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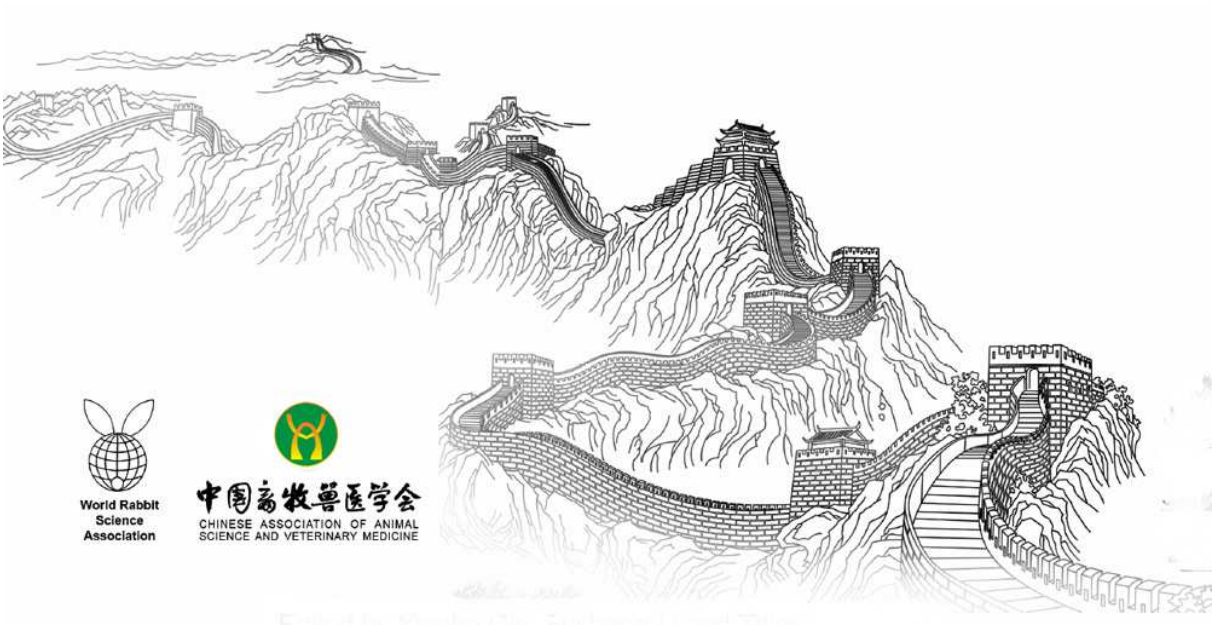

ACETATE INHIBITS HYPOTHALAMIC JNK SIGNALING IN RABBITS

Liu L.[1], Sui X. [1], Li F. [1,*]

[1] Department of Animal Science, Shandong Agricultural University, Taian, Shandong China
* Corresponding author: Li F, Emal: chlf@sdau.edu.cn

ABSTRACT

We investigated the effects of acetate on hypothalamic mitogen-activated protein kinases (MAPKs) levels and (an)orexigenic neuropeptides. Sixty rabbits (Hyla, 35-d old) were allocated to two groups (n=30/group): intravenous injection of acetate (0.5 mg/day/kg body weight) or vehicle (control). Acetate treatment for 0 d (4 h after first injection), 4 d or 7 d decreased (P<0.05) food intake compared with control. Acetate treatment had no effect on mRNA levels of hypothalamic neuropeptide Y, agouti-related protein, cocaine-amphetamine-regulated, G-protein-coupled receptors (GPR) 41, acetyl-CoA carboxylase, fatty acid synthase and carnitine palmitoyltransferase-1, but treatment for 4 h increased (P<0.05) pro-opiomelanocortin (POMC) and GPR43 gene expressions. Acetate treatment did not alter protein levels of p-ERK and p-p38 MAPK compared with the control group, but decreased (P<0.05) the phosphorylated c-Jun NH2-terminal kinases (JNK) protein. In conclusion, acetate administration induced anorexia via increasing the gene expression of POMC, which was associated with GPR43 and intracellular JNK signaling.

