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Localization of cholecystokinin-like immunoreactivity in the central nervous system of *Triatoma infestans* (Insecta: Heteroptera)

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Abstract

The distribution of cholecystokinin-like immunoreactivity was studied in the central nervous system of the heteropteran insect *Triatoma infestans* using high-sensitivity immunocytochemistry. In the protocerebrum, CCK-IR somata were observed in the anteromedial, anterolateral and posterior cell-body layers. The neuropils displayed different densities of immunoreactive neurites. Few immunoreactive somata were found in the optic lobe in both the medial and lateral soma rinds, as well as in the proximal optic lobe. Immunoreactive fibers were present in the medulla and lobula neuropils. The sensory deutocerebrum contained a higher number of immunopositive perikarya than the antennal mechanosensory and motor center. The antennal lobe glomeruli displayed a moderate density of immunoreactive fibers.

With regard to the subesophageal ganglion, numerous CCK-IR somata were found close to the root of the mandibular nerve; others were present in the soma rind of the remaining neuromeres.

CCK-IR perikarya were present in both thoracic ganglia, with the abdominal neuromeres containing the highest number of positive somata. The neuropils of both ganglia showed moderate densities of immunopositive processes.

The distribution of CCK-LI in somata and neuropils of central nervous system of *T. infestans* is widespread suggesting that a CCK-like peptide may act mainly as a neuromodulator in the integration of information from distinct sensory receptors.

Keywords: Insect; CCK; Immunocytochemistry; Insect central nervous system; Chagas' disease

1. Introduction

The subfamily *Triatominae* (Heteroptera: Reduviidae) includes several species with the capacity of vectorizing the parasite *Trypanosoma cruzi*, the causative agent of Chagas' disease. The overall prevalence of this infection is estimated at 16–18 million cases, and about 120 million people representing 25% of the inhabitants of this region, are at risk of contracting the disease (WHO, 2005). *Triatoma infestans* is the main vector of the parasite in southern-cone countries. Transmission of the causative agent by this insect vector occurs when the parasites are deposited above the skin of the host, a fact which happens soon after feeding. The identification of factors and mechanisms involved in the control of host-finding and feeding behaviors in *T. infestans* and related species may provide a basis for the design of new drugs to combat these vectors. Moreover, blocking or diminishing the amount of food intake may alter the growth of the vector populations as it might hinder molting, egg formation and egg laying (Regis, 1979).

The presence of CCK-like immunoreactive (LI) material has been reported in several insect species (El-Salhy et al., 1980, 1983; Duve and Thorpe, 1981; Veenstra et al., 1985; Tamarelle et al., 1988). Distribution studies of neurotrasmitters and neuropeptides in the central nervous system (CNS) of *T. infestans* have revealed the colocalization of neuroactive molecules with CCK-LI (Villar et al., 1994; Settembrini et al., 2003). Thus, using high-sensitivity immunocytochem-

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istry, we have demonstrated the colocalization of CCK-LI with nitric oxide synthase-, NPY- and NPYY1-LIs in distinct cell bodies of the CNS (Villar et al., 1994; Settembrini et al., 2003). Some of these immunostained perikarya were located in regions of the CNS which are known to innervate organs and tissues involved in feeding and defecation as the salivary glands, the mouthparts and the abdominal muscles (Insausti, 1994). However, the distribution and functional role of CCK-like peptides in triatomines is still unknown. Here, we describe the distribution pattern of CCK-LI in perikarya and neuropils of the brain and ganglia of *T. infestans*. The widespread distribution of CCK-LI in the brain and ganglia of this species suggests that a CCK-like peptide may act as a neuromodulator in integrative functions or have a neurohormonal role in reproductive and excretory processes.

