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# SENSITIVITY OF RENAL BRUSH-BORDER $Na^+$-COTRANSPORT SYSTEMS TO ANIONS

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**The effect of anions on $Na^+$-cotransport of succinate, lactate, glucose, and phenylalanine was studied under voltage clamped conditions in brush-border membrane vesicles prepared from rabbit renal cortex. The initial rate of succinate uptake varied by an order of magnitude depending on the anion: the highest rates were obtained with fluoride and gluconate, and the lowest with iodide. The anion sequence corresponded with the inverse of the anion hydration energies. The kinetics of succinate uptake were measured in the presence of fluoride and chloride. There was no difference in the maximal rates of uptake, but the $K_t$ in fluoride (0.30 mM) was less than half that in chloride (0.70 mM), i.e. $Cl^-$ behaved as a competitive inhibitor of succinate transport with a $K_i$ of 150 mM. The uptake of L-lactate, D-glucose and L-phenylalanine was less sensitive to anions, and there was no correlation with hydration energies. We conclude that the anion effects on sugar and amino acid uptakes measured under open-circuit conditions are largely due to variations in membrane potential, but in the case of the dicarboxylate transporter anions behave as weak competitive inhibitors. The specificity of the anion inhibition suggests that the dicarboxylate binding sites have a weak field strength relative to water.**

It is generally held that the anion dependence of $Na^+$-cotransport of organic solutes (sugars, amino acids, and carboxylic acids) into brush-border membrane vesicles is simply due to variations in membrane potential. In renal brush-border membrane vesicles sugars, amino acids and carboxylic acids depolarise the membrane in the presence of $Na^+$ [1–4] and variations in membrane potential influence the kinetics of solute uptake [5–7]. Therefore, replacing poorly permeant anions (e.g., $SO_4^{2-}$) with more highly permeant anions (e.g., $Cl^-$ or $SCN^-$) should hyperpolarize the membrane and increase the rate of solute uptake. To determine the validity of this assumption, we measured the effect of anions on renal brush-border cotransport systems under voltage clamped conditions. Initial rates of solute uptake were measured as a function of anion composition when the membrane potential was short circuited with $K^+$ and valinomycin.

Rabbit renal cortex brush-border membrane vesicles were prepared as described previously [7], and were suspended in 222 mM mannitol, 100 mM $K^+$, 25 mM Tris-Hepes (pH 7.5), and 25 μg/ml valinomycin. Membranes were then stored for up to 48 h in liquid nitrogen [8]. Before each experiment the membranes were thawed and pre-incubated at 22–23°C for 15 min in buffer containing 75 μM FCCP. Uptakes of radioactive tracers were measured by a rapid filtration method [7], where the final composition of the uptake buffer was 100 mM $Na^+$, 100 mM $K^+$, 25 mM Hepes-Tris (pH 7.5), 25 μg/ml valinomycin, 75

Abbreviations: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone.

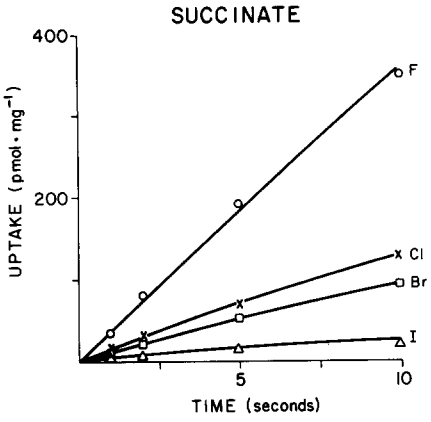


Fig. 1. Succinate uptake as a function of anion composition. Uptake of 15 $\mu$M succinate was measured over 1–10 s as described in the text. The uptake buffer consisted of 100 mM NaF, NaCl, NaBr or NaI; 100 mM KF, KCl, KBr or KI; 222 mM mannitol 25 mM Tris-Hepes (pH 7.5), 25 $\mu$g/ml valinomycin and 75 $\mu$M FCCP. The vesicles were loaded with 100 mM KF, KCl, KBr or KI, 25 mM Tris-Hepes (pH 7.5); and 25 $\mu$g/ml valinomycin. The uptake data were fitted to a quadratic equation by an iterative nonlinear regression program. All estimates were in triplicate and standard deviations are indicated when they exceed the size of the points. The 1-s uptakes give good estimates of initial rates [7]. The voltage across the membrane was shunted by $K^+$ and valinomycin and FCCP.

$\mu$M FCCP, and $^{14}$C-labelled substrate. Previous experiments have shown that the membrane potential is short circuited under these experimental conditions [7]. The final osmolarity of the uptake medium was iso-osmotic with the membrane suspension medium. The $^{14}$C-labelled substrates were obtained from Amersham (Arlington Heights, IL), and valinomycin and FCCP from Sigma Chemical Co. (St. Louis, MO). All other reagents used were of the highest grade commercially available.

Fig. 1 shows the first 10 s of succinate uptake in the presence of the halide salts of sodium. The initial rate of succinate uptake decreased by an order of magnitude from NaF to NaI even though the membrane potential was short circuited. The relative rates of succinate and L-lactate uptakes with each anion are summarized in Table I. With $Na^+$/succinate cotransport the highest rates were obtained with fluoride, isethionate and gluconate, the lowest rate with iodide, while $Cl^-$, $NO_3^-$ and $Br^-$ gave intermediate values. A different pattern emerged with lactate, where there was less than a 2-fold variation in rate; $NO_3^-$ gave a significantly higher rate of lactate uptake than the other five anions. As with lactate, the anion effects on D-glucose (5 $\mu$M) and L-phenylalanine (5 $\mu$M) uptakes were less marked than with succinate and there was little, if any, correlation between substrates and the relative rates with each anion: for glucose–gluconate (2.5) > $I^-$, isethionate (1.7) > $Br^-$ (1.6 > $F^-$(1.4) > $Cl^-$(1) > $NO_3^-$(0.6); and for phenylalanine–gluconate (2.6) > $F^-$(1.9) > $Br^-$ (1.7) > $NO_3^-$ (1.4) > $Cl^-$ (1).

