INFLUENCE OF INSUFFICIENCY AND EXCESS OF THYROID HORMONES IN THE RABBIT BODY UPON THE ACTIVITY OF A NONSPECIFIC INHIBITOR OF HYALURONIDASE

(UDC 612.444 : 612.015.16 : 577.154.365.025.3)

P. I. Fogel

Transcarpathian Scientific Research Institute for the Protection of Mothers and Children (Director, Candidate of Medical Sciences Ya. V. Stovbunenko-Zaichenko),* Mukachevo
(Presented by Member of the Academy of Medical Sciences of the USSR V. V. Parin)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 61, No. 5, pp. 51-53, May, 1966
Original article submitted November 21, 1964

Recently more and more communications on the characteristic changes in the amount and distribution of mucopolysaccharides in the ground substance of connective tissue and blood serum during disfunctions of the thyroid gland have appeared [1-3, 5, 9, 14, 19]. Researchers note that in the presence of thyroid gland insufficiency, an accumulation of hyaluronic acid in the connective tissue is observed, while the hyperthyroid state of the organism is accompanied by intensified decomposition of it. These changes are associated with the regulating influence of the thyroid hormones upon the mucopolysaccharide metabolism [15, 16, 19].

It has been established that the only specific factor regulating the decomposition and depolymerization of hyaluronic acid is the enzyme hyaluronidase [9, 11, 17, 18]. However, in blood serum and ascitic fluid of man and animals, a protein substance evidently possessing the properties of an enzyme, which inhibits the action of hyaluronidase, is present [10, 12, 13]. This substance is denoted in the literature as a nonspecific inhibitor of hyaluronidase (or nonspecific antihyaluronidase), in contrast to the specific antihyaluronidase, which neutralizes hyaluronidase manufactured by a strictly definite type and species of bacterial culture [8]. Consequently, the physiological activity of hyaluronidase in the organism is directly related to the activity of its inhibitor – nonspecific antihyaluronidase.

The question of the mechanism of the action of the thyroid hormones upon the mucopolysaccharide metabolism has been little studied. In particular, it has been insufficiently determined whether the thyroid hormones influence the polysaccharides directly or through hyaluronidase.

The purpose of this work was to study the influence of insufficiency and excess of thyroid hormones in the rabbit organism upon the activity of the nonspecific inhibitor of hyaluronidase.

EXPERIMENTAL METHOD

The experiments were conducted on 40 sexually mature female rabbits weighing 2-2.7 kg, which were divided into four groups (10 animals in each). The first group was the control. The rabbits of the other three groups for a period of three weeks daily received peroral administrations of 0.15 g of 6-methylthiouracil per kg of weight (second group) or thyroidin in doses of 0.1 g (third group) and 0.2 g per kg of body weight (fourth group).

The effectiveness of the administration of 6-methylthiouracil and thyroidin was monitored by determining the weight of the thyroid gland and weight of the animal, as well as by investigating the amount of protein-bound iodine (PBI) in the blood plasma according to the method of I. I. Litvin [6].

* Deceased.

TABLE 1. Activity of Nonspecific Hyaluronidase Inhibitor (in %) in the Blood Serum of Hypothyroid Animals (M ± m)

Group of animals	No. of ani.	Fluctuations	Activity of inhibitor	P
First (control)	10	25,9—36,8	30,0±1,17	
Second	10	33,5—48,6	39,7±1,46	<0,001

TABLE 2. Activity of Nonspecific Hyaluronidase Inhibitor (in %) in Blood Serum of Hyperthyroid Animals (M ± m)

Group of animals	No. of ani.	Fluctuations	Activity of inhibitor	P*
First (control)	10	25,9—36,8	30,0±1,17	
Third	10	18,3—30,3	26,5±1,32	<0,1
Fourth	10	7,2—28,3	20,3±1,77	<0,001

* With respect to control.

The activity of the nonspecific hyaluronidase inhibitor in the blood serum was established according to the viscosimetric method of Haas [13] in the modification of M. S. Ignatova [4]. The result was expressed in percent inhibition of serum hyaluronidase in comparison with a sample containing no antihyaluronidase, and calculated according to the modified [12] Haas formula:

$$A = \frac{P - P_0}{P} \cdot 100\% ,$$

where A is the activity of nonspecific antihyaluronidase; P_0 is the half-time of decrease in the viscosity of hyaluronic acid under the influence of hyaluronidase; P is the time in which the viscosity of hyaluronic acid decreases also by half, but under the influence of hyaluronidase whose action is inhibited by the test blood serum.

The dilutions of hyaluronidase and hyaluronic acid were selected so that the half-time would always be equal to 300 sec ± 10%. Soviet preparations of lidase and luronite were used as the sources of hyaluronidase and hyaluronic acid. The blood for investigation of the antihyaluronidase activity was obtained from the marginal vein of the ear of the rabbit, and that for the determination of the PBI content from the carotid artery. The results were treated statistically [7].

RESULTS

Signs of hypofunction of the thyroid gland appeared in the experimental animals of the second group from the eighth to tenth days from the beginning of administration of 6-methylthiouracil; the rabbits were lethargic and lost their appetite. By the end of the experiment, the weight of the animals of this group had increased by an average of 8.4%, while the ratio of the weight of the thyroid gland (in milligrams) to their body weight (in kilograms) had increased to 120 ± 10.7, while the weight of the control animals was essentially unchanged, and the indicated coefficient was only 45.4 ± 1.34 ($P < 0.001$). The PBI content in the blood plasma of the animals of the second group had decreased almost four-fold by the end of the experiment in comparison with its concentration in the control (0.9 ± 0.22 μg % and 3.3 ± 0.18 μg %, respectively; $P < 0.001$).

