Fine Structure of the Perivascular Space of the *Gecko japonicus* Subcommissural Organ

The secretory ependymal cells constituting the subcommissural organ (SCO), which is situated on the roof of the third ventricle and immediately beneath the posterior commissure, have been the subject of many electron microscopic studies, and it is recently confirmed that in vertebrates the secretory substances characteristic of the SCO are first produced in the ergastoplasmic cisternae located in the perinuclear region and then transported to the apical part of the cell, via or not via the Golgi apparatus for their release in the fashion of micro-merocrine into the ventricular lumen (apical secretion)[1–10]. On the other hand, ultrastructural evidence demonstrating the discharge of the secretory substances via the basal processes of the SCO cells into the capillaries (basal secretion) seems to be scanty.

In the course of a study on the fine structure of the gecko subcommissural organ, large perivascular spaces filled with substances similar to the secretory ones of the SCO, and containing occasionally striated structures, were found between the SCO and the capillaries.

Material and methods. As material 10 adult geckos (*Gecko japonicus*) of both sexes were used, which were collected during the summer in the vicinity of the author's laboratory. The animal was decapitated and a small block containing the subcommissural organ was quickly obtained. The blocks were fixed in a cold buffered 2% formol-glutaraldehyde solution followed by refixation with 1% osmium. After brief dehydration with acetone the blocks were embedded in Epon 812. The thin section were made with glass knives, stained with uranyl acetate and lead citrate and examined with a JEM-7 or a HU-11A electron microscope.

Results and discussion. The basal processes of the SCO extending to the capillaries contained, like the other cytoplasmic parts of the cells, both many secretory sacs filled with flocculent substances of medium electron density and secretory vacuoles with somewhat denser content which are supposed to be drived from the former. Between such basal processes and the capillaries the large perivascular spaces were usually encountered the dimension of which was up to several microns, and within which occasionally a small number of collagen fibrils and mesenchymal cells with dark or light appearance could be seen. These perivascular spaces have 2 characteristics. The first is that this space does not appear to be electron lucent but is filled entirely with flocculent substances, quite similar to those of either secretory sacs or vacuoles in the SCO basal processes in which the secretory vacuoles exist very often in close contact with the plasma membrane facing the perivascular space (Figure 1). There seems to be no doubt that such flocculent substances filling the perivascular spaces may be nothing but the secretory substances discharged from the SCO basal processes into the perivascular spaces,

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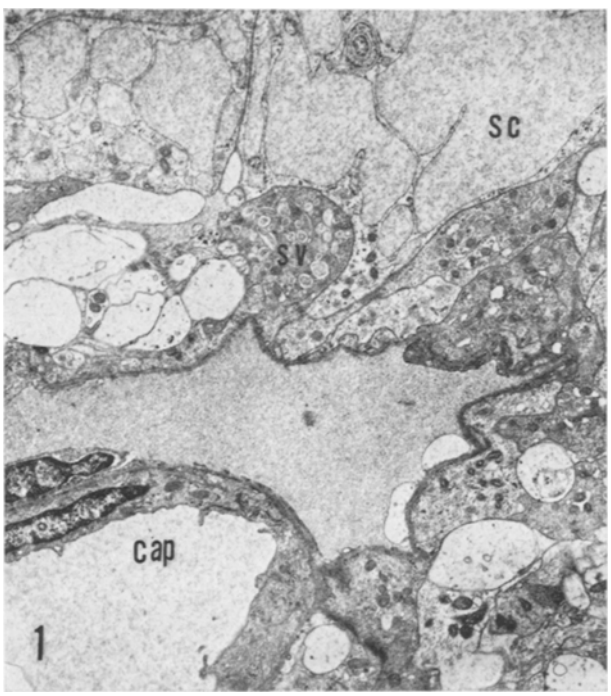

Fig. 1. Electron micrograph showing a large perivascular space between SCO basal processes and a capillary. Note that the perivascular space is filled entirely with flocculent substances similar to those of the secretory sacs (SC) or vacuoles (SV) in the SCO basal processes. Cap, capillary lumen. × 4600.

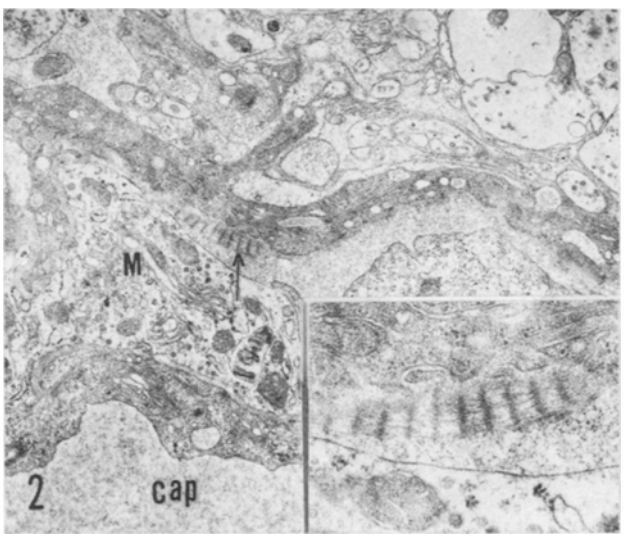

Fig. 2. Electron micrograph showing a striated structure (arrow) in the perivascular space of the SCO. M, mesenchymal cell; Cap, capillary lumen. × 9300. Insert: Higher magnification of the same striated structure. × 23,800.

although openings of the secretory vacuoles or sacs into the perivascular spaces could not be observed. The second characteristic finding in the perivascular spaces is the occasional presence of fusiform striated structures, which are found exclusively in an intimate association with the perivascular basement membrane near the SCO basal processes. These structures are oriented parallel to the perivascular basement membrane and composed of numerous fine filaments about 80 Å in diameter showing cross striations with a repeating periodicity of about 1200 Å (Figure 2). Similar striated structures have been hitherto disclosed in the perivascular space of the SCO of some mammals[11–13] and are postulated to be atypical collagenous elements. The significance of these striated structures is still not completely elucidated, but according to some authors[11,12] it is assumed that they might play an important role in metabolic interaction between the SCO and the capillaries.

