Korean J Physiol Pharmacol
Vol 17: 493−497, December, 2013
http://dx.doi.org/10.4196/kjpp.2013.17.6.493

Repaglinide, but Not Nateglinide Administered Supraspinally and Spinally Exerts an Anti-Diabetic Action in D-Glucose Fed and Streptozotocin-Treated Mouse Models

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We have recently demonstrated that some anti-diabetic drugs such as biguanide and thizolidinediones administered centrally modulate the blood glucose level, suggesting that orally administered anti-diabetic drugs may modulate the blood glucose level by acting on central nervous system. The present study was designed to explore the possible action of another class of anti-diabetic drugs, glinidies, administered centrally on the blood glucose level in ICR mice. Mice were administered intracerebroventricularly (i.c.v.) or intrathecally (i.t.) with 5 to 30 μg of repaglinide or nateglinide in D-glucose-fed and streptozotocin (STZ)-treated models. We found that i.c.v. or i.t. injection with repaglinide dose-dependently attenuated the blood glucose level in D-glucose-fed model, whereas i.c.v. or i.t. injection with nateglinide showed no modulatory action on the blood glucose level in D-glucose-fed model. Furthermore, the effect of repaglinide administered i.c.v. or i.t. on the blood glucose level in STZ-treated model was studied. We found that repaglinide administered i.c.v. slightly enhanced the blood glucose level in STZ-treated model. On the other hand, i.t. injection with repaglinide attenuated the blood glucose level in STZ-treated model. The plasma insulin level was enhanced by repaglinide in D-glucose-fed model, but repaglinide did not affect the plasma insulin level in STZ-treated model. In addition, nateglinide did not alter the plasma insulin level in both D-glucose-fed and STZ-treated models. These results suggest that the anti-diabetic action of repaglinide appears to be, at least, mediated via the brain and the spinal cord as revealed in both D-glucose fed and STZ-treated models.

Key Words: Blood glucose, Glinides, Spinal, Streptozotocin, Supraspinal

INTRODUCTION

Diabetes mellitus is one of the chronic diseases and is rapidly growing in prevalence worldwide. The complications of diabetes mellitus contribute to a major health problem in modern societies. Type 2 diabetes mellitus is characterized by highly elevated concentrations of glucose in the blood, which is caused by decreased secretion of insulin from the β-cells of pancreas and diminished action of insulin. Glinides are widely used oral drugs for the treatment of type II diabetes mellitus. Glinides exert their anti-diabetic pharmacological effects in type 2 diabetes mellitus by enhancing insulin release from the β-cells of pancreas, thereby increasing circulating insulin to act [1-5]. Furthermore, several studies have reported that repaglinide bind to the "B" site in sulfonylurea receptor 1 (SUR1), and nateglinide bind to the "A" site bind to SUR1 subunit, in turn, inhibiting the β-cell K^+/ATP channel. This action leads to the attenuated K^+ conductance, finally resulting in an increase of membrane depolarization of the β-cells [6-8]. In addition, the depolarization of plasma membrane opens voltage-gated Ca^{2+} channels, and finally, causes the release of insulin from pancreatic β-cells [9,10].

Accumulating data have shown that the central nervous system plays an important role for the regulation of the blood glucose level. The numerous studies have demonstrated that the several regions of the brain also play crucial roles to keep the homeostatic status of the blood glucose level [11]. Supraspinal injection of certain drugs such as atropine methyl bromide (methylatropine) produces modulatory action in the regulation of the blood glucose and insulin levels [12]. In addition, supraspinal administration of IL-1 alpha produces a hyperglycemia, hyperglucagonemia

Received March 28, 2013, Revised September 26, 2013, Accepted October 17, 2013

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ABBREVIATIONS: i.c.v., intracerebroventricular; i.t., intrathecal; STZ., streptozotocin.

and hyperinsulinemia that resulted from an early increase in hepatic glucose production [13]. Several lines of evidence have demonstrated that the spinal cord is one of the important sites for the regulation of the blood glucose level. In an earlier clinical study, Sala et al. [14] have reported that less dose of insulin is used to regulate the blood glucose level in patients with spinal cord injury. Furthermore, several studies have reported that spinal administration of the saporin (anti-D beta H-SAP) such as ribosome inactivating protein modulate of the blood glucose level [15]. Furthermore, we have recently reported that substance P or several pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ) and interleukin-1beta (IL-1β) administered spinally cause an elevation of the blood glucose level [16,17]. Taken together, both the brain and the spinal cord are important sites for the regulation of the blood glucose homeostasis.

