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Chromatin structure and the state of human organism

Yuriy G. Shckorbatov*, Lyubov A. Zhuravlyova, Valeria V. Navrotskaya, Elena V. Miroshnichenko, Pavel Y. Montvid, Valery G. Shakhbazov, Takhir A. Sutushev

Ins l i title of Biology, Kharkov National University, Kharkov 61077, pi. Svobody 4, Ukraine

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Abstract

The state of chromatin in human buccal epithelium cell nuclei upon the influence of sport trainings was investigated. Chromatin state was evaluated in interphase buccal cell nuclei after orcein staining. The heterochromatin granule quantity (HGQ) was estimated in 30 nuclei per sample, and for every donor the mean HGQ value per 30 cells was determined. Donors of masculine sex, aged from 18 to 48 years performed training walks and samples of buccal epithelium were collected. Sportive charges induced the process of chromatin condensation in cell nuclei. After the period of repose (24—48 h) the HGQ decreased to control level therefore the process of chromatin decondensation was observed. The state of chromatin changes in connection with circadian rhythm. Chromatin became more condensed at nighttime and less condensed in the morning. Hormones such as adrenaline, noradrenaline, and hydrocortisone in vitro induced the increase of HGQ.

Keywords: Nucleus; Human cell; Buccal epithelium; Heterochromatin; Sportive training; Adrenaline; Hydrocortisone; Circadian rhythm

1. Introduction

The unique cell as a part of the whole organism represents the general state of the organism and its properties change with changes in the physiological state of the organism. The state of cell may be adequately assessed. We propose to use for these purposes the determination of the state of condensation of chromatin in interphase cell nucleus.

The problem of adequate assessment of training charge influence on human organism is very acute in sport medicine. This problem has also application in different spheres of human activity connected to work in extreme conditions. The cell and cell nucleus integrate different signals from the organism level and thereafter we supposed that investigation of cell reactions could give important information about the state of whole organism in stress conditions. In this connection we investigated the state of chromatin in interphase buccal epithelium nuclei after training loads of different intensity.

The main nuclear component, chromatin, may be divided into two parts: euchromatin and heterochromatin. Euchromatin is the decondensed, functionally active chromatin and heterochromatin being condensed, functionally inactive part of chromatin that is stained in different way (heteropycnotic) as compared with euchromatin. The increase of the facultative heterochromatin quantity in cell nucleus may be an evidence of decrease of nuclear functional activity (Therman and Susman, 1993).

We investigated the hetrochromatin granule quantity (HGQ) in buccal epithelium interphase cell nuclei. The

Abbreviations: HGQ; heterochromatin granule quantity.

* Corresponding author.

E-mail address: yury.g.shckorbatov(« univer.kharkov.ua (Y.G. Shckorbatov).

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HGQ changes reflect the changes in the state of chromatin condensation. Previously the changes of the HGQ under the influence of in vitro treatment of cells with stress hormones adrenaline, noradrenaline, and hydrocortisone were demonstrated (Shckorbatov et al., 1999a). We supposed that the analogous changes may occur in vivo in human cells after performing the sportive exercises. The purpose of this work was to investigate changes in the state of chromatin in human buccal epithelium cells in the course and after training walks.

2. Materials and methods

2.1. Cells

In our experiments cells of human buccal epithelium of male donors of different age were investigated. Cells were obtained from the inner surface of donor's cheek by light scraping with sterile spatula. This operation is absolutely bloodless and painless. The goodwill donors of cells were students and lecturers of the Kharkov Institute of Air Forces of Ukraine. Cell were obtained from donors in field conditions and analyzed in laboratory.

2.2. Training charges

The training walks lasted for 8—10 h. The mean speed during every walk was approximately 5—6 km/h. In Tables 1—4 we present the results obtained in four training walks. As a rule while performing training walks in every stage of the walk the energy waste for one person was approximately 1 MJ. Only in the training walk No. 2 at the first stage of training the energy waste per person was 2 MJ. The method of evaluation of the energy waste was described by Ivaschenko and Strapko (1988).

2.3. Hormone treatment

Cells were treated with solutions of hormones of different concentrations, prepared in the buffer solution as described above. Adrenaline hydrochloride was used in concentrations of 0.25, 0.5, 1.0, 10.0, 50.0 ug/1, that equals to molarity 1.19, 2.28, 4.55, 45.5, 228 nM. Noradrenaline (noradrenaline, arterenol) bitartrate salt was applied in the same molar concentrations. Hydrocortisone (Cortisol, hydroxycorticosterone) was applied in concentrations 1,10, 50, 100, 150 ug/1, that equals to 2.76, 27.6, 138, 276, 414 nM. Treatment of cells in different experiments lasted from 5 to 120 min.