The capillary endothelial cell contains abundant, probably pinocytotic, vesicles and well-developed organelles such as the Golgi apparatus and the rough endoplasmic reticulum. In its cytoplasm also homogenously dense granules about 2000 Å in diameter enclosed by the limiting membrane were often seen. However, it has not been determined whether or not they are absorbed secretory substances of the SCO cells. No fenestration of the endothelial cells was noticed.

The aspects of the perivascular spaces described above may serve as morphological evidence for the existence of the possible basal secretion in the gecko[14].

Zusammenfassung. Im Verlauf von elektronenmikroskopischen Untersuchungen am Subkommissuralorgan (SKO) von *Gecko japonicus* wurden zwischen den SKO-Zellen und den Kapillaren breite perivaskuläre Spalträume beobachtet, die mit flockigen Substanzen gefüllt sind und mitunter quergestreifte Strukturen aufweisen. Die funktionelle Bedeutung dieser perivaskulären Spalträume wurde im Hinblick auf die basale Sekretion des SKO diskutiert.

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[14] This paper is dedicated to Professor Dr. E. TONUTTI, Director of the Department of Clinical Morphology, University Ulm (Germany), on the occasion of his 60th birthday.

Lack of Effect of Ciliarectomy on the Fine Structure of the Small Multiple Endings in the Extraocular Muscles of the Rat

The extraocular muscles of the rat are innervated in part by ordinary myoneural junctions arising from myelinated nerves and in part by multiple small junctions from unmyelinated nerves[1]. These 'small multiple endings' exhibit acetylcholinesterase activity, as has been observed with both the light[1] microscope and the electron[2] microscope, and their fine structure is similar to that of a typical cholinergic excitatory synapse[1]. Neither the site of the nerve cell bodies connected with these unmyelinated nerves nor their physiological function is known (for references see TERÄVÄINEN[1]). Because extirpation of the ganglion ciliare is reported to result in progressive atrophy of the extraocular muscles[3], and electrical stimulation of the ciliary ganglion is stated to cause contraction of the extraocular muscles[4], we decided to study the fine structure of the small multiple endings after removal of the ganglion ciliare.

The ganglion ciliare of adult Sprague-Dawley rats was either removed or electrocoagulated under ether anaesthesia. The rectus superior and lateralis muscles were prepared in ether anaesthesia for 24 h, 2, 4, 6, 9, and 21 days after the operation and fixed immediately at 4 °C and pH 7.2 for $2^1/_2$ h with 2.5% glutaraldehyde[5] buffered with phosphate. The contralateral unoperated side served as a control. After postfixation with 1% osmium tetroxide in the phosphate buffer, the specimens were dehydrated in graded series of ethyl alcohol and embedded in Epon 812[6]. The sections were counterstained with lead citrate[7].

Small terminals of unmyelinated axons were apposed to the electrondense postsynaptic membrane of the muscle fibres on the unoperated control side (Figure 1), as earlier described[1]. We did not observe degenerative

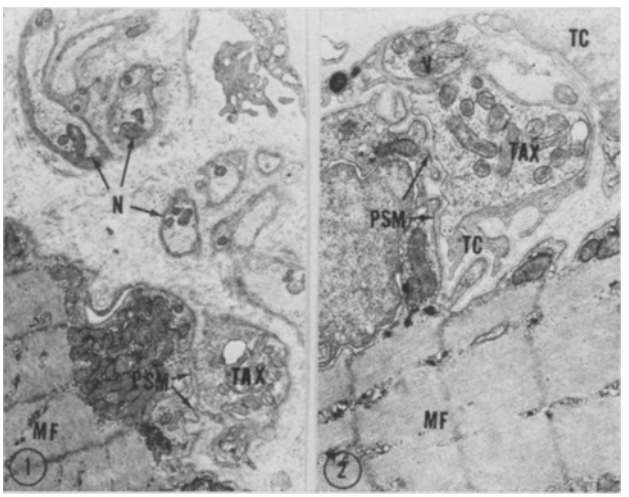

Fig. 1. Electron micrograph of a small myoneural junction in the extraocular muscle of the unoperated side. One axon terminal (TAX), filled with vesicles and mitochondria, is seen to be apposed to the electron-dense postsynaptic membrane (PSM) of the muscle fibre (MF). The section also passes through the unmyelinated nerves (N) from which the terminal is derived. The structure of the muscle fibre is of the slow type, with small mitochondria and a relatively weakly developed sarcoplasmic reticulum incompletely separating the muscle fibrils. × 10,000.

Fig. 2. Small myoneural junction 21 days after removal of the ganglion ciliare. No degenerative changes are present (compare with Figure 1). × 15,000.