Atti Accademia Nazionale dei Lincei

Classe Scienze Fisiche Matematiche Naturali

Rendiconti

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Posthypophyseal hormone action: discussion on Ginetzinsky's hypothesis on the urinary bladder of Bufo bufo

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8, Vol. **51** (1971), n.5, p. 429–433.

Accademia Nazionale dei Lincei

<http://www.bdim.eu/item?id=RLINA_1971_8_51_5_429_0>

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Accademia Nazionale dei Lincei, 1971.

Fisiologia. — *Posthypophyseal hormone action: discussion on Ginetzinsky's hypothesis on the urinary bladder of* Bufo bufo (*). Nota di DARIO CREMASCHI, SILVIO HÉNIN e MARINA CALVI, presentata (**) dal Corrisp. V. CAPRARO.

RIASSUNTO. — La ialuronidasi, secondo Ginetzinsky, è il mediatore dell'azione permeabilizzante degli ormoni postipofisari. Gli esperimenti riportati dimostrano che nella vescica urinaria di *Bufo bufo* l'enzima non è implicato nell'azione ormonale.

Posthypophyseal hormones perform an anti-diuretic action by increasing the permeability of the kidney, distal tubule and collecting duct and also of the urinary bladder of *Bufo marinus* to water, urea and sodium [1, 2, 3, 4, 5].

The intracellular mediator of these hormones has been shown to be 3′5′ cyclic adenosinemonophosphate [6, 7]. Many hypotheses have been postulated to explain how these hormones affect permeability. Amongst them, Ginetzinsky's hypothesis in 1958: the hormone induces hyaluronidase secretion in the lumen, thus depolymerising the hyaluronic acid in the intercellular cement and therefore increasing the permeability of the membrane.

Ginetzinsky [8], Dicker and Eggleton [9] and Thorn, Knudsen & Koefoed [10] have found a connection between antidiuresis and the presence of hyaluronidase in the urine of rat and man.

On the other hand Ginetzinsky [11] and Cort [12] by injecting commercial hyaluronidase did not report any change in dog kidney diuresis. Likewise, Leaf treating *Bufo marinus* urinary bladder with a high concentration (125 U/ml) of commercial hyaluronidase, did not observe any increase in permeability [3]. It has been noted however by Cobbin & Dicker [13] that hyaluronidase (a bovine testis extract) has a pH optimum between 6.6–6.8 and that therefore could be completely inactive at the pH values found in urine: in urine, these Authors have found the hyaluronidase action optimum to be at pH 4.1–4.2.

In our work we have tested the validity of Ginetzinsky's hypothesis on *Bufo bufo* in a study on the function of the urinary bladder of this amphibian.

The experiments were carried out in September at room temperature (24–25° C) using killed and pithed *Bufo bufo* females, from which the bladder was immediately removed.

In a first series of experiments, one lobe of the bladder was isolated, cannulated and perfused on the serosal side with frog Ringer solution

(*) Lavoro eseguito nell'Istituto di Fisiologia generale di Milano.

(**) Nella seduta del 13 novembre 1971.

