EFFECT OF ADRENALIN ON CELL REPRODUCTION IN THE CORNEAL EPITHELIUM OF RATS AT DIFFERENT AGES

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UDC 612.841.1.014.3:612.6].014.46:
615.357.452

The effect of adrenalin on cell division was studied in the corneal epithelium of rats at 12 different ages. In 21-day fetuses and animals aged 3-4 days an increase in the mean mitotic index (MI) was observed 45 min after its administration, caused by dealy in the passage of the cells through the middle and late stages of division. In rats aged 7-57 days MI was unchanged by adrenalin. In animals aged 70 days the mitotic activity was sharply reduced as a result of delay in the onset of mitosis by the cells. In animals killed 2 h after injection of adrenalin an antimitotic effect was observed starting from the age of 4 days. This effect was due to inhibition of passage of the cells from the G_2-period of the mitotic cycle into mitosis. The results are evidence of differences in the character of action of adrenalin on the early stages of ontogeny.

Many investigations have shown that injection of adrenalin into animals leads to a decrease in the number of cell divisions in ectodermal tissues [1, 2, 5, 6, 9, 11]. Since the decrease in the number of mitoses arises soon after the injection of adrenalin and begins with a decrease in the number of the early stages of division it is generally considered that adrenalin blocks the passage of cells from the G_2-period of the mitotic cycle into mitosis. Only a few investigators have postulated that this hormone may affect the course of individual phases of mitosis or of mitosis as a whole [4, 10]. It has also been shown [7, 8] that in some tissues of fetuses and of mice aged under 6-14 days there is no decrease in the number of mitoses in response to injection of adrenalin.

It was therefore decided to study the development and ontogeny of tissue reactions to adrenalin and the differences in its action at different age periods.

TABLE 1. Changes in Mitotic Activity of Corneal Epithelium of Fetuses after Administration of Adrenalin

Group of animals	Number of rats	Mean MI (in %)	P	Mean number of phases of mitosis					
				EP	P	M	A	T	R
Control	4	2,3	0,001	3,0	0,7	4,5	0,2	7,3	5,2
Experimental	7	10,8		2,0	3,2	30,4	0,2	33,3	11,8

Legend: here and in Tables 2-4: EP) early prophase, P) prophase, M) metaphase, A) anaphase, T) telophase, R) reconstruction.

Department of General Biology, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 77, No. 4, pp. 102-105, April, 1974. Original article submitted July 20, 1973.

TABLE 2. Changes in Mitotic Activity of Corneal Epithelium of Fetuses after Injection of Adrenalin into Pregnant Rats

Group of animals	Number of rats	Mean MI (in %)	P	Mean number of phases of mitosis					
				EP	P	M	A	T	R
Control	5	9,2	0,001	3,9	13,6	37,8	2,5	10,7	2,6
Experimental	6	6,2		3,3	1,7	9,2	1,0	20,5	14,3

TABLE 3. Changes in Mitotic Activity of Corneal Epithelium of Rats of Different Ages 45 min after Injection of Adrenalin

Age (in days)	Group of animals	Number of rats	Mean MI (in %)	P	Mean number of phases of mitosis					
					EP	P	M	A	T	R
1	Control	5	5,3	0,8	2,8	5,3	14,6	0,6	14,0	3,4
	Experimental	5	5,2		0,4	3,4	12,6	1,0	16,8	8,2
3	Control	4	4,1	0,002	1,5	0,8	5,0	0,8	12,0	14,7
	Experimental	5	9,6		1,4	1,6	24,2	2,4	33,8	21,0
4	Control	5	5,3	0,001	2,0	0,6	6,6	1,0	18,4	16,8
	Experimental	5	11,1		2,8	3,2	31,4	3,2	39,2	14,0
7	Control	4	3,4	0,15	5,7	9,5	2,3	0	5,3	9,0
	Experimental	5	5,7		1,0	1,2	8,4	2,2	29,4	14,2
15	Control	4	3,2	0,45	7,5	16,7	12,5	0	2,5	3,0
	Experimental	4	2,7		1,2	1,2	1,8	0	15,0	14,5
20	Control	4	2,0	0,04	0,2	0,3	1,5	0,5	14,2	15,5
	Experimental	4	4,4		0,2	0	28,0	2,5	34,5	5,5
25	Control	5	3,8	0,20	2,2	8,0	4,8	1,8	32,4	16,6
	Experimental	5	5,4		1,0	0,4	10,0	3,4	44,2	29,6
30	Control	3	3,0	0,06	0,7	0,7	3,3	0,3	21,3	20,7
	Experimental	3	5,2		0	0,4	5,3	1,7	40,3	30,3
45	Control	4	3,0	0,80	2,0	9,5	21,0	1,5	10,8	9,0
	Experimental	4	3,4		1,2	0	3,8	1,3	30,3	22,2
57	Control	4	2,5	—	2,0	0,7	7,8	0,5	22,5	9,0
	Experimental	5	2,5		0	0,4	2,2	0,8	27,8	11,0
70	Control	4	5,6	0,02	4,7	17,8	48,2	2,2	21,8	10,8
	Experimental	4	2,8		0	0	5,2	0,8	35,0	11,0

