Histochemistry (1990) 93:433–438

Histochemistry

Gastrin-cholecystokinin-like and neuroparsin-like immunoreactivities in the brain and retrocerebral neuroendocrine complex of the cockroach *Blattella germanica*

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Accepted October 7, 1989

Summary. Using single and double labelling techniques respectively, brain-corpora cardiaca-corpora allata complexes of the cockroach *Blattella germanica* have been immunohistochemically investigated with antisera raised against either the vertebrate peptide gastrin-cholecystokinin (CCK-8(s)) and/or the locust neurohormone neuroparsin (NPA).

Single immunolabelling with anti-CCK-8(s) reveals immunoreactive perikarya and processes in median and lateral parts of protocerebrum, optic lobes, deutocerebrum and tritocerebrum. Some fibres originating in median and lateral protocerebrum are intrinsic to the brain, whereas others terminate in the nervous areas of the corpora cardiaca. Single immunolabelling with anti-NPA reveals immunoreactive cell bodies in the median part of the protocerebrum and their processes terminate both in the nervous area of the corpora cardiaca and between the intrinsic secretory cells of this neurohaemal organ. Double immunolabelling with anti-CCK-8(s) and anti-NPA enables a description of the anatomical relations between the processes and the endings of these two neurosecretory systems.

Introduction

Gastrin-cholecystokinin was the first of the gut peptide family to be reported in vertebrate brain (Vanderhaeghen et al. 1975). Using antibodies recognizing the sequence common to both cholecystokinin and gastrin, previous immunohistochemical studies have demonstrated immunoreactivity in the central nervous system of several species of insects (Yui et al. 1980; Duve and Thorpe 1981; Dhainaut-Courtois et al. 1984; Hansen et al. 1987; Tamarelle et al. 1988). However no study of this substance has been performed to date on the complete brain-retrocerebral neuroendocrine complex of a dictyopteran species.

Neuroparsin is a neurohormone produced in the A1 type protocerebral median neurosecretory cells of the migratory locust (Girardie et al. 1986). Using specific anti-neuroparsin serum (Bourême et al. 1987), Tamarelle and Girardie (1989) demonstrated neuroparsin-like immunoreactivity in several insect orders, including some dictyopteran species.

In the present study, we report on the immunohistochemical exploration for gastrin-cholecystokinin-like and neuroparsin-like products in the brain-corpora cardiaca-corpora allata complexes of the cockroach *Blattella germanica* using single and double labelling procedures.

Material and methods

Material and immunohistological procedure. Adult cockroaches *Blattella germanica* of both sexes were reared at 26 (+1° C) and 60–70 RH in constant darkness, as described elsewhere (Bellés and Piulachs 1983).

Brains were dissected and fixed for 4–6 h in Bouin Holland mixture without acetic acid and to which saturated mercuric chloride solution was added (10% v/v). After dehydration through alcohol steps, sections were embedded in paraffin and serially sectioned at 7 microns. Some sections were mounted alternately to compare and identify immunostaining and Victoria blue-paraldehyde fuchsin staining of Ganagajarah and Saleuddin (1970) and to control the specificities of immunostaining.

Immunohistochemical procedure and double immunostaining method. For simple immunohistochemical detection of each antigen (CCK-8(s) or NPA) on different slides of brains, it was used the peroxidase antiperoxidase procedure (PAP), as described by Sternberger et al. (1970). Sequential detection of the two related antigens on the same brain section was performed using the double staining immunoenzymatic labelling technique (Nakane 1968; Vandesande 1988). Following this technique, both CCK-8(s) and NPA related antigens are labelled using the PAP procedure. Two different enzyme substrates were applied: diaminobenzidine (DAB) for the first antigen (brown colour) and DAB+nickel chloride for the second (blue colour).

Antisera. Gastrin-cholecystokinin antiserum (anti-CCK-8(s)) was a gift of Prof. J.J. Vanderhaeghen. Antiserum was obtained with terminal carboxyl octapeptide of cholecystokinin in its complete sulfated form (Squibb) after linking to thyroglobulin by use of carbodiimide (Sigma) (Vanderhaeghen et al. 1980) and used at 1/8000 dilution. The antiserum against locust neuroparsin (anti-NPA) was raised in rabbits in the Laboratory of Neuroendocrinology at Talence (Bourême et al. 1987) and used at 1/4000 dilution. Conventional staining controls were carried out as suggested by Sternberger (1986).

