PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 53, 284-288 (1976)

The Search for Submammalian Gastrins: The Identification of Amphibian Gastrin[1] (39529)

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Little information is available as to the phylogenetic distribution of the gastrointestinal hormone gastrin. It is hoped that by studying the phylogenetic evolution of this hormone one will be able to provide some insight into the development of hormone heterogeneity. Gastrin-like materials have been previously reported in nonmammalian species such as molluscs (1), elasmobranchs (2), reptiles, and birds (3, 4). This study represents the initial finding and characterization of the molecular heterogeneity of immunoreactive gastrin activity in the order Amphibia.

Methods. The gastrointestinal tracts (including liver and pancreas) of 30 bullfrogs (*Rana catesbeiana*), 10 *R. pipiens*, and 8 *Necturus* were dissected out quickly after the animals were killed by decapitation. The gastrointestinal tracts were sectioned into esophageal, fundic, antral, duodenal, midgut, and terminal intestinal components. The muscularis of each of these tissues was dissected away as much as possible in a petri dish in oxygenated amphibian Ringer's solution at pH 7.4. The mucosa of each of the above tissues as well as the liver and pancreas were then weighed and separately homogenized. The homogenates were then diluted in 20 ml of H_2O and boiled for 15 min. The solutions were allowed to cool for 10 min and then centrifuged and the supernatant solutions quantitated and stored at $-20°$ until they were assayed for gastrin content.

Samples of blood and tissue extracts were fractionated by gel chromatography on Sephadex G-50 superfine columns (1×100 cm) using 0.02 *M* sodium barbital, pH 8.4, containing 0.2 g/liter of sodium azide as the elution buffer. Each sample (1 ml) was applied to the column with approximately 1000 cpm of monoiodinated, ^{125}I-labeled G-17 and 2000 cpm of ^{131}I (to indicate salt peak). The flow rates of the columns were adjusted to 6 ml/hr with fraction volumes of 1 ml.

Calibration of the Sephadex columns was accomplished using pure human G-13, G-17, and G-34 gastrins.[4] In the situation where tissue samples were fractionated, 10 μl of human serum albumin was added to the sample prior to chromatography to provide a protein marker.

Gastrin content was determined via radioimmunoassay. Each tissue or column eluate sample was assayed in duplicate and the data expressed in terms of synthetic human gastrin 17-I equivalents in picograms (pg) per milliliter.

Briefly, the procedure for the radioimmunoassay is as follows: One-hundred microliters of sample (at various dilutions) was incubated at 4° for 72 hr in a mixture consisting of 900 μl of Veronal buffer (pH 8.4), 800 μl of monoiodinated synthetic human gastrin 17-I[5] in buffer (~2000 cpm/tube), and 200 μl of antibody (Ab 1296, 1:1,000,000 final dilution) specific for the C-terminal region of G-17 (5).

At the end of 72 hr free gastrin was adsorbed onto Amberlite IRP-58M resin and separated from the antibody-bound fraction by centrifugation and decantation. Both free and antibody-bound fractions were counted in a gamma scintillation counter and the results are expressed as B/F ratio. The sensitivity of this assay was found to be 1 pg/ml.

Results. All three species of Amphibia

[1] Supported by Public Health Service NIH Training Grant 2A05286, NIH Research Grant AM09260, and VA Project No. 3649-01.

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[4] Obtained courtesy of the Center for Ulcer Research and Education (CURE), Los Angeles, Calif.

[5] Kindly supplied by Schwarz/Mann Pharmaceuticals, Orangeburg, N.Y.

tested contained measurable quantities of gastrin-like immunoreactivity (Table I). In both species of *Rana* studied, the highest tissue concentrations of gastrin immunoreactivity were found in the antrum, followed by the duodenum and pancreas, in order of tissue content. *Necturus*, however, was found to have the largest concentration of gastrin immunoreactivity in the portion of intestine just distal to the pyloric sphincter, the area which would correspond to the duodenum in mammals. The antrum and pancreas, in decreasing order, were also found to contain assayable quantities of gastrin.

Figure 1 shows the elution profile of the three standard gastrins used to calibrate the Sephadex columns. Fractionation of serum from *Rana catesbeiana* (Fig. 2) produced two peaks of immunoreactivity corresponding to the void volume and the G-34 elution peak. The void volume peak may correspond to the "big big gastrin" (BBG) described in man (5) and was present in all frog sera chromatographed.

Immunoreactive gastrin in antral mucosa from *Rana catesbeiana* (Fig. 3) was found to correspond primarily to G-34-I, with a small amount of immunoreactivity present as smaller fragments. *Necturus*, in addition to the G-34 peak, was found to possess gastrin immunoreactivity in smaller fragments, some of which corresponded to G-17 and G-13.

Duodenal mucosa extracts from *R. catesbeiana* and *Necturus* yielded substantial gastrin immunoreactivity in the eluted void volume of the columns (Fig. 4). *R. catesbeiana* was found also to possess substantial gastrin immunoreactivity in the elution region corresponding to G-34. *Necturus*, in addition to the void volume peak, was found to possess gastrin immunoreactivity which corresponded to G-34 and G-17 elution volumes.