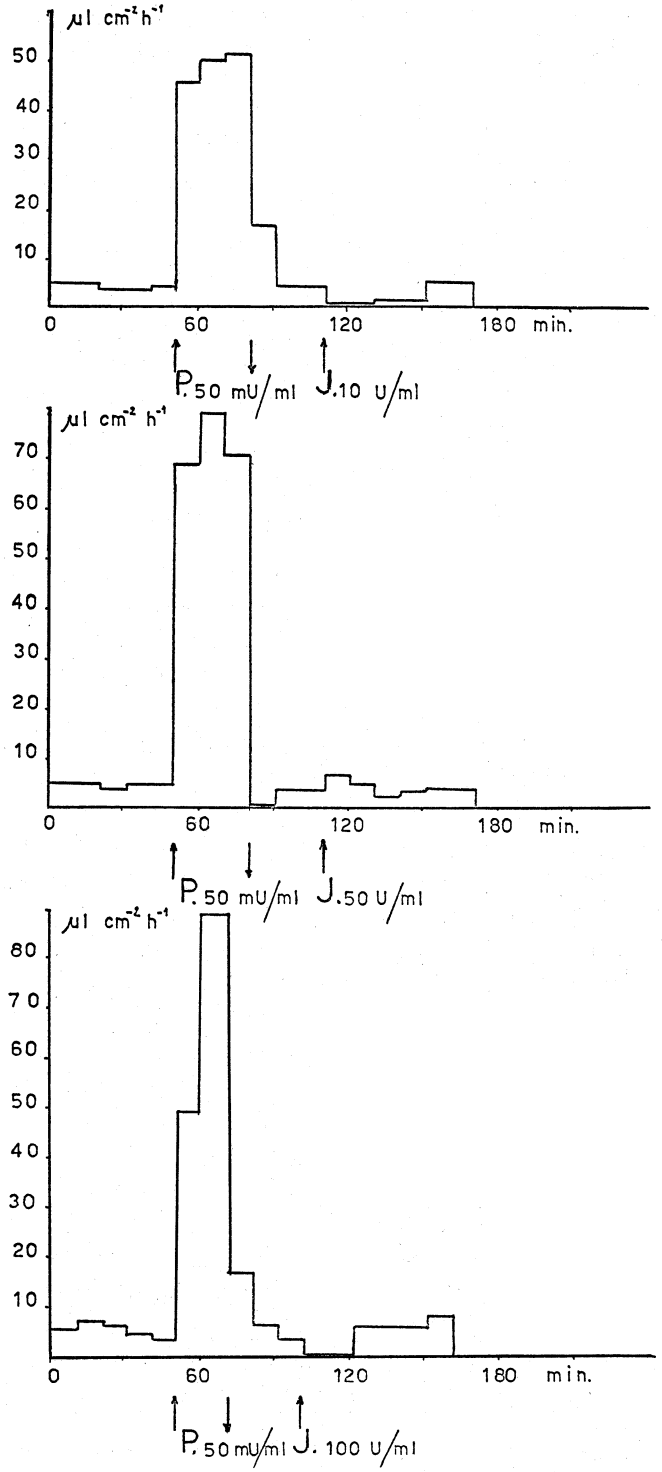

Fig. 1. – Net osmotic water transport in the urinary bladder of *Bufo bufo*. Treatment with Pituitrin (50 mU/ml) and with hyaluronidase (10–50–100 I.U./ml) (shown by the arrows). P = Pituitrin; J = hyaluronidase.

(NaCl 110 mM, KCl 2 mM, $CaCl_2$ 2.5 mM, Na_2HPO_4 3.7 mM, NaH_2PO_4 1.7 mH, pH 7).

The lumen of the bladder, was kept in a diluted Ringer solution (1 : 10) at pH 5.9 (Buffer Na_2HPO_4 0.67 mM, NaH_2PO_4 6.0 mM). The net osmotic water transport was measured gravimetrically and was expressed in $\mu l \cdot cm^{-2} \cdot h^{-1}$, assuming the bladder to be spherical. After five ten-min. experimental periods, posthypophyseal hormones (Pituitrin, Parke & Davies, 50 mU/ml) were added to the serosal medium, and the transport progress was followed for 2–3 periods. Afterwards the transport was reduced to its basal values, perfusing with a hormone-free solution (3 periods) and the tissue was treated on its mucosal side with hyaluronidase in varying concentrations (10, 50, 100 U/ml) (6 periods).

Hyaluronidase (Hyaluronate lyase BDH) ovine testis extract, was used with an action optimum at pH 5.5–6.2. Therefore under our experimental conditions the pH used coincided with the pH optimum of the enzyme.

However despite the favourable pH conditions and the marked effect of the hormone on the tissue, the water permeability was not even altered by high hyaluronidase concentrations (cf. fig. 1).

To confirm these results a second set of experiments was carried out on bladders of toads which had been deprived of water for 60 hrs. and were therefore under anti-diuresis.

The blood and urine of 5 dehydrated toads was collected, the blood was treated with an anti-coagulant, (sodium citrate). The blood and urine osmolarities were measured using a Knauer osmometer and isosmotic solutions (A) to the urine and (B) to the blood were prepared by adding NaCl to an ordinary Ringer solution.

One lobe of the bladder was isolated, cannulated and perfused, water transport was measured under the following conditions:

a) five ten-min. periods, with solution A in the lumen and B in the serosal medium.

b) 3 periods, with solution A in the lumen and blood as the serosal medium.

c) 5 periods under the initial conditions.

d) 5 periods with urine in the lumen and B in the serosal medium.

e) 3 periods under the initial conditions.

f) 2 periods with solution A in the lumen and solution B in the serosal medium with the addition of Pituitrin (50 mU/ml).

g) 3 periods under initial conditions.

As can be seen in fig. 2, the presence of blood or Pituitrin in the serosal medium causes an increase in water transport while urine in the lumen has no effect. Since the toads have been dehydrated, their blood presumably contained high levels of the hormones as shown by the results obtained. Assuming Ginetzinsky's hypothesis to be true, the urine should contain hyaluronidase and therefore increase the permeability of the bladder.

This was not so, as seen in fig. 2.

