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Evidence for Basal Secretion in the Subcommissural Organ of the Adult Rabbit*

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Summary. Morphological evidence is presented supporting the possibility of basal secretion into hypendymal capillaries of the adult rabbit subcommissural organ (SCO). The synthetic apparatus of the SCO cell is described as well as the heterogeneous granules and vesicles which are concentrated in the basal processes bordering a widened perivascular space. The origin of the electron dense granules, of which two fairly distinct subgroups are found, is discussed.

A binding of secretory sacs to the lateral plasma membrane is seen. The possibility of a lateral secretion is supported by the presence of a system of extracellular channels between SCO cells which are filled with a flocculent material resembling that of the secretory sacs.

Nerve perikarya which are separated from the SCO by only a few glial fibers are demonstrated. Synapses are described in nerve fascicles bordering on the hypendymal capillaries. The possibility of an innervation of the hypendymal region is discussed as well as possible nervous connections with the pineal gland.

Key words: Subcommissural organ (SCO) — Basal secretion, Lateral secretion — Extracellular spaces (ECS) — Innervation — Electron microscopy.

Introduction

The subcommissural organ (SCO) is a member of a group of specialized areas of the ependyma situated in various parts of the IIIrd and IVth ventricles. These areas are collectively called circumventricular organs. The SCO consists of two parts: 1) a pseudo-stratified or stratified layer of high columnar ependymal cells covering the anterior and inferior surfaces of the posterior commissure and extending from the pineal recess in the third ventricle to the mesocoelic recess in the cerebral aqueduct, and 2) a hypendyma consisting of glial cells, SCO cells, and processes of glial, SCO and nerve cells, which underlies the ependyma throughout the extent of the SCO. The function of the SCO remains unclear. Reviews covering the attempts to establish the function of the SCO include Palkovits (1965), Oksche (1969), and Herrlinger (1970).

The ultrastructure of the SCO has been studied extensively in vertebrates. A few of the more recent and comprehensive include studies of fish (Murakami and Tanizaki, 1966; Stanka, 1967), amphibia (Murakami and Tanizaki, 1963; Rodríguez, 1970a, b), reptiles (Murakami *et al.*, 1970), mammals – rat (Stanka *et al.*, 1964; Schwink and Wetzstein, 1966), guinea pig (Vigh *et al.*, 1967; Papacharalampous *et al.*, 1968), mouse (Herrlinger, 1970). For a more thorough review of the literature, see Herrlinger, 1970.

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The presence of tight junctions between SCO ependymal cells at the apical surface has been recently demonstrated by the use of ruthenium red, perfused intraventricularly *in vivo* (Kimble, Sørensen and Møllgård, 1973). The sealing off of the SCO from the cerebrospinal fluid (CSF) by tight junctions suggests the possibility of a basal secretion as discussed in the aforementioned study. We have, therefore, made a detailed examination of the hypendymal region with particular emphasis on the area in the immediate vicinity of the capillaries. The present study has not only amplified the findings of a previous study on the adult rabbit SCO (Schmidt and D'Agostino, 1966), but has also revealed several features possibly related to the secretory mechanisms involved in the SCO which have not been described before.

Material and Methods

Four male rabbits weighing 2.5–3.0 kg were anaesthetized with Nembutal ® (30 mg/kg), curarized with Gallamonium iodide (Relaxan ®) and artificially respirated with a small animal respirator (Harvard apparatus).

A catheter was placed in the abdominal aorta of two of the rabbits, and the brains were fixed by perfusing a solution containing 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH = 7.4) through the aortic catheter at a constant pressure of 150 mm Hg. In the other two rabbits the ventricular system was perfused from one lateral ventricle to the cisterna magna. The ventriculo-cisternal perfusion was performed according to the method described by Oldendorf and Davson (1967). Intracranial pressure and arterial blood pressure were monitored continuously in order to assure that these parameters remained normal during the ventriculo-cisternal perfusion. The ventricular system was first perfused with mock cerebrospinal fluid (CSF) at a rate of 0.35 cc/min for 1 hour. Thereafter, the brains were fixed by ventriculo-cisternal perfusion with 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH = 7.4). The osmolarities of the above perfusates were as follows: mock CSF, 294 m.osm. and glutaraldehyde + buffer, 526 m.osm.

Following fixation by intra-arterial or ventriculo-cisternal perfusion, the brains were immersed for 8 hours in the fixative. Tissue blocks containing the subcommissural organ were isolated and postfixed for 2 hours in 2% OsO_4 in 0.1 M cacodylate buffer. Specimens were block-stained with uranyl acetate for 1 hour, quickly dehydrated in increasing concentrations of ethanol and embedded in Epon ®. Areas suitable for electron microscopy were localized in 1 micron sections stained with toluidine blue. Silver-to-grey thin sections were stained with uranyl acetate and lead citrate.

Results

The SCO cells are seen both as elongated ependymal cells in contact with the cerebrospinal fluid apically, and as basal or hypendymal cells which are probably detached from the ventricular surface. In addition to SCO cells, astrocytes and nerve cells contribute to the hypendyma.

