THE EFFECTS OF ADRENALIN ON MITOSIS, RESPIRATION AND GLYCOLYSIS IN THE CORNEA AND INTESTINAL MUCOSA OF RATS

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The question of the effect of adrenalin on the mitotic activity of tissues in the organism was dealt with extensively in the experimental works of a number of authors.

In experiments involving removal and subsequent reimplantation of the suprarenals [2, 5, 8] and injection of a solution of adrenalin, it was shown that adrenalin inhibits mitotic division in the epithelium of the cornea, tongue, duodenum and epidermis and does not effect appreciably mitoses in cells of lymph nodes, thyroid, uterus and metastatic or recurrent tumors [1-4]. Adrenalin does not effect the mitotic activity of the epithelium of the small intestine either [8].

In view of the fact that much data found in the literature points to the intimate relationship between cellular growth and division, and carbohydrate – phosphorus metabolism, it was expedient to investigate the effect of adrenalin on the processes of respiration and glycolysis in various tissues, taking into consideration the definite role of adrenalin in these processes. It is known that in the presence of intense cellular division aerobic glycolysis or incomplete oxidation of carbohydrates, producing not only the necessary energy for the cells but plastic material as well, is quite typical for the tissues of the organism.

The purpose of this work was the comparative study of the effect of adrenalin on cellular division, aerobic glycolysis and consumption of oxygen in the cornea and the epithelium of the small intestine of rats.

EXPERIMENTAL METHOD

Investigations were carried out on white male rats weighing 150-200 g. Tissues were fixed in Bouin's solution prior to histological examination. 200-250 visual fields were examined in each preparation of the entire cornea stained with Carazzi's hematoxylin – eosin. A segment of the small intestine (10 cm from its gastric junction) was sectioned 8 μ in thickness after it was embedded in paraffin. In each case mitoses were counted in 50 crypts. In part of a crypt the total number of cells was counted.

In the biochemical investigations the corneas were dissected from other tissues of the eye along their edges in a cold 0.9% solution of NaCl immediately after the rats were killed. After drying the corneas with filter paper they were cut in half, weighed on torsion scales and placed immediately into an incubating medium. The corneas from left eyes (30-40 mg) were used for tests prior to incubation and from right eyes for tests after incubation. The mucosa of the small intestine was obtained by scraping in the cold with a glass slide with polished edges [11]. The tissue was gently stirred with a scalpel, weighed on torsion scales and promptly placed in the incubating medium. The rate of glycolysis was determined by the increase in the amount of lactic acid after incubation. Incubation was carried out for 1 hour in a Warburg vessel at air temperature of 37°. A 1.2 ml (pH = 7.4) freshly prepared solution [90 ml of 0.9% solution of NaCl, 8 ml solution of Na_2HPO_4 (0.4 M), 2 ml of solution of KH_2PO_4 (0.4 M) and 100 mg of glucose] was used as an incubating medium. Oxygen

absorption was measured manometrically, the lactic acid concentration – colorimetrically using p– oxydiphenyl following the method of Barker and Sammerson modified by S. L. Bonting. Proteins were precipitated with hot acetic acid. In order to make extraction of acetic acid more complete the contents of the Warburg vessel was placed into centrifuge tubes, the corneas macerated with a glass rod and the tubes allowed to stand for 10-15 minutes. After the material was centrifuged and 2 ml of corneal filtrate or 1 ml of intestinal filtrate obtained, 0.2 ml of 10% solution of NaCl was added to the former or 0.1 ml to the latter; the following were added to either: 0.5 ml of 20% of $CuSO_4$, 0.5 g CaO and distilled water to make 5 ml. The concentration of lactic acid was determined electrophotocolorimetrically. The quantity of oxygen absorbed was expressed in cubic millimeters to 100 mg of wet weight (QO_2), the concentration of lactic acid – in v/mg of wet tissue weight.

