SIZE AND CHARGE HETEROGENEITY OF IMMUNOREACTIVE GASTRIN IN EXTRACTS OF PORCINE ANTRA

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SUMMARY: Two components immunoreactive to porcine gastrin antisera have been found in porcine antral extracts by starch gel electrophoresis and gel filtration. One corresponds to the heptadecapeptide hormone gastrin. The other displays the same size and charge characteristics reported by Yalow and Berson for BG in human plasma and human tissue extracts. Dialysis was of considerable value in separating the two.

In hypergastrinemic states, the major fraction of immunoreactive human plasma gastrin is larger and more basic (1) than the gastrin heptadecapeptide isolated by Gregory and Tracy (2). The physiologic function of the latter molecule in exocrine gland secretion has been extensively studied (3). However, the recent discovery of a larger gastrin-like molecule, known as BG (1), and the determination of its distribution in plasma and gastrointestinal tissues (4,5) has thrown into question the role played by gastrin in gastric physiology. In plasma, there appears to be more BG than gastrin but in tissue extracts it is the heptadecapeptide that is found in excess. The relative abundance of BG in gastrointestinal tissues has been found to increase as one proceeds distally however, and it is the only detectable component in proximal jejunal mucosal extracts (5). The origin of BG, its function, and its relation ship to gastrin are unresolved problems in gastric physiology.

Although BG has been demonstrated in human antral extracts, it has not yet been identified in other animals. It is the purpose of this communication to demonstrate the existance of BG in porcine antral extracts. By use of the techniques described herein it may be possible to isolate BG in quantities sufficient to elucidate its chemical and physiologic properties.

METHODS

Gastrin concentrations were determined by the radioimmunoassay method of Yalow and Berson (6). Porcine gastrin antisera (1:5000 titer) was obtained from Wilson Laboratories. Five picograms of gastrin produced a 10% drop in the B/F ratio.

An extract of hog pyloric linings treated by the method of Gregory and Tracy (2) was obtained from Wilson Laboratories. This material was an acetone precipitate of the pH 4 supernatant remaining after heptadecapeptide gastrin has been precipitated. The acetone precipitate was dissolved in either .05M acetic acid (HOAC), pH 5, containing 0.1% human serum albumin (HSA) or 0.02M veronal buffer, pH 8.4, containing 0.1% HSA. After centrifugation at 85,000 g for 30 min, 4 ml of supernatant was applied to a column (2.6 x 85 cm bed) containing Sephadex G-50, medium. Elution was with the same solution used to dissolve the acetone precipitate. Fractions corresponding to elution volumes of 250 to 300 ml and 320 to 370 ml were pooled, concentrated by lyophilization, and redissolved in minimal volumes of water. These were designated PI and PII respectively. Aliquots of PI and PII were then rechromatographed on the same column and the fractions assayed for gastrin by radioimmunoassay. Ribonuclease A (RNase A) and pure porcine gastrin were also chromatographed to calibrate the column.

Forty microliter samples of PI, PII, pure porcine gastrin, free bromphenol blue dye, and bromphenol blue-stained serum albumin were placed in alternate slits of the starch gel and subjected to electrophoresis. After the free dye had migrated about 9 cm from the origin, sections were cut from the block at 1 cm intervals and frozen overnight. After thawing, gastrin was eluted by maceration in 2 ml of veronal buffer (.02M, pH 8.4) containing 1% HSA. Gel particles were removed by centrifugation and the supernatant solutions were radio immunoassayed for gastrin.

Three ml of the acetone precipitated material dissolved in .05M HOAC - 0.1% HSA (pH 5) was placed in a boiled dialysis sac and dialyzed against the

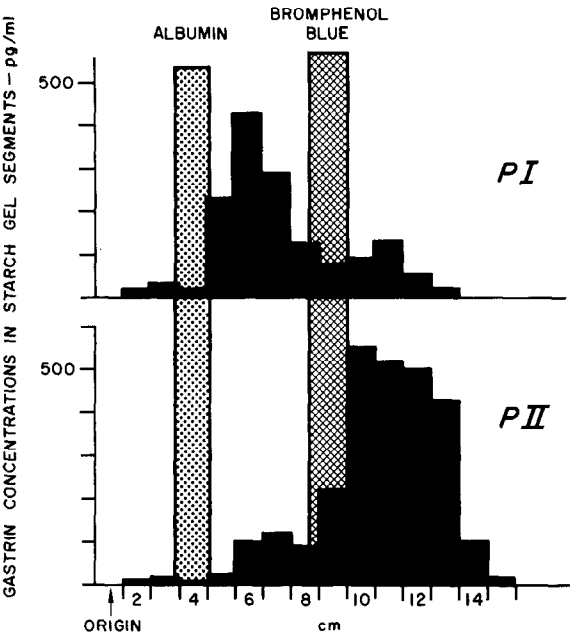

Fig. 1. Starch gel electrophoresis of PI and PII.

same solution with frequent changes at 4°C. The dialyzed solution was then subjected to gel filtration as described above and the fractions assayed for gastrin.

RESULTS AND DISCUSSION

Starch gel electrophoresis of porcine antral extracts revealed two regions of gastrin-like activity. The material in PII migrated immediately in front of free bromphenol blue dye, displaying the same electrophoretic mobility as porcine heptadecapeptide gastrin (Fig. 1). In contrast, the material in PI demonstrated less acidic character migrating immediately in front of serum albumin and behind the free dye (Fig. 1). BG, the gastrin-like component demonstrated in human plasma and tissue (4,5), displayed similar electrophoretic characteristics, i.e., migrating immediately in front of serum albumin.

