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Effect of cell sodium on Na^+/K^+-ATPase-dependent sodium efflux in cortical collecting tubule of rabbits under different aldosterone status

Marcel Blot-Chabaud, Frédéric Jaisser, Jean-Pierre Bonvalet and Nicolette Farman

INSERM U 246 Département biologie / SBCe, CEN Saclay, Gif / Yvette (France)

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Aldosterone increased the tubular volume in cortical collecting tubules (CCD) of rabbit kidney. It modulated the rate of cell sodium accumulation, under condition of ATPase inhibition (4°C, in the absence of K^+). In contrast, the relationship between Na^+/K^+-ATPase-dependent Na^+ extrusion rate and intracellular Na^+ concentration (Na_i^+) was similar in control, adrenalectomized, and aldosterone-treated adrenalectomized animals: Na^+ extrusion rate increased with Na_i^+, up to 70 mM Na_i^+, and then plateaued. This indicates that aldosterone does not modify the characteristics of Na_i^+-dependent Na^+ extrusion rate by the Na^+/K^+-ATPase pump in CCD.

Aldosterone enhances sodium reabsorption in the cortical collecting tubule (CCD) of the kidney through coordinated actions, including a stimulation of sodium entry via apical membrane and sodium extrusion from the cell via Na^+/K^+-ATPase pumps at the basolateral side [1]. Sodium concentration has been shown to modulate the activity of purified Na^+/K^+-ATPase [2]. In a previous study [3], we set up a method that measures Na^+/K^+-ATPase-dependent Na^+ extrusion rate as a function of intracellular sodium concentration (Na_i^+) in intact rabbit CCD. As recently pointed out [4], the respective effects of aldosterone and cell sodium with regards to the modulation of Na^+/K^+-ATPase activity are yet in debate. This prompted us to examine whether aldosterone modifies the relationship between Na_i^+ and Na^+/K^+-ATPase-dependent Na^+ extrusion rate in CCD.

Experiments were performed in three groups of New Zealand rabbits: adrenalectomized 6 days before experiments (Adx, $n = 13$), sham-operated control (Sham, $n = 6$) and Adx infused (minipumps) with aldosterone, 24 μg or 120 μg per day for 6 days (Aldo, $n = 7$). The methods used have been previously described in detail [3]. Microdissected CCD were Na^+-loaded in the presence of $^{22}Na^+$ and [^{3}H]sorbitol in a K^+-free solution at 4°C. The length and volume of CCD were determined using an image analyser (Biocom, France). A superfusion method was applied to Na^+-loaded CCD (8–10 mm length) in order to measure the kinetics of Na^+ efflux at 37°C, in the presence or absence of 10^{-4} M ouabain. In the presence of ouabain, choline chloride was used as superfusion solution, in order to avoid isotopic dilution. On the whole, 63 Na^+ efflux curves were obtained. For each experimental curve, a computer analysis was used to determine the gradual decrease in Na_i^+ and the instantaneous Na^+ extrusion rate at each Na_i^+. From these data, the relationship between Na_i^+ and Na^+ extrusion rate was established. Na^+/K^+-ATPase-dependent Na^+ extrusion rate was obtained by subtracting data in the presence of ouabain from those in the absence of ouabain.

Aldosterone plasma concentrations (in pg/ml) were 645 ± 97 in Sham, 55 ± 16 in Adx, 534 ± 49 in Adx treated with 24 μg/day aldosterone and 1513 ± 35 in Adx treated with 120 μg/day aldosterone.

Tubular volume (in nl/mm tubular length) was 0.68 ± 0.04 in Sham. Adrenalectomy reduced it (0.52 ± 0.03) and aldosterone treatment increased it (24 μg aldo: 0.93 ± 0.10; 120 μg aldo: 0.85 ± 0.08). These variations are in accordance with previous reports on the effects of aldosterone on cell volume regulation in CCD [5–7].

During Na^+ loading at 4°C in absence of external K^+, Na_i^+ gradually increased with incubation time, as previously reported [3]. This increase was slower in Adx

Correspondence: Marcel Blot-Chabaud, INSERM U 246, Département Biologie/SBCe, CEN Saclay, 91191 Gif/Yvette, France.

