Cell Tissue Res (1990) 261:573–581

Cell and Tissue Research

Immunoreactivity to the pancreatic polypeptide family in the nervous system of the adult human blood fluke, *Schistosoma mansoni*

P.J. Skuce[1], C.F. Johnston[1], I. Fairweather[2], D.W. Halton[2], C. Shaw[1], and K.D. Buchanan[1]

[1] Department of Medicine and [2] School of Biology and Biochemistry, The Queen's University of Belfast, Belfast, Northern Ireland, UK

Accepted April 30, 1990

Summary. The presence and distribution of neuropeptides belonging to the pancreatic polypeptide family have been demonstrated by an indirect immunofluorescence technique in the nervous systems of adult male and female *Schistosoma mansoni*. Seven antisera of differing regional specificity to pancreatic polypeptide (PP), peptide YY (PYY) and neuropeptide Y (NPY) were employed on both whole-mount and cryostat-sectioned material. Positive immunoreactivity (IR) was obtained with all antisera except an N-terminally-directed antiserum to NPY. In the CNS, immunoreactivity was restricted to cell bodies and nerve fibres in the anterior ganglia, central commissure and dorsal and ventral nerve cords of both sexes, whereas, in the PNS, positive-IR was present in the plexuses innervating the subtegumental musculature and the oral and ventral suckers. Intense immunoreactivity was observed in a plexus of nerve fibres and cell bodies in the lining of the gynaecophoric canal and in fine nerve fibres innervating the dorsal tubercles of the male. In contrast, in the female, strong immunoreactivity was evident in nerve plexuses innervating the lining of the ovovitelline duct and in the wall of the ootype, but most notably in a cluster of cells in the region of Mehlis' gland. Results suggest that molecules with C-terminal homology to the PP-family are present in *S. mansoni*. These peptides would appear to be important regulatory molecules in the parasite's nervous system and may play a role in the control of egg production.

Key words: Neuropeptides (pancreatic polypeptide, peptide YY, neuropeptide Y) – Immunocytochemistry – Confocal scanning laser microscopy – *Schistosoma mansoni* (Scolecida, Trematoda)

The human blood fluke, *Schistosoma*, afflicts over 250 million people in the tropics as the causative agent of schistosomiasis (bilharzia). The worm is dioecious, male and female *S. mansoni* living in permanent copulation within the hepatic portal vein and mesenteric blood vessels.

The nervous system of adult *S. mansoni* comprises cholinergic, aminergic and peptidergic components. Acetylcholine is the major inhibitory neurotransmitter in the parasite (Barker et al. 1966; Hillman 1983; Mellin et al. 1983; Pax et al. 1984). The biogenic amines 5-hydroxytryptamine (5-HT or serotonin), noradrenaline and dopamine have been demonstrated in the adult worm by use of a variety of techniques (Bennett et al. 1969; Bennett and Bueding 1971; Chou et al. 1972; DeiCas et al. 1979, 1981; Gianutsos and Bennett 1977; Gustafsson 1987; Machado et al. 1972). Serotonin is believed to act as an excitatory neurotransmitter, whereas noradrenaline and dopamine are considered as inhibitory neurotransmitters (Hillman 1983; Mellin et al. 1983; Pax et al. 1984; Tomosky et al. 1974).

However, relatively little attention has been paid to the peptidergic (or neurosecretory) component of the schistosome nervous system. In the absence of a circulatory system and true endocrine glands, this system is likely to play an important integrative role in the neurobiology of *S. mansoni*. To date, immunoreactivity to 7 regulatory peptides has been demonstrated in the nervous system of adult male *S. mansoni:* they are the vertebrate neuropeptides substance P, leu-enkephalin, growth hormone-releasing factor, gastrin-17, cholecystokinin and luteinizing hormone-releasing hormone, human chorionic gonadotropin, and the native invertebrate peptide FMRFamide (Basch and Gupta 1988; Gupta and Basch 1989; Gustafsson 1987).

These two investigations concerned sectioned material from adult male worms. The present study involves paired adult male and female worms, together with immature females from unisexual infections. Specimens have been imaged by confocal scanning laser microscopy. Evidence is presented for immunoreactivity to 3 further peptides in *S. mansoni*, namely, pancreatic polypeptide (PP), peptide YY (PYY) and neuropeptide Y.

Send offprint requests to: Dr. P.J. Skuce, Department of Medicine, The Queen's University of Belfast, Belfast BT7 1NN, Northern Ireland, UK

Table 1. Antisera employed in immunocytochemical studies of the nervous system of the human blood fluke *Schistosoma mansoni*

Antiserum raised against	Regional specificity	Working dilution	Code	Source	Staining intensity
C-terminal hexapeptide amide of pancreatic polypeptide (PP)	C-terminal	1:200	PP221(6)	A	+++
Natural bovine pancreatic polypeptide (PP)	mid-to-N-terminal	1:200	PP204(7)	A	+
Synthetic polypeptide YY (PYY)	non-C-terminal	1:250	PYY301(3)	A	+++
Synthetic polypeptide YY (PYY)	non-C-terminal	1:500	PYY297(4)	A	++
Synthetic porcine neuropeptide Y (NPY)	–	1:400	RPN.1702	B	–
C-terminal decapeptide of neuropeptide Y (NPY)	C-terminal	1:1600	GNPY.163(2)	A	+++
Synthetic NPY	C-terminal tyrosine amide dependent	1:400	NPY8999(6)	C	+++

A Department of Medicine, The Queen's University, Belfast, Northern Ireland; B Amersham International plc, Aylesbury, Buckinghamshire, England; C Dr M.M.T. O'Hare, Laboratory of Molecular Endocrinology, University Department of Clinical Chemistry, Rigshospitalet, Copenhagen, Denmark

+++ = intense immunoreactivity; ++ = moderate immunoreactivity; + = weak immunoreactivity; – = no immunoreactivity

Materials and methods

Infections of *S. mansoni* (Puerto Rican strain) were maintained in Swiss TO mice. Adult worm pairs were recovered from the hepatic portal vein and mesenteric vessels at autopsy and prepared by the indirect immunofluorescence technique of Coons et al. (1955) for use as whole-mounts or for cryostat sectioning. For whole-mounts, paired worms or separate males and females were lightly flattened between glass slides in 4% (w/v) paraformaldehyde (PFA) in 0.1 M phosphate-buffered saline (PBS), pH 7.4, for 4 h at 4° C, then washed for 24 h at 4° C in PBS containing 0.1% (w/v) bovine serum albumin (BSA), 0.2% (v/v) Triton X-100 and 0.2% (w/v) sodium azide. Specimens were then incubated for 24–48 h at 4° C in primary antiserum diluted in PBS (for details of antisera used see Table 1) and washed for a further 24 h in PBS prior to incubation for 24 h at 4° C in swine anti-rabbit or rabbit anti-guinea pig secondary antiserum conjugated to fluorescein isothiocyanate (SWAR-FITC and RAGP-FITC, respectively) (Dako Ltd, High Wycombe, Buckinghamshire, England, UK). After a final wash, specimens were mounted in Apathy's neutral mounting medium and examined in a MRC-500 confocal scanning laser microscope (Bio-Rad Lasersharp Ltd., Abingdon, Oxfordshire, England, UK).

