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PROSTAGLANDIN I_2 SYNTHASE IN THE LYMPHATIC ENDOTHELIUM OF RAT LIVER AS REVEALED BY PREEMBEDDING IMMUNOELECTRON MICROSCOPY

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ABSTRACT

Localization of prostaglandin I_2 synthase-like immunoreactivity (PGI_2SI) in the lymphatic endothelium of rat liver was investigated by means of preembedding immunoelectron microscopy. Vesicular and multivesicular bodies with PGI_2SI were closely apposed to Golgi complexes of the endothelial cells. Membrane-bounded PGI_2SI structures including vesicular and dense bodies were also present beneath the endothelial plasmalemma on both luminal and abluminal sides. Other organelles within the endothelial cells did not exhibit PGI_2SI. These findings are consistent with previous biochemical and physiological studies suggesting prostaglandin I_2 is produced in the Golgi apparatus and released into luminal and abluminal spaces through membrane-bounded structures containing PGI_2SI.

Prostaglandin I_2 (PGI_2) is thought to participate in the regulation of lymph and blood flow by antagonizing vasocontractile stimulants such as thromboxane A_2 and leukotrienes (1-4). Several biochemical and physiological studies suggest that PGI_2 is produced in the walls of blood and lymphatic vessels (2,3,5-9). In support of this concept, a light microscopic immunohistochemical study using monoclonal antibodies against PGI_2 synthase has demonstrated localization of the enzyme in the endothelium and smooth muscle layer of blood vessels (10,11). It also seems of interest to investigate the sites of PGI_2 generation in the lymphatics. The present study describes PGI_2 synthase-like immunoreactivity (PGI_2SI) in lymphatic capillaries of the rat liver as demonstrated by preembedding immunoelectron microscopy.

MATERIALS AND METHODS

Seven Wistar male rats weighing 200g were housed under constant temperature (20°C) in a 12 + 12h light-dark cycle, and given food and water ad libitum. Each rat was deeply anesthesized with pentobarbital and perfused transcardially with 100ml of saline, followed by 200ml of 4%paraformaldehyde-0.05% glutaraldehyde-0.2% picric acid in 0.1M phosphate buffer (PB) (pH 7.4) (12). Following perfusion, the liver was quickly removed and cut into tissue blocks at the interlobular connective tissue regions. The tissue blocks were sectioned at 60μm with a Dosaka microslicer. Then the sections were collected, washed five times with phosphate buffered saline (PBS) (10 min for each washing), and processed for PGI synthase immunohistochemistry with the use of a monoclonal antibody against the enzyme

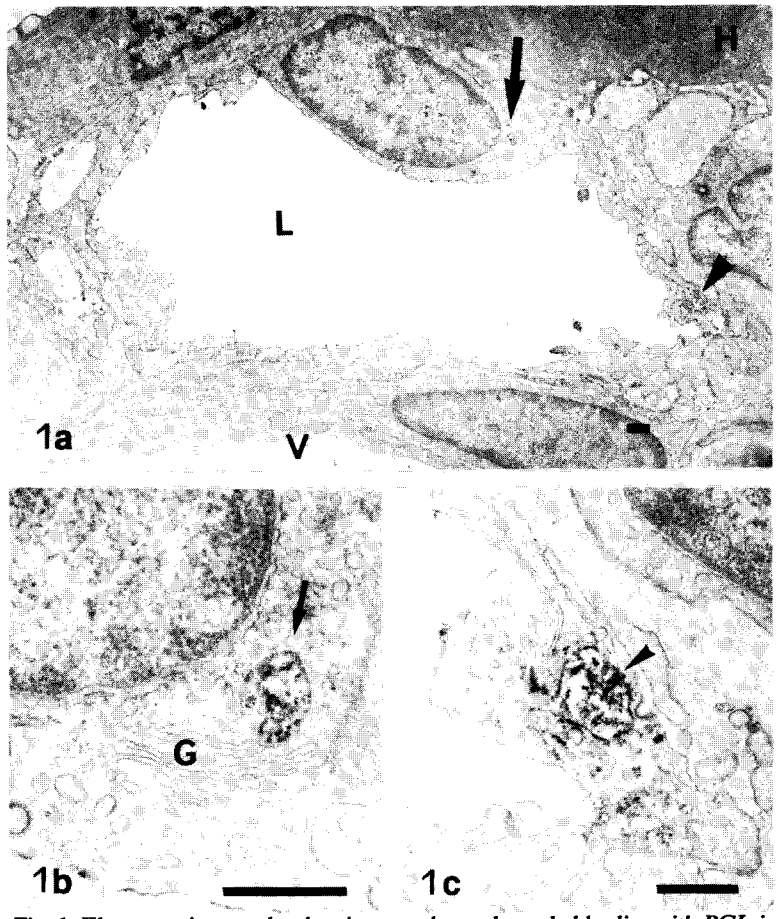

Fig. 1. Electron micrographs showing membrane-bounded bodies with PGI_2 in the perikaryon of a lymphatic endothelial cell (a, b, arrows) and in an area beneath the abluminal plasmalemma of the cell (a, c, arrowheads), at low magnification (a) and at high magnification (b, c). Note that the immunoreactive membrane-bounded body in the perikaryon is closely apposed to a Golgi complex (G). Abbreviations: H, hepatocyte; L, lumen of interlobular lymphatic capillary; V, interlobular vein; G, Golgi complex. Bars = 0.5μm; a, x7000; b, x40000; c, x26000.

