Kinetic Properties of the Sodium Bicarbonate (Carbonate) Symport in Monkey Kidney Epithelial Cells (BSC-1)

INTERACTIONS BETWEEN Na⁺, HCO₃⁻, AND pH*

The abbreviations used are: pHᵢ, intracellular pH; pHₑ, extracellular pH; SBS, standard bicarbonate saline; DIDS, 4,4'-disothiocyanostilbene-2,2'-disulfonic acid (DIDS)-sensitive "Na⁺" uptake into confluent monolayers of BSC-1 is measured in the presence of ouabain (10⁻⁴M) and amiloride (10⁻³M) to define the interactions between Na⁺ and HCO₃⁻ binding and pH. Dependence of DIDS-sensitive "Na⁺" fluxes on either Na⁺ or HCO₃⁻ can be described by Michaelis-Menten kinetics. External apparent Kₘ for HCO₃⁻ decreases with increasing Na⁺ concentration (Kₘapp (HCO₃⁻) = 36 ± 10, 18 ± 5, and 9 ± 3 mM at 20, 45, and 151 mM Na⁺, respectively (pHᵢ = 7.4)). Similarly, external apparent Kₘ for Na⁺ decreases with increasing HCO₃⁻ concentration (Kₘapp (Na⁺) = 73 ± 22, 28 ± 8, and 14 ± 4 mM at 6, 17, and 56 mM HCO₃⁻, respectively (pHᵢ = 7.4)). Vmax remains constant within the experimental error. When data are replotted as a function of calculated NaC₀₂ concentration, they can be approximated by a single Michaelis-Menten equation. DIDS-sensitive uptake at constant Na⁺ and HCO₃⁻ displays a broad pH optimum in the range between 7.2 and 7.6. The data are compatible with the ion pair model in which the transported species, NaC₀₂, binds to the transport site with Kₘ = 15.3 ± 4 μM. However, the data may also be fitted by either a random or ordered bicaque system. Sets of parameters necessary for these fits are given.

In recent years, regulation of intracellular pH (pHᵢ) and, intimately linked to it, transport of acid equivalents have received widespread interest (Boron, 1983). Increasing evidence suggests an important role for an electroneutral symport of Na⁺ and HCO₃⁻ in a variety of cells involved in transcellular transport of bicarbonate (Alpern, 1985; Biagi and Sothell, 1986; Boron and Boulpaep, 1983; Jentsch et al., 1984a, 1985b, 1985c; Yoshitomi et al., 1985). This symport is thought to tightly couple the movement of one Na⁺ ion to the transport of two or three HCO₃⁻ ions, thus leading to a transfer of net negative charge. The cotransporter is inhibitable by DIDS or SITS, compounds known to inhibit anion exchange of erythrocytes (Cabantchik and Rothstein, 1972) and several other anion transport processes in a variety of tissues. The first known example of such symport was described in the basolateral membrane of the proximal tubule (Boron and Boulpaep, 1983) of the salamander (Ambystoma tigrinum), where it was postulated to mediate net efflux of HCO₃⁻ and Na⁺, giving rise to transcellular transport of HCO₃⁻. We then identified a similar process in a mammalian tissue, the bovine corneal endothelium (Jentsch et al., 1984a, 1985a, 1985c), which also performs transepithelial transport of HCO₃⁻. This suggested its presence in the mammalian nephron, too. This was confirmed by recent evidence demonstrating similar symports in BSC-1 cells (Jentsch et al., 1985b), an established epithelial cell line derived from the African green monkey (Cercopithecus aethiops) (Hopps et al., 1963), and in proximal tubular cells of the rat (Alpern, 1985; Yoshitomi et al., 1985) and the rabbit (Biagi and Sothell, 1986; Sasaki et al., 1985). A potentially related mechanism is a Na⁺HCO₃⁻/Cl⁻⁻H⁺ exchanger, which is involved in the regulation of intracellular pH in several invertebrates (Boron, 1985; Boron et al., 1979; Moody, 1981; Thomas, 1977). Recently, such process has also been suggested for A431 cells (a human epidermoid cancer cell line) (Rothenberg et al., 1983) and for fibroblasts (L'Allemand et al., 1985). This process is also inhibitable by SITS or DIDS, but is electroneutral due to the countertransport of chloride. An intriguing transport model for this process (Becker and Duhm, 1978) suggests that the compound ion NaC₀₂⁻, rather than sodium and two bicarbonate ions (or Na⁺ and HCO₃⁻ with a countertransport of H⁺) is transported in exchange for Cl⁻. This has been originally described for the anion exchanger of the red blood cell (Becker and Duhm, 1978; Funder et al., 1978; Wieh, 1979). Indeed, this "ion pair model" is consistent with kinetic data on the Na⁺HCO₃⁻/Cl⁻⁻H⁺ exchanger from the squid axon (Boron, 1985). The larnacal muscle system, however, is incompatible with this model (Boron et al., 1981), and no such information is available for a mammalian system.

