SYMPATHETIC NEUROCYTE TRANSCRIPTION AND SURVIVAL PARAMETERS IN FRACTIONALLY AND PARTIALLY CHEMICALLY DESYMPATHIZED ACEPHEN-TREATED RATS

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The mechanisms of restructuring of protein biosynthesis in nerve cells and, in particular, the activity of their genetic apparatus, leading to changes in the volume and spectrum of proteins synthesized, in the presence of fluctuations of their functional loads and during aging still remain inadequately studied [1, 4]. Research has recently been published which indicates that the psychoenergizer acephen* (centrophenoxine, Lucidryl) [2, 9] exerts its effect on the life span of mammals [6] through nerve cells, delaying their age-induced changes, reducing the rate of lipofuscin accumulation [8, 10], improving acetylcholine metabolism [11], and increasing mRNA synthesis [12].

The aim of this investigation was an autoradiographic analysis of transcription in nuclei of sympathetic nerve cells of aging, normally developed rats and fractionally and partially chemically desympathized rats, treated with acephen, and also to study correlation between the transcription parameters and kinetics of mortality of the animals.

EXPERIMENTAL METHOD

Experiments were carried out on 225 noninbred albino rats aged 22 months, divided into seven groups: 1 and 2 (control) — normally developed rats, receiving and not receiving acephen respectively, 3-6) rats with a varied degree of desympathization, 7) rats with the maximal degree of desympathization for these experiments, and receiving acephen (control for the group of rats desympathized in the course of 14 days). Desympathization was produced chemically by means of guanethidine (isobarin, from Pliva, Yugoslavia), which was injected in sterile physiological saline in a dose of 15 mg/kg into newborn rats a few hours after birth, and thereafter daily for 3, 5, 10, and 14 days; this agent causes death of 26.7% of neurocytes in the cranial cervical sympathetic ganglion of the rat when given for 3 days, 60.2% if given for 10 days, and 75.2% if given for 14 days, whereas the rate of somatic development of the animals, tested by measuring the time course of body weight, remains comparable [4]. Animals of the control group were given an injection of physiological saline. Acephen was given with the drinking water in a dose of 1 mg/ml starting from 12 months. The kinetics of mortality of the rats in the groups was recorded after the same age. The maximal life span (MLS), i.e., the time of existence of the longest-living individual in each experimental group, was determined in each group. To assess the state of transcription in the neurocytes studied, autoradiography was used to detect activity of endogenous RNA-polymerases in cell nuclei of fixed sections [7]. For this purpose sections through the cranial cervical sympathetic ganglion, 8μ thick, cut on a freezing microtome at -20°C, were dried in air and fixed in a system of alcohol and acetone (1:1 by volume) for 5 min at 4°C. The sections were kept at −20°C until use. Next, 0.02 ml of incubation mixture of the following composition was applied to the sections beneath a coverslip (in μ moles/ml): Tris-HCl buffer (pH 7.9) — 100, sucrose — 150, ammonium sulfate — 80, 2-mercaptoethanol — 12, ^{3}H-uridine triphosphate (specific activity 27 Ci/mmole) — 0.02, unlabeled triphosphate (ATP, GTP, CTP) — 0.6 of each, $MgCl_2$ — 8, $MnCl_2$ — 2. After application of the mixture the preparations were incubated at 37°C in vapor of Tris-HCl buffer with the addition of 2-mercaptoethanol for 30

*Meclofenoxate

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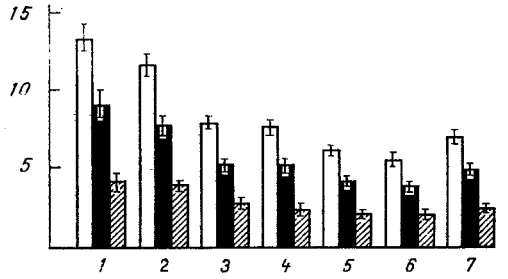

Fig. 1. Intensity of transcription in nuclei of control and fractionally (chemically) desympathized rats, receiving and not receiving acephen. Abscissa: unshaded columns — nuclear labeling, black columns — nucleolar labeling, obliquely shaded columns — nucleoplasmic labeling; ordinate, mean number of grains of reduced silver beneath nuclear structures. 1) Control + acephen; 2) control; 3-6) desympathization for 3, 5, 10, and 14 days, respectively; 7) desympathization for 14 days + acephen.

