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Various pain stimulations cause an increase of the blood glucose level

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The relationship between pain stimulation and the blood glucose level was studied in ICR mice. We examined the possible change of the blood glucose level after the pain stimulation induced by acetic acid injected intraperitoneally (i.p.),, formalin injected subcutaneously (s.c.) into the hind paw, or substance P (SP), glutamate, and pro-inflammatory cytokines (TNF-α and IFN-γ) injected intrathecally (i.t.). We found in the present study that acetic acid, formalin, SP, TNF-α, and IFN-γ increased the blood glucose level. The blood glucose level reached at maximal state 30 min and returned to normal level 2 h after the pain stimulation in a fasting group. Furthermore, acetic acid, formalin, SP, TNF-α, and IFN-γ caused the elevation of the blood glucose level in D-glucose-fed group only in an additive manner. However, i.t. injection of glutamate did not alter the blood glucose level in a fasting group. In contrast, i.t. injection of glutamate enhanced the blood glucose level in the D-glucose-fed group. Our results suggest that the blood glucose level appears to be differentially regulated by various pain stimulation induced by acetic acid, formalin, SP, glutamate, and pro-inflammatory cytokines.

Keywords: pain; blood glucose level; acetic acid; formalin; substance P; glutamate; pro-inflammatory cytokine

Introduction

The hyperglycemic effect induced by the stress has been known for many years. It has been characterized that stress influences brain activity and promotes long-term changes in various neural systems. Stress therefore elicits a cluster of neuronal disorders that is implicated in cognitive, endocrinal, and psychiatric problems (Mazzon and Cuzzocrea 2008). It is widely accepted that stress can elevate blood glucose level and worsen glycemic control in patients with diabetes. Physical stressors such as hypoxia, hypothermia, and sepsis have shown to produce hyperglycemia (Wing et al. 1985). Previous studies have demonstrated that the elevation of stress state usually activates the neural systems. Increased activity of the neural systems involves the increment or impairment in blood pressure, heart rate, body temperature, and plasma glucose (Uresin et al. 2004).

The experience of pain in response to noxious stimuli serves a crucial biological purpose: it alerts a living organism to environmental dangers, inducing behavioral responses that protect the organism from additional damage (Santos-Arteaga et al. 2003). Pain is a multi-dimensional process involving the physical, emotional, and perceptual integration of noxious information. The physical component is relayed via the spinal cord to several brain areas to initiate the detection of pain. The emotional aspect is encoded by the limbic system and encapsulates the relationship between pain and mood (Blackburn and Blackburn 2003). The recent studies have suggested that pain response induced by the activation of nociceptors belongs to stress-induced behavior (Seo et al. 2006). One of the responses to stress is the elevation of the blood glucose level (Yamada et al. 1993). Although pain stimulation may be related to stress-induced response, the regulation of the blood glucose level in acute pain model has not been well characterized. Thus, the present study was designed to examine the regulation of the blood glucose level in response to various types of pain stimulations.

Materials and methods

Animal experiments were approved by the Hallym University Institutional 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 and the ethical guidelines of the International Association for the Study of Pain.

Experimental animals

Male ICR mice (MJ Co., Seoul, Korea) weighing 23–25 g were used for all the experiments. Animals were housed five per cage in a room maintained at $22 \pm 0.5^{\circ}$C with an alternating 12-h light–dark cycle.

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Food and water were available ad libitum. The animals were allowed to adapt to the laboratory for at least 2 h before testing. Experiments were performed during the light phase of the cycle (10:00–17:00). The animals were fasted for 16 h.

Oral administration and intrathecal (i.t.) injection

Oral administration was performed with gavage in a volume of 1 ml/kg body weight. The i.t. administration was performed in conscious mice following the method of Hylden and Wilcox (1981) using a 30-gauge needle connected to a 25-μl Hamilton syringe. 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.

Acetic acid-induced pain

For the acetic acid-induced pain model (Koster and Beer 1959), a mouse was administered intraperitoneally (i.p.) with 0.5 ml of 1% acetic acid dissolved in saline.

Formalin-induced pain

Formalin-induced pain, previously published by Hunskaar et al. (1985), was examined in mice. Of 1.0% formalin solution in physiologic saline (0.9% NaCl), 10 μl was injected subcutaneously (s.c.) under the plantar surface of the left hind paw. The number of animals used for each group was 17–20.

