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USE OF CHEMILUMINESCENCE ANALYSIS TO DETERMINE ACTIVITY OF HEPATIC ANTICHALONE AND CHALONE

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Chalones — tissue-specific inhibitors of DNA synthesis and mitosis — are found in all tissues so far studied [1, 5]. Many investigators have found not only chalones, but also tissue-specific stimulators of DNA synthesis and mitosis, often called antichalones [1, 10, 12]. It has been suggested that these stimulators and inhibitors act together [2]. Methods of obtaining and purifying chalones and antichalones have been developed and the class of chemical compounds to which they belong has been established (glycoproteins [7]). However, the method of determining activity or quantity of antichalone and chalone in an isolated preparation still remains the most difficult problem. Meanwhile, no method of determination of antichalone and chalone activity per unit mass of an organ has yet been developed. We know that DNA synthesis is coupled with the state of many factors. For example, if the level of cell proliferation falls the intensity of free-radical reactions increases, and the quantity of natural inhibitors is reduced. Opposite changes take place in cases of stimulation of cell proliferation and, in particular, after partial hepatectomy [8]. Thus the state of proliferation, including under conditions when factors regulating it are acting, can be judged from changes in the parameters of free-radical reactions.

The aim of this investigation was to determine the antiradical activity of hepatic antichalone and chalone by the use of chemiluminescence analysis for this purpose.

EXPERIMENTAL METHOD

Antichalone and chalone were isolated from bovine liver and from the liver of 27 noninbred male albino rats weighing 130-150 g [13]. The writers' previous investigations showed that preparations obtained from the liver and partially purified stimulate intrahepatic DNA synthesis and cell division (antichalone) and inhibit these processes (chalone), and their optimal doses also were established [1, 4]. Quenching of chemiluminescence by antichalone and chalone was determined in a system generating free radicals (luminol — riboflavin in carbonate buffer, pH 10.0, irradiated by ultraviolet light at 365 nm) [11].

Chemiluminescence was recorded on a KhLMITs-01 chemiluminometer, with continuous flow cuvette, in which the components of the system forming free radicals circulated. No free radicals were generated without irradiation of the luminol-riboflavin system. Luminescence reached a maximum 1-2 min after irradiation. If under these circumstances antichalone or chalone was added, chemiluminescence was quenched. The degree of quenching was expressed as a percentage. Antichalones and chalones are known to be thermolabile. They were inactivated by heating their solutions for 15 min at 65°C. Partial hepatectomy (PHE) was performed on the male albino rats by the method in [9]. The operation was performed on the animals in the morning. The rats were killed by decapitation 24 h (six animals), 72 h (seven animals), and 14 days (five animals) after the operation. Intact animals (nine rats) served as the control. Antichalone and chalone were isolated from the liver by the method in [13]. The alcoholic residue was redissolved and applied to a chromatography column with Sephadex G-50. Eluates from the chromatography column were recorded in the cuvette at 280 nm. Their activity was determined in pooled fractions of antichalone

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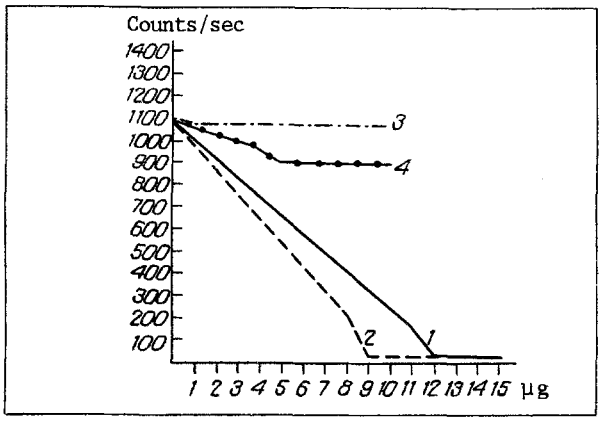

Fig. 1. Dependence of intensity of chemiluminescence (ordinate, counts per second) on quantity of hepatic antichalone and chalone (abscissa, μg) in a system generating free radicals. 1) Native antichalone, 2) native chalone, 3) inactivated antichalone, 4) inactivated chalone.

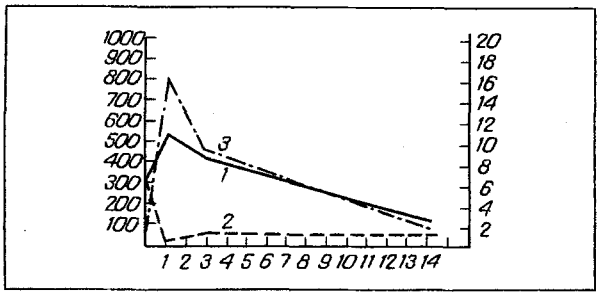

Fig. 2. Activity of antichalone and chalone in regenerating rat liver based on results of chemiluminescence analysis. Abscissa, time after PHE (in days); ordinate: on left, activity (in units SOD/g tissue), on right – ratio of activities of antichalone to chalone (in relative units). 1) Activity of antichalone, 2) activity of chalone, 3) ratio of antichalone to chalone.

and chalone respectively, without lyophilization, according to the degree of quenching of chemiluminescence in the system generating free radicals. Activity of antichalone and chalone was expressed in units of superoxide dismutase (SOD) per gram of liver, assuming that 1 unit (1 U) SOD quenches chemiluminescence by 50%.

The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The study of the effect of antichalone and chalone on chemiluminescence showed that addition of chalone and antichalone to the system reduced luminescence in proportion to the quantity of the preparation added (Fig. 1). Chemiluminescence was quenched by 30% by 3 μg of chalone ($p < 0.01$). The relationship remained linear for chalone until addition of 8 μg per sample. For antichalone the linear relationship was preserved until addition of 10 μg per sample. A further increase in the amount of preparation in the cuvette completely quenched chemiluminescence and the effect could not be assessed quantitatively. Thus native antichalone and chalone isolated from the liver extinguish chemiluminescence in a system generating free radicals.

The results obtained in a study of hepatic antichalone and chalone, after heating, indicate that irrespective of the amount of the preparation added to the system generating free radicals, they lost their ability to quench chemiluminescence ($p < 0.01$). This property correlates with data showing that antichalones and chalones are thermolabile compounds. After heating they lose their ability to regulate cell multiplication [1, 7].

The property of antichalone and chalone established in these experiments was used to study the process of regeneration of the liver after PHE. The results of experiments with reparative regeneration of the liver (Fig. 2) indicate that during the first day after PHE, when intensive DNA synthesis takes place [6], which is followed by increased mitotic activity, activity of antichalone in the liver also increases (by 1.87 times, $p < 0.01$). Chalone activity at this time was considerably (sixfold, $p < 0.01$) depressed. On the 3rd day after PHE a tendency was noted for antichalone activity to fall by 20% ($p > 0.05$) compared with the 1st day, and chalone activity began to rise (Fig. 2, $p > 0.05$). At this same time evidence of repair of the liver structure begins to appear [3]. By the 14th day antichalone activity was 47% lower than normally ($p < 0.05$) and chalone activity was 61% lower ($p < 0.05$). Thus, first, partially purified antichalone and chalone possess antiradical activity in a system generating free radicals, and second, the dynamics of antiradical activity of antichalone and chalone coincides with the dynamics of cell proliferation after PHE.

It was next decided to analyze changes in the ratio of antichalone activity to chalone activity as a parameter reflecting changes in this system, in the direction of one or another factor. It will be clear from Fig. 2 that after 1 and 3 days of regeneration of the liver there was a shift in the value of this parameter toward antichalone. After 14 days of the experiment the antichalone/chalone ratio was virtually back to normal. This indicates that 2 weeks after removal of the greater part of the liver, regeneration of the organ takes place against the background of normal quantitative relations between the stimulator and inhibitor of proliferation, whereas before this time the process of regeneration of the liver has taken place with the distinct predominance of stimulation of proliferation.

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