Vasoactive Intestinal Polypeptide (VIP) in Epithelial Cells of the Gut Mucosa of an Elasmobranchian Cartilaginous Fish, the Ray

STURE FALKMER^{1*}, JAN FAHRENKRUG², JAN ALUMETS³,
ROLF HÅKANSON⁴, AND FRANK SUNDLER³

¹Department of Pathology, University of Lund, Malmö General Hospital, Malmö, Sweden. ²Department of Clinical Chemistry, Bispebjerg Hospital, Copenhagen, Denmark. ³Department of Histology, University of Lund, Lund, Sweden. ⁴Department of Pharmacology, University of Lund, Lund, Sweden

Synopsis

As part of study of the phylogeny of VIP, it was observed that the colonic mucosa of the cartilaginous fish, *Raja radiata* and *Raja clavata*, contained numerous epithelial cells, obviously of closed type, displaying intense VIP immunoreactivity. Accordingly, high concentrations of VIP were found by radioimmunoassay. At the ultrastructural level, the VIP cells were characterized by the presence of highly electron dense secretory granules.

The colonic mucosa of the ray may be a good source for the isolation of an early VIP.

In a recent review of the evolution of the gastro-entero-pancreatic (GEP) neuroendocrine system (Van Noorden and Falkmer, 1980) it was stated that the phylogeny of the vasoactive intestinal polypeptide (VIP) is poorly known. In addition to a report from one of our laboratories a couple of years ago on VIP immunoreactive cells in nerve fibres and cell bodies in ganglia of the annelid, *Lumbricus terrestris*, the common earthworm (Sundler *et al.*, 1977), and another, more recent one, on the GEP neuroendocrine system in mammals, a bird, and in a bony fish (Larsson *et al.*, 1979), we are only aware of three recent meeting

abstracts, reporting the occurrence of VIP as studied by immunocytochemistry (IMC) in a mollusc (Van Noorden *et al.*, 1980a), eleven species of teleost fish (Van Noorden *et al.*, 1980b), and seven species of urodelan and anuran amphibia (Buchan *et al.*, 1980). No VIP was found in the mollusc but in nerves and epithelial cells of the gastrointestinal mucosa of all fish and amphibia species investigated. The IMC observations in the amphibia were corroborated by radioimmunoassay (RIA). No reports seem to exist of the occurrence of VIP cells in the gut of the most original of the gnathostomian vertebrates, *i.e.*, the cartilaginous fish.

VIP belongs to the secretin/glucagon family and is the only one which can carry out all the biological actions of the group (*cf.* Van Noorden and Falkmer, 1980). VIP may, therefore, be an early development in the evolution of these peptides.

In a pilot study of a more systematic investigation of the occurrence of VIP in the GEP neuroendocrine system in lower

* Supported by grants from the Swedish Medical Research Council (Projects Nos. 4×4499 and 12×718) and from the Swedish Cancer Society (Project No. 806-05×A).

An unpublished preliminary report was given (S. Falkmer) at the Satellite Symposium "Paraneurons: New Concepts on Neuroendocrine Relatives" on April 1st, 1977, to the 82nd Annual Meeting at Morioka of Japanese Anatomists, April 2-4, 1977.

vertebrates and in invertebrates, we observed that the ray, an elasmobranchian cartilaginous fish, harboured numerous epithelial cells, containing immunoreactive VIP in the mucosa of the lower parts of the gut.

Materials and Methods

Animals

The fish were caught by traps or trawling outside the Kristineberg Marine Biology Station at Fiskebäckskil on the Swedish West Coast and were kept in large aquaria or basins equipped with running sea water. Two closely related species of ray were studied, viz. *Raja radiata* and *Raja clavata*. The gross anatomy and the microscopic structure of the gut are both the same in the two species. In all, 7 adult fish of both sexes were studied. Both fed and fasting fish were investigated. The studies were made at Autumn and the water temperature was about 8°C.

Tissue Processing

For IMC, specimens from various parts of the gut were rapidly frozen to the temperature of liquid nitrogen in a mixture of propane and propylene. After freeze-drying, the specimens were fixed with diethylpyrocarbonate vapours at 55°C for 3 hrs. All specimens were embedded in paraffin *in vacuo* and sectioned at 5 µm.

For electron microscopy, specimens were fixed by immersion over night in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2. Postfixation was made in 1% osmium tetroxide for one hr. The specimens were dehydrated, contrasted with uranyl acetate and lead citrate and embedded in epon. For cell identification purposes, consecutive thin/semi-thin sections were examined after immunostaining of the semi-thin section, as previously described (Larsson *et al.*, 1976).

IMC

Deparaffinized sections were carried down to water in graded ethanol solutions and processed for the IMC demonstration of VIP, as described in detail elsewhere (Sundler *et al.*, 1979). Analogous protocols were followed for the glutaraldehyde-fixed, epon-embedded specimens (Larsson *et al.*, 1976). The indirect immunofluorescence method and the peroxidase-anti-peroxidase procedure were both used. The VIP antiserum (code No. 5603) was applied at dilutions of 1:80 and 1:800, respectively. Control sections were incubated with VIP antiserum, inactivated by the addition of excess antigen (10 µg pure

pig VIP per ml diluted antiserum). VIP was a generous gift from Prof. V. Mutt, Department of Biochemistry, Karolinska Institute, Stockholm, Sweden.

RIA

Acid ethanol extracts of various parts of the gut were prepared as previously described (Falkmer *et al.*, 1977). The extracts were kept frozen or freeze-dried for several months until finally analyzed by the RIA procedure for VIP, described in detail elsewhere (Fahrenkrug and Schaffalitzky de Muckadell, 1977; 1978).

