Biochimica et Biophysica Acta, 689 (1982) 161–164
Elsevier Biomedical Press

BBA Report

BBA 70043

DETERMINATION OF THE COUPLING RATIO FOR Na^+-H^+ EXCHANGE IN RENAL MICROVILLUS MEMBRANE VESICLES

JAMES L. KINSELLA and PETER S. ARONSON *

Departments of Medicine and Physiology, Yale School of Medicine, New Haven, CT 06510 (U.S.A.)

(Received December 21st, 1981)

Key words: Na^+ transport; H^+ transport; Cotransport; Ion exchanger; Acid-base regulation; (Renal microvillus membrane)

We evaluated the H^+:Na^+ coupling ratio of the Na^+-H^+ exchanger present in microvillus membrane vesicles isolated from the rabbit renal cortex. Our approach was to impose transmembrane Na^+ and H^+ gradients of varying magnitude and then to measure the net flux of Na^+ over the subsequent 5-s period. The Na^+-H^+ exchanger was observed to be at equilibrium (i.e. no significant net Na^+ flux) whenever $[Na^+]_i/[Na^+]_o$ was equal to $[H^+]_i/[H^+]_o$. Moreover, under all conditions the magnitude and direction of net Na^+ flux was independent of changes in the transmembrane electrical potential difference. These results are consistent with a value of 1.0 for the coupling ratio of Na^+-H^+ exchange in renal microvillus membrane vesicles.

The plasma membranes of diverse tissues possess Na^+-H^+ exchangers that participate in the regulation of intracellular pH [1]. Microvillus membrane vesicles isolated from the rabbit renal cortex contain such an Na^+-H^+ exchange system as indicated by the observations that imposing an inside-acid, transmembrane pH gradient drives uphill Na^+ accumulation, and that imposing an inwardly directed Na^+ gradient drives uphill H^+ extrusion [2]. Because the rate of Na^+ uptake by renal membrane vesicles is unaffected by maneuvers that alter the transmembrane electrical potential difference, it has been presumed that Na^+-H^+ exchange is electroneutral with a H^+:Na^+ coupling ratio of 1.0 [2,3]. However, evaluating the voltage-sensitivity of the rate of transport of a solute is not necessarily a valid means for determining whether the transport of that solute is rheogenic (i.e. associated with a flow of charge) [4].

Clearly, the most direct approach for evaluating the coupling ratio of the renal microvillus membrane Na^+-H^+ exchanger would be to measure the efflux of H^+ as a function of Na^+ influx. Unfortunately, application of this approach is made difficult by the existence of leak pathways for Na^+ and H^+. Although at Na^+ concentrations below 1 mM the Na^+-H^+ exchanger is essentially the only route for Na^+ transport in renal microvillus membranes [2,5], at the higher Na^+ concentrations necessary to generate measureable changes in extravesicular pH [3], significant Na^+ flux occurs via other pathway(s) (Kinsella, J.L. and Aronson, P.S., unpublished data). Similarly, renal microvillus membrane vesicles have been shown to possess a significant leak pathway for H^+ [6].

An alternative strategy for determining the coupling ratio of Na^+-H^+ exchange can be based on equilibrium thermodynamics. If n represents the H^+:Na^+ coupling ratio, then the conditions under which the Na^+-H^+ exchanger will be at equilibrium (i.e. there are no net fluxes of Na^+ or H^+) are given by Ref. 4.

$$\frac{[Na^+]_i}{[Na^+]_o} = \left(\frac{[H^+]_i}{[H^+]_o}\right)^n \exp\left(\frac{F}{RT}(\psi_o - \psi_i)(1-n)\right)$$

* To whom all correspondence and reprint requests should be addressed.

Abbreviations: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; ADA, *N*-(2-acetamido)iminodiacetic acid; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenyl hydrazone.

