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Detection and *in vitro* regulation of NHE1 and NHE3 mRNA expression in rumen epithelia of cows*

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ABSTRACT

NHE (Na^+/H^+ exchanger) mediates electroneutral Na transport across rumen epithelium by ejection of intracellular H^+ in exchange of external Na^+ with a ratio of 1:1. It contributes to the absorption of Na^+ and maintenance of intracellular pH homeostasis of rumen epithelium. This study explored the existing of NHEs in rumen epithelia of cows and the responses of NHE1 and NHE3 mRNA to IGF-1. The results, obtained by semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR), illustrated that NHE1 and NHE3 mRNA is present in rumen epithelia of cows. The NHE1 and NHE3 mRNA expression were promoted by IGF-1 treatment (25 ng/ml) *in vitro*. The abundance of NHE1 and NHE3 mRNA were significantly higher in IGF-1 treated groups ($P<0.05$). Taking together, the current study revealed that the mRNA of NHE1 and NHE3 in rumen epithelia of cows could be positively regulated by IGF-1.

KEY WORDS: rumen epithelium, NHE, IGF-1, cow

INTRODUCTION

Changes of morphology and absorption occurred in rumen epithelia in response to feeding regime are probably mediated by IGF system. Energy-rich diet causes a marked increment of cell proliferation in rumen papillae and, consequently leads to an enlarged rumen papillae size and surface area for absorption (Dirksen

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et al., 1984; Gabel et al., 1987; Shen et al., 2004). Our previous study reported that enhanced Na^+ absorptive activity, performed by Na^+/H^+ exchanger (NHE), is associated with augmented IGF-1 concentration in plasma and increased IGF type 1 receptor in rumen papillae (Shen et al., 2004).

NHEs are membrane antiporters, with 9 isoforms in a protein transporter family, distributed to almost all of the mammalian cell types. The NHEs exert multiple effects on mammalian physiology: maintaining the intracellular pH homeostasis, regulating the cell volume, being the carrier of transepithelial Na^+ transport by ejection of intracellular H^+ in exchange for external Na^+ at a ratio of 1:1. Though it is well established that in ruminants the electroneutral Na transport across rumen epithelium is mediated by NHE (Martens et al., 1991), the stimulating effects of rumen microbial fermentation products like SCFA (Gabel et al., 1991), ammonia (Abdoun et al., 2003), as well as dietary energy intake (Shen et al., 2004) on rumen epithelial NHE activity is well documented. The knowledge of existing of NHE protein and its encoding gene in forestomach has not been completely understood. Furthermore, it is not yet very clear whether feeding regime induced up-regulation of NHE activity in rumen epithelia is mediated by IGF-1. The present study, therefore, a. detect the expression of NHE1 and NHE3 in rumen epithelia of cows and b. studies the effect of IGF-1 on the expression of NHE1 and NHE3 mRNA in rumen epithelia of cows.

MATERIAL AND METHODS

Sample collection

The epithelia for mRNA detection were removed from the ventral rumen of 6 cows in local slaughter house immediately after stunning and exsanguination. The samples were stored at -80°C for analysis. For the epithelial tissue culture the fresh rumen epithelia were taken from the caudal blind sac of 4 cows, kept on ice and transferred to laboratory.

Tissue culture and IGF-1 treatment

Epithelia were cut into small pieces of about 1-2 mm^3 and planted to the culture flask. They were allowed to attach at 37°C in an incubator with 5% CO_2. DMEM medium (Invitrogen, USA) with 8% foetal calf serum, 2 mmol/l L-glutamine and antibiotics was used, together with administration of IGF-1 at 25 ng/ml (Exp) or 0 ng/ml (Control). The cells were incubated for 24 h. After incubation the tissue samples were harvested and homogenized for RP-PCR.

RT-PCR

The mRNA expression was measured by semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was isolated by guanidinium isothiocyanate/phenol/chloroform extraction from rumen epithelia. 2 μg of this RNA, 0.04 μg/l of oligo (dN10) and 0.8 mmol/l of dNTP primer reverse-transcribed with 200U M-MLV reverse transcriptase (Promega) following the manufacture's instructions. Reverse transcriptase-generated cDNA were amplified using PCR with 18S rRNA as an internal standard. Oligonucleotide primers for NHE1 and NHE3 were designed by software (Primer Premier 5.0) followed the gene sequence source of GenBank (No.U49432 and No.AJ131764).

Statistical analysis

Data were expressed as means±SEM. The significance was determined by Student`s t-test or one-way ANOVA, LSD. $P<0.05$ was considered to be significant. The program of SPSS 11.0 for windows was used for statistical calculation.

RESULTS

mRNA of NHE1 and NHE3 were detected in rumen epithelia of all 6 cows (Figure 1). The sequences of PCR generated gene fragments were analysed. The obtained NHE1 gene fragment contains 427 bp (Figure 1 A) and the NHE3 gene fragment contains 223 bp (Figure 1 B).

The mRNA abundance of NHE1 (Figure 2) increased by 35% in IGF-1 treated group, compared with control group (1.12±0.06 vs 0.83±0.08; P=0.03). In IGF-1 treated group the mRNA abundance of NHE3 (Figure 3) was 43% higher than that in control (1.26±0.07 vs 0.88±0.09; P=0.01).

