CONNECTION BETWEEN CHANGES IN MEMBRANE POTENTIAL AND DNA, RNA, AND PROTEIN SYNTHESIS IN REGENERATING LIVER CELLS AFTER PARTIAL HEPATECTOMY

V. V. Frol'kis, N. M. Turaeva, Kh. K. Muradyan, and E. L. Levitskii

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Much information is available on changes in the state of the various stages of protein biosynthesis during depolarization and hyperpolarization of the cell membrane. Another fundamentally important problem is that of how changes in protein biosynthesis affect the electrical properties of the plasma membrane.

The writers' previous studies showed that during activation of protein biosynthesis by various hormones, blood loss, and hypertrophy, similar changes took place in the level of membrane potential (MP) of the cells studied, hyperpolarization developed, and the membrane resistance and thresholds of excitability of the cells were modified [3, 5-7].

Regeneration of the liver arising after partial hepatectomy is a convenient model with which to study correlation between activation of the genetic apparatus of the cell and the level of polarization of its membranes.

The object of this investigation was to study the relationship between changes in MP of the liver cells and DNA, RNA, and protein synthesis during regeneration induced by partial hepatectomy.

EXPERIMENTAL METHOD

Albino rats weighing 300-350 g were used. Partial hepatectomy was carried out by the method of Higgins and Anderson [8]. Intracellular measurement of MP was carried out by the usual microelectrode technique [1]. The rate of synthesis of total RNA and protein was judged from the changes in relative specific radioactivity (RSR), determined as the ratio between the specific radioactivity (SR) of the acid-insoluble and acid-soluble materials of liver homogenates in 10% TCA. Orotate-^{14}C and leucine-^{3}H, injected intraperitoneally in a dose of 0.5 mCi/kg 30 min before sacrifice of the animal, were used as labeled precursors of RNA and protein. The radioactivity of RNA and protein was determined after purification on nitrocellulose membrane filters (from Oxoid Great Britain), by Kennel's method [9]. Radioactivity of the samples was measured on a type SL-30 scintillation counter (France). The level of DNA replication was determined by the method suggested by Obolenskaya and Levitskii [2]. These parameters were studied for 48 h after hepatectomy.

EXPERIMENTAL RESULTS

The experiments showed that during regeneration of the liver after partial hepatectomy the value of MP of the liver cells rose and hyperpolarization developed. For instances, the initial MP of the liver cells was 38.03 ± 0.57 mV, 6 h after removal of two-thirds of the liver MP reached 48.4 ± 0.53 mV ($P < 0.001$), falling after 24 h to 46.1 ± 0.73 mV ($P < 0.001$), and it remained at this high level throughout the 48 h of the investigation (Table 1).

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TABLE 1. Changes in MP (in mV) of Liver Cells after Partial Hepatectomy

Statistical index	Initial values of MP	MP after hepatectomy		
		6 h	24 h	48 h
M	38,3	48,4	46,1	46,2
$\pm m$	0,57	0,53	0,73	0,93
n	8	7	6	6
P		<0,001	<0,001	<0,001

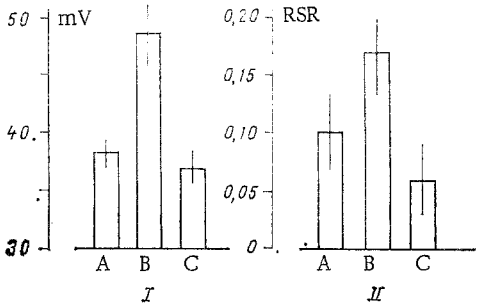

Fig. 1. Effect of actinomycin D on changes in MP and RNA synthesis in liver cells after partial hepatectomy. I) MP of liver cells; II) RSR of RNA. A) Control; B) hepatectomy; C) hepatecomy under actinomycin D protection.

Simultaneously with the development of hyperpolarization, activation of the genetic apparatus of the cell took place, as shown by an increase in RSR of total RNA and protein and an increase in the level of DNA replication (Table 2). By 6 h after hepatectomy, for instance, RSR was increased by 32%. By 12 h it was increased by 58%, and it remained at this high level for 48 h. Protein synthesis also was activated under these circumstances: RSR of protein by 12 h after hepatectomy was increased by 17%, by 24 h by 64%, and after 48 h it amounted to 119% relative to the control.

