Cell Tissue Res (1988) 254:189–195

Cell and Tissue Research

Immunohistological demonstration of a substance related to neuropeptide Y and FMRFamide in the cephalic and thoracic nervous systems of the locust *Locusta migratoria*

C. Rémy[1], J. Guy[2], G. Pelletier[2], and H.H. Boer[3]

[1] Laboratoire de Zoologie expérimentale, UA 1138 CNRS, Université de Bordeaux I, Bordeaux, France;
[2] MRC group in molecular endocrinology, Le centre hospitalier de l'Université Laval, Québec, Canada;
[3] Biological Laboratory, Vrije Universiteit, Amsterdam, The Netherlands

Summary. A neuropeptide related to the mammalian neuropeptide Y (NPY) is present in various neurosecretory cells (NSC) of the cephalic and thoracic nervous systems of the insect *Locusta migratoria*. Immunoreactive perikarya are detected in the protocerebrum, tritocerebrum, optic lobes and the suboesophageal and thoracic ganglia. They give rise to many immunoreactive processes that ramify extensively throughout the neuropiles. In the brain, prominent axon bundles tightly surround the tractus I to the corpora cardiaca. This fiber pattern suggests that the NPY-like substance may have a neuromodulator and/or neurotransmitter function. This substance may also have a neurohormonal role, since some immunoreactive tracts penetrate into neurohaemal organs via the nervi corporis cardiaci II and the thoracic median nerves. NCS containing NPY-like neuropeptide also display an FMRFamide-like immunoreactivity (except for the abdominal part of the metathoracic ganglion). NPY or FMRFamide antisera are not inactivated after preabsorption with FMRFamide or NPY, respectively. It might therefore be inferred that in locust NSC these two antisera recognize two distinct antigenic sites belonging either to a large polypeptide, or to two distinct neuropeptides.

Send offprint requests to: Prof. C. Rémy, Laboratoire de Zoologie expérimentale, Université de Bordeaux I, Avenue des facultés, F-33405-Talence-Cedex, France

Key words: Immunohistology – Insects – FMRFamide – Neuropeptide Y – Neurosecretory cells – *Locusta migratoria* (Insecta)

The existence of immunological relationships between invertebrate and vertebrate neuropeptides has been demonstrated repeatedly over the past ten years. By use of antisera raised against vertebrate neuropeptides, immunohistological experiments have been carried out in various species belonging to different classes of invertebrates, more particularly in insects (for a review, see Rémy and Vieillemaringe 1987). Antisera raised against FMRFamide (a molluscan cardio-excitatory peptide originally extracted by Price and Greenberg 1977) revealed a related neuropeptide in the central nervous system of insects (Boer et al. 1980). More recent results suggested a possible relationship of some neurosecretory products of insects with bovine pancreatic polypeptide (BPP) and FMRFamide. Antisera raised against FMRFamide revealed immunoreactivity in many insect neurons which also showed BPP-like immunoreactivity and vice versa (Veenstra 1984; Veenstra and Schooneveld 1984; Myers and Evans 1985a, b; Verhaert et al. 1985). Various interpretations have been put forward in an attempt to explain this cross-reactivity.

Neuropeptide Y (NPY) is a 36-amino-acid peptide of porcine brain purified and characterized by Tatemoto

Table 1. Amino acid sequences of NPY, BPP and FMRFamide

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
NPY	Tyr	Pro	Ser	Lys	Pro	Asp	Asn	Pro	Gly	Glu	Asp	Ala	Pro	Ala	Glu	Asp	Leu	Ala
BPP	Ala	Pro	Leu	Glu	Pro	Glu	Tyr	Pro	Gly	Asp	Asn	Ala	Thr	Pro	Glu	Gln	Met	Ala

	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
NPY	Arg	Tyr	Tyr	Ser	Ala	Leu	Arg	His	Tyr	Ile	Asn	Leu	Ile	Thr	Arg	Gln	Arg	Tyr-NH2
BPP	Gln	Tyr	Ala	Ala	Glu	Leu	Arg	Arg	Tyr	Ile	Asn	Met	Leu	Thr	Arg	Pro	Arg	Tyr-NH2

