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Note

Non-effect of Hexamethonium, a Ganglionic Blocker, on the Response of Ileal Apolipoprotein A-IV mRNA Following a Massive Small Bowel Resection in Rats

Kei SONOYAMA,† Reiko FUJIWARA, and Takanori KASAI

Laboratory of Food Biochemistry, Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan

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An intravenous infusion of hexamethonium, a ganglionic blocker, did not affect the increase in the apolipoprotein A-IV mRNA level in the residual ileum following a massive small bowel resection in unrestrained conscious rats. The result suggests that upregulation of the apolipoprotein A-IV gene in the residual ileum is not mediated by a neural pathway, including the nicotinic synapse route.

Key words: apolipoprotein A-IV; mRNA; small intestine; small bowel resection; hexamethonium

Apolipoprotein (apo) A-IV is a component of triglyceride-rich lipoproteins that is mainly synthesized by enterocytes in the small intestine.[1] Although the precise function of apo A-IV is not known, it has been shown to modulate lipoprotein metabolism,[2-5] food intake,[6,7] and gastric functions.[8,9] For this reason, information on the expression of apo A-IV in the small intestine should lead to a better understanding of the regulation of lipoprotein metabolism, feeding behavior, and gastric functions.

We have previously reported that a massive small bowel resection resulted in a rapid increase in the apo A-IV mRNA and protein levels in the ileum remnant of fasted rats.[10] In addition, the plasma apo A-IV concentration following a small bowel resection was transiently decreased before recovering to the control level.[11] These observations suggest that enterocytes in the residual ileum responded to the loss of proximal intestine by a compensatory increase in apo A-IV expression at the pretranslational stage. However, the mechanism underlying the upregulation of the apo A-IV gene following a small bowel resection is not entirely clear. We have recently demonstrated that the intravenous infusion of either hexamethonium or atropine, but not of propranolol, diminished the basal expression of ileal apo A-IV mRNA in unrestrained conscious rats, suggesting that apo A-IV gene expression in the ileum was regulated in part by a cholinergic neuron including at least one nicotinic synapse.[12] Although a number of studies have been focused on the innervation of motility and secretion in the intestine, there have been few reports showing the neural regulation of gene expression in intestinal epithelial cells. One of these few reports has demonstrated that gene expression of the human intestinal trefoil factor in human colon cancer cell line HT-29 was stimulated by carbachol, an analog of acetylcholine, and by the neuroendocrine peptides, somatostatin and vasoactive intestinal polypeptide, suggesting regulation of the trefoil factor gene by the intestinal neuroendocrine system.[13] In the present study, to make clear whether the neural factor is involved in the upregulation of the ileal apo A-IV gene following a massive small bowel resection, we examined the effect of the intravenous administration of hexamethonium, a ganglionic blocker, on the apo A-IV mRNA level in the residual ileum following a massive small bowel resection in unrestrained conscious rats.

Male Wistar rats (Japan SLC, Hamamatsu, Japan), which were 7 wk old at the start of the experiment, were housed in individual cages in a temperature-controlled (23 ± 2°C) room with a dark period from 19:00 to 5:00 h. Twenty-four rats were subjected to cannulation into the right cervical vein as previously described.[12] After recovering for 2 days, the rats were fasted for 18 h and then subjected to either a massive small bowel resection or transection as previously described.[10] The massive small bowel resection left a 10-cm portion of the terminal ileum, and the transection was done at a point 10 cm proximal to the ileocecal valve as a control. After the surgery, six rats in both the resected and transected groups were intravenously infused for 22 h with hexamethonium bromide (10 mg/kg·h, 1 ml/h, Wako Pure Chemical Industries, Osaka, Japan) under unrestrained and fasted conditions. The remaining six rats in both groups were intravenously infused with a vehicle (0.15 M NaCl, 1 ml/h). At the end of the infusion period, the rats were anesthetized by an in-

† To whom correspondence should be addressed. Fax: +81-11-706-2496; E-mail: ksnym@chem.agr.hokudai.ac.jp

traperitoneal injection of Nembutal. After a laparotomy, two 10-cm portions of the intestine were excised, one 2 cm distal to the ligament of Treitz as the jejunal segment, and the other just proximal to the ileocecal valve as the ileal segment, and the luminal contents were washed with 10 ml of ice-cold saline. The mucosa was scraped with a glass slide and immediately plunged into liquid nitrogen. It was then stored at −80°C until RNA was isolated.

This study was approved by the Hokkaido University Animal Use Committee, and the animals were maintained in accordance with the guidelines for the care and use of laboratory animals of Hokkaido University.

Total RNA was isolated from intestinal mucosa by using Isogen (Nippon Gene, Tokyo, Japan) according to the manufacturer's protocol and then subjected to a Northern hybridization analysis to determine the relative levels of apo A-IV and apo A-I mRNA as previously described.[12]

Each result is expressed as the mean ± SEM. A statistical comparison of the mean was carried out by Duncan's multiple-range test. Differences are considered significant if $P<0.05$.

