Summary. A second population of cytoplasmic microvesicles was constantly recognized in the SGC (small granule chromaffin) cells of the mouse adrenal medulla by means of transmission electron microscopy in glutaraldehyde/osmium tetroxide-fixed material. The microvesicles were rounded in shape and of mean profile diameter of between 30, 40 nm; some contained several dense precipitates. The vesicles were usually dispersed throughout the cytoplasm among the typical secretory granules of 100-230 nm in profile diameter, though they occasionally formed aggregations. The SGC cells were also characterized by a high nucleus to cytoplasm ratio, rich innervation, and long cytoplasmic processes which were traced up to 30 μm.

Co-existence of the synaptic-like vesicles and secretory granules in the SGC cells suggests that they may represent an intermediate position between the chromaffin and sympathetic nerve cells.

Based on his light microscopic investigation in some mammalian species including the mouse, BÄNDER (1950) distinguished two kinds of chromaffin cells which corresponded to adrenaline-storing (A) cells and noradrenaline-storing (NA) cells in the adrenal medulla. This was confirmed by electron microscopists when WOOD and BARNETT (1964) in glutaraldehyde/dichromate-fixed material and COUPLAND, PYPER and Hopwood (1964) in glutaraldehyde/osmium tetroxide-fixed material reported that the NA cells possessed membrane-bound secretory granules with a solid and dense core, while those of the A cells had a finely-granulated and light core. However, it has been still open to question whether the adrenal medulla contains more than two kinds of chromaffin cells.

With this point in mind, we recently investigated the fine structure of the chromaffin cells in the mouse adrenal medulla by electron microscopy, and it was found that a third type of chromaffin cell was distinguishable. The cell was characterized by the smallness of its secretory granules, hence called the SGC (small granule chromaffin) cell. We further found that the SGC cell contained peculiar synaptic vesicle-like structures in addition to the known secretory granules. The purpose of the present paper is to report the fine structure of the SGC cells with particular reference to the synaptic-like vesicles and to discuss the nature and significance of the SGC cell.

Materials and Methods
Adult, male mice of dd-strain were used in the present study. Under ether
anesthesia, the whole animal body was perfused through the left ventricle of the heart for several minutes with 2.5% glutaraldehyde in a 0.1 M phosphate buffer of pH 7.2. Ten to 30 min later, adrenal glands were removed and further fixed in the same fixative for several hours and then post-fixed in a 1.0% osmium tetroxide solution. After dehydration through a graded ethanol series and treatment with propylene oxide, the fixed adrenal glands were embedded into Epon 812. Thin sections of silver to gold interference color were stained with both 0.5% uranylacetate and Millonig's lead and then observed with a Hitachi HU 125ds electron microscope.

Measurement of the vesicle diameters was carried out using a morphometric analyser, Kontron MOP/AM Ol-System (a semi-automatic count- and storage unit for quantitative image analyzing), on the electron micrographs at a final magnification of ×56,000. The vesicle diameter meant the straight line, drawn in a random direction, passing from one side to another side of the limiting membrane through the central area of each microvesicle. It should be mentioned that the resolution of the measuring system was not better than 0.25 mm on the photograph.

Observations

Extensive studies of previous electron microscopists have revealed the fine structure of the adrenal chromaffin cells of various vertebrate species (for reference, see Coupland, 1972; Grynszpan-Winograd, 1975). Chromaffin cells of the mouse adrenal medulla have been classified into two types. The predominant ones were A cells possessing, as in other mammalian species, rounded secretory granules with fine-granular and moderately-osmiophilic contents. The second type, NA cells, occupied less than 30 percent of the total population and were characterized by the membrane-bound secretory granules of irregular shape which contained an intensely-osmiophilic substance.

In addition to these two types of chromaffin cells, we distinguished in the present study a third type of chromaffin cells which are tentatively called SGC (small granule chromaffin) cells. The most distinctive feature of the SGC cells was the smallness of the secretory granules which tended to line the cell membrane. The content of the secretory granules in the SGC cells was intermediate in density between that in the A cells and that in the NA cells. No fundamental differences were noticed among the three types of chromaffin cells with regard to the fine structure and distribution of the nucleus, mitochondria, free ribosomes and granular endoplasmic reticulum, microtubules, Golgi complex and lysosomes. The cytoplasm to nucleus ratio was much smaller in the SGC cells than that in the other types of chromaffin cells.

