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Chromaffinity, uranaffinity and argentaffinity of small granule-containing (SGC) cells in rat superior cervical ganglia

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Summary. A systemic examination on the small granule-containing (SGC) cells in rat superior cervical ganglia was conducted by conventional and cytochemical electron microscopy including chromaffin, argentaffin and uranaffin reactions. According to the fine structure of dense cored vesicles (DCVs) in the cytoplasm, three types of small granule-containing (SGC) cells were revealed — Type I: 90 - 160 nm vesicles with cores of moderate or low electron density; Type II: 130 - 330 nm vesicles, polymorphic with highly electron dense cores; Type III: elongated vesicles (170 nm x 60 nm) with cores of moderate to low electron density. The majority of SGC cells were the Type I cells (78%) and Type II and III cells made up 13% and 9% of SGC cell population, respectively. Cytochemical results demonstrated that only the Type II cells displayed a positive chromaffin reaction and all three types of SGC cells showed argentaffinity and uranaffinity. The present study is the first to demonstrate the argentaffin reaction at ultrastructural level in SGC cells of sympathetic ganglia. Based on the results of the present study we also concluded that (1) the DCVs of Type II SGC cells contained noradrenaline and (2) biogenic amines and nucleotides (ATPs) coexisted in the DCVs of all three types of SGC cells.

Key words: SGC cells, Chromaffinity, Uranaffinity, Argentaffinity, Superior cervical ganglia

Introduction

Sympathetic ganglia contain, in addition to typical principal neurons, clusters of small parenchymal cells which exhibit a variable chromaffinity after treatment with fixative containing potassium dichromate (Böck, 1982). On the other hand, cluster of small intensely fluorescent (SIF) cells which exhibit an intense fluorescence induced by formaldehyde vapor were also identified in rat superior cervical ganglia (Eränkö and Härkönen, 1965). Chromaffin positive (CH + 've) small parenchymal cells were soon identified as small granule-containing (SGC) cells which were characterized by the presence of numerous dense-cored vesicles (DCVs) in their cytoplasm. Cross identifications between CH + 've small cells, SIF cells and small granule-containing (SGC) cells by chromaffin reaction, fluorescence and electron microscopy have been established (Siegrist et al., 1968; Grillo et al., 1974; Matthews, 1989).

Various types of SGC cells have been described, using the criteria such as cell morphology, ultrastructure and intraganglionic distribution. Watanabe (1971) demonstrated four types of SGC cells in the guinea-pig hypogastric ganglia, Dail et al. (1975) two types in rat pelvic plexus, Elfvin et al. (1975) two types in the guinea-pig inferior mesenteric ganglia and Lu et al. (1976) three types in rat superior cervical and coeliac-mesenteric ganglia (Taxi, 1979; Böck, 1982; Taxi et al., 1983). The heterogeneity and often inconsistent distribution of CH + 've, SIF or SGC cells in the sympathetic ganglia render morphological and cytochemical investigations at ultrastructural level difficult and may explain why only a few cytochemical studies have been conducted on these cells compared with the cells in adrenal medulla or carotid body.

Recent studies on SGC cells in autonomic ganglia were concentrated on the development with ganglia and the identification of the chemical substance within SGC and/or the enzymes responsible for the synthesis of these substances by immunohistochemical and immunocytochemical methods (Fuxe et al., 1971; König and Heym, 1978; Verhofstad et al., 1981; Järvi et al., 1987; Soinila et al., 1989; for review Heym, 1987). Nonetheless, most of traditional cytochemical studies on SGC cells in sympathetic ganglia at electron microscopic level were rather incomplete due to the multiplicity of SGC cells in sympathetic ganglia. So far, chromaffin reaction of the SGC cells has been studied

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by Lever's group (Lever et al., 1974, 1977; Santer et al., 1978), and to our knowledge, uranaffin reaction has only shown in one type of SGC cells (Richards and DaPrada, 1980) and no report on the argentaffinity of SGC cells is available. In order to establish a systemic information on the cytochemical nature of various types of SGC cells at the ultrastructural level, the present study investigate the SGC cells in rat superior cervical ganglia by chromaffin, argentaffin and uranaffin reactions.

