

Intracerebroventricular Kainic Acid-Induced Damage Affects Blood Glucose Level in d-glucose-fed Mouse Model

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We have previously reported that the intracerebroventricular (i.c.v.) administration of kainic acid (KA) results in significant neuronal damage on the hippocampal CA3 region. In this study, we examined possible changes in the blood glucose level after i.c.v. pretreatment with KA. The blood glucose level was elevated at 30 min, began to decrease at 60 min and returned to normal at 120 min after D-glucose-feeding. We found that the blood glucose level in the KA-pretreated group was higher than in the saline-pretreated group. The up-regulation of the blood glucose level in the KA-pretreated group was still present even after 1~4 weeks. The plasma corticosterone and insulin levels were slightly higher in the KA-treated group. Corticosterone levels decreased whereas insulin levels were elevated when mice were fed with D-glucose. The i.c.v. pretreatment with KA for 24 hr caused a significant reversal of D-glucose-induced down-regulation of corticosterone level. However, the insulin level was enhanced in the KA-pretreated group compared to the vehicle-treated group when mice were fed with D-glucose. These results suggest that KA-induced alterations of the blood glucose level are related to cell death in the CA3 region whereas the up-regulation of blood glucose level in the KA-pretreated group appears to be due to a reversal of D-glucose feeding-induced down-regulation of corticosterone level.

Key words: kainic acid, neuronal cell death, blood glucose, D-glucose-fed model

INTRODUCTION

Kainic acid (KA) is an excitotoxin in a variety of brain regions, especially in the hippocampus, where it has been repeatedly observed to induce the loss of neurons [1]. KA-induced seizures in rats result in delayed neuronal necrosis in the hippocampus and limbic cortex [2]. Delayed neuronal losses were also reported in the

rat hippocampal CA1 and CA3 regions following intraperitoneal (i.p.) [3] or intracerebroventricular (i.c.v.) administration of KA [4, 5]. KA, administered i.c.v. to mice, also induces significant lesion of CA3 pyramidal neurons [6-11]. It is generally agreed that KA-induced hyperexcitability and subsequent neuronal damage is converged on the hippocampal formation.

Several lines of evidence have demonstrated that seizure elevates cerebral metabolic rates [12] and glycolysis [13], which may lead to the accumulation of metabolic intermediates such as lactate and adenosine. In addition, Uysal et al. [14], reported that insulin reduces KA-induced seizure activity. Furthermore, Koenig and Cho [15] showed that hypothalamic KA receptor mRNA levels are elevated in insulin-induced hypoglycemic rats, suggesting that

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KA receptor expression may be dynamically regulated depending on the level of blood glucose. We have recently found that the acute supraspinal administration of KA produces a hyperglycemia effect. This finding suggests that the up-regulation of blood glucose level during the activation of central kainite receptors may be associated with hippocampal neuronal cell death, especially in the CA3 region. However, possible changes in the regulation of blood glucose level after damage to the hippocampal cells by KA have not been characterized yet. Thus, in the present study, we intended to investigate the blood glucose level changes induced by i.c.v. administered KA in a D-glucose-fed animal model. Twenty-four hours after the i.c.v. treatment with KA or saline, mice were fed with D-glucose and the blood glucose level was determined. Furthermore, changes in plasma corticosterone and insulin levels in saline- and KA-pretreated groups were evaluated.

MATERIALS AND 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 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 each mouse was only used once. Experiments were performed during the light phase of the cycle (10:00~17:00).

Intracerebroventricular (i.c.v.) injection

I.c.v. administration was performed according to Haley TJ's [16] method. Each mouse was grasped firmly without anesthesia by the loose skin behind the head. The skin was pulled taut. A 30-gauge needle attached to a 25 µl syringe was inserted perpendicularly through the bregma of the skull into the brain with the depth of 2.4 mm and solution was injected. The injection site was 2 mm from either side of the midline. 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 experiments were performed only when the success rate of i.c.v. injection was over 95%.

Measurement of blood glucose level

The blood glucose level was measured at 30, 60 and 120 min after the D-glucose administration. As much blood as possible was collected from the tail vein with a minimum volume of 1 µl. The glucose level was measured using Accu-Chek Performa blood glucose monitoring system (glucometer) (Mannheim, Baden-Württemberg, Germany).

