Involvement of a Na-K-ATPase in Sodium Transport by Fish Urinary Bladder

The isolated urinary bladder of various teleosts reabsorbs water from the mucosal border and this process is dependent upon an active transepithelial sodium transport[1–4].

In vitro, the trout urinary bladder actively transports Na^+ and Cl^- ions in equal amounts, as a highly hyperosmotic solution with respect to the luminal fluid, so that a significant osmotic gradient is created but no potential difference appears across the bladder wall[4].

The present studies consider the possibility that a Na-K-ATPase is involved in this process. The amount of enzyme in the membrane and the rate of sodium transport have been measured, and in addition these parameters have been modified by adapting the fish to saline environments.

Methods. Trouts (*Salmo irideus*) weighing 250–300 g purchased from a hatchery were initially kept in freshwater (FW) at 13 °C. Some were subsequently transferred to $^1/_3$ sea water ($^1/_3$ SW, approximately isosmotic to the blood) and some of these fish were submitted to stepwise increments in salinity until full sea water (SW) prevailed.

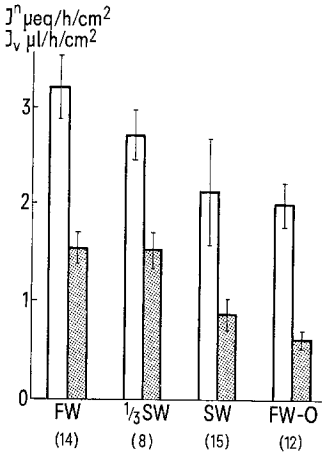

Fig. 1. Water (J_v, open columns) and Na (J^n, cross-hatched columns) transport rates across bladders with Ringer solution on both sides, over a 2-h period. FW, 1/3 SW and SW: animals adapted to freshwater, 1/3 sea water and sea water respectively. FW–O, freshwater fish bladders in presence of 10^{-4} *M* ouabain in the serosal fluid. Numbers in parentheses indicate number of bladders used.

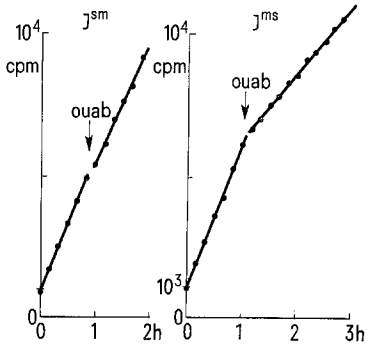

Fig. 2. Release of ^{22}Na into the mucosal (left) or the serosal (right) solutions as a function of time. The unidirectional fluxes of Na (J^{sm} and J^{ms} respectively) are proportional to the slopes. After addition of ouabain 10^{-4} *M* (ouab) J^{ms} is inhibited by 45% while J^{sm} s unchanged.

Animals were considered completely adapted to their particular media after 1 week had elapsed.

The net transports of sodium (J^n Na) and water (J_v) by bladders removed from variously adapted fish were studied as described previously[4], using a sac technique, over a 2-hour period. A modified Forster's bicarbonate Ringer solution was present on both sides.

To measure a unidirectional flux of sodium, ^{22}Na was added to one compartment and samples were taken every 10 min from the other. The bladder was mounted with a plastic cannula in either end of the organ and these connected to an external resevoir. The mucosal fluid consisted of 12 ml of Ringer solution. Circulation of the fluid as well as gassing were achieved by an air-lift using 95% O_2–5% CO_2 mixture. The outer, or serosal, solution bathing the bladder was 15 ml of an identical Ringer, gassed in a similar way and gently stirred with a magnet. All the above experiments were carried out at 18 °C.

