Z. Zellforsch. 113, 39–43 (1971)

Presynaptic Tubular Structures in Photoreceptor Cells*

Amanda Pellegrino de Iraldi and Angela María Suburo**
Instituto de Anatomía General y Embriología, Facultad de Medicina,
Universidad de Buenos Aires, Argentina

Received September 8, 1970

Summary. After the application of fixatives including phosphotungstic acid or a mixture of osmium tetroxide and zinc iodide, complex tubular structures are evident in the presynaptic side of the synapses between photoreceptor and bipolar cells of the rat's retina. In the first case only the limiting membranes are visualized, while in the second only the content of the tubules is stained. These tubules seem to be related, on a morphological ground, with the formation of synaptic vesicles. These tubular structures are not observed when fixation is done with osmium tetroxide or glutaraldehyde-osmium tetroxide.

Key-Words: Photoreceptor cells—Presynaptic tubular structures—Fixation.

The ultrastructure of the synapses between photoreceptor and bipolar cells has been extensively studied in different species (Sjöstrand, 1956; De Robertis and Franchi, 1956; Ladman, 1958; Dowling and Boycott, 1967) giving special consideration to the presence of synaptic vesicles and ribbons in the presynaptic side. The presence of vacuoles of 800–1200 Å has also been described and they have been considered as belonging to the smooth endoplasmic reticulum.

In this paper we will show that the use of certain fixatives allows to see some presynaptic tubular structures in the rat's retina. These seem to be related with the formation of synaptic vesicles.

Material and Methods

The retina of adult Wistar rats were carefully dissected and fixed according to the following schedules:

1. G-O-E. PTA: 3% glutaraldehyde in 0.1 M phosphate buffer pH 7.2 for 22 hours, washing in 4% sucrose in the same buffer for one hour and postfixation in 1.5% osmium tetroxide in phosphate buffer for two hours. The blocks were dehydrated in graduated ethanol until the 100%. After 5 minutes in the 100% ethanol they were immersed in 1% phosphotungstic acid solution in absolute ethanol prepared according to Bloom and Aghajanian (1968).

2. ZIO: osmium tetroxide-zinc iodide mixture for 2 hours (Pellegrino de Iraldi and Gueudet, 1968).

3. G-O-ZIO: In some cases the ZIO mixture was applied after a previous fixation in glutaraldehyde-osmium tetroxide (Pellegrino de Iraldi and Gueudet, 1969b).

For comparison, retinae were also fixed in osmium tetroxide in "Periston" (Bayer) and in glutaraldehyde-osmium tetroxide (Pellegrino de Iraldi and Gueudet, 1969 a).

In all cases the dehydration was done in ethanol and embedding in Epon 812. Some sections were stained with uranyl acetate and/or lead citrate. Sections were examined under a Siemens Elmiskop 1.

* This work has been supported by grants from the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina, and from National Institutes of Health, U.S.A., (5 RO1 NS 06953-05 NEUA).

** We want to express our gratitude to Mrs. Haydée Agoff de Zimman and Mr. Alberto Saénz for their skillful technical assistance.

