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Response of the gut neuroendocrine system of *Leuciscus cephalus* (L.) to the presence of *Pomphorhynchus laevis* Müller, 1776 (Acanthocephala)

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Summary. Immunohistochemical tests were applied to sections of intestine of uninfected and *Pomphorhynchus laevis* Müller-infected chub, *Leuciscus cephalus* (L.) using 15 different antisera. Nerve cell bodies and fibres immunoreactive (IR) to the anti-bombesin, -Cholecystokinin-8 (CCK-8), -galanin, -Gastrin-Releasing Peptide (-GRP), -Nitric Oxide Synthase (-NOS), -Substance P (-SP), and -Vasoactive Intestinal Peptide (-VIP) sera were observed in the myenteric plexus of uninfected chub. The density of nerve components immunoreactive to these antisera was high in the intestine of the infected fish, especially near the site of attachment. Moreover, numerous nerve fibres, immunoreactive to anti-bombesin, -GRP, -galanin, -SP, and -VIP sera, were encountered in the connective tissue capsule surrounding the bulb and proboscis of *P. laevis*. The occurrence of *P. laevis* in the chub gut significantly increased the number of endocrine cells per intestinal fold immunoreactive to galanin, met-enkephalin and leu-enkephalin antisera. CCK-8, Neuropeptide Y and glucagon-like immunoreactive cells were less numerous in the intestine of infected chub. A large number of cells in the tunica propria-submucosa of *L. cephalus* infected with *P. laevis* were immunoreactive to anti-serotonin and -leu-enkephalin sera.

Key words: Immunohistochemistry, Neuroendocrine system, Digestive tract, *Leuciscus cephalus,* Acanthocephalan infection

Introduction

Pomphorhynchus laevis is a common parasite of several species of freshwater fish but achieves sexual maturity only in a few of these (Hine and Kennedy, 1974). The effects of acanthocephalans on the fish digestive tract and associated organs have been studied by McDonough and Gleason (1981), Dezfuli (1991), Dezfuli et al. (2000, 2003), Taraschewski (2000). The extent of damage caused by acanthocephalans is related to the intensity of infection and depth of proboscis penetration into the host tissues (Bullock, 1963). With regard to this second factor, species belong to genera, *Acanthocephalus*, (Taraschewski, 1989), *Pomphorhynchus* (Dezfuli, 1991; Dezfuli et al., 2003) and *Southwellina* (Dezfuli et al., 1998) penetrate deeply through the tissue and cause extensive damage to the host alimentary canal.

In vertebrates, enteric helminths induce structural modification of host tissues, and provoke alterations to the normal intestinal physiology (Castro, 1992; Fairweather, 1997). It is well known that the nervous, diffuse endocrine and immune systems cooperate to elicit host responses to these helminths (Fairweather, 1997) and for this purpose, signal molecules, namely neuromodulators, are involved in the communication among cells of these three systems (O'Dorisio and Panerai, 1990). Immunohistochemical studies revealed the occurrence and distribution of many neuromodulators in the enteric neuroendocrine system of different vertebrate species infected with intestinal parasites. Nonetheless, these reports are mainly focused on mammals (Palmer and Greenwood-Van Meerveld, 2001; De Man et al., 2002; Gay et al., 2003).

The present investigation is part of a programme of research on the effect of helminth parasites on the enteric neuroendocrine systems of different fish species. Previously we have studied the effect of two intestinal helminths, *P. laevis* and the cestode *Cyathocephalus truncatus* Pallas, 1781 on the neuroendocrine system of brown trout, *Salmo trutta* L., 1758 (Dezfuli et al., 2000, 2002a, 2003). Data purpose of this study was to detect the presence and distribution of several neuromodulators in the digestive tract of *L. cephalus* naturally infected with *P. laevis*, compared to uninfected fish.

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Materials and methods

From June to September 2003, 58 *L. cephalus* (total length 22-43 cm) were caught by electrofishing from two tributaries of the River Brenta (north of Padua, Italy). Pieces of intestine from 28 infected and twelve uninfected chub were prepared for immunohistochemical studies as follows. Tissue sections were processed using the indirect immunohistochemical method (peroxidase-anti-peroxidase immunocomplex) (Dezfuli et al., 2002a,b, 2003). The antisera used are listed in Table 1. Controls for the specificity of the immunohistochemical reactions were performed by preabsorption of each antiserum with the corresponding antigen (Table 2). Mammalian (swine, rat) tissue sections were used as positive controls.

Tissue samples from *L. cephalus* infected with 15 to 38 *P. laevis* per fish, were fixed for comparison of the number of endocrine cells in intestinal folds of infected and uninfected chub. This range of intensity is based on a previous study (Dezfuli et al., 2002b), which showed that there was no significant difference in the number of endocrine cells in fish with less than 15 worms or in those with more than 38 worms. Ten intestinal folds in two sections from 12 uninfected chub and 12 chub infected with *P. laevis* were examined (240 folds counted from each of the two groups). Comparable intestinal regions were examined from both chub groups. The

Table 1. List of primary antisera used in this study.

