MITOTIC ACTIVITY OF ADRENAL CORTICAL CELLS IN RATS DURING PROLONGED HYPOKINESIA

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Male Wistar rats weighing 95-100 g were placed in small containers restricting their movements. Experimental and control rats were sacrificed in groups after 1 and 12 h and 2,5, 9,14, and 19 days. Mitotic division in the adrenal cortex was sharply inhibited after 1 h. Subsequently (from 12 h to 9 days) the mitotic index in the zona glomerulosa returned to the control level, but in the outer zona fasciculata it was considerably higher than in the control. After 14 and 19 days, when signs of exhaustion of the animals had developed and hypertrophy of the adrenals was less marked, the mitotic index in both zones was again reduced. The presence of a phase of stimulation of mitosis in the outer zona fasciculata indicates that adrenal hypertrophy under stress conditions takes place not only through hypertrophy of the cells, as is usually considered, but also on account of stimulation of mitotic division.

It was shown previously that in rats swimming repeatedly for 3 h daily for a period of 30 days the mitotic activity of the adrenal cortical cells undergoes phasic changes, including one clearly distinct phase during which stimulation of cell division is observed [6] . Since the attention of workers studying the adrenal cortex during the action of harmful agents has so far been concentrated mainly on reactive inhibition of mitosis [1, 2-4] , the results of the earlier experiments described above are in some disagreement with other work published in the literature. Nevertheless the presence of a phase of stimulation of mitosis, in the writers' opinion, is of definite theoretical interest for it shows that the adrenal hypertrophy which develops during stress does so not only on account of cell hypertrophy, as most workers believe, but also on account of stimulation of mitotic division. However, before drawing such far-reaching conclusions it was necessary to determine whether the stimulation of mitosis is not specific purely for muscular work and not connected with the action of other harmful factors.

To answer this question experiments were carried out to study the action of hypokinesia, which acts in a diametrically opposite manner to muscular exertion.

EXPERIMENTAL METHOD

Male Wistar rats weighing 95-100 g were divided into experimental and control groups. The experimental rats were placed in small transparent plastic containers which sharply restricted their freedom of movement. The walls of the containers had many ventilation holes. In the front of the container were bowls of food and water. In the floor of the container holes were cut out for the evacuation of urine and feces. The floor also was moveable so that the container could be cleaned without removing the rat. Only a few of the animals occasionally succeeded in turning over around the longitudinal axis; rats which accomplished this frequently were excluded from the experiments.

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TABLE 1. Mitotic Index (%) of Adrenal Cortical Cells in Control and Immobilized Rats

Time of experiment	Animals	Number of rats	Zona glomerulosa	Outer zona fasciculata
1 h	Control	5	0,24±0,050	0,12±0,033
	Experimental	5	0,00	0,00
			0,001	0,007
12 h	Control	6	0,21±0,054	0,13±0,073
	Experimental	7	0,14±0,042	0,07±0,036
			0,389	0,500
2 days	Control	11	0,27±0,051	0,12±0,025
	Experimental	12	0,16±0,033	0,33±0,066
			0,071	0,007
6 »	Control	10	0,20±0,038	0,12±0,026
	Experimental	13	0,14±0,039	0,32±0,050
			0,297	0,002
9 »	Control	7	0,24±0,048	0,13±0,054
	Experimental	7	0,27±0,038	0,64±0,204
			0,626	0,031
14 »	Control	8	0,22±0,046	0,08±0,029
	Experimental	9	0,04±0,023	0,07±0,047
			0,003	0,844
19 »	Control	7	0,26±0,036	0,09±0,027
	Experimental	8	0,03±0,019	0,00
			0,000	0,006

The rats of the experimental and control groups were sacrificed in batches of 5-13 at a time 1 and 12 h, and also 2, 5, 9, 14, and 19 days after the beginning of the experiment, always at 7 A.M. to exclude changes depending on the diurnal periodicity of adrenal function. The left adrenal was fixed in Carnoy's fluid and embedded in paraffin wax and sections were stained with hematoxylin and eosin. The number of mitoses was counted for each zone separately in 10,000 cells.

EXPERIMENTAL RESULTS

In the rats exposed to hypokinesia the weight of the adrenals was increased at the above-mentioned times of sacrifice by 9, 33, 41, 30, 17, 8, and 5%, i.e., adrenal hypertrophy was well-marked at the beginning of the experiment and then diminished. The decrease in hypertrophy in the late stages was attributed to the onset of general exhaustion of the animals.

Mitoses were found in the control and experimental rats only in the zona glomerulosa and the outer zona fasciculata. No dividing cells could be found in the inner zona fasciculata and the zona reticularis.

One hour after the beginning of immobilization no mitoses were found in the outer zona fasciculata of the experimental rats, evidently on account of reactive inhibition of mitoses (Table 1). Mitotic division was resumed after 12 h, but there were fewer mitoses than in the controls. Next followed a relatively long period during which mitotic activity in the immobilized rats greatly exceeded the control level. After two days the mitotic index was increased by 2.8 times, after 6days by 2.7 times, and after 9 days by 4.9 times. On the 14th day, when signs of general exhauston of the animals were apparent, the mitotic index fell again. On the 19th day mitoses were completely absent.

Reactive inhibition of mitosis 1 h after the beginning of exposure to hypokinesia was also observed in the zona glomerulosa. Later, figures of division reappeared in the experimental animals, although their total number after 12 h and 2 and 6 days was somewhat lower than in the control, the difference was not statistically significant. After 9 days the mitotic index in both groups was almost identical, while on the 14th and 19th days, just as in the outer part of the zona fasciculata, the number of mitoses in the zona glomerulosa was reduced.

Changes in mitotic activity in the adrenal cortex during prolonged hypokinesia were thus phasic in character. The phasic properties were most clearly marked in the outer zona fasciculata, where reactive inhibition was immediately followed by a period of stimulation of mitosis, but at the end of the experiment the mitotic index had fallen again. In the zona glomerulosa no phase of stimulation of mitosis was present, but here also reactive inhibition of mitosis continued only in the first few hours, after which mitotic division was resumed and returned almost to the control level. It may be recalled once again that similar changes in mitotic activity, with a phase of increase in the number of mitoses, were observed previously by the writers in rats during repeated swimming. Since this phase was equally well marked during two situations

so opposite in character as muscular work and hypokinesia, it can be concluded that stimulation of mitotic division is a nonspecific mechanism of hypertrophy of the adrenals during stress.

Some workers have described an increase in the number of mitoses in the recovery period after cessation of the action of unfavorable factors [3, 7]. The stimulation of mitoses observed in the present experiments differs in principle, it will be noted from those results because it takes place, not during the period of rest, but actually during stress when the action of the harmful agent is still in progress. Reactive inhibition of mitoses is also observed initially during prolonged administration of ACTH but it is then followed by an increase in mitotic activity [1, 3]. An increase in the number of mitoses is also observed after unilateral adrenalectomy [5, 8, 9]. From a consideration of all these facts, stimulation of mitotic activity can be regarded as a mechanism of hypertrophy of the adrenal cortex which is just as important as hypertrophy of the cells.

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