Materials and methods

Adult male rats (Long Evans strain) ranging from 150 - 250 g in body weight were used in this investigation. All animals were maintained in natural temperature and photoperiodicity. Food and water were supplied *ad libitum*. All animals were sacrificed by cardiovascular perfusion with a fixative consisting of 2.5% glutaraldehyde and 0.2% paraformaldehyde (for modified chromaffin reaction and argentaffin reaction) or 2% glutaraldehyde and 2% paraformaldehyde (for ultrastructural observations and uranaffin reaction) in 0.067 M cacodylate buffer (pH 7.4) under sodium pentobarbital anaesthesia. After perfusion the superior cervical ganglia were dissected out and immersed in the same fixative for an additional 4 h. SIF cell groups were identified by fluorescence microscopy of 50 µm sections from the superior cervical ganglia cut on an Oxford vibratome. Areas containing SIF cell groups were then excised and stored in cacodylate buffer for further processing.

(A) Conventional Electron Microscopy (Grillo et al., 1974)

Thick sections were then postfixed in 1% aqueous osmium tetroxide for 1 h, stained *en bloc* with 0.5% uranyl acetate for 30 min, dehydrated in alcohol and then embedded in Epon-Araldite mixture.

(B) Modified Chromaffin Reaction (Lever et al., 1974; Santer et al., 1975)

Thick sections containing SGC cell areas were fixed in 3% glutaraldehyde in cacodylate buffer for an additional 4 to 6 h, rinsed twice briefly in cacodylate

