

Short Communication

Synaptophysin: A Novel Marker for Human and Rat Hepatic Stellate Cells

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Synaptophysin is a protein involved in neurotransmitter exocytosis and is a neuroendocrine marker. We studied synaptophysin immunohistochemical expression in 35 human liver specimens (normal and different pathological conditions), in rat models of galactosamine hepatitis and carbon tetrachloride-induced cirrhosis, and in freshly isolated rat stellate cells. Synaptophysin reactivity was present in perisinusoidal stellate cells in both human and rat normal liver biopsies. The number of synaptophysin-reactive perisinusoidal cells increased in pathological conditions. Double staining for α -smooth muscle actin and synaptophysin, detected by confocal laser scanning microscopy, unequivocally demonstrated colocalization of both markers in lobular stellate cells. In addition, freshly isolated rat stellate cells expressed synaptophysin mRNA (detected by polymerase chain reaction) and protein. Finally, electron microscopy showed the presence of small electron translucent vesicles, comparable to the synaptophysin-reactive synaptic vesicles in neurons, in stellate cell projections. We conclude that synaptophysin is a novel marker for quiescent as well as activated hepatic stellate cells. Together with the stellate cell's expression of neural cell adhesion molecule, glial fibrillary acidic protein, and nestin, this finding raises questions about its embryonic origin and its differentiation. In addition, the presence of synaptic vesicles in stellate cell processes suggests a hitherto unknown mechanism of interaction with neighboring cells. (*Am J Pathol* 1999, 155:1831–1839)

Hepatic stellate cells have been demonstrated to be involved in conditions ranging from acute necrotizing and regenerating liver disorders^{1–3} to chronic and fibrosing

hepatic diseases.^{4–6} Essential functions exerted in these conditions are the production of extracellular matrix components,^{7–9} the production of transforming growth factor β 1 (TGF- β 1)^{10,11} and hepatocyte growth factor (HGF),^{12–16} and the role of effector cell responsible for microvascular tone regulation in the liver.^{17,18}

Based on their expression of vimentin, desmin, and α -smooth muscle actin (α -SMA),^{19–24} stellate cells have been considered to be of mesenchymal origin. In addition, several markers of neural/neuroectodermal differentiation have been found. Stellate cells express the intermediate filament glial fibrillary acidic protein (GFAP),^{25–27} and activated rat stellate cells express the intermediate filament nestin.²⁷ Human stellate cells are immunoreactive with neural cell adhesion molecule (N-CAM) antibody,²⁸ whereas rat stellate cells become positive for N-CAM on activation.²⁹

Synaptophysin (SYN) is a major transmembrane glycoprotein of small (30–80 nm) electron-translucent (SET) vesicles. This class of vesicles includes the presynaptic vesicles in neuronal cells and the somewhat larger synaptic-like microvesicles in neuroendocrine cells.³⁰ The SYN protein is implicated in the control of exocytosis³⁰ and neurotransmitter release, eg, in the neuromuscular synapse.³¹ Immunohistochemical detection of SYN is commonly used in conjunction with chromogranin-A, neuron-specific enolase, and Leu-7 immunohistochemistry to determine neuroendocrine origin or differentiation in tissues and tumors throughout the body.^{32,33} To the best of our knowledge, only neural and neuroendocrine cell types have been shown to express SYN,³⁴ with the exception of rabbit thrombocytes.³⁵

In the present study we demonstrated expression of SYN in quiescent as well as activated human and rat hepatic stellate cells.

Supported by a grant from the Fonds voor Wetenschappelijk Onderzoek-Vlaanderen.

Accepted for publication August 24, 1999.

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Materials and Methods

Animals

Male Wistar-Kyoto rats (Harlan, The Netherlands), 250 to 300 g, were used in D-galactosamine (Gal) intoxication experiments and for isolation of stellate cells. Male Wistar rats, 250 to 300 g body weight, were used for carbon tetrachloride (CCl₄) fibrogenic regimens. All animals were fed *ad libitum* and received care in accordance with the institution's guidelines for the care and use of laboratory animals in research. Gal hepatitis was induced by a single intraperitoneal (i.p.) injection of 500 mg/kg Gal (Sigma-Aldrich, Steinheim, Germany). Animals (14 intoxicated, 2 controls) were killed at 12, 24, 36, 48, and 72 hours and 7, 14, and 21 days after i.p. injection. CCl₄ cirrhosis was induced by gastric gavage of CCl₄, together with administration of phenobarbital (350 mg/l) in the drinking water, starting 7 days before CCl₄ administration. CCl₄ was mixed 1:1 with corn oil and started at a dose of 80 μ l CCl₄. Subsequent doses were adjusted to percentage of weight loss, as described by Proctor and Chatamra,³⁶ with modifications according to Fischer-Nielsen et al.³⁷ A total of 5 rats were killed, one animal each at 2, 4, 6, 8, and 10 weeks. Liver specimens were fixed in B5 fixative and further specimens were snap-frozen in liquid nitrogen-cooled isopentane. The frozen specimens were stored at -70°C until use.