Figure 1 shows the relative mRNA levels of apo A-IV in the jejunum and ileum of the massive small bowel-resected and transected rats that had been infused intravenously with either hexamethonium or the vehicle. In the transected control animals, the ileal apo A-IV mRNA level was significantly lower in the hexamethonium-infused rats than in the vehicle-infused rats. The ileal apo A-IV mRNA level was significantly increased by the massive small bowel resection in both the hexamethonium- and vehicle-infused rats. In the bowel-resected rats, there was no significant difference between the hexamethonium- and vehicle-infused groups. The jejunal apo A-IV mRNA level was not changed by the hexamethonium. In contrast to the apo A-IV mRNA level, neither the massive small bowel resection nor intravenous hexamethonium influenced the apo A-I mRNA level in either the jejunum or ileum. These results clearly indicate that the increase in the apo A-IV mRNA level in the residual ileum following the massive small bowel resection was not affected by the intravenous administration of the ganglionic blocker. The hexamethonium-induced decrease in the ileal apo A-IV mRNA level in the transected control animals is consistent with that in our previous observations.[12] Thus, the neural factor is thought to be involved in the basal but not resection-stimulated expression of the ileal apo A-IV gene.

Our previous studies suggest that the biliary component is a luminal factor mediating the post-resectional increase in the apo A-IV mRNA level in the residual ileum.[10,14,15] In addition, our recent report has suggested that peptide YY (PYY), a gastrointestinal hormone, acts as a humoral factor mediating the

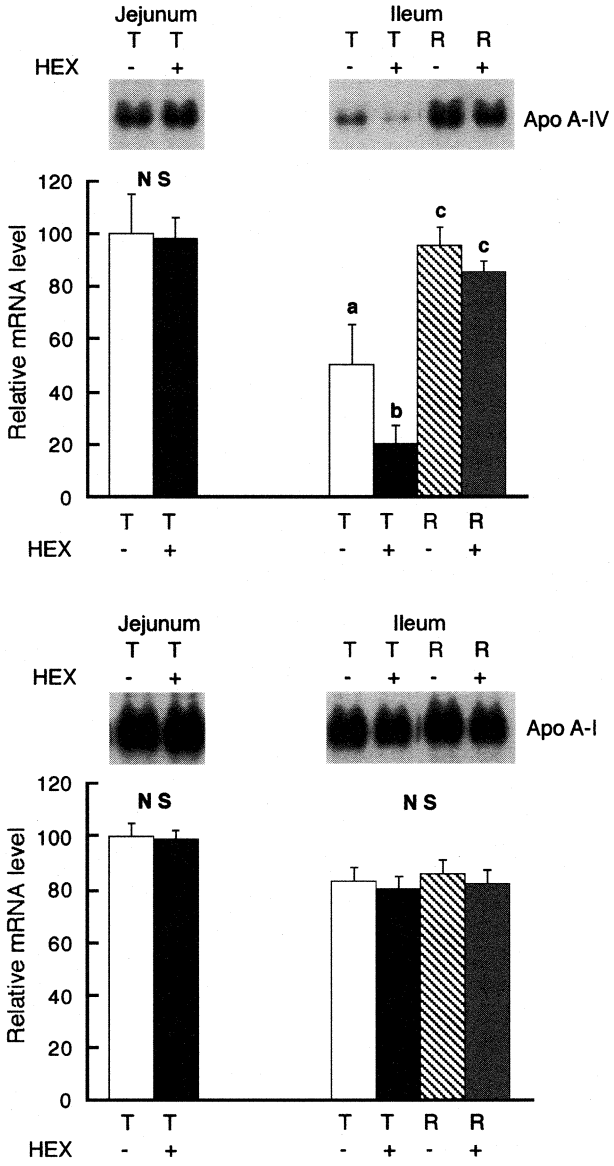

Fig. 1. Effect of an Intravenous Infusion of Hexamethonium (HEX) on the Small Intestinal Apo A-IV (upper) and Apo A-I (lower) mRNA Levels in Small Bowel-resected (R) and -transected (T) Rats.

Each value is the mean ± SEM, n = 6; values with different letters are significantly different ($P<0.05$). The values of apo A-IV and A-I mRNA were normalized to the value of 18s rRNA, and these values are expressed relative to the average values for the jejunum in the transected rats that were infused with the vehicle, which is set to 100. Insets illustrate representative Northern blots of intestinal RNA.

upregulation of ileal apo A-IV expression following a massive small bowel resection.[16] Therefore, it is of interest whether these factors interplay in regulating the apo A-IV expression in the intestine. At present, however, there is no evidence to demonstrate that PYY is associated with the bile-dependent increase in apo A-IV expression. In addition, our preliminary investigation has demonstrated that intravenous hexamethonium did not affect the increase in the ileal apo A-IV mRNA level in unrestrained conscious rats

whose pancreaticobiliary secretion had been diverted into the ileum (unpublished data). Thus, it is unlikely that stimulation of the ileal apo A-IV gene by the biliary component was mediated by neural factors.

In conclusion, we propose that the upregulation of the apo A-IV gene in the residual ileum following a massive small bowel resection was not mediated by a neural pathway, including the nicotinic synapse route.

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