RENAL SECRETION OF HYALURONIDASE IN DIFFERENT CLASSES OF VERTEBRATES

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One of the most important adaptations involved in the transition from aquatic to terrestrial life is the evolution of an effective method of preventing water loss. An important factor in such a system is the antidiuretic hormone (ADH). It is found in the neurohypophysis of all vertebrate classes [5]. A. G. Ginetsinskii and his coworkers have recently shown that in the mammalian kidney, the action of the ADH is mediated by the enzyme system hyaluronidase – hyaluronic acid [2, 3].

From among the thousands of foreign and Russian papers dealing with hyaluronidase and hyaluronic acid, only a few report the presence of the enzyme in the kidney of various mammalian species [1, 6].

It seemed to us essential to discover whether hyaluronidase occurs in the kidney of all vertebrates.

METHOD AND RESULTS

The hyaluronidase was obtained from minced renal tissue, and the activity was determined by our own modification of the viscosimetric method [4]. The determinations were made on 25 species of animals (Table 1).

It can be seen from Table 1 that although there are quite big differences in activity within each class, considerable quantities of the enzyme were found in the kidneys of most animals. Those of the bullhead and pike contained such a large amount, that the tissue was scarcely less active than the commercial preparation "Ronidase". In the roach kidney, no enzyme was found at a dilution of 1 : 50. Similar variation occurred in birds: a high concentration of the enzyme was found in the kidneys of the wagtails and crossbills, while only small amounts were present in the gull and loon. It was difficult to find any relation between the activity of the enzyme and the ecological or physiological conditions of the species.

The substance which we extracted from vertebrate kidneys and which caused depolymerization of solutions of hyaluronic acid is certainly an enzyme. It was destroyed by boiling. Its activity fell logarithmically with the dilution.

To determine the part played by hyaluronidase in kidney function, we followed the excretion of the enzyme in water and osmotic diuresis. Bird urine, collected at low diuretic levels, always shows hyaluronidase activity, and this falls rapidly when urinary excretion rises after a water load (Fig. 1). The results obtained with osmotic diuresis also agree completely with those obtained by other authors in mammalian experiments [2]. When diuresis increases after intravenous injection of a hypertonic sodium chloride solution, the activity of the urine falls much less than when the urine secreted initially is diluted with distilled water (Fig. 2). In experiments where the water diuresis was arrested by injecting an extract of chick hypophysis, the concentration of hyaluronidase in the urine immediately rose.

TABLE 1

Activity of Renal Hyaluronidase in Different Vertebrates

Number, in order	Species	Temperature during test	Dilution	Activity (in arbitrary units)
1	River minnow, Lampetra fluviatilis	34°	1:50	12
2	The pike, Esox lucius	34°	1:50	40
3	Crucian carp, Carassius carassius	34°	1:50	11
4	The perch, Perca fluviatilis	34°	1:50	7
5	Bream, abramis brama	34°	1:50	7
6	The rudd, Scardinius erythrophthalmus	34°	1:50	5
7	The roach, Rutilus rutilus	34°	1:50	0
8	North Atlantic bullhead, Myoxocephalus scorpius	24°	1:50	57
9	The flounder, Limanda limanda	24°	1:50	32
10	The cod, Gadus morhua	24°	1:50	30
11	The White Sea herring, Clupea harengus	24°	1:50	32
12	The navaga , Eleginus navaga	24°	1:50	26
13	The frog, Rana temporaria	34°	1:50	5
14	The adder, Vipera berus	34°	1:50	5
15	The white wagtail, Motacilla alba	24°	1:50	36
16	The crossbill, Loxia pityopsittacus	24°	1:50	35
17	The oyster catcher, Haematopus ostralegus	24°	1:50	20
18	The black grouse, Lyrurus tetrix	24°	1:50	10
19	The redstart, Phoenicurus phoenicurus	24°	1:20	18
20	The tern, Sterna paradisaea	24°	1:20	12
21	The red throated loon, Gavia stellata	24°	1:20	10
22	The herring gull, Larus argentatus	24°	1:20	6
23	The chick, Gallus domesticus	34°	1:50	11
24	The common gull, Larus canus	24°	1:20	0
25	The white rat , Rattus novegicus albino	34°	1:50	20