	1	2	3	4
FMRFamide	Phe	Met	Arg	Phe-NH2

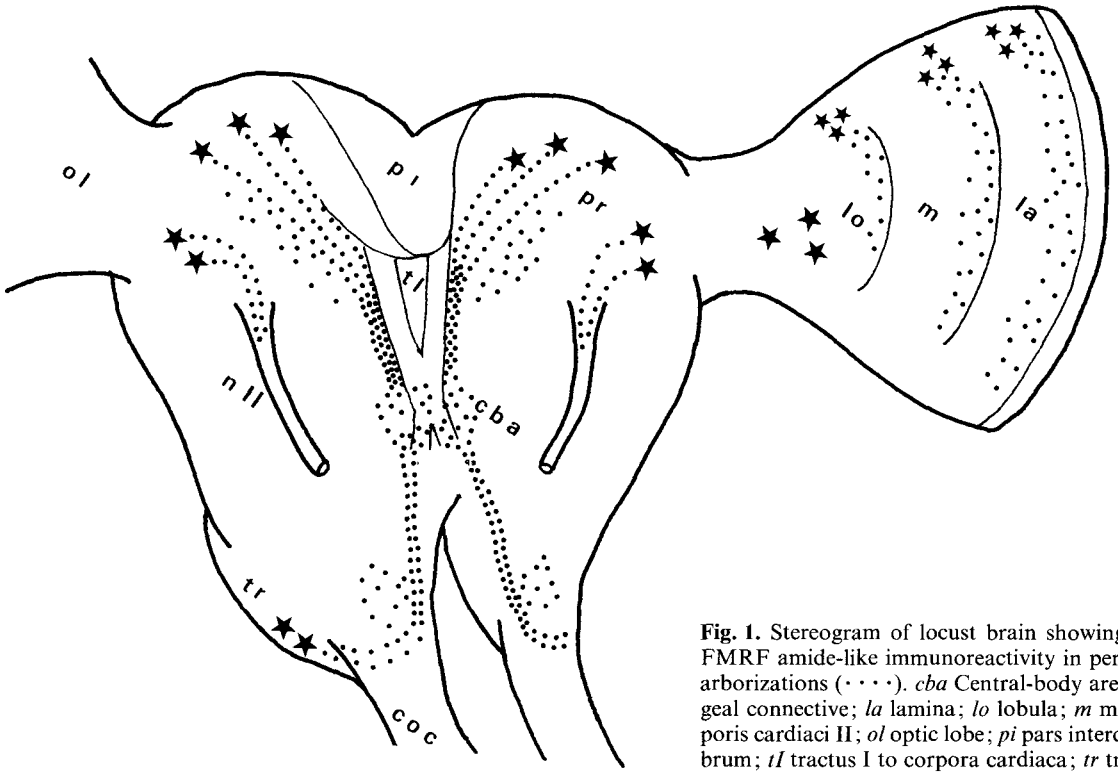

Fig. 1. Stereogram of locust brain showing distribution of NPY/FMRF amide-like immunoreactivity in perikarya (★), fibers, and arborizations (· · · ·). *cba* Central-body area; *coc* circum-oesophageal connective; *la* lamina; *lo* lobula; *m* medulla; *n II* nervus corporis cardiaci II; *ol* optic lobe; *pi* pars intercerebralis; *pr* protocerebrum; *tI* tractus I to corpora cardiaca; *tr* tritocerebrum

(1982). This neuropeptide, though displaying a high degree of homology with BPP, also shows a few differences in its amino acid sequence (Table 1). As far as we know, no report concerning the existence of immunological relationships between NPY and invertebrate neurosecretory products has been published to date. In the insect *Schistocerca gregaria*, using an NPY antiserum, Myers and Evans (1985a) failed to obtain positive results. An immunohistological investigation was therefore conducted in the cephalic and thoracic central nervous systems of *Locusta migratoria* with an NPY antiserum, and numerous controls were performed on adjacent sections with an FMRFamide antiserum.

Materials and methods

Animals and tissue preparation

This study was carried out on forty-five 10- to 20-day-old female adult locusts, *Locusta migratoria migratorioides* R and F. The tissues were fixed for 24 h in Bouin-Hollande fixative without acetic acid, to which a saturated mercuric chloride solution was added (10% v/v). Brains and subesophageal and thoracic ganglia were washed overnight, embedded in paraffin and cut into 7 μm serial sections.

Antisera

The antisera used were anti-porcine NPY, anti-FMRFamide, anti-methionine enkephalin and anti-neurophysin II. The preparation and specificity of these antisera have been described in detail in previous reports: anti-porcine NPY (Pelletier et al. 1984), anti-FMRFamide (Boer et al. 1980; Schot et al. 1984), anti-met-enkephalin (Rémy and Dubois 1981) and anti-neurophysin II (Rémy et al. 1979).

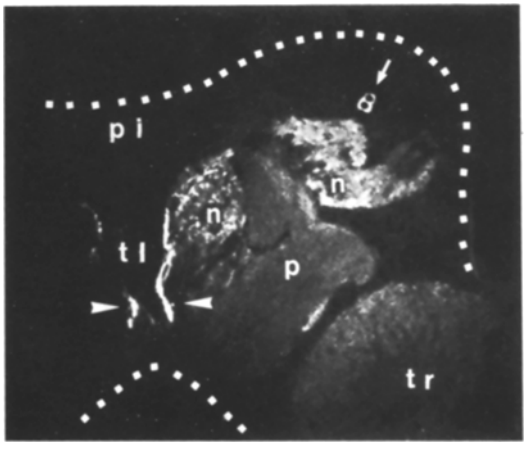

Fig. 2. Treatment with NPY antiserum, right side of protocerebrum. Two immunoreactive perikarya (*arrow*) in outer part of lateral NSC area; fluorescent network in neuropile and two immunoreactive tracts (*arrowheads*) surrounding tractus I to corpora cardiaca. *n* Neuropile; *p* mushroom-body pedunculus; *pi* pars intercerebralis; *tI* tractus I to corpora cardiaca; *tr* tritocerebrum. × 75

Immunoreaction

Rehydrated sections were stained according to the indirect fluorescent antibody technique (Coons et al. 1955). Incubations were carried out for 24 h at 4° C in a moist atmosphere with primary antisera diluted in veronal buffer (0.1 M, pH 7.2), anti-NPY: 1:400 + 2‰ bovine serum albumin, anti-FMRFamide: 1:400 + 2‰ thyroglobulin, anti-met enkephalin: 1:150 + 2‰ human serum albumine, anti-neurophysin II: 1:150. To obtain reliable staining with the NPY or FMRFamide antisera, the limit of dilution was about 1:2000. After washing, the sections were treated with fluorescein isothiocyanate-conjugated sheep anti-rabbit-Ig

