Effect of Chalone and Antichalone on Free Radical Processes in Rat Liver

A. N. Pashkov and A. Yu. Maslov

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Effect of the proliferative regulators chalone and antichalone on the free radical system is studied on isolated and perfused rat liver under normal conditions and after ammonium chloride load (5 mM). It is shown that chalone suppressed both lipid peroxidation and antiradical system, while antichalone exhibits opposite effects on these processes. Ammonium chloride abolishes the effect of antichalone and does not change the effect produced by chalone. A hypothesis is proposed that specific function of the organ predominates over its response to local regulatory stimuli.

Key Words: *chalone; antichalone; isolated liver; lipid peroxidation; antiradical activity*

The majority of studies in the field of regulation of cell homeostasis are aimed at investigating final effects of regulatory factors and usually employ morphological methods. Such an approach ignores cell processes which occur after the regulatory stimulus reaches the cell to the appearance of morphological changes triggered by this signal. These processes occurring during the lag-period prepare the target cell to competent response to the action of growth factors.

MATERIALS AND METHODS

Isolated livers from random-bred albino rats of both sexes (221±3 g) were used in the experiments. The animals were sacrificed in the morning after 24-h fasting. The liver of ketamine-narcotized and heparinized rat was connected via *v.portae* to a perfusion system as described previously [2]. Then it was isolated from ligaments, removed from the abdominal cavity, and placed into a thermocontrolled perfusion chamber. At the first stage the liver was perfused for 30 min with Hank's medium (37°C, pH 7.45, 5 ml/min) for stabilization of metabolic processes. During the next 30 min the liver was perfused under the same conditions with experimental solutions containing chalone, antichalone, 5 mM NH_4Cl, or a combination of growth factor with NH_4Cl. The preparations were added in a proportion of one dose per 10 ml. The dose was calculated on the basis of the antioxidant properties of chalone and antichalone discovered by A. N. Pashkov [4]. The amount of preparation inducing 50% quenching of chemiluminescence was taken as one dose.

After the end of perfusion, the liver was frozen in liquid nitrogen and then homogenized in physiological saline containing Triton X-100 and centrifuged for 10 min at 15.000 g min. The contents of protein (by the method of Lowry) and malonic dialdehyde (MDA) [7] in the supernatant were measured. Antiradical activity of the supernatant was evaluated by chemiluminescence quenching on an KhLM1Ts-01 chemiluminometer [3].

The significance of differences was evaluated by one-factor dispersion analysis.

RESULTS

Our results show that cell proliferation regulators specifically modulate such phylogenetically old process as lipid peroxidation (LPO).

As seen from Fig. 1, chalone and antichalone had opposite effects on the intensity of LPO, judging from the content of MDA in isolated liver. Chalone more than 2-fold reduced the intensity of LPO in liver

Department of Medical Biology and Genetics, N. N. Burdenko State Medical Academy, Voronezh

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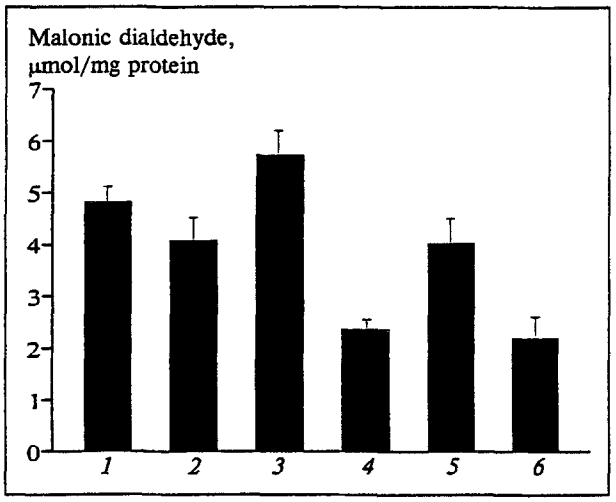

Fig. 1. Effect of chalone and antichalone on the intensity of lipid peroxidation in isolated rat liver under different perfusion conditions. Here and on Fig. 2: *1*) Hank's medium (HM), control; *2*) HM+ NH_4Cl (5 mM); *3*) HM+antichalone; *4*) HM+chalone; *5*) HM+antichalone+ NH_4Cl (5 mM); *6*) HM+chalone+NH_4Cl (5 mM).