Glutamate, substance P, and pro-inflammatory cytokines-induced pain

Mice were injected i.t. with glutamate (20 μg), substance P (SP) (0.7 μg), TNF-α (100 pg/5 μl), or IFN-γ (100 pg/5 μl). The dose of glutamate, SP, and cytokines, which equally affected pain response, was determined by our previous studies (Choi et al. 2003; Kwon et al. 2005a, 2005b; Park et al. 2010).

Measurement of the blood glucose level

Blood glucose level was measured at 30, 60, and 120 min after various pain stimulations ($n = 8$–10). Blood was collected shortly as much as possible with a minimum volume (1 μl) from the tail vein. Glucose level was measured using Accu-Chek Performa blood glucose monitoring system (glucometer) (Mannheim, Baden-Württemberg, Germany).

Drugs

D-glucose, acetic acid, formalin, and glutamate were purchased from Sigma Chemical Co. (St Louis, MO, USA). SP was purchased from Tocris Cookson Ltd (Bristol, UK). TNF-α and IFN-γ were purchased from R and D Systems Inc. (Minneapolis, MN, USA). All drugs were prepared just before use.

Statistical analysis

Statistical analysis was carried out by student *t*-test using GraphPad Prism Version 4.0 for Windows (GraphPad Software, San Diego, CA, USA). *P*-values < 0.05 were considered to indicate statistical significance. All values were expressed as the mean $\pm$ SEM. 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

It was examined whether various types of pain stimulations in a fasting group evoked alteration of the blood glucose level. SP and glutamate are important neurotransmitters for the transmission of the pain in the spinal cord. In addition, pro-inflammatory cytokines are believed to be involved in the production of neuropathic pain pathology (Linda and Steven 2002). As shown in Figures 1a and c, the blood glucose level was increased in acetic acid-induced pain stimulation after mice were fasted for 16 h. The acetic acid-induced pain model can produce the peritoneal inflammation (acute peritonitis), which causes a response characterized by contraction of the abdominal muscles accompanying an extension of the forelimbs and elongation of the body. In addition, the blood glucose levels were elevated in formalin (Figures 2a and c), SP (Figures 3a and c), TNF-α (Figures 5a and c), and IFN-γ (Figures 6a and c) pain models. The blood glucose level induced by pain stimulation in a fasting group reached at maximum 30 min after and returned to the basal level 120 min after various types of pain stimulations. However, the blood glucose level was not altered in glutamate pain model (Figures 4a and c).

As shown in Figures 1b and c, the blood glucose level in the D-glucose-fed group reached at maximum 30 min and returned to the basal level 2 h after mice were fed orally with 2 g/kg D-glucose. The blood glucose level was increased in acetic acid-induced pain stimulation in D-glucose-fed group in an additive manner. In addition, the blood glucose levels were,

