

## AGE-DEPENDENT NEUROSECRETION RELEASE INDUCED BY DOPAMINE IN THE CORPORA CARDIACA OF *BLATTELLA GERMANICA* (L.) (DICTYOPTERA : BLATTELLIDAE)

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**Abstract** — In addition to glial cells, intrinsic glandular cells and ordinary axons, the corpora cardiaca of *Blattella germanica* (L.) (Dictyoptera : Blattellidae) contain 4 types of neurosecretory fibers originating from the brain (types 1, 2, 3, and 4), which can be recognized on the basis of the size, form, and electron density of their neurosecretory granules. A comparative ultrastructural study of the corpora cardiaca from normal females and from dopamine-treated (1 µg) females has been carried out at different stages within the first ovarian cycle (freshly emerged: day 0; 2-day-old: pre-vitellogenesis; 4-day-old: beginning of vitellogenesis; 6-day-old: full vitellogenesis; 8-day-old: post-vitellogenesis, period of ootheca transport). Quantitative data on the exocytotic configurations observed in each type of fiber (1–4) have led to the following conclusions: (a) the exocytotic configurations in control specimens are too infrequent to prompt any inference about the dynamics of neurosecretion release in the different types of fibers; (b) dopamine treatment induces a stimulation of the exocytosis phenomenon, whose extent depends on the specimen age and on the type of fiber. Therefore, on days 2 and 6, dopamine preferentially stimulates neurosecretion release in the fibers of types 3 and 4 respectively.

**Index descriptors** (in addition to those in title): Juvenile hormone, ultrastructure.

### INTRODUCTION

THE corpora cardiaca (CC) of insects are complex neurohemal organs in which neurohormones of both extrinsic and intrinsic origins are stored and released into the hemolymph at appropriate times (Gupta, 1983; Sedlak, 1985; Raabe, 1989). Extrinsic neurosecretory fibers are located in the so-called neurohemal portion of the CC, which is constituted by the axons of the neurosecretory cells spread over the cerebral lobes, and which reach the CC via the nervi corporis cardiaci (NCC). Intrinsic neurohormones are

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produced in glandular neurosecretory cells located in the CC. In several species, such as locusts, both neurohemal and glandular portions are structurally well-differentiated (Cassier and Fain-Maurel, 1970a, b; Cassier, 1983), whereas in others, such as cockroaches, the axons arriving through the NCC intermingle with the intrinsic glandular cells within the CC (Scharrer, 1963).

Morphological and immunocytochemical studies on the CC neurosecretory products have led to the classification of different aminergic and peptidergic types of neurosecretory axons (Scharrer, 1989) and also to the identification of fibers in which amines and peptides are co-localized (Raabe, 1989). Furthermore, the 3 most important biogenic amines in insects, i.e. octopamine, dopamine and serotonin, have been unequivocally identified in the CC of different species of insects (Raabe, 1989), where it is assumed that they may act as neurotransmitters or neuromodulators (Evans, 1980). The first experimental indication of this neuromodulatory role was furnished by Scharrer and Wurzelmann (1978), when they showed that exogenous serotonin induces the release of neurosecretory material in the CC fiber endings of the cockroach *Leucophaea maderae*. More recently, Pannabecker and Orchard (1986) have demonstrated in *Locusta migratoria* the releasing activity of octopamine on the CC cells producing adipokinetic hormones. It has also been suggested that this amine could be involved in the release of neurosecretions modulating corpora allata (CA) activity and juvenile hormone (JH) production in *L. migratoria* (Lafon-Cazal and Baehr, 1988) and *Diploptera punctata* (Thompson *et al.*, 1990).

In the cockroach *Blattella germanica*, we have previously shown (Pastor *et al.*, 1991a) that brain dopamine levels experience remarkable fluctuations during the first ovarian cycle. In short, brain dopamine levels show an amine accumulation until days 2–3 and a sudden drop on day 4, just when a significant increase of oocyte length simultaneously occurs; then, dopamine levels increase on day 5 and suddenly decrease on day 6, just before the beginning of chorionation. In addition, we have also shown that exogenous dopamine has a differential age-dependent effect on oocyte growth and JH production, being stimulatory at the beginning of vitellogenesis and inhibitory at the end of this process (Pastor *et al.*, 1991b).

