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IMMUNOCYTOCHEMICAL DEMONSTRATION OF SOME VERTEBRATE PEPTIDE HORMONE-LIKE SUBSTANCES IN THE MIDGUT ENDOCRINE CELLS IN *TRAMEA VIRGINIA* (RAMBUR) (ANISOPTERA: LIBELLULIDAE)

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The present immunocytochemical study reveals the presence of well-defined endocrine cells, intermingled with the columnar cells of the epithelium in the midgut region of the alimentary canal of *T. virginia*. The midgut endocrine cells are of 2 types, the open-type midgut endocrine cells (OMEC) with a long tubule opening into the lumen of the midgut and close-type midgut endocrine cells (CMEC) which are spherical in shape and devoid of extending tubules. Various gastrointestinal hormone-like substances are localized in respective types of midgut endocrine cells in different regions of the midgut i.e. anterior, middle and posterior. The NPY, FMRFamide and β-endorphin were localized in the open-type while substance P, gastrin, CCK and VIP in the close-type midgut endocrine cells. The midgut endocrine cells in *T. virginia* differ from each other in their location, cytomorphological and immunocytochemical characteristics representing different types of endocrine cells. Functional significance of these myotropic and vertebrate gastrointestinal hormone-like substances in the midgut endocrine cells of *T. virginia* is discussed.

INTRODUCTON

With the help of electronmicroscopic and immunohistochemical techniques, the endocrine cells in the midgut of several insect species have been localized showing immunoreactivity with antibodies of various vertebrate peptidergic and aminergic hormonal substances (FUJITA et al., 1981; BROWN, 1986; SCHOLS et al., 1987; CRIM et al., 1992; LUNDQUIST et al., 1994; NIJHOUT, 1994; LEHANE,

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1998; VEENSTRA et al., 1995; LANGE, 2001; WANG et al., 2001; NEVES et al., 2002). Although the functional significance of the midgut endocrine cells and their secretory material is still obscure, some evidences have been accumulated suggesting their involvement in the control of transport and digestion of food material by inducing peristaltic contraction in the foregut and stimulating release of digestive enzymes from columnar cells into the lumen of midgut, respectively (FUJITA et al., 1981; NIJHOUT, 1994; MURALEEDHARAN, 1995; NACHMAN et al., 1997; FUSE et al., 1999; PRABHU & SREEKUMAR, 1999; WEI et al., 2000; HILL & ORCHARD, 2005).

Besides some information on *Aeshna cyanea* (ANDRIES & TRAMU, 1985b), no comprehensive efforts have been made to demonstrate cytomorphological characteristics and immunocytochemical localization of various vertebrate hormones in Odonata. The present study was, therefore, undertaken to elucidate cytomorphology and immunoreactivity of the midgut endocrine cells in *Tramea virginia.*

MATERIAL AND METHODS

HISTOLOGICAL TECHNIQUES. – Adult dragonflies were collected from the university campus premises and their alimentary canals were dissected and fixed immediately for 18-20 h in aqueous Bouin's fluid, dehydrated in ethanol, cleared in xylene and embedded in paraffin wax at 58-62°C. The 4μm thick sections were stained with Ehrlich's Hematoxylin-Eosin and Heidenhain's Iron-Hematoxylin-Orange G.

IMMUNOCYTOCHEMICAL TECHNIQUES. – For immunocytochemical studies, the midgut was fixed in cold Bouin's fluid, Zambonin or 4% paraformaldehyde fixatives for 24 h. Thereafter, overnight treatment of cold sucrose solution was given for cryoprotection of tissue and frozen sections of 15 μm thickness were cut on the cryostat (Lieca). The sections were affixed to poly-L-lysin coated or gelatin subbed slides.

The slides were preserved in deep freezer and proceeded for immuno-staining.