For cryostat sectioning, adult worm pairs or separate males and females were fixed in 4% (w/v) PFA in PBS for 4 h at 4° C, transferred to 5% (w/v) sucrose in PBS overnight at 4° C, then placed in 30% (w/v) sucrose in PBS until submerged for the purposes of cryoprotection. Specimens were then cryostat frozen (−20° C) in Cryo-m-bed and sections (10 µm in thickness) cut on a Reichert CryoCut E cryostat and mounted on gelatin-coated coverslips. The sections were incubated for 24 h at 4° C in primary antiserum, washed in PBS and incubated in secondary antiserum at room temperature for 30 mins. The sections were washed again prior to mounting in Apathy's neutral mounting medium and viewing in an Olympus BH-2 incident fluorescence microscope fitted with a supplementary EY455 excitatory filter.

Controls included (i) omission of primary antiserum, (ii) replacement of primary antibody with non-immune rabbit serum, and (iii) liquid-phase preabsorption of primary antibody by the addition of 1 ng–100 µg pure peptide/ml diluted antiserum. Peptides employed in preabsorption studies included the C-terminal hexapeptide of bovine PP and intact synthetic PYY, NPY and FMRFamide.

Results

The CNS of adult *S. mansoni* consists of a circumoesophageal commissure joining a pair of anterior ganglia from which emanate the main nerve cords. Nerve fibres extend a short distance from the anterior ganglia to innervate the oral and ventral suckers, but the main dorsal and ventral nerve cords extend the full length of the body. The arrangement of the CNS is essentially the same in both male and female worms. The PNS comprises plexuses of cell bodies and nerve fibres serving to innervate the main body muscles, the oral and ventral suckers, the gastrodermis and also the musculature associated with the reproductive organs and their accessory ducts (for references see Bennett and Bueding 1971).

Positive immunoreactivity was obtained with all the antisera employed, except that raised to NPY (antiserum

Figs. 1–5. Confocal scanning laser micrographs of whole-mount preparations of adult male and female *S. mansoni*

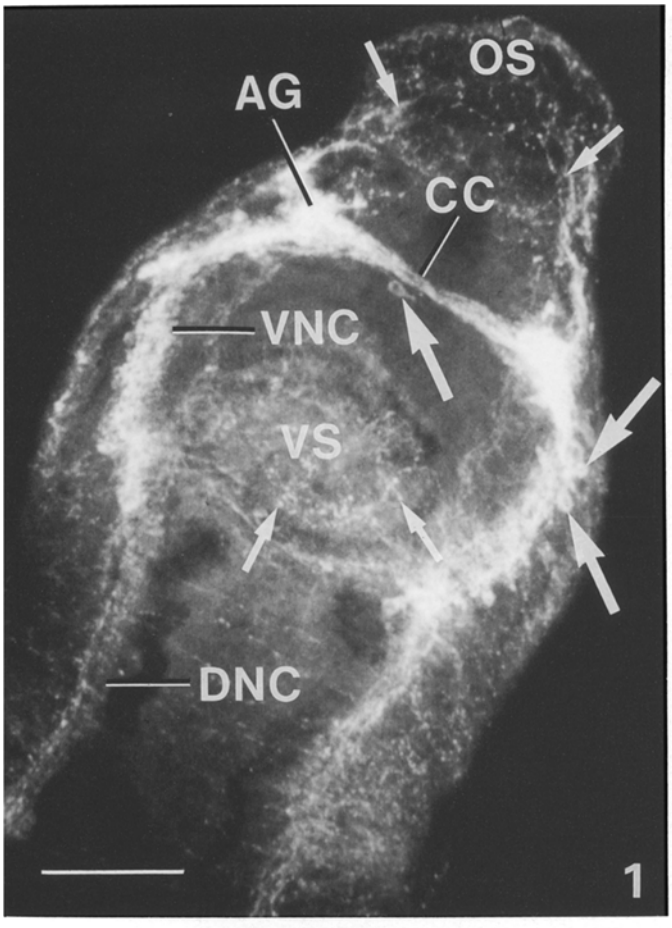
Fig. 1. Anterior region of an adult male, showing immunoreactivity to PP in the paired anterior ganglia (*AG*), central commissure (*CC*) and main dorsal (*DNC*) and ventral (*VNC*) nerve cords. Nerve cell bodies (*large arrows*) are associated with the ganglia and central commissure and immunoreactive nerve fibres (*small arrows*) innervate the oral (*OS*) and ventral (*VS*) suckers. *Scale bar*: 200 µm

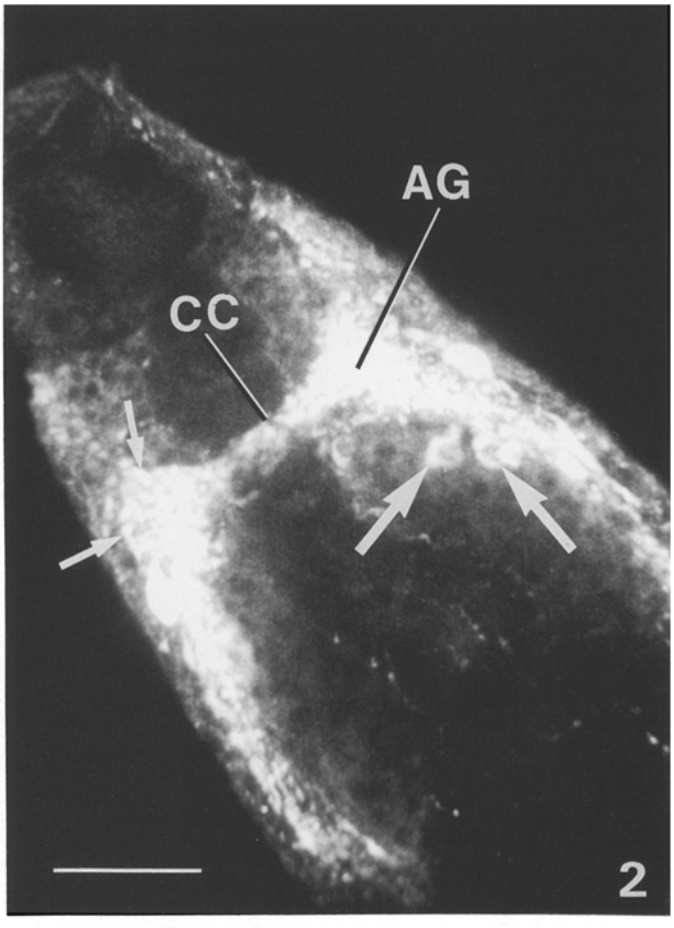
Fig. 2. Anterior region of an adult female, showing immunoreactivity to PYY in nerve cell bodies (*large arrows*) at the periphery and in nerve fibres (*small arrows*) in the central neuropile of the anterior ganglia (*AG*). *CC* central commissure. *Scale bar*: 100 µm

