Immunohistochemical Identification of Androgen Receptors in the Liver of Pubertal Male Rats

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Androgen receptors in the liver of pubertal male rats are identified by an indirect immunoperoxidase method. Hepatocyte nuclei predominate among positively stained structures. The intensity of staining varies from hepatocyte to hepatocyte and does not depend on its position in the liver lobule.

Key Words: *hepatocytes; androgen receptors*

Androgen receptors (AR) belong to the superfamily of ligand-dependent transcription factors. Hormone binding induces transformation of the receptor providing its interaction with hormone-sensitive components of the competent genes with subsequent modulation of their transcriptional activity [2]. There is controversy over the intracellular localization of AR [11]. Recent studies showed that AR are present in the liver [1,7]. It was demonstrated that the activity of some metabolic systems is regulated by androgens (AG) at the level of hepatic AR [13]. However, biochemical data on the liver content of AR provide no information regarding cellular mechanisms of the sex-differentiating activity of AG.

The concentration of AR in the liver of intact male rats determined by the radioligand method is 2- to 3-fold lower than that in the prostate [3,7]. It is unclear whether this is due to the fact that fewer cells express AR in the liver than in the prostate or to low AR expression in all hepatocytes. Gradient distribution of some AR-dependent proteins (α_{2u}-globulin and specific estrogen-binding protein) in the rat liver with the maximum expression at the central veins [4,5] supports the hypothesis on a gradient sensitivity of hepatocytes to AG.

The distribution of the target cells for AG in the rat liver was studied by immuhistochemical identification of AR. Our objective was to optimize this method, localize AR in hepatocytes, evaluate AR expression, and assess the distribution of liver cells sensitive to direct effect of AG.

MATERIALS AND METHODS

Experiments were performed on pubertal outbred albino male rats. In order to increase the amount of AR firmly bound to the nucleus, some animals were injected the synthetic AG methyltrienolone (R1881, 100 μg in 0.4 ml propylene glycol, one hour prior to sacrifice). Specific anti-AR antibodies PG-21 (affinity purified rabbit IgG against the N-terminal synthetic AR peptide) were kindly supplied by Dr. G. Greene (USA).

Frozen liver sections 6 μ thick were fixed with 4% parafomaldehyde (Sigma) in 0.1 M phosphate buffer for 5 min. Then they were successively treated with 3% H_2O_2 (Merck) and 0.2% Triton X-100 (Sigma) and washed with phosphate buffered saline (PBS). In order to reduce nonspecific background the sections were incubated for 30 min with 2% nonimmune goat serum (Cardiology Research Center, Russian Academy of Medical Sciences) and then with primary antibodies (10-30 μg/ml) at 4°C overnight. After washing with PBS, the sections were incubated with the Ig fraction of goat antiserum to rabbit IgG (1/50, Miles-Yeda) for 45 min at room temperature and for 45 min with rabbit peroxidase-antiperoxidase

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complex (1/100, Arnel). After washing, the sections were incubated for 15 min with 0.05% 3,3-diaminobenzidine tetrahydrochloride (Sigma) with 0.01% H_2O_2. In the control series, PBS or Ig-fraction of nonimmune rabbit serum was used instead of the primary affinity-purified antibodies.

The intensity of staining was measured cytospectrophotometrically at a wavelength of 550 nm in a LYuMAM I-3 luminescent photometric microscope using a light probe 0.1 arb. units in size. Measurements were performed in at least three animals in 100 points for each parameter. The results were statistically processed using the STADIA software.

RESULTS

The AR-specific immunostaining was detected predominantly in the nuclei and cytoplasm of hepatocytes of intact male rats (Fig. 1, *a*). Visual observations were confirmed by cytospectrophotometric studies which revealed a significant difference between the optical density of experimental and control sections (Fig. 1, *b*) both in the nuclei and cytoplasm. The intensity of nuclear staining varied. Based on the data of discrimination analysis, we specified four classes of cells differing in the extent to which AR-positive staining of the nuclei was more intense than that of the cytoplasm: class 0 cells showed no difference, class 1 slight difference, class 2 considerable difference, and class 3 pronounced difference. About 60% of hepatocytes in intact adult males had class 2 and 3 nuclei, the majority of AR-positive cells belonging to class 2 (Fig. 2, *a*).

