Morphological evidence for calcium storage in the chromatoid body of rat spermatids

M. D. Andonov and G. N. Chaldakov

Department of Anatomy and Histology and Laboratory of Electron Microscopy, Varna Institute of Medicine (VIM), 9002 Varna (Bulgaria)
Received 26 August 1988; accepted 5 January 1989

Summary. Morphological evidence for probable Ca^{2+} storage in the vesicular elements of the rat spermatid chromatoid body is documented using the K-pyroantimonate method, combined with EDTA chelation. Some vesicles are related to the microtubules associated with the chromatoid body. A possible involvement of Ca^{2+} in the intracellular movement and/or structural integrity of the chromatoid body is discussed.
Key words. Chromatoid body; spermatids; calcium; microtubules; morphology; pyroantimonate; rat.

The chromatoid body (CB) in rat spermatids is a two-component organelle consisting of a) electron-dense material, and b) numerous smooth-surfaced vesicles [1–4]. In this report, we present data demonstrating Ca^{2+} within the CB vesicles of rat spermatids, using a selective K-pyroantimonate method combined with EDTA chelation [5].

Materials and methods. Pieces from testes of adult Wistar rats were fixed in unbuffered 3% glutaraldehyde containing 2% K-pyroantimonate, pH 7.3, at room temperature for 2 h. After triple washing in 2% K-pyroantimonate, the specimens were postfixed in unbuffered 1% OsO_4 containing 2% K-pyroantimonate, pH 7.3, at room temperature for 2 h. Control specimens were fixed in the same solutions without K-pyroantimonate. After ethanol dehydration, samples were embedded in Durcupan ACM (Fluka). Ultrathin sections, unstained or stained with uranyl acetate and lead citrate, were examined in a JEM 7A or EM 108 Turbo (Opton) electron microscope. A postsectioning chelation of antimonate precipitates was made using unstained sections treated with 10.0 mM EDTA, pH 7.5 (adjusted with KOH), at 60 °C for 30 min [5]. After this treatment, which resulted in destaining of antimonate deposits, uranyl acetate-lead citrate staining was carried out to improve the contrast of cellular components.

Results and discussion. Antimonate precipitates were located within the vesicles associated with the electron-dense material of all the CB observed (figs 1–3). No precipitates were found in control specimens. The diameter of an individual precipitate was 20–30 nm. Antimonate-containing vesicles were closely related to the CB-associated microtubules (MT) (fig. 3). The latter

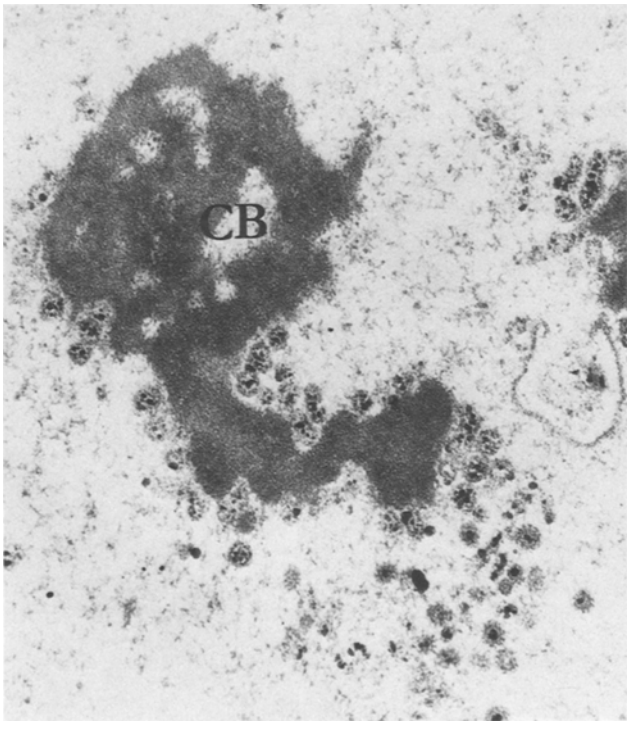

Figure 1. Chromatoid body (CB) in a spermatid of the rat. K-pyroantimonate-containing glutaraldehyde-OsO_4 fixation; uranyl acetate-lead citrate staining. All the vesicles contain antimonate precipitates. x 16,000.