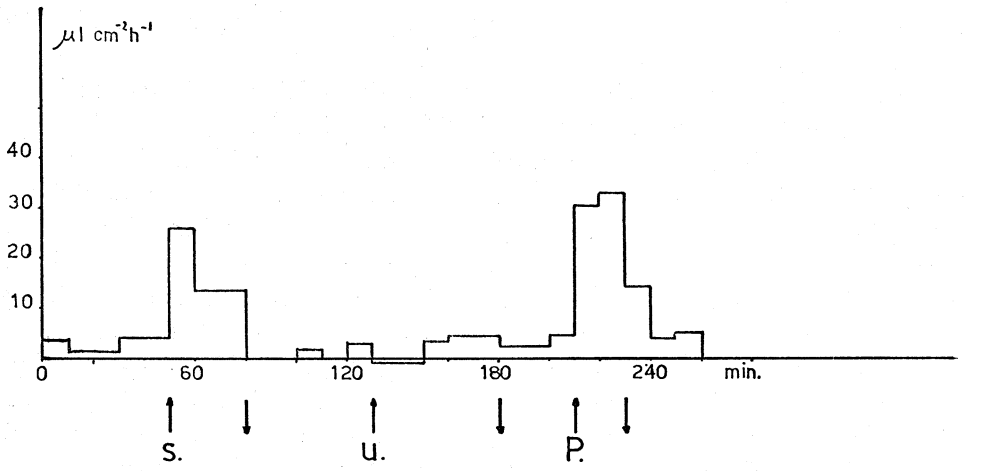

Fig. 2. – Net osmotic water transport in the urinary bladder of *Bufo bufo*. Treatment with blood (S) and urine (U) of dehydrated toad (shown by the arrows). The control is done using Pituitrin (50 mU/ml) (P).

To confirm the presence of active hyaluronidase in the urine so obtained, a test described in "Methods in Enzymology" [14] was used. Briefly the torbidity of a solution at 600 mμ of highly polymerized hyaluronic acid, precipitated with albumin, was measured; any hyaluronidase present would cause a decrease of torbidity.

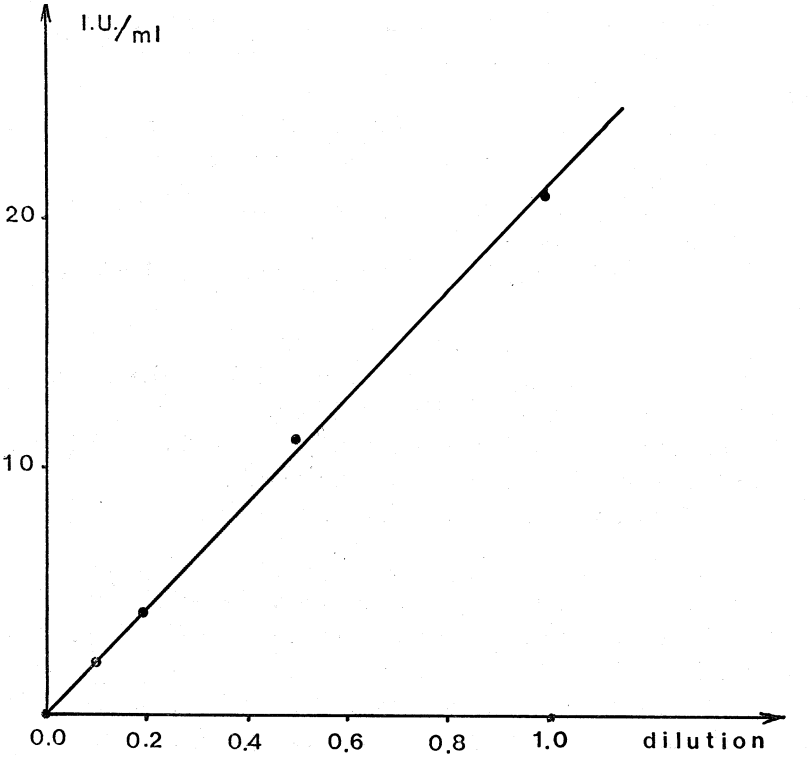

Fig. 3. – Correlation between hyaluronidase acvitity and urine dilution.

The solution was buffered at pH 7 (phosphate buffer 0.07 M), since urine tests on *Bufo bufo* had given this pH value.

The calibration curve was obtained using an ovine testis hyaluronidase (hyaluronate lyase B.D.H.). A control free from hyaluronidase was set up using urine which had been boiled for 3 min and centrifuged.

As seen in fig. 3, in the urine used in the experiments, hyaluronidase was present (about 20 W.H.O.I.U./ml).

The number of units reported must be regarded only as approximative since the hyaluronidase used as a standard and that in the urine probably differ and have a different pH optimum.

In fig. 3 moreover, one notes that by diluting the urine a proportional dilution of the activity of the enzyme is obtained. This therefore excludes the presence of hyaluronidase inhibitors in toad urine, since by diluting the urine, they would become diluted thus leading to a disappearance of their inhibitory action. In man however, inhibitors are present in the urine, as noted by Knudsen and Koefoed [15].

We must conclude therefore from these experiments that in *Bufo bufo* hyaluronidase, although present in the urine, does not seem to be important as a mediator in the mechanism of action of posthypophyseal hormones.

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