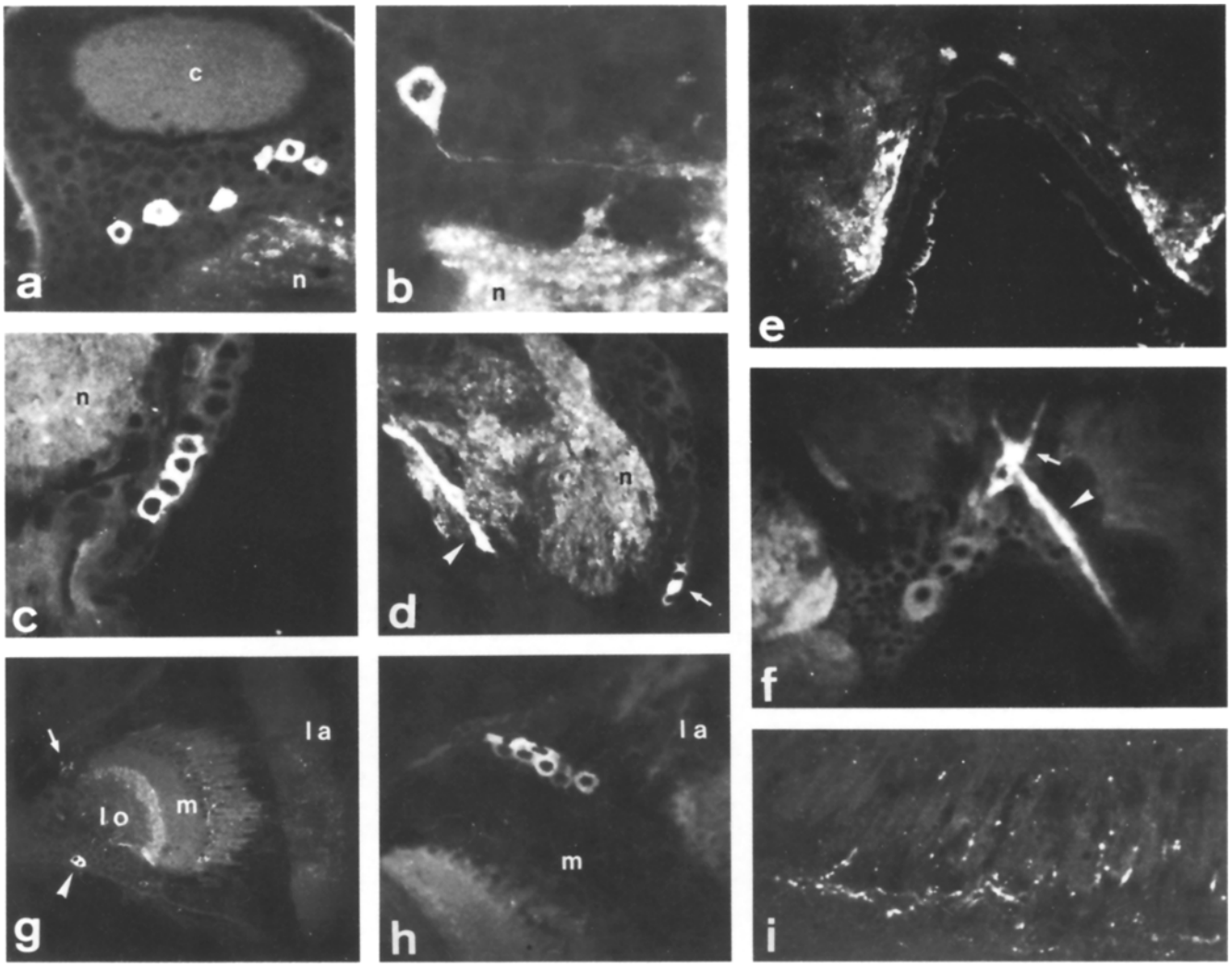

Fig. 3a–b. Protocerebrum, **c–f** Tritocerebrum, **g–i** Optic lobes. **a** Perikarya immunoreactive with FMFRamide antiserum in inner part of lateral NSC area. ×180. **b** NPY antiserum. Inner part of lateral NSC area, showing immunoreactive perikaryon and its axon running towards neuropilar fluorescent network. ×250. **c** FMRFamide antiserum, right side. Four of five immunoreactive perikarya on lateral face. ×250. **d** NPYantiserum, right side. Immunoreactive perikarya on lateral face (*arrow*) and immunoreactive tract on medial face (*arrowhead*). ×125. **e** FMRFamide antiserum. Immunoreactive tracts and fiber networks on medial faces. ×75. **f** NPY antiserum. Fluorescent fiber networks ending protocerebral (*arrow*) and tritocerebral (*arrowhead*) tracts in central body area. ×100. **g** FMRFamide antiserum, right optic lobe. Two round cell bodies (*arrowhead*) and cell cluster (*arrow*) in basal part. ×50. **h** NPY antiserum. Immunoreactive cell cluster between medulla and lamina (anterior edge). ×160. **i** FMRFamide antiserum. Tiny processes in outer chiasma. ×175. *c* Mushroom-body calyx; *la* lamina; *lo* lobula; *m* medulla; *n* neuropile; *p* protocerebrum

immunoglobulin (Institut Pasteur, France) at a concentration of 1:50 for 1 h at room temperature (20° C). Evans blue (0.01%) was used to obtain better contrast.

