Procolipase mRNA: tissue localization and effects of diet and adrenalectomy

Shuichi OKADA, David A. YORK* and George A. BRAY
Louisiana State University, Pennington Biomedical Research Center, 6400 Perkins Road, Baton Rouge, LA 70808-4124, U.S.A.

Northern blot analysis has identified procolipase mRNA in rat pancreas, stomach and duodenum. Pancreatic colipase mRNA was increased by high-fat diets. Adrenalectomy increased pancreatic procolipase mRNA, an effect enhanced by high-fat diets. The results suggest that colipase is not unique to the pancreas and that diet and glucocorticoids interact in regulating the transcription of its gene.

INTRODUCTION

Enterostatin is the N-terminal pentapeptide derived from pancreatic procolipase by trypsin digestion in the small intestine [1–3]. This pentapeptide has an anorectic effect on rats after administration either peripherally [4] or centrally [5]. Recently we have shown that this peptide specifically decreases fat intake, and that enterostatin may be a satiety factor by acting as a feedback signal to regulate fat intake [5–7]. Since enterostatin is produced from pancreatic procolipase on an equimolar basis with pancreatic colipase, endogenous enterostatin production can be estimated from either colipase activity or procolipase mRNA expression.

The cDNA sequence for pancreatic procolipase has been described in a number of species, including the rat [8]. From analysis of human cDNA libraries, it has been suggested that procolipase mRNA expression is restricted to the pancreas [9]. There is little information on the regulation of this gene. Dietary fat, insulin and adrenal steroids have all been reported to affect pancreatic colipase activity or mRNA levels [10–13]. In this paper we report for the first time that procolipase mRNA is present in the stomach and duodenum in addition to the pancreas, and provide evidence to suggest that glucocorticoid hormones and dietary fat may interact in the regulation of pancreatic procolipase mRNA levels.

EXPERIMENTAL

Materials

All reagents were purchased from Sigma (St. Louis, MO, U.S.A.) and, unless otherwise stated, were of molecular biology grade. The cDNA probe for β-actin was purchased from CLONTECH (Palo Alto, CA, U.S.A.). The probe was the 2.0 kb insert from Bluescript II at the *Eco*RI site [14]. The oligonucleotide probe used for pancreatic colipase was based on the amino acid sequence Tyr^{58}-Tyr-Arg-Cys-Pro-Cys-Glu^{64}, which is highly conserved in porcine, equine and human colipases [1–3]. The probe used was 5′-TCACAGGGACACCTGTAGTA-3′, which was based upon the cDNA analysis of rat colipase [8], and was synthesized by the Advanced DNA Technologies Laboratory (Texas A & M, College Town, TX, U.S.A.).

Animals

Sprague–Dawley and Osborne Mendel rats used in these studies were purchased from Charles River Company (Indianapolis, IN, U.S.A.). They were 7–9 weeks old at the start of the study. The Sprague–Dawley rats were fed on rat chow *ad libitum* and were used for Northern blot analysis of the tissue distribution of procolipase mRNA.

The Osborne Mendel rats used to study the effects of adrenalectomy and dietary fat on procolipase mRNA levels were fed either a high-fat diet (HF; 56% of energy supplied as fat) or a low-fat diet (LF; 10% of energy supplied as fat). The compositions of these diets have been described previously [15]. Rats were adapted to these diets for 2 weeks, after which time they were either adrenalectomized bilaterally or received sham operations [16]. After a further 2 weeks the rats were killed by decapitation, and tissues were cleanly dissected, frozen in liquid nitrogen and stored at −80 °C until used for RNA isolation.

RNA isolation

Total RNA was isolated by the method of Chirgwin et al. [17]. A 50 μg portion of total RNA was run on 1.5% (w/v) agarose gels before transfer to nylon zetaprobe membranes.

Northern blot analysis with oligonucleotide probe for colipase

The oligonucleotide probe was labelled with ^{32}P by a DNA 5′-terminus labelling system (BRL, Gaithersberg, MD, U.S.A.). Hybridization to the labelled probe was performed in a solution containing 5 × SSC (0.75 M NaCl, 75 mM sodium citrate), 20 mM sodium phosphate buffer, pH 7, 7% (w/v) SDS, 10 × Denhardt's solution and denatured herring sperm DNA (100 μg/ml) at 50 °C overnight. The membranes were washed twice for 30 min with 3 × SSC, 10 × Denhardt's solution, 5% (w/v) SDS and 25 mM sodium phosphate buffer, pH 7.5, at 50 °C.