SGC cells in the mouse were most frequently located in the zone of the adrenal medulla near the cortico-medullary junction. In this zone, the SGC cells were usually seen in the periphery of the NA cell groups. The SGC cells existed either as a single cell between other kinds of chromaffin cells or in small groups. Careful visual observations gave us the impression that the SGC cells accounted for less than 4 percent of the whole chromaffin cell.

Figure 1 illustrates the size distribution of the secretory granules in the A, NA and SGC cells. It shows that the secretory granules in the SGC cells are remarkably smaller than those of the A and NA cells. The usual size of the secretory granules in the SGC cells was 100 to 230 nm in profile diameter, while those in the A and NA cells were 170 to 350 nm and 185 to 495 nm in profile diameter, respectively. The variation
in size, shape and density of the content was noted within different secretory granules in a single SGC cell.

As shown in Figures 5-A, B, C, the SGC cells contained, in addition to the above-mentioned secretory granules, a smaller population of membrane-bound cytoplasmic vesicles. They measured 40 to 70 nm in profile diameter forming an independent group of vesicular structures (Fig. 2) and contained two to several dense asymmetrically located precipitates as illustrated in Figures 5-B, C. These synaptic-like vesicles were scattered among the large secretory granules throughout the cytoplasm (Fig. 5-B), but frequently formed clusters (Fig. 5-A, C). Thus, the distribution of the large secretory granules did not always correspond to that of the synaptic-like vesicles.

The SGC cells had one or more cytoplasmic processes of various thickness and length (Fig. 3, 4). These cytoplasmic processes may reach up to 30 μm in length and run along the periphery of an islet of NA cells and of the A cell group, in the space between chromaffin cells and satellite cells, and along the non-myelinated nerve fibers. The fine structures in the cytoplasmic processes, including those of the synaptic-like vesicles and secretory granules, were similar to those in the paranuclear cytoplasm, except that the Golgi complex was seen only in the latter.

The SGC cells were partly surrounded by satellite cells showing the same relationship to these as A and NA cells. The area of the surface directly facing the connective tissue space was covered by the basal lamina. Many profiles of non-myelinated axons and nerve endings were located around the SGC cells. Nerve endings which were partially embraced by satellite cells were frequently in direct contact with the SGC cells. Asymmetrical membrane thickenings were formed between the
Fig. 3. Montage of 4 electron micrographs showing a long cytoplasmic process of an SGC cell (SGC). The extent of the cell is outlined. The two small profiles of the granule-containing cytoplasm seen on the top of the micrograph possibly belong to the same SGC cell. One of them is in direct contact with a nerve ending (ne). A A cell, NA NA cell, a non-myelinated axons. Notice the difference in the size of the secretory granules of the three kinds of chromaffin cells. Compare also the density of the content of the secretory granules in these chromaffin cells. This electron micrograph was unexpectedly obtained from an autoradiogram of the mouse adrenal medulla prepared after 3H-dopamine injection. The black figure indicated by the arrow is a specific silver grain due to 3H-dopamine. × 9,000
Fig. 4. An electron micrograph illustrating the nucleus and perinuclear cytoplasm of an SGC cell, satellite cell (S) enclosing both axons (a) and cytoplasmic processes (p) of the SGC cell, thin capillary endothelial cell (E) and profiles of connective tissue cells in the pericapillary space. ×17,000

Inset: An exocytotic figure (e), possible gap junctions (g) and interdigitations (i) seen between two SGC cells. ×20,000
Fig. 5.  **A.** A portion of an SGC cell showing a cytoplasmic process containing both synaptic-like vesicles and secretory granules. An aggregation of small synaptic-like vesicles is seen in the thin cytoplasmic process. × 33,000  **B.** A portion of the perinuclear cytoplasm of the SGC cell showing the fine structure of the two populations of the cytoplasmic vesicles. × 52,000  **C.** A higher magnification picture of the cytoplasmic process of the SGC cell shown in A. Notice that the synaptic-like vesicles contain dense precipitations (arrowhead). × 52,000
SGC cells and nerve terminals (Fig. 6). Small synaptic vesicles were accumulated on the nerve side at a location associated with the membrane thickening. No conspicuous assemblies of synaptic-like vesicles and membrane thickening were demonstrated on the side of the SGC cell. Symmetrical membrane thickenings (possible gap junctions) and interdigitations were seen between the SGC cells and the adjoining chromaffin cells (Fig. 4, inset). A single cilium associated with a deep indentation of the cell surface was often seen. Coated pits and occasional exocytotic invaginations were seen on the plasma membrane (Fig. 4, inset).