0005-2736/82/0000–0000/$02.75

When n has a value of 1.0, equilibrium will occur when $[Na^+]_i/[Na^+]_o$ equals $[H^+]_i/[H^+]_o$ and will be unaffected by alterations in the transmembrane electrical potential difference, $(\psi_o - \psi_i)$.

Our experimental approach was to impose transmembrane Na^+ and H^+ gradients of varying magnitude and then to monitor the net flux of Na^+ over the subsequent 5 s. The combinations of $[Na^+]_i/[Na^+]_o$ and $[H^+]_i/[H^+]_o$ causing the Na^+-H^+ exchanger to be at equilibrium could thus be defined. The effect of altering the transmembrane electrical potential difference was also evaluated. The contribution of Na^+ leak pathways was minimized by our employing Na^+ concentrations ≤ 1 mM, under which conditions $\geq 90\%$ of Na^+ transport has been shown to occur via the Na^+-H^+ exchanger in these membrane vesicles [5]. Dissipation of imposed pH gradients was minimized by measuring net Na^+ flux over only a 5-s interval. Although a component of Na^+ uptake measured after prolonged incubations at low ionic strength may represent intravesicular binding [2], it is extremely unlikely that Na^+ binding and debinding contribute significantly to the apparent rates of Na^+ influx and efflux. The finding that both the rate of uptake and the rate of release of 1 mM Na^+ can be almost completely inhibited by amiloride [5] argues that measured rates of Na^+ uptake and release must represent the transmembrane influx and efflux of Na^+ rather than the process of binding and debinding.

Microvillus membrane vesicles were isolated from the rabbit renal cortex, as previously described [7], and then suspended in an isosmotic mannitol medium buffered with 10 mM Tris − 16 mM Hepes, pH 7.5 or 16 mM Tris − 10 mM ADA, pH 6.5. The membrane vesicles were preloaded with 1 mM NaCl and tracer ^{22}Na by preincubation for 1 h at 20°C. Intravesicular content of ^{22}Na was then measured by a rapid filtration technique [8] both before and 5 s after dilution of the vesicles into media of varying pH and Na^+ concentration. In each experiment, the specific activity of ^{22}Na in the diluting media was the same as that in the preloading medium so that changes in intravesicular ^{22}Na would be precisely proportional to changes in Na^+ content. The pH values of all solutions were checked daily. Each experiment was performed in triplicate on three separate occasions using different membrane preparations. The data represented in each figure are the means ± S.E. for three experiments. Further details of the experimental methods are given in the figure legends.

In the experiment illustrated in Fig. 1, the vesicles were pre-equilibrated with 1 mM Na^+ at pH 6.5 and then net Na^+ flux was assayed in the presence of a medium containing 0.1 mM Na^+ at pH 6.5, 7.5 or 8.5. At an external pH of 6.5, when $[H^+]_i/[H^+]_o$ was equal to 1.0, net efflux of Na^+ down its concentration gradient was observed. At an external pH of 7.5, when $[H^+]_i/[H^+]_o$ was 10 and equal to $[Na^+]_i/[Na^+]_o$, there was no net

