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Activation of κ-Opioid Receptor Exerts the Glucose-Homeostatic Effect in Streptozotocin-Induced Diabetic Mice

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ABSTRACT

Opioid and its receptors play important roles in glucose homeostasis. However, few reports were available for the study of κ-opioid receptor in glucose regulation. In our study, we found that the blood glucose of diabetic mice dropped significantly following the treatment with U50,488H (a selective κ-opioid receptor agonist). This phenomenon was time-dependent and associated with the coincident alteration of Glut4 translocation in the skeleton muscles. U50,488H increased the serum adiponectin, but not serum insulin in diabetic mice. U50,488H increased the AdipoR1 expression at both mRNA and protein levels. It also promoted AMPK phosphorylation and Glut4 translocation. All these effects were abolished by nor-BNI (a selective κ-opioid receptor antagonist). These findings suggest that activation of κ-opioid receptor reduces hyperglycemia in streptozotocin-induced diabetic mice. This effect is associated with the translocation of Glut4 and might be relevant to increased adiponectin, AdipoR1, and AMPK phosphorylation. J. Cell. Biochem. 116: 252–259, 2015. © 2014 Wiley Periodicals, Inc.

KEY WORDS: DIABETES; OPIOIDS; ADIPONECTIN; ADIPOR1; AMPK; GLUT4

Glucose homeostasis is the consequence of combined regulation of nervous system, hormone secretion, and target organs. Patients with diabetes are generally characterized by either impaired insulin secretion or reduced sensitivity to insulin. It has been known that hyperglycemia and hyperglycemia-related metabolic disorders result in various complications, including coronary artery diseases, cerebrovascular disorders, renal failure, and diabetic retinopathy [Hunter et al., 2011; Tabak et al., 2011]. Besides insulin, various endogenous hormones have been studied on their roles in glucose homeostasis, of which opioid and its receptors attract great interest.

Three classes of opioid receptors have been cloned, including μ-, δ-, and κ-opioid receptors [Minami and Satoh, 1995; Satoh and Minami, 1995]. Opioid receptors mediate the biological functions of opioids and are selectively expressed on different organs. It is

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generally thought that β-endorphin, enkephalin, and dynorphin are selective endogenous agonists for the μ-, δ-, and κ-opioid receptors, respectively [Minami and Satoh, 1995; Satoh and Minami, 1995]. Researches showed that μ- and δ-opioid receptors might play important roles in the glucose homeostasis. Stimulation of μ-opioid receptor (μ-OR) has been reported to lower plasma glucose by increasing either glycogen synthesis in liver or glucose utilization in peripheral tissues [Tzeng et al., 2003; Amirshahrokhi et al., 2008; Yang et al., 2009]. Enkephalin, an agonist for the μ- and δ-receptors increased insulin levels in both lean and obese mice (Bailey and Flatt, 1987). Even though there were some reports about the activation of κ-opioid receptor in glucose homeostasis [Green et al., 1983; Ishizuka et al., 1986; Khawaja et al., 1990a; Josefsen et al., 1998], these results were isolated and limited work was done to systemically illustrate this issue. As a result, it remains difficult to conclude the effects of κ-opioid receptor in the regulation of glucose homeostasis.

Besides enhancing insulin secretion, increasing the sensitivity to insulin is another strategy in treating patients with diabetes. Adiponectin has been reported to improve the sensitivity to insulin, and plasma levels of adiponectin have been reported to be significantly reduced in obese/diabetic mice and humans [Arita et al., 1999; Yatagai et al., 2003; Takashi and Toshimasa, 2005]. Till now, two adiponectin receptors have been identified: adiponectin receptor 1 (AdipoR1) and adiponectin receptor 2 (AdipoR2). They both are seven-transmembrane spanning receptors but with differential features from the superfamily of G protein-coupled receptors [Yamauchi et al., 2003]. It has been demonstrated that AdipoR1 is abundantly expressed in skeletal muscle, whereas AdipoR2 is mainly located in the liver [Yamauchi et al., 2003]. Adiponectin binding to AdipoR1 on the membrane of skeletal muscle has been shown to exert anti-diabetic effects [Kadowaki et al., 2006]. In AdipoR1$^{-/-}$ mice, glucose tolerance and energy expenditure have been demonstrated to be decreased. However, glucose tolerance was significantly improved in AdipoR2$^{-/-}$ mice and those mice were resistant to high-fat diet-induced obesity [Bjursell et al., 2007]. Glucose transporter 4 (Glut4) is the major insulin-responsive GLUT isoform only expressed in muscle and fat cells and it regulates glucose transportation into the cell [Niu et al., 2010; Nolan and Elmendorf, 2011]. The activation of AMPK via the binding of adiponectin to AdipoR1 also results in the translocation of Glut4 [Takashi and Toshimasa, 2005; Kadowaki et al., 2006]. However, no research has been reported about the connection between opioid receptors and adiponectin.

We undertook the present study to determine whether U50,488H could reduce blood glucose concentration in streptozocin (STZ)-induced diabetic mice. To elucidate this mechanism of the effect on glucose homeostasis mediated by κ-OR, radioimmunoassay, enzyme linked immonusorbent assay, western blotting, immunohistochemistry, and reverse transcription–polymerase chain reaction (RT-PCR) were used to detect the changes of insulin, adiponectin, AdipoR1, phosphorylation of AMPK, and the translocation of Glut4 in diabetic mice. Interestingly, we found that stimulation of κ-OR improved hyperglycemia in STZ-induced diabetic mice, and it was associated with the translocation of Glut4. Meanwhile, elevated phosphorylation of AMPK and increased expression of adiponectin and AdipoR1 might be relevant to this effect.