Human Liver Specimens

A series of 35 human liver specimens, taken for diagnostic purposes or resected before transplantation, was used for this study: 5 near-normal liver biopsies, 5 specimens showing regeneration after submassive necrosis, 15 specimens with chronic fibrotic disease in a noncirrhotic stage (hepatitis B, $n = 3$; hepatitis C, $n = 5$; autoimmune hepatitis, $n = 2$; chronic biliary subobstruction, $n = 2$; alcoholic hepatitis, $n = 3$) and 10 specimens with cirrhosis (posthepatitis C, $n = 5$; alcoholic, $n = 3$; primary biliary cirrhosis, $n = 2$). The diagnoses were based on histopathological examination of routinely processed tissue and on clinical and laboratory data. Each specimen was received freshly. One part was fixed in formalin or B5 fixative for diagnostic purposes, one part was snap-frozen in liquid nitrogen-cooled isopentane and stored at -70°C until use, and one small part was immediately fixed in 2.5% glutaraldehyde 0.1 mol/L phosphate buffer and further treated for routine electron microscopy.

Isolation of Stellate Cells

Rat stellate cells were isolated by collagenase/pronase digestion as described by De Bleser et al,³⁸ followed by density gradient centrifugation with Optiprep (Nycomed, Sweden). Collagenase type IV was purchased from Sigma (St. Louis, MO), and applied in a concentration of 0.05% (w/v) for reperfusion and afterward for digestion in a shaking suspension. Pronase E was purchased from

Merck (Darmstadt, Germany) and was applied in a concentration of 0.2% (w/v) for reperfusion and 0.05% (w/v) for digestion. Cells were harvested at densities <1.053 (9% Optiprep), according to Alpini et al.³⁹ Purity as determined by desmin, vimentin, α -SMA, and oil red O staining on cytopins of freshly isolated cells was 75 to 85%, and viability was systematically over 95%.

Cytopins

Freshly isolated rat stellate cells (1×10^5 cells per slide) were spun onto glass slides for 10 minutes at a speed of 500 rpm. The glass slides were washed in PBS, left to dry, and subsequently fixed in acetone.

Immunohistochemistry

Immunohistochemistry was performed on cryosections of rat and human liver biopsies and on cytopins of rat stellate cells fixed in acetone. The polyclonal synaptophysin antibody was purchased from Dako (Copenhagen, Denmark) and applied in a 1/50 dilution. The monoclonal anti- α -SMA antibody was purchased from Sigma-Aldrich (Steinheim, Germany) and applied in a 1/400 dilution. Desmin antibody from Boehringer Mannheim (Mannheim, Germany) and vimentin monoclonal antibody from Amersham (Roosendaal, The Netherlands) were applied in a 1/20 dilution. Polyclonal TGF- β 1 antibody from Ciba (Groot-Bijgaarden, Belgium) was applied in a 1/200 dilution. For polyclonal antibodies, a three-step unlabeled peroxidase-anti-peroxidase method was used, as described previously.⁴⁰ For monoclonal antibodies, a three-step indirect immunoperoxidase procedure was used, as described previously.⁴¹

Double-staining was performed with a sequential fluorescent method on 20- to 60- μm -thick cryostat sections on 3-aminopropyltriethoxysilan-coated slides. The cryostat sections were dried overnight, fixed in acetone for 10 minutes, and washed in PBS. Then, the sections were incubated with a mixture of normal rabbit serum and normal swine serum diluted 1/5 in PBS for 45 minutes. The polyclonal SYN antibody was incubated at room temperature for 1 hour, followed by fluoresceine isothiocyanate (FITC) or tetramethylrhodamine isothiocyanate (TRITC) labeled swine-anti-rabbit antibodies (Dakopatts, Copenhagen, Denmark). Subsequently, the monoclonal antibody anti- α -SMA was incubated for 1 hour at room temperature, followed by FITC- or TRITC-labeled rabbit-anti-mouse antibodies (Dakopatts). The antibodies were diluted in PBS containing 0.3% Triton X100. All incubation steps were followed by a wash in three changes of PBS, pH 7.4, for 5 minutes. Sections were mounted with glycerol/PBS with para-phenylene-diamine. Controls consisted of omission of the first step (polyclonal SYN antibody) and/or omission of the anti- α -SMA monoclonal antibody and were consistently negative. Additional controls consisted of incubation of the polyclonal antibody with the rabbit-anti-mouse secondary antibody and incubation of the monoclonal antibodies with the swine-anti-rabbit antibody. No nonspecific labeling could be de-