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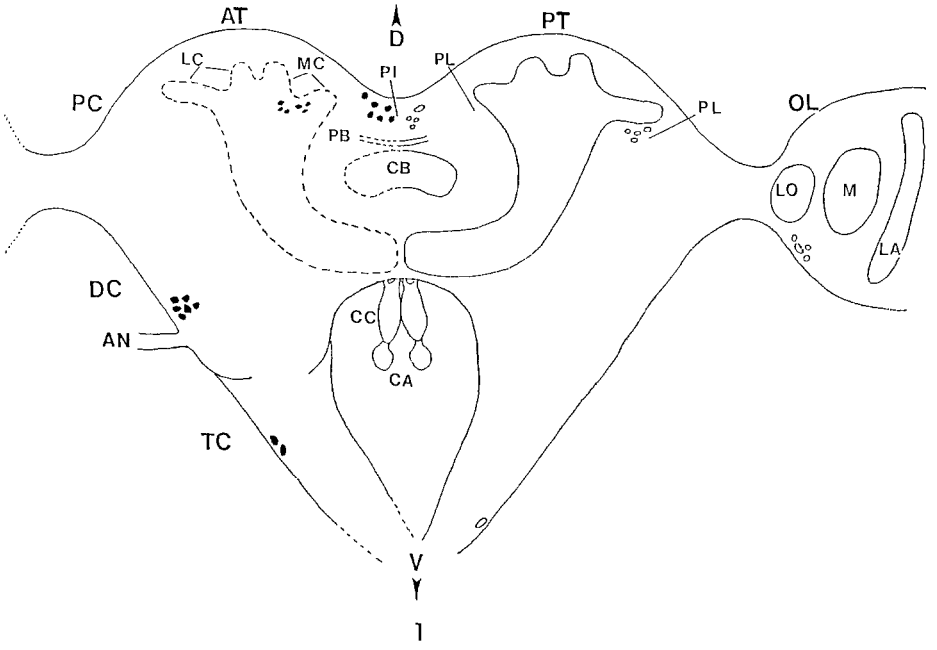


Fig. 1. Schematic view of the brain of *B. germanica* showing the distribution of CCK-8(s) immunoreactive perikarya in anterior position (*filled circles*) and in posterior position (*open circles*). *AN* antennal nerve; *AT* half anterior view of the brain; *CA* corpora allata; *CB* central body; *CC* corpora cardiaca; *D* dorsal part; *DC* deutocerebrum; *LA* lamina; *LC* lateral calyx; *LO* lobula; *M* medulla; *MC* medial calyx; *OL* optic lobe; *PB* protocerebral bridge; *PC* protocerebrum; *PI* pars intercerebralis; *PL* pars lateralis; *PT* half posterior view of the brain; *TC* tritocerebrum; *V* ventral part

Results

Immunohistochemical detection of CCK-8(s)like material

No staining was observed when anti-CCK-8(s) serum was replaced either by control serum or by serum previously inactivated with CCK-8(s) (4.5 µg/ml). Likewise no staining occurred when the secondary antibody PAP or DAB, respectively are omitted from the immunohistochemical procedure. The diagramatic scheme shown in Fig. 1 summarizes the distribution of CCK-8(s) immunoreactive cell bodies observed in the brain.

Protocerebrum

Each hemisphere of the pars intercerebralis presents anteriorly about six to seven strongly immunoreactive perikarya (10–12 µm in diameter). These cell bodies are unstained when using the Victoria blue-paraldehyde fuchsin technique which indicates that they do not belong neither to A1 nor A2 type. Posteriorly, scattered clusters of two to three small immunoreactive perikarya (6–7 µm in diameter) are present, and more posteriorly two symmetrical perikarya (12 µm in diameter) are located. Processes from the anterior and posterior median cell bodies run towards protocerebral neuropile in which they profusely branch to surround alpha and beta lobes of the corpora pedunculata (Fig. 2). Processes from anterior median cell bodies run closely parallel to the median tract but no fibres penetrate the tract (Fig. 3).