MATERIALS AND METHODS

ANIMAL PREPARATION AND REAGENTS

Ten-week old male BALB/C mice (22–25 g) were obtained from Fourth Military Medical University, Xi'an, China. Male mice were housed in colony cages and maintained on a 12 h light/12 h dark cycle. Mice that were fasted for 12 h were intraperitoneally injected 0.1–0.2 ml of 50 mM sodium citrate solution (pH 4.5) containing STZ (Sigma Chemical, St Louis, MO). STZ was given at a dosage of 60 mg/kg for three consecutive days. Fasted blood glucose was measured by Bayer's BREEZE2 meter (Bayer Health Care LLC, Mishawaka, IN) 7 days after injection. Mice with fasted glucose concentration over 16.7 mmol/L were selected for the diabetic mice model and were raised under the standard condition. Eight weeks later, U50,488H (Tocris, 1.5 mg/kg; Tocris Cookson Inc., St. Louis, MO) was given through the caudal vein (Zhang QY et al., 2010). The blood glucose was measured every 10 min after the injection of U50,488H for 60 min. The solei muscle was isolated after the mice were sacrificed. The tissues were stored at −80°C till used. Next, U50,488H was given to diabetic mice, and nor-BNI (Tocris, 2 mg/kg) was intravenously given 10 min before U50,488H. The blood samples and solei obtained 20 min after the injection of U50,488H. All the studies were performed in accordance with the guidelines described in the National Animal Protection Law, and the use of animal tissues was approved by the Fourth Military Medical University Internal Review Committee.

MEASUREMENT OF SERUM INSULIN AND ADIPONECTIN

The blood samples were placed at 25°C for 30 min. Serum was separated after centrifugation at 3,000 g for 20 min and the supernatant was stored at −80°C until used. Total serum adiponectin concentration was measured by ELISA (Westang Biotechnology, Shanghai, China). Serum insulin was measured by radioimmunoassay (Beifang Biotechnology, Beijing, China).

WESTERN BLOTTING

Tissues were solubilized in PBS containing protease inhibitors (1 mM each: antipain, benzamidine, leupeptin, pepstatin A, and phenylmethyl sulfonylfluoride, 1% sodium dodecyl sulfate, and 5 mM

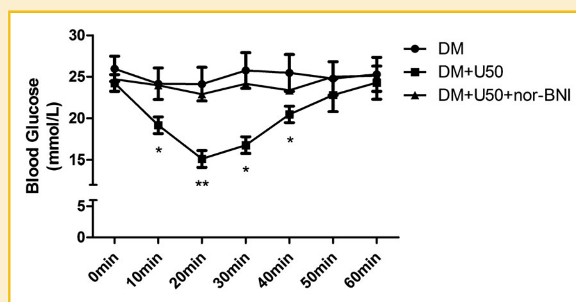

Fig. 1. Effects of intravenous U50,488H administration (1.5 mg/kg) on blood glucose levels in STZ-induced diabetic (DM) mice. Mice were fasted for 12 h before the injection. All data are shown as means ± SE. n = 15 in each group. *$P < 0.05$ versus DM 0 min group; **$P < 0.01$ versus DM 0 min group.

ethylene diamine tetraacetic acid). Membranous protein was extracted using Membranous Protein Extraction Kit (Beyotime, Shanghai, China). Protein concentration of extracts was determined by BCA assay (Pierce, Rockford, IL). Equal amounts of protein (40 μg) were resolved on SDS-PAGE, and transferred onto nitrocellulose membranes. The membranes were probed with primary antibodies (Anti-AdipoR1 Antibody [goat anti-rat, sc-46748]; Santa Cruz Biotechnology, Inc., CA; Anti-phospho/total AMPK Antibodies [rabbit anti-rat, 2535/2532]; Cell Signaling Technology Inc., Denver; Anti-Glut4 Antibody [rabbit anti-rat, BA1626]; Boster, Wuhan, China; Anti-tubulin Antibody [mouse antirat, AT819]; Beyotime, Shanghai, China). Then, membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (goat anti-mouse/rabbit; donkey antigoat; Beyotime, Shanghai, China). Immunoreactive signals were detected using the enhanced chemiluminescence Western Blotting Detection System (Bio-Rad, Hercules, CA). Quantification of protein bands was done by densitometry with the use of Quantity One Software (Bio-Rad).

RNA ISOLATION AND RT-PCR ANALYSES

Total RNA was isolated from solei using Trizol (Takara Bio Inc, Otsu, Japan) according to the manufacturer's instructions. cDNA was amplified using a two-step reverse transcription system (Takara Bio Inc). Primer sequences were as follows: AdipoR1—5'-CAC CTA TGC CCT CCT TTC GG-3', 5'-CAC CAC TCA AGC CAA GTC CC-3'; Glut4—5'-GCC CGA AAG AGT CTA AAG-3', 5'-AGA GCC ACG GTC ATC AAG-3'; GAPDH—5'-AGG CCG GTG CTG AGT ATG TC-3', 5'-TGC CTG CTT CAC CAC CTT CT-3'. PCR conditions were as follows: AdipoR1—94°C for 5 min, 94°C for 45 s, 56°C for 60 s, and 72 °C for 60 s for 28 cycles, and finally 72°C for 10 min; Glut4—94°C for 5 min, 94°C for 45 s, 56°C for 60 s, and 72 °C for 60 s for 28 cycles, and finally 72°C for 10 min; GAPDH—95°C for 3 min, then 95°C for 90 s, 60°C for 120 s for 22 cycles, and finally 60°C for 10 min. Samples were resolved on 1.5% agarose gels. RT-PCR bands were analyzed using Quantity One software (Bio-Rad). Relative mRNA levels were calculated using standard curves, with the PCR product for each primer set normalized to GAPDH.

