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• BASIC RESEARCH •

Changes of biological functions of dipeptide transporter (PepT1) and hormonal regulation in severe scald rats

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Abstract

AIM: To determine the regulatory effects of recombinant human growth hormone (rhGH) on dipeptide transport (PepT1) in normal and severe scald rats.

METHODS: Male Sprague-Dawley rats with 30 % total body surface area (TBSA)IIIdegree scald were employed as the model. In this study rhGH was used at the dose of 2 IU.kg^{-1}d^{-1}. An everted sleeve of intestine 4 cm long obtained from mid-jejunum was securely incubated in Kreb's solution with radioactive dipeptide (^{3}H-glycylsarcosine, ^{3}H-Gly-Sar, 10 μCi/ml) at 37 ℃ for 15 min to measure the effects of uptake and transport of PepT1 of small intestinal epithelial cells in normal and severe scald rats.

RESULTS: Abundant blood supply to intestine and mesentery was observed in normal and scald rats administered rhGH, while less supply of blood to intestine and mesentery was observed in rats without rhGH. Compared with controls, the transport of dipeptide in normal rats with injection of rhGH was not significantly increased (P=0.1926), while the uptake was significantly increased (P=0.0253). The effects of transport and uptake of PepT1 in scald rats with injection of rhGH were significantly increased (P=0.0082, 0.0391).

CONCLUSION: Blood supply to intestine and mesentery of rats was increased following injection of rhGH. The effects of uptake and transport of dipeptide transporters in small intestinal epithelial cells of rats with severe scald were markedly up-regulated by rhGH.

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INTRODUCTION

Small intestine is the major site of dietary protein absorption, the main route of absorption protein is transport of protein in the form of small peptides (di/tripeptide) across the small intestinal wall. H^+-coupled dipeptide transporter, PepT 1, is known to be located in the intestine and kidney, and plays an important role in the absorption of di/tripeptide. In addition, it mediates the intestinal absorption of β-lactam antibiotics, angiotension–converting enzyme inhibitors, and other peptide–like drugs[1].

Knowledge about the regulation of PepT 1 activity is limited. A number of studies have shown that dietary protein load causes an increase in di/tripeptide transport in small intestine of rats and mice[2,3]. Recent studies have shown that PepT1 in rat intestine is upregulated after a short period of fast via an increase in gene expression[4-6]. Another interesting regulation of PepT 1 expression is that PepT1 in rat' s small intestine is resistant to tissue damage induced by 5-flourouracil, whereas other markers such as sucrase activity, D-glucose uptake are significantly decreased[7]. This suggests that expression of PepT 1 is robust towards cellular damage.

Studies showed that some hormones metabolically regulated the expression of intestinal dipeptide transporter[8,9]. For example, insulin could increase the membrane population of PepT 1 by increasing its translocation from a preformed cytoplasmic pool[9]. Our previous study[10] showed that rhGH markedly stimulated the uptake and transport of cephalexin in Caco-2 cells with normal or anoxia/reoxygenation management. These results indicate that rhGH greatly upregulates the physiological functions of dipeptide transporters (PepT1) of human cell line. Although rhGH has been shown to be a major regulator of peptide transport activity[10], little is known about rhGH in regulation of peptide absorption *in vivo*, especially in rats with severe scald.

In this study, we determined whether rhGH could stimulate uptake and transport of small intestinal epithelial cells in normal or severe scald rats. We also investigated the *in vivo* application of 3H-glycylsarcosine (3H-Gly-Sar) as an ideal substrate for PepT1.

MATERIALS AND METHODS

Materials

[3H]-glycysarcosine (special activity of 1Ci (37GBq)/mmol, radiochemical purity >=97 %, work concentration in this study: 10 μCi/ml) was purchased from Moravek Biochemicals,USA. Recombinant human growth hormone (rhGH, 2 IU.kg^{-1}d^{-1}) was from Serono, Switzerland, Temperature-controlled surge culture device from Taicang Medical Instrument Co. Ltd, China. All other reagents were of analytical grade at least.

Animals

Adult male Sprague–Dawley (SD) rats (weighing 200±20 g) were housed in individual stainless steel cages in an air–conditioned room at 23±2 ℃ with a 12: 12-h light schedule and were fed normally. The weight of rats was measured daily during an experiment. The animals were treated in accordance with European Community Standards concerning the care and use of laboratory animals (INSERM and Ministere de 1' Agriculture et de la Foret, Paris, France).

