 30 min for insulin analyses using radioimmunoassay (11). IP pyruvate tolerance (IPPTT; 2 mg sodium pyruvate/g BW) and s.c. insulin tolerance (SCTTT; 0.5 mU insulin/g BW) tests were carried out in overnight fasted GK rats.

Glucose Oxidation

After overnight culture in RPMI, GK rat islets were preincubated for 30 min in KRB buffer supplemented with 3.3 mmol/L glucose. Ten islets were placed in glass incubation vials with either 3.3 mmol/L or 16.7 mmol/L glucose in 100 μL KRB (pH 7.4), with or without 10 ng/mL ARA290 and also containing 1 μCi D-[U-14C]glucose (PerkinElmer). Incubation vials were then placed in 20 mL scintillation bottles containing 1.5 mL water and sealed with a rubber-membrane equipped cap under 95% O2, 5% CO2 and incubated for 2 h in 37°C in a water bath. The incubation was terminated by injecting through the rubber membrane 100 μL of 0.05 mmol/L antimycin in 70% ethanol, followed by 250 μL of hyamine in the scintillation bottles and 100 μL of 0.4 mmol/L sodium phosphate buffer, pH 6.0, in the incubation vials. The 14CO2 was allowed to absorb overnight into the hyamine. Incubation vials were discarded and 5 mL of scintillation liquid (Ultima Gold, Perkin Elmer) were added in the scintillation bottles. Radioactivity in 14CO2 was measured with a Liquid Scintillator Analyzer (Tricarb 1900-TR, Packard), and results were expressed as pmoles glucose oxidized/islet per 2 h.

ATP Determination

After preincubation as above, batches of 20 islets were incubated for 1 h in 300 μL KRB with either 3.3 mmol/L or 16.7 mmol/L glucose, and with or without 10 ng/mL ARA290, at 37°C in a shaking water bath. ATP levels were measured using the ATP Bioluminescence Assay Kit.
ARA290 treatment had no effect on fasting plasma glucose levels in GK rats (Figures 1A–C). Moreover, in the insulin sensitivity test (SCITT; Supplementary Figure 3A) and the IP pyruvate tolerance test, (IPPTT; Supplementary Figure 3B) performed after 4 wks, results were similar in ARA290-treated and placebo groups, suggesting that altered extrahepatic and hepatic insulin sensitivity do not contribute to the improvement in plasma glucose levels.

**ARA290 Improves β Cell Secretory Function in GK Islets**

To assess whether ARA290 exerts a direct effect on β-cell secretory function, which may account for the improved glucose homeostasis in GK rat, we assessed effects of ARA290 on insulin secretion by performing GSIS experiments on islets isolated after 4 wks of ARA290 treatment in W and GK rats as well as batch incubations and islets perfusion experiments. In islets from ARA290-treated GK rats, insulin responses to 16.7 mmol/L glucose, in relation to basal (3.3 mmol/L) glucose, were significantly increased about two-fold compared with responses in islets from the placebo-treated rats, the fold increase in response being 3.8 ± 0.5 versus 2.0 ± 0.4, respectively (p < 0.05).

Islets isolated from untreated W and GK rats were exposed to ARA290 in the range of 1 to 10 ng/mL at both 3.3 and 16.7 mmol/L glucose. In W rat islets, ARA290 did not change insulin secretion during either basal or high glucose stimulation (Figure 2A). In GK rat islets, exposure to ARA290 at basal glucose conditions did not enhance insulin secretion, although in the presence of the high glucose concentration, 1 ng/mL ARA290 significantly improved insulin secretion 2.7-fold compared with 16.7 mmol/L glucose alone (Figure 2B). Higher concentrations of ARA290 further increased insulin secretion.