The Streptavidin-biotin-peroxidase (Sigma) method was employed during the present study. The sections were washed in PBS (pH- 7.4) for 15 min and treated with 1% bovine serum albumin (BSA) in PBS containing 0.3% Triton X-100. The polyclonal antibodies against FMRFamide (Incstar; Cat. No. 20091, dilution-1:1000), neuropeptide Y (NPY) (Sigma; Cat No. N-9528, dilution-1:800), β-endorphin (Sigma; Cat. No. E1520, dilution 1:1000), substance-P (ICN; Cat. No. 11820, dilution-1:1000), gastrin (Sigma; G-0785, dilution-1:800), cholecystokinin (CCK) (Sigma; Cat. No. C-2581, dilution-1:800) and vasoactive intestinal peptide (VIP) (ICN; Cat. No. P-2026, dilution-1:800) were diluted in PBS in respective concentrations containing 0.3% Triton X-100 and 1% BSA and the sections were then incubated for 2 h at 25°C with each antibody separately. Excess antibody was rinsed in PBS and incubated with biotinylated secondary antibody for 40 min, followed by Streptavidin-peroxidase conjugate (Sigma). 3-Amino-9-ethyl Carbazole (AEC101) was used as chromogen to visualize the reddish brown reaction product. Sections were again washed in distilled water to stop reaction and mounted in glycerol gelatin. The specificities and cross reactivities of the above antisera were tested by liquid-phase preabsorption of the diluted antisera with respective concentrations separately and was found that it abolished all immunostainings in the midgut sections of *T. virginia.*

RESULTS

The midgut wall is composed of an outer layer of longitudinal muscles, middle layer of circular muscles and inner layer of epithelium consisting two types of cells; the tall columnar cells and tiny regenerative cells. Besides the above cell types, the immunocytochemical studies reveal the presence of endocrine cells intermingled with the columnar epithelial cells. They can be classified into two principal types; the open and close-types of midgut endocrine cells. The open--type midgut endocrine cells are closely associated with the inner surface of the circular muscle layer and provided with the distal cytoplasmic tubule opening into the lumen, while, the close-type midgut endocrine cells are devoid of the distal cytoplasmic tubular process (Fig. 1). The midgut endocrine cells vary in location, shape, size and immunoreactivity from each other and can be classified in various sub-types (Tab. I).

THE OPEN-TYPE MIDGUT ENDOCRINE CELLS (OMEC)

The OMEC are oval, conical or elliptical in shape and each cell bears a bunch of dendrite-like processes apically and a long tubular process distally leading to the lumen. They showed immunoreactions with the NPY, FMRFamide and β-endorphin antisera. The NPY-positive OMEC are observed in large number in the anterior and middle regions of the midgut (Fig. 2) and the FMRFamide-positive OMEC are densely distributed in the anterior and posterior regions of

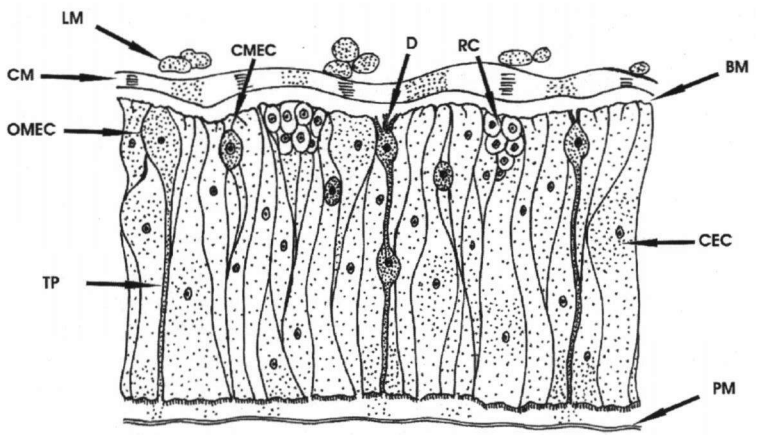


Fig. 1. Diagrammatic representation of the midgut histological structure showing - columnar epithelial cells (CEC), regenerative cells (RC) and open-type midgut endocrine cells (OMEC) and close-type midgut endocrine cells (CMEC) and other histological structures. [LM = longitudinal muscles, – CM = circular muscles, – BM = basement membrane, – PM = peritrophic membrane, – D = dendrites, – TP = tubular processes]

Table I
Cytomorphological details of midgut endocrine cells with respect to different vertebrate peptide hormone-like substances in *Tramea virgina*

