EFFECT OF THYROXINE ON THE ACTION OF ADRENALIN ON CELL DIVISION

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After administration of thyroxine for three days to rats, depression of mitosis by adrenalin in the corneal epithelium developed later than in animals not receiving thyroxine, while in the small intestine and bone marrow the depression of mitosis became more marked. The action of adrenalin on division of the cells of the small intestine was shown to be dependent on the location of the cells in the crypt.

Although the action of adrenalin on cell division in various tissues has often been investigated, very little work has been done to study the possible effects of other hormones on it. Yet it is quite obvious that hormonal correlations are one of the most important aspects of the study of hormonal regulation of cell reproduction in the organism.

The writers have shown previously [8] that disturbance of thyroid function in rats by methylthiouracil leads to changes in the action of adrenalin on cell division.

In the investigation described below the effect of adrenalin was studied on cell division in the epithelium of the cornea and small intestine and in the bone marrow after preliminary administration of thyroxine to rats.

EXPERIMENTAL METHOD

Male albino rats with a mean weight of 170 g were used in the experiment. The control consisted of 10 animals (group 1), 5 of which were sacrificed at the beginning and 5 at the end of the experiment. Ten animals received physiological saline and were sacrificed 15 (group 2) and 30 min (group 3) after the beginning of the experiment. Twenty animals received an intraperitoneal injection of adrenalin in a dose of 2 μg/g body weight at 9 a.m. and they were sacrificed 15 (group 4), 30 (group 5), 50 min (group 6) and 1 h 50 min (group 7) after injection of the hormone. An intraperitoneal injection of L-thyroxine in a dose of 10 μg/100 g body weight was given daily for 3 days to 25 rats, of which 5 (group 8) were the control, while the rest received adrenalin in the above-mentioned dose on the 4th day of the experiment and were sacrificed 15 (group 9), 30 (group 10), 50 min (11) and 1 h 50 min (group 12) after the beginning of the experiment.

The mitotic index (MI) in the cornea was calculated for 25,000-30,000 epithelial cells in each case in total two-dimensional preparations. MI for the bone marrow was calculated for 4000-5000 cells in impression preparations stained with aceto-orcein. The overall MI (OMI) for the epithelium of the small intestine was calculated for 4000-5000 cells in 50 longitudinally divided crypts. In addition, MI was calculated separately in the lower, middle, and upper third of the crypt in the direction from its base to its neck (MI_1, MI_2, and MI_3, respectively). MI was expressed in promille. In each case the number of cells in individual phases of mitosis was determined.

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TABLE 1. MI (in ‰) and Index of Phases of Mitosis (PI) in Tissues after Injection of Adrenalin into Rats Receiving and Not Receiving Thyroxine

Group of animals	Cornea		Bone marrow		Small intestine	
	MI	PI	MI	PI	MI	PI
1st	7,87	4,9	18,5	1,3	19,7	1,0
2nd	8,18	2,1	18,0	1,8	19,7	1,0
3rd	6,96	4,3	20,0	4,0	20,6	0,9
4th	4,70 P_1=0,050	0,6	15,8 P_1=0,011	0,6	20,3	1,0
5th	2,59 P_1=0,008	0,5	17,2	0,7	9,9	0,9
6th	2,15 P_1=0,001	0,2	23,9 P_1=0,001	0,9	15,0 P_1=0,009	1,1
7th	4,43 P_1=0,050	0,6	21,1	0,9	24,7 P_1=0,050	0,8
8th	10,68 P_1=0,050	6,3	21,2 P_1=0,027	2,2	23,3 P_1=0,050	1,1
9th	7,11	0,7	17,7 P_8=0,002	0,5	16,5 P_8=0,007	0,8
10th	8,17	0,8	20,9	1,3	11,8 P_8=0,001	1,2
11th	2,33 P_8=0,023	0,6	18,1 P_8=0,025	1,9	11,3 P_8=0,001	1,7

Note. Here and in Table 2 the number equated with P shows the level of significance of the difference between the result and that for the group whose number is indicated by the subscript to P.

EXPERIMENTAL RESULTS

It will be seen from Table 1 that 15 min (group 4) after injection of adrenalin the mitotic activity in the corneal epithelium was considerably reduced. After the experiment had continued for 50 min (group 6), inhibition of cell division was at its maximum. Inhibition of mitosis was accompanied by a sharp decrease in the number of prophases and metaphases. A slight increase in MI and a simultaneous increase in the number of the first two phases of mitoses were observed 1 h 50 min after the beginning of the experiment (group 7). Administration of thyroxine to the rats for three days caused an increase in MI in the corneal cells. Injection of adrenalin into these animals led to a slight decrease in mitotic activity in the cornea after 30 min (groups 9 and 10), but this decrease was not significant. Great variability in MI was observed at these periods of the experiment (from 0.35 to 15.00 ‰). Marked inhibition of mitosis by the action of adrenalin in animals receiving thyroxine was observed only 50 min after injection of the hormone (group 11), i.e., 35 min later than in animals not receiving thyroxine. From 50 min to 1 h 50 min after injection of adrenalin into animals receiving thyroxine 4 of the 5 rats died with manifestations of severe dyspnea, apathy, and adynamia and increased perspiration. The value of MI for the corneal cells of the surviving rats was 0.20 ‰ (phase index 2.0).