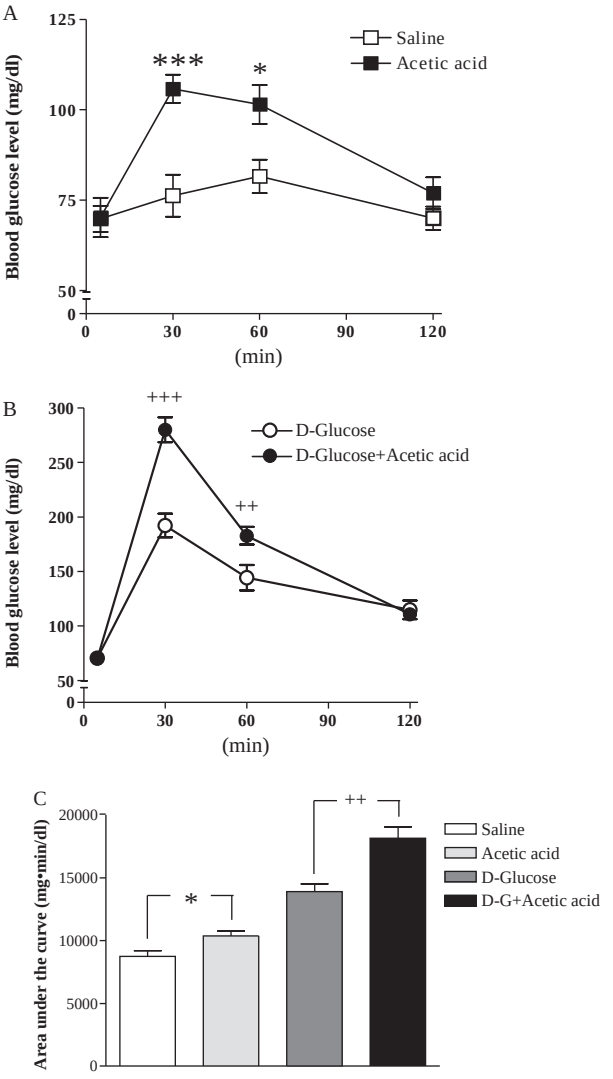

Figure 1. The alteration of blood glucose levels and total area under curve (c) by i.p. acetic acid administration in fasting (a) for 16 h and D-glucose-fed (b) groups. Blood was collected at 30, 60, and 120 min after various pain stimulations from tail-vein and, blood glucose level was measured by Accu-Chek Performa blood glucose monitoring system (glucometer) (Roche Diagnostics, USA). The vertical bars indicate the standard error of the mean. The number of animals used for each group was 8–10. $^{*}P<0.05$ and $^{***}P<0.001$, compared to saline group; $^{+}P<0.05$ and $^{++}P<0.01$, compared to D-glucose group.

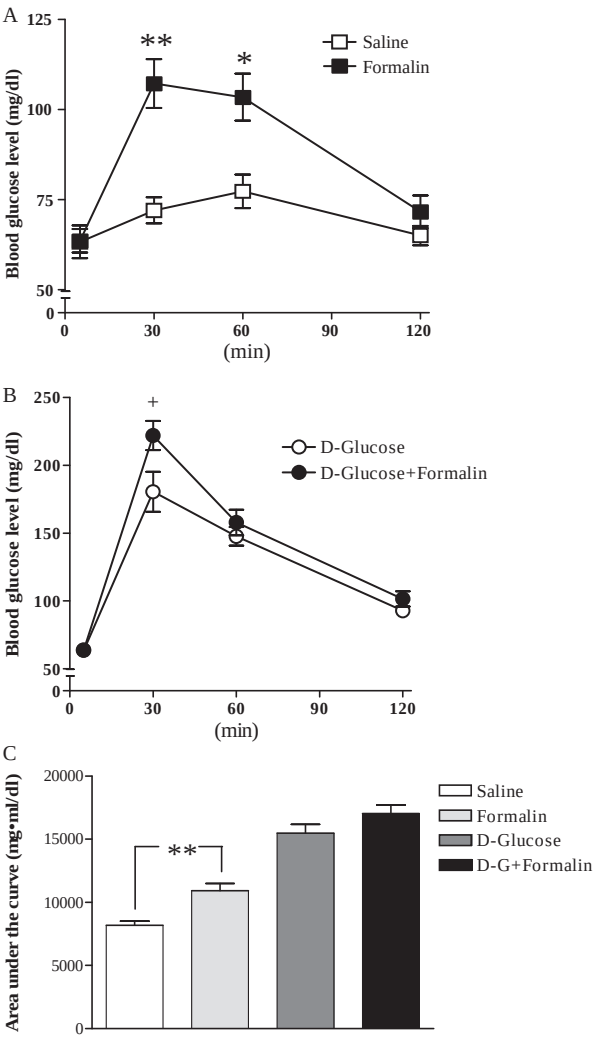

Figure 2. The alteration of blood glucose levels and total AUC (c) by s.c. formalin injection in fasting (a) for 16 h and D-glucose-fed (b) groups. The vertical bars indicate the standard error of the mean. The number of animals used for each group was 8–10. $^{*}P<0.05$ and $^{**}P<0.01$, compared to saline group; $^{+}P<0.05$, compared to D-glucose group.

in an additive manner, also elevated by pain stimulation induced by formalin (Figures 2b and c), SP (Figures 3b and c), glutamate (Figures 4b and c), TNF-α (Figures 5b and c), and IFN-γ (Figures 6b and c) in the D-glucose-fed group. However, glutamate administrated i.t. increased the blood glucose level in the D-glucose-fed group in a potentiative manner (Figures 4b and c).

