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Induction of hypothalamic serum- and glucocorticoid-induced protein kinase-1 gene expression and its relation to plasma des-acyl ghrelin in energy homeostasis in mice

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Abstract

Serum- and glucocorticoid-induced protein kinase (SGK) is a serine/threonine-specific protein kinase that is transcriptionally regulated by serum, glucocorticoids, and mineral corticoids. Here, we report that fasting or obesity with hyperphagia increased hypothalamic SGK-1 gene expression. Hypothalamic SGK-1 mRNA levels were proportional to daily food intake and body weights in C57BL6J mice, KK mice, and KKA^y mice matched for age. Plasma des-acyl ghrelin, but not active ghrelin, levels were inversely proportional to daily food intake and body weights among these animals. The increases in hypothalamic SGK-1 gene expression in these animals were not accompanied by increases in plasma corticosterone levels. Under conditions of increased energy usage such as fasting, hypothalamic SGK-1 gene expression and plasma des-acyl ghrelin levels were positively correlated while during conditions of increased energy storage, they were negatively correlated. These results suggest that hypothalamic SGK-1 gene is a novel candidate gene involving in energy homeostasis in mice.

Keywords: SGK-1; Corticosterone; Ghrelin; Hypothalamus; Energy homeostasis; Ay mice; NPY; POMC; Fasting; Obesity; Hyperphagia

Serum- and glucocorticoid-induced protein kinase (SGK) is a serine/threonine-specific protein kinase that is transcriptionally regulated by serum, glucocorticoids, and mineral corticoids [1]. Sgk has a catalytic domain homologous to that of Akt, but it lacks the pleckstrin homology domain present in Akt [2]. SGK is related to protein kinase B, which is expressed in all tissues, including the brain. SGK-1 belongs to a family of kinases including SGK-2 and SGK-3, which are not under strong genomic regulation by cell stress or hormones [3]. SGK-1 is involved in the stimulation of salt intake, kidney growth, proteinuria, and renal K(+) excretion during mineralocorticoid excess, glucocorticoid-induced inhibition of insulin secretion, and the regulation of blood pressure and glucose metabolism in peripheral tissues [4–7].

SGK-1 gene expression in brain tissue such as the hippocampus and cortex is induced by various stimuli such as restraint stress, water-immersion, elevated plus maze exposure, and transient ischemia [8–10]. The hypothalamus is important in the regulation of energy homeostasis via feeding, the autonomic nervous system, and the neuroendocrine system. Hypothalamic SGK-1 gene expression in relation to energy homeostasis, however, has not been evaluated. To examine the induction of hypothalamic SGK-1 gene expression during changes in feeding and body weight, we investigated: (1) the induction of hypothalamic SGK-1 gene expression in age-matched C57BL6J, KK, and KKA^y mice with different body weights, (2) alterations of hypothalamic SGK-1 gene expression induced by fasting and feeding, and (3) the relationship between hypothalamic SGK-1 gene expression and plasma levels of ghrelin, an orexigenic peptide secreted from the stomach [11], in the regulation of energy homeostasis in mice.

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Materials and methods

General procedure. Animals were purchased from Japan CLEA. Mice were housed in individual cages with free access to water and chow pellets in a light—(12 h on/12 h off; lights on at 08:00 h and lights off at 20:00 h) and temperature—(20–22 °C) controlled environment. Animals were acclimatized to these conditions for 1 week before beginning the experiment.

To determine the relationship between hypothalamic SGK-1 mRNA levels and plasma active and des-acyl ghrelin levels under increased energy storage, we examined hypothalamic SGK-1 mRNA levels and plasma active ghrelin and des-acyl ghrelin levels in male 8-week-old C57BL/6J mice, KK mice, and KKA^y mice. Animals were decapitated and blood was collected for measurement of plasma active and des-acyl ghrelin levels, and corticosterone levels under a fed state at 10:00 to 11:00 h. The hypothalamic tissues were removed for total RNA extraction.

To determine the relationship between hypothalamic SGK-1 mRNA levels and plasma des-acyl ghrelin levels under increased energy usage, we examined hypothalamic SGK-1 mRNA levels and plasma des-acyl ghrelin levels in fed and 24-h fasted C57BL6J mice. C57BL6J mice (6 weeks old) were decapitated and blood was collected for measurement of plasma active and des-acyl ghrelin levels under a fed state and after a 24-h fast at 10–11 a.m. The hypothalamic tissues were similarly removed for total RNA extraction.

Ghrelin and corticosterone assay. Ghrelin has two forms, active *n*-octanoyl-modified ghrelin and non-active des-acyl ghrelin [11]. Plasma active and des-acyl ghrelin levels were measured by enzyme-linked immunosorbent assay (ELISA; active ghrelin and des-acyl ghrelin ELISA kit, Mitsubishi Kagaku Iatron Inc., Japan). Plasma corticosterone levels were measured by radioimmunoassay (Linco, St. Louis, MO).