Control tests

Controls were performed on adjacent sections with nonimmune rabbit serum and with antigen-inactivated antisera (2 μg of antigen/ml of diluted antiserum 1:400, 4 h, at room temperature). Other controls included the use of the NPY antiserum preabsorbed with synthetic FMRFamide (Sigma) and the FMRFamide antiserum preabsorbed with synthetic NPY.

Results

Immunoreactive cells were found in the brain and in the suboesophageal and three thoracic ganglia. Except for the metathoracic ganglion, anti-NPY and anti-FMRFamide sera stained the same cells.

Brain

Perikarya containing NPY-like material are located in the protocerebrum, the tritocerebrum and the optic lobes (Fig. 1).

Protocerebrum. In the lateral neurosecretory cell (NSC) area, 12–14 pearshaped reactive perikarya are visible in each brain half. Six of them are scattered in the outer part of this area (mean diameter: 30 μm) (Fig. 2); the immunoreactive fibers found close to the root of the nervus corporis cardiaci II (NCC II) probably belong to these cells. These fibers penetrate the NCC II and reach the neural portion of the corpora cardiaca where small amounts of NPY-like

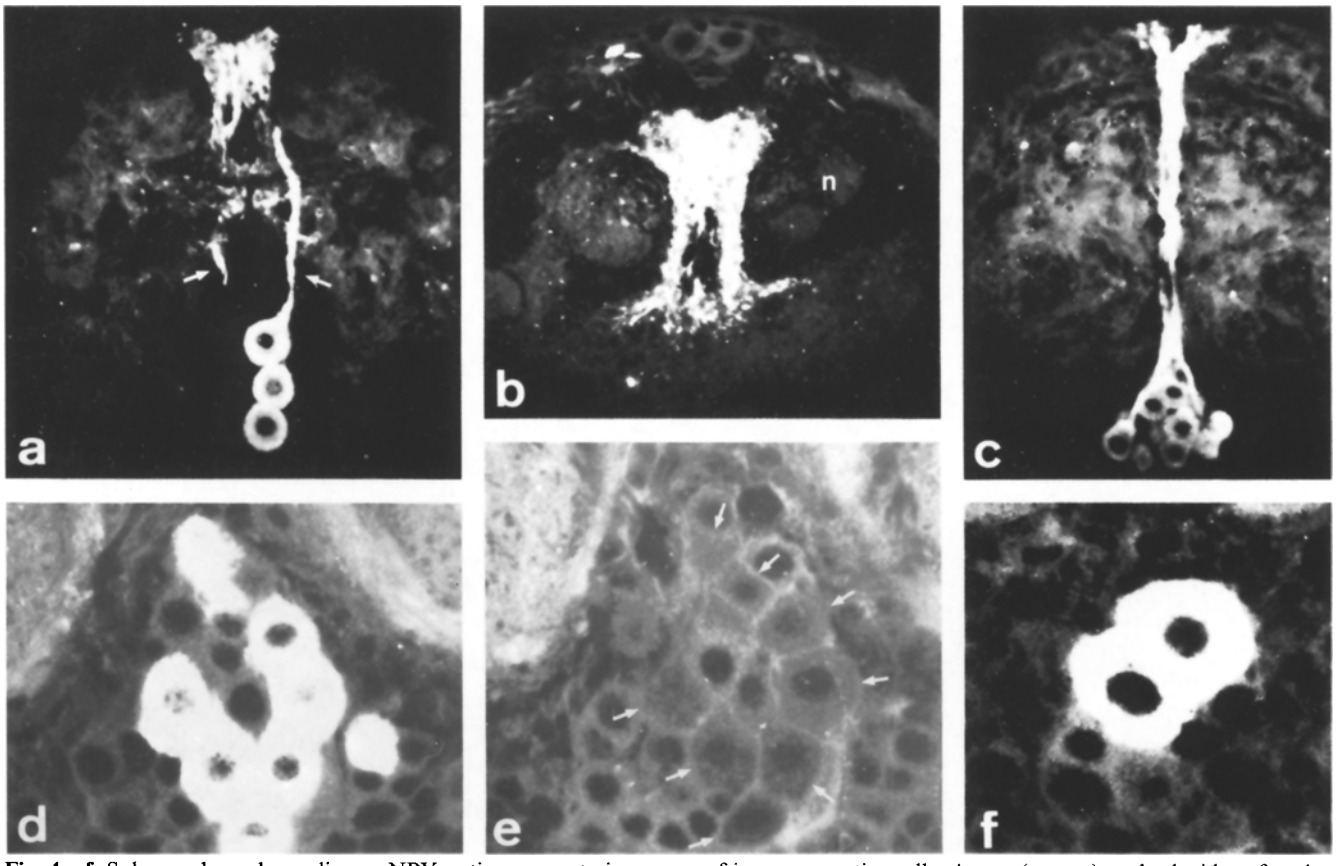

Fig. 4a–f. Suboesophageal ganglion. **a** NPY antiserum, anterior group of immunoreactive cells. Axons (*arrows*) on both sides of sagittal plane giving rise to fiber network (*arrowhead*). × 160. **b** NPY antiserum. Transverse section of immunoreactive network in dorsal neuropile (n). × 200. **c** NPY antiserum. Second group of immunoreactive cells in median part of ganglion. Axons cross in sagittal plane. × 140. **d–e** Two adjacent sections of second group of immunoreactive perikarya. × 300. **d** NPY antiserum. **e** NPY antiserum preincubated with NPY. No reaction at level of perikarya (*arrows*) stained in **d**. **f** NPY antiserum. Two large posterior immunoreactive perikarya. × 300

