EFFECT OF REPEATED SUBLETHAL HYPERTHERMIA ON CYTOGENETIC PROCESSES IN THE CORNEAL EPITHELIUM AND BONE MARROW CELLS OF ALBINO RATS

M. I. Radivoz, S. S. Timoshin, A. P. Baranov, and N. B. Murzina

UDC 612.841.1.014.2:612.6/-06:612.591

KEY WORDS: sublethal hyperthermia; cytogenetic processes; corneal epithelium; bone marrow cells.

The writers showed previously [3] that a single exposure of male albino rats to sublethal hyperthermia up to 40-41.5°C causes inhibition of mitotic activity (MA) of the corneal epithelium by 1.3-3 times compared with the control, and is accompanied by a two- to fourfold increase in the number of pathological mitoses (PM).

The object of this investigation was to study the character of the response of cytogenetic processes in the corneal epithelium and bone marrow cells during repeated exposure to sublethal hyperthermia.

EXPERIMENTAL METHOD

Experiments were carried out on 200 male albino rats weighing 150-200 g. The animals were exposed for 1.5 h to hypothermia at 41-41.5°C by the method in [3] daily, at the same time of day (from 10 a.m. to noon) for a period of 5 days. The tests were carried out immediately after the final exposure and 2, 6, 12, and 24 h later. The mitotic cycle was determined and autoradiographs prepared also by the method in [3]. To assess the role of the adrenal glands in changes in the mitotic cycle during hyperthermia, parameters of the stress reaction were studied: The adrenals and thymus were weighed and the concentrations of cholesterol in the adrenals [6] and 11-hydroxycorticosterone in the blood plasma and the adrenalin content in the liver were determined [7]. In parallel experiments the animals were subjected to bilateral adrenalectomy. To rule out any possible change in the duration of mitosis itself, experiments also were carried out at the same time on animals receiving an injection of colchicine, in a dose of 4 μg/g, intraperitoneally 2 h before sacrifice, and 0.1 ml of a 0.1% solution of colchicine was applied simultaneously to the cornea. To assess the mutagenic effect of the high temperature metaphase analysis of bone marrow cell chromosomes was carried out 3 h after the end of exposure to hyperthermia by the method in [8].

EXPERIMENTAL RESULTS

The results are evidence that the animals developed a marked stress reaction immediately and 6 h after the final exposure to a high temperature. This was shown by a decrease in the body weight of the animals and in the weight of their thymus, an increase in the weight of their adrenals accompanied by a decrease in their cholesterol concentration, and an increase in the plasma 11-hydroxycorticosterone level (Fig. 1). These results agree with data in the literature indicating that sublethal hyperthermia is a severe form of stress [1].

The time course of the reaction of the mitotic cycle of the corneal epithelium to repeated hyperthermia differed considerably from that to a single exposure to heat. Whereas with a single exposure deep inhibition of proliferation was observed for 12 h after the end of exposure to heat, in the case of repeated exposure to hyperthermia depression of MA was observed only immediately after the end of heating. By 2 h after the end of exposure there was a tendency for the number of dividing cells to increase: 6 and 12 h after the last exposure the number of mitoses was increased by 4 and 1.7 times, respectively. After 24 h MA was the same in the experimental and control animals (Fig. 2). The experiments with colchicine showed that

Central Research Laboratory, Khabarovsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. E. Kulagin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 4, pp. 98-100, April, 1982. Original article submitted June 15, 1981.

 0007-4888/82/9304-0508$07.50

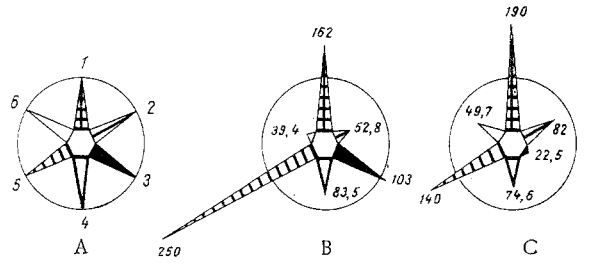

Fig. 1. Parameters of the general adaptation syndrome (in %) in rats after repeated exposure to sublethal hyperthermia. A) Control; B) after hyperthermia; C) 6 h after hyperthermia. 1) 11-Hydroxycorticosterone (17.24 μg%); 2) cholesterol (4.73 μg/mg); 3) adrenalin (2.36 μg/g); 4) body weight of animal (230 g); 5) weight of adrenals (8.78 mg/100 g); 6) weight of thymus 450.6 mg/100 g).

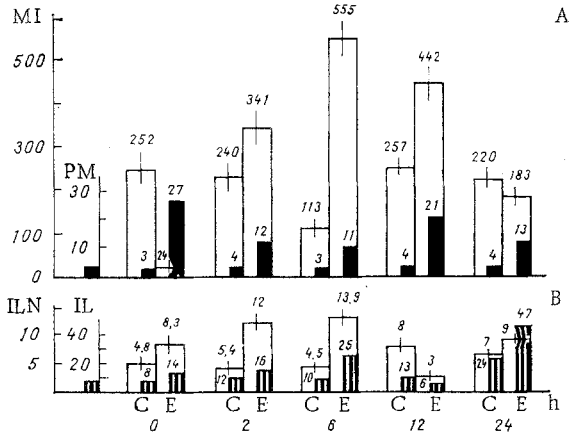

Fig. 2

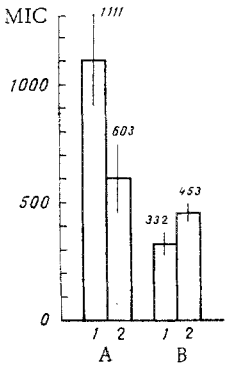

Fig. 3

Fig. 2. Time course of proliferation of corneal epithelium of albino rats after time of investigation relative to end of large exposure to hyperthermia (in h); ordinate: MI) number of mitoses in 100 fields of vision, PM) percent of pathological mitoses; ILN) index of labeled nuclei (in %), IL) intensity of labeling (number of tracks above the nucleus). *) $P < 0.05$.

