Kallikrein-gene expression in the rat gastrointestinal tract

Peter J. FULLER, Karen VERITY, Bronwyn A. MATHESON and Judith A. CLEMENTS
Medical Research Centre, Prince Henry's Hospital, St. Kilda Road, Melbourne, Victoria 3004, Australia

The serine proteinase glandular kallikrein has been demonstrated in the gastrointestinal tract, although there is some doubt as to whether it is synthesized there or derives from exocrine-gland secretions. Using a rat pancreatic kallikrein cRNA probe we have demonstrated kallikrein-like gene expression in the corpus, duodenum, jejunum, ileum, caecum and colon, and compared the pattern of expression with that of the gastrointestinal peptides somatostatin, gastrin and glucagon. In addition, using a panel of oligonucleotide probes specific for various members of the rat kallikrein-gene family, we have shown that the kallikrein-like gene expressed appears to be expressed as true kallikrein.

INTRODUCTION

Glandular kallikreins are arginine-bond-specific esteropeptidases involved in the processing of prohormone precursors to their bioactive end products [for reviews, see Schachter (1980), Fuller & Funder(1986), Isackson et al. (1987) and MacDonald et al. (1988)]. Kallikrein-like enzyme activity has been demonstrated in various regions of the rat gastrointestinal tract (Zeitlin et al., 1986). This activity has been purified from rat and human gastric mucosa (Uchida et al., 1980; Uetsuji et al., 1982). More recently, kallikrein-like immunoreactivity and kallikrein-like (kininogenase) enzyme activity have been demonstrated in mucous cells of the colon of several species (Schachter et al., 1983, 1986) as well as in mucous cells elsewhere in the gastrointestinal tract (Schachter et al., 1986). In addition, the inactive pro-form of kallikrein has been previously demonstrated in the colon of various species (Seki et al., 1972). Although such studies suggest that kallikrein is synthesized in the gastrointestinal mucosa, Skagen & Andersen (1986) found levels of kallikrein to be decreased in surgically isolated loops of bowel. Since experimentally administered porcine kallikrein has been shown to be absorbed in the rat gastrointestinal tract (Overlack et al., 1983), Skagen & Andersen (1986) argued that kallikrein seen in the gut might be of submaxillary or pancreatic origin.

Swift et al. (1982) used a rat pancreatic kallikrein cDNA probe to demonstrate kallikrein-like gene expression in the pancreas, kidney, submaxillary gland, parotid, spleen and prostate. Clements et al. (1986) and, more recently, Pritchett & Roberts (1987) have extended these data to include the rat anterior pituitary gland. The rat kallikrein/arginine esteropeptidase family probably consists of eight (Ashley & MacDonald, 1985b) to 17 (Gerald et al., 1986) genes compared with 24 in the mouse (Evans et al., 1987) and possibly only three in the human (Baker & Shine, 1985) genome. Of the rat genes which have been characterized (Swift et al., 1982; Ashley & MacDonald, 1985a,b; Gerald et al., 1986; Chen et al., 1988), only one would appear to be a pseudogene (Gerald et al., 1986). mRNA sequences for six transcribed genes have been derived from submaxillary-gland and/or pancreatic cDNA libraries (S or PS series) and kidney (K) or prostatic (P) cDNA libraries (Swift et al., 1982; Ashley & MacDonald, 1985a; Clements et al., 1988). Thus, in addition to PS, which encodes true kallikrein, and S2, which encodes tonin, several other similar but distinct arginine-esteropeptidase genes have been sequenced. These have been designated kallikrein-like (S1, K1, P1) or tonin-like (S3) with respect to amino acid sequence, although their specific enzymic function is as yet unknown.

In the present study we have shown that the gastrointestinal tract is a site of kallikrein-like gene expression by demonstrating the presence of kallikrein-like mRNA throughout the gastrointestinal tract. In addition, we have used oligonucleotide probes specific for six rat arginine esteropeptidase genes, namely PS, S1, S2, S3, K1 and P1, to identify the specific gene expressed as true kallikrein.

