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# Distribution of Prohormone Convertase-6 Expression in the Gastrointestinal Tract and Effects of a Fasting-Refeeding Regimen and a High-Fat Diet on Ileal Prohormone Convertase-6 Expression

Vidyavathi Udupi, Guillermo Gomez,
Isabel Lambertz, Xiang Qi, Jason Reed, and George H. Greeley, Jr.

*Department of Surgery, The University of Texas Medical Branch, Galveston, TX*

**Proforms of gastrointestinal peptide hormones and neuropeptides are processed, in part, by prohormone convertases (PCs) to mature, biologically active peptides. The purpose of this study was to characterize the mRNA levels of PC-6 isoforms, PC-6A and PC-6B, in the gastrointestinal tract and pancreas of the rat, and to investigate the effects of a fasting and refeeding regimen, and a high-fat diet on ileal PC-6A expression. PC-6A mRNA is expressed throughout the entire gastrointestinal tract with the highest levels in the small intestine. Multiple-sized transcripts are present. PC-6B mRNA is expressed in the antrum and fundus of the stomach, in the small intestine, and colon. Ileal PC-6A mRNA expression increases significantly with fasting and then declines with refeeding toward control levels. Increased dietary fat increases PC-6A mRNA levels in the ileum. Since PC-6 is found throughout the entire gastrointestinal tract, it is likely that PC-6 participates in the processing of proforms of gastrointestinal peptides. The two isoforms of PC-6 have different patterns of distribution in the gastrointestinal tract and pancreas, suggesting that they process proforms of different gut peptides.**

**Key Words:** Alimentary tract; mRNA; peptide.

## Introduction

Proforms of gastrointestinal peptide hormones and neuropeptides, such as gastrin, gastric-inhibitory polypeptide (GIP), pituitary adenylate cyclase activating peptide (PACAP), vasoactive polypeptide (VIP), peptide YY (PYY), glucagon-like peptides, and others, are processed in part by a selective cleavage at the C-terminal side of paired basic amino acids sites by specific endoproteases *(1–3)*. A family of endoproteases has been characterized by cDNA cloning that is homologous to Kex2, the yeast endoprotease that cleaves the α-maturing factor precursor *(1–3)*. There are presently six characterized endoproteases; they are called prohormone convertase-1 (PC-1, also called PC-3), PC-2, PC-4, PC-6 (also called PC-5), PACE 4, and furin *(1–13)*. Furin is expressed ubiquitously and is believed to participate in the activation of a variety of proteins *(1–3)*. PC-1, PC-2, and PACE 4 have been shown to be involved in the processing of several endocrine/neuroendocrine peptide hormones and neurotransmitters. PC-6 is a recently described prohormone convertase that occurs in two isoforms: PC-6A and PC-6B *(5,6,14)*. PC-6B has an exceptionally large Cys-rich region and a putative transmembrane domain not found in PC-6A *(14)*. Although one of the original reports on PC-6 tissue expression showed the highest levels in the mouse small intestine and a widespread distribution of PC-6 in the rat gastrointestinal tract, the comparative distributions of PC-6A and PC-6B in the rat gastrointestinal tract and pancreas have not been described. The large similarities in the catalytic regions of PC-6 with those of PACE 4, PC-1, PC-2, and furin suggest that PC-6 is involved in the processing of gut peptides. The purpose of this article is to compare the distributions of PC-6A and PC-6B mRNA expression in the rat gastrointestinal tract and pancreas. Because food intake and diet can affect gut peptide expression, changes in the level of food intake and diet may also affect processing enzymes potentially involved in expression of gut peptides. Therefore, a second objective of this study was to characterize the effects of a fasting and refeeding regimen and a high-fat diet on PC-6A mRNA expression in the small intestine of the rat.

