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Intralobular Innervation and Lipocyte Contractility in the Liver

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ABSTRACT

In the liver of humans, guinea pigs, cats, and tupaia, nerve endings are distributed all over the hepatic lobules from the portal spaces to the centralobular spaces. Nerve endings in the intralobular spaces are located mainly in the space of Disse, and are closely related to lipocytes. In the human liver, various neurotransmitters such as substance P (SP) exist in the nerve endings. Lipocytes are believed to contract through these substances. In fact, the contraction of lipocytes is induced by SP. Moreover, lipocytes possess endothelin (ET) receptors (ET_A, ET_B), and the cells are contracted by ET-1 by way of ET receptors in the autocrine or paracrine mechanism. Contraction of lipocytes seems to be related to the enhancement of the intracellular Ca^{2+} and inositol phosphates. In addition, α-smooth muscle actin, which is a contractile protein, exists in the cytoplasm of lipocytes. Lipocyte contractility may be similar to that of vascular smooth muscle cells. On the other hand, prostaglandin E_2, Iloprost, and adrenomedullin cause the elevation of c-AMP levels in lipocytes and relax the cells. In addition, lipocytes produce nitric oxide (NO) and inhibit contractility by an autocrine mechanism related to NO. In this way, lipocytes appear to be associated with the regulation of hepatic sinusoidal microcirculation by contraction and relaxation. In the cirrhotic liver, intralobular innervation is decreased or absent, but ET-1 and NO are overexpressed. These phenomena indicate that lipocytes may play an important role in the sinusoidal microcirculation through these agents rather than through intralobular innervation in liver cirrhosis. ©*Elsevier Science Inc.* 1997 *Nutrition* 1997;13:141–148

Key words: hepatic innervation, lipocyte (hepatic stellate cell), contractility

INTRODUCTION

Lipocytes located in the space of Disse are also called fat-storing cells, Ito cells, stellate cells, and perisinusoidal cells.[1–4] These cells have many cytoplasmic processes and surround hepatic sinusoidal endothelial cells. The structure of the hepatic sinusoid resembles that of a capillary (Fig. 1).[5] Hepatic sinusoidal endothelial cells are homologous to capillary endothelial cells, and lipocytes are homologous to pericytes located around capillaries. Pericytes regulate the microcirculation of the capillary by contraction or relaxation in response to neurotransmitters released from nerve endings or peptides such as endothelin (ET).[6] Lipocytes, as well as pericytes, appear to be associated with nerves and function to regulate the hepatic sinusoidal microcirculation by contraction and relaxation. In this review, we discuss the relationship between intralobular innervation and lipocytes, and the contractility of lipocytes.

INTRALOBULAR INNERVATION AND LIPOCYTES IN THE LIVER

With regard to intrahepatic innervation, previous reports have indicated a wide variation in the distribution of nerves according to species studied and methods employed.[7–14] In the human liver, nerve endings are distributed all over the hepatic lobules from the portal spaces to the centrolobular spaces.[15–23] In the portal and centrolobular spaces, naked endings devoid of a Schwann sheath appose some mesenchymal cells such as fibroblasts, myofibroblasts, and smooth muscle cells, which form part of the adventitial wall of blood vessels and bile ducts (Figs. 2 and 3). These cell types are believed to control some aspects of hemodynamics in the portal and centrolobular spaces.

Nerve endings in the intralobular spaces are located mainly in the space of Disse. Nerve endings at this site are in close proximity to lipocytes and hepatocytes.[15,19,21] Moreover, nerve endings are seen near hepatic sinusoidal endothelial cells (Fig. 4).[19] In 1968, Ito and Shibasaki[15] first reported the close relationship between