We have recently reported that i.c.v. injection with metformin or rosiglitazone modulates the blood glucose level in D-glucose-fed and STZ-treated models, suggesting that the anti-diabetic actions of metformin and rosiglitazone appear to be centrally mediated [18]. However, central action of another class of anti-diabetic drugs, glinides, in the regulation of the blood glucose level has not been well studied yet. Thus, in the present study, we assessed the effects of glinides administered spinally or supraspinally on the regulation of the blood glucose level in D-glucose-fed and streptozotocin (STZ)-treated models.

METHODS

These experiments were approved by the Hallym University Animal Care and Use Committee (Registration Number: Hallym 2009-05-01). All procedures were conducted in accordance with the 'Guide for Care and Use of Laboratory Animals' published by the National Institutes of Health.

Experimental animals

Male ICR mice (MJ Co., Seoul, Korea) weighing 20~25 g were used for all the experiments. Animals were housed 5 per cage in a room maintained at 22±0.5°C with an alternating 12 h light-dark cycle. Food and water were available *ad libitum*. The animals were allowed to adapt to the laboratory for at least 2 h before testing and were only used once. Experiments were performed during the light phase of the cycle (10:00~17:00).

Oral, intracerebroventricular (i.c.v.), and intrathecal (i.t.) administrations

Oral administration was performed with gavage in a volume of 1 ml/kg body weight. Mice were fasted overnight (16 h) and D-glucose (2 g/kg body weight) administered orally once. The blood glucose level was measured at 0, 30, 60, and 120 min after D-glucose administration.

I.t. administration was performed in conscious mice following the method of Hylden and Wilcox using a 30-gauge stainless-steel needle attached to a 25 μl Hamilton microsyringe was inserted between the lumbar 5 and lumbar 6 segments in unanesthetized mice [19]. The i.t. injection volume was 5 μl and the injection site was verified by injecting a similar volume of 1% methylene blue solution and determining the distribution of the injected dye in the spinal cord. The dye injected i.t. was distributed both rostrally and caudally but with short distance (about 0.5 cm) and no dye was found in the brain. The success rate for the injections was consistently found to be over 95%, before the experiments were done.

I.c.v. administration followed the method described by Haley [20]. Each mouse was grasped firmly without anesthesia by the loose skin behind the head. The skin was pulled taut. A 30-guage needle attached to a 25 μl syringe was inserted perpendicularly through the skull into the brain and solution was injected. The injection site was 2 mm from either side of the midline on a line drawn through the anterior base of the ears. The i.c.v. injection volumes were 5 μl, and the injection sites were verified by injecting a similar volume of 1% methylene blue solution and determining the distribution of the injected dye in the ventricular space. The success rate for prior injections with this technique was over 95%.

STZ-induced diabetic mice

Diabetic mice were induced by a single intraperitoneal injection of STZ (150 mg/kg in citrate buffer, pH 4.5). Normal groups received the buffer only. On the 6th day after STZ administration, animals with non-fasting blood glucose concentration above 400 mg/dl were considered to be diabetic and used in current study.

Measurement of blood glucose level

Blood glucose measurements were obtained using blood samples collected by lateral tail vein laceration. The blood was collected shortly as soon as possible with a minimum volume (1 μl). The glucose level was measured using Accu-Chek Performa blood glucose monitoring system (glucometer) (Mannheim, Baden-Württemberg, Germany).

Insulin ELISA assay

In Mouse Insulin ELISA, biotin conjugated anti insulin, and standard or sample were incubated in monoclonal anti-insulin-coated wells. After horse radish peroxidase (HRP) conjugated streptavidin remaining in the wells were reacted with a substrate chromogen reagent, and the reaction was stopped by addition of an acidic solution, and absorbance was measured spectrophotometrically at 450 nm.