Keywords: acetate, appetite, GPR43, MAPKs, rabbits

INTRODUCTION

Energy intake and expenditure are tightly regulated in mammals and the hypothalamus plays a central role in the integration of nutritional status with control of feeding and energy homeostasis. Several neuronal populations, particularly in the hypothalamic arcuate nucleus (ARC), are involved in the regulation of energy homeostasis. These include neurons that release orexigenic peptides (e.g., neuropeptide Y (NPY)/agouti-related protein (AgRP)) or anorexigenic peptides (e.g., pro-opiomelanocortin (POMC)/cocaine-amphetamine-regulated transcript (CART))
In the hypothalamus, key signaling molecules in the transduction of trophic signals are Mitogen-activated protein kinases (MAPKs). The MAPKs are serine/threonine-specific protein kinases and include the extracellular signal-regulated kinases ERK1/2, the c-Jun NH2-terminal kinases (JNK), and the p38 MAPK. Fasting activated ERK and p38 MAPK signaling in the mouse hypothalamus (Morikawa et al., 2004). Inhibiting the hypothalamic ERK1/2 and p38 pathways prevented NPY synthesis and secretion (Kim et al., 2010). Tsaousidou et al. (2014) demonstrated that activation of JNK induced hyperphagia via up-regulation of AgRP gene expression.

Short-chain fatty acids (SCFAs) increase the rate of lipolysis via activating p38 MAPK signaling (Rumberger et al., 2014). SCFAs can increase energy expenditure and improve glucose tolerance to increase energy utilization via binding G-protein coupled receptors (GPR) 41 or GPR43 (Kimura et al., 2014). Acetate has a direct role in the central regulation of appetite, is higher than that of other SCFAs (e.g., butyrate and propionate) in rabbits (Rabbani et al., 1999). Although the role of SCFAs and specially acetate on the energy homeostasis in mammalian species has been studied widely, however it needs to be elucidated in rabbits. Hence, in the present study we investigated whether the hypothalamic MAPK signaling is modulated by acetate to determine the relationship between this process and hypothalamic orexigenic or anorexigenic peptides.

MATERIALS AND METHODS

Experimental protocol and sample collection

At 35 days of age, 60 rabbits (Hyla) of similar body weight (980 ± 40 g) were divided into 2 groups (30 replicates per group). Rabbits after fasting for 6 h were randomly subjected to one of the following two treatments: intravenous injection of acetate (0.5 mg/day/kg body weight, one injection per day) or vehicle-saline (control). Food intake was recorded after injection. At days of 0, 4, and 7, 8 rabbits per group were electrically stunned and sacrificed 4 h after treatments. After being snap-frozen in liquid nitrogen, the hypothalamic arcuate nucleus tissue samples were stored at -80℃.

Chemical Analyses

Western blotting and quantitative real-time PCR were performed according to Liu et al. (2014). The comparative CT method ($2^{-\Delta\Delta CT}$) was used to quantitate mRNA expression. ℃

Statistical analysis

The data are means ± SME. Homogeneity of variances among treatments was confirmed using Bartlett's test. Data were subjected to ANOVA to test the effect of treatment. Differences between means were assessed by Duncan's multiple range analysis. $P<0.05$ was considered significant.

RESULTS

Acetate treatment significantly inhibited food intake within 5 h after first injection (Figure 1A). Acetate treatment for 4 or 7 days decreased the average daily food intake (Figure 1B). Compared with control, acetate treatment had no effect on hypothalamic NPY, AgRP, and CART mRNA levels (Figure 1C, D and F, respectively). Acetate treatment increased ($P<0.05$) the POMC and GPR43 gene expressions 4 h after the first injection (Figure 1E), but did not alter the mRNA levels of hypothalamic GPR41, acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), and carnitine palmitoyltransferase-1 (CPT1) (Figure 1G, H). Although no differences were observed in protein levels of p-p38 MAPK and p-ERK (Figure 1J and K, respectively) between treatment groups, hypothalamic p-JNK protein level was significantly decreased after acetate injection (Figure 1I).

