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Existence of VIP and PHI-Like Immunoreactivities in the Amphibian Gut

Shinya KISHIMOTO, Tatsuya KOSAKA, Norio TAKABA, Mitsuyo OGAWA, Reiko KONEMORI, Kazuma OKAMOTO, Satoru SHIMIZU, Toshiro KUNITA, Kunihiko DAITOKU, Goro KAJIYAMA and Akima MIYOSHI

Department of Internal Medicine, Hiroshima University School of Medicine, 1-2-3, Kasumi, Minami-ku, Hiroshima 734, Japan

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ABSTRACT

The present paper provides the first definitive evidence on the presence and possible co-existence of VIP- and PHI-like peptides in the peripheral nervous system of the amphibian (bullfrog) gut wall. The possibility of co-existence of the peptides suggests that VIP- and PHI-like peptide may be synthesized from the same precursor protein in the amphibian.

Vasoactive intestinal peptide (VIP), a 28 amino acid peptide[4], is present in the neuronal cell bodies and fibers in both the central and peripheral nervous systems[9]. In the periphery, VIP-immunoreactive fibers are widely distributed particularly in the gastrointestinal[12], genitourinary[1], cerebrovascular[8], and respiratory systems[7] where they innervate various tissues, including the smooth muscles, exocrine and endocrine glands. Peptide HI (PHI), N-terminal histidine and C-terminal isoleucine amide was recently isolated from porcine intestinal extracts. PHI was found to correspond to a linear heptacosapeptide amide characterized by a pronounced sequence homologuous to other previously known members of the glucagon family[14,15]. Close similarities of its amino acid sequence particularly with VIP of the members were noted[16]. Both peptides VIP and PHI (/PHM) have been found to originate from the same precursor protein, prepro VIP[6,11,16]. The structure of the prepro VIP molecule has recently been disclosed by nucleotide sequence analysis[2,6,11,16].

It has been disclosed both by radioimmunoassay and immunochemistry using specific antibodies to PHI (/PHM) that there is a pattern of distribution close to that observed for VIP and further coexistence of VIP and PHI (/PHM) in the mammalian brain and gut[3,13,18,19].

The purpose in the present study, is to know whether or not VIP and PHI-like peptide were present and to establish by the use of immunocytochemistry the anatomical relationship between VIP and PHI-like immunoreactivities in the amphibian gut, if both peptides existed.

EXPERIMENTAL PROCEDURE

Experimental animals

Bullfrogs (Rana Catesbiana), being in hibernation and weighing about 80 g, were used in this study.

Preparation for morphological and immunocytochemical study

After the brain stem was cut and the frog pitched, the abdomen and chest were opened and the whole gut from the esophagus to the distal colon was removed. Each part (2.0 × 2.0 cm) of the lower esophagus, stomach, proximal and distal small intestine, and proximal and distal large intestine was cut and each tissue specimen was divided into two specimens (1.0 × 1.0 cm), one of which was immersed in Bouin's solution for morphological study and the other was immersed in p-benzoquinone solution for immunocytochemical study. These manipulations

were made as soon as possible after removal of the tissue specimens.

Conventional histology

The tissue specimens fixed in Bouin's solution at 4°C for 4 hr were prepared to obtain three micron-wax paraffin tissue sections. The tissue sections were stained with hematoxylin and eosin for histology.

Indirect immunofluorescent method[4].

To ascertain the existence of VIP or PHI, the tissue specimens fixed in p-benzoquinone solution at 4°C for 4 hr were washed in 7% sucrose in phosphate buffered saline (PBS) at pH 7.2. Serial twenty micron-frozen tissue sections were prepared for immunostaining thereafter. The frozen tissue sections were left to react with the primary antiserum (R502) of VIP (dilution 1:200) or with the primary antiserum (938) of PHI (dilution 1:200) for 18 hr at 4°C. The labelled second layer was applied for 1 hr at room temperature. FITC conjugated goat antirabbit globulin (Behring Institute) was used at a dilution of 1:100. Each immunofluorescence reacted in the serial sections was photographed and compared VIP immunoreactivities with PHI Immunoreactivities in the nerves in the tissue of adjasent sections.

Control for immunocytochemistry

In order to demonstrate that the immunocytochemical reactions were specific, the following tests were performed. (1) Prior to immunostaining, the diluted antiserum to VIP or PHI was absorbed with synthetic VIP (10 ng, Shizuoka or synthetic PHI (Pennisula). (2) Normal rabbit serum was used instead of the primary antiserum as the first layer. (3) FITC second layer was applied alone.