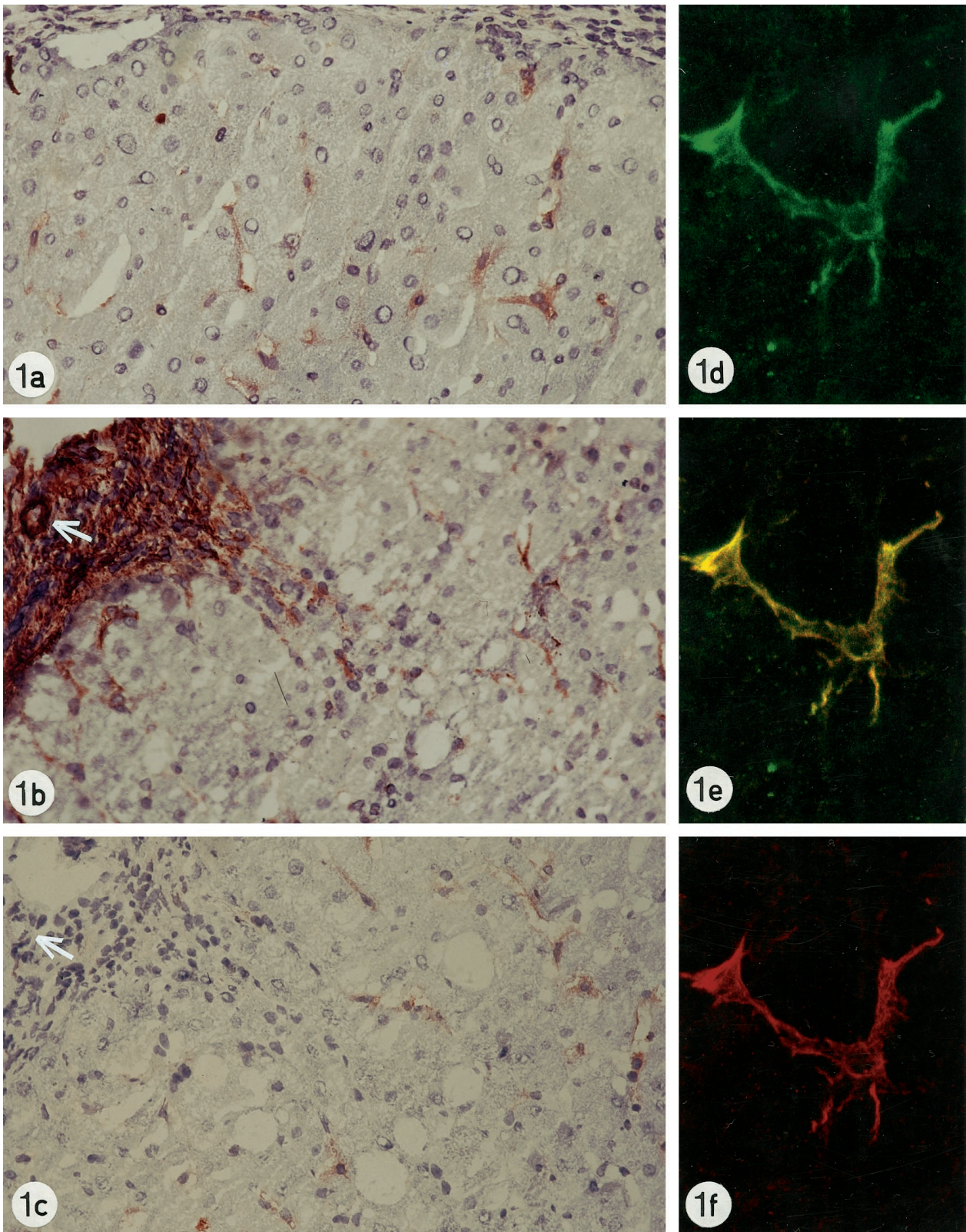
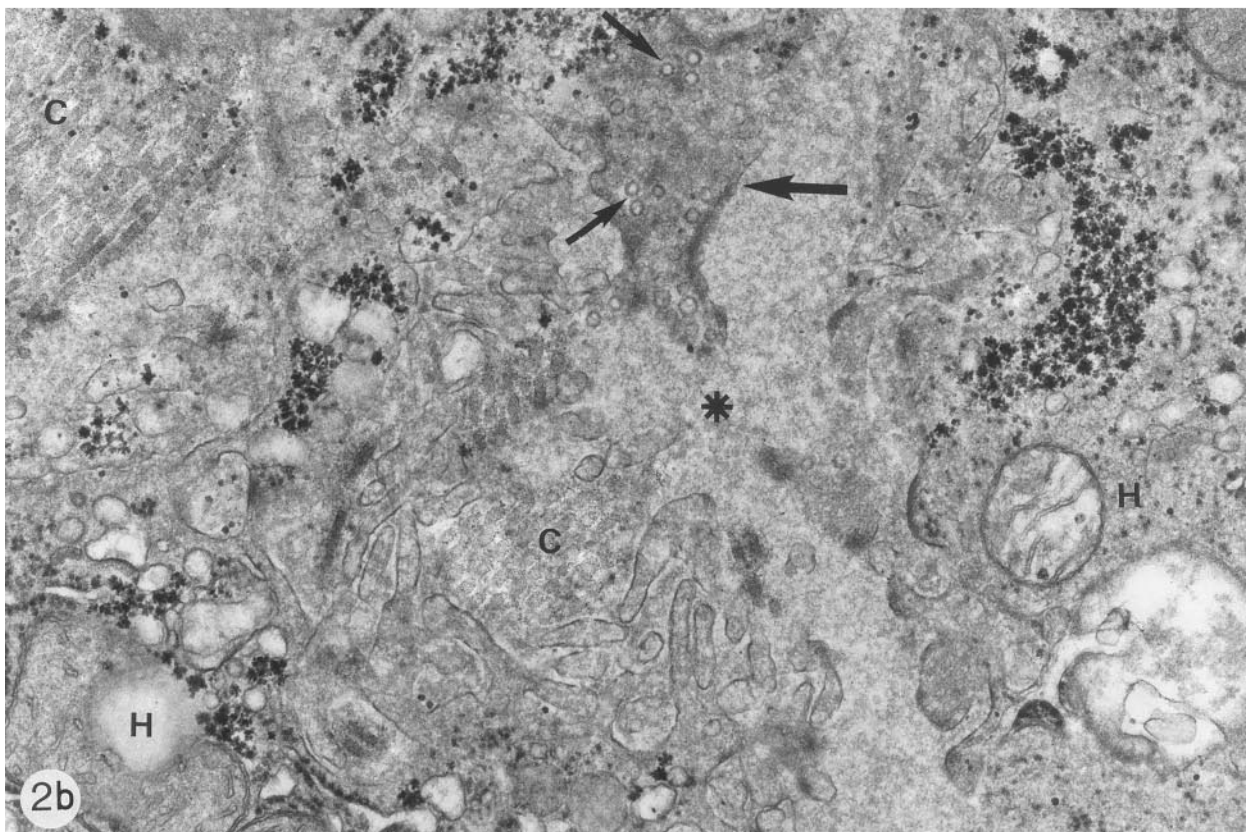