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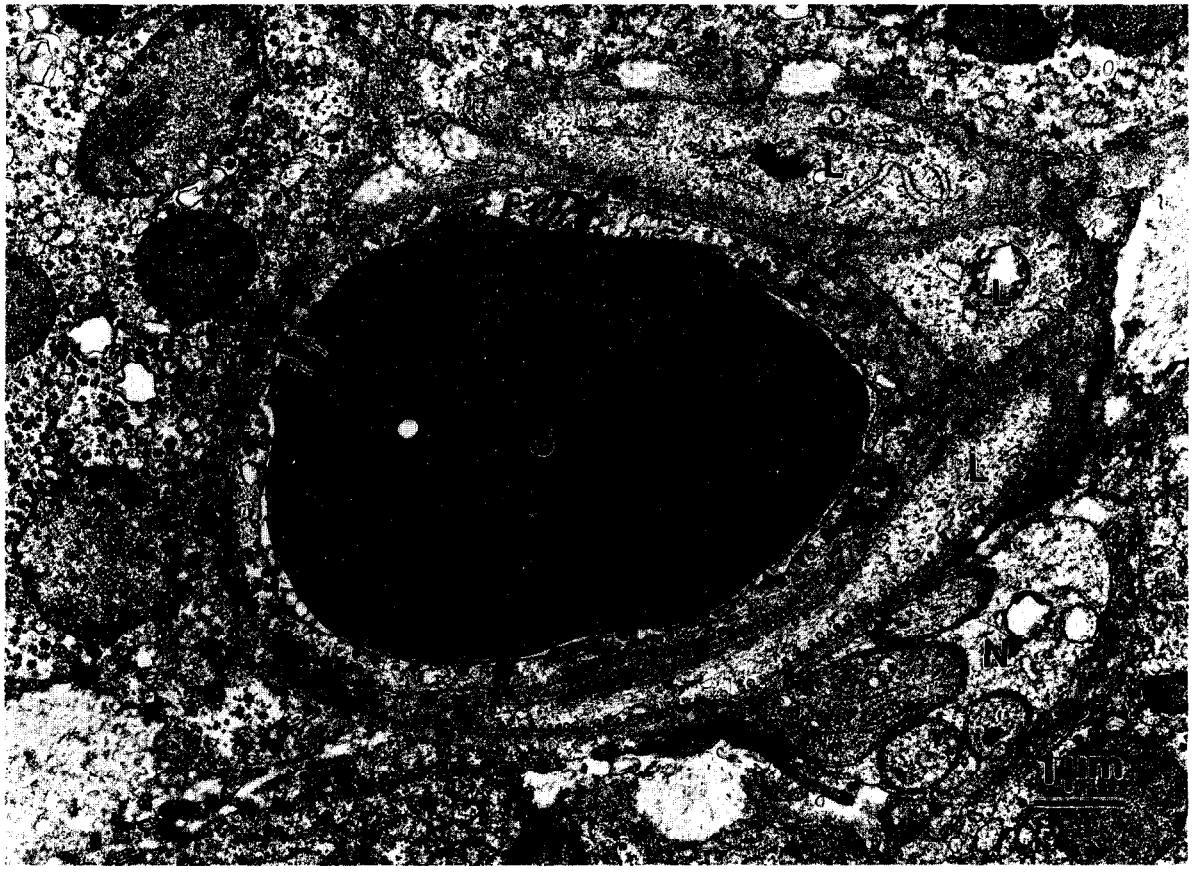

FIG. 1. Electron micrograph (× 11,000) showing a sinusoid in the human liver. Hepatic sinusoidal endothelial cells with fenestrae (arrows) in the cytoplasm are enfolded by the cytoplasmic processes of lipocytes (L). A nerve bundle (N) closely apposes the process of a lipocyte. S, sinusoid.

nerve endings and lipocytes in the human liver. More recently, Bioulac-Sage et al.[20] also showed that nerve endings are located close to lipocytes in the human liver. Guinea pigs, cats, and tupaia have an intralobular innervation similar to that of humans.[8,10] However, in the livers of mice and cats, intralobular innervation is not as abundant, and light and electron microscopic observation of such innervation is difficult.[7-14]

Generally, branches of the vagus nerve (a parasympathetic nerve), the splanchnic nerve (a sympathetic nerve), and sometimes the phrenic nerve enter the human liver. In the human liver, an adrenergic type of innervation has been suggested mainly at the lobular level,[16,18] although some cholinergic innervation, based on cytochemical studies, has also been suggested.[17] Moreover, innervation mediated by various neurotransmitters such as substance P (SP), vasoactive intestinal peptide (VIP), neuropeptide Y, somatostatin, cholecystokinin, and neurotensin has been reported to exist in the human liver.[21-23] The majority of studies have been conducted in attempts to correlate neurophysiologic data with specific metabolic functions of hepatocytes, glucose metabolism, biliary secretion, and so forth.[24,25] Studies related to the autonomic control of hemodynamic, baroreceptive, and osmoreceptive functions have been based on macroscopic extrinsic innervation of the liver.[26,27] Little attention has been paid to the intrinsic innervation and its physiologic implications for cells other than hepatocytes.

BACKGROUND OF LIPOCYTE CONTRACTILITY

In intralobular spaces, nerve bundles are observed along the space of Disse. Nerve endings with vesicles containing neurotransmitters such as SP or VIP are often visible close to lipocytes.[19] In certain respects, lipocytes around the sinusoids resemble pericytes around a capillary. Consequently, lipocytes have the potential to contract and may be involved in the regulation of hepatic sinusoidal microcirculation.

