avirulent phenotype and is capable of limited multiplication in parenteral lymphoid tissues after oral administration.

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Immunohistochemical Localization of Somatotropin Receptors in Rat Liver Cells. Effect of Sex and Hormonal Status

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Peculiarities of cell and tissue distribution of somatotropin receptors in liver cells of male rats and intact, pregnant, and estrogenized female rats is studied using an indirect immunohistochemical method. Experiments reveal equal and positively regulated by female hormones expression of somatotropin receptors in all hepatocytes.

Key Words: *somatotropin receptors; rat liver; immunohistochemical analysis; sex-dependent expression*

Somatotropic hormone receptors (STHR) in rat liver are translated from at least two mRNA types with different 5'-flanking regions, arising due to initiation of transcription from two different promoters of the STHR gene [9]. A question arises of whether these processes can proceed simultaneously on one cell. One approach to solving this problem is an immunohistochemical analysis of STHR distribution in liver cells of animals with different levels of the STHR type I mRNA expression (intact males and females, and pregnant and estrogenized females [9]). This analysis is of interest in view of a comparison of liver cell sensitivity to STH and expression of STH-dependent proteins.

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MATERIALS AND METHODS

Experiments were carried out on 14 mature albino rats weighing 200-250 g (2 normal males, 5 normal and 2 pregnant (gestation days 15-18) females, and 5 females intramuscularly injected with estradiol in propylene glycol (15 μg/0.5 ml once a day for 10 days). The animals were decapitated, and the liver was fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 for 20 min at 4°C. Sections (3-μ thick) of liver tissue were embedded in Paraplast,

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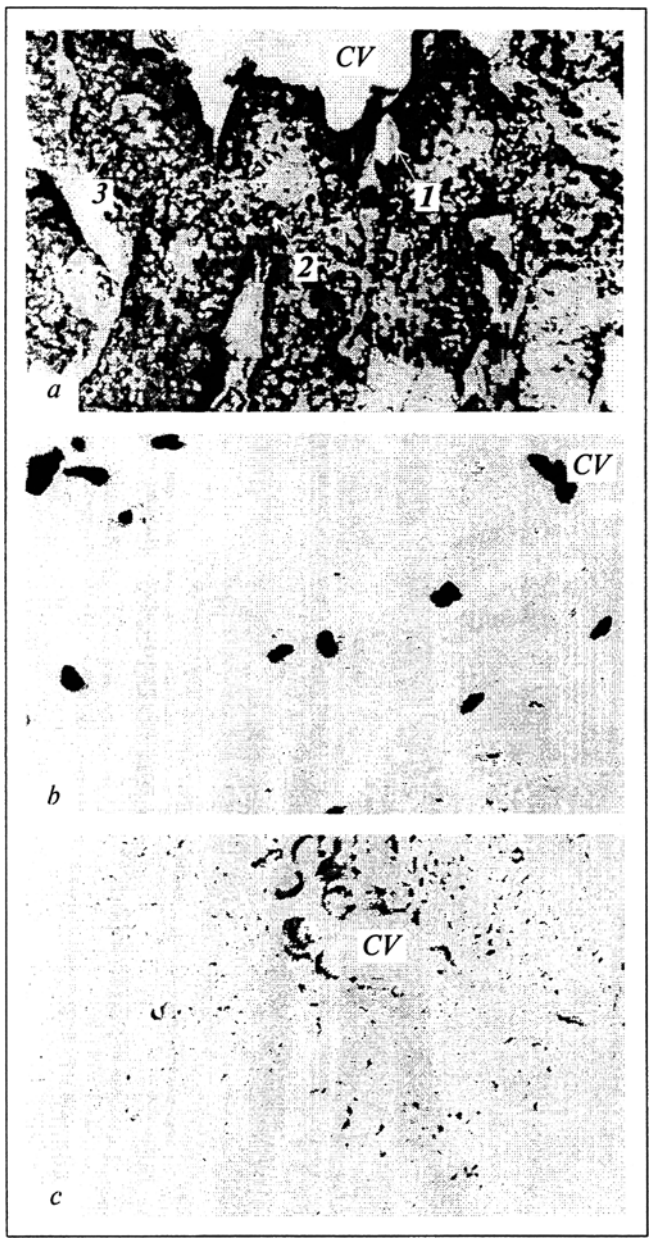

Fig. 1. Immunoperoxidase identification of subcellular distribution of somatotropic hormone receptors (STHR) in pericentral zone of hepatic lobule from estrogenized female rats. *a*) treatment with guinea pig antiserum containing polyclonal antibodies to human STHR cross-reacting with rat STHR; *b*) treatment with nonimmune guinea pig serum; *c*) treatment with dilution buffer. STHR-positive staining of sinusoidal domains of cell membranes (*1*); cytoplasmic granules (*2*); perinuclear space (*3*); ×787.5; *CV*: central vein.

transferred to slides precoated with 0.1% poly-L-lysine, and dried at 37°C for 2 h.