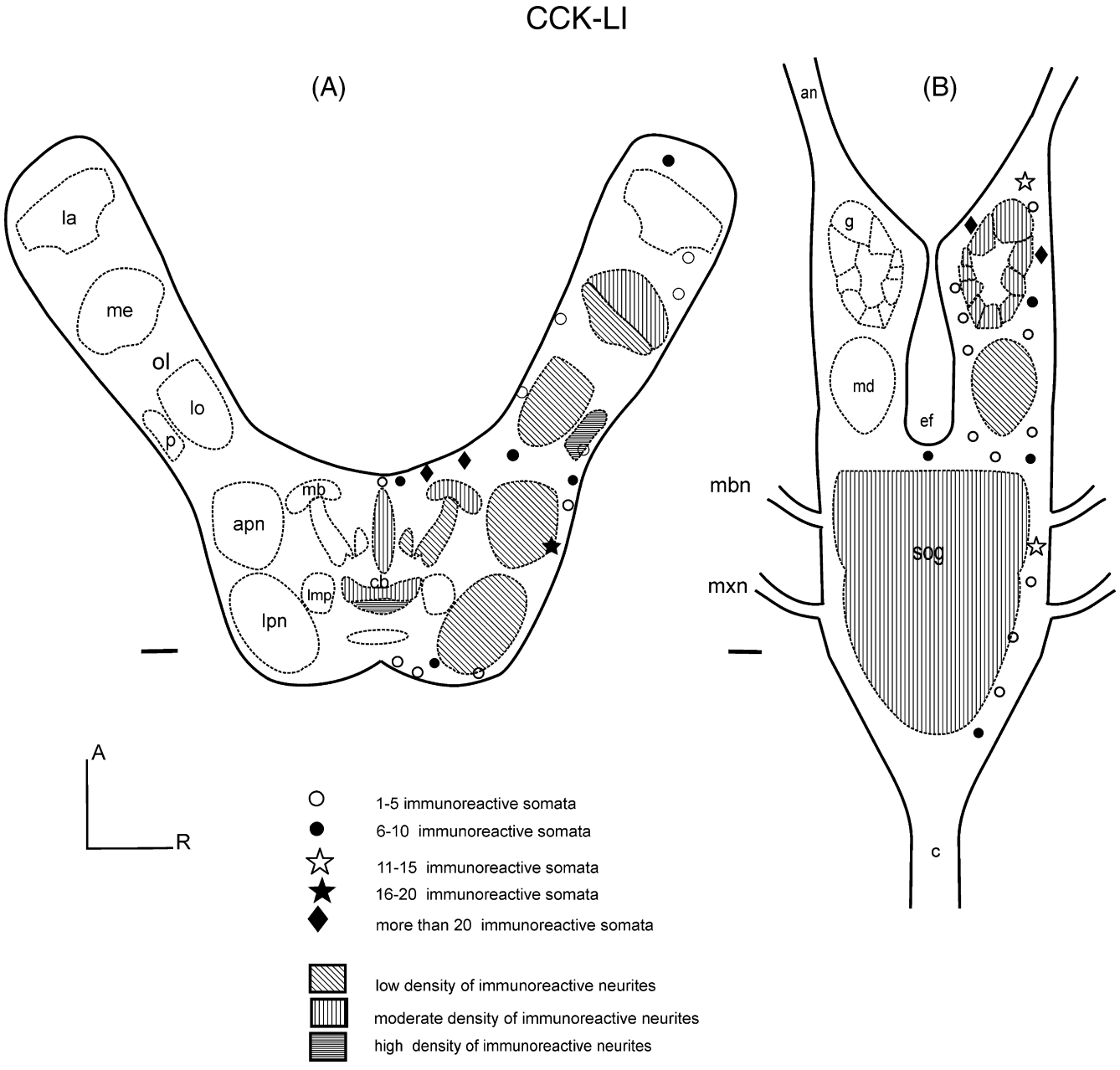
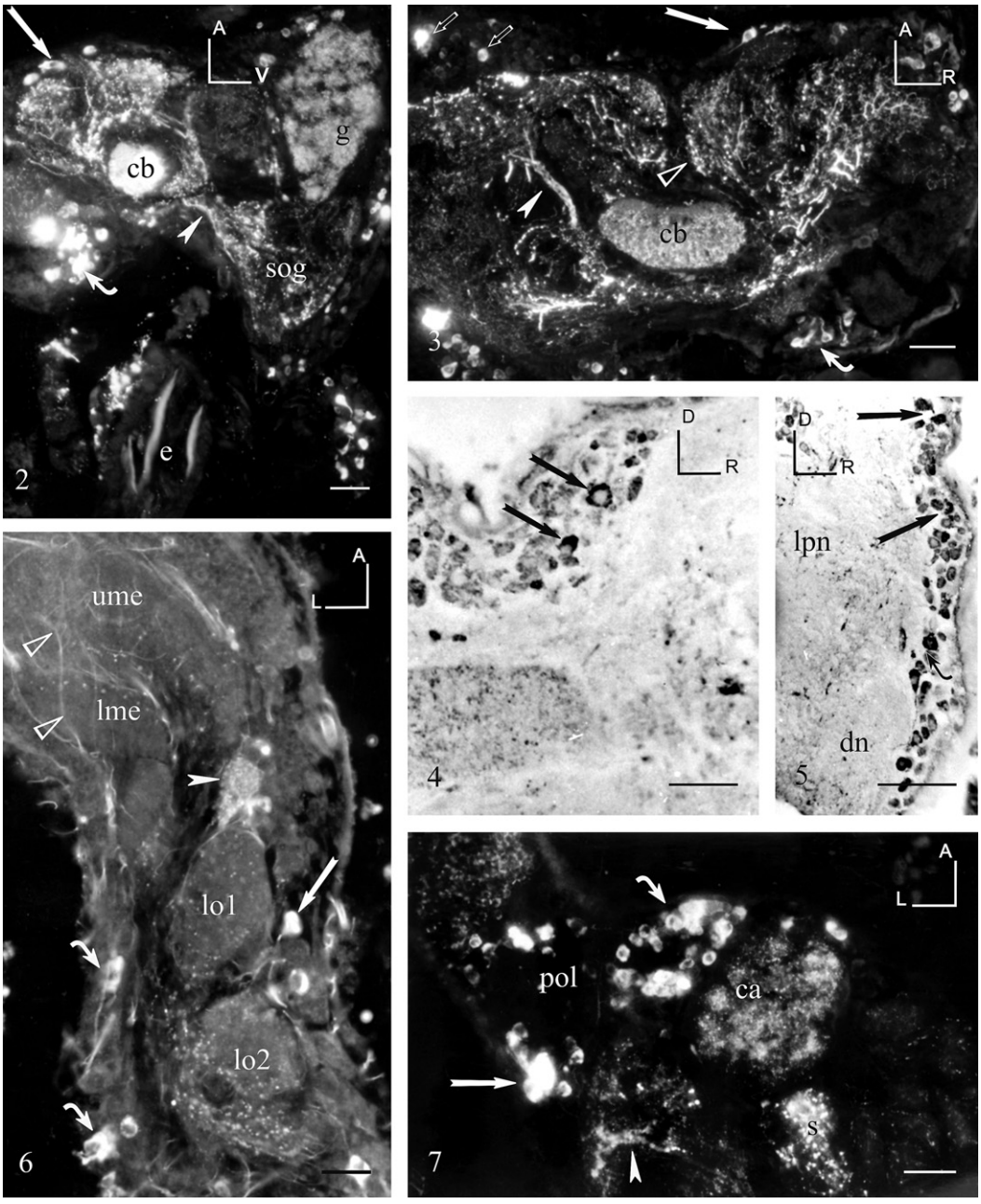