Drugs

Repaglinide, nateglinide and D-glucose were purchased from Sigma Chemical Co. (St. Louis, MO, USA). STZ was purchased from USB Co. (Cleveland, OH, USA). Repaglinide and Nateglinide were prepared following steps: (A) 1 g of repaglinide and nateglinide were dissolved in 0.5 ml of ethanol plus 0.5 ml of polyethylene glycol 400. (B) Separately, 100 mg of sodium carboxymethylcellulose was dissolved in 9 ml of distilled water. (C) Finally, Solution (A) and Solution (B) were vigorously mixed. This solution (PEC) excluding repaglinide and nateglinide were used as vehicle control. All drugs were prepared just before use. Blood glucose meter, lancing device and strips were purchased from Roche Diagnostics (Accu-Chek Performa, Germany). The mouse insulin ELISA kit was purchased from Shibayagi Co. (Shibukawa, Japan).

Statistical analysis

The statistical significance of differences between groups was assessed with one-way ANOVA with Bonferroni's post-hoc test using GraphPad Prism Version 4.0 for Windows XP (GraphPad Software, San Diego, CA, USA). p-values less than 0.05 were considered to indicate statistical significance. All values were expressed as the mean±S.E.M. In our study, we established the mean blood glucose value of the control group through many experiments under matching conditions. Selected mice of established blood glucose level were then used in replication experiments.

RESULTS

Effects of repaglinide and nateglinide administered i.c.v. or i.t. on the blood glucose level in D-glucose-fed model

Mice were pretreated i.c.v. or i.t. with 5, 20 or 30 μg of repaglinide or nateglinide for 10 min. Then 2 g/kg of D-glucose were orally fed. The blood glucose level was measured at 30, 60 and 120 min after D-glucose feeding. As shown in Fig. 1A (F=0.7531; p=0.4984), 1B (F=1.141; p=0.3616), 2A (F=0.02923; p=0.9713) and 2B (F=0.06676; p=0.9359), repaglinide administered i.c.v. or i.t. dose-dependently attenuated the blood glucose level in D-glucose-fed model. However, nateglinide administered i.c.v. or i.t. did not affect the blood glucose level in D-glucose-fed model.

Effects of repaglinide administered i.c.v. or i.t. on the blood glucose level in STZ-treated model

Since repaglinide administered was effective in attenuating the blood glucose level in D-glucose-fed model, we assessed if repaglinide produces also anti-diabetic effect in STZ-treated model. Mice were pretreated intraperitoneally with STZ (150 mg/kg) for 6 days. And then, 5, 20 or 30 μg/5 μl of repaglinide were administered i.c.v. or i.t. As shown in Fig. 3, repaglinide administered i.c.v. (F=0.8940; p=0.4424) slightly enhanced the blood glucose level in STZ-treated model. On the other hand, repaglinide administered i.t. (F=3.988; p=0.0575) dose-dependently attenuated the blood glucose level in STZ-treated model.

Effects of repaglinide and nateglinide administered i.c.v. or i.t. on the plasma insulin level in D-glucose-fed and STZ-treated models

Mice were pretreated i.c.v. or i.t. with 30 μg of repaglinide or nateglinide for 10 min. Then 2 g/kg of D-glucose were orally fed. The plasma insulin level was measured at 30 min after D-glucose feeding. As shown in Fig. 4A (F=9.579; p=0.0016) and 4B (F=1.557; p=0.2393), repaglinide administered i.c.v. or i.t. markedly enhanced the