Discussion. Considerable debate has occurred as to where the hormone gastrin first appears on the phylogenetic tree. Gastrin immunoreactivity or biological activity has been found in molluscan species (1), in elasmobranchs (2), and in mammals such as pig, dog, cat, rabbit, and man (6).

The current study found gastrin immunoreactivity to be present in the serum, antrum, duodenum, and pancreas of each amphibian species examined. Amphibians are monogastric and have a histologically distinct antrum (7). The sequence of tissue gastrin content was found to be similar to

TABLE I. THE TISSUE CONTENT OF IMMUNOREACTIVE GASTRIN ACTIVITY IN THREE SPECIES OF AMPHIBIA.

Species	Number of Animals	Gastrin content (ng/g ± SE)				
		Serum	Antrum	Duodenum	Pancreas	Remaining gut
Rana catesbeiana	30	33.71 ± 19.43	40.22 ± 13.3	5.22 ± 1.36	0.62 ± 0.12	0
Rana pipiens	10	–	10.34 ± 5.24	3.67 ± 3.14	0.41 ± 0.20	0
Necturus	8	–	2.27 ± 0.62	14.04 ± 2.83	0.13 ± 0.01	0

[a] The results are reported as nanograms of gastrin activity, expressed in synthetic human gastrin I equivalents, per gram wet tissue weight.

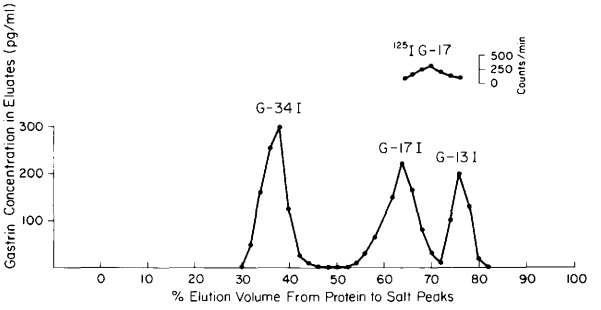

FIG. 1. Elution profile of standard human gastrins after filtration on Sephadex G-50 (superfine). Percentage of elution volume is measured from protein peak (0%) to the salt peak (100%).

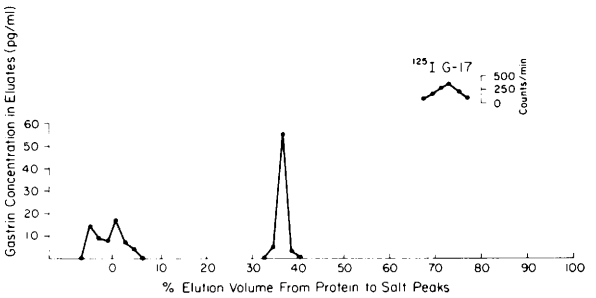

FIG. 2. Sephadex G-50 (superfine) gel filtration of immunoreactive gastrin in the blood (serum) of *Rana catesbeiana*.

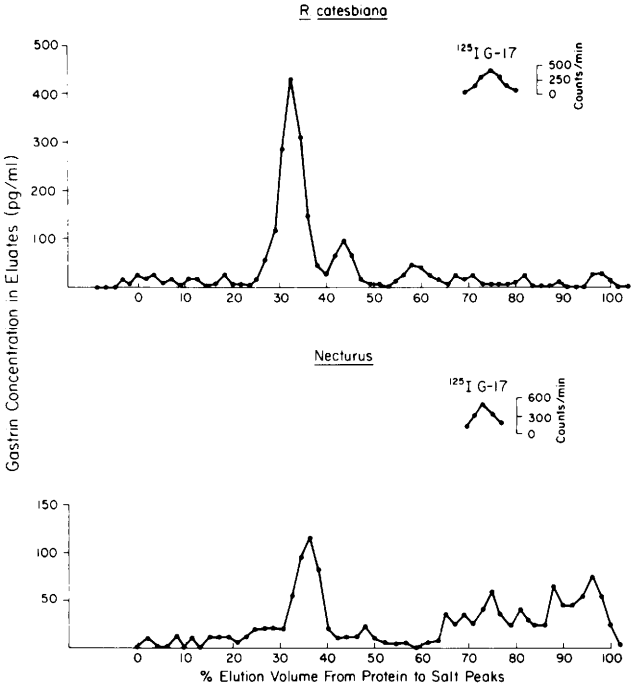

FIG. 3. Sephadex G-50 (superfine) elution profile of antral mucosa extracts from *R. catesbeiana* (top panel) and *Necturus* (lower panel).

that previously described in the cat, dog and man; viz., the antrum contained the largest concentration of gastrin, followed by the duodenum and pancreas. *Necturus*, however, was found to be an exception in that the duodenum was found to contain the largest tissue concentration of gastric activity, followed by the antrum and then pancreas.