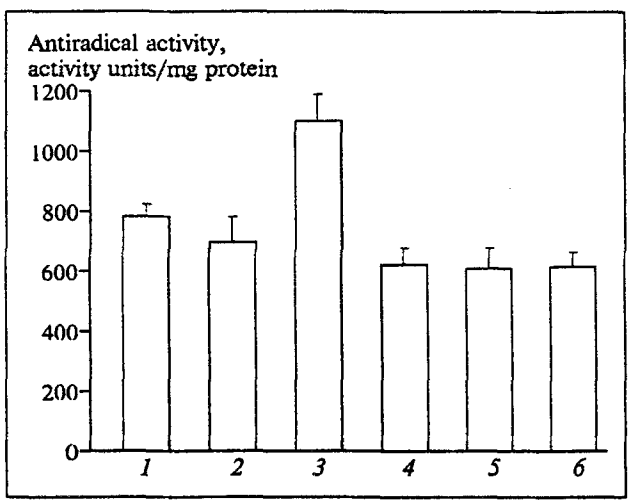

Fig. 2. Effect of chalone and antichalone antiradical activity in isolated rat liver under different perfusion conditions.

homogenate in comparison with the control ($p<0.05$), while antichalone considerably enhanced this process. It should be emphasized that the differences in experimental series with chalone and antichalone are significant with a probability of more 95%, i.e., inhibitors and activators of cell proliferation had opposite effects on the intensity of LPO in isolated liver.

Addition of 5 mM NH_4Cl had no effect on LPO.

Inhibitory effect of chalone on the intensity of LPO persisted in the presence of ammonium ions: the content of MDA in the homogenate was significantly decreased in comparison with both control series and series with NH_4Cl ($p<0.05$). The effect of antichalone in combination with NH_4Cl did not differ from that of NH_4Cl alone, i.e., the presence of ammonium ions masked the effect of antichalone.

Our findings suggest that the effect of chalone practically does not depend on the presence of ammonium ions, while the effect of antichalone is inverted by NH_4Cl: activation of LPO gives place to its inhibition, and the content of MDA in liver homogenate in the presence of antichalone in a combination with NH_4Cl significantly decreases in comparison with the effect of antichalone alone ($p<0.05$). It should be noted that the data of both series ("antichalone" and "antichalone+ammonium") did not differ significantly from the control.

The state of antioxidant system was evaluated by superoxide dismutase activity (SOD) in liver homogenate. Being the major type II antioxidant, SOD interrupts chain reaction of free radical propagation.

As seen from Fig. 2, SOD activity increased in the presence of chalone and decreased in the presence of antichalone in comparison with the control. The differences between these series were significant ($p<0.05$).

Addition of NH_4Cl (5 mM) to the perfusate had no significant effect of antiradical activity of liver homogenate: however, a tendency toward a decrease in comparison with the control was noted. Chalone and antichalone potentiated inhibiting effect of NH_4Cl on SOD, the effect of chalone was significant ($p<0.05$).

As seen from Fig. 2, the effect of chalone was practically independent on the presence of ammonium in the perfusate, while stimulating effect of antichalone on SOD disappeared in the presence of NH_4Cl ($p<0.05$).

According to the concept of three-component tissue structure [5,6], all tissues of an organism consist of proliferative, differentiating, and fixed cell pools. It can be assumed that biochemical effects of chalone and antichalone are characteristic of proliferating or specialized cells.

The observed resistance of the liver to antichalone in the presence of NH_4Cl is probably associated with the necessity to mobilize functional reserves under adverse cultural conditions. Antichalone shifts the balance between cell pools toward proliferating cells which are evidently unable to execute specific functions of hepatocytes [8]. It can be hypothesized that in the evolution of Metazoa a priority system has been developed: specific functions of the organ or tissue as the component of the integral system (organism) predominate over their responses to local regulatory stimuli. This conclusion is confirmed by experimental data on inhibition of regeneration in the liver after subtotal (to 90%) resection [8] and original theoretical study of T. V. Antipova and A. I. Yakovleva [1], who estimated the

number of hepatocyes (10%) critical for the maintenance of vital functions of the organism.

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