In the present work, using the BSC-1 system, we investigate the interaction between Na⁺, HCO₃⁻, and pH in DIDS-sensitive "Na⁺" transport in an attempt to elucidate the molecular transport mechanism of the Na⁺HCO₃⁻ symport. We show
that the dependence of DIDS-sensitive $^{22}\text{Na}^+$ fluxes on either Na$^+$ or HCO$_3^-$ can be described by simple Michaelis-Menten kinetics, with an apparent $K_m$ for Na$^+$ decreasing with increasing HCO$_3^-$ concentration, whereas $V_{	ext{max}}$ (HCO$_3^-$) decreases with increasing Na$^+$ concentration. This allows accurate estimates of the initial uptake rate to be made.

**EXPERIMENTAL PROCEDURES**

**Materials**—Cell culture media were from Biochrom AG, Berlin, Federal Republic of Germany; tissue culture plasticware was from NUNC A/S, Roskilde, Denmark. $^{22}\text{Na}^+$ was obtained as carrier-free NaCl solution from Amersham Buchler, Braunschweig, FRG. DIDS was from Fluka, Neu-Ulm, FRG, ouabain from Merck, Darmstadt, FRG, and amiloride was a generous gift from Merck Sharp and Dohme, Munich, FRG.

Cells—BSC-1 cells were of the same strain as described previously (Jentsch et al., 1983b) (obtained from Flow Laboratories, Irvine, United Kingdom, passage 52, lot 37944). In some experiments (not shown), we also used BSC-1 obtained from the American Type Culture Collection, Bethesda, MD (CCL26, obtained at passage 43, batch F-2695), and noted that DIDS-sensitive $^{22}\text{Na}^+$ uptake was significantly lower in that strain. Therefore, BSC-1 obtained from Flow Laboratories were used throughout this work. Cells were maintained as described previously (Jentsch et al., 1983b). For the experiments, confluent monolayers covering 25-cm$^2$ flasks were used 5 days after seeding. The cells used in this study were from passage 58-69.

**$^{22}\text{Na}^+$ Uptake Studies**—Uptake of $^{22}\text{Na}^+$ was performed exactly as described previously (Jentsch et al., 1985b) by preincubating the cells in saline containing ions and inhibitors as specified in the figure legends, adding uptake saline containing 10-30 kBq/ml $^{22}\text{Na}^+$, and incubating the flasks for the desired time at 37°C. Uptake was terminated by three rapid washes with 100 mM MgCl$_2$, 20 mM HEPES/Tris, pH 7.4. Cells were dissolved and radioactivity was determined and referred to the number of cells as described previously (Jentsch et al., 1985c). Measurements were performed at least in quadruplicate. The standard bicarbonate saline, SBS, contained 151 mM Na$^+$, 5 mM K$^+$, 0.9 mM Mg$^{2+}$, 1.7 mM Ca$^{2+}$, 130 mM Cl$^-$, 28 mM HCO$_3^-$, 1 mM H$_2$PO$_4^-$, 0.9 mM SO$_4^{2-}$, and 5 mM glucose and was gassed with 5% CO$_2$ in air to pH 7.4. When bicarbonate concentration exceeded the one of SBS, it was substituted for Cl$^-$. Within each experiment in which HCO$_3^-$ concentration was varied, HCO$_3^-$ was replaced by bicarbonate to keep Cl$^-$ concentration constant. Na$^+$ concentration was varied by substitution with NMDG and choline, which was added as choline bicarbonate. Thus, in experiments where Na$^+$ or HCO$_3^-$ can be described by simple Michaelis-Menten kinetics, and that apparent $K_m$ for Na$^+$ decreases with increasing HCO$_3^-$ concentration, whereas $V_{	ext{max}}$ (HCO$_3^-$) decreases with increasing Na$^+$ concentration. This allows accurate estimates of the initial uptake rate to be made.