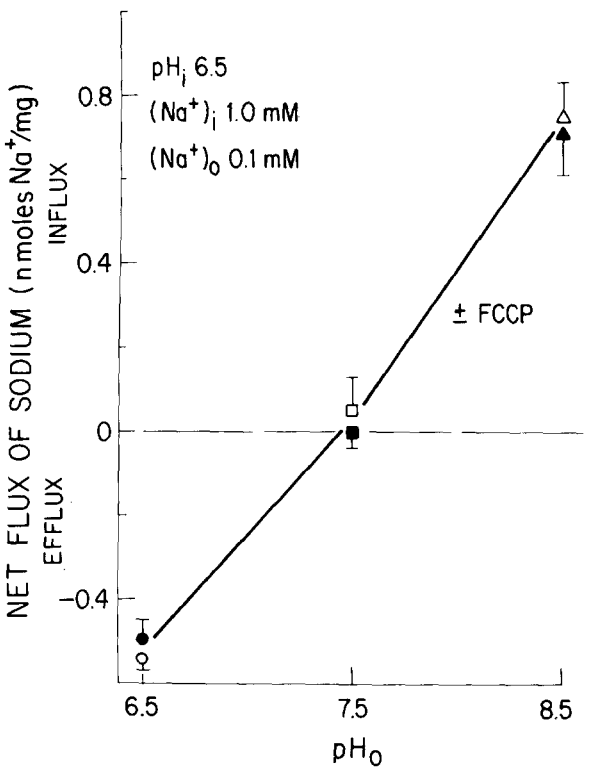

Fig. 1. Net flux of Na^+ at varying external pH. Membrane vesicles were pre-equilibrated in 298 mM mannitol, 1 mM NaCl, 10 mM ADA, 16 mM Tris, pH 6.5, and then the 5-s net flux of Na^+ determined in the presence of 300 mM mannitol, 0.1 mM NaCl, 10 mM ADA, 16 mM Tris, pH 6.5 (○, ●); 299 mM mannitol, 0.1 mM NaCl, 1.0 mM ADA, 14 mM Hepes, 10 mM Tris, pH 7.5 (□, ■); or 267 mM mannitol, 0.1 mM NaCl, 1.0 mM ADA, 13 mM Hepes, 42 mM Tris, pH 8.5 (△, ▲). The experiment was performed in both the absence (●, ■, ▲) and the presence (○, □, △) of 22.4 μg/ml FCCP.

flux of Na^+. At an external pH of 8.5, when $[H^+]_i/[H^+]_o$ was 100 and exceeded $[Na^+]_i/[Na^+]_o$, net influx of Na^+ occurred. The finding that equilibrium occurred when $[H^+]_i/[H^+]_o$ was equal to $[Na^+]_i/[Na^+]_o$ indicates that the coupling ratio for Na^+-H^+ exchange must be 1.0.

To confirm this conclusion, we examined whether altering the transmembrane electrical potential difference would affect the results in Fig. 1. The experiment was repeated in the presence of FCCP, an agent that increases the H^+ conductance of membranes [9]. In particular, we [2] and others [10] have previously shown that in the presence of an inside-acid pH gradient, FCCP will shift the membrane potential toward greater inside-negativity in rabbit renal microvillus membrane vesicles. Despite this effect of FCCP to alter the membrane potential, there was no effect of the ionophore to displace the equilibrium that occurred at an external pH of 7.5 in Fig. 1. Again, this result supports the conclusion that the coupling ratio for Na^+-H^+ exchange is 1.0.

In the experiment illustrated in Fig. 2, the vesicles were pre-equilibrated with 1 mM Na^+ at pH 6.5 and then net Na^+ flux was measured in the presence of a medium containing 0.01, 0.10, or 1.0 mM Na^+ at pH 7.5. At 0.01 mM external Na^+, when $[Na^+]_i/[Na^+]_o$ was 100 and exceeded the $[H^+]_i/[H^+]_o$ of 10, net Na^+ efflux was observed. At 0.10 mM external Na^+, when $[Na^+]_i/[Na^+]_o$ was 10 and equal to $[H^+]_i/[H^+]_o$, there was no significant net flux of Na^+. At 1.0 mM external Na^+, when $[Na^+]_i/[Na^+]_o$ was 1.0 and less than $[H^+]_i/[H^+]_o$, net Na^+ influx occurred. Addition of FCCP to alter the transmembrane electrical potential difference had no effect on these results. The findings in this experiment thus provide additional support for the concept that the $H^+:Na^+$ coupling ratio is 1.0.

