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ACTION OF HUMAN CHORIONIC GONADOTROPHIN ON PROLIFERATION OF HYPATOCYTE ORGANELLES FROM THE NORMAL AND PATHOLOGICALLY CHANGED RAT LIVER

T. F. Zhdanova and A. A. Chernikov

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KEY WORDS: liver; proliferation of organelles; chorionic gonadotrophin.

One manifestation of intracellular regeneration is proliferation of the cytoplasmic organelles [9]. Previous investigations [5-7] have shown an increase in the intensity of proliferation of the rough endoplasmic reticulum (RER), the smooth endoplasmic reticulum (SER), and mitochondria (M) of the hepatocytes of the pathologically changed human and animal liver under the influence of the human placental hormone chorionic gonadotrophin (CG). On injection of CG into albino rats with experimental chronic hepatitis, the stimulating action of the hormone lasted throughout the period of observation (60 days). A marked increase in the number of structures in the treated animals receiving the stimulator was observed 48 h after two injections of the hormone. The character of the proliferative processes was pulsed. The stages of proliferation of the organelles was preceded by a state of their hypertrophy. Hypertrophy of the structures was the starting stage for the measurement of proliferation. It was postulated on this basis that the healthy and pathologically changed organ respond differently to the action of the hormone. The direction of action of CG on hepatocytes of the intact liver is unknown.

The purpose of this investigation was a comparative study of proliferation of the reticulum and mitochondria of hepatocytes of the normal and pathologically changed rat liver under the influence of CG.

EXPERIMENTAL METHOD

Experiments were carried out on 30 noninbred albino rats weighing 180-200 g, divided into two groups: 1) healthy animals, 2) animals with chronic hepatitis induced by CCl_4. The animals of both groups were given CG in a dose of 150 i.u. per rat subcutaneously at 8 a.m. on 2 consecutive days. The rats were decapitated 0, 12, and 48 h after the beginning of CG injections. Before decapitation the animals were given trimeperidine in a dose of 10 mg/kg body weight. Pieces of tissue were fixed by Caulfield's method at pH 7.2 (the working solution of the fixative was made up as required), stained with uranyl acetate, dehydrated in alcohols, and embedded in Araldite by the usual methods. Serial sections were cut on the LKB-800 ultramicrotome, counterstained by Reynolds' method, and examined in the UÉMV-100K

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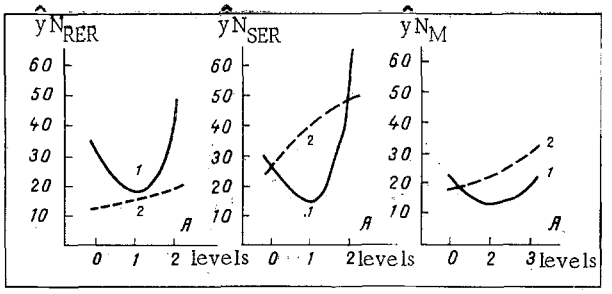

Fig. 1 Fig. 2 Fig. 3

Fig. 1. Changes in a relative number of structures of rough endoplasmic reticulum in hepatocyte cytoplasm as a function of time and number of injections of CG. Here and in Figs. 2 and 3: 1) healthy animals, 2) animals with hepatitis.

Fig. 2. Changes in relative number of structures of smooth endoplasmic reticulum in cytoplasm of hepatocyte as a function of time and number of injections of CG.

Fig. 3. Changes in relative number of mitochondria in cytoplasm of hepatocyte depending on time and number of injections of CG.

electron microscope under a magnification of 10,000-12,000. The necessary number of sections, their orientation, and the number of electron-microscopic negatives were determined as described previously [1, 2]. The resulting negatives were examined morphometrically under a magnification of 50,000 by Weibel's method in Stefanov's modification, using equal- and random-step grids [10, 11]. The results were subjected to statistical analysis by Student's t-test at the P = 0.05 level of significance. The mean total (ΣP) and single (P) volumes of the RER, SER, and mitochondria in a definite volume of hepatocyte cytoplasm were calculated for the rats of each group. Individual variations in morphometric indices for different animals were disregarded. On the basis of the results the mean conventional number of organelles in a definite volume of cytoplasm was calculated: $N = \Sigma\overline{P}/\overline{P}$. Changes in N_{RER}, N_{SER}, and N_M in healthy and affected animals were investigated by means of regression equations adquately describing the objects in the assigned factor space.