The pars lateralis presents two lateral groups of cell bodies: one internal, the other one external. The internal group is composed by six to eight perikarya (10 µm in diameter) whose processes project into the protocerebral neurophile and intermingle with processes originating from antero-median cell bodies. The external group has three to four cell bodies (8 µm in diameter) (Fig. 4), and their fibres project into the protocerebral neuropile and some of them penetrate into the external nervus corpora cardiaca (NCCII). At the level of the protocerebral bridge, immunoreactive fibres are seen running towards the respective contralateral part (Fig. 8a). The central body complex shows immunoreactive fibre tracts in its superior arch and in its concave part arranged as vertically parallel columns (Fig. 8b).

Optic lobes

At the frontal base of the optic lobes, four cell bodies are scattered under the lobula. One of them (12 µm in diameter) is strongly immunostained and slight fibres are observed in the outer chiasma. Fine fibres are revealed between lobula and medulla and several varicosities can be seen in extra lateral part of the protocerebral neuropile (Fig. 7).

Fig. 2–10a. Immunostaining with CCK-8(s) antiserum

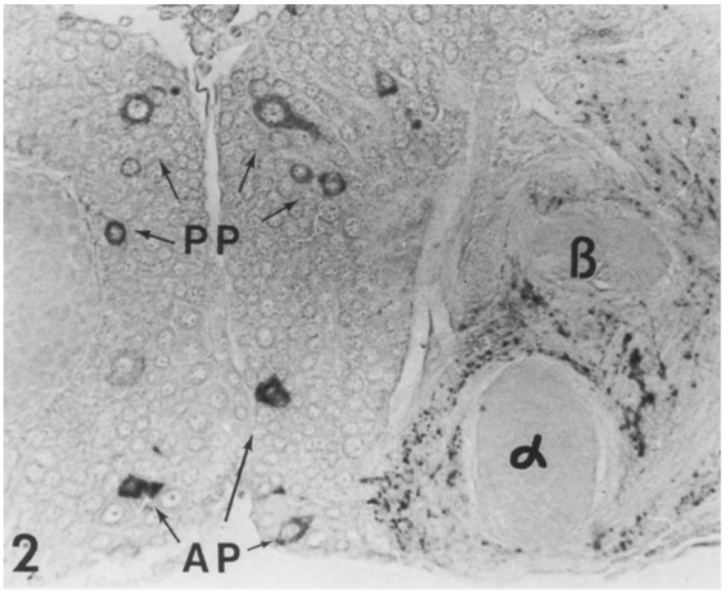
Fig. 2. Protocerebrum: median part (dorso-ventral section). Median immunoreactive perikarya in anterior position (*AP*) and in posterior position (*PP*). On the right side, immunoreactive fibres are seen around α and 3 lobes of corpus pedunculata. × 290

Fig. 3. Protocerebrum: median part (antero-posterior section). Median immunoreactive cell bodies (*M*) and processes in protocerebral neuropile (*P*). *MT* unstained median tract. × 290

Fig. 4. Protocerebrum: left hemisphere (antero-posterior section). *C* calyx; *LE* lateral external immunoreactive perikarya; *LI* lateral internal immunoreactive perikarya; *M* median immunoreactive perikarya. × 290

Fig. 5. Deutocerebrum: left side (antero-posterior section). Two perikarya and their processes reaching antennal glomeruli (*GL*). *AN* antennal nerve. × 350

