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EFFECT OF pH ON THE TRANSPORT OF KREBS CYCLE INTERMEDIATES IN RENAL BRUSH BORDER MEMBRANES

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Lowering extravesicular pH stimulated Na^+-dependent citrate transport in renal brush border membrane vesicles: e.g., at $pH_{out} = 5.5$, the initial rate of citrate uptake was increased 10-fold compared to parallel control experiments at pH 7.5. The same experimental conditions had little effect on succinate uptake. The influence of pH on citrate transport is a product of the extravesicular H^+ concentration; pH gradients did not potentiate the effects nor were proton gradients capable of driving transport in the absence of Na^+. The effect of pH is adequately explained if only the mono- and divalent species of citrate (Cit^{1-}, Cit^{2-}) are considered acceptable substrates for transport. The stimulatory influence of pH on transport correlated quite well with pH-related increases in the concentrations of Cit^{1-} and Cit^{2-}, and over the same pH range [Cit^{3-}] was inversely related to citrate uptake. A model of the Na^+-dependent dicarboxylate transport system is discussed in which three sodium ions are translocated per molecule of dicarboxylic acid.

Reabsorption of polyvalent organic acids from the renal tubular filtrate is mediated by a common Na^+-dependent transport system in proximal tubule brush borders [1,2]. Competition studies revealed that this system handles 4-carbon *trans*-terminal dicarboxylic acids and several related structural analogs, and is therefore capable of transporting most of the intermediates of the Krebs cycle [2]. Recently it was observed that the transport of both succinate, a dicarboxylic acid, and citrate, a tricarboxylic acid, via this system produced a depolarization of the brush border membrane [3]. The transport of succinate/citrate is sensitive to the electrical potential across the brush border membrane in a manner consistent with the translocation of net positive charge [4]. We have direct evidence that three sodium ions are coupled to the translocation of both succinate and citrate [4]. This stoichiometry is adequate to explain the electrogenicity of succinate transport (net charge of -2 at pH 7.5), however, the predominant ionic form of citrate at pH 7.5 is the trivalent species. A coupling of three sodium ions per molecule of citrate would be expected to produce an electroneutral transport event. The present study was designed to investigate this paradox by examining the role of hydrogen ions in the transport of succinate and citrate.

Transport measurements were performed using purified rabbit renal brush border membrane vesicles, prepared as described [2]. Uptakes were determined using a rapid filtration procedure similar to that described by Kessler et al. [5], permitting estimates of the initial rate of transport based

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

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on 1-s uptakes. Experimental details are given in the figure legends. As previously noted [1,2], the transport of both succinate and citrate is dependent on the presence of Na^+ in the extravesicular solution. Except where stated, all uptake measurements were performed under Na^+-gradient conditions (i.e., $[Na^+]_{out} = 100$ mM, $[Na^+]_{in} = 0$ mM), with a substrate concentration of 0.1 mM. Statistical errors are ± 1 S.E. of the mean of triplicate samples. The results presented here are from representative single experiments; all observations were verified by at least three independent experiments.