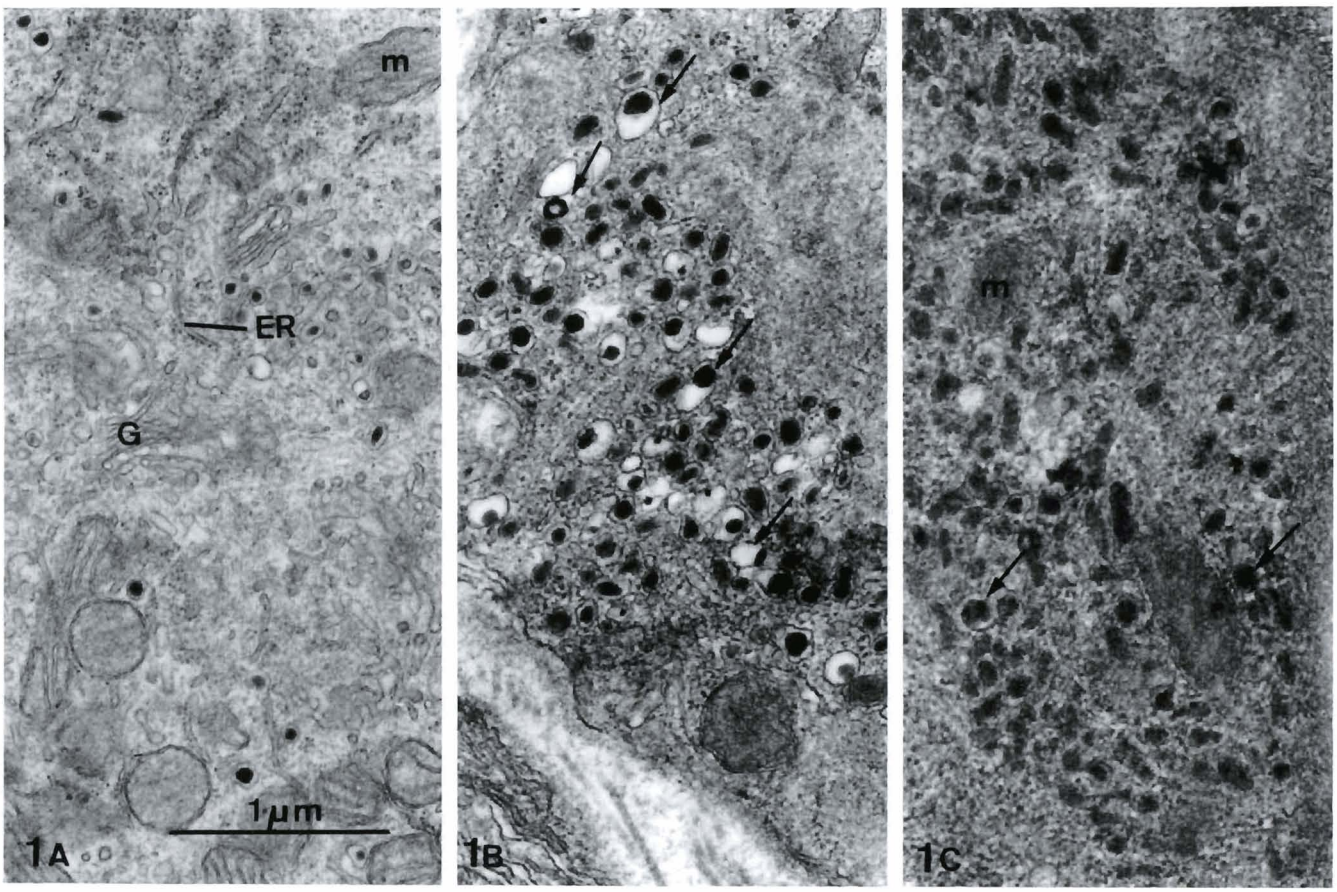

Fig. 1. Electron micrographs of SGC cells in rat superior cervical ganglia illustrating ultrastructural difference between the cytoplasmic DCVs suggesting a multiplicity of SGC cells. All magnifications x 30,000. In Fig. 1A (Type I) DCVs are moderately electron dense, rounded and of 90-160 nm in diameter; in Fig. 1B (Type II) DCVs are polymorphic, more electron dense and larger, 130 - 330 nm, arrows indicate some irregular profiles of DCVs; in Fig. 1C DCVs (Type III) moderately electron dense and predominantly oblong, 170 (100 - 230 nm long axis) x 60 (55-90 nm short axis) nm. Note the occasional rounded profiles (arrows) in Fig. 1C. ER: endoplasmic reticulum, G: Golgi complex, m: mitochondria.