The distribution of hepatocytes with stained nuclei in the lobule was mosaic, with no differences in the distribution near the central vein (Fig. 1, *c*), portal vein (Fig. 1, *d*), and in the intermedial region (Fig. 1, *a*). Administration of R1881 (an agent enhancing the binding between AR and the nucleus) to intact males led to an increase in the number of hepatocytes with intensely stained nuclei to 80% predominantly due to increased number of class 3 hepatocytes and decreased number of class 0 and 1 hepatocytes (Fig. 2, *b*). Hepatocytes with AR-positive nuclei were randomly distributed over the lobule.

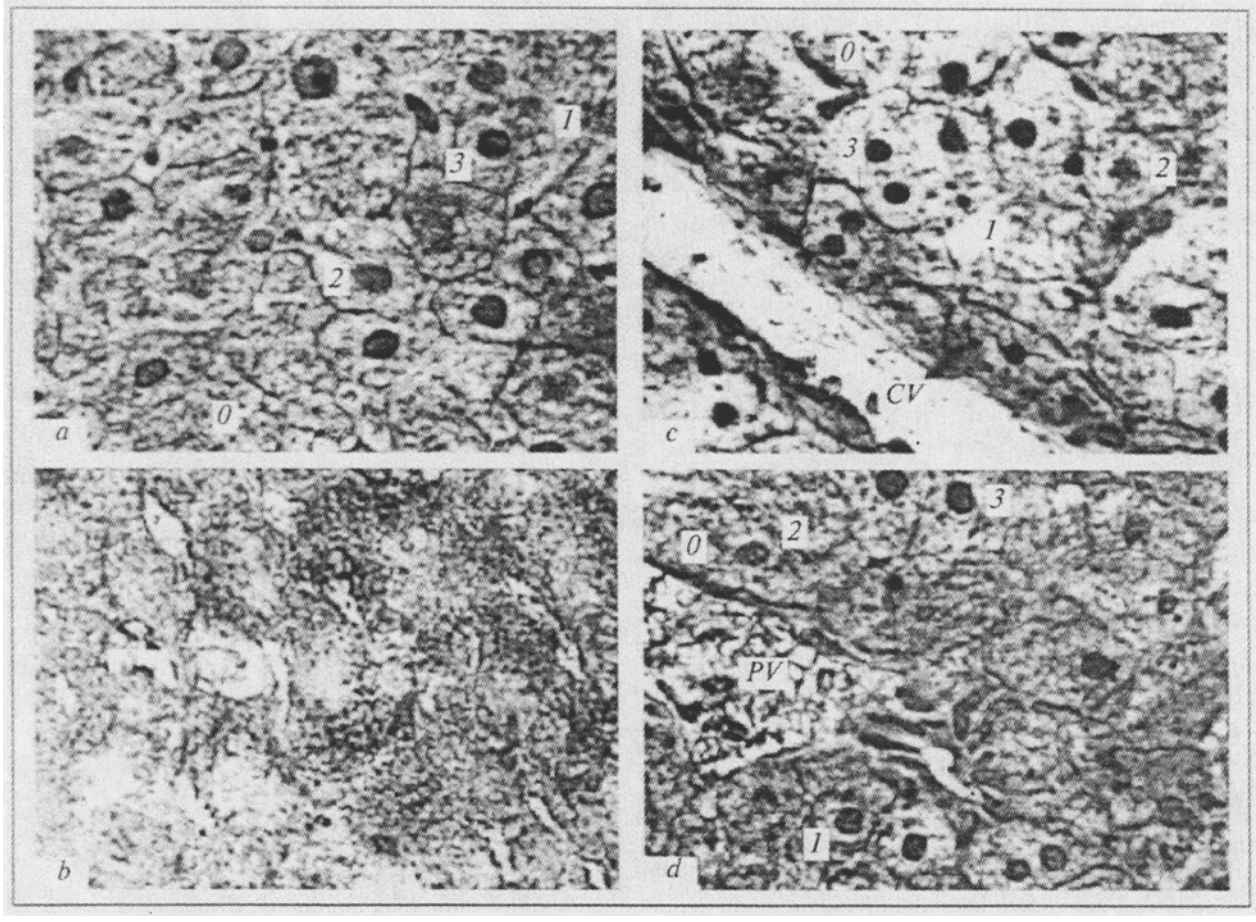