ANTISERA RAISED IN RABBIT	CODE	SOURCE	DILUTION
Bombesin	IHC 7113	Peninsula Lab., Inc., Belmont, CA, USA	1:200
CCK-8[a]	IHC 7181	Peninsula Lab., Inc., Belmont, CA, USA	1:600
CGRP[a]	IHC 6006	Peninsula Lab., Inc., Belmont, CA, USA	1:600
Galanin	AB 1985	Chemicon Int., Temecula, CA, USA	1:250
Gastrin	AB 930	Chemicon Int., Temecula, CA, USA	1:200
GRP[a]	4620-3104	Biogenesis Ltd, Poole, UK	1:200
Glucagon	AB 932	Chemicon Int., Temecula, CA, USA	1:500
Leu-enkephalin	CA-08-235	Genosys Biotechnologies, Cambridge, UK	1:1000
Met-enkephalin	IHC 8602	Peninsula Lab., Inc., Belmont, CA, USA	1:1000
NOS[a]	sc-648	Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA	1:200
NPY[a]	IHC 7180	Peninsula Lab., Inc., Belmont, CA, USA	1:600
Secretin	IHC 7313	Peninsula Lab., Inc., Belmont, CA, USA	1:1000
Serotonin	61066	Chemicon Int., Temecula, CA, USA	1:1000
SP[a]	IHC 7451	Peninsula Lab., Inc., Belmont, CA, USA	1:600
VIP[a]	CA-08-340	Genosys Biotechnologies, Cambridge, UK	1:1000

[a]: CCK-8, cholecystokinin-8; CGRP, calcitonin gene-related peptide; GRP, gastrin-releasing peptide; NOS, nitric oxide synthase; NPY, neuropeptide Y; SP, substance P; VIP, vasoactive intestinal peptide.

Table 2. List of peptides used for absorption controls.

PEPTIDE	CODE	SOURCE
Bombesin	B 4272	Sigma Chemicals, St. Louis, MO (USA)
CCK-8[a]	H 2085	Bachem AG, Bubendorf, Switzerland
CGRP[a]	H 4924	Bachem AG, Bubendorf, Switzerland
Galanin	H 1365	Bachem AG, Bubendorf, Switzerland
Gastrin	G 3131	Sigma Chemicals, St. Louis, MO (USA)
GRP[a]	H 3120	Bachem AG, Bubendorf, Switzerland
Glucagon	G 7774	Sigma Chemicals, St. Louis, MO (USA)
Glucagon	H 6790	Bachem AG, Bubendorf, Switzerland
Leu-enkephalin	H 2740	Bachem AG, Bubendorf, Switzerland
Met-enkephalin	H 2785	Bachem AG, Bubendorf, Switzerland
NOS[a]	sc-648 P	Santa Cruz Biotechnologies, Inc., Santa Cruz, CA, USA
NPY[a]	H 6375	Bachem AG, Bubendorf, Switzerland
Secretin	S 7147	Sigma Chemicals, St. Louis, MO (USA)
Serotonin	H 9523	Sigma Chemicals, St. Louis, MO (USA)
SP[a]	H 1890	Bachem AG, Bubendorf, Switzerland
VIP[a]	V 3628	Sigma Chemicals, St. Louis, MO (USA)

[a]: CCK-8, cholecystokinin-8; CGRP, calcitonin gene-related peptide; GRP, gastrin-releasing peptide; NOS, nitric oxide synthase; NPY, neuropeptide Y; SP, substance P; VIP, vasoactive intestinal peptide.

mean number of enteroendocrine cells per intestinal fold that were immunoreactive to Cholecystokinin-8, Calcitonin Gene-Related Peptide, galanin, gastrin, glucagon, leu-enkephalin, met-enkephalin, Neuropeptide Y and Substance P antisera between uninfected and infected chub were compared using Student's t-test. The level of significance was set at P=0.05.

Stained sections were examined by standard Olympus BX51 light microscopy and digital images were obtained using the program DP-Soft (Olympus).

Results

Forty-two (72.41%) of 58 chub were infected with *P. laevis*. The intensity of infection was 3-85 worms per host, commonly occurring at a density of 6 worms per cm^2. Fore-gut and mid-gut were the most infected regions. Both female and male worms penetrated deeply through all the layers of the intestinal wall by means of their slender neck, bulb and proboscis. During the present study, 15 antisera were tested on sections of intestinal tissues of uninfected and infected fish (Table 1). Seven sera revealed positive responses in nerve cell bodies and fibres of the enteric nervous system (Table 3), moreover, positive responses to 9 sera were observed in epithelial cells of enteric diffuse endocrine system (Table 4). The nerve fibres (NF) of the myenteric plexus of the chub intestine were immunoreactive to -Cholecystokinin-8 (-CCK-8),-galanin and Substance P (-SP) antisera (Fig. 1A,C, Table 3). Additionally with anti-Vasoactive Intestinal Peptide (-VIP) and -Nitric Oxide Synthase (-NOS) sera, some nerve cell bodies rather than NF appeared to be immunoreactive (IR) (Fig. 1B). The presence of the worm did not alter the number of nerve structures that were positive to anti-CCK-8, -galanin and -SP sera within the tunica muscularis of zones distant from the site of attachment of the parasite. In contrast, NF IR to these antibodies were much more numerous especially in myenteric plexus of the areas near the bulb of *P. laevis* (Fig. 2A, Table 3). Furthermore, the nerve components IR to VIP, and NOS antisera were numerous in the gut of infected chub in comparison to uninfected fish (Fig. 2B, C, Table 3). Nonetheless, NF IR to anti-galanin, -SP, -VIP, -bombesin, and -Gastrin-Related Peptide (-GRP) sera were observed in the inflammatory response connective tissue capsule surrounding the bulb and proboscis of *P. laevis* (Fig. 2A, D, E).

