ELECTRON-MICROSCOPIC IDENTIFICATION OF ENDOCRINE SECRETORY GRANULES IN ENDOTHEIAL CELLS

I. M. Kvetnoi and R. P. Manokhina

UDC 612.135.018-086.3.

KEY WORDS: endothelial cells; endocrine granules; electron-microscopic identification; hormonal regulation

Thanks to the widespread introduction of immunohistochemical and electron-microscopic research techniques into biology and medicine hitherto unknown endocrine cells have been discovered; these cells, located in nonendocrine organs, produce highly active chemical substances (biogenic amines and peptide hormones) that are responsible for the local regulation of homeostasis [1, 16]. There have also been individual reports that hormones can be synthesized in cells for which an endocrine function had not hitherto been known. For example, endocrine secretory granules, which are the site of production of various peptides, are found in myocardial cells [5, 10, 13, 17]. The biological significance of this effect is evidently autoregulation by hormones of intracellular processes essential for the performance of specific function.

There have been a few investigations aimed at discovering argyrophilic granular cells in the wall of the aorta and other vessels [11, 12, 15]. The presence of serotonin and melatonin has been demonstrated immunohistochemically in the endothelium of arterioles of the kidneys [2] and angiotensin has been found in the wall of the aorta [3]. Fairly high renin concentrations in the vascular wall have been recorded by radioimmunoassay [6].

These facts, together with the familiar vasotropic effects of biogenic amines and certain peptide hormones, suggest that the hypothesis of the existence of secretory endocrine granules in the walls of blood vessels, the morphological reflection of the existence of local neuroendocrine mechanisms for regulation of vascular tone, vascular permeability, the blood flow, and other manifestations of homeostasis, rests on a firm biological basis. The question whether hormone production in the endothelium is linked with specific endocrine cells, or whether this function may be performed by certain endothelial cells, remains unanswered. The aim of this investigation was an electron-microscopic study of the possible cellular sources of hormone synthesis in the vascular wall.

EXPERIMENTAL METHOD

Capillaries of organs with a well-developed blood supply, namely the liver, spleen, and bone marrow, from Wistar rats were the test objects. Fragments of the organs were fixed in a mixture of 2.5% glutaraldehyde and 2% formaldehyde (1:2) in 0.1 M Sorensen's phosphate buffer for 1 h, followed by postfixation for 1 h in 1% OsO_4 solution in the same buffer (pH 7.2). After treatment with a saturated solution of uranyl acetate in 70° alcohol the material was dehydrated in alcohols of increasing strength and embedded in a mixture of epoxy resins. Sections were cut on the LKB-4800-A Ultrotome, stained with lead citrate by Reynolds' method, and examined in the JEM-100C and JEM-5Y electron microscopes.

EXPERIMENTAL RESULTS

The experiments showed the presence of polymorphic osmiophilic granules of different sizes in the capillary endothelium of the liver, spleen, and bone marrow (Fig. 1 and 2).

Granules were distributed singly and in groups in the cytoplasm of the endothelial cells among other organoids (mitochondria, ribosomes, lysosomes, cisterns of the rough and smooth endoplasmic reticulum, and vacuoles). Elements of the lamellar complex were frequently found in the immediate vicinity of the granules. The granules differed in size, shape, and structure, but granules of the following three types were found most frequently: type I) homogen-

Research Institute of Medical Radiology, Academy of Medical Sciences of the USSR, Obninsk. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 101, No. 1, pp. 116-119, January, 1986. Original article submitted April 26, 1985.

