MICROSPECTROPHOTOMETRIC STUDY OF CHANGES IN HEPATOCYTE NUCLEAR DNA CONTENT IN DOGS WITH CHRONIC SOFT TISSUE CRUSH SYNDROME

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KEY WORDS: hepatocyte; DNA; microspectrophotometry; decompression period.

In the chronic soft tissue crush syndrome metabolic disturbances affecting nucleic acid metabolism in the cell nuclei develop in the organs and, in particular, in the liver [4-7]. Immediately after trauma the RNA content in the hepatocytes is increased [4, 7]. Meanwhile processes determining DNA metabolism in the liver are less subject to the influence of trauma [5, 7, 8]. The combination of changes in the genetic material of the cells during traumatic processes has not yet been adequately studied. There are no data in the literature on microspectrophotometric analysis of the DNA content in mononuclear and binuclear hepatocytes of dogs with an experimental chronic crush syndrome of average severity. Such information could be used for a differential metabolic protection of the organ in this form of pathology specifying times and doses.

EXPERIMENTAL METHOD

Altogether 27 mature male mongrel dogs weighing 18-20 kg were used. A chronic soft tissue crush syndrome (CCS) of average severity was produced by the method adopted at the Laboratory of Pathophysiology, S. M. Kirov Military Medical Academy [6, 10, 11]. At each time of the experiment and for the comparison group (normal animals) three dogs were used. After compression of the soft tissues of the left thigh for 5 h the animals were killed 1, 3, 6, 12, 24, 48, 72, and 168 h after decompression.

Since the main aim of the investigation was the comparison, i.e., relative evaluation of the dynamics of changes in the DNA content in a single nucleus, the method of microspectrophotometry of histological sections was used. Pieces of liver from the right medial lobe were fixed in Carnoy's mixture and embedded in paraffin wax. Sections up to 5 μm thick were hydrolyzed in 1 N hydrochloric acid at 60°C for 15 min and then stained by the Feulgen reaction. The sections were dehydrated, cleared, and mounted in Canada balsam.

The DNA content in the hepatocyte nuclei was studied on a modified scanning integrating digital microspectrophotometer [1] with 10×10 μm^2 frame, and with a probe 0.5 μm in diameter giving a 20-line scan in a monochromatic beam with wavelength 560 nm, and with a linear stepwise scanning time of the whole frame of 1 sec. The optical magnification was 600. Logarithms were taken of the optical density values of the test substance along the line of scan and all data were integrated and expressed in conventional units on the display. In the normal group and at each time of the experiment 100 arbitrarily chosen nuclei of binuclear hepatocytes and 100 nuclei of mononuclear hepatocytes were investigated microspectrophotometrically from each animal, close to the central veins. In preparations of normal liver of the dogs the DNA content was determined by the same method in the nuclei of 30 small lymphocytes and the averaged results of the measurements were taken as the standard for a diploid cell. To judge the total quantity of genetic material in the hepatocyte nuclei, a general index of the kinetics of the DNA content was used: the "index of DNA accumulation" [1] in conventional units, namely the arithmetic mean weighted DNA content per nucleus. The numerical results were subjected to statistical analysis [1, 3]. The results were compared by Student's t test [12].

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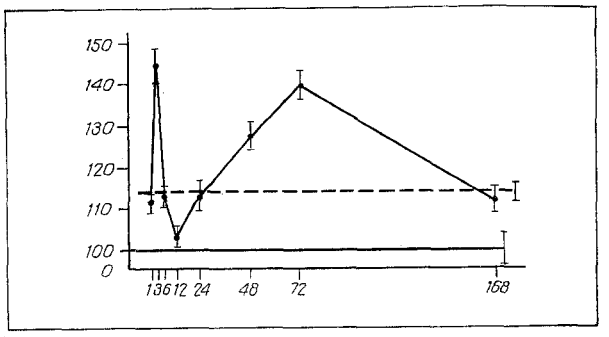

Fig. 1

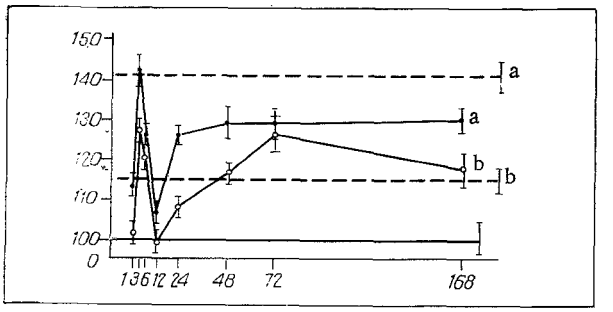

Fig. 2

Fig. 1. DNA content in nuclei of mononuclear hepatocytes of dogs at different times after decompression. Abscissa, time after decompression (in h); ordinate, DNA content per nucleus (in % of that in nuclei of small lymphocytes, shown as a straight line). Broken line gives DNA content in normal nuclei. Short vertical lines represent error of means.

Fig. 2. DNA content in nuclei of binuclear hepatocytes of dogs at different times after decompression. a) First nucleus; b) second nucleus. Remainder of legend as to Fig. 1.