ET is a peptide composed of 21 amino acids that has been isolated from the supernatant after culture of porcine aortic endothelial cells.[28] Of the known vasoactive substances, ET has the most potent vasoconstrictive effects on smooth muscles.[28] The physiology of ET is currently the focus of much research. ET has three isoforms (ET-1, ET-2, and ET-3) with different sequences of amino acids.[29] There are at least two subtypes of ET receptors[30,31]: ET_A and ET_B. According to the relative binding affinities and biological activities of the three isopeptides for their receptors, ET-1 is equipotent to ET-2 but more potent than ET-3 for ET_A receptor, whereas ET-1 is equipotent to ET-2 and ET-3 for ET_B receptor.[32] Although a third receptor type (ET_C) has been pharmacologically and biochemically characterized, the cDNA encoding of this receptor type has not been identified.[31,33] Receptors have been found not only in vascular smooth muscle but also in myocardium, brain, kidney, lung, adrenal gland, intestine, and liver.[34,35] In the rat liver, ^{125}I-ET-1 binding sites, which localize ET receptors, are located mainly in the sinusoidal lining cells of hepatic lobules and are weakly expressed in the luminal space of the portal and central veins.[36,37] In the intralobular spaces, the density is greatest in the periportal region and recedes progressively from the midzonal to the pericentral region (Fig. 5).[36] Ultrastructural autoradiographic observation mainly reveals grains in lipocytes, as

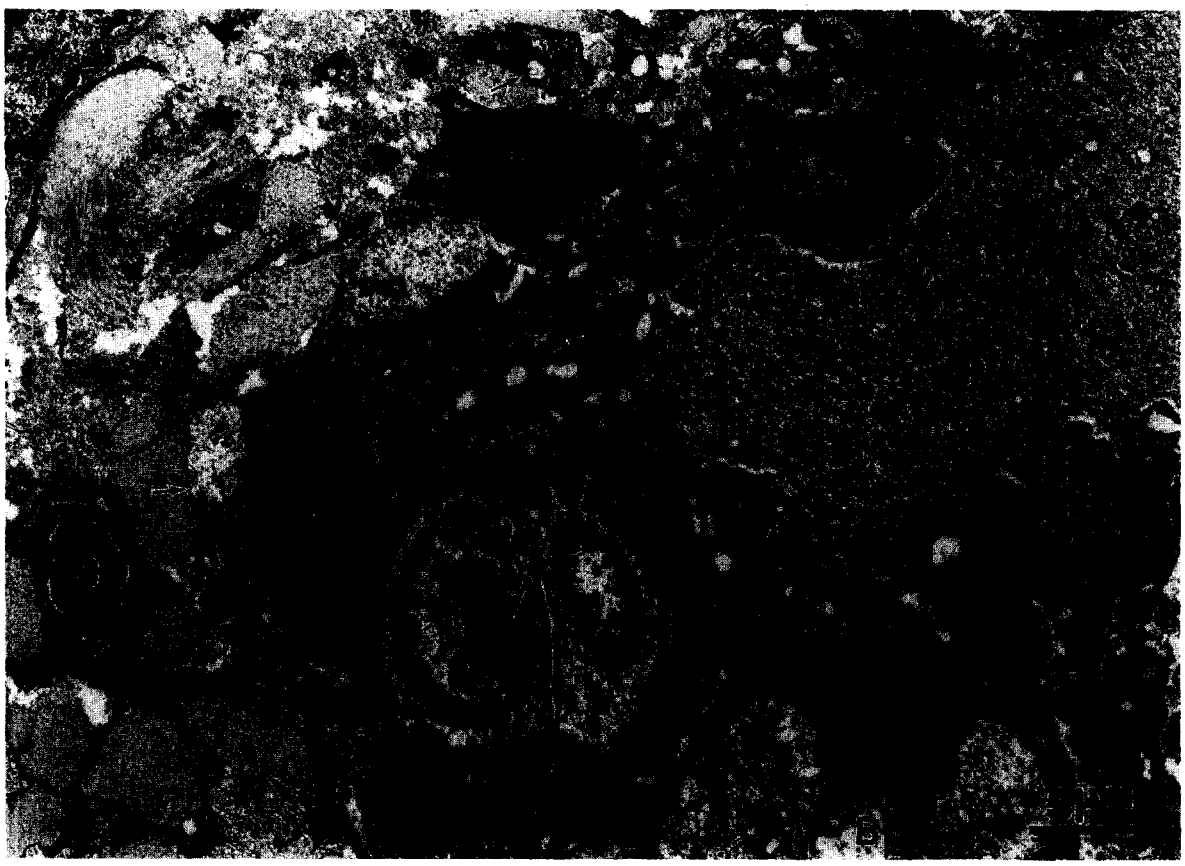

FIG. 2. Electron micrograph (× 6,500) showing the portal area in the human liver. Nerve bundles (arrows) closely appose fibroblasts (F) localized around the portal veins (P), hepatic artery (A), and bile duct (B). S, Schwann cell.