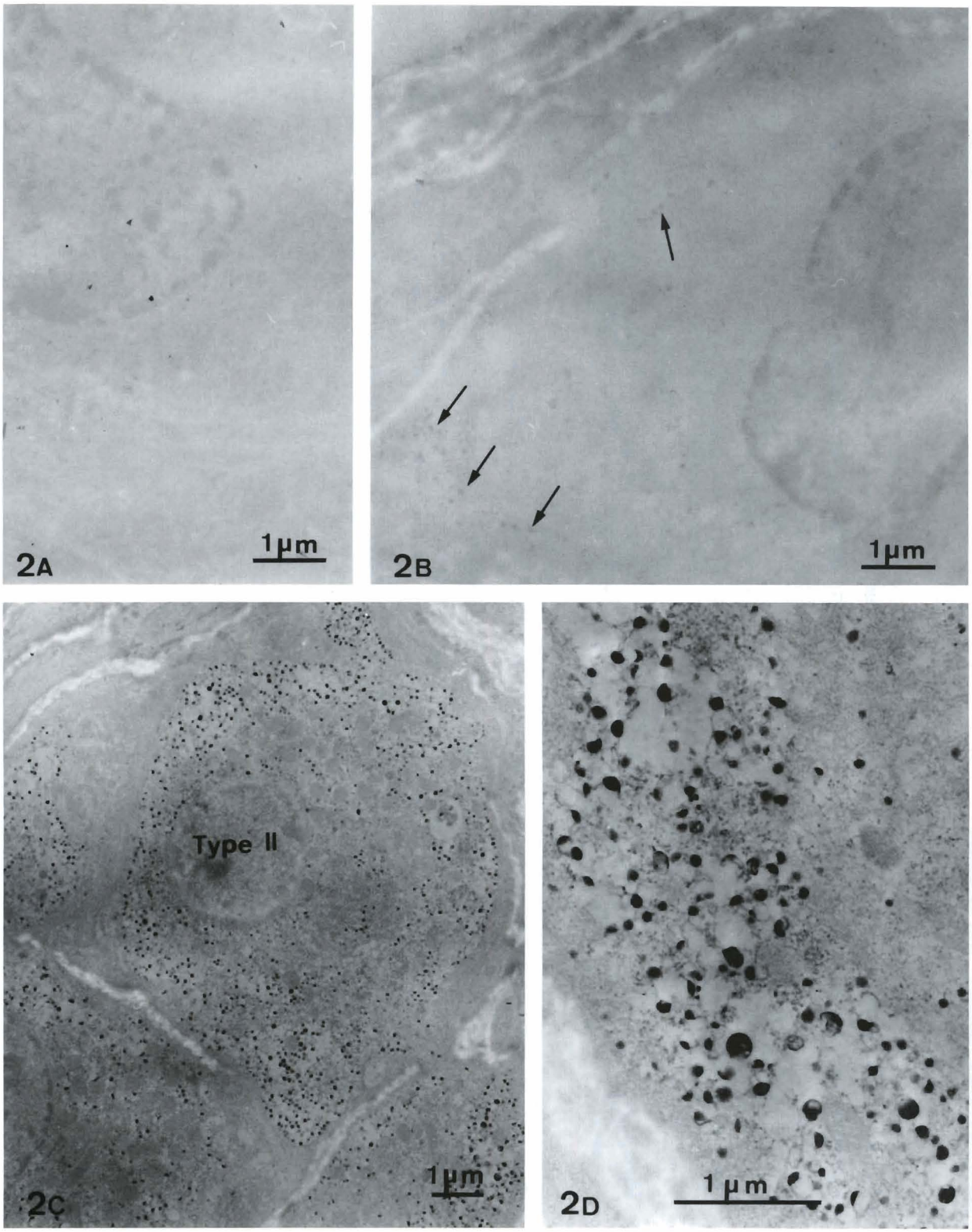

Fig. 2. Modified chromaffin reaction, SGC cells in rat superior cervical ganglia. In nonchromated control sections (Fig. 2A) only very faintly stained nucleus can be detected and all the other organelles are hardly visible. x 12,000. In chromated specimens some nonchromaffin SGC cells as shown in Fig. 2B, which is similar to control sections, may be type I or type III SGC cell. Arrows indicate the DCVs. x 12,000. Compare to Figs. 2A and 2B, chromaffin-positive Type II cells are readily distinguishable at lower power in chromated sections as shown in Fig. 2C. x 8,000. Fig. 2D shows strong chromaffinity of the dense core in the characteristic DCVs of Type II cells at higher magnification. x 24,000

