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A gastrin gene is expressed in both porcine pituitary and antral mucosal tissues

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ABSTRACT

By employing S1 nuclease mapping of total RNA isolated from porcine cerebral cortex, cerebellum, hypothalamus, pituitary, kidney, liver, pancreas, intestine, and antral mucosa, we have investigated gastrin gene expression in these tissues. Our results show that a gastrin gene is expressed only in the antral mucosal and pituitary tissues. Based on the amount of gastrin specific probe protected from S1 nuclease digestion in the presence of a given weight of total RNA, the amount of gastrin mRNA present in pituitary is approximately 330 times lower than in antral mucosa. These findings help establish the tissue distribution of gastrin gene expression.

INTRODUCTION

Gastrin, a peptide hormone, is one of the most studied gastrointestinal hormones for its chemistry and physiology (1). The major physiological actions of gastrin are regulation of gastric acid secretion, antral smooth muscle activity, and growth of gastric mucosa (2). The primary physiological source of the hormone is the pyloric antral mucosa in the mammalian stomach. Relatively smaller amounts of gastrin have been found in the small intestine (chiefly in duodenum)(3) and in the pituitary (4). The localization of gastrin in these tissues was made immunologically by radioimmunoassay and by immunocytochemistry of the respective tissues. Since the gastrin specific antibodies employed in these studies could have crossreacted with cholecystokinin (CCK), which is present in small intestine and brain, the significance of these results have been viewed with extreme caution (5).

To determine whether a gastrin gene is expressed in any of the major tissues of the brain and gut other than antral mucosa, we employed S1 nuclease mapping of total RNA as a means of detecting gastrin specific mRNA in several porcine tissues. In this paper, we report our finding that a gastrin gene is expressed only in the pituitary and antral mucosal tissues, among the several tissues examined. Our results are consistent with the previous contention that gastrin is present in the porcine pituitary as well as in antral mucosal tissues.

EXPERIMENTAL

Animals

Male domestic swine weighing 50-60 pounds were housed under standard conditions and provided with food and water ad libitum. The animals were anesthetized with 700-800 mg of ketimine followed by 5-7 ml pentobarbital, tissues were removed as quickly as possible and immediately frozen in liquid nitrogen.

RNA isolation

Total RNA was extracted from a given tissue by homogenization at 14°C in 5M guanidinium isothiocyanate containing 50mM Tris-Cl, pH 7.5, 10mM EDTA, 4% (wt/vol) N-laurylsarcosine, and 5% (v/v) 2-mercaptoethanol using a polytron in 30 second bursts (12). The solution was adjusted to 15% (Wt/vol) CsCl and centrifuged at 8,000 rpm at 20°C for 20 min. to remove insoluble material. The supernatant was centrifuged through a 5.7 M CsCl cushion as described (6). The pelleted RNA was extracted with n-butanol - $CHCl_3$ (7:3) and then ethanol precipitated. The RNA pellet was suspended in 2mM EDTA pH 7.0 and stored at -20°C.

Preparation of single-stranded labeled probe

Single-stranded labeled probe, complementary to gastrin mRNA, was prepared by the following primer extension-restriction enzyme digestion method. A recombinant M13 phage DNA (2µg), containing the coding region (PstI-SstI) of hog gastrin mRNA (7), sequencing primer (0.5 pmol 15-mer), dCTP, dTTP, dGTP (each 20µM), and (α^{32}P)dATP(15pmol, 3000 Ci/mmol) were treated in 12µl of sequencing buffer (10mM TrisHCl, pH7.5, 5mM $MgCl_2$, 1mM DTT) containing 1 unit of DNA polymerase I (large fragment) at 23°C for 30 min. Then dATP was added to a final concentration of 20µM and the reaction continued for 5 min. at 23° followed by inactivation of DNA polymerase by heating at 65°C for 10 min. The reaction mixture was adjusted to the salt concentration required for the appropriate restriction endonuclease cleavage. After incubation for 2 hr. at 37°C, the reaction mixture was extracted with an equal volume of phenol/$CHCl_3$/isoamylalcohol (25:24:1, vol/vol/vol) and precipitated with ethanol. Strands were denatured by heating in the presence of DMSO and then separated by electrophoresis on a 5% acrylamide gel as described (8). The radioactive band was electroeluted and recovered after precipitation with ethanol.