In the bone marrow 15 min after injection of adrenalin into the animals of group 4, a definite decrease in mitotic activity and a decrease in the number of the first two phases of mitosis were observed (Table 1). The mitotic index of the bone marrow cells 50 min after the end of the experiment (group 2) was increased above the control value, and the normal ratio between the phases of mitosis was restored. The mitotic index 1 h 50 min after injection of the hormone (group 7) was indistinguishable from the control. Injection of adrenalin into animals receiving thyroxine likewise led to inhibition of mitotic activity in the bone marrow and to a decrease in the number of prophases and metaphases, but in this case the effect was observed much sooner than in animals not receiving thyroxine: 15 min after the beginning of the experiment (group 9). After 30 min of action of the hormone (group 10) the normal MI was restored, but after 50 min of the experiment (group 11) inhibition of cell division in the bone marrow had again developed. In an animal which survived 1 h 50 min from the beginning of the experiment MI was 15.9 ‰ (phase index 0.5).

After injection of adrenalin into rats not receiving thyroxine, a decrease in MI of the epithelial cells of the small intesting was observed, starting 50 min after the beginning of the experiment (group 6, Table 1). At the next time of observation (1 h 50 min after the beginning of the experiment) the number of mitoses in

TABLE 2. MI (in ‰) in Different Parts of the Crypt of the Small Intestine after Injection of Adrenalin into Rats Receiving and Not Receiving Thyroxine

Group of animals	MI_1	MI_2	MI_3
1st	27,5	22,7	10,3 $P_{MI_{1,2}}=0,006$
6th	22,6 (—18)	18,7 (—18)	3,7 (—64) $P_{MI_{1,2}}=0,001$ $P_1=0,032$
7th	32,7 (+19)	29,4 (+29)	11,8 $P_{MI_{1,2}}=0,001$
8th	33,4 (+21) $P_1=0,050$	27,3 (+20)	9,5 (—8) $P_{MI_{1,2}}=0,001$
10th	18,6 (—44) $P_s=0,005$	14,2 (—48) $P_s=0,005$	2,8 (—71) $P_{MI_{1,2}}=0,001$ $P_s=0,013$
11th	19,5 (—42) $P_s=0,013$	11,9 (—56) $P_{MI_1}=0,050$ $P_s=0,007$	2,5 (—74) $P_{MI_1}=0,003$ $P_s=0,013$

Note. Numbers in parentheses denote degree of changes (in %) compared with control (intact animals and animals receiving thyroxine only).

the crypts was higher than normal (group 7). No significant changes in the relative percentages of the phases of mitosis could be seen. Injections of thyroxine into the animals stimulated division of the crypt cells. Injection of adrenalin into animals after injections of thyroxine caused a decrease in OMI in the crypt cells as early as 15 min after the beginning of the experiment (group 9), and later (30 and 50 min) the inhibition of cell proliferation in the epithelium of the small intesting was more marked still. In the rats which still survived after 1 h 50 min, OMI was 17.6 ‰ (phase index 1.7). It is clear from Table 2 that the inhibition of mitotic activity in animals not receiving thyroxine after administration of adrenalin was greatest in the upper third of the crypts of the small intestine. It may be considered that a decrease in the number of mitoses 50 min after the beginning of the experiment also occurred in other parts of the crypt, because 1 h 50 min after injection of the hormone not only MI_3, but also MI_1 and MI_2 were significantly increased. In animals receiving thyroxine, a marked decrease in mitotic activity after injection of adrenalin was observed in all parts of the crypt, but the decrease was greatest in the upper third.

The results of this investigation are in agreement with existing ideas of the inhibition of cell division in the corneal epithelium of normal animals by adrenalin. They also confirmed the fact previously discovered by the writers that brief suppression of cell division by adrenalin takes place in the bone marrow [7]. It must be pointed out that inhibition of mitosis by adrenalin in the bone marrow was much shorter in duration than in the corneal epithelium, and an increase in mitotic activity took place when the $G_2 \rightarrow M$ block had ended. The character of the effect of adrenalin on cell division in the epithelium of the small intestine is still a matter for discussion. Some investigators [1, 2, 4, 5, 9] did not observe a decrease in mitotic activity in this tissue in normal animals under the influence of adrenalin. Other workers [3, 6], however, did observe a decrease. Results of the present investigation indicate that adrenalin can inhibit cell division in the crypts of the small intestine, but that the decrease in activity in the intestine continues for only a short time, not as long as in the corneal epithelium, and it is followed by a fresh wave of increase in the number of mitoses. It was also shown that the changes in cell reproduction in the epithelium of the small intestine induced by adrenalin depend on differentiation of the crypt cells. The action of adrenalin in inhibiting mitosis is manifested much more strongly in the upper third of the crypt where, according to some observations [10-13], the most highly differentiated cells are found.

Thyroid hormone is thus a factor capable of modifying the response of cell division to adrenalin; the end result of the interaction between these hormones differs in different tissues.

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