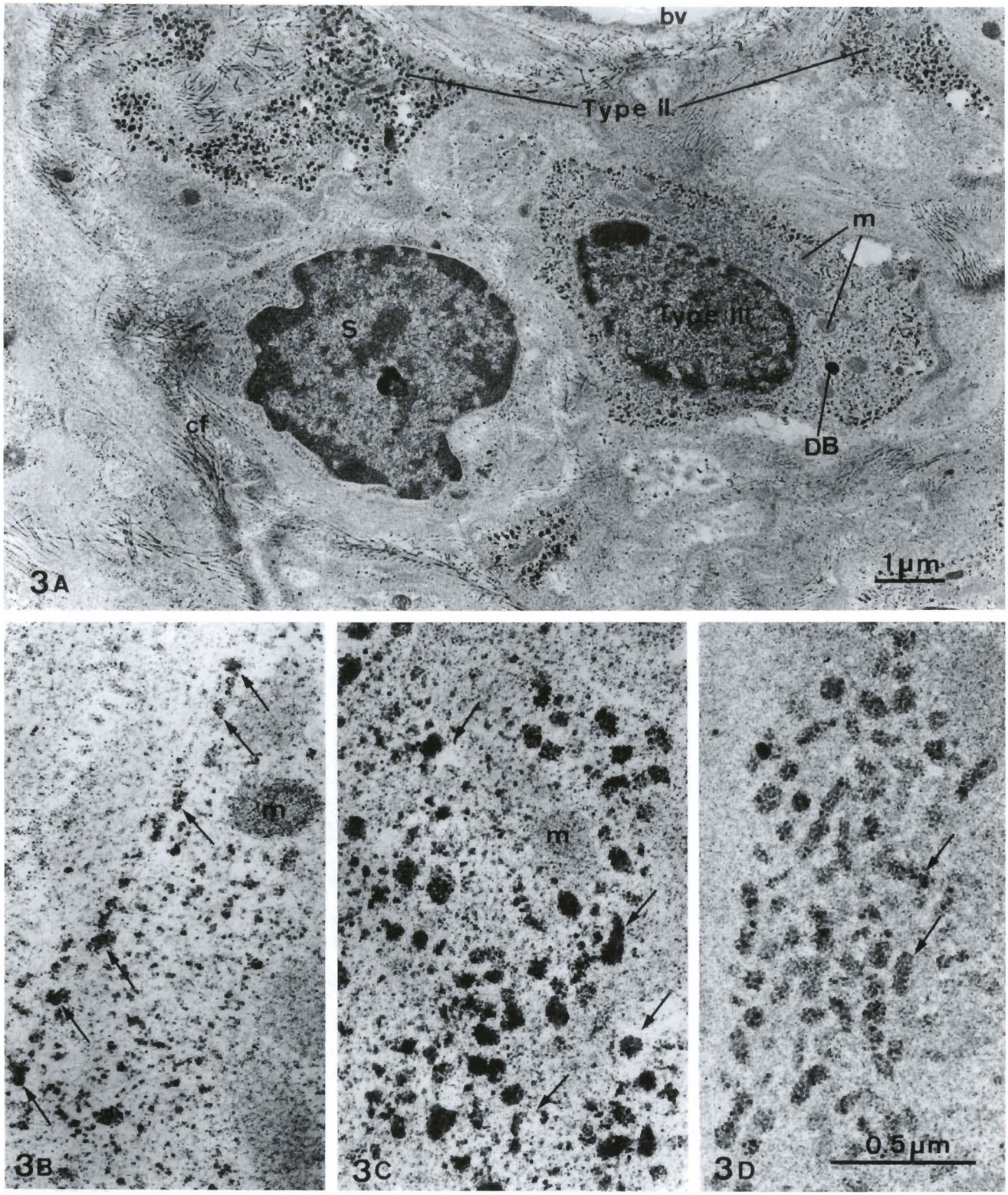

Fig. 3. Argentaffin reaction. Fig. 3A is a lower power electron micrograph showing the argentaffinity of the SGC cells in the rat superior cervical ganglia. Silver precipitates are selectively over the nucleus, mitochondria (m), dense body (DB) and the DCVs. Note the peripheral margination of the DCVs in Type III (Type III) cell. Randomly distributed DCVs in some profiles of the cytoplasm of Type II cells (Type II) are also seen at the upper right and left corners. No argentaffin DCVs are seen over the cytoplasm of Schwann cell (S). Cf: collagen fibrils. x 12,000. Figs. 3B - D, arrows indicate the selective argentaffinity over the specific types of DCVs (arrows) in various types of SGC cell. m: mitochondria x 50,000