RESULTS

There were submucosal nerve and myenteric nerve plexuses in the frog gastrointestinal wall as in the case of the mammalian gastrointestinal wall (Fig. 1). Myenteric nerve plexuses, however, were more abundant than submucosal nerve plexuses. Myenteric nerve plexuses were stretched in a row in the intestine (Fig. 2).

VIP-like immunoreactivities were noted mainly in nerve cell bodies and fibers at various sites of the gastrointestinal tract (Fig. 3), while VIP-like immunoreactive nerves and varicose nerve fibers were also observed in the proximal small intestine (Fig. 4) and the colon.

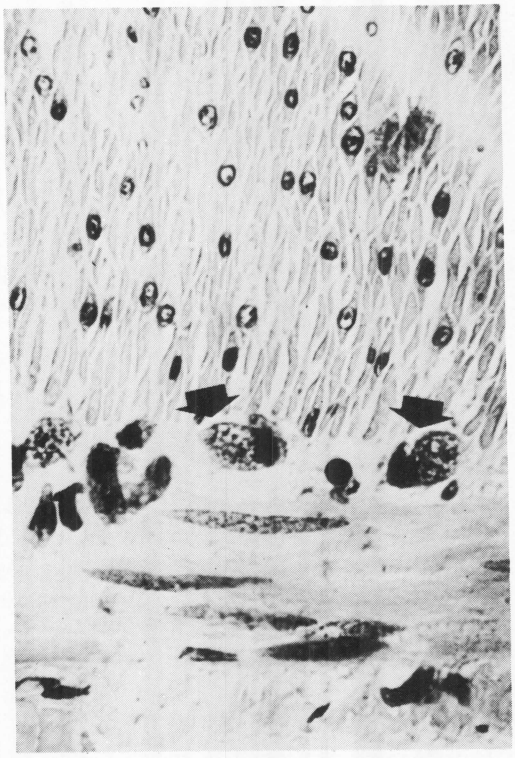

Fig. 1. Submucosal and myenteric plexuses in the bullfrog small intestinal wall (HE stain, original magnification, ×100).

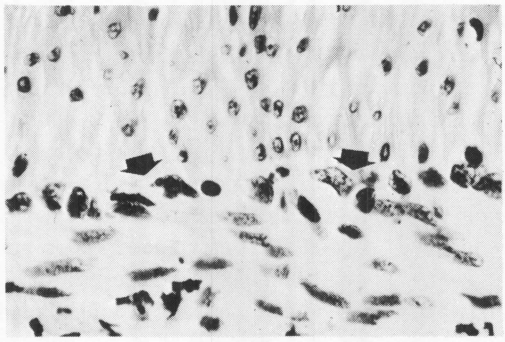

Fig. 2. Myenteric plexuses stretched in a row in the small intestinal wall (HE stain, original magnification, ×100).

Nerve cell bodies and fibers containing PHI-like immunoreactivities were also observed mostly in the myenteric plexuses of the gastrointestinal wall (Fig. 5). A bundle of nerve fibers containing PHI-like immunoreactivities was lo-

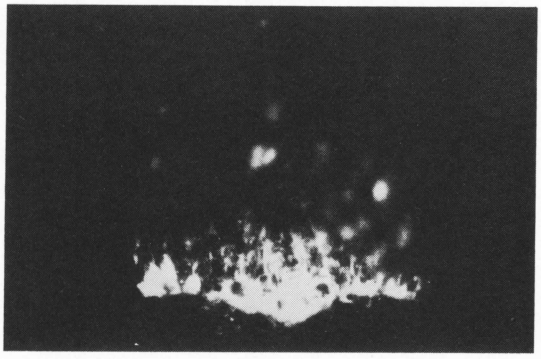

Fig. 3. VIP-like immunoreactive nerve cell bodies and fibers in the lower small intestine (IF: FITC, original magnification, ×400).

Fig. 5. Nerve cell bodies and fibers containing PHI-like immunoreactivities in the myenteric plexus of the small intestine (IF: FITC, original magnification, ×400).

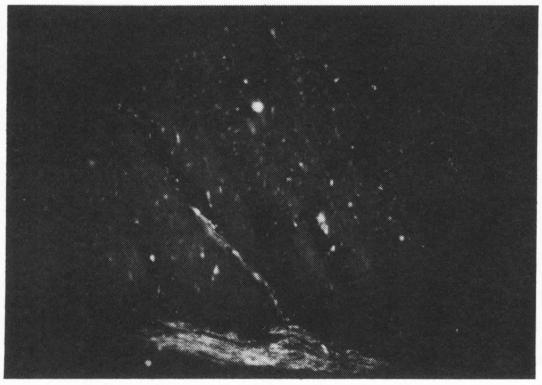

Fig. 6. A bundle of nerve fibers containing PHI-like immunoreactivities in the gastric antrum (IF: FITC, original magnification, ×400).

cated between the circular and longitudinal muscle layers of the gastric antrum (Fig. 6). Fine varicose nerve fibers showing PHI-like immunoreactivities were abundantly present and these terminated at the apical portion of villi in the mucosa of small intestines (Fig. 7).

