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CHANGES IN PHYSICO-CHEMICAL PROPERTIES OF CHROMATIN IN WHITE RAT BRAIN AT UNILATERAL GANGLIOSYMPATHECTOMY

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Discovery of molecular mechanisms of adaptational trophic effect of CNS, especially of SNS, on brain, particularly on genetic apparatus of nerve cells, is of biological and neurochemical importance.

Based on the data obtained at unilateral gangliosympathectomy of ganglion (removal of the upper cervical sympathetic ganglion) for studying the qualitative and quantitative changes of the content of nucleic acids, histone and non-histone proteins of brain cells genetic apparatus we have investigated the changes in physico-chemical properties, such as fluorescent characteristics (proper fluorescence, quenching of fluorescence) and infrared spectra of chromatin.

Material and Methods

The experiments were carried out on mongrel male and female white rats weighing 180-200 g with general feeding ration. The right upper sympathetic ganglion was removed under slight ether anesthesia with K.Bernard-Horner syndrome statement on the ectomized hemisphere. The animals were decapitated 7 days later (it is a period of more accomplished changes of chromatin) under slight ether anesthesia. The nuclei of brain tissue were obtained by Dingman and Sporn method [5], of chromatin by Shaw and Huang method [12], protein concentration was determined by Lowry et al. method [8] as well as spectrophotometrically [7]. Chromatin fluorescence was studied in 0.001 M Tris-HCl buffer, pH 8.0 in concentrations 25 and 50 *μg/ml* in right-angle quartz cuvettes 1×1 *cm* at room temperature on fluorescent spectrophotometer MP-2A (Hitachi, Japan) at maximal sensitivity of the apparatus, and the lenght of monochromator holes was 6-10 *mμ*.

Determination of quantum yield of fluorescence was realized using the relative method [9], the registration of shifts of fluorescence maximums was made by two-waves method [14]. The fluorescence of tryptophanils in chromatin proteins was studied by excitation at wave length 292 *nm*, chromatin complexes formation was studied by fluorescence of cation stain acridine-orange (AO) adsorbed on it; the quenching of fluorescence of tryptophanils of chromatin proteins by use of $CaCl_2$ solvents in final concentration 3×10^{-2} M; chromatin fluorenscence dissociated in 2 M NaCl prepared in 0.01 M Tris-HCl buffer (pH 8.0), infrared spectra of disoriented films of chromatin were studied using KRS glasses on spectrophotometer Specord-75IR (Germany) by Tsuboi method [13]. The isolated chromatin had the following spectral characteristics:

$A_{230\text{-}260} = 1.21$; $A_{280\text{-}260} = 0.75$; $A_{320\text{-}260} = 0.29$.

Results and Discussion

The results obtained have demonstrated relative increase of chromatin fluorescence intensity in both hemispheres of gangliosympathectomized brain (Fig.1), accompanied by inhibition of quantum yield and some shift of excitation spectrum (287 *nm* and 288 *nm* in the right ectomized and left intact hemispheres, respectively, against 292 *nm* in control) and emission (325 *nm* and 328 *nm* in the right and left hemispheres against 330 *nm* in control) into a short wave region.