In uninfected fish, the antibodies anti-bombesin and -GRP were recognized in numerous NF situated in connective axis of the intestinal folds and not in the typical site among muscle fibres of the tunica muscularis. In chub infected with *P. laevis*, NF IR to GRP and bombesin antisera were numerous especially in connective axis of the intestinal folds adjacent to the site of worm attachment (Fig. 3A,B).

The presence of acanthocephalans did not significantly affect the mean number of endocrine cells per intestinal fold IR to anti-Calcitonin Gene-Related Peptide (-CGRP), -gastrin, and -SP sera (Table 4). Whereas, the mean number of endocrine cells per intestinal fold increased significantly with met-enkephalin antiserum (Fig. 3C, Table 4), and anti-galanin and -leu-enkephalin sera significantly augmented the number of cells (Fig. 3D, Table 4). The occurrence of *P. laevis* significantly decreased the mean number of endocrine cells IR to Neuropeptide Y and CCK-8 antisera in intestinal folds (Fig. 3E, Table 4), indeed, a highly significant reduction in cell number with anti-glucagon serum was also recorded (Table 4).

In infected *L. cephalus*, a large number of cells IR to leu-enkephalin and serotonin antisera were observed in the lamina propria-submucosa as well as in the connective tissue of the capsule around the bulb and proboscis of *P. laevis* (Fig. 2D, 4A, B), the same cells were noticed in low numbers in uninfected chub.

Table 3. Frequencies of nerve cell bodies and fibres immunoreactive to primary antisera in the intestine of *Leuciscus cephalus* uninfected and infected with *Pomphorhynchus laevis*. +++: high presence, ++: medium presence, and +: low presence of nervous components immunoreactive to the specified antiserum.

ANTISERUM	UNINFECTED FISH	INFECTED FISH
Bombesin	+	+
CCK-8[a]	+	++
Galanin	+	++
GRP[a]	+	+
NOS[a]	+	++
SP[a]	+	+++
VIP[a]	+	++

[a]: CCK-8, cholecystokinin-8; GRP, gastrin-releasing peptide; NOS, nitric oxide synthase; SP, substance P; VIP, vasoactive intestinal peptide.

Table 4. Mean number of endocrine cells per intestinal fold immunoreactive to antisera in the intestine of *Leuciscus cephalus* infected with *Pomphorhynchus laevis* (240 intestinal folds from 12 uninfected and 12 infected fish were counted). Mean values are given ± mean standard error. Student's t-test was performed using SAS.

ANTISERUM	UNINFECTED FISH	INFECTED FISH	t-TEST	P VALUE
CCK-8[a]	0.78±0.14	0.35±0.02	3.041	0.002*
CGRP[a]	0.20±0.05	0.18±0.06	0.256	0.798
Galanin	3.20±0.22	4.59±0.34	-3.432	0.000**
Gastrin	0.14±0.05	0.04±0.03	1.715	0.087
Glucagon	1.46±0.07	0.61±0.07	8.586	0.000**
Leu-enkephalin	0.36±0.12	2.36±0.36	-5.270	0.000**
Met-enkephalin	1.24±0.18	3.71±0.93	-2.608	0.009*
NPY[a]	1.70±0.08	1.37±0.13	2.162	0.031*
Substance P	0.41±0.08	0.46±0.06	-0.500	0.617

[a]: CCK-8, cholecystokinin-8; CGRP, calcitonin gene-related peptide; NPY, neuropeptide Y. Differences between mean numbers of endocrine cells from uninfected and infected fish are **: highly significant and *: significant.

No immunoreactivity was encountered in sections treated with preabsorbed antisera and the swine and rat positive control sections gave the expected immunoreactivity.