The endoplasmic reticulum (ER) of the SCO cells is often engorged with a flocculent material, and is very extensive in both apical and basal halves. Ribosomes are most often distributed on its membranes in groups rather than being evenly spaced. The endoplasmic reticulum forms a very complex network of membranes in SCO cells which can appear in several patterns: 1) stacked cisternae studded with ribosomes, 2) concentric lamella, or "Nebenkern"-like structures (Fig. 9), 3) enclosed rings, the ribosomes of which are located on the inner membrane (Fig. 1), 4) sacs exhibiting sparse ribosomes (Fig. 1). Adjacent cisternae

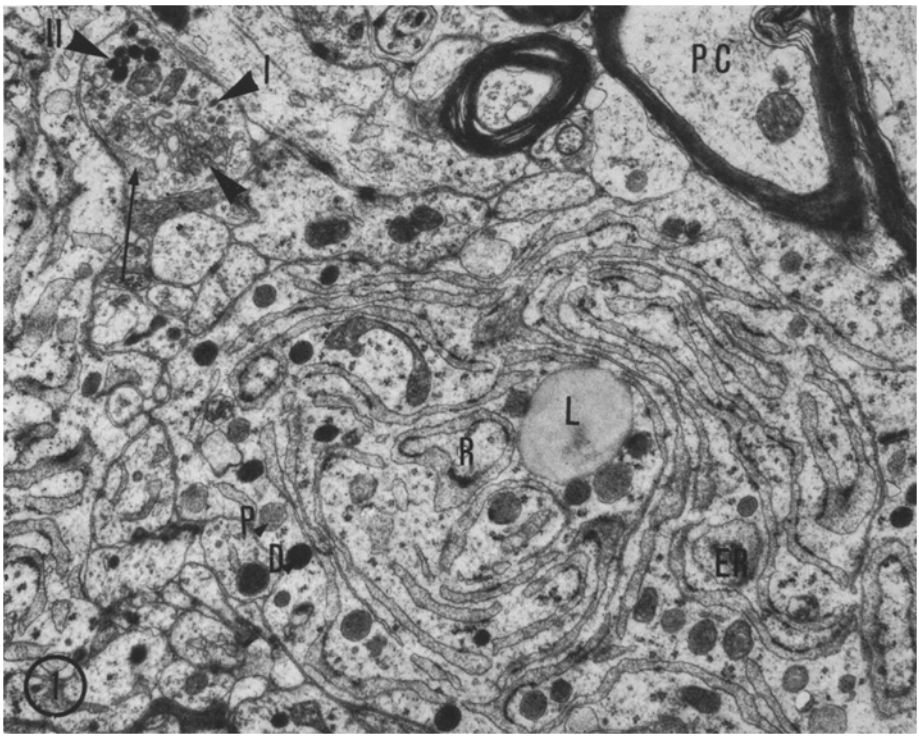


Fig. 1. Section through hypendymal region bordering the posterior commissure (*PC*). The flocculent material-filled cisternae of endoplasmic reticulum (*ER*) exhibit a complex array which encircles a lipid droplet (*L*). A ring (*R*) of ER is probably due to the invagination of a cisterna into itself. In one case a sac of ER can be seen aligned with the plasma membrane (*long arrow*). The difference between granules found in the cell body and those associated with the basal processes can be seen. A basal process exhibits type I (*arrowhead I*), type II (*arrowhead II*), and a cluster of electron lucent vesicles (*arrowhead*), which may be part of a Golgi apparatus. In the cell body, larger dense (*D*) granules are found as well as pale granules (*P*). ×13200

are occasionally flattened against each other with only a dense filamentous network between them (Fig. 2). Greatly enlarged, flocculent material containing sacs may be found in the most basal part of SCO cells. These can exhibit cilia within them, and can show continuity with the extracellular space (Fig. 3). Pale and dense granules can be seen in the vicinity of ER cisternae. The density of the pale granules is approximately the same as that of the material in the ER, and these granules or secretory sacs can sometimes be seen to be continuous with the cisternae of ER (Fig. 3). Lipid droplets can sometimes be encircled by some of the more complex arrays of cisternae.

The Golgi apparatus is usually found apically or perinuclearly, but can also occasionally be seen basally. Often several Golgi profiles are seen within the same cell. Dense bodies with a homogeneous granular content can sometimes be seen budding off the membranes of the Golgi apparatus.

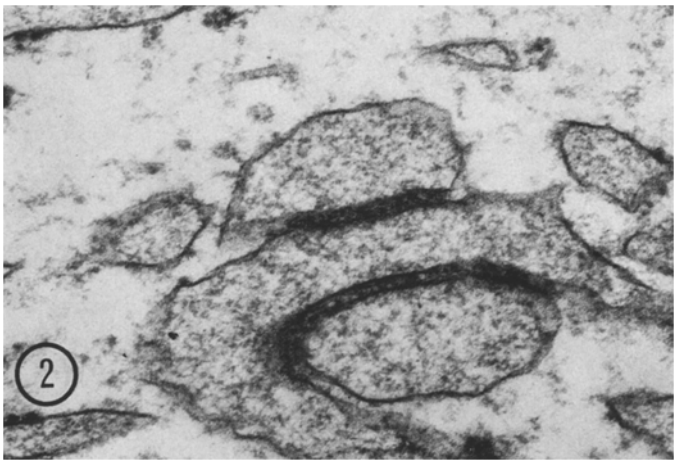


Fig. 2. Cisternae of the endoplasmic reticulum sometimes become flattened against each other, with only a dense filamentous network separating the membranes of the individual sacs. This configuration resembles the lamellar bodies (Herndon, 1964) of neurons. ×57000

The highest concentrations of mitochondria in the SCO cells are observed at the ventricular surface and in the basal processes, although they are found in lower number throughout the cell. A single basal process may contain as many as nine mitochondria and groups of two to three are common.

Microtubules are common elements of the SCO cell both in the apical and basal processes. Microfilaments have not been identified in SCO ependymal cells.