Fig. 6a and b. Tritocerebrum: left side (antero-posterior section). **a** Perikaryon and its processes in anterior lateral position; **b** Perikaryon in posterior lateral position. *Pr* processes; *T* tritocerebral neuropile. × 300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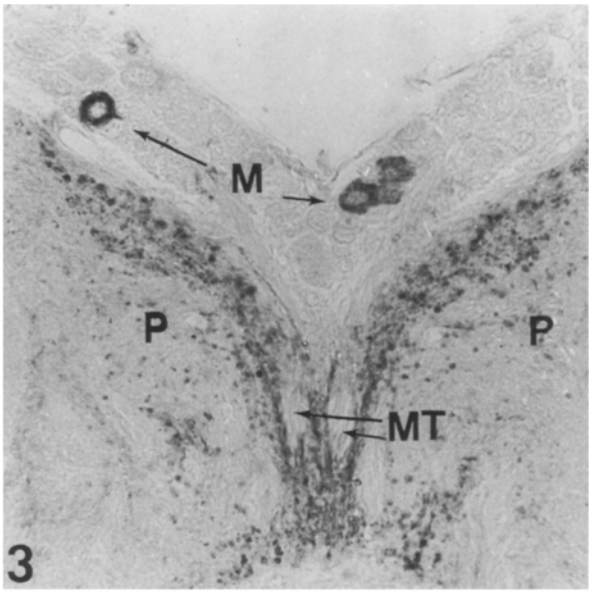
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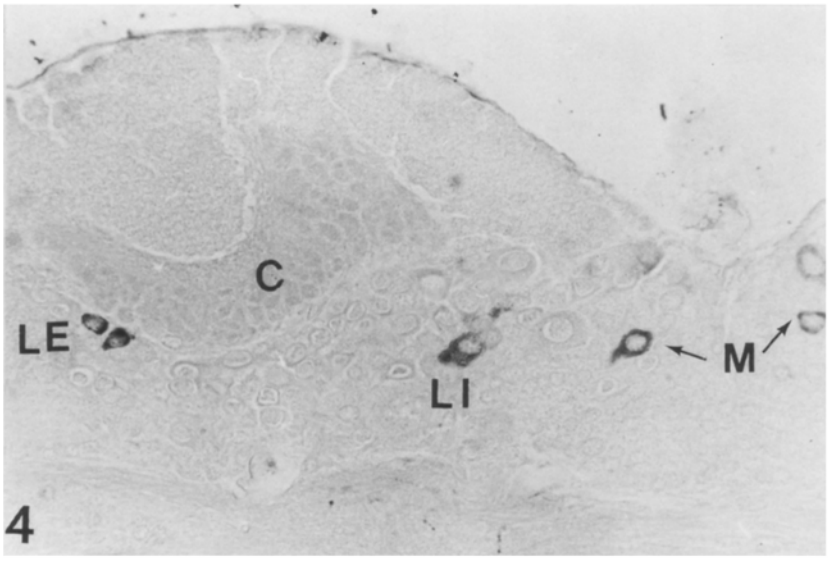
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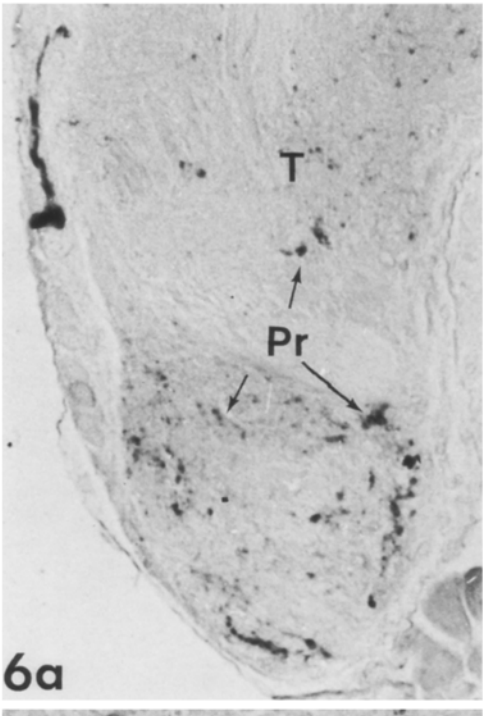
