THE EFFECT OF GLUCOCORTICOIDS ON THE GENE EXPRESSION OF NUTRIENT TRANSPORTER IN DIFFERENT RABBIT INTESTINAL SEGMENTS

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ABSTRACT

In rabbits, stress disrupts nutrient digestion and absorption.However, the underlying molecular mechanism is not clearly understood. The objective of this study was to investigate if the stress alter the nutrient transporter expression in different segments in small intestine. We analysed the effects of 3 h (short-term) or 7 d (long-term) dexamethasone (DEX) (2mg/kg body weight) treatment on the gene expression of most nutrient transporters. The results showed that short-term DEX treatment significantly decreased PepT1, B0AT, rBAT and SGLT1 expression in small intestinal segments (P<0.05). Long-term DEX treatment also significantly decreased PepT1,CAT1, B^0AT, rBAT and SGLT1 in small intestinal segments (P<0.05). In conclusion, DEX could decrease the gene expression of most nutrient transporters affect the transport of intestinal amino acids and monosaccharides.

Key words:Stress,glucocorticoids,peptide transporter,amino acid transporter,glucose transporter

INTRODUCTION

There are various stressors in intensive breeding production of rabbits, such as high temperature, noise, transportation, feeding restriction,feed exchange,immune stress and fright.The stress response induced by these stressors will reduce the production performance of rabbits and affect the feed reward. (Hu *et al*.,2010;Song *et al*., 2013). Glucocorticoids (GCs), which are considered to be the end products of hypothalamic–pituitary–adrenal (HPA) axis stimulation, are counterregulatory hormones with broad effects on carbohydrate, lipid and protein metabolism (Bamberger et al., 1996).

The small intestine is one of the main sites of nutrient digestion and absorption. Although the histological characteristics of duodenum, jejunum and ileum mucosa are similar, the digestion and absorption capacity of nutrients in different segments of the small intestine is different. The products of dietary protein digestion in the small intestine are mainly absorbed in the form of small peptides and amino acids. Rabbits intestinal uptake of small peptides, which primarily consist of 2 or 3 amino acids, is mediated by the H^+-dependent peptide transporter, i.e.,PepT1, localized at the brush-border membranes of intestinal epithelial cells (Freeman *et al*., 1995). Free amino acids are transported by amino acid transporters. Several distinct amino acid transport systems have been identified and characterized in the small intestine. For instance, the cationic amino acid transporter CAT1(y+ transport system),neutral amino acid transporter B0AT(B0AT transport system)and facultative amino acid transporter rBAT(b0,+ transport system) (Broer, 2008). The absorption of carbohydrates into the enterocytes of the small intestine is mediated by sugar transporters, such as sodium-glucose transporter 1 (SGLT1) (Thorens, 1996), which diffuses monosaccharides into the extracellular fluid and then into the blood (Wrigh and Turk, 2004). Metabolic and endocrine functions induce adaptive responses in the absorptive capacity of the intestine. Nutrient transporters in the small intestine are responsible for dietary nutrient assimilation, therefore, stress-related changes in the expression of these transporters affect the availability of nutrients and energy to the animal for growth and development. However, the effects of stress on the nutrient transporters in the small intestine of rabbits are unclear.

Therefore, this study aims to investigate the effects of glucocorticoids to reproduction of rabbits and expression levels of glucose, amino acid, and fatty acid transporters in the intestine of rabbits exposed to glucocorticoids,which provided theoretical basis for the development of some anti-stress drugs or additives and the improvement of feed conversion efficiency.

MATERIALS AND METHODS

Animals and experimental design

At 40 days of age, 80 rabbits (Hyla, male-female ratio of 1:1) with similar body weight (1388 ± 8.5 g) were divided into 2 groups: one received subcutaneous injection of dexamethasone (synthetic glucocorticosteroid, DEX, 2 mg/kg ,body weight, 8:00 am per day) for 7 d and the other received sham-treatment (Control). The body weights and feed consumption of individual rabbits were recorded daily at 8:00 am.After injection at first day, 8 rabbits with fasting for 3 h were electrically stunned and slaughtered. Mucosa samples from duodenum, jejunum and ileum were collected. After the last injection, 8 rabbits with similar weight were fasted for 3 h and then electrically stunned and slaughtered. Mucosa samples from the duodenum, jejunum and ileum were collected.

RNA isolation and analysis

Total RNA extraction and quantitative reverse transcription-PCR (**qRT-PCR**) of mucosa samples were performed as described previously. The relative amount of mRNA of a gene was calculated according to the 2 delta delta CT method (Livak and Schmittgen, 2001) using glyceraldehyde 3-phosphate dehydrogenase (***GAPDH***) as normalizing gene and the control group as calibrator group.