Discussion

In the present study, the relationship between pain stimulation and the blood glucose level was investigated in various types of pain models. The acetic acid-induced writhing response is considered as a visceral inflammatory pain model (Koster and Beer 1959). Formalin pain model is widely agreed that the nociceptive behaviors manifested during the acute first phase and tonic second phase (Hunskaar et al. 1985; Choi et al. 2001). We found in the present study that the pain stimulation induced by acetic acid and formalin increases the blood glucose level in a fasting group. In the D-glucose-fed group, the blood glucose

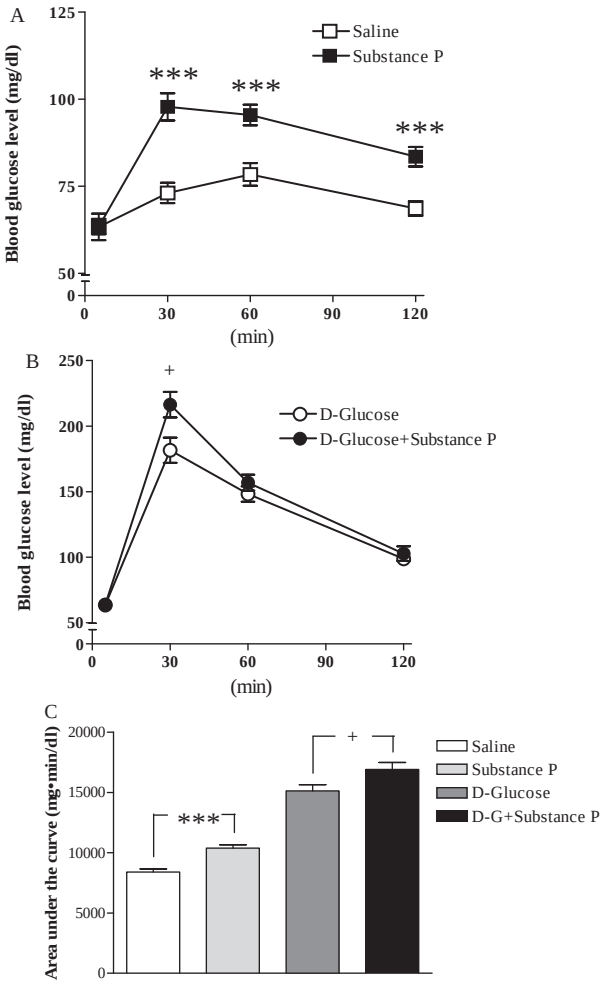

Figure 3. The alteration of blood glucose levels and total AUC (c) by i.t. substance P administration in fasting (a) for 16 h and D-glucose-fed (b) groups. The vertical bars indicate the standard error of the mean. The number of animals used for each group was 8–10. $^{***}P<0.001$, compared to saline group; $^{+}P<0.05$, compared to D-glucose group.