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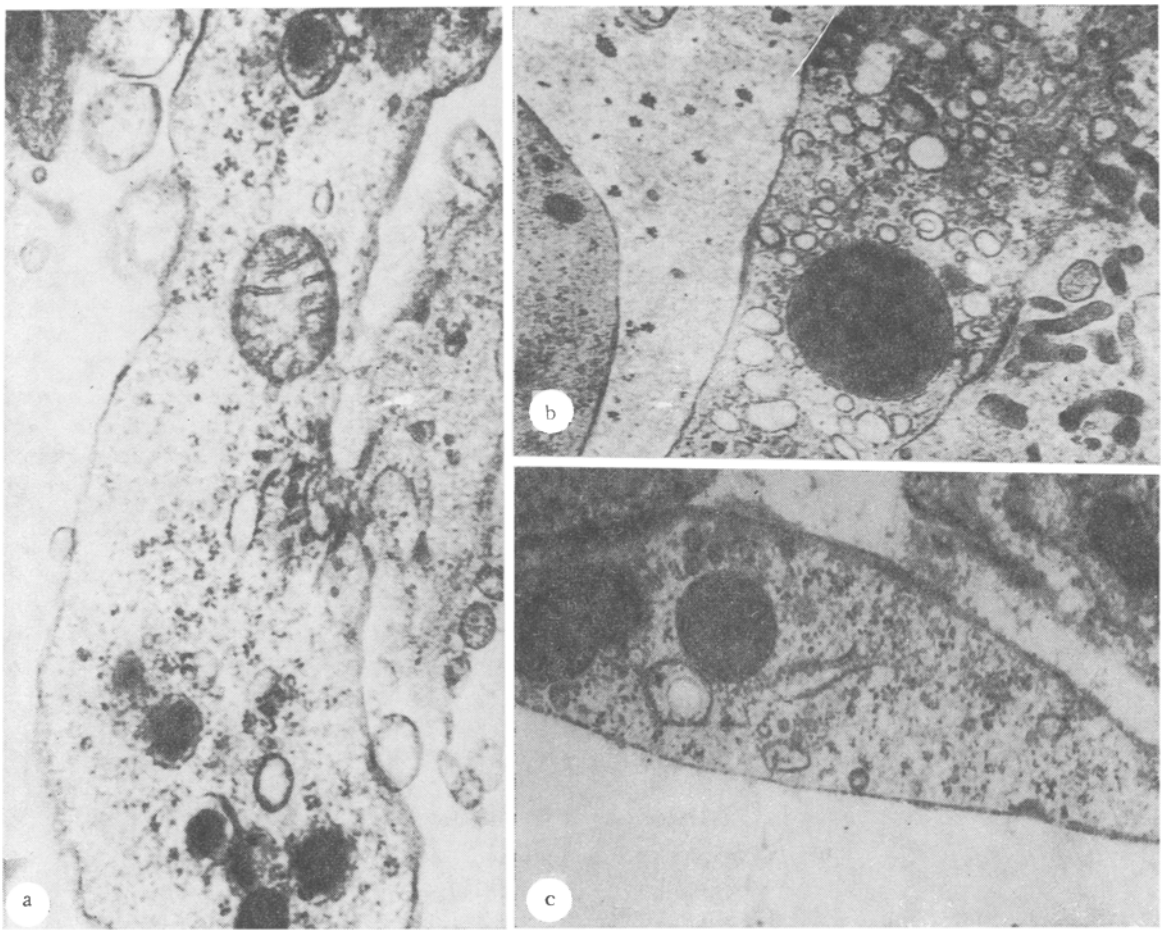

Fig. 1. Endocrine granules in capillary endothelium of various organs: a) serotonin- and insulin-containing granules in endothelial cell of splenic capillary. 45,500 ×; b) Large catecholamine-containing secretory granule in capillary endothelium of the liver. 55,000 ×; c) bean-shaped (serotonin) and round (catecholamine) granules in bone marrow capillary. 16,750 ×.

eous electron-dense oval or bean-shaped granules with a very narrow pale border around the electron-dense core, or without a border (Fig. 1a, c; Fig. 2a, d-f); type II) "signet-ring" granules with an electron-dense core, located centrally or displaced toward one pole, and with a wide, pale halo around it (Fig. 1a; Fig. 2b, e); type III) large round granules with an electron-dense core and a thin, pale halo around it (Fig. 1b, c; Fig. 2a-d). Granules in some endothelial cells were located in the immediate vicinity of the cytoplasmic membrane, facing the lumen of the vessel (Fig. 2a-c). Areas of endothelium forming bud-shaped evaginations, with one or more secretory granules in their apices, were found (Fig. 2d, e). The membrane of one of them formed a single entity with the cytoplasmic membrane of the endothelial cell, and in that way the electron-dense granule limited communication between the cytoplasm of the endothelial cell at the site of the evagination and the capillary lumen, like a "cork" (Fig. 2d, e).

Objective ultrastructural criteria have now been drawn up [1], according to which the granules described above in the endothelial cells can be regarded as endocrine in nature, and the type of the hormones synthesized in them can be judged. For instance, the type I oval or bean-shaped granules are characteristic of serotonin and its metabolites — melatonin. Similar granules are found in large numbers in the enterochromaffin cells of the intestine, where they are the main site for biosynthesis of these substances in the body. The "signet-ring" type II granules are identical in their structure with the insulin-producing granules of the parncreatic B-cells. The large round type III granules resemble catecholamine granules of cells of the adrenal medulla.

The electron-microscopic investigations thus established for the first time that the sites of hormone production in the vascular wall are secretory granules, located actually within

