Journal of Neural Transmission 38, 149—157 (1976)

Quantitative Changes in Number of "Synaptic" Ribbons in Rat Pinealocytes after Orchidectomy and in Organ Culture*

M. Karasek**

Laboratory of Electron Microscopy, Research Centre, Medical Academy, Łódź, Poland, and the Netherlands Central Institute for Brain Research, Amsterdam, The Netherlands

With 11 Figures

Received January 12, 1976

Summary

A quantitative study of "synaptic" ribbons in rat pinealocytes was performed after orchidectomy and in organ culture. Both orchidectomy and culturing the pineal organ caused an increase in number and size of these structures.

Present study indicate that microtubular sheaves and/or microtubules may represent precursors of "synaptic" ribbons.

It is suggested that "synaptic" ribbons of rat pinealocytes, far from being phylogenetic relics of no functional significance play a role in the function of the pineal organ. An exact functional interpretation of the "synaptic" ribbons, however, is still a matter of conjecture.

Introduction

"Synaptic" ribbons, functionally enigmatic components, are quite often observed in the cell bodies and the processes of the mammalian pinealocytes (*Kappers*, 1971; *Karasek*, 1976; *Wurtman et al.*, 1968). These structures have also been termed "vesicle-crowned rodlets" (*Wolfe*, 1965) and "vesicle-crowned lamellae" (*Arstila*, 1967). They

* In gratitude and with admiration this paper is dedicated to Professor Dr. J. Ariëns Kappers.

** IBRO/UNESCO fellow.

are seen in longitudinal sections as three dense laminae with less dense zones sandwiched between and are surrounded by small vesicles. "Synaptic" ribbons lie singly or in groups, very often close to the cell membrane. They have been interpreted by *Hopsu* and *Arstila* (1965) as organelles involved in functional neurohumoral synaptic contacts between pinealocytes. This hypothesis has not been generally accepted, because of the endocrine rather than neuronal nature of mammalian pinealocytes (*Kappers*, 1969, 1971). "Synaptic" ribbons are not restricted to the pineal organ. They were first demonstrated in photoreceptor cells of the retina and then also found in other sensory organs, *e.g.* the organ of Corti, the vestibular organ and the electrical receptor organs of teleost fishes, in which they play a role in synaptic transmission (for references see *Vollrath, Huss*, 1973). "Synaptic" ribbons are also permanent components of photoreceptor cells of the pineal organ of non-mammalian vertebrates (*Collin*, 1969; *Oksche*, 1971). In the mammalian pineal organ they were often regarded as non-functioning phylogenetic relics (*Kappers*, 1971; *Karasek*, 1973; *Wurtman et al.*, 1968).

Recent studies, however, have demonstrated striking changes in number and arrangement of "synaptic" ribbons of the guinea-pig pineal organ during the circadian rhythm as well as when animals were kept under continuous illumination (*Vollrath*, 1973; *Vollrath, Huss*, 1973). *Vollrath* (1973) concluded that "synaptic" ribbons of the mammalian pineal organ represent cell organelles of definite, but as yet unrevealed, functional significance. He suggested that they may be involved in intercellular communication. Their function would be either to enhance the secretory activity of the pineal organ or to establish circuits within the organ between adjacent pinealocytes, similar to neuronal circuits.

Since in previous qualitative studies of rat pineal in organ culture and after orchidectomy we found an increase in number of "synaptic" ribbons (*Karasek*, 1974; *Karasek et al.*, 1976) while, in contrast, *Arstila et al.* (1971) reported that these structures were absent in the pineal organ cultured *in vitro*, we decided to perform quantitative investigations of "synaptic" ribbons in rat pineal organ cultured *in vitro* and after orchidectomy.

Material and Methods

In present study, 40 adult male Sprague-Dawley rats weighing 120 to 200 g were used. The pineals of 6 rats were cultured for 12 hours, while those of 8 other rats were cultured for 48 hours. Details of the technique of the rat pineal organ culture have been previously described (*Karasek*, 1974).

Six pineals were obtained from rats 2 weeks after orchidectomy and 10 other pineals from rats 3 weeks after orchidectomy. Ten pineals of intact rats served as controls.