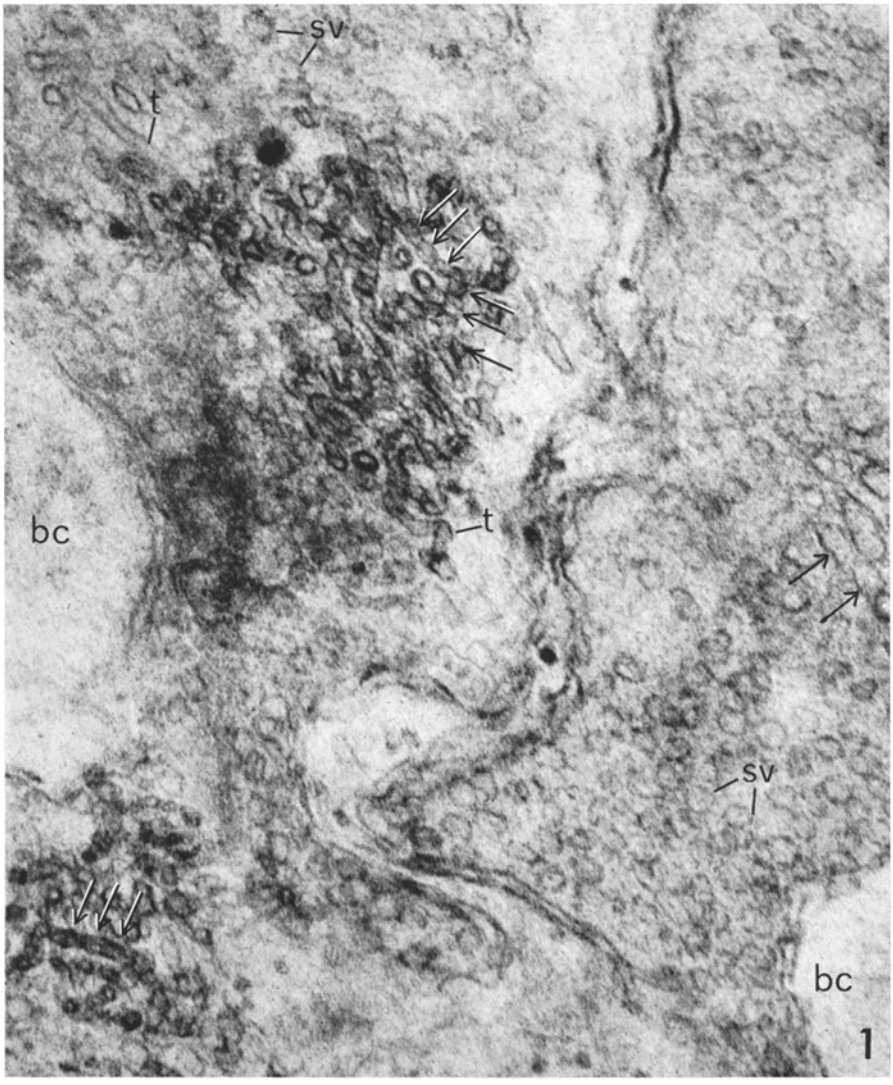

Fig. 1. Synapses of the photoreceptor and bipolar cells in the rat's retina fixed in glutaraldehyde-osmium tetroxide-phosphotungstic acid. Tubules, most of them showing varicosities (arrows), placed in close vicinity to the synaptic vesicles, are observed in the photoreceptor terminals. ×100000. Abbreviations for all figures: *bc* dendrite of bipolar cell; *mi* mitochondria; *sv* synaptic vesicle; *t* tubule

Results

When G-O-E. PTA is used (Fig. 1) convoluted tubular structures can be observed in the presynapses of the photoreceptor cells. Some of them have a diameter of about 200 Å while others have a diameter of about 350 Å. Although they are in a close relationship with synaptic vesicles they can be easily distin-