VIP-like immunoreactive nerve cells and fibers also showed PHI-like immunoreactivities in the

Fig. 4. VIP-like immunoreactive nerve fibers in the lamina propria mucosae of the proximal small intestine (IF: FITC, original magnification, ×200).

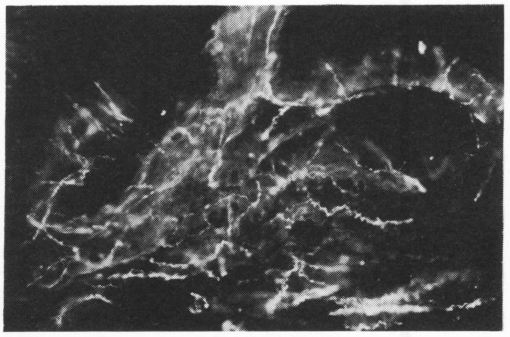

Fig. 7. Numerous PHI-like immunoreactive nerve fibers in the lamina propria mucosae of the proximal small intestine (IF: FITC, original magnification, ×200).

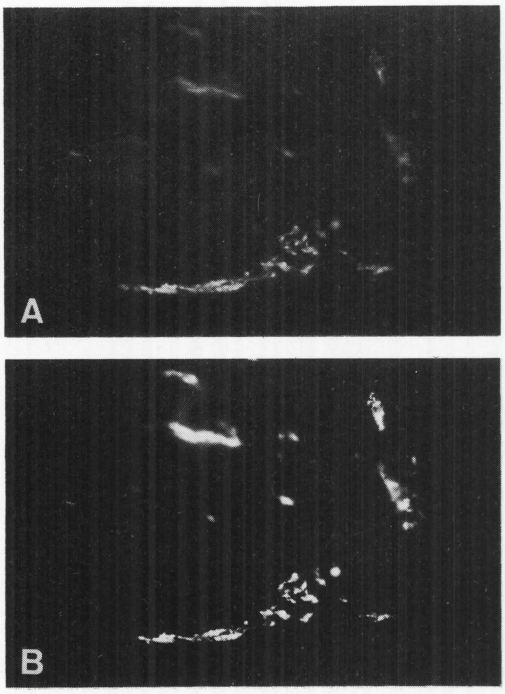

Fig. 8. VIP-like immunoreactive nerve cell bodies and fibers, and PHI-like immunoreactive nerve cells and fibers in the myenteric plexus of the distal small intestine in the adjacent section (original) magnification, both ×200).

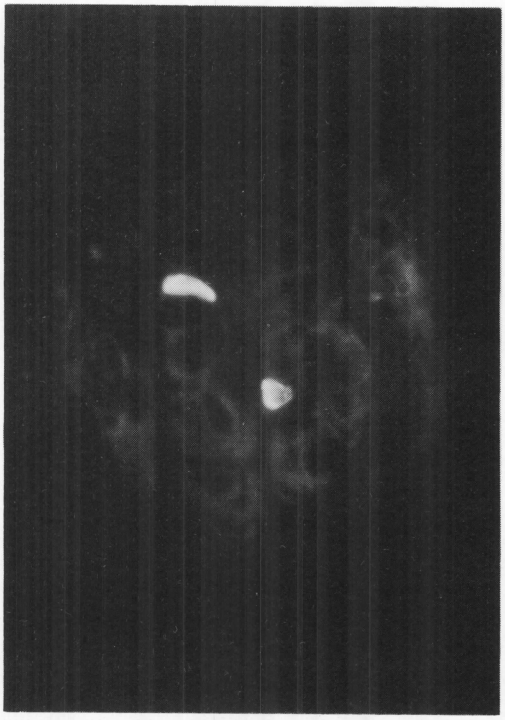

Fig. 10. Endocrine cells containing PHI-like immunoreactivities in the distal colon (IF: FITC, original magnification, ×200).