All animals used in the present experiments were exposed to 14 hours light daily (7 a.m.—9 p.m.). They were supplied with standard rat pellets and tap water *ad libitum*. All animals were sacrificed unanaesthetized by decapitation between 9 and 10 a.m. The pineals were fixed in *Karnovsky* fixative, followed by post-fixation in 1 % osmium tetroxide, and embedded in Epon 812. Sections were made with a LKB Ultrotom III, double stained with uranyl acetate and lead citrate and examined under a Philips 200 electron microscope.

For the quantitative evaluation of "synaptic" ribbons, thin sections were taken from different areas of the pineal organ. Since "synaptic" ribbons lie singly or in groups of two, three or more, "ribbon fields" including all types of ribbon grouping were counted. In each animal "ribbon fields" lying inside 30 grid apertures were counted. The data obtained are expressed as mean ± standard error of the mean (SEM) per unit area. The unit area corresponds to an area of pineal tissue fully covering 1 grid aperture measuring 45×45 um (2024 um^2). In order to avoid counting the same structures twice, only one section out of a series was used. For statistical analysis Student's t-test was used.

Results

"Synaptic" ribbons were rather rarely observed in the pinealocytes of control animals (1.24 ± 0.16 ribbon fields/unit area) (Fig. 1). They occurred in the cell body as well as in cell processes. Very frequently they were situated close and perpendicular to the cell membrane (Fig. 2). In most cases the "ribbon fields" consisted of one or two rods only. Sometimes, however, three rods were present. Very rarely "ribbon fields" consisting of more rods were seen.

In the cultured pineals an increase in number of "ribbon fields" was observed after 12 as well as after 48 hours of incubation (Fig. 1). Moreover, fields consisting of more than three rods were often observed (Fig. 3).

In the pinealocytes of orchidectomized rats the numbers of "ribbon fields" were considerably increased (Fig. 1). Very often they consisted of numerous rods, some of which were frequently elongated (Fig. 4). Sometimes, ribbons in adjacent cells were placed opposite to each other (Fig. 5). Moreover, an increased numbers of microtubules and structures described by *Lin* (1970, 1972) as microtubular sheaves were observed (Figs. 6 and 7). Frequently microtubules and microtubular sheaves were demonstrated in the vicinity of "synaptic" ribbons (Figs. 8, 9 and 10). Some of them showed structures similar to ribbons, but they were deprived of surrounding vesicles (Figs. 7 and 11).

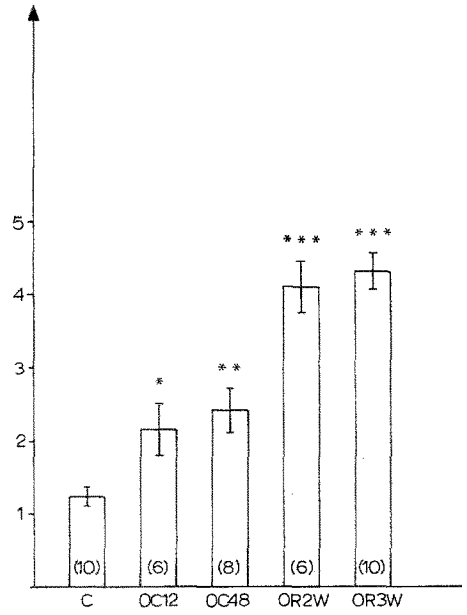

Fig. 1. Diagram of "synaptic ribbon fields" per unit area under different conditions. C — control rats; OC 12 — organ culture for 12 hours; OC 48 — organ culture for 48 hours; OR 2W — 2 weeks after orchidectomy; OR 3W — 3 weeks after orchidectomy. Means ± SEM are indicated. Significance: *$p < 0.02$; **$p < 0.005$; ***$p < 0.001$ as compared to controls. Number of animals in parentheses

Discussion

The results of the present study show that the number of "synaptic" ribbons in the rat pinealocytes undergoes prominent changes of a similar nature in such different experimental conditions as orchidectomy and organ culture. It is note worthy that, in the present quantitative studies we found an increase in number of "synaptic" ribbons in cultured pineals, which is in contrast to the qualitative findings of *Arstila et al.* (1971).