Partition coefficients (K_{av})(7) of 0.34 and 0.65 were found for RNase A and porcine gastrin upon gel filtration. When PI was rechromatographed on

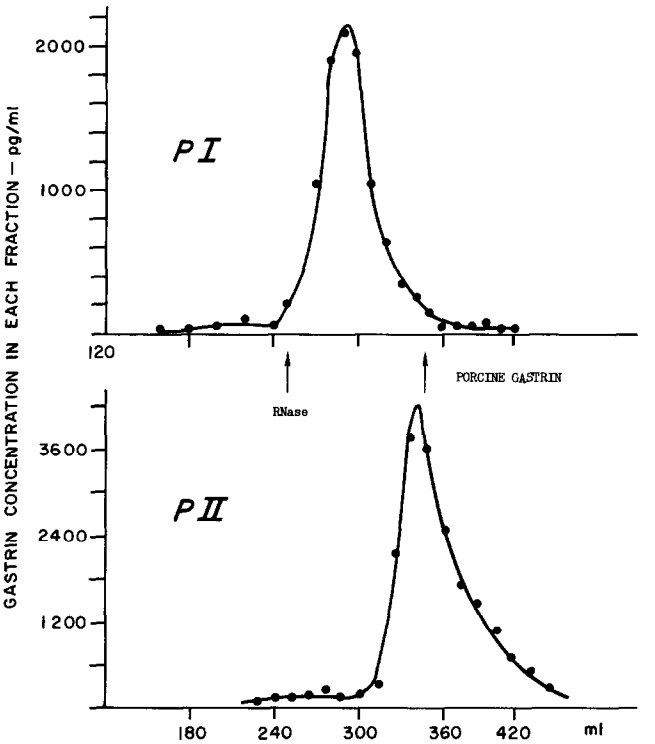

Fig. 2. Gel filtration of PI and PII. Elution peaks of RNase and pure porcine gastrin are indicated.

this column a single peak with a K_{av} = 0.47 was found (Fig. 2). By relating the partition coefficients of RNase and porcine gastrin to their molecular weights, 13,800 and 2100 respectively, we estimate that the gastrin-like material in PI has a molecular weight of about 6200. Approximately the same molecular weight (7000) was found for the more basic gastrin component (BG) in human plasma and human tissue extracts (1). The material in PII, which behaved like heptadecapeptide gastrin on electrophoresis, emerged as a single peak with some trailing upon rechromatography (Fig. 2). Its partition coefficient of 0.63 corresponds to that found for the heptadecapeptide (0.65).

By both electrophoretic and gel filtration techniques we have demonstrated in porcine antral extracts the existence of two components which react with porcine gastrin antisera. The component that migrated immediately in front

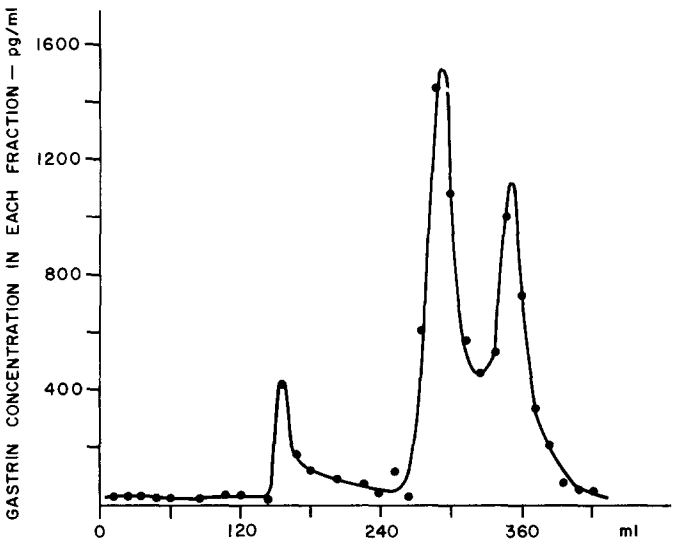

Fig. 3. Gel filtration of the dialyzed acetone precipitate.

of free bromphenol blue on electrophoresis and emerged upon gel filtration with a partition coefficient of 0.63 corresponds to the heptadecapeptide first isolated by Gregory and Tracy (2). The larger and more basic component has the same size and charge characteristics found for BG in human plasma and human tissue extracts (1,4,5). This similarity suggests that the gastrin-like material found in PI may be identified as porcine BG.

The acetone precipitate contained 5 to 10 times more gastrin than BG as estimated by radioimmunoassay.* It was not possible because of this large difference to resolve both gastrin and BG peaks by directly assaying the original gel chromatography fractions. Dialysis of the acetone precipitate resulted, however, in the selective removal of more than 80% of the heptadecapeptide. Gel filtration of the dialyzed solution produced two clearly resolved peaks (Fig. 3).

*The amounts of BG reported here are only relative. The radioimmunoassay system utilized in this work has been developed for heptadecapeptide gastrin. Although cross immunoreactivity is unquestionable, one cannot predict "a priori" if the antisera is more, less, or equally as immunoreactive to BG as to gastrin. The resolution of this problem must await the isolation of sufficient quantities of pure BG to allow for the development of a specific radioimmunoassay.

The first major peak eluted at a volume that corresponded to that found for BG in Fig. 2; the second to that found for gastrin. (The small peak at 150 ml contained immunoreactive material that was bound to serum albumin and eluted in the void volume). It seems likely from these results that dialysis may be of significant value in separating these two gastrins.

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