The origin of the anion effect on succinate was examined by measuring the kinetics of succinate uptake in the presence of fluoride and chloride

TABLE I

INITIAL RATES OF SUCCINATE AND L-LACTATE UPTAKE AS A FUNCTION OF ANION COMPOSITION

Experiments were carried out as described in Fig. 1 and initial rates were obtained from triplicate 1-s uptakes. Note that the membrane potential was short circuited with $K^+$ and valinomycin. Rates are quoted as the mean ± S.D. in pmol·mg$^{-1}$·s$^{-1}$ and the numbers in parenthesis are the rates relative to those in chloride.

| Succinate (15 $\mu$M) uptake (pmol·mg$^{-1}$·s$^{-1}$) | | | | | | | |
|---|---|---|---|---|---|---|---|
| | $F^-$ | $Cl^-$ | $Br^-$ | $NO_3^-$ | $I^-$ | Isethionate | Gluconate |
| Mean ± S.D. | 35 ± 2.5 (227) | 15.5 ± 2.4 (100) | 4.5 ± 1.3 (75) | 10.5 ± 1.5 (68) | 4.5 ± 0.4 (29) | 30 ± 2.4 (197) | 36.5 ± 2.2 (236) |
| L-Lactate (8 $\mu$M) uptake (pmol·mg$^{-1}$·s$^{-1}$) | | | | | | | |
| | $F^-$ | $Cl^-$ | $Br^-$ | $NO_3^-$ | $I^-$ | Isethionate | Gluconate |
| Mean ± S.D. | 8.8 ± 1.1 (113) | 7.8 ± 0.2 (100) | 9.6 ± 0.8 (124) | 14.2 ± 0.8 (182) | – – | 10.3 ± 1.2 (132) | 9.8 ± 1.6 (127) |

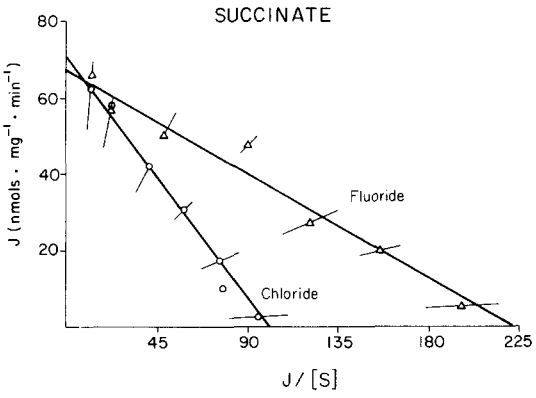


Fig. 2. Kinetics of succinate uptake in chloride and in fluoride solutions. Experimental conditions are as in Fig. 1, except that the succinate concentration ranged from 30 $\mu$M to 5 mM. In NaCl the $J_{max}$ was $71 \pm 4$ nmol·mg$^{-1}$·min$^{-1}$ and the $K_t$ was $0.70 \pm 0.07$ mM, while in NaF the $J_{max}$ was $67.5 \pm 2$ nmol·mg$^{-1}$·min$^{-1}$ and the $K_t$ was $0.30 \pm 0.02$ mM. The chloride results are consistent with those published previously [7].

(Fig. 2). The maximum rates of uptake were identical ($68 \pm 2$ vs. $71 \pm 4$ nmol · mg$^{-1}$ · min$^{-1}$), but the $K_t$ in fluoride solutions was less than half that in chloride ($0.30 \pm 0.02$ vs. $0.70 \pm 0.07$ mM), i.e., the anions appear to act as competitive inhibitors of $Na^+$/succinate transport. The $Cl^-$ inhibitor constant ($K_i$) is 150 mM (where $K_i = (K_t \cdot I)/(K_a - K_t)$, where $K_t = 0.30$ mM, $I = 200$ mM, and $K_a = 0.70$ mM), indicating that $Cl^-$ is a very weak competitive inhibitor of succinate transport. As judged from the rates of uptake at concentrations much less than the succinate $K_t$ (Table I), the inorganic anion affinities for the dicarboxylate carrier are $I^-$ (3.3) > $NO_3^-$ (1.5) > $Br^-$ (1.4) > $Cl^-$ (1) > $F^-$ (0.4). This sequence is the inverse of the anion hydration energies (see Ref. 9), suggesting that the free energy of anion interaction with the succinate-binding site is weak relative to the ion hydration energies. That is, the largest anion, $I^-$, has the highest affinity for the site because it has the lowest hydration energy and it takes the least work to transfer the anion from water to the binding site. Consistent with this interpretation is the fact that the hydration energies for the inorganic anions (50–110 kcal/mol, see Ref. 9), are two orders of magnitude greater than for the carboxylic acids (0.5–1 kcal/mol, see Ref. 10). Similar anion effects were not observed with lactate, which also carries a net negative charge at pH 7.5. This suggests that the effective field strength of the lactate-binding sites are quite different from the succinate sites.

We conclude that under open-circuit conditions the variation in uptakes of D-glucose, L-phenylalanine and L-lactate with anion composition reflect the effect of membrane potential on cotransport. In the case of succinate, however, there is an additional effect due to weak competitive inhibition between the anions and the dicarboxylate for the transport sites. This latter effect can be minimized by using fluoride, gluconate or isethionate salts.

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