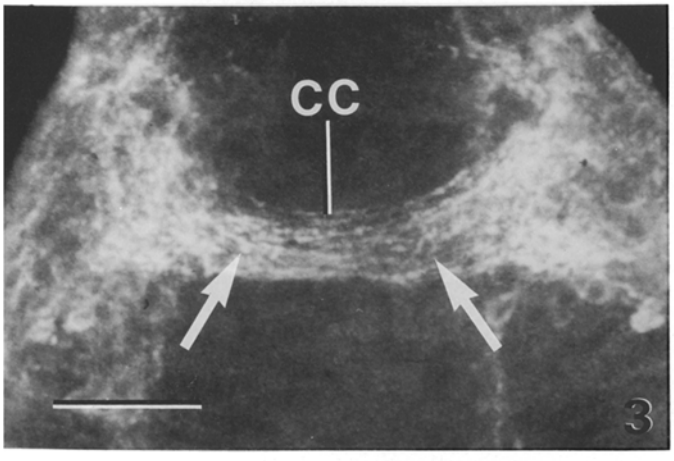
Fig. 3. Adult male. The central commissure (*CC*) is composed of numerous fine nerve fibres (*arrows*) immunoreactive to PP. *Scale bar*: 100 µm

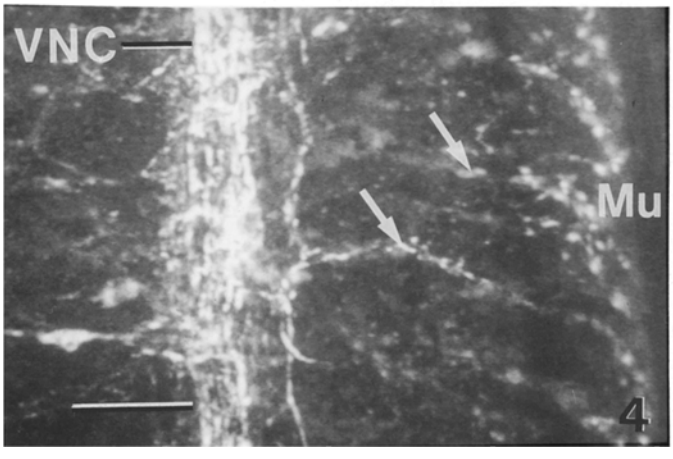
Fig. 4. Adult male. NPY-immunoreactive nerve fibres are present within the ventral nerve cord (*VNC*) and fine nerve fibres (*arrows*) emanate from the cord to innervate the subtegumental musculature (*Mu*). *Scale bar*: 100 µm

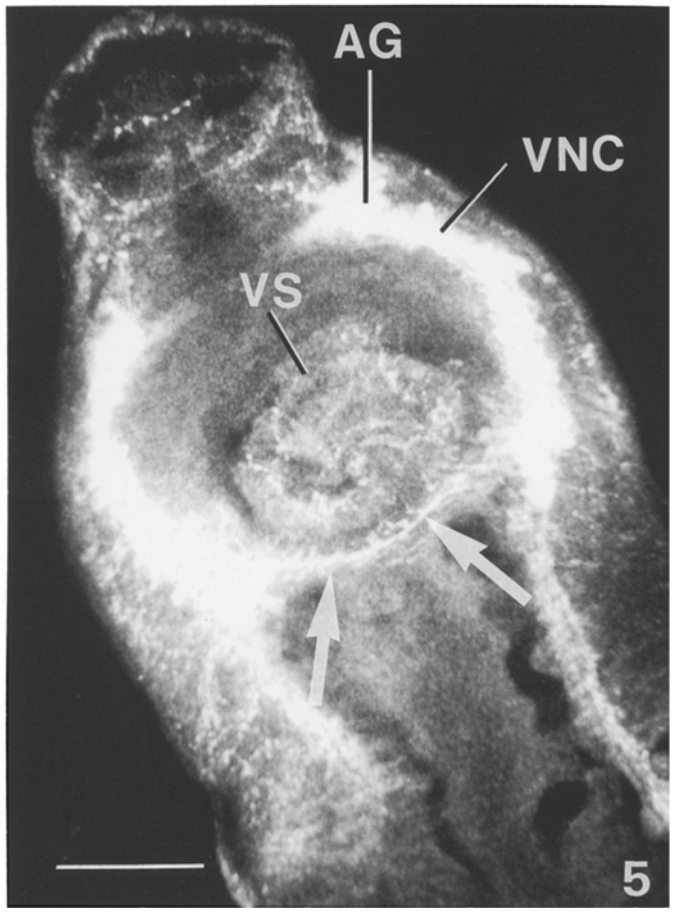
Fig. 5. Anterior region of an adult male, showing the origins of the well-developed ventral nerve cord (*VNC*) from the anterior ganglia (*AG*). A cross-connective (*arrows*) unites the nerve cords just posterior to the ventral sucker (*VS*). *Scale bar*: 200 µm


AG
OS
CC
VNC
VS
DNC
1


AG
CC
2


CC
3


VNC
Mu
4


AG
VNC
VS
5

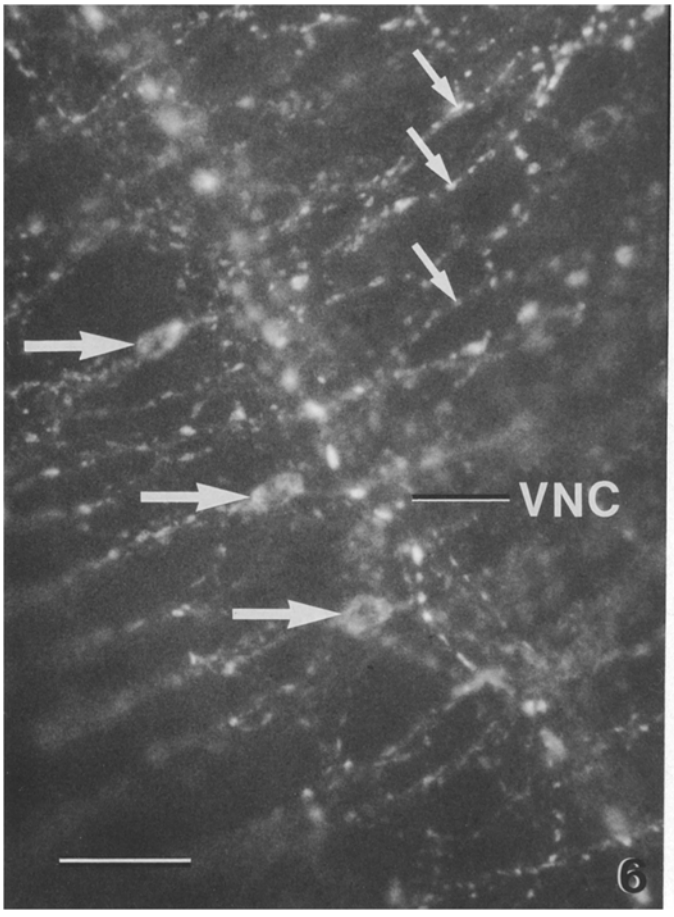


Fig. 6. Fluorescence micrograph of the ventral midbody region of an adult male whole-mount preparation, showing PYY-immunoreactivity in an extensive plexus of varicose nerve fibres (*small arrows*) and associated nerve cell bodies (*large arrows*) in the lining of the gynaecophoric canal. Note the path of the ventral nerve cord (VNC) which runs above. *Scale bar*: 100 μm