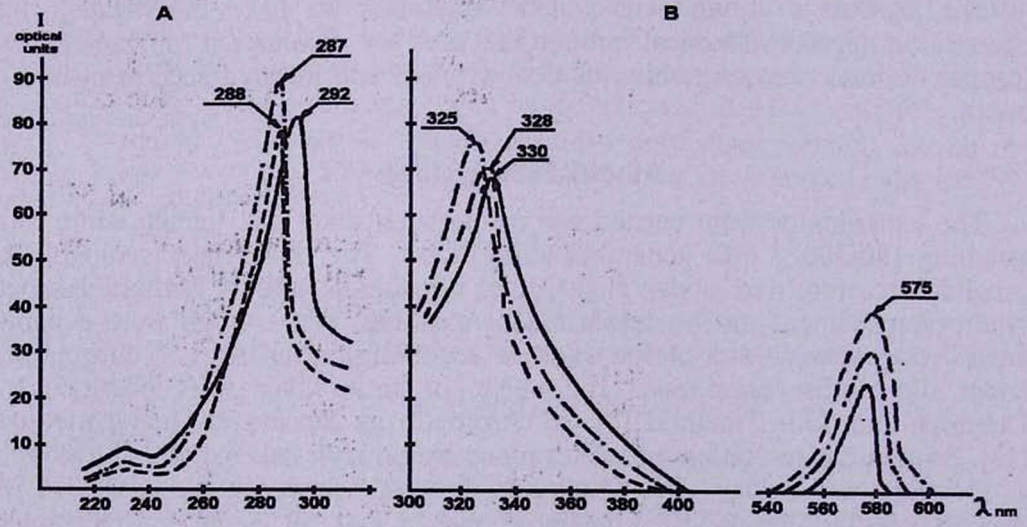

Fig. 1 The excitation (A) and emission (B) spectra of chromatin obtained from control (—), intact (----) and ectomized (-.-.-) rat brain hemispheres at EmW-330*nm* and ExW-292*nm*

Dissocation of chromatin by 2 M of NaCl has caused a relative increase of the intensity of contorol fluorescence in comparison with the chromatin of ectomized and intact hemispheres to the increase of quantum yield as well as to the shift of irritation and emission spectra to the short wave region (289 *nm* against 292 *nm*, and 325 *nm* against 330 *nm*, respectively), but without overstepping the limits characteristical to tryptophanil residues [4]. On the ectomized hemisphere we have stated a shift of chromatin emission spectrum to the long wave region (328 *nm* against 325 *nm*) which testifies to the change of microenvironment polarity around the tryptophanils of chromatine proteins [3]. It is noticeable that the existence of positive charge causes a transformation of radiation spectrum into short wave region. So, the changes in chromatin are not of a simple character, and in connection with this the fluorescence of AO cation stain adsorbed on chromatin has been studied.

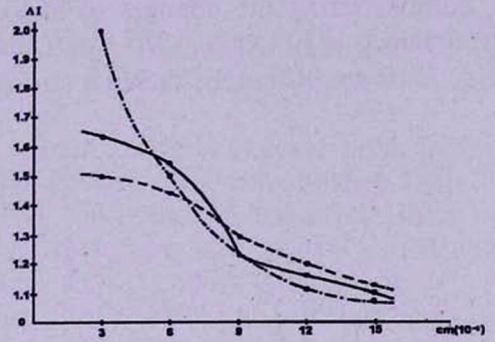

Fig. 2. The relationship between calcium chloride concentration and relative intensity of fluorescence (ΔI) of chromatin obtained from rat brain (control (—), intact (---) and ectomized (-.-.-.)) hemispheres

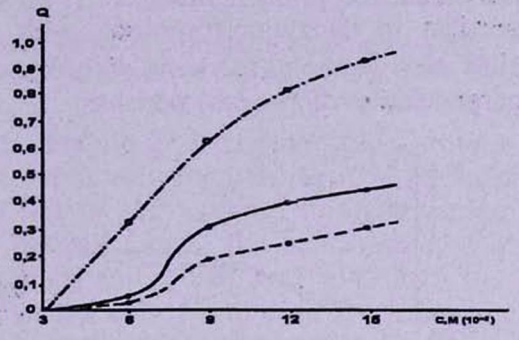

Fig. 3. The relationship between calcium chloride concentration and efficiency of fluorescence extinction of chromatin obtained from rat brain (control (—), intact (---) and ectomized (-.-.-.)) hemispheres in Stern-Folmer coordinates

For the complexes AO + chromatin there are peculiar slight short wave shifts to fluorescence spectrum, increase of intensity of stain fluorescence with more expressed manifestation in brain tissue chromatin of the intact hemisphere, as well as in both hemispheres of control animals. These data testify to the accessibility of the smaller part of DNA of the ectomized hemisphere chromatin to interrelation with stain [1]. The mentioned changes are combined with some shifts in the image of Ca^{2+} interrelations with chromatin existing in norm. Addition of $CaCl_2$ in the final concentration 3×10^{-2} M is accompanied by a sharp increase of the intensity at light-diffusion fluorescence, which is a phenomenon of diffusion of Relay and Tindal excitation light of the second order [15]. The data studied are well expressed in the chromatin of ganglioectomized hemisphere. The further addition of $CaCl_2$ is characterized by devel-

opment of the effect of fluorescence quenching. At increasing Ca^{2+} concentration (Fig.2) there take place intermittent changes of fluorescence parameters which reflect the structural state of protein chromatin from brain tissue of control animals and at some extent of the intact hemisphere of the desympathized animals.