Discussion

Pomphorhynchus laevis is site specific in the host alimentary canal, a phenomenon which is widespread

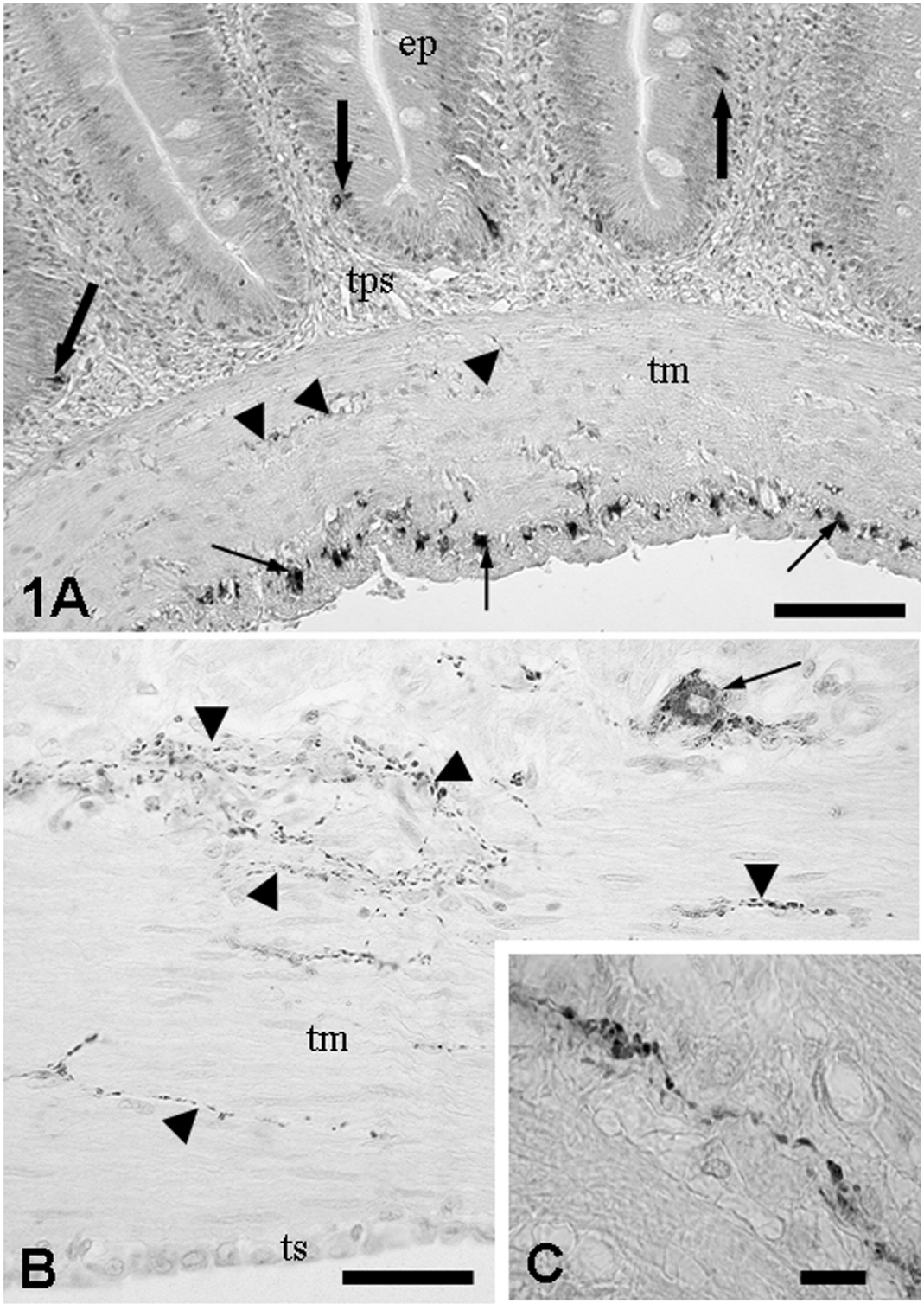

Fig. 1. A. Occurrence of galanin-like immunoreactivity in endocrine cells (thick arrows), in neurons (thin arrows) and in nerve fibre (arrowheads) of the proximal intestine of *Leuciscus cephalus*. ep: epithelium; tps: tunica propria-submucosa; tm: tunica muscularis. Bar: 200 μm. **B.** Nerve body (arrow) and several nerve fibres (arrowheads) immunopositive to VIP antiserum in the muscular layer of the intestine of uninfected chub. tm: tunica muscularis; ts: tunica serosa. Bar: 100 μm. **C.** High magnification of CCK-8-like nerve fibres in the tunica muscularis of the intestine of uninfected fish. Bar: 20 μm.

and due to several factors (Crompton, 1973). *P. laevis* is mostly distributed in the anterior portion of the host's alimentary canal (Kennedy et al., 1976; Munro et al. 1989; Dezfuli, 1991).

Several immunohistochemical studies on the localization, distribution and physiology of the neuromodulators in digestive tract of fishes refer only to healthy animals (Domeneghini et al., 2000; Loretz and Pollina, 2000; Olsson and Holmgren, 2000; Radaelli et al., 2001; Kurokawa and Suzuki, 2002; Shahbazi et al., 2002). Little is known about the morphological alterations in the gut neuroendocrine system of fishes infected with helminths apart from studies on the effect of the cestode, *Cyathocephalus truncatu*s, and on the acanthocephalan *P. laevis* on neuropeptides of *Salmo trutta* digestive tract (Dezfuli et al., 2000, 2002a, 2003). Moreover, the presence and distribution of neuropeptide within the body wall of three-spined stickleback, *Gasterosteus aculeatus* (L.) naturally infected with the protozoan *Glugea anomala* Moniez, 1887 was reported by Dezfuli et al. (2004).

