IDENTIFICATION OF DIGITONIN-BINDING SITES OF THE ENDOTHELIOCYTE PLASMA MEMBRANE OF THE RABBIT AORTA

E. G. Popova, T. M. Povalii,
S. A. Gusev, I. A. Gritskova,
and E. M. Kogan

UDC 616.132-018.74-008.939.22-074

KEY WORDS: digitonin, cholesterol, endotheliocyte, cholesterolosis.

The important role of endothelium in the development of lesions of the vascular wall in cholesteroloses of varied etiology has now been established. The modern concept of cholesteroloses is based on ideas regarding cholesterolaccumulation in cell membranes, leading to significant changes in the dynamic characteristics of these membranes [1]. The study of the quantity and the stereotopography of cholesterol in the membrane cells accordingly assumes particular urgency, for it can make an important contribution to the elucidation of the mechanisms of atherosclerosis and may enable methods of early diagnosis of this disease at the cellular level to be developed. The possibility of determining the cholesterol content in the plasma membrane of a single cell by scanning electron microscopy (SEM), and with the aid of an x-ray microanalyzer and a specially synthesized substance whose molecule consists of the ligand digitonin, of a polymer carrier polyvinylpyrrolidone, and silver as the "signal" element [2], was discovered previously. Methods of identifying and determining the topography of components of the cell surface for SEM with the aid of discrete carriers are currently being intensively developed [4-7]. In the present investigation this approach was used in order to mark the sites of localization of cholesterol in endotheliocyte membranes with the aid of latex particles visible in the SEM.

EXPERIMENTAL METHOD

Experiments were carried out on rabbits weighing 3.5-4 kg. After perfusion fixation with 2.5% glutaraldehyde solution for 10-15 min the thoracic part of the aorta was excised from the animals and placed in a fresh portion of the same fixative for 12 h at 4°C. The preparation was then washed to remove the fixative in 0.1 M phosphate buffer and placed in 0.1 M glycine solution for 30 min. After removal of the glycine fragments of the aorta were placed in a suspension of latex particles, containing digitonin molecules on their surface, for 1-4 h at room temperature with constant agitation. As the control of the specificity of binding, some fragments of the aorta, before incubation in the suspension of latex particles, were placed in 0.4% digitonin solution for 24 h. The material was postfixed in 1% OsO_4 solution in phosphate buffer for 1 h. The preparations were dehydrated in alcohols of increasing concentration, dried at the critical point with CO_2 in an HCP-2 apparatus, mounted on the specimen holders, sprayed with gold in the EIKO IB-3 apparatus, and examined in the S-570 scanning electron microscope.

EXPERIMENTAL RESULTS

On electron-microscopic analysis of the preparations, nonadherent latex particles were found (Fig. 1a) after exposure of the aorta for 1-4 h with latex on the surface of the endotheliocyte plasmalemma. The cell surface, incidentally, was covered unevenly with latex particles. Some regions of the plasmalemma had a high density of particles, whereas others were completely free from them. Several particles located very close to each other often formed a straight or slightly curved line. In the region of contact between two adjacent endotheliocytes, latex particles were localized along the edge of the marginal region of the zone of the endothelial cell around the junction.

Laboratory of Cytology, Research Institute of Physicochemical Medicine, Ministry of Health of the RSFSR. Department of Polymer Synthesis, M. V. Lomonosov Institute of Fine Chemical Technology, Moscow. Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 107, No. 1, pp. 96-97, January, 1989. Original article submitted October 17, 1986.