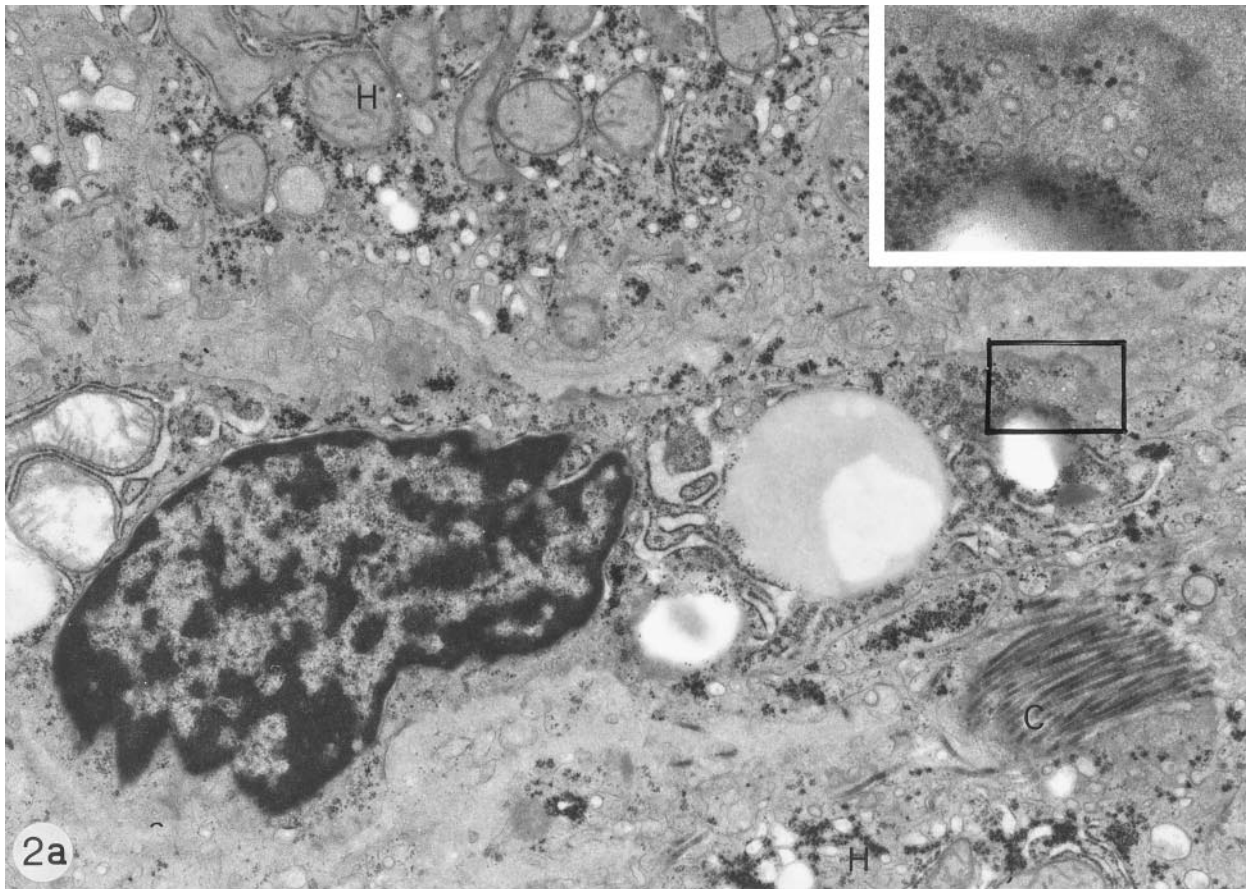


