THE ROLE OF HYALURONIDASE IN THE PROCESS OF URINARY EXCRETION

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The problem of mucolytic enzymes, particularly the enzyme system of hyaluronidase-hyaluronic acid, has attracted the attention of research workers from various points of view. Published experimental and clinical data provide evidence for the role of this system in membrane permeability, in the physiology and pathology of connective tissue, etc.

The least known role of hyaluronidase is its participation in the regulation of water-salt metabolism. Only isolated observations are available in this respect which indicate that the mucolytic system is involved in the active binding of water and electrolytes [2-6].

As already reported [1] the enzyme hyaluronidase has been found in the urine of man and animals; this work was carried out in the laboratory of A. G. Ginetsinskii. It was established that the hyaluronidase activity of the urine was strictly dependent on the extent of diuresis, showing a decline along a characteristic curve following hydration.

The present investigation is concerned with further studies of this question.

EXPERIMENTAL METHODS

Experiments were performed on dogs with ureters exteriorized in abdominal skin flaps. The hyaluronidase activity of the urine was determined viscosimetrically, the method being based on the ability of hyaluronidase to depolymerize hyaluronic acid with consequent lowering of its viscosity. The enzyme activity was expressed in conventional units: 1 conventional unit corresponding to the activity of a preparation causing a 1% drop in the viscosity of the initial solution of hyaluronic acid in the course of 20 minutes.

EXPERIMENTAL RESULTS

In attempting to analyze the cause of decline in hyaluronidase activity of the urine with increase in diuresis, the idea first presents itself that the activity drops as the result of dilution of the enzyme entering the lumen of the nephron in some constant concentration. However, appropriate investigation showed that lowering of enzyme activity observed during increasing urinary excretion could not be explained by a concentration effect. The hyaluronidase activity of urine obtained with small diuresis remained relatively high on dilution of urine in vitro and dropped to zero only when a considerably greater dilution than that observed in natural increase of diuresis was employed (Fig. 1).

Nor is the value of hyaluronidase activity appreciably altered by concentration of urine collected during massive diuresis. If the initial sample is inactive, no activity can be detected on concentrating the urine to a volume corresponding to a low level of urinary excretion.

Experiments in which urine was dialyzed and reduced under vacuum to a volume corresponding to a diuresis of 0.2 ml/min × m^2 also indicated that the curve denoting the dependence of hyaluronidase activity did not result from changes in concentration. The use of this method of analysis excluded the influence of dilution and of a possible salt effect in every sample.

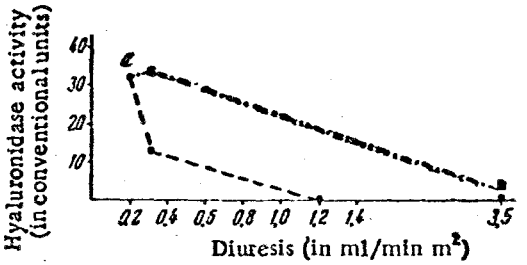

Fig. 1. Effect of dilution on the hyaluronidase activity of urine.
O) Hyaluronidase activity of urine under natural conditions; □) hyaluronidase activity of sample a after dilution of the urine to volumes corresponding to diuresis of 0.3; 1.2 and 3.5 ml/min × m^2.

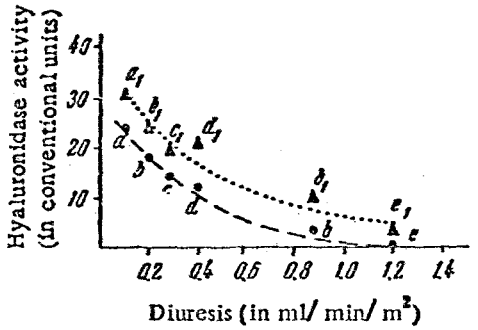

Fig. 2. Dependence of hyaluronidase activity on the magnitude of diuresis after dialysis and concentration of urine to a constant volume.
O) Hyaluronidase activity of urine under natural conditions; Δ) hyaluronidase activity of urine under standardized conditions. Sample a_1 obtained by dialysis of urine collected during diuresis of 0.12 ml/min × m^2 and addition to it of distilled water to correspond to the volume 0.2 ml/min × m^2. Sample b_1 obtained by dialysis of urine collected during diuresis of 0.2 ml/min × m^2. Samples c, d, e_1 obtained by dialysis of corresponding samples of urine collected during diuresis exceeding 0.2 ml/min × m^2 and reduced to this volume by concentration under vacuum.