In the experiment illustrated in Fig. 3, the vesicles were pre-equilibrated with 1 mM Na^+ at pH 7.5 and then net Na^+ flux was measured in the presence of a medium containing 0.01, 0.10, or 1.0 mM Na^+ at pH 8.5. At 1.0 mM external Na^+, when $[Na^+]_i/[Na^+]_o$ was 1.0 and less than the $[H^+]_i/[H^+]_o$ of 10, net Na^+ influx was observed. At 0.10 mM external Na^+, when $[Na^+]_i/[Na^+]_o$ was 10 and equal to $[H^+]_i/[H^+]_o$, there was no measurable net flux of Na^+, consistent with a

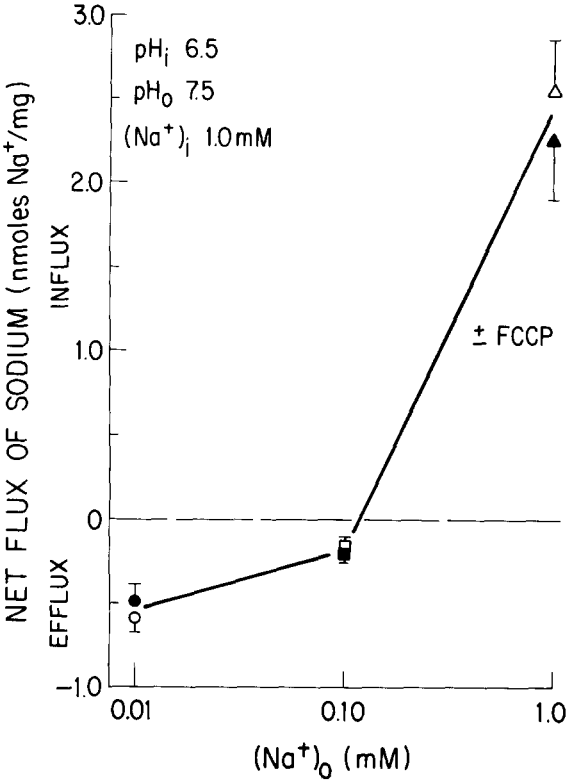

Fig. 2. Net flux of Na^+ at varying external Na^+ concentration. Membrane vesicles were pre-equilibrated in 298 mM mannitol, 1 mM NaCl, 10 mM ADA, 16 mM Tris, pH 6.5 and then the 5-s net flux of Na^+ was determined in the presence of 300 mM mannitol, 0.1 mM ADA, 10 mM Hepes, 16 mM Tris, pH 7.5 with 0.01 mM NaCl (○, ●), and in the presence of 299 mM mannitol, 1.0 mM ADA, 14 mM Hepes, 10 mM Tris, pH 7.5 with 0.10 mM NaCl (□, ■) or 1.0 mM NaCl (△, ▲). The experiment was performed in both the absence (●, ■, ▲) and the presence (○, □, △) of 22.4 μg/ml FCCP.

coupling ratio of 1.0 as previously discussed. However, at 0.01 mM external Na^+, when $[Na^+]_i/[Na^+]_o$ was 100 and exceeded $[H^+]_i/[H^+]_o$, there was also no measurable net flux of Na^+. If one were to assume that the Na^+-H^+ exchanger was at equilibrium under these latter conditions, then the $H^+:Na^+$ coupling ratio would have to be 2.0. However, the lack of any significant effect from altering the membrane potential with FCCP argues that the coupling ratio is indeed 1.0. It is therefore probable that the finding of no net Na^+ flux at 0.01 mM external Na^+ resulted from the fact that net Na^+ efflux was present but too low to be