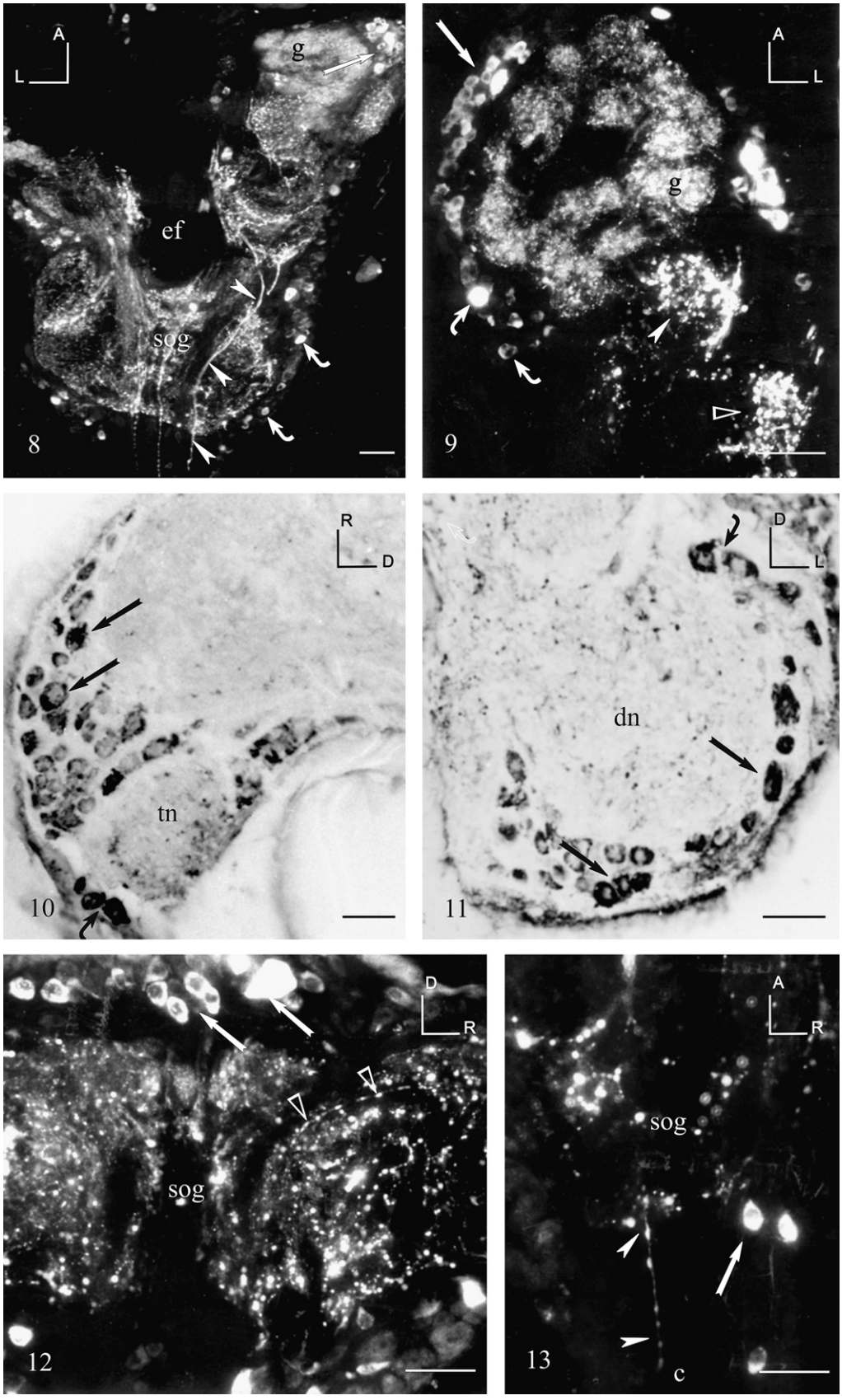