The effect of pH on the time course of Na^+-dependent citrate and succinate transport is shown in Fig. 1. Under pH-gradient conditions (i.e., $pH_{out} = 5.5$, $pH_{in} = 7.5$) the one second uptake of citrate was stimulated 10-fold compared to the control where $pH_{out} = pH_{in} = 7.5$ (1.09 ± 0.08 vs. 0.11 ± 0.01 nmol/mg protein). There was no stimulation in the rate of succinate uptake under these same conditions. In fact, a slight inhibition of succinate transport was sometimes noted at pH 5.5. This may be due to a proton diffusion potential, consistent with the effect on transport of K^+ diffusion potentials [4]. The effect of a broad range of pH on citrate and succinate transport is presented in Fig. 2. Increasing $[H^+]$ from 0.05 to 2 μM (i.e., pH 7.3 to 5.7) had little effect on the transport of succinate. Citrate transport, however, increased 10-fold, from 10.6 ± 0.5 to 99.3 ± 3.7 nmol/mg per min over this range. The stimulatory effect of pH on citrate transport did not require gradients of pH across the vesicle membrane. For example, when $pH_{out} = pH_{in} = 5.5$ the initial rate of citrate transport increased 12-fold over the control value ($pH_{out} = pH_{in} = 7.5$). Succinate transport was identical under these two conditions (data not shown). A pH gradient was incapable of driving citrate or succinate transport, however, in the absence of Na^+: e.g., at $pH_{out} = 5.5$, $pH_{in} = 7.5$, and $[Na^+] = 0$, the initial rate of citrate transport was 0.49 ± 0.09 nmol/mg per min compared to 41.6 ± 0.45 in the presence of a 100 mM Na^+ gradient. These observations indicate that the effect of pH on transport is specific for citrate, and because proton gradients do not potentiate the effect, nor are they in and of themselves capable of driving transport, the effect is due to the H^+ concentration in the extravesicular solution.

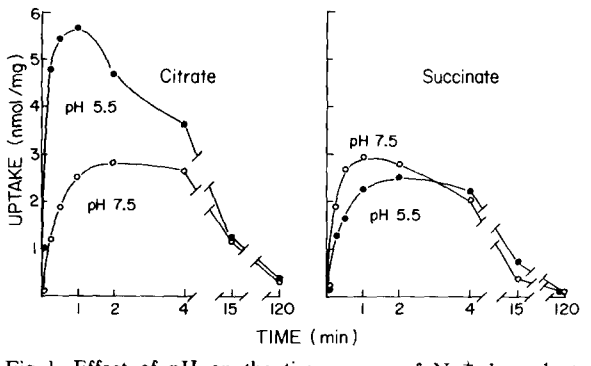

Fig. 1. Effect of pH on the time course of Na^+-dependent citrate and succinate transport in renal brush border membranes. Vesicles were preequilibrated in 300 mM mannitol, 1 mM Tris-Hepes, pH 7.5. Solid circles represent uptakes from a transport buffer producing final extravesicular concentrations of the appropriate ^{14}C-labeled substrate (0.1 mM), 100 mM NaCl, 90 mM mannitol and 10 mM Hepes, buffered to pH 5.5 with Tris. Open circles are uptakes from a similar transport buffer, pH 7.5. Points are the mean of duplicate samples, with the exception of triplicate samples at 1 s; the range of the points was less than 10% of the mean.

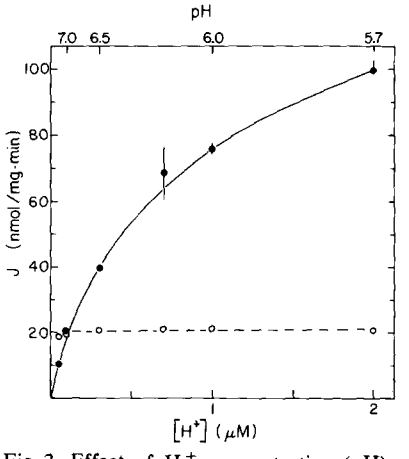

Fig. 2. Effect of H^+ concentration (pH) on Na^+-dependent citrate and succinate transport. Conditions were as described for the experiment described in Fig. 1, except transport buffers were titrated to six different values of pH (7.3, 7.0, 6.5, 6.2, 6.0, 5.7). Solid circles represent initial rates of 0.1 mM citrate transport, estimated from 1-s uptakes; open circles represent succinate transport. Vertical bars signify ± 1 S.E. of triplicate samples; where vertical bars are absent, the standard error was smaller than the graphical representation of the mean.