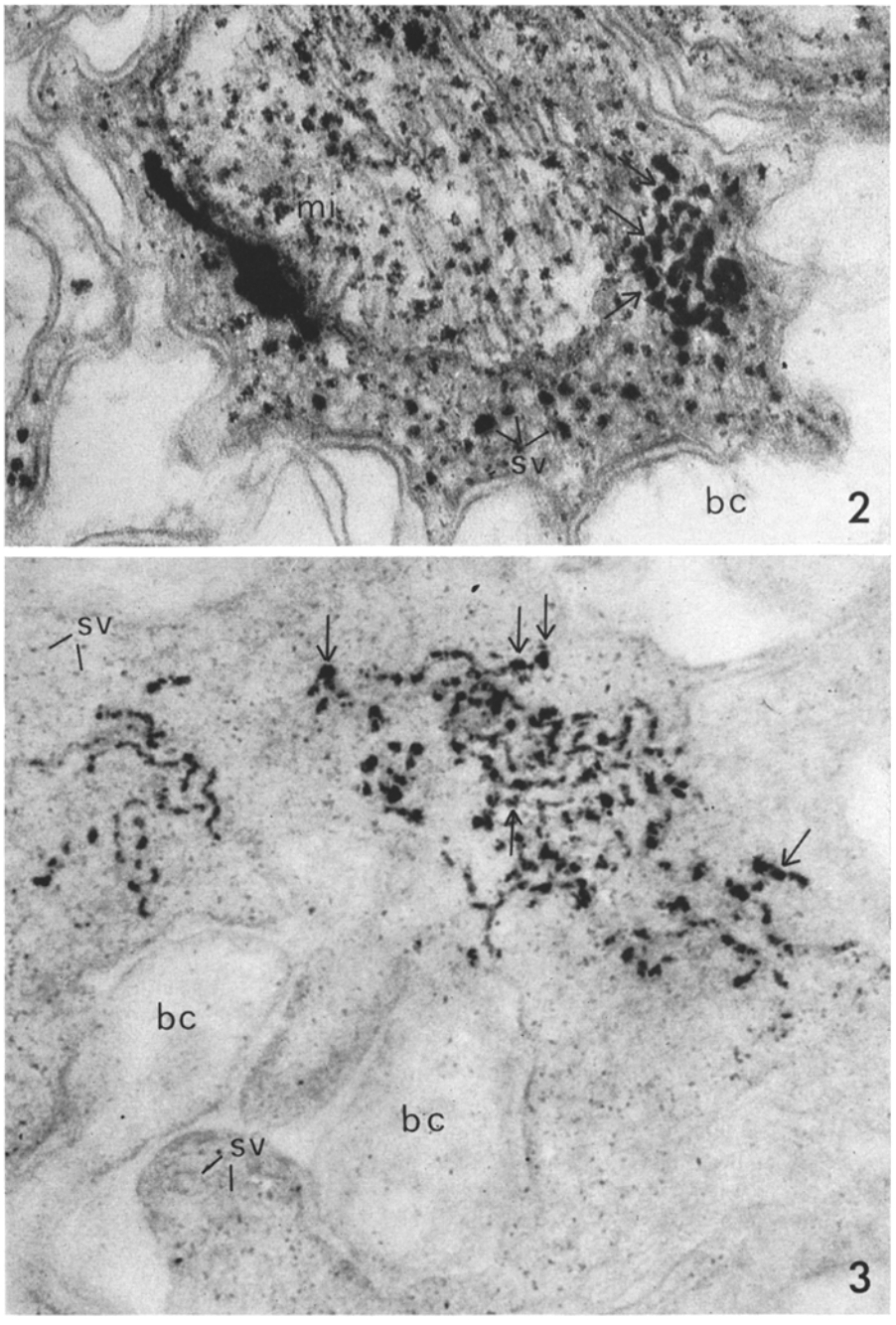

Fig. 2. The same as Fig. 1, fixed in osmium tetroxide-zinc iodide. Electron dense material may be observed in tubular structures and their varicosities (arrows). Many synaptic vesicles show a similar electron dense content. $\times 100000$

Fig. 3. The same as Fig. 1, treated with the osmium tetroxide-zinc iodide mixture after a previous fixation in glutaraldehyde-osmium tetroxide. Electron dense material partially remains in the tubular structures and their varicosities (arrows) but has disappeared in synaptic vesicles whose ghosts can be seen. $\times 100000$

guished because the tubular membranes are more intensely stained than those of the synaptic vesicles. Most of the tubules show varicosities along their course.

When fixation is done with ZIO (Fig. 2) similar tubular structures can be observed. These tubules as well as many of the synaptic vesicles show a very electron dense content. When G-O-ZIO is used the electron dense material disappears from synaptic vesicles but partially remains in the tubular structures and their varicosities (Fig. 3).

After fixation in osmium tetroxide or glutaraldehyde-osmium tetroxide, some short tubules, previously described by other authors can be observed. However, the extensive tubular structures here described are not visualized with these fixatives.

In every case the inner plexiform layer was thoroughly examined but no tubular structures were observed in their synapses.

Discussion

We have shown the existence of complicated tubular structures in the synapses between photoreceptor and bipolar cells of the rat's retina. These structures remain unseen with common fixatives but they are revealed by phosphotungstic acid after glutaraldehyde-osmium tetroxide fixation and also with the osmium tetroxide-zinc iodide mixture, directly or after glutaraldehyde-osmium tetroxide. Phosphotungstic acid stains the membranes of these tubules, while the osmium tetroxide-zinc iodide mixture reveals an electron dense material inside them. In both cases these tubular structures show a different reactivity towards these fixatives than do the synaptic vesicles. The tubular membrane has a greater affinity to phosphotungstic acid than the synaptic vesicle membranes, and the electron dense material inside the tubules is not affected by a previous fixation in glutaraldehyde-osmium tetroxide, as is the case for the electron dense material inside the synaptic vesicles. However, the presence of varicosities in the tubular structures and their vicinity to synaptic vesicles, suggest that the latter could be formed by a process of dilatation and pinching off from these tubules. If this were the case, it is necessary to admit that the formation of synaptic vesicles implies a transformation in the membrane structure and in the content of these tubules.