MATERIALS AND METHODS

Tissue preparation

Male Sprague–Dawley rats weighing 120–180 g from a pathogen-free colony bred in the Central Animal House of Monash University were used in all experiments. Rats were maintained on water and standard rat chow ad libitum. Animals were killed by cervical dislocation, and the relevant tissues were dissected, snap-frozen in liquid N2 and stored at –70 °C. The specific regions examined were the corpus of the stomach, antrum, duodenum, jejunum (a 2 cm segment 20 cm from the duodenum), terminal ileum, caecum and the ascending, transverse and descending colon.

mRNA analysis

Total RNA was isolated by the method of Chirgwin et al. (1979). Polyadenylated RNA was prepared by the method of Aviv & Leder (1972). Northern-blot analysis was performed as previously described (Clements et al., 1986; Fuller et al., 1986). Briefly, 12.5 μg of total RNA was denatured in 1 M-glyoxal/50% dimethyl sulphoxide, electrophoresed in a 1.2%–agarose gel and transferred to Hybond nylon membranes (Amersham International) (Thomas, 1983). The membranes were baked at 80 °C for 2 h, u.v.-cross-linked for 10 min and prehybridized at 42 °C in hybridization solution [50% formamide/5 x SSPE (1 x SSPE is 0.15 M-NaCl/10 mM-sodium phosphate/1 mM-EDTA, pH 7.4), 5 x Den-
hardt's solution (1 × Denhardt's solution is 0.02% bovine serum albumin/0.02% Ficoll 400/0.02% polyvinylpyrrolidone), herring sperm DNA (100 μg/ml) and Escherichia coli tRNA (200 μg/ml)] before hybridization at 65°C for 20 h with the cRNA probes or at 42°C for 24–36 h with the cDNA probe. The blots were then washed once at room temperature in 2 × SSC (1 × SSC is 0.15 M- NaCl/0.015 M-sodium citrate, pH 7.0), 0.1% SDS and then twice for 15 min at 65°C (cRNA probes) or 50°C (cDNA probe) in 0.2 × SSC/0.1% SDS. They were then blotted dry and exposed to Kodak X-AR (Eastman–Kodak, Rochester, NY, U.S.A.) or Fuji X-ray film with a Cronex Lightning-Plus intensifying screen (du Pont, Wilmington, DE, U.S.A.) at −80°C. For hybridization with oligonucleotide probes, blots were prehybridized at 42°C in 5 × SSC/50 mM-sodium phosphate (pH 8.0)/10 × Denhardt's solution/0.1% SDS/herring sperm DNA (100 μg/ml), hybridized for 24–48 h at 37°C, washed in 0.1 × SSC/0.1% SDS at room temperature and then at 37°C. Before rehybridization with a different probe, blots were placed in boiling distilled water for 2 min, re-exposed to determine the adequacy of removal of the previous probe, then prehybridized and hybridized as before.

32P-labelled probes

The kallikrein cRNA probe was constructed by subcloning a 300 bp restriction-endonuclease-HindIII-BgII fragment of the rat pancreatic kallikrein cDNA clone pXP39 (Swift et al., 1982) into the plasmid pGEM-4 (Promega Biotech, Madison, WI, U.S.A.) with the 3'-end of the sense strand of the cDNA contiguous with the SP6 promoter. This construct was linearized with HindIII, enabling synthesis of an ~300-base 32P-labelled cRNA probe in a reaction mixture containing 1 μg of linearized plasmid DNA, 10 units of SP6 polymerase (Promega), 40 mM-Tris/HCl, pH 7.5, 6 mM-MgCl2, 1 mM-spermidine, 10 mM-NaCl, 500 mM-ATP, -GTP and -CTP, 12 μM-UTP, 10 mM-dithiothreitol, RNasin (an RNAase inhibitor; 1 unit/μl; Promega) and [α-32P]UTP (> 400 Ci/mm; BRESA, Adelaide, South Australia, Australia) (Melton et al., 1984). The somatostatin, gastrin and glucagon cRNA probes have been described previously (Fuller et al., 1987; Brand & Fuller, 1988). Relative levels of kallikrein mRNA between tissues was determined by scanning the autoradiograph with a densitometer (ISCO gel scanner 1312).

The rat tubulin cDNA probe (Lemischka et al., 1981) was labelled by nick translation (Amersham Nick Translation Kit; Amersham International) with [α-32P]dCTP (1800 Ci/mm; BRESA).

