A Freeze-Fracture Study of the Plasma Membrane of the Ito Cell in the Normal Rat Liver

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Summary. The plasma membrane of the Ito cell in the normal rat liver was studied by freeze-fracture electron microscopy. Ito cells appeared to adhere to endothelial cells or to be embedded in the microvilli of hepatocytes. Ramified processes with a few microvilli of the cell extended along the endothelial cells. Caveolae were constantly seen on the plasma membrane, but their numbers varied among cells. Two different patterns of intramembranous particles were found on the plasma membrane of the Ito cells: most cells showed an even distribution of the particles, but the others, aggregates of them. Particle-free domains were seen on the plasma membrane in some Ito cells. Piles of concave or convex sheets were sometimes seen in the freeze-fractured lipid droplets.

The Ito cell (fat-storing cell), which was initially described in human liver by light microscopy (Ito, 1951), is dispersed in the perisinusoidal space of Disse and is characterized by the presence of lipid droplets containing vitamin A (Ito and Shibasaki, 1968; Ito, 1978; Wake, 1980). The number of lipid droplets in the Ito cell has been correlated to the nutritional status of animals, especially to the amount of vitamin A intake (Kobayashi and Takahashi, 1971; Wake, 1971). With the freeze-fracture technique, studies have been done on lipid droplets in the Ito cell after the administration of a large dose of vitamin A (Kobayashi, 1981a,b, 1982; Yamamoto and Ogawa, 1984). However, detailed freeze-fracture profiles of the plasma membrane of the Ito cell in normal rats have yet to be thoroughly described.

The present study will demonstrate en face freeze-fracture profiles of Ito cells in the normal rat liver.

MATERIALS AND METHODS

Ten adult Wistar strain rats, five male and five female animals fed laboratory chow and water ad libitum, were used in this study. The animals were killed by decapitation. Excised liver blocks were cut into small pieces and immersed in 2.5% glutaraldehyde, 2% paraformaldehyde and 2% acrylamide in 0.1M cacodylate buffer, pH 7.4 for 3 hrs at 4°C. Other animals were infused with the above-mentioned fixative via the portal vein immediately after decapitation. Liver blocks were cut and fixed in the same manner as described above.
Thin-section electron microscopy

After several washings in the buffer, small tissue pieces were immersed in 1% osmium tetroxide in 0.1M phosphate buffer containing 0.5% glucose for 3 hrs at 4°C, dehydrated with a graded series of ethanol, embedded in Epoxy resin and sectioned with a diamond knife equipped in an ultramicrotome, LKB-8800 (LKB, Bromma, Sweden). Thin sections were stained with uranyl acetate and lead hydroxide.

Freeze-fracture electron microscopy

Other tissue pieces were washed in 0.1M cacodylate buffer containing 7.5% sucrose, soaked in cacodylate-buffered 30% glycerol for about 12 hrs and placed on a copper plate. The copper plates with the tissue pieces were frozen on a brass-block apparatus, HFZ-1 (Hitachi, Tokyo, Japan), cooled in liquid nitrogen, fractured with the apparatus in a vacuum evaporator, HUS-5 (Hitachi, Tokyo, Japan) at about -110°C, 1 × 10⁻⁶ Torr, shadowed with platinum-carbon, and backed with carbon. Replicas were immersed in a commercial bleach to remove the organic materials, washed several times with 50% ethanol, and mounted on copper grids.

Freeze-fracture replicas and thin sections were examined in electron microscopes, JEM-100U and JEM-100C (JEOL, Tokyo).

Fig. 1. A freeze-fractured cytoplasmic face of an Ito cell with numerous lipid droplets (L). An outer leaflet of the nuclear envelope is pressed upon by lipid droplets. The arrowhead points to a bundle of collagen fibers. S sinusoid, H hepatocyte. ×11,000
RESULTS

Thin-section electron microscopy

Ito cells in the normal rat liver were located in the perisinusoidal space and, therefore, had an endothelial side facing the endothelial cells and a hepatocytic side facing the microvilli of the hepatocytes. The Ito cells extended subendothelial processes along the endothelial cells. These processes sometimes ended in microvillous projections as thick as those of the hepatocytes. The processes occasionally contained lipid droplets, some of which were surrounded by an electron dense rim. A few caveolae were occasionally seen along the plasma membrane.

Freeze-fracture electron microscopy

In freeze-fracture replicas, the hepatocytes were clearly identified by the profiles of their cytoplasm. They were characterized by numerous microvilli facing the perisinusoidal space and the adluminal junctional complex facing adjacent hepatocytes. Sinusoidal endothelial cells were identified by their cytoplasmic sheet provided with numerous fenestrae which were partly grouped to form the sieve plates.