Enlarged, often triangular-shaped extracellular spaces (ECS) are found lateral to the basal half of the SCO cells (Fig. 4). In sections which are cut parallel to the longitudinal axis of the cell, the spaces are most often seen in an elongated form, suggesting that they run along the long axis of the cell. An opening of the ECS onto the perivascular space is seen only rarely, and a basal lamina always separates the ECS from the perivascular space. The plasmalemmata of the cells bordering these spaces often exhibit pinocytotic profiles, and the limits of the spaces are frequently marked by the presence of puncta adherentia-like contacts. The ECS contain a flocculent material of approximately the same density as that seen within the secretory sacs of the endoplasmic reticulum. These secretory sacs are often associated with the spaces, and can be seen attached to the plasma membrane by a series of electron dense particles (Fig. 5A). In one case, the release of the flocculent material from one of the sacs could be visualized (Fig. 5B).

Capillaries, Basal Processes, and Basal Granules. The rich vascular network supplying the SCO is found in the hypendymal region, though occasional branches penetrate into the ependyma. In the rostral half of the SCO the small vessels are characterized by a widened perivascular space exhibiting vascular and parenchymal basal laminae. The parenchymal basal lamina often extends away from the immediate vicinity of the vascular wall, following the borders of a widened,

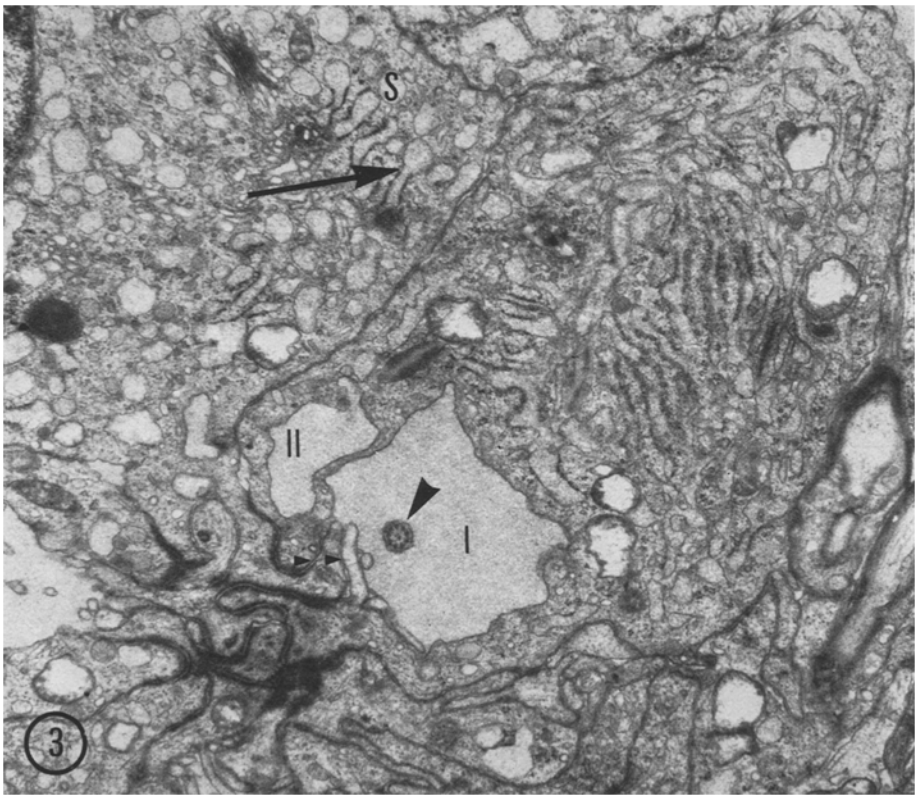


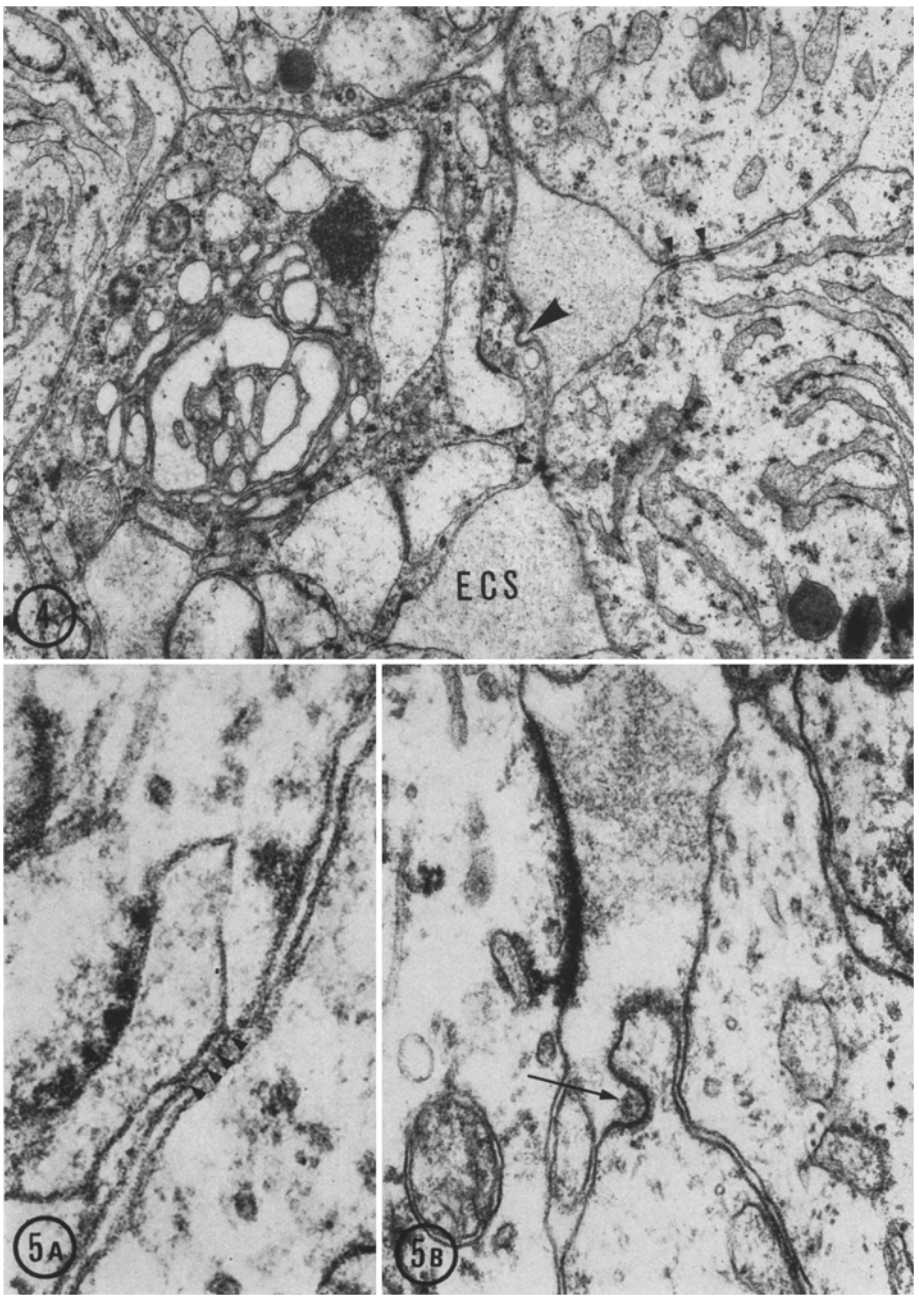
Fig. 3. Two cases (I and II) of the enlarged, flocculent material-filled, basal secretory sacs are shown. One (*I*) contains a cilium (*large arrowhead*) in cross section. It can be seen that the membrane enclosing the sac is continuous with the plasma membrane bordering the cell (*small arrowheads*). It is suggested that this sac is continuous with the extracellular space, and that the cilium found within it has grown out into the space. Note secretory sacs (*S*) and their relation to the endoplasmic reticulum (*arrow*). ×13200

