Ultrastructure of Avian Gastrin Cell Granules

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Received May 7, 1979

Summary. Gastrin cells in the pigeon, quail, gull and kite were identified at ultrastructural level by immunocytochemistry, using the consecutive semithin/ultrathin section technique. In contrast to the gastrin granules known in mammals, the avian gastrin granules generally contained a consistent dense core accompanied by a clear halo between the core and limiting membrane. The mean diameters of gastrin granules in the pigeon, quail, gull and kite were 211 ± 37 , 247 ± 45 , 331 ± 61 and 353 ± 73 nm respectively. The carnivorous birds seemed to possess larger gastrin granules than the grain-eating birds. The species differences in the size and fine structure of the gastrin granules were discussed.

The existence of gastrin-immunoreactive cells (gastrin cells) in the gastrointestinal tract has been reported in the chicken and quail (POLAK et al., 1974; LARSSON et al., 1974; YAMADA et al., 1979). Especially numerous gastrin cells were found in the pyloric region, in a narrow zone joining the gizzard and the duodenum of the chicken (LARSSON et al., 1974) and the quail (YAMADA et al., 1979).

The ultrastructure of the gastrin cells in mammals and birds has been defined by immunocytochemistry (BUSSOLATI and CANESE, 1972; GREIDER et al., 1972; CANESE and BUSSOLATI, 1974; SUNDLER et al., 1977). The granules of the gastrin cells in the chicken have a homologous and an electron dense core in contrast to the flocculent and electron low-dense core of the mammalian gastrin granules. However, it is still uncertain whether this ultrastructural characteristic of the gastrin granules is valid for other species of birds or not. It seems worthwhile to study the possible ultrastructural difference in the avian gastrin cell granules based on the species and nature of feed. The present report deals with this problem using the pigeon, quail, gull and kite.

MATERIALS AND METHODS

Grain-eating birds represented by five fantails (pigeon, *Columba livia*) and five Japanese quails (quail, *Coturnix coturnix japonica*), and carnivorous birds, five black-tailed gulls (gull, *Larus crassirostris*) and five black-eared kites (kite, *Milvus migrans*) were

used in this study. The birds killed by bloodletting had an empty stomach. The pyloric regions were dissected out for electron microscopy and immunocytochemistry. The samples were fixed by immersion for 2 to 3 hrs in a fixative containing 3% formaldehyde and 1% glutaraldehyde in a 0.1 M phosphate buffer, pH 7.2, with and without 2% parabenzoquinone, one each. All specimens were postfixed for 1.5 hrs in 1% osmium tetroxide in the same buffer, dehydrated in graded ethanol solutions and embedded in Epon-Araldite.

Identification of the gastrin cells at ultrastructural level was made by the consecutive semithin/ultrathin section technique. An indirect technique for the peroxidase-labelled antibody methods (KAWARAI and NAKANE, 1975) was used in this study. Antisera used in this study were similar to those of the previous report (YAMADA et al., 1979). Semithin sections were mounted on glass slides. Resin was removed with 3% potassium hydroxide in ethanol. Osmium deposits were removed with 1% periodic acid. The semithin sections were then exposed to the gastrin antiserum in dilution 1:40 for 2 hrs at room temperature. The site of the antigen-antibody reaction was revealed by peroxidase-labelled rabbit anti-guinea pig IgG serum applied at 1:20 dilution for 1 hr at room temperature. Peroxidase activity was demonstrated by the method of GRAHAM and KARNOVSKY (1966). Controls were run as recommended by STERNBERGER (1974).

Adjacent ultrathin sections were placed on grids and contrasted with lead citrate and uranyl acetate and examined in an electron microscope. The granule size of gastrin cells was decided by measuring the diameter of 1,050 cytoplasmic granules in at least ten identified gastrin cells from each species. All the photographs were reproduced at magnification of $24,000\times$. The data obtained were analyzed by various analytical means.