material are clearly visible. In the inner part of the lateral NSC zone, 6–8 cell bodies (30 μm) are located under the mushroom-body calyx (Fig. 3a), in the vicinity of the perikaryon containing met-enkephalin-like neuropeptide previously described in *Locusta* (Rémy and Dubois 1981). Anti-NPY immunoreactive cells and anti-met-enkephalin immunoreactive cells are distinct, as confirmed by the use of antisera against NPY or met-enkephalin on adjacent sections. The axons of these 6–8 NPY perikarya generally exhibit a bright fluorescence and can be easily traced in the protocerebral neuropile (Fig. 3b). They cross in the protocerebrum, just behind the mushroom-body pedunculus and give rise to various immunoreactive collaterals and arborizations. These arborizations form an important neuropilar network (Fig. 2), from the calyx to the pars intercerebralis edge and the root of the tractus I to the corpora cardiaca. The axons join in a fluorescent tract which curves inwards in the central part of the brain, joins the tractus I to the corpora cardiaca, and tightly follows the same pathway (Fig. 2). Except for a few very thin collaterals that enter the tractus I, no ramification is visible along the fluorescent bundle which terminates under the central body in a fiber network (coupled with tractus I). The small size of the fiber network is in striking contrast with that of the tract.

Tritocerebrum. In each half of the posterior tritocerebrum, NPY-like immunoreactivity is detected at the level of five round-shaped perikarya (20 μm) forming a linear pattern on the lateral face, close to the circumoesophageal connective (Fig. 3c). Their axons come together in an important tract running along the medial face of the tritocerebrum towards the central-body area (Fig. 3d, e). This tract runs parallel to the posterior part of tractus I to the corpora cardiaca and terminates in a small network, in the immediate vicinity of the immunoreactive protocerebral tract. When the plane of section is adequate, the right and left proto- and tritocerebral bundles are almost perfectly X-shaped and the centre of the X is situated right below the central body (Fig. 3f). In the dorso-lateral tritocerebrum, the anti-NPY serum reacts, on each side, with the neurosecretory product of three scattered cells of larger size (35 μm) whose axons do not seem to be part of the tritocerebral immunoreactive tracts described above.

Optic lobes. In the basal portion of each optic lobe, between the posterior protocerebrum and the lobula, 7–8 round-shaped perikarya (35 μm) showing NPY-like material were found in the vicinity of a perikaryon immunoreacting with an anti-met-enkephalin serum (Rémy and Dubois 1981). Some axons of these cells run to the posterior protocerebral neuropile; they could not be traced any farther. In each optic lobe, three cell clusters of 20–30 immunoreactive small spherical perikarya (20 μm) lie at the anterior edge, behind the lobula, between the lobula and the medulla, and be-