collagen-containing perivascular space which can either take the shape of large outpocketings, or long narrow channels (Fig. 6). The capillary wall itself is always non-fenestrated, but often exhibits numerous pinocytotic vesicles (Fig. 10).

In contrast to the vessels in the rostral part of the SCO, the vessels examined in the caudal part of the SCO show a narrow perivascular space, or a partial fusion of the parenchymal and vascular basal laminae. The partial fusion results in a thicker than normal lamina of irregular density.

The perivascular space is bordered by glial and SCO cell processes. The basal processes of the SCO cells are usually filled with a heterogeneous population of granules and vesicles which, though difficult to characterize, seem to fall into the following major categories:

1) Type I granules (Figs. 1, 6, 7A and B) are small (60–120 nm in width), membrane-bounded dense-cored vesicles exhibiting a halo. The shape of these granules is quite irregular, varying from round to oval to dumbbell-shaped. The



Figs. 4 and 5

Fig. 4. The extracellular space (*ECS*) expands at certain points forming a series of channels which contain a flocculent material. Note the pinocytotic profile (*large arrowhead*) and the puncta adherentia-like contacts (*small arrowheads*) of the membranes bordering the space. ×24600

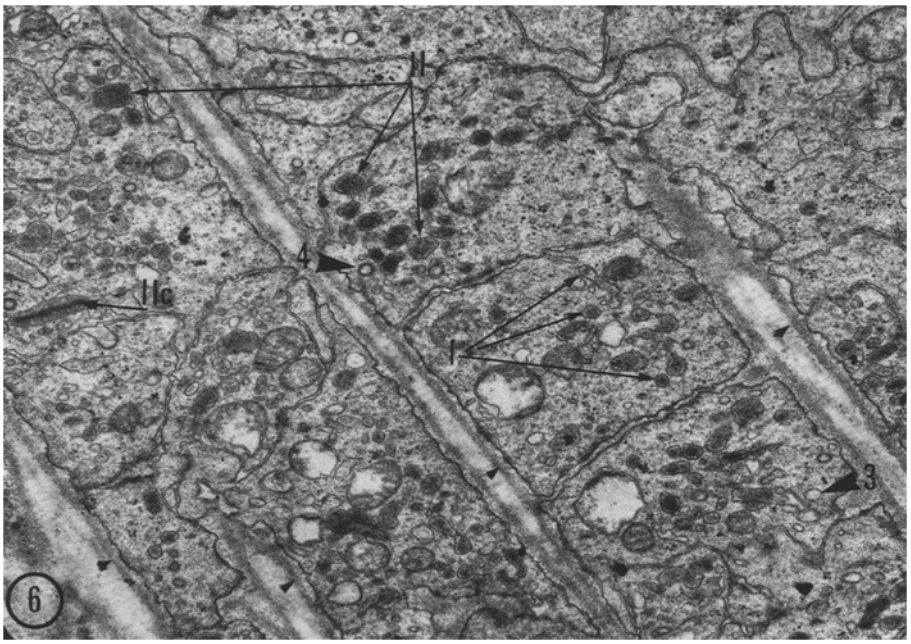


Fig. 6. Narrow channels lined by a parenchymal basal lamina (*small arrowheads*) sometimes extend between basal processes of the SCO, providing a surface on each side of the process for possible secretion. Note the different kind of granules: (*I*) type I electron dense granules, (*II*) type II electron dense granules, (*IIc*) same exhibiting a crystal, (3) electron lucent vesicles, (4) coated vesicles. ×16700

density also varies from granule to granule, and is often very weak. These are most numerous within the basal processes, and are very rarely found in other areas of the SCO cell. They have not been seen associated with any particular cell organelle.