EXPERIMENTAL RESULTS

An orthogonal factor plan of type 3^k [4, 8] was drawn up, on the basis of which a regression equation was constructed of the following type:

$$\hat{y} = b_0 + b_1x_1 + b_2x_2 + b_{12}x_1x_2 + b_{22}z_2 + b_{22}x_1z_2,$$

$$\text{where } x_i = k(\chi_i + A);\ z_i = k(x_1^2 + bx_1 + c);$$

where χ_1 is a factor determining the type of organ (the pathological or healthy liver); χ_2 the time factor, dependent on the number of injections of the hormone, defined by three levels: A_0) the liver without injections, A_1) the liver with one injection of hormone, 12 h after the beginning of the injection, and A_2) the liver with two injections of hormone, 48 h after the beginning of injection; x_0, x_1, x_2) coded values of the test factors in the 1st and 2nd degrees; b_0, b_1, b_2, b_{12}, b_{22}) coefficients of the regression equation.

The repeated responses were done with allowance for the requirement of randomization, so that homogeneity of dispersion could be checked by Cochrane's criterion, and after verification of significance of the coefficients, the regression equation could be tested for adequacy at the P = 0.05 level of significance [3].

After all calculations had been done changes in the index N_{RER} were described by the equations:

$$\hat{y}_{N\,RER\,(1)} = 28.42 + 3.83x_2 + 6.87z_2$$

in healthy animals, and by the equations

$$\hat{y}_{N\,RER\,(2)} = 15.01 + 3.83x_2 + 1.09z_2,$$

in the animals with hepatitis, where $x_2 = \chi_2 - 1$, $z_2 = 3x^2_2 - 2$; the equations are illustrated graphically in Fig. 1. The equations for N_{SER} had the form:

$$\hat{y}_{N\,SER\,(1)} = 36.83 + 17.70x_2 + 10.33z_2$$

for the healthy animals, and

$$\hat{y}_{N\,SER\ (2)} = 36{,}83 + 10{,}05x_2 - 1{,}22z_2$$

for the animals with hepatitis, where $x_2 = \chi_2 - 1$, $z_2 = 3x^2 - 2$.

The equations for $\hat{y}N_{SER(1)}$ and $\hat{y}N_{SER(2)}$ are illustrated in Fig. 2.

The equations for N_M had the form:

$$\hat{y}_{NM\ (1)} = 15{.}93 - 2{.}75x_2 + 1{,}48z_2$$

for healthy animals and

$$\hat{y}_{N_M\ (2)} = 22{,}23 + 4{.}3x_2 + 1{,}48z_2$$

for animals with hepatitis, where $x_2 = \chi_2 - 1$, $z_2 = 3x^2_2 - 2$. This relationship is shown graphically in Fig. 3. On animals of the changes in $\hat{y}N_{RER}$, $\hat{y}N_{SER}$, and $\hat{y}N_M$ in the sick and healthy animals the response of the organ to injection of the hormone was observed to be different, although the final result of the action of CG on hepatocyte organelles of the rats of groups 1 and 2 consisted of intensification of proliferation of the reticulum and mitochondria.

Analysis of changes in $\hat{y}N_{RER}$ gave the following results. The original points A_0 had different levels in the sick and healthy animals, evidence of suppression of the formation of structures of the rough reticulum in chronic hepatitis. Injection of CG sharply reduced the value of $\hat{y}N_{RER}$, it rose sharply after two injections of the hormone at level A_2, higher than its initial value, but remained much below $\hat{y}N_{RER}$ at the end point for healthy animals.

Changes in $\hat{y}N_{SER}$ were similar to changes in $\hat{y}N_{RER}$. At the A_0 level the values of $\hat{y}N_{SER}$ for the healthy and sick animals were almost the same. Injection of CG sharply increased the value of $\hat{y}N_{SER}$, evidence of the marked action of CG on the number of structures of the smooth reticulum; $\hat{y}N_{SER}$ of the healthy animals at the A_1 level was much lower than the values of $\hat{y}N_{SER}$ for the sick animals. At the end point at the A_2 level the value of $\hat{y}N_{SER}$ of the animals of group 1 was significantly higher than that for the animals of group 2. The response of the smooth membranes to injection of the hormone was stronger than that of the rough membranes. This phenomenon confirms the view of Lowe, who considered that proliferation of the rough reticulum is impossible without preliminary activation of synthesis of components of the smooth membranes.

