The Renal Cortical Na-HCO_3 Cotransporter: V. Expression in Xenopus Oocytes (43962)

OFELIA S. RUIZ, YI-YONG QIU, AND JOSE A. L. ARRUDA[1]
Section of Nephrology, University of Illinois at Chicago and West Side VAMC, Chicago, Illinois 60612

Abstract. **The Na-HCO_3 cotransporter is the main system responsible for HCO_3 transport from proximal tubule cells into the blood. The expression of the renal cortical basolateral Na-HCO_3 cotransporter was studied in *Xenopus laevis* oocytes. Injection of mRNA prepared from renal cortex into oocytes enhanced HCO_3-dependent ^{22}Na uptake, on average 6-fold, (range: 2–44) compared with vehicle-injected oocytes, without altering ^{22}Na uptake in presence of gluconate. These findings indicate that mRNA selectively expresses Na-HCO_3 cotransporter activity without altering diffusive ^{22}Na uptake. The expressed Na-HCO_3 cotransporter activity had a K_m for Na of 20 m*M*, was electrogenic and was sensitive to disulfonic stilbenes. In presence of HCO_3, the expressed Na-HCO_3 cotransporter activity was like the native cotransporter, enhanced by carbonate or sulfite, a finding compatible with the existence of distinct sites for HCO_3 and carbonate on the transport system. After fractionation by a sucrose density gradient, the mRNA fraction responsible for the Na-HCO_3 cotransporter was found to be between 1.50 and 3.48 kb classes.** [P.S.E.B.M. 1996, Vol 211]

HCO_3 transport from proximal tubule cells into the blood is mediated by a basolateral electrogenic Na-HCO_3 cotransporter (1–4). The ionic mechanism of the Na-HCO_3 cotransporter has been well characterized, and it has been well established that the system functions via 1:1:1 cotransport of CO_3^{2-}, HCO_3^-, and Na^+ on different sites (2). This system functions in an integrated fashion with the brush border Na-H antiporter and the activities of these two systems vary in parallel (5, 6). Despite the importance of this system in the transport of HCO_3 in the kidney and in other organs (7, 8), little is known about the factors that regulate the activity of this system. It has been well established that the activity of the renal Na-HCO_3 cotransporter is stimulated by acidosis and inhibited by alkalosis (5, 6). In addition, cyclic AMP and calmodulin inhibit while protein kinase C stimulates the cotransporter (9). Little is known about this transport protein, but recent studies in our laboratory have solubilized and partially purified a protein fraction that has been suggested to represent the Na-HCO_3 cotransporter (10). The *Xenopus laevis* oocytes has been utilized to express different transport proteins (11–17). In the present study, we utilized this system to express the mRNA that encodes the renal Na-HCO_3 cotransporter protein. Our results demonstrate that renal cortical poly (A^+) RNA injected with frog oocytes result in expression of the Na-HCO_3 cotransporter. The expressed protein has kinetic characteristics, anion dependence, and inhibitor sensitivity similar to the native Na-HCO_3 cotransporter. The mRNA encoding the Na-HCO_3 cotransporter corresponds to a class size between 1.50 and 3.48 kb, which should allow cloning of the gene responsible for the Na-HCO_3 cotransporter.

Materials and Methods

Isolation and Purification of Renal Cortical mRNA. Total RNA from renal cortical tissue taken from New Zealand white rabbits were prepared by phenol/chloroform extraction using the method of Chomczynski and Sacchi (18) with some modifica-

[1] To whom requests for reprints should be addressed at Section of Nephrology, University of Illinois at Chicago, 840 S. Wood Street, Chicago, IL 60612.

Received April 12, 1995. [P.S.E.B.M. 1996, Vol 211]
Accepted September 12, 1995.

0037-9727/96/2112-0199$10.50/0

tions. After the second ethanol precipitation step, the pellet was washed twice with 75% ethanol to remove excess salt. Total RNA was resuspended in DEPC-treated water and stored precipitated in ethanol at −20°C.

Poly (A^+) mRNA was purified by affinity chromatography on oligo (dT) cellulose (19) using oligo (dT) spun column kit. About 200 μg total mRNA were size-fractionated on a linear 5%–25% (w/w) sucrose gradient containing 10 m*M* Tris-Cl, pH 7.50, 100 m*M* KCl, and 1 m*M* EDTA as described by McGookin (20). After heating for 2 min at 65°C, the gradient was spun at 200,000*g* for 4 hr at 4°C using SW 41 Beckmann rotor. Twenty 0.5-ml fractions were collected. Each fraction was precipitated with 0.1 volume of 3 *M* Na acetate, pH 5.2, and 2.5 volumes of 95% ethanol at −20°C. Then each fractionated mRNA was resuspended in DEPC-treated H_2O at concentrations needed for microinjection.

Expression of mRNA in Xenopus Oocytes. Adult female Xenopus laevis were anesthetized with 0.2% of 3-amino-benzoic acid ethyl ester methane sulfonate salt for 20–30 min, and oocytes were prepared as described by Colman (21) and Markus-Sekura (22). Oocytes from three to five ovarian lobes were dissected and defolliculated by 2–3 hr incubation in 2 mg/ml collegenase in Ca^{++}-free modified Barth's solution containing 88 m*M* NaCl, 1 m*M* KCl, 2.4 m*M* $NaHCO_3$, 0.82 m*M* $MgSO_4$, and 7.5 m*M* Tris, pH 7.60, with HCl. Stages V and VI oocytes (23) were carefully selected and incubated for 15–20 hr in modified Barth's solution containing Ca^{++}, 100 U/ml penicillin, 1 mg/ml streptomycin, 50 μg/ml gentamicin, and 0.25 μg/ml amphotericin B. Healthy oocytes were injected with 10 ng of rabbit cortical mRNA (0.2 mg/ml) or an equivalent volume of water. Injected oocytes were cultured for three to five days with daily changes of Barth's solution.

