Subsurface tubular system in the outer sensory cells of the rat cochlea

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Summary. The system of subsurface tubules in the outer sensory cells of the rat cochlea consists of tubules lying immediately beneath the cell membrane. The tubules extend from the region below the cuticular plate, follow without interruption along the contours of the cell membrane, and terminate as a complicated, branched system under the nucleus. Several mitochondria are found on the cytoplasmic side of the tubules. Microtubules are observed in the space between the subsurface tubules and the mitochondria, in contact with the membranes of the subsurface system and, rarely, in contact with small, stalked vesicles.

Current theories on the role of the subsurface system are discussed and a new one, considering possible involvement of the system in transport of the synaptic transmitter, is proposed.

Key words: Organ of CORTI – Sensory cells – Microtubules – Synaptic transmitter

A subsurface membrane system in the distal sensory cells of the organ of Corti has been described several times (Engström and Wersäll 1958; Engström and Ades 1973; Angelborg and Engström 1973; Engström et al. 1970; Iurato 1961, 1967; Spoendlin 1957–1970). It is present on the underside of the cell membrane as a single layer in man, the cat (Spoendlin 1968) and the rat (Iurato 1961) and as a single or double layer in the squirrel monkey (Engström and Ades 1973), and has a multilayer structure in the guinea pig (Spoendlin 1959; Engström and Ades 1973). Although no direct studies of morphology or function of the subsurface tubular system have been made, a number of hypotheses concerning its possible role have been considered in recent years (Spoendlin 1966, 1968; Engström and Ades 1973; Vinnikow 1974).

In this study the ultrastructural morphology of the subsurface membrane system is described.

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Materials and methods

12 Wistar laboratory rats were used. The animals were anesthetised with intraperitoneally injected Nembutal® (35-40 mg/100 g body weight). The cochleae were fixed in two ways. In the direct method, the tympanic membrane was opened under the microscope and both malleus and incus were removed. The opening of the tympanic bulla was enlarged and overhanging bone removed with a dental drill. After exposing both round and oval windows, the stapes was removed with a stapedectomy hook and a small bore, steel tube was inserted into the oval window. The tube was connected either to a perfusion pump or to a syringe filled with the fixative. The fixative was injected for 4 min. In 3 rats a simultaneous, direct fixation of both cochleae was performed with good results.

In the second method, the cochleae were fixed by means of general systemic perfusion. The perfusate was circulated for 20 min by means of a perfusion pump. The 3-aldehyde mixture of formaldehyde, glutaraldehyde and acrolein (Kalt and Tandler 1971) was used as the fixative with both methods.

After initial fixation the cochleae were quickly dissected free, a hole was made in the apical coil and the organs were immersed in cold fixative for 3 h. Subsequent dissection was carried out under a dissecting microscope with the organ immersed in 0.1 M cacodylate buffer. The bony capsule was thinned down with a dental drill and then broken away with watchmakers' forceps and microhooks. After the entire organ of Corti was exposed, small pieces of tissue were cut, washed in fresh buffer solution and osmicated in cold 2% OsO₄. After 1 h in osmium the tissue was again briefly washed in buffer, dehydrated through ascending concentrations of alcohol, and embedded in either Spurr's low viscosity resin (Spurr 1969) or Epon 812 (Luft 1961). Plastic blocks were cut on a Reichert OmU-3 ultramicrotome, stained with uranyl acetate and lead citrate and examined in a Zeiss EM-9 electron microscope.

Results

Depending on the section angle, the system of "subsurface membranes" in the outer sensory cells of the rat cochlea appears either as rounded, vesicle-like profiles or as short, flattened cisternae (Figs. 1, 2). Only sections tangential to and just beneath the cell membrane allow observation of the form and arrangement of the membranes, which comprise a series of 40-50 nm diameter tubules located beneath the membrane of the cell (Fig. 3). The tubules continue uninterruptedly from under the cuticular plate to the region just below the nucleus. A 50 nm space separates neighbouring tubules the lumens of which are filled with a finely granular electron dense material (Fig. 3). A substance with similar appearance is contained within the rough endoplasmic reticulum (RER) located in the apical part of the cell.

At the upper end of the cell the tubules are almost parallel to the cuticular plate. Serial reconstruction showed presence of irregular elements connecting the tubules with the endoplasmic reticulum (ER) (Fig. 4). The connecting tubules are slightly wider than the members of the subsurface system and follow a complicated and often twisting course within the apical cytoplasm. Ribosomes were often attached to the interconnecting segments. Beneath the apical region, the subsurface tubules tilt gradually downwards and follow a spiralling course towards the base of the cell (Fig. 1). Their inclination with respect to the longitudinal axis of the cell varies between 15 and 40 degrees. At the level of the nucleus, the angle of incidence becomes steeper (down to 5 degrees). At several points along the height of the cell, the tubules send off collateral branches which establish contact with neighbouring members of the system. The branches are commonly located on the cytoplasmic face of the subsurface elements which accounts for local 2-layered appearance of the system. Similar but less extensively branching regions were also observed in the