These findings suggested that dopamine could be involved in the release of neurosecretions modulating JH production in *B. germanica*, and motivated the present study, which deals with the cytological changes in the CC of this species in response to dopamine treatment.

The study was carried out on virgin females representing 5 developmental stages within the first ovarian cycle. The observations on the CC ultrastructure will show that, in addition to glial cells, ordinary fibers and intrinsic glandular cells, there are 4 types of neurosecretory fibers originating from the brain, and that the administration of dopamine stimulates a differential neurosecretion-releasing activity, which depends on the type of fiber and also on the physiological stage of the female.

## MATERIAL AND METHODS

### *Insects*

Cultures of *B. germanica* were maintained at 30°C. Freshly ecdysed virgin females removed from the colony were used at the following ages: freshly ecdysed, basal oocyte length (BOL): 0.42–0.47 mm; 2-day-old

(preparation for vitellogenesis), BOL: 0.53–0.55 mm; 4-day-old (beginning of vitellogenesis), BOL: 0.99–1.28 mm; 6-day-old (full vitellogenesis), BOL: 1.76–1.94 mm; 8-day-old (post-vitellogenesis, females carrying the ootheca), BOL (new batch): 0.47–0.50 mm. These stages are well-characterized in terms of oocyte length, CA volume, and JH biosynthetic activity (Bellés *et al.*, 1987), CA ultrastructure (Piulachs *et al.*, 1989), brain dopamine levels (Pastor *et al.*, 1991a) and dopamine activity on oocyte growth and JH production (Pastor *et al.*, 1991b).

#### *Dopamine treatment*

Each specimen received 1 µg of dopamine in a volume of 2 µl of Ringer's solution injected between the 3rd and the 4th abdominal sternites. The dose of 1 µg was chosen as this had been the effective dose in previous studies of the activity of dopamine on oocyte growth (Pastor *et al.*, 1991b). Controls received the same volume of solvent. Fixation of the CC–CA complex was carried out 5 min after the injection.

#### *Electron microscopy*

The CC–CA complexes were fixed in 0.2 M cacodylate-buffered 3% glutaraldehyde (pH 7.3) with 5% sucrose for 30 min. Post-fixation was carried out in cacodylate buffered 2% OsO<sub>4</sub> for 2 hr. Dehydration was in ethanol and embedding in Epon–Araldite medium (Piulachs *et al.*, 1989). Semi-thin sections for sample orientation were stained with uranyl acetate and lead citrate (Reynolds, 1963). Observations were carried out with a Philips electron microscope (EM 300 or EM 201), at 80 kV. Observations mainly concerned the latero-ventral and ventral parts of the CC. For each CC, 4 grids were prepared with about 15 ultra-thin sections each.

#### *Ultrastructural observations*

For quantitative studies of exocytotic configurations 100 micrographs (magnification: × 32,000 or × 40,000) from dopamine-treated material, representing the different ages and specimens, were used (4 micrographs selected at random of each specimen × 5 treated specimens × 5 ages studied). Each selected area containing various types of fibers had a surface area of 7–10 µm<sup>2</sup>. An equivalent series of 100 micrographs was used in the case of Ringer's solution-treated controls.