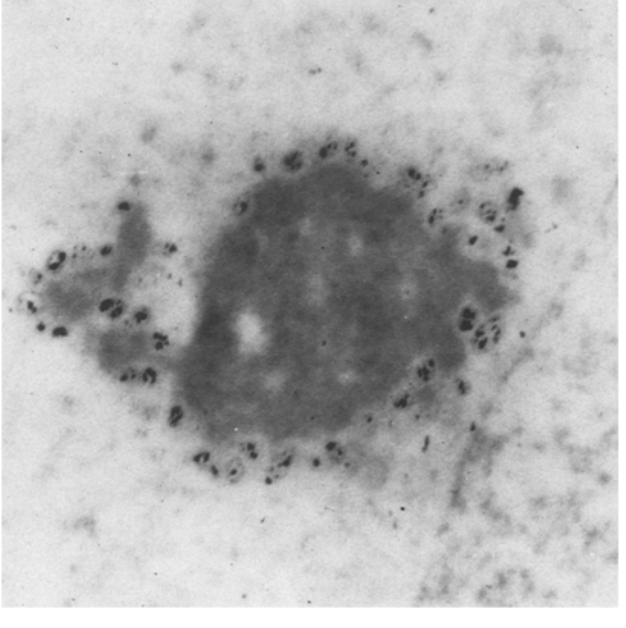

Figure 2. Chromatoid body in a spermatid of the rat. K-pyroantimonate-containing glutaraldehyde-OsO_4 fixation; unstained with uranyl acetate-lead citrate section. All the vesicles contain antimonate deposits. x 16,000.

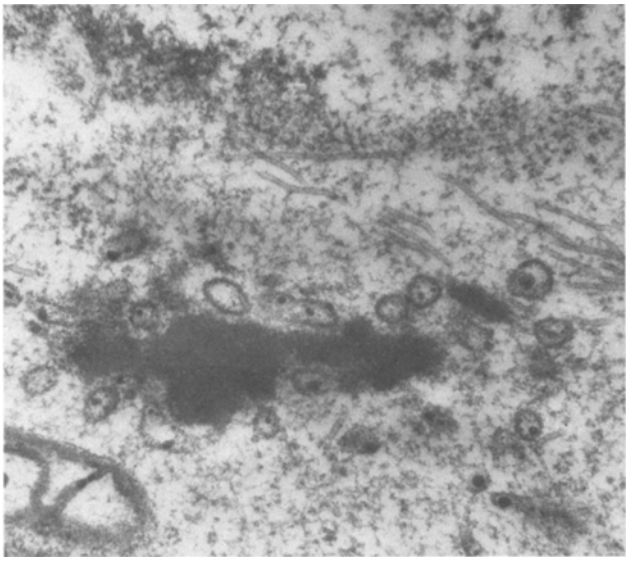

Figure 3. Chromatoid body and the associated microtubules, in a spermatid of the rat. K-pyroantimonate-containing glutaraldehyde-OsO_4 fixation; uranyl acetate-lead citrate staining. Microtubules located near antimonate precipitate-containing vesicles. x 16,000.