well as in endothelial cells of the portal vein, central vein, and hepatic sinusoids, and Kupffer cells.[36,37] Furuta et al.[37] reported that about 35% of all silver grains were located on the lipocytes. Moreover, rat lipocytes express both ET_A and ET_B receptors.[38] The mRNA for ET_B receptor, but not for ET_A receptor, is present in sinusoidal endothelial cells and Kupffer cells. The heterogeneity of ET-1–binding sites in hepatic lobules appears to parallel the blood flow pattern in hepatic sinusoids: the portal veins branch out into the hepatic sinusoids (6–7 cm H_2O blood pressure[39]), and the hepatic arterial branches also drain into the sinusoids either directly (12–15 cm H_2O blood pressure[40]) via the peribiliary plexus or through the portal venules. Thus, stable regulation of blood flow in the hepatic sinusoids is required predominantly in the periportal spaces compared with that in the mid-zonal and centrolobular spaces. According to McCuskey,[41] sinusoidal endothelial cells and Kupffer cells are responsive to a wide variety of pharmacodynamic substances, and by contracting or relaxing, these cells regulate the rate and distribution of blood flow through the sinusoids. Although the participation of lipocytes remains to be clarified, these cells may play a leading role in the regulation of the hepatic sinusoidal microcirculation.

Lipocytes, as well as sinusoidal endothelial cells and Kupffer cells, contain a cytoskeleton that may be related to contractile activity.[42] After contractile agents such as SP or ET-1 attaches to these receptors, the intracellular Ca^{2+} content increases, and the increased Ca^{2+} binds to calmodulin.[43] The Ca^{2+}-calmodulin complex enhances myosin-light chain kinase activity, and its activity affects contractile proteins such as actin and myosin.[44] Through these reactions, lipocytes seem to contract. Recently, it was reported that lipocytes contain α-smooth muscle actin, which is closely involved in the contractility of smooth muscle cells.[45–47]

CONTRACTION OF LIPOCYTES

Lipocytes contract or relax in response to various agents. Kawada et al.[48] recently reported that ET-1 is a lipocyte contraction agonist that concomitantly stimulates the formation of inositol phosphates and a transient increase in Ca^{2+} content. They also noted that contraction is induced but less markedly by prostaglandin (PG) $F_2\alpha$ and a thromboxane A_2 analogue. Since $PGF_2\alpha$ is released from lipocytes after stimulation with noradrenaline, lipocytes may contract in response to an autocrine mechanism under the influence of noradrenaline released from adrenergic nerve endings.[49] We reported the contractile response of cultured rat lipocytes to ET-1 and SP.[50] Even though lipocytes contract in response to treatment with ET-1 or SP, the extent and onset of the contraction differ in response to the two peptides (Fig. 6). That is, the effect of ET-1 has a stronger and more prolonged effect compared with that of SP. Yanagisawa et al.[28] reported that ET-1 induces contraction of vascular smooth muscle cells lasting for more than 1 h. Therefore, ET-1 may play a role in prolonging tension in the hepatic sinusoidal walls.

Pinzani et al.[51] demonstrated that the response of cultured human lipocytes to ET-1 includes an increase in intracellular free calcium content. An increase in Ca^{2+} content of more than four-fold was associated with a simultaneous and transient reduction in cell area. In cultured vascular smooth muscle cells