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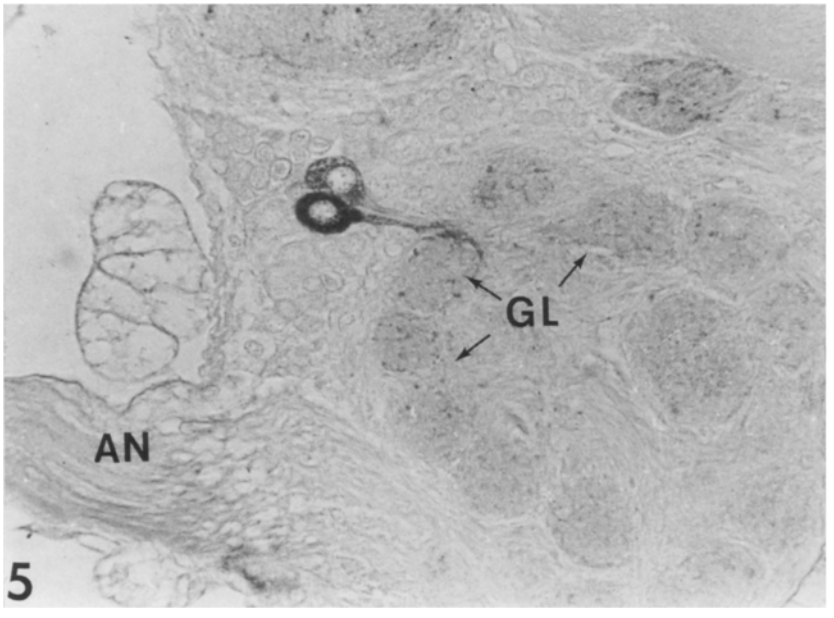
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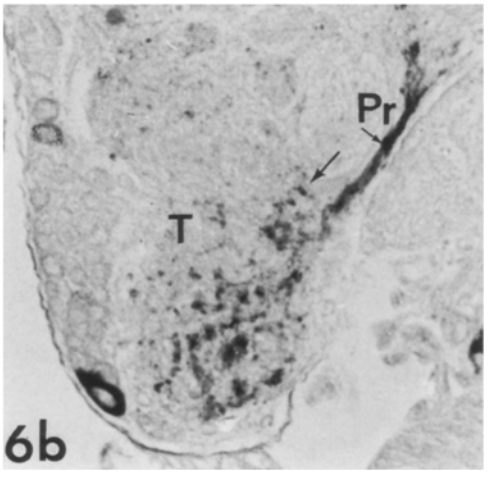
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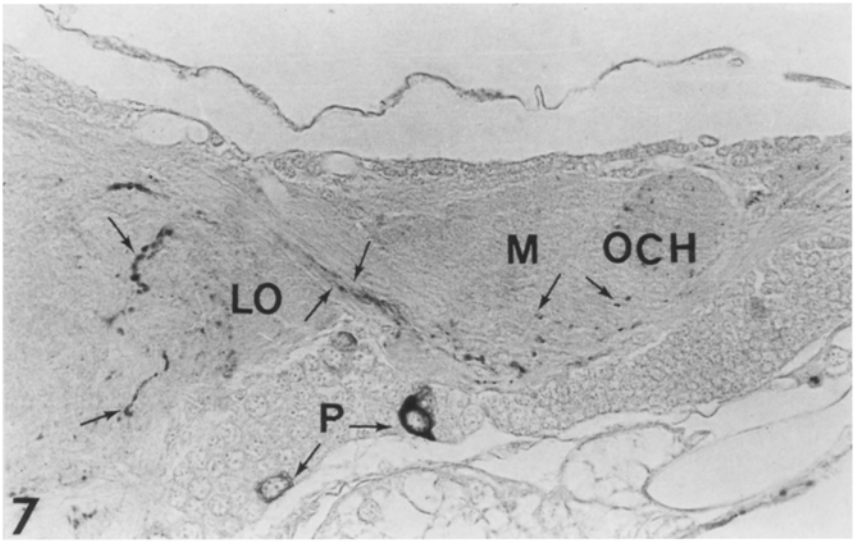
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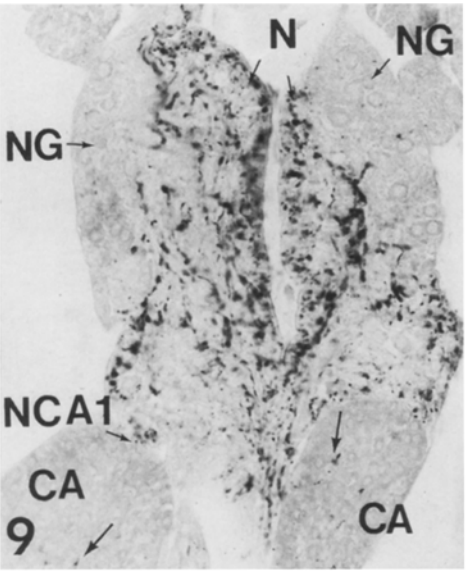
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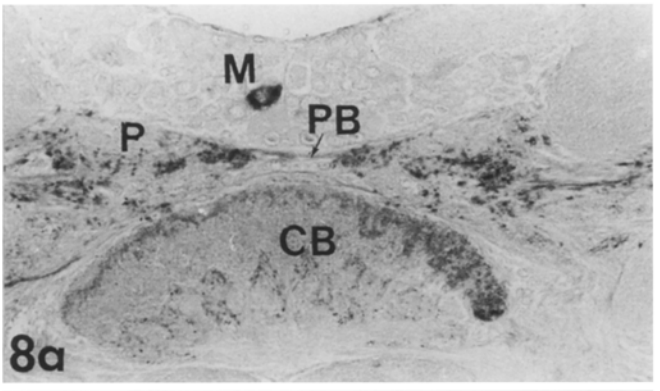