These changes testify to the cooperativity of shifts in proteins, demonstrating rather high degree of sensibility to conformational state of protein macromolecule [3]. The same cannot be said about brain tissue chromatin of the gangliosympathectomized hemisphere, which strongly differs by its quenching effectivity in Stern-Folmer coordinates [2] from the brain chromatin of control animals and chromatin of intact hemisphere on the background of unilateral gangliosympathectomy (Fig. 3).

The changes in physico-chemical properties of chromatin are also testified by the results of infrared spectra of disoriented films of native chromatin from the ectomized brain hemisphere (Fig. 4), demonstrating the changes of bands peculiar to deoxynucleoproteids with frequencies 1710 cm^{-1}, 1225 cm^{-1} and 1084 cm^{-1}. Namely, the band with frequency 1710 cm^{-1} (demonstrating a strong perpendicular dichroism) perishes.

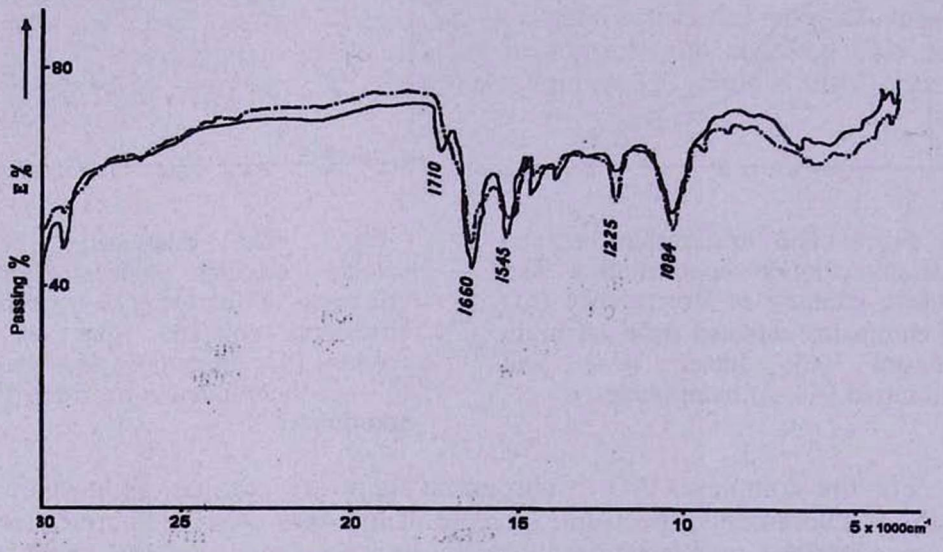

Fig. 4. The infrared spectra of disorientated films of chromatin obtained from control (—), ectomized (-.-.-.) rat brain hemispheres (5-30x10^3 cm^{-1})

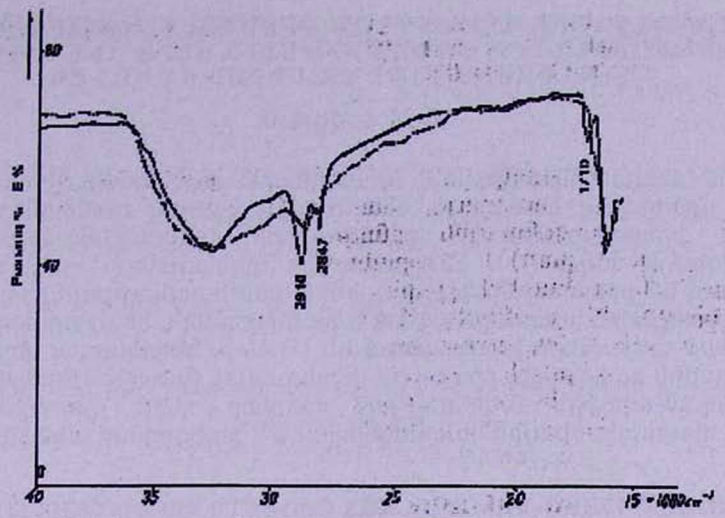

Fig. 5. The infrared spectra of disorientated films of chromatin obtained from control (—), ectomized (-.-.-.) rat brain hemispheres (15-40x10^3 cm^{-1})