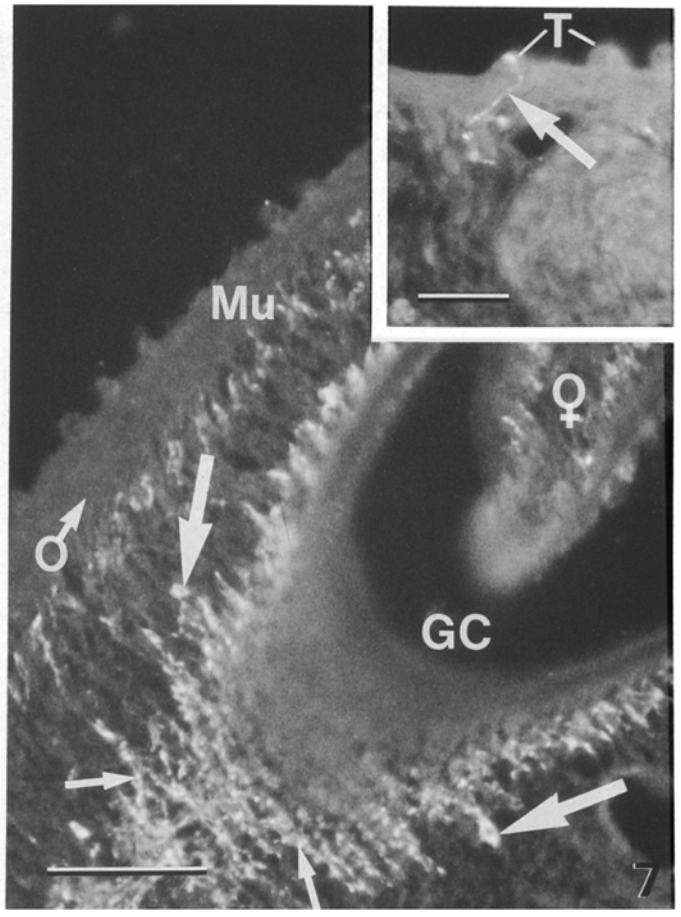


Fig. 7. Cryostat section of an adult worm pair, showing PYY-immunoreactivity in an extensive plexus of nerve fibres (*small arrows*) and associated nerve cell bodies (*large arrows*) in the lining of the gynaecophoric canal (*GC*) of the male worm (♂). Note also the PYY-immunoreactive nerve fibres associated with the subtegumental muscle layers (*Mu*) on the dorsal surface of the male. ♀ female. *Scale bar:* 100 μm. The *inset* shows a distinct PYY-immunoreactive nerve fibre (*arrow*) extending into one of the spiny tubercles (*T*) on the dorsal surface of the male. *Scale bar*: 100 μm

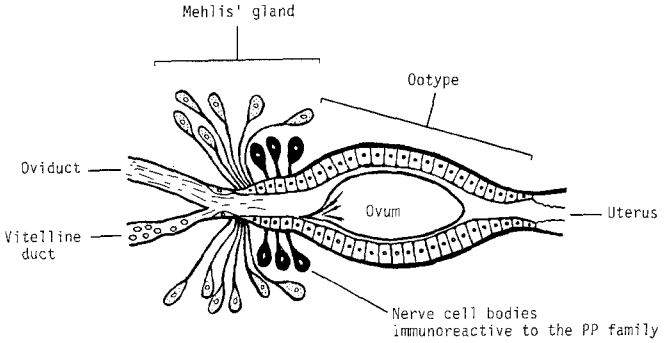
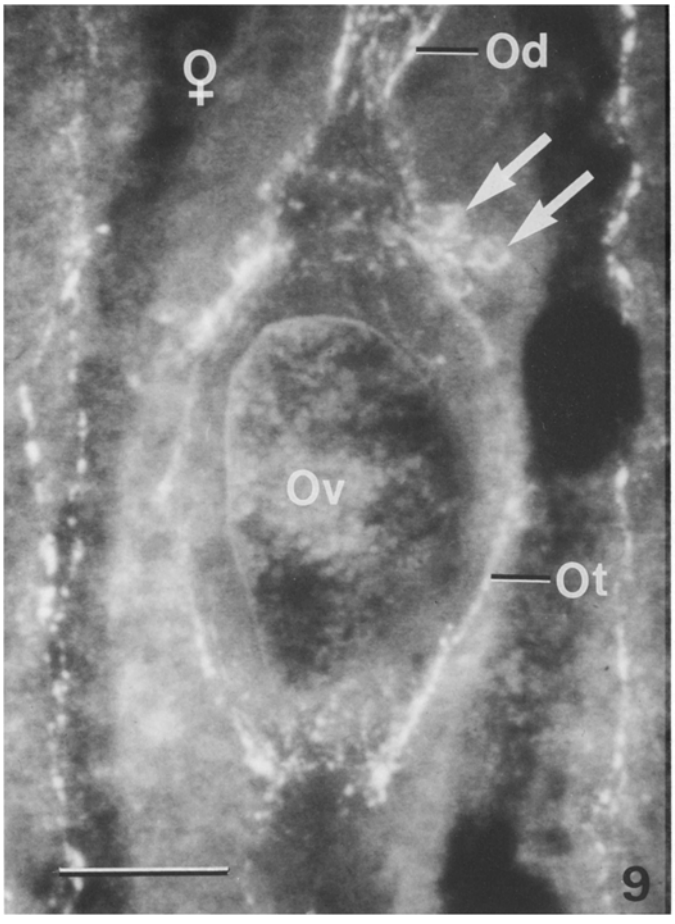


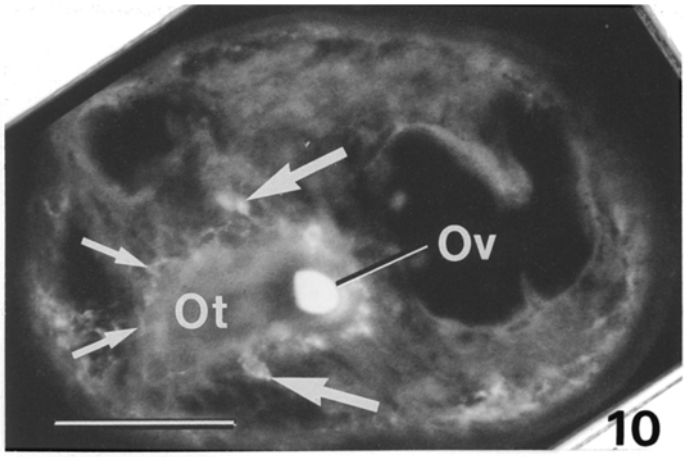
Fig. 8. Diagram of the ootype/Mehlis' gland complex of *S. mansoni* (based on Gönnert 1955; Silk and Spence 1971). Included is the collar of nerve cell bodies immunoreactive to the PP family

RPN.1702). However, of the remaining antisera that gave consistently positive results, there was considerable variation in the degree of immunostaining, depending on the regional specificity of the antiserum (see Table 1).