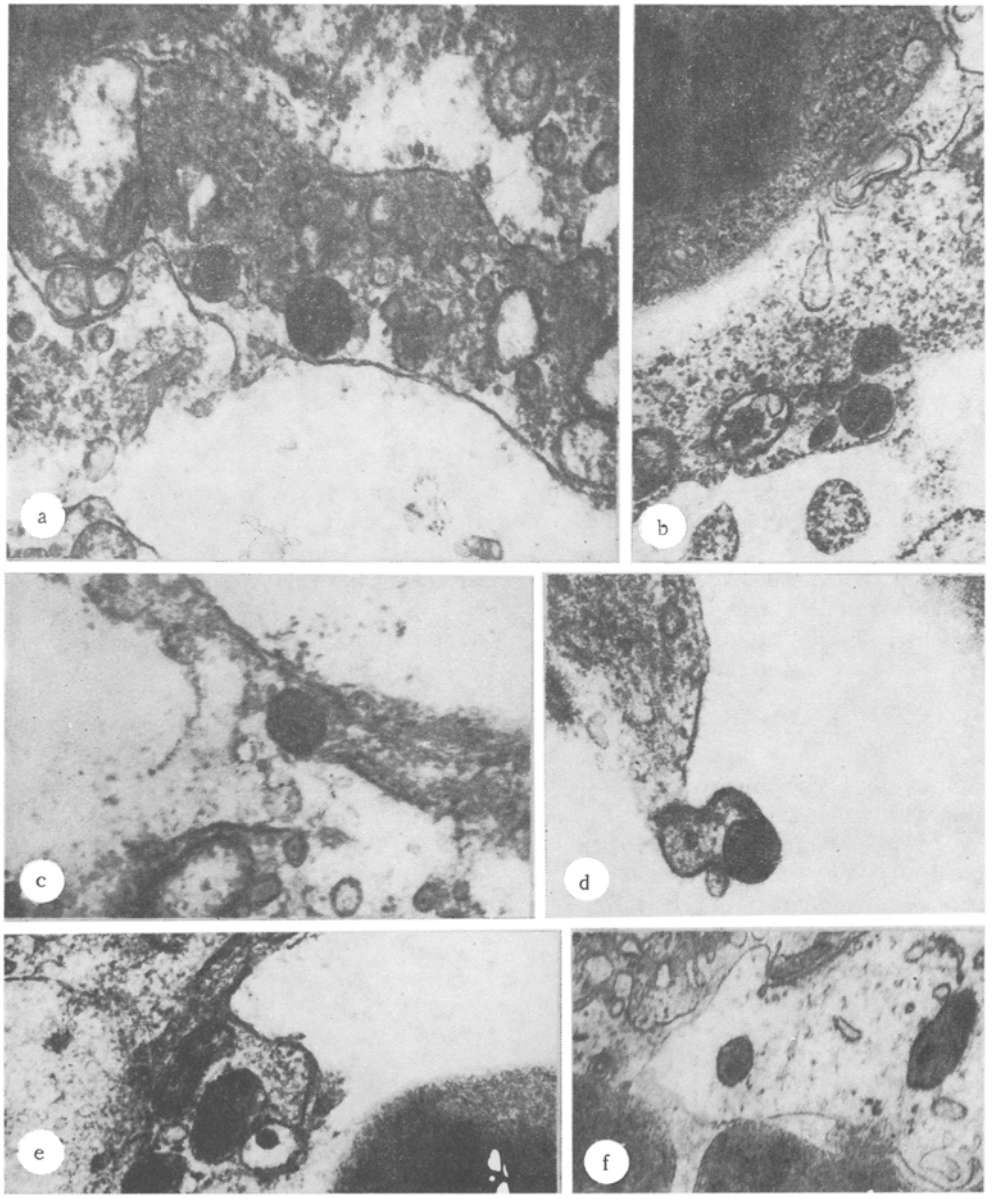

Fig. 2. Polymorphism and exocytosis of secretory granules in endothelial cells. a) Serotonin-containing secretory granules in immediate vicinity of cytoplasmic membrane of endothelial cell. Spleen b) Insulin- and serotonin- containing granules in endothelium of bone marrow capillary; c, d, e) different stages of exocytosis of secretory granules in endothelial cells of capillaries of bone marrow (c) and spleen (d, e); f) bean-shaped (serotonin) and club-shaped (melatonin) secretory granules in endothelium of capillary of the liver. Magnification: a-c, e) 31,250, d) 45,500, f) 28,250.

individual endothelial cells, and not special endocrine cells incorporated in the endothelium.

Identification of the secretory granules indicates that biologically active substances are synthesized actually within the endothelial cells, and their synthesis is not linked solely with the presence of mediators in neurons [4] and peptidergic nerve fibers, found in the vascular wall [7-9].

The bud-shaped evaginations of the endothelium, containing secretory granules in intimate contact with the cytoplasmic membrane, are the morphological manifestation of exocytosis, the characteristic mechanism whereby hormones enter the blood stream through rupture of the granules membrane at the site of its contact with the lumen of the vessel [14]. This mechanism of an immediate change in hormone concentrations in the blood stream of a particular organ is

physiologically rational and can ensure the essential biological action of the hormone in the shortest time directly on those effector components whose activation is needed in whatever situation has arisen.

The further study of local endocrine mechanisms of vascular activity will provide further opportuninties for the elucidation and clarification of important factors in regulation of the blood flow, processes of hemostasis, transport of biologically active substances, and an adequate blood supply.

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