Such an effect is observed at drying or treating of deoxyribonucleo-proteids (DNP) with deoxyribonuclease [13]. The bands with frequencies 1225 cm^{-1} and 1084 cm^{-1} become intensive, which testifies to the changes in hydrogenium bonds of phosphate groups [13,16]. On the other hand the bands amide-1 at 1660 cm^{-1} (valency variation of C=0 groups), and amide-2 at 1545 cm^{-1} (deformational variations of N-H groups) become less intensive, testifying to the change of DNA-protein interralation [6]. There are definite changes in the intensity of bands 2910 cm^{-1} and 2847 cm^{-1} (Fig. 5), which are like the perished bands with frequency 1710 cm^{-1}, prompting about the changes in DNA-lipid interrelations [6,10,11]. Chromatin of the brain intact hemisphere does not undergo such notable changes.

The results obtained allow to conclude that at unilateral gangliosympathectomy there are developed principally new disorders (by the degree of their expresssion) of physico-chemical properties of chromatin, DNA-protein, DNA-lipid interrelations, more seldom manifested in the ectomized hemisphere, and testifying to the desarrangement of the structural organization, e.g. of functional activity of brain chromatin.

Поступила 23.07.99

ՍՊԻՏԱԿ ԱՌՆԵՆՏՆԵՐԻ ԳԼԽՈՒՂԵՂԻ ՔՐՈՄԱՏԻՆԻ ՖԻԶԻԿԱՔԻՄԻԱԿԱՆ ՀԱՏԿՈՒԹՅՈՒՆՆԵՐԻ ՓՈՓՈԽՈՒԹՅՈՒՆՆԵՐԸ ՄԻԱԿՈՂՄԱՆԻ ԳԱՆԳԼԻՈՍԻՄՊԱԹԷԿՏՈՄԻԱՅԻ ԺԱՄԱՆԱԿ

Գ.Ա.Հովեյան

Յույց են տրված միակողմանի գանգլիոսիմպաթէկտոմիայի պայմաններում (աջ վերին սիմպաթիկ հանգույցի հեռացում) սպիտակ առնետների գլխուղեղի քրոմատինի ֆլուորեսցենտային բնութագրերի (սեփական ֆլուորեսցենցիա, ֆլուորեսցենցիայի մարում) և ինֆրակարմիր սպեկտրների փոփոխությունները, որոնք վկայում են քրոմատինի ֆիզիկաքիմիական հատկությունների, ինչպես նաև նրա կառուցվածքային կազմակերպման և ֆունկցիոնալ տեղաշարժերի մասին:

Ստացված տվյալները հաստատում են ՄՆՀ–ի, հատկապես վերին պարանոցային սիմպաթիկ հանգույցի, որպես ծայրամասային նյարդա-էնդոկրին կենտրոնի, հարմարողական-տրոֆիկ ազդեցության կարևոր դերը գլխուղեղի բջիջների քրոմատինի կառուցվածքային կազմակերպման և ֆունկցիոնալ ակտիվության մեջ:

ИЗМЕНЕНИЯ ФИЗИКО-ХИМИЧЕСКИХ СВОЙСТВ ХРОМАТИНА ГОЛОВНОГО МОЗГА БЕЛЫХ КРЫС ПРИ ОДНОСТОРОННЕЙ ГАНГЛИОСИМПАТЭКТОМИИ

Г.А.Овеян

Показаны изменения флуоресцентных характеристик (собственная флуоресценция, тушение флуоресценции), инфракрасных спектров хроматина головного мозга белых крыс при односторонней ганглиосимпатэктомии (удаление правого верхнего шейного симпатического ганглия), что свидетельствует об изменении физико-химических свойств, а также структурной организации и функциональной активности хроматина.

Полученные результаты подтверждают важную роль адаптационно-трофического действия СНС, в частности верхнего шейного симпатического ганглия, как периферического нейроэндокринного центра на субклеточные структуры клеток головного мозга.

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