Odonatologica 37(2): 119-130 *June 1, 2008*

IMMUNOCYTOCHEMICAL LOCALIZATION OF SOME AMINERGIC AND PEPTIDERGIC NEUROSUBSTANCES IN THE CEPHALIC NEUROSECRETORY SYSTEM OF THE DRAGONFLY, *TRAMEA VIRGINIA* (RAMBUR) (ANISOPTERA: LIBELLULIDAE)

N.V. PATANKAR and D.B. TEMBHARE*
Department of Zoology, Nagpur University Campus, Amravati Road, Nagpur-440033, India

Received November 6, 2006 / Revised and Accepted October 15,2007

An immunocytochemical study showed the presence of 7 neurosubstance-like materials: FMRFamide, neuropeptide-Y (NPY), substance-P, serotonin, gastrin, cholecystokinin (CCK) and vasoactive intestinal peptide (VIP) in the median, lateral, ventral and optic neurosecretory cells groups (MNC, LNC, VNC and ONC, respectively) in the brain and in the corpora cardiaca (CC) of the adult, *T. virginia*. In the MNC cell type A showed NPY- and serotonin- while B and C cell types showed NPY-, serotonin-, substance P- and CCK-like positive immunoreaction. The B cell type in LNC showed FMRFamide-, NPY- and serotonin- and the C cell type showed only NPY and serotonin-like positive immunoreaction. In VNC group, the B cell type showed substance P- and gastrin-, while the C cell type showed substance P- and gastrin- and VIP- like positive immunoreaction. B and C cell types of ONC group showed substance P- and serotonin-like positive immunoreaction. The CC showed only NPY-like positive immunoreactive intrinsic neurosecretory cells. The functional significance of these myotropic and vertebrate gastrointestinal hormone-like substances in the cephalic neurosecretory system of *T. virginia* is discussed.

INTRODUCTION

Recently, a number of workers (KRAMER, 1984; RAABE, 1989; GÄDE, 1992; NIJHOUT, 1994; TEMBHARE, 1995; GÄDE et al., 1997; NÄSSEL, 1999, 2000, 2002; PREDEL, 2001; SCHOOFS et al., 2001, NICHOLS, 2003; TAGHERT & VEENSTRA, 2003) have reviewed the accumulated information on localization,

* Address for correspondence: Prof. D.B. Tembhare, 44 Vijaya Nagar, South Ambazari Road, Nagpur – 440 022, India; – dr.nitisha@gmail.com

isolation, characterization and functional significance of several neurosubstances in the insects.

Apart from several histomorphological, histochemical, ultrastructural and experimental studies exploring the structure and functions of the neuroendocrine system in various odonates (ARVY & GABE, 1962; SCHALLER & MEUNIER, 1968; GILLOTT, 1969; THAKARE & TEMBHARE, 1975a, 1975b; TEMBHARE & THAKARE, 1976; TEMBHARE, 1980, 2002) and a study on the immunocytochemical localization of some vertebrate peptide hormones in the nervous system of *Aeshna cyanea* (ANDRIES et al., 1991), no thorough immunocytochemical studies have yet been made on the localization of the aminergic and peptidergic neuroactive substances in the neurosecretory cells (NSC) in the brain of the dragonflies. The present study was, therefore, undertaken to localize the presence of some aminergic and peptidergic neurohormone-like substances in the NSC in the brain and in intrinsic neurosecretory cells (INC) and extrinsic neurosecretory axons in the corpora cardiaca (CC) of the dragonfly, *Tramea virginia*.

MATERIAL AND METHODS

HISTOLOGICAL TECHNIQUES – Adult dragonflies were collected from the university campus, dissected immediately in Bouin's fluid and fixed for 18-24 hours. The material was dehydrated in ethanol, cleared in xylene and embedded in paraffin wax at 58-62°C. The sections were cut at 4-5 µm thickness, fixed to Mayer's albumenized slides and stained with Bargman's Chrome alum, Hematoxylin Phloxin (CHP) or Ewen's Aldehyde Fuchsin (AF).

IMMUNOCYTOCHEMICAL TECHNIQUES – For immunocytochemical studies, the brain was fixed in cold Bouin's fluid, Zambonin or 4% paraformaldehyde for 24 hours. Thereafter, overnight treatment in cold sucrose solution was given for cryoprotection of the tissue. Frozen sections of 15 µm thickness were cut on a cryostat (Leica). The sections were fixed to poly-L-lysin coated or gelatin subbed slides. The slides were preserved in a deep freezer and proceeded for immuno-staining.

The Streptavidin-biotin-peroxidase (Sigma) method was used. 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), serotonin (5-HT) (Zymed; Cat. No. 080077, 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 the respective concentrations containing 0.3% Triton X-100 and 1% BSA. The sections were then incubated for 2 hrs 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 a chromogen to visualize the reddish brown reaction product. Sections were washed in distilled water to stop the 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 the respective concentrations separately, which inhibited positive immunoreactions in the brain sections of *T. virginia*.