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Author to whom all correspondence and reprint requests should be addressed: George H. Greeley, Jr., Ph.D., Department of Surgery, The University of Texas Medical Branch, 301 University Boulevard, Galveston, TX. 77555-0725. E-mail: ggreeley@mspo2.med.utmb.edu

## Results

The distributions of PC-6A and 6B in the rat gastrointestinal tract and pancreas are shown in Fig. 1. With the

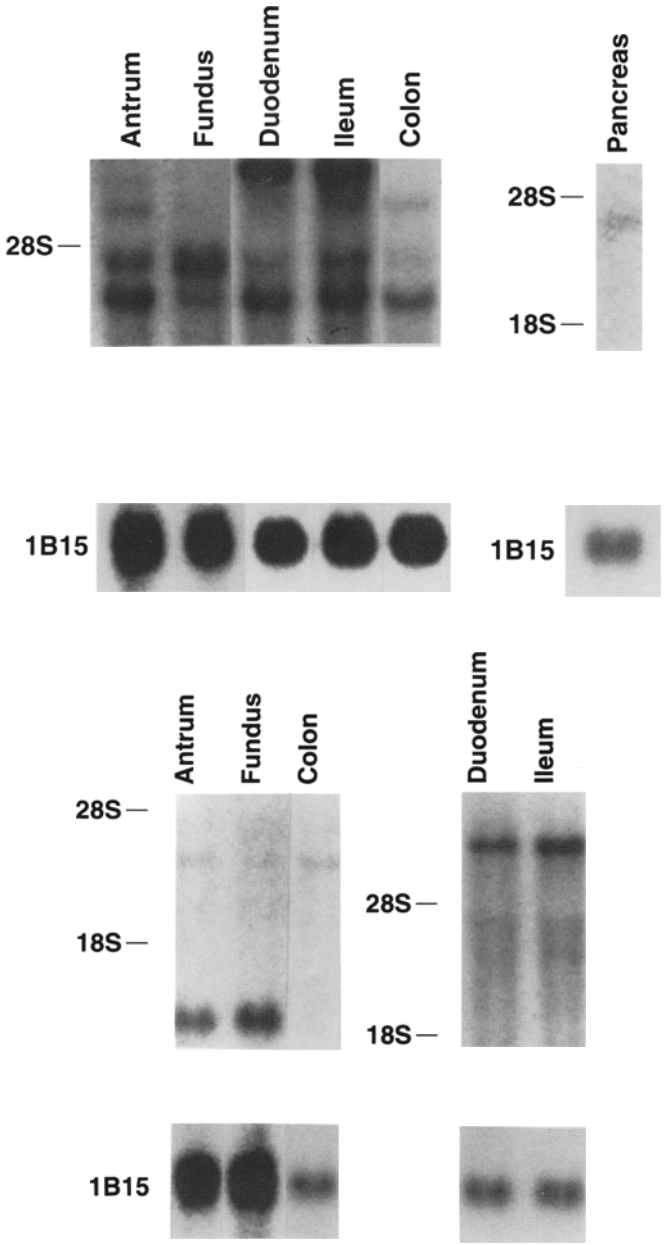


**Fig. 1.** Northern blot analysis of rat gastrointestinal tract and pancreas (10 μg Poly $[A]^+$) using riboprobes specific for PC-6A (top) and PC-6B (bottom). Representative lanes are shown; N ≥ 3 rats/tissue; RNA was analyzed from three separate rats, or three separate RNA pools prepared from several rats. In some cases, blots were overexposed to enhance visualization of the less abundant transcripts.