Results

Cells, displaying intense VIP immunoreactivity, were found in the mucosa of both the spiral fold gut and colon-rectum. They were particularly numerous in the proximal colon, where they formed an almost continuous layer at the basal membrane (Fig. 1). The cells were polygonal and did not show any apical processes. VIP immunoreactivity was also observed in scattered nerves in the submucosa and in the muscular layers.

Due to this rather distinct topographical localization of the VIP immunoreactive cells, it was easy to identify them in the electron micrographs. The cells were found to be equipped with mostly spherical secretory granules of moderate size with a highly electron dense core, separated from the membrane by a distinct electron lucent halo (Fig. 2). Often the secretory granules were accumulated in the basal portion of the cells. Also ultrastructurally, the cells were devoid of apical processes.

The IMC observations were corroborated by the results of RIA of the extracts of various parts of the gut. The results of the RIA in two rays are given in Table 1. The highest VIP concentrations were found in the proximal colon.

Fig. 1. Medium-power photomicrograph of the colonic mucosa in the ray, *Raja radiata*, showing an almost continuous layer of intensely immunofluorescent VIP cells, situated close to the basal membrane. The cells seemed to be devoid of apical processes. (The gut lumen is in the upper right corner of the picture). $\times 300$

Discussion

VIP, first thought of as a gut hormone candidate (Said and Mutt, 1970), has recently been found to be a neuronal peptide with a ubiquitous distribution in the body of homothermic animals. In the chicken gut, VIP seems to have a dual distribution, occurring both in nerves and in endocrine cells (Sundler *et al.*, 1979). The present IMC observations made in cartilaginous fish, as well as those previously made in teleost fish by Van Noorden *et al.* (1980b) and in amphibia by Buchan *et al.* (1980), indi-

Table 1. VIP immunoreactivity, as determined by RIA, in acid-ethanol extracts of different parts of the lower alimentary tract of two adult fasting rays of both sexes. The values are given in pmol/g wet weight.

Fish	Sex	Spiral Fold Gut			Colon-Rectum	
		Mid- dle	Distal	Junction to colon- rectum	Prox- imal	Anal
<i>Raja radiata</i>	M	26.3	48.4	56.8	138.1	15.1
<i>Raja radiata</i>	F	34.3	63.5	42.1	95.4	—

cate that VIP has this dual distribution also in poikilothermic vertebrates, which, consequently, may be a general vertebrate feature, at least in the gnathostomian species. Pilot studies of the GEP neuroendocrine system of agnathan vertebrates, such as the cyclostomes *Myxine glutinosa* and *Lampetra fluviatilis*, have, so far, given no proof that VIP occurs in parenchymal cells of the islet organ or in epithelial cells in the gut mucosa (Falkmer, Alumets, Hakanson & Sundler, unpublished) in these species of the most original vertebrates. If subsequent studies confirm these preliminary observations, VIP would then be of about the same evolutionary age as PP in the GEP neuroendocrine system (Van Noorden and Falkmer, 1980). It has already been shown that immunoreactive VIP and PP both appear early in evolution, *i.e.*, already in primitive invertebrates (Sundler *et al.*, 1977), when localized to the nervous system.

The physiological role of the abundantly occurring VIP epithelial cells in the ray colon mucosa is completely unknown. The high VIP content implies that this tissue may be a good source for the isolation and molecular characterization of another early GEP hormonal polypeptide, perhaps permitting studies analogous to those of insulin (Emdin and Falkmer, 1977).

Acknowledgement

Thanks are due to the staff of the Kristineberg Marine Biological Station for excellent working facilities.

References

- Buchan, A. M. J., M. C. Bryant, J. M. Polak, S. R. Bloom and A. G. E. Pearse (1980). *Gen. Comp. Endocrinol.* 40, 349.
- Emdin, S. O. and S. Falkmer (1977). *Acta Paediatr. Scand. Suppl.* 270, 15.
- Fahrenkrug, J. and O. B. Schaffalitzky de Muckadell (1977). *J. Lab. Clin. Med.* 89, 1379.
- Fahrenkrug, J. and O. B. Schaffalitzky de Muckadell (1978). *J. Neurochem.* 31, 1445.
- Falkmer, S., R. P. Elde, C. Hellerström, B. Petersson, S. Efendić, J. Fohlman and J.-B. Siljevall (1977). *Arch. Histol. Jpn.* 40, suppl. 99.
- Larsson, L. I., F. Sundler and R. Hakanson (1976). *Diabetologia* 12, 211.
- Larsson, L. I., J. M. Polak, R. Buffa, F. Sundler and E. Solcia (1979). *J. Histochem. Cytochem.* 27, 936.
- Said, S. I. and V. Mutt (1970). *Science* 169, 1217.
- Sundler, F., R. Hakanson, J. Alumets and B. Walles (1977). *Brain Res. Bull.* 2, 61.
- Sundler, F., J. Alumets, J. Fahrenkrug, R. Hakanson and O. B. Schaffalitzky de Muckadell (1979). *Cell. Tissue Res.* 196, 193.
- Van Noorden, S., H. A. R. Fritsch, T. A. I. Grillo, J. M. Polak and A. G. E. Pearse (1980a). *Gen. Comp. Endocrinol.* 40, 375.
- Van Noorden, S., M. Langer, J. M. Polak and A. G. E. Pearse (1980). *Gen. Comp. Endocrinol.* 40, 351.
- Van Noorden and S. Falkmer (1980). *Invest. Cell. Path.* 3, 21.

Fig. 2. Electron micrograph of a cell, identified as a VIP immunoreactive cell by the thin/semi-thin technique, situated close to the basal membrane (lower left corner). Parts of adjacent VIP cells are seen to the left and right.

The secretory granules represent the most distinctive feature of the VIP cells with their electron dense core and distinct electron lucent halo. $\times 15,000$