(Cayman Chemical Company). The sections were postfixed with 1% osmium tetraoxide in 0.1M PB (pH 7.4), dehydrated in a graded series of ethanol while being stained with 1% uranyl acetate in 70% ethanol, transferred to propylene oxide and embedded in an epoxy resin. Ultrathin sections were made with a Porter-Blum MT2-B ultramicrotome and observed in Hitachi H-300 and Hatachi H800 electron microscopes.

Control sections were first incubated with the monoclonal antibody absorbed with an excess of PGI_2 synthase, and then they were processed as described above.

RESULTS

The lymphatic endothelium of the liver, which was identified in accordance with the criteria of Leak and Burke (13,14) and Magari et al (15), frequently exhibited PGI_2SI. Significant numbers of vesicular and multivesicular bodies with PGI_2SI were seen in close association with the Golgi complexes of lymphatic

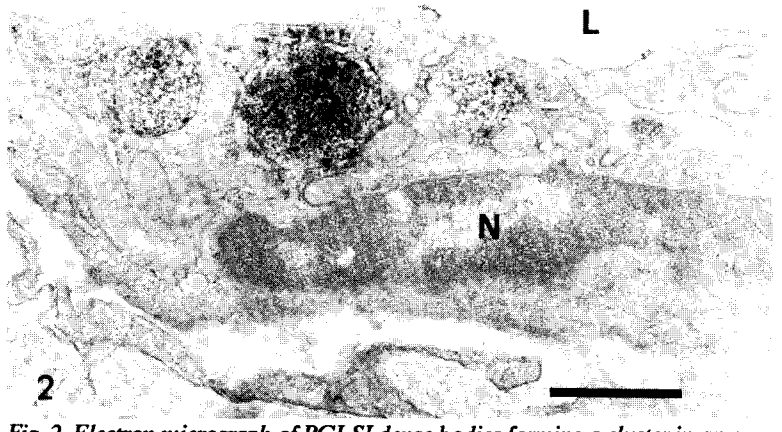

Fig. 2. Electron micrograph of PGI_2SI dense bodies forming a cluster in an area beneath the luminal plasmalemma of an endothelial cell. Abbreviations: N, nucleus of endothelial cell; L, lumen of interlobular lymphatic capillary. Bar = 1.0μm; x22000.

endothelial cells in the interlobular connective tissue *(Fig. 1a,b)*. They occasionally appeared to be in contact with the periphery of the Golgi complexes. In addition to the immunoreactive structures in the perikarya of the endothelial cells, PGI_2SI vesicular bodies were frequently observed in areas subjacent to the abluminal plasmalemma of the lymphatic endothelium *(Figs. 1a,c)*. Dense bodies containing PGI_2 were also present in proximity to the luminal surface of the lymphatic endothelium *(Fig. 2)*. Other organelles within the endothelial did not show PGI_2SI. A small number of nonmyelinated nerve fibers were closely apposed to the immunoreactive lymphatic endothelium without any apparent membrane specialization. Although most of the PGI_2SI was located in the endothelium, it was also detected in the rough endoplasmic reticulum of some plasma cells *(Fig. 3)*. These plasma cells did not contain membrane-bounded bodies with PGI_2SI (see Discussion).

Pretreatment of the monoclonal antibody against PGI_2 synthase with an excess of the homologous antigen eliminated immunostaining throughout the experiments.

DISCUSSION

We investigated the cellular distribution of PGI_2 synthase on the assumption that it represents the location of PGI_2 in tissues. However, the location of PGI_2 synthase may not always coincide with that of PGI_2 per se, as is known to occur in cells that contain glutamate decarboxylase and/or gamma-amino butyrate (16). With this caveat in mind, the implications of the present results are discussed.

PGI_2SI was identified in the lymphatic endothelium of the liver in agreement with previous biochemical and physiological studies suggesting PGI_2 synthesis within lymphatics (2-4,6,8). The presence of PGI_2SI in the perikarya of the lymphatic endothelial cells within the liver further provides morphological evidence that the Golgi apparatus is the site of PGI_2 production. Although the present electron micrographs do not show release of membrane-bounded structures with PGI_2SI, the contents of the immunoreactive vesicular and dense bodies located close to the endothelial plasmalemma suggest PGI_2 may eventually be liberated into both luminal and abluminal spaces in response to specific stimuli (4,8).