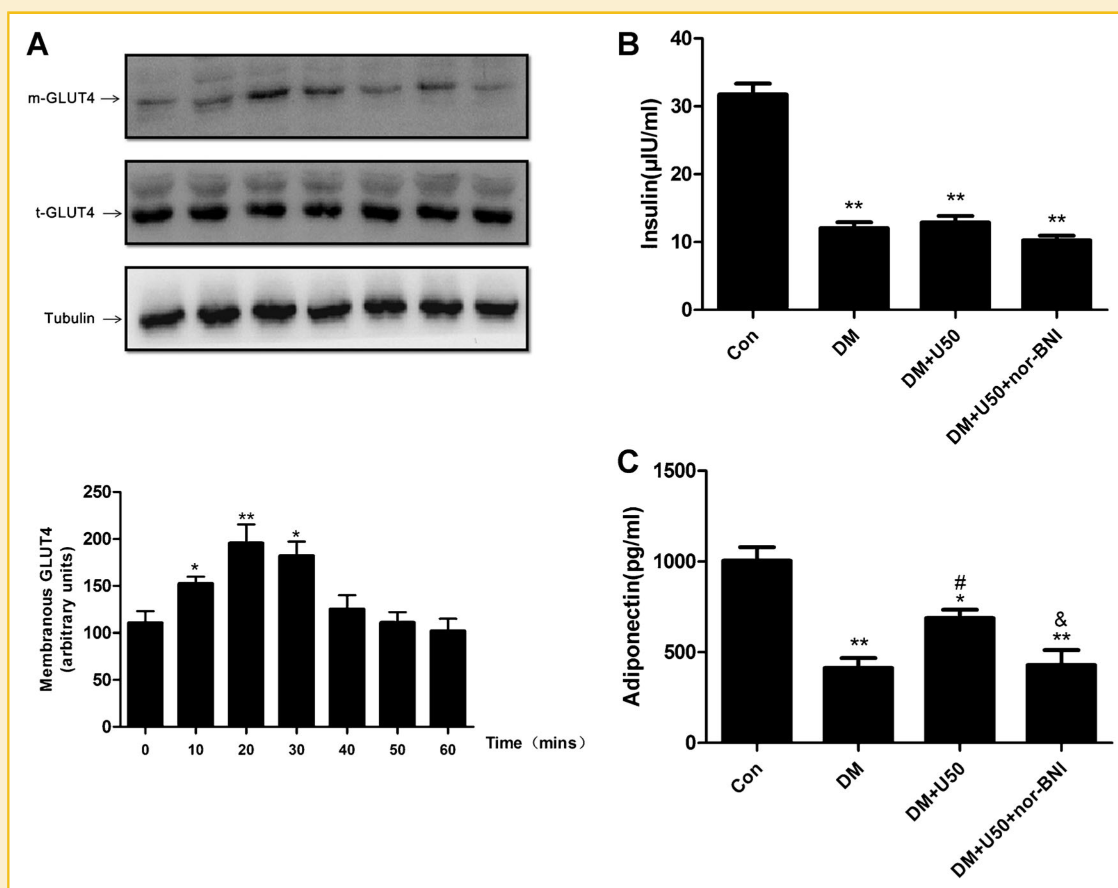

Fig. 2. U50,488H-stimulated Glut4 translocation and adiponectin elevation in diabetic (DM) mice. A: Mice were fasted for 12 h, intravenously injected with U50,488H (1.5 mg/kg), and sacrificed at the indicated time points. Representative total and membranous Glut4 immunoblots of solei lysates are shown. Each lane contained in the gel image showing the change of Glut4 immunoblots with the time as indicated in the bottom part of the figure. Ratio of membranous versus total Glut4 was calculated and is shown as means ± SE. n = 5 in each group. $^{*}P < 0.05$ versus DM group; $^{**}P < 0.01$ versus DM group. B: Serum insulin of diabetic mice treated with U50,488H. $^{**}P < 0.01$ versus Con group. C: Serum adiponectin of normal and diabetic mice treated with U50,488H. $^{*}P < 0.05$ versus Con group; $^{**}P < 0.01$ versus Con group; $^{\#}P < 0.05$ versus DM group; $^{\&}P < 0.05$ versus DM + U50 group. All data are shown as means ± SE. n = 8 in each group. Con, control; U50, U50,488H.

IMMUNOHISTOCHEMISTRY

Three micrometer slices from solei were fixed in glass slides and placed in a bathing solution of 3% H_2O_2 and 60% methanol phosphate-buffered saline (PBS) for 30 min and then treated with 0.01 mol/L sodium citrate buffer at 95°C in a microwave oven for 20 min. Thereafter, specimens were treated with 5% normal goat serum. Before each step, sections were rinsed three times in PBS buffer. Incubation with anti-Glut4 antibody (Boster, Wuhan, China) was performed in a PBS-based solution for 12 h at 4°C in the recommended dilutions. And then, sections were incubated with the corresponding secondary biotinylated goat anti-rabbit antibody (Histostain-plus Kit; ZSGB-BIO Company, China) for 1 h at 25°C. A streptavidin/horseradish peroxidase complex was then applied as a detection system for 40 min. Finally, staining was developed with 3,3'-diaminobenzidine tetra-hydrochloride (DAB kit; ZSGB-BIO Company, China) in 0.05 mol/L Tris–HCl buffer and 0.1% H_2O_2. Negative control sections were incubated without the primary antibody. All dates in this study were analyzed by software Image Pro Plus (Media Cybernetics Corporation, Washington, DC).

STATISTICAL ANALYSIS

Data are expressed as mean ± SE. Differences between sample populations in glucose concentrations, immunoblotting studies, RT-PCR studies, and immunohistochemical signal intensities were determined with analysis of variance (one-way) followed by using Tukey post hoc testing. $^{*}P < 0.05$ and $^{**}P < 0.01$ was considered statistically significant.