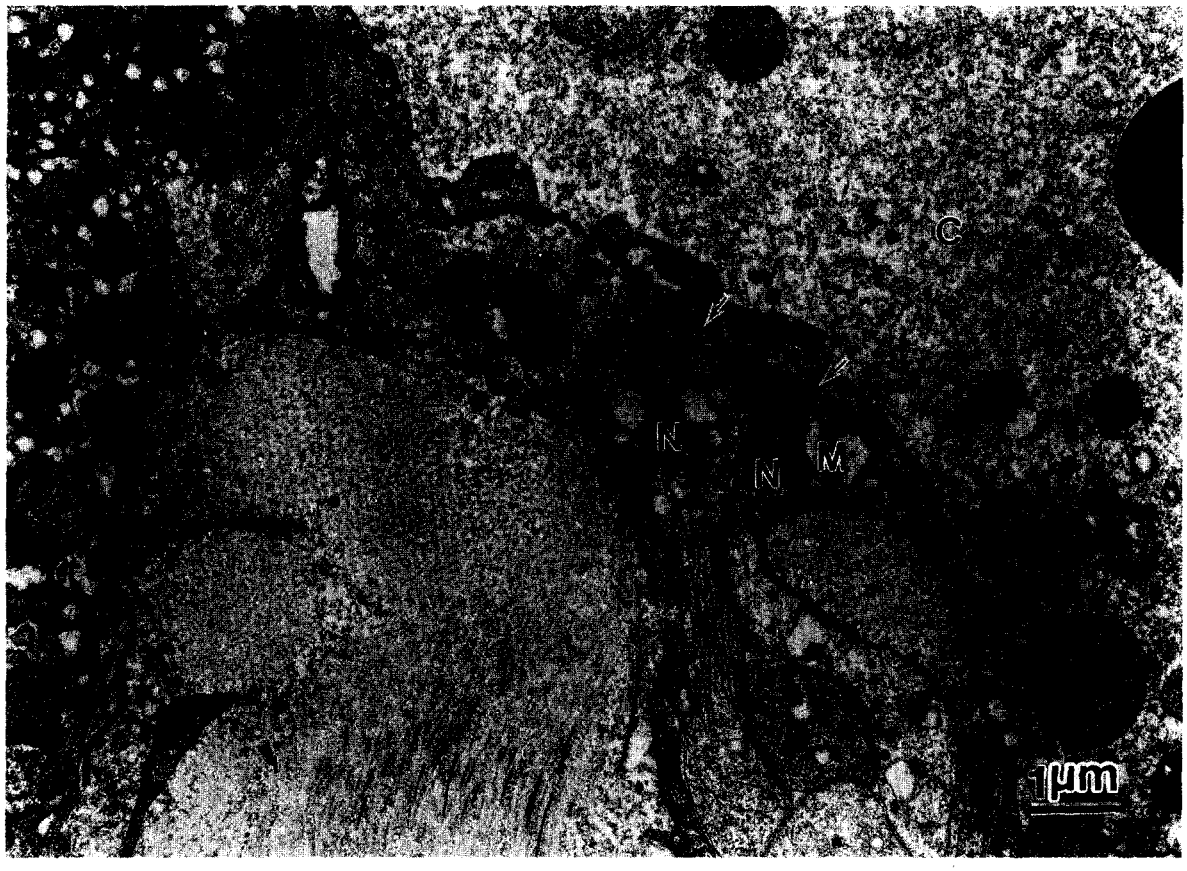

FIG. 3. Electron micrograph (× 11,000) showing the central vein in the human liver. Enlarged nerve endings (N) contact the surface of myofibroblasts (M). The distal end of the nerve ending is closely associated with the basal lamina (arrows) of the endothelial cell of the central vein (C).

and glomerular mesangial cells, ET-1 activates phospholipase C after binding to its receptors, leading in turn to the formation of inositol triphosphate (IP_3) and diacylglycerol.[52,53] Thus, the rise in cytosolic Ca^{2+} induced by the peptide is derived mainly from intracellular stores due to the formation of IP_3. The contraction of lipocytes may be similar to that of vascular smooth muscle cells and glomerular mesangial cells. Zhang et al.[54] studied sinusoidal and extrasinusoidal constrictor responses of the hepatic microcirculation to ET-1 and ET-3, and the possible roles of lipocytes, Kupffer cells, and sinusoidal endothelial cells in mediating this response using isolated rat livers under high-power intravital microscopy. ET-1 was found to induce significant sinusoid constriction at the sites containing lipocytes but not at those including Kupffer cells or endothelial cells, whereas ET-3 had no effect on sinusoid narrowing. Moreover, they noted that ET-1-induced sinusoid constriction was mediated by ET_A receptor on lipocytes.[54] Sinusoidal constriction by ET-1 in the perfused liver reaches a maximum in a short time compared with the time required for maximal contraction of cultured lipocytes by ET-1.[48,50,54] A few factors such as the dose of ET-1 and differences in experimental circumstance between in vivo and in vitro appear to be related to the discrepancy. That is, since lipocytes in vivo are located in three-dimensional spaces such as the space of Disse through limited cell-to-cell contact or cell-to-matrix contact, the cells appear to be able to contract promptly in concert with hepatic sinusoidal endothelial cells in response to lower concentrations of ET-1.[55]