Fig. 1. Schematic drawings of CCK-LI in the protocerebrum and optic lobes (A), deutocerebrum (B, upper part) and subesophageal ganglion (lower part of B). Symbols represent the number of immunoreactive somata whereas shadowings indicate density of immunolabeled processes in neuropils. Abbreviations: an, antennal nerve; apn, anterolateral protocerebral neuropil; c, connectives; cb, central body; ef, esophageal foramen; g, antennal lobe glomeruli; la, lamina ganglionaris; lmp, lateromedial protocerebal neuropil; lo, lobula complex; lpn, lateral protocerebral neuropil; mb, mushroom body; me, medulla; md, mechanosensory and motor deutocerebrum; mdn-mxn, mandibular and maxillary nerve roots; ol, optic lobe; p, lobula plate; sog, subesophageal ganglion. Bars = 100 μm.



Figs. 2–7. Immunofluorescence (2, 3, 6, 7) and bright field (4, 5) micrographs of the protocerebrum (PC) and the optic lobe (OL). (2) Tangential section of the PC plus the antennal lobe glomeruli (g) and the subesophageal ganglion (SOG). Straight arrow points to CCK-IR perikarya of the anterior soma rind and a curved arrow marks somata of the posterior soma rind; arrowhead points to fibers connecting the PC with the SOG (sog). cb, central body; e, foregut. (3) Horizontal section of the PC showing the central body (cb) intensively stained. Open arrows point to CCK-IR somata of the anterior soma rind while a filled arrow points to an immnunolabed perikaryon located close to the median furrow. Note stained fibers close to the median furrow (open arrowhead). A curved arrow marks positive cell bodies of the posterior PC slope while a filled arrowhead points to thick labeled processes. (4) Frontal section showing immunolabeled cell bodies in the vicinity of the median furrow (straight arrows). (5) Frontal section of the lateral soma rind showing CCK-IR somata (straight arrows); a curved arrow points to cells in the protocerebral-deutocerebral crest. lpn, lateral PC neuropil; dn, deutocerebral neuropil. (6) Horizontal section of the OL showing immunostained somata of the medial (straight arrow) and lateral (curved arrows) cell-body layers. Open arrowheads mark fibers connecting the upper (ume) to the lower (lme) divisions of the medulla neuropil. A filled arrowhead points to a region with a high density of immunostained fibers. lo1 and lo2 are divisions of the lobula neuropil. (7) Horizontal section of the PC and proximal OL (pol). The straight arrow points to a cluster of cell bodies in the lateral cell-body layer while the curved one marks a group of immunopositive somata above the calycal neuropil (ca). CCK-IR processes are also visible in the lateral PC neuropil (arrowhead) and in the pedunculus (s) of the mushroom bodies. Abbreviations in orientation lines: A, anterior; D, dorsal; R, right; V, ventral. Bars = 50 μm.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2. Materials and methods

2.1. Insects

Adult male *T. infestans,* free of *T. cruzi* and *Blastocrithidia triatomae*, were used in the experiments. These insects, originated from bugs provided by the Center for the Control of Chagas' disease (Santa María, Córdoba, Argentina), were maintained under controlled conditions of light (L:D = 12:12, lights on at 6 a.m.), temperature (27 ± 1 °C) and relative humidity (70–80%). They were fed on the shaved thorax of chicken every 15 days, during 1 h and under dim light (Settembrini, 1984). Experiments were carried out 1 week after their last feeding.

2.2. Immunocytochemistry

Eighty insects were processed for immunohistochemistry according to Settembrini and Villar (1999, 2004). In brief, bugs were cold-anesthetized and after that, their legs were secured to a wax lamina. The dorsal cuticle of the head was removed and the tissues were immediately flushed with cold fixative, a mixture of formalin and picric acid (4% paraformaldehyde and 0.4% picric acid) in 0.16 M sodium phosphate buffer (PB), pH 6.9. Thoracic tergites were quickly dissected out, and soft tissues were also flushed with cold fixative. Dissection of brain and ganglia out of remaining tissues proceeded with the tissues bathed in cold fixative. Nervous tissue remained in the fixative overnight at 4 °C. After that, they were transferred to 0.01 M PB saline (PBS, pH 7.4) containing 15% sucrose, 0.02% bacitracin (Sigma, St. Louis, MO, USA) and 0.01% sodium azide (Merck, Darmstadt, Germany) for at least 48 h. Frontal and horizontal 18 μm serial sections were obtained with a cryostat (Microm, Waldorf, Germany) and processed either for indirect immunofluorescence histochemistry or following the avidin–biotin peroxidase protocol. Sections were incubated with mouse monoclonal antibodies directed against the N-terminal portion CCK-8 (MAB2D4.19 from J. Walsh, University of California, Los Angeles) for 24 h at 4 °C.

For indirect immunofluorescence histochemistry the sections were incubated with mouse monoclonal CCK-8 antiserum diluted 1:400, rinsed in 0.01 M PBS and further incubated with lissamine rhodamine B sulfonyl chloride (LRSC)-conjugated goat anti-mouse (1:80, Jackson ImmunoResearch Laboratories, PA, USA) secondary antibodies for 30 min at 37 °C. After that, the sections were rinsed in PBS, mounted in a mixture of glycerol and PBS (3:1) containing *p*-phenylenediamine and examined in a Nikon Eclipse 800 epifluorescence microscope (Nikon, Tokyo, Japan) equipped with filter for LRSC-induced fluorescence. Kodak TriX black and white film was used for photography.

For the ABC method (Hsu et al., 1981), the sections were incubated with mouse monoclonal CCK-8 antiserum diluted 1:2000, rinsed in PBS, incubated at room temperature for 30 min in biotinylated goat anti-mouse secondary antibodies (1:100, Vector Laboratories, Burlingame, CA, USA), rinsed again in PBS and further incubated for 1 h in the ABC reagent (Vectastain Elite kit, Vector Laboratories). Peroxidase activity was revealed by reaction with 3,3′-diaminobenzidine tetrahydrochloride (Sigma) using glucose oxidase (Sigma) and nickel salts for enhancement of the reaction product (Shu et al., 1988). The sections were mounted with permount (Fluka, Buchs, Switzerland) and photographed with Agfapan APX 25 (Agfa Gevaert AG, Leverkusen, Germany).