Corticosterone assay and blood sampling

The plasma corticosterone level was determined using the fluorometric determination method [17]. Four hundred microliters of blood were collected by puncturing the retro-orbital venous plexus. Plasma was separated by centrifugation and stored at -80°C until assayed.

Insulin ELISA assay

In Mouse Insulin ELISA, biotin conjugated anti insulin, along with a standard or the sample, is incubated in monoclonal anti-insulin-coated wells to capture insulin bound with biotin conjugated anti insulin. After 2 h incubation and washing, HRP (horse radish peroxidase) conjugated streptavidin is added, followed by another 30 min of incubation. After washing, HRP conjugated streptavidin remaining in wells is reacted with a substrate chromogen reagent (TMB) for 20 min. The reaction is stopped by addition of an acidic solution and the absorbance of the yellow product is measured spectrophotometrically at 450 nm. The absorbance is proportional to the insulin concentration. The standard curve is prepared by plotting absorbance against standard insulin concentrations. Insulin concentrations in unknown samples are then determined using this standard curve.

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

Drugs

Kainic acid and D-glucose were purchased from Sigma chemical Co. (St. Louis, MO, USA). D-glucose was dissolved in sterile saline (0.9070 NaCl solution) and kainic acid was prepared in phosphate-buffered saline (PBS) as the vehicle.

Statistical analysis

Statistical analysis was carried out by the student t test using GraphPad Prism Version 4.0 for Windows (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 several experiments under matching conditions. Selected mice with the established blood glucose levels were then used in replication experiments.

RESULTS

Effects of i.c.v. pretreatment with KA on the blood glucose level in D-glucose-fed mice model

After i.c.v. pretreatment with KA (from 0.01 to 0.1 µg) for 24 h, mice were fed orally with D-glucose (2 g/kg). The blood glucose level was measured at 30, 60 and 120 min after D-glucose feeding. As shown in Fig. 1A, the blood glucose was elevated at 30min and returned to base line after 2 hours. This level was maintained up