In contrast, PGE_2, Iloprost, which is a PGI_2 analogue, sodium nitroprusside, and adrenomedullin derived from the human adrenal medulla promote the relaxation of lipocytes.[48,56] PGE_2, Iloprost, and adrenomedullin cause the elevation of c-AMP levels in lipocytes, whereas sodium nitroprusside triggers cGMP accumulation. In addition, lipocytes produce nitric oxide (NO) and inhibit contractility by an autocrine mechanism related to NO.[57,58] In this way, lipocytes appear to be associated with the regulation of hepatic sinusoidal microcirculation by contraction and relaxation. Moreover, hepatic sinusoidal endothelial cells are closely related to lipocytes with respect to hepatic sinusoidal microcirculation. These cells possess a cell body with a nucleus and slender extended processes containing pores (fenestrae) that are arranged in so-called sieve plates and allow direct contact between the plasma and its solutes and cells, such as hepatocytes or lipocytes, which are located behind the endothelial barrier. That is, the fenestrated endothelial lining forms a barrier excluding direct contact of cells in the space of Disse with larger particles such as blood cells or large chylomicrons, and is involved in the transfer of substances such as nutrients between the sinusoidal and the space of Disse by way of the fenestrae.[59]

Fenestrae can be altered by various factors. Enlargement of fenestrae diameter has been reported after alcohol abuse,[60] sinusoidal pressure,[61] hypoxia, and endotoxins,[62] while application of serotonin and noradrenalin decreases fenestrae diameter.[63] In addition, the relative sizes of the sinusoidal diameter and blood cells flowing into the sinusoids are closely related to the transfer between the sinusoids and the space of Disse. Commonly, a considerable number of blood cells are too large to fit into sinusoids. However, red blood cells pass in a single row and adapt their shapes to various

FIG. 4. Electron micrograph (× 15,000) showing the sinusoidal area in the human liver. Nerve endings (arrows) with many vesicles are observed close to a lipocyte (L) and hepatocyte (H). The nerve ending is associated with the sinusoidal endothelial cell (E) for a short distance. S, sinusoid.

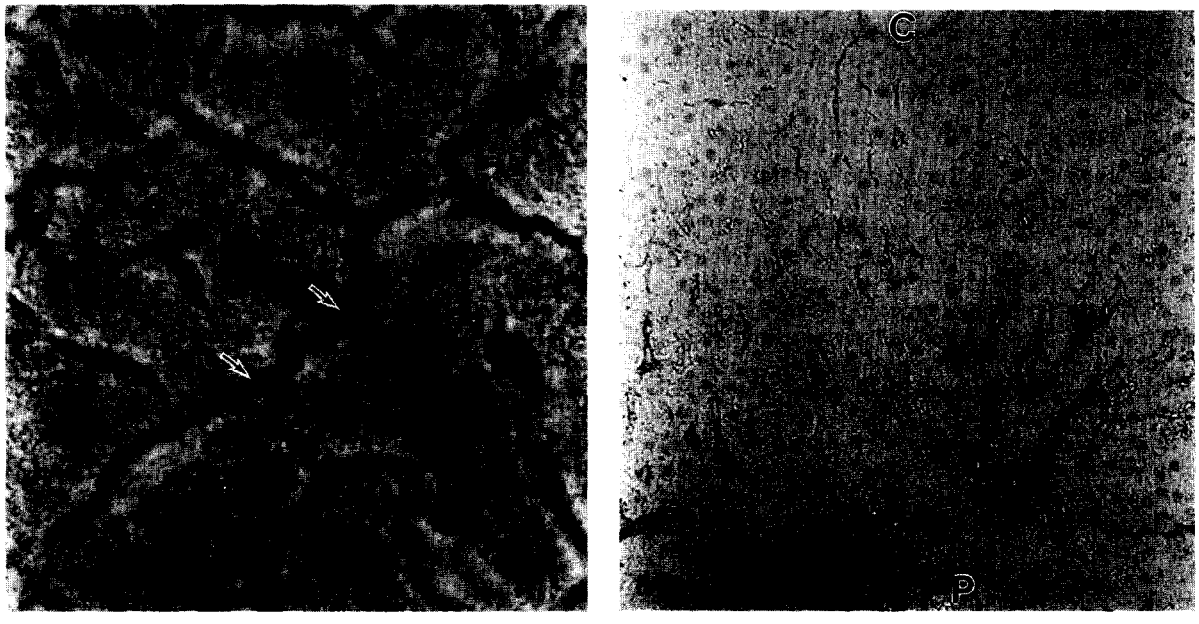

FIG. 5. Light microscopic autoradiographs showing ^{125}I-SP (a) and ^{125}I-ET-1 (b) binding sites in the normal human liver. (a) A concentrated accumulation of ^{125}I-SP grains is observed in lipocytes (arrows) of the perisinusoidal walls. × 800. (b) Many more ^{125}I-ET-1 grains are observed in sinusoidal lining cells of the periportal area as compared with the midzonal or pericentral areas. C, central vein × 200; ET, endothelin; P, portal vein; SP, substance P.