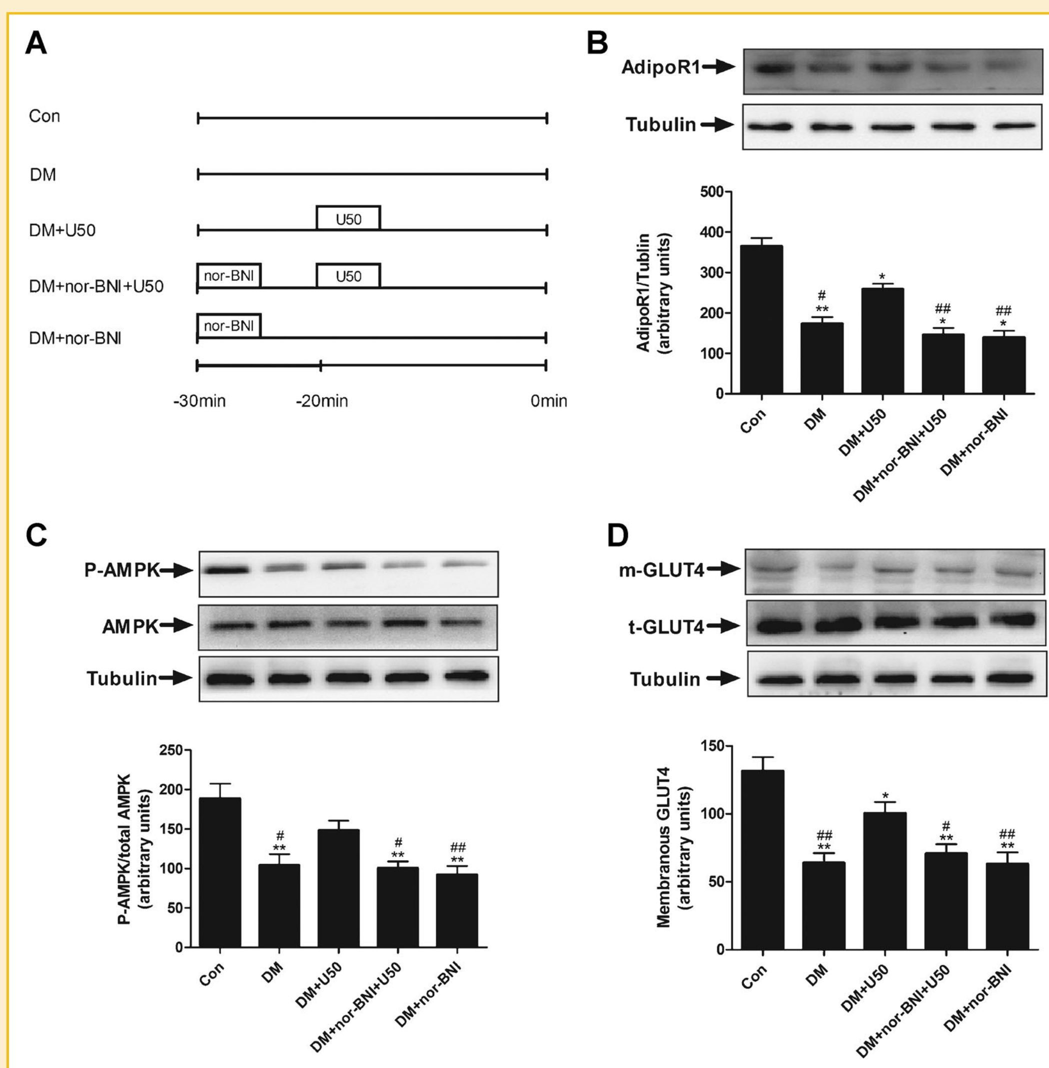

Fig. 3. Western blotting of solei in diabetic mice. A: Protocols of this study. Con, normal mice; DM, STZ-induced diabetic mice; DM + U50, diabetic mice subjected to U50,488H; DM + nor-BNI + U50,488H, diabetic mice subjected to U50,488H at the presence of nor-BNI; DM + nor-BNI, diabetic mice subjected to nor-BNI. U50,488H was injected intravenously (completed in 10 s) 20 min before muscle isolation; nor-BNI was injected intravenously (completed in 10 s) 10 min before U50,488H. Samples were probed with anti-tubulin, anti-AdipoR1, anti-p/total AMPK, and anti-Glut4 antibodies. Representative immunoblots are respectively shown in B, C, and D. Quantitative densitometric analysis of each blot was performed and all the results are shown as means ± SE. n = 5 in each group. $^{*}P < 0.05$ versus Con group; $^{**}P < 0.01$ versus Con group; $^{\#}P < 0.05$ versus DM + U50 group; $^{\#\#}P < 0.01$ versus DM + U50 group. Con, control; U50, U50,488H.

RESULTS

EFFECTS OF U50,488H ON BLOOD GLUCOSE AND MEMBRANOUS GLUT4 LEVELS

A marked decrease in glucose levels was observed at 10, 20, 30, and 40 min following the U50,488H treatment in STZ-induced diabetic mice ($P < 0.01$). Glucose concentrations of diabetic mice returned to original levels at 50 min, and remained stable afterwards (Fig. 1). This reduction to glucose was attenuated by nor-BNI ($P < 0.01$). The Western blotting data showed that the amount of membranous Glut4 was correlated with the reduction of hyperglycemia. The amount of total Glut4 remained unchanged in this process (Fig. 2A). Our study demonstrated a time-dependant improvement of hyperglycemia by U50,488H, and the increased membranous Glut4 during κ-OR stimulation contributed to this effect.

EFFECTS OF U50,488H ON SERUM INSULIN AND ADIPONECTIN IN DIABETIC MICE

As the blood glucose was most significantly reduced at 20 min after the U50,488H injection, we collected the blood samples of mice subjected to different treatment. Serum insulin levels were decreased in diabetic mice and U50,488H did not cause any alteration to insulin levels (Fig. 2B). In diabetic mice, adiponectin was decreased, and the improvement of its level was found in diabetic mice treated with U50,488H (Fig. 2C).