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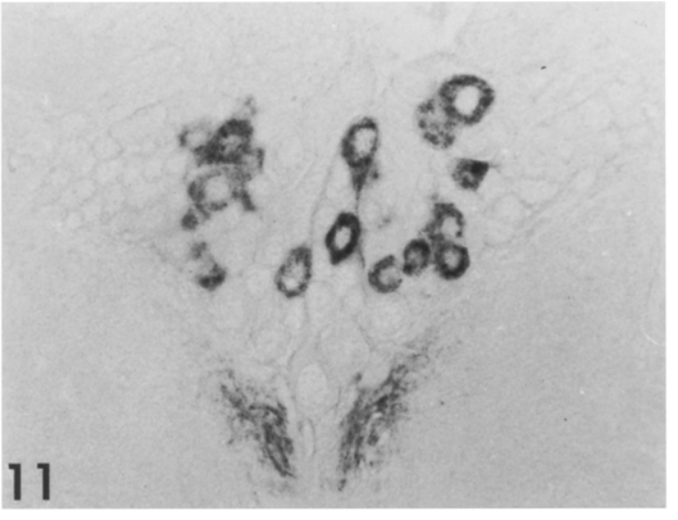
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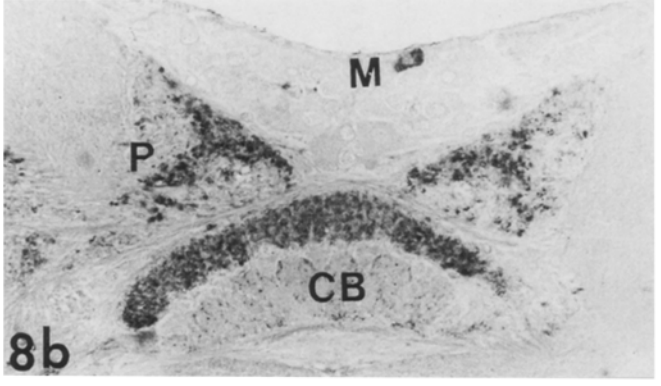
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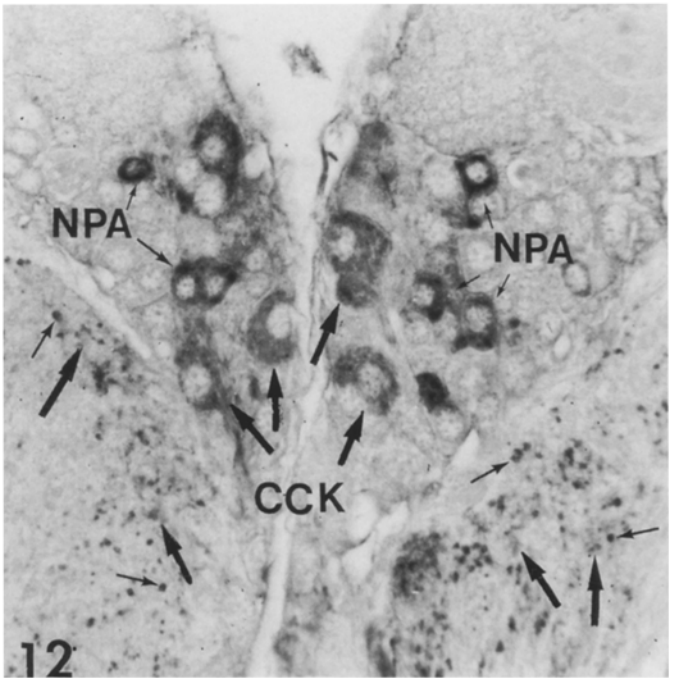
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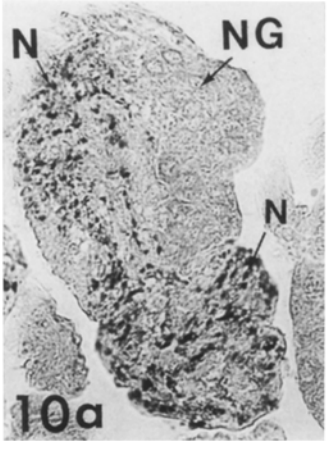
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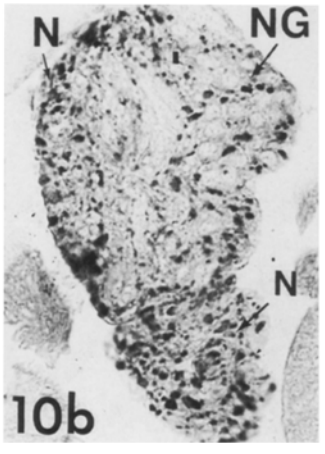
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Deutocerebrum