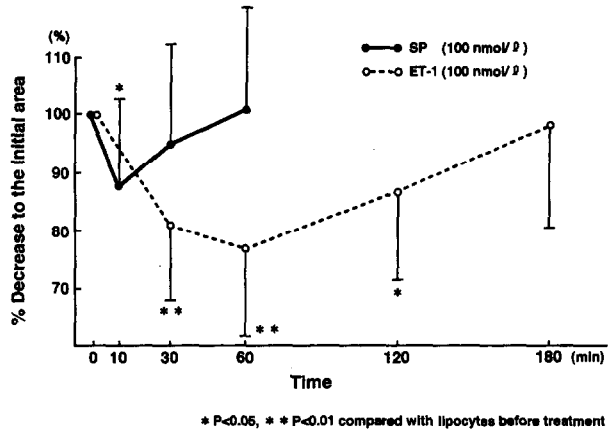

FIG. 6. Comparison of the time course of changes in lipocyte contraction after SP or ET-1 treatment. Rat lipocytes cultured for 24 h at 37°C with 5% CO_2 were used in this study. The contractility of lipocytes is estimated by the change in the area of lipocytes after the addition of 100 nmol SP in culture medium or 100 nmol ET-1. That is, the area of a lipocyte before SP or ET-1 treatment is expressed as A, and its area after treatment as B. The percentage of change after treatment is calculated as follows: (B/A × 100)%. Maximal reduction occurs 60 min after ET-1 treatment and 10 min after SP treatment. However, the contraction in response to ET-1 is more marked compared with that in response to SP. ET, endothelin; SP, substance P.

differences. Soft red blood cells enhance fluid or solid phase particles such as lipoproteins in the space of Disse.[59] Through fenestrae, particles escape to the space of Disse. White blood cells are less plastic and do not easily adapt to obstacles or diameter changes of sinusoids. One of the effects of compressing the space of Disse by moving white blood cells is displacement of fluids within this space in a downstream direction. When fenestrae are encountered, fluid flows out of the space of Disse and when white blood cells move along, the microvilli and endothelial lining return to position.[59] Consequently, uptake and exchange of various substances containing nutrients in the liver are deeply involved in the hepatic sinusoidal microcirculation through the contraction and relaxation of lipocytes or sinusoidal endothelial cells.

LIVER INJURY AND LIPOCYTE CONTRACTION

In mammals, lipocytes play an important role as a major storage site for vitamin A. Kent et al.[64] observed an increase in vitamin A-containing cells in the injured areas following carbon tetrachloride administration. During the ensuing liver fibrosis, the increased deposition of extracellular matrix was clearly correlated with the proliferation and transformation of lipocytes showing a well-developed endoplasmic reticulum (ER).[65] These "activated" lipocytes have lost vitamin A and fat droplets; they show prominent ER and microfilaments. The cells have become long, flat, and spindle-shaped. The microfilaments consist mainly of α-smooth muscle actin.[46] Lipocytes containing abundant α-smooth muscle actin may demonstrate increased contraction.[66,67] During liver injury, the blood concentrations of endotoxins, tumor necrosis factor-α, interleukin-1, and other factors are elevated,[34,35,68] and these substances seem to enhance ET-1 production in sinusoidal endothelial cells or lipocytes.[69] The contraction of "activated" lipocytes may be augmented through increased ET-1 production.