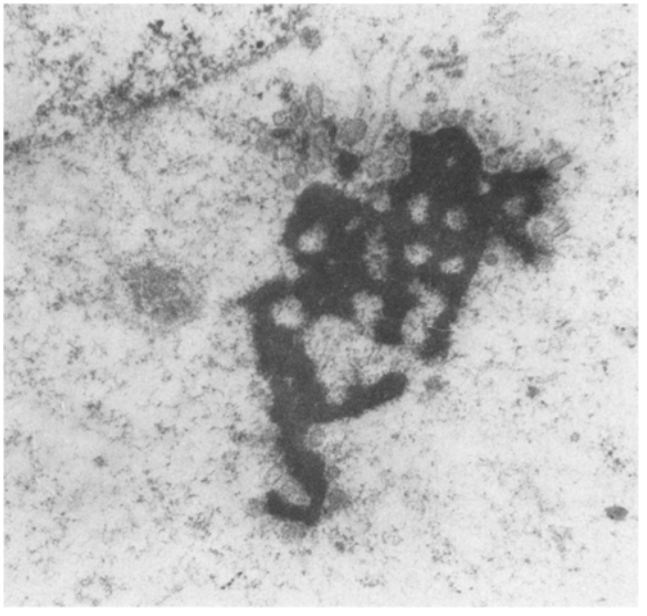

Figure 4. Chromatoid body in a spermatid of the rat. K-pyroantimonate-containing glutaraldehyde-OsO_4 fixation; these sections were not first stained with uranyl acetate-lead citrate; they were treated with 10 mM EDTA, at 60 °C for 30 min, followed by uranyl acetate-lead citrate staining. All the vesicles, that contained antimonate precipitates prior to EDTA treatment, are free of any precipitates after EDTA. x 16,000.

were previously described[2,6]. Multivesicular bodies, which commonly accompanied the CB, also contained antimonate deposits (not shown). In all the sections treated with a Ca^{2+} chelator, EDTA, an evident destaining of the antimonate precipitates was documented (fig. 4).

Since the K-pyroantimonate method is a selective electron microscopic stain for localization of Ca^{2+} [5], it is possible that through this study we are detecting Ca^{2+} storage in the rat spermatid CB for the first time. Thus, these vesicles may originate from the smooth endoplasmic reticulum[3], a major site of Ca^{2+} storage in various cells[7], including male germ cells[8] and Sertoli cells[9]. The relationship between vesicles and MT described here may represent an example of smooth membrane-MT microdomains[8,10]: these vesicles may create a local Ca^{2+} environment controlling the assembly-disassembly of MT[11,12] associated with the CB. We conclude that the CB probably possesses structural elements capable of Ca^{2+} storage and release. This may be involved in the intracellular motion and/or structural integrity of this particular organelle. The associated MT could contribute to this phenomenon[2,6].

Acknowledgements. The authors thank Dr S. Donev, Department of Anatomy and Histology, Medical Academy, Sofia, Bulgaria, for stimulating discussions and Miss L. Pastarmadgieva, Mrs P. Philipova and Mrs S. Pavlova for the skillful technical assistance.

1 Anton, E., Experientia *39* (1983) 393.
2 Andonov, M. D., and Chaldakov, G. N., Verb. Anat. Ges. *80* (1986) 527.
3 Clermont, Y., in: Spermatogenesis, p. 293. Eds R. O. Greep and M. A. Koblinsky. Cambridge 1977.
4 Söderström, K.-O., Exp. Cell Res. *131* (1981) 488.
5 Wick, S., and Hepler, P. K., J. Histochem. Cytochem. *30* (1982) 1190.
6 Kalt, M. R., Pinney, H. E., and Graves, K., Cell Tiss. Res. *161* (1975) 193.
7 Somlyo, A. P., Nature *30* (1984) 516.
8 Wise, D. A., and Wolniak, S. M., J. Cell Biol. *97* (1983) 41A.
9 Franchi, E., and Camatini, M., Cell Biol. int. Rep. *9* (1985) 41.
10 Chaldakov, G. N., and Vankov, V. N., Atherosclerosis *61* (1986) 175.
11 Keith, C., DiPaola, M., Maxfield, F. R., and Shelanski, M. L., J. Cell Biol. *97* (1983) 1918.
12 Matsuoka, T., and Shigenaka, Y., J. Electron Microsc. *34* (1985) 33.

0014-4754/89/040377-02$1.50 + 0.20/0