Table 1
The HGQ changes in the course of the training walk No. 1

Variant of experiment	Donors (age)			
	A (26)	B(27)	C (29)	D(30)
Control	13.0 ± 0.5	14.9 ± 0.4	12.7 ± 0.4	11.7 ± 0.6
After training	18.5 ± 0.3*	21.5 ± 0.6*	21.6 ± 0.8*	19.5 ± 0.7*
Repose. 45 min	20.5 ± 0.7*	18.5 ± 0.3*	23.8 ± 0.6*	23.2 ± 0.7*

*Difference from control $P < 0.05$.

2.4. Evaluation of the state of chromatin

Heterochromatin granule quantity (HGQ) was estimated in cell nuclei after staining of cells with orcein. The method of HGQ determination was described by Shckorbatov (1999). In brief, staining of cells with 2% orcein solution in 45% acetic acid was applied. HGQ in interphase nuclei was estimated at magnification X700. HGQ was determined in 30 nuclei and the mean HGQ value was calculated. Standard error in every experiment not exceeded 5% of the measured value. The quantity of cells analyzed in each experiment (30) seems to be near optimal, because further increase of analyzed cells does not significantly decrease standard error. In Tables 1—8 the mean values of HGQ ± standard error are presented.

3. Results and discussion

The results of the first experiment are presented in Table 1. The training charges induce the significant increase in HGQ in cells of all four tested donors. After the short period of repose (45 min) the HGQ in the cells of some donors increases (cells of donors A, C, and D) or decreases but remains on the elevated level as compare to control (cells of donor B).

In experiments No. 2—4 (Tables 2—4), we investigated HGQ changes in different stages of training. The data presented in Table 2 show that after the first stage of training HGQ significantly increases in cells of two donors (E and F). In cells of donor G after the first stage of training HGQ remains unchanged and in cells of donor H the HGQ level decreases. It may be mentioned that donor H is in a very good sportive form, go in for

Table 2
The HGQ changes in the course of the training walk No. 2

Variant	Donors (age)			
	E(18)	F(18)	G (20)	H (48)
Control	14.5 + 0.5	13.4 + 0.5	18.3 + 0.5	18.0 + 0.4
Training, stage 1	23.2 + 0.7*	15.8 + 0.5*	17.9 + 0.7	14.3 + 0.6*
Training, stage 2	22.7 + 0.4*	25.7 + 0.7*	21.3 + 0.6*	20.6 + 0.6*
Training, stage 3	26.2 + 0.8*	21.4 + 0.5*	24.0 + 0.5*	21.7 + 0.5*
Repose, 9 h	-	20.4 ± 0.6*	23.9 ± 0.5*	22.2 ± 0.8*

*Difference from control $P < 0.05$.

Table 3
The HGQ changes in the course of the training walk No. 3

Variant	Donors (age)					
	0(21)	P (21)	Q(21)	R (21)	S (21)	T(21)
Control	14.9 ± 0.4	14.9 + 0.6	15.6 ± 0.6	15.6 ± 0.6	13.3 + 0.4	15.6 ± 0.5
Training, stage 1	16.9 ± 0.6*	16.1 ± 0.6	17.5 ± 0.6*	17.5 ± 0.6*	15.1 ± 0.5*	15.0 ± 0.4
Training, stage 2	19.6 ± 0.4*	18.0 ± 0.8*	19.7 + 0.4*	19.7 + 0.4*	16.9 ± 0.5*	15.9 + 0.5
Training, stage 3	20.5 ± 0.5*	16.9 + 0.6*	19.1 ± 0.5*	19.1 +0.5*	16.1 ±0.4*	18.6 ± 0.4*
Training, stage 4	17.5 ± 0.6*	18.2 ± 0.4*	18.2 ± 0.4*	18.4 ± 0.6*	17.8 + 0.4*	19.4 ± 0.5*
Repose, 16 h	14.0 + 0.4	15.5 + 0.4	22.0 ± 0.4*	16.4 ± 0.9	14.7 + 0.3	-

*Difference from control $P < 0.05$.

heavy athletics, and the absence of HGQ increase and even HGQ decrease after the first stage of training may be connected to this fact. After the second and third stages of training HGQ significantly exceeded the control level in cells of all donors. The repose during 9 h did not significantly change HGQ, it remained on the elevated level.