Ito cells were identified by their localization in the perisinusoidal space and by

![Image: A freeze-fractured cytoplasmic face and the P-face plasma membrane (P) of an Ito cell. Note the uneven distribution of caveolae (arrowheads) on the cell body and cell processes (asterisks). The E-face plasma membrane of an endothelial cell (E) showing pores (arrow) covers these cell processes. L lipid droplet. ×11,000]
Fig 3. and 4. Legends on the opposite page
their possession of lipid droplets. When the fracture plane hit their cytoplasm, various organelles were revealed (Fig. 1, 2). The nucleus was usually indented, and impressions due to lipid droplets were sometimes seen on the nuclear envelope. The lipid droplets appeared as spherical bodies containing a homogeneous material and piles of concave or convex sheets. Occasionally, particles resembling intramembranous particles were seen on the surface of the piled sheets in the lipid droplets. The lipid droplets with piled sheets were also seen in the freeze-fractured cytoplasm of the subendothelial processes. The hepatocytes also contained lipid droplets with piles of concave or convex sheets. When the fracture plane hit the hepatocytic side of the plasma membrane, the Ito cells appeared to be attached to the endothelial cells. When the fracture plane hit the endothelial side of the plasma membrane, Ito cells appeared to be embedded in the microvilli of the hepatocytes. Branched processes of the Ito cells, sometimes terminating on few microvilli of the hepatocytes, were extend-

Fig. 3. A cytoplasmic face and the P-face plasma membrane of an Ito cell (I) located between an endothelial cell (E) and a hepatocyte (H). Arrowheads point to invaginations of caveolae. L lipid droplet, S sinusoid. ×23,000

Fig. 4. Ramified processes of an Ito cell (asterisks) cover the microvilli (M) of hepatocytes (H). Note the presence of caveolae on the P-face plasma membrane and lipid droplets (arrowheads) in the cytoplasmic face. ×14,000

Fig. 5. P- and E-face plasma membrane of an Ito cell. Note the presence of piled sheets in a lipid droplet (L) and an even distribution of intramembranous particles. H hepatocyte. ×27,000
ed along the endothelial cells and into the space between adjoining hepatocytes (Fig. 3, 4). Impressions of the lipid droplets were sometimes identified as oval hillocks on the P-face plasma membrane or as oval dimples on the E-face plasma membrane. These oval domains were devoid of intramembranous particles. Particle-free oval domains were also found on the occasional nuclear envelopes. Caveolae were constantly seen on the plasma membrane of the cell body as well as the processes, but their numbers varied among cells. They were unevenly distributed on the plasma membrane (Fig. 2) and appeared to be more numerous on the cell body than on the processes. Microvilli were few on the endothelial side of the Ito cell surfaces. Therefore, at low power views of freeze-fracture replicas, Ito cells were identified by the smooth profiles of their surfaces facing the endothelial cells (Fig. 2). Most of the Ito cells showed evenly distributed intramembranous particles on the plasma membrane (Fig. 5), whereas some had aggregates of intramembranous particles on the plasma membrane (Fig. 6, 7). The aggregates of particles were also seen on the cell body, processes and microvilli. The number of the particles in an aggregate varied considerably. Some aggregate were composed of a few particles, while others, of numerous particles. Particle groups indicating desmosomes and gap junctions could not be found on the plasma membrane of the Ito cells.

**DISCUSSION**

The present observations indicate that most of the Ito cells possess evenly distributed intramembranous particles on the plasma membrane, and a few of them show aggregates of intramembranous particles. Previously KOBAYASHI (1981b) demonstrated particle aggregates on the plasma membrane of the Ito cells in the liver of vitamin A-administered rats, and he speculated that these are implicated in the process of vitamin A uptake. Such particle aggregates have been shown in pancreatic B cells (ORCI, PERRELET and FRIEND, 1977), neurohypophysial cells (THEODOSIS, DREIFUSS and
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ORCI, 1978) and anterior pituitary cells (ISHIMURA, EGAWA and FUJITA, 1980). In addition, particle aggregates have been demonstrated during the phospholipase A₂-induced granule secretion from mast cells (CHI, HENDERSON and KLEBANOFF, 1982) and the action of an antidiuretic hormone on amphibian bladder cells and on mammalian renal collecting duct cells by a rapid-freeze technique (HAYS et al., 1985). Therefore, these differences in the intramembranous particle distribution pattern may represent functional differences in the Ito cells. Indeed, it has been widely known that the size and number of the lipid droplets in the Ito cells gradually decrease from the periportal zone toward the centrilobular zone in the hepatic lobule. Caveolae on the plasma membrane of the Ito cell have been shown in thin-section electron microscopy of the human liver (ITO and SHIBASAKI, 1968; TANUMA, ITO and SHIBASAKI, 1982) and in the liver of vitamin A-administered rats by the freeze-fracture technique (KOBAYASHI, 1981b). The present study shows a variation in the number of caveolae in the Ito cells. Moreover, zonal diversities among hepatocytes in the hepatic lobule have been well established. This also may possibly correlate with the functional diversities in the Ito cells.

Some freeze-fractured lipid droplets in the Ito cells revealed piles of concave or convex sheets. Similar structures in lipid droplets have been shown in the Ito cells of the vitamin A-administered rat liver (KOBAYASHI, 1981a), brown fat cells (TAIRA and SHIBASAKI, 1984) and cardiac muscle cells (BRUNEVAL, YOUNG and HUTTNER, 1985). The occurrence of piled sheets in freeze-fractured lipid droplets has been considered to be caused by artefacts induced by immersion fixation, since no lipid droplets in the Ito cells show such structures following perfusion fixation (YAMAMOTO and OGAWA, 1984). In the present study, however, similar piled sheets were usually seen in freeze-fractured lipid droplets in the Ito cells as well as those in the hepatocytes after immersion fixation or intraportal fixative infusion immediately following the decapitation of the animals. Therefore, the occurrence of piled sheets in freeze-fractured lipid droplets is not believed to be attributable to an artefact induced by the procedures of immersion fixation.

REFERENCES