RESULTS

HISTOMORPHOLOGY OF THE CEPHALIC NEUROENDOCRINE SYSTEM

The cephalic neurosecretory system of the adult *T. virginia,* consists of the neurosecretary cells (NSCs) in the brain, and a pair of corpora cardiaca (CC).

NEUROSECRETORY CELLS IN THE BRAIN – There are five paired groups of neurosecretory cells in the brain: the medial (MNC), mid-dorsal (MDNC) and lateral groups in the protocerebrum, a ventral group (VNC) in the tritocerebrum and an optic group (ONC) in the basal region of the optic lobes (Fig. 1) (TEMBHARE & ANDREW, 1995; PATANKAR, 2004). The axons of each group of MNC, MDNC, LNC and VNC form the medial (MNSP), mid-dorsal (MDNSP), lateral (LNSP) and ventral (VNSP) neurosecretory pathways respectively. They run dorso-ventrally and emerge as a pair of fine nerves, the nervi corporis cardiaci (NCC,) from the ventero-posterior region of the brain. The two NCC run parallel to each other on either side of the aorta, dorsal to the oesophagus and enter the anterior end of the CC. The axons of the ONCs form the optic neurosecretory pathways (ONSP), which run in an antero-posterior direction towards the medulla interna and terminate in the close vicinity of the inner chiasma within the optic lobes where they appear to store and release the neurosecretory material.

CORPORA CARDIACA – The CC is a paired, elongated, fusiform glistering white body lying just beneath the brain on the mid-dorsal region of the oesophagus, intimately associated with the dorsal aorta. Anteriorly the two lobes are free from one another but fused to form a single lobe posteriorly. A pair of lateral nerves, the nervi cardiostomatogastrici connects the CC ventrally to the hypocerebral ganglion. Internally, the CC consists of a large number of extrinsic axonal end-

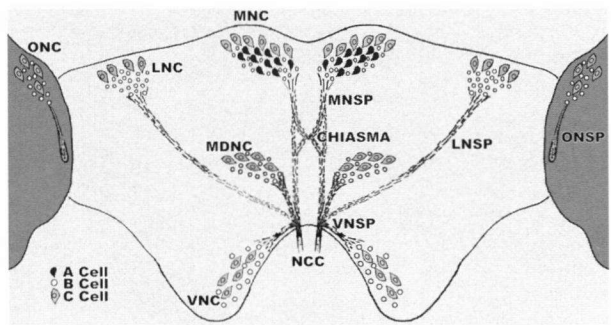


Fig. 1. Diagrammatic representation of the cephalic neurosecretory system depicting the distribution of neurosecretory cell groups and their neurosecretory pathways in the brain of *Tramea virginia.* – LNC: lateral neurosecretory cells; – LNSP: lateral neurosecretory pathways; – MDNC: mid-dorsal neurosecretory cells; – MNC: medial neurosecretory cells; – MNSP: medial neurosecretory pathways; – ONC: optic neurosecretory cells; – ONSP: optic neurosecretory pathways; – VNC: ventral neurosecretory cells; – VNSP: ventral neurosecretory pathways.

ings of the NSCs of the brain, some intrinsic neurosecretory cells (INC) dispersed randomly (Fig. 2) and a few ordinary neurons.

IMMUNOCYTOCHEMISTRY

The NSCs were classified into A, B and C cells based on their staining affinities with the chrome-alum hematoxylin phloxine (CHP) and aldehyde fuschin (AF) stains (Tab. I).

FMRF-AMIDE POSITIVE NEUROSECRETORY CELLS – Three types of B cell in the LNC, one type of B and one type of C cell in the VNC and two types of B and three types of C cell in the ONC, along with their axons in the brain, showed strong immunoreactivity with the FMRFamide antisera (Figs 3-5).

THE NEUROPEPTIDE Y POSITIVE NEUROSECRETORY CELLS – In the brain the cell bodies of one A, eight B and four C cell types in the MNC and six B and four C cell types in the LNC showed strong reaction with the neuropeptide-Y antibody (Figs 3, 6, 7). The INC in the CC also exhibited prominent neuropeptide-Y positive immunoreaction (Fig. 8).