In the guinea-pig pineal organ it has been previously shown that "synaptic" ribbons increase in number during the night or after exposure of the animals to continuous illumination, while they decrease in number during the day (*Vollrath,* 1973; *Vollrath, Huss,* 1973). In the rat pineal organ rhythmic changes in number of these structures have also been observed (*Vollrath et al.*, 1975). In the rabbit pineal organ *Romijn* (1975) found an increase in number and size of "synaptic" ribbons after sympathectomy or continuous illumination.

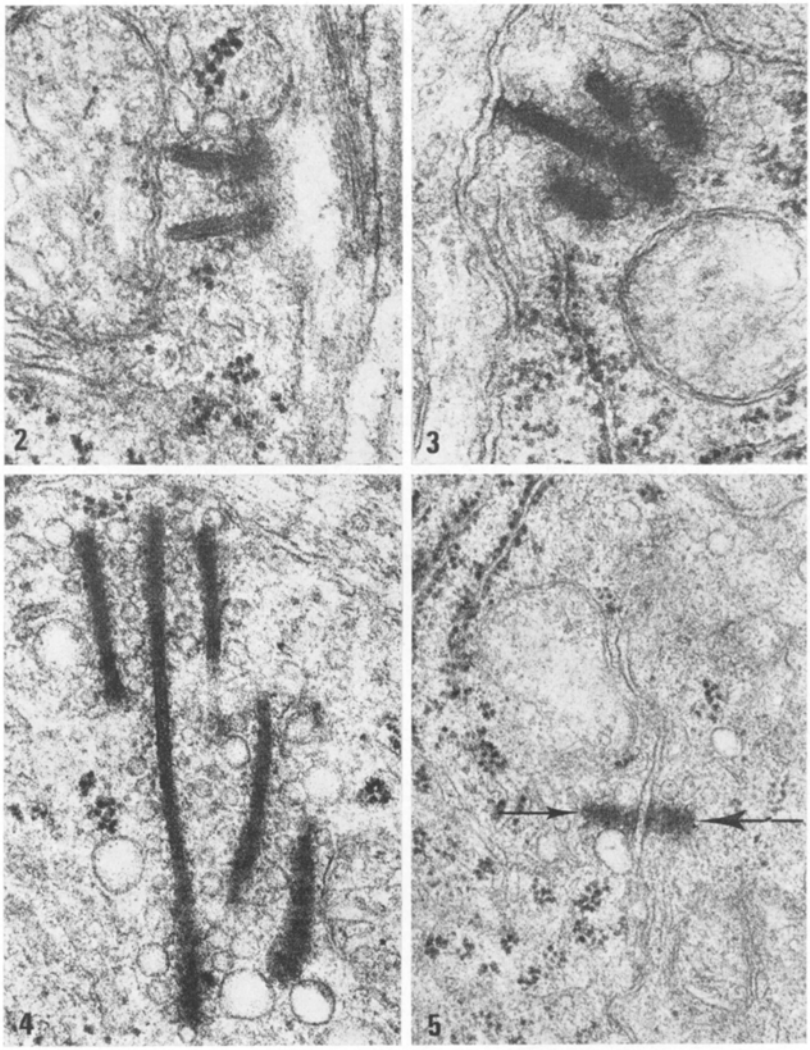

Fig. 2. Control rat. "Synaptic ribbon field" containing two rods, which are composed of three dense laminae with less dense zones sandwiched between, lying close and perpendicular to the cell membrane. ×47,500

Fig. 3. Rat pineal organ culture after 48 hours. "Synaptic ribbon field" consisting of 4 rods in the cell body of a pinealocyte, lying close to the cell mebrane. ×40,000

Fig. 4. Three weeks after orchidectomy. "Synaptic ribbon field" containing 5 elongated rods in the process of a pinealocyte. ×40,000