The data of Table 3 show that training charges induced a significant increase of HGQ in cells of four donors among six tested in the first stage of training. On the second stage of training the HGQ increased in cells of five donors. On the third and fourth stages HGQ increased in cells of all tested donors. After the period of repose during 16 h (sleep) the HGQ decreased in cells of four donors to the control level but increased in cells of donor Q.

In Table 4 are presented the data obtained in cells of six very good trained donors (heavy and light athletics, sportive running). As one can see no HGQ increase was detected in cells of donors of this group after the first stage of training. In cells of donor V was even observed the decrease of HGQ after first and second stages of the training walk. After the second stage of training the HGQ increased in cells of two donors of six tested. After the third stage of training the HGQ was elevated in cells of three donors. After the fourth stage of training the HGQ level was elevated in cells of four donors. Repose during 48 h induced the decrease of HGQ to control level in cells of three donors. In cells of one donor (donor U) HGQ was elevated as compare to control, and in cells of other (donor W) even less than in control. Summing up the results of the experiment No. 4 one can say that in cells of the well-trained donors the changes induced by the training walk are less pronounced. Nevertheless, in cells of five donors among six the training charge induced the increase of HGQ at different stages of sport training.

The results obtained in this work show the close connection between the state of chromatin in human buccal epithelium cells and the state of tiredness induced by sportive training. The sport charges induce chromatin condensation, and this process depends upon the state of human organism. In well-trained people the process of chromatin condensation begins later in the process of training and is less pronounced. In some cases decondensation of chromatin was detected after the first stages of training in cells of well-trained persons.

The mechanisms of chromatin condensation induced by sport training are not explicitly clear now. We suppose that in this process the definite role play so called stress hormones — catecholamines and corticosteroids. These hormones induce chromatin condensation in human buccal cell nuclei in vitro (Shckorbatov et al., 1999a).

Some experimental data concerning the action of stress hormones: adrenaline, noradrenaline, and hydrocortisone are presented in Tables 5—7.

We investigated the action on cells of adrenaline and noradrenaline in different concentrations (Tables 5 and 6). The concentration of hormones 2.28 and 4.55 nM are close to concentrations in human blood after fulfillment of mild and moderate sportive exercises (Hartley et al., 1972).

Table 4
The HGQ changes in the course of the training walk No. 4

Variant	Donors (age)					
	U(18)	V(22)	W(23)	X(30)	Y (31)	Z (32)
Control	10.7 ± 0.3	14.5 ± 0.6	18.7 ± 0.6	8.1 + 0.3	9.8 ± 0.4	16.4 ± 0.6
Training, stage 1	10.2 ± 0.4	11.8 + 0.3*	18.9 ± 0.4	8.3 ± 0.4	8.4 ± 0.3	15.8 ± 0.5
Training, stage 2	10.0 + 0.4	11.3 ± 0.5*	17.0 ± 0.6	11.9 + 0.5*	14.3 ± 0.5*	15.2 ± 0.4
Training, stage 3	13.0 ± 0.5*	12.6 + 0.4	18.3 ± 0.4	11.9 ± 0.4*	14.5 ± 0.6*	16.4 ± 0.3
Training, stage 4	16.6 ± 0.6*	17.2 ± 0.4*	19.0 ± 0.4	12.3 + 0.5*	11.8 ± 0.5	18.6 ± 0.3*
Repose, 48 h	15.8 ± 0.7*	15.1 ± 0.6	12.8 ± 0.4*	8.6 ± 0.3	9.4 ± 0.4	-

*Difference from control $P < 0.05$.

Table 5
The HGQ changes in cells of donor Z' (23 years) after adrenaline treatment

Treatment time (min)	Adrenaline concentration (nM)	
	2.28	4.55
0 (control)	11.5 ± 0.3	11.4 ± 0.3
5	12.5 ± 0.3*	12.6 ± 0.3*
10	12.8 ± 0.3*	13.3 ± 0.2*
15	13.8 ± 0.4*	14.0 ± 0.3*
30	14.5 ± 0.4*	14.4 ± 0.4*
60	15.1 ± 0.4*	14.5 ± 0.4*

*DifTerence from control $P < 0.05$.