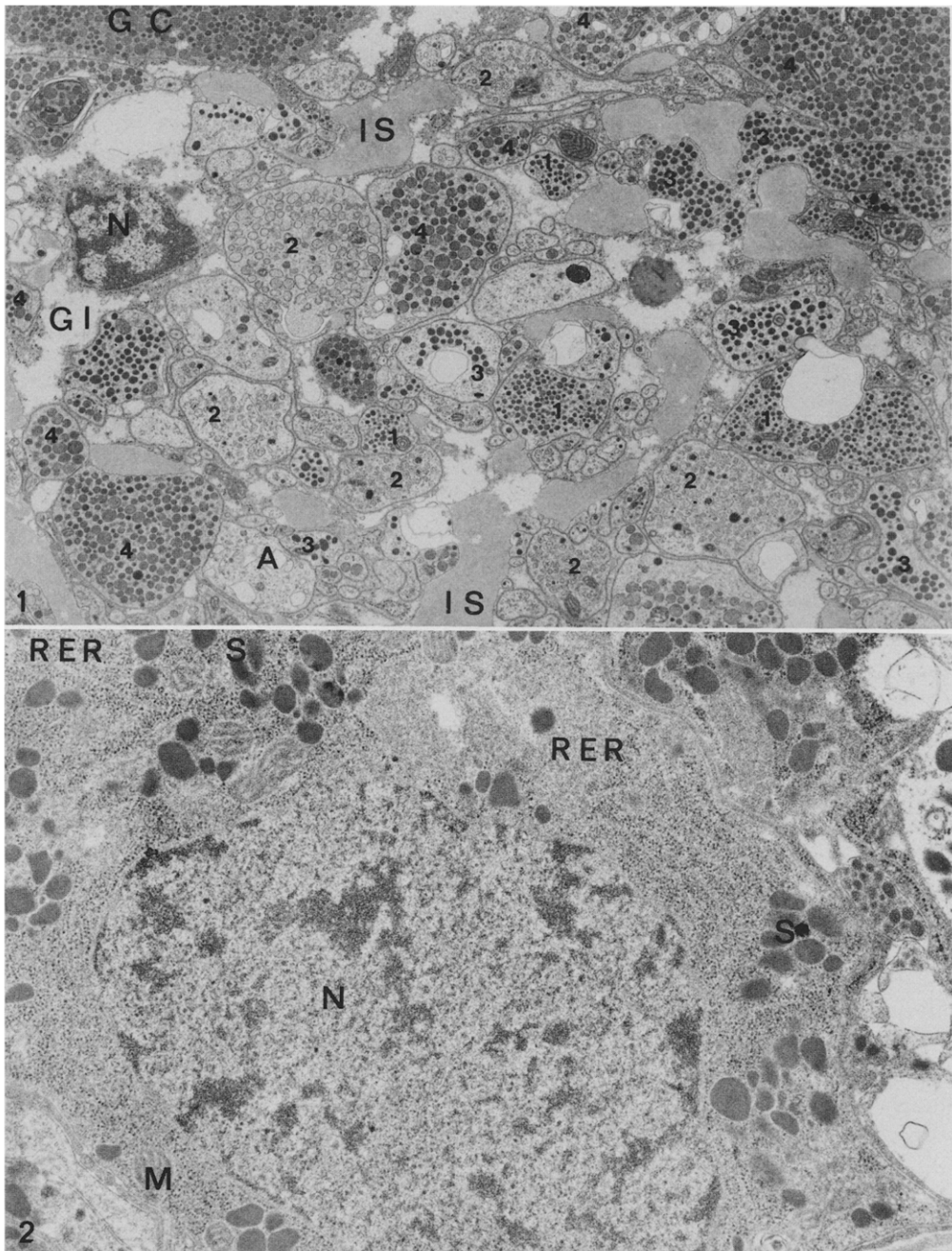
Exocytotic configurations taken into account were either membrane-bound granules indicative of a pre-exocytotic process, omega figures (indicative of a recent exocytotic process) or granules in the extracellular stroma, devoid of their bounding membrane and showing various degrees of breakdown, lying in close proximity to the axolemma (indicative of a recent release) (see Scharrer and Wurzelmann (1978)). With quantitative data on the number of exocytotic configurations (EC) observed in each type of fiber and the number of fiber sections of the corresponding type (F), we defined an exocytotic ratio (ER) for each type of fiber,  $ER = EC/F$ .