Figure 2 demonstrates one of the typical experiments of this series. It shows that hyaluronidase activity of urine under standardized conditions changes with the same consistency as under natural conditions but the curve is situated at a higher level owing to removal of concentration and salt effects. This indicates that true diminution of enzyme liberation by renal tissue occurs as increased urinary excretion develops. In order to discover the nature of the processes underlying changes in hyaluronidase activity of urine we were interested in finding out whether the relationship established by us persisted during development of diuresis induced by other factors than hydration. An osmotic diuretic agent – urea – was used for this purpose.

The osmotic load was given to the dogs per os on the basis of 0.5 g urea per 1 kg body weight. The hyaluronidase activity was determined after dialysis and adjustment of the urine to a volume corresponding to a diuresis of 0.2 ml/min × m^2.

It is known that water diuresis develops as the results of diminished facultative reabsorption of water. Increased diuresis following urea administration however, has a different mechanism. Urea which enters the filtrate in large quantities binds the water osmotically, causing its retention in the tubules. In this case the extent of diuresis is determined by the degree of diminution of obligatory reabsorption.

Urinary excretion under the influence of osmotic diuretics increases not as the result of reduced facultative reabsorption as in water diuresis but as a consequence of inflow into the distal segment of the nephron of a volume of fluid in excess of that which can be reabsorbed despite the maximal intensity of processes occurring there.

The fall in hyaluronidase activity of the urine is thus connected not with the fact of increased urinary excretion itself but with the physiologic mechanism responsible for the development of increased diuresis. Low hyaluronidase activity – and at a certain critical level, zero activity – is associated with water diuresis which occurs under conditions of suppressed facultative reabsorption. Osmotic diuresis, on the other hand, occurs under conditions of intensive facultative reabsorption and is associated with high hyaluronidase activity of urine (Fig. 3).

These data permit the conclusion that the system hyaluronidase-hyaluronic acid participates in the process of water reabsorption which takes place in the distal segment of the nephron.

The main factor responsible for stimulation of water transport in the distal segment is known to be the antidiuretic hormone of the hypophysis. It therefore appeared wise to investigate the influence of this hormone on the hyaluronidase activity of urine.

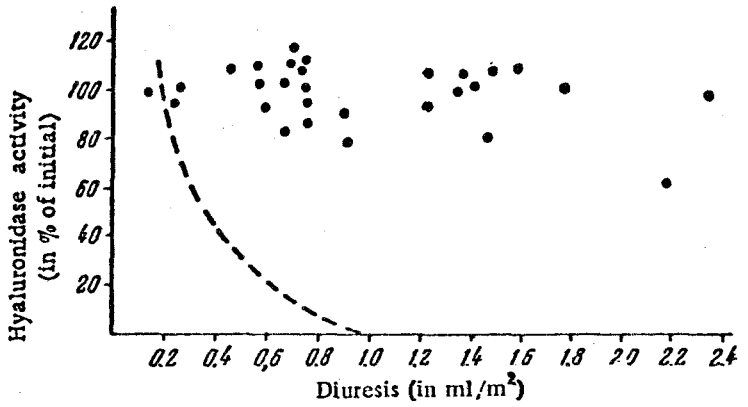

Fig. 3. Relation of hyaluronidase activity of urine to magnitude of urinary excretion during osmotic diuresis.
Broken line denotes this relationship during water diuresis. Data obtained in 11 experiments on 2 dogs.

Pituitrin P was used as a preparation containing antidiuretic hypophiseal hormone; it was injected intravenously in the dose of 0.005 units per 1 kg body weight. In the first series of experiments the effect of pituitrin was studied with administration of the preparation after 3% water loading against the background of diuresis of such extent that no hyaluronidase activity could be detected in the urine. It was found that following pituitrin injection the diminution in diuresis was accompanied by a definite increase in hyaluronidase activity of urine, the activity changing in accordance with the magnitude of urinary excretion and coinciding exactly with the typical curve obtained in control experiments.

TABLE 1

Influence of Hypophyseal Antidiuretic Hormone and of In Vitro Concentration on the Hyaluronidase Activity of Urine

Original sample		Pituitrin administration		Activity of urine concentrated in vitro
diuresis	activity	diuresis in ml/min · m²	activity	
0.82	0	0.21	12	0
0.82	0	0.36	7	0
0.83	0	0.40	15	0
0.83	0	0.45	13	0
0.83	0	0.33	14	0
0.94	0	0.7	9	0
0.94	0	0.56	11	0
0.94	0	0.23	12	0
0.98	0	0.26	13	0
0.98	0	0.55	4	0
1.6	0	0.36	12	0
1.6	0	0.6	7	0
1.6	0	0.7	9	0

Special experiments established convincingly that the antidiuretic hormone exerted no effect on hyaluronidase in vitro. Consequently, the increase in hyaluronidase activity of urine following introduction of pituitrin cannot be the result of direct hormonal influence on the activity of the enzyme.