Table 7
The HGQ changes in cells of donor Z' (23 years) after hydrocortisone treatment

Treatment time (min)	Hydrocortisone concentration (nM)		
	138	276	414
0 (control)	17.4 ± 0.4	16.8 ± 0.4	15.5 ± 0.4
5	17.8 ± 0.4	17.6 ± 0.5	16.0 ± 0.4
10	18.8 ± 0.5	17.1 ± 0.4	16.2 ± 0.3
15	18.8 ± 0.4	18.6 ± 0.6*	17.0 ± 0.3*
30	19.8 ± 0.4*	19.4 ± 0.5*	17.8 ± 0.4*
60	20.0 ± 0.5*	18.9 ± 0.4*	18.2 ± 0.4*

*Difference from control $P < 0.05$.

The results of hydrocortisone action on cells are presented in Table 7. Hydrocortisone concentration in human blood in the state of rest is close to 138 nM (Hartley et al., 1972).

All tested hormones induce in cell nuclei the process of heterochromatinization that is expressed in the rise of HGQ.

The role of these hormones in reaction of human organism to sportive charges is well defined (Gorostiaga et al., 1999). In well-trained people the increase of the level of these hormones induced by sport exercise is less than in less trained people (Hartley et al., 1972; Shoemaker et al., 1998; Uusitalo et al., 1998). This may be connected to the less expressed reaction of chromatin condensation induced by sport training in cells of well-trained donors.

The mechanisms of chromatin condensation may be closely related to the process of decrease of electric charge of cell nuclei. The decrease of negative electric charge of human buccal cell nuclei induced by sportive exercises was described earlier (Shakhbazov et al., 1986; Shckorbatov et al., 1999b). The electric charge of chromatin is necessary for its conservation in decondensed state (Leake et al, 1972).

We suppose that the processes of chromatin condensation and decrease of negative electric charge of nuclei in buccal cells of donors performing sportive exercises reflect the process of decrease of the negative electric charge of chromatin in living cell.

Thereafter, our results make it possible to conclude that training charges as a rule induce the condensation of chromatin in buccal epithelium cells. The degree of chromatin condensation depends on the intensity of training charge and on the physical state of donor. On the base of this observation the method for evaluation of the state of tiredness was proposed (Shckorbatov et al., 2001). After the short period of repose (9 h) the level of chromatin condensation remains unchanged but after the long periods of repose (16—48 h) the chromatin becomes less condensed in cells of the most part of donors.

In experiments with cells obtained from donors at different stages of circadian rhythm all studies were conducted on cells of four donors of feminine sex of age 21. The results of experiments are presented in Table 8. It is shown that HGQ changes depending on time of the day. HGQ is minimal at the morning and in cells of three donors it decreases at 17 h. This may be connected with the depressed state of organism and feeling of tiredness in these donors in this period. HGQ is maximal in night at 1—5 h. Possibly that phenomenon of chromatin condensation at night is connected with reduction of the activities of the organism in this period.

Therefore our results indicate the strong correlation between the general state of human organism and the state of chromatin in cells of buccal epithelium. State of tiredness and decrease of activity at nighttime are connected with chromatin condensation. The results of in vitro experiments give an opportunity to connect

Table 6
The HGQ changes in cells of donor Z' (23 years) after noradrenaline treatment

Treatment time (min)	Noradrenaline concentration (nM)	
	2.28	4.55
0 (control)	11.9 ± 0.3	11.9 ± 0.3
5	10.9 ± 0.4	11.5 ± 0.3
10	11.7 ± 0.4	12.4 ± 0.4
15	12.9 ± 0.5	13.3 + 0.4
30	13.3 ± 0.5	13.2 ± 0.4
60	13.3 ± 0.5	15.8 ± 0.4*

*Difference from control $P < 0.05$.

Table 8
The HGQ changes in cells of four female donors (21 year) during different periods of day and night

Time	Donors			
	A	B	C	D
9 (control)	19.3 ±0.4	15.4 ±0.3	17.3 ± 0.6	21.1 ±0.8
13	19.8 ±0.5	15.0 ±0.2	14.1 ±0.5*	20.3 ± 0.7
17	23.0 ±0.5*	18.1 ±0.7*	15.2 ± 0.5	23.4 ± 0.8*
21	19.4 ±0.6	15.1 ±0.5	18.9 ± 0*7	25.1 ± 0.7*
1	23.5 ±0.4*	18.2 ±0.2*	24.1 ±0.7*	26.7 ± 0.6*
5	23.6 ±0.7*	17.7 ±0.2*	25.9 ± 0.6*	26.3 ± 0.7*

*Difference from control $P < 0.05$.

these changes with changes in hormonal situation in the organism.

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