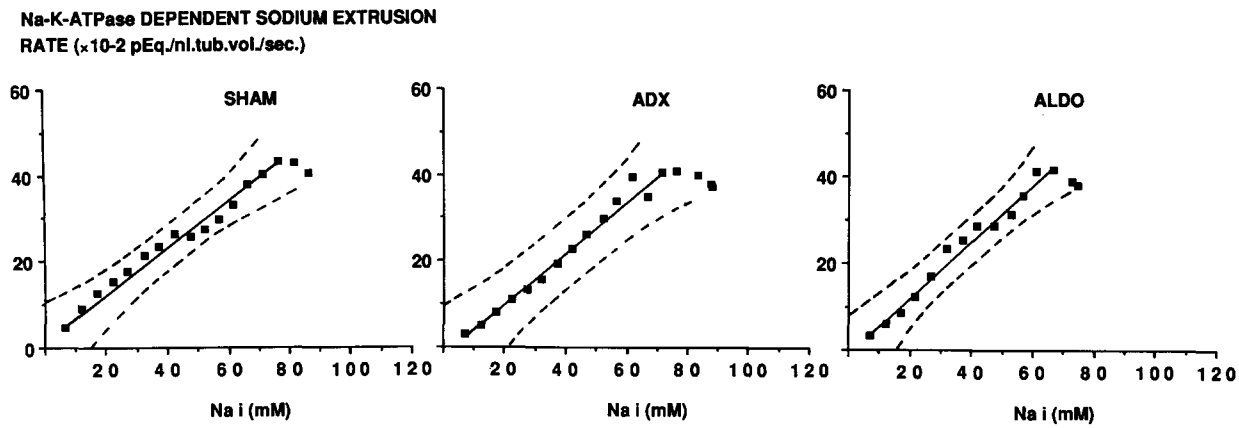

Fig. 1. Relationship between Na^+/K^+-ATPase-dependent sodium extrusion rate and intracellular sodium concentration (Na_i^+) in CCD from sham-operated (SHAM), adrenalectomized (ADX) and Adx rabbits receiving aldosterone (ALDO). 24 μg and 120 μg Aldo yielded similar results and were pooled. Mean values of Na^+ extrusion rate were calculated from 63 kinetics of sodium efflux, at 5 mM Na_i^+ intervals. Each point gives the difference between the mean values in presence or absence of ouabain. Dotted lines give the confidence belts of regression lines (full lines). No significant difference in slope was present between groups (covariance analysis).

group than in the other ones. At 300–400 min, Na_i^+ was 80–90 mM in Adx, whereas it reached values close to the external Na^+ concentration (149 mM) in the other groups. Using the function $Na_i^+(t) = 149[1 - \exp(k_{app} \cdot t)]$, where $Na_i^+(t)$ is Na_i^+ at the time t, we determined the apparent rate constant (k_{app}) of Na^+ entry into the cell. The k_{app} was (in 10^{-3} min^{-1}) 5.4 ± 0.5 in Sham. It was largely reduced in Adx (3.0 ± 0.1) and restored by aldosterone administration (24 μg aldo: 5.1 ± 0.7; 120 μg aldo: 4.4 ± 0.6). The reduced rate of Na^+ entry into CCD cells from Adx could be accounted for by the well-documented reduction in basolateral membrane surface area observed in parallel with the reduction in cell volume after adrenalectomy [5–8].

Fig. 1 represents the relationship between Na^+/K^+-ATPase-dependent Na^+ extrusion rate and Na_i^+ in Sham, Adx and Aldo animals. Aldosterone status did not influence this relationship: in all groups, Na^+ extrusion rate gradually increased with Na_i^+ up to ≈ 70 mM Na_i^+, with an apparent $K_{1/2}$ of ≈ 35–40 mM. It is unlikely that the observed plateau of Na^+ extrusion rate should be due to a limitation in ATP supply. In the presence of 3 mM ATP, Jörgensen [2] observed, on purified Na^+/K^+-ATPase, an activation curve by Na^+ concentration similar to that in our experiments. Moreover, several lines of arguments indicate that in vitro transepithelial Na^+ transport is not limited by ATP content, in the presence of adequate substrates [9,10].