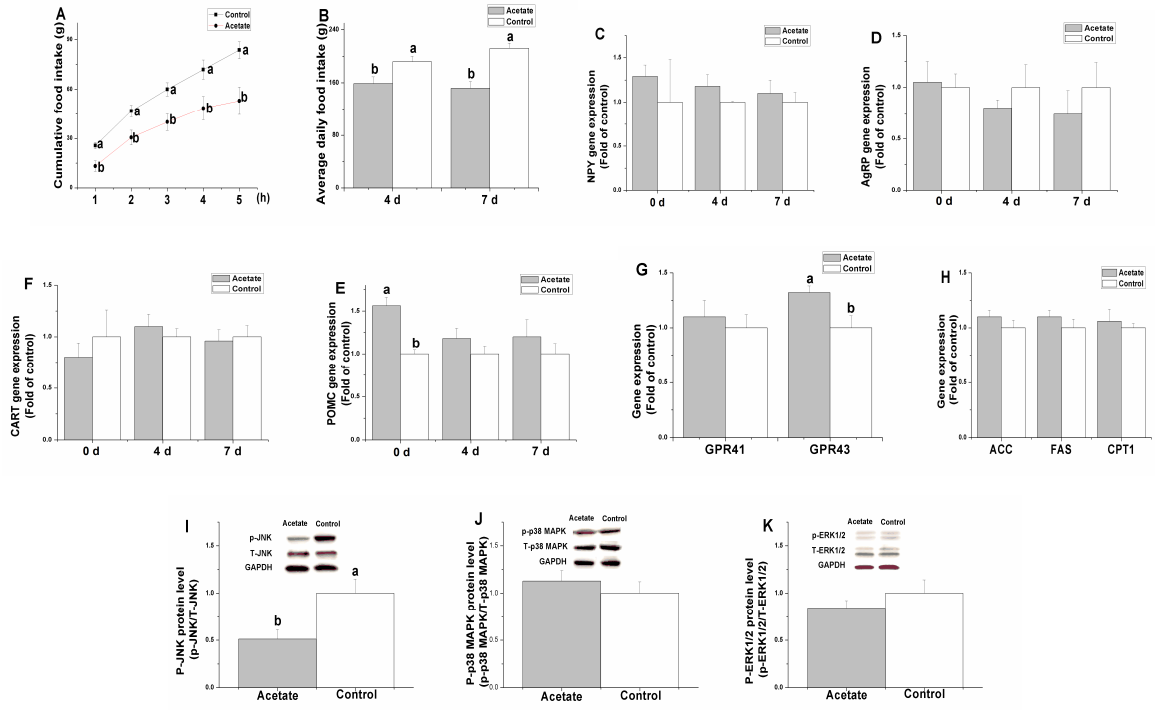

Figure 1 Effects of acetate injection on food intake, appetite-related genes, and protein expression.

DISCUSSION

In the present study, we examined the effect of acetate on appetite and related control pathways. Our results showed that up-regulated hypothalamic POMC gene level was responsible for the lower food intake caused by acetate and that the process was associated with membrane GPR43 and intracellular JNK signaling.

Acetate induced anorexia by up-regulating the hypothalamic POMC gene expression

In mice, acetate is a well-known anorectic agent, which acts on the brain following colon production or exogenous administration (Frost et al., 2014). In the present study, we further evaluated the gene expressions of hypothalamic orexigenic and anorexigenic neuropeptides. In mammalians, it is demonstrated that POMC decreases appetite (Tritos et al., 1998). In line with results obtained in rats (Frost et al., 2014), we have demonstrated that acetate treatment increased hypothalamic POMC levels in rabbits, suggesting that the up-regulation of hypothalamic POMC was responsible for the acetate-induced anorexia. In our study, the expression of other appetite regulatory genes such as AgRP, CART, and NPY were not altered by acetate administration, suggesting that these appetite regulating neurotransmitters were not the main targets in acetate-induced anorexia when acetate was administrated peripherally.