0007-4888/89/0001-0113$12.50

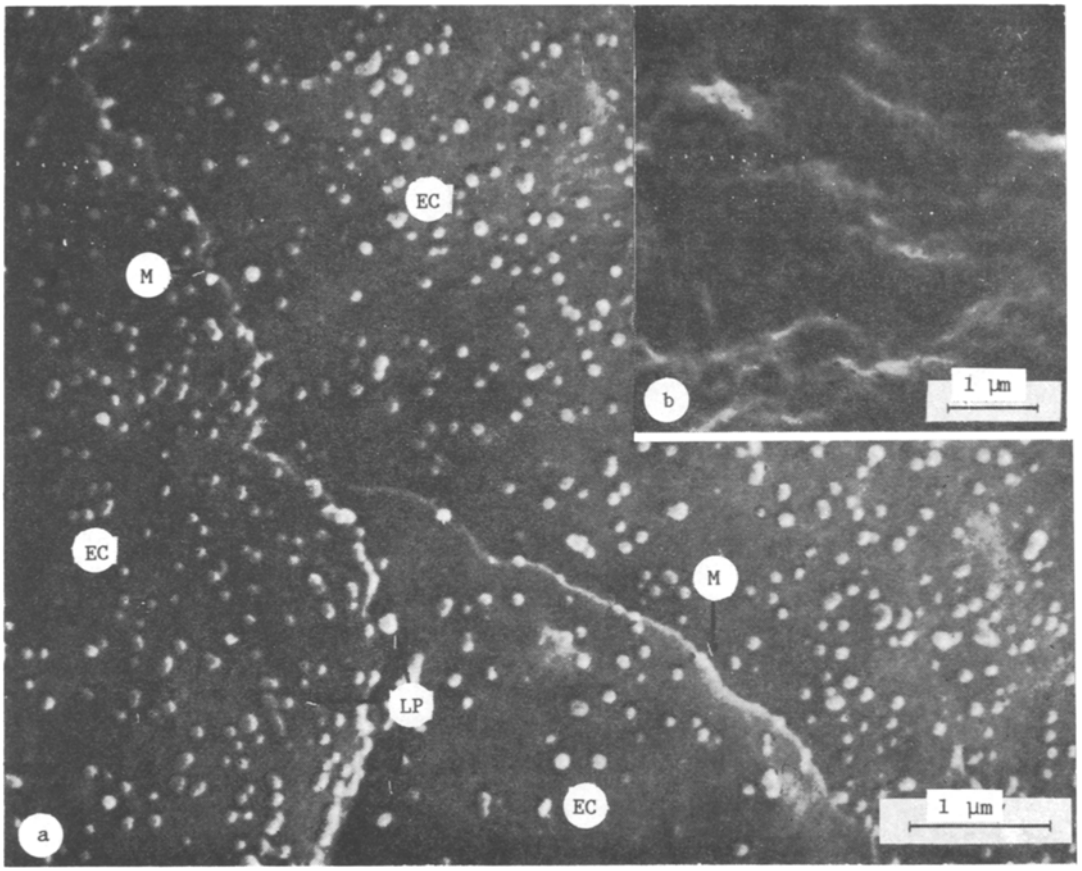

Fig. 1. Stereotopography of digitonin-binding sites of endotheliocyte plasma membrane of rabbit aorta (a) and surface of endothelium of aorta in a control preparation (b). EC) Endothelial cells; LP) latex particles; M) marginal region of contact zone of endothelial cell.

A study of the control preparations showed that, after preliminary treatment of the endothelial cells with digitonin solution, latex particles do not react with the surface of endotheliocytes (Fig. 1b).

The results are thus evidence that the latex particles used bind specifically with the cholesterol of the endothelial cell plasmalemma of the rabbit aorta. This suggests that the particles used are specific markers for cholesterol. The varied density of distribution of the marker over the surface of the cell membrane probably reflects the presence of heterogeneities in the arrangement of certain components of the endotheliocyte plasmalemma. Regions of the plasmalemma free from marker particles are perhaps characterized by a higher content of protein components in the membrane [3].

LITERATURE CITED

1. Yu. M. Lopukhin, Yu. A. Vladimirov, A. I. Archakov, and É. M. Kogan, Cholesterolosis [in Russian], Moscow (1983).
2. T. M. Povalii and S. A. Gusev, Proceedings of the 4th All-Union Symposium on Scanning Electron Electron Microscopy and Analytical Methods of Investigation [in Russian], Moscow (1984), p. 52.
3. R. M. Elias, D. S. Frien, and I. J. Goerki, J. Histochem. Cytochem., 27, No. 9, 1247 (1979).
4. E. Harven and M. K. Nemanic, Cell Surface Labeling (1979).
5. S. Margel, Ing. Eng. Chem. Prod. Res. Dev., 21, 343 (1982).
6. R. S. Moldey and P. Maher, Histochem. J., 12, 273 (1980).
7. P. Pinto de Silva and W. K. Kan, J. Cell Biol., 99, 1156 (1984).