THE RELATIONSHIP OF THE HYALURONIDASE AND ANTIHYALURONIDASE ACTIVITY OF THE RETINA TO THE INTENSITY OF A LIGHT STIMULUS

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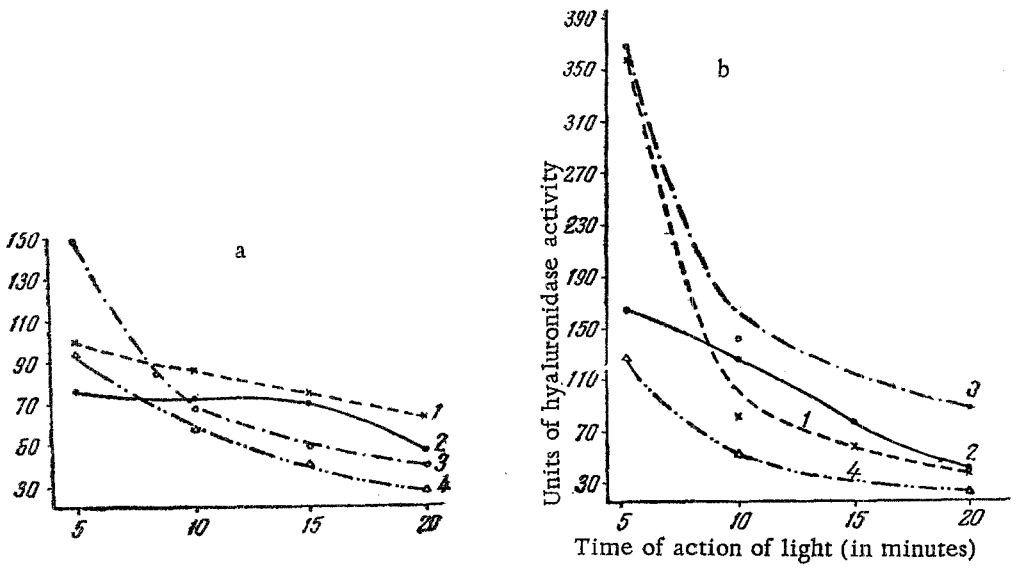
(Received March 5, 1958. Presented by Active Member Acad. Med. Sci. USSR, S. E. Severin)

The enzyme hyaluronidase takes an active part in many processes in the body associated with the application of the permeability of the "basic substance" of connective tissue and with a reduction in the viscosity of certain fluids. The hyaluronidase of bacteria is widespread as a factor facilitating their entry into the animal body. A connection has been found between hyaluronidase and fertilization (McClean [6]; Duran-Reynals [5]), and it has been found to affect the permeability of the capillaries (B. N. Mogil'nitskii [3]).

The hyaluronidase of the ciliary body is important in the regulation of the circulation of the intraocular fluid and also in the maintenance of the transparency of the optic media of the eye (Meyer and Palmer [7]; Meyer, Smyth and Gallardo [8]).

Kh. S. Koshtoyants [2] demonstrated that hyaluronidase took part in nerve excitation. We ourselves reported [1] the secretion of hyaluronidase by the retina of the frog.

In the present investigation we studied the connection between the hyaluronidase activity of the retina and its functional state, and also the causes of the fall in the hyaluronidase activity of the retina during prolonged, continuous action of a light stimulus.



Changes in the activity of the hyaluronidase secreted by the retina of frogs (a) and rabbits (b) depending on the intensity of the light stimulus and the time of its action. Conventional signs: 1) during strong stimulation by a 15 w lamp; 2) 1 w; 3) 40 w; 4) 150 w.

EXPERIMENTAL METHOD

The material used in the investigation was prepared from the enucleated eyes of animals (frogs and rabbits). By an incision along the limbus the posterior half was removed, consisting of part of the eyeball, in the form of a shallow cup inside which were the retina and the pigmented membrane. The final preparation from the eye was placed in a vessel containing a nutrient solution.

