Tubular lysosomes in rat and gerbil pinealocytes

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Summary. Ultrastructural demonstration of acid phosphatase (AcPase) in pinealocytes of rat and Mongolian gerbil (Meriones unguiculatus) has revealed an existence of tubular lysosomes (30–200 nm in diameter and more than 5 μm long) in their cytoplasm. The tubular lysosomes arise by bulging from GERL cisternae (Golgi apparatus, endoplasmic reticulum, lysosomes) and spread throughout the whole cell body without forming an anastomosing network. Numerous varicosities are characteristic for the tubular lysosomes whose similarity with grumose bodies has lead to conclusion that the vermiculate variety of the latter are almost certainly tubular lysosomes. The role of these organelles is unknown. Their possible engagement in a rapid cytoplasmic remodelling of the pinealocyte body in answer to various stimuli has been discussed.

Introduction

Until recently the pinealocyte lysosomes have been neglected in the pinealogic research. Beside the study of Arstila (1967), there was for a long time no other studies about this topics. It is therefore not surprising that the pinealocyte lysosomes have been considered to be too rare to be of great cytophysiologic significance (Vollrath 1981). However, in the light of new data this opinion should be revised. Indeed, it has been recently demonstrated (Krstić 1985) that aside from classic autophagocytosis in rat and gerbil pinealocytes, lysosomes develop a particular type of autophagocytosis very similar to the lysosome wrapping mechanism (Mayahara and Ogawa 1972, Ogawa 1981). Still more recently the existence of GERL (Golgi apparatus, endoplasmic reticulum, lysosomes) in gerbil pinealocytes has been described (Krstić 1986). All these data speak in favour that production and cytophysiologic activity of pinealocyte lysosomes are considerably more important than hitherto believed.

Continuing research in this direction, the present paper intends to show a particular form of rat and gerbil pinealocyte lysosomes and to discuss their possible significance.

Materials and methods

For the present study a group of 3 adult male Wistar rats weighing 180 g and 5 adult male Mongolian gerbils Meriones unguiculatus) of 80 g body weight were used. Animals were anesthetized at 09:00 h with ether and intracardially perfused for 3 min with Ringer solution followed by fixation solution consisting of 2% glutaraldehyde and 1% formaldehyde in 0.15 M cacodylate buffer at pH 7.2 for 3 min; the pineal bodies were excised and fixed by immersion for 45 min in the same mixture at 4 °C. After washing in 0.1 M cacodylate buffer containing 5% sucrose for 120 min at 4 °C, the 30 μm thick Vibratome sections of pineal bodies were incubated for 60–90 min in a β-glycerophosphate-cerium medium according to Robinson and Karnovsky (1983) for ultracytochemical demonstration of acid phosphatase (AcPase).

In order to control the exact localization of this enzyme, simultaneous incubations of sections was effected in media for ultracytochemical demonstration of glucose-6-phosphatase (Robinson and Karnovsky 1983) and thiamine pyrophosphatase (Angermuller and Fahimi 1984).

For control of ultracytochemical reactions, Vibratome slices of liver and kidneys of the same animals were incubated together with those of pineal bodies. In addition, pineal, liver, and kidney slices were incubated in media without corresponding substrates as suggested by Robinson and Karnovsky (1983).

Following described treatments, the slices were washed overnight in 0.15 M cacodylate buffer at 4 °C, postfixed for 45 min in 1% OsO4-0.15 M cacodylate at room temperature, dehydrated and embedded in Epon. Ultrathin and 0.3–0.6 μm thick sections were cut with a Reichert Ultracut ultramicrotome and observed with a Zeiss EM-IOA electron microscope at 100 kV. Only ultrathin sections were stained with uranyl acetate and lead citrate. Some thick sections were tilted with a goniometric stage at ±7° from point zero to obtain three dimensional view.

Results

Aside from round or spherical primary lysosomes described by Arstila (1967), one can observe on ultrathin sections of the rat and gerbil pinealocytes clusters of moderately strongly AcPase reactive tubuli 30–200 nm in diameter and up to 3 μm long in the plane of the section (Fig. 1). Unreactive tubules have been rarely observed. The contents of all tubules was a finely granular material in which occasional highly dark dots 30 nm in diameter and structures resembling lipid droplets of the same dimensions were scattered. The AcPase reactive tubules were obviously found near the Golgi apparatus but also distributed in cell processes.

Thick sections have furnished much better information about morphology, dimension, and extension of tubuli. As shown in Fig. 2, they can be straight, more than 5 μm long and display numerous varicosities. In other pinealocytes, tubuli cross under various angles (Fig. 3) and reveal round
Fig. 1. Acid phosphatase (AcPase) reactive tubuli in a rat pinealocyte. Ultrathin section stained with uranyl acetate and lead citrate. Bar = 1 μm

Fig. 2. AcPase reactive tubuli in a thick section. Numerous varicosities (arrowheads) can be observed along the relatively straight tubulus which ends with enlargements (arrows). At the right the tubuli seem to form a coil. Mongolian gerbil, 0.6 μm thick section, unstained. Bar = 1 μm

Fig. 3. AcPase reactive and almost rectilinear tubuli crossing each other under various angles. Transversally sectioned tubuli display small round profiles (arrows). Mongolian gerbil, 0.6 μm thick section, unstained. Bar = 1 μm

Fig. 4. Numerous tubuli with irregular and tortuous courses from which at least one ends with an enlargement (arrow). Mongolian gerbil, 0.6 μm thick section, unstained. Bar = 1 μm

Fig. 5. One of the two AcPase reactive cisternae (arrow) adjacent to the Golgi apparatus (G) seems to be continuous with the beginning of an AcPase reactive tubulus (arrowhead). Rat, ultrathin section, stained as in Fig. 1. Bar = 0.5 μm

Fig. 6a and b. Stereopair of a 0.6 μm thick section. (To obtain a three-dimensional view, the distance of stereoscopic lenses should be adjusted to 65 mm). Arrows indicate the continuities between AcPase positive cisternae and the tubuli. Mongolian gerbil, unstained. Bar = 1 μm