Occurrence of polypeptide YY (PYY) and pancreatic polypeptide (PP) in the gastrointestinal tract of the bony fish

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ABSTRACT
The gastrointestinal tract of two bony fish species, the daddysculpin (Cottus scorpius) and the cod (Gadus callarias), was investigated immunocytochemically for the occurrence of polypeptide YY (PYY) and pancreatic polypeptide (PP). In C. scorpius, PYY-immunoreactive cells were found in the cardiac portion of the stomach. Beaded PYY-immunoreactive nerve fibers were located in the muscle layers of the stomach as well as under the mucosal basement membrane of the pyloric portion of the stomach. In G. callarias, PYY-immunoreactivity was not detected in any part of the gut. In C. scorpius, PP-immunoreactive cells were observed in the gastric caeca and duodenum. In G. callarias, PP-immunoreactive cells were distributed widely in the stomach, gastric caeca, duodenum, ileum and colon. All the endocrine cells observed here were of the open-type.

Polypeptide YY (PYY) is a candidate gut hormone isolated from the pig duodenum (12), and consists of a linear chain of 36 amino acids (11). PYY-Immunoreactivity has been shown in endocrine cells in the mucosa of the gastrointestinal tract in mammals (1, 3, 4, 6, 8), birds (5), reptiles and amphibians (2). However, occurrence of this polypeptide in the bony fish gut has not been examined. In this study, the gut of two species of bony fish was examined for PYY. Due to a structural similarity between PYY and pancreatic polypeptide (PP) (11), location of PP was also examined.

Animals were captured in August at the Kristineberg Marine Biological Station at Fiskebäckskil on the Swedish west coast. They were killed by a blow on the head, and specimens were dissected out from various parts of the gut. Tissues were fixed in Bouin's fluid for 24 h, and embedded in paraffin. Sections of 5 μm thick were cut. In order to detect PYY- and PP-immunoreactive cells the peroxidase-antiperoxidase (PAP) method (10) was used. Rabbit antiserum against porcine PYY (No. 69D; from Dr L. Terenius, Department of Pharmacology, University of Uppsala, Uppsala, Sweden) and rabbit antiserum against bovine PP (BPP) (No. 615-R-110-146-6; from Dr R. E. Chance, Eli Lilly Research laboratories, Indianapolis, U.S.A.) were applied at the dilution of 1:2,500 and 1:4,000, respectively. Characterization of these antisera has been given in detail elsewhere (3, 8). As specificity controls: 1) normal rabbit serum was used as the first layer instead of the antisera; 2) the first layer antiserum was pre-incubated with 100 μg of the corresponding peptide per ml diluted antiserum for 24 h at 4°C; 3) the anti-PYY was pre-incubated with 75–100 μg per ml diluted antiserum for 24 h at 4°C with the following peptides: porcine neuropeptide Y (NPY) (from Dr K. Tatemoto, Department of Biochemistry, Karolinska Institute, Stockholm, Sweden), avian PP (APP) (from Dr J. R. Kimmel, University of Kansas, Medical Center, Kansas City, U.S.A.), bovine PP (BPP) (from Dr R. E. Chance) and porcine PP (PPP) (Novo Research Institute, Copenhagen, Denmark, Lot No. PPP 79062); and 4) the anti-BPP was pre-incubated with 75–100 μg of porcine PYY and of porcine NPY for 24 h at 4°C.

In Cottus scorpius a moderate number of PYY-immunoreactive cells were seen in the cardiac portion of the stomach. They were located in the deep parts of the glandular tissues and exhibited a patchy distribution in the sense that
they were numerous in some sections and were completely absent in others. These cells were basal-granulated and of the endocrine type (Fig. 1). Beaded PYY-immunoreactive nerve fibers were located in the muscle layers of the stomach (Fig. 2). In the pyloric portion of the stomach, similar nerve fibers were found to run under the mucosal basement membrane (Fig. 3). No PYY-immunoreactivity was observed in other parts of the gut. PP-Immunoreactive cells were found in the gastric caeca and duodenum. These cells were few in the duodenum and numerous in the gastric caeca. They occurred in the lower, middle or deep parts of the villi and were basal-granulated (Fig. 4).

In the cod neither PYY-immunoreactive cells nor nerve fibers were detected in any parts of the gastrointestinal tract. On the other hand, basal-granulated PP-immunoreactive cells of the endocrine type were found in mucosa of the cardiac and pyloric portion of the stomach, of the gastric caeca, of the duodenum, of the ileum and of the colon. They were numerous in the stomach and duodenum, but few in the gastric caeca, ileum and colon. In the stomach they occurred mostly in the glandular part and were round or flask-shaped. In the intestine they were located mostly in the deep parts of the glandular tissue and rarely in the lower or middle part of the villi, and were tall and thin (Fig. 5). All the endocrine cells observed were of the open type, i.e. extended from the basal lamina to the gut lumen.

No immunostaining was seen when the antisera were replaced by normal rabbit serum. Preincubation of the antisera with the corresponding peptides completely abolished the immunoreactivity. Preincubation of anti-PYY with NPY, APP, BPP and PPP, and of anti-BPP with PYY and NPY had no effect on the immunostaining.

Thus, in addition to earlier observations (1–6, 8) in other vertebrates, PYY-immunoreactive cells also exist in the stomach of C. scorpion. The absence of these cells in the cod gut is rather puzzling. This may be due to a real absence of this polypeptide or to a large difference in the molecular structure between mammalian PYY and PYY in this species. The presence of PYY-immunoreactive nerve fibers in the muscle layers and in the submucosa in C. scorpion is rather interesting, because such nerves have not been previously observed in the gastrointestinal tract of other vertebrates (1–6, 8). As the immunoreactivity of these nerves was not abolished after pre-incubation of the anti-PYY with NPY, it is unlikely that this antiserum cross-reacts with NPY. However, the possibility that NPY in this species has an amino-acid sequence different from that in mammals and is more close to mammalian PYY can not be completely ruled out.

PP-Immunoreactive cells have been reported to occur in the stomach, gastric caeca and duodenum of C. scorpion (9). In the present study, however, PP-immunoreactive cells were not detected in the stomach of this species. PP-Immunoreactive cells in the cod seem to show a wider distribution than in the daddy sculpin. It is worthy of note that in a previous study (7), PP-immunoreactive cells were found in the mid-gut in only 4 of 11 teleost species investigated.

Thus, the difference found between the daddy sculpin and the cod regarding PYY- and PP-immunoreactivity may be another example of species variation (7) in the occurrence of the gut neurohormonal peptides in the bony fish.

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