In uninfected chub, nerve fibres positive to Cholecystokinin- (CCK-8-), galanin-, and Substance P- (SP-) like substances were observed in the myenteric

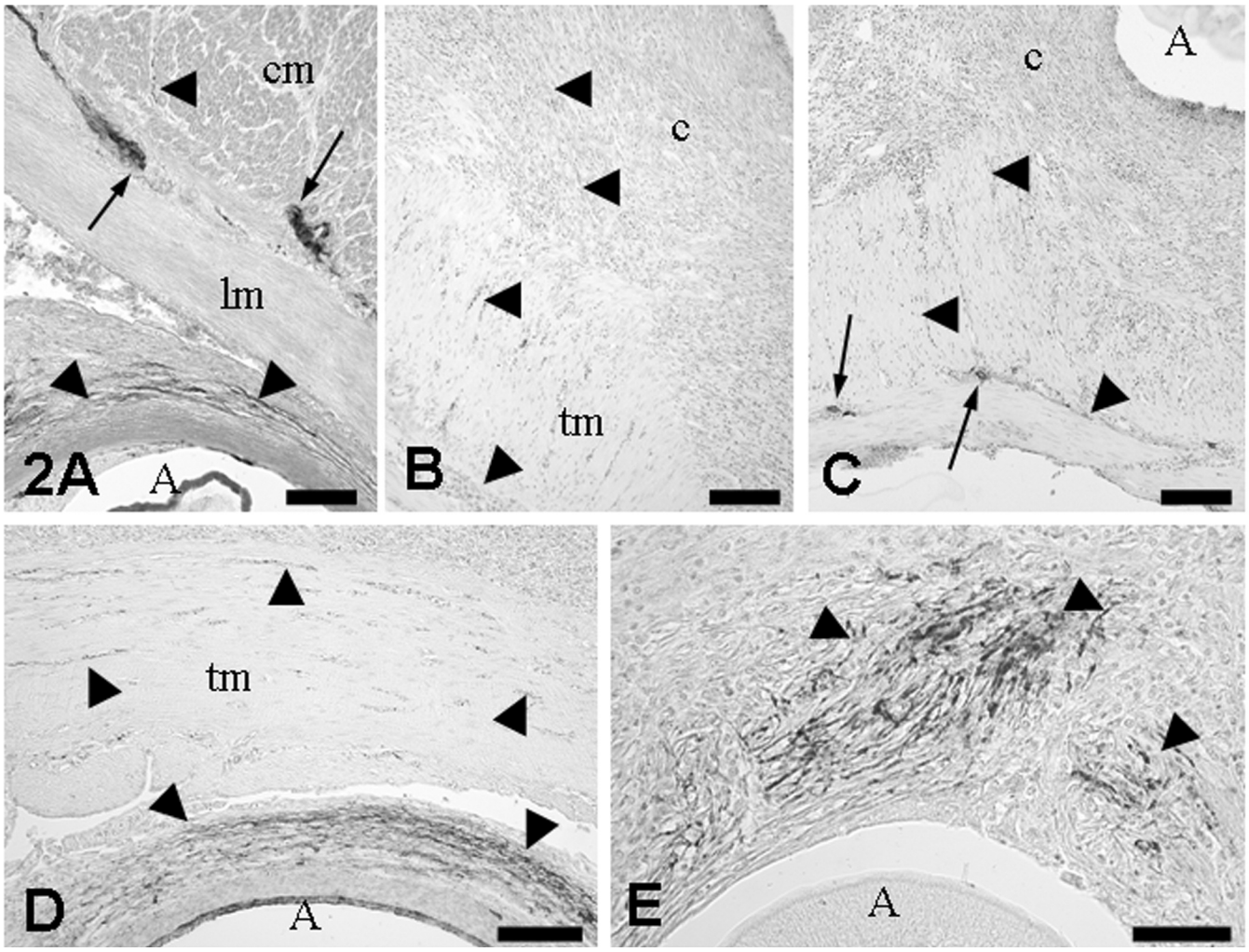

Fig. 2. A. Intense immunoreactivity to galanin antiserum in nerve bodies (thin arrows) and fibres (arrowheads) of the myenteric plexus of *L. cephalus* parasitized with *Pomphorhynchus laevis*. Galanin-like nerve fibres (arrowheads) in the connective inflammatory tissue surrounding the parasite. cm: circular muscle; lm: longitudinal muscle; A: acanthocephalan. Bar: 100 μm. **B.** Large number of nerve fibres (arrowheads) immunoreactive to VIP antiserum within the capsule around the *P. laevis* praesoma. tm: tunica muscularis; c: capsule. Bar: 100 μm. **C.** NOS-like immunoreactivity in the myenteric plexus of *L. cephalus* infected with *P. laevis*. Arrows show nerve bodies positive to the antiserum, and arrowheads indicate marked nerve fibres. c: capsule; A: acanthocephalan. Bar: 100 μm. **D**. Section of infected chub intestine showing nerve fibres (arrowheads) immunoreactive to SP antiserum in the muscular layer and in the connective inflammatory tissue around the parasite praesoma. tm: tunica muscularis; A: acanthocephalan. Bar: 100 μm. **E.** A dense network of nerve fibres (arrowheads) reactive to the anti-bombesin serum within the host inflammatory tissue around the praesoma of *P. laevis*. A: acanthocephalan. Bar: 20 μm.