Northern blot analysis with cDNA probe for β-actin

This cDNA probe was labelled with ^{32}P using the multiprime DNA labelling system (Amersham Corporation, Arlington Heights, IL, U.S.A.). Hybridization to the labelled probe was performed in solution containing 50% formamide, 0.25 M sodium phosphate buffer, pH 7.2, 0.25 M NaCl, 7% SDS and 1 mM EDTA at 43 °C overnight. The membranes were washed twice for 15 min each time with 2 × SSC/0.1% SDS at room

Abbreviations used: HF diet, high-fat diet; LF diet, low-fat diet.
* To whom correspondence should be addressed.

temperature, and once for 15 min with 0.1 × SSC/0.1 % SDS at 65 °C.

Radioimmunoassay

Corticosterone levels were assayed using a radioimmunoassay kit (ICN Diagnostic, Anaheim, CA, U.S.A.) in order to confirm the success of the bilateral adrenalectomies.

Data analysis

The Northern blot signals were analysed by laser beam densitometry and quantified by comparison of the colipase hybridization signal with the β-actin signal. Data were analysed by two-way ANOVA.

RESULTS

A single mRNA band of approx. 0.6 kb in size was identified by the oligonucleotide probe for procolipase in the pancreas, duodenum and stomach, but not in a range of other tissues (Figure 1). In the stomach, the procolipase mRNA appeared to be regionally distributed, being abundant in the antrum and body of the stomach, but absent from the fundus (Figure 2).

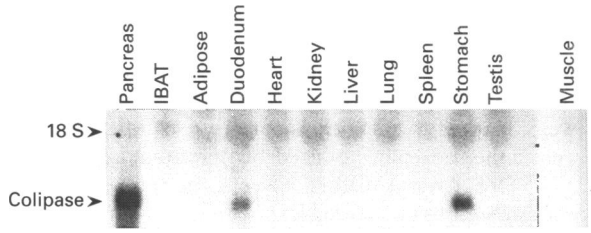

Figure 1 Tissue distribution of colipase mRNA

Tissues were dissected and washed in sterile saline before extraction of total RNA. The tissues analysed were as shown. IBAT (interscapular brown adipose tissue), adipose tissue of the epididymal depot, the duodenum (intestine from pylorus to ligament of Trietz) and muscle from the hind limb were used.

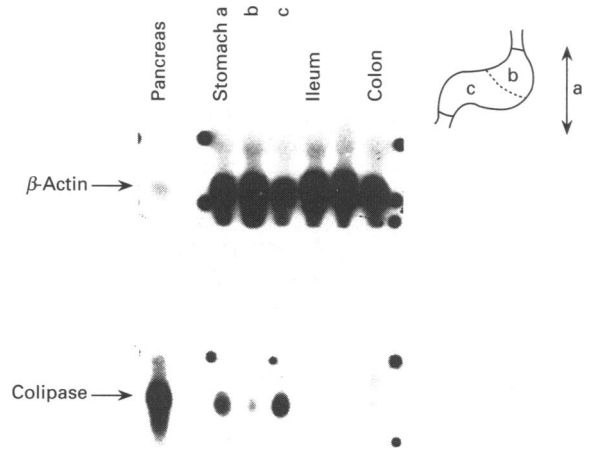

Figure 2 Regional distribution of colipase mRNA in stomach

The stomach was carefully dissected free from all surrounding tissues, opened longitudinally and washed in three changes of sterile saline to remove all contents. Antral (b) and fundic (c) regions were separated along their clear anatomical boundary and RNA was extracted from each portion. A second whole stomach (a) was also used for extraction of RNA. The intensity of the actin and colipase mRNA signals was compared with that of pancreatic RNA. The results are indicative of the relatively low abundance of colipase mRNA in stomach regions.

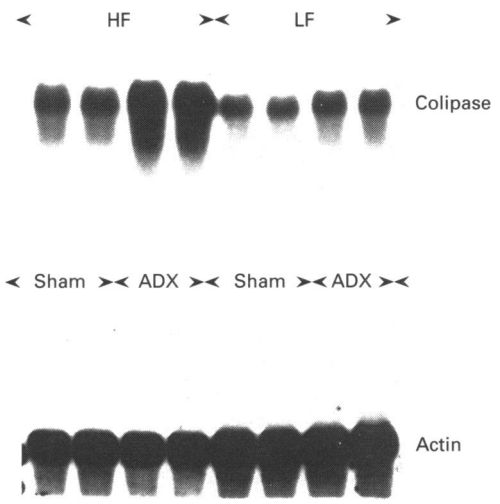

Figure 3 Representative Northern blot of pancreatic colipase mRNA showing the effects of diet and adrenalectomy

Osborne Mendel rats adapted to the two diets for a 2 week period were either adrenalectomized (ADX) or sham-operated (Sham) before being killed a further 14 days later. Densitometric data for all experimental animals are given in the text.