Figure 1. a: Liver biopsy of patient with chronic biliary disease showing synaptophysin reactivity in perisinusoidal cells with stellate projections. Original magnification, $\times 250$. **b:** Liver biopsy of patient with chronic hepatitis C showing α -smooth muscle actin-reactivity in lobular perisinusoidal stellate cells, in septal myofibroblasts (left upper side of figure) and in smooth vessel cells of an arterial wall (**arrow**) Original magnification, $\times 250$. **c:** Liver biopsy of patient with chronic hepatitis C showing synaptophysin-immunoreactivity in lobular perisinusoidal cells with stellate projections. In the fibrotic septum (upper left side of figure), no reactivity is present. Arterial wall, **arrow**. Original magnification, $\times 250$. **d-f:** Liver biopsy of patient with chronic hepatitis C: double staining for synaptophysin and α -smooth muscle antigen, detected by the confocal laser scanning microscope: Lobular stellate cell showing reactivity for synaptophysin (green labeling, **d**), α -smooth muscle actin (red labeling, **f**) and colocalization for synaptophysin and α -smooth muscle actin (yellow double-labeling, **e**). Original magnification, $\times 1000$.



tected. Double staining was detected using confocal laser scanning microscopy (Zeiss 410 inverted laser scan microscope).

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted according to Chomczynski and Sacchi.⁴² RT-PCR was performed using Promega *Taq* polymerase, 0.5 U per 50 μ l reaction. Cycling was performed in a Biometra Trio cycler (Westburg, The Netherlands). Total RNA was reverse transcribed in a reaction volume of 50 μ l, using murine leukemia virus reverse transcriptase at 37°C. Thirty-four cycles of PCR (1 minute at 94°C, 1 minute at 68°C, and 1 minute at 72°C), followed by 10 minutes at 72°C, were performed, using a forward primer 5'-TGTACTTTGATGCACCCWCCT-GCS-3' (SYNP5) and a reverse primer 5'-CAGCCTGTCTCCTTRAACACGAACC-3' (SYNM2). These primers are complementary to the published cDNA sequence of rat SYN⁴³ (accession number X06388), positions 262 to 285 and 685 to 709, respectively. The PCR product was calculated to a length of 448 bp, as was confirmed on agarose gel electrophoresis stained with ethidium bromide. Identification of the PCR product as the rat SYN cDNA was achieved by sequencing.

FTO-2B rat hepatoma cell line was used as negative control; rat brain was used as positive control. RT-PCR for detection of G3PDH mRNA (accession number X02231) was performed on the cDNA samples, to control cDNA quality. Primers used were 5'-ACCACAGTCCATGCCATCAC-3' and 5'-TCCACCACCCTGTTGCTGTA-3'. The cycling program was 5 minutes at 94°C, followed by 28 cycles (1 minute at 94°C, 30 seconds at 65°C, 2 minutes at 72°C) and 10 minutes at 72°C (adapted from Clontech, Palo Alto, CA). Expected length of the amplified region was 452 bp.

Results

In Vivo Studies

Human Liver Biopsies/Immunohistochemistry

In normal human liver, perisinusoidal cells were immunoreactive to SYN in a discontinuous pattern. Immunoreactive cells were evenly distributed throughout the lobule. Sometimes fat vacuoles indenting the nucleus were recognized, suggesting the immunoreactive cells were stellate cells. In portal tracts, nerve bundles were immunoreactive as described,⁴⁴ serving as an internal control.

In submassive necrosis, SYN reactivity was abundant in spindle cells in and around necrotic areas. In addition, SYN-positive perisinusoidal cells with stellate projections were seen, scattered in the surviving parenchyma. The

number of SYN-reactive cells was markedly higher in submassive necrosis specimens than in normal human liver. Sparse immunoreactive cells were seen in portal tracts. Portal and lobular nerve bundles and nerve fibers were immunoreactive.

In all chronic liver diseases, a higher number of SYN-reactive perisinusoidal cells were seen compared to the number seen in normal biopsies. Immunoreactive cells were found mainly throughout the lobule and near fibrotic septa (Figure 1, a and c). α -SMA immunoreactivity paralleled the lobular staining pattern of SYN (Figure 1b); septa showed only occasional SYN-reactive cells, whereas myofibroblasts in fibrous septa and portal tracts showed abundant α -SMA reactivity (Figure 1b). Nerve fibers were strongly immunoreactive for SYN and negative for α -SMA; smooth muscle cells in vessel walls were immunoreactive for α -SMA and negative for SYN staining.

Double staining for α -SMA and SYN detected by confocal laser scanning microscopy proved colocalization for both markers in lobular stellate cells. Lobular staining showed complete overlap, confirming that SYN-immunoreactive cells were hepatic stellate cells (Figure 1, d-f). Septal myofibroblasts, vessel walls, and nerve fibers showed no overlap.

Electron microscopy showed scattered SET vesicles in the cytoplasm of hepatic stellate cells of normal (Figure 2a) as well as diseased human liver (Figure 2b). In the cytoplasmic processes of stellate cells many SET vesicles, comparable to the synaptic vesicles found in neurons, were observed (Figure 2). The processes of stellate cells could be distinguished from neural endings because of the presence of submembranous dense plaques and the absence of neurotubules (Figure 2).

Rat Liver Biopsies/Immunohistochemistry

Biopsies from normal rats showed immunoreactivity for SYN in perisinusoidal cells in a discontinuous pattern (Figure 3a). The reactive cells were evenly distributed and scattered throughout the lobule. Cells stained by SYN antibody contained a nucleus, differentiating them from nerve endings; this nucleus was often indented by fat vacuoles (Figure 3a).