Islet perfusion experiments showed that ARA290 greatly augmented the first phase insulin response (control 0.076 ± 0.017 versus ARA290 0.303 ± 0.184 µU/islet/min at 34 min, p < 0.01) (Figure 2C). By contrast,
ARA290 improves islet glucose metabolism

To address whether improved first phase insulin secretion could result from a beneficial effect of A290 on glucose metabolism, we assessed effects of A290 on islet glucose oxidation and ATP production. Under hyperglycemic conditions, A290 significantly increased glucose oxidation (Figure 3A) in GK islets. Treatment by A290 for 1 h also improved ATP production in GK islets (Figure 3B). These results suggest that A290 has a direct effect on glucose metabolism by increasing Krebs cycle activity and ATP production by the mitochondria.

ARA290 did not exert a significant effect on the second phase insulin secretion. However, addition of KCl and ARA290 increased insulin secretion further (Figure 2C), suggesting that ARA290 affects not only the glucose stimulatory pathway but also the amplification pathway.

ARA290 Augments the Insulin Secretion Pathway

To investigate whether ARA290 has effects on the islet \( K_{\text{ATP}} \) channel in the insulin secretion pathway, we used the \( K_{\text{ATP}} \) channel opener diazoxide. At 3.3 mmol/L glucose, diazoxide did not suppress basal insulin secretion, and the stimulation of insulin secretion by addition of diazoxide and KCl was not significantly modulated by ARA290 (Figure 4A). However, in the presence of 16.7 mmol/L glucose, coinubcation of
ARA290 has an additional effect in the insulin secretion pathway that is distal of the K\textsubscript{ATP} channels. Indeed, when GK islets were coincubated with 10 ng/mL ARA290 and 0.25 mmol/L diazoxide neutralized the stimulatory effect of ARA290 on GSIS (Figure 4B). This suggests that the stimulatory effect on GSIS by ARA290 is mediated through K\textsubscript{ATP} channels. ARA290 has an additional effect in the insulin secretion pathway that is distal of the K\textsubscript{ATP} channels. Indeed, when GK islets were coincubated with 10 ng/mL ARA290 and 10 \textmu mol/L of the protein kinase A (PKA) inhibitor...
ARA290 IMPROVES INSULIN SECRETION IN DIABETIC GK RATS

ARA290 Improves Islet [Ca\textsuperscript{2+}] Oscillations in the GK Rat

Figures 5A and 5B show representative traces of glucose-stimulated [Ca\textsuperscript{2+}]\textsubscript{i} and [Ca\textsuperscript{2+}] oscillations in isolated islets from GK rats treated with the vehicle (PBS) and ARA290, respectively. There was no statistically significant difference between the two groups in basal [Ca\textsuperscript{2+}]\textsubscript{i} in islets perfused with 3 mmol/L glucose (Figure 5C). We observed an increase in peak [Ca\textsuperscript{2+}]\textsubscript{i} values, that is, fura-2 ratio, after 16.7 mmol/L glucose stimulation in ARA290-treated islets as compared with vehicle-treated islets (Figure 5D). We also found increases in carbamylcholine- and KCl-induced [Ca\textsuperscript{2+}]\textsubscript{i} in ARA290-treated islets (Figures 5E, F). Furthermore, we performed power spectrum analysis for the [Ca\textsuperscript{2+}]\textsubscript{i} oscillation data and observed that islets from ARA290-treated GK rats displayed an increased frequency in slow [Ca\textsuperscript{2+}]\textsubscript{i} oscillations compared with controls (Figures 5A, B, G). The average period for slow [Ca\textsuperscript{2+}]\textsubscript{i} oscillations in ARA290-treated GK rats was 178.5 s, whereas the average period in islets from ARA290-treated GK rats was 101.5 s, which corresponded to a 43% increase in [Ca\textsuperscript{2+}] oscillation frequency versus the control islets. However, there was no difference in amplitude of slow [Ca\textsuperscript{2+}] oscillations between the groups (Figure 5H). Also, there was no difference in frequency and amplitude of fast [Ca\textsuperscript{2+}] oscillations (Figures 5I, J).