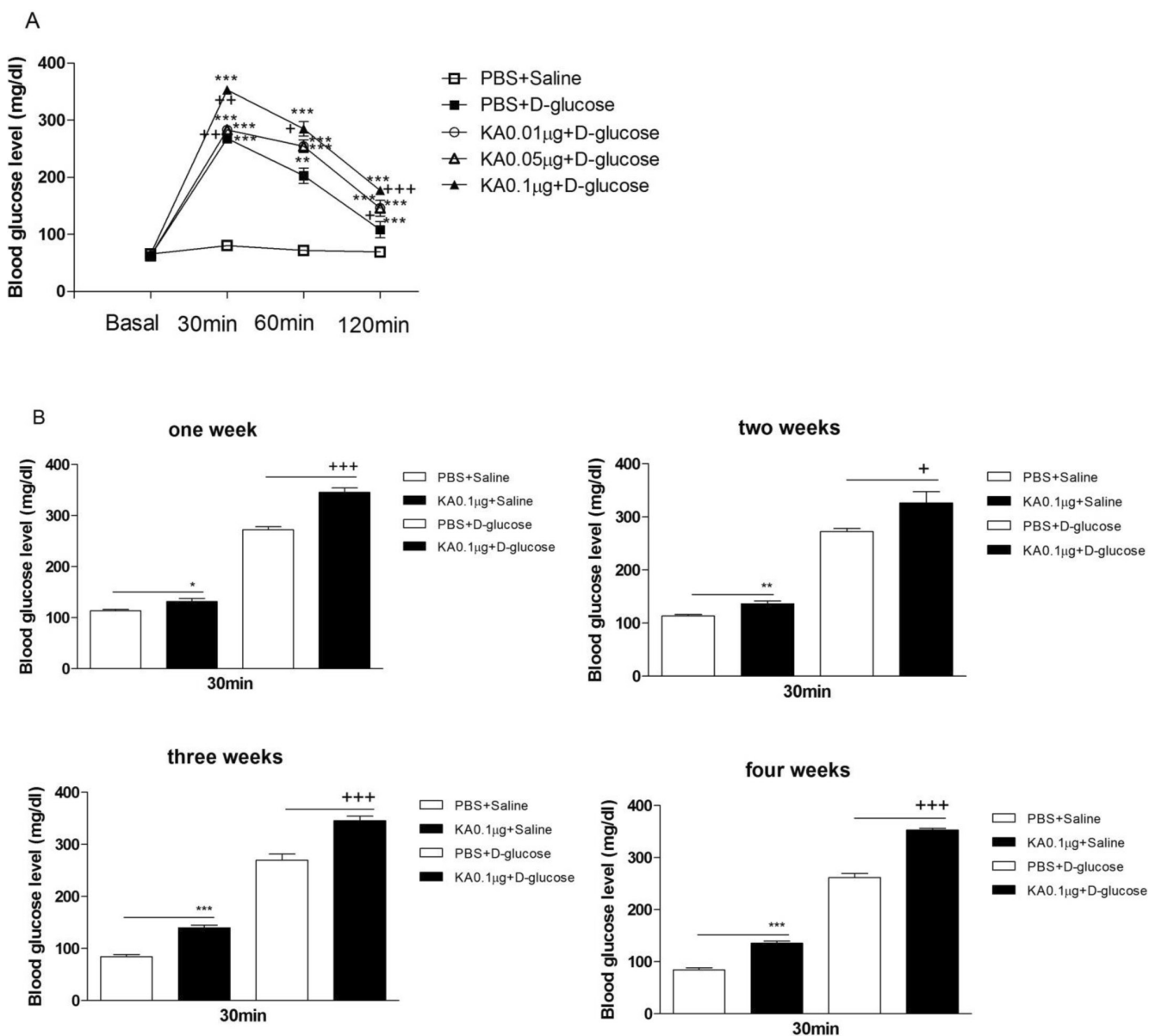


Fig. 1. Effect of i.c.v. pretreatment with KA on the blood glucose level in D-glucose-fed model. (A) After mice were pretreated i.c.v. with KA (from 0.01 to 0.1 µg) for 24 h, they were fed orally with D-glucose (2 g/kg). The blood glucose level was measured at 30, 60 and 120 min after D-glucose feeding. (B) After mice were pretreated i.c.v. with KA (0.1 µg) for 1, 2, 3, or 4 weeks, they were fed orally with D-glucose (2 g/kg). The blood glucose level was measured at 30 min after D-glucose feeding. The vertical bars indicate the standard error of mean (**p<0.01, ***p<0.001, *p<0.05; compared to PBS+Saline, +++p<0.001, **p<0.01, *p<0.05; compared to PBS+D-glucose). The number of animals used for each group was 8~10.