The importance of the fixative employed is in agreement with the observations of Birks (1966) in the neuromuscular junction of the frog. This author could observe tubular structures only when fixatives containing acrolein were used, while synaptic vesicles can also be observed with common fixatives. These tubules, which seem to originate synaptic vesicles, were morphologically considered as belonging to the smooth endoplasmic reticulum. Tubular structures resembling the Golgi complex have been described in central synapses (Andres, 1964, 1965) and in the mammalian endplate (Düring, 1967), and they have also been related to the formation of synaptic vesicles.

The origin of synaptic vesicles has been placed in the endoplasmic reticulum (De Robertis and Bennett, 1955; Palay, 1956, 1958) and also in the neurotubules (De Robertis, 1964). In regenerating nerves, Pellegrino de Iraldi and De Robertis (1968) have observed that neurotubules seem to participate in the formation of clear and granulated vesicles through their connections with cisterns similar to

those of the Golgi complex. In the present case, the nature of these tubules cannot be discerned because although they have a diameter similar to that of neurotubules they do not show their other morphological characteristics. A morphological likeness to the smooth endoplasmic reticulum cannot be discarded.

The fact that no tubular structures were observed in the inner plexiform layer could indicate a functional difference between both synaptic layers related to the vesicle forming activity and to the synaptic transmission.

References

Andres, K. H.: Mikropinozytose im Zentralnervensystem. Z. Zellforsch. **64**, 63–67 (1964).

— Der Feinbau des Bulbus olfactorius der Ratte unter besonderer Berücksichtigung der synaptischen Verbindungen. Z. Zellforsch. **65**, 530–561 (1965).

Birks, R. I.: The fine structure of motor nerve endings at frog myoneural junctions. Ann. N.Y. Acad. Sci. **135** art. 1, 8–26 (1966).

Bloom, F. E., Aghajanian, G. K.: Fine structural and cytochemical analysis of the staining of synaptic junctions with phosphotungstic acid. J. Ultrastruct. Res. **22**, 361–375 (1968).

De Robertis, E.: Histophysiology of synapses and neurosecretion. London: Pergamon Press 1964.

— Bennett, H. S.: Some features of the submicroscopic morphology of the synapses in frog and earthworm. J. biophys. biochem. Cytol. **1**, 47–56 (1955).

— Franchi, C. M.: Electron microscope observations on synaptic vesicles in synapses of the retinal rods and cones. J. biophys. biochem. Cytol. **2**, 307–317 (1956).

Dowling, J. E., Boycott, B. B.: Organization of the primate retina: electron microscopy. Proc. roy. Soc. B No 1002, **166**, 80–111 (1967).

Düring, M. v.: Über die Feinstruktur der motorischen Endplatte von höheren Wirbeltieren. Z. Zellforsch. **81**, 74–90 (1967).

Ladman, A.: The fine structure of the rod-bipolar synapse in the retina of the albino rat. J. biophys. biochem. Cytol. **4**, 459–466 (1958).

Palay, S. L.: Synapses in the central nervous system. J. biophys. biochem. Cytol., Suppl. **2**, 193–202 (1956).

— The morphology of synapses in the central nervous system. Exp. Cell Res., Suppl. **5**, 275–293 (1958).

Pellegrino de Iraldi, A., De Robertis, E.: The neurotubular system of the axon and the origin of granulated and non granulated vesicles in regenerating nerves. Z. Zellforsch. **87**, 330–344 (1968).

— Gueudet, R.: Action of reserpine on the osmium tetroxide-zinc iodide reactive site of synaptic vesicles in the pineal nerves of the rat. Z. Zellforsch. **91**, 178–185 (1968).

— Catecholamine and serotonin in granulated vesicles of nerve endings in the pineal gland of the rat. Int. J. Neuropharmac. **8**, 9–14 (1969a).

— Zinc iodide-osmium tetroxide reactive sites in the photoreceptors cells of the retina of the rat. Z. Zellforsch. **101**, 203–211 (1969b).

Sjöstrand, F. S.: Ultrastructure of cells as revealed by the electron microscope. Int. Rev. Cytol. **5**, 455–533 (1956).

Prof. Dr. Amanda Pellegrino de Iraldi
Instituto de Anatomía General y Embriología
Facultad de Medicina
Universidad de Buenos Aires
Paraguay 2155, Buenos Aires/Argentina