**RESULTS**

**Apparent Binding Constants for HCO$_3^-$ at Different Values of Na$^+$**—Previously, we have reported the apparent external $K_m$ for HCO$_3^-$ of the Na$^+$-HCO$_3^-$ symport (where $n$ designates the apparent number of transported HCO$_3^-$ ions) of BSC-1 to be in the 7-14 mM range (at 151 mM Na$^+$). In this work, we have explored the effect of Na$^+$ concentration on $K_{	ext{app}}$ (HCO$_3^-$). A fixed uptake time of 3.5 min was chosen since this allows accurate estimates of the initial uptake rate to be made (Jentsch et al., 1985b). Uptakes were studied at three fixed levels of Na$^+$-concentration. Mean cell density was 1.1 x 10$^5$ cells/cm$^2$. Points are the mean value of five determinations, with S.E. being below 8% for all points (typically 3%). $D$, Lineweaver-Burk plot of DIDS-sensitive uptake taken from experiments A-C. The lines were drawn using kinetic parameters determined from Edie-Scatchard plots: for 20 mM Na$^+$, $K_{\text{app}}$ = 35 mM, $V_{\text{max app}}$ = 2.9 nmol/cm$^2$/3.5 min; for 45 mM Na$^+$, $K_{\text{app}}$ = 18 mM, $V_{\text{max app}}$ = 2.8 nmol/cm$^2$/3.5 min; and for 151 mM Na$^+$, $K_{\text{app}}$ = 10 mM, $V_{\text{max app}}$ = 2.9 nmol/cm$^2$/3.5 min. Curves displayed in parts A-C were calculated from the Michaelis-Menten equation using these parameters. $E$, Hill plot of the data from A-C. The lines were calculated from the Hill equation using $n_H = 0.96$. $F$, Hill plot of the data from A-C. The lines were calculated from the Hill equation using $n_H = 0.89$.
FIG. 2. $^{22}$Na* dependence of DIDS-sensitive $^{22}$Na* uptake at different fixed levels of HCO$_3$; $A$–$C$. $^{22}$Na* uptake (3.5 min) at 6, 17, and 56 mM HCO$_3$; respectively, at near constant pH$_7$ (7.4), D, DIDS-pretreated cells. $Q$, untreated cells. Mean cell density was $7.5 \times 10^6$ cells/cm$^2$. Points are the mean value of five determinations. $A$, the point representing uptake at 7 mM Na* in cells not treated with DIDS. HCO$_3$; had no effect on uptake into cells pretreated with DIDS. The increase in DIDS-sensitive uptake is a saturable function of HCO$_3$; Most importantly, at higher Na* concentration, less HCO$_3$; is needed to reach saturation. This is substantiated by Fig. 1D, a Lineweaver-Burk plot of DIDS-sensitive uptake from experiments $A$–$C$. The experimental points may be approximated by straight lines, which is compatible with Michaelis-Menten kinetics. The parameters of the lines have been obtained by linear regression analysis with Eadie-Scatchard plots (not shown) to avoid errors inherent to the Lineweaver-Burk method. The lines intersect at a common point located to the 1/2 axis, indicating that changes in Na* do not significantly change apparent $V_{max}$ (calculated $V_{max}$ specific $= 2.8$ nmol/cm$^2$ x 3.5 min). In contrast, Na* concentration has a large effect on apparent $K_m$ (HCO$_3$;), as already suggested by the plots in Fig. 1, $A$–$C$. $K_{app}$ (HCO$_3$;) decreases from 35 mM at 20 mM Na* to $K_{app}$ (HCO$_3$;) = 18 mM at 45 mM Na* and to $K_{app}$ (Na*) = 10 mM at 151 mM Na*. This suggests that the presence of Na* increases the apparent affinity of HCO$_3$; to the cotransporter. Furthermore, the data are formally compatible with Michaelis-Menten kinetics. Construction of Hill plots for the data of Fig. 1, $A$–$C$. The indicated value of $V_{max}$ yielded Hill coefficients between 0.85 and 0.96 (Fig. 1E).