EXPERIMENTAL METHOD

Cell division was studied in the corneal epithelium of rats at 12 different ages. Two series of experiments were carried out on fetuses. In series I the adrenalin was injected directly into the fetuses. For this purpose laparotomy was performed on pregnant rats 1-2 days before parturition, adrenalin was injected subcutaneously into each fetus through the wall of the uterine cornua in a dose of 2 μg/g, and the operation wound was then closed. Control females underwent the same operation but physiological saline was injected into the fetuses. In series II adrenalin was injected subcutaneously into pregnant rats; control animals received physiological saline. In both cases the animals were killed 45 min after injection of the adrenalin. In series III and IV animals of different ages were studied 45 min and 2 h respectively after injection of adrenalin in a dose of 2 μg/g.

The cornea was fixed in Carnoy's fluid and total preparations were stained with Carazzi's hematoxylin. In each case 100 fields of vision of the microscope were examined. The mean mitotic index (MI) was calculated per 1000 cells. All experiments were carried out at the same time of day – from 5 to 8 p.m., i.e., at a time when mitotic activity in the cornea does not change significantly but remains at a high level [3].

EXPERIMENTAL RESULTS

Series I. Injection of Adrenalin Directly into Fetuses

The results given in Table 1 show that injection of adrenalin led to a significant increase in the mean MI. Analysis of the phases of mitosis showed that this increase did not reflect the stimulant action of adrenalin on mitotic activity because the number of early prophases was not increased. On the other hand, the mean number of later phases of mitosis (excluding anaphases) was sharply increased in the experimental

TABLE 4. Changes in Mitotic Activity of Corneal Epithelium of Rats of Different Ages 2 h after Injection of Adrenalin

Age (in days)	Group of animals	Number of rats	Mean MI (in %)	P	Mean number of phases of mitosis					
					EP	P	M	A	T	R
2	Control	4	4,5	—	1,5	3,7	17,0	1,8	6,3	2,5
	Experimental	6	4,7		1,8	2,2	5,2	0,8	12,5	7,5
4	Control	4	7,2	0,001	3,0	10,0	34,8	5,0	15,7	9,0
	Experimental	3	2,4		1,7	0	0	0	3,3	19,3
7	Control	3	10,2	0,001	1,0	8,0	26,3	7,0	44,7	33,3
	Experimental	4	1,4		0,2	0,3	0	0	3,8	11,2
25	Control	2	4,9	—	3,0	11,5	30,0	1,5	30,5	7,5
	Experimental	3	0		0	0	0	0	0	0

group (P = 0.001). It can be concluded from these results that adrenalin affects the course of mitosis by delaying the passage of the cells through its middle and late phases (especially meta- and telophases).

Series II. Injection of Adrenalin into Pregmant Rats

A significant decrease in the mean MI was found in the cornea of the experimental fetuses (Table 2). It was accompanied by a decrease in the mean number of prophases and metaphases (P = 0.001), indicating delay in the onset of mitosis in the cells. A marked increase also was observed in the number of telophases (P = 0.01) and reconstructions (P = 0.01) and this can be regarded as inhibition of the course of these stages of division by adrenalin.

Series III. Animals of Different Ages Killed 45 min after Injection of Adrenalin

In rats aged 1 day no significant changes in the mean MI were found (Table 3). In animals aged 3 and 4 days MI was sharply increased. This was not due to stimulation of the entry of the cells into mitosis; this is shown by the absence of any increase in the number of early prophases in the cornea of the experimental animals. Since the number of divisions in the later stages increased during administration of adrenalin, the increase in the mean MI in the experimental group was evidently due to delay in the passage of the cells chiefly through the middle and late phases of mitosis.

In rats between the ages of 7 and 57 days some increase in the mean MI was found under the influence of adrenalin, but the increase was not significant. With an increase in age there was an increasing tendency toward inhibition of the entry of the cells into mitosis: a sharp decrease in number or even the complete disappearance of the early prophases and prophases was observed in the experimental animals. Although the number of metaphases and, in particular, of telophases and reconstructions in some cases was higher than in the control, the differences were not significant.

Series IV. Animals of Different Ages Killed 2 h after Injection of Adrenalin

No significant changes in mitotic activity were found in the corneal epithelium of the experimental rats aged 2 days (Table 4). In animals aged 4, 7, and 25 days a sharp decrease in the mean MI due to a decrease in the number or disappearance of the early stages of division was observed; mitosis was completely absent in the 25-day rats.

It can be concluded from these results that the action of adrenalin on cell division is biphasic in character. It affects the course of mitosis itself by delaying the passage of the cells through certain phases, as is seen most clearly in young animals in the early periods after injection of the hormone. With age another aspect of its action is intensified: delay of the entry of the cells into mitosis. The results showed that in order to assess the character of action of adrenalin on proliferative processes correctly it is not sufficient simply to determine the mean value of MI, for its changes under the influence of the hormone are the result of inhibition of entry of the cells into mitosis and a disturbance of the course of the individual phases of mitosis. The role of each of these aspects of the action of adrenalin differs at different stages of ontogeny of the animals.

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