2) Type II granules (Figs. 1, 6 and 7C) are membrane-bounded electron dense granules with or without a halo and which exhibit a granular, uniform content. The diameter of the granules usually ranges from about 160–260 nm, though cases of both greater and lesser magnitude can be found. Dense granules with a similar granular content are frequently found outside the basal processes, though they are quite often larger when they appear in other areas of the cell. Material

Fig. 5A. A common phenomenon in the SCO is the attachment of the secretory sacs to the plasma membrane by regularly spaced particles (*arrowheads*). Ribosomes are often attached to the opposite, free side of the sac. ×102000

Fig. 5B. One of the secretory sacs sometimes seen attached to the plasma membrane has probably just released its contents into one of the extracellular spaces. Note the pinocytotic profile (*arrow*). ×57000

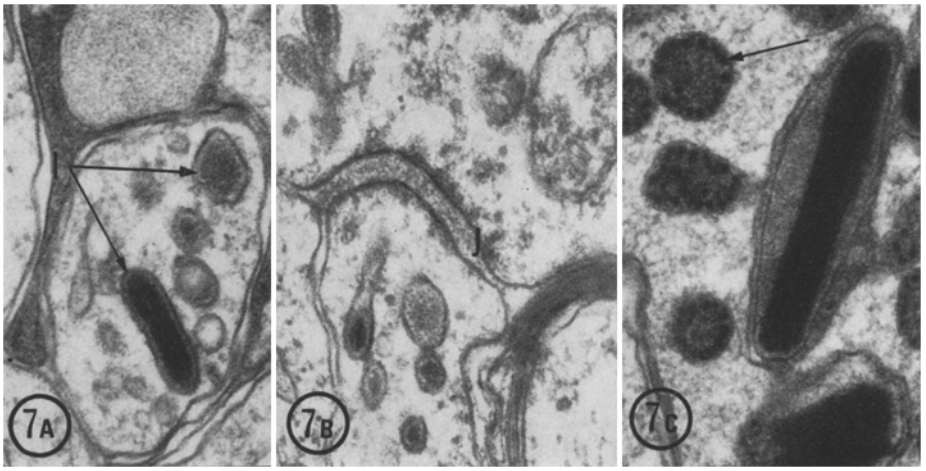


Fig. 7 A—C. Higher magnification of the electron dense granules found in basal processes. A The small type I granules (*I*), show not only different sizes and shapes, but also different affinities for staining media. ×57000. B Type I granules are shown as well as the fine structure of the junction (*J*) typical of SCO cell processes. ×57000. C The crystal bearing granules are shown here in cross- and longitudinal section. A pattern is revealed in the arrangement of the crystals shown in cross-section (*arrow*). ×57000

of this granular character has also been seen in association with the Golgi membranes. Crystals are sometimes found in granules of this type, both in those in the basal processes (Fig. 6) and those outside (Fig. 7C).

3) Electron lucent vesicles 50–100 nm in diameter. These can be seen either individually among the electron dense granules or in groups of their own (Fig. 1).

4) Coated vesicles.

The basal processes of the SCO cells are often joined by desmosome-like junctions which exhibit: a widened, regular intercellular space, localized cytoplasmic densities bilaterally, and an intercellular substance which often is condensed into a line approximately midway between the two plasma membranes (Fig. 7B). Filaments are not associated with these junctions, however, and the junctions can extend over considerable surfaces, both characteristics differentiating these junctions from desmosomes.

Nervous Elements of the Hypendyma and Subependymal Neuropil. Myelinated and unmyelinated axons from the posterior commissure enter the SCO hypendymal region and penetrate between hypendymal cells to reach the capillary region. Though single axons are seen among SCO cells, most axons run in bundles. Upon reaching the capillary region, the bundles break up into fascicles which distribute themselves around the capillary (Fig. 10). Synapses are identified within the pericapillary groups of nerve fibers (Fig. 11). The synaptic vesicles seen within the presynaptic terminals are clear; however, dense-cored vesicles are sometimes seen in longitudinal sections of axons. The axons are never seen to end directly on the perivascular space, nor have synaptic varicosities beer found in the perivascular space.

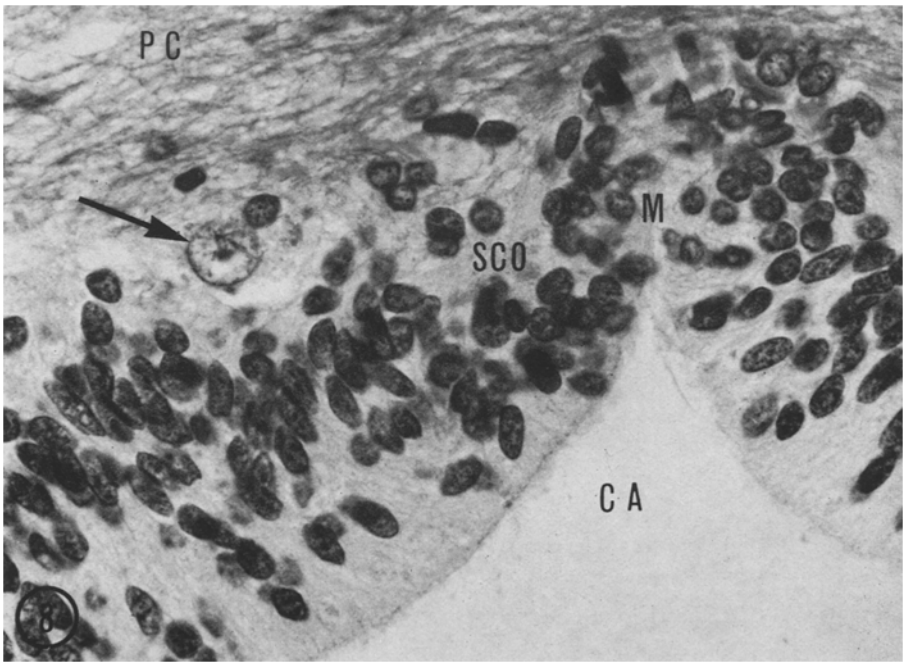


Fig. 8. Frontal section through SCO as seen light microscopically. *Arrow* indicates a nerve cell nucleus located at the edge of the hypendyma. The indentation in the middle represents the midline (*M*). *CA* cerebral aqueduct. *PC* posterior commissure. ×600

Postsynaptic-like densities have been seen on identifiable SCO processes which are in direct apposition to unmyelinated axons, but vesicles were never seen in either process.