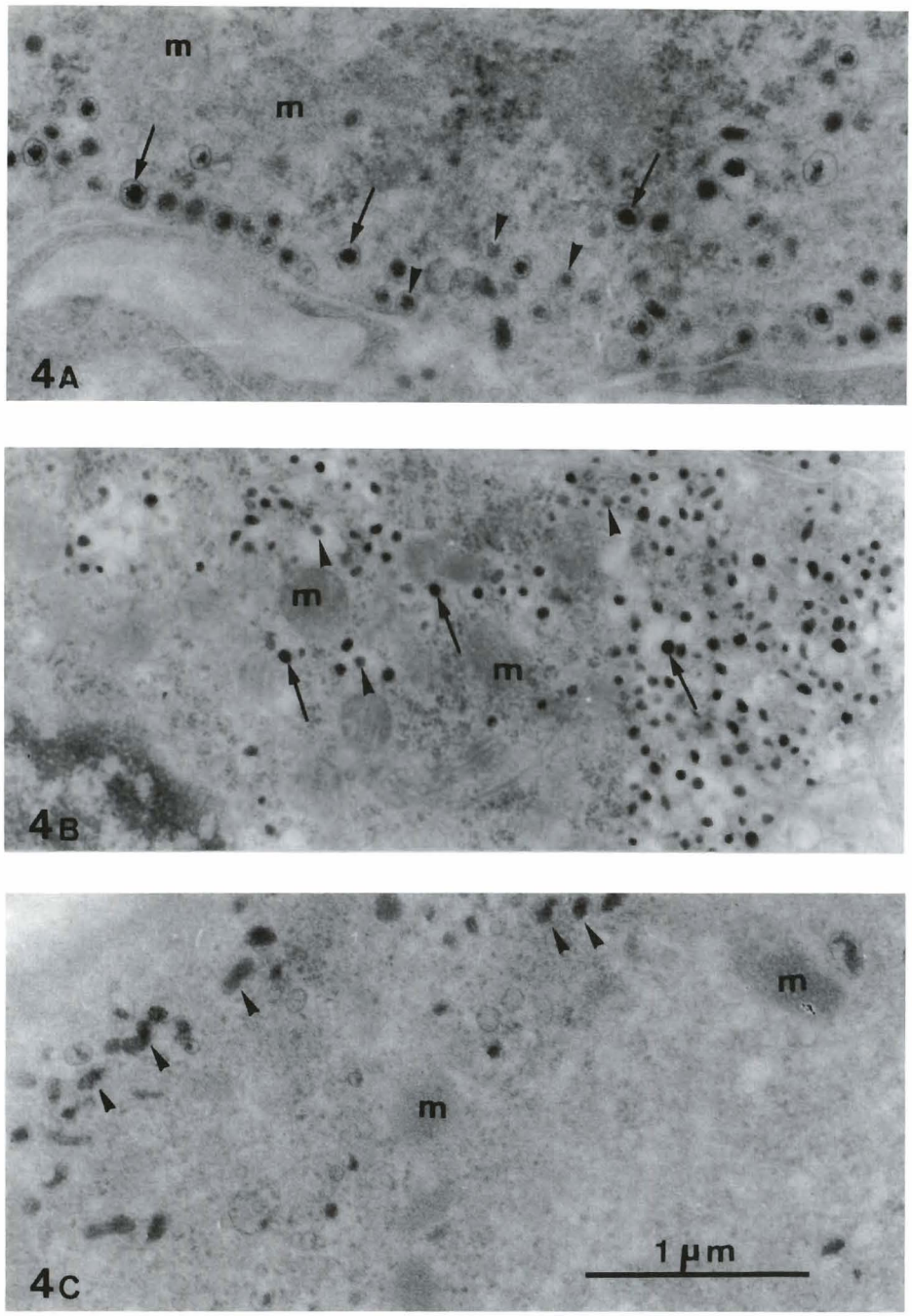

Fig. 4. Uranaffin reaction of SGC cells in rat superior cervical ganglia. In Figs. 4A - C, selective binding of uranium ions to DCVs, ribosomes and nuclear chromatin is evident and mitochondria (m) remain unstained. Arrows indicate various types of DCVs in each type of SGC cells showing strong uranaffinity while the arrowheads indicate the weakly-reacted DCVs. x 30,000

buffer and then treated with 2.5% sodium dichromate for 18 to 24 h. After thoroughly wash with buffer, the blocks were dehydrated and embedded in epoxy resin without osmication. Control sections were obtained from some blocks which, after completion of aldehyde fixation, were dehydrated and embedded as described above without chromation.

(C) Argentaffin Reaction (Singh, 1964; Håkånson et al., 1971)

Thick sections containing SGC cells were fixed in 2.5% glutaraldehyde for additional 4 h in 0.2 M sodium cacodylate buffer at room temperature. The blocks were briefly washed in buffer, then dehydrated and embedded in epoxy resin without osmication. Thin sections mounted on the nickel grids were floated on Fontana's silver solution for 1 to 4 h at 60°C and then were throughly washed. The ammoniacal silver nitrate solution of Fontana was prepared in the following way: strong ammonia solution was added dropwise to a 10% silver nitrate solution until the precipitation first formed was re-dissolved. Fresh silver nitrate solution was then added until a slight opalescence appeared and persisted. To each volume of this solution, 9 volumes of re-distilled water was added.

(D) Uranaffin reaction (Richards and DaPrada, 1977)

After fixation, the thick sections were washed thoroughly with 0.85% sodium chloride and then treated with 4% uranyl acetate aqueous solution for 18 h at room temperature. After thoroughly washing in 0.85% sodium chloride, the blocks were dehydrated and then embedded in epoxy resin without further treatment. Thin sections obtained from blocks without uranium-treatment were served as controls.

Semithin sections from all blocks cut and stained with 1% toluidine blue for correlative study by light microscopy. Thin sections were either doubly stained with uranyl acetate and lead citrate (for conventional electron microscopy) or unstained (for all cytochemical studies) before examination in a Hitachi-HU12A electron microscope.

Results

(A) Ultrastructure (Fig. 1)

Three types of SGC cells were observed in rat superior cervical ganglia (Fig. 1).

Type I SGC cells (Fig. 1A) were characterized by the presence of numerous round DCVs 90 to 160 nm in diameter, in which a centrally located core of moderate to low electron density was observed. The intracellular distribution of the DCVs was random throughout the cytoplasm is highly granulated Type I cells but showed a characteristic peripheral margination in Type I cells of low granularity.

Type II SGC cells (Fig. 1B) were characterized by numerous randomly distributed and highly electron-dense, polymorphic DCVs whose limiting membranes ranged in diameter from 130 - 330 nm. The core of these DCVs was centrally or eccentrically located and consistently more electron dense than that of Type I and III cells.

Type III SGC cells (Fig 1C) were characterized by a population of DCVs of variable moderate to low electron density which appeared oblong or circular in profile. The average dimensions of these DCVs were 170 nm x 60 nm.