Midgut Endocrine Cells	Antibodies applied	Size (μm) Cell diameter	Nucleus diameter	Dendrite	Tubule length	Location
OMEC	NPY	13.5 ± 2.78	6.80 ± 1.92	18.23 ± 2.36	70.52 ± 7.73	AMG, MMG,
	FMRFamide	14.82 ± 1.78	7.25 ± 1.40	------	52.36 ± 4.95	AMG, PMG
	β-Endorphin	18.15 ± 1.72	7.08 ± 2.44	11.26 ± 2.36	44.26 ± 5.04	AMG
CMEC	VIP	6.38 ± 2.32	3.12 ± 1.94	------	------	MMG
	Gastrin	7.26 ± 2.12	3.56 ± 2.36	------	------	AMG
	Substance P	8.52 ± 2.10	4.46 ± 1.92	------	------	MMG
	CCK	10.74 ± 2.42	5.18 ± 2.64	------	------	MMG

(AMG- Anterior midgut; MMG- Middle midgut; PMG- Posterior midgut).

the midgut (Fig. 3) while the β-endorphin immunopositive OMEC are confined to the anterior part of the midgut only (Fig. 4).

THE CLOSE-TYPE MIDGUT ENDOCRINE CELLS (CMEC)

The CMEC are spherical in shape but devoid of any kind of processes. They showed immunoreaction with the VIP, gastrin, substance P and CCK antisera. The VIP, substance P and CCK- positive CMEC are observed in the middle region (Figs 5, 7 & 8) while the gastrin-positive CMEC in the anterior region of the midgut (Fig. 6).

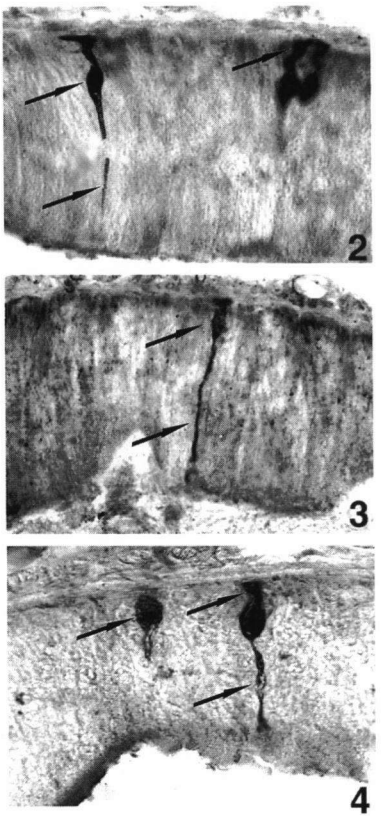
DISCUSSION

The midgut endocrine cells are well distinct in the dragonfly, *Tramea virginia* and on the basis of shape, size and immunoreactivity to the antibodies of various vertebrate hormones, they can be classified into various cell-types similar to that in other species of insects (MONTUEGA et al., 1989; SIVASUBRAMANIAN, 1992; ŽITŇAN et al., 1993; VEENSTRA et al., 1995; NACHMAN et al., 1997; PREDEL et al., 1999, 2001; NICHOLS et al., 2003; HILL & ORCHARD, 2005). MONTUEGA et al., (1989) noticed ten types of midgut endocrine cells immunoreactive to ten different types of neuropeptides in *Aedes aegypti* while during the present study seven types of midgut endocrine cells immunoreactive to seven different types of neuropeptides can be recognized in *Tramea virginia.*

The NPY, FMRFamide and β-endorphin immunoreactive open-type endo-

crine cells are well distinct in *T. virginia*. The neuropeptide Y (NPY) immunoreactive peptide was demonstrated in *Drosophila melanogaster* (BROWN et al., 1999). FMRFamide-like immunoreaction was reported in the midgut in the larvae of *Helicoverpa zea* (CRIM et al., 1992), bee *Melipona quadrifasciata anthidiodes* (NEVES et al., 2002) and locust *Locusta migratoria* (LANGE, 2001). The immunocytochemical localization of β-endorphin-like substance has been noticed in the gut tissues of *Periplaneta americana* (SCHOLS et al., 1987).