Nerve cells are seen in the subependymal neuropil, most often in the near vicinity of a hypendymal capillary. These are identifiable both light microscopically (Fig. 8) and electron microscopically (Fig. 9). Numerous synapses are found on both perikaryon and dendrites. Most of the presynaptic endings contain either clear vesicles, or clear and dense-cored vesicles. The honeycomb arrangement of synaptic vesicles described by Murakami *et al.* (1970) was only seen once. Presynaptic terminals containing flattened vesicles have also been identified, but rarely. The hypendymal nerve cells are always separated from the SCO cells in the hypendyma by at least a few glial fibers.

Discussion

The cells of the subcommissural organ (SCO cells) exhibit an ultrastructure which clearly sets them apart from normal ependymal cells, neurons and glial cells, and which strongly supports the possibility of a secretory function for the SCO. The profuse endoplasmic reticulum and numerous profiles of Golgi membranes indicate a very high degree of synthetic activity, and the high concentration

Fig. 9. The relationship of various nervous elements to the hypendymal region is shown here. A bundle of myelinated axons approaches an arteriole. A large dendrite (*D*), is seen basally, separated from an SCO cell by a few glial processes (*G*). The abundance of synapses (*s*) appearing above the dendrite indicates its probable course. Further notation: *A* astrocytic process, *E* endothelial cell, *M* smooth muscle cell, *N* axon, *Nk* Nebenkern-like configuration of ER, *P* pericyte. ×6300

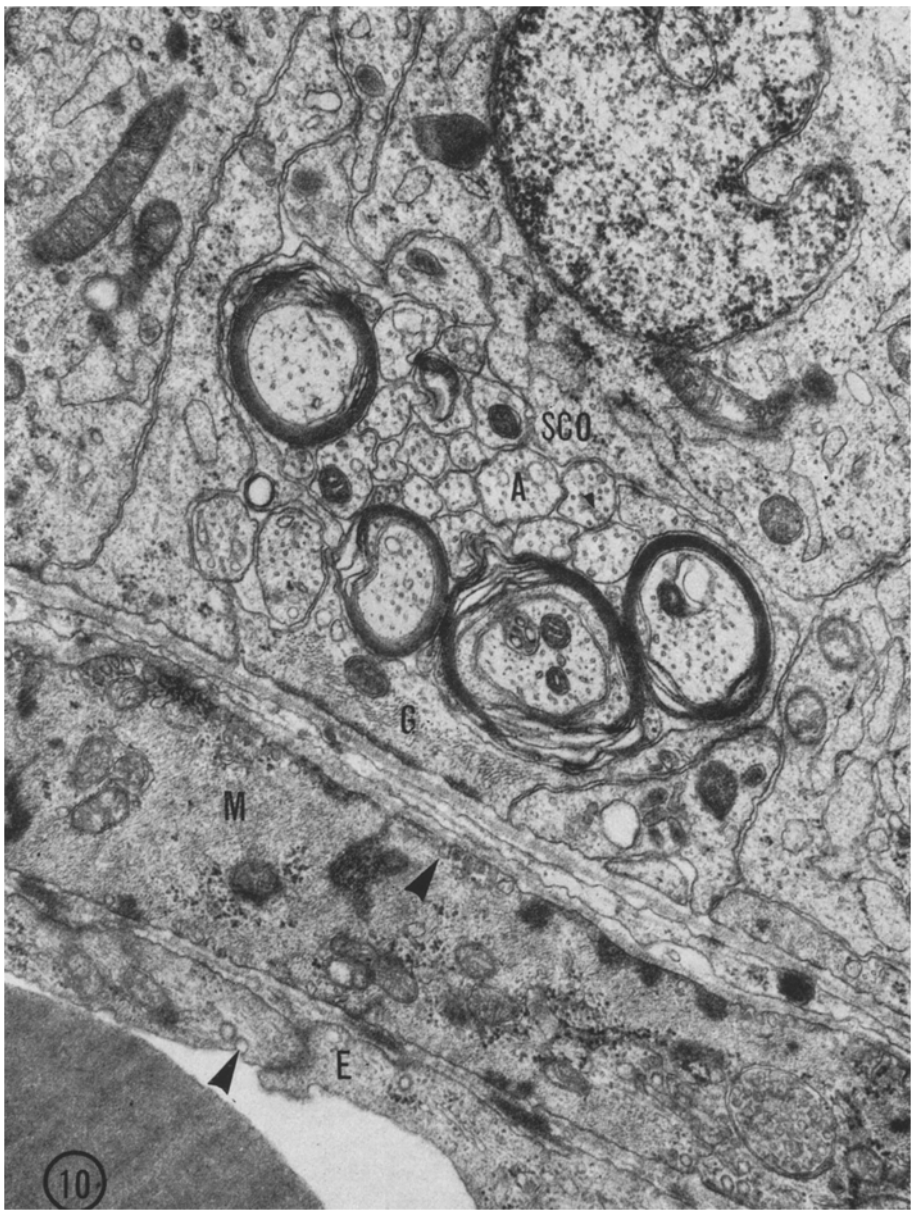


Fig. 10. A hypendymal nerve fascicle containing myelinated and unmyelinated axons (*A*) is separated from the arteriole by a glial process (*G*), whereas an SCO cell (*SCO*) lies directly adjacent to the axons. Note pinocytotic activity (*large arrowheads*) in the muscle cell (*M*) and the endothelial cell (*E*) comprising the arteriole wall. Also note that in one of the axons, a synaptic vesicle (*small arrowhead*) can be seen in association with a slightly specialized membrane indicating that this section probably passes through the periphery of a synaptic terminal. ×24600