Oligonucleotide probes specific for the previously described (Swift et al., 1982; Ashley & MacDonald, 1985a) rat kallikrein genes (PS, S1, S2 and S3), together with probes specific for two additional rat kallikrein-like cDNAs recently cloned from kidney (K1) and prostate (P1), which have been described in detail elsewhere (Clements et al., 1988), were provided by Dr. Ray MacDonald (Southernwestern Medical Center, Dallas, TX, U.S.A.). To ensure hybridization and wash conditions were sufficiently stringent to distinguish the different kallikrein-gene-specific mRNAs, dot-blots of 5 ng of denatured cDNA (PS, S1, S2, S3, P1 and K1) were included in each Northern hybridization. Probes were end-labelled with [γ-32P]ATP (~ 2000 Ci/mm; BRESA) to a specific radioactivity of ~106 c.p.m./μg.

RESULTS

As Fig. 1(a) shows, the kallikrein cRNA probe hybridizes to kidney RNA as reported previously (Swift et al., 1982; Fuller et al., 1986). Hybridization to transcripts of equivalent size is also seen in the various regions of the gastrointestinal tract examined, with the probable exception of the gastric antrum. The level of kallikrein mRNA is highest in the caecum (~ 64% of the level in the kidney), slightly lower in the corpus (~50%) and colon (ascending ~60%, transverse ~56%, and descending ~43%), much lower in the duodenum (~23%) and very low, but clearly detectable, in the jejunum (~3%) and terminal ileum (~6%). Although a very faint band can be seen in the gastric antrum (Fig. 1),
Kallikrein-gene expression

Hybridization of the kallikrein-gene-specific oligonucleotide probes (Fig. 2) shows that the kallikrein gene expressed in the gastrointestinal tract is true kallikrein, since only the PS-specific oligonucleotide hybridizes to the corpus, duodenal and colonic RNA. Whereas all of the probes hybridize with the submaxillary-gland RNA, only K1 and PS hybridize with the kidney RNA; only S3 and P1 hybridize with the prostate RNA, as has been previously described (Ashley & MacDonald, 1985b; Clements et al., 1988).

DISCUSSION

By using a kallikrein cRNA probe derived originally from rat pancreas (Swift et al., 1982), we have obtained hybridization, on Northern blots, with a species of mRNA in the gastrointestinal tract of equivalent size to that seen in the kidney. In a previous report (Swift et al., 1982) in which kallikrein mRNA was not detected with a cDNA probe in the 'intestine', only the small intestine was studied. Even with the highly sensitive cRNA probe, levels of kallikrein mRNA are found to be very low in both jejnum and ileum.

The kallikrein-like activity previously detected in the stomach (Uchida et al., 1980; Schachter et al., 1986; Kobayashi & Ohata, 1984) has been suggested to be derived from the salivary glands (Fuller & Funder, 1986; Skagen & Andersen, 1986). The present results, however, leave no doubt that synthesis of kallikrein does occur in the corpus of the stomach, though not in the gastric antrum. Schachter et al. (1986) have localized gastric kallikrein by immunocytochemistry and enzyme histochemistry to the surface epithelial mucous cells of the rat stomach. The histochemical studies by Schachter et al. (1986), and our mRNA data, clearly indicate local synthesis of kallikrein in the small intestine, albeit at lower levels than those observed in the corpus, caecum or colon. Miller et al. (1984) have demonstrated incorporation of [35S]methionine into kallikrein-like immunoreactivity in the rat colon; Seki et al. (1972) have shown that the inactive zymogen (precursor) form of kallikrein is present in the colon of various species. Schachter et al. (1983, 1986) have localized kallikrein-like enzymes in the colon to the goblet cells, as is the case in stomach and intestine. The demonstration of local synthesis does not preclude the possibility that some portion of the kallikrein-like activity reported in the gastrointestinal tract (Uchida et al., 1980; Uetsuji et al., 1982; Schachter et al., 1983, 1986; Zeitlin et al., 1986), particularly the upper region, is derived from exocrine-gland secretions.