Fig. 1. Immunohistochemical localization of AR in rat liver. Intact male: *a*, *c* and *d*) intermedial, central, and periportal zones of liver lobule, respectively; *b*) control section treated with buffer instead of primary antibodies; Central (*CV*) and portal (*PV*) veins. *0-3*) class of nuclear staining. ×500.

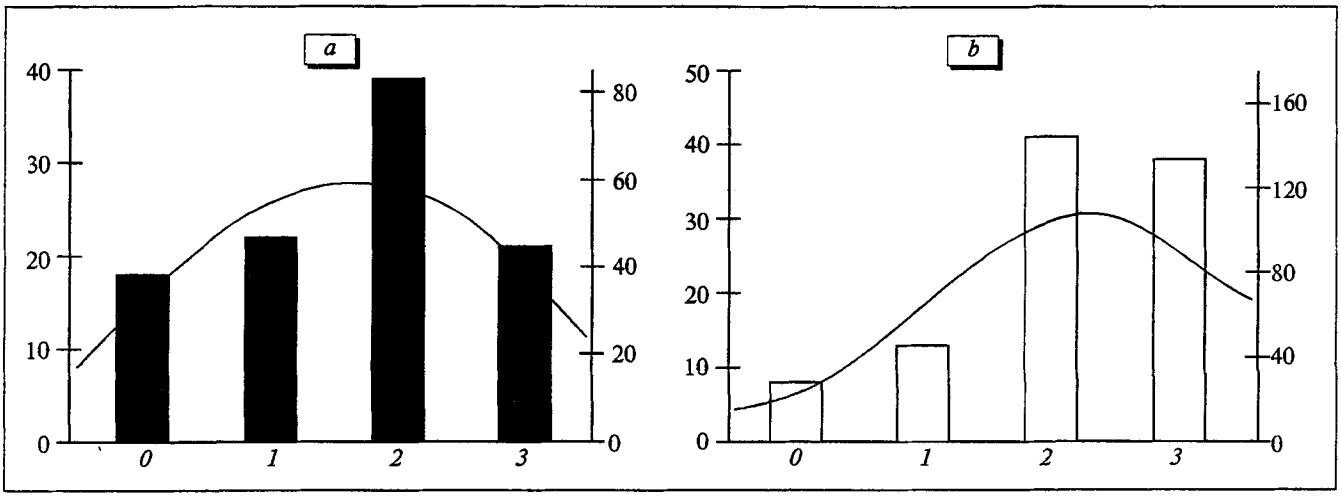

Fig. 2. Distribution of AR-positive hepatocytes according to four classes specified by the discrimination analysis. *a*) intact male; *b*) R1818-treated male. Abscissa: classes according to the increase in the degree of AR-positive staining of the nuclei relative to that of the cytoplasm: no difference (*0*); slight (*1*), medium (*2*), and marked (*3*) differences. Ordinate: left, proportion of cells of the given class, % of total cell number; right, number of determinations.

