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SODIUM UPTAKE MECHANISMS IN BRUSH-BORDER MEMBRANE VESICLES PREPARED FROM RABBIT RENAL CORTEX

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Amiloride-sensitive and amiloride-insensitive components of $^{22}Na^+$ uptake were examined in brush-border membrane vesicles prepared from rabbit renal cortex. Both components could be stimulated by interior-negative electrical potentials, demonstrating a sodium conductance pathway and an effect of electrical potential on the initial rate of Na^+/H^+ exchange.

Na^+/H^+ exchange has previously been examined in brush-border membrane vesicles prepared from renal cortex [1,2]. These studies described an electrically neutral antiporter which transports Na^+ ions across the luminal membrane in exchange for H^+. In addition, amiloride has been shown to inhibit this transport system [2]. The present study examined the effects of pH gradients and electrical potentials on the amiloride-sensitive and amiloride-insensitive components of $^{22}Na^+$ uptake in vesicles prepared from rabbit renal cortex.

Experimental methods are described in a previous publication [3]. In brief, brush-border membrane vesicles were prepared by homogenization, Mg^{2+} precipitation and differential centrifugation [4]. Rates of $^{22}Na^+$ uptake were measured with a rapid filtration assay at 0.1 min and 0.5 min. Vesicles were prepared in internal media containing (in mM); sucrose 100; potassium gluconate 50; and Hepes 1.0. The pH was adjusted to 6.0 or 7.5 with 0.2 M H_2SO_4 or Tris. Valinomycin (100 μg/ml), when present, was added to vesicles at least 30 min before use. Uptake studies were begun by adding aliquots of vesicles to external media which contained (final concentrations in mM): $^{22}Na^+$ as the gluconate salt 2; potassium gluconate 10; sucrose 180; hepes 10; and Tris 7. The external pH was adjusted to 6.0 or 7.5 before addition of vesicles. Amiloride (0.3 mM), when present, was added to the external buffer before the vesicles.

The average results (±S.E.) for five separate vesicle preparations are presented in Fig. 1. The control rates of $^{22}Na^+$ uptake (nmol/mg protein) are presented with open bars in each series. The external and internal pH are indicated below each series. The effects of valinomycin (V), amiloride (A) or both (VA) are denoted by the appropriate letters in the bars. The statistical comparisons were made with paired-*t* tests, and were considered as significant (*) for $P < 0.05$ and non-significant (N.S.) if P was > 0.05.

The control rate of $^{22}Na^+$ uptake was greater when the external pH was 7.5 than when external pH was 6.0. At constant external pH, the control $^{22}Na^+$ uptake rate was greater when the internal pH was more acid. These results are consistent

Abbreviation: Hepes, *N*-2-Hydroxyethylpiperazine-*N'*-ethanesulfonic acid.

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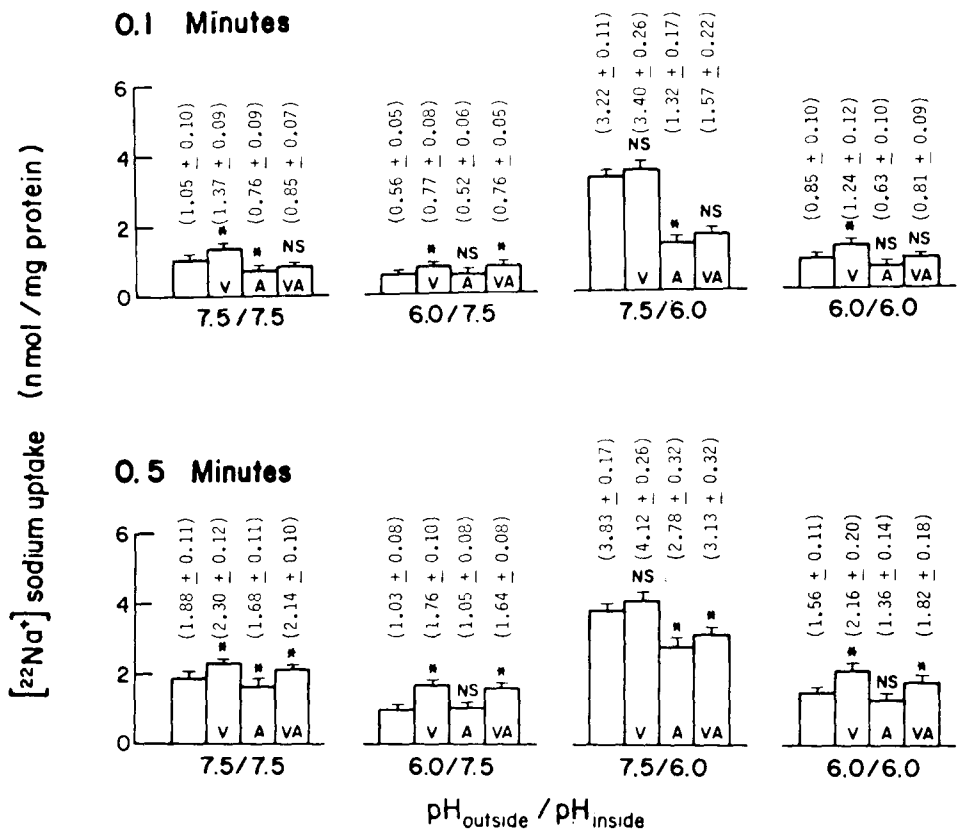

Fig. 1. Effect of valinomycin (V) and amiloride (A) on $^{22}Na^+$ uptake. Mean values (±S.E.) at 0.1 min and 0.5 min for five separate brush-border vesicle preparations are indicated in parentheses above the bars. The paired statistical comparisons (* or N.S.) were made between the V series and control (open bars), between the A series and control (open bars), and between the VA series and the A series.

with the operation of a Na^+/H^+ antiporter [1,2].