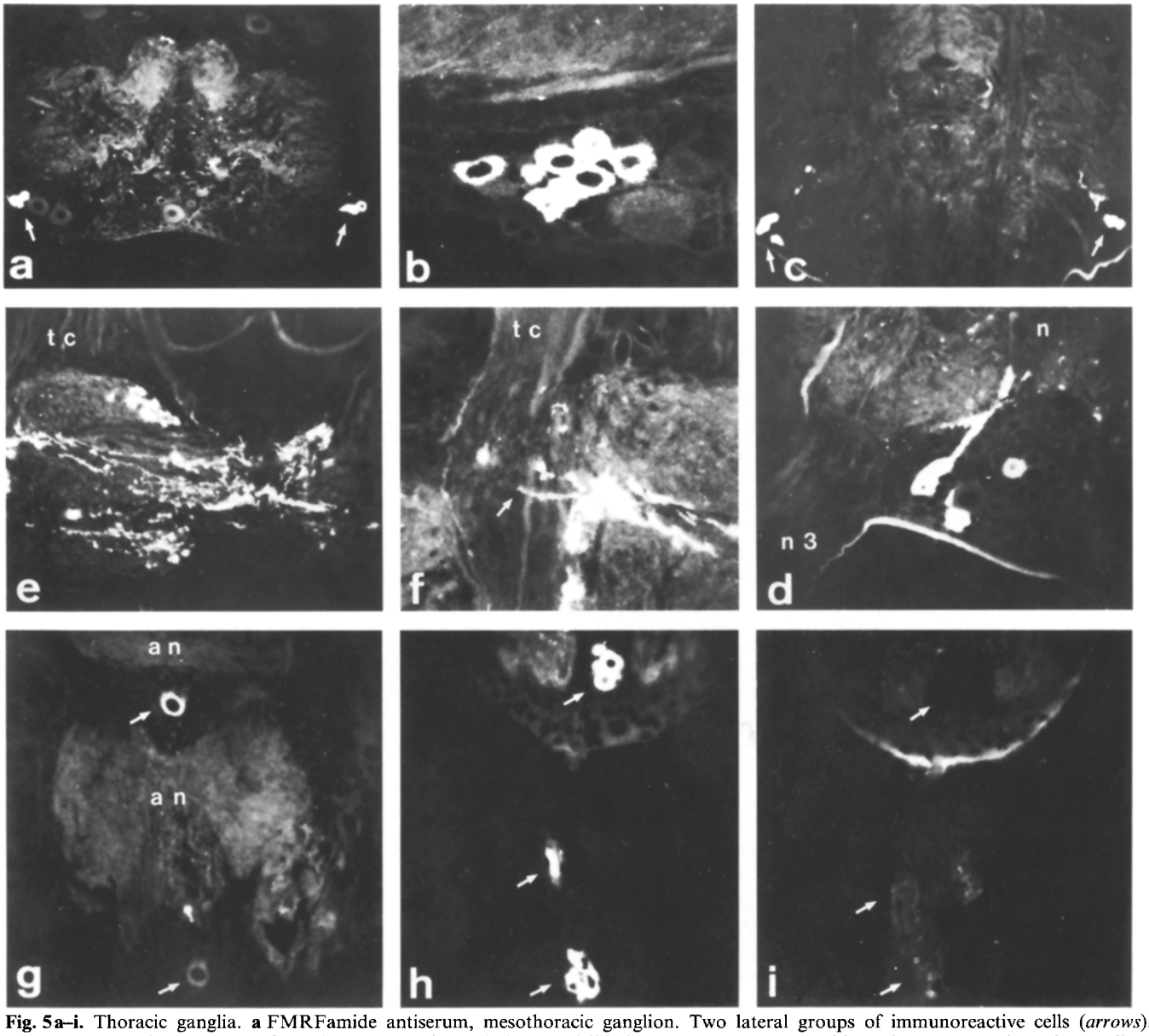

Fig. 5a–i. Thoracic ganglia. **a** FMRFamide antiserum, mesothoracic ganglion. Two lateral groups of immunoreactive cells (*arrows*). ×60. **b** NPY antiserum. One of two immunoreactive cell clusters visible in **a**. ×250. **c** FMRFamide antiserum, metathoracic ganglion. Two lateral groups of immunoreactive perikarya (*arrows*). ×60. **d** NPY antiserum. One of two immunoreactive cell groups visible in **c**. Axons enter neuropile diagonally. ×150. **e–f** NPY antiserum, mesothoracic ganglion. **e** Immunoreactive ramifications near root of thoracic connective. ×150. **f** Some tiny immunoreactive collaterals (*arrows*) penetrating perpendicularly into root of thoracic connective. ×300. **g** NPY antiserum, abdominal part of metathoracic ganglion. Immunoreactive perikarya (*arrows*) behind neuropile of abdominal neuromeres. ×150. **h–i** Two adjacent sections in abdominal part of metathoracic ganglion. **h** NPY antiserum. Immunoreactive material in perikarya and in abdominal median nerve (*arrows*). i FMRFamide antiserum. NPY-like material not related to FMRFamide (*arrows*). ×140. *an* Abdominal neuromere; *n* neuropile; *n3* segmental nerve 3; *tc* thoracic connective

tween the medulla and the lamina, respectively (Fig. 3g, h). Very tiny processes originate from these perikarya, run in a transverse direction and contain very small fluorescent swellings throughout the outer chiasma and the postretinal fibers (Fig. 3i).

Suboesophageal ganglion

Cells showing NPY-like immunoreactive material are found in three defined regions of the ventral midline. The most anterior group, located at one fifth of the ganglion, consists of four rounded cells (40 μm) whose axons run on both sides of the sagittal plane to the dorsal edge of the neuropile where they generate a longitudinal fiber network (Fig. 4a, b). In the median part of the ganglion, about 15 immunoreactive pearshaped perikarya (30 μm) form a large cell cluster adjacent to the two perikarya containing a vasopressin-neurophysin-like neuropeptide (Rémy et al. 1979) (Fig. 4c, d). All axons originating in this large cluster form an immunoreactive bundle crossing the ganglion in the sagittal plane and reaching the dorsal edge of the neuropile where it joins the longitudinal fiber network. The last group, situated at four-fifths of the ganglion's length, consists of two spherical very large perikarya (60 μm) (Fig. 4f). Their axons run in

the sagittal plane to the dorsal zone where they curve inwards on each side, forming an arc in the dorso-lateral neuropile.

Thoracic ganglia

As in the brain and suboesophageal ganglion, antisera raised against NPY or FMRFamide react with the same cells in the pro- and mesothoracic ganglia. In the metathoracic ganglion, these antisera identify two distinct types of immunoreactive cells.

The patterns of immunoreactive perikarya and processes are quite similar in the pro- and mesothoracic ganglia. NPY-like immunoreactivity is found in 4–5 rounded cell bodies (25 μm) in the ventral midline of the ganglia and in two symmetrical lateral groups of cells (Fig. 5a, c). Each of these two groups that occupy a position immediatly posterior to the root of the last segmental nerve (nerve 3, Albrecht 1953) consists of 8–9 rounded or elliptical perikarya (20 × 30 μm) (Fig. 5b). Their axons enter the neuropile diagonally (Fig. 5d) and form a large network of ramifications visible in almost the entire part of this neuropile. Immunoreactive ramifications are visible mainly in the midline of the ganglia, around the roots of the thoracic connectives and close to several nonimmunoreactive fiber bundles (Fig. 5e). Some tiny immunoreactive processes run forward from the network and penetrate these fiber bundles perpendicularly (Fig. 5f). Further down, the axons containing NPY-like material gather in the midline of the dorsal neuropile and form a fluorescent tract which joins the median sympathetic nerve.