S1 nuclease mapping of the total RNA

The labeled probe was mixed with an appropriate amount of total RNA and nucleic acids were ethanol precipitated. The precipitate was resuspended in 2x hydridization buffer (1.0M NaCl, 2mM EDTA, and 80-mM Pipes, pH 6.4) by incubation at 37° for 10 min. Freshly deionized formamide (7µl) was added, the solution was mixed by vortexing, heated at 70° for 10 min. and then hybridized at 50° for 12 hr.

200µl of cold 1xS1 buffer (0.05M Na acetate, pH 4.5, 200 mM NaCl, 1 mM $ZnSO_4$ and 1% glycerol) and 100 units of S1 nuclease were added. The reaction mix was incubated at 37° for 5 min. followed by further incubation at room temperature for 45 min. The reaction was stopped by adding 10µl of 200mM EDTA, pH 7.0 followed by extraction with phenol/$CHCl_3$/isoamylalcohol/2-mercaptoethanol (250:250:25:1). 5µg of yeast tRNA was added and the mixture was ethanol precipitated. The pellet was resuspended in 10mM NaOH, formamide dye mix was added and the samples were analyzed by 6% acrylamide urea gels.

Northern blot hybridizations

Appropriate amounts of total RNA were incubated with glyoxal and DMSO and electrophoresed on a 1.5% agarose gel as described previously (9). The RNA was transferred from the gel to nitrocellulose and hybridized to a ^{32}P-labeled single-stranded probe as described previously (10).

RESULTS AND DISCUSSION

Previously we have shown that a gastrin gene is expressed in porcine mucosal tissue by successful isolation and characterization of a gastrin cDNA clone (7) and northern hybridization of mRNA isolated from this tissue (11). In the present studies, the porcine gastrin cDNA clone, pPG3282 (7) was employed as a radioactive probe in screening the total RNAs for gastrin mRNA. The reason for employing total RNA instead of poly(A)-RNA was to avoid possible loss of gastrin mRNA, whch may be unadenylated in brain tissue (13). In addition, selection of poly(A)-RNA from total RNA by oligo(dT)-cellulose is difficult to correlate quantitatively from experiment to experiment.

Since the goal of our present studies is to detect gastrin mRNA which is in low abundance in the total RNA population, a highly sensitive and reliable assay involving protection of the labeled gastrin cDNA probe when it hybridizes to gastrin mRNA, from S1 nuclease was employed. Total cellular RNA was isolated from 10 adult tissues by the guanidinium isothiocyanate procedure, which is known to result in a comparatively low level of RNA degradation (12). For generation of a high specific activity radiolabeled probe, we subcloned the PstI-SstI fragment of clone pPG3282 (7), which contains the entire gastrin coding sequence, into M13 mp8 RFDNA. By employing a primer-extension and restriction digestion method, a single stranded probe of high specific activity (1-2x10^8cpm/µg of DNA) was isolated and used in the S1 nuclease experiments. The conditions selected for hybridization and for S1 nuclease treatment were such that no non-gastrin specific hybridization leading to gastrin probe protection was observed. The results of the S1 nuclease mapping studies are displayed in Fig. 1 and summarized in Table 1. Since the probe employed contains

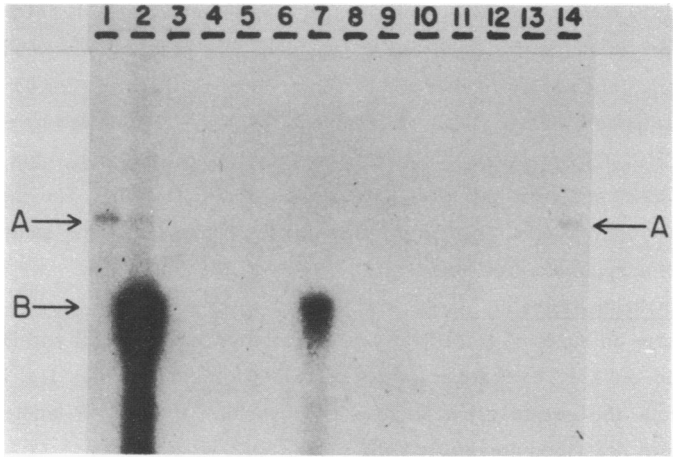

Figure 1. S1 nuclease mapping of total RNA with the gastrin specific probe.