JNK signaling is involved in acetate-induced anorexia

The nutritional status modifies the expression of hypothalamic neuropeptides through various signaling molecules, including extracellular MAPKs. Fasting activates ERK in ARC and p38 MAPK in paraventricular nucleus (Morikawa et al., 2004). Furthermore, melanocortin, corticotropin-releasing hormone, leptin, and insulin regulate appetite via hypothalamic MAPK signaling (Refojo et al., 2005). The activation of MAPKs is depended on regions and condition of energy balance (Morikawa et al., 2004). Similarly, in the present study we found that acetate could inhibit the JNK activation in ARC of rabbit's hypothalamus, but not the p38 MAPK and ERK. The results suggest that the hypothalamic JNK signaling is associated with the acetate-induced anorexia. Moreover, the increased POMC gene expression may be relative to the inhibited JNK. Chai et al. (2009) found that the activation of POMC receptor and melanocortin-4 receptor could inhibit JNK activity in HEK293 cells.

Acetate induced anorexia in a GPR43-dependent manner

A recent study employing a "reverse pharmacology" approach identified short-chain fatty acids, including acetate, propionate, and butyrate, as ligands for orphan GPR41 and GPR43 (Nilsson et al., 2003). In the present study, the acetate treatment significantly increased the hypothalamic GPR43 gene expression, but not GPR41. This result suggests that acetate causes anorexia in a GPR43-dependent manner. Acetate had a higher binding potency for GPR43 than GPR41 (Bindels et al., 2013). It is reported that the activation of GPR43 can reduced lypolysis and these effects were abolished in GPR43-deficient mice (Ge et al., 2008). Besides, high fat diet can decrease the food intake by up-regulating hypothalamic POMC gene expression (Lin et al., 2000). In mice, it is shown that GPR43-knockout mice had significantly higher food intake when fed high fat diet compared with wild type mice (Bjursell et al., 2011). Hence, it is possible that in rabbits, acetate up-regulates POMC gene transcription via GPR43. In addition, the alteration of intracellular JNK activity in acetate-treated rabbits may be induced via GPR43.

CONCLUSIONS

Acetate induces anorexia via increasing the gene expression of POMC, which was associated with membrane GPR43 and intracellular JNK signaling.

ACKNOWLEDGEMENT

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Methods

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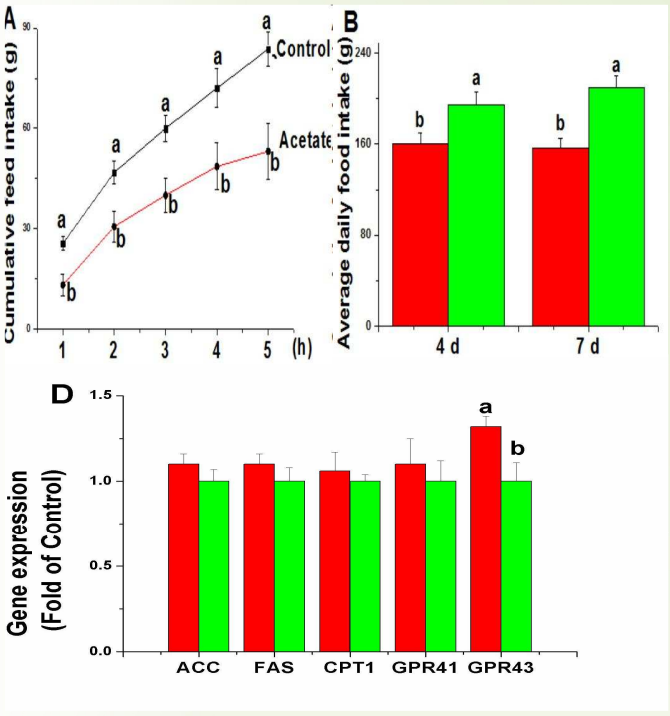
Results

