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SPECIFICITY OF THE EFFECT OF GROWTH HORMONE ON THE DNA CONTENT IN LYMPHOCYTE NUCLEI OF HYPOPHYSECTOMIZED RATS

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The effect of growth hormone on the DNA content in lymphocyte nuclei in the thymus, spleen, and lymph nodes was investigated cytophotometrically. In hypophysectomized rats growth hormone was shown to increase the DNA content in nuclei of medium lymphocytes of these organs but did not change its content in small lymphocytes. Lymphocytes of the thymus were most sensitive to the action of growth hormone. The DNA content in the nuclei of these cells increased as early as 1 h after injection of the hormone and reached its maximum after 4 h. Other hormones with anabolic action (insulin, thyroxine, testosterone) caused no increase in DNA in the thymocyte nuclei during this period. It is concluded that growth hormone has high affinity for cells of the lymphoid organs and, in particular, for thymocytes (medium lymphocytes of the thymus).

KEY WORDS: growth hormone; nuclei of lymphocytes; thymocytes; hypophysectomy.

Investigations in the writers' laboratory have shown that a few months after hypophysectomy in rats only growth hormone and, to a far lesser degree, thyrotropic hormone can increase the weight of the thymus significantly when administered daily for 10 days [2,3]. No other hormones had this property. The special role of growth hormone in relation to lymphoid tissue is reflected in the stimulation of incorporation of labeled precursors into proteins and nucleic acids of lymphocytes [8] and also the increase in the mitotic activity of lymphocytes [9]. It has recently been shown that thymocytes specifically bind growth hormone [4].

In this investigation a comparative cytophotometric analysis was made of the specificity of the early effect of growth hormone on DNA synthesis in different types of cells in several lymphoid organs.

EXPERIMENTAL METHOD

Female Wistar rats weighing 65-70 g were hypophysectomized by the transauricular route [1]. The animals were used in the experiments 14 days after the operation. In the experiments of series I the rats were divided into four groups with 10 animals in each group. Group 1 served as the control, the animals of groups 2 and 3 received a single intraperitoneal injection of growth hormone in a dose of 200 µg, dissolved in 0.25 ml physiological saline and were killed 4 and 6 h later, respectively, and the rats of group 4 were intact animals of

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TABLE 1. Effect of Growth Hormone on DNA Content (in conventional units) in Lymphocyte Nuclei from Different Organs ($M \pm m$)

Animals	Thymus		Spleen		Lymph nodes	
	Medium lymphocytes	Small lymphocytes	Medium lymphocytes	Small lymphocytes	Medium lymphocytes	Small lymphocytes
Hypophysectomized	0,375 \pm 0,014	0,329 \pm 0,018	0,302 \pm 0,024	0,363 \pm 0,024	0,247 \pm 0,014	0,324 \pm 0,017
Hypophysectomized, 4 h after injection of hormone p	0,824 \pm 0,051 <0,001	0,380 \pm 0,028 >0,05	0,533 \pm 0,041 <0,01	0,390 \pm 0,012 >0,05	0,465 \pm 0,029 <0,001	0,357 \pm 0,046 >0,05
Hypophysectomized, 6 h after injection of hormone p	0,851 \pm 0,068 <0,001	0,367 \pm 0,031 >0,05	0,485 \pm 0,027 <0,01	0,387 \pm 0,019 >0,05	0,640 \pm 0,052 <0,001	0,377 \pm 0,023 >0,05
Intact P	0,790 \pm 0,025 <0,001	0,421 \pm 0,031 >0,05	0,446 \pm 0,038 <0,05	0,380 \pm 0,032 >0,05	0,305 \pm 0,024 <0,05	0,371 \pm 0,036 >0,05

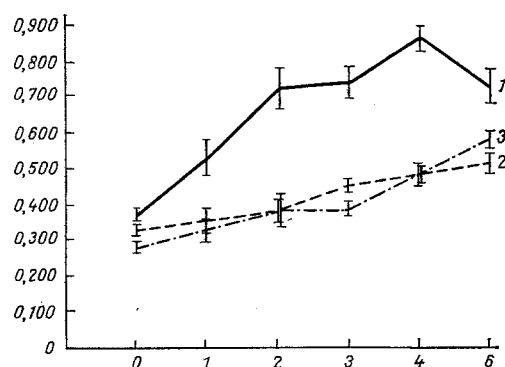


Fig. 1. Dynamics of DNA content in nuclei of medium lymphocytes from various organs of hypophysectomized rats after injection of growth hormone: 1) thymus, 2) spleen, 3) lymph nodes. Ordinate, DNA content (in conventional units); abscissa, time (in h).