Fig. 3. Effect of repeated exposure to hyperthermia on cell division in the corneal epithelium of intact (A) and adrenalectomized (B) rats. 1) Control; 2) experiment. Ordinate: MIC) number of colchicine mitoses in 100 fields of vision. Tests carried out 2 h after end of last exposure to hyperthermia.

the increase in the number of dividing cells was due, not to a change in the time of mitosis, but to an increase in the number of cells starting mitosis. Just as in the case of a single exposure, in this series of experiments a significant rise in the PM level was observed immediately after hyperthermia, and also 2, 6, 12, and 24 h after the last exposure (Fig. 2). The results of autoradiographic analysis are evidence that the **increase in the number of dividing cells** after the 5th exposure to **hyperthemia was due to activation of DNA synthesis. Inhibition of DNA** synthesis after 12 h was probably the result of the differential sensitivity of the various phases of the cell cycle to the action of sublethal hyperthermia. According to data in the literature, the G_1 period is the most sensitive to the action of a high temperature. The absence of inhibition of cell division 2, 6, and 12 h after five exposures to sublethal hyperthermia was due not only to a decrease in the adrenalin concentration, but also, probably, to massive death of the cells following repeated hyperthermia [9], which leads to a fall in the concentration of **chalones** — tissue regulators of cell division. Another contributory factor is inhibition of protein synthesis under conditions such as these [10]. However, this hypothesis requires further experimental verification.

TABLE 1. Frequency of Chromosomal Aberrations in Bone Marrow Cells of Intact and Adrenalectomized Rats after Repeated Exposure to Hyperthermia

Experimental conditions	No. of metaphases	Aneuploid cells, %			Types of aberrations					Cells with aberrations, %	Cells with delections
		hypodiploid	hyperdiploid	total	ACF	CCF	CE	DC	AC		
Intact rats											
control	600	4,4	0,3	4,7	1	1	—	—	—	0,3	0,5
experiment	600	4,5	0,5	5,0	2	—	—	—	1	0,5	0,5
Adrenalectomy											
control	600	3,9	0,3	4,2	2	—	—	—	—	0,3	0,17
experiment	600	4,7	0,3	5,0	7	—	1	1	—	1,5*	0,00

Legend. *) $P < 0.05$ relative to control; ACF) acentric chromatid fragment; CCF) centric fragment (chromatid deletion); CE) chromatid exchange; DC) dicentric chromosome· AC) atypical chromosome.

Repeated exposure of adrenalectomized animals to sublethal hyperthermia was not accompanied by stimulation of cell division in the corneal epithelium. Meanwhile, in rats with their adrenals intact, the number of colchicine mitoses was increased 3 h after hyperthermia, whereas in adrenalectomized animals their number was reduced 3 h after exposure (Fig. 3). The PM level in the adrenalectomized animals 6 h after exposure to hyperthermia was 25%, whereas in animals with intact adrenals it was only 15.3%. This is in agreement with previous observations showing the protective character of the action of increased secretion of adrenal hormones for cell division during stress [4, 5]. Further confirmation of this view is given by the results of metaphase analysis of bone marrow cell chromosomes of albino rats exposed to these conditions of hyperthermia. Whereas five exposures to sublethal hyperthermia caused no change in the level of aneuploid cells or in the number of chromosomal aberrations in animals with intact adrenals, in adrenalectomized animals the number of aberrant cells was increased sixfold (Table 1).

The results of these experiments are evidence of the different character of response of the mitotic cycle of the corneal epithelium to single and repeated exposure to hyperthermia. The results confirm the view that high temperatures exert a mutagenic action and they demonstrate once again the protective effect of increased secretion of adrenal hormones for cytogenetic processes in the somatic cells of homoiothermic animals.

LITERATURE CITED

1. V. D. Lindenbraten, "Data on the action of heat on the body," Author's Abstract of Doctoral Dissertation, Leningrad (1968).
2. S. V. Rudneva, F. V. Sushkov, S. I. Polikarpova, et al., Byull. Eksp. Biol. Med., No. 11, 1365 (1976).
3. M. I. Radivoz and S. S. Timoshin, Byull. Eksp. Biol. Med., No. 8, 222 (1980).
4. S. S. Timoshin, in: Mechanisms of Injury, Resistance, Adaptation, and Compensation [in Russian], Vol. 1, Tashkent (1976), p. 342.
5. S. S. Timoshin, Byull. Eksp. Biol. Med., No. 7, 103 (1980).
6. Yu. A. Pankov and I. Ya. Usvatova, in: Methods of Investigation of Some Hormones and Mediators [in Russian], No. 3, Moscow (1965), p. 137.
7. E. Sh. Matlina, Z. N. Kiseleva, and I. Z. Sofieva, in: Methods of Investigation of Some Hormones and Mediators [in Russian], No. 3, Moscow (1965), p. 25.
8. C. E. Ford et al., Stain Technol., 31, 247 (1956).
9. M. Ponchelet, Exp. Cell Res., 83, 207 (1974).
10. J. M. Voaden, Exp. Eye Res., 11, 714 (1971).