Pancreatic procolipase mRNA levels were significantly increased in rats fed on the HF diet. Adrenalectomy increased the pancreatic procolipase mRNA level in the HF group, but there was little effect of adrenalectomy in rats fed on the LF diet. Representative Northern blots from two rats per group are presented in Figure 3. The densitometric analysis for all animals (four per group) indicated procolipase/β-actin mRNA ratios (means ± S.E.M.) of 0.78 ± 0.01 (sham/HF diet), 1.34 ± 0.02 (adrenalectomy/HF diet), 0.41 ± 0.02 (sham/LF diet) and 0.51 ± 0.01 (adrenalectomy/LF diet). Two-way ANOVA showed significant effects of diet, adrenalectomy and the diet–adrenalectomy interaction (diet, F [1,20] = 25.4; $P < 0.05$; adrenalectomy, F [1,20] = 26.7, $P < 0.05$; adrenalectomy–diet interaction, F [1,20] = 20.9, $P < 0.05$). Analysis of serum corticosterone levels confirmed the success of the adrenalectomies (ng/ml: HF/sham, 40.0 ± 11.3; HF/adrenalectomy, < 0.6; LF/sham, 35.1 ± 9.8; LF/adrenalectomy, < 0.1).

DISCUSSION

Rat pancreatic procolipase cDNA was sequenced by Wicker and Puigserver [8]. They reported that the entire cDNA clone contained 525 nucleotides, including a poly(A) tail of 80 nucleotides [8]. Our observation that the oligonucleotide probe hybridized with a single band of size approx. 0.6 kb suggested that the probe recognized procolipase mRNA. In order to confirm this specificity, we studied the tissue distribution of this hybridization signal. Surprisingly, a single procolipase mRNA band was detected in the duodenum and stomach in addition to the pancreas, but not in a range of other tissues, including skeletal muscle, liver, adipose tissue and other regions of the intestine. We have also screened RNA and mRNA from whole brain, cortex, cerebellum, hippocampus, hypothalamus, striatum and pituitary gland, but failed to identify the presence of procolipase mRNA in any of these tissues (results not shown).

Lowe et al. [9] suggested from their studies of human cDNA libraries that procolipase mRNA was unique to the pancreas.

They failed to detect procolipase mRNA in the stomach. It is not clear whether this reflects a species difference between rat and man or a methodological difference. However, the discrepancy may be explained by our reported observation that the colipase mRNA is localized to the antral/body regions of the stomach and is not present in the fundus. Our mRNA was extracted initially from total stomach, whereas the human cDNA library screened by Lowe et al. [9] was undoubtedly prepared from mRNA isolated from a small sample of human stomach. They may thus have failed to observe colipase mRNA in the stomach because of its regional specificity.

Recently we reported that voluntary fat intake in rats was correlated with endogenous enterostatin levels, as estimated from colipase activity [15]. In addition to its role in fat digestion, pancreatic procolipase may play a pivotal role in initiating the satiety sequence to fat feeding by the release of enterostatin from its N-terminal end. We confirm previous observations that procolipase mRNA expression is increased by feeding HF diets [12,13], but extend this observation to a different species of rat (the Osborne Mendel rat), and we show that the effect of adrenalectomy on procolipase mRNA was diet-dependent, being pronounced in rats fed the HF diet but absent in rats fed the LF diet. HF diets appear to alter responsiveness to glucocorticoids and the control of the hypothalamic–pituitary axis, but the mechanism of these effects is not clear [18]. It may involve changes in glucocorticoid receptor number [19]. The response of procolipase mRNA level to adrenalectomy may reflect the direct consequence of removal of glucocorticoid hormones, or alternatively an indirect effect of changes in insulin levels. Pancreatic colipase activity is increased in streptozotocin-diabetic rats [7] and reduced by administration of insulin. In our experiment with Osborne Mendel rats, there was no significant difference in insulin level between the HF and LF groups, although there could be differences in their insulin sensitivity. Likewise, type II glucocorticoid receptor blockade does not affect insulin levels in HF- or LF-fed Osborne Mendel rats [20]. Thus it seems likely that the effect of adrenalectomy on procolipase mRNA is independent of changes in insulin levels.

We have recently reported abnormal regulation of pancreatic procolipase mRNA levels in the genetically obese *fa/fa* rat [21]. In contrast to lean rats, the *fa/fa* rat has low levels of procolipase mRNA that are restored to normal after adrenalectomy. From these studies we suggested that a reduction in enterostatin secretion may underlie the hyperphagia and fat preference of these obese rats.

To date, the significance of the presence of pancreatic procolipase mRNA in the stomach is unknown, and we have yet to identify colipase protein, colipase activity or enterostatin peptide in this tissue. However, cholecystokinin, which is secreted from the pancreas, is also found in the stomach, where it is thought to regulate antral function [22]. It is possible that if enterostatin is released from stomach, colipase may also regulate antral function or enhance efferent vagus activity to convey information on feeding to the brain.

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