RESULTS

In the chicken and quail, the gastrin cells have been known to be abundant in the pyloric region (LARSSON et al., 1974; SUNDLER et al., 1977; YAMADA et al., 1979), and this portion (gizzard-duodenal junction) was used in this study for identification of the gastrin cells.

The ultrastructure of the gastrin cells in the pigeon, quail, gull and kite was defined using the consecutive semithin/ultrathin section technique (Fig. 1); the semithin section was used for immunocytochemical analysis and the ultrathin section for electron microscopy. In the pigeon, quail, gull and kite, the gastrin cells were more numerous in the lower half portion than in upper portion of pyloric glands. Their shapes were mainly pyramidal or barrel-like among the exocrine cells. They were often seen to reach the glandular lumen with a short process, usually furnished with microvilli and rarely with a cilium. The gastrin cells contained numerous round and cored granules which displayed varying granular sizes and internal structures according to the species.

In the pigeon (Fig. 2A), the granules of the gastrin cells were round and were the smallest in this study. The mean diameter of the gastrin granules was 211 ± 37.3 nm, ranging between 125–363 nm. They showed a round core separated from an evident membrane by a narrow halo filled with low electron-dense materials. Their

cores displayed a variation from low to high electron density, but their highly dense cores were somewhat lower than those of the quail and kite. Electron lucent granules empty in appearance were never found in the gastrin cells of pigeons. The ultrastructures of the pigeon gastrin granules were stable to the two fixatives in this study.

Fig. 1. Immunocytochemical identification of gastrin cell in the kite pyloric region. **A**, **B** and **C** are serial sections. **A** shows an immunoperoxidase-positive cell in the semithin section. **B** demonstrates ultrastructure of the same cell in the ultrathin section. **C** is a control exposed to antigen-inactivated antiserum in the semithin section. Gastrin cell (G) is identified clearly by the consecutive semithin/ultrathin section technique. A and C: $\times 1,300$, B: $\times 4,000$

Fig. 2. Ultrastructures and size distribution histograms of gastrin granules in the pigeon (**A**), quail (**B**), gull (**C**) and kite (**D**). Note the differences in the granular size and internal structure among the species. $\times 20,000$. *Mean $\pm$ S.D.

In the quail (Fig. 2B), the granules of the gastrin cells were round in shape and high in electron density. Usually a narrow halo filled with low electron-dense materials occurred between the limiting membrane and the dense core. The mean diameter of the quail gastrin granules was 247 ± 45.0 nm, ranging between 142–428 nm. The granule profiles were stable to the two fixatives used in this study. Electron lucent granules were never found.

In the gull (Fig. 2C), the gastrin granules were round in shape, somewhat granular in texture, and variable in electron density. Sometimes, electron lucent granules were intermingled. The electron lucent halos between the core and the limiting membrane varied in their width according to the fixative used. With the fixative containing parabenzoquinone, the halos were relatively wide and clear, while without parabenzoquinone, the halos were very narrow and sometimes the limiting membranes appeared adhered to the cores. The mean diameter of the gull gastrin granules measured 331 ± 61.2 nm, ranging between 156–536 nm. This figure is significantly larger than those of the two above mentioned grain-eating birds.

In the kite (Fig. 2D), the granules of the gastrin cells were round in shape and high in electron density. They usually displayed a wide electron lucent zone between the limiting membrane and the dense core. Sometimes, vesicular granules were found with a small dense core eccentrically located in the wide electron lucent zone. Electron lucent granules were found constantly among the granules. The mean diameter of the gastrin granules was 353 ± 72.7 nm and their range was 178–571 nm. They were the largest of all in the species of birds examined.

The differences among the sizes of the gastrin granules in all the avian species examined were statistically significant ($p < 0.001$).