the same weight and sex. The rats of groups 1 and 4 each received in injection of 0.25 ml physiological saline. In the experiments of series II hypophysectomized rats were given the same dose of growth hormone and were killed 1,2,3,4, and 6 h later, eight animals at each time. In the experiments of series III (eight animals in each group) hypophysectomized rats of group 1 received an injection of physiological saline, those of group 2 received growth hormone (200 μ g), group 3 insulin (2 units), group 4 thyroxine (30 μ g), and group 5 testosterone propionate (5 mg). The animals were killed 4 h after receiving the hormone. In the experiments of series I and II pieces of thymus and spleen and lymph nodes were fixed in Carnoy's fluid and embedded in paraffin wax, after which sections were cut to a thickness of 5 μ . In the experiments of series III squash preparations were made from the organs and fixed in methanol. DNA in the lymphocyte nuclei was revealed by Feulgen's and Rossenbeck's methods. Cytophotometric determination of the DNA content in the nuclei of the lymphocytes (small cells 5-6 μ and medium-sized 8-9 μ in diameter) was carried out on the MUF-5 instrument at a wavelength of 546 nm by a probe method and expressed in conventional units relative to a standard. Fifty cells from each animal were investigated photometrically. The significance of differences between the results was assessed by Student's criterion.

EXPERIMENTAL RESULTS

In the control rats a decrease in the DNA content (Table 1) was observed after hypophysectomy in the medium lymphocytes of the thymus, spleen, and lymph nodes ($P < 0.001$). The results reflect delay of synthetic processes in the nuclei of the medium lymphocytes and are in agreement with observations of other workers who found a decrease in proliferative activity, lengthening of the mitotic cycle, and a decrease in the number of cells ready for division after hypophysectomy [5,6]. No statistically significant changes in the DNA content were found in the small lymphocytes of any of the organs studied. After injection of growth hormone into the hypophysectomized rats the DNA content was increased in the medium lymphocytes of the thymus, spleen, and

lymph nodes both 4 and 6 h after injection of the hormone ($P < 0.001$). No difference was found in the DNA content in the small lymphocytes. The results are in agreement with investigations by other workers who observed an increase in DNA synthesis in the thymus, spleen, and lymph nodes autoradiographically 6 h after injection of growth hormone [8]. These workers did not carry out any earlier tests.

In the experiments of series II an attempt was made to discover the earliest manifestations of the action of growth hormone in the nuclei of medium lymphocytes of the thymus, spleen, and lymph nodes (Fig. 1). Injection of growth hormone into the hypophysectomized rats caused a significant increase in the DNA content in the medium lymphocytes of the spleen and lymph nodes, starting from 2-3 h after injection of the hormone and reaching a maximum at 6 h. In the thymocytes a statistically significant increase in the DNA content was observed as early as 1 h after injection of the hormone, and the increase reached a maximum after 4 h.

Activation of DNA synthesis by growth hormone in cell nuclei of different tissues in hypophysectomized animals is known to begin after different time intervals. This effect was observed, in particular, by Goldspink and Goldberg [7], who investigated DNA synthesis in nuclei of hepatocytes, epithelial cells of the kidney tubules, and muscle fibers with the aid of thymidine- ^3H . However, in the present experiments the cells were from an identical tissue.

In the experiments of series III the effect of growth hormone was compared with that of other hormones with an anabolic action on the DNA content in medium lymphocytes of the thymus. The DNA content in the nuclei was determined 4 h after injection of the hormones. Injection of insulin, testosterone, and thyroxine into hypophysectomized rats caused no appreciable increase in the DNA content in the thymocyte nuclei and only growth hormone gave a significant increase in the DNA content (0.367 ± 0.027 compared with 0.170 ± 0.022 in the control).

Growth hormone thus stimulates DNA synthesis only in nuclei of medium lymphocytes of hypophysectomized rats and has no appreciable effect on the DNA level in the nuclei of small lymphocytes. Activation of DNA synthesis arises at different times depending on the organ to which the medium lymphocytes belong. The action of the hormone is manifested soonest, 1 h after injection, in thymocytes.

The high sensitivity of thymocytes to growth hormone deserves special attention, considering the important role of the thymus in the lymphoid system. In the period of strongest action of growth hormone (4 h after injection) other hormones with anabolic properties have no effect on the DNA content in the medium lymphocytes of the thymus, thus showing that growth hormone exhibits definite specificity toward lymphoid tissue.

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