Evidence of the increase in the intensity of DNA synthesis during regeneration of the liver was given by data showing elevation of the level of DNA replication by 95% 20-24 h after hepatectomy (SR of DNA from intact liver 6386 cpm/mg DNA, of regenerating liver 156,740 cpm/mg DNA). After hepatectomy an increase was thus observed in MP of the cells and DNA, RNA, and protein synthesis was activated.

In the next series of experiments the effect of actinomycin D, which delays the progress of RNA-polymerase along the DNA chains to be transcribed, on changes in MP, and on RNA and protein synthesis in the liver cells was studied after hepatectomy. The antibiotic was injected intraperitoneally in a dose of 1 mg/kg 2 h before hepatectomy.

As Fig. 1 shows, injection of actinomycin D prevented the development of hyperpolarization of the cell membranes. For instance, the initial value of MP of the liver cells was 38.3 ± 0.5 mV, and after hepatectomy under actinomycin D protection it was 36.8 ± 0.73 mV. Meanwhile actinomycin D prevented intensification of synthesis of total RNA and protein arising after hepatectomy.

The development of hyperpolarization of the plasma membrane during activation of protein biosynthesis is connected with the formation of a special hyperpolarizing factor [4]. It might be supposed that this mechanism also determines the increase in MP of the liver cells during regeneration.

In a special series of experiments the effect of liver tissue homogenate from intact and hepatectomized animals (6 and 24 h after hepatectomy) on MP of the liver cells of recipient rats was studied. A chamber with a volume of 0.1 ml, through which the liver tissue filtrate from the donor animal was passed, was secured to the surface of the liver of recipient rats. As Fig. 2 shows, the liver tissue filtrate from intact animals caused no change in MP of the recipient's liver cells, whereas liver filtrate from hepatectomized animals led to marked hyperpolarization of the liver cells of the recipient rats. Consequently, the hyperpolarization effect arising after hepatectomy can be transferred from one animal to another. This suggests that during activation of protein biosynthesis occurring during regeneration a

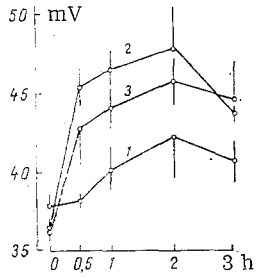

Fig. 2. Effect of application of liver homogenate of intact and hepatectomized donor rats on changes in MP of liver cells of recipient rats. 1) Intact rats; 2) 6 h after hepatectomy; 3) 24 h after hepatectomy.

TABLE 2. Effect of Hepatectomy on Synthesis of Total RNA and Protein in Rat Liver

Time after operation, h	RNA			Protein			RSR of protein/RSR of RNA
	SR of pool	SR of preparation	RSR	SR of pool	SR of preparation	RSR	
Control (n = 6)	4213±383	436±38	0,107±0,017	3 457±121	218±38	0,063±0,007	0,522±0,083
6 h (*n* = 6)	5555±420	790±112	0,141±0,015	11 932±1004	614±61	0,051±0,003	0,372±0,025
12 h (*n* = 6)	5652±494	925±70	0,169±0,022	12 027±983	872±63	0,074±0,005	0,455±0,053
24 h (*n* = 7)	6693±135	837±158	0,121±0,012	5 688±891	567±115	0,101±0,013	0,855±0,117
48 h (*n* = 7)	8180±201	984±156	0,126±0,011	6 825±1024	535±97	0,079±0,005	0,620±0,084

factor modifying the properties of the cell membranes is synthesized.

These findings prove that the development of hyperpolarization of liver cells in the course of regeneration is connected with changes in the system of protein biosynthesis. This is shown, first, by elevation of MP against the background of RNA, DNA, and protein synthesis and, second, by the absence of changes in MP when RNA synthesis is blocked by a specific inhibitor (actinomycin D). It can be tentatively suggested that this connection is effected through the formation of a special hyperpolarizing factor.

The relationship described between activity of the cell genetic apparatus and changes in MP also was observed by the writers under different conditions of stimulation of protein biosynthesis (during the action of hormones, blood loss, hypertrophy), and on other types of cells (neurons, muscle fibers, myocardial fibers, acinar cells of salivary glands). During the development of hyperpolarization of the cell membranes the properties of the cells changed significantly: transport of materials inside the cell, ionic asymmetry, processes of energy generation, excitability of the cell, resistance of the membrane. It can be tentatively suggested that the hyperpolarization of the cell membranes developing during regeneration, by modifying the membrane mechanisms regulating the state of the cells, is of great importance from the point of view of adaptation and regulation.

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