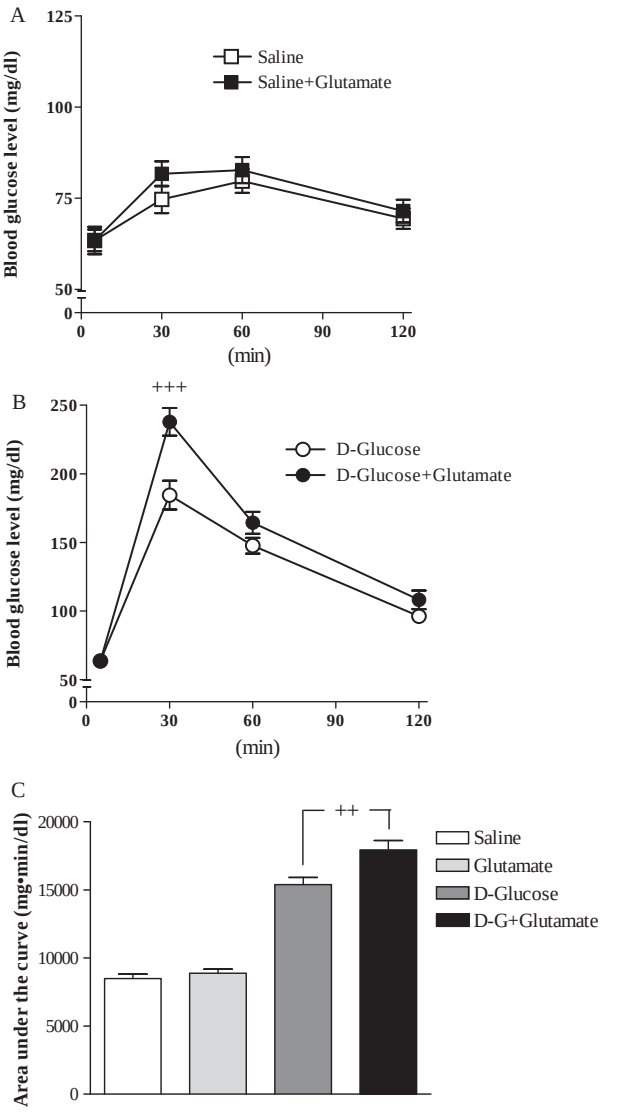

Figure 4. The alteration of blood glucose levels and total AUC (c) by i.t. glutamate injection in fasting (a) for 16 h and D-glucose-fed (b) groups. The vertical bars indicate the standard error of the mean. The number of animals used for each group was 8–10. $^{+++}P<0.001$, compared to D-glucose group.

level was further increased by acetic acid or formalin administered spinally, but just in an additive manner, suggesting that acetic acid- and formalin-induced pain stimulations appear not to exert the modulatory role in the regulation of the blood glucose level in D-glucose-fed group, while acetic acid- and formalin-induced pain stimulations increase the blood glucose level in a fasting group.

In the present study, we found that the pain stimulation induced by SP administered spinally causes an elevation of the blood glucose level in a fasting group. However, the blood glucose level was not altered by the pain stimulation induced by glutamate administered spinally in a fasting group. The exact reasons of no alteration in the blood glucose level in response to glutamate-induced pain stimulation should be clarified in the future. The findings of the present study suggest that SP- and glutamate-induced pain stimulations differentially regulate the blood glucose level in a fasting group. In addition, SP-induced pain stimulation further enhanced the blood glucose level in the D-glucose-fed group in an additive manner, while glutamate-induced pain stimulation further enhanced the blood glucose level in the D-glucose-fed group in a potentiative manner. Although SP-induced pain stimulation increases the blood glucose level, it appears not to modulate the blood glucose level in the D-glucose-fed model. However, glutamate-induced

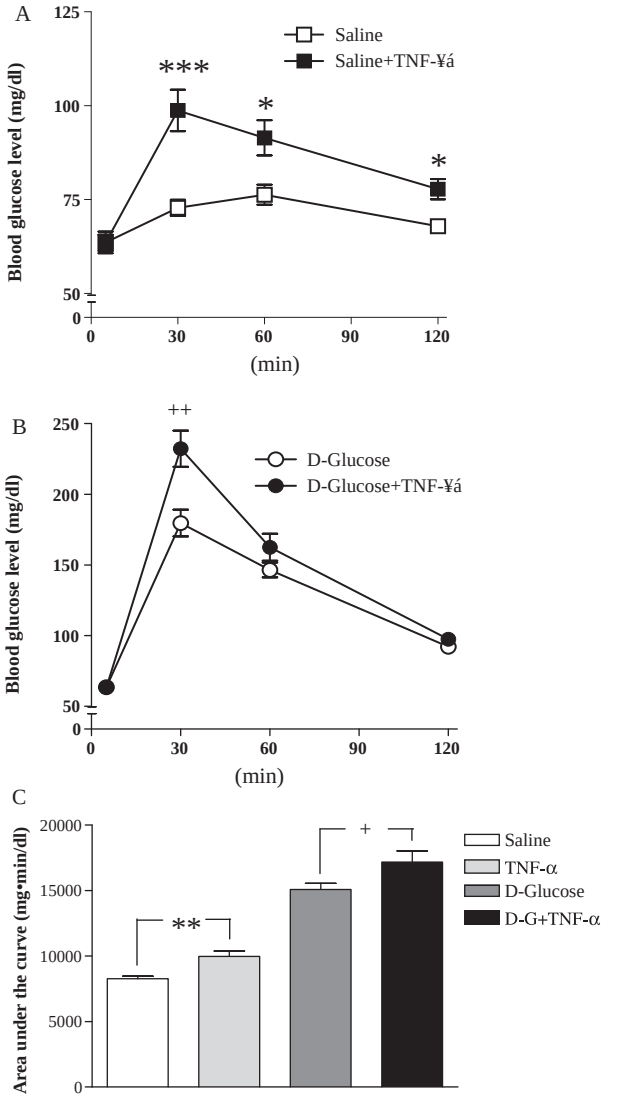

Figure 5. The alteration of blood glucose levels and total AUC (c) by i.t. TNF-α administration in fasting (a) for 16 h and D-glucose-fed (b) groups. The vertical bars indicate the standard error of the mean. The number of animals used for each group was 8–10. $^{*}P<0.05$ and $^{***}P<0.001$, compared to saline group; $^{++}P<0.01$, compared to D-glucose group).