In liver injury, NO in blood and liver tissue is enhanced.[70-72] One cause of increased NO production could be the high levels of circulating endotoxin and tumor necrosis factor-α.[73-75] NO as well as ET-1 may regulate hepatic vascular tone in response to various stimulants.[76,77] Hyperdynamic circulation occurs during liver cirrhosis,[77] and involves systemic cardiovascular complications such as general vasodilation manifested clinically as hypotension, decreased systemic vascular resistance, increased cardiac output, and an increased rate of blood flow through the tissues. Hartleb et al.[78] reported that pressor responses to ET-1 alone were significantly lower in cirrhotic rats than in normal rats. In cirrhotic rats, however, pressor responses to high doses of ET-1 (6–10 μg/kg) were significantly higher in the presence rather than absence of an NO synthesis inhibitor (N^G-nitro-L-arginine methyl ester).[78] That is, in normal animals the administration of ET-1 induced a vasoconstrictor effect in the systemic territory, while in cirrhotic animals, the administration of ET-1 did not affect systemic or regional hemodynamics. This phenomenon is probably responsible for the decreased pressor response to ET-1 due to NO hyperproduction in cirrhotic animals. In addition, NO

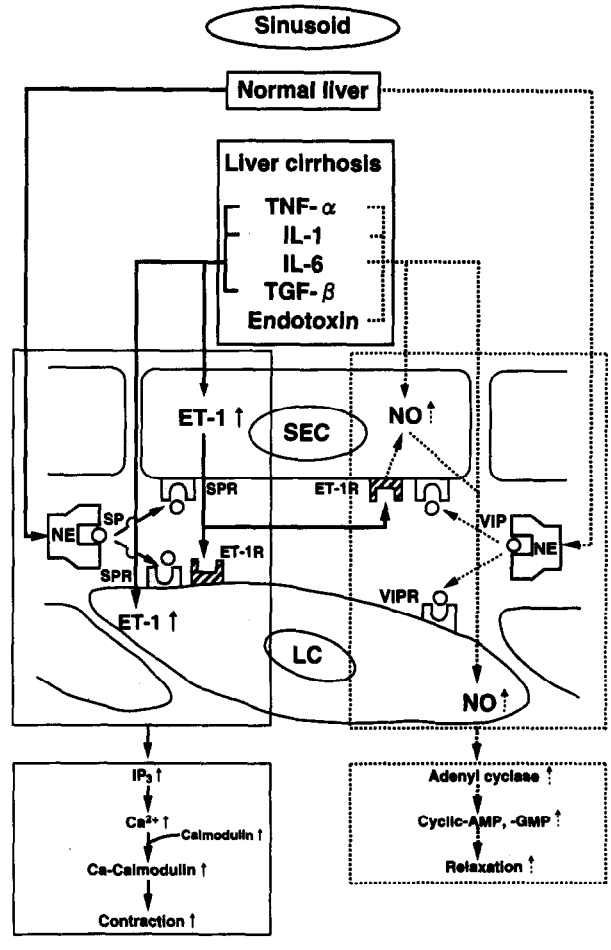

FIG. 7. Diagram showing the concept of the relationship between hepatic sinusoidal cells and vasoactive agents in normal and cirrhotic livers. It is suggested that sinusoidal microcirculation through lipocytes or hepatic sinusoidal cells in the normal liver is regulated mainly by intralobular innervation, while sinusoidal microcirculation in the cirrhotic liver is regulated primarily by vasoactive agents such as ET-1 or NO in a paracrine or autocrine manner. ET, endothelin; ET-1 R, endothelin-1 receptor; IL, interleukin; IP_3, inosital-3-phosphate; LC, lipocyte; NE, nerve ending; NO, nitric oxide; SEC, sinusoidal endothelial cell; SP, substance P; SPR, substance P receptor; TGF, transforming growth factor; TNF, tumor necrosis factor; VIP, vasoactive intestinal peptide; VIPR, vasoactive intestinal peptide receptor.

seems to contribute to vascular hyporeactivity in the presence of high doses but not low doses of ET-1.[71]

Autonomic hyperactivity with increased cardiac output and other manifestations is observed in patients with cirrhosis,[77] whereas intralobular innervation is decreased or absent in the cirrhotic liver. However, the nerve fiber density is increased around the portal vein branches and vessels in the fibrous septa.[79–81]

In addition, liver fibrosis is characterized by the accumulation of extracellular matrix components in the space of Disse, forming a so-called "capillarization" of the sinusoids.[82] At this stage, resistance in the sinusoidal walls is increased, and the hepatic sinusoidal microcirculation may be limited.[83] With the progression of liver fibrosis, intralobular innervation becomes scant, whereas the role of lipocytes in the hepatic sinusoidal microcirculation may become more prominent. As shown in Figure 7, the regulation of blood flow, resistance, pressure, osmolarity and glucose metabolism, and so on in the sinusoids of normal liver is believed to be performed mainly by neuropeptides such as SP or VIP released from nerve endings. However, the regulation of these functions in sinusoids of the cirrhotic liver seems to be regulated by agents such as ET-1 or NO in a paracrine or autocrine manner. Regardless, the relationship between intralobular innervation and the contractility of lipocytes in liver disorders requires further investigation.

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