EFFECTS OF U50,488H ON ADIPOR1, AMPK AND MEMBRANOUS GLUT4 IN DIABETIC MICE

Adiponectin binding to AdipoR1 can activate AMPK which leads to the translocation of Glut4 in skeletal muscle. To investigate whether this pathway was involved in the reduction of hyperglycemia, five groups of mice with different treatment were present in our following studies, including normal mice (Con), diabetes mellitus (DM), diabetes mellitus + U50,488H (DM + U50), diabetes mellitus + nor-BNI + U50,488H (DM + nor-BNI + U50), and diabetes mellitus + nor-BNI (DM + nor-BNI). The RT-PCR and Western blotting analyses showed that both mRNA and protein levels of AdipoR1 were significantly decreased in diabetic mice (Figs. 3B and 4), indicating that reduced AdipoR1 in skeletal muscle were correlated with hyperglycemia. The activation of AMPK was examined by detecting phosphorylated AMPK through Western blotting, and it was showed to be suppressed in STZ-induced diabetic mice (Fig. 3C). RT-PCR and western blotting showed that mRNA and protein levels of total Glut4 were not changed among the groups (Fig. 3D and 4). The western blotting showed the impaired translocation of Glut4 in DM group, and as what we have demonstrated, administration of U50,488H increased membranous Glut4 in skeletal muscle (Fig. 3D). Meanwhile, U50,488H elevated the phosphorylation of AMPK and expression of AdipoR1 (Fig. 3B,C). In immunohistochemical study, Glut4 was detected around the plasma membrane in normal gastrocnemius muscles. In diabetic mice, a great amount of stained particles were observed in the cytoplasma of skeletal muscles, indicating that Glut4 was quantitatively stored in the plasma during STZ-induced hyperglycemia. U50,488H could improve the translocation of Glut4 (Fig. 5). All these effects of U50,488H were eliminated by the κ-OR antagonist nor-BNI, which showed that κ-OR played an important role in the regulation of glucose homeostasis in STZ-induced diabetic mice subjected to U50,488H.

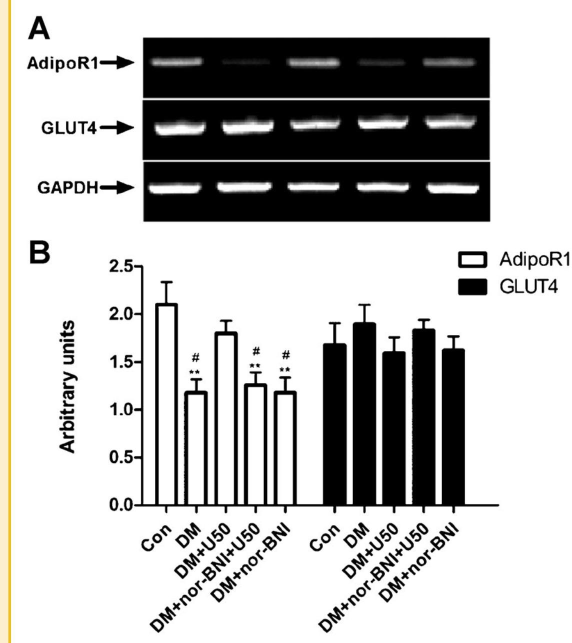

Fig. 4. Reverse transcription–polymerase chain reaction (RT-PCR) study of solei in STZ-induced diabetic (DM) mice. A: Representative RT-PCR bands of samples from solei of diabetic mice. B: Quantitative densitometric analysis of mRNA of AdipoR1 and Glut4 in five groups (protocols in Fig. 3A). All the data are shown as means ± SE. n = 6 in each group. $^{*}P < 0.05$ versus Con group; $^{**}P < 0.01$ versus Con group; $^{\#}P < 0.05$ versus DM + U50 group. Con, control; U50, U50,488H.

DISCUSSION

The efforts of exploring the connection between opioid and glucose homeostasis have been made for decades but this issue remains ambiguous. To our knowledge, our study is the first to demonstrate the reduction of hyperglycemia following the κ-OR stimulation in STZ-induced diabetic mice. We also observed the association between glucose changes and translocation of Glut4, and proved the positive correlation of them. This phenomenon might be relevant to the increased adiponectin, AdipoR1, and phosphorylation of AMPK.

We assessed the effect of κ-OR stimulation on the levels of adiponectin, and the decreased levels of adiponectin in STZ-induced diabetic mice were found. In addition, we observed that the serum adiponectin was greatly increased during κ-OR stimulation. Besides the circulating level of adiponectin in plasma, the quantity and function of adiponectin receptors were also critical to the function of adiponectin. Therefore, decreased expression or impaired function of adiponectin receptors could lead to the reduced adiponectin bioactivity and insulin sensitivity [Tsuchida et al., 2004; Bruce et al., 2005; Kadowaki et al., 2006; Lin et al., 2007]. Adiponectin binding to AdipoR1/R2 stimulates fatty acid oxidation in liver,