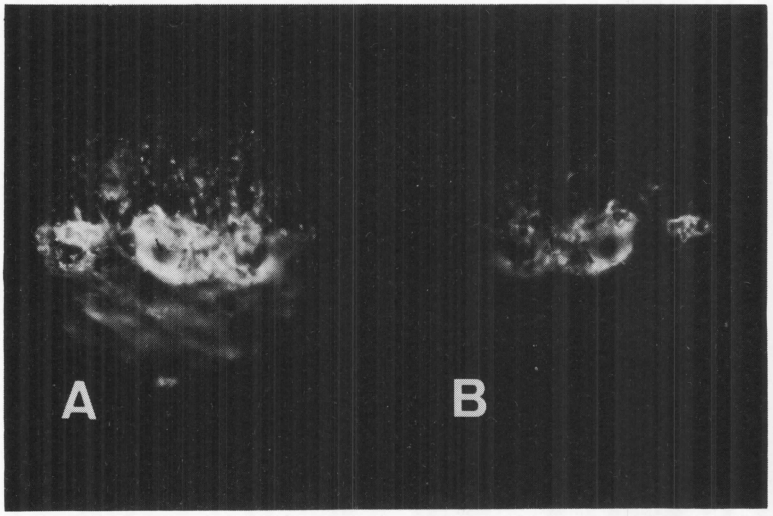

Fig. 9. PHI-like immunoreactive nerve cell bodies and fibers, and VIP-like immunoreactive nerve cell bodies and fibers in the myenteric plexus of the proximal small intestine (original magnification, ×400).

myenteric plexus of the distal small intestine in the adjasent tissue section (Fig. 8A and B). Similarily, VIP-like immunofluorescent nerve cells in the myenteric plexus of the proximal small intestines in the serial tissue section (Fig. 9A and B). The immunoreaction produced by

PHI antibodies was absorbed by incubation with synthetic PHI similarly, the immunoreaction produced by VIP antibodies was obsorbed by incubation with synthetic VIP.

Occasionally, a couple of epithelial cells containing VIP or PHI-like immunoreactivities in the large intestinal mucosa were noted (Fig. 10).

DISCUSSION

The present results showed that not only VIP-like but PHI-like peptides (or substances) are present in the neuronal cell bodies and fibers of the frog gut. The distribution of the peptides was similar to that in the mammalian gut wall (peripheral nerves) as reported previously in several works. This is an interesting finding because the structure of PHI is known to differ among various mammals. VIP- and/or PHI-like immunoreactivities were densely noted in the myenteric nerve plexuses in particular of the gut wall. Numerous fine varicose nerve fibers containing VIP- as well as PHI-like immunoreactivities were abundantly distributed in the lamina propria mucosae of the small intestine and less abundantly in the large intestine. These evidences suggest that VIP- as well as PHI-like peptides play some role on the function of the frog gut similar to the functions of the mammalian gut.

The possibility of co-existence of VIP- with PHI-like immunoreactive nerves was observed in the study using the serial sections, indicating that PHI-like peptide shares receptors and functions of VIP. It should be considered that the putative co-existence of PHI- with VIP-like peptides could be attributed to cross-reactivity. The PHI antibodies employed in the present study, however, did not cross-react with VIP on immunocytochemistry after preincubating the antibodies with synthetic VIP.

The molecular biological data which have been reported to date indicate that PHI and VIP have considerable structural homology and they are encoded by the same gene and co-synthesized from the same precursor molecule[6,11,16].

The distribution of PHI-like immunoreactivity is identical to or parallel with that of VIP-like immunoreactivity in the central and peripheral nervous systems of several mammals and peripheral nerves in the amphibian of the present study. Radioimmunoassay of VIP and PHI, however, has demonstrated that relative amounts of the peptides vary markedly among different tissues[3]. Furthermore, PHI nerve fibers are completely distinct from VIP nerve fibers in the central nervous system of rats[5]. These reports suggest that post-transitional processing of the precursor may be highly tissue specific. In the present data, however, the distribution of VIP- and PHI-like immunoreactivities were almost identical in the nerves of the upper to lower parts of the gut wall. Thus, the data and possible co-existence of VIP- and PHI-like peptides led to the assumption that, even in amphibians as well as in mammalians, VIP- and PHI-like peptides (or substances) share a common precursor protein and post-transitional processing of the precursor may not be tissue specific, at least in the perpheral nervous system. Further studies of chemistry and radioimmunoassay, however, will be necessary to clarify these points.

Immunostaining of endocrine cells with PHI and/or VIP antibodies showed that the distal colon was probably attributed partially to cross-reactivity with other peptides of the secretin family as well as to the possible presence of VIP- and PHI-like substances, distinct from the amino acid sequences of VIP or PHI, in these cells[7].

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