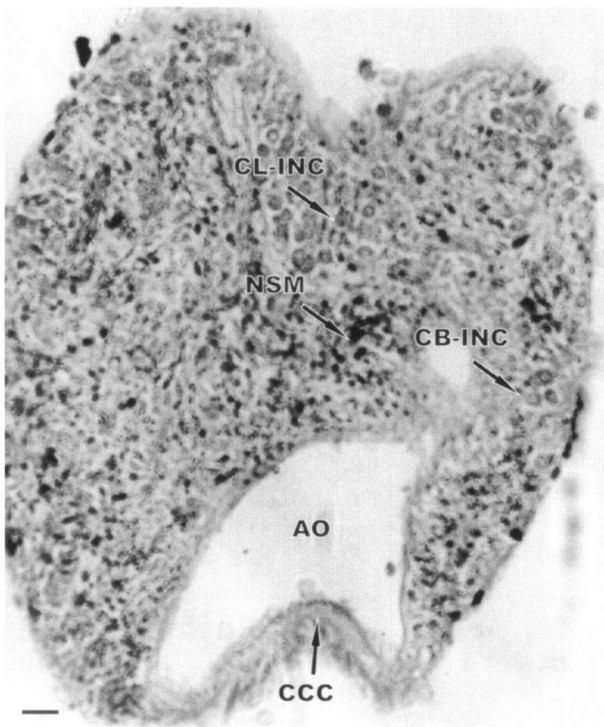


Fig. 2. Section through the corpora cardiaca of *Tramea virginia* showing the intrinsic neurosecretory cells. – AO: aorta; – CCC: corpora cardiaci connectives; – CB-INC: chromophobic intrinsic neurosecretory cells; – CL-INC: chromophillic intrinsic neurosectretory cells; – NSM-neurosecretory material. – [Scale bar: 1000 μm]

THE SUBSTANCE-P POSITIVE NEUROSECRETORY CELLS – Ten A and two C cell types in the MNC and all B and C cell types in the VNC and ONC, along with their neurosecretory pathways, showed intense immunoreaction with the substance-P antisera (Figs 9-11).

THE SEROTONIN POSITIVE NEUROSECRETORY CELLS – The

Table I
Cytomorphological characteristics of the cerebral neurosecretory cells in *Tramea virginia*

S#	Cytomophological Characteristics	Neurosecretory Cell Type A-cell	B-cell	C-cell
1.0		STAINING AFFINITIES		
1.1	Chrome-alum haematoxylin phloxine (CHP)	Blue black	Red	Blue black inclusion, red cytoplasm
1.2	Aldehyde fuchsin (AF)	Dark purple	Greenish	Purple inclusion, brown cytoplasm
2.0		SHAPE		
		Pyriform	Spherical	Oval
3.0		SIZE		
3.1	Cell diameter(μm)	1063 ± 027	960 ± 080	1862 ± 056
3.2	Nuclear diameter (μm)	572 ± 032	540 ± 027	920 ± 036
4.0		DISTRIBUTION		
4.1	Medial group	40 - 45	25 - 30	8 - 10
4.2	Mid-dorsal group	-	10 - 15	10 - 12
4.3	Lateral group	-	15 - 18	4 - 6
4.4	Ventral group	-	15 - 18	10 - 12
4.5	Optic group	-	8 - 12	10 - 12

serotonin-positive immunoreactivity was observed distinctly in one A, two B and two C cell types in the MNC, four B cell types in the LNC and six B cell types in the ONC and their axons in the brain (Figs 12-14).

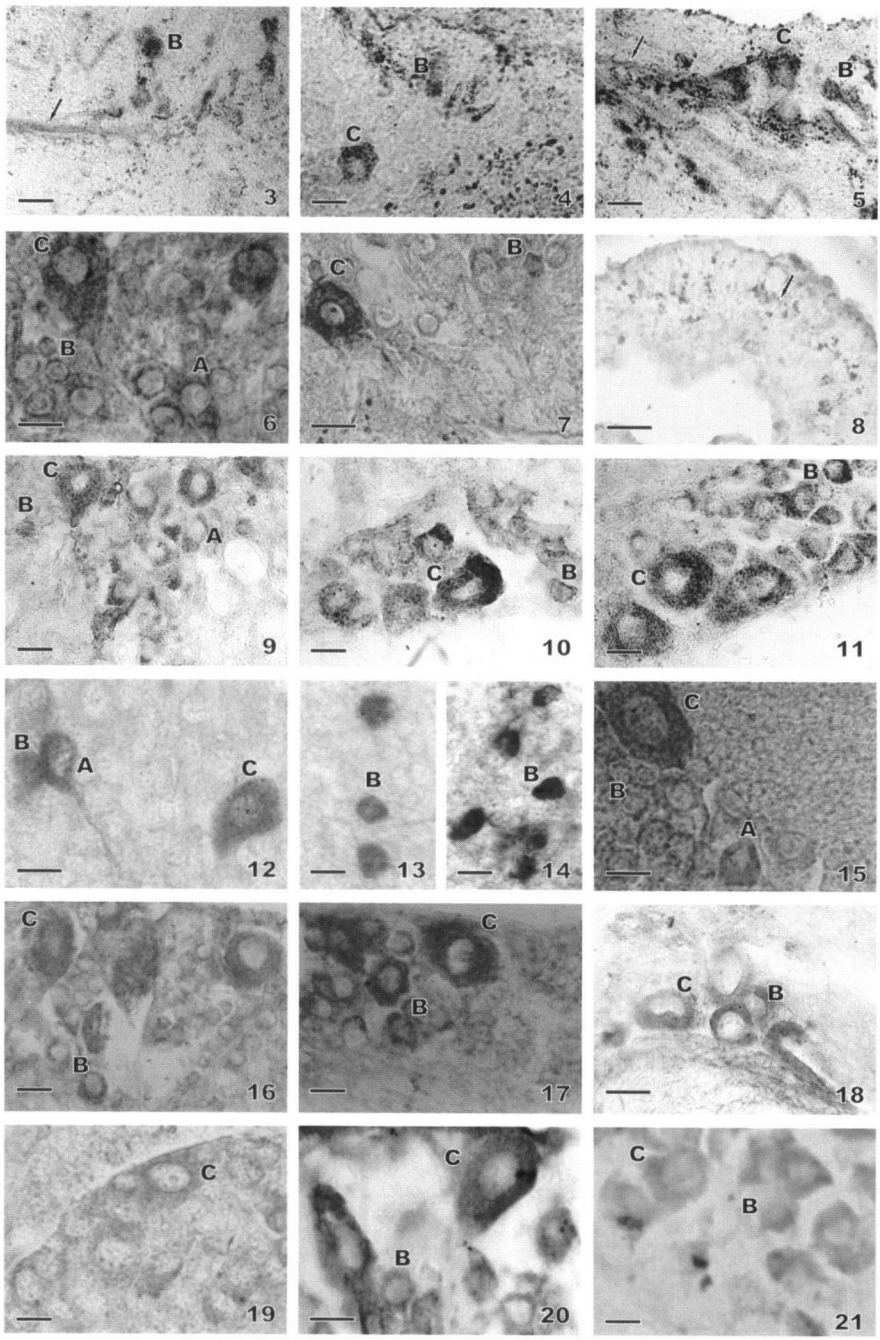
THE GASTRIN POSITIVE NEUROSECRETORY CELLS – One B and one C cell type in the MNC, three B and C cell types in the LNC and five B and one C cell types in the VNC showed the immunoreaction with the gastrin antibody (Figs 15-17).