Discussion

The presence of synaptic-like vesicles, in addition to the large-cored vesicles, has been reported in many neurosecretory cells such as those in the neurohypophysis (Douglas, Nagasawa and Schulz, 1971) in the SIF (small intensely fluorescent) cells in sympathetic ganglia (Williams, 1967), and in the chief cells of the carotid body (Kobayashi and Uehara, 1970). Furthermore, Unsicker (1976) found in the tortoise adrenal gland that the cell process with adrenaline containing-granules sometimes included aggregations of synaptic-like vesicles.

Among the synaptic or synatic-like vesicles mentioned above, those in the chief cells of the carotid body contain dense inclusions (Kobayashi and Uehara, 1970) whereas those in the neurosecretory cells in the posterior pituitary gland (Douglas, Nagasawa and Schulz, 1971) and tortoise adrenal gland (Unsicker, 1976) are devoid of visible content in the published electron micrographs. The synaptic-like vesicles seen in the SGC cells of the mouse adrenal medulla contained several dense precipitations. Furthermore, the size and shape of these microvesicles are wholly comparable to
those of the SIF cells in the sympathetic ganglia and the chief cells of the carotid body.

In the SIF cells in the sympathetic ganglia and chief cells of the carotid body, the small vesicles tend to accumulate immediately beneath the asymmetrical membrane thickenings between the cell and the nerve ending (WILLIAMS, 1967; KOBAYASHI and UEHARA, 1970). Therefore, it has been suggested that the small vesicles are involved in the transmission of signals from the cell to the nerve ending (KOBAYASHI and UEHARA, 1970). However, in the SGC cells of the mouse adrenal, the occurrence of the synaptic-like vesicles is not restricted to the subsynaptic area of the cytoplasm. Therefore, it seems unlikely that they play a role in the synaptic transmission.

The presence of two populations of microvesicles is well-known with respect to cholinergic and adrenergic nerve terminals (for reference, see PETERS, PALAY and WEBSTER, 1976). The small population of microvesicles seen in the adrenal SGC cells bear some resemblance to the appearance of the synaptic vesicles in the adrenergic nerve terminals, though the latter usually possess only a single electron dense inclusion and these are usually paracentral in position. Further studies are needed to establish whether the synaptic-like vesicles in the SGC cell contain catecholamines.

As is widely accepted, the adrenal chromaffin cells and sympathetic ganglion cells are derived from the same precursor of the neural crest origin (COUPLAND, 1965). Thus, it might be reasonable to assume that every transitional type is possible between adrenal chromaffin cells and sympathetic ganglion cells. Although the real function of the SGC cells in the mouse adrenal medulla is still open to question, it is tempting to suggest, on the basis of the occurrence of the characteristic synaptic-like vesicles together with the dense-cored secretory granules, that the SGC cells occupy an intermediate position between the chromaffin cells of purely endocrine nature and adrenergic nerve cells. The occurrence of cytoplasmic processes of considerable length revealed in the present study seems to strengthen this view.

**Addendum.** After this manuscript was processed to the press, a publication by K. UNSICKER and J. H. CHAMLEY (Cell Tiss. Res. 177: 247-268, 1977) arrived. In addition to the ordinary “chromaffin” granules, they demonstrated small, clear and dense-cored vesicles (40-60 nm) both in the somata and processes of the cultured rat chromaffin cells. These vesicles in cultured rat cells apparently correspond to the synaptic-like vesicles in mouse SGC cells reported in the present paper.
SGC Cell of the Mouse Adrenal Medulla

SGC 細胞は、従来知られていた 2 種類のクロム親和細胞 (A と NA) と 交感神経節細胞との 中間に分類されるべき細胞と思われる。

References