Before incubation with antibodies, each section was treated with 10 mM sodium periodate and 0.01% sodium borohydride for 10 min for inhibition of endogenous peroxidase [2].

Guinea pig antiserum containing polyclonal antibodies to the extracellular domain of human STHR cross-reacting with rabbit and rat STHR (kindly provided by Dr. M.-C. Postel-Vinay, Paris) were used as primary antibodies. The sections were incubated for 48 h (4°C) with the primary antibodies diluted 1:10 with a buffer (0.9% NaCl in Tris-buffer containing 0.1% bovine serum albumin). Bridge antibodies (rabbit antibodies to anti-guinea immunoglobulins) were incubated with rat serum (1:2) for 2 h at room temperature.

The sections were successively incubated with rabbit antibodies to guinea pig immunoglobulins (1:50), goat biotin-conjugated antibodies to rabbit immunoglobulins (1:1600), and extravidin peroxidase (1:800) for 30 min each at room temperature. The sections were washed with 0.1 M buffered physiological saline containing 0.1% Triton X-100. Peroxidase was visualized with 0.05% 3'3-diaminobenzidine in 0.05 M Tris-HCl containing 0.03% hydrogen peroxide.

In order to verify the specificity of primary antibodies, in parallel with incubation with guinea pig antiserum containing primary antibodies the sections were incubated with the same dilution of nonimmune guinea pig serum or dilution buffer.

Diaminobenzidine oxidation product was intensified with 0.4% OsO_4, and the sections were examined under a light microscope.

RESULTS

In all experimental groups STHR-specific staining was observed in all hepatocytes but not in the lithoral cells, the intensity of staining was the same in all zones of hepatic lobule.

Within cells STHR positive staining was seen in sinusoidal domains of cell membranes and cytoplasmic granules, while in estrogenized females it was found in the perinuclear space and/or in nuclear membranes of some cells in the pericentral zone of hepatic lobule (Fig. 1, *a*).

It was found that expression of STHR is sex-dependent: the intensity of STHR-positive staining in females was higher than in males and was associated primarily with cytoplasmic granules, whereas in males it was more pronounced in cell membranes (Fig. 2, *a*, *c*).

In pregnant females, the intensity of STHR-specific staining was markedly increased in comparison with normal females (Fig. 2, *e*). In estrogenized females, the STHR-positive staining apart from its general enhancement, was observed in the perinuclear space of some hepatocytes in the pericentral zone, some hepatocytes in this zone were stained more intensively than others (Fig. 1, *a*).

The observed compartmentalization of STHR in cells is a common feature of receptors of the STH