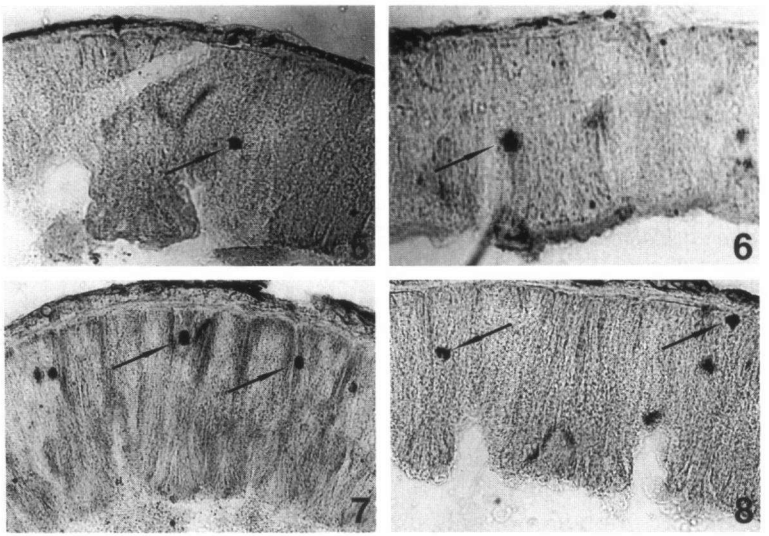
Using various radioimmunoassay, immunohistochemical and immunofluoresence techniques, it has been found that FMRFamide related peptides not only modulate midgut contraction (NICHOLS, et al., 1999) but are also directly involved in the control of digestive process (NEVES et al., 2002) and gut motility (RANKIN, 2001). HILL & ORCHARD (2003) found that the gut tissue and associated nervous system of *Locusta migratoria*, contained FMRFamide-like immunoreactive (FLI) material throughout the five larval instars and adult stage in both male and female and revealed the significant difference in the content of FLI in anterior and posterior midgut by radioimmunoassay with respect to the tissue size.



Figs 2-4. Immunocytochemistry of the midgut endocrine cells: (2) OMEC stained with NPY antibody (arrows) ×450; – (3) OMEC stained with FMRFamide antibody (arrows) ×450; – (4) OMEC stained with β-Endorphin antibody (arrows) ×400.

Similarly, gastrin, CCK, substance P and VIP immunnoreactive close-type midgut endocrine cells are observed in *T. virginia*. The vertebrate-like gut-neuropeptides *viz.* gastrin, CCK, substance P and VIP-like materials have been described in the midgut of some insects (ANDRIES & TRAMU, 1985a, 1985b; LUNDQUIST et al., 1994).

Several myotropic factors were reported in the midgut endocrine cells of three lepidopteran species: *Manduca sexta*, *Agrotis segetum* and *Spodoptera exempta* (YI et al., 1992). These peptides have FMRFamide C-terminus and thus have homology with FMRFamide. Recombinant DNA techniques revealed a gene in *Drosophila melanogaster* that encodes two additional members of the gastrin/CCK family. These are termed as drosulfakinin I and II (NICHOLS et al., 1988).



Figs 5-8. Immunocytochemistry of the midgut endocrine cells: (5) CMEC stained with VIP antibody (arrow) ×400; – (6) CMEC stained with Gastrin antibody (arrow) ×400; – (7) CMEC stained with Substance P antibody (arrows) ×400; – (8) CMEC stained with CCK antibody (arrows) ×400.

According to the current research findings, Gastrin/CCK-like immunoreactivity is attributed, at least in part, to the sulfakinins (NICHOLS et al., 1988; FONAGY et al., 1992; PREDEL et al., 1999). Sulfakinins have been shown to stimulate digestive enzyme secretion in the weevil, *Rynchophorus ferragineus* (NACHMAN et al., 1997). In the opinion of some workers, neuropeptides, such as sulfakinins, myosuppressins and allatostatins alter digestive enzyme activity in vitro (NACHMAN et al., 1997; FUSE et al., 1999; WEI et al., 2000; HARSHINI et al., 2002; HILL & ORCHARD, 2005).

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