For ATPase determinations, bladders were carefully dissected under a microscope, the attached outer muscle was removed as completely as possible and the bladders were stored at −18 °C for a few days. Pooled membranes (2 or 3 bladders per sample) were then homogenized in a Potter tissue grinder using a teflon pestle. The homogenizing solution contained sucrose 0.25 *M*, deoxycholate 2.5 m*M*, EDTA 1.5 m*M*, pH 7.2, *tris*-acetate buffer. The final whole tissue homogenate contained 2 to 6 mg protein per ml. A 30 min incubation was carried out at 25 °C. Either 62.5 m*M* Na and 25 m*M* K was present, and this gave total ATPase, or the incubation medium contained 87.5 m*M* Na, no K but 10^{-3} *M* ouabain, giving the Mg-ATPase. The difference between these two values was the Na-K dependent ATPase for the sample.

Inorganic phosphate was determined with Biochemica Test Combination Kits (Boehringer, Mannheim GMBH). Proteins were estimated using LOWRY'S[5] method. The enzyme concentration was expressed in each case as µ*M* ATP hydrolyzed (or inorganic phosphate, P_i, released) per h/mg protein in the homogenate.

Results. Rates of transport. Comparing bladders removed from FW animals with those from SW fish, there is a reduced rate of transport (Figure 1). This is significant ($P < 0.01$) for net Na flux, J^nNa, which drops from 1.55 ± 0.16 to 0.87 ± 0.16 µEq/h cm², but is less obvious ($0.1 < P < 0.2$) for the solute-linked water transport, J_v, which is 3.21 ± 0.33 and 2.14 ± 0.55 µl/h cm² in FW and SW respectively.

Both reabsorptive activities are unchanged in fish adapted to $^1/_3$ SW when compared with FW (J^nNa: 1.53 ± 0.19; J_v: 2.72 ± 0.26). Thus only adaptation to a hypertonic medium (SW) diminishes the absolute rate of Na transport in the trout bladder.

Effects of ouabain. Using the sac technique, ouabain 10^{-4} *M* lowers J_v and J^nNa ($P = 0.01$ and $P < 0.001$) when it is applied to the serosal side of FW animal bladders. For the 2-hour period of these experiments, these net fluxes drop 27% and 61% respectively and are brought to

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levels slightly lower than those observed in SW fish (Figure 1).

In other experiments, where the drug was left overnight (17 h) in contact with the membrane, further transport was blocked and no osmotic gradient was created across the bladder at the end of the assay. Figure 2 shows that ouabain 10^{-4} *M* immediately reduces the mucosal to serosal flux of sodium, $J^{ms}Na$ (45%, 35% and 66% inhibition in 3 separate experiments). Since spontaneous contractions of the bladder are occasionally observed when the drug is added, it is possible that folding of the bladder surface is responsible for the reduction in this flux. This does not appear to be so, however, since the reverse flux, $J^{sm}Na$, remains unchanged (less than 5% diminution) with this treatment. Thus ouabain inhibits the net transport of sodium and the subsequent absorption of water by a specific effect on an active component and not on passive permeability to sodium.

Since ouabain inhibits the Na-K exchange at the serosal side of transporting membranes, a reduction in Na flux would be expected to occur when the bladder is bathed by a K^+-free solution at its serosal side. Figure 3 shows that this does indeed occur: $J^{ms}Na$ is partially inhibited (by 41%, 42% and 48% in 3 individual experiments) and this effect is reversible. These actions of ouabain and K^+ removal, i.e. reduction of J^{ms} by about 45% and of J^{sm} by 5%, mean that net Na flux drops 85% since the former unidirectional flux is approximately twice as great as the latter in the freshwater trout bladder (4 and unpublished results).

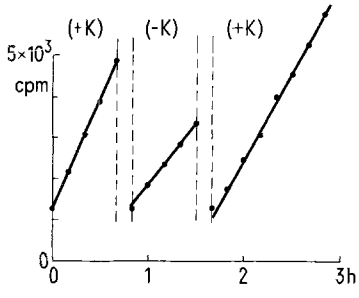

Fig. 3. Inhibition of the mucosal to the serosal (J^{ms}) Na flux when K^+-free (− K) replaces normal Ringer (+ K) solution on the serosal side. The flux (proportional to the slope) is reduced by 44% in (− K). Initial value obtains on addition of K^+ (extreme right of the figure).

