CHALONE-ANTICHALONE SYSTEM OF THE LIVER IN VARIOUS ANIMALS AND ITS STATE IN RELATION TO PHYSIOLOGICAL RHYTHMS IN AMPHIBIANS

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Compounds which take part in events concerned with the regulation of cell growth are an inseparable part of eukaryotes. However, it is only with the appearance of multicellular highly differentiated organisms that tissue-specific regulators of cell proliferation, namely chalones and antichalones, have appeared. Antichalones and, in particular, chalones have now been found in many mammalian tissues [1, 6, 7]. Chalones and antichalones are known to be polyfunctional compounds. They take part in the regulation of metabolism, of cell division and differentiation, and the formation of the tissue level of organization, and they also possess antioxidative properties [5]. The chalone system is the oldest system of regulation [4]. However, the phylogenesis of the chalone–antichalone system has not been investigated. We know that the state of the chalone–antichalone system is influenced by the functional state of the organ [2].

We accordingly have studied the biological activity of chalone and antichalone, and also its ratio in the liver of representatives of all classes of vertebrates, and in one representative invertebrate, *Anadonta cygnea.* We also studied the same parameters in the liver of the frog (*Rana temporaria*) in relation to the functional state of the organ, which is linked with a change of body temperature.

EXPERIMENTAL METHOD

Preparations of chalone and antichalone were obtained from the animals' liver by Verli's method [8].

The following animals were used in the experiments: *Anodonta* (five), roach (four), carp-bream (five), frog (15), grass snake (five), pigeon (five), and rat (five). Activity of the chalone and antichalone preparations was determined from the degree of quenching of chemiluminescence on a KhLM 1Ts-01 chemiluminometer. The results were expressed in units of superoxide dismutase (SOD) per gram of liver. The quantity of the protein component in antichalone and chalone preparations was recorded in a continuous flow cuvette at 280 nm, in the course of elution from the chromatographic column. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Both chalone and antichalone were present always in the liver of all the animals studied in the spring and summer seasons (Fig. 1). Activity of the chalone and antichalone preparations in different animals varied from 20 to 280 U SOD/g liver. Lowest activity of chalone (12.8 ± 0.20) and antichalone (22.8 ± 2.96) were observed in the liver of *Anodonta.* The highest activity of chalone (193.7 ± 7.0) and antichalone (280.4 ± 2.51) was identified in the rat liver. Quite high activity of chalone and antichalone was present in the liver of all vertebrates. A difference was found in members of the Pisces class. For instance, chalone activity was greater than antichalone activity ($p < 0.05$) in the roach liver. In the carp-bream, however, antichalone activity was greater ($p < 0.05$). This may be a species-dependent difference: it may perhaps be connected with the fact that roach and carp-bream used in the experiments were of the same calendar age (4-6 years) but

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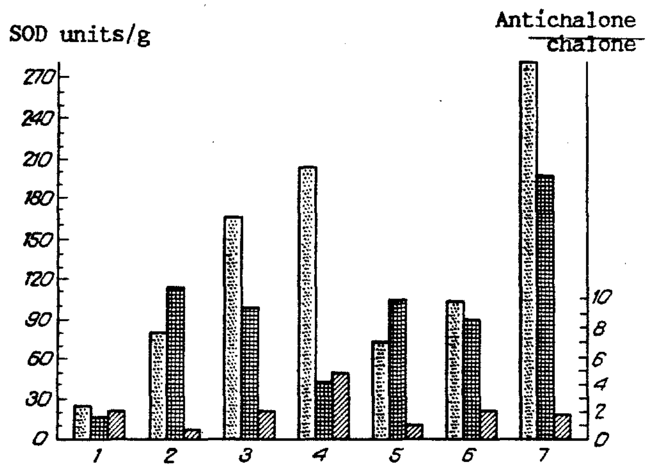

Fig. 1. Antichalone and chalone activity in animals' liver. 1) *Anodonta*, 2) roach, 3) carp-bream, 4) frog, 5) grass snake, 6) pigeon, 7) rat. Dotted columns — antichalone, cross-hatched columns — chalone. Obliquely shaded column — antichalone/chalone ratio.