Routine examination of whole-mount and sectioned material showed that the antisera to PP, PYY, and NPY were staining essentially the same elements, albeit to differing degrees (see Table 1). Therefore, the main immunocytochemical features of the peptidergic nervous systems of adult male and female worms will be described collectively.

The strongest immunostaining was observed in the paired anterior ganglia, the commissure and the dorsal and ventral nerve cords, which together comprise the CNS. This configuration is essentially the same in both sexes (Figs. 1 and 2), but is especially evident in the larger male. Each anterior ganglion comprises a distinct central neuropile of nerve fibres surrounded by as few as four small (5 μm by 5 μm) unipolar cell bodies (Figs. 1 and 2). Immunoreactive nerve fibres with associated cell bodies also make up the broad commissure uniting the two anterior ganglia (Fig. 3) and the dorsal and ventral nerve cords which emanate from them (Fig. 4). The ventral pair of nerve cords is better developed than the dor-





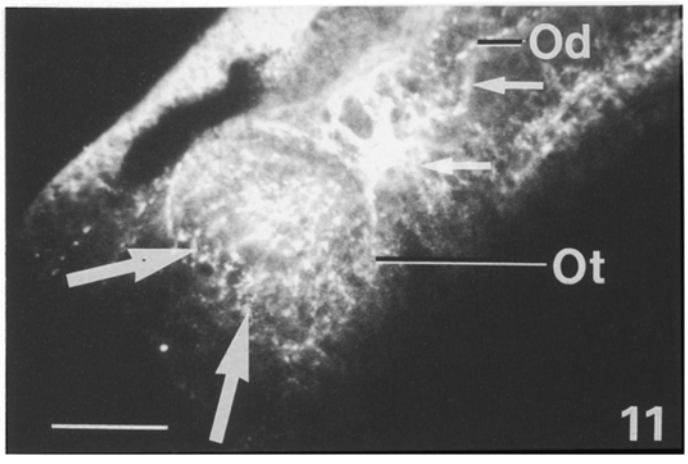


Fig. 9. Confocal scanning laser micrograph of an intact worm pair showing PP-immunoreactivity in the ootype/Mehlis' gland complex of the female worm (♀). PP-immunoreactive nerve fibres innervate the lining of the ovovitelline duct (*Od*) and the wall of the ootype (*Ot*). A collar of PP-immunoreactive nerve cell bodies (arrows) surrounds the neck of the ootype. *Ov* ovum forming within ootype. *Scale bar*: 200 μm

Fig. 10. Cryostat section through the neck of the ootype (*Ot*) of an adult female, showing intense PYY-immunoreactivity in nerve plexuses (*small arrows*) lining the ootype and in a number of nerve cell bodies (*large arrows*) with cytoplasmic processes extending into the ootype. *Ov* ovum forming within ootype. *Scale bar*: 200 μm

Fig. 11. Confocal scanning laser micrograph of the reproductive system of an immature female from a unisexual infection, showing PP-immunoreactivity in a nerve plexus (*small arrows*) innervating the lining of the ovovitelline duct (*Od*) and in a plexus of nerve fibres (*large arrows*) in the wall of the ootype (*Ot*). *Scale bar*: 200 μm

sal pair and is joined by a distinct cross-connective at a level just posterior to the ventral sucker (Fig. 5). Immunoreactive nerve fibres also extend from the anterior ganglia to innervate the oral and ventral suckers (Fig. 1).

In the PNS, intense immunoreactivity was observed in an extensive plexus of nerve fibres and associated elongated unipolar cell bodies (7 μm by 3.5 μm) in the lining of the gynaecophoric canal of the male (Figs. 6 and 7). This network was evident in both whole-mount (Fig. 6) and sectioned material (Fig. 7). Immunoreactive nerve fibres were also associated with the innervation of the subtegumental musculature (Figs. 4 and 7) and with the muscular oral and ventral suckers in both sexes (Figs. 1 and 5). In addition, fine immunoreactive nerve fibres extend through the subtegumental muscle layer to innervate the spiny tubercles which project from the dorsal surface of the male (inset Fig. 7).

No immunoreactivity was observed in any of the male reproductive structures or their associated ducts, although strong immunoreactivity was obtained with all antisera (except NPY antiserum RPN.1702 and PP antiserum PP204) in the region of the ootype/Mehlis' gland complex, which is responsible for egg production in the female (Fig. 8). Nerve plexuses in the lining of the common ovovitelline duct and the muscular wall of the ootype were strongly immunoreactive, and a cluster of not more than six unipolar cell bodies (5 μm by 5 μm) was evident where the ovovitelline duct leads into the ootype (Fig. 9). These cell bodies had short cytoplasmic extensions (about 7.5 μm in length) leading into the ootype wall itself (Fig. 10). In immature female worms from unisexual infections, the nerve plexuses in the lining of the ovovitelline duct and the wall of the ootype were consistently immunoreactive (Fig. 11), although there was no evidence of the immunoreactive cell bodies previously described in the region of Mehlis' gland.

The controls involving omission or substitution of primary antiserum were consistently negative. The re-

Table 2. Preabsorption studies: minimum quantity of peptide antigen which abolishes immunostaining

Antiserum	C-terminal hexapeptide of PP (pmol/ml)	Synthetic PYY (pmol/ml)	Synthetic NPY (pmol/ml)	Synthetic FMRFamide (pmol/ml)
PP221(6)	12.5	25	250	–
PP204(7)	125	–	–	–
PYY301(3)	1250	25	2500	–
PYY297(4)	1250	2.5	–	–
GNPY163(2)	1250	25	25	–
NPY8999(6)	1250	NT	NT	NT
FMRFamide 494.2	–	–	–	150

NT = not tested; – = immunoreactivity not blocked

sults of the preabsorption studies are summarised in Table 2.

Discussion

The present investigation has demonstrated, for the first time, the presence and distribution of immunoreactivity to three members of the PP family in the nervous systems of adult male and female *S. mansoni*. The finding of peptides in the nervous system suggests that these molecules play an important neurotransmitter/neuromodulatory role in the parasite. Immunoreactivity was observed throughout the CNS in both sexes; its distribution is identical to that of the cholinergic component of the nervous system (Bueding et al. 1967; Fripp 1967). In the PNS, immunoreactive fibres innervate the subtegumental musculature and the muscular oral and ventral suckers of both sexes. Immunoreactivity to the PP family was especially evident in an extensive plexus of nerve fibres and associated cell bodies in the lining of the gynaecophoric canal (ventral surface) of the male. This concentration of peptidergic material, where the male and female are in most intimate contact, could suggest a sensory role for these peptides, for example in the orientation of male and female worms during pairing or in the stimulation of the sexual development of the female. A neurosecretory involvement in these processes has been proposed (Michaels 1969; Popiel 1986). Another sensory role is suggested by the innervation of the spiny tubercles which project from the dorsal surface of the male worm. Innervation of the tubercles by peptidergic fibres has been described in previous immunocytochemical studies on *S. mansoni*, together with peptide immunoreactivities in the ciliated sensory receptors that are distributed throughout the general body tegument (Basch and Gupta 1988; Gustafsson 1987). Innervation of the latter type of receptor was not observed in the present study.