## RESULTS

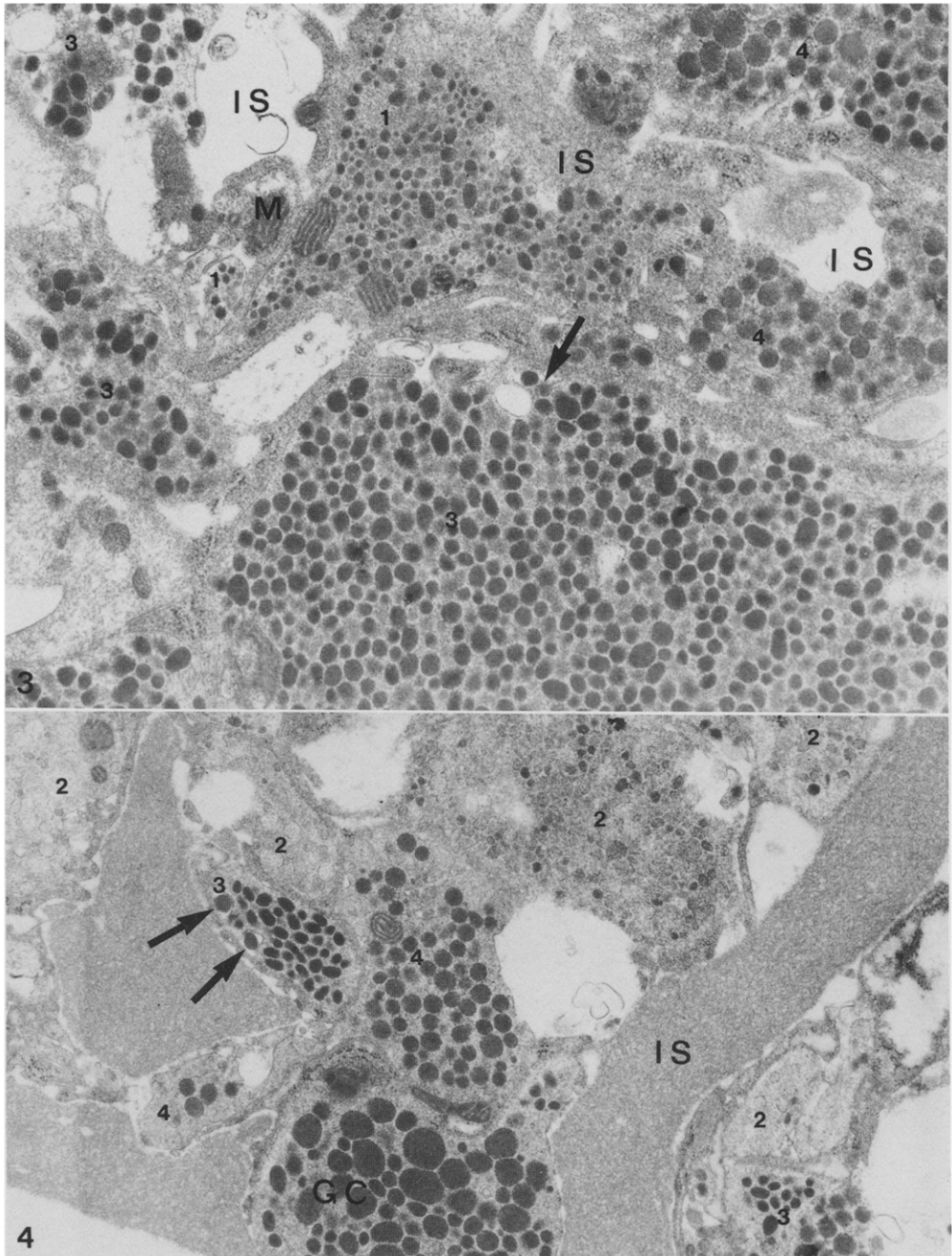
### *The corpora cardiaca of B. germanica*

The CC of *B. germanica* are paired, ellipsoidal organs that lie in the wall of the dorsal vessel, in a retrocerebral position. The ultrastructural characteristics are similar to those described in detail for other cockroaches, such as *Leucophaea maderae* (Scharrer, 1963) or *Periplaneta americana* (Scharrer and Kater, 1969). They are separated from adjacent structures and from the surrounding hemocoel by a basement membrane giving rise to branching processes dividing the CC into irregular compartments. Four different categories of intermingled cellular structures can be recognized (see Figs 1–6): (a) glial cells with long and intricately ramifying processes; (b) ordinary axons (with neurotubules and synaptic vesicles measuring about 60 nm); (c) intrinsic glandular cells not preferentially arranged and markedly ramified, with a well-developed ergastoplasm (RER) and rich in electron-dense and polymorphic (subspherical or elongate, 200–250 µm × 250–400 µm) neurosecretory granules; and (d) neurosecretory axons originating from the brain and which penetrate into the CC via the NCC.