Each batch of oocytes was pre-screened for baseline Na-HCO_3 cotransporter activity before microinjection as described below. Only those batches of oocytes with activity of less than 1 nmol/oocyte/hr were used for the study.

^{22}Na Uptake Assay. ^{22}Na uptake assay previously described in our laboratory (6) was modified for the oocyte experiment. In brief, oocytes were pre-incubated for 1 hr at room temperature in a solution containing 200 m*M* sucrose, 50 m*M* Hepes, pH 7.50, with Tris, and 1m*M* Mg gluconate. The reaction was started by incubating the oocyte in an uptake medium consisting of 40 m*M* Na gluconate, 60 m*M* K gluconate, 1 m*M* Mg gluconate, 0.25 μCi ^{22}NaCl, and 25 m*M* of $KHCO_3$ or K gluconate. The reaction was stopped by addition of 4 ml ice-cold solution containing 200 m*M* sucrose and 50 m*M* Hepes, pH 7.50 with Tris. The stop solution was then carefully pipetted out, and the oocyte was washed and then rinsed three times more. Each oocyte was then dissolved in 1 ml 10% Na lauryl sulfate and radioactivity was measured by scintillation spectroscopy.

HCO_3-dependent ^{22}Na uptake, a measure of Na-HCO_3 cotransporter activity, was taken as the difference in ^{22}Na uptake in the presence and in the absence of an inwardly directed HCO_3-gradient. Statistical significance was calculated using the paired *t* test. Values are expressed as mean ± SEM.

Results

To look for the existence of endogenous of Na-HCO_3 cotransporter activity in the oocytes, we performed paired experiments in which we measured ^{22}Na uptake in presence of HCO_3 or gluconate. Preliminary experiments showed that in uninjected oocytes and in oocytes injected with vehicle, ^{22}Na uptake in the presence of gluconate was highly variable. However, in these paired experiments, ^{22}Na uptake in presence of HCO_3 tended to be higher than in the presence of gluconate, suggesting the presence of endogenous Na-HCO_3 cotransporter (expressed as the difference in ^{22}Na uptake in the presence of HCO_3 and gluconate). This endogenous Na-HCO_3 cotransporter activity was not always present and when present its activity was of small magnitude (less than 1 nmol/oocyte/hr). In contrast, in oocytes injected with mRNA, ^{22}Na uptake in the presence of HCO_3 was always higher than in the presence of gluconate suggesting expression of the Na-HCO_3 cotransporter. Time curve experiments indicated that ^{22}Na uptake increased with time and plateaued at 60 min and therefore all experiments were performed at one hr. Preliminary experiments showed that ^{22}Na uptake was highest after 3–5 days injection of mRNA and therefore, this time interval was used in all subsequent experiments. Table I shows ^{22}Na uptake in the presence of HCO_3 and in the presence of gluconate in oocytes injected either with the vehicle or with 10ng mRNA. It can be seen that in the mRNA-injected oocytes, ^{22}Na uptake increased in the presence of HCO_3 but not in

Table I. ^{22}Na Uptake (nmol/oocyte/hr) in the Presence of HCO_3^- or Gluconate in Vehicle and mRNA-Injected Oocytes

	HCO_3^-	Gluconate
Vehicle	13.6 ± 1.0	12.9 ± 1.1
	$P < 0.01$	NS
mRNA	17.8 ± 1.0	13.2 ± 1.1

Note. ^{22}Na uptake in vehicle and mRNA-injected oocytes. Oocytes were incubated at room temperature for one hour in a buffer containing HCO_3^- or gluconate and ^{22}Na uptake was measured as described in Materials and Methods. Values are means ± SEM from 10 experiments done in triplicates from 10 separate oocyte preparations. Statistical significance was analyzed using paired *t* test. NS, not significant.

the presence of gluconate, suggesting that mRNA-injected oocytes selectively expressed the Na-HCO_3 cotransporter without altering diffusive ^{22}Na uptake. This latter finding suggests that ^{22}Na uptake is not mediated by expression of a Na^+ channel. Further evidence against the expression of Na channel is the fact that amiloride did not inhibit HCO_3-dependent ^{22}Na uptake (in nmols/oocyte/hr: control, 2.05 ± 0.64; amiloride, 2.54 ± 0.13, n = 3). In Figure 1, HCO_3-dependent ^{22}Na uptake (difference in ^{22}Na uptake in the presence of HCO_3 and gluconate) was increased, on the average 6-fold (range: 2–44), in mRNA-injected as compared with vehicle-injected oocytes. This variable rate of enhancement of Na-HCO_3 cotransporter could be related either to variable degradation of mRNA because it is not capped or alternatively because of variable expression of different batches of oocytes. Injection of higher concentrations (25 or 50 ng) of mRNA (data not shown) induced greater increases in Na-HCO_3 cotransporter activity, but we chose to use only 10 ng for all subsequent experiments.

Since in the kidney the Na-HCO_3 cotransporter functions to extrude HCO_3 and Na from the cell, it was important to demonstrate that mRNA-injected oocytes can extrude ^{22}Na at a rate greater than vehicle-injected oocytes. In this experiment, oocytes injected with vehicle or mRNA, were incubated with ^{22}Na and HCO_3 in presence of 1 mM ouabain and 1 mM amiloride. After 1 hr, they were removed from the medium, washed several times, and ^{22}Na was allowed to efflux in a Na-free medium. It can be seen that in oocytes injected with mRNA, the rate of ^{22}Na efflux is greater