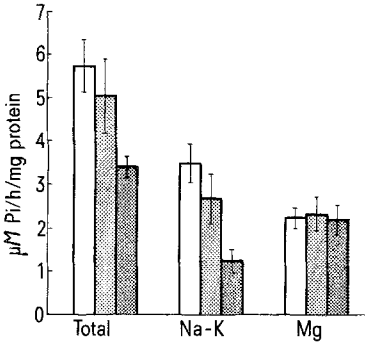

Fig. 4. Total, Na-K-dependent and Mg-dependent ATPase activities in homogenized pooled bladders taken from trout adapted to freshwater (open columns, n = 14 measurements), 1/3 sea water (dotted columns, n = 8) and sea water (cross-hatched columns, n = 7). The total and Na-K-ATPase activities significantly decrease in sea water compared with that of other media. The Mg-ATPase is unchanged.

Variations in ATPase content. In FW bladders, Na-K-ATPase represents more than 50% of total enzyme activity (Na-K: 3.49 ± 0.47; Mg: 2.29 ± 0.22; total: 5.57 ± 0.58 µ*M* P_i/h mg protein). No change is observed in 1/3 SW (2.69 ± 0.54; 2.35 ± 0.37 and 5.04 ± 0.84 respectively), but adaptation to SW induces a significant decrease in Na-K-ATPase (1.26 ± 0.27; $P < 0.01$) and total ATPase (3.40 ± 0.22; $P < 0.02$), while Mg-ATPase is unchanged (2.21 ± 0.37).

Discussion. The diminution in water and sodium transport by the bladder after adaptation of trout to salt (Figure 1) is in contrast with the increase in water reabsorption which occurs in that medium in such fishes as the flounder[1], and indicates that the trout does not behave as a typically euryhaline species in this respect.

The trout bladder transfers Na^+ and Cl^- against an electrochemical gradient, as an electrically neutral solution[4]. This is similar to the gall bladder[6], with the remarkable exception that in the trout bladder transport is hyperosmotic and not isosmotic. Van Os and Slegers[7] have shown that the intimate association which exists between Na^+ and Cl^- transports in gall bladder is compatible with the participation of a Na-K-ATPase. The present studies demonstrate that this enzyme is present in fish urinary bladder.

The decrease in enzyme activity in parallel with Na transport following salt adaptation (Figures 1 and 4) is similar to the diminution in renal Na-K-ATPase observed in *Fundulus heteroclitus* upon transfer to sea water[8]. It is consistent with what may be regarded as the 'physiological need' in saline environments.

The inhibitory action of ouabain on transport provides more direct evidence for the involvement of Na-K-ATPase in bladder function. This effect has also been observed in the flounder[3]. In addition, since J^{sm} does not change while J^{ms} drops immediately (Figure 2), this effect is not artifactual and ouabain readily penetrates the outer layers of the bladder wall. There is virtually no net transfer of K^+ through the trout bladder (unpublished observations). Removal of K^+ from the serosal fluid however inhibits Na transport to the same extent as ouabain (Figure 3). This is additional evidence for a direct participation of Na-K-ATPase in Na transport and indicates that a Na^+/K^+ ion exchange may take place at the serosal border. Experiments are in progress to examine this point and to define more precisely the role of Cl^- ions in this process.

Résumé. La Na-K-ATPase sensible à l'ouabaine diminue parallèlement au transport du sodium in vitro dans la vessie urinaire de la truite (*Salmo irideus*) au cours de l'adaptation des animaux aux milieux salés et semble directement impliquée dans le mécanisme de ce transport.

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[9] This work is supported by the Délégation Générale à la Recherche Scientifique et Technique (Contrat No. 71.7.3180).