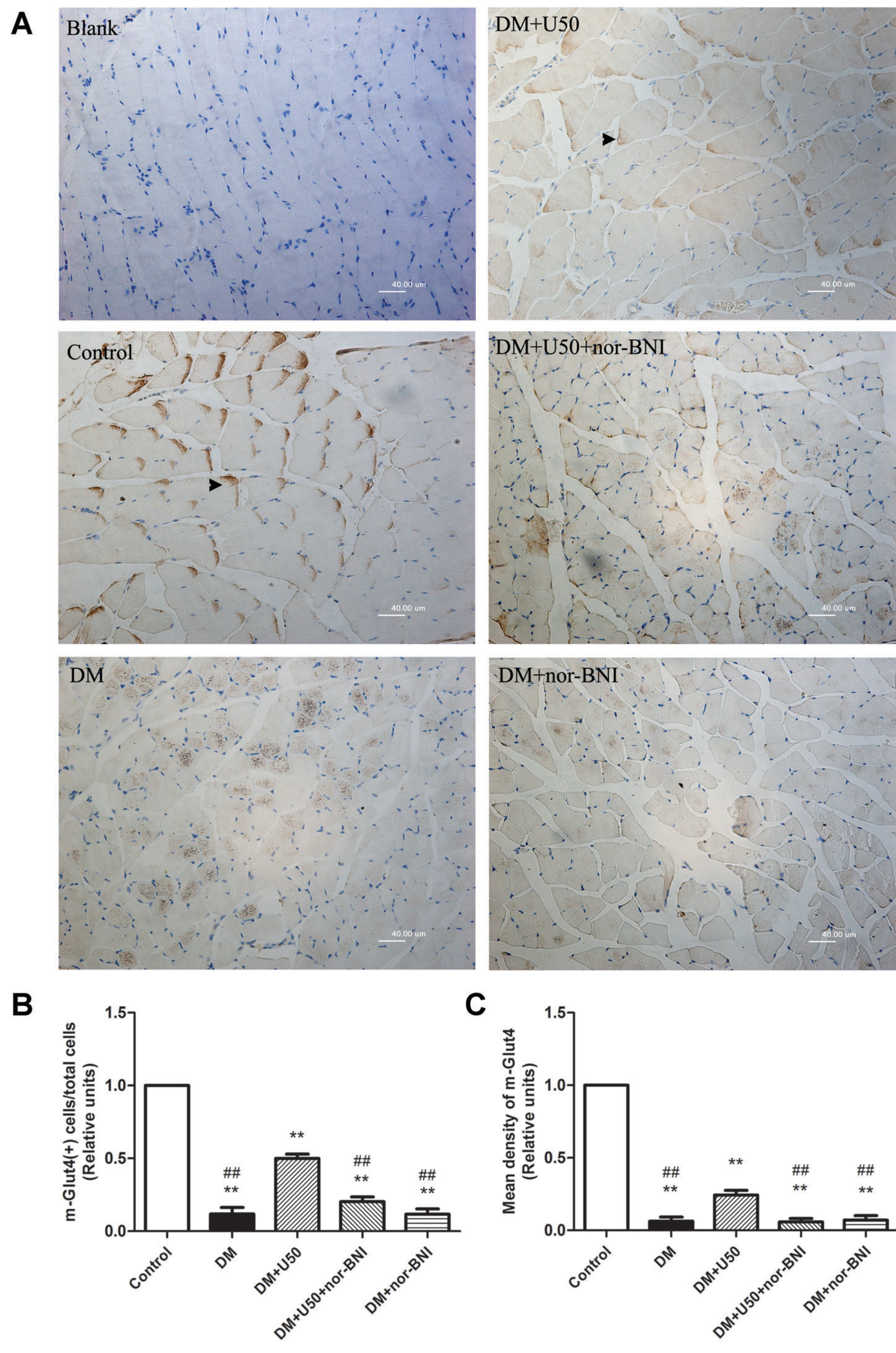

Fig. 5. Immunohistochemical study of translocation of Glut4. The protocol was as Fig. 3A showed. A: Slices incubated without primary antibody was used as the negative control. Solei of mice with different treatment were stained with anti-Glut4 antibody. Arrows indicated the membrane location of Glut4 in skeletal muscle cells. B: The ratio of cells with membranous Glut4 in total cells. C: The mean density of membranous Glut4 in solei of mice with different treatment. All the data are shown as means ± SE. n = 10 in each group. $^{**}P < 0.01$ versus Con group; $^{\#\#}P < 0.01$ versus DM + U50 group.

increases glucose uptake in skeletal muscle and suppresses gluconeogenesis [Fruebis et al., 2001; Josefsen et al., 1998; Hotta et al., 2001]. Iwabu et al. [2010] showed that muscle-specific disruption of AdipoR1 suppressed the adiponectin-mediated activation of AMPK, in which insulin resistance was involved. This indicated that levels of adiponectin and AdipoR1 may have crucial roles for insulin resistance. So far, no agreement in the assessment of AdipoR1 in diabetes was reached. Compared with patients with type 2 diabetes, mRNA of AdipoR1 was found to be lower in people with normal glucose tolerance [Blüher et al., 2006]. In addition, AdipoR1

was up-regulated in skeletal muscle in people with type 2 diabetes treated with rosiglitazone but was decreased following the treatment of pioglitazone [Tan et al., 2005; Coletta et al., 2009]. There were also reports demonstrating that no significant alteration of AdipoR1 between patients with diabetes and normal people [McAinch et al., 2006; Kuoppamaa et al., 2008]. Combined previous and our studies, STZ-induced hypoinsulinmia appears to affect the expression of AdipoR1 in a time dependent manner. It was reported that the mRNA level of AdipoR1 was significantly increased in diabetic mice 4 days after the STZ treatment [Eriksson et al., 1992]. In fasted mice, the reduction in blood insulin could increase AdipoR1 at mRNA level, and re-feeding of these mice could rapidly restore AdipoR1 expression to its original state [Eriksson et al., 1992]. These facts demonstrated the initial reduction in AdipoR1 during insulin deprivation. However, AdipoR1 expression was reported to be positively correlated with insulin secretion in human. Also, we observed the decreased expression of AdipoR1 in mice 8 weeks following the STZ treatment. These facts provided the fundamental evidence for our speculation that expression of AdipoR1 was up-regulated at the early stage of insulin deficiency but was down-regulated during the long-term insulin insufficiency. However, the underlying mechanism of this possible time-related effect was unknown and our speculation needs to be further tested. We also demonstrated that κ-OR stimulation in STZ-induced diabetic mice increased both mRNA and protein levels of AdipoR1. Increased AdipoR1 expression was beneficial in the binding of adiponectin, and this might improve the insulin sensitivity in diabetic mice which partly contributed to reduction in hyperglycemia.

AMPK is a key regulator in energy balance and its activation could lower plasma glucose by increasing glucose uptake and repressing the expression of gluconeogenesis-associated enzymes in the liver [Hardie, 2008]. U50,488H increased the phosphorylation of AMPK in diabetic mice, and this might be also involved in the reduction of glucose. Glut4 plays a crucial role in glucose metabolism. It is sequestered in the interior of muscle and fat cells within lipid bilayers of vesicles and transported to plasma membrane when stimulated [Grillo et al., 2009]. As what was demonstrated in an earlier report [Eriksson et al., 1992], we also found that impaired glucose uptake in skeletal muscle was associated with decreased Glut4 translocation rather than the change in its total quantity. In our present study, the altered membranous Glut4 levels were consistent with the time dependent change of glucose concentrations following the administration of U50,488H, suggesting that the reduction of hyperglycemia was associated with the translocation of Glut4 in skeletal muscle. Further studies should be performed to elaborate whether AMPK activation and Glut4 translocation were two separate or sequential processes during κ-OR stimulation in STZ-induced diabetic mice.