Fig. 5. Two weeks after orchidectomy. "Synaptic ribbon fields" in the cell body of a pinealocyte (small arrow) and in the process of a pinealocyte (large arrow) lying opposite to each other. ×40,000

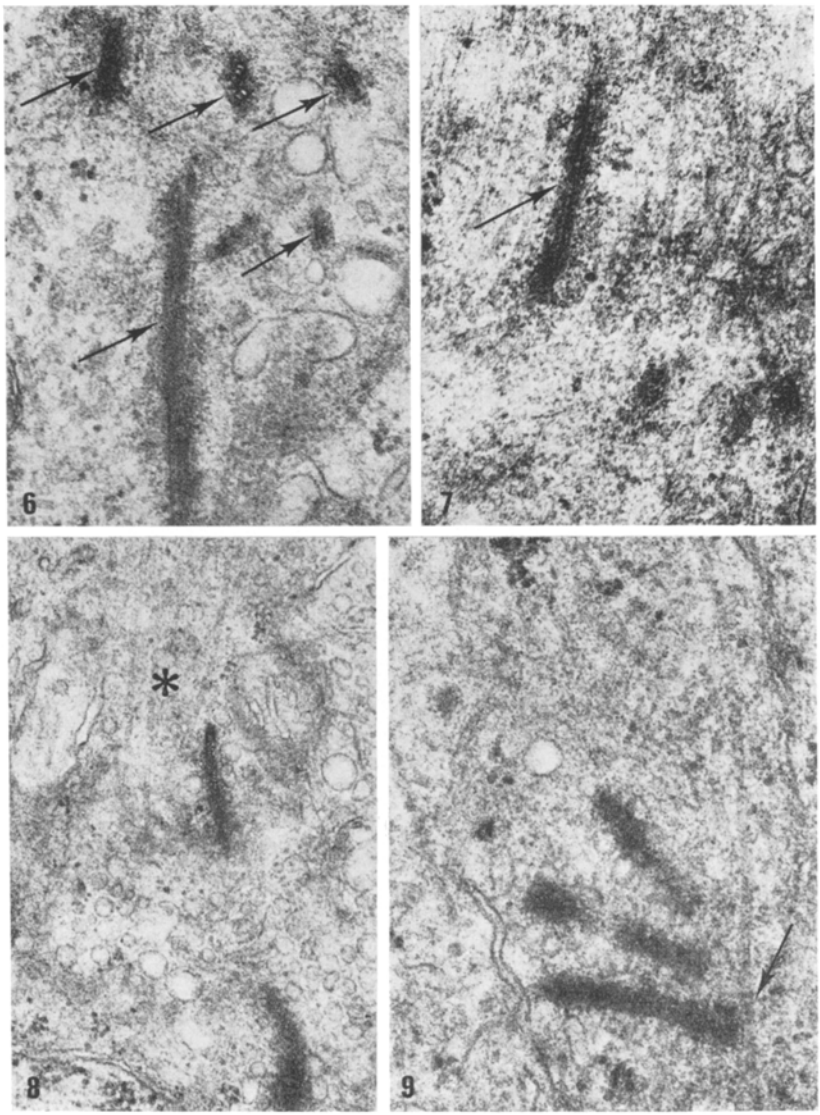

Fig. 6. Two weeks after orchidectomy. Microtubular sheaves in longitudinal, oblique and cross section (arrows). ×39,500

Fig. 7. Three weeks after orchidectomy. A dense rod consisting of three dense laminae with less dense zones sandwiched between (arrow) lying among numerous microtubules. ×46,000

Fig. 8. Three weeks after orchidectomy. Numerous microtubules (asterisk) lying in close vicinity to a "synaptic" ribbon. ×30,000

Fig. 9. Two weeks after orchidectomy. A long microtubule in close contact with a "synaptic ribbon field" (arrow). ×46,000

Thus it appears that "synaptic" ribbons in the mammalian pineal organ, far from being phylogenetic relics of no functional significance, may play a role in the functioning of the gland. A functional interpretation of the "synaptic" ribbons, however, is so far, difficult and a matter of conjecture. *Vollrath* (1973) suggested that "synaptic" ribbons are excitatory structures altering the physico-chemical properties of neighbouring pinealocytes, as a result of which the secretory activity of the pineal organ as a whole is increased. *Romijn* (1975) hypothesized that "synaptic" ribbons function as pineal receptors for the sympathetic neurotransmitter noradrenaline and that their increase after sympathectomy is of a compensatory nature. In previous studies, however, we have not been able to note any difference in number of "synaptic" ribbons in the pineal organ cultured in the presence of noradrenaline, compared with control cultures (*Karasek*, 1974).