The distribution of three types of SGC cells was quantified by counting totally 129 SGC cells from 15 blocks of 7 rats and the results showed that the majority (78%) of SGC cells in rat superior cervical ganglia were the Type I cells. Type II (13%) and Type III (9%) SGC cells made up the rest of the SGC cell population.

(B) Modified chromaffin reaction (Fig. 2)

In sections from control blocks (with chromation, osmication and staining omitted), DCVs and other organelles were hardly visible and only nuclei were visible in all types of SGC cells in rat superior cervical ganglia (Fig. 2A). After chromation but without any electron staining, only the DCVs in type II cells were identified (Fig. 2C). Fine metallic precipitates of chromium ions were deposited homogeneously over the dense core of the DCVs (Fig. 2D). Type I or type III cells showed little or no chromaffinity in their cytoplasm and the electron micrograph of these cells (Fig. 2B) was similar to the controls.

(C) Argentaffin reaction (Fig. 3)

All three types of SGC cells in the sections treated with silver ammoniacal silver nitrate solution showed a positive argentaffin reaction (Fig. 3A). Heavy silver precipitates selectively accumulated over the heterochromatin, some dense bodies, mitochondria and the DCVs in all types of SGC cells (Figs. 3B - D). Prolonged incubation times gave a weak background staining in the form of fine silver grains diffusely scattered throughout the cytoplasm.

(D) Uranaffin reaction (Fig. 4)

Specific uranaffin reactivity was clearly demonstrated by a significantly enhanced electron density of chromatin, ribosomes and specific amine-storing DCVs in all three types of SGC cells (Figs. 4A - C). In control sections, however, only nuclear heterochromatin was faintly stained and therefore, all three types of SGC cells were hardly visible. After uranaffin reaction the SGC cells were readily distinguished from the other cells in the thin sections. It is clear from figures 4A - 4C that variation in the uranaffin reactivity of DCVs was significant in each individual SGC cell and/or between different types of SGC cells: the DCVs in Type II cells showed the strongest while in Type III cells the weakest reactivity toward the uranaffin reaction.

Discussion

Fine structure

Based on the fine structure of DCVs in the cytoplasm, three types of SGC cells have been reported in rat superior cervical and coeliac-mesenteric ganglia (Lu et al., 1976; Hsiao and Lu, 1982). Our present data confirm this previous findings of a multiplicity of SGC cells in sympathetic ganglia (Böck, 1982; Taxi et al., 1983). This multiplicity suggests that different types of SGC cells might store different biogenic amines and play different roles within the ganglia. However, the exact functional significance for each type of SGC cells in sympathetic ganglia remains an enigma.

Lu (1976) and Lu et al. (1976) reported that of 59 individual SGC cells in the superior cervical ganglia of Wistar rats, the majority (93%) were the Type I cells and that Type II and Type III cells made up only 7% of the SGC cell population. Different data were obtained from the quantitative analysis on the distribution of SGC cells in the present study (78% vs. 93%, 22% [13% + 9%]vs. 7% for Type I and [II and III] cells respectively). This variation may be due to the difference in animal strain (white Wistar vs. Long-Evans) or other factors.

Chromaffin Reaction

When applying the chromaffin reaction at light microscopic level in studies on sympathetic ganglia, contradictory results have often been obtained: the chromaffinity of small parenchymal cells (equivalent to SGC cells at ultrastructural level) varies greatly not only in the same ganglia from different or same species but also differs from cell to cell within same cluster of small parenchymal cells (Lever et al., 1974; Santer et al., 1975). The small cells within the same ganglia can be found alternating to be chromaffin or non-chromaffin (Santer et al., 1975; Böck, 1982). Species variations and functional variations between different small cells of

the same ganglia can also account for different results on chromaffinity.

Lu (1976) demonstrated that the noradrenaline-containing cells in the rat adrenal medulla and the Type II cells in rat superior cervical ganglia and coeliac-mesenteric ganglion complex showed chromaffinity at light and electron microscopic level by this modified chromaffin reaction. Subsequently Lever et al. (1977) and Santer et al. (1978) proposed that the Type II SGC cells may contain noradrenaline. This speculation is further supported by X-ray micropreobe analysis. Our results in the present study that only Type II SGC cells in rat superior cervical ganglia displayed chromaffinity at electron microscopic level are in accord with these previous findings and therefore, we assumed that our Type II SGC cells also contain a high concentration of noradrenaline.