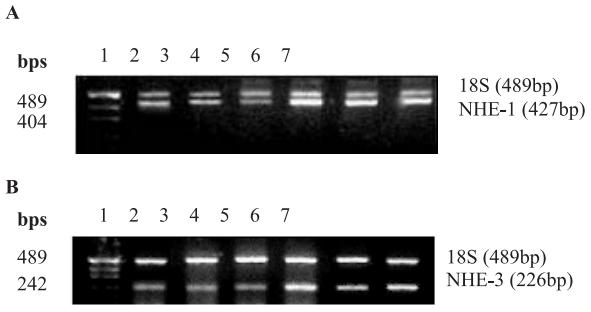

Figure 1. RT-PCR products of NHE1 (A) and NHE3 (B) mRNA in rumen epithelia of cows. Lane 1: marker of PUC19; Lane 2~7: ruminal epithelia of individual animals. 18S:18S rRNA (an intra-standard)

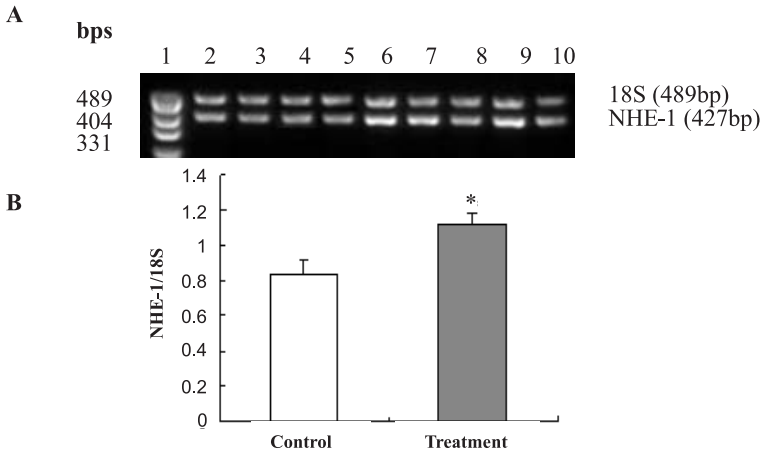

Figure 2 Effects of IGF-1 on NHE-1 mRNA expression in ruminal epithelium of cows *in vitro* *: P<0.05; (A): electrophoresis photo, Lane 1: DNA marker of PUC19; Lane 2~5: control group; Lane 6~9: treatment group; Lane 10: ruminal epithelium *in vivo*. (B): statistical analysis results

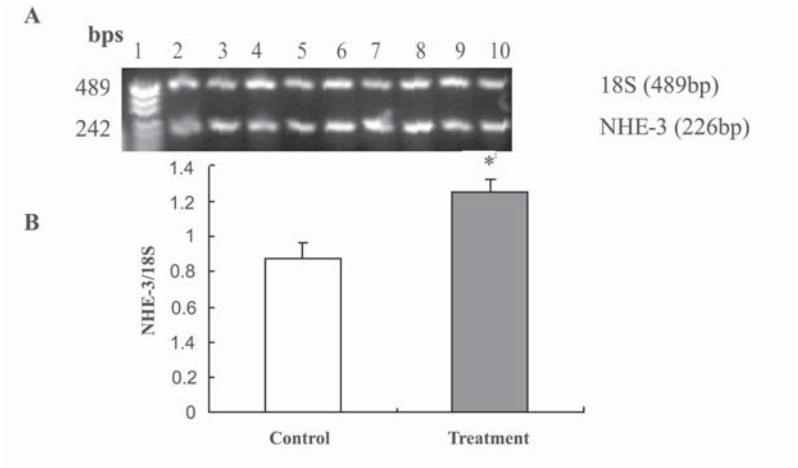

Figure 3. Effects of IGF-1 on NHE3 mRNA expression in ruminal epithelium of cows *in vitro*. *: P<0.05; (A): electrophoresis photo, Lane 1: DNA marker of PUC19; Lane 2~5: control group; Lane 6~9: treatment group; Lane 10: ruminal epithelium *in vivo*. (B): statistical analysis results

DISCUSSION

It is well documented that in mammals NHE1 is expressed in almost all cell types and tissues, and NHE3 locates in kidney, intestine and many other tissues. But, so far the evidence of NHE in rumen epithelium is insufficient. This paper,

provided data that mRNA of NHE1 and NHE3 are expressed in rumen epithelium of cows, confirmed the studies (Schweigel et al., 2005; Graham et al., 2007), which reported mRNA of NHE in bovine and ovine rumen epithelium.

The present observation of *in vitro* IGF-1 enhanced NHE1 and NHE3 mRNA abundance supports our previous hypothesis (Shen et al., 2004): in goat intake of energy-rich diet the dietary effect of stimulating ruminal NHE activity was mediated by increased plasma IGF-1 concentration and enhanced ruminal IGF-1 type receptors numbers.

CONCLUSIONS

The mRNA of NHE1 and NHE3 are existing in rumen epithelia of cow. The expression of these NHE1 and NHE3 mRNA could be positively regulated by IGF-1.

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