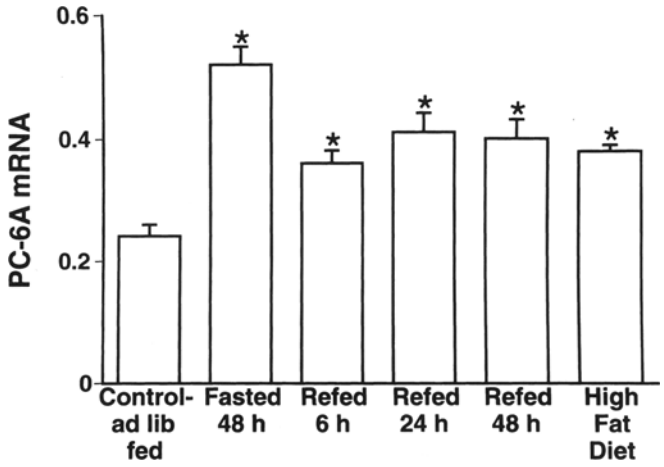


**Fig. 2.** Effects of a fasting (48 h) refeeding regimen and a high-fat diet on PC-6A mRNA levels in the rat ileum ($n = 6$ rats/group; ratio of ileal PC-6A mRNA over 18S ribosomal mRNA densitometric readings; * = $p < 0.05$ vs control ad lib-fed rats). The ileal ~3.7- and ~8.0-kb transcripts were measured; however, only the data of a single transcript (~8.0 kb) are shown, since both transcripts showed similar patterns. Northern and slot blots were hybridized with a specific $^{32}$P-labeled PC-6A cRNA probe. Blots were also hybridized with $\alpha^{32}$P-labeled 18S ribosomal mRNA probe to monitor mRNA loading and were visualized by autoradiography.

PC-6A-specific probe, transcripts of ~3.7, ~4.9, and ~6.5 kb were detected in the antrum, fundus, and colon. In the fundus and colon, the ~6.5-kb and the ~4.9- and ~6.5-kb transcripts, respectively, are less abundant. In the antrum and fundus, the ~3.7- and ~4.9-kb transcripts are the most abundant. In the duodenum and ileum, transcripts of ~3.7, ~4.9, ~6.5, and ~8.0 kb were detected. The ~3.7- and ~8.0-kb transcripts are expressed abundantly when compared to the others, and the ~6.5-kb transcript is least abundant. The cecum shows the same PC-6A mRNA pattern as the colon (not shown). In the pancreas, only a single, faint ~3.7-kb transcript is detected. With the PC-6B-specific probe, a single, abundantly expressed ~1.0-kb transcript is detected in the antrum and fundus. In the duodenum and ileum, an ~8.0-kb transcript is detected. A faint ~3.5-kb transcript is detected in the antrum, fundus, and colon. PC-6B mRNA is not detected in the pancreas (not shown).

With fasting, ileal PC-6A mRNA levels increased approximately twofold ($p < 0.05$) when compared to control ad lib-fed rats (Fig. 2). Refeeding for 6–48 h lowered ileal PC-6A mRNA levels toward those levels measured in control ad lib-fed rats. A high-fat diet increased ileal PC-6A mRNA levels significantly 1.5-fold ($p < 0.05$) when compared to control rats.

## Discussion

The present study demonstrates that PC-6A is expressed throughout the gastrointestinal tract and pancreas, whereas PC-6B is expressed only in the antrum, fundus, small intestine, and colon of the rat. With the PC-6A-specific probe, multiple transcripts were detected in the antrum, fundus, small intestine, and colon. With the PC-6B specific probe, an ~8-kb transcript was detected in the intestine, an ~1-kb transcript in the antrum and fundus of the stomach, and a 3.5-kb transcript in the colon. The present data also indicate that a fasting and feeding regimen, and a high-fat diet can influence the magnitude of PC-6A expression in the small intestine.

In an earlier mouse study *(14)*, expression of PC-6A and 6B was examined using polyadenylated (Poly $[A]^+$)RNA and specific PC-6A and 6B probes as in the present study. A single transcript (~6.5 kb) was found in the mouse small intestine with the PC-6B probe. With the PC-6A probe, three transcripts (~3.5, ~5.5, and ~6.5 kb) were detected in the mouse small intestine. Other regions of the gastrointestinal tract and pancreas were not described. There are some differences in the sizes of the more abundant transcripts in the mouse and rat small intestine. For example, with the PC-6B probe, an ~8-kb transcript is found in the rat small intestine, whereas an ~6.5-kb transcript is detected in the mouse small intestine. Additionally, it has been reported earlier that the predominant species of PC-6A in the rat gastrointestinal tract are 3.8- and 7.5-kb forms, with no expression of PC-6A in the stomach *(5)*. These differences in the stomach findings between the two studies may be owing, in part, to the fact that (Poly$[A]^+$) RNA was used in the present study, whereas the earlier report used total cellular RNA. We cannot offer an explanation for the size differences in the major rat PC-6A species. The significance of the multiple transcripts for PC-6 in the gut has not been examined, and which of these isoforms produces active enzymes is not clear presently. However, the clear abundance of the ~8.0-kb transcript in the small intestine suggests that this is the primary transcript for PC-6A in the rat. In the earlier mouse paper *(14)*, it was suggested that PC-6B is encoded by the ~6.5-kb transcript, and that PC-6A is encoded by the ~6.5-, ~5.5-, and 3.5-kb transcripts in the small intestine. One group of investigators *(14)* proposed that alternative splicing of the primary transcript and differences in the length of the 3'- and 5'-untranslated regions gave rise to the multiple PC-6 transcripts in the mouse. Similar transcriptional and posttranscriptional events may explain the different transcripts in the rat gut. The mouse PC-6 cDNAs were identified originally as corresponding to only ~3.5- and ~6.5-kb transcripts *(6,14)*, therefore, cloning and sequencing of the various transcript species are needed to understand their relevance.