In the external cortex of the antennal lobe a cluster of about 6 large round shaped cell bodies (15 µm in diameter) is observed. Their processes reach the antennal glomeruli where thin fibres are revealed (Fig. 5).

Tritocerebrum

Anteriorly on the lateral external margin of the tritocerebrum, two strongly immunostained ovoid (18 µm in large axe) cell bodies are detected. Their processes run along the margin and project in the upper part of the tritocerebral neuropile (Fig. 6a). More posteriorly, near the circumoesophageal connective an ovoid cell body (20 µm in large axe) is observed. In addition, a network of varicosities is revealed in the tritocerebral neuropile. Immunoreactive fibres form a tract running along the internal medial face of the tritocerebral lobe (Fig. 6b).

Corpus cardiacum-corpus allatum complex

A substantial amount of immunoreactive material appears in the neural zone facing the dorsal blood vessel and in the path of the NCCII. Large immunoreactive deposits are observed in the posterior ventral lobes of the corpora cardiaca (CC). A small amount of immunoreactive material enters into the nervi corporis allati (NCAI) and terminates in the corpora allata (CA) (Fig. 9). No endings are seen in the intrinsic secretory cells area of the CC (Figs. 9 and 10a).

Fig. 7. Optic lobe: right side (antero-posterior section). Immunoreactive perikarya (*P*). Immunoreactive fibres (arrows) in lobula (*LO*) between lobula and medulla (*M*), in outer chiasma (*OCH*). × 350

Fig. 8a and b. Protocerebrum: median part (antero-posterior section). At the level of central body (*CB*). **a** Contralateral fibres in protocerebral bridge (*PB*). **b** More posterior section, immunoreactive fibres in superior arch of central body. *M* median perikaryon; *P* protocerebral neuropile. × 350

Fig. 9. Corpora cardiaca-corpora allata complex. Immunoreactive endings (arrows) in the nervous areas (*N*) of corpora cardiaca and in the corpora allata (*CA*). *NCA1* nervus corpora cardiaca-corpora allata 1; *NG* neuroglandular areas of corpora cardiaca. × 350

Fig. 10a and b. Corpora cardiaca (left parts). Two adjacent sections. **a** Reactive endings in nervous part (*N*) immunostained with CCK-8(s) antiserum. Unstained neuroglandular part (*NG*). **b** Reactive endings in nervous and neuroglandular (*NG*) parts immunostained with NPA antiserum. × 350

Fig. 11. Protocerebrum: pars intercerebralis (dorso-ventral section). Cell bodies and processes immunostained with NPA antiserum. × 300

Fig. 12. Protocerebrum: pars intercerebralis (dorso-ventral section). Double immunostaining procedure. Cell bodies immunostained with CCK-8(s) antiserum (*CCK*), *thick arrows*. Cell bodies immunostained with NPA antiserum (*NPA*), *thin arrows*. In the upper of the protocerebral neuropile *CCK* (*thick arrows*) and NPA (*thin arrows*) processes. × 380

Immunohistochemical detection of NPA-like material

The same controls as for CCK-8(s) detection were applied, except that 1 µg/ml neuroparsin was used in previous inactivated serum. No staining was observed in any case.