ARA290 Treatment Increases Intracellular Ca\textsuperscript{2+} Mobilization after Cholinergic Stimulation

To investigate effects of ARA290 treatment on Ca\textsuperscript{2+} mobilization from intracellular stores, we studied the direct effects of carbamylcholine, a cholinergic agonist that activates the acetylcholine receptor, on PLC/Ins\textsubscript{P}\textsubscript{3} mediated Ca\textsuperscript{2+} release in islets from ARA290-treated GK rats as compared with vehicle-treated GK rats. We observed an increase in peak [Ca\textsuperscript{2+}] values in ARA290-treated islets during stimulation by 200 μmol/L carbamylcholine, in the presence or in the absence of extracellular Ca\textsuperscript{2+}, as compared with the vehicle-treated GK rats. We observed an increase in peak [Ca\textsuperscript{2+}] values in ARA290-treated islets during stimulation by 200 μmol/L carbamylcholine, in the presence or in the absence of extracellular Ca\textsuperscript{2+}, as compared with the vehicle-treated islets (Phase I and Phase III in Figure 6). These results were from islets perfused with basal glucose (3 mmol/L) and were compatible to the increase in [Ca\textsuperscript{2+}] stimulated by 100 μmol/L carbamylcholine in the presence of 16.7 mmol/L glucose in ARA290-treated islets (Figure 5E). There was no statistically significant difference in Ca\textsuperscript{2+} entry over the plasma membranes between the two groups (Phase II in Figure 6).

Figure 3. Effect of ARA290 on glucose metabolism in GK rat islets. (A) After 30 min preincubation of islets in KRB supplemented with 3.3 mmol/L G, glucose oxidation was measured in batches of 10 GK islets during incubation for 2 h with 3.3 or 16.7 mmol/L G alone (○) or in the presence of 10 ng/mL ARA290 (◼). Results represent means ± SEM of n = 6 experiments. *P < 0.05 versus 3.3 mmol/L G control, #P < 0.02 versus 16.7 mmol/L G control. (B) Islets were preincubated for 30 min in KRB supplemented with 3.3 mmol/L G, batches of 20 islets were the incubated for 1 h with 3.3 or 16.7 mmol/L G alone (○) or in the presence of 10 ng/mL ARA290 (◼) and ATP content was measured. Results are expressed as pmol of ATP normalized by the total protein content of islets. Results represent means ± SEM of n = 6 experiments. *P < 0.05 versus 3.3 mmol/L G control, #P < 0.05 versus 16.7 mmol/L G control.
DISCUSSION

The results of these experiments show that ARA290 prevents the progressive worsening of glucose tolerance that occurs in the GK rat, a nonobese model of type 2 diabetes characterized by impaired insulin secretion (2). Notably, although fasting PG concentrations remained normal in both treatment groups, IPGTT showed that the glucose responses were significantly lower after 2 and 4 wks of treatment in the ARA290 group. This was supported by a significantly reduced HbA1c concentration after 4 wks of treatment in ARA290-treated GK rats compared with control.

The improved glucose tolerance observed in ARA290-treated GK rats could be due to enhancement of insulin sensitivity and/or increased insulin secretion. Since there were no significant
ARA290 improves insulin secretion in diabetic GK rats

976 | Muller et al. | Mol Med 21:969-978, 2015

differences between ARA290-treated and placebo-treated rats regarding their responses in the pyruvate tolerance and the insulin sensitivity tests, it is not plausible that ARA290 exerts its primary action on insulin sensitivity in this model. This is further supported by similar fasting plasma insulin levels in treated and control rats, suggesting that the analogue does not improve glucose homeostasis by acting on the liver. Thus, ARA290-induced improvement of glucose tolerance in the GK rat can be accounted for by a direct effect on the pancreatic β cells.

This conclusion is supported by the observation that islets isolated from GK rats exhibit an increased glucose-induced insulin secretion when compared with control.