Acute acetate treatment significantly inhibited the food intake within 5 h after first injection (P<0.05). Acetate treatment for 4 d or 7 d decreased the average daily food intake compared with the control (P<0.05). Compared with the control, acetate treatment for 4 h, 4 d or 7 d, had no effect on hypothalamic neuropeptide Y (NPY) , agouti-related protein (AgRP) and cocaine-amphetamine-regulated transcript (CART) mRNA levels (P>0.05). Although acetate treatment for 4 d or 7 d did not significantly affect the hypothalamic pro-opiomelanocortin (POMC) gene expression (P>0.05), acetate treatment for 4 h significantly increased the POMC gene expression (P<0.05). Besides, acetate injection did not alter the mRNA levels of hypothalamic G-protein-coupled receptors (GPR) 41, acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), and carnitine palmitoyltransferase-1 (CPT1) compared with the control (P>0.05), but the GPR43 gene expression was significantly increased after 4h with acetate injection (P<0.05). Although no significant difference was observed in protein levels of p-AMPK (Figure 4 A), p-p38 MAPK (Figure 4 C) and p-ERK (Figure 4 D) in acetate group rabbits compared with the control (P>0.05), hypothalamic p-JNK protein level were significantly decreased after acetate injection (P<0.05, Figure 4 B).



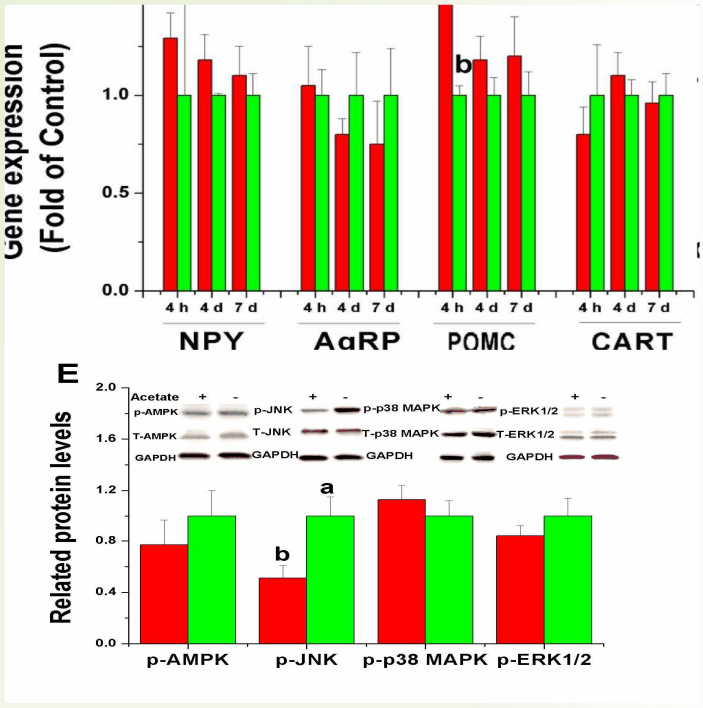


Figure 1 Effects of acetate treatment on food intake (A, B), hypothalamic appetite-related genes expression(C: NPY, AgRP, POMC and CART; D: GPR41, GPR41, ACC, FAS and CPT1) and related protein levels (E: p-AMPK, p-JNK, p-p38 MAPK and p-ERK1/2).Values are shown as the mean ± SE (n=10). a, b Means with different superscripts are significantly different (P<0.05).

Conclusions

Acetate induces anorexia via increasing the gene expression of POMC, which was associated with membrane GPR43 and intracellular JNK signaling.

Acknowledgements

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