Thus, at any given Na_i^+, the rate of Na^+/K^+-ATPase-dependent Na^+ extrusion rate by the functional pumps inserted in the membrane was unaffected by aldosterone. On the other hand, it has been shown [11] that the stoichiometry between Na^+/K^+-ATPase activity (as measured by the rate of ATP hydrolysis) and the number of functional pumps inserted in the membrane (as determined by ouabain binding) is not modified by aldosterone. Thus, the reported effects of aldosterone on Na^+/K^+-ATPase activity [11,12] might reflect changes in the number of functional pumps rather than modifications of the characteristics of Na^+ transport by the pump.

Several studies (reviewed in Ref. 12) indicate that the Na^+/K^+-ATPase activity, as expressed per mm tubular length of CCD is decreased by adrenalectomy and increased by aldosterone. However, Fig. 2 shows that the measured activity per unit tubular length is highly dependent on the tubular volume. If the present results were expressed per mm tubular length instead of per unit tubular volume, then the Na^+/K^+-ATPase-dependent Na^+ extrusion rate as a function of Na_i^+ would appear to be reduced in Adx (by about 20%) and increased by aldosterone (by about 30%). This points

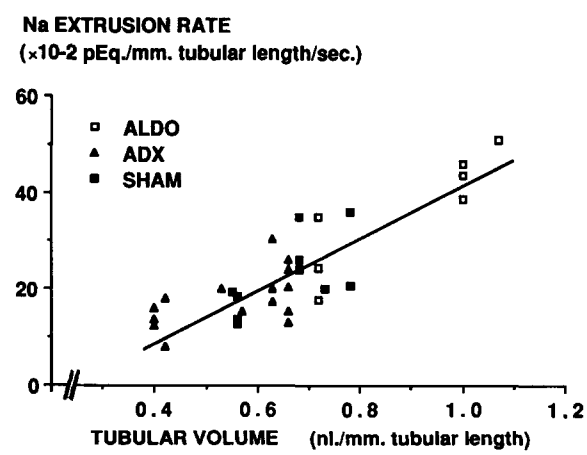

Fig. 2. Relationship between tubular volume and Na^+ extrusion rate per unit tubular length in CCD from sham-operated (SHAM), adrenalectomized (ADX) and aldosterone-treated Adx (ALDO) rabbits. Each point is Na^+ extrusion rate at the apparent $K_{1/2}$ (35–40 mM Na_i^+), in the absence of ouabain. Na^+ extrusion rate per mm tubular length is highly correlated to tubular volume ($r = 0.85$, $P < 0.001$).

out the importance of taking into account cell volume in the evaluation of Na^+/K^+-ATPase activity.

In conclusion, the present study indicates that aldosterone does not modify the characteristics of Na_i^+-dependent Na^+ extrusion rate by the Na^+/K^+-ATPase pump in CCD.

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References

1 Garty, H. (1986) J. Membr. Biol. 90, 193–205.
2 Jörgensen, P.L. (1986) Kidney Int. 29, 10–20.
3 Blot-Chabaud, M., Jaisser, F., Gingold, M. , Bonvalet, J.P. and Farman, N., (1988) Am. J. Physiol. 255 (Renal Fluid Electrolyte Physiol. 24), F605–F613.
4 Stanton, B.A. and Kaissling, B. (1989) Am. J. Physiol. 257 (Renal Fluid Electrolyte Physiol. 26), F1–F10.
5 Kaissling, B. and Le Hir, M. (1982) Cell Tissue Res. 224, 469–492.
6 Stanton, B.A. (1985) Fed. Proc. 44, 2717–2722.
7 Kashgarian, M., Ardito, T., Hirsch, D. and Hayslett, J.P. (1987) Am. J. Physiol. 253 (Renal Fluid Electrolyte Physiol. 22), F8–F14.
8 Wade, J.B., O'Neil, R.G., Pryor, J.L. and Boulpaep, E.L. (1979) J. Cell Biol. 81, 439–445.
9 Grasl, M., Krivanek, P. and Turnheim, K. (1982) Pflügers Arch. 395, 257–259.
10 Uchida, S. and Endou, H. (1988) Am. J. Physiol, 255 (Renal Fluid Electrolyte Physiol. 24), F977–F983.
11 El Mernissi, G. and Doucet, A. (1984) Pflügers Arch. 402, 258–263.
12 Katz, A.I. (1986) Kidney Int. 29, 21–31.