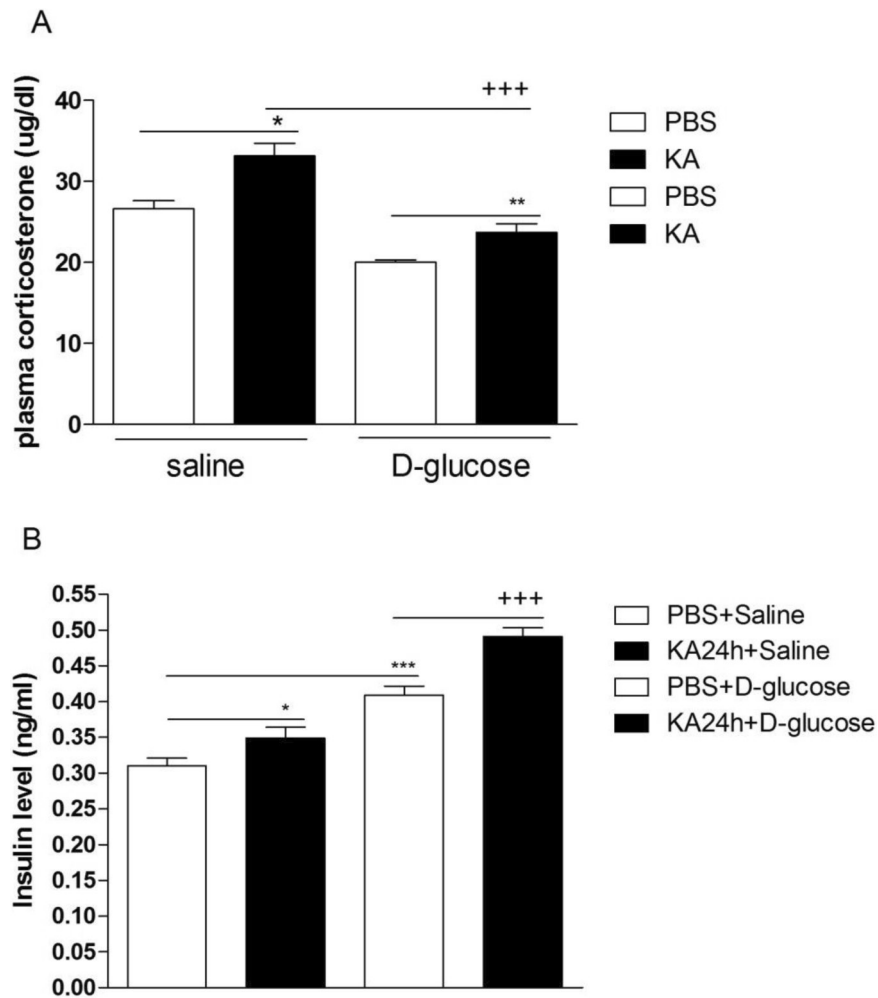


Fig. 2. Effect of i.c.v. pretreatment with KA on the plasma corticosterone (A) and insulin levels (B) in D-glucose-fed model. After mice were pretreated i.c.v. with KA (0.1 μ g) for 24 h, they were fed orally with D-glucose (2 g/kg). The plasma corticosterone and insulin levels were measured before and 30 min after D-glucose feeding. The vertical bars indicate the standard error of mean (** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$; compared to PBS+Saline, *** $p < 0.001$; compared to PBS+D-glucose). The number of animals used for each group was 8–10.

to 2 h after D-glucose feeding in the control group. KA pretreated i.c.v. for 24 h caused an up-regulation of the blood glucose level in a dose-dependent manner.

Effects of various periods of i.c.v. pretreatment with KA on the blood glucose level in D-glucose-fed model

After i.c.v. pretreatment with KA (0.1 μ g) for 1, 2, 3, or 4 weeks, mice were fed orally with D-glucose (2 g/kg). The blood glucose level was measured at 30 min after D-glucose feeding. As shown in Fig. 1B, the blood glucose level in the i.c.v. KA-pretreated group for weeks 1, 2, 3, and 4 was higher than that of the saline-pretreated group after the mice were orally fed with D-glucose.

Effects of i.c.v. pretreatment with KA on the plasma corticosterone and insulin levels in D-glucose-fed model

To determine whether the glucocorticoid and insulin systems are involved in the up-regulation of blood glucose level in the KA-pretreated group, the effect of i.c.v. pretreatment with KA (0.1 μ g) on plasma corticosterone level in the D-glucose-fed group was

investigated. The plasma corticosterone and insulin levels were measured before and 30 min after D-glucose feeding. As shown in Fig. 2, the plasma corticosterone and insulin levels were higher in the KA-treated group. When the mice were orally fed with D-glucose, the plasma corticosterone decreased slightly whereas the plasma insulin level was elevated. KA i.c.v. pretreatment for 24 hr caused a significant reversal of D-glucose-induced down-regulation of corticosterone level (Fig. 2A). However, the plasma insulin level was further enhanced in the KA-pretreated group compared to the vehicle-treated group when mice were fed with D-glucose as shown in Fig. 2B.

DISCUSSION

KA is a well-known excitatory, neurotoxic substance. In mice, morphological damage of the hippocampus induced by KA administered intracerebroventricularly (i.c.v) was markedly concentrated in the CA3 pyramidal neurons [10]. Several lines of evidence indicate that the activation of kainate receptors

located in the hippocampal formation plays an important role in synaptic physiology and plasticity [18, 19]. Previously, KA-induced alterations of nocifensive behaviors were correlated with the neuronal death of the hippocampal formation, especially CA3 pyramidal neurons [20]. KA is a well-known potent agonist of the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate class of glutamate receptors, which, when injected directly into the brain or systemically, induces a characteristic pattern of persistent seizure activity [21]. KA activates ionotropic glutamate receptors, and selectively induces cell death in postsynaptic neurons in the CA3 and CA1 hippocampal regions. This may be the possible mechanism through which KA causes brain damage and seizure [22-26].