Sephadex-gel chromatography of the immunoreactive gastrin in the blood and tissues of these amphibian species demonstrated molecular heterogeneity. Gastrin molecular heterogeneity has previously been demonstrated in both the plasma and tissues of mammals (8).

In both the serum and duodenal extracts of *R. catesbeiana* and *Necturus*, a considerable portion of the gastrin immunoreactivity was found to be present in the void volume of the Sephadex G-50 column. This component of gastrin immunoreactivity has been termed "big, big gastrin" (BBG) and has been identified in both serum and duodenal

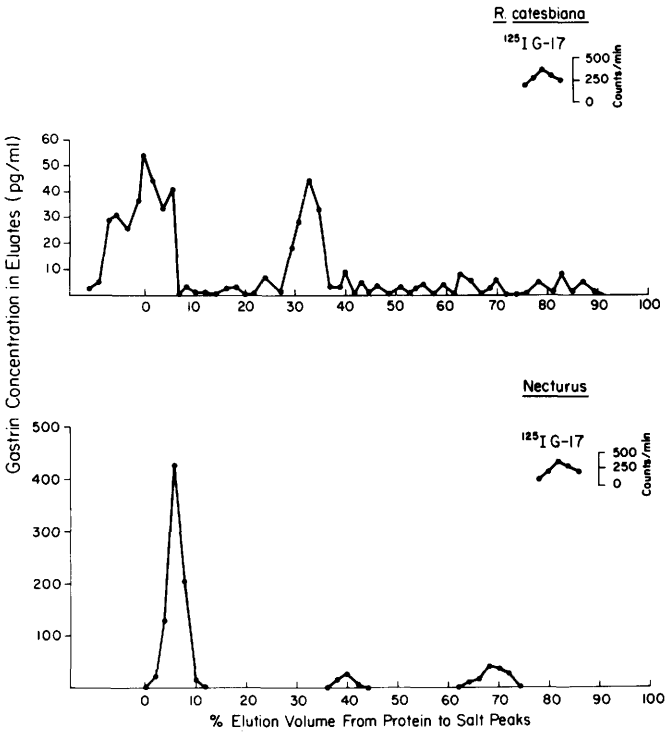

FIG. 4. Sephadex G-50 (superfine) elution profile of immunoreactive components from duodenal mucosa extracts from *R. catesbeiana* (top) and *Necturus* (bottom).

extracts of humans (9). The origin and biological activity of this immunoreactive component are yet unknown. It has been shown in man (9) that the fraction of the gastrin immunoreactivity present in the BBG form increases as one goes distally down the gastrointestinal tract, from being almost absent in the antrum to comprising a substantial portion of the gastrin immunoreactivity of the proximal jejunum. This same pattern was found to be present in *Necturus* and *R. catesbeiana*.

The predominant gastrin immunoreactivity in the sera and antral tissue extracts of *R. catesbeiana* was found in the elution volumes corresponding to "big gastrin," the 34-amino acid form of approximately 3900 MW (10). The heptadecapeptide (G-17) form of gastrin which has been found in man (10) could not be identified in *R. catesbeiana sera*.

Gastrin immunoreactivity corresponding to the G-17 molecular form was identified in both antral and duodenal tissue extracts of *R. catesbeiana*. The G-17 component was found to comprise a significant portion of the antral gastrin immunoreactivity. Gastrin immunoreactivity corresponding to G-17 could be demonstrated in the antral tissue extracts of *Necturus*, with an additional portion of the gastrin immunoreactivity being present in elution volumes corresponding to the G-13 molecular form and possibly smaller fragments. Only trace quantities of G-17 immunoreactivity could be located in *Necturus* duodenal extracts.

The finding that gastrin immunoreactivity can be detected in Amphibia with an antibody with COOH-terminal specificity indicates that a certain similarity in the structure or amino acid sequence exists between this portion of amphibian gastrins and those of mammalian species. In addition, the observation that amphibian gastrins can be segregated into molecular species with molecular weights similar to those of mammalian gastrins lends some support to the hypothesis that this hormone in both species probably evolved from a similar precursor.

Gastrin has been found to be an effective

stimulus for gastric H^+ secretion in the bullfrog (11), and we presume that the endogenous gastrin activity we are describing serves as a physiological stimulus for H^+ secretion in amphibia.

Summary. Extracts of the digestive tracts of three amphibian species were examined by radioimmunoassay for gastrin-like materials. Gastrin was detected in all three species of amphibia examined. In *R. catesbeiana* and *R. pipiens* the largest tissue concentrations of gastrin were found in the antrum, followed by the duodenum and pancreas, respectively, in order of decreasing gastrin content. In the urodele *Necturus* the duodenum contained the highest tissue content of gastrin followed by the antrum and then the pancreas.

Molecular heterogeneity of gastrin was found in the sera of *R. catesbeiana* and in tissue extracts of *R. catesbeiana* and *Necturus*.

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Received May 23, 1975. P.S.E.B.M. 1976, Vol. 153.