Changes in $\hat{y}N_M$ for the animals of groups 1 and 2 differed from changes in $\hat{y}N_{RER}$ and $\hat{y}N_{SER}$. The mitochondria reacted less actively to injection of CG in hepatocytes of both the healthy and the pathologically changed liver, although the general direction of the process remained the same. The value of $\hat{y}N_M$ at the three different levels changed only very little in the hepatocytes of both groups of rats, but proliferation of the mitochondria took place more intensively in the pathologically changed hepatocytes than in the intact liver. Proliferation of the mitochondria in the healthy animals under the influence of CG fell at level A_1, then rose, but remained below the initial value of $\hat{y}N_M$ at level A_2 in the hepatocytes in chronic hepatitis.

Injection of CG thus stimulates proliferation of the reticulum and mitochondria in the hepatocytes of the pathologically changed organ. Intensification of proliferation of organelles of the hepatocytes of the normal liver takes place after the creation of a pathological situation in the organ, which may be called the phase of excitation. The structures of the endoplasmic reticulum respond more actively to injection of CG than the mitochondria.

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BIOLOGICAL ACTIVITY OF CHALONES ISOLATED FROM THE NORMAL AND REGENERATING LIVER

S. A. Ketlinskii and E. V. Parfenova

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KEY WORDS: liver chalones; partial hepatectomy; regulation of mitotic homeostasis of the liver.

It has now been shown that an essential role in the regulation of mitotic homeostasis of the liver is played by tissue-specific inhibitors of proliferation, or chalones [1, 4, 6]. After partial hepatectomy, leading to intensive proliferation of hepatocytes in the residual part of the organ, chalone activity disappears: The chalone-containing extract isolated from regenerating liver 24 h after the operation cannot inhibit growth of hepatoma cells in an *in vitro* system [5]. The mechanism of disappearance of biological activity of the chalones has not yet been explained.

The object of this investigation was to study the effect of chalones, isolated from the regenerating liver at various times after partial hepatectomy, on the entry of hepatocytes into the phase of DNA synthesis and mitotic division in an *in vivo* system.

EXPERIMENTAL METHOD

Aqueous extracts of normal and regenerating liver, fractionated with ethanol, were used as the chalones; the method used to obtain them was described previously [3]. The fraction precipitated between 70 and 81% saturation with ethanol possessed activity. Analytical disc electrophoresis of the resulting extracts was carried out by Maurer's method [2] in 7.5% polyacrylamide gel at pH 8.9. Preparative electrophoresis was carried out using the same system of buffers in a block of polyacrylamide gel measuring 160 × 230 × 4 mm for 14 h with a current of 50 mA. After the end of electrophoresis the block of gel was cut into four regions (Fig. 1A-D), corresponding to groups of proteins with different mobility, and after elution, concentration, and dialysis against physiological saline, the effect of these groups of proteins on the level of hepatocyte proliferation was determined in an *in vivo* system. For this purpose two series of experiments were conducted on noninbred male rats from the Rappolovo Nursery, Academy of Medical Sciences of the USSR, weighing 70-80 g, hepatectomized by the method of Higgins and Andersen. In the experiments of series I, simultaneously with colchicine (0.2 mg/kg body weight), chalones isolated from the normal or regenerating liver 22 h and 28 days after the operation (in a dose of 15 mg/100 g body weight) were injected into the hepatectomized animals 25 h after the operation. In the experiments of series II, chalones isolated from normal or regenerating liver 28 days after the operation (15 mg/100 g body weight per animal) and also various protein components of these chalones (equivalent to the protein content in 15 mg of whole extract per 100 g body weight) were injected into hepatectomized rats 21 h after the operation. One hour before sacrifice, ^{3}H-thymidine was injected (1 μCi/g body weight) intraperitoneally into the animals of this group. Instead of chalones, physiological saline in the same volume was injected into the control hepatectomized rats in both series. All the animals were killed 29 h after hepatectomy. Activity of hepatic chalones was assessed by the decrease in number of C-mitotic activity, and in the fraction of ^{3}H-thymidine-labeled hepatocytes (in 3000 cells from each animal).

Laboratory of Experimental Histology, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR A. N. Klimov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 7, pp. 96-98, July, 1981. Original article submitted April 11, 1980.

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