Fig. 11. Close-up of a synapse such as those found within the fascicles bordering a capillary. There is no ultrastructural detail which might indicate that the post-synaptic process (*P*) is other than a dendrite. *PRE* presynaptic process. ×57000

of dense granules basally suggests that the synthesized product may be destined for secretion. Although there are several configurations of endoplasmic reticulum in SCO cells, as well as a heterogeneity of dense granules, no definite correlation could be observed between any one of the membrane patterns and a specific synthetic product. Although the Golgi membranes found outside the basal processes were occasionally associated with a dense granular material, those profiles found in the basal processes never displayed such an association. The function of the basal Golgi apparatus, thus, is enigmatic.

The secretory product of the SCO cells is usually described as consisting of two populations of granules—light secretory sacs, or pale granules, and dense granules (e.g. Papacharalampous *et al.*, 1968; Rodríguez, 1970; Murakami *et al.* 1972). There is general agreement among the above-mentioned authors that the pale granules are derived from the endoplasmic reticulum, though their fate is a matter of controversy. Rodríguez (1970a) reports that the pale granules bypass the Golgi apparatus, whereas Papacharalampous *et al.* (1968) describe a deliverance of the flocculent material to the Golgi complex for a synthesis into dense granules. Our findings show no association of the secretory sacs with the Golgi membranes. Although the secretory nature of the flocculent material has been questioned (Smith, 1970), many findings support the idea that the light secretory sacs release their contents apically, whereas concrete evidence for basal secretion (cf. Oksche, 1969), i.e. presence of flocculent material in the perivascular space, has been found only in the case of lower vertebrates (Murakami *et al.*, 1969, 1970).

In support of the fact that this flocculent material may be a secretory product of the SCO, we have shown the binding of these sacs to the lateral membranes by means of small, electron dense particles, and the presence of the flocculent material in a system of extracellular spaces to be discussed further below. Although the binding of these sacs was not observed at the basal membrane directly bordering the perivascular space, the presence of tight junctions at the apical surface (Kimble *et al.*, 1973) suggests that a lateral secretion could not provide the secretory product with a channel to the ventricular system.

The second population of granules, the dense granules, have also been the subject of much dispute. Some authors (Vigh *et al.*, 1967; Herrlinger, 1970) consider the electron dense granules to be the EM correlate of the Gomori-positive neurosecretory substance seen at the light microscopic level, while Smith (1970) implies that what Vigh *et al.* (1967), for example, call secretory granules are most likely lysosomes. A recent histochemical study (Murakami *et al.*, 1972) has shown at the EM level that few of the dense granules react positively for acid phosphatase, and therefore, that most of them are not of lysosomal nature.

The origin of the dense granules has been shown in the Golgi apparatus by some authors (Murakami and Tanizaki, 1963; Murakami *et al.*, 1970; Papacharalampous *et al.*, 1968), whereas others (Oksche, 1969; Rodríguez, 1970a) have never seen an association of the dense granules with the Golgi membranes. Rodríguez (1970a) shows a budding of the dense granules from the endoplasmic reticulum. It has been suggested that this controversy over the origin of the dense granules is the result of a species difference (Murakami *et al.*, 1970). It is worth mentioning here that a difference in physiological activity can also apparently alter completely the synthetic pathway in secretory cells. Farquhar (1969) has shown that the TSH-secreting cells in the anterior pituitaries of thyroidectomized animals exhibit an engorged endoplasmic reticulum filled with a flocculent material, the secretory product of which is not seen in the Golgi membranes in contrast to the normal pathway of product synthesis.

Of special interest is the fact that we observe two, fairly distinct subgroups of electron dense, membrane bound granules. Type I prevails in the basal processes and is very rarely encountered elsewhere in the SCO cell, so the maturation of these granules probably takes place *in situ*. The most likely precursors are the cisternal sacs of flocculent material which are also found in the cell processes bordering capillaries. The frequent elongated and dumbbell shapes observed among this type of granules could indicate that they are the product of a condensation of the material in the sacs to a much more compact form. In addition the lower reactivity often observed of the granules for osmium-uranyl-lead staining (OsUL) may indicate a relation with the Gomori-positive secretory product seen with the light microscope. The affinity of uranyl acetate for anionic groups has been suggested (e.g. Hodge and Schmitt, 1960) and supported by the fact that a weakening of the reactivity of tissue with OsUL is seen after a lowering of pH of the uranyl solution (Pfenninger, 1973). Since the substance in the SCO has been shown to be a *neutral* mucopolysaccharide-protein complex (Bargmann and Schiebler, 1952; Wislocki and Leduc, 1952), a weak or variable reactivity of this substance could be expected with OsUL staining, although this can not be considered as proof that the substance is neutral.

The type II electron dense granules, with their homogeneous, granular content resemble both lysosomes (dense bodies) and neurosecretory granules. A morphological differentiation can not be made between these two possibilities from the present data, nor can the possibility be discarded that the structurally equal granules could represent functional entities of more than one type. The possibility that type II granules include large type I granules as well as lysosomes can not be excluded.