Rats with Gal hepatitis showed SYN reactivity in cells along the sinusoids, periportally, and in and around necrotic areas. The number of SYN-positive stellate cells increased parallel to the severity of necrosis, attaining a maximum around 48 hours after intoxication (Figure 3, b and c) and decreasing to normal intensity during the following weeks. The SYN immunoreactivity paralleled the staining pattern and the increased number of α -SMA-, TGF- β 1- and desmin-reactive cells.

Biopsies from CCl₄-intoxicated rats showed SYN reactivity in cells along the sinusoids and in occasional cells in fibrotic septa. The overall number of SYN-positive cells

Figure 2. a: Electron micrograph of normal human liver biopsy showing a stellate cell in the Disse space, flanked by part of two hepatocytes (H). The stellate cell contains fat droplets. Collagen bundles (C). Original magnification, $\times 14,500$. **Inset:** Detail of a cytoplasmic process containing many small clear vesicles, comparable to the synaptic vesicles found in neurons. Original magnification, $\times 46,000$. **b:** Electron micrograph of human liver biopsy with chronic subobstruction. Disse space (*) and sinusoidal recess between two hepatocytes (H). Part of a cytoplasmic process of a stellate cell (arrow) containing many small clear vesicles (small arrows). Collagen bundles (C). Original magnification, $\times 36,800$.