In pancreatic β cells, glucose stimulates insulin secretion by virtue of its metabolism. Following rapid transport through the β-cell plasma membrane, the hexose is phosphorylated to glucose-6-phosphate, and then further metabolized through glycolysis and citric acid cycle to yield ATP (15). The subsequent increase in the cytosolic ATP/ADP ratio leads to closure of the ATP-regulated K⁺ channels, plasma membrane depolarization and opening of voltage-dependent L-type Ca²⁺ channels (13,14). The resulting increase in [Ca²⁺]i stimulates the exocytosis of insulin granules (16). Our results show that the effect of ARA290 on β cells occurs via an improvement of glucose metabolism as evidenced by increased glucose oxidation and ATP production. Additional data show that the increase in insulin secretion also depends on the activation of PKA-dependent pathways, that is, the effects of ARA290 occur via known insulin secretory pathways.

Interestingly, ARA290 did not affect glucose concentrations in W rats or stimulate glucose-induced insulin release in W islets. This may imply that ARA290 specifically improves the mechanisms responsible for defective insulin secretion in the GK rat.

In this animal model of type 2 diabetes, reduced β-cell mass associated with defective insulin secretion is a hallmark of its phenotype and can be attributed to several abnormalities in the β cell function, similar to what is found in human type 2 diabetes (2).
Measurements of Ca\(^{2+}\) mobilization from intracellular stores and Ca\(^{2+}\) influx over the plasma membrane. (A) Effects of 0.2 mmol/L CCh on changes in [Ca\(^{2+}\)]\(_i\) in islets from GK rats treated with ARA290 compared with placebo treatment. Bars above the traces indicate the duration of stimulation. The values 0 CaCl\(_2\) and 2.56 CaCl\(_2\) indicate 0 mmol/L and 2.56 mmol/L CaCl\(_2\), respectively. The concentration of CCh is 0.2 mmol/L. Phase I shows changes in [Ca\(^{2+}\)]\(_i\) in response to CCh in the absence of extracellular Ca\(^{2+}\). Phase II shows effects of adding 2.56 mmol/L CaCl\(_2\) in the perfusion chamber on changes in [Ca\(^{2+}\)]\(_i\), including Ca\(^{2+}\) influx over the plasma membrane. Phase III shows changes in [Ca\(^{2+}\)]\(_i\), in response to CCh in the presence of extracellular Ca\(^{2+}\). The displayed traces are representatives of 13 traces from the placebo-treated rats (n = 4) and 19 traces from ARA290-treated rats (n = 4). (B) Phase I: Average Δ peak [Ca\(^{2+}\)]\(_i\), values, that is, fura-2 ratio, in islets stimulated with CCh in the Ca\(^{2+}\)-free buffer; Phase II: Average basal Ca\(^{2+}\) influx over the plasma membrane; Phase III: Average Δ peak fura-2 ratio in islets stimulated with CCh in the presence of extracellular Ca\(^{2+}\). Data are shown as means ± SEM. P values between the two groups (PBS placebo, black bars, versus ARA290, red bars) are shown. *P < 0.05, **P < 0.01, N.S. indicates no statistical significance.