It should be emphasized here that a clear distinction between type I and type II granules can be made in most cases, but not all. There exist granules in the basal processes which exhibit, for example, an intermediate diameter (120–160 nm), but many of these can be classified according to staining characteristics and/or shape. These intermediates may, therefore, be considered as the result of biological variation, i.e. larger-than-average type I, or smaller-than-average type II granules. The value of the classification into granule types lies in the relatively high constancy of multiple characteristics exhibited by each granule type.

Extracellular Spaces. Extracellular spaces (ECS) have been described before in the SCO (Murakami and Tanizaki, 1963; Oksche and Vaupel-von Harnack, 1965), but doubt has been cast on the validity of these reports (Rodríguez, 1970a). Although only one fixation procedure was used in our investigation, there are several ultrastructural details of the ECS which support the fact that these spaces exist under physiological conditions rather than being fixation artefacts. First the plasma membranes bordering the ECS appear to have a high activity, as they frequently exhibit omega figures, which indicate a probable exchange between the limiting cells and the space. In addition, puncta adherentia-like contacts (Peters *et al.*, 1970) often demarcate the boundaries of the space. The fact that a flocculent material of approximately the same density as that found in the secretory sacs occupies these spaces, when coupled with the fact that profiles of secretory sacs are often seen associated with these membranes via electron dense particles, suggests that a lateral secretion occurs directly from the ER sac into the ECS. In one case we even saw what appeared to be one of these sacs releasing its contents into the space.

Papacharalampous *et al.* (1968) have described large secretory sacs containing cilia which they interpret as a coalescence of the smaller secretory sacs into which a cilium has grown. Though we have also seen these sacs, our work has revealed points at which these sacs are continuous with the ECS. Moreover, frequent basal cilia extend between the cells in the hypendymal region. This seems to indicate that a basal coalescence of secretory sacs may result in an extrusion of this flocculent material into the ECS, or that, in some cases, these large basal secretory sacs containing cilia may actually be the ECS which has been sectioned in such a way as to be surrounded by one cell's cytoplasm.

The spaces seem to belong to a system of channels which may provide a means of communication among SCO cells. Though a continuation between the ECS and perivascular space was seen only infrequently, this may provide a communication between the two compartments, which are, however, always separated from each other by the parenchymal basal lamina. Although the extracellular

channels described here may function towards the similar end of directing the flocculent material to the perivascular space, they establish the possibility of a new pathway of secretion.

Nervous Elements of the Hypendymal Region of the SCO. Many studies have described light and/or electron microscopically the entrance of nerve fibers from the posterior commissure into the SCO (e.g. Dendy, 1907; Marburg, 1920; Olsson, 1958; Rodríguez, 1970b). Our findings show from a study of serial sections that these nerve bundles break up into small groups of axons which distribute themselves around hypendymal capillaries. Furthermore, we demonstrated synapses occurring within these smaller nerve fascicles, whereas no endings were found on or in the perivascular space. Although the postsynaptic elements were unidentifiable, the existence of chemical synapses so close to the capillary provides support for the possibility of an innervation in the SCO basal region.

Further evidence for the innervation of the SCO is the presence of nerve cells found basally in the hypendymal region. Yamada *et al.* (1957) report nerve cells located above and laterally to the SCO of the dog, which they term "pericommissural ganglion cells". Isomäki *et al.* (1965) also describe nerve cells situated deep in the hypendyma of the SCO of the calf. Rodríquez (1970b) describes an abundance of synapses in the posterior commissure below the hypendyma of the toad SCO, the postsynaptic elements of which are dendrites. We confirm in the present study the finding of many synapses, both with and without dense core vesicles, in the subhypendymal region, but we find that the postsynaptic elements include nerve cell perikarya as well as dendrites. By means of fluorescence microscopy, Fuxe (1965) has shown a strong reaction for serotonin below the rat SCO which may identify the nature of the dense core vesicles seen with the electron microscope.

The pineal gland is very closely associated with the most rostral area of the SCO, and a nervous connection between them has been suggested in lower vertebrates by way of the pineal tract (Dendy, 1907; Oksche, 1955; for critical comments, see Oksche, 1969). In a recent publication, Møllgård and Møller (1973) have demonstrated in human fetuses a miniature sensory nerve including a bipolar cell ganglion leading from the pineal gland to a small meso-diencephalic group of nerve cells adjoining the SCO. This nerve cell group is located in a cluster at the rostral end of the SCO and has now also been located in the fetal rabbit SCO (unpublished results). However, the nerve cells described here in the adult rabbit SCO, are spaced fairly evenly from the rostral to the caudal extremities of the SCO and are nestled down very close to the hypendyma, being separated from it by only a few glial processes. The destination of the axons and dendrites of these nerve cells are the subject of a present investigation in our laboratory, and should be of considerable interest in light of the possible connections to the pineal body, as indicated by the discovery in higher vertebrates of a specific nervous connection between the two regions.

In conclusion, the present data must be considered as strong evidence for a basal secretion occurring in the SCO, though only an autoradiographic study can give unequivocal evidence for such secretion. The morphology of the cells shows the presence of many factors which might be involved in basal secretion: 1) electron dense granules in high concentration in the basal processes, 2) increased

concentration of mitochondria in the basal processes; 3) a possible mode of secretion for the membrane bounded flocculent material by binding of the sac by small particles to the lateral membrane and subsequent release into a system of extracellular spaces, 4) widened perivascular space which extends out in sacs and channels among the basal processes, 5) increased pinocytotic activity in the hypendymal capillary wall, 6) nerve cells found in close association to the capillary region which can provide a control mechanism for secretion, 7) chemical synapses between nervous elements within fascicles bordering on the perivascular space which indicate nervous activity in the pericapillary region other than that involved in an innervation of smooth muscle.

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