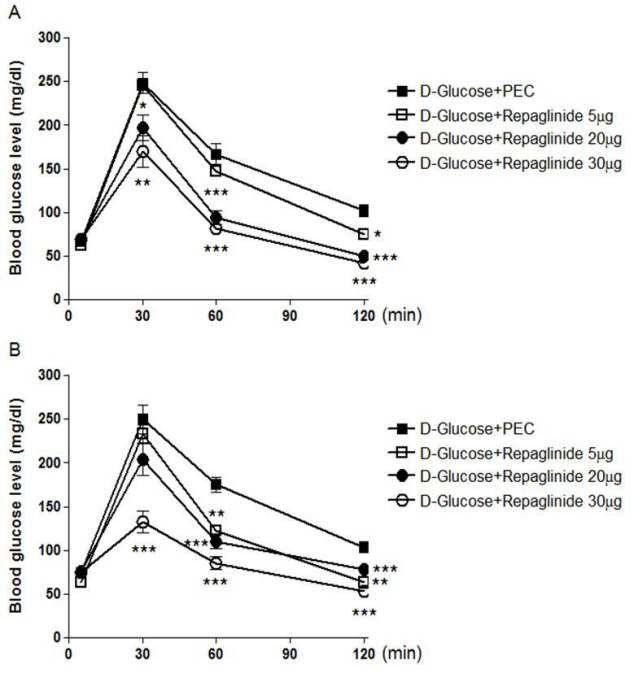

Fig. 1. Effects of repaglinide administered i.c.v. or i.t. on the blood glucose level in D-glucose-fed model. Mice were pretreated i.c.v. (A) and i.t. (B) with 5, 20 or 30 μg of repaglinide for 10 min. Then 2 g/kg of D-glucose were orally fed. The blood glucose level was measured at 30, 60 and 120 min after D-glucose administration. The blood was collected from tail-vein. The vertical bars indicate the standard error of mean. Each quantified result was analyzed by one-way ANOVA with a Bonferroni post hoc test (**$p<0.01$, ***$p<0.005$; compared to D-Glucose+PEC group). The number of animal used in each group was 8~10.

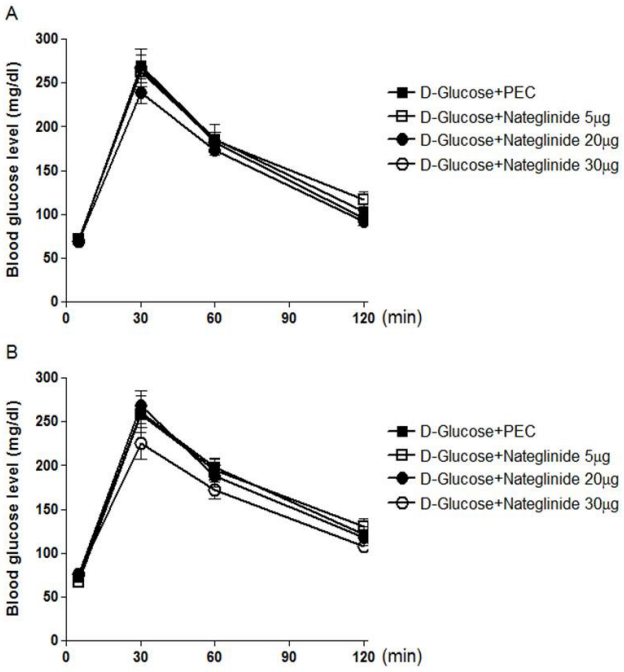

Fig. 2. Effects of nateglinide administered i.c.v. or i.t. on the blood glucose level in D-glucose- fed model. Mice were pretreated i.c.v. (A) and i.t. (B) with 5, 20 or 30 μg of repaglinide or nateglinide for 10 min. Then 2 g/kg of D-glucose were orally fed. The blood glucose level was measured at 30, 60 and 120 min after D-glucose administration. The blood was collected from tail-vein. Each quantified result was analyzed by one-way ANOVA with a Bonferroni post hoc test. The number of animal used in each group was 8~10.