The gastrointestinal tract and pancreas synthesize at least 30 different peptides *(15)*. The involvement of specific PCs in the processing for some of these enteric peptides has been described. For instance, PC-1 and PC-2 are involved in processing proinsulin to insulin *(16,17)*. Although the involvements of PC-1 and PC-2 in the processing of some gut peptides has been described, precursor processing for many gut peptides has not been examined. Because PC-6 is expressed throughout the gut, it is a candidate endoprotease for gut peptide processing. The exact cellular distribution of PC-6A/B in the gut has not been investigated in detail, although one paper mentions that PC-6A is found in the epithelial layer, which includes sparsely dispersed enteroendocrine cells *(5)*.

Interestingly, ileal PC-6A mRNA expression is responsive to fasting and feeding, and to a high-fat diet. Our laboratory and others have reported previously that fasting can influence intestinal gut peptide levels in the rat *(18–20)*. Because fasting results in a decrease of food-induced gut peptide release, the increase in ileal PC-6A mRNA levels is opposite to what we would predict. This finding suggests that PC-6A may be involved in the processing of gut peptides that exert an inhibitory influence on other peptides that are normally synthesized, processed, and released by regular food intake. Processing of peptides that act as mitogens in the gut may be inhibited as well during fasting. The ileum contains several different peptides (i.e., PACAP, CCK, VIP, substance P, and so forth) *(21–24)*, and PC-6 may participate in the processing of these peptide proforms.

Interestingly, the increase in ileal PC-6A mRNA levels with fasting did not return to control levels with 48-h refeeding. Although speculative, a transient stabilization of PC-6A mRNA to a higher level of expression may occur, and a longer refeeding period may be required to restore fasted PC-6A mRNA levels to normal.

Ileal PC-6A mRNA expression also increases significantly with increased dietary fat. Increased dietary fat can elevate expression of PYY (Greeley et al., unreported findings) and proglucagon expression *(20)*. Changes have been described for PC-1 and PC-2 translation with glucose exposure in an insulinoma cell line *(25)*. In addition, PACE4 mRNA levels in the anterior pituitary and PC-1 and PC-2 mRNA levels in the stomach change with alterations in thyroid status, and with fasting and inhibition of stomach acid secretion in the rat, respectively *(26,27)*. Furthermore, in the rat, inhibition of stomach acid secretion and fasting also affect stomach gastrin and somatostatin (SRIF) mRNA and peptide levels *(28–32)*. Together, these studies as well as the present data indicate that functional changes in gut peptide secretion are paralleled by changes in their mRNA expression as well as translation, and in expression of processing enzymes potentially involved in processing of gut peptides.

