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The modulatory role of alpha-melanocyte stimulating hormone administered spinally in the regulation of blood glucose level in D-glucose-fed and restraint stress mouse models

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ABSTRACT

Alpha-melanocyte stimulating hormone (α-MSH) is known as a regulator of the blood glucose homeostasis and food intake. In the present study, the possible roles of α-MSH located in the spinal cord in the regulation of the blood glucose level were investigated in D-glucose-fed and immobilization stress (IMO) mouse models. We found in the present study that intrathecal (i.t.) injection with α-MSH alone did not affect the blood glucose level. However, i.t. administration with α-MSH reduced the blood glucose level in D-glucose-fed model. The plasma insulin level was increased in D-glucose-fed model and was further increased by α-MSH, whereas α-MSH did not affect plasma corticosterone level in D-glucose-fed model. In addition, i.t. administration with glucagon alone enhanced blood glucose level and, i.t. injection with glucagon also increased the blood glucose level in D-glucose-fed model. In contrasted to results observed in D-glucose-fed model, i.t. treatment with α-MSH caused enhancement of the blood glucose level in IMO model. The plasma insulin level was increased in IMO model. The increased plasma insulin level by IMO was reduced by i.t. treatment with α-MSH, whereas i.t. pretreatment with α-MSH did not affect plasma corticosterone level in IMO model. Taken together, although spinally located α-MSH itself does not alter the blood glucose level, our results suggest that the activation of α-MSH system located in the spinal cord play important modulatory roles for the reduction of the blood glucose level in D-glucose fed model whereas α-MSH is responsible for the up-regulation of the blood glucose level in IMO model. The enhancement of insulin release may be responsible for modulatory action of α-MSH in down-regulation of the blood glucose in D-glucose fed model whereas reduction of insulin release may be responsible for modulatory action of α-MSH in up-regulation of the blood glucose in IMO model.

1. Introduction

Alpha-melanocyte stimulating hormone (α-MSH) is known as a processed bioactive form of pro-opiomelanocortin (POMC) in the brain and peripheral tissues such as the pituitary gland, immune system, and skin. α-MSH is primarily located at various tissues such as gut and several regions of the brain (Bjartell et al., 1990; Coveñas et al., 2000; Lynd-Balta et al., 1996). In addition, α-MSH exerts a variety of physiological and pharmacological functions such as modulation of inflammatory bowel diseases, antimicrobial effects, and allergic reaction changes (Cutuli et al., 2000; Orita et al., 2011; Rajora et al., 1997).

One of important functions of α-MSH is the regulation of glucose and metabolism. For example, peripheral administration of α-MSH is associated with glucose regulation (Brennan et al., 2003). In addition, α-MSH produces an anorexigenic effect (Cline et al., 2008). Intravenous injection of α-MSH increases the plasma levels of glucagon and insulin (Knudtzon, 1984). In addition, Katsuki et al., 2000 have previously demonstrated that the circulating concentration of α-MSH is significantly increased and correlated with insulin resistance in obese men.

In addition to the involvement of α-MSH in the peripheral system, several lines of evidence have suggested that α-MSH system located in the brain also appears to be involved in the glucose homeostasis and metabolism. The activation of α-MSH system in the hypothalamus is associated with regulation of the appetite and obesity (Yu and Kim, 2012). The earlier studies have demonstrated that neuronal pathways containing α-MSH extend from hypothalamic area to the spinal cord (Köhler et al., 1984; Shiosaka et al., 1985), suggesting that the receptors acted by α-MSH are located at the spinal cord. Immunoreactivity for the