Addition of valinomycin to vesicles with an outwardly-directed K^+ gradient created an interior-negative electrical potential [1–3]. There was a significant increase in rate of $^{22}Na^+$ uptake (bars labelled V) in every series at 6 s and 30 s except when the external pH was 7.5 and the internal pH was 6.0. In this instance, the outwardly-directed proton gradient had already maximally stimulated $^{22}Na^+$ uptake in comparison to the other series.

Amiloride (0.3 mM) was added to inhibit the uptake of $^{22}Na^+$ by the antiporter [2]. No effect of amiloride was observed if the external pH was 6.0, but there was a significant decrease in the rate of $^{22}Na^+$ uptake (bars labelled A) if the external pH was 7.5. When the different series were compared, the magnitude of the amiloride effect (i.e., the difference between the control series and the A series) was found to be proportional to the control rate of $^{22}Na^+$ uptake. The effects of amiloride were not as great at 0.5 min as at 0.1 min, indicating that the amiloride-sensitive component of $^{22}Na^+$ uptake had a relatively short time course compared to the amiloride-insensitive component.

Amiloride also blocked the valinomycin effect at 0.1 min if the internal and external pH were equal (bars labelled VA). Amiloride did not block the valinomycin effect at 0.1 min when the external pH was 6.0 and internal pH was 7.5. Furthermore, amiloride did not block the valinomycin effect in any series at 0.5 min. This finding reflects the short time course of the amiloride-sensitive component relative to the amiloride-insensitive

component of $^{22}Na^+$ uptake that was stimulated by valinomycin. Valinomycin did not affect $^{22}Na^+$ uptake when external pH was 7.5 and internal pH was 6.0; consequently, there was no further effect of amiloride under this condition.

Previous workers [1,2] concluded that the Na^+/H^+ antiporter of renal cortical brush border membrane vesicles was electrically-neutral because K^+ and valinomycin did not affect the rate of H^+ ejection or $^{22}Na^+$ uptake. In agreement with these reports, our findings indicate that Na^+/H^+ antiporter is electrically-neutral when maximally stimulated by an outwardly-directed proton gradient (inside pH = 6.0, outside pH = 7.5). With other pH conditions, our results demonstrate an effect of electrical potential on the initial rate of $^{22}Na^+$ uptake. Amiloride inhibits the Na^+/H^+ antiporter in rabbit renal cortical brush-border membrane vesicles [2], and was used in the present study to distinguish between $^{22}Na^+$ uptake by the Na^+/H^+ antiporter and by other, amiloride-insensitive pathways. Stimulation of $^{22}Na^+$ uptake by interior-negative electrical potentials would be predicted for electrogenic sodium co-transport [3,5–7]. The present studies demonstrate a stimulation of $^{22}Na^+$ uptake by interior-negative electrical potential, but were conducted in the absence of co-transported substrates. This electrical effect could be observed when $^{22}Na^+$ uptake was not maximally stimulated by an outwardly-directed proton gradient, and was more prominent at 0.5 min. Our results demonstrate a conductive pathway for $^{22}Na^+$ uptake in renal cortical brush-border membrane vesicles, in agreement with the recent findings of Wright et al. [8]. It is of interest that this pathway is insensitive to amiloride, in contrast to sodium conductance pathways in distal segments of the nephron [9–11].

We have also observed an effect of valinomycin on the amiloride-sensitive component of $^{22}Na^+$ uptake at 0.1 min. This results indicates that the rate of Na^+/H^+ exchange may be influenced by electrical potentials when the internal and external pH are equal. One explanation for this effect is that the Na^+/H^+ antiporter is not electrically-neutral under all conditions. For example, the stoichiometric ratio may not be fixed at 1:1 so that each cycle or turnover of the antiporter translocates net charge. Other possibilities can also explain an effect of electrical potential upon the initial rate of Na^+/H^+ exchange. If the unloaded carrier has a net negative charge, then interior-negative electrical potentials could initially distribute the carriers at the outer aspect of the membrane. As a result the initial rate of Na^+/H^+ exchange would be increased, even though the Na^+/H^+ antiporter had a 1:1 stoichiometry and was, therefore, electrically neutral. A third possibility is that the rate of exchange is limited by substrate availability, and that access of $^{22}Na^+$ to the carrier is diffusion-limited. This concept is analogous to the 'proton well' discussed by Mitchell [12] and West [13]. On the other hand, availability of protons at the inner aspect of the membrane could be rate limiting. Our studies were done with low internal buffer strength (1 mM Hepes) so that Na^+/H^+ exchange may have depleted the internal volume of protons in the absence of a preformed outwardly-directed proton gradient. If these membranes have a proton conductance in parallel with the Na^+/H^+ antiporter [14], then the interior-negative electrical potential could accelerate H^+ entry and, therefore, provide additional substrate (i.e., internal protons) for Na^+/H^+ exchange by the antiporter. The present studies do not distinguish between these possibilities, but do demonstrate an amiloride-insensitive, conductive pathway for $^{22}Na^+$ uptake and an effect of electrical potential on the initial rate of Na^+/H^+ exchange.

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