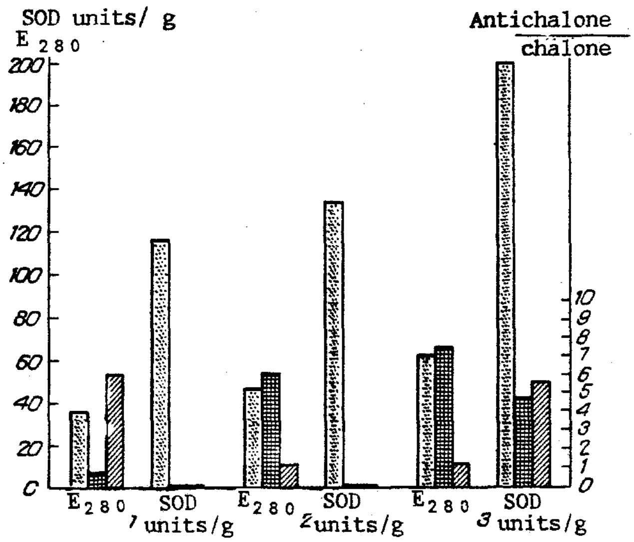

Fig. 2. Antichalone and chalone activity in frog liver: 1) anabiosis, 2) 5 days at 25°C, 3) spring animals. Dotted column — antichalone, cross-hatched column — chalone, obliquely shaded column — antichalone/chalone ratio.

of different biological age. At this age the roach has reached its definitive size and has ceased to grow. The carp bream at this age has yet attained its largest size, and both its organs and its body as a whole continue to grow, possibly in connection with the level of antichalone activity in the liver.

Not only the presence of chalone and antichalone in the liver, but also the ratio between them, is an important parameter, for the proliferation of the cells and, correspondingly, growth of the organ depend on predominance of activity of one or other component.

Values of the ratio of antichalone to chalone activity in the animals' liver are given in Fig. 1. It follows from these results that although activity of antichalone and chalone in the liver of different animals varies within wide limits, the antichalone/chalone ratio is about equal in most of them and does not exceed 2.0. The exception is the frog liver, in which the ratio of antichalone to chalone activity is higher, namely 4.86 ± 0.3. In this experiment the preparations of chalone and antichalone were isolated from the liver of spring frogs, which were in the most active state. It was thus necessary to study biological activity of the chalone and antichalone preparations and the ratio between them under conditions of hibernation (a state close to anabiosis), which is accompanied by a considerable fall of body temperature and of the intensity of metabolism, and also during awakening from hibernation, when the frogs were kept at a temperature of 25°C for 5 days. In this particular experiment we determined not only activity of the antichalone and chalone preparations, but also the light absorbance of their eluates from chromatography columns at 280 nm, which reflects the content of the polypeptide in antichalone and chalone preparations.

It will be clear from Fig. 2 that in a state of anabiosis the content of the polypeptide component in the antichalone preparation was lower than in animals of the other two groups. In waking frogs, kept for 5 days at 25°C, its content increased and reached the peak value in spring frogs. Antichalone activity also was lowest (120 SOD units/g) in frogs in a state of anabiosis. During awakening, besides synthesis of the polypeptide component of the antichalone, its activity also increased.

In the chalone preparation from the liver of frogs in a state of anabiosis the polypeptide component was present in trace amounts, and in some individuals it could not be detected at all. During the 5 days of awakening, its synthesis intensified rapidly and reached a value close to that observed in spring (65.0 ± 8.8). However, despite the formation of a large quantity of polypeptide in the fraction corresponding to the chalone preparation, it was still inactive. Only in spring frogs did the chalone preparation acquire biological activity. Because of the absence of chalone activity in the preparation from the liver of animals in a state of anabiosis and in the period of awakening, it was impossible to determine the ratio of antichalone to chalone activity. The absence of chalone activity in the liver of frogs kept in a state of hibernation is evidently connected with its biochemical adaptation, for the metabolism of ectothermal animals is subject to considerable seasonal changes. Before hibernation, with lowering of the body temperature synthesis of the chalone is probably inhibited to a greater degree than synthesis of the antichalone. We know that before hibernation the intensity of lipid peroxidation and concentrations of cholesterol and phospholipids in frogs undergo considerable changes [3]. Evidently loss of chalone activity by the hepatocytes and the comparatively low antichalone activity under conditions of inhibition of physiological processes are advantageous for the animal. Cellular proliferation and synthetic processes under these circumstances may be blocked by a lowered external environmental temperature, and the need for synthesis of specific inhibitors of proliferation is no longer present.

Maintenance of the stimulator of proliferation at the optimal level is evidently essential, for reserves of it are necessary, for use during awakening from hibernation for urgent stimulation of metabolism and proliferation.

The investigations thus demonstrated the existence of antichalone and chalone activity and established the ratio between them in the liver of invertebrates and vertebrates, and also shed light on functioning of the chalone-antichalone system of the liver in relation to the rhythm of physiological activity of frogs.

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