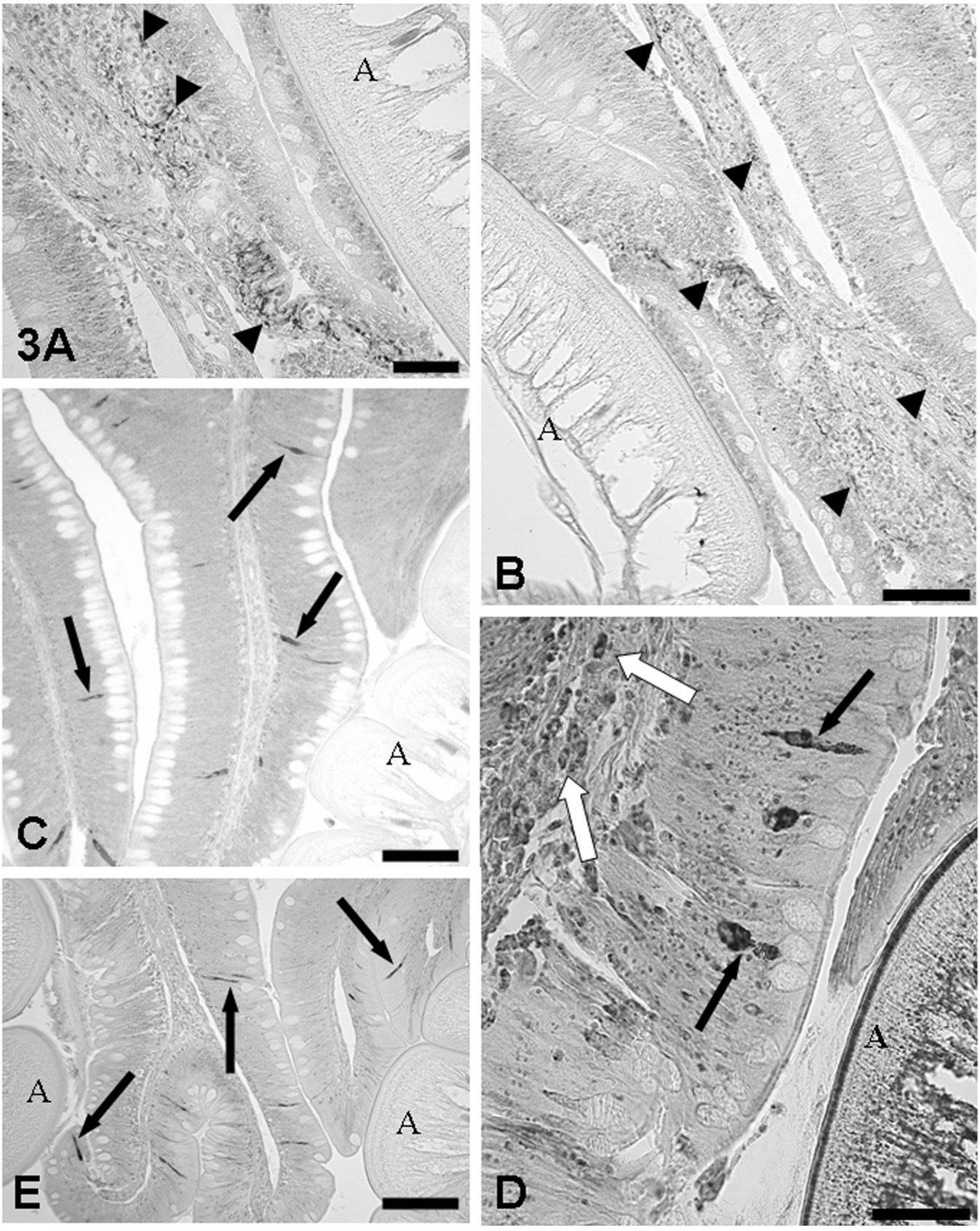

Fig. 3. A. GRP-like immunoreactivity in nerve fibres (arrowheads) placed in the connective axis of an intestinal fold beside *P. laevis* tegument. **A.** acanthocephalan. Bar: 100 μm. **B.** Nerve fibres (arrowheads) in the connective axis of an intestinal fold immunoreactive to the bombesin antiserum. A: acanthocephalan. Bar: 100 μm. **C.** Numerous endocrine cells (arrows) containing a met-enkephalin-like substance in the intestine of infected chub. A: acanthocephalan. Bar: 100 μm. **D.** Endocrine cells (black arrows) immunoreactive to the leu-enkephalin antiserum in the intestinal epithelium of *L. cephalus* infected with *P. laevis*. Large number of likely immune cells (white arrows) also marked with this antibody in the tunica propria-submucosa. A: acanthocephalan. Bar: 100 μm. **E.** Endocrine cells (arrows) containing a CCK-8-like substance in the tunica mucosa of the parasitized fish. A: acanthocephalan. Bar: 100 μm.