## Materials and Methods

### *Experimental Protocols*

*Experiment 1*

The purpose of this experiment was to characterize the expression of PC-6A and PC-6B mRNA in the gastrointestinal tract and pancreas of the rat. Adult male Sprague-Dawley rats (200–225 g) were sacrificed in the ad lib-fed condition. The gastrointestinal tract (antrum, fundus, duodenum, ileum, cecum, and colon) and pancreas were harvested for examination of PC-6A and PC-6B mRNA distribution by Northern blotting analysis. Excised tissues were cleaned carefully of food and feces and then homogenized immediately in 4 *M* guanidine thiocyanate (including 25 m*M* sodium citrate [pH 7.0], 0.5% sodium *N*-lauroylsarcosine, and 0.1 *M* 2-mercaptoethanol). Homogenates were stored frozen (–80°C) until centrifugation (18 h,

~100,000*g*) through a cesium chloride cushion (2 mL, 5.7 *M*) as described previously *(33,34)*. (Poly $[A]^+$) RNA then was prepared for all tissues from total cellular RNA by means of oligo(deoxythymidylate) cellulose column chromatography *(35)*. For preparation of pancreatic RNA, the pancreas was homogenized in 5 *M* guanidine, Tris-HCl (0.05 *M*), EDTA (0.01 *M*), 1.0% sodium *N*-lauroylsarcosine, and 1% 2-mercaptoethanol. LiCl (5 *M*, sevenfold dilution, v/v) was then added, and RNA was precipitated overnight at 4°C. Samples were pelleted by centrifugation, and the pellet was resuspended in 5 *M* guanidine. Pancreatic RNA was then extracted twice using a standard phenol/chloroform extraction procedure *(31)*. (Poly$[A]^+$) RNA was then prepared. For Northern blotting analysis, 10–20 μg of (Poly $[A]^+$) RNA were separated by electrophoresis using denaturing formaldehyde gels and transferred onto nitrocellulose membranes as described in detail previously *(33–35)*. In order to characterize the PC6A/B transcript sizes, RNA size markers were also electrophoresed in one lane. Membranes were hybridized with either specific $^{32}$P-labeled PC-6A or PC-6B cRNA probes. RNA loading was monitored by IB15-cyclophilin mRNA levels.

*Experiment 2*

The purpose of this experiment was to examine the effects of a fasting and refeeding regimen and a high-fat diet on PC-6A expression in the ileum. Control adult male Sprague-Dawley rats were fed a standard rat diet (AIN-76, [% by weight: protein-17; fat-5; carbohydrate-65] (Bio-Serv, Frenchtown, NJ) and the high-fat diet-fed rats were given an AIN-76 diet with fat replacing carbohydrates (% by weight: protein 41; fat 27; carbohydrate 23). In the high-fat diet arm of this experiment, rats fed the high-fat and AIN-76 (controls) diets consumed equal amounts of protein daily. AIN-76-fed rats were given AIN-76 for 2 wk before fasting; the high-fat-fed rats were fed the high-fat diet for 2 wk. In the fasting-refeeding part of this experiment, AIN-76-fed rats were fasted in wire-bottomed cages for 48 h with access to water only. Rats then were refed and sacrificed at different times after refeeding. Groups of the rats fed AIN-76 and high-fat diets were sacrificed without fasting. The ileum was harvested, and total cellular and (poly $[A]^+$) RNA was prepared as described above. Changes in PC-6A mRNA expression were quantitated by means of Northern (10 μg, (poly $[A]^+$) RNA) and slot-blot analyses (5 μg, total RNA) *(33,34)*. Slot-blot and Northern blots were processed simultaneously. To examine for specificity in changes of ileal PC-6A expression, membranes were hybridized with an appropriate mRNA cyclophilin 1B15 or a 18S ribosomal probe. The relative amounts of mRNA were calculated in arbitrary densitometric units.

***Analysis of RNA***

Appropriate RNAs were visualized using specific PC-6A and PC-6B cRNA probes labeled with [$^{32}$P]CTP. Mouse PC-6A and 6B full-length cDNAs were subcloned into the *Not* I site of pRcCMV (Invitrogen, San Diego, CA). To make full-length antisense probes, the plasmid was digested with *Hin*dIII and the transcription reaction done with SP6 polymerase. The PC-6A and 6B plasmids were given to our laboratory by K. Nakayama *(6,14)*. Hybridization was done as described previously *(6,14,33,34)*.

***Statistical Analyses***

Results are means ± SEM. Data were analyzed by analysis of variance followed by the Newman-Keuls test. Differences with a $p < 0.05$ were considered significant.

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