Increase in urinary activity after injection of pituitrin cannot be explained by a simple increase in hyaluronidase concentration during small diuresis either; this is clearly demonstrated by experiments with parallel investigation of urine concentrated in vitro under vacuum and in vivo as the result of antidiuretic hormone action.

The experimental procedure of appropriate experiments, whose data are presented in Table 1, consisted of the following: 2 samples of urine were collected from the animal, during extensive diuresis after hydration and after administration of pituitrin when the diuresis was considerably reduced. Part of the urine obtained during extensive diuresis was concentrated under vacuum at a temperature of 37° to a degree corresponding to the diminution of diuresis under the influence of pituitrin as compared with the original. The hyaluronidase activity was determined in all 3 samples.

As Table 1 shows, considerable increase in activity is found in the urine obtained after injection of pituitrin, whereas urine of the same volume but concentrated in vitro possesss no hyaluronidase activity. It follows therefore that increase of hyaluronidase activity under the influence of the anitdiuretic hormone is not determined by concentration changes and is, evidently, the result of increased liberation of the enzyme by renal tissue elements.

TABLE 2

Effect of Water-Osmotic Load and Pituitrin on the Hyaluronidase Activity of Urine

Water load		Administration of urea		Administration of pituitrin	
diuresis in ml/min · m^2	activity	diuresis in ml/min · m^2	activity	diuresis in ml/min · m^2	activity
Experiments on the dog Molli					
0.98	14	3.6	14	2.9	44
4.5	0	6.0	0	2.8	30
2.8	0	6.0	0	5.5	28
2.8	0	6.0	0	2.8	31
3.0	2	12.0	2	7.0	13
Experiments on the dog Kutsei					
1.2	0	2.5	0	1.9	11
1.8	0	7.0	0	1.1	11
1.5	0	5.5	0	2.2	13
4.5	0	11.0	0	2.8	16

However, in the experiments described, the effect of the antidiuretic hormone was investigated against the background of considerable reduction of diuresis when the urine usually shows hyaluronidase activity. In order to trace the action of antidiuretic hormone without appreciable fall in diuresis a combined water-osmotic load was used. The animal was given urea in the dose of 0.5 per 1 kg body weight against the background of developed water diuresis. When the diuresis increased still further the animal was given pituitrin intravenously.

The hyaluronidase activity was determined in the urine which was preliminarily dialyzed and adjusted to the volume corresponding to a diuresis of 0.2 ml/min × m^2 to exclude in all samples the possibility of salt or concentration effect.

As can be seen from Table 2, in which the data of this series of experiments is presented, water loading increases diuresis to such an extent that the urine either loses its hyaluronidase activity completely or only exhibits slight activity.

Administration of urea after water loading increases diuresis but exerts no influence on the magnitude of enzyme activity, since under conditions of hyperhydration of the organism the facultative reabsorption does not rise. Following injection of pituitrin, which increases distal reabsorption of water, the diuresis diminishes somewhat but thanks to the osmotic activity of urea, continues to maintain a high level considerably in excess of that during the initial water diuresis. Despite this, the urine acquires considerable hyaluronidase activity.

The experiments carried out suggest the following conclusions:

1. The dependence of hyaluronidase activity of urine on the extent of diuresis cannot be explained in terms of simple dilution, salt effect or change in the volume of urine in which the enzyme is distributed. The characteristic curve of hyaluronidase activity of urine depends on the differences in intensity of liberation of the enzyme by renal tissue elements associated with changes in the extent of urinary excretion.

2. The urine shows high hyaluronidase activity when the animal's blood stream has a high concentration of the antidiuretic hormone and when distal reabsorption of water is intense. Reduction in concentration of the antidiuretic hormone and of distal reabsorption of water, as is the case after water loading, is accompanied by a corresponding change in the enzyme activity of urine.

3. Liberation of the hyaluronidase enzyme by renal tissue elements is related directly to the process of distal reabsorption of water and is regulated by the antidiuretic hormone of the pituitary.

SUMMARY

The activity of hyaluronidase which was detected in the urine of man and animals in weak diuresis is changed according to a characteristic curve when urine excretion is increased. Dependence of its activity on the diuresis cannot be explained by dilution, salt effect or by the change of the volume of urine in which the enzyme is distributed. Evidently the curve of hyaluronidase activity is connected with varying intensity of liberation of the enzyme by the elements of kidney tissue.

High hyaluronidase activity is found in cases when the concentration of the antidiuretic hormone in the blood is high and intense reabsorption of water takes place in the distal segment of the nephron. This suggests that excretion of hyaluronidase by the elements of kidney tissue has a direct relationship to the process of active reabsorption of water. It is regulated by the antidiuretic hypophyseal hormone.

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