DISCUSSION

The gastrin cells were first identified by MCGUIGAN (1968) by a direct immunofluorescence method using rabbit anti-human gastrin antiserum. The ultrastructure of the gastrin cells was confirmed by applying immunofluorescence and electron microscopy respectively to adjacent semithin and ultrathin sections of Epon-embedded antropyloric mucosal tissue (BUSSOLATI and CANESE, 1972; CANESE and BUSSOLATI, 1974). It was decided that the mammalian gastrin cells were in accordance with G-cells, which had been classified ultrastructurally (see the review of SOLCIA et al., 1975).

It was meanwhile noticed that in the chicken the appearance of the gastrin granules is markedly different from that of the mammals. The chicken G cells have granules with a homologous, electron dense core in contrast to the flocculent, less dense core of mammalian G cell granules (LARSSON et al., 1974; SUNDLER et al., 1977).

In mammals, specific difference has been noted in the ultrastructural appearance of the G-cells (LECHAGO and WEINSTEIN, 1978). In birds, however, no report is available concerning the species difference of the ultrastructure of the gastrin cells. This study revealed that the fundamental ultrastructure of gastrin granules is a cored granule associated with a halo between the core and limiting membrane. Thus the ultrastructural difference in the gastrin granules of mammals and birds (LARSSON et al., 1974; SUNDLER et al., 1977) is strongly supported and extended by the present study. However, there are differences in size and internal structure of the gastrin

granules among the species examined.

In this study, the diameters of the gastrin granules in the pigeon, quail, gull and kite were determined. The gastrin granules were relatively small in the pigeon and quail, and quite large in the gull and kite. The mean diameter of chicken gastrin granules (230 ± 54 nm, SUNDLER et al., 1977) was somewhat larger than that of the pigeon (211 ± 37 nm), but smaller than those of the quail, gull and kite. The ultrastructure of the gastrin granules showed more or less noticeable differences among the avian species. The quail gastrin cells corresponded to the type II endocrine cells which have been classified ultrastructurally in our previous report (YAMADA et al., 1978).

Ultrastructural classification of the gastrointestinal endocrine cells in mammals could not be applied directly to the gastrointestinal endocrine cells of birds. According to the mammalian classification, the gastrin cells of the pigeon, gull and kite would bear resemblance to the mammalian D₁, A and ECL cells respectively.

When we discuss the species difference of the gastrin granules, there are three noteworthy reports (FORSSMANN and ORCI, 1969; McC. MORTENSEN and MORIS, 1977; TRACK et al., 1978). These papers emphasize that the ultrastructure of the gastrin granules may be profoundly affected by functional activity of the gastrin cells or fixation conditions of the specimen. Since all the specimens were dissected on the empty stomach of birds and they were fixed with the same condition and procedures in our study, factors which might cause some change in the ultrastructure of the granules may be excluded.

It is apparent that the gastrin granules of carnivorous birds are larger than those of grain-eating birds. However, it is uncertain whether this difference is caused by the difference of feed or not. The species difference in the fine structure of the gastrin granules might also be reflected from their different chemical composition. Furthermore, phylogenetical analysis may be worthwhile to clarify this problem. Unfortunately the phylogeny of birds is not yet established.

鳥類のガストリン細胞顆粒の微細構造

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ハト, ウズラ, ウミネコ および トビのガストリン細胞が, 厚切り切片 (免疫細胞化学的観察用) と超薄切片 (微細構造観察用) との連続切片法によって同定された。哺乳類で知られているガストリン細胞顆粒とは対照的に, 鳥類のそれは 一般的に 高電子密度の芯と限界膜との間に明調なハローを有する顆粒として認められた。ハト, ウズラ, ウミネコおよびトビのガストリン細胞顆粒の平均直径は, それぞれ, 211 ± 37 , 247 ± 45 , 331 ± 61 および 353 ± 73 nm であった。肉食鳥のガストリン細胞顆粒は, 穀食鳥のそれよりも大きいとの傾向がうかがわれた。

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