No immunoreactivity to the PP family was observed in the musculature surrounding the gastrodermis in which immunoreactivity to gastrin and cholecystokinin (CCK) has been described (Basch and Gupta 1988). Similarly, no immunoreactivity was evident in the reproductive organs or associated ducts of the male worm. The ootype/Mehlis' gland complex (Gönnert 1955; see Fig. 8), which is responsible for egg production in the female, was consistently immunoreactive for the PP family. The nerve plexus in the lining of the common ovovitelline duct, which serves to convey the products of the ovary and vitelline gland, respectively, into the egg-forming chamber or ootype, was consistently immunoreactive, as was that in the muscular wall of the ootype itself. At these sites, the neuropeptides may be involved in regulating the movement of material along the ovovitelline duct and/or co-ordinating the contractions of the ootype wall, thereby controlling the movement of ova through the ootype and the moulding of the characteristic shape of the schistosome egg. However, of more interest was the cluster of not more than six immunoreactive cell bodies at the proximal (posterior) end of the ootype in the region of Mehlis' gland, with cytoplasmic extensions leading into the wall of the ootype. These cell bodies presumably correspond to the six perikarya observed by Basch and Gupta (1988) in the region between the ovary and common vitelline duct, which were interpreted as equivalent to the nerve plexuses described by Gönnert (1962) in *F. hepatica*. It is interesting to note in this respect that two groups of PP-immunoreactive cell bodies have been observed in *F. hepatica* in sites corresponding to nerve plexuses I and II (Gönnert 1962), but they are distinct from a cluster of some 20–30 PYY- and FMRFamide-immunoreactive cell bodies that lie amongst the cells of Mehlis' gland around the ootype (Magee et al. 1989). The immunoreactive cell bodies observed in the present study on *S. mansoni* more closely resemble the latter and in such a location could exert a paracrine action, possibly influencing the release of the secretions of Mehlis' gland and/or the secretions of the ootype itself. Basch and Gupta (1988) observed immunoreactivity to CCK in the innervation of the uterine wall and the vitelline follicles, but no such immunoreactivity to the PP family was evident in the present study. The finding that, in immature females from unisexual infections, the lining of the common ovovitelline duct and the wall of the ootype were consistently immunoreactive to the PP family, was surprising because these structures are not fully developed in such individuals (Erasmus 1987). In contrast, there was no evidence of the immunoreactive cell bodies in the region of Mehlis' gland, which are such a prominent feature of the peptidergic system of the mature female.

There was a marked difference in the intensity of immunostaining produced by the various antisera. For example, of the two PP antisera used, PP221, which was raised to the C-terminal hexapeptide of bovine PP, produced the strongest immunostaining, whereas, PP204, which was raised to the whole PP molecule and likely to be more mid-to N-terminally-directed, consistently produced a weaker reaction. The immunostaining produced by PP204 could be quenched by >125 pmol/ml of the C-terminal hexapeptide of PP, suggesting that there is at least a small sub-population of C-terminally-

directed antibodies in the PP204 antiserum. Similarly, the immunostaining produced by all the other antisera, except PYY297, could also be quenched by the C-terminal hexapeptide of PP, albeit at the slightly higher concentration of 1250 pmol/ml, suggesting that these antisera cross-react with the PP-like material in the schistosome's nervous system. Several workers have expressed concern about cross-reactivity between PP and antisera raised to the invertebrate peptide FMRFamide (Triepel and Grimmelikhuijzen 1984; Grimmelikhuijzen and Graff 1985; Grimmelikhuijzen 1986). The latter shares a penultimate arginine and an amidated aromatic amino acid in common positions with the C-terminal hexapeptide of PP to which antiserum PP221 was raised. Cross-reactivity between the two peptides does not appear to have been a problem in the present study, since the immunostaining produced by PP221, and the other antisera to the PP family for that matter, could not be quenched by up to 150 nmol/ml of FMRFamide antigen. Conversely, the immunostaining produced by the FMRFamide antiserum (antiserum 494.2) could not be quenched by the addition of up to 125 nmol/ml of the C-terminal hexapeptide of PP, nor indeed by up to 25 nmol/ml of intact PYY or NPY. The fact that immunostaining with the more PYY-specific antiserum PYY297 could be blocked by 1250 pmol/ml of the C-terminal hexapeptide of PP suggests that PYY297 cross-reacts slightly with PP, although within the constraints of the preabsorption studies it would appear that PYY297 is more specific for PYY than PP by a factor of approximately 500. The preabsorption studies suggest that antiserum GNPY 163(2), raised to the C-terminal decapeptide of NPY, is equally specific for PYY and NPY and more specific for these than the C-terminal hexapeptide of PP by a factor of approximately 50. Therefore, it is possible that the immunoreactivity obtained with GNPY 163(2) is due to cross-reactivity of the antiserum with a more PYY-like molecule. Consequently, there may be little or no NPY-like material present in *S. mansoni*. The N-terminally-directed NPY antiserum (RPN.1702) yielded a consistently negative reaction. This is not surprising since it is likely that there will have been numerous amino acid substitutions during evolution between the schistosome and mammalian peptides in the mid-to-N-terminal region of the molecule, away from the highly-conserved and biologically active C-terminus. This point is borne out by the strong immunoreactivity obtained with the other NPY antisera (GNPY 163(2) and NPY 8999(6)), which were raised to the C-terminus of mammalian NPY. Indeed, the immunostaining obtained with the amide-requiring, C-terminally-directed, antiserum NPY 8999(6) would suggest that the parasite peptide may be amidated, although this would require further validation under competitive assay conditions.