EXPERIMENTAL RESULTS

Data on the measurement of the mitotic coefficient in the cornea and intestine summarized in Table 1 shows that when an intramuscular injection of adrenalin is given (2.5 mg/kg of animal weight), a significant lowering of the mitotic coefficient in the corneal epithelium of rats takes place and lasts not less than 12 hours after injection. Forty-five minutes after injection of adrenalin the ratio of the absolute number of early phases of mitosis (prophase and metaphase) to later phases (anaphase and telophase) is equal to 1.3; in the corneas of rats sacrificed in 3 hours this ratio is equal to 2.2. In control animals which were given an injection into the soft tissues of the thigh and in all experimental animals sacrificed later on this ratio, with rare exceptions, was close to 3.0.

TABLE 1

Average Mitotic Coefficient in the Epithelium of the Cornea and Crypts of the Intestine at Different Periods Following Injection of Adrenalin and in Control Rats

Adrenalin injections			Soft tissue puncture		
period of sacrifice after injection	mitotic coefficient (in ‰)		period of sacrifice after puncture	mitotic coefficient (in ‰)	
	cornea	intestine		cornea	intestine
45 minutes	3,7	25,9	45 minutes	9,9	42,4
3 hours	2,2	48,1	—		
7 "	2,5	31,2	—		
12 "	1,3	35,4	12 hours	9,6	38,6
18 "	16,9	31,8	—		
1 day	8,7	28,3	1 day	13,4	32,5
2 days	17,6	36,7			

Entirely different results were obtained in the case of the epithelium of the crypts of the small intestine. The lowest mitotic coefficient (25.9‰) was obtained in rats sacrificed 45 minutes after adrenalin injection. For all subsequent periods the mitotic coefficient, in experimental as well as control rats, remained fairly high and not subject to any regular changes. Neither were any essential differences observed in the ratio between the absolute amount of early phases of mitosis and later phases.

In order to make the data obtained more precise a second series of experiments was carried out in which, besides adrenalin injection, injections of colchicine were also given. The animals in this group were divided into 4 groups with 7-8 rats in each. The rats were sacrificed 5½ hours after injection. The results from the study of mitotic coefficients presented in Table 2 indicate that injections of 1.3 mg of adrenalin per 1 kg weight also results in abrupt lowering of the mitotic coefficient in corneal epithelium and does not cause lowering in the number of mitoses in the epithelium of intestinal crypts.

The results of experiments with subcutaneous injection of colchicine (0.2 mg per 150 g body weight) were unexpected. Typical pictures of metaphase arrested by colchicine were observed in the corneal epithelium of the animals in this group. In some animals the amount of mitosis was insignificant and for this reason the average mitotic coefficient for the entire group was found to be even lower than in controls. The number of mitoses in

the stage of metaphase increased in the epithelium of intestinal crypts. In those animals which were given simultaneously an injection of colchicine in one extremity and adrenalin in the other, the mitotic coefficient in the corneal epithelium was very low (3.2‰) while that in the intestine was unusually high (248.3 ‰).

TABLE 2

Average Mitotic Coefficient (in ‰) in the Epithelium of the Cornea and Crypts of the Small Intestine of Rats

Experiment No.	Substances administered	Mitotic coefficient	
		corneal epithelium	epithelium of intestinal crypts
1	Physiological salt solution	12.5	32.0
2	Adrenalin	2.7	30.2
3	Colchicine	11.0	211.5
4	Colchicine and adrenalin	3.2	248.3

Thus, the experimental data obtained indicates that adrenalin depresses the entry of the epithelial cells of the cornea into mitotic division and does not influence significantly mitotic division of the epithelial cells in the intestinal crypts.