**Apparent Binding Constants for Na* at Different Values of HCO$_3$;** Whereas $K_{app}$ (HCO$_3$;) decreases with increasing Na* concentration, $K_{app}$ (Na*) increases with increasing HCO$_3$; concentration (Fig. 2). Fig. 2, $A$–$C$ displays $^{22}$Na* uptake in the presence of amiloride (1 mM) and ouabain (10$^{-4}$ M) as a function of Na* concentration at 6, 17, and 56 mM HCO$_3$; respectively. Uptake was studied under steady-state conditions for HCO$_3$; and solutions were gassed with appropriate partial pressures of CO$_2$ (as specified in the legend) to keep pH$_7$ constant (7.4). With DIDS-pretreated cells, uptake increased to a first approximation linearly with Na* concentration, as is expected e.g. with unspecific binding. The slight saturation of uptake occurring also with DIDS-pretreated cells might mean that other specific ion transport processes contribute to total uptake even with DIDS-pretreated cells (e.g. Na*/H*-antiport, which is presumably not totally blocked with 1 mM amiloride (Benos, 1982)). A similar increase of $^{22}$Na* uptake with Na* was seen with DIDS-controls in Fig. 1 (compare experiments $A$–$C$. DIDS-sensitive $^{22}$Na* uptake, however, is a saturable function of Na* at all bicarbonate concentrations tested (Fig. 2, $A$–$C$). A Lineweaver-Burk plot (Fig. 2D) using DIDS-sensitive uptake from the experiments $A$–$C$ reveals that uptake kinetics may be described by simple Michaelis-Menten kinetics. This has been confirmed by Hill plots which yielded slopes near 1 (not shown). Fig. 2, $E$ and $F$ are Lineweaver-Burk plots from other, similar experiments. In each of these experiments, the apparent $K_m$ for Na* decreased with increasing bicarbonate concentration. Whereas $V_{max}$ was constant within the experimental error for the experiments of Fig. 2, $E$ and $F$, the lines in Fig. 2D intersect at a point left from the 1/2 axis, indicating an increase of $V_{max}$ with increasing HCO$_3$; This, however, was not statistically significant and may be due to a lower accuracy of $^{22}$Na* uptake data obtained at 6 mM HCO$_3$; where $V_{max}$ is $10^{-4}$ M ouabain and $10^{-3}$ M amiloride. 30 s prior to $^{22}$Na* uptake, the flask was rinsed twice with saline containing the indicated values of Na* and HCO$_3$; ouabain ($10^{-4}$ M) and amiloride ($10^{-3}$ M), equilibrated with CO$_2$ as above. Uptake was measured in identical saline containing additional $^{22}$Na*. In uptake saline, HCO$_3$; was replaced by cyclamate to keep extracellular Cl$^-$ constant.
DIDS-sensitive uptake is small. The apparent binding constants for Na⁺ decrease with increasing HCO₃⁻ (Fig. 2D; $K_{\text{app}}$ (Na⁺) = 73, 28, and 19 mM at 6, 17, and 56 mM HCO₃⁻, respectively; other kinetic data are summarized in the figure legends and in Table I). This indicates that the apparent affinity for Na⁺ to the Na⁺HCO₃⁻ symport increases with increasing HCO₃⁻.

**Effect of pH on DIDS-sensitive ²²Na⁺ Uptake**—The pH-dependence of transport via the Na⁺HCO₃⁻ symport was studied at constant Na⁺ and HCO₃⁻ concentration (10 mM each). At these concentrations, the symport operates reasonably far from saturation, and effects of pH are expected to be most easily observable. pH was varied between pH 6.5 and 8.25 by equilibrating the solutions with appropriate partial pressures of CO₂, as specified in the legend. The cells were equilibrated with the respective pH for 30 min prior to ²²Na⁺ uptake. Under these conditions, DIDS-sensitive sodium uptake displays a broad maximum as a function of pH (Fig. 3), maximal uptake rates occurring between pH 7.2 and 7.6.

**DISCUSSION**

Since electrogenic Na⁺HCO₃⁻ symport has been discovered only recently (Alpern, 1985; Biagi and Sohtell, 1986; Boron and Bouapae, 1983; Jentsch et al., 1984a, 1984b, 1985b, 1985c; Sasaki et al., 1985; Yoshitomi et al., 1985), no detailed information on its molecular mechanism (including the stoichiometry of apparent HCO₃⁻ to Na⁺ coupling) is available. In the present study we have used ²²Na⁺ uptake technique into confluent monolayers of BSC-1 cells to obtain kinetic data for the Na⁺HCO₃⁻ symport expressed by these cells (Jensch et al., 1985b). The main findings are (a), the dependence of transport via the Na⁺HCO₃⁻ symport was studied at constant Na⁺ and HCO₃⁻ concentration (10 mM each). At these concentrations, the symport operates reasonably far from saturation, and effects of pH are expected to be most easily observable. pH was varied between pH 6.5 and 8.25 by equilibrating the solutions with appropriate partial pressures of CO₂, as specified in the legend. The cells were equilibrated with the respective pH for 30 min prior to ²²Na⁺ uptake. Under these conditions, DIDS-sensitive sodium uptake displays a broad maximum as a function of pH (Fig. 3), maximal uptake rates occurring between pH 7.2 and 7.6.