Table 1 :Primers of the houskeeping GAPDH gene and transporter genes for Real Time PCR

Gene	Primer sequence		Product size
GAPDH	F:TGCCACCCACTCCTCTACCTTCG	R:CCGGTGGTTTGAGGGCTCTTACT	163bp
PepT1	F:CAGCCACCATGGGAATGTCT	R:GATGACCGTGGACAGGTTGT	173bp
CAT1	F:CCAGTCTATTAGGTTCCATGTTCC	R:CGATTATTGGCGTTTTGGTC	117bp
rBAT	F:ACACCAGTGATAAACACGCTTG	R:ACCAGTTGTTGGGTGGAGTG	123bp
B^0AT	F:CCCGCCAAGTTCCCTAAG	R:GAGCCCGCGTATCTGTCC	125bp
SGLT1	F:GATTTCCCGTATGATTACCGAG	R:AAGAGGGAGACAACCACAACG	153bp

Statistical analysis

All of the data collected were subjected to one-way ANOVA analysis with the Statistical Analysis Systems statistical software package (Version 8e, SAS Institute). Homogeneity of variances among groups was confirmed using Bartlett's test (SAS Institute). When significant difference was observed in the ANOVA analysis, differences between means were assessed by unpaired t-tests. $P<0.05$ was statistically significant.

RESULTS AND DISCUSSION

Using qRT-PCR we observed lower PepT1,CAT1,B^0AT,rBAT and SGLT1 mRNA expression in different segments of small intestine of rabbits, obtained from injected with dexamethasone rabbits to sham-treated rabbits.

Glucose absorption in the intestine is important for energy balance and glucose homeostasis (Mithieu *et al.*, 2009). The absorption of carbohydrates from the lumen of the small intestine is influenced by several factors, including luminal digestion, apical membrane digestion, and transport into the enterocytes (Sklan *et al.*, 2003). Moreover, the expression levels of intestinal glucose transporters influence the absorption and uptake of glucose in the small intestine (Rodriguez *et al.*, 2004).Glucocorticoids may also reduce glucose delivery to some tissues by impairing local blood flow (Mangos *et al.*, 2000).Previous researches showed that glucocorticoids could decrease glucose utilization, and we found that injection of dexamethasone decreased the expression of SGLT1 mRNA was remarkable decreased in small intestine after injection of dexamethasone .

Little is known about the influence of stress on intestinal nutrient absorption in rabbits.In this study,PepT1 mRNA decreased with injection of dexamethasone,which consistently with results in brioler(Jiaju *et al.*, 2010).Uptake of dipeptides also caused stimulation of AA uptake by the bo,+

system (Wenzel *et al.*, 2001), suggesting that free AA uptake may also be regulated by PepT1 activity.So we observed the expression of rBAT, CAT and B0AT mRNA decreased with injection of dexamethasone.

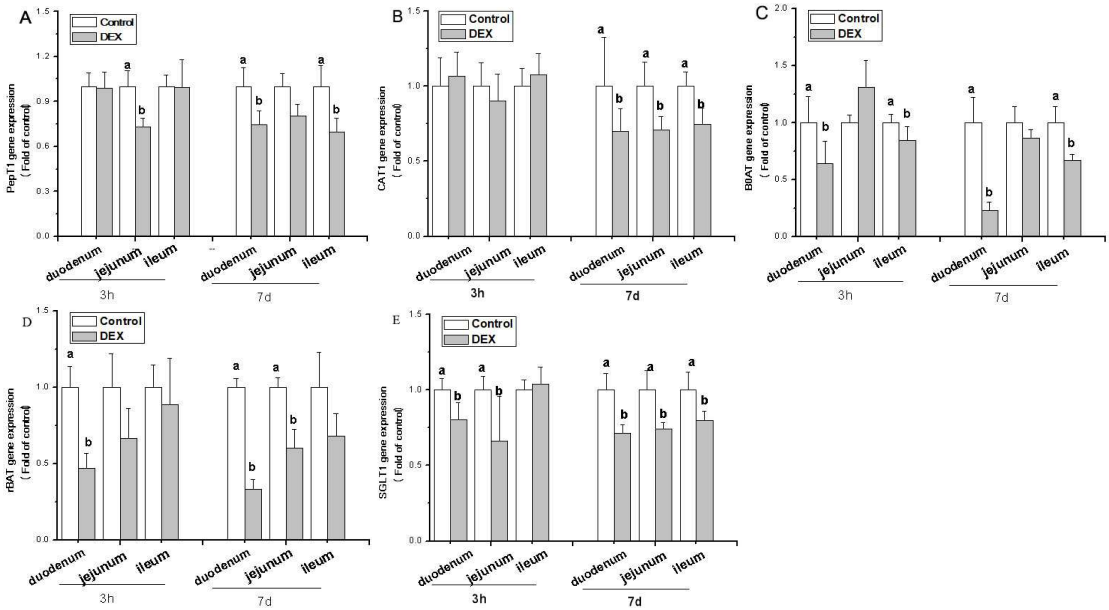

Figure 2*:*Effect of DEX treatment for short-term (3 h) and long-term (7 d) on PepT1 (A), CAT1 (B), B^0AT (C), rBAT (D, SGLT1 (E) in the gastrointestinal tract of rabbitsThe values are expressed as the mean ± SEM (n=8). [a, b] The means differ significantly (P<0.05).

CONCLUSIONS

The present study showed that glucocorticoids alter the expression of nutrition transporter,decreased the expression of peptide transporter ,amino acid tansporter and carbohydrates transporter, effect the reproduction of rabbits.The stress is thus important factors for restricting the development of rabbit breeding industry.

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