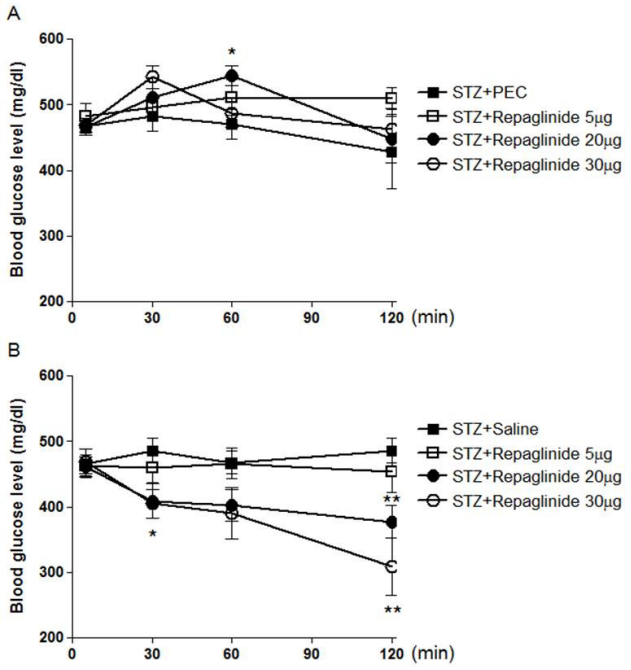

Fig. 3. Effects of repaglinide administered i.c.v. or i.t. on the blood glucose level in STZ-treated model. Mice were pretreated intraperitoneally with STZ (150 mg/kg) for 6 days. And then, 5, 20 or 30 μg/5 μl of repaglinide was administered treated i.c.v. (A) or i.t. (B) The blood glucose level was measured at 30, 60 and 120 min after repaglinide injection. The blood was collected from tail-vein. The vertical bars indicate the standard error of mean. Each quantified result was analyzed by one-way ANOVA with a Bonferroni post hoc test (*$p<0.05$, **$p<0.01$; compared to STZ+PEC group). The number of animal used in each group was 8~10.

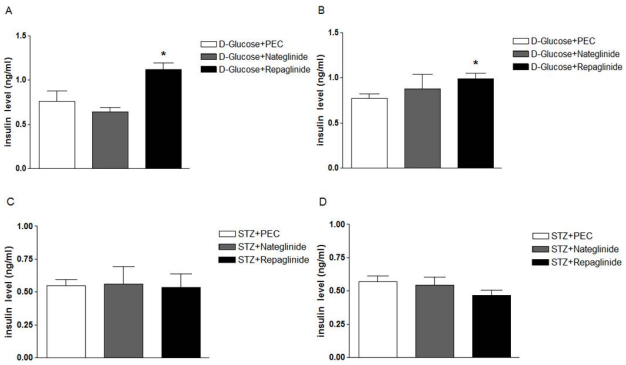

Fig. 4. Effects of repaglinide and nateglinide administered i.c.v. or i.t. on the plasma insulin level in D-glucose-fed and STZ-treated models. Mice were pretreated i.c.v. (A) and i.t. (B) with 30 μg of repaglinide and nateglinide for 10 min. Then 2 g/kg of D-glucose were orally fed. The plasma insulin level was measured at 30 min after D-glucose administration. In another experiment, mice were pretreated intraperitoneally with STZ (150 mg/kg) for 6 days. And then, 30 μg/5 μl of repaglinide and nateglinide was administered treated i.c.v. (C) or i.t. (D) The plasma insulin level was measured at 30 min after repaglinide and nateglinide injection. The vertical bars indicate the standard error of mean. Each quantified result was analyzed by one-way ANOVA with a Bonferroni post hoc test. The number of animal used in each group was 6~8.

plasma insulin level in D-glucose-fed model. However, nateglinide administered i.c.v. or i.t. did not affect the plasma insulin level in D-glucose-fed model. Next, mice were pretreated intraperitoneally with STZ (150 mg/kg) for 6 days. And then, 30 μg/5 μl of repaglinide and nateglinide was administered i.c.v. or i.t. As shown in Fig. 4C ($F=0.013$; $p=0.9871$) and 4D ($F=1.159$; $p=0.3403$), repaglinide and nateglinide administered i.c.v or i.t. did not alter the plasma insulin level in STZ-treated model.