Experimental groups

Rats were randomly divided into groups A, B, C and D. Group A(control group): normal feed rats, Group B: normal feed + injection of rhGH (2 IU.kg^{-1}d^{-1}) rats, Group C: scald rats and Group D: scald + injection of rhGH (2 IU.kg^{-1} d^{-1}) rats. The indices were observed on postburn days (PBDs) 0, 1, 3, 5 and 7 (n=4), respectively. Rats were killed by decapitation at every time point.

Scald injury models

Rats were anaesthetized with 2 % pentoburbite (30 mg.kg^{-1} body weight) and scald on the back to 30 % total body surface area (TBSA) III degree, and 30 min later, they were resuscitated with Ringer' s solution (2 ml.kg^{-1} per 1 % body surface area).

Preparation of everted sleeve of rat small intestine

The rats were fasted overnight and water was available ad libitum throughout the study. The rat was killed by decapitation, a laparotomy was performed. We defined the region approximately 6 cm below the ligament of Treitz, then a 4-cm long segment of small intestine (mid-jejunum) was removed, ringed immediately with Kreb' s buffer. One end of the intestinal fragment was ligated, an everted process was securely made by small tweezers, then an intact everted sleeve was formed after another terminal ligation. Each sleeve was weighed.

Uptake and transport measurement

We measured 3H-Gly-Sar taken up into intestinal epithelial cells of the everted sleeve across the brush–border membrane. The everted sleeve was rinsed with Kreb' s buffer, 0.2 ml Kreb' s buffer was injected slowly into the lumen of the everted intestinal sleeve. The whole segment was then immersed into a 50 ml flask containing dipeptide (3H-Gly-Sar) solution (10 μCi) while 5 % CO_2 and 95 % O_2 were filled into the flask. The uptake and transport experiments were performed when the device was surged continually with a frequency of 100 r/min at 37 ℃ for 15 min, then the everted sleeve was rinsed immediately with cold (4 ℃) Kreb' s buffer to stop subsequent transport and uptake of PepT1 in epithelial cells. The transport sample was harvested from the lumen of the sleeve, a 0.5 cm×0.5 cm segment was removed from the middle of the sleeve, weighed and digested with HCl_4 to obtain the uptake sample. All samples were mixed with 10 ml of scintillation cocktail and the radioactivity was determined by liquid scintillation counter.

Statistical analysis

Data were expressed as mean ±SD. Differences between groups were assessed by analysis of variance. Values less than 0.05 were considered statistically significant.

RESULTS

Blood Supply in bowel of rats

After killed by decapitation, a laparotomy was performed immediately at the different time point (0, 1, 3, 5 and 7 days) in rats (normal or scald) with or without injection of rhGH. Direct appearance of blood supply was observed in mesentery and the wall of intestine of rats. Abundant blood supply was shown in rats after injection of rhGH, while less blood supply was observed in rats without injection of rhGH (Figure 1, 2).

Uptake and transport in everted sleeve of normal rats after injection of rhGh

In comparison with the control, the transport of dipeptide (3H-Gly-Sar) in normal rats after injection of rhGH was not significantly increased (P=0.1923) while the uptake were markedly increased (P=0.0253) (Figure 3, 4).

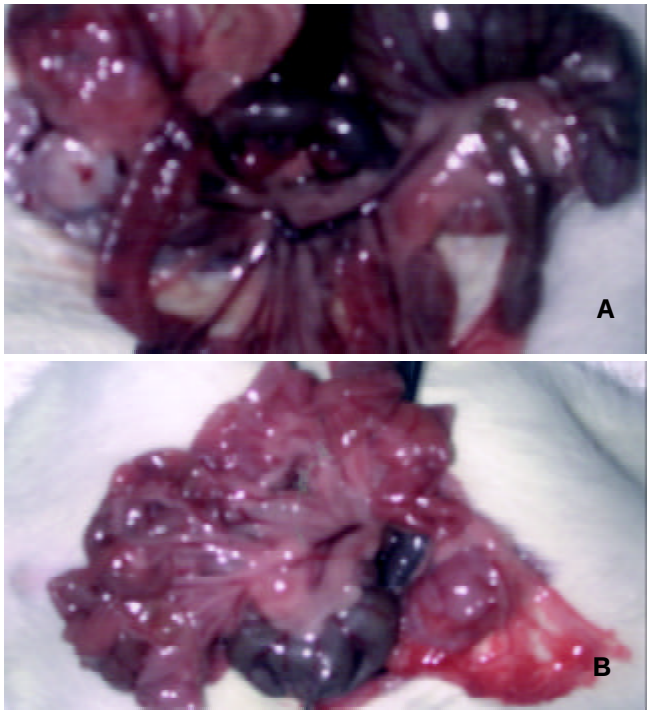

Figure 1 Blood supply to intestine and mesentery of rats 7 days after injection of rhGH was significantly abundant compared with controls. (A: rhGH group, B: control).