**TABLE I**

Comparison of observed and calculated values of $K_{\text{app}}$

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**Fig. 3.** ²²Na⁺ uptake (4 min) in bicarbonate saline as a function of pH. A, uptake of DIDS-pretreated cells (○) and untreated cells (□). B, DIDS-sensitive uptake, calculated as difference in uptake between untreated and DIDS-treated cells. Mean cell density was 1.2 × 10⁶ cells/cm². Points are the mean of eight determinations, with S.E. being below 4% for all points. The experimental procedures were as follows: Cells were preincubated at 37 °C for 120 min in saline containing 10 mM HCO₃⁻ (gassed with 2% CO₂ to pH 7.4), and 10⁻⁴ M ouabain. Control cells were incubated in this saline for 30 min, followed by a 30-min incubation in an identical saline containing 1 mM DIDS. This was followed by a 30-min incubation in 10 mM HCO₃⁻ saline containing 10⁻⁴ M ouabain and 10⁻⁴ M amiloride, equilibrated with 0.25, 0.5, 1, 2, 3, 5, 9, and 10% CO₂ in air to obtain a pH of 8.55, 7.95, 7.65, 7.35, 7.17, 6.95, and 6.69, and 6.47, respectively. Uptake saline contained identical concentrations of inhibitors, bicarbonate, and CO₂, but Na⁺ was reduced to 10 mM (replaced by NMDG). Prior to uptake, the monolayers were rinsed twice with an identical saline (but free of ²²Na⁺).

**Validation of Approach**

Before discussing the implications of these data, we should note that values of apparent $K_m$ for Na⁺ and HCO₃⁻ were obtained under slightly different conditions. Throughout this work, we have used ouabain-pretreated cells to obtain a uniform high concentration of intracellular Na⁺. By possibly saturating the intracellular transport site of the symport, this may minimize trans-acting effects of Na⁺ on uptake rates. Furthermore, this allows a direct comparison with previously published studies (Jentsch et al., 1985b, 1985c). Values of $K_{\text{app}}$ (Na⁺) were obtained starting with a constant ionic composition of the cell interior, whereas values of $K_{\text{app}}$ (HCO₃⁻) were obtained with HCO₃⁻ varied symmetrically on both sides of the plasma membrane. This was done to avoid abrupt changes of pH induced by changing pCO₂ during the uptake period. In addition, lowering external Na⁺ concentrations during uptake (as done e.g., for determinations of $K_{\text{app}}$ (Na⁺)) should lead to an intracellular acidification and to a depolarization of the plasma membrane, which might also affect transport rates. To some extent, these possible problems may be reduced by using K⁺ and H⁺-ionophores. Since the presence of high K⁺ concentrations together with a K⁺ ionophore would have restricted the range of Na⁺ concentration amenable to variation, and since the additional manipulations necessary with ionophores are likely to affect the accuracy of the data, we have adopted the methods presented here. The resulting data may be fitted by simple Michaelis-
Menten kinetics, making it unlikely that the above-discussed mechanisms exert a large influence on uptake rates.

In the proximal renal tubule, the putative sodium bicarbonate symport is located at the basolateral membrane (Alpern, 1985; Boron and Boulpaep, 1983; Yoshitomi et al., 1985). No information on the location is available for BSC-1. In addition, several characteristics of this cell line are atypical for proximal tubular cells. The fast uptake of $^22Na^+$ by a DIDS-sensitive mechanism in the present study suggests that in BSC-1 the symport is either located apically, or the intercellular junctions allow a fast access of $^22Na^+$ to the basolateral membrane.