plexus, and anti-Nitric Oxide Synthase (-NOS) and -Vasoactive Intestinal Peptide (-VIP) sera were seen in nerve cell bodies and nerve fibres in the myenteric plexus. Most of the antisera used in the present study were reported for enteric nerve fibres and cell bodies in other uninfected cypriniformes species (Rombout and Reinecke, 1984; Burkhardt-Holm and Holmgren, 1989; Kiliaan et al., 1993; Brüning et al., 1996). In the present study, *P. laevis* induced an increase in number of nerve cell bodies and nerve fibres immunoreactive to the NOS and VIP antisera in the gut myenteric plexus. Indeed, with reference to VIP-like peptide, a large number of nerve fibres positive to VIP were encountered also in the myenteric plexus of *S. trutta* infected with *P. laevis* (Dezfuli et al., 2002a).

NOS is the cytoplasmic enzyme responsible for the intracellular production of nitric oxide. In mammals, this molecule is a neurotransmitter involved in several physiological mechanisms, such as smooth muscle activity and regulation of inflammatory reactions (Balemba et al., 2002). Mourad et al. (2003) showed that NOS enhances the release of VIP from nerve endings and vice versa in the rat jejunum, and this potentiates the mechanisms inducing intestinal fluid secretion. A significant increase in NOS-immunoreactive nerve cell body was reported for the enteric nervous system of pigs infected with the trematode *Schistosoma japonicum* (Balemba et al., 2002). Apparently, the worm provoked synthesis or increase in production of those neuromodulators (i.e., NOS, VIP) which exert primarily an inhibitory effect on intestinal muscle of fish. Furthermore, in the fish gut, NOS and VIP were indicated as neurotransmitters, co-stored in a neuron population, and with an inhibitory function on smooth muscle contraction (Olsson and Holmgren, 1997).

According to Ogilvie and Jones (1971) a parasite prevents its detachment and rejection from the host digestive tract by means of a so-called "biochemical holdfast". Supporting this interpretation Foster and Lee (1996) found a homologue of VIP porcine peptide synthesized by *Nippostrongylus brasiliensis* which reduced the amplitude of muscular contraction of the intestine of uninfected rat *in vitro*. The peptide possibly prevents the rejection of the worm from the host intestine *in vivo*.

Nerve components positive to -VIP and -NOS were found in uninfected and infected chub, but their number was higher in infected fish. It was not within the scope of this study to detect neuropeptides produced by acanthocephalans, nevertheless, it is reasonable to presume that *P. laevis* most likely uses a "biochemical holdfast" of unknown mechanism for its survival.

During the present investigation, anti-bombesin and -Gastrin-Related Peptide (-GRP) sera were revealed in nerve fibres of the connective axis of several intestinal folds localized near the site of *P. laevis* attachment. The same was noticed in the intestinal folds of *S. trutta* parasitized with *C. truncatus* and *P. laevis* (Dezfuli et al., 2000, 2003). Bombesin and GRP belong to the same peptide family, and are closely related to similar peptides found in other vertebrate groups (Holmgren and Jensen, 1994; Jensen, 2001). In mammals, GRP/bombesin regulate the ion transport in the small and large intestine (Kachur et al., 1982; Brown and O'Grady, 1997). Further information is needed to determine if GRP/bombesin have the same function in fish.

In *S. trutta*, nerve fibres immunoreactive to bombesin, met-enkephalin, SP, and VIP were recognized within the inflammatory connective capsule which surrounds the bulb and proboscis of *P. laevis* (Dezfuli et al., 2002a). In infected chub, nerve fibres positive to bombesin, GRP, galanin and SP were noted. In *Gasterosteus aculeatus* infected with *G. anomala* occurrence of nerve fibres positive to bombesin-and galanin-like substances was reported by Dezfuli et al. (2004). On the evidence presented here and based on our earlier results (Dezfuli et al., 2004), it could be postulated that in the inflammatory tissues of fish, the neo-formed network of nerve fibres uses a particular group of neuropeptides as neurotransmitters.

