

THE EFFECT OF HYALURONIDASE INHIBITORS ON URINARY EXCRETION

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Recently, our laboratory has issued reports [1,2,3,4,5] which have shown that the facultative reabsorption of water which has been excreted from the distal part of a nephron under the influence of the antidiuretic hormone is due to the action of hyaluronidase. Through the depolymerization of hyaluronic acid, which is a component of the intercellular cement of the nephron's wall, the cement is made permeable to water, which is then reabsorbed osmotically.

If this hypothesis is correct, substances which inactivate hyaluronidase would be expected to restore the impermeability of the walls of the tubules to water, and so prevent facultative reabsorption. Antihyaluronidases should therefore increase diuresis.

The present work has been undertaken to test the correctness of this conclusion, which follows from our hypothesis on the mode of action of the antidiuretic hormone.

METHOD

The work was done on white rats. In one set of experiments, a fistula was established in the urinary bladder which allowed urine to be collected continuously. In another set, four rats were placed in a cage having a base made of wire mesh, and the urine was collected through a funnel placed beneath it. In these experiments, a record was made of the amount of urine excreted over a period of several hours when the excretion was voluntary. As hyaluronidase inhibitors we used heparin and ascorbic acid. The antihyaluronidases were injected intraperitoneally. The ascorbic acid was first neutralized with sodium bicarbonate, and was given as a dose of 0.1 g per 100 g weight. The amount of heparin injected was 1000 units per 100 g weight. The concentration of sodium in the urine was measured by flame photometry and by the inulin-resorcin method.

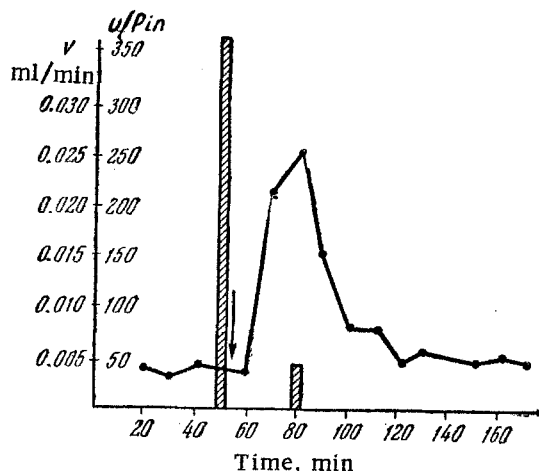
RESULTS

The rats were injected with inulin before the experiment. After a few control portions of the urine had been collected, the ascorbic acid was injected intraperitoneally. Immediately after the injection, the diuresis rose from 0.005 to 0.025 ml/minute—a fivefold increase. When the effect of the single injection of the antihyaluronidase agent had worn off, urinary excretion returned to normal.

The results of a typical experiment are given in the figure.

Measurement of the inulin concentration ratio shows that the polyuria produced by the ascorbic acid is caused by a marked reduction in facultative reabsorption. In the control period, the ratio of concentration of inulin in the urine and plasma exceeded 350, which represented a condition of oliguria. After the ascorbic acid had been injected, the ratio was reduced more than seven times, indicating a great reduction in the reabsorption of water, despite the fact that the rat still has a water deficit. A similar effect was induced by injecting heparin.

In the second set of experiments, 1 ml of 10% ascorbic acid per 200 g weight was injected hourly for five hours. The results obtained are shown in the table, which gives the mean values of all the determinations.



The effect of ascorbic acid on the excretion of urine in a rat with a urinary fistula. The columns indicate the concentration index of inulin. Ordinate-diuresis in ml/minute (V) and the ratio of the inulin concentration in the urine to that in the plasma (u/P index); abscissa-time in min. Time of injection of ascorbic acid shown by arrow.

Before testing the action of hyaluronidase inhibitors, a control determination was first made in all the animals. All the results concerning the excretion of water and sodium were obtained under standard conditions of moderate water deficiency, induced by keeping the animals without food or drink for 18 hours.

Ascorbic acid injected into the same animals and under the same experimental conditions increased urinary excretion five times. At the same time there was a marked increase in the amount of sodium excreted, and the concentration in the urine did not fall, as it would if the increased diuresis were the only effect, but it actually rose. In order to eliminate any possible error due to the fact that some sodium would be introduced together with the ascorbic acid, a special control experiment was arranged. As would be expected, injecting sodium bicarbonate in the same amount as was required for neutralization caused only a very small and statistically insignificant increase in urinary excretion. At the same time sodium excretion was increased 60%, whereas after the injection of ascorbic acid, the amount of this ion excreted increased eight times.

Results of the Second Set of Experiments

Experiment	Number of animals	Diuresis (in ml per 100 g weight per hour)	Urinary Na (in m-equivalents per liter)	Sodium excretion (in μ -equivalents per hour per 100g weight)
Control determination	32	0.049 ± 0.022	106	4.4
10% ascorbic acid, 1 ml per 200 g weight	32	0.200 ± 0.015	175	35.0
1% NaHCO ₃ solution, 1 ml per 200 g weight	16	0.056 ± 0.030	131	7.3
Water load of 5% body weight	16	0.58 ± 0.034	9	5.2

The effect of ascorbic acid is therefore specific, and is most likely due to its antihyaluronidase effect.

At present we can put forward no explanation of the mechanism of the increased sodium excretion caused by the antihyaluronidases. It is certain however that it is not due to a simple increase in urinary excretion. As the results given in the table show, a diuresis two and a half times that due to the action of ascorbic acid, induced by a water load, does not cause any increased excretion of sodium. The concentration of sodium in the urine during excretion following increased water intake is reduced approximately in proportion to the increased urinary excretion, so that there is practically no change in the amount of salt excreted.

SUMMARY

Experiments on albino rats have shown that the antihyaluronidases (ascorbic acid and heparin) increase diuresis by reducing water reabsorption. The effect accords with the idea that enzymatic depolymerization of the hyaluronic complexes in the walls of the tubules is the cause of facultative reabsorption. Neutralized ascorbic acid markedly increases the excretion of sodium, but the mechanism is obscure.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