Immunoreactive cell bodies are detected only in the pars intercerebralis. These neurosecretory cell bodies are also stained by Victoria blue which indicates that they belong to the A1 cellular type. Their processes enter the internal nervus corpora cardiaca (NCCI) (Fig. 11) and terminate as reactive endings in the entire CC (nervous and intrinsic secretory cells areas) (Fig. 10b).

Sequential immunohistochemical detection of CCK-like and NPA-like products

Double immunoperoxidase labelling shows immunoreactive CCK-8(s)-like and NPA-like cell bodies lying closely but not co-localized. This confirms the results obtained through dye staining which indicates that the respective reactive cell bodies belong to two different cellular types. This procedure also shows the intermingling of the two types of fibres in the upper part of the protocerebral neuropile (Fig. 12). Moreover the double labelling enhances the difference between CCK-8(s) endings in the nervous part of the CC and NPA endings both in the nervous and glandular parts of the CC.

Discussion

These results describe clearly a widespread distribution of the CCK-8(s)-like perikarya and nerve fibres within the three ganglia of the brain of *B. germanica*. It is shown, in this insect, that there exist in the median part of the protocerebrum two categories of immunoreactive cell bodies which are not fuchsinophilic. The first is located in the pars intercerebralis and is constituted by median anterior cell bodies; the second is situated more posteriorly and includes grouped or isolated perikarya. No processes from these cell bodies penetrate the median tract but they intermingle intrinsically in the protocerebral neuropile. We also demonstrate the immunolabelling of two groups of cell bodies in the pars lateralis: an internal group whose processes intermingle closely with those coming from median cell bodies, and an external group whose processes project to the nervous part of the CC via NCCII and even to CA via NCA1. Some of these latter cell bodies which extend into the CC-CA could belong to the same category of those identified by Pipa (1978) in *Periplaneta americana* using iontophoretic techniques.

The pattern of the CCK-8(s) distribution within the brain-retrocerebral neuroendocrine complex of *B. germanica* is similar to that described for *Locusta migratoria* by Tamarelle et al. (1988). In comparison with the CCK immunolabelling carried out on the CC-CA complex of *Leucophaea maderae* by Hansen et al. (1987), it is worth noting that we did not find immunostaining in the intrinsic glandular cells of the CC. This difference possibly could be explained by the distinct specificities of the two antisera used. Whatever the case, there is a homology between the structure of CCK-8 and that of leucosulfakinin, a peptide obtained from CC extracts of the cockroach *Leucophaea ma-*

derae (Nachman et al. 1986a and b). This resemblance suggests that the CCK-8(s)-like substance revealed in *B. germanica* could belong to the leucosulfakinin family of peptides.

The pattern of NPA-like immunoreactivity shows that only the A1 type of protocerebral median neurosecretory cells are immunostained, as is the case in *Locusta* (Bourême et al. 1987) and in some other insect species (Tamarelle and Girardie 1989). In *B. germanica* we additionally observe the presence of NPA-like endings via NCC1 both in the nervous part and between the intrinsic neuroglandular cells of the CC. Furthermore, it is reasonable to suggest that the neuroparsin-like product revealed in *B. germanica* could be related to cockroach neuroparsin-like proteins containing disulfide bonds (Bourême et al. 1989).

The sequential immunohistochemical application of the two antisera on brain of *B. germanica* shows two distinct neurosecretory systems in the median protecerebrum: the median CCK-like system intrinsic to the brain and the median NPA-like system projecting in nervous and neuroglandular parts of the corpora cardiaca. In the protocerebral neuropile, this procedure also enables to enhance the visualization of the intermingling of NPA-like processes originating from median cell bodies with CCK-like fibres originating from median, internal lateral and probably some external lateral CCK-like cell bodies. Our study also demonstrates the close juxtaposition of CCK-like endings (lateral protocerebral origin) with NPA-like endings (median protocerebral origin) in the nervous part of the CC. These two types of endings originate from processes running via two different nerves (NCCI and NCCII). The close vicinity of CCK and NPA-like processes and endings suggests functional interrelations between these two neurosecretory identified systems.

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