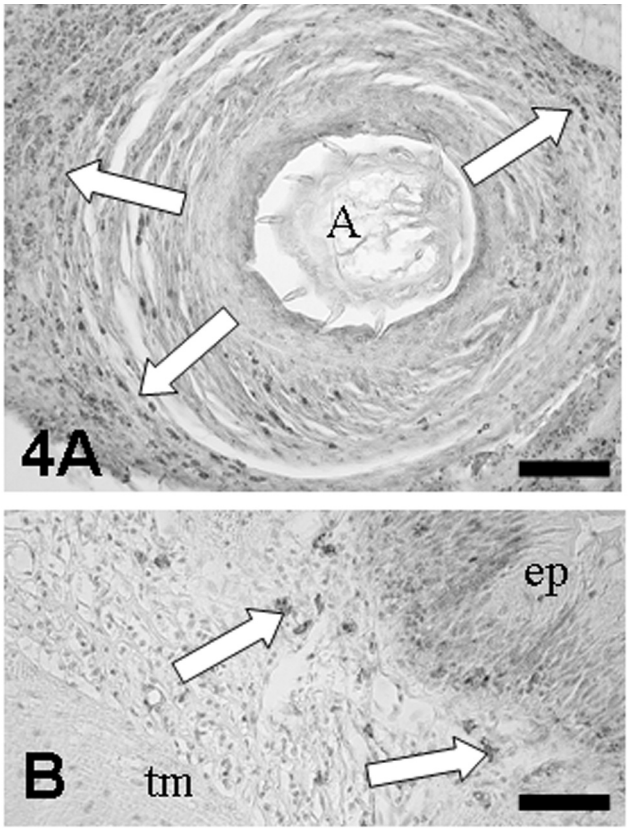

Fig. 4. A. Large number of cells (white arrows) immunoreactive to leu-enkephalin antiserum in the host inflammatory capsule around the proboscis of *P. laevis*. A: acanthocephalan. Bar: 100 μm. **B.** Cells (white arrows) containing a serotonin-like substance in the tunica propria-submucosa of the chub infected with *P. laevis*. tm: tunica muscularis; ep: epithelium. Bar: 100 μm.

Several endocrine cell populations have been reported in cyprinid species using anti-Calcitonin Gene-Related Peptide (-CGRP), -CCK-8, -galanin, -gastrin, -glucagon, -leu- and -met-enkephalin, -Neuropeptide Y (-NPY) and -SP sera. Regarding VIP and serotonin, they were not found in chub, although they were reported in two other cyprinid species, *Barbus conchonius* Hamilton, 1822 and *Leuciscus idus* (L.) (Rombout et al., 1986; Burkhardt-Holm and Holmgren, 1989).

Galanin was reported to be absent in Cyprinidae (Rombout and Reincke, 1984; Rombout et al., 1986; Burkhardt-Holm and Holmgren, 1989; Kiliaan et al., 1993), whereas, in chub galanin was found in endocrine cells of the intestinal folds, and infected fish presented higher number of immunoreactive cells. With reference to met-enkephalin, an increase in the number of cells immunoreactive to this antiserum was reported in the intestine of goldfish experimentally intoxicated by lead (Pederzoli et al., 1996); nevertheless, in infected chub the occurrence of this peptide was high. Galanin and met-enkephalin in mammals have an inhibitory effect on electrolyte secretion (Kiliaan et al., 1993; Pederzoli et al., 1996), but, our knowledge is too limited to assume that these peptides have the same function in fish.

In intestinal folds of infected chub in comparison to uninfected fish, a low number of endocrine elements containing CCK-8-, and glucagon-like substances was recorded, nevertheless, the number of endocrine cells immunoreactive to NPY antiserum was the same in uninfected and parasitized chub. These three peptides are involved in the control of food intake in fish (Lin et al., 2000; Gay et al., 2003).

The present investigation shows that the response of the chub enteric diffuse nervous system to *P. laevis* is not the same as documented for *S. trutta* against the same parasite (Dezfuli et al., 2000, 2002a, 2003). Accordingly, in brown trout infected with *P. laevis* and/or *C. truncatus*, high number of nerve fibres in the myenteric plexus appeared to be immunoreactive to bombesin, CGRP, met-enkephalin, and SP antisera (Dezfuli et al., 2000, 2002a). Among these antisera, only bombesin and SP revealed the occurrence of nerve structures in the intestinal wall of uninfected chub. Except for CGRP and NPY, other antisera were found in a low mean number of chub cells in comparison to that observed in the intestine of *S. trutta* (Dezfuli et al., 2000, 2002a, 2003). Several studies on teleosts, have indicated that the occurrence and distribution of different immunoreactive substances are species-specific (see Elbal et al., 1988).

In chub infected with *P. laevis,* several cells of the lamina propria-submucosa appeared to be immunoreactive to serotonin antiserum. The same finding was noticed in *S. trutta* infected with *C. truncatus* (Dezfuli et al., 2000) and it is known that this antiserum reveals fish mast cells (Khan and Deschaux, 1997). Immunoreactive cells positive to leu-enkephalin were observed among the intestinal fold cells and inside the capsule surrounding the *P. laevis* bulb and proboscis. The same neuropeptide was encountered in the tunica propria-submucosa of *S. trutta* infected with this acanthocephalan (Dezfuli et al., 2002a).

The present study and our previous studies (Dezfuli et al., 2000, 2002a, b, 2003) show that intestinal helminths induce morphological lesions that are responsible for biochemical and physiological alterations of the host digestive tract (Castro, 1989). Furthermore, worms provoke the involvement of vertebrate enteric neuroendocrine and immune systems (Castro, 1989, 1992; Fairweather, 1997; Palmer and Greenwood-Van Meerveld, 2001).

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