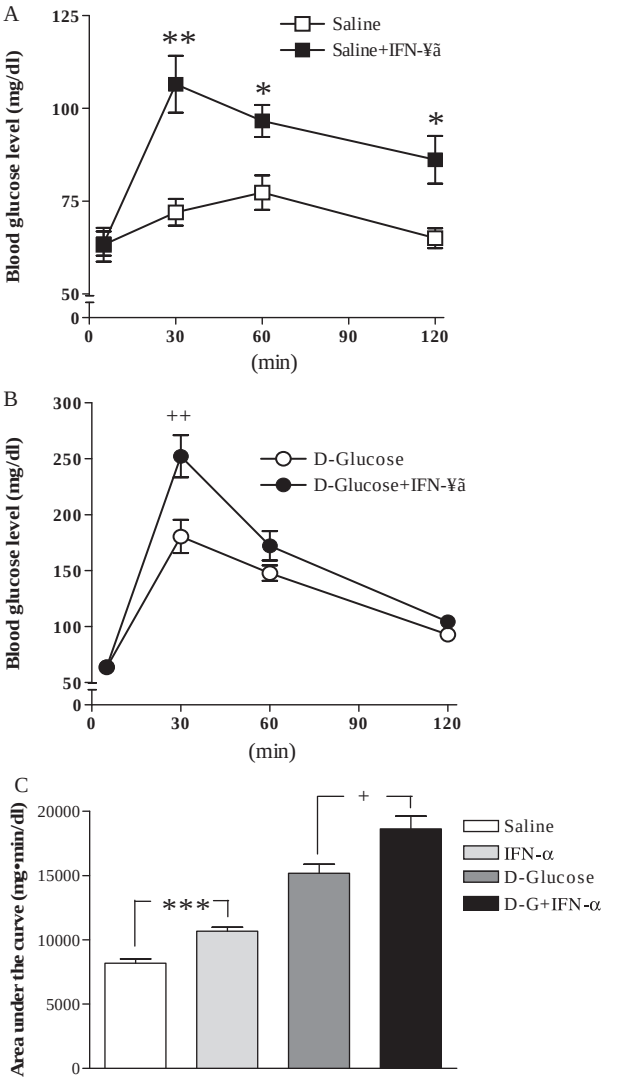

Figure 6. The alteration of blood glucose levels and total AUC (c) by i.t. IFN-γ injection in fasting (a) for 16 h and D-glucose-fed (b) groups. The vertical bars indicate the standard error of the mean. The number of animals used for each group was 8–10. $^{*}P<0.05$ and $^{**}P<0.01$, compared to saline group; $^{++}P<0.01$, compared to D-glucose group).

pain stimulation may exert a modulatory role in the regulation of the blood glucose level in the D-glucose-fed model.

A previous study has shown that i.t. injection of pro-inflammatory cytokines evokes nociceptive behaviors (Choi et al. 2003). Thus, in the present study, we assessed the possible regulation of the blood glucose level by pro-inflammatory cytokines such as TNF-α and IFN-γ administered spinally. We found that the pain stimulation induced by TNF-α and IFN-γ elevated the blood glucose level in a fasting group and the D-glucose-fed group in an additive manner. Thus, it is suggested that the pain stimulation induced by TNF-α and IFN-γ clearly causes alteration of the blood glucose level in a fasting group, while the pain stimulation induced by TNF-α and IFN-γ appears not to exert the modulatory role in the regulation of the blood glucose level in the D-glucose-fed group.

In summary, our results suggest that the pain stimulations induced by acetic acid, formalin, SP, TNF-α, and IFN-γ clearly produce hyperglycemic effect in fasting mice. However, glutamate-induced pain stimulation is not involved in the regulation of the blood glucose level in a fasting group. Furthermore, the pain stimulation induced by glutamate, but not acetic acid, formalin, SP, TNF-α, and IFN-γ, exerts the

modulatory role in the regulation of the blood glucose level in the D-glucose-fed group.

Acknowledgements

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