The seven members of the rat kallikrein-gene family so far characterized show extensive similarity in their nucleotide sequences. Though the cRNA probe used in these studies encodes true kallikrein, it will readily cross-hybridize with the other genes (Ashley & MacDonald, 1985a). The mRNAs for the various genes may be

pattern of expression, being localized predominantly to terminal ileum, caecum and colon. The similar levels of non-specific binding to the 28S ribosomal RNA seen with the various cRNA probes demonstrates the relatively uniform loading of RNA. This is reinforced by equivalent hybridization with the tubulin cDNA probe (Fig. 1e) to the various gastrointestinal-tract RNAs, except the jejunal RNA, which may be loaded at slightly lower levels.

Hybridization with the kallikrein-specific oligonucleotide probes (Fig. 2) shows that the kallikrein gene expressed in the gastrointestinal tract; small (results preparations this band; 3 days).

The probe used for panel PS is specific for the true glandular kallikrein gene (PS). The other probes are specific for P1 (a kallikrein-like gene), K1 (a kallikrein-like gene), S1 (a kallikrein-like gene), S2 (true tonin gene) and S3 (a tonin-like gene). The position of the 18 S and 28 S ribosomal bands is indicated in the top panel. Autoradiography was for 3 (PS), 1 (P1), 4 (K1), 3 (S1), 3 (S2) and 3 (S3) days.

this band has been absent from blots of other preparations (results not shown) and may thus represent a small amount of contamination by corpus RNA.

The somatostatin cRNA probe (Fig. 1b) yields a very different pattern of hybridization along the gastrointestinal tract; note particularly the high levels observed in the gastric antrum. The gastrin cRNA probe (Fig. 1c) hybridizes almost exclusively with the antral RNA (lane 3). The glucagon cRNA probe (Fig. 1d) yields a distinct
distinguished on blots or by ‘in situ’ hybridization (van Leeuwen et al., 1987) by the use of short synthetic oligonucleotide probes directed at regions where the sequences are maximally different between family members. A panel of oligonucleotide probes specific for the six members of the rat kallikrein-gene family characterized so far (PS, S1, S3, K1, P1) detected expression in the gut of only PS, true kallikrein (Ashley & MacDonald, 1985a).

In vitro, kallikrein and/or tonin have been shown to cleave prorenin to yield renin (Sealey et al., 1978), pro-opiomelanocortin to yield adrenocorticotrophic hormone (Seidah et al., 1979) and atriopeptinogen to yield atrial natriuretic factor (Currie et al., 1984). The different pattern of expression of the kallikrein gene compared with that for the precursors of somatostatin, gastrin, glucagon and cholecystokinin (results not shown) suggests that they are unlikely to be necessarily subject to processing by kallikrein in the gastrointestinal tract. Schachter et al. (1986) postulate that kallikrein may have a role in goblet cells, processing the mucoprotein peptide segments, and that abnormal kallikrein function may contribute to the pathogenesis of cystic fibrosis. Similar speculation has related the possible role of kallikrein in epithelial chloride-ion transport to the pathogenesis of cystic fibrosis (Fuller & Funder, 1986). Since the only proven physiological substrate for true kallikrein is kininogen, the role of gastrointestinal kallikrein is probably to process circulating kininogen [kininogen mRNA has not been detected in the rat gastrointestinal tract (G. Schreiber, unpublished work)], to yield bradykinin. Kinins are thought to be involved in the regulation of local blood flow (Overlack et al., 1983) and/or chloride-ion transport (Musch et al., 1983; Cuthbert & MacFinish, 1986).

Identification of the expression of the kallikrein gene in the gastrointestinal tract establishes local synthesis as a source of the observed kallikrein-like activity; this should enable further studies of the regulation of its expression. Though the physiological substrate is yet to be identified, it is likely to be circulating kininogen, resulting in the generation of kinins.

We thank Dr. Ray MacDonald for the synthesis of the kallikrein oligonucleotide probes and his encouragement of this work. We also thank Professor John Funder for his critical reading of the text, and Mrs. Sue Smith and Ms. Sue Panckridge for preparation of the manuscript. This work was supported by the National Health and Medical Research Council of Australia. P.J.F. is a Wellcome Australian Senior Research Fellow in Medical Science.

REFERENCES

Ashley, P. L. & MacDonald, R. J. (1985a) Biochemistry 24, 4512–4520
Ashley, P. L. & MacDonald, R. J. (1985b) Biochemistry 24, 4520–4527

Received 25 April 1989; accepted 4 July 1989