The activity of the hyaluronidase thus secreted was determined at definite intervals of time in the nutrient solution by means of viscosimetry, and was expressed in conventional units.

Determination of the activity of the antihyaluronidase factor was based on its power to reduce the activity of a standard solution consisting of testicular extract with a certain concentration of the enzyme hyaluronidase.

Nutrient salt solution taken from that in which the retinas were kept, and tested for antihyaluronidase activity, was mixed with testicular extract and allowed to stand for 15-20 minutes.

The hyaluronidase in the mixture was determined before and after this operation; the antihyaluronidase activity was estimated by the difference and was expressed as conventional units and as true units of concentration of enzyme and antifactor. We made use of the graph of the relationship between units of activity and the logarithm of the enzyme concentration, as drawn up by Swyer and Emmens [9].

TABLE 1

The Effect of Alternate Stimulation and Rest on the Hyaluronidase Activity of the Retina

Record no.	Hyaluronidase activity in conventional units							
	right retina				left retina			
	light	dark	light	dark	light	dark	light	dark
	exposure for 5 minutes							
97	149	30	127	33	24	130	30	116
98	120	50	106	47	39	128	47	120
99	134	46	110	46	32	110	43	105
100	137	20	106	18	35	106	18	104
101	118	23	95	22	30	98	17	92

TABLE 2

Hyaluronidase and Antihyaluronidase Activity of the Nutrient Solution with Illumination for 20 Minutes

Record no.	Without changing the nutrient solution		Nutrient solution with exposure for 10 minutes	
	concentration		concentration	
	of hyaluronidase	of antifactor	of hyaluronidase	of antifactor
44	—	—	1,09	2,12
56	1,57	1,80	1,35	1,18
59	1,66	1,32	1,37	1,66
108	1,43	0 76	1,22	1,64
110	1,68	2.10	1,60	2,00
113	1,35	1 40	1,35	1,60
118	1,41	1,70	1,26	1,60
119	1.40	1,80	1,30	2.00
Mean	1,50	1,55	1,32	1,80

TABLE 3

Hyaluronidase and Antihyaluronidase Activity of the Nutrient Solution in Succesive Determinations in the Course of 20 Minutes

Record no.	Enzyme	Exch. of nutrient solution		No change of nutrient solution
		5 minutes (the first)	10 minutes (the last)	
56	Hyaluronidase	2,50	1,35	1,57
	Antihyaluronidase	3,0	1,8	1,8
111	Hyaluronidase	2,13	1,35	1,35
	Antihyaluronidase	2,14	1,60	1,4

EXPERIMENTAL RESULTS

The retina of the frogs was stimulated by interrupted light, alternating every 5 minutes with darkness. In this way the repeated, brief action of the stimulus together with equal intervals of rest was achieved. The results of some of the experiments are shown in Table 1.

The hyaluronidase activity of the retina was increased during illumination and decreased when the retina was darkened. These results suggested that the hyaluronidase activity depends on the functional state of the retina.

The relationship of the hyaluronidase activity of the retina to the intensity of the light stimulus was next studied. The source of light used was electric lamps of different power: 1, 15, 40 and 150 w. The intensity of illumination created by the lamps was measured with an illuminometer, and was respectively 14-18, 140-240, 380-540 and 1143-1900 lux.

In 54 experiments with preparations of frogs' eyes and 21 experiments with preparations of rabbits' eyes it was shown that the activity of the secreted hyaluronidase depends on the intensity of the light stimulus.

With an increase in the intensity of illumination from 14 to 540 lux the hyaluronidase acitivy increased. The highest hyaluronidase activity was observed at an intensity of illumination of 380-540 lux.

The results obtained may be compared with those of Z. V. Smelyanskii and V. V. Meshkova [4], according to whom an intensity of illumination of 400-500 lux is optimal for the eye.

A further increase in the intensity of illumination to 1143-1900 lux led to a considerable fall in the activity of the secreted hyaluronidase. We looked upon this as the manifestation of a specific physiological state of the nerve cells in response to the action of a strong stimulus.