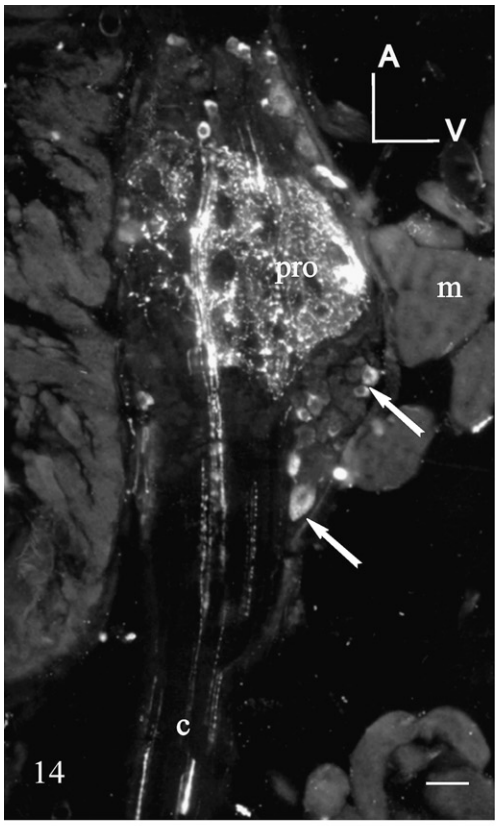
2.3. Controls

The specificity of the CCK-8 antibodies was tested by overnight preincubation of the antiserum with CCK-8 (Peninsula Laboratories, CA) at 10^{-6} M in the diluted antiserum. After preabsorption of the antiserum with the peptide, the sections were incubated with the preabsorbed antiserum and processed following the protocols stated above. Other controls were performed by incubating the sections without the first or the second antibodies. No immunostaining was seen.

3. Results

The central nervous system of *T. infestans* is composed by the brain and the subesophageal ganglion, both located within the cephalic capsule, and also by the prothoracic ganglion and the posterior ganglion which are situated in the thorax. The brain includes the optic lobe and the protocerebrum, deutocerebrum and tritocerebrum.

Figs. 8–13. Immunofluorescence (8, 9, 12, 13) and bright field (10, 11) micrographs of the deutocerebrum (DC; 8–11), tritocerebrum (TC), and subesophageal ganglion (SOG; 8, 12–13). (8) Horizontal section of the DC showing also part of the SOG. CCK-IR cell bodies in the lateral soma rind (straight arrow) around intensively stained antennal lobe glomeruli (g). Immunopositive fibers from the posterior DC run laterally reaching the cephalic connective (arrowheads), while in the center of the SOG (sog) beaded processes also run towards the connectives. Curved arrows mark immunostained cell bodies of SOG; ef, esophageal foramen. (9) The antennal lobe glomeruli (g) exhibit CCK-LI except for the central core. CCK-IR perikarya are present in the anterior (straight arrow) and medial soma rinds (curved arrows). Patches of CCK-IR fibers posterior to the glomeruli are marked by arrowheads. (10) Frontal section showing CCK-IR somata of the DC (straight arrows) and TC (curved arrow); tn, tritocerebral neuropil. (11) Frontal section showing immunopositive somata in the protocerebral-deutocerebral crest (curved arrow) and around the neuropil of the antennal mechanosensory and motor DC (straight arrows); dn, deutocerebral neuropil. (12) Frontal section of the SOG (sog). Arrows point to immunostained somata of the dorsal cell-body layer and open arrowheads mark beaded fibers of the neuropil. (13) Horizontal section of the caudal SOG (sog) showing immunostained somata (straight arrow) and beaded processes (arrowheads). c, cephalic connectives. Abbreviations in orientation lines: A, anterior; D, dorsal; R, right; V, ventral. Bars in 7, 8, 9, 12 and 13 = 50 μm; bars in 10 and 11 = 25 μm.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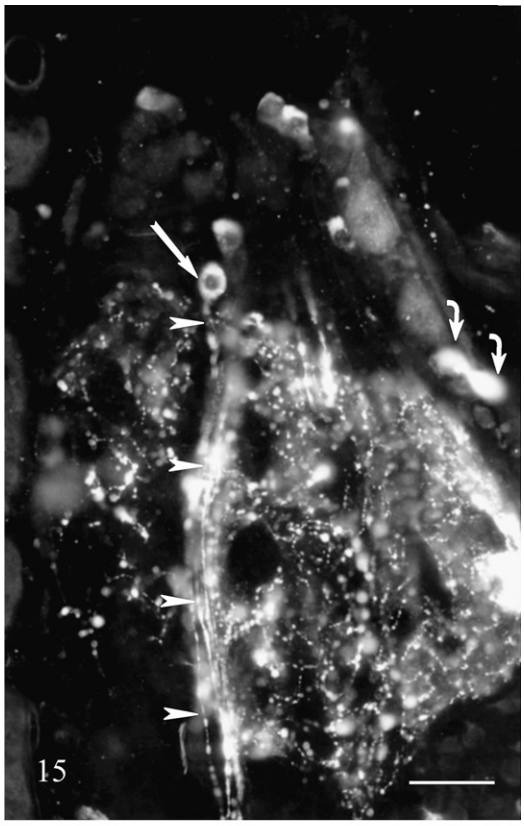
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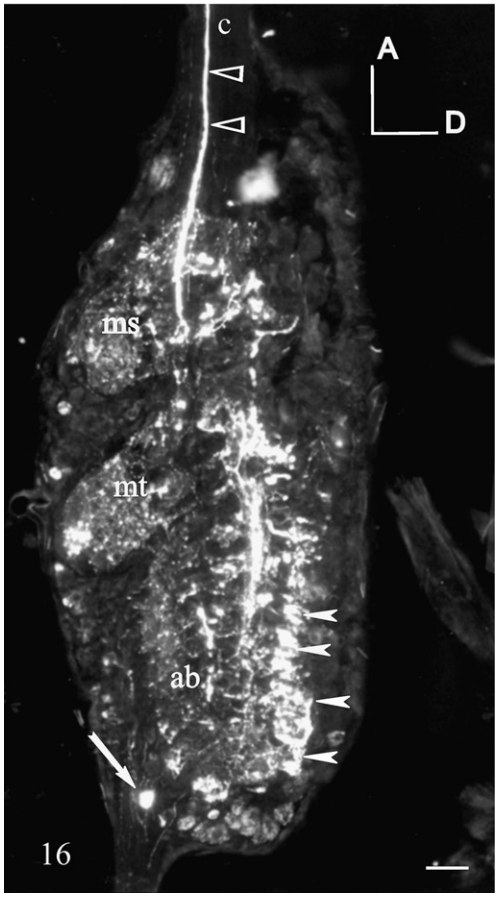
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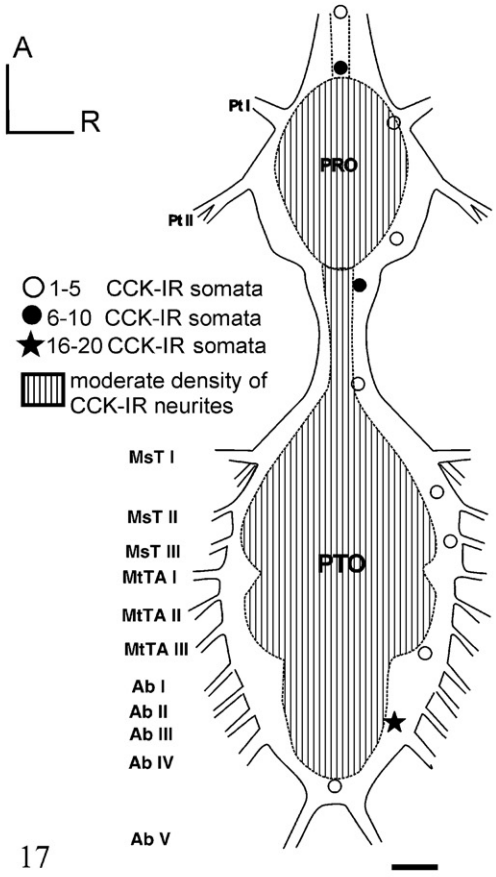
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3.1. Brain