THE CCK POSITIVE NEUROSECRETORY CELLS – Two B and C cell types of MNC and three C and all B cell type in the VNC displayed a strong immunoreaction with the CCK antibody (Figs 18-19).

THE VIP POSITIVE NEUROSECRETORY CELLS – Six B and four C cell types in the VNC and all B cell types in the ONC showed the immunoreaction with VIP antibody (Figs 20-21).

DISCUSSION

The histomorphological organization of the cephalic neuroendocrine system in the adult dragonfly, *Tramea virginia* was described by TEMBHARE & ANDREW (1994) and PATANKAR (2004) and is basically similar to that in *Aeshna cyanea* (CHARLET, 1972a, 1972b; TEMBHARE, 1980) and *Orthetrum chrysis*


B
3
B
C
4
C
B
5
C
B
A
6
C
B
7
8
C
B
A
9
C
B
10
B
C
11
B
A
C
12
B
13
B
14
C
B
A
15
C
B
16
C
B
17
C
B
18
C
19
C
B
20
C
B
21

(TEMBHARE & THAKARE, 1976). The present study has localized the presence of FMRFamide-, NPY-, serotonin-, substance P-, gastrin-, CCK- and VIP-like substances in the NSCs of different groups in the brain and CC of the adult *T. virginia*.

The FMRFamide-like positive immunoreaction has been noticed in the NSC in the brain of several insect species. In the locust, *Schistocerca gregaria* MYERS & EVANS (1985) tested BPP antisera not specific for the C terminal hexapeptide, along with liquid preabsorption experiments with BPP and FMRFamide, and suggested that the endogenous peptide antigen contained in the stained neurones may belong to the pancreatic polypeptide family or to the FMRFamide family. WHITE et al. (1986) characterized neuropeptide-FMRFamide-like immunoreactivity in the fruit fly; *Drosophila melanogaster* and suggested its significance during development. EICHMÜLLER et al. (1991) analysed the brain and suboesophageal ganglion of the honeybee *Apis mellifera* L. immunocytochemically and observed FMRFamide-like immunoreactivity in the cells projecting to the CC from the median, lateral and suboesophageal ganglion, suggestng their neurosecretory nature. Definitive evidence for the presence of FMRFamide in insects was provided by KINGAN et al. (1990) who extracted and purified this peptide from the central nervous system of the hawkmoth, *Manduca sexta*. HEWES et al. (1998) analyzed the effect of eight FMRFamide-related peptides expressed by neurosecretory cells on nerve-stimulated contraction (twitch tensions) of *Drosophila* larval body wall muscles encoded by Drosophila FMRFamide gene. They suggested that FMRFamide functions as a neurohormone to modulate the strength of contraction at the larval neuromuscular junction in which the functional role of the seven peptides appear to be functionally redundant. ROBB & EVANS (1990) studied the quantitative distribution of FMRFamide-like peptides in the nervous system and in their putative target sites in the locust *Schistocerca gregaria* and suggested that FMRFamide-like peptides in the locust function both as circulating neurohormones and as locally released neuromodulators or neurotransmitters. RANKIN & SEYMOUR (2001), immunocytochemically observed a material similar to that of FMRFamide in the neurosecretory system of the earwig, *Eubeorellia annulipes* and suggested its functional significance. Recently, NICHOLS (2003), reviewed structures, precursors, organizations, distributions and