Each lane corresponds to a reaction mixture containing 1 x 10^6 cpm of ^{32}P labelled probe with total RNA from a specific tissue or a control reaction. Lanes 2, 4-8 and 10-12: reaction mixtures containing hog antral mucosa (10µg), upper intestine (75µg), lower intestine (75µg), liver (75µg), pituitary (75µg), hypothalamus (75µg), cortex (75µg), cerebellum (75µg), and pancreas (75µg), RNA respectively. Lane 1: probe, no RNA, no S1 nuclease. Lane 3: probe, S1 nuclease, no RNA. Lane 9: no RNA, no probe, no S1 nuclease and Lane 13: probe, S1 nuclease, no RNA. Arrow A denotes the size of the full length probe (373 nucleotides) of gastrin coding sequence plus 46 nucleotides of M13 phage and polylinker sequence. Arrow B denotes the size of the probe fragment (327 nucleotides) protected from S1 nuclease in lanes 2 and 7. The autoradiogram was exposed for 96 hours, at -80° with two intensifiers.

TABLE I

Relative levels of gastrin mRNA in adult porcine tissues.

Tissue	Amount of RNA used (µg)	Radioactivity Protected (cpm)	Amount of gastrin mRNA relative to pyloric antral mucosa (%)
Pyloric Antral Mucosa	10.0	1536	100
Small Intestine	75.0	< 8	-
Large Intestine	75.0	< 8	-
Liver	75.0	< 8	-
Kidney	75.0	< 8	-
Pancreas	75.0	< 8	-
Spleen	75.0	< 8	-
Cerebral Cortex	75.0	< 8	-
Pituitary	75.0	108	0.94
Hypothalamus	75.0	< 8	-

The data shown represent the quantitative estimation of radioactivity present in the region of the gel where S1 protected radioactivity is present.

a 46 nucleotide sequence of M13 phage and polylinker, the probe length of 373 nucleotides should be reduced to 327 nucleotides upon treatment with S1 nuclease if the gastrin coding region is protected by gastrin mRNA. The presence of the uncomplementary sequence of 46 nucleotides in the probe also serves as a control for determining the activity of the S1 enzyme. The probe, in the absence of any added RNA, is completely digested by S1 nuclease and shows very little background on the autoradiogram (Fig. 1, lane 13) whereas as few as 15 cpm of ^{32}P in a band (Fig. 1, lane 1, Band A) could be detected on an autoradiogram exposed 96 hr. at -80° with two intensifiers. When 10 µg of antral mucosal total RNA was used, an intense band of 327 nucleotides with substantial tailing was observed (Fig. 1, lane 2). This tailing radioactivity appears to be due to the presence of shorter fragments of the gastrin mRNA in the total RNA because it is resistant to S1 digestion, and similar tailing is observed when relatively larger amounts of antral mucosal RNA are used in Northern hybridizations (data not shown). Futhermore, the amount of radioactivity present in the entire tailed area corresponds to only 1.2% of the total radioactivity indicating that a very small fraction of the total gastrin mRNA is degraded in these preparations.