Note: In cases where the activity of the hyaluronidase of test samples diluted 1 in 50 was low or absent, dilutions of 1:20 were used. Tests were made at 24° during an expedition to Ryazhkov Island, on the Kandalakshskii Reserve.

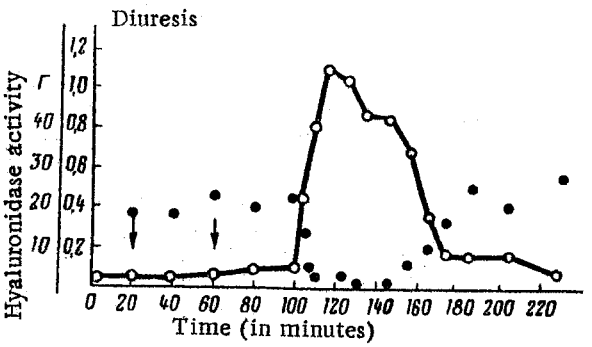

Fig. 1. Changes in hyaluronidase activity (in arbitary units) in chicken urine in water diuresis (in ml per minute). Points – hyaluronidase activity, curve – urine excreted, arrow indicates time at which 50 ml per kg of water were introduced into the intestine.

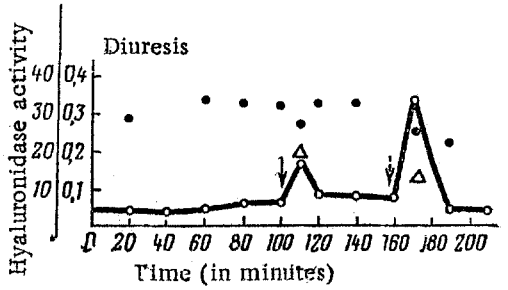

Fig. 2. Effect of osmotic diuresis on the excretion of hyaluronidase by the chick kidney. Triangle – activity of the initial urine sample, diluted with water to correspond to the degree of diuresis; ↓ – intravenous injection of 0.2 g per kg

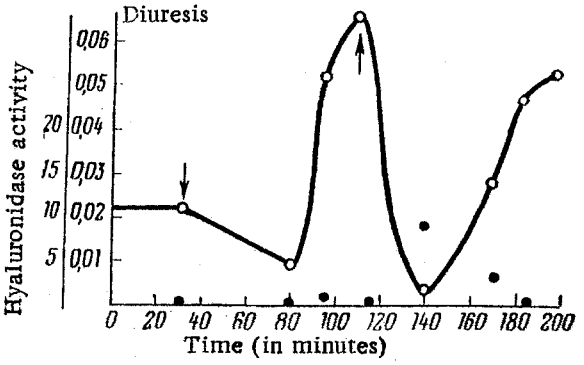

Fig. 3. Secretion of hyaluronidase by the kidney of the bog-turtle after injecting a turtle hypophyseal extract. ↓ – introduction of 50 ml per kg water into the intestine; ↑ – intravenous injection of turtle hypophysis extract. Curves as in Fig. 1.

The results obtained with osmotic diuresis also agree completely with those obtained by other authors in mammalian experiments [2]. When diuresis increases after intravenous injection of a hypertonic sodium chloride solution, the activity of the urine falls much less than when the urine secreted initially is diluted with distilled water (Fig. 2). In experiments where the water diuresis was arrested by injecting an extract of chick hypophysis, the concentration of hyaluronidase in the urine immediately rose.

From the results obtained it can be seen that just as in mammals, excretion of hyaluronidase by the renal parenchyma cells constitutes an essential link in the ADH mechanism.