DISCUSSION

In the present study, the possible anti-diabetic action of glinides administered supraspinally or spinally was studied in ICR mice. We found for the first time that repaglinide administered supraspinally or spinally attenuates the blood glucose level in D-glucose-fed model. However, nateglinide, another glinide, administered supraspinally or spinally did not alter the blood glucose level in D-glucose-fed model. These results clearly suggest that repaglinide exerts its hypoglycemic effects, at least by acting on both the brain and the spinal cord. In contrasted to the effect of repaglinide, we found in the present study that surpraspinal or spinal administration of nateglinide is not effective for regulating the blood glucose level in D-glucose-fed model. These findings indicate that all glinides do not act by the same mechanism in the regulation of the blood glucose level when they are administered supraspinally or spinally. The exact reasons of the differences between repaglinide and nateglinide in the regulation of blood glucose in D-glucose fed model are not currently clear. It has been well accepted that glinides produce their pharmacologic effects by acting on peripheral pancreatic β-cells. It has been well known that glinides stimulate insulin release from the β-cells of pancreas [1-5]. At the molecular level, it is well described that glinides inhibit the β-cell K^+/ATP channel, ultimately resulting in membrane depolarization of the β-cells [6]. Although the exact mechanism involved in down-regulation of the blood glucose level induced by repaglinide administered supraspinally or spinally is currently obscure, a previous study have demonstrated that hypothalamic K^+/ATP channel plays an important role for the regulation of the blood glucose level induced by repaglinide or glibenclamide [21]. Thus, it can be speculated that repaglinide administered supraspinally or spinally may lower the blood glucose level by acting on K^+/ATP channel located centrally. Additional studies to delineate the exact mechanism involved in centrally administered repaglinide-induced hypoglycemia in D-glucose-fed model are required in the future. However, we found in the present study that supraspinal or spinal administration with nateglinide, another glinide, did not affect the blood glucose level in D-glucose-fed model. Although the exact reasons for the differential action between repaglinide and nateglinide administered centrally are not currently known, we found a similar result in our previous study in that centrally administered rosiglitazone lowers the blood glucose level, whereas pioglitazone administered centrally did not affect the blood glucose level in D-glucose-fed model [18].

Since repaglinide administered supraspinally or spinally exerted the hypoglycemic effect in D-glucose-fed model, we assessed if repaglinide administered supraspinally or spinally can regulate the blood glucose in STZ-treated model. We found in the present study that repaglinide admini-

stered spinally significantly and dose-dependently attenuates the blood glucose level in STZ-treated model. This result is in line with a finding that spinally administered metformin also lowers the blood glucose level in STZ-treated model [18]. It has been well known that STZ destroys the β-cells of pancreas, resulting in the lack of insulin, in turn, leading to finally a type I diabetes mellitus [22,23]. Although repaglinide belongs to a class of anti-diabetic drugs to treat type II diabetes mellitus, the results of the present study suggest that the regulation of the blood glucose level induced by repaglinide administered supraspinally or spinally appear to be different in STZ-treated diabetic model, and repaglinide administered spinally can be effective to reduce the blood glucose in type I diabetes mellitus. However, we found in the present study that supraspinal administration of repaglinide did not cause any decrease of the blood glucose level in STZ-treated model. These findings suggest that repaglinide administered into the brain sites and the spinal cord may differentially regulate the blood glucose level in STZ-treated model.

In an attempt to characterize the mechanism involved in repaglinide-induced hypoglycemic effect, we measured the plasma insulin level in repaglinide-treated group. We found in the present study that supraspinal or spinal treatment with repaglinide increases the plasma insulin level in D-glucose-fed model. However, repaglinide administered supraspinally or spinally does not alter the plasma insulin level in STZ-treated model, suggesting that repaglinide-induced reduction of the blood glucose level in D-glucose-fed model appears to be due to an increment of the plasma insulin level, but lowering blood glucose level effect of repaglinide administered spinally in STZ-treated model does not appeared to be mediated by insulin system. In addition, we additionally found that nateglinide administered supraspinally or spinally does not affect the plasma insulin level in both D-glucose-fed and STZ-treated models, further suggesting that the blood glucose level is not modulated by nateglinide administered supraspinally or spinally.

In conclusion, although orally administered repaglinide is wide used as an anti-diabetic drug, our results suggest that the anti-diabetic action of repaglinide administered supraspinally or spinally exerts the anti-diabetic action as revealed in both D-glucose-fed and STZ-treated models. The detailed study on anti-diabetic mechanism of repaglinide administered centrally should be carried out further in the future.

ACKNOWLEDGEMENTS

This research was supported by Priority Research Centers (NRF-2009-0094071) and Basic Science Research (NRF-2011-0011156) Programs through the National Research Foundation of Korea (NRF) funded by the Ministry ofEducation, Science and Technology.

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