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 x m² 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.
Figure 1. Effect of dilution on the hyaluronidase activity of urine.

- Hyaluronidase activity of urine under natural conditions;
- Hyaluronidase activity of the urine after dialysis to volumes corresponding to diuresis of 0.3, 1.2, and 3.5 ml/min x m².

Figure 2. Dependence of hyaluronidase activity on the magnitude of diuresis after dialysis and concentration of urine to a constant volume.

- Hyaluronidase activity of urine under natural conditions;
- Hyaluronidase activity of urine under standardized conditions. Sample a obtained by dialysis of urine collected during diuresis of 0.12 ml/min x m² and addition to it of distilled water to correspond to the volume 0.2 ml/min x m².
- Sample b obtained by dialysis of urine collected during diuresis of 0.2 ml/min x m². Samples c, d, e obtained by dialysis of corresponding samples of urine collected during diuresis exceeding 0.2 ml/min x m² and reduced to this volume by concentration under vacuum.

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. Hence it appeared wise to investigate the influence of this hormone on the hyaluronidase activity of urine.

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 x m².

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).