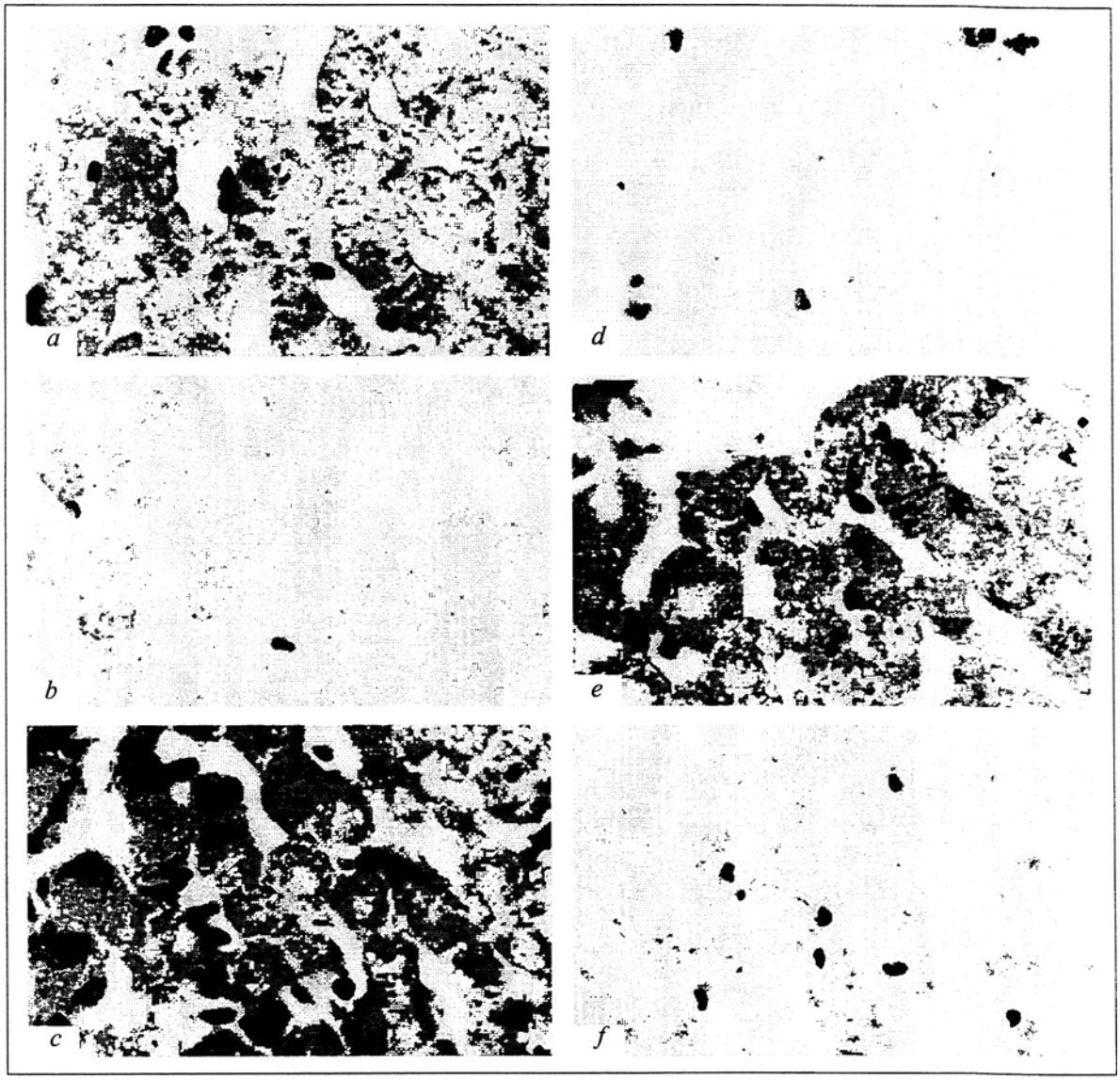

Fig. 2. Immunoperoxidase identification of somatotropic hormone receptors (STHR) in hepatocytes from rats with different hormone status. Treatment with guinea pig antiserum containing polyclonal antibodies to human STHR cross-reacting with rat STHR (*a*, *c*, *e*); treatment with nonimmune guinea pig serum (*b*, *d*, *f*); *a* and *b*) normal male rat; *c* and *d*) normal female rat; *e* and *f*) pregnant rat; ×500.

family. Our previous investigation showed a similar cell and tissue distribution of prolactin receptors in rat liver [10].

Large pool of cytoplasmic receptors is a characteristic feature of hormones of the STH family [5,8]. Our experiments agree with previous data demonstrating a high content of STH-binding sites in the microsomal fraction from the liver [6], which were either internalized hormone-receptor complexes or newly synthesized receptors translocated to the cell membrane.

Intense STHR-positive staining of the perinuclear space in estrogenized females is in conformity with the data on translocation of STHR complexes into nucleus and the presence of STH-binding sites in the nucleus [7], which is probably associated with direct effect of STH on nuclear system of signal transduction.

Previously, we showed that the higher content of STHR in the liver of female rats results from a more intense expression of these receptors in all hepatocytes, but not from an increased number of STHR-expressing cells.

Our data on equal (except of some hepatocytes in the pericentral zone) distribution of STHR-positive staining in hepatocytes of different location within the hepatic lobule in animals with different intensity of the STHR type I mRNA expression (minor expression in males, moderate expression in intact females, and very high expression in pregnant

and estrogenized females [9]) suggest the presence of both types of STHR mRNA in one cell. Direct evaluation of the content and synthesis of both STHR mRNA types is required for verification of this assumption.

When comparing the distribution of STHR and STH-dependent proteins (some of these proteins are characterized by unequal expression within a cell [3,4]) it can be assumed that cell sensitivity to STH is not the only factor responsible for this nonuniformity. This agrees with previous data that nonuniform distribution is not due to different sensitivity of hepatocytes to prolactin and androgens [1,10].

Thus, the intensity of STHR expression in rat hepatocytes is regulated in a sex-dependent manner, while pregnancy and estrogenization enhance STHR expression.

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