Figs 3-21. Immunocytochemistry of the neurosecretory cells in the brain and corpora cardiaca (CC) of *Tramea virginia*: (3-5) brain NSC stained with FMRFamide antibody: (3) B-LNC and LNSP (arrow), (4) C- VNC, (5) B and C- ONC and ONSP (arrow); – (6-8) NSC stained with NPY antibody: (6) B and C-MNC, in the brain, (7) C-LNC, in the brain, (8) Extrinsic axonal fibers in CC (arrow); – (9-11) NSC in the brain stained with Substance P antibody: (9) B and C- MNC, (10) B and C- VNC, (11) B and C- ONC; – (12-14) NSC in the brain stained with Serotonin antibody: (12) A, B and C-MNC, (13) B - LNC, (14) B – ONC; – (15-17) NSC in the brain stained with Gastrin antibody: (15) B and C- MNC, (16) C- LNC, (17) B and C- VNC; – (18-19) NSC in the brain stained with CCK antibody: (18) A, B and C- MNC, (19) B and C- VNC; – (20-21) NSC in the brain stained with VIP antibody: (20) B and C- VNC, (21) B and C- ONC. – [Scale bars: 1800 μm]

activities of FMRFamide related peptides (FaRPs) encoded by the melanogaster FMRFamide (dFMRFamide), myosuppressin (Dms) and sulfakinin (Dsk) genes and predicted their signaling pathways and biological functions. DUTTLINGER et al. (2003) observed the structure of the FMRFamide receptor and the activity of the cardioexcitatory neuropeptide in a mosquito. They concluded that its structure and activity are conserved in the mosquito, which may help to increase the frequency of spontaneous contractions of the larval heart, thus increasing heart rate.

REMY et al. (1988) demonstrated a neuropeptide related to mammalian neuropeptide Y (NPY) in *Locusta migratoria* in various neurosecretory cells which also display an FMRFamide-like immunoreactivity in the cephalic and thoracic nervous systems. They inferred that in locust NSC these two antisera recognize two distinct antigenic sites belonging either to a large polypeptide, or to two distinct neuropeptides. SETTEMBRINI et al. (2003) observed NPY and NPY-Y1 receptor-like immuno-reactivity in its receptors and colocalization with cCK-LI, in the central nervous system of *Triatoma infestans* and analysed its distribution patterns, suggesting that peptide and receptor are mainly involved in the processing of information coming from sensory receptors

SCHURMANN & KLEMM (1984), observed serotonin immunoreactive neurons in the brain of the honeybee and concluded that the serotonin- and catecholamine-containing neurons often occur together in the same brain areas. KLEMM et al. (1986) observed neurons reactive to antibodies against serotonin in the stomato-gastric nervous system and in the alimentary canal of locust and crickets and suggested that serotonin acts as a neurotransmitter and/or neuromodulator on intestinal and some somatic muscles and glandular cells, and also acts as a neurohormone released from the neurohaemal sites into the body fluid. GRANGER et al. (1989) observed serotonin-immunoreactive neurons in the brain of *Manduca sexta* during larval development and larval-pupal metamorphosis and suggested the possible relationship of serotonin to cerebral neuroendocrine functions during the postembryonic development. According to NÄSSEL (1995), in *Drosophila melanogaster* these peptides, like their mammalian counter parts, act as neuromodulators (neurohormones) through a second-messenger and facilitate release of hormones, such as prothoracicotropic (PTTH), allatotropic (ATH), allatostatic (ASH) and eclosion hormone (EH), PREDEL et al. (2001a, 2001b) discussed the structure, distribution, pharmacological activities and mimetic analogs of the myoinhibitory and myostimulatory neuropeptidesin the American cockroach, *Periplaneta americana*. The distribution of cholin acetyltransferase (ChAT); GABA, histamine and octapamine and serotonin have been reported in the larval chemosensory system of *Drosophila melanogaster* (FRANCIOSE et al., 2002) suggesting their role as neurotransmitters. NÄSSEL (2002) suggested that the neuropeptides in the nervous system of *Drosophila* and other insects act as a neuromodulator and neurohormones. LANGE (2004) observed

that serotonin increases the frequency and amplitude of spontaneous contractions and leads to an increase in the basal tonus of locust oviducts suggesting the neurohormonal role for serotonin in the control of locust oviducts. MARTINI et al. (2004), observed the possible regulation by serotonin (5-HT) to detect the presence of an aquoporin-like water channel in the malphigian tubules (MT) of the hematophagous insect *Rhodnius prolixus*, and suggested that the up-regulated expression of MT MIP mRNA after a blood meal is probably due to the action of 5-HT *via.* a cyclic AMP dependent pathway.