It is well known that seizures result in altered glucose metabolism, the reduction of intracellular energy metabolites such as ATP, ADP and phosphocreatine as well as the accumulation of metabolic intermediates such as lactate and adenosine. In particular, it has been suggested that the duration and extent of glucose dysregulation may be a predictor of the pathological outcome of status. Although the direct effects of glycemic control on brain metabolism and the effects of managing systemic glucose concentrations in epilepsy have been characterized [27], the direct effect of KA on the blood glucose level is not yet well characterized. Previous studies have demonstrated that the hypoglycemic condition prevents neuronal cell death in stroke animal models whereas the hyperglycemia condition aggravates neuronal cell death in animal stroke models [28, 29]. Johansen and Diemer [30] demonstrated that blood glucose level may influence KA-induced neurotoxicity. They found that systemic injection of KA in hyperglycemic rats resulted in higher lethality as well as more severe hippocampal CA1 damage, whereas hypoglycemia protected against KA-induced hippocampal CA1 damage [30]. A recent study demonstrated that KA administration leads to hyperglycemia. This may cause CA3 damage. Administration of repaglinide, PTX (pertussis toxin), and sulfonylureas also had neuroprotective effects against KA induced CA3 damage [31-33].

In the present study, up-regulation of the blood glucose level in the D-glucose-fed group was more pronounced in the KA-pretreated group compared to the saline pretreated group. The up-regulation of blood glucose level in the D-glucose-fed mice model was maintained in the KA-pretreated group for up to 4 weeks. Taken together, the results from all of these studies, including the present study, suggest that neurodegeneration in the CA3 region of the hippocampus by KA is associated with up-regulation of the blood glucose level when mice are fed with D-glucose.

The present study revealed that i.c.v. pretreatment with KA for 24 hr elevates plasma corticosterone level and insulin level. This

finding suggests that i.c.v. KA administration continues to activate the HPA axis 24 hr after KA administration. Our finding is partially in line with a previous report that an acute intrahippocampal injection of KA elevated plasma corticosterone level in rats [34], indicating that the glucocorticoid system is strongly activated when animals are injected with KA. In addition, D-glucose feeding causes the down-regulation of corticosterone and the up-regulation of insulin levels. This finding suggests that the down-regulation of plasma corticosterone and the up-regulation of insulin can, on the whole, lower blood glucose level after D-glucose ingestion. Additionally, the corticosterone level in the KA-pretreated group was higher than in the saline-treated group when mice were fed with D-glucose. This up-regulation of corticosterone might be responsible for the up-regulation of blood glucose in the KA-pretreated group after D-glucose feeding. Furthermore, D-glucose-induced up-regulation of insulin was further enhanced in the KA-pretreated group, suggesting that up-regulation of blood glucose in the KA-pretreated group may result in further enhancement of insulin level in the KA-pretreated group to maintain glucose homeostasis.

Some preclinical studies have reported that hyperglycemia is often observed in focal ischemia animal models [35, 36]. In addition, several lines of evidence have demonstrated that, in some clinical studies, hyperglycemia or abnormal glucose regulation is often observed in stroke patient groups. For example, Jia et al [37] reported that diabetic-like symptoms were observed in about 45% of ischemic stroke patients examined using an oral glucose tolerance test. In addition, Els et al. [38] reported that 58% of stroke patients showed diabetes mellitus-like symptoms up to 12 weeks after ischemic attack. Although it is too early to make an assumption at the present time, it can be speculated that the HPA axis might be in an activated state in stroke survivors. The neuronal damage in some areas of the brain may activate the HPA axis in these individuals. Thus, it might be necessary in future studies to check blood glucose and glucocorticoid hormone levels in post-stroke patients to determine whether the hyperglycemic state present in some patients is correlated with the up-regulated glucocorticoid system.

In conclusion, the present study demonstrates that supraspinal pretreatment with KA for 24 h causes an up-regulation of the blood glucose level in a D-glucose-fed mice model. The up-regulation of blood glucose in the KA-pretreated group appears to be due to the activation of the glucocorticoid system.

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