In the opinion of the present author it cannot, however, be excluded that "synaptic" ribbons play a role in the pineal secretory process. It should be emphasized that after orchidectomy we demonstrated ultrastructural features indicating increased secretory activity in the rat pinealocytes (*Karasek et al.*, 1976).

The question also rises whether or not there are structures in the pinealocytes which could be regarded as precursors of "synaptic" ribbons. *Vollrath* (1973) has stated the absence of any such structures. Our present results, however, indicate the possibility that microtubular sheaves arising from centrioles (described by *Lin* [1970, 1972] in the rat and guinea-pig pinealocytes) may be somehow transformed into "synaptic" ribbons. Quite often we observed microtubules or microtubular sheaves lying in close vicinity to "synaptic" ribbons. It could be that two groups of microtubule fascicles become opposed and then transformed into the two laminae that make up the ribbon. This would account for the fact that each lamina has the same diameter as a microtubule.

It is of interest that *Gray* (1976), using an albumin technique, has shown that in synapses in the outer and inner plexiform layers of the frog retina, microtubules can be seen running up to, and lying in close topographical relationship with the synaptic ribbons.

It is obvious that the results of the present study do not solve the problem of the functional significance of "synaptic" ribbons in the mammalian pineal organ. However, the observation that these structures respond not only to changes in environmental illumination and innervation but also to hormonal imbalances is of some importance and point the way to further studies.

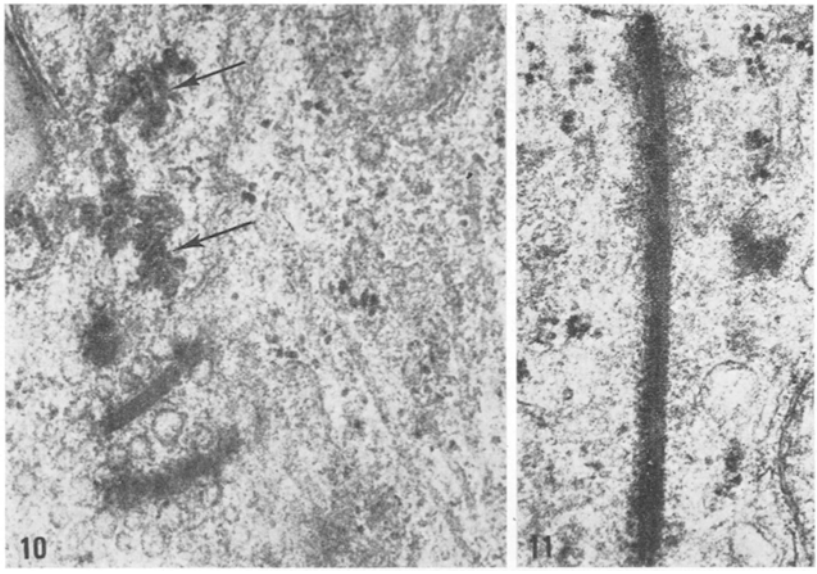

Fig. 10. Two weeks after orchidectomy. Cross sections of numerous microtubules lying in close vicinity to a "synaptic ribbon field" (arrows). ×46,000

Fig. 11. Three weeks after orchidectomy. A long dense rod consisting of three dense laminae with less dense zones sandwiched between. ×39,500

Acknowledgements

I wish to express my gratitude to Professor E. G. Gray and Professor J. Ariëns Kappers for fruitful discussions and their advice in preparing the manuscript. I am also indebted to Dr. G. Boer for his help in statistical analysis and to Miss M. T. Mud and Mr. P. S. Wolters for their skilful technical assistance.