After an initial transient rise in [Ca\(^{2+}\)]\(_i\), stimulated by glucose, [Ca\(^{2+}\)]\(_i\), in pancreatic islets normally oscillates due to a sophisticated interplay between Ca\(^{2+}\) entry through voltage-activated Ca\(^{2+}\) channels and Ca\(^{2+}\) mobilization from intracellular stores. In the present study, we aimed at determining whether changes in [Ca\(^{2+}\)]\(_i\) dynamics, that is, [Ca\(^{2+}\)]\(_i\), oscillations, in pancreatic β cells contribute to the significant improvement on insulin secretion and glucose homeostasis after ARA290 treatments. Although 16.7 mmol/L glucose-induced [Ca\(^{2+}\)]\(_i\), oscillations occurred in both placebo- and ARA290-treated islets in GK rats, power spectrum analysis found that ARA290 treatment increased [Ca\(^{2+}\)]\(_i\), oscillation frequency in GK rat β cells. Results from the [Ca\(^{2+}\)]\(_i\) experiments suggest that the enhanced [Ca\(^{2+}\)]\(_i\), dynamics in pancreatic β cells, that is, increased initial peak [Ca\(^{2+}\)]\(_i\), values and faster [Ca\(^{2+}\)]\(_i\), oscillation frequency in response to glucose, is a key feature of the increased insulin secretion (17,18) and therefore improved glucose tolerance after ARA290 treatments in GK rats. The improved [Ca\(^{2+}\)]\(_i\), oscillation frequency observed in the isolated islets were mirrored by increased glucose-induced insulin secretion in vivo.

To determine how ARA290 treatment increases [Ca\(^{2+}\)]\(_i\), oscillation frequency in GK rat β cells, we measured changes in CCh-stimulated Ca\(^{2+}\) mobilization from intracellular stores and Ca\(^{2+}\) influx over the plasma membrane. We first observed increased amounts of PLC/InsP\(_3\)-generated signals in ARA290-treated islets induced by 0.1 mmol/L CCh, a cholinergic agonist, in the presence of 16.7 mmol/L glucose. We thereafter found increased CCh-stimulated Ca\(^{2+}\) mobilization from intracellular stores. These findings are compatible with more releasable Ca\(^{2+}\) in the endoplasmic reticulum (ER) pool in β cells after ARA290 exposure and may be explained by an increased basal Ca\(^{2+}\) influx over the plasma membrane in ARA290-treated islets (Phase II in Figures 6A, B). These results suggest that, in ARA290-treated islets, more Ca\(^{2+}\) was available to fill the ER pool, and, there was also an increase in the ER Ca\(^{2+}\) uptake, which leads to a significant increase in the peak [Ca\(^{2+}\)]\(_i\), values and an increase in [Ca\(^{2+}\)]\(_i\), oscillation frequency in response to glucose. Moreover, to determine whether ARA290 enhanced depolarization-induced Ca\(^{2+}\) influx over plasma membrane, we found that ARA290-treated islets displayed a 39% increase in peak [Ca\(^{2+}\)]\(_i\), values after 30 mmol/L KCl treatment versus control islets (Figure 5D). The latter finding is compatible with an increased activity of the voltage-activated Ca\(^{2+}\) channels in plasma membrane and that, in turn, contributes to the enhancement in β cell [Ca\(^{2+}\)] dynamics.

Overall, these experiments demonstrate for the first time that the enhancement in [Ca\(^{2+}\)]\(_i\), oscillations in pancreatic β cells of GK rats treated with the non-hematopoietic erythropoietin analogue, ARA290, is associated with increased insulin secretion and improved glucose tolerance. ARA290-induced fine tuning of the [Ca\(^{2+}\)]\(_i\), signal, that is, increased [Ca\(^{2+}\)]\(_i\), oscillation frequency, in GK islets may be further explained by activation of PLC/InsP\(_3\)-mediated Ca\(^{2+}\) mobilization from intracellular stores and enhancement of membrane depolarization-induced Ca\(^{2+}\) influx.
Altogether, these observations mean that ARA290 could act on insulin secretion by a mechanism involving both Ca\(^{2+}\)-independent mechanisms through the PKA activation and Ca\(^{2+}\)-dependent mechanisms, the latter supported by the fact that the Ca\(^{2+}\) channel blocker nimodipine suppressed the effect by ARA290 on insulin release.

Our findings are in agreement with the recently published study of db/db mice treated with rhEPO, resulting in improvement of insulin secretion (7). However, in this study the authors failed to show a direct effect of EPO on the β cell secretory function, and rather showed that EPO rescued diabetic mice in the db/db mouse by an effect on the proliferation and antiapoptotic mechanisms and therefore on β cell mass. The discrepancy in the results may be explained by the specific pathophysiologic mechanisms of diabetes in the GK rats, that is, that the defect in these rats is not primarily due to an increased rate of apoptosis in the pancreatic β cells but rather to molecular defects in these cells (2).