In a series of experiments the activity of the secreted hyaluronidase was determined repeatedly, every 5 minutes during the interrupted action of light for 20 minutes (see Figure).

In 34 experiments with preparations from frogs' eyes and in 19 experiments with preparations from rabbits' eyes it was found that the prolonged, interrupted action of light gradually reduced the hyaluronidase activity in the nutrient solution. The rate of fall of this activity depended on the intensity of light used.

Exposure to a light stimulus of 14 to 240 lux gave, in the case of the frog's retina, a low initial hyaluronidase activity, which fell slowly and slightly during the 20 minutes of illumination. When the eye preparation was illuminated by 380-540 lux the rate of fall of hyaluronidase activity was noticeably altered: the high initial activity fell rapidly and considerably during the period of time.

A light stimulus of 1143-1900 lux also shoed a rapid fall in the initial activity.

In the experiments with the preparations from rabbits' eyes similar results were obtained (see Figure).

Thus the higher the initial activity of the hyaluronidase, the quicker its subsequent fall. We thought that the hyaluronidase activity of the solution containing the eye preparations illuminated interruptedly for 20 minutes would be higher than that of the solution in which the preparation had been illuminated only for the first 5 minutes. The activity was found to be lower, however, and in the majority of the experiments it was lower than during the first 5 minutes of action of the light.

In a series of experiments, lengthening the period of light stimulation to 30 minutes led to a further fall in the hyaluronidase activity.

To explain this phenomenon it was necessary to determine the antihyaluronidase factor in the nutrient solutions. For this purpose solutions were used which had contained preparations from rabbits' eyes exposed for 20 minutes to the action of light, or in which the preparations had spent only the last 10 minutes of a total exposure of 20 minutes to the light (Table 2).

It can be seen from Table 2 that antihyaluronidase activity was present in the nutrient solution containing the illuminated eye preparation, and as investigation showed, this factor was thermolabile.

In a series of experiments the activity of hyaluronidase and antifactor was determined several times in succession in the course of 20 minutes. The results of two of these experiments are shown in Table 3.

The gradual fall in the hyaluronidase activity of the nutrient solution in response to interrupted exposure of the retina to light for 20-30 minutes was not connected with loss of viability. This was shown by experiments in which light and dark were alternated every 5 minutes in the course of 20 minutes, in which corresponding fluctuations of hyaluronidase activity took place: light increased the hyaluronidase activity of the retina, but darkness reduced it. The level of activity of the hyaluronidase reaction in response to illumination remained within the usual limits (see Table 1).

The gradual fall in hyaluronidase activity of the nutrient medium during continuous exposure to light may be explained by the secretion by the retina of an antihyaluronidase factor, which we have discovered. The concentration of antifactor in the nutrient solution in which the surviving preparations of animals' eyes were kept underwent regular changes.

This antifactor is capable of inactivating hyaluronidase, by destroying it or by combining with it to form an inactive complex. The fall in the concentration of antifactor as well as enzyme suggests that they do combine together to form an inactive complex.

From the results obtained it may be postulated that the retina possesses hyaluronidase and antihyaluronidase activity. The hyaluronidase activity of the retina of animals depends on the intensity of the light stimulus and fluctuates regularly, being reduced in response to prolonged and continuous illumination. The antihyaluronidase factor has the power to inactivate hyaluronidase by combining with it to form an inactive complex.

SUMMARY

The retina possesses hyaluronidasic and antihyaluronidasic activity which depends on the strength of the light stimulus. In prolonged continuous light stimulation the retinal hyaluronidasic activity is diminished. The dynamics of decrease of the hyaluronidasic activity depends on its initial value: the higher the initial activity, the quicker its decrease.

The activity of the antihyaluronidasic factor, as well as the activity of hyaluronidase gradually decreases in continuous action of the light. The antifactor promotes the inactivation of the hyaluronidase combining with it into an inactive complex.

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