**Implications of Michaelis-Menten Kinetics**

The Michaelis-Menten-type kinetics observed with variation of both $Na^+$ and $HCO_3^-$ concentration under a variety of conditions agrees well with our previous, more limited observations for the bovine corneal endothelium (Jentsch et al., 1985c) and BSC-1 (Jentsch et al., 1985b). To account for the observed transport of negative charge and for the transport direction, $Na^+$/$HCO_3^-$ symport has been postulated to involve the transport of two or three $HCO_3^-$ ions together with one $Na^+$ ion (Boron and Boulpaep, 1983; Jentsch et al., 1984a, 1985b; Yoshitomi et al., 1985). In that case, one would expect a Michaelis-Menten-type kinetics when $Na^+$ concentration is varied. However, if bicarbonate ions bind individually to the transporter with similar values of $K_m$, a sigmoidal dependence of uptake rates on $HCO_3^-$ concentrations and a Hill coefficient greater than 1 would be expected. Thus, for BSC-1, such a model is excluded by the present data. However, Michaelis-Menten kinetics are compatible with transport of $Na^+ CO_3^-$, as discussed below, or with a separate binding of $Na^+$ and $CO_3^-$.

These two models are equivalent to $2 HCO_3^-$ to $1 Na^+$ stoichiometry, as far as thermodynamics and the transfer of charge is concerned. On the other hand, if the values of apparent $K_m$ for the respective $HCO_3^-$ binding sites are largely different, the largest $K_m$ will dominate the overall effect and Michaelis-Menten kinetics will be observed (Jentsch et al., 1985b). This may even accommodate a $3:1$ stoichiometry.

**Dependence of $K_m$ on the Concentration of the Cotransported Ion**

A prominent finding of this work is that $K_m$ for $Na^+$ decreases with increasing $HCO_3^-$, whereas $K_m$ for $HCO_3^-$ decreases with increasing $Na^+$, with $V_{max}$ remaining constant. We will restrict the following discussion to three simple kinetic models, namely (i) a transport of the ion pair $Na^+ CO_3^-$, (ii) a transport of $Na^+$ and $HCO_3^-$ (or $CO_3^-$) binding individually and randomly to the transporter, and (iii) a transport of $Na^+$ and $HCO_3^-$ (or $CO_3^-$) binding individually in an ordered fashion to the symport.

(i) Transport of $Na^+ CO_3^-$ (Ion Pair Model)—As first discussed for the red blood cell (Becker and Duhm, 1978; Funder et al., 1978; Wieth, 1970), the ion pair model postulates a transport of the compound ion $Na^+ CO_3^-$, which is accepted by a (DIDS-sensitive) anion transport protein (e.g. band 3 of the erythrocyte). Using the data of Garrels et al. (1961), such ion pair is expected to be present under control conditions ($151$ mM $Na^+$, $28$ mM $HCO_3^-$, pH $7.4$) at an ion activity of about $115$ $\mu$M. A natural consequence of this model is the existence of DIDS-sensitive bicarbonate-dependent $^22Na^+$ fluxes, as observed with the corneal endothelium (Jentsch et al., 1985c) and BSC-1 cells (Jentsch et al., 1985b). Since $Na^+ CO_3^-$ concentration is linked to $Na^+$, $HCO_3^-$, and $H^+$ concentration by law of mass action, apparent $K_m$ values for $Na^+$ and $HCO_3^-$ may be given in terms of $K_m$ for $Na^+ CO_3^-$:

\[
K_m \text{ app (Na)} = \frac{\beta \times K_m (Na^+ CO_3^- \times [H^+]^a)}{[HCO_3^-]^a}.
\]

\[
K_m \text{ app (HCO_3^-)} = \frac{\beta \times K_m (Na^+ CO_3^- \times [H^+]^a)}{[Na^+]^a}.
\]

\[
V_{max} = V_{max app (Na^+)} = V_{max app (HCO_3^-)},
\]