Among all the other RNAs examined only the pituitary total RNA showed a radioactive band similar to the 327 nucleotide band (Fig. 1, lane 7) but the intensity of this band was considerably weaker than the band observed with the antral mucosal RNA. Protection of the 327 nucleotide band in the pituitary RNA indicates the presence of gastrin mRNA in the total pituitary RNA while the absence of this band (Fig. 1, lane 7) in all other RNAs tested strongly suggests the absence of gastrin mRNA in these tissues. Thus, these results suggest that the gastrin gene is expressed only in the antral mucosal and pituitary tissues and not in all the other tissues examined. These findings are consistent with the previously published results of Rehfeld (4) that gastrin is localized in the pituitary tissue. In addition, these results establish that the presence of gastrin in the pituitary tissue is due to the expression of the gastrin gene and not to the exogeneous delivery of gastrin to this tissue.

The relative amounts of gastrin mRNA present in the antral mucosal and pituitary tissue total RNAs was determined by quantitative estimation of the radioactivity present in each lane in the region of the 327 nucleotide species and below. As shown in Table 1, 1536 cpm were protected by 10µg of antral mucosal RNA whereas only 108 cpm were protected by 75 µg of pituitary RNA. Based on our calculations, the level of gastrin mRNA present in the pituitary RNA is approximately 1% of the level in antral mucosal RNA. This low level of gastrin mRNA in pituitary may be due to either a substantially lower abundance of gastrin producing cells in this tissue or a lower efficiency of gastrin gene expression in this tissue.

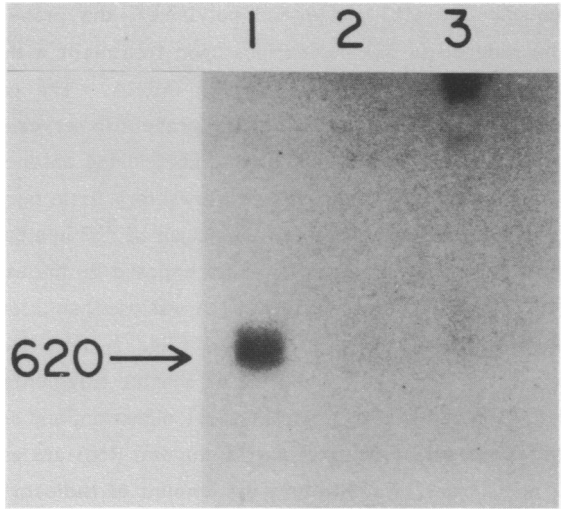

Figure 2. Northern hybridization of total RNA with the gastrin specific probe. RNA was electrophoresed as described previously (9). The filter was hybridized to ^{32}P-labelled gastrin specific complementary probe (1 x 10^6 cpm/ml hybridization buffer) as described previously (10) and exposed to X-ray film for 136 hr. at -80° with two intensifiers. The porcine total RNA samples are: Lane 1: antral mucosa, 5µg RNA; Lane 2: distal duodenal mucosa, 20µg RNA; La 3: pituitary, 20µg RNA.

Our attempts to detect gastrin mRNA in the pituitary total RNA by northern blot hybridization were unsuccessful. As shown in Fig. 2, lane 1, gastrin mRNA can be detected in the antral mucosal total RNA (5µg), but no gastrin mRNA is detectable when 4 times more lower duodenal and pituitary total RNA is used (Fig. 2, lanes 2 and 3, respectively). However, detectable hybridization in the form of a streak of larger size RNAs was detected with both duodenal and pituitary RNAs. The signal in the uppermost part of these 2 lanes appears to be due to nonspecific binding of the probe to regions of high RNA concentration. These radioactive counts could be slowly washed from the filter by extensive washing without reducing the intensity of the gastrin band in the lane 1. These results show that very low abundant mRNA species, which cannot be detected in total RNA by the northern hybridization method, can be readily detected by the S1 mapping procedure. The drawback of the S1 procedure is that interspecific probes cannot be employed whereas such probes are suitable for the northern blot hybridization method.

In summary, of the ten porcine tissues examined, the gastrin gene is expressed only in the pituitary and antral mucosal tissues. The level of gastrin gene expression in the pituitary is approximately 1% of the level in antral mucosal tissue. The expression of a gastrin gene in pituitary tissue allows the use of pituitary derived cell-

lines in the study of gastrin gene regulatory sequences. Studies along these lines are presently being pursued.

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