Of the reptiles, we used the bog-turtle. No hyaluronidase was found in urine samples taken directly from the ureters in turtles during water diuresis, or in other samples taken from the bladder after cutting through the shell. However, if during the water diuresis the animal is injected with an extract of turtle hypophysis, the diuresis is inhibited, and hyaluronidase appears in the urine (Fig. 3). Some minutes later, the antidiuretic effect became weaker, urinary excretion increased, and the enzyme disappeared from the urine. Thus, it appears that in bog-turtles, ADH stimulates hyaluronidase secretion.

In frogs, whose bodies contain a lot of water, the rate of urinary excretion is high, and no hyaluronidase appears in the urine. If the animals are placed in a solution of sodium chloride, in order to cause a dehydration, diuresis is reduced, and some enzymatic activity is found in the urine (Table 2).

TABLE 2

Hyaluronidase Activity in Frog Urine

Number of test	Diuresis (in ml/hour per 100 g body weight)	Activity	Remarks
1	3,3	0	Summer frog taken from water
2	2,8	0	» » » »
3	1,37	0	Winter frog from water
4	1,22	0	» » » »
5	1,15	0	» » » »
6	1,06	0	» » » »
7	1,02	0	» » » »
8	1,05	2	Summer frog from NaCl solution
9	1,05	3	» » » » »
10	0,61	5	» » » » »

TABLE 3

Relationship of Hyaluronidase Activity in Pike Urine and Diuresis

Number of test	Diuresis (in ml/hour per 100g body weight)	Activity	Remarks
1	0,48	0	Pike from fresh water
2	0,32	0	» » » »
3	0,19	0	» » » »
4	0,15	0	Pike from NaCl solution
5	0,095	0	Pike from fresh water
6	0,085	3	Pike from NaCl solution
7	0,08	5	» » » »
8	0,06	7	» » » »
9	0,06	8	» » » »

From some of the sea fish, it was sometimes possible to obtain a sample of urine immediately after the animals were caught. This urine showed considerable activity; for instance in the bullhead it was 49 units, and in the cod 16 units. A systematic study of urinary hyaluronidase activity was made in the perch and pike. Studies were also made of the enzymatic activity of the urine of fish kept in fresh water, or dehydrated by immersion in various strengths of salt solution, which reduced diuresis. No hyaluronidase was found in perch taken from fresh water or from sodium chloride solutions. No enzyme was found in pike taken from fresh water. After being left for 3-5 hours in 1-1.3% NaCl solution, urinary excretion was reduced to a small fraction of its fresh water value. In most cases, hyaluronidase then appeared in the urine (Table 3), and the enzymatic activity was greater, the lower the diuresis. It is important to note, that according to V. F. Vasil'eva and M. M. Sokolova, under these conditions, the reduction of diuresis in the pike results not only from a reduced filtration rate, but also from an increase in facultative reabsorption, as can be deduced from the reduced excretion of the osmotically free water. Therefore, in some fish (pike), the reabsorption of water in the distal part of the tubule involves the excretion of hyaluronidase into urine.

Thus, hyaluronidase was found in representatives of all classes of vertebrates. It was shown that when diuresis was low, hyaluronidase activity was high, that in water diuresis the enzyme disappeared from the urine and that its activity was maintained during osmotic diuresis. We may conclude, therefore, that just as in mammals, facultative reabsorption of water in the tubules is associated with the secretion of hyaluronidase.

SUMMARY

At low diuresis rates, hyaluronidase was found in the kidneys and urine of representatives of all vertebrate classes. The enzyme was not present during water diuresis, but continued to be excreted during osmotic diuresis.

Its excretion into the urine was stimulated by the injection of hypophyseal extract. The results showed that facultative water reabsorption in the renal tubules of all vertebrates, including mammals, is connected with hyaluronidase secretion.

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