Another possible mechanism of the anti-hyperglycemic effect of U50,488H is related to hormone secretion. Radionuclides labeled opioids for μ-, δ-, and κ-opioid receptors were used to examine the opioid binding sites in pancreas and liver. δ- and κ-opiate sites of high affinity were identified in crude membrane preparations of islets of Langerhans, but no specific opiate-binding sites could be demonstrated in liver membrane preparations [Khawaja et al., 1990b]. In α-TCL-6 cells, κ-OR was also detected on the membrane [Jacobson et al., 2006]. However, there is no evidence for the existence of κ-OR on pancreatic β cells. Moreover, in a previous study, both dynorphin and U50,488H were showed to raise plasma insulin in ob/ob mice [Khawaja et al., 1990a]. Due to the previous reports mentioned above, it was necessary to examine the serum insulin level to check whether the blood glucose lowering effect of U50,488H was associated with insulin secretion. We found that insulin was not affected during κ-OR stimulation and this result excluded the possibility that the improved adiponectin, AdipoR1, AMPK, and translocation of Glut4 was the result of increased insulin secretion. Meanwhile, we have to notice that both AdipoR1 and AdipoR2 are abundantly expressed on pancreatic β cells in human and rats, but their function remained unknown [Kharroubi et al., 2003]. Our data revealed that U50,488H significantly increased the expression of AdipoR1 in skeletal muscle, but we did not check the altered expression of AdipoR1 in islets. It would be of great interest to further study whether κ-OR stimulation also acts via AdipoR1 in islets.

In conclusion, our study reported here demonstrates that κ-OR activation time dependently reduces hyperglycemia in STZ-induced diabetic mice. During κ-OR activation, glucose uptake in skeletal muscle is improved resulting from the translocation of Glut4. This effect might be relevant to the increased adiponectin, AdipoR1 and phosphorylation of AMPK. Further studies should be carried out to deeply explain the mechanisms underlying this phenomenon and it would be interesting to study the function of κ-OR and AdipoR1/R2 in the pancreas islet.

REFERENCES

Amirshahrokhi K, Dehpour AR, Hadjati J, Sotoudeh M, Ghazi-Khansari M. 2008. Methadone ameliorates multiple-low-dose streptozotocin-induced type 1 diabetes in mice. Toxicol Appl Pharmacol 232:119–124.

Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y. 1999. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun 257:79–83.

Bailey CJ, Flatt PR. 1987. Increased responsiveness to glucoregulatory effect of opiates in obese-diabetic ob/ob mice. Diabetologia 30:33–37.

Bjursell M, Ahnmark A, Bohlooly YM, William OL, Rhedin M, Peng XR, Ploj K, Gerdin AK, Arnerup G, Elmgren A, et al. 2007. Opposing effects of adiponectin receptors 1 and 2 on energy metabolism. Diabetes 56:583–593.

Blüher M, Bullen JW, Jr., Lee JH, Kralisch S, Fasshauer M, Klöting N, Niebauer J, Schön MR, Williams CJ, Mantzoros CS. 2006. Circulating adiponectin and expression of adiponectin receptors in human skeletal muscle: Associations with metabolic parameters and insulin resistance and regulation by physical training. J Clin Endocrinol Metab 91:2310–2316.

Bruce CR, Mertz VA, Heigenhauser GJ, Dyck DJ. 2005. The stimulatory effect of globular adiponectin on insulin-stimulated glucose uptake and fatty acid oxidation is impaired in skeletal muscle from obese subjects. Diabetes 54:3154–3160.

Coletta DK, Sriwijitkamol A, Wajcberg E, Tantiwong P, Li M, Prentki M, Madiraju M, Jenkinson CP, Cersosimo E, Musi N, DeFronzo RA. 2009. Pioglitazone stimulates AMP-activated protein kinase signalling and increases the expression of genes involved in adiponectin signalling, mitochondrial function and fat oxidation in human skeletal muscle in vivo: A randomised trial. Diabetologia 52:723–732.

Eriksson J, Koranyi L, Bourey R, Schalin-Jäntti C, Widén E, Mueckler M, Permutt AM, Groop LC. 1992. Insulin resistance in type 2 (non-insulin-

dependent) diabetic patients and their relatives is not associated with a defect in the expression of the insulin-responsive glucose transporter (GLUT-4) gene in human skeletal muscle. Diabetologia 35:143–147.

Fruebis J, Tsao TS, Javorschi S, Ebbets RD, Erickson MR, Yen FT, Bihain. BE, Lodish HF. 2001. Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. Proc Natl Acad Sci U S A 98:2005–2010.

Green IC, Perrin D, Penman E, Yaseen A, Ray K, Howell SL. 1983. Effect of dynorphin on insulin and somatostatin secretion, calcium uptake, and c-AMP levels in isolated rat islets of Langerhans. Diabetes 32:685–690.

Grillo C, Piroli A, Hendry GG, Reagan RM. 2009. Insulin-stimulated translocation of GLUT4 to the plasma membrane in rat hippocampus is PI3-kinase dependent. Brain Res 1296:35–45.

Hardie DG. 2008. AMPK: A key regulator of energy balance in the single cell and the whole organism. Int J Obes (Lond) 4:7–12.

Hotta K, Funahashi T, Bodkin NL, Ortmeyer HK, Arita Y, Hansen BC, Matsuzawa Y. 2001. Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. Diabetes 50:1126–1133.

Hunter JD, Ravert PK, Lassetter JH, Williams M. 2011. Renal protection in diabetes. Adv NPs PAs 2:25–29.

Ishizuka J, Toyota T, Ono T, Sasaki M, Yanaihara C, Yanaihara N. 1986. Inhibitory effects of rimorphin and dynorphin on insulin secretion from the isolated, perfused rat pancreas. Tohoku J Exp Med 150:17–24.

Iwabu M, Yamauchi T, Okada-Iwabu M, Sato K, Nakagawa T, Funata M, Yamaguchi M, Namiki S, Nakayama R, Tabata M, et al. 2010. Adiponectin and AdipoR1 regulate PGC-1alpha and mitochondria by Ca2+ and AMPK/SIRT1. Nature 464:1313–1319.