PP has not been detected in coelenterates (Grimmelikhuijzen 1984, 1986). The platyhelminths are the lowest group in which PP-related molecules are present. Within the group, PP-immunoreactivity has been demonstrated in a range of free-living and parasitic species (Gustafsson et al. 1986; McKay et al. 1990; Magee et al. 1989; Maule et al. 1989a; Maule et al. 1989b; Reuter and Palmberg 1987; Reuter et al. 1988; Yui et al. 1985). In higher organisms, PP has been demonstrated in the nervous system of annelids and molluscs (Curry et al. 1989; Sundler et al. 1977; Van Noorden 1984), and in the "brain and gut" of arthropods and protochordates (Falkmer et al. 1984, 1985; Thorndyke 1986; Van Norden 1984). In contrast, PYY-immunoreactivity has only been recorded in the annelid *Lumbricus terrestris* (Curry et al. 1989) and a number of parasitic flatworms (Fairweather et al. 1988, 1990; McKay et al. 1990; Magee et al. 1989; Maule et al. 1989a). For NPY, apart from one study of an insect (Rémy et al. 1988) and its localisation in *L. terrestris* (Curry et al. 1989), this is the only description of NPY-immunoreactivity in a non-chordate species. So, relatively little is known about the distribution in invertebrates of the three members of the PP family, and the present results have added to the body of knowledge. Moreover, most of the previous invertebrate studies on PP-immunoreactivity used commercial antisera of uncharacterised specificity. Indeed, lack of reactions to antisera against PP, PYY, and NPY have been reported in a number of studies on flatworms (Gustafsson et al. 1986; Reuter 1987; Wikgren and Reuter 1985; Yui et al. 1985). Our investigation has employed a range of antisera of defined specificities, in combination with a comprehensive series of preabsorption studies, to characterise as fully as possible (by means of immunocytochemistry) the nature of the PP-like material(s) in *S. mansoni*.

The present results also have a bearing on the evolution of the PP family. The three members of the family are known to have differential distributions within mammals, with PP being located in endocrine cells in the pancreas and PYY in endocrine cells in the large intestine and colon (El-Salhy et al. 1987); NPY is confined to the nervous system (Gray and Morley 1986; Sundler et al. 1986). By virtue of its location, NPY would appear to be evolutionarily the most ancient member and hence the prototype of the PP family, the differentiation of the sequences of PP and PYY following with the advent of more sophisticated function of the gastrointestinal tract (Scharrer 1987). A similar neuronal ancestry has been proposed for several other brain-gut peptides (Falkmer 1985). In *S. mansoni*, immunoreactivity to the PP family had a purely neuronal distribution and so may have a neuropeptide-like function more analogous to that of mammalian NPY than either PP or PYY. However, the use of a broad spectrum of antisera of different regional specificities to the individual members of the PP family, in conjunction with the preabsorption studies, indicates that the peptide(s) of this parasite may have more sequence homology with PP and PYY than with the postulated prototype molecule, NPY. In any event, this immunoreactive material must be characterised further and ultimately sequenced if the nature of the peptide(s) concerned is to be determined. Since it is proposed that these neuropeptides may act as important neurotransmitters/neuromodulators in the parasite and may even be involved in the regulation of egg production, sequence data would permit the synthesis of peptide analogues which might prove of use as novel chemotherapeutic agents against schistosomiasis.

Acknowledgements. The authors wish to thank the Medical Research Council for the provision of a Postdoctoral Research Fellowship (to PJS) and also the following for the gifts of antisera: Dr. Mairead O'Hare (PP and NPY), Ann O'Neill (PYY) and Raymond Magee (FMRFamide).

References

Barker LR, Bueding E, Timms AR (1966) The possible role of acetylcholine in *Schistosoma mansoni.* Br J Pharmacol 26:656–665

Basch PF, Gupta BC (1988) Immunocytochemical localization of regulatory peptides in six species of trematode parasites. Comp Biochem Physiol (C) 91:565–570

Bennett J, Bueding E (1971) Localization of biogenic amines in *Schistosoma mansoni.* Comp Biochem Physiol (A) 39:859–867

Bennett J, Bueding E, Timms AR, Engstrom RG (1969) Occurrence and levels of 5-hydroxytryptamine in *Schistosoma mansoni.* Mol Pharmacol 5:542–545

Bueding E, Schiller EL, Bourgeois JG (1967) Some physiological, biochemical, and morphologic effects of tris(p-aminophenyl) carbonium salts (TAC) on *Schistosoma mansoni.* Am J Trop Med Hyg 16:500–515

Chou T-CT, Bennett J, Bueding E (1972) Occurrence and concentrations of biogenic amines in trematodes. J Parasitol 58:1098–1102

Coons AH, Leduc EH, Connolly JM (1955) Studies on antibody production. I. A method for the histochemical demonstration of specific antibody and its application to a study of the hyperimmune rabbit. J Exp Med 102:49–60

Curry WJ, Fairweather I, Johnston CF, Halton DW, Buchanan KD (1989) Immunocytochemical demonstration of vertebrate neuropeptides in the earthworm *Lumbricus terrestris* (Annelida, Oligochaeta). Cell Tissue Res 257:577–586

Dei-Cas E, Dhainaut-Courtois N, Dhainaut A, Vernes A (1979) Ultrastructural localization of tritiated 5-HT in adult *Schistosoma mansoni.* A preliminary report. Biol Cell 35:321–324

Dei-Cas E, Dhainaut-Courtois N, Biguet J (1981) Contribution à l'étude du système nerveux des formes adultes et larvaires de *Schistosoma mansoni* Sambon, 1907 (Trematoda Digenea). II. Rôle de la sérotonine et de la dopamine. Ann Parasitol Hum Comp 56:271–284

El-Salhy M, Grimelius L, Emson PC, Falkmer S (1987) Polypeptide YY- and neuropeptide Y-immunoreactive cells and nerves in the endocrine and exocrine pancreas of some vertebrates: an onto- and phylogenetic study. Histochem J 119:111–117

Erasmus DA (1987) The adult schistosome: structure and reproductive biology. In: Rollinson D, Simpson AG (eds) The Biology of Schistosomes: from genes to latrines. Pergamon Press, London, pp 51–82

Fairweather I, Macartney GA, Johnston CF, Halton DW, Buchanan KD (1988) Immunocytochemical demonstration of 5-hydroxytryptamine (serotonin) and vertebrate neuropeptides in the nervous system of excysted cysticercoid larvae of the rat tapeworm, *Hymenolepis diminuta* (Cestoda, Cyclophyllidea). Parasitol Res 74:371–379

Fairweather I, Mahendrasingham S, Johnston CF, Halton DW, Shaw C (1990) Peptidergic nerve elements in three developmental stages of the tetraphyllidean tapeworm, *Trilocularia acanthiaevulgaris:* an immunocytochemical study. Parasitol Res (in press)

Falkmer S (1985) Phylogenetic aspects of the brain-gut axis, with special reference to islet hormones in invertebrates and lower vertebrates. In: Kobayashi H, Bern HA, Urano A (eds) Neurosecretion and the Biology of Neuropeptides. Springer, Berlin Heidelberg New York, pp 317–325

Falkmer S, El-Salhy M, Titlbach M (1984) Evolution of the neuroendocrine system in vertebrates. A review with particular reference to the phylogeny and postnatal maturation of the islet parenchyma. In: Falkmer S, Håkanson R, Sundler F (eds) Evolution and Tumour Pathology of the Neuroendocrine System. Elsevier Science Publishers B.V., Amsterdam New York Oxford, pp 59–87

Falkmer S, Dafgard E, El-Salhy M, Engstrom W, Grimelius L, Zetterberg A (1985) Phylogenetical aspects on islet hormone families: a minireview with particular reference to insulin as a growth factor and to the phylogeny of PYY and NPY immunoreactive cells and nerves in the endocrine and exocrine pancreas. Peptides 6 (suppl 3): 315–320