TABLE 3

Effect of Adrenalin on Aerobic Glycolytic Action and Oxygen Consumption in the Cornea of Rats

Expt. No.	Control				Experiment			
	QO_2 (in mm^3 per 100 mg of raw tissue)	lactic acid in γ/mg of raw weight			QO_2 (in mm^3 per 100 mg of raw tissue)	lactic acid in γ/mg of raw weight		
		prior to incubation	after incubation	difference		prior to incubation	after incubation	difference
1	24,20	0,371	1,823	1,452	21,90	0,522	2,03	1,508
2	17,21	0,00	1,06	1,06	13,57	0,150	1,14	0,99
3	18,63	0,341	2,02	1,679	21,66	0,60	2,45	1,85
4	16,28	0,154	3,22	3,066	17,80	0,142	3,58	3,438
5	10,03	0,451	2,973	2,522	15,99	1,038	2,41	1,372
6	21,06	0,216	1,701	1,485	18,81	0,33	2,12	1,79
7*	23,65	1,056	2,564	1,508	26,66	0,933	2,744	1,811
Average	17,90	—	—	1,877	18,29	—	—	1,825

*Removal of corneas 30 minutes after injection of adrenalin (not taken into account in calculating averages).

In the first series of biochemical investigations of the effect of adrenalin on oxygen consumption and aerobic glycolysis in the rat cornea, intramuscular injections of adrenalin were given in the amounts of 1.3 mg per 1 kg body weight. Animals which were given 0.2 ml of 0.9% solution of NaCl served as controls. Corneas were excised 2 hours after injection during the period of maximal inhibition of mitotic division of epithelial cells.

Results of the experiments presented in Table 3 indicate that intramuscular introduction of adrenalin (1.3 mg per 1 kg body weight) does not produce a noticeable effect on oxygen consumption and lactic acid accumulation in the rat cornea under aerobic conditions. According to the data of experiment No. 7, no

noticeable changes in the indicated tests were demonstrable 30 minutes after the injection of adrenalin. However, we consider it premature to conclude that the effect of adrenalin on mitotic division of cells in corneal epithelium is not conditioned by its influence on the processes of respiration and glycolysis. Additional biochemical investigations using not only whole corneas but also a single layer of its epithelium are needed.

TABLE 4

Effect of Adrenalin on Aerobic Glycolytic Action and Oxygen Consumption in the Epithelium of the Small Intestine of Rats

Expt. No.	Control				Experiment			
	QO_2 (in mm^3 per 100 mg of raw tissue)	lactic acid in γ/ mg of raw weight			QO_2 (in mm^3 per 100 mg of raw tissue)	lactic acid in γ/ mg of raw weight		
		prior to incubation	after incubation	difference		prior to incubation	after incubation	difference
1	64,20	1,85	4,62	2,77	54,20	2,45	4,62	2,17
2	43,20	0,97	5,82	4,85	59,30	1,00	4,83	3,83
3	48,10	2,08	4,70	2,62	52,30	4,00	8,11	4,11
4	58,40	1,37	6,10	4,73	42,30	2,35	8,28	5,93
5	58,40	0,76	5,59	4,83	50,70	3,54	8,09	4,55
6	35,52	1,62	3,37	1,75	44,20	2,00	3,49	1,49
7	47,97	1,86	4,18	2,32	44,40	2,67	3,27	0,60
8	41,91	0,00	1,35	1,35	38,39	0,00	1,59	1,59
Average	49,34	—	—	3,15	48,22	—	—	3,03

The results of the second series of biochemical investigations carried out on the mucosa of the small intestine of rats whose mitotic activity does not change under the action of adrenalin are presented in Table 4. As one can see from this table, introduction of adrenalin intramuscularly does not produce any noticeable effect on oxygen consumption and lactic acid accumulation under aerobic conditions 2 hours after injection.

Thus, during the period of maximal inhibition of mitoses in corneal epithelium by adrenalin, oxygen consumption and lactic acid accumulation under aerobic conditions in the cornea as well as in the intestine remain unchanged. Further studies of the effect of adrenalin on individual aspects of metabolism in its relation to its varied action on mitotic activity of tissue are necessary.

SUMMARY

Intramuscular injection of adrenalin causes a significant decrease of mitotic activity in the cornea of rats and produces no effect on the epithelium of the crypts of the small intestine. Similar results were obtained after injection of adrenalin simultaneously with colchicine. During the period of maximal inhibition of mitoses in the corneal epithelium by adrenalin the consumption of oxygen and accumulation of lactic acid under aerobic conditions remain unchanged both in the cornea and in the intestine.

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