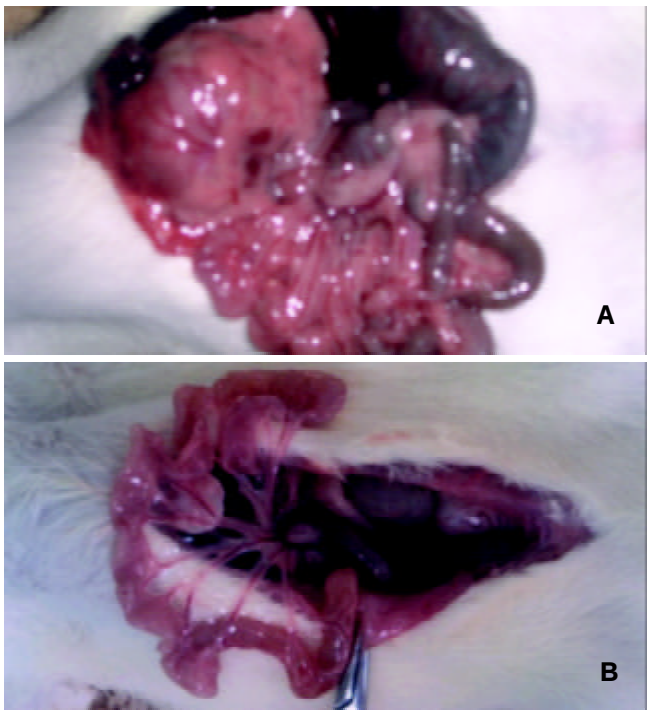

Figure 2 Blood supply to intestine and mesentery of rats with severe scald 7 days after injection of rhGH was significantly abundant compared with controls. (A: rhGH group, B: control).

Uptake and transport in everted sleeve of severe scald rats after injection of rhGH

The effects of transport and uptake of PepT1 in everted sleeve of severe scald rats after injection of rhGH were greatly increased compared with controls (P=0.0082, 0.0391) (Figure 5, 6).

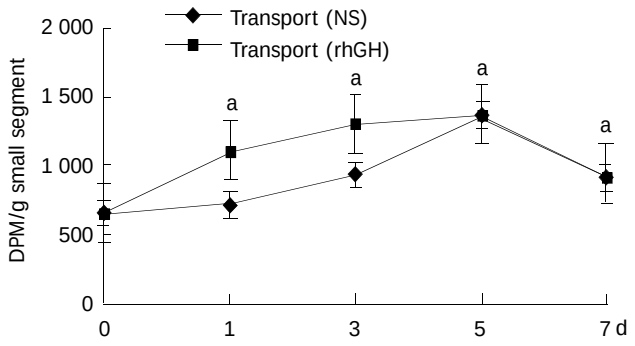

Figure 3 Transport of PepT1 in normal rats administered rhGH. Each point represents mean ±s, n=4, ^aP>0.05 *vs* control.

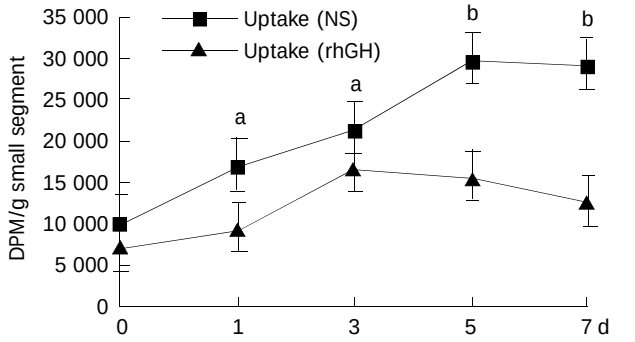

Figure 4 Uptake of PepT1 in normal rats administered rhGH. Each point represents mean ±s, n=4, ^aP<0.05, ^bP<0.01 *vs* control.

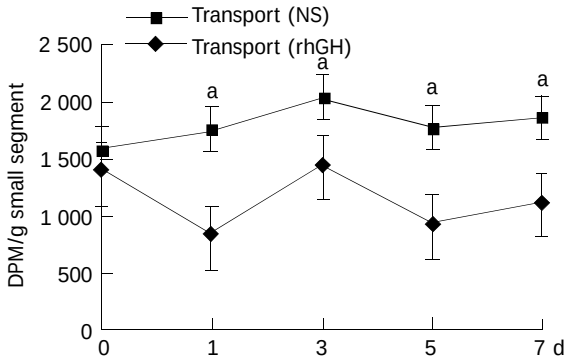

Figure 5 Transport of PepT1 in scald rats administered rhGH. Each point represents mean ±s, n=4, ^aP<0.05 *vs* control.