The existence of PGI_2SI in some plasma cells raises two possibilities: first, the plasma cells are capable of synthesizing PGI_2; second, they have PGI_2 synthase--like materials including precursors of the enzyme and other unknown substances with the epitope recognized by the mono-

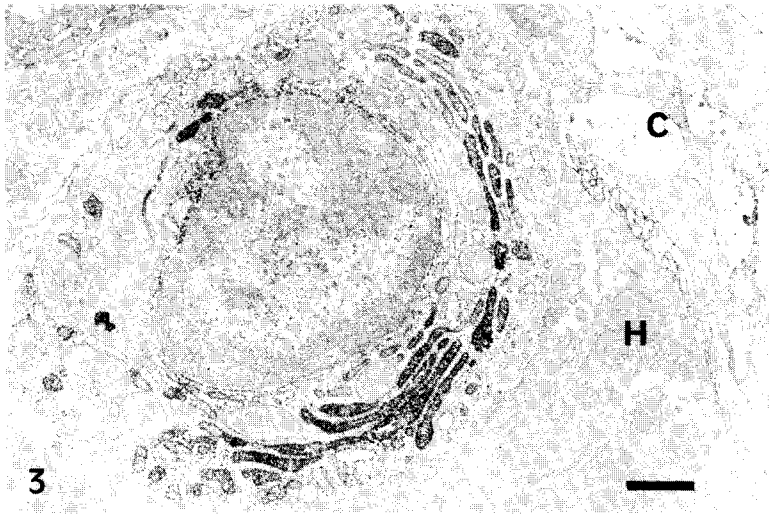

Fig. 3. Electron micrograph of a plasma cell with PGI_2SI in the endoplasmic reticulum. Abbreviations: C, interlobular connective tissue; H, hepatocyte. Bar=1.0μm; x11000.

clonal antibody, but do not produce PGI_2. Development of a method for visualizing PGI_2 would, at least in part, clarify the uncertainty of the present immunoelectron microscopic study.

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REFERENCES

1. Ally, AL, DB Horrobin: Thromboxane A_2 in blood vessel walls and its physiological significance: Relevance to thrombosis and hypertension. Prostaglandins and Medicine 4 (1980), 431.
2. Sinzinger, H, J Kaliman, E Mannheimer: Arachidonic acid metabolites of human lymphatics. Lymphology 17 (1984), 39.
3. Sinzinger, H, J Kaliman, E Mannheimer: Regulation of human lymph contractility by prostaglandins and thromboxane. Lymphology 17 (1984), 43.
4. Sinzinger, H, J Kaliman, E Mannheimer: Effect of leukotrienes C_4 and D_4 on prostaglandin I_2-liberation from human lymphatics. Lymphology 19 (1986), 79.
5. Moncada, S, AG Herman, EA Higgs, et al: Differential formation of prostacyclin (PGX or PGI_2) by layers of arterial wall. An explanation for the anti-thrombotic properties of vascular endothelium. Thrombosis Research 11 (1977), 323.
6. Mannheimer, E, H Sinzinger, R Oppolzer, et al: Prostacyclin synthesis in human lymphatics. Lymphology 13 (1980), 44.
7. Salzman, PM, JA Salmon, S Moncada: Prostacyclin and thromboxane A_2 synthesis by rabbit pulmonary artery. J. Pharmacol. Exp. Ther. 215 (1980), 240.
8. Sinzinger, H, J Kaliman, E Mannheimer: Enhanced prostaglandin I_2-formation of human lymphatics during pulsatile perfusion. Lymphology 19 (1986), 153.
9. Brunkwall, J, D Berggvist, ULF Stjernquist: Prostacyclin and thromboxane release from the vessel wall--comparison between an incubation and a perfusion model. Prostaglandins 34 (1987), 467.
10. DeWitt, DL, JS Day, WK Sonnenburg, et al: Concentration of prostaglandin endoperoxide synthase and prostaglandin I_2 synthase in the endothelium and smooth muscle of bovine aorta. J. Clin. Invest. 72 (1983), 1882.
11. Smith, Wl, DL DeWitt, ML Allen: Bimodal distribution of the prostaglandin I_2 synthase antigen in smooth muscle cells. J. Biol. Chem. 258 (1983), 5922.
12. Somogyi, P, H Takagi: A note on the use of picric acid paraformaldehyde-glutaraldehyde fixative for correlated light and electron microscopic immunocytochemistry. Neuroscience 7 (1982), 1779.
13. Leak, LV, JF Burke: Fine structure of

lymphatic capillary and the adjoining connective area. Am. J. Anat. 118 (1966), 785.
14. Leak, LV, JF Burke: Ultrastructural studies on the lymphatic anchoring filaments. J. Cell Biol. 36 (1968), 129.
15. Magari, S, K Fujikawa, A Nishi: Form, distribution, fine structure and function of hepatic lymphatics with special reference to blood vessels and bile ducts. Asian Med. J. 24 (1981), 254.
16. Agardh, E, A Bruun, B Ehinger, et al: Gamma-aminobutyric acid--and glutamic acid decarboxylase--immunoreactive neurons in the retina of different vertebrates. J. Comp. Neurol. 258 (1987), 622.

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