3.1.1. Immunoreactive cell bodies

The distribution pattern of CCK-immunoreactive (IR) cell bodies in the protocerebrum, deutocerebrum and tritocerebrum is depicted in Fig. 1A and B (upper part). In the protocerebrum, immunolabeled somata were usually found forming clusters. They were located in the anterior, lateral and posterior cell-body layers (Figs. 1A and 2). In the anterior soma rind several immunoreactive somata were seen (Figs.1A and 3); large-sized (16–20 μm) CCK-IR perikarya were observed close to the median furrow between the protocerebral hemispheres (Figs. 1A and 4), while immunostained cell bodies were found more laterally to these perikarya (Fig. 3). CCK-IR perikarya were also detected in the lateral soma layer (Figs. 1A and 5). The posterior slope of the protocerebrum housed four clusters of immunoreactive perikarya either close the median furrow or in posteromedial position (Figs. 1A, 2 and 3). A cell-body cluster formed by medium-sized (11–15 μm) immunolabeled somata was observed above the mushroom body calyces (Figs. 1A and 7).

In the optic lobe, scattered immunopositive somata were detected at the level of the first optic chiasma and around the neuropil of the lamina ganglionaris (Fig. 1A). Other cell-body groups were seen in the medial soma rind at the level of the second chiasma, whereas CCK-IR somata of the lobula were present in both the lateral and the medial soma rinds (Fig. 6). Numerous CCK-IR perikarya of various sizes (9–25 μm) were found at the boundary of the protocerebrum with the optic lobe, an area which will be termed as the proximal optic lobe (Fig. 7).

With regard to the sensory deutocerebrum, CCK-IR somata were observed mostly in the anterior and lateral soma rinds (Fig. 1B upper part, 8–9). Immunostained somata of the lateral layer showed size uniformity (about 13 μm). CCK-IR perikarya were also detected surrounding the neuropil of the antennal mechanosensory and motor center (Fig. 1B upper part, 8, 11) whereas scattered cell-body profiles were observed in the tritocerebrum (Fig. 10) and around the esophageal foramen (Fig. 1B).

3.1.2. Immunoreactive fibers

The distribution of CCK-IR fibers in the brain is represented in Fig. 1. Thus, the anterolateral and antero-medial protocerebral neuropil contained immunopositive fibers (Figs. 1A and 3). Thick CCK-IR fibers formed a crescent-shaped area at the anterior and lateral borders of the anterolateral neuropil; tiny processes were seen emerging from this fiber tract. The central body (Fig. 3) contained immunopositive neurites, with the lower division of this neuropil showing a higher degree of immunostaining than the upper part. Immunostained fibers were seen entering the upper division of the central body. The mushroom body calyces (Fig. 7) displayed a patchy pattern of immunostaining. Beaded positive processes were present in the stalk and in the α, β and γ lobes. The inner antenno-cerebral tract contained CCK-IR neurites; the fibers which ran close to the central body complex sent branches to the calycal neuropil. The ventromedial neuropil and the posterior protocerebral commissure were also stained (Fig. 3).

In the optic lobe, stained processes were detected in the medulla and lobula neuropils (Figs. 1A and 6). The outer medulla neuropil showed higher immunostaining than the inner medulla. The serpentine layer, located between both divisions was also stained. Within the lobula neuropil, the lobula plate showed the highest degree of immunostaining. A varicose fiber was observed emerging from the lobula plate, one of its branches ascended towards the medulla neuropil. The descending branch was observed running towards the protocerebrum and entering the anterior protocerebral neuropil at approximately its medial part.

With regard to the antennal lobe glomeruli (Figs. 2, 8 and 9), the basal portion of some glomeruli exhibited a higher density of immunoreactive fibers than the medial and inner areas. The antennal mechanosensory and motor center and the tritocerebrum contained few varicose stained processes (Figs. 8 and 11). Fibers emerging from the posterior antennal mechanosensory and motor center were observed travelling across the subesophageal ganglion towards the cephalic connectives (Fig. 3).

3.2. Subesophageal ganglion

CCK-IR perikarya of varied sizes were observed in the subesophageal ganglion (Fig. 12). They were located in the anterior part of the ganglion, close to the esophageal foramen and in the lateral soma rind of all the neuromeres, with the mandibular neuromere containing the highest number of immunostained perikarya (Fig. 1B, lower part).

Figs. 14–17. Immunofluorescence micrographs (14–16), and schematic representation (17) of CCK-LI in the thoracic ganglia. (14) Tangential section of the prothoracic ganglion (pro) showing immunostained somata (arrows) of the posterior soma layer; c, connectives to the posterior ganglion; m, muscle. (15) Higher magnification of Fig. 14. Note a CCK-IR perikaryon (straight arrow) sending a process into a fiber tract (arrowheads). Curved arrows mark immunopositive somata. (16) Tangential section of the posterior ganglion showing a immunostained soma around the abdominal neuropil (ab). Open arrowheads mark the course of immunopositive fibers in the posterior connectives (c) which end in the mesothoracic neuropil (ms). Filled arrowheads point to patches of CCK-LI in a dorsal area of the abdominal neuropil; mt, metathoracic neuropil. (17) CCK-LI in the thoracic ganglia. Open circles, filled circles and star indicate 1–5, 6–10 and 16–20 immunoreactive somata, respectively, whereas shadowing in neuropils indicate density of immunolabeled processes. Abbreviations: ab I–V, abdominal nerves I–V; ms I–III, mesothoracic nerves I–III; mt I–III, metathoracic nerves I–III; pro, prothoracic ganglion; pt I–II, prothoracic nerves I–II; pto, posterior ganglion. Abbreviations in orientation lines: A, anterior; D, dorsal; R, right; V, ventral. Bars in 14–16 = 50 μm, bar in 17 = 100 μm.

Varicose immunolabeled neurites were seen running medially within this ganglion and in the cephalic connectives (Figs. 8 and 13). Immunolabeled processes were also seen around the esophageal foramen (Fig. 8).

3.3. Thoracic ganglia

The distribution of CCK-IR cell bodies in the prothoracic and posterior ganglia is portrayed in Fig. 17. In the prothoracic ganglion, immunopositive somata were observed in the anteriormedial soma rind (Figs. 14 and 15); others were present in the lateral and posterior soma rinds (Fig. 17). A fiber tract formed by varicose positive neurites was observed running medially from the anterior part of the ganglion to the posterior connectives (Fig. 14). Connections between this tract and the ventral neuropil exhibited a peculiar arrangement (Fig. 14).

The abdominal neuromeres contained the highest number of immunostained perikarya (Fig. 17). Cell-body clusters were found close to the roots of mesothoracic nerves II and III, metathoracic nerve III and abdominal nerves IV and V. The neuropil of this ganglion was traversed by thick CCK-IR processes. A patchy pattern in the immunostaining was visible in the abdominal neuromeres of this ganglion (Fig. 16). Immunostained neurites were observed within the abdominal nerve trunks.

3.4. Controls

Control incubations of the sections in which the antiserum had been preabsorbed with CCK-8 resulted in the absence of immunostaining. No immunostaining was seen in the sections when the first or the second antibodies were omitted.

4. Discussion

Using high-sensitivity immunocytochemistry and monoclonal antibodies against CCK-8, we have shown that the distribution pattern of CCK-LI in cell bodies and neuropils of *T. infestans* CNS is widespread. The specificity of these antibodies has been already tested in mammals (Hökfelt et al., 1988) and in colocalization experiments performed with this insect species (Villar et al., 1994; Settembrini et al., 2003).

Although different antisera have been used in distribution studies of CCK-LI, it appears that some staining patterns are conserved among the insect species tested so far. Differences in the number and shape of the immunoreactive somata located in the pars intercerebralis and the pars lateralis of *T. infestans* arise when compared with previous reports in *Calliphora erythrocephala* (Duve and Thorpe, 1981), *Blatella germanica* (Tamarelle et al., 1990) and *Manduca sexta* (Homberg et al., 1991). Discrepancies may be due to differences in the antisera and/or the sensitivity of the immunocytochemistry protocols employed in the aforementioned studies. It is also possible that they may represent true species-specific differences, as these species belong to separate orders. The projections of immunoreactive somata located close to the median furrow could not be traced within the median bundle. However, the presence of positive neurites in the median bundle, the corpora cardiaca-corpora allata complex and above the cephalic aorta suggests that these perikarya might be of the neurosecretory type like the gastrin/CCK-IR IIa perikarya of *M. sexta* (Homberg et al., 1991). Thus, a CCK-8-like peptide might be acting as a neurohormone in these triatomine insects. In this regard, detection of this molecule in the hemolymph may be helpful to assign a neurohormonal function to the peptide in this pathway.

The optic lobe of *T. infestans* housed CCK-IR somata around the three main neuropil regions. Immunolabeled neurites were detected except for the unstained lamina ganglionaris neuropil. These results are in line with previous reports in insects (Duve and Thorpe, 1981; Andries et al., 1991; Tamarelle et al., 1990). Immunopositive neurites were observed in both the inner and the outer divisions of the optic medulla. Regularly-spaced varicosities were seen in the vicinity of the serpentine layer. A layered organization of the immunoreactive fibers like those reported for *Aeschna cyanea* was not evident in *T. infestans*. The observation of projections from immunostained optic lobe somata within the optic lobe or to the protocerebrum suggests the possibility of a neurotrasmitter role in the integration of visual information.

Triatomine insects are highly dependent on olfactory cues for the location of the appropriate blood source (Guerenstein and Guerin, 2001). The antennal nerve of *T. infestans* was unstained but the sensory deutocerebrum housed numerous immunoreactive somata and fibers. Seven clusters of immunolabeled perikarya were observed; three of them contained numerous cell bodies. This contrasts with previous reports in which only a few stained cells were found (Duve and Thorpe, 1981; Tamarelle et al., 1990). Double labeling experiments demonstrated the coexistence of CCK-LI with other immunoreactivities in this species. CCK-IR somata of the lateral soma rind outnumbered those expressing NOS- (Villar et al., 1994) and NPY-LIs. The antennal lobe glomeruli immunostaining may be originated from local somata of the lateral and medial cell-body clusters or from centrifugal neurons (Homberg et al., 1989; Ignell, 2001). The basal portion of some glomeruli showed a high density of stained processes suggesting that projection neurons also contribute to the immunostaining. These findings suggest that a CCK-8-like peptide may also have a neurotrasmitter role in the processing of olfactory information.

T. infestans nerves from the subesophageal ganglion innervate the mandibular and maxillary stylets, associated muscles as well as the salivary glands (Insausti, 1994). During the feeding process all these structures need to act in coordination. CCK-LI was present in cell bodies and fibers of the subesophageal ganglion with the most numerous group of immunoreactive somata located close to the roots of the maxillary nerve. However, no immunoreactive fibers were

observed within the root of this nerve or in the mandibular or labial nerve trunks. On the other hand, the subesophageal ganglion receives fibers from first-order ocellar interneurons (Insausti and Lazzari, 1996) and from cephalic mechanoreceptors (Insausti and Lazzari, 2000). It is possible that CCK-IR elements of the subesophageal ganglion might be related to the integration of information from various sensory modalities originated in head structures.

CCK-IR somata were observed in the thoraco-abdominal ganglia of the flies *Calliphora vomitaria* and *Drosophila melanogaster* (Lundquist and Nässel, 1990). These authors report that immunostained cell bodies did not display stained neurites. In *T. infestans*, the prothoracic and posterior ganglia contained immunoreactive perikarya and neurites. Varicose CCK-IR processes were observed in abdominal nerve trunks. Abdominal nerves send branches to the reproductive organs and to the rectal sac as well as to other abdominal structures (Insausti, 1994). These observations raise the possibility of a neurohormonal role for CCK-IR elements in reproductive and excretory functions.

In this study we have used heterologous monoclonal antibodies against rat CCK-8. Vertebrate CCK is closely related to the sulfakinin family of peptides (Hewes and Taghert, 2001; Maestro et al., 2001; Vanden Broeck, 2001; Homberg, 2002; Nässel, 2002). Sulfakininins share a sulphated tyrosine and a Gly-His-Met-Arg-Phe-amide C-terminus. Differences in the amino-acid sequence among CCK-8 and insect sulfakinins reported so far, are present particularly at the N-terminus, a region to which the monoclonal antibodies used in this study were raised. These differences do not suggest that the immunostaining pattern here described might be due to sulfakinins only. In this regard, it should be noted that data from RIA, chromatographic, immunohistochemical and in situ hybridization experiments suggested the existence of gastrin/CCK material in insects (Duve et al., 1995). However, the possibility that the distribution pattern of CCK-LI here reported might include also some immunoreactivity to unknown *T. infestans* sulfakinin-related peptides cannot be disregarded.

In conclusion, these results show a widespread distribution of CCK-LI in *T. infestans* CNS. CCK-IR somata arborize profusely in most neuropil regions of the brain suggesting that a CCK-8 like peptide might be involved mainly in the processing of sensory inputs from visual, chemosensory and mechanosensory receptors.

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