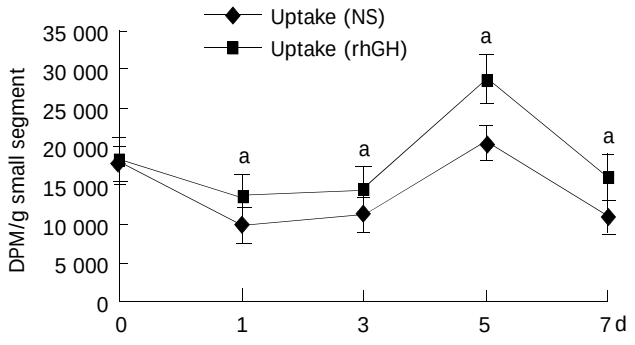

Figure 6 Uptake of PepT1 in scald rats administered rhGH. Each point represents mean ±s, n=4, ^aP<0.05 *vs* control.

DISCUSSION

We found in this study that rhGH could significantly increase the transport and uptake of peptides across intestinal epithelial barrier via proton–dependent transporter PepT1, suggesting that rhGH might be an important parameter in hormonal regulation of this transporter. It is well known that dietary proteins are absorbed as di – and tripeptides rather than free amino acids[11-14]. This absorption process is carried out by intestinal brush board transporter PepT1, which transfers peptides from a region with low dipeptidase activity (intestinal lumen) to a region with high dipeptidase activity (enterocyte cytoplasm)[15]. As a member of a family of transport proteins, PepT1 is located at the brush-border membranes of absorptive epithelial cells along the small intestine but absent in crypt and goblet cells[16,17]. PepT1 allows the use of small peptides as a source of nitrogen for enteral feeding and the route for delivery of peptidomimetic drugs such as β-lactam antibiotics. Therefore, PepT1 appears to be essential for the efficient absorption of dietary proteins[18]. Most studies on PepT1 have focused on its fundamental kinetic properties and its functional and structural characterization[19-21].

Previous studies have shown that the functions of intestine (including PepT1) were changed under the influence of many factors[22,23]. However, few reports have dealt with the hormonal regulation of PepT1. Insulin could stimulate dipeptide transport by increasing membrane insertion of PepT1 from a preformed cytoplasmic pool[9], and choleratoxin could decrease dipeptide transport by inhibiting the activity of PepT1 through an increase in intracellular concentration of adenosine 3', 5'-cyclic monophosphate[24]. Strong evidences have demonstrated that growth hormone (GH) was an important growth factor for intestine[25]. Complete GH depletion due to hypophysectomy could cause pronounced hypoplasia of small intestinal mucosa with decreased villus height and reduced crypt cell proliferation[26]. Simple replacement of GH could restore mucosal proliferative activity[27], rhGH could promote normal growth and development in the body by changing chemical activity in cells. It activates protein production in muscle cells and release of energy from fats. rhGH could significantly improve anabolism in parenteral feeding[28]. It has been typically used to stimulate growth of children with hormone deficiency, or to treat people with severe illness, burns or sepsis where destruction of human tissues and muscle occurs[29-31]. It remains unclear, however, whether the key hormone, human growth hormone (hGH) also shows some significant importance in transport and uptake of PepT1. To examine the functional changes of PepT1, everted sleeves of small intestine were used as *in vivo* intestinal model, and severe scald (30 % TBSA III degree) rats with or without injection of rhGH were employed as animal model. The results in this study indicated that the blood supply in mesentery and the wall of rat's intestine (normal or severe scald) with injection of rhGH was abundant compared with the controls. It was suggested that rhGH could increase blood supply of animal bowel, therefore, upregulate directly the physiological functions of PepT1 of small intestine.

The data in this study confirmed that both transport and uptake of PepT1 in everted sleeves of severe scald rats administered rhGH were significantly increased compared with controls. It indicated that rhGH upregulated the biological functions of PepT1. This result was in accordance with our previous research[10]. In our study, however, the transport of dipeptide in normal rats treated with rhGH was not markedly increased, while the uptake was greatly increased compared with controls. It might be due to the cytoplasmic level of dipeptidases, or a short period of experiment.

In conjunction with previous results[10], the present study further testified the enhancement effect of peptide transport by rhGH. The biological mechanism might involve increased translocation of the cytoplasmic pool of PepT1 to the apical membrance, or increased level of PepT1 mRNA. Clearly,

further study on physiology and biology of PepT1 is required to clarify the mechanism of rhGH in upregulating the functions of PepT1.

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