Real-time quantitative RT-PCR. Total RNA was isolated from mouse hypothalamic tissue using the RNeasy Midi kit (Qiagen, Hilden, Germany) according to the manufacturer's directions, and cDNA synthesis was performed using a Super Script III First-Strand Synthesis System for RT-PCR Kit (Invitrogen, Rockville, MD) using 1 μg total RNA. cDNA synthesized from total RNA was evaluated in a real-time polymerase chain reaction (PCR) quantitative system (Light Cycler Quick System 350S; Roche Diagnostics, Mannheim, Germany). The primers used were as follows. For mouse SGK-1, sense, 5′-ACC CTT ACC TAC TCC AGA ATG-3′, antisense, 5′-GCT GGC AAT CTT CTG AAT A-3′; for mouse pro-opiomelanocortin (POMC), sense, 5′-ATA GAT GTG TGG AGC TGG TG-3′, antisense, 5′-GGC TGT TCA TCT CCG TTG-3′; for mouse neuropeptide Y (NPY), sense, 5′-GCT TGA AGA CCC TTC CAT TGG TG-3′, antisense, 5′-GGC GGA GTC CAG CCT AGT GG-3′; for mouse ghrelin, sense, 5′-GAA AGG AAT CCA AGA AGC CA-3′, antisense, 5′-GCT TGA TGC CAA CAT CGA A-3′; and for mouse β-actin, sense, 5′-TTG TAA CCA ACT GGG ACG ATA TGG-3′, antisense, 5′-GAT CTT GAT CTT CAT GGT GCT AGG-3′. The relative amount of mRNA was calculated with β-actin mRNA as the invariant control. The data are shown as the fold change of the mean value of the control group, which received saline.

Data are presented as mean values ± SEM. Comparisons between two groups were performed using Student's *t* test. Comparisons among more than two groups were performed using analysis of variance with Bonferroni's correction for multiple comparisons. The presence of a linear correlation was assessed using a parametric (Pearson's) correlation test. A *P* value of less than 0.05 was considered statistically significant.

Results and discussion

Plasma des-acyl ghrelin levels were lowest in KKA^y mice, then KK mice, and highest in C57BL6J mice (Fig. 1B). There were no differences in plasma active ghrelin levels among these animals (Fig. 1A). Hypothalamic SGK-1 mRNA levels were lowest in C57BL6J mice, then KK mice, and highest in KKA^y mice (Fig. 1C). There were no correlations, however, between hypothalamic NPY, POMC, or ghrelin mRNA levels and plasma des-acyl ghrelin levels among these mice (Fig. 1C). Hypothalamic SGK-1 mRNA levels and plasma des-acyl ghrelin levels were negatively correlated ($r = -0.8903$, two-tailed $P < 0.0001$) (Fig. 1D). Daily food consumption and body weights were lowest in C57BL6J mice, then KK mice, and highest in KKA^y mice (Figs. 1E and F). Moreover, plasma corticosterone levels were 39.5 ± 3.1 ng/ml in C57BL6J mice, 32.4 ± 2.7 ng/ml in KK mice, and less than 25.0 ng/ml in KKA^y mice ($n = 7$ for each group). These results indicate that increases in hypothalamic SGK-1 gene expression are not induced by increased plasma corticosterone levels in age-matched C57BL6J, KK, and KKA^y mice. These findings also suggest that reduced levels in des-acyl ghrelin, but not active ghrelin, contribute to decreases in plasma total ghrelin levels in obesity.

On the other hand, hypothalamic SGK-1 mRNA levels were significantly increased in 24-h fasted mice compared with fed mice (4.5-fold; Fig. 2A). Plasma des-acyl ghrelin levels were significantly increased in 24-h fasted mice compared with fed mice (3.1-fold; Fig. 2B). Plasma des-acyl ghrelin levels and hypothalamic SGK-1 mRNA levels were positively correlated (correlation coefficient $r = 0.9226$; $P < 0.0001$; Fig. 2C). Plasma active ghrelin levels were also significantly increased (2.5-fold) as described previously [12].

The present study demonstrates that the induction of hypothalamic SGK-1 gene expression is likely to increase in mice that consume more food and gain more body weight among C57BL6J, KK, and KKA^y mice. In addition, the induction of hypothalamic SGK-1 gene expression is increased under fasting conditions. Moreover, the present results demonstrate that plasma des-acyl ghrelin levels decreased under increased energy storage conditions and increased under fasting conditions. The induction of hypothalamic SGK-1 gene expression was positively proportional to plasma des-acyl ghrelin levels under conditions of increased energy usage, whereas it was inversely proportional to plasma des-acyl ghrelin levels under conditions of increased energy storage. Thus, the relationship between hypothalamic SGK-1 gene expression and plasma des-acyl ghrelin levels can be altered by changes in energy balance.

Although SGK-1 is considered a primary glucocorticoid-induced gene [1], the results of the present study demonstrate that increases in hypothalamic SGK-1 gene expression are not always accompanied by increases in plasma corticosterone levels. Rather, plasma corticosterone levels were lower in obese KKA^y mice, which had the highest induction of hypothalamic SGK-1 gene expression. Hypothalamic SGK-1 gene expression, therefore, might not be regulated by serum glucocorticoids. The increased hypothalamic SGK-1 gene expression might be a compensatory response to increased energy storage in KKA^y mice.

In summary, these results suggest that the hypothalamic SGK-1 gene is a novel candidate gene involved in energy homeostasis and its relation to plasma des-acyl ghrelin levels can be altered by changes in the energy balance in mice.