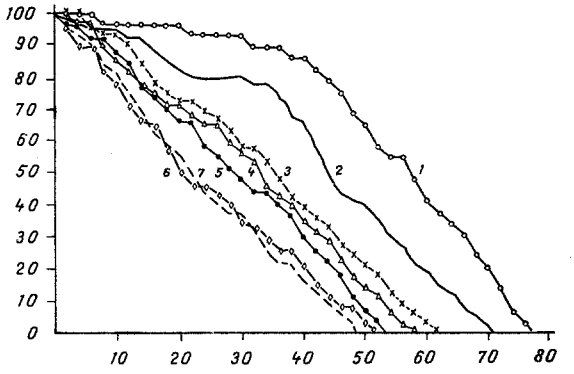

Fig. 2. Kinetics of survival of control and fractionally (chemically) desympathized rats receiving and not receiving acephen starting from the age of 12 months. Abscissa, time (in weeks) from beginning of experiment; ordinate, number of animals remaining alive (in %). 1) Control + acephen; 2) control; 3) desympathization for 3 days, 4) desympathization for 5 days; 5) desympathization for 14 days + acephen, 6) desympathization for 10 days, 7) desympathization for 14 days.

min. The reaction was stopped by washing the preparations thoroughly in distilled water, after which the sections were postfixed for 30 min in a mixture of ethanol and acetic acid (3:1 by volume). Unincorporated triphosphates were removed by means of 5% TCA (15 min at 4°C), after which the sections were washed (60 min) in running water and then rinsed in distilled water. The sections were dried, coated with type M photographic emulsion, and exposed for 14 days. The intensity of incorporation of the precursor was assessed by counting visually the number of grains of reduced silver above the nucleoplasm and nucleoli separately. Some of these values characterized the level of nuclear labeling. Altogether 150 neurocytes from each group of animals were analyzed. The level of template activity was characterized by mean values. The background value was determined separately for each case by counting the number of grains of reduced silver above the area free from the preparation, which was about equal to the average area of section of the nucleus. According to data in the literature [3], the number of grains of reduced silver detected in the nucleus by this method is proportional to the number of transcription termination points, so that it is possible to estimate the number of DNA regions counted at the moment of fixation, and also to some degree, the relative value of that part of the genome from which the information is read.

To determine the significance of differences between the average parameters, Student's or Wilcoxon's tests were used, depending on the significance of asymmetry and excess of the distribution of labeling intensity in each case, separately.

EXPERIMENTAL RESULTS

The writers showed previously that the natural process of aging of sympathetic nerve cells in rats is accompanied by a regular decline in the template activity of their nuclear chromatin [3]. Comparison of the template activity of the test nerve cells from intact and desympathized rats at the age of 22 months, tested with respect to autoradiographically detectable activity of endogeneous RNA-polymerases, showed that desympathization leads to a reduction of both nucleolar and nucleoplasmic labeling; moreover, the degree of reduction correlates with the degree of desympathization (Fig. 1). Injection of guanethidine for 3, 5, 10, and 14 days caused, in particular, a reduction of nucleolar labeling by 34.1, 31.5, 45.5, and 51.4% respectively. The reduction of template activity of the nucleoplasmic sites of DNA amounted to 29.2, 35.9, 46.7, and 49.7%, respectively.

Administration of acephen both to control rats and to rats desympathized for 14 days increased the intensity of labeling of the nucleoli of the neurocytes compared with the control by 18.8 and 25.1%, respectively. The increase in transcription of nucleoplasmic sites under the influence of acephen was not significant in either case.

The kinetics of mortality of the different groups of animals between 12 and 30 months is shown in Fig. 2.

MLS was then calculated. Compared with the control rats and rats desympathized for 14 days and not receiving acephen, MLS in analogous groups of animals receiving the geroprotector increased by 10.9 and 10.0%. With an increase in the degree of desympathization, MLS was reduced compared with the control by 12.6, 17.8, 27.3, and 31.2% respectively. The greatest MLS was observed in the animals receiving acephen.

The following conclusions can be drawn from these results. First, acceleration of aging of sympathetic neurocytes in desympathized animals is accompanied by a decrease in MLS of the animals. In both cases a definite positive dose dependence was observed. Second, the geroprotector acephen delays some manifestations of aging of sympathetic nerve cells while at the same time increasing MLS of the rats.

The trend of the age-induced changes in the sympathetic nerve cells of the desympathized animals may reflect overstraining of the compensatory mechanisms of the neurocytes, the earlier using up of their functional potential, in accordance with the views of those pathologists who have described the transition of stable and lasting compensation on the basis of cellular hypertrophy into decompensation [4]. According to our own data, this outcome is observed after a prolonged increase in the load on the population, the number of cells in which may be reduced by half or more.

Thus definite correlation is observed between changes in the state of the genome of the long-living cells and the life span of the individual animal.

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