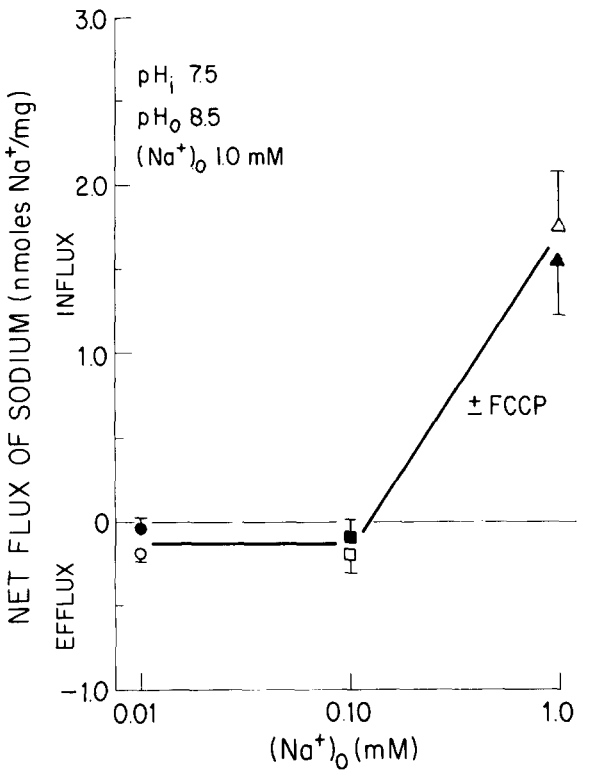

Fig. 3. Net flux of Na^+ at varying external Na^+ concentration. Membrane vesicles were pre-equilibrated in 298 mM mannitol, 1 mM NaCl, 10 mM $MgSO_4$, 10 mM Tris, 16 mM Hepes, pH 7.5 and then the 5-s net flux of Na^+ was determined in the presence of 259 mM mannitol, 10 mM $MgSO_4$, 42 mM Tris, 13 mM Hepes, pH 8.5 with 0.01 mM NaCl (○, ●), 0.10 mM NaCl (□, ■), or 1.0 mM NaCl (△, ▲). The experiment was performed in both the absence (●, ■, ▲) and the presence (○, □, △) of 22.4 μg/ml FCCP.

measurable rather than from the fact that the Na^+-H^+ exchanger was actually at equilibrium. Despite a favorable thermodynamic driving force, Na^+ efflux was likely to have been impeded kinetically by the relative unavailability of external H^+ for exchange at pH 8.5.

In summary, these studies indicate that the Na^+-H^+ exchanger in rabbit renal microvillus membrane vesicles has a H^+:Na^+ coupling ratio of 1.0. Such a coupling ratio is consistent with H^+:Na^+ stoichiometries of 1:1, 2:2, 3:3, etc. However, we have previously observed that Na^+ transport via the rabbit renal Na^+-H^+ exchanger conforms to simple Michaelis-Menten kinetics [5], suggesting that each transport event is associated with the binding of a single Na^+. We therefore conclude that the stoichiometry of Na^+-H^+ exchange in renal microvillus membrane vesicles is 1:1.

The excellent technical assistance of Gregory Martin and Joyce Elwell and secretarial assistance of Lori Testori are gratefully acknowledged. This work was supported by U.S. Public Health Service research grant AM-17433. J.L.K. was a NIH Postdoctoral Research Fellow (AM-06102) and P.S.A. an Established Investigator of the American Heart Association.

References

1 Roos, A. and Boron, W.F. (1981) Physiol. Rev. 61, 296–434
2 Kinsella, J.L. and Aronson, P.S. (1980) Am. J. Physiol. 238, F461–F469
3 Murer, H., Hopfer, U. and Kinne, R. (1976) Biochem. J. 154, 597–604
4 Aronson, P.S. (1981) Am. J. Physiol. 240, F1–F11
5 Kinsella, J.L. and Aronson, P.S. (1981) Am. J. Physiol. 241, F374–F379
6 Burnham, C., Muenzesheimer, T., Rabon, E. and Sachs, G. (1981) Fed. Proc. 40, 462
7 Aronson, P.S. (1978) J. Membrane Biol. 42, 81–88
8 Aronson, P.S. and Bounds, S.E. (1980) Am. J. Physiol. 238, F210–F217
9 Hopfer, U., Lehninger, A.L. and Thompson, T.E. (1968) Proc. Natl. Acad. Sci. U.S.A. 59, 484–490
10 Beck, J.C. and Sacktor, B. (1975) J. Biol. Chem. 250, 8674–8680