These results show that AR in the hepatocytes of male rats are localized predominantly in the nuclei. Comparative studies of the specific staining of the nuclei in the liver and prostate of intact rat males (data not shown) revealed that in the prostatic endothelium the nuclei are stained uniformly and intensely. By contrast, the specific staining of hepatocyte nuclei varied considerably. In some hepatocytes the staining was confined to the cell periphery, reflecting the pool of imported or exported AR which are not bound to DNA (Fig. 1, *a*). From these findings it can be concluded that the expression of AR in the liver is lower than in the prostate. This may partially explain the difference in the AR content between these organs in intact animals.

Specific staining of higher intensity (compared with that in the control group, where PBS was added instead of primary anti-AR antibodies) was revealed in the cytoplasm of hepatocytes. It did not disappear after administration of R1881. In the presence of androgens AR were detected in the cytoplasm in rats with AG-independent carcinoma of the prostate [9] and in the spermatids at the late stage of development [14]. In addition, it is known that intracellular localization of AR depends on cell type and the presence of ligand [8,11]. The cytoplasmic staining of cells from reproductive organs and brain as well as of cells cultured from some internal organs was observed only in the absence of ligand [11,15]. The possibility that the presence of AR is specific for the liver due to the low rate of AR turnover cannot be ruled out. Cytoplasmic receptors may represent the pool of newly synthesized or exported from the nucleus AR.

It was interesting to compare the localization of AR and AG-dependent proteins in the liver lobule. It is known that the high content of specific estrogen-binding protein in the liver of intact males is a consequence of programming and regulatory activity of AG. The distribution of estrogen-binding protein and α_{2u}-globulin, which is also positively regulated by AG, was stepwise with the maximum at the central veins [4,13]. At the same time, pericentral and periportal zones of the liver lobule did not differ in the content of class 1-4 hepatocytes. There was no correlation between the distribution of AR and prolactin receptors, which are negatively regulated by AG. Although the content of prolactin receptors was low, they were uniformly expressed by all hepatocytes of intact males [12].

Thus, it can be concluded that the response of hepatocytes to AG depends not only on the presence of AR, but is probably associated with the presence of factors participating in the transduction of the AG signal in the liver from more to less sensitive cells, thus modifying cell reaction.

Our results indicate that AR is localized in the nuclei and cytoplasm of rat hepatocytes, and the degree of AR expression varies from cell to cell. The distribution of AR-positive hepatocytes in the lobule is irregular.

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Anticoagulant Activity of Naturally Occurring Anticardiolipin Antibodies

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Natural antibodies isolated from a commercial immunoglobulin preparation react with cardiolipin *in vitro* and exhibit anticoagulant activity in the thromboplastin suppression and kaolin time tests performed with the use of Wistar rat plasma.

Key Words: *antiphospholipid syndrome; antibodies; cardiolipin; anticoagulant activity*

The antiphospholipid syndrome (APS), a thrombotic state associated with hereditary or acquired deficiency of blood coagulation inhibitors and fibrinolytic disorders, has been recently discovered. Two APS types have been defined: anticoagulant-thrombotic lupoid and anticardiolipin-thrombotic, manifesting themselves as certain differences in the thrombosis of cardiac, cerebral, and renal arteries and veins, repeated spontaneous abortions, and thrombocytopenia. APS is developed against the background of lupus erythematosus, in other connective tissue diseases, autoimmune and malignant disorders, in response to some drugs for syphilis and some infectious diseases in persons below 50 as a secondary syndrome, and as a primary syndrome in patients healthy in all other respects [7]. The APS has been associated with the emergence of antibodies against IgG, IgM, and IgA. Previously, we showed that latent anticardiolipin antibodies (IgG and IgM) are present in the serum of healthy subjects [4].

In this study a possible role of these antibodies in the development of APS is evaluated.

MATERIALS AND METHODS

Experiments were performed *in vitro* on citrated platelet-free plasma of intact Wistar rats.

Commercial Ig preparations for intramuscular administration were used as a source of antibodies. These preparations were fractionated by ion exchange chromatography on QAE A-50 Sephadex in 0.01 M potassium phosphate buffer (pH 7.3) as described elsewhere [5]. The fraction of positively

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