Eur. J. Entomol. **96**: 279–285, 1999
ISSN 1210-5759

Immunolocalization of two types of allatostatins in the central nervous system of the cricket *Gryllus bimaculatus* (Ensifera: Gryllidae)*

Günther WITEK[1], Peter VERHAERT[2], Matthias W. LORENZ[1] and Klaus H. HOFFMANN[1]**

[1]Tierökologie I, Universität Bayreuth, D-95440 Bayreuth, Germany
[2]Zoölogisch Instituut van de Universiteit, Naamsestraat 59, B-3000 Leuven, Belgium

Key words. Allatostatins, anti-peptide antibody, immunocytochemistry, brain/retrocerebral complex, Gryllidae *Gryllus bimaculatus*

Abstract. Two anti-peptide antisera (anti-A and anti-B) raised against *Gryllus bimaculatus* allatostatin A1 (Grb-AST A1) and B1 (Grb-AST B1), respectively, were applied in the peroxidase-antiperoxidase (PAP) immunohistochemical technique on complete series of sections from the brain, suboesophageal ganglion, corpora cardiaca (CC), and corpora allata (CA) of *G. bimaculatus.* Both antisera yielded intense staining of numerous cells and nerve fibres. Serial sections, alternately stained with anti-A and anti-B confirmed that the anti-B generally stained more cells and nerves. Extensive immunoreactivity in the retrocerebral complex suggests that the CC and CA may represent a storage and/or release site for both allatostatin types or allatostatin-like molecules produced in the brain.

INTRODUCTION

Cricket corpora allata (CA) synthesize juvenile hormone III (JH III), which plays a major role in regulating vitellogenesis (Koch & Hoffmann, 1985; Hoffmann & Sorge, 1996). JH biosynthesis in the insect CA may be controlled by stimulatory and inhibitory signals which reach the glands either via the haemolymph or via nervous connections (Goodman, 1990). Brain neuropeptides that either stimulate (allatotropin) or inhibit (allatostatin) JH biosynthesis have been described in a few insect species (for review see Stay et al., 1994; Gäde et al., 1997). Grb-AST A1 belongs to the so-called *Diploptera* (cockroach) A-allatostatin family (allatostatin superfamily) having a Y/FXFGL/I-amide C-terminus whereas Grb-AST B1 belongs to the B-allatostatin family (W^2W^9 peptides) with sequence homology to the myoinhibiting peptides from *Locusta migratoria* (Lom-MIP) and *Manduca sexta* (Mas-MIP I, II). So far, five A-allatostatins (Grb-AST A1–A5) and five B-allatostatins (Grb-AST B1–B5) have been isolated from 3-day old female *G. bimaculatus* brains (Lorenz et al., 1995a, b; Lorenz et al., 1999; Hoffmann et al., 1998). All these peptides are potent inhibitors of cricket JH III biosynthesis in vitro. The inhibitory effect is fully reversible. IC_{50} values for the inhibitory action range from 4 to 8 × 10^{-9} M for the A-peptides and from 1 to 7 × 10^{-8} M for the B-peptides (Lorenz et al., 1995a, b). Whole mounts of *G. bimaculatus* brains were previously immunostained with an antibody raised against *Diploptera* allatostatin 7 (Dip-AST 7, APSGAQRLYG- FGL-NH_2) by Neuhäuser et al. (1994). Dip-AST 7 is also a potent inhibitor of *G. bimaculatus* JH biosynthesis in vitro. The present paper describes the immunostaining of sections of the *G. bimaculatus* brain/retrocerebral complex with antibodies raised against Grb-AST A1 and Grb-AST B1 by using the peroxidase-antiperoxidase immunocytochemical technique.

MATERIAL AND METHODS

Animals and tissue sectioning

Mediterranean field crickets, *G. bimaculatus* de Geer (Ensifera: Gryllidae), were reared at 30°C under a natural, late spring photoperiod of 16L : 8D. Animals were fed with water and a mixed standard diet (Altromin GmbH, Lage, Germany) consisting of rabbit (2021), rat/mouse (1311) and cat (5031) food powder. Males and females were separated during the penultimate larval stage and those that underwent an imaginal moult on the same day (day 0) were grouped.

Complete brain-corpora cardiaca (CC) – corpora allata (CA) – suboesophageal ganglion (SOG) complexes of 3d adult females were dissected under 0.9% saline and immediately transferred to Bouin Hollande 10% sublimate fixative for 24 h. After dehydration in ethanol and clearing in histosol, tissues were embedded in paraplast and thereafter cut with a Historange microtome (Pharmacia/LKB, Uppsala, Sweden) in 5 µm sections. This procedure for tissue preparation has previously been successfully used for immunocytochemical investigations of peptidergic neurons in the insect brain (De Loof et al.,1984; Verhaert et al., 1984 a, b).

Antisera

Two polyclonal antisera (anti-A and anti-B) were raised in rabbits by SNPE Neosystem, Strasbourg, France. Immunization was done with Grb-AST A1 (AQHQYSFGL-NH_2) and Grb-AST B1 (GWQDLNGGW-NH_2) respectively, aminoterminally coupled to keyhole limpet haemocyanine (KLH) via glutaraldehyde. Both synthetic peptides were also supplied by SNPE Neosystem.

Sensitivity and selectivity of the two anti-peptide antisera were tested by indirect ELISA, although immunoreactivity in

---

* This paper is based on a lecture presented at the 6th European Congress of Entomology held in České Budějovice, Czech Republic, August 1998.

** Corresponding author; e-mail: klaus.hoffmann@uni-bayreuth.de.

ELISA may be different from that in immunocytochemistry. The anti-A was found not to cross-react with Grb-AST B1 nor did the anti-B cross-react with Grb-AST A1. However, anti-B cross-reacted with Lom-MIP (AWQDLNAGW-NH_2) for 7% and with *G. bimaculatus* adipokinetic hormone (Grb-AKH, pQVNFSTGW-NH_2) for 1% (due to some obvious C-terminal sequence homologies). Anti-A cross-reacted with Dip-AST 7 and Grb-AST A2 (AGGRQYGFGL-NH_2) at 60% and 13% levels, respectively, compared to the autologous immunoreaction of anti-A towards Grb-AST A1. Anti-A did not show any cross-reaction with Grb-AKH in ELISA.

The rabbit antisera were used in the peroxidase-antiperoxidase (PAP) procedure as described by Vandesande & Dierickx (1976) at dilutions between 1 : 2,000 and 1 : 10,000. Specificity of the anti-A and anti-B was checked by liquid phase as well as by solid phase preabsorption. For liquid phase preabsorption, 1 µg peptide per µl undiluted serum was preincubated for 2 h (serum dilution 1 : 100) before subjecting it to a normal PAP staining on sections of *G. bimaculatus* nervous tissue. The anti-B serum appeared to be specific for its antigen as there was only negligible staining left after the preabsorption experiment. In the case of the anti-A serum, however, preabsorption studies indicated the presence of some unspecific immunostaining as there were still a few cells and nerve fibres stained after preabsorption. To confirm these results, liquid preabsorption was repeated with 10 µg peptide per µl undiluted serum for both antisera. Also a solid phase preabsorption was performed on anti-A and anti-B. For this aim, 500 µg of peptide was covalently coupled to 100 mg Sepharose 4B (Pharmacia). After incubation of 50 µl 1 : 100 diluted antiserum with the Sepharose-bound peptide, antigen-specific antibodies were removed from the serum by spinning down the Sepharose beads. The supernatant was then used in the appropriate dilution as the primary antiserum in a normal PAP staining. In the case of anti-B, this procedure led to the complete absence of immunostaining, whereas for the anti-A the presence of some unspecific antibodies was still obvious. Finally, antiserum specificity was controlled by replacement of the primary antisera by the respective rabbit preimmune sera. This treatment resulted in a complete absence of immunostaining for both antisera. Complete series of sections from the brain, SOG, CC and CA were used for identification of total nerve and cell staining. Some serial sections were alternately stained with the two antisera.

RESULTS

Retrocerebral complex

CC-CA complexes of 3-day old *G. bimaculatus* females showed intense nerve staining with both antisera, anti-A (Fig. 1) and anti-B (Fig. 2). Most pronounced staining was observed in the CC, the nerve connections between CC and CA (nervus corpus allatum 1, NCA 1) and between CA and SOG (NCA 2) as well as in the connection between NCA 1 and NCA 2 running on the surface of the CA (Fig. 3). Inside the CA, nerve staining was weaker. CC staining with anti-B showed, in addition to the normal nerve staining, some intensely stained big round shaped areas which were not visible in CC stained with anti-A (compare Fig. 1 with 2). No cell staining could be detected inside CC and CA.

Specificity tests showed that the anti-B staining was highly specific (not shown here), whereas with the anti-A only the staining inside the CA vanished after preabsorption studies (compare Fig. 4 with Fig. 5).

Suboesophageal ganglion: Anti-A

In the middle of the SOG, a group of 6 large cells were strongly immunolabelled (Fig. 6). Another four large cells were immunopositive in the median, cranial SOG between the entrance of the circumoesophageal connectives (not seen in this section). However, the staining of these large cells did not disappear when sections were exposed to antibody preabsorbed with Grb-AST A1 (see Figs 14–15). Neuropil staining occurred inside the SOG, inside the circumoesophageal connectives and also in the connectives running to the abdominal ganglia. Not all these neuropil stainings disappeared after preabsorption.

Suboesophageal ganglion: Anti-B

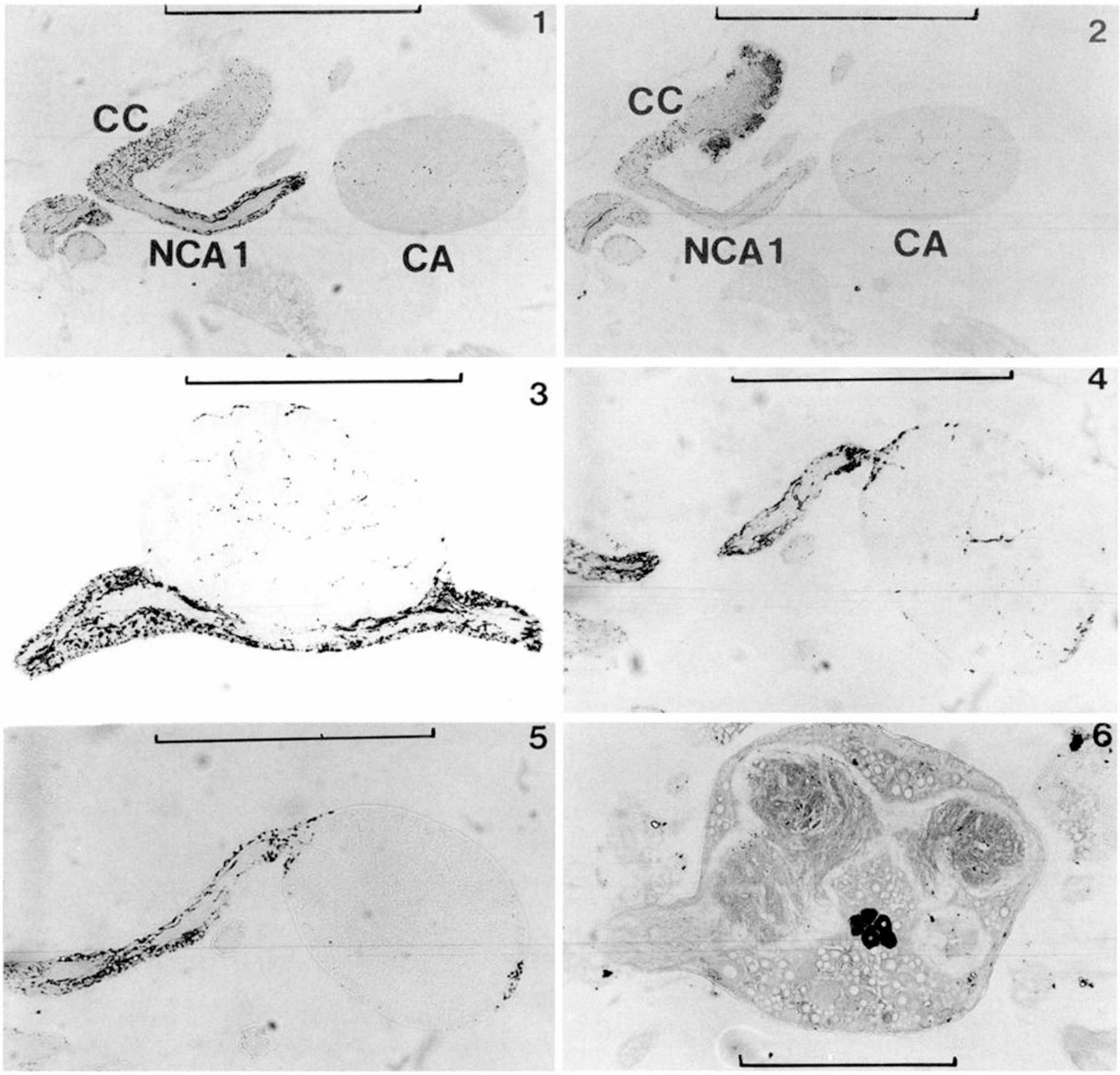
Neuropil staining was visible in the SOG and the connectives to the SOG. Several normal (ca. 15 µm in diameter) and small sized cells were stained all over the SOG (Fig. 7). In the case of anti-B, all SOG-staining was specific. When comparing serial sections alternately stained with anti-A and anti-B, no cells could be detected that were stained by both antisera (see Figs 6–7).

Brain: Anti-A

Four immunoreactive cells in the pars intercerebralis (PI, Fig. 8) and four lateral cells in each hemisphere (Fig. 9) were observed (not all these cells can be seen in a single section). In the tritocerebrum, 4–5 median tritocerebral neurosecretory cells per hemisphere were immunopositive (Fig. 10), as well as 4–5 cells per hemisphere along the lateral side of the tritocerebrum. Immunostaining could be seen in the whole neuropil area of the brain (Fig. 11), in the central body, in the antennal glomeruli, in the optic nerves, in the tritocerebrum and also in the calyces, but not in the mushroom bodies. Apart from the median tritocerebral cells (Figs 12–13), the brain staining with anti-A was specific as could be shown by a liquid phase preabsorption experiment with a brain-SOG complex. However, note the unspecific staining of the SOG-cells mentioned above (Figs 14–15).

Brain: Anti-B

Generally, brain sections stained with anti-B showed more immunopositive cells and more nerve staining at lower background staining compared to the results of the anti-A treatment. In the PI, approximately 40 normal sized cells were immunopositive (Fig. 16). In the lateral area about 30 cells per hemisphere were stained. In the tritocerebrum 4–5 median tritocerebral neurosecretory cells per hemisphere were immunopositive. Comparing serial sections alternately stained with both antisera showed that these tritocerebral cells were stained by both antisera (Figs 10 and 17). Along the lateral side of the tritocerebrum more cells (at least 8 cells per side, Fig. 18) were stained by the anti-B serum compared to anti-A. Extensive nerve staining occurred in the whole neuropil area of the brain, in the central body, in the antennal glomeruli, in the optic nerves, in the tritocerebrum and also in the calyces. Again, no staining could be found in the mushroom body stalks (Fig. 18).



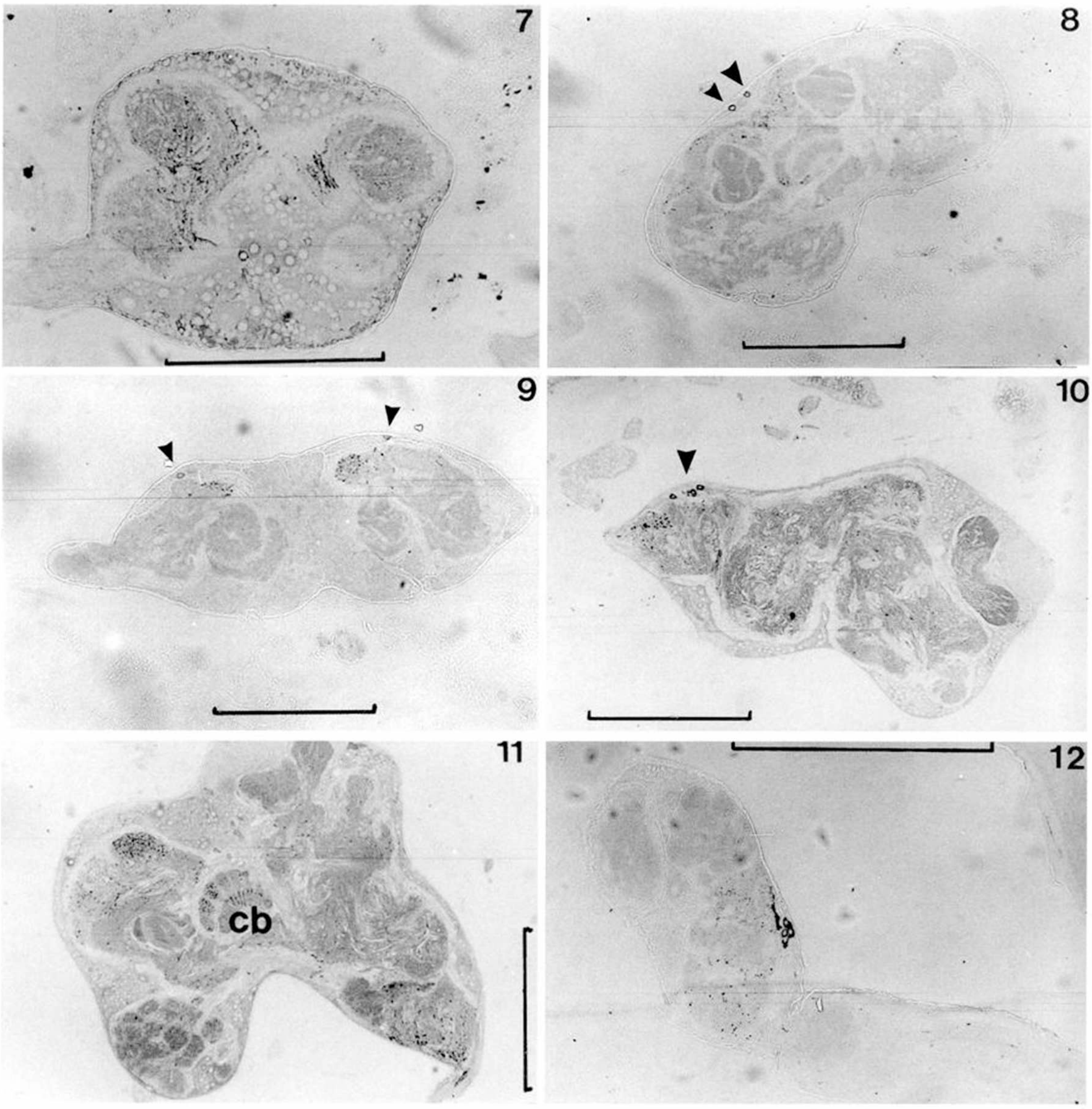
Figs 1–6: Brain/retrocerebral complex sections of 3-day old female crickets (*G. bimaculatus*) immunoreacted with two anti-peptide antisera (anti-A and anti-B) raised against Grb-AST A1 and Grb-AST B1, respectively. 1 – CC-CA complex immunostained with anti-A; 2 – CC-CA complex immunostained with anti-B; 3 – CA with NCA 1 and NCA 2 connections immunostained with anti-A; 4 – CA-NCA 1 immunostained with anti-A; 5 – CA-NCA 1 immunostained with anti-A preabsorbed with Grb-AST A1 (liquid phase preabsorption, 10 µg/µl undiluted serum), note the specific staining of nervous arborizations between the CA-cells; 6 – SOG immunostained with anti-A. Scales: 1, 2 – 0.5 mm; 3, 4, 5 – 0.3 mm; 6 – 0.4 mm. CA – corpus allatum; CC – corpus cardiacum; NCA 1 – nervus corpus allatum 1.

All the anti-B staining turned out to be highly specific. Serial sections alternately stained with both antisera showed that different PI and PL (pars lateralis) cells were apparently stained by the two antisera (Figs 16 and 19).

DISCUSSION

Anti-A staining

The present results obtained by staining sections of brain-CC-CA-SOG complexes with allatostatin A antiserum are similar to previous immunocytochemical studies on *G. bimaculatus* wholemount brain-retrocerebral complex preparations (Neuhäuser et al., 1994). Although these authors used an antiserum raised against Dip-AST 7 for immunostaining, it is not surprising that the results are similar. Both allatostatins used are members of the allatostatin superfamily, and in both cases these peptides were coupled through their N-terminus to the carrier proteins for immunization, thus the antigenic determinant is very likely to include the common Phe-Gly-Leu-amide C-terminus. As expected, ELISA studies showed a strong cross-reaction of the present anti-A serum with Dip-AST 7. Therefore, it can be assumed that the antisera against Dip-AST 7 and Grb-AST A1 recognize, at least partly, the same antigens. In their whole mount studies on cricket brain-retrocerebral complexes, Neuhäuser et al. (1994) found 4 strongly immunoreactive cells in the PI, and nerve staining in the neuropil, the tritocerebrum, the CC and the CA, similar to those found in this work. However,



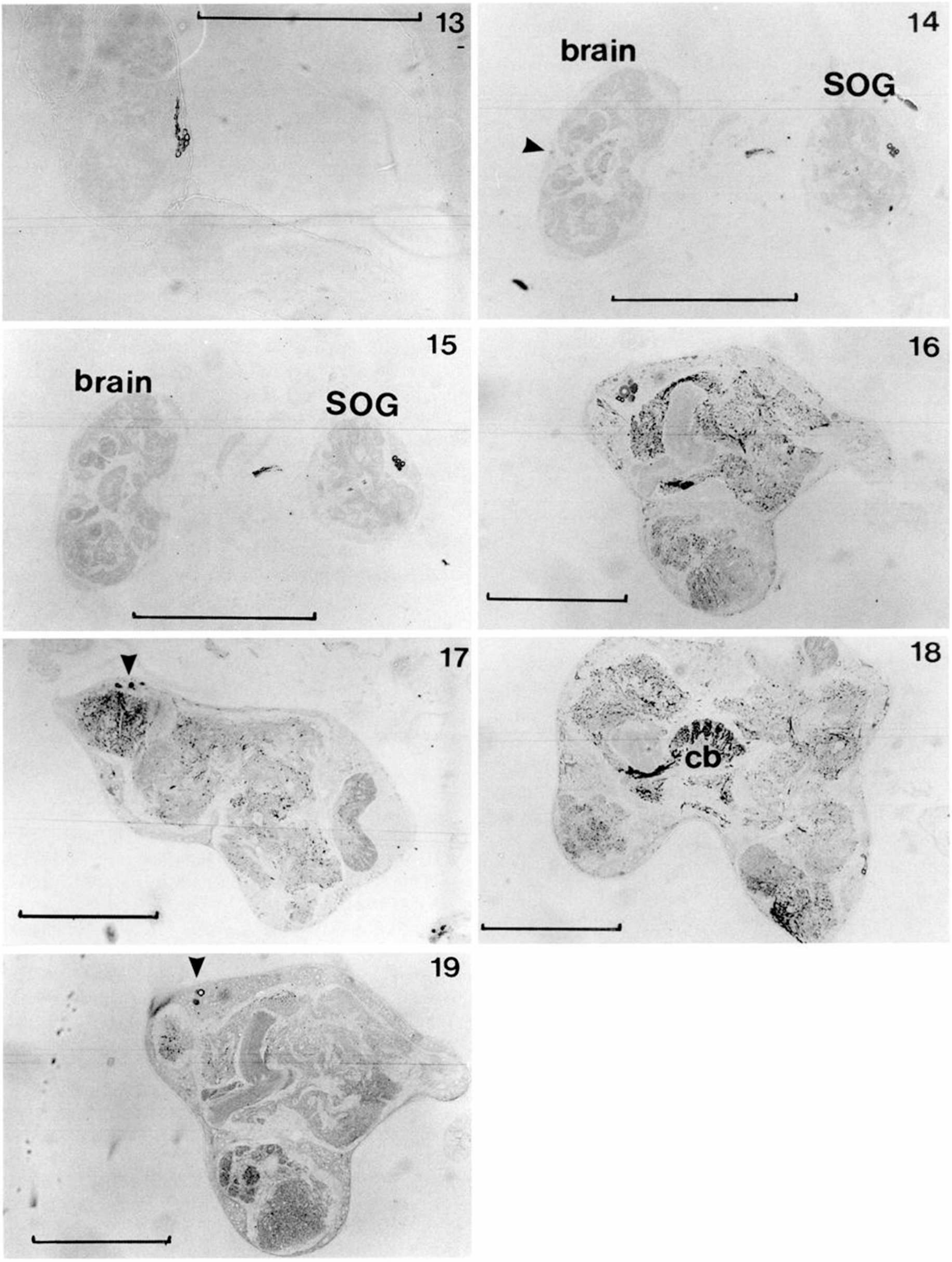
Figs 7–12: Brain/retrocerebral complex sections of 3-day old female crickets (*G. bimaculatus*) immunoreacted with two anti-peptide antisera (anti-A and anti-B) raised against Grb-AST A1 and Grb-AST B1, respectively. 7 – SOG immunostained with anti-B; 8 – Brain immunostained with anti-A showing 2 of the 4 immunopositive PI-cells (arrows); 9 – Brain immunostained with anti-A showing 1 of the 4 PL-cells per hemisphere (arrows); 10 – Brain immunostained with anti-A showing median tritocerebral neurosecretory cells (arrows); 11 – Brain immunostained with anti-A; 12 – Tritocerebrum immunostained with anti-A showing median tritocerebral neurosecretory cells. Scales: 8, 9, 10, 11, 12 – 0.5 mm; 7 – 0.4 mm. cb – central body.

these authors found more immunopositive lateral cells and additional immunoreactive cells in the protocerebrum, and also more immunopositive cells in the tritocerebrum, compared to our results. Probably several weakly stained cells were missed by only counting the unambiguously stained cells in our sections. It is also possible that weakly stained cells display a deeper colour in whole mounts than in sections, due to the longer path of light going through entire cells in whole mounts. In their whole mounts, Neuhäuser et al. (1994) also found strong immunoreactivity in all three nerves (NCC 1–3) connecting the brain with the CC. Although in our sections several stained nerve fragments lying between CC and brain were found, it was not possible to assign them clearly to the NCC 1, 2 or 3.

Schildberger & Agricola (1992) showed that some lateralis cells send their axons into the CA by using double labelling with the antibody against Dip-AST 7 and a lucifer yellow backfill of the NCA 1.

Specificity of anti-A staining

Anti-A staining in this work proved to be partially unspecific, as was shown in preabsorption studies. Generally, a polyclonal antiserum as used in this study contains many different antibody populations and not all of them bind a possible antigenic determinant of the immunogen (in the present case an allatostatin). The antiserum can also contain antibodies which are binding compounds that are not related to the allatostatins at all. There are several possibilities for the presence of unspecific antibodies



Figs 13–19: Brain/retrocerebral complex sections of 3-day old female crickets (*G. bimaculatus*) immunoreacted with two anti-peptide antisera (anti-A and anti-B) raised against Grb-AST A1 and Grb-AST B1, respectively. 13 – Tritocerebrum immunostained with anti-A preabsorbed with Grb-AST A1 (liquid phase preabsorption, 10 µg/µl undiluted serum), note the unspecific staining of the median tritocerebral neurosecretory cells; 14 – Brain-SOG complex immunostained with anti-A; note the specific staining of the PI-cell (arrow) in comparison to 15; 15 – Brain-SOG complex immunostained with anti-A preabsorbed with Grb-AST A1; note the unspecific staining of the SOG-cells; 16 – Brain immunostained with anti-B; note the PI-cells (arrow); 17 – Brain immunostained with anti-B, showing median tritocerebral neurosecretory cells (arrow); 18 – Brain immunostained with anti-B; 19 – Brain immunostained with anti-A; note the two PI-cells (arrow). Scales: 13, 16, 17, 18, 19 – 0.5 mm; 14, 15 – 1.0 mm. SOG – suboesophageal ganglion.

(Tips et al., 1997). However, the observation of unspecific nerve process or cell body staining must not necessarily fully exclude the presence of the antigen one is looking for. It may be possible that one cell generates more than one immunoreactive substance recognized by different antibody populations in the antiserum. The very strong immunoreactive SOG cells found in this work by using the anti-A serum could contain a majority of those cross-reacting substances compared to the proper allatostatin.

Anti-B staining

Generally, the anti-B serum immunoreacted with more cells and nerves at lower background staining than the anti-A. Preabsorption studies on the anti-B serum showed the high specificity of this antiserum for Grb-AST B1 epitopes. After liquid phase preabsorption, virtually all immunoreactions within the cricket sections disappeared. Weak remaining staining is probably due to the formation of soluble antigen-antibody complexes during liquid phase preincubation and their subsequent dissociation in favour of the tissue antigen (Swaab & Pool, 1975; Swaab et al., 1975). Solid-phase preabsorption totally abolished the positive immunoreaction.

Lorenz et al. (1995b) calculated the allatostatin content in cricket brains to be 0.18 pmol/brain for Grb-AST A1 and 0.73 pmol/brain for Grb-AST B1. However, we do not yet know how many members of each of the two allatostatin families are present in the cricket brain and in which concentrations. Even if there is no cross-reaction at all between different peptides belonging to the same family, the Grb-AST B1 content in the brain is 4 times higher than that of the Grb-AST A1. This could be one possible explanation for the higher degree of immunoreaction found in the brains with anti-B compared to anti-A. Of course, this observation could also be due to the higher affinity of the anti-B serum, although the dilution of the two antisera used for staining purposes was the same. Another conspicuous difference in the staining patterns of the two antisera is the existence of intensively stained, huge round shaped areas in the CC, visible only with the anti-B serum. The CC is known to be the production and release site of AKH. Although the sensitivity of Grb-AKH to anti-B was only 1% compared to Grb-AST B1 in ELISA studies, we suspect the antibody to stain AKH. The AKH-content in CC of 4-day old female crickets was determined to be 42 pmol/CC (Woodring & Hoffmann, 1997), which is rather high compared to the allatostatin content in the brain. An HPLC-analysis of the CC revealed that the content of AKH in the CC is at least 100 times higher compared to the content of any allatostatin (Lorenz, unpublished observation).

CONCLUSIONS

Members of two families of allatostatic neuropeptides (A and B allatostatin family) have been detected in cricket (*G. bimaculatus*) brain by chromatographic (Lorenz et al., 1995a, b) as well as by immunological methods (this paper). These brain factors could reach the CA either directly through nerves (NCC 1-3, NCA 1) or via the haemolymph. Another possible source of CA regulating neuropeptides is the SOG which is connected to the CA through the NCA 2. In this work a rather comparable extent of immunostaining in the brain/retrocerebral complex of crickets was found when using two antisera raised against Grb-AST A1 and B1, respectively. Both antisera, for example, immunoreacted with cells in the pars intercerebralis and the pars lateralis of the brain, and showed nerve staining in the CC as well as in the CA. In most cases the two antisera stained different cells of the PI, PL or SOG. Some median neurosecretory tritocerebral cells, however, contained immunoreactive material recognized by both antisera. Strong Grb-AST A1 and B1 immunoreactivity in the CC suggests that this neurohaemal organ will be a site of release for both the allatostatins into the haemolymph. Yu et al. (1993) demonstrated the presence of A allatostatin-immunoreactive material in the haemolymph of *Diploptera punctata*, in favour of the humoral pathway for the action of these peptides. Highly specific staining of nerve endings among JH producing cells within the CA, however, strongly indicates that both types of allatostatic neuropeptides may reach the CA directly through nerve connections. The present results corroborate the assumption that members of the two allatostatin families will also act as potent inhibitors of JH biosynthesis in crickets in vivo.

ACKNOWLEDGEMENTS. PV is senior research associate of the Fund for Scientific Research-Flanders and presently on a one-year sabbatical leave at innogenetics, Gent, Belgium.

REFERENCES

De Loof A., Vandesande F., Geysen J. & Verhaert P. 1984: Immunocytochemical localization of CRF, neurophysin I and II, oxytocin and vasopressin in the brain and corpora cardiaca of the cockroach Periplaneta americana. *Gen. Comp. Endocrinol.* **53**: 484.

Gäde G., Hoffmann K.H. & Spring J.H. 1997: Hormonal regulation in insects: Facts, gaps, and future directions. *Physiol. Rev.* **77**: 963–1032.

Goodman W.G. 1990: Biosynthesis, titer regulation, and transport of juvenile hormones. In Gupta A.P. (ed): *Morphogenetic Hormones of Arthropods. 1.* Univ. Press, New Brunswick, pp. 83–124.

Hoffmann K.H. & Sorge D. 1996: Vitellogenin levels in allatectomized female crickets, Gryllus bimaculatus de Geer (Ensifera, Gryllidae). *Arch. Insect Biochem. Physiol.* **32**: 549–558.

Hoffmann K.H., Lorenz M.W. & Witek G. 1998: Neuropeptides that influence juvenile hormone (JH) biosynthesis in Gryllus bimaculatus. In Vaudry H., Tonon M.C., Roubos E.W. & De Loof A. (eds): *Trends in Comparative Endocrinology and Neurobiology. Ann. N.Y. Acad. Sci. Vol. 839.* New York Academy of Sciences, New York, pp. 297–300.

Koch P.B. & Hoffmann K.H. 1985: Juvenile hormone and reproduction in crickets, Gryllus bimaculatus de Geer: corpus allatum activity (in vitro) in females during adult life cycle. *Physiol. Entomol.* **10**: 173–182.

Lorenz M.W., Kellner R. & Hoffmann K.H. 1995a: Identification of two allatostatins from the cricket, Gryllus bimaculatus (Ensifera, Gryllidae): additional members of a family of neuropeptides inhibiting juvenile hormone biosynthesis. *Regul. Pept.* **57**: 227–236.

LORENZ M.W., KELLNER R. & HOFFMANN K.H. 1995b: A family of neuropeptides that inhibit juvenile hormone biosynthesis in the cricket, Gryllus bimaculatus. *J. Biol. Chem.* **270**: 21103–21108.

LORENZ M.W., KELLNER R., HOFFMANN K.H. 1999: Allatostatins in Gryllus bimaculatus (Ensifera: Gryllidae): New structures and physiological properties. *Eur. J. Entomol.* **96**: 267–274.

NEUHÄUSER T., SORGE D., STAY B. & HOFFMANN K.H. 1994: Responsiveness of the adult cricket (Gryllus bimaculatus and Acheta domesticus) retrocerebral complex to allatostatin-1 from a cockroach, Diploptera punctata. *J. Comp. Physiol. (B)* **164**: 23–31.

SCHILDBERGER K. & AGRICOLA H. 1992: Allatostatin–like immunoreactivity in the brains of crickets and cockroaches. In Elsner N. & Richter D.W. (eds): *Rhythmogenesis in Neurons and Networks.* Georg Thieme, Stuttgart, 489 pp.

STAY B., TOBE S.S. & BENDENA W.G. 1994: Allatostatins: Identification, primary structures, functions and distribution. *Adv. Insect Physiol.* **25**: 267–337.

SWAAB D.F. & POOL C.W. 1975: Specificity of oxytocin and vasopressin immunofluorescence. *J. Endocrinol.* **66**: 263–272.

SWAAB D.F., POOL C.W. & NIJVELDT F. 1975: Immunofluorescence of vasopressin in the rat hypothalamo-neurohypophyseal system. *J. Neural Transm.* **36**: 195–215.

TIPS A., SCHOOFS L., PAEMEN L., HENDRICKX K. & DE LOOF A. 1997: False positive immunostaining of Locusta neurosecretory cells with a variety of preimmune sera. *Gen. Comp. Endocrinol.* **106**: 231–240.

VANDESANDE F. & DIERICKX K. 1976: Immunocytochemical demonstration of separate vasotocinergic and mesotocinergic neurons in the amphibian magnocellular neurosecretory system. *Cell Tissue Res.* **179**: 289–296.

VERHAERT P., MARIVOET S., VANDESANDE F. & DE LOOF A. 1984a: Localization of CRF immunoreactivity in the central nervous system of three vertebrate and one insect species. *Cell Tissue Res.* **238**: 49–53.

VERHAERT P., GEYSEN H., DE LOOF A. & VANDESANDE F. 1984b: Immunoreactive material resembling vertebrate neuropeptides and neurophysins in the brain, suboesophageal ganglion, corpus cardiacum and corpus allatum of the dictyopteran Periplaneta americana L. *Cell Tissue Res.* **238**: 55–59.

WOODRING J. & HOFFMANN K.H. 1997: Apparent inhibition of in vitro juvenile hormone biosynthesis by the corpus cardiacum of adult crickets (Gryllus bimaculatus and Acheta domesticus) is due to juvenile hormone esterase. *Arch. Insect Biochem. Physiol.* **34**: 19–29.

YU C.G., STAY B., JOSHI S. & TOBE S.S. 1993: Allatostatin content of brain, corpora allata and haemolymph at different developmental stages of the cockroach, Diploptera punctata: Quantitation by ELISA and bioassay. *J. Insect Physiol.* **39**: 111–122.

Received January 4, 1999; accepted May 5, 1999