with $\beta = [Na^+][HCO_3^-]/([Na^+ CO_3^-][H^+])$. Thus, this model predicts Michaelis-Menten kinetics for both $Na^+$ and $HCO_3^-$. Furthermore, $V_{max}$ should remain constant, and $K_m$ for $Na^+$ should decrease with increasing $HCO_3^-$, and vice versa. All these predictions have been qualitatively verified in this study. For a more quantitative comparison, we have replotted experiments from Fig. 1 and Fig. 2 as a function of $Na^+ CO_3^-$ (Fig. 4). $Na^+ CO_3^-$ activity was calculated from the stability constant given by Garrels et al. (1961) as stated in the figure legend. Experiments are shown individually since $V_{max}$ varies between different experiments by a factor of 2. It is obvious from Fig. 4 that the data may be described by a single Michaelis-Menten equation if transport of $Na^+ CO_3^-$ is assumed. Most importantly, the calculated value of $K_m$ for $Na^+ CO_3^-$ is reasonably constant with different experimental approaches. Thus, the data are compatible with a transport of $Na^+ CO_3^-$ binding to a single site with $K_m = 15.3 \pm 4$ $\mu$M $Na^+ CO_3^-$ activity (S.D., $n = 5$). Furthermore, Table I gives experimental values of $K_m$ for $Na^+$ and $HCO_3^-$, and, for comparison, those resulting from the ion pair hypothesis and other models, as discussed below. The ion pair model additionally predicts that apparent $K_m$ values for both $Na^+$ and $HCO_3^-$ should increase proportionally to $[H^+]$. Therefore, if $V_{max}$ remains constant with pH and if uptake is studied under conditions excluding a saturation of the symport, one should expect an increase in uptake with increasing pH. This is in clear contrast to the experiment shown in Fig. 3, where uptake displays a broad maximum in the pH 7.2 to 7.6 range. However, since the transporter is expected to be a membrane protein, titration of amino acids may lead to changes in kinetic behavior difficult to predict. Indeed, if one assumes that the negatively charged $Na^+ CO_3^-$ ion binds to a positively charged group generated by titration with $H^+$, acidic pH should increase the concentration of transport sites, whereas alkaline pH should increase the concentration of the transported species. This would result in an ordered bireactant system where $H^+$ binds before $Na^+ CO_3^-$. Furthermore, $^22Na^+$ uptake rates might be influenced by pH, which was changed in parallel with pH. Thus, the pH dependence of DIDS-sensitive $^22Na^+$ uptake shown in Fig. 3 cannot be used to rule out the ion pair model.

(ii) Random Binding of $Na^+$ and $HCO_3^-$ (or $CO_3^-$)—We may also consider a random bireactant system, where $Na^+$ and $HCO_3^-$ (or $CO_3^-$) bind to separate sites, with arbitrary sequence of binding (Segel, 1975). Binding of one ion may affect the dissociation constant of the other, which is formally expressed by a factor $\alpha$ ($\alpha = 1$, no interaction; $\alpha < 1$, increase in binding). Since the observed values of $K_m$ depend on the concentration of the cotransported ion, we can clearly exclude a random model with $\alpha = 1$. However, a reasonable fit may be obtained using a low value for $\alpha$. In Table I we have calculated apparent $K_m$ values from this model using a set of constants (given in the table) obtained from reploting the data as described by Segel (1975). The calculated values...
agree reasonably well with the experimental results. In contrast to the NaCO\(_3\) model, the random bireactant system predicts an increase in \(V_{\text{max}}\) with increasing ion concentration, and the lines in Lineweaver-Burk plots should intersect at a point left to the \(1/v\) axis (at \(1/K_m\)). Although \(V_{\text{max}}\) is constant within the experimental error a slight increase of \(V_{\text{max}}\) with the respective ion concentrations cannot be excluded. Thus, a random bireactant system cannot be ruled out using the present data, but is clearly restricted to specific combinations of kinetic parameters.

(iii) Ordered Binding of Na\(^+\) and HCO\(_3^-\) (or CO\(_3^{2-}\))—With this model, binding of one ion is the prerequisite for binding of the second one (Segel, 1975), which (unlike the random system) automatically results in a dependence of \(K_{\text{app}}\) (HCO\(_3^-\)) on [Na\(^+\)] and vice versa. Arbitrarily assuming Na\(^+\) to bind first, we have (by replotting) obtained a set of parameters (given in Table I), yielding values of apparent \(K_m\) which are in reasonable agreement with the experimental ones (see Table I). Although that model predicts that either for Na\(^+\) or for HCO\(_3^-\) the lines in Lineweaver-Burk plots should intersect to the left of the \(1/v\) axis, we may not rule out the ordered bireactant system.

Thus, within the experimental error, the data may be described by either the ion pair model, the random bireactant or the ordered bireactant system. Bearing in mind the replots in terms of NaCO\(_3\) concentration, it is tempting to speculate that the most simple and elegant system, namely the transport of NaCO\(_3\), is realized with BSC-1. Indeed, to obtain the observed kinetics, only one parameter is needed for a fit to the ion pair model (namely \(K_m\) (NaCO\(_3\))). For the two remaining models, a fortuitous agreement of 2 parameters (ordered bireactant system) or 3 parameters (random bireactant system) is necessary.