The effect of pH on citrate transport can be explained if the transported species of citrate are limited to the divalent and monovalent forms. Fig. 3 is a graphical comparison of the effect of pH on citrate transport and the relative concentrations of the mono-, di- and trivalent forms of citrate over the same range. The correlation between the increase in transport and the increase in concentration of the -2 and -1 species is quite good, and the inverse relationship between the availability of Cit^{3-} and the rate of transport argues convincingly against this species as a substrate for transport. At lower pH values (pH 5.7), however, there is a deviation between the increase in concentration of the -2 and -1 species of citrate and the stimulation of transport, and we cannot exclude the influence of direct effects of $[H^+]$ on either the membrane or the transport system itself. The inclusion of both Cit^{1-} and Cit^{2-} as acceptable substrates is the result of the observation that succinate transport is essentially unaffected by decreases in pH. The pK_{a2} of succinate is 5.6 ($pK_{a1} = 4.2$). Thus by pH 5.7 the availability of Suc^{2-} is reduced about 2-fold with no concomitant decrease in the rate of uptake. The acceptability of both the -1 and -2 species is an adequate explanation for this observation, as well as for the effect of pH on citrate transport.

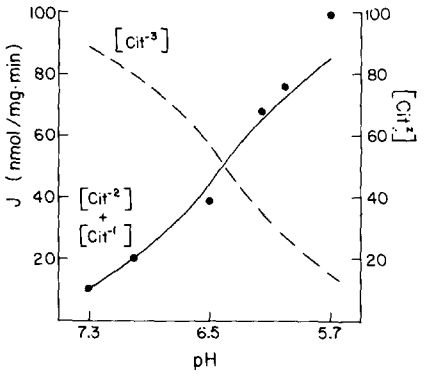

Fig. 3. Relationship between pH-dependence of citrate transport and the relative concentrations of the ionic species of citrate. The left ordinate refers to the data presented as solid circles representing the rates of citrate transport described in Fig. 2. The right ordinate indicates the concentration (μM) of citrate, net charge Z; the dashed line describes the pH-dependent change in concentration of Cit^{3-}, while the solid line represents the concomitant changes in combined concentration of Cit^{2-} and Cit^{1-} species. Lines calculated from concentrations predicted by the Henderson-Hasselbach equation; $pK_{a1} = 3.1$, $pK_{a2} = 4.8$, $pK_{a3} = 6.4$ [6].

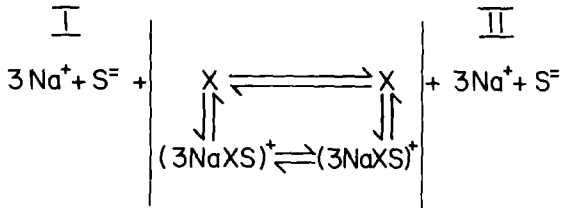

Fig. 4. Model of events associated with the Na^+-dependent transport of dicarboxylic acids in the renal brush border. Roman Numerals I and II represent the extra- and intravesicular faces of the brush border vesicle, though no asymmetry of the transport mechanism is implied by this notation. X represents the membrane-associated dicarboxylate transporter; S^{2-} represents dicarboxylate substrates (substrate specificity discussed in Ref. 2).

The basic events involved in the translocation of dicarboxylic acids across the renal brush border membrane are summarized in the model presented in Fig. 4. The coupling of three sodium ions per molecule of dicarboxylate is based on direct measurements of the simultaneous fluxes of ^{14}C-labelled substrates and $^{22}Na^+$, and is consistent with the kinetic characteristics of the interaction of Na^+ with succinate/citrate transport [4]. The present report indicates that at pH 7.5 the predominant transported species of both succinate and citrate have a net charge of -2. Coupling between the fluxes of three sodium ions and either a succinate or citrate molecule results in the net movement of positive charge across the membrane and is sufficient to explain our earlier report of the electrogenicity of succinate/citrate transport [3]. At present we have insufficient information to suggest a possible sequence of substrate binding, and this topic is currently under investigation.

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