Argentaffin Reaction

The present investigation is the first to study the argentaffinity of SGC cells in sympathetic ganglia at ultrastructual level. Argentaffin reaction is a traditional cytochemical reaction for demonstration of biogenic amines and can be employed for ultrathin sections (Häkänson et al., 1971). However, this reaction has never been aplied to the SGC cell in the sympathetic ganglia at ultrastructural level. This reaction is very frequently used in the study of enterochromaffin cells and other amine-containing cells and is very useful clinically for the diagnosis of small cell-derived carcinoma (Inokuchi et al., 1983; Lundqvist and Wilander, 1984; Portela-Gomeo et al., 1987; Watanabe et al., 1987; Shaw, 1989).

Kobayashi et al. (1978) demonstrated argentaffin substances in the SGC cells in mouse adrenal medulla and their results showed that not only the usual large dense cored vesicles but also the small synaptic vesicles were found to react. Although argentaffin reaction does not permit a distinct differentiation between various types of biogenic amines including catecholamine and indoleamines, most investigators assent to the view that argentaffin reaction in certain neurons and endocrine cell systems reflects a high concentration of a highly reducing compound such as catecholamine or 5-hydroxytryptamine (5-HT) (Inokuchi et al., 1983; Lundqvist and Wilander, 1984; Watanabe et al., 1987). The reaction mechanism of the argentaffin reaction has been investigated by Cannata et al. (1968) and according to their interpretation the terminal aminogroup ($-NH_2$) in the catecholamine or indoleamine is responsible for the reduction of silver. The present findings that small granules similar in size and distribution to the DCVs in all three types of SGC cells displayed a positive argentaffin reaction suggest that the DCVs in SGC cells may contain catecholamines or indoleamines.

Uranaffin Reaction

Tranzer (1971) observed that the amine storing organelles of plates became intensely stained with uranyl acetate when the reaction was carried out after glutaraldehyde fixation but before dehydration. Richards and DaPrada (1977) postulated the uranaffin reaction as an ultracytochemical procedures for localization of adenine nucleotides in organelles storing biogenic amines. It is now well established that uranyl ions had an extremely high affinity ATP- or ADP-rich organelles in megakaryocyte, adrenomedullary cells, sympathetic ganglion cells, SIF cells and adrenergic nerves (Richards and DaPrada, 1980; Richards, 1983). Payne et al. (1984) and Payne and Cromey (1987) applied this reaction to the neurosecretory granules in normal, non-neoplastic and neoplastic human tissue and the neurosecretory granules within the epidermis and gastrodermis of Hydra. They emphasized that the uranaffin reaction is considered to identify a nucleotide elements (ATP, ADP and AMP) of packaging structure within neurosecretory granules and not any specific product. They further proposed that protein-nucleotide-neurohormone (or -neurotransmitter) complexes are formed within the neurosecretory granules and these complexes may represent a highly efficient and basic mechanism of neurohormone (or neurotransmitter) packaging and storage.

Richards and DaPrada (1980) demonstrated the uranaffin reaction in only one of SGC cells (=SIF cells) in rat superior cervical ganglia in Figure 11 of their paper. The present study herein not only confirmed their findings but also reported the uranaffin reaction in all three types of SGC cells in rat superior cervical ganglia. Moreover, different reactive intensity of uranaffin reaction was noted in the present study (Fig. 4), we suggest this difference in the reactive intensity may reflect the quantity and/or quality of the nucleotides storing in different types of amine-storing dense-cored vesicles in SGC cells.

From the results of the present study that the DCVs exhibited both uranaffinity and argentaffinity, we confirmed that biogenic amines and nucleotides (ATPs) co-store in the DCVs of all three types of SGC cells in rat superior cervical ganglia. We also confirmed that the Type II SGC cells in rat superior cervical ganglia contain noradrenaline because these cells display their unique chromaffinity by this modified chromaffin reaction, although we were unable to identify the exact biogenic amines in other types of SGC cells in the present investigation.

In summary, we demonstrated (1) the presence of three types of SGC cells and (2) the argentaffinity and uranaffinity in all three types of SGC cells and the chromaffinity only in Type II SGC cells, and we confirmed (3) the coexistence of biogenic amines and nucleotides (ATPs) in the DCVs of SGC cells and (4) the presence of noradrenaline in the Type II SGC cells in rat superior cervical ganglia.

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