References

Arstila, A. U.: Electron microscopic studies on the structure and histochemistry of the pineal gland of the rat. Neuroendocrinology 2, Suppl. 1—101 (1967).

Arstila, A. U., Kalimo, H. O., Hyyppä, M.: Secretory organelles of the rat pineal gland: electron microscopic and histochemical studies *in vivo* and *in vitro*. In: The Pineal Gland, a CIBA Foundation Symposium (*Wolstenholme, G. E. W., Knight, J.*, eds.), pp. 147—164. Edinburgh: Churchill Livingstone. 1971.

Collin, J. P.: Contribution a l'étude de l'organe pinéal. De l'épiphyse sensorielle a la glande pinéale: modalités de transformation et im-

plications fonctionnelles. Annls Stn Biol. Besse-en-Chandesse Suppl. *1*, 1—359 (1969).

Gray, E. G.: Microtubules in synapses of the retina. J. Neurocytol. (1976, in press).

Hopsu, V. K., Arstila, A. U.: An apparent somato-somatic synaptic structure in the pineal gland of the rat. Exp. Cell Res. *37*, 484—487 (1965).

Kappers, J. Ariëns: The mammalian pineal organ. J. Neuro-visc. Rel. Suppl. *9*, 140—184 (1969).

Kappers, J. Ariëns: The pineal organ: an introduction. In: The Pineal Gland, a CIBA Foundation Symposium (*Wolstenholme, G. E. W., Knight, J.*, eds.), pp. 3—25. Edinburgh: Churchill Livingstone. 1971.

Karasek, M.: Ultrastruktura szyszynki szczura białego w różnych warunkach doświadczalnych. Praca habilitacyjna. Łódź. 1973.

Karasek, M.: Ultrastructure of rat pineal gland in organ culture; influence of norepinephrine, dibutyryl cyclic adenosine 3′, 5′-monophosphate and adenohypophysis. Endokrinologie *64*, 106—114 (1974).

Karasek, M.: Szyszynka. Warszawa: PZWL. 1976.

Karasek, M., Pawlikowski, M., Ariëns Kappers, J., Stepień, H.: Influence of castration followed by LH-RH administration on the ultrastructure of rat pinealocytes. Cell Tiss. Res. (1976, in press).

Lin, H. S.: The fine structure and transformation of centrioles in the rat pinealocyte. Cytobios *2*, 129—151 (1970).

Lin, H. S.: Transformation of centrioles in pinealocytes of adult guinea-pigs. J. Neurocytol. *1*, 61—68 (1972).

Oksche, A.: Sensory and glandular elements of the pineal gland. In: The Pineal Gland, a CIBA Foundation Symposium (*Wolstenholme, G. E. W., Knight, J.*, eds.), pp. 127—146. Edinburgh: Churchill Livingstone. 1971.

Romijn, H. J.: The ultrastructure of the rabbit pineal gland after sympathectomy, parasympathectomy, continuous illumination and continuous darkness. J. Neural Transm. *36*, 183—194 (1975).

Vollrath, L.: Synaptic ribbons of a mammalian pineal gland. Circadian changes. Z. Zellforsch. *145*, 171—183 (1973).

Vollrath, L., Huss, H.: The synaptic ribbons of the guinea-pig pineal gland under normal and experimental conditions. Z. Zellforsch. *139*, 417—429 (1973).

Vollrath, L., Kantarjian, A., Howe, C.: Mammalian pineal gland: 7-day rhythmic activity? Experientia *31*, 458—460 (1975).

Wolfe, D. E.: The epiphyseal cell: an electron-microscopic study of its intercellular relationships and intracellular morphology in the pineal body of the albino rat. Progr. Brain Res. *10*, 332—386 (1965).

Wurtman, R. J., Axelrod, J., Kelly, D. E.: The Pineal. New York-London: Academic Press. 1968.

Author's address: Ass. Prof. Dr. *M. Karasek*, Laboratory of Electron Microscopy, Research Centre, Medical Academy, ul. Sterlinga 5, 91425 Łódź, Poland.