Fripp PJ (1967) Histochemical localization of esterase activity in schistosomes. Exp Parasitol 21:380–390

Gianutsos G, Bennett JL (1977) The regional distribution of dopamine and norepinephrine in *Schistosoma mansoni* and *Fasciola hepatica.* Comp Biochem Physiol (C) 58:157–159

Gönnert R von (1955) Schistosomiasis-studien. II. Über die Eibildung bei *Schistosoma mansoni* und das Schicksal der Eier im Wirtsorganismus. Z Tropenmed Parasitol 6:33–52

Gönnert R von (1962) Histologische Untersuchungen über den Feinbau der Eibildungsstätte (Oogenotop) von *Fasciola hepatica.* Z Parasitenkd 21:475–492

Gray TS, Morley JE (1986) Neuropeptide Y: anatomical distribution and possible function in mammalian nervous system. Life Sci 38:389–401

Grimmelikhuijzen CJP (1984) Peptides in the nervous system of coelenterates. In: Falkmer S, Håkanson R, Sundler F (eds) Evolution and Tumour Pathology of the Neuroendocrine System. Elsevier Science Publishers B.V., Amsterdam New York Oxford, pp 39–58

Grimmelikhuijzen CJP, Graff D (1985) Arg-Phe-amide-like peptides in the primitive nervous systems of coelenterates. Peptides 6 (Suppl 3):477–483

Grimmelikhuijzen CJP (1986) FMRFamide-like peptides in the primitive nervous systems of coelenterates and complex nervous systems of higher animals. In: Stefano GB (ed) Handbook of Comparative Opioid and Related Neuropeptide Mechanisms. Vol 1. CRC Press, Inc, Boca Raton, Florida, pp 103–115

Gupta BC, Basch PF (1989) Human chorionic gonadotropin-like immunoreactivity in schistosomes and *Fasciola.* Parasitol Res 76:86–89

Gustafsson MKS (1987) Immunocytochemical demonstration of neuropeptides and serotonin in the nervous system of *Schistosoma mansoni.* Parasitol Res 74:168–174

Gustafsson MKS, Lehtonen MAI, Sundler F (1986) Immunocytochemical evidence for the presence of "mammalian" neurohormonal peptides in neurones of the tapeworm *Diphyllobothrium dendriticum.* Cell Tissue Res 243:41–49

Hillman GR (1983) The neuropharmacology of schistosomes. Pharmacol Ther 22:103–115

Machado CRS, Machado ABM, Pellegrino J (1972) Catecholamine-containing neurons in *Schistosoma mansoni.* Z Zellforsch 124:230–237

Magee RM, Fairweather I, Johnston CF, Halton DW, Shaw C (1989) Immunocytochemical demonstration of neuropeptides in the nervous system of the liver fluke, *Fasciola hepatica* (Trematoda, Digenea). Parasitology 98:227–238

Maule AG, Shaw C, Halton DW, Johnston CF, Fairweather I (1989a) Localization, quantification and characterization of pancreatic polypeptide immunoreactivity in the parasitic flatworm *Diclidophora merlangi* and its fish host (*Merlangius merlangus*). Gen Comp Endocrinol 74:50–56

Maule AG, Halton DW, Johnston CF, Fairweather I, Shaw C (1989b) Immunocytochemical demonstration of neuropeptides in the fish-gill parasite, *Diclidophora merlangi* (Monogenoidea). Int J Parasitol 19:307–316

McKay DM, Halton DW, Johnston CF, Fairweather I, Shaw C (1990) Occurrence and distribution of putative neurotransmitters in the frog lung parasite, *Haplometra cylindracea* (Trematoda: Digenea). Parasitol Res (in press)

Mellin TN, Busch RD, Wang CC, Kath G (1983) Neuropharmacol-

ogy of the parasitic trematode, *Schistosoma mansoni*. Am J Trop Med Hyg 32:83–93

Michaels RM (1969) Mating of *Schistosoma mansoni* in vitro. Exp Parasitol 25:58–71

Pax RA, Siefker C, Bennett JL (1984) *Schistosoma mansoni:* differences in acetylcholine, dopamine and serotonin control of circular and longitudinal parasite muscles. Exp Parasitol 58:314–324

Popiel I (1986) The Reproductive Biology of Schistosomes. Parasitol Today 2:10–15

Rémy C, Guy J, Pelletier G, Boer HH (1988) Immunohistological demonstration of a substance related to neuropeptide Y and FMRFamide in the cephalic and thoracic nervous systems of the locust *Locusta migratoria*. Cell Tissue Res 254:189–195

Reuter M, Palmberg I (1987) An ultrastructural and immunocytochemical study of gastrodermal cell types in *Microstomum lineare* (Turbellaria, Macrostomida). Acta Zool (Stockh) 68:153–163

Reuter M, Lehtonen M, Wikgren M (1988) Immunocytochemical evidence of neuroactive substances in flatworms of different taxa – a comparison. Acta Zool (Stockh) 69:29–37

Scharrer B (1987) Neurosecretion: beginnings and new directions in neuropeptide research. Ann Rev Neurosci 10:1–17

Sundler F, Håkanson R, Alumets J, Walles B (1977) Neuronal localization of pancreatic polypeptide (PP) and vasoactive intestinal peptide (VIP) immunoreactivity in the earthworm (*Lumbricus terrestris*). Brain Res Bull 2:61–65

Sundler F, Håkanson R, Ekblad E, Uddman R, Wahlestedt C (1986) Neuropeptide Y in the peripheral adrenergic and enteric nervous systems. Int Rev Cytol 102:243–269

Thorndyke MC (1986) Immunocytochemistry and evolutionary studies with particular reference to peptides. In: Polak JM, Van Noorden S (eds) Immunocytochemistry. Modern Methods and Applications. Second Edition. John Wright and Sons Ltd, Bristol, pp 308–327

Tomosky TK, Bennett JL, Bueding E (1974) Tryptaminergic and dopaminergic responses of *Schistosoma mansoni*. J Pharmacol Exp Ther 190:260–271

Triepel J, Grimmelikhuijzen CJP (1984) Mapping of neurons in the central nervous system of the guinea pig by use of antisera specific to the molluscan neuropeptide FMRFamide. Cell Tissue Res 237:575–586

Van Noorden S (1984) The neuroendocrine system in protostomian and deuterostomian invertebrates and lower vertebrates. In: Falkmer S, Håkanson R, Sundler F (eds) Evolution and Tumour Pathology of the Neuroendocrine System. Elsevier Science Publishers B.V., Amsterdam New York Oxford, pp 7–38

Wikgren MC, Reuter M (1985) Neuropeptides in a microturbellarian-whole mount immunocytochemistry. Peptides 6 (Suppl 3):471–475

Yui R, Iwanaga T, Kuramoto H, Fujita T (1985) Neuropeptide immunocytochemistry in protostomian invertebrates, with special reference to insects and molluscs. Peptides 6 (Suppl 3):411–415