SEVERINI et al. (2002) stated that tachykinins have been recognized to have a variety of effects in physiological and pathological conditions, and there is evidence suggesting intrinsic neuroprotective and neurodegenerative properties of these neuropeptides. This review provides an update on the current body of knowledge regarding the occurrence and distribution of tachykinin in the animal kingdom and the physiological and pharmacological actions of tachykinins, outlining the importance of this large peptide family. LUNDQUIST & NASSEL, (1990) demonstrated neurons displaying substance P- (SPLI), FMRFamide-(FLI), and cholecystokinin-like (CCKLI) immunoreactivity in the thoraco-abdominal ganglia of the dipterans *Drosophila melanogaster* and *Calliphora vomitoria.*

Gastrin/CCK-like immunoreactivity have been observed in the NSC and CC of various insects. DUVE & THORPE (1984) studied its distribution in the retrocerebral complex of *Calliphora vomitoria* and observed that CC contains COOH--terminal specific gastrin/CCK-like material within the intrinsic cells and in the neuropile, suggesting its co-existence in the cells of CC. HANSEN et al. (1987) observed COOH-terminal specific gastrin/CCK-like material in CC-CA complex, suggesting the appearance of these antibodies early in evolution within neural elements and being conserved during phylogeny. ANDRIES et al. (1991) studied antisera gastrin/CCK, VIP immunohistochemically in the brain, suboesophageal ganglion and corpora cardiaca of *Aeshna cyanea* and found multiple peptide immunoreactivities. TAMARELLE & VANHEMS, (1997) characterized a new neurosecretory cell-type in the locust *Schistocerca gregaria* pars intercerebralis, immunolabelled with an antiserum against a vertebrate peptide related to gastrin-cholecystokinin (CCK-8 (s)), both in situ and in primary cell cultures. On the basis of their number, size and localization they suggested the possibility of CCK-like neurosecretory cells corresponding to a neurosecretory cell type which has not to date been identified at the fine structural level. HEWES & TAGHERT (2001) scanned the recently completed *Drosophila* genome sequence for G protein-coupled receptors sensitive to bioactive peptides (peptide GPCRs) and also scanned for genes encoding potential ligands. They described 22 bioactive peptide precursors and found that at least 32 *Drosophila* peptide receptors appear to have evolved from common ancestors of 15 monophyletic vertebrate GPCR subgroups (e.g., the ancestral gastrin/cholecystokinin receptor), shedding light on the evolutionary history of peptide GPCRs and providing a template for physi-

ological and genetic analyses of peptide signaling in *Drosophila*.

The present paper provides information based on immunocytochemical studies regarding the presence of some vertebrate hormone-, neurotransmitter- and neuromodulator-like substances in the cerebral neurosecretory system which may represent co-localization with the intrinsic insect neuropeptides and may exert a vital role in the life of an insect.

ACKNOWLEDGEMENTS

We are grateful to Prof. N.K. SUBHEDAR for providing training in immunocytochemical techniques and permitting to work in his laboratory. The "University Research Fellowship" awarded to NVP by the Nagpur University is also acknowledged.

REFERENCES

ANDRIES, J.C., G. BELLEMTOUGRI & G. TRAMU, 1991. Multiple peptide immunoreactivities in the nervous system of Aeschna cyanea (Insecta: Odonata). An immunohistochemical study using antisera to cholecystokinin, octapeptide, somatoliberin, vasoactive intestinal peptides, motilin and proctolin. *Histochemistry* 96: 139-148.

ARVY, L. & M. GABE, 1962. Histochemistry of the neurosecretory product of the pars intercerebralis of pterygote insects. *In:* L. Heller & K. Clark, [Eds.], Neurosecretion. *Mem. Soc. Endocrinol.* 12: 331-344.

CHARLET, M., 1972a. Etude du systeme neurosecreteur d 'Aeschna cyanea Müll. (Insecta: Odonata). PhD thesis, Univ. Strasbourg.

CHARLET, M., 1972b. Etude histologique de ls pars intercerebralis de la larva d'Aeschna cyanea Müll. *C. R. Acad. Sci. Paris,* 257: 1047-1050.

DUTTLINGER, A., M. MISPELON & R. NICHOLS, 2003. The structure of the FMRFamide receptor and activity of the cardioexcitatory neuropeptide are conserved in mosquito. *Neuropeptides* 37: 120-126.

DUVE, H. & A. THORPE, 1984. Immunocytochemical mapping of gastrin/CCK-like peptides in the neuroendocrine system of the blowfly: Calliphora vomitoria (Diptera). *Cell Tissue Res.* 237: 309-320.

EICHMÜLLER, S., M. HAMMER & S. SCHAFER, 1991. Neurosecretory cells in the honeybee brain and suboesophageal ganglion show FMRFamide-like immunoreactivity. *J. comp. Neurol.* 312: 164-174.

FRANCIOSE, V., G. PYTHON, F. REINHORD & J. STOCKNER, 2002. Immunoreactivity against choline acetyltransferase, amino butyric acid, histamine, octopamine and serotonin in the larval chemosensory system of Drosophila melanogaster. *J. comp. Neurol.* 453: 157-167.