The metathoracic ganglion of the locust results from the fusion of the third thoracic ganglion and the first three abdominal ganglia. The distribution of cells reacting with antisera against NPY or FMRFamide in the thoracic portion of the ganglion is quite similar to that in the first two thoracic ganglia. In the abdominal part, no immunoreaction could be obtained with the anti-FMRFamide serum (Fig. 5i). Only the anti-NPY serum reacts with three cell clusters, each of them containing 5–6 spherical perikarya situated in the midline, respectively, behind the neuropile of each abdominal neuromere (Fig. 5g, h). These perikarya (35 μm) are larger than the thoracic cell bodies showing NPY/FMRFamide-like immunoreactivity; nevertheless, the intensity of their immunoreaction was slightly weaker. The axons originating in each of the three cell clusters enter the first, second, and third abdominal median nerves, respectively, where they form a dense network of immunofluorescent swellings (Fig. 5h).

Control and cross-absorption tests

Sections treated with antisera preabsorbed with their homologous antigens gave negative results, as did sections treated with a nonimmune serum (Fig. 4d, e). However, the activity of the NPY antiserum was not blocked by preincubation with FMRFamide. Likewise, the activity of the FMRFamide antiserum was not suppressed by preincubation with NPY.

Discussion

In the brain and the suboesophageal and thoracic ganglia of *Locusta migratoria*, a large number of NSC synthetize a neuropeptide related to NPY and FMRFamide. A few of these cells, located in the suboesophageal ganglion, have been previously described by Chalaye (1967) as azocarminophil cells, then characterized by Veenstra (1984) by use of BPP or FMRFamide antisera. In the brain, the NPY-like immunoreactivity revealed for the first time a particular category of NSC in the protocerebrum, tritocerebrum and optic lobes. The axons of some protocerebral immunoreactive cells enter the NCC II and reach the neurohaemal corpora cardiaca. This suggests a neurohormonal function for the NPY/FMRFamide-like neuropeptide. However, this immunoreactive neuropeptide may also act as a neuromodulator or a neurotransmitter within the brain, as suggested by the large immunoreactive network of fibers in the protocerebral neuropile. These fibers are not only coupled with the tractus I to the corpora cardiaca but also reach the central-body area, a major meeting point of fibres originating in several parts of the insect brain. A similar diversity of functions can be attributed to the suboesophageal and thoracic NPY/FMRFamide-like material present in large fiber networks and in tracts penetrating the thoracic neurohaemal organs, via the median sympathetic nerves. Finally, the existence of numerous immunoreactive neurons in the optic lobes suggests that this locust neuropeptide could be concerned with visual function.

The distribution of the NPY-like material within the cephalic and thoracic nervous systems of *Locusta* is comparable to the distribution of the BPP/FMRFamide-like neuropeptide observed in insects belonging to distinct orders: *Periplaneta americana*, (Dictyoptera, Endo et al. 1942; Verhaert et al. 1985), *Manduca sexta*, (Lepidoptera, El-Salhy et al. 1983), *Calliphora erythrocephala* and *C. vomitoria*, (Diptera, Duve and Thorpe 1980, 1982), *Leptinotarsa decemlineata*, (Coleoptera, Veenstra and Schooneveld 1984). In orthopteran species, only the ventral nerve cord of *Schistocerca gregaria* has been studied (Myers and Evans 1985a, b). The distribution of the BPP/FMRFamide-like immunoreactivity in this species is, in this part of the nervous system, the same as the NPY immunoreactivity in *Locusta*. The controls with the FMRFamide antiserum show that the anti-NPY immunoreactive cells of *Locusta* correspond to the anti-BPP immunoreactive cells observed in other insects. Consequently, it can be inferred that a neurosecretory product related to the vertebrate pancreatic polypeptide family is commonly found in the insect world.

The immunoreactivity of the BPP antiserum is suppressed by absorption with FMRFamide (Verhaert et al. 1985). The results we obtained with the cross-absorption tests are markedly different. In *Locusta* the immunoreactivity of the NPY antiserum preabsorbed with FMRFamide is not affected; similarly the FMRFamide antiserum is not inhibited after preabsorption with NPY. On the other hand, similar concentrations of the antigens thoroughly inhibit the homologous antisera. This would then mean that, in the locust antigen, the antibodies of the NPY antiserum recognize antigenic sites that are not related to FMRFamide. The fact that the immunoreactivity of the FMRFamide antiserum is not suppressed after preabsorption with NPY seems surprising; yet the same results were obtained by Myers and Evans (1985b) with an FMRFamide antiserum preabsorbed with BPP. The anti-FMRFamide serum could therefore recognize, in the locust neurosecretory product, antigenic sites much more closely related to FMRFamide than to NPY, these sites being distinct from

those recognized by the NPY antiserum. Consequently, it seems logical to conclude that the locust immunoreactive neurosecretory product is made up of (1) either a large neuropeptide with antigenic sites identical to those of the NPY-BPP family, and other antigenic sites similar to those of FMRFamide, (2) or two neuropeptides simultaneously synthetized, one related to the NPY-BPP family, the other to FMRFamide. The latter assumption seems to receive further support from the fact that some locust metathoracic cells crossreact only with the NPY antiserum (in these cells, the FMRFamide-like material may therefore be absent). Further evidence for this assumption was provided by Duve et al. (1981, 1982) who were able to identify a neuropeptide with a primary structure similar to that of BPP in *Calliphora vomitoria*.