Jacobson DA, Cho J, Landa LRJ, Tamarina NA, Roe MW, Buxbaum JD, Philipson LH. 2006. Downstream regulatory element antagonistic modulator regulates islet prodynorphin expression. Am J Physiol Endocrinol Metab 291:587–595.

Josefsen K, Buschard K, Sùrensen LR, Wùllike M, Ekman R, Birkenbach M. 1998. Glucose stimulation of pancreatic beta-cell lines induces expression and secretion of dynorphin. Endocrinology 139:4329–4336.

Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. 2006. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest 116:1784–1792.

Kharroubi I, Rasschaert J, Eizirik DL, Cnop M. 2003. Expression of adiponectin receptors in pancreatic beta cells. Biochem Biophys Res Commun 312:1118–1122.

Khawaja XZ, Green IC, Thorpe JR, Bailey CJ. 1990a. Increased sensitivity to insulin-releasing and glucoregulatory effects of dynorphin A1–13 and U 50488 h in ob/ob versus lean mice. Diabetes 39:1–13.

Khawaja XZ, Green IC, Thorpe JR, Titheradge MA. 1990b. The occurrence and receptor specificity of endogenous opioid peptides within the pancreas and liver of the rat. Comparison with brain. Biochem J 267:233–240.

Kuoppamaa H, Skrobuk P, Sihvo M, Hiukka A, Chibalin AV, Zierath JR, Koistinen HA. 2008. Globular adiponectin stimulates glucose transport in type 2 diabetic muscle. Diabetes Metab Res Rev 24:554–562.

Lin HV, Kim JY, Pocai A, Rossetti L, Shapiro L, Scherer PE, Accili D. 2007. Adiponectin resistance exacerbates insulin resistance in insulin receptor transgenic/knockout mice. Diabetes 56:1969–1976.

McAinch AJ, Steinberg GR, Mollica J, O'Brien PE, Dixon JB, Macaulay SL, Kemp BE, Cameron-Smith D. 2006. Differential regulation of adiponectin receptor gene expression by adiponectin and leptin in myotubes derived from obese and diabetic individuals. Obesity (Silver Spring) 14:1898–1904.

Minami M, Satoh M. 1995. Molecular biology of the opioid receptors: Structures, functions and distributions. Neurosci Res 23:121–145.

Niu W, Bilan PJ, Ishikura S, Schertzer JD, Contreras-Ferrat A, Fu Z, Liu J, Boguslavsky S, Foley. KP, Liu Z, Li J, Chu G, Panakkezhum T, Lopaschuk GD, Lavandero S, Yao Z, Klip A. 2010. Contraction-related stimuli regulate GLUT4 traffic in C2C12-GLUT4myc skeletal muscle cells. Am J Physiol Endocrinol Metab 298:1058–1071.

Nolan JH, Elmendorf JS. 2011. Signaling, cytoskeletal and membrane mechanisms regulating GLUT4 exocytosis. Trends Endocrinol Metab 22:110–116.

Satoh M, Minami M. 1995. Molecular pharmacology of the opioid receptors. Pharmacol Ther 68:343–364.

Tabak O, Gelisgen R, Erman H, Erdenen F, Muderrisoglu C, Aral H, Uzun H. 2011. Oxidative lipid, protein, and DNA damage as oxidative stress markers in vascular complications of diabetes mellitus. Clin Invest Med 34:163–171.

Takashi K, Toshimasa Y. 2005. Adiponectin and Adiponectin Receptors. Endocr Rev 26:439–451.

Tan GD, Debard C, Funahashi T, Humphreys SM, Matsuzawa Y, Frayn KN, Karpe F, Vidal H. 2005. Changes in adiponectin receptor expression in muscle and adipose tissue of type 2 diabetic patients during rosiglitazone therapy. Diabetologia 48:1585–1589.

Tsuchida A, Yamauchi T, Ito Y, Hada Y, Maki T, Takekawa S, Kamon J, Kobayashi M, Suzuki R, Hara K, Hara K, Kubota N, Terauchi Y, Froguel P, Nakae J, Kasuga. M, Accili D, Tobe K, Ueki K, Nagai R, Kadowaki T. 2004. Insulin/Foxo1 pathway regulates expression levels of adiponectin receptors and adiponectin sensitivity. J Biol Chem 279:30817–30822.

Tzeng TF, Liu IM, Lai TY, Tsai CC, Chang WC, Cheng JT. 2003. Loperamide increases glucose ultilization in streptozotocin-induced diabetic rats. Clin Exp Pharmacol Physiol 30:734–738.

Yamauchi T, Kamon. J, Ito Y, Tsuchida A, Yokomizo T, Kita S, Sugiyama T, Miyagishi M, Hara K, Tsunoda M, Murakami K, Ohteki T, Uchida S, Takekawa S, Waki H, Tsuno NH, Shibata Y, Terauchi Y, Frogue P, Tobe K, Koyasu S, Taira K, Kitamura T, Shimizu T, Nagai R, Kadowaki T. 2003. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature 423:762–769.

Yang PS, Lee JJ, Tsao CW, Wu HT, Cheng JT. 2009. Stimulatory effect of stevioside on peripheral mu opioid receptors in animals. Neurosci Lett 454:72–75.

Yatagai T, Nagasaka S, Taniguchi A, Fukushima M, Nakamura T, Kuroe A, Nakai Y, Ishibashi S. 2003. Hypoadiponectinemia is associated with visceral fat accumulation and insulin resistance in Japanese men with type 2 diabetes mellitus. Metabolism 52:1274–1278.

Zhang QY, Wang W, Shi QX, Li YL, Huang JH, Yao Y, Li J, Zhang SM, Fan R, Zhou JJ, Guo HT, Wang YM, Yin W, Pei JM. 2010. Antiarrhythmic effect mediated by κ-opioid receptor is associated with Cx43 stabilization. Crit Care Med 38:2365–2376.

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