Comparison with Other Systems Involved in Na\(^+\) and HCO\(_3^-\) Transport

The ion pair hypothesis has originally been proposed to explain the DIDS-sensitive and bicarbonate-dependent transport of Li\(^+\) and Na\(^+\) by the erythrocyte (Becker and Duhm, 1978; Funder et al., 1978; Wieth, 1970). Later, analysis of acid extrusion rates in the squid axon similar to the one performed in this work led Boron (1985) to suggest that also the DIDS-sensitive Na\(^+\)HCO\(_3^-\)/Cl\(^-\)/H\(^+\) exchange (a pH\(_i\)-regulating process) performs an exchange of extracellular NaCO\(_3\) for intracellular Cl\(^-\). In analogy to the present study, however, he was unable to exclude other possible models. Interestingly, the ion pair model has been excluded using the same kinetic approach for a similar pH\(_i\)-regulating process of the barnacle muscle (Boron et al., 1981). At present, no data for such a process in mammals is available.

In the erythrocyte, no saturation of Li\(^+\) or Na\(^+\) transport has been observed even with high concentrations of these ions or HCO\(_3^-\) (Becker and Duhm, 1978; Funder et al., 1978; Wieth, 1970), suggesting that \(K_m\) (NaCO\(_3\)) is considerably higher
than that assumed for the squid axon system (80 ± 8 μM (Boron, 1985)) or the one proposed here for the BSC-1 system (15.3 ± 4 μM). For the red blood cell, several additional lines of evidence pointed to a transport of ion pairs: (a) there was a bicarbonate-dependent DIDS-sensitive transport of Li+ and Na+, but not of K+, with Li+ being preferred over Na+ (Becker and Duhm, 1978; Wieth, 1970); instead, Li+ and Na+, but not K+, form complexes with CO32- in aqueous solutions (Becker and Duhm, 1978; Garrels et al., 1961); (b) the CO32- ion could be substituted by other divalent anions of similar electronic structure, in particular SO42- and PO43- (Becker and Duhm, 1978). In contrast to the erythrocyte, however, the squid axon system is known not to accept Li+ (Boron, 1985). We have reached a similar conclusion for BSC-1, since DIDS-sensitive 22Na+ uptake could not be cis-inhibited by Li+ (Jentsch et al., 1985b). As to SO42-, we are not aware of any published report describing its use as substitute for CO32- in a system other than the red blood cell. This may be due to unspecified effects of this substance. Indeed, we have previously tested this anion on the Na+-HCO3- symport of cultured bovine corneal endothelial cells. We could obtain no evidence from either micro-electrode studies or 22Na+ uptake measurements that it could efficiently substitute for HCO3- (or CO32-) in that system. We rather observed a poorly reversible inhibition of the electrical responses to applied Na+ or HCO3 gradients. This is again in contrast to the red blood cell system, where SO42- has been shown to act reversibly.

Implications of Stoichiometry for Net Transport Direction

A transport of NaCO3 is thermodynamically equivalent to a cotransport of two HCO3 with one Na+ ion, and this in turn determines the net transport direction of the symport. In the proximal tubule of the kidney, the physiological role of the symport is assumed to be an efflux of bicarbonate and sodium across the basolateral membrane. For the salamander kidney, Boron and Boulpaep (1983), knowing intra- and extracellular ion concentrations, demonstrated that an apparent coupling ratio of bicarbonate to sodium of 2:1 is compatible with this transport direction. Similar conclusions have been reached by Alpern (1985) for the rat, whereas Yoshitomi et al. (1985) postulate a stoichiometry of 3:1 to account for an efflux of both ions in the same species. Although the data for the corneal endothelium are compatible with both a 2:1 or 3:1 stoichiometry (Jentsch et al., 1984a), the symport of BSC-1 seems to mediate an inward transport, as suggested by electrophysiological studies, and thus might be different from the proximal tubular symport. With the plasma membrane voltage of BSC-1 (about -55 mV), the values of pH1 determined in our laboratory (about 7.2), and reasonable assumptions on intracellular Na+ concentration (about 15 mM), only with a 2:1 (and not with a 3:1) stoichiometry an inward transport would be expected. Thus, the data for BSC-1 are in principle compatible with the ion pair hypothesis.

In summary, the kinetic characteristics of the Na+HCO3- symport of BSC-1 cells are, within the experimental error, compatible with a transport of the ion pair NaHCO3. Other models (random or ordered bireactant systems) may not be excluded, but are restricted to specific combinations of 2 or 3 kinetic parameters.

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