The antihyaluronidase activity of the blood serum of hypothyroid animals increased (Table 1).

As can be seen from Table 1, the administration of 6-methylthiouracil to the experimental rabbits induced a significant increase in the activity of the nonspecific antihyaluronidase in the blood serum. Consequently, under conditions of insufficiency of thyroid hormones in the animal, the hyaluronidase-inhibiting property of the blood serum is intensified.

Feeding the experimental animals with thyroidin led to the opposite results. Rabbits that received this preparation were already distinguished from the controls on the seventh to ninth days of the experiment by great mobility and restlessness; the fur became disheveled, and lost its usual luster; the animals exhibited tachycardia and tremors; some of them were aggressive. These changes depended upon the dose of thyroidin. At a dose of the preparation of 0.1 g per kg of weight, the symptoms of hyperthyroidism appeared to a moderate degree, while at twice the dose (0.2 g per kg), the signs of thyrotoxicosis were pronounced. By the end of the experiment, the weight of the rabbits of the third and fourth groups had decreased in comparison with the original by 14.9 and 20%, respectively. The ratio of the weight of the thyroid gland to the body weight also decreased (30.4 ± 1.87 and 27.0 ± 1.26; $P < 0.001$) in comparison with the control group. The amount of PBI in the blood plasma of these animals increased on the average to 8.9 ± 0.97 μg % ($P < 0.001$) in the third group and to 27.9 ± 2.37 μg % ($P < 0.001$) in the fourth group, i.e., moderate hyperthyroidization of the rabbits led to a 2.7-fold increase in the PBI level, while intensified hyperthyroidization led to an 8.4-fold increase in comparison with the control.

The activity of nonspecific antihyaluronidase in the hyperthyroid animals decreased in comparison with the controls (Table 2).

From Table 2 it is evident that in the case of moderate hyperthyroidization of the animals, only some tendency toward a decrease in the antihyaluronidase activity of the blood serum was noted, while their intensified hyperthyroidization led to a pronounced inhibition of this activity. Thus, the results of our experiments indicated that there is an inverse relationship between the antihyaluronidase activity of the blood serum and the concentration of thyroid hormones in the animal's body. Under conditions of insufficiency of thyroid hormones in the rabbit organism, the antihyaluronidase activity increases, while in the presence of a pronounced excess of these hormones in the blood and tissues of the animal, the activity of the hyaluronidase inhibitor, on the contrary, decreases. The data obtained permit us to assume that the hormones of the thyroid gland take part in regulating the mucopolysaccharide metabolism by changing the activity of the hyaluronidase inhibitor.

SUMMARY

The object of study was the influence of thyroid gland hormones on the activity of nonspecific antihyaluronidase in rabbits. For this purpose, a hypothyroid state of thyrotoxicosis of a variable degree was induced in the animals. It was found that there was an inverse relationship between the antihyaluronidase activity of the blood serum and the concentration of protein-bound iodine in the blood plasma. With a decrease in protein-bound iodine, the activity of hyaluronidase grew, and with an increase in its level the activity of the inhibitor lessened. Apparently, thyroid hormones take part in the regulation of mucopolysaccharide metabolism through a change in the activity of the nonspecific inhibitor of hyaluronidase.

LITERATURE CITED

1. G. V. Alekseeva, Izv. Sibirsk. Otdeleniya AN SSSR, No. 8, Seriya Biol.-Med. Nauk, No. 2 (1963), p. 108.
2. M. E. Barabash, In book: Modern Problems of Dermatology [in Russian], Kiev, Vol. 2 (1958), p. 191.
3. S. R. Barvinskii and V. M. Urin, Probl. Éndokrinol., No. 6 (1963), p. 61.
4. M. S. Ignatova, Pediatriya, No. 11 (1957), p. 40.
5. V. P. Leitan, Arkh. Anat., Gistol. i Émbriol., No. 2 (1961), p. 24.
6. I. I. Litvin, Ukr. Biokhim. Zh., No. 3 (1954), p. 343.
7. I. A. Oivin, Pat. Fiziol., No. 4 (1960), p. 76.
8. N. N. Rubel', In book: Droplet Infections of Children [in Russian], Leningrad (1953), p. 108.
9. E. Chain and E. S. Duthie, Brit. J. Exp. Path., Vol. 21 (1940), p. 324.
10. A. Dorfman, M. L. Ott, and R. Whitney, J. Biol. Chem., Vol. 174 (1948), p. 621.
11. J. H. Fessler, Biochem. J., Vol. 76 (1960), p. 132.
12. D. Glick and F. Gollan, J. Infect. Dis., Vol. 83 (1948), p. 200.
13. E. Haas, J. Biol. Chem., Vol. 163 (1946), p. 63.
14. S. Krompecher et al., Nature, Vol. 189 (1961), p. 670.
15. S. Krompecher, M. B. Lázlo, and E. H. Oláh, Ibid., Vol. 194 (1962), p. 778.
16. M. B. Lázlo and S. Krompecher, Z. Anat. Ent.-Wickl.-Gesch., Bd. 123, S. 87 (1962).
17. D. McClean and C. W. Hale, Biochem. J., Vol. 35 (1941), p. 159.
18. D. McClean, Ibid., Vol. 37 (1943), p. 169.
19. S. Schiller, J. Chron. Dis., Vol. 16 (1963), p. 291.