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POMC-derived peptides such as α-MSH and ACTH has been described in rat spinal cord (Gutstein et al., 1992; Tsou et al., 1986). Several studies related to functions of α-MSH on spinal system have been demonstrated. For example, the expression of melanocortin receptor protein in spinal cord areas is commonly associated with nociception (van der Kraan et al., 1999). α-MSH induces a cAMP increase in cultured rat spinal cord cells (Hol et al., 1993). Neurological and electrophysiological improvement is evidenced in animals treated with α-MSH in spinal cord injury model (van de Meent et al., 1997). Furthermore, α-MSH promotes the recovery of locomotor activity in spinal cord injury animal model (Bharne et al., 2011). Although the involvement of α-MSH located peripherally as well as in the brain sites in the regulation of the blood glucose level have been well demonstrated as revealed in numerous previous studies, the functional roles of spinal α-MSH system in the regulation of the blood glucose level have not been well characterized yet. Thus, in the present study, the effects of α-MSH administered spinally on the blood glucose level in mice were examined. In addition, since the sources and the regulation of the blood glucose in D-glucose-fed and immobilization stress (IMO) models are different, the possible modulatory roles of α-MSH on these two animal models were investigated.

2. Material 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 and the ethical guidelines of the International Association for the Study of Pain.

2.1. Experimental animals

Male Hsd: CD-1 (ICR) [Charles River, USA] mice, weighing 24–26 g, were used for all the experiments. Five mice were housed 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).

2.2. Drugs

D-glucose, α-MSH and glucagon were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All drugs were prepared just before use. Blood glucose meter, lancing device and strips were purchased from Roche Diagnostics (Sandhofer Strasse, Mannheim, Germany). The mouse insulin ELISA kit was purchased from Shibayagi Co. (Shibukawa, Japan).

2.3. Intrathecal (i.t.) injection

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 which was inserted into the tissue to one side of the L5 or L6 spinous process so that it slipped into the groove between the spinous and transverse processes (Hylden and Wilcox, 1981). 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.

2.4. Oral glucose tolerance test (OGTT)

After 16 h of fasting (n = 8–10), OGTT was performed. D-glucose solution (2 g/kg body weight) was administered orally, and the blood glucose level was measured from the tail vein at 30, 60, and 120 min after the glucose feeding.

2.5. Measurement of blood glucose level

Blood glucose measurements were obtained using blood samples collected by lateral tail vein laceration. A minimum volume (1 μl) of blood was collected as quickly as possible. Glucose level was measured using Accu-Chek Performa blood glucose monitoring system (Sandhofer Strasse, Mannheim, Germany).

2.6. Insulin ELISA assay

Measurement of serum levels of insulin was performed according to the manufacturer's manual. The levels of insulin in the serum were evaluated by measuring the absorbance at 450 nm using a microplate spectrophotometer Epoch (Biotek, Winooski, VT).

2.7. Corticosterone assay

Plasma corticosterone levels were measured at 30, 60 and 120 min after i.t. injection with clonidine. Four hundred microliters of blood was collected by puncturing the retro-orbital venous plexus. Plasma was separated by centrifugation and stored at −80 °C until assayed. Plasma corticosterone levels were determined by the fluorometric determination method (Glick et al., 1964).

2.8. Immobilization stress (IMO)

The IMO was conducted by placing the mouse in 50-ml corning tubes from 9:00 to 11:00. Adequate ventilation was provided by means of holes at the sides of the tubes (Suh et al., 2000). The control group was not subjected to IMO and was merely placed inside mouse cages.

2.9. Statistical analysis

The statistical significance of differences between groups was assessed with one-way ANOVA with repeated measures and Bonferroni's post hoc test using GraphPad Prism Version 4.0 for Windows XP (GraphPad Software, San Diego, CA, USA). P-values of 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 the established blood glucose level were then used in replication experiments.

3. Results

3.1. The effect of α-MSH administered i.t. on the blood glucose level in D-glucose fed model

Mice were treated i.t. with α-MSH (0.1–1 μg/5 μl) and the blood glucose level was measured at 30, 60 and 120 min after α-MSH treatment. As shown in Fig. 1A (F = 0.0769; P = 3.193), i.t. administration of α-MSH at the doses from 0.1 to 1 μg did not change the blood glucose level. In addition, i.t. with α-MSH and D-glucose (2 g/kg) was fed orally, the blood glucose level was significantly