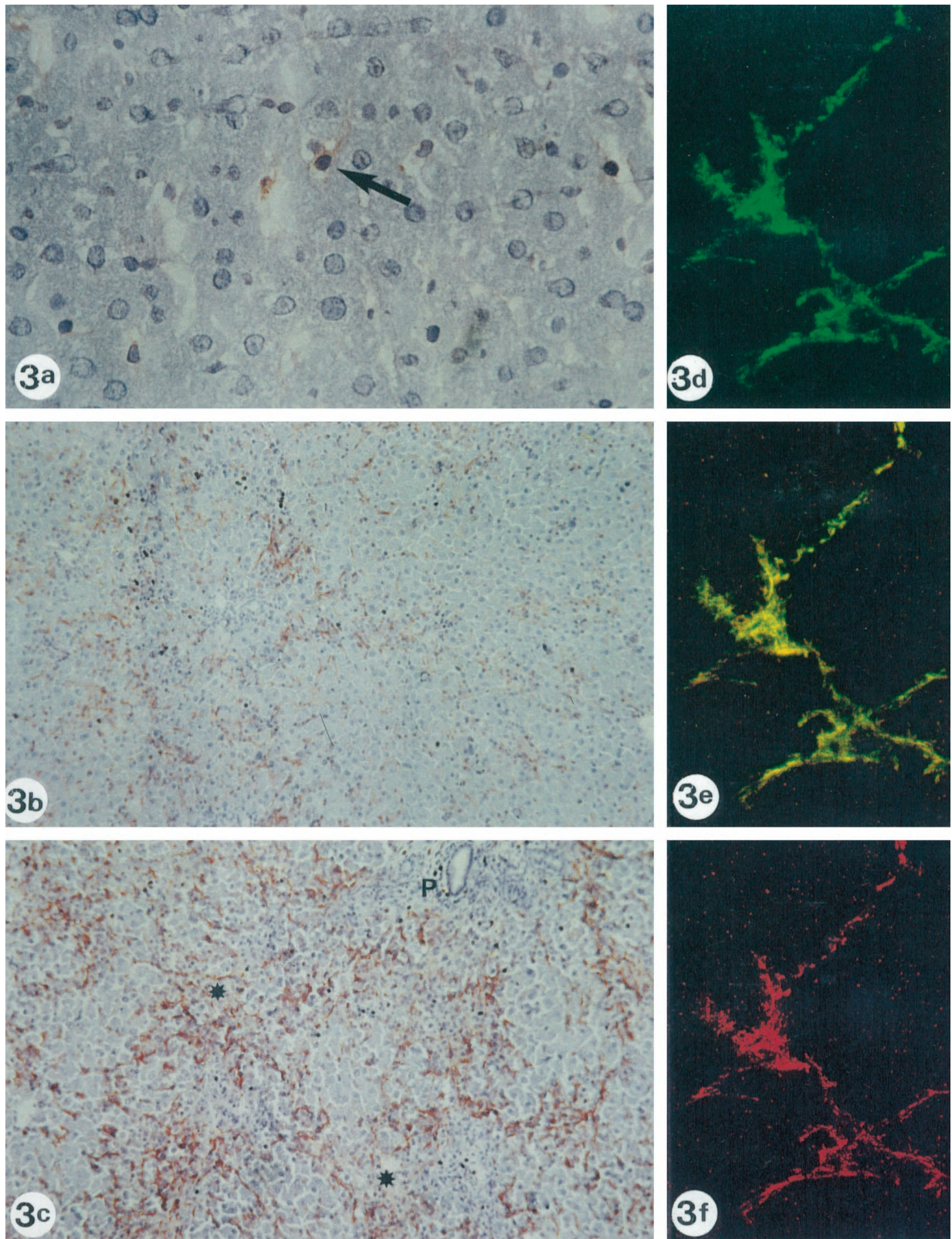


Figure 3. **a:** Normal rat biopsy immunostained for synaptophysin, showing reactivity in perisinusoidal stellate cells. Fat droplets can be recognized in the cytoplasm of some immunoreactive cells (arrow; original magnification, $\times 400$). **b:** Rat liver biopsy 36 hours after galactosamine intoxication. Synaptophysin reactivity is seen in a higher number of stellate cells, compared with the normal rat liver shown in **a**. Stellate cells are clustered around necrotic areas. Original magnification, $\times 100$. **c:** Rat liver biopsy 48 hours after galactosamine intoxication. Synaptophysin reactivity is seen in stellate cells. The number of stellate cells reaches its maximum at 48 hours. Stellate cells are clustered around necrotic areas. P, portal tract; *, necrotic areas. Original magnification, $\times 100$. **d-f:** Rat liver biopsy 36 hours after galactosamine intoxication. Double staining for synaptophysin and α -smooth muscle antigen, detected by the confocal laser scanning microscope: lobular stellate cell showing reactivity for synaptophysin (green labeling, **d**), α -smooth muscle actin (red labeling, **f**) and colocalization for synaptophysin and α -smooth muscle actin (yellow double-labeling, **e**). Original magnification, $\times 1000$.

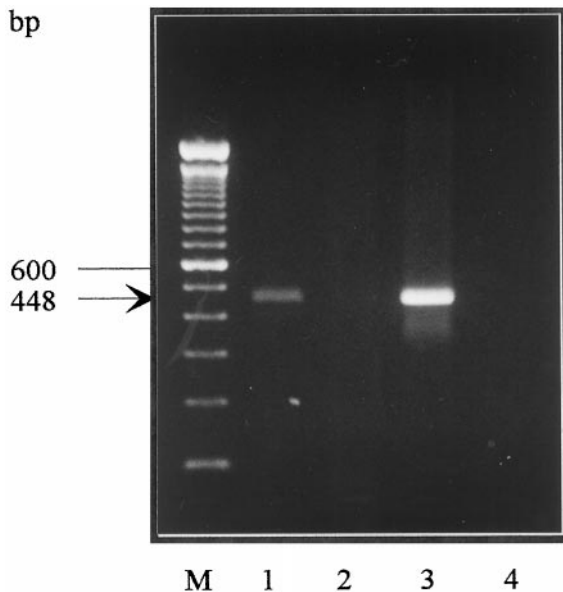


Figure 4. Transcription of the SYN gene in freshly isolated rat liver stellate cells was demonstrated by RT-PCR. The PCR product was calculated to a length of 448 bp, as was confirmed on agarose gel electrophoresis stained with ethidium bromide. Rat hepatic stellate cells (**lane 1**) show transcription; FTO-2B rat hepatocyte cell line (**lane 2**) showed no transcription; rat brain (**lane 3**) showed abundant transcription. **Lane M:** 100-bp ladder. **Lane 4:** negative control (no sample). Identification of the PRC product as rat synaptophysin cDNA was achieved by sequencing.

did not reach levels similar to the number of immunoreactive cells in Gal hepatitis. Repeating the dose of CCl₄ induced formation of fibrotic septa and development of cirrhosis after 6 weeks, as described previously.³⁷

Double staining for α -SMA and SYN detected by confocal laser scanning microscope proved colocalization in rat lobular stellate cells, confirming their stellate cell nature (Figure 3, d-f).

In Vitro Studies

Cytospins of freshly isolated rat stellate cells, isolated from normal rat livers as well as livers from rats intoxicated with Gal, were reactive on SYN immunohistochemical staining. SYN reactivity of varying intensity was found in the majority of cells; the number of positive cells was compatible with purity of 75 to 85%.

RT-PCR

Transcription of the SYN gene in freshly isolated rat liver stellate cells was demonstrated by RT-PCR. FTO-2B rat hepatocyte cell line showed no transcription; rat brain showed abundant transcription, as expected (Figure 4). Control RT-PCR detecting G3PDH mRNA showed satisfactory quality of synthesized cDNA (results not shown).

Discussion

In this study we have shown that quiescent as well as activated human and rat hepatic stellate cells express SYN. The SYN protein is known to be present in mem-

branes of neuronal synaptic vesicles containing neurotransmitters; SYN-positive secretory granules in pancreatic tissue have been demonstrated to contain glucagon or insulin⁴⁵; tumors of bronchial epithelium and gastrointestinal mucosa can also demonstrate SYN reactivity.^{46,47} Expression of SYN is advocated as a sign of neuroendocrine differentiation or subdifferentiation.^{32,33} Functionally, SYN is most probably involved in membrane fusion leading to exocytosis.³⁰

Based on the expression of vimentin, desmin, and α -SMA,^{19–21,23,24} hepatic stellate cells have been considered to be of mesenchymal origin. Since Kupffer's first description of *Sternzellen* (hepatic stellate cells) in liver, using gold chloride staining in search of nerve fibers,⁴⁸ indications of neural/neuroendocrine differentiation of hepatic stellate cells have been accumulating. Stellate cells show GFAP reactivity,^{25–27} N-CAM expression,^{28,29} nestin reactivity,²⁷ and SYN expression. All these can be considered as arguments in favor of a neural/neuroendocrine differentiation or origin. Neural tissue originates from the ectodermal layer. Neuroendocrine tissue is thought to originate from either the neural crest, migrating out of the ectodermal layer, or from the endodermal layer.⁴⁹ Expression of so-called mesenchymal intermediate filaments (vimentin, desmin, α -SMA) appears not to be contradictory to the suggested neural/neuroendocrine origin, because, for example, in the aorticopulmonary septum, smooth muscle cells supposed to originate from the neural crest also express α -SMA⁵⁰ and desmin.⁵¹ In view of the evidence mentioned above, unavoidable questions arise about the mesenchymal origin and functional differentiation of hepatic stellate cells. As a consequence, new studies focusing on the expression of neural and neuroendocrine markers in embryonic as well as adult liver become necessary.