CONCLUSION

Treatment with ARA290 significantly improved glucose tolerance in diabetic GK rats but had no effects on nondiabetic Wistar rats. The beneficial effect in glucose tolerance in GK rats results from an increase in insulin release. ARA290 increases GSIS by a sequence of events focused on the β cell that culminates in improved glucose oxidation and ATP production, reflecting enhanced mitochondrial oxidation. This in turn leads to K\(_{\text{ATP}}\) channel closure and an increase in Ca\(^{2+}\) entry through voltage-dependent Ca\(^{2+}\) channels. Further, the mechanism behind the increased insulin secretion also appears to be mediated by improved β cell [Ca\(^{2+}\)] handling and thus of the insulin exocytotic machinery.

ACKNOWLEDGMENTS

This study was supported by grants from the Swedish Research Council, the Swedish Diabetes Association, the Strategic Research Program in Diabetes at Karolinska Institutet, the ERC-2013-AdG 338936-Betamage, the Novo Nordisk Foundation, Knut and Alice Wallenberg Foundation, Berth von Kantzow’s Foundation, The Skandia Insurance Co. Ltd, the Family Erling-Persson Foundation and the Stichting af Jochnick Foundation. The skilled technical assistance of Elisabeth Nørén-Krog and Yvonne Strömberg is acknowledged. AC and MB are officers of Arai Pharmaceuticals and own stock or stock options. All other authors declare no conflict of interest.

DISCLOSURE

The authors declare that they have no competing interests as defined by Molecular Medicine, or other interests that might be perceived to influence the results and discussion reported in this paper.

REFERENCES

1. Frayling TM. (2007) Genome-wide association studies provide new insights into type 2 diabetes aetiology. Nat. Rev. Genet. 8:657–62.
2. Ostenson CG, Efendic S. (2007) Islet gene expression and function in type 2 diabetes; studies in the Goto-Kakizaki rat and humans. Diabetes Obes. Metab. 9 Suppl 2:180–6.
3. Homo-Delarche F, et al. (2006) Islet inflammation and fibrosis in a spontaneous model of type 2 diabetes, the GK rat. Diabetes. 55:1625–33.
4. Donath MY, Sterling J, Maedler K, Mandrup-Poulsen T. (2003) Inflammatory mediators and islet beta-cell failure: a link between type 1 and type 2 diabetes. J. Mol. Med. (Berl) 81:455–70.
5. Fenjves ES, et al. (2003) Human, nonhuman primate, and rat pancreatic islets express erythropoietin receptors. Transplantation. 75:1356–60.
6. Brines M, Cerami A. (2006) Discovering erythropoietin’s extra-hematopoietic functions: biology and clinical promise. Kidney Int. 70:246–50.
7. Choi D, et al. (2010) Erythropoietin protects against diabetes through direct effects on pancreatic beta cells. J. Exp. Med. 207:2831–42.
8. Brines M, et al. (2008) Nonerythropoietic, tissue-protective peptides derived from the tertiary structure of erythropoietin. Proc. Natl. Acad. Sci. U. S. A. 105:10925–30.
9. Brines M, Cerami A. (2012) The receptor that tames the innate immune response. Mol. Med. 18:486–96.
10. Brines M, et al. (2015) ARA 290, a nonerythropoietic peptide engineered from erythropoietin, improves metabolic control and neuropathic symptoms in patients with type 2 diabetes. Mol. Med. 20:658–66.
11. Herbert V, Lau KS, Gottlieb CW, Bleicher SJ. (1965) Coated charcoal immunoassay of insulin. J. Clin. Endocrinol. Metab. 25:1375–84.