Refernces

Albrecht F (1953) The anatomy of the migratory locust. Athlone Press, London

Boer H, Schot L, Veenstra J, Reichelt D (1980) Immunocytochemical identification of neural elements in the central nervous systems of a snail, some insects, a fish, and a mammal with an antiserum to the molluscan cardio-excitatory tetrapeptide FMRF-amide. Cell Tissue Res 213:21–27

Chalaye D (1967) Neurosécrétion au niveau de la chaîne nerveuse ventrale de *Locusta migratoria migratoriodes* R et F (Orthoptère Acridien). Bull Soc Zool (France) 92:87–108

Coons A, Leduc E, Connoly J (1955) Studies on antibody production. I. Method for the histochemical demonstration of specific antibody and its application to the study of the hyperimmune rabbit. J Exp Med 102:49–59

Duve H, Thorpe A (1980) Localization of pancreatic polypeptide (PP)-like immunoreactive material in neurones of the brain of the blowfly, *Calliphora erythrocephala* (Diptera). Cell Tissue Res 210:101–109

Duve H, Thorpe A (1982) The distribution of pancreatic polypeptide in the nervous system and gut of the blowfly, *Calliphora vomitoria* (Diptera). Cell Tissue Res 227:67–77

Duve H, Thorpe A, Neville R, Lazarus N (1981) Isolation and partial characterization of pancreatic polypeptide-like material in the brain of the blowfly *Calliphora vomitoria*. Biochemical jal 197:767–770

Duve H, Thorpe A, Lazarus N, Lowry P (1982) A neuropeptide of the blowfly *Calliphora vomitoria* with an amino acid composition homologous with vertebrate pancreatic polypeptide. Biochem J 201:429–432

El-Salhy M, Falkmer S, Kramer K, Speirs R (1983) Immunohistochemical investigations of neuropeptides in the brain, corpora cardiaca, and corpora allata of an adult lepidopteran insect, *Manduca sexta* (L.). Cell Tissue Res 232:295–317

Endo Y, Iwanaga T, Fujita T, Nishiitsutsuji-Uwo J (1982) Localization of pancreatic polypeptide (PP)-like immunoreactivity in the central and visceral nervous systems of the cockroach *Periplaneta*. Cell Tissue Res 227:1–9

Myers C, Evans P (1985a) The distribution of bovine pancreatic polypeptide-FMRFamide-like immunoreactivity in the ventral nervous system of the locust. J Comp Neurol 234:1–16

Myers C, Evans P (1985b) An FMRFamide antiserum differentiates between populations of antigens in the ventral nervous system of the locust, *Schistocerca gregaria*. Cell Tissue Res 242:109–114

Pelletier G, Desy L, Kerkerian L, Cote J (1984) Immunochemical localization of neuropeptide Y (NPY) in the human hypothalamus. Cell Tissue Res 238:203–205

Price D, Greenberg M (1977) Structure of a molluscan cardioexcitatory neuropeptide. Science 197:670–671

Rémy C, Dubois M (1981) Immunohistological evidence of methionine enkephalin-like material in the brain of the migratory locust. Cell Tissue Res 218:271–278

Rémy C, Vieillemaringe J (1987) Immunocytology of insect peptides and amines. In: Thorndyke MC, Goldsworthy GJ (eds) Amines and peptides in invertebrates. Cambridge University Press, Cambridge (in press)

Rémy C, Girardie J, Dubois M (1979) Vertebrate neuropeptide-like substances in the suboesophageal ganglion of two insects: *Locusta migratoria* R. and F. (Orthoptera) and *Bombyx mori* L. (Lepidoptera). Immunocytological investigation. Gen Comp Endocrinol 37:93–100

Schot L, Boer H, Montagne-Wajer C (1984) Characterization of multiple immunoreactive neurons in the central nervous system of the pond snail *Lymnaea stagnalis* with different fixatives and antisera adsorbed with the homologous and the heterologous antigens. Histochemistry 81:373–378

Tatemoto K (1982) Neuropeptide Y: complete amino acid sequence of the brain peptide. Proc Natl Acad Sci USA 79:5485–5489

Veenstra J (1984) Immunocytochemical demonstration of a homology in peptidergic neurosecretory cells in the suboesophageal ganglion of a beetle and a locust with antisera to bovine pancreatic polypeptide, FMRFamide, vasopressin and -MSH. Neurosci Lett 48:185–190

Veenstra J, Schooneveld H (1984) Immunocytochemical localization of neurons in the nervous system of the Colorado potato beetle with antisera against FMRFamide and bovine pancreatic polypeptide. Cell Tissue Res 235:303–308

Verhaert P, Grimmelikhuijzen C, De Loof A (1985) Distinct localization of FMRFamide- and bovine pancreatic polypeptide-like material in the brain, retrocerebral complex and suboesophageal ganglion of the cockroach *Periplaneta americana* L.. Brain Res 348:331–338

Accepted January 20, 1988