Close contacts between hepatic stellate cells and bare nerve endings have been demonstrated ultrastructurally.^{52–54} Functional complexes of myofibroblasts, mast cells, and cholinergic nerve terminals have been suggested to play a role in the development of cirrhosis.⁵⁵ Vagotomy was reported to significantly slow down hepatic regeneration.^{56–59} Stimulation of sympathetic nerves was described as having a deteriorating effect on Gal hepatitis in rats.⁶⁰ Taken together with the evidence on the role of hepatic stellate cells in regeneration^{1–3} and fibrosis,^{4–6} it is tempting to suggest that the central nervous system influences regeneration and fibrosis through hepatic stellate cells. The central nervous system could, for instance, influence the stellate cells' production of extracellular matrix components or the secretion of growth factors from stellate cells, with stellate cells functioning as the end organ effector cells of the central nervous system.

Similar interactions of hepatic stellate cells with liver innervation have been postulated in other settings. Glycogenolysis in hepatocytes was proposed to be modulated in a paracrine way through adrenergic stimulation of hepatic stellate cells.⁶¹ Hepatic stellate cells were hypothesized to influence sinusoidal microcirculation, controlled by endocrine and paracrine substances (eg, endothelins and nitric oxide^{18,54}) as well as under central

nervous control.⁵²⁻⁵⁴ The demonstration of SYN expression in hepatic stellate cells (Figures 1 and 3) adds credibility to these hypotheses, SYN being a synaptic vesicle protein. In addition, we were able to demonstrate the ultrastructural presence of SET vesicles in cytoplasmic processes of human stellate cells, comparable to the SYN-reactive synaptic vesicles found in neurons (Figure 2).

Recently, Knittel et al demonstrated two morphologically and functionally different cell populations of the fibroblast lineage in fibrotic rat liver.⁶² They describe, on the one hand, hepatic stellate cells (P100-, desmin-, and GFAP-positive) located at the scar-parenchymal interface, and on the other hand myofibroblasts (P100-, desmin-, and GFAP-negative) located within the septa. The two distinct populations were suggested to play similar, but not identical, roles in matrix production. Our finding that α -SMA is strongly reactive in septa as well as in lobular (parenchymal) stellate cells, but that SYN marks only the lobular stellate cells, is an additional argument in favor of two distinct (portal-septal and parenchymal) types of myofibroblasts (Figure 1, b and c).

The demonstration of SYN reactivity in hepatic stellate cells may represent a useful immunohistochemical marker for human hepatic stellate cells (Figure 1). Desmin staining has been demonstrated to be positive in only a minor fraction of human hepatic stellate cells. Vimentin staining is too nonspecific, because endothelial cells as well as Kupffer cells are positive. α -SMA staining only becomes positive on switching of the hepatic stellate cells to the so-called myofibroblast cell phenotype.²⁴ GFAP was demonstrated to be positive in only a periportal subpopulation of human stellate cells.²⁷ N-CAM is rather nonspecific in that it also stains reactive bile ductules⁴¹ and nerve endings.²⁸ Nestin, finally, stains bile duct cells and vascular endothelium in rat livers and has not been tested on human liver biopsies yet. *In vitro*, however, nestin staining of human stellate cells was negative.²⁷ SYN is immunoreactive in human stellate cells (Figure 1) and nerve bundles. *In vivo*, nerve endings and stellate cells can be distinguished by their morphology: stellate cells contain a nucleus that in the resting state is indented by lipid droplets; parenchymal nerve endings as a rule do not contain a nucleus.

Whether SYN stains every single hepatic stellate cell in human and rat specimens is a question that cannot be solved. There is no marker for quiescent human stellate cells. As a consequence, double labeling cannot be performed. The number of SYN-positive cells in normal human liver is concordant with the number of stellate cells that was previously described, based on electron microscopic studies.⁵² In addition, SYN and α -SMA double staining showed overlap in all lobular stellate cells, in pathological specimens.

Because SYN is immunoreactive in quiescent as well as activated stellate cells and in rat as well as human stellate cells, it is a potentially useful marker for future diagnostic use.

In conclusion, SYN staining is a promising tool for diagnostic practice and fundamental research in the field of hepatology and hepatic stellate cells. Experimental evidence on the interaction between stellate cells and

hepatic innervation and accumulating evidence of neural/neuroendocrine differentiation of stellate cells awaits further research. The data presented here challenge current views on the origin and function of hepatic stellate cells.

Acknowledgments

We thank Paula Aertsen for her excellent technical support.

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