GADE, G., 1992. Insect neuropeptides. *In*: B. Bennettóva, I .Geibic & T. Soldán, [Eds], *Advances in regulation of insect reproduction*, pp. 67-80, Inst. Ent., Czech Acad. Sci., Ceské Budejovice.

GÄDE, G., K.H. HOFFMANN & J.H. SPRING, 1997. Hormonal regulation in insects: facts, gaps, and future directions. *J. Physiol.* 77: 963-1032.

GILLOTT, C., 1969. Morphology and histology of the cephalic endocrine glands of the damselfly, Coenagrion angulatum Walker. *Can. J. Zool.* 47: 1187-1192.

GRANGER, N.A., U. HOMBER, P. HENDERSON, A. TOWLE & J.M. LAUDER, 1989. Serotonin-immunoreactive neurons in the brain of Manduca sexta during larval development and larval-pupal metamorphosis. *Int. J. Dev. Neurosci.* 7: 55-72.

HANSEN, B., G. HANSEN & B. SCHARRER, 1987. Gastrin/CCK-like immunoreactivity in the

corpus cardiacum-corpus allatum complex of the cockroach Leucophaea maderae. *Cell Tissue Res.* 248: 595-598.

HEWES, R.S & P.H. TAGHERT, 2001. Neuropeptides and neuropeptide receptors in the Drosophila melanogaster genome. *Genome Res.* 11: 1126-1142.

HEWES, R.S., E.C. SNOWDEAL, M. SAITOE & P.H. TAGHERT, 1998. Functional redundancy of FMRFamide-related peptides at the Drosophila larval neuromuscular junction. *J. Neurosci.* 18: 7138-7151.

KINGAN, T.G., D.B. TEPLOW, J.M. PHILLIPS, J.P. REIHM, K.R. RAO, J.G. HILDEBRAND, U. HOMBERG, A.E. KAMMER, I. JAEDINE, P.R. GRIFFIN & D.F. HUNT, 1990. A new peptide in the FMRFamide family isolated from the CNS of the hawkmoth, Manduca sexta. *Peptides* 11: 849-856.

KLEMM, N., R. HUSTERT, R. CANTERA & D.R. NÄSSEL, 1986. Neurons reactive to antibodies against serotonin in the stomatogastric nervous system and in the alimentary canal of locust and crickets (Orthoptera, Insects). *Neuroscience* 17: 247-261.

KRAMER, K.J., 1984. Vertebrate hormones in insects. *In*: G.A. Kerkut & L.I. Gilbert, [Eds], *Comprehensive insect physiology, biochemistry and pharmacology*, Vol. 7, pp. 511-536, Pergamon Press, Oxford.

LUNDQUIST, T. & D.R. NASSEL, 1990. Substance P-, FMRFamide-, and gastrin/cholecystokinin-like immunoreactive neurons in the thoraco-abdominal ganglia of the flies Drosophila and Calliphora. *J. comp. Neurol.* 294: 161-78.

LANGE, A.B., 2004. A neurohormonal role for serotonin in the control of locust oviducts. *Arch. Insect Biochem. Physiol.* 56: 179-190.

MARTINI, S.V., R.C. GOLDENBERG, F.S. FORTES, A.C. CAMPOS-DE-CARVALHO., D. FALKENSTEIN & M.M. MORALES, 2004. Rhodnius prolixus Malpighian tubule's aquaporin expression is modulated by 5-hydroxytryptamine. *Arch. Insect Biochem. Physiol.* 57: 133-141.

MYERS, C.M. & P.D. EVANS, 1985. The distribution of bovine pancreatic polypeptide / FMRFamide-like immunoreactive neurons in the ventral nervous system of the locust. *J. comp. Neurol.* 234: 1-16.

NÄSSEL, D.R., 1995. Neuropeptide diversity in the insect nervous system. Facts and speculations. *In*: D. Muraleedharan & M. Jacob, [Eds], *Recent advances in insect endocrine research*, pp. 1-38, Assn. Advt Ent., India.

NÄSSEL, D.R., 1999. Tachykinin-related peptides in invertebrates: a review. *Peptides* 20: 141-158.

NÄSSEL, D.R., 2000. Functional role of neuropeptides in the insect central nervous system. *Naturwissenschaften* 87: 439-449.

NÄSSEL, D.R., 2002. Neuropeptides in the nervous system of Drosophila and other insects: multiple roles as neuromodulators and neurohormones. *Prog. Neurobiol.* 68: 1-84.

NICHOLS, R., 2003. Signaling pathways and physiological functions of Drosophila melanogaster FMRFamide-related peptides. *Annu. Rev. Ent.* 48: 485-503.

NIJHOUT, H.F., 1994. *Insect hormones*. Princeton Univ. Press.

PATANKAR, N.V., 2004. *Studies on neuroendocrine control of digestion in the dragonfly, Tramea virginia (Rambur) (Odonata: Libellulidae)*. PhD thesis, Univ. Nagpur, India.

PREDEL, R., 2001. Peptidergic neurohemal system of an insect: mass spectrometric morphology. *J. comp. Neurol.* 436: 363-375.

PREDEL, R., J. RAPUS & M. ECKERT, 2001a. Myoinhibitory neuropeptides in the American cockroach. *Peptides* 22: 199-208.

PREDEL, R., R.J. NACHMAN & G. GADE, 2001b. Myostimulatory neuropeptides in cockroaches: structures, distribution, pharmacological activities, and mimetic analogs. *J. Insect. Physiol.* 47: 311-324.

RABBE, M., 1989. *Recent developments in insect neurohormones*. Plenum Press, New York.

RANKIN, S.M & S.M. SEYMOUR, 2001. FMRFamide-like material in the earwig, Euborellia annulipes, and its fun ctional significance. *Arch. Insect. Biochem. Physiol.* 47: 189-197.

REMY, C., J. GUY, G. PELLETIER & H.H. BOER, 1988. Immunohistological demonstration of a substance related to neuropeptide Y and FMRFamide in the cephalic and thoracic nervous system of the locust Locusta migratoria. *Cell Tissue Res.* 254: 189-195.

ROBB, S. & P. D. EVANS, 1990. FMRFamide-like peptides in the locust: distribution, partial characterization and bioactivity. *J. exp. Biol.* 149: 335-360.

SCHALLER, F. & J. MEUNIER, 1968. Etude systeme neurosecteur cephalique des insectes odonates. *Bull. Soc. Zool.* 93: 233-249.

SCHOOFS, L., E. CLYNEN, A. CERSTIAENS, G. BAGGERMAN, Z. WEI, T. VERCAMMEN, R. NACHMAN, A. DE LOOF & S. TANAKA, 2001. Newly discovered functions for some myotropic neuropeptides in locusts. *Peptides* 22: 219-227.

SCHURMANN, F.W. & N. KLEMM, 1984. Serotonin-immunoreactive neurons in the brain of the honeybee. *J. comp. Neurol.* 225: 387-395.

SETTEMBRINI, B.P., S. NOWICKI, T. HOKFELT & M.J. VILLAR, 2003. Distribution of NPY and NPY-Y1 receptor-like immunoreactivities in the central nervous system of Triatoma infestans (Insecta: Heteroptera). *J. comp. Neurol.* 460: 141-154.

SEVERINI, C., G. IMPROTA, G. FALCONIERI-ERSPAMER, S. SALVADORI & V. ERSPAMER, 2002. The tachykinin peptide family. *Pharmacol. Rev.* 54: 285-322.

TAGHERT, P.H. & J.A. VEENSTRA, 2003. Drosophila neuropeptide signaling. *Adv. Genet.* 49: 1-65.

TAMARELLE, M. & E. VANHEMS, 1997. Characterization of a new neurosecretory cell type containing gastrin-cholecystokinin-like peptide in the locust pars intercerebralis: ultrastructural and immunocytochemical studies, in situ and in vitro. *J. Neurocytol.* 26: 399-406.

TEMBHARE, D.B., 1980. An electron microscopic study of the neurosecretory pars intercerebralis-corpus cardiacum system in the larvae of the dragonfly, Aeschna cyanea (Müller). *Z. mikrosk.-anat. Forsch.* 94: 60-70.

TEMBHARE, D.B., 1995. Molecular chemistry of insect hormones. *In*: D. Muraleedharan & M. Jacob, [Eds], *Recent advances in insect endocrine research*, pp. 39-68, Assn Advt Ent., India.

TEMBHARE, D.B., 2002. Endocrinology of Odonata. *In*: Arvind Kumar, [Ed.], *Current trends in odonatology*, pp. 83-114, Daya, Delhi.

TEMBHARE, D.B. & R.J. ANDREW, 1994. Activity of the cephalic neuroendocrine system during development of the last instar nymph of Tramea virginia (Rambur). *In*: V.K. Srivastava, [Ed.], *Advances in oriental odonatology*, pp. 31-40, Allahabad [Proc. 4th Sth. Asian Symp. Odonatol.]

TEMBHARE, D.B. & V.K. THAKARE, 1976. The cephalic neuroendocrine system of the dragonfly Orthetrum chrysis (Selys). *Odonatologica* 5: 355-370.

THAKARE, V.K. & D.B. TEMBHARE, 1975a. Histochemistry of the neurosecretory cells of the pars intercerebralis of the dragonfly Orthetrum chrysis (Selys). *Odonatologica* 4: 225-235.

THAKARE, V.K. & D.B. TEMBHARE, 1975b.The effect of electrical stimulation on the cephalic neurosecretory system in the last instar nymph of the dragonfly, Orthetrum chrysis (Selys). *Experientia* 31: 693-694.

WHITE, K., T. HURTEAU & P. PUNSAL, 1986. Neuropeptide FMRFamide-like immunoreactivity in Drosophila: development and distribution. *J. comp. Neurol.* 247: 430-538.