EXPERIMENTAL RESULTS

Analysis of the time course of the DNA content in mononuclear hepatocytes of the dogs during the experiment revealed a definite rhythm characterized by a bimodal curve (Fig. 1). The first peak of rise of the DNA accumulation index was observed 3 h, the second 72 h after decompression. The increase in the index was due to an increase in the number of DNA-synthesizing cells. The sharp drop in this index at the 12th hour of the decompression period was evidently due to exhaustion of the intracellular reserve metabolic mechanisms, and this was followed successively (24, 48, and 72 h after decompression) by a rise coupled with the process of intracellular regeneration, and later by cyclic changes in the systems during the period of extinction of compensation. At nearly all stages of the experiment, except 12 h after decompression, the DNA accumulation index in mononuclear hepatocytes was higher than in nuclei of small lymphocytes. At this time of the experiment the nuclei became predominantly diploid as a result of utilization of their genetic material.

As regards binuclear hepatocytes, the DNA accumulation index of one of the nuclei under normal conditions was 22.6% higher than for the second nucleus (Fig. 2). The index of the first nucleus at all times of the experiment except 3 h after decompression was below normal, but higher than in nuclei of small lymphocytes. The curve of DNA content in this nucleus also was characterized by a bimodal curve: A sharp drop was observed 1 and 12 h, and a sharp rise 3 and 48 h after decompression. The DNA accumulation index 72 and 168 h after decompression was unchanged compared with the previous period of the experiment. Whereas the DNA content in the nuclei of mononuclear hepatocytes toward the end of the period of observation was close to normal, it did not reach normal in the corresponding cell population of binuclear hepatocytes.

The time course of the DNA content in the second nucleus of binuclear hepatocytes also revealed a definite rhythm, expressed by a bimodal curve (Fig. 2). The peak of rise of the DNA accumulation index in the second nucleus corresponded in its time interval to that observed in nuclei of mononuclear hepatocytes, but it was not as high. A sharp drop of the curve was found at the 1st and 2nd hour of the decompression period, just as in the first nucleus of binuclear hepatocytes. At subsequent stages of the experiment (24, 48, and 72 h after decompression), due to intercellular regeneration, a rise in the mean DNA content per nucleus was observed. Toward the end of the experiment this parameter came close to normal. At all periods of the experiment, except 1 and 12 h after decompression, the DNA accumulation index in this particular nuclear population was higher than in nuclei of small lymphocytes.

The rhythm of the change in DNA content observed in both mononuclear and binuclear hepatocytes is in agreement with the law of biological structural and functional discreteness in

time, which embodies two other laws — that of structural-temporal "quantization" of biological processes and that of intermittent activity of functioning structures [2, 9].

LITERATURE CITED

1. G. G. Avtandilov, Morphometry in Pathology [in Russian], Moscow (1973).
2. G. G. Avtandilov, Arkh. Patol., No. 4, 3 (1975).
3. G. G. Avtandilov, Introduction to Quantitative Pathological Morphology [in Russian], Moscow (1980).
4. A. P. Gordeeva, in: Mine Rescue Medicine (Proceedings of the 1st All-Union Conference) [in Russian], Donetsk (1966), p. 90.
5. V. M. Gukasov and L. F. Frantsev, in: Tashkent Postgraduate Medical Institute. Scientific and Practical Conference to Celebrate the 20th Anniversary of the Department of Pathological Anatomy. Proceedings [in Russian], Tashkent (1970), p. 26.
6. I. V. Diasamidze, "Structure and function of certain organs in the chronic crush syndrome," Candidate's Dissertation, Tbilisi (1973).
7. A. A. Zor'kin, in: Shock and Collapse [in Russian], Kishinev (1970), p. 76.
8. A. A. Zor'kin, in: Pathological Physiology of Some Extremal States [in Russian], Kishinev (1980), p. 3.
9. G. N. Kryzhanovskii, in: Biological Rhythms in Mechanisms of Compensation of Disturbed Functions [in Russian], Moscow (1973), p. 20.
10. M. I. Kuzin, Clinical Features, Pathogenesis, and Treatment of the Chronic Crush (Traumatic Toxicosis) Syndrome [in Russian], Moscow (1959).
11. L. M. Nebol'sina, Soobshch. Akad. Nauk Gruz. SSR, 78, No. 3, 717 (1975).
12. I. A. Oivin, Patol. Fiziol., No. 4, 76 (1960).

ACTION OF MICROWAVES ON BIOLOGICALLY ACTIVE POINTS IN RABBITS WITH ACUTE EMOTIONAL STRESS

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KEY WORDS: microwaves; acupuncture; biologically active points; emotional stress.

Acupuncture is an effective method of protecting an organism against stress [2, 3, 5, 10]. Even in terminal states acupuncture can be used for resuscitation [3]. Generalized irradiation of experimental animals by a modulated electromagnetic field also protects them against acute emotional stress [8, 9]. It has been suggested that when electromagnetic waves act on the living organism, biologically active points also are excited [4, 7, 11]. It is also known that exposure of the body surface to microwaves leads to normalization of physiological functions [6]. However, the question of how effective is the action of microwaves on biologically active points during acute emotional sttess has not yet been settled. The present investigation is devoted to a study of this problem.

EXPERIMENTAL METHOD

Experiments were carried out on 25 male chinchilla rabbits weighing 1.5-2.5 kg. Bipolar nichrome electrodes were implanted into the ventromedial nuclei of the hypothalamus of the animals and their electrical stimulation evoked a passive defensive response. Steel electrodes, inserted subcutaneously into one of the animal's hind limbs were used for electrodermal stimulation. Emotional stress was induced in the immobilized rabbits by aperiodic stimulation of negative emotiogenic centers of the hypothalamus and electrodermal stimula-

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