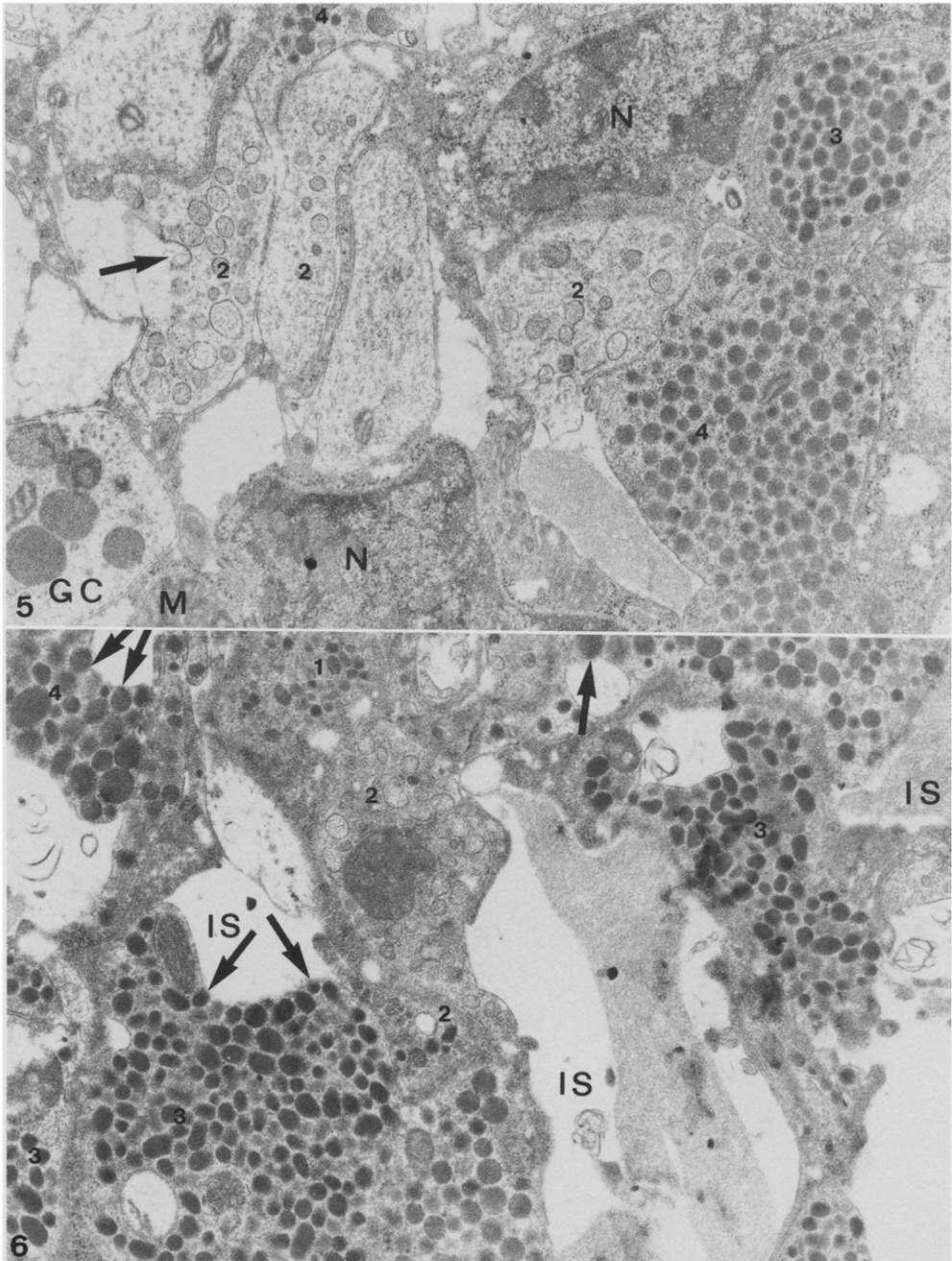
The neurosecretory axons contain a large number of neurosecretory granules showing a variety of size, form, electron density and internal structure. Considering these parameters, they fall into 4 major categories:



FIGS 1, 2. General organization of corpora cardiaca of *Blattella germanica* (6-day-old adult female). FIG. 1. Neurohemal area with large cellular spaces (IS), sections of axons (A) and neurosecretory fibers (types 1, 2, 3, 4) and expansions of a glandular cell (GC). Fibers are deprived of, or surrounded by, glial expansions. GI = glycogen.  $\times 7500$ . FIG. 2. Nuclear (N) and perinuclear parts of an intrinsic glandular cell showing a well-developed rough endoplasmic reticulum (RER), numerous mitochondria (M) and granules of secretion (S).  $\times 21,500$ .



FIGS 3, 4. Corpora cardiaca of a 2-day-old female of *Blattella germanica*. FIG. 3. Control specimen. Neurohemal part showing glial expansions surrounding neurosecretory fibers (types 1, 3, 4) in vicinity of intercellular spaces (IS). Arrow = membrane-bound granule. M = mitochondria.  $\times 21,000$ . FIG. 4. Dopamine-treated female. Exocytotic processes (arrows) are enhanced mainly in fibers of type 3. 2, 3, 4 = fibers of types 2, 3 and 4. GC = expansions of a glandular cell. IS = intercellular space.  $\times 17,000$ .



FIGS 5, 6. Corpora cardiaca of a 6-day-old female of *Blattella germanica*. FIG. 5. Control specimen showing a characteristic omega configuration (arrow). 2, 3, 4 = fibers of types 2, 3 and 4. GC = expansion of a glandular cell. M = mitochondria. N = nuclei of glial cells.  $\times 22,000$ . FIG. 6. Dopamine-treated female. Exocytotic processes (arrows) are enhanced mainly in fibers of type 4. 1-4 = fibers of types 1-4. IS = intercellular spaces.  $\times 27,000$ .

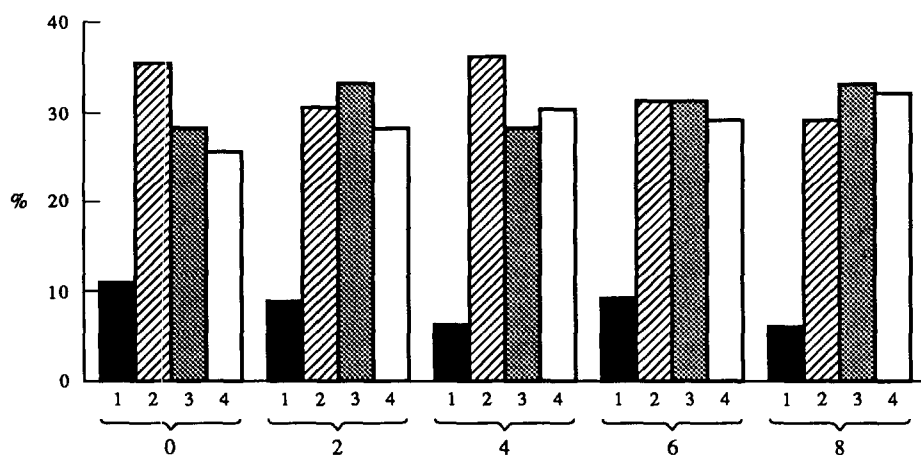


FIG. 7. Percentage of each type of neurosecretory fibers (1-4) observed in corpora cardiaca of *Blattella germanica* on different days (0, 2, 4, 6 and 8) studied within first ovarian cycle. Data based on 100 micrographs representing different ages and specimens used (4 micrographs per specimen and 5 specimens per age).

Type 1. Fibers of small granules (100–120 nm), generally electron-dense, subspherical or elliptical (Figs 1, 3, 6).

Type 2. Fibers containing spherical granules (diameter between 120 and 130 nm), surrounded by a limiting membrane, which is sometimes discontinuous, and showing a granular content moderately electron-dense, appearing in various shades of grey. These granules are associated with small vesicles (50–60 nm), electron transparent or opaque (Figs 1, 4–6).

Type 3. Fibers containing elliptical (diameters 150–200 nm × 100–120 nm) and electron-dense granules (Figs 1, 3–6).

Type 4. The same general features as in type 3, but the neurosecretory granules are spherical or subspherical (Figs 1, 3–6).

Whether granules are of type 1 or 2 is easily recognizable because of their small size and granular content, respectively. Conversely, those of type 3 or 4 are quite similar to each other. However, type 3 granules are clearly elliptical, whereas type 4 granules are subspherical. This difference is well apparent if the diameter ratio (DR: shorter/larger granule diameter) in both types of fiber is compared. The average value of DR in type 3 granules was  $0.78 \pm 0.13$  ( $n = 83$  granules measured at random on different fibers from 5 specimens corresponding to the 5 ages studied), whereas in type 4 it was  $0.95 \pm 0.05$  ( $n = 135$  granules measured as in type 3). Differences ( $t$ -test) are statistically significant ( $P < 0.001$ ).

The 4 types of fibers have been observed wedged in between the intrinsic glandular cells of the CA. Moreover, it is worth noting that these 4 types of fibers and granules are present in similar proportions in the 5 stages studied within the cycle (Fig. 7). Type 1 fiber is the least frequent (10% or less of the total fibers), whereas the great majority belong to the types 2, 3 and 4 (with a frequency of about 30% each).

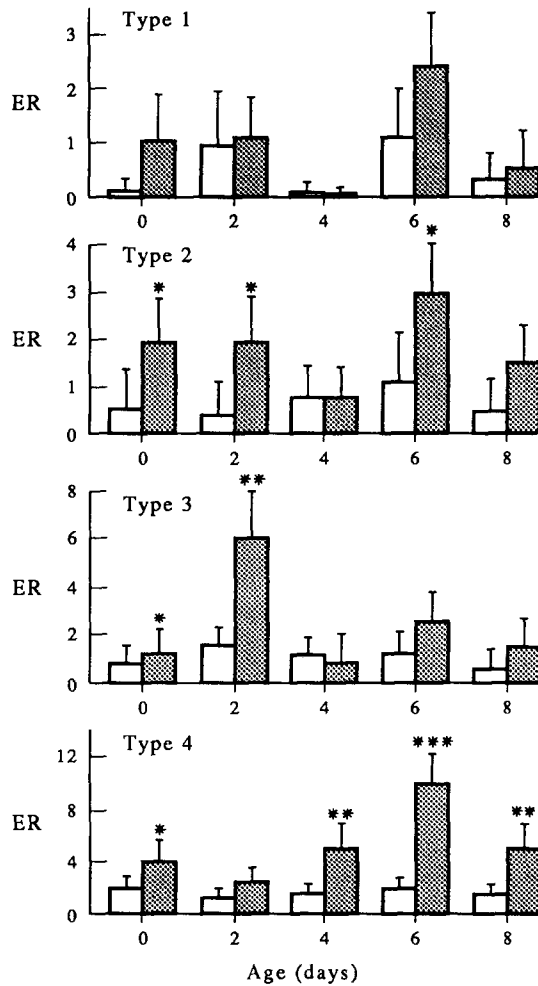


FIG. 8. Exocytotic ratio (ER) measured (mean  $\pm$  SEM) in each type of neurosecretory fibers in corpora cardiaca of control (white columns) and dopamine-treated (shaded columns) specimens of *Blattella germanica* in different ages studied within first ovarian cycle. Data based on 100 micrographs representing the different ages and specimens used (4 micrographs per specimen and 5 specimens per age). Results of *t*-test (from comparison between dopamine-treated and control specimens at each age and type of fiber) are summarized as follows: \* ( $P < 0.05$ ); \*\* ( $P < 0.01$ ); \*\*\* ( $P < 0.001$ ).

#### *Influence of dopamine treatment*

In untreated or control specimens (Fig. 8) exocytotic configurations are infrequent, especially in the fibers of types 1 and 2, where a maximum of one exocytotic configuration per fiber section was observed. Signs of granule extrusion were slightly more frequent in the fibers of types 3 and 4, but, in any case, the ratio was never above 2 exocytotic configurations per fiber section.

The equivalent profiles corresponding to dopamine-treated specimens (Fig. 8) clearly



indicate that dopamine stimulated the process of neurosecretion release in almost all cases. The fibers of types 1 and 2 appear to be less stimulated, and the number of exocytotic configurations is still quite low, thus making it difficult to interpret the fluctuations observed. The effects of dopamine and the influence of age are more apparent in the fibers of types 3 and 4. The most noticeable result concerning the type 3 fiber, is the sharp ER peak observed on day 2. Interestingly, the ER fluctuations in relation to age in dopamine-treated specimens follow a pattern equivalent to that of controls, although with the peak on day 2 greatly increased in the former. A similar situation occurred in type 4 fibers. A sharp ER peak was also observed in dopamine-treated specimens, although in this case it appeared on day 6. Thus it seems that type 4 fibers become more sensitive to the stimulatory action of dopamine at this age.

#### DISCUSSION

The general organization of the CC of *B. germanica* follows a similar pattern to that described for other cockroaches (see Scharrer, 1963; Scharrer and Kater, 1969). The extrinsic axonal processes coming from the brain through the NCC intermingle with the intrinsic CC glandular cells to form a compact organ with no distinct neurohemal or glandular parts. The study of the ultrastructural features of the extrinsic axonal processes has furnished a diversity of types rarely observed in insects (Gupta, 1983). Four different types of fibers (1–4) defined on the basis of the ultrastructural features of their neurosecretory granules were recognized.

Our previous studies on *B. germanica* indicated that brain dopamine levels underwent significant fluctuations throughout the first ovarian cycle (Pastor *et al.*, 1991a) that could be correlated with fluctuations in JH production (Bellés *et al.*, 1987). Therefore, we hypothesized that dopamine could act as a releasing factor of the neurosecretions modulating the biosynthetic activity of the CA. In general, neurosecretory granules are released by exocytosis (Scharrer, 1989) and, among other mechanisms, the involvement of biogenic amines (especially dopamine and noradrenaline) has been suggested in this release in insects (Raabe, 1989).

In the context of this hypothesis, our first idea was to compare the dynamics of neurosecretion release in the different types of CC fibers at different stages within the ovarian cycle, i.e. on days 0, 2, 4, 6, and 8, which were well characterized in terms of brain dopamine levels (Pastor *et al.*, 1991a) and CA activity (Bellés *et al.*, 1987). However, the exocytotic configurations observed in control specimens were too infrequent (Fig. 8) to prompt any inference in relation to brain dopamine contents or JH production. Thus, another approach was followed using dopamine-stimulated specimens, in keeping with experiments described by Scharrer and Wurzelmann (1978). As expected, the treated specimens showed an increased number of exocytotic configurations but, interestingly, these effects were not only age-dependent, but also fiber-dependent (Fig. 8). The most significant differences between the controls and dopamine-stimulated specimens were found on day 2 for type 3 fiber, and on day 6 for type 4. In other words, it appeared as if the fibers of types 3 and 4 at these stages were more sensitive to the releasing effect of dopamine.

The possible physiological significance of these observations remains obscure. However, it is worth remembering that day 2 represents the starting point for a fast

increase of JH biosynthesis and the beginning of the vitellogenesis process, and that day 6 precedes the decline of JH production and the termination of that process (Bellés *et al.*, 1987). In addition, dopamine (1 µg/cockroach) slightly enhances oocyte growth when injected on day 2 or 3, whereas it induces the inverse effect on day 6 or 7; and *in vitro*, dopamine (10<sup>-4</sup> M) stimulates JH production by incubated CA from 2-day-old females, and inhibits it on 6-day-old glands (Pastor *et al.*, 1991b). The results as a whole, therefore, suggest the hypothesis that type 3 fibers could contain a factor stimulating CA activity, whereas those of type 4 could contain an inhibitory one.

However, the diversity of fibers encountered within the CC of *B. germanica* cannot plausibly explain the great number of functions assumed to be regulated by this organ; thus it rather seems that each type of fiber controls different functions. Indeed, cytochemical studies in very diverse insect species have afforded some indications in this regard, as different peptides, co-localized in the same fiber, have been detected (see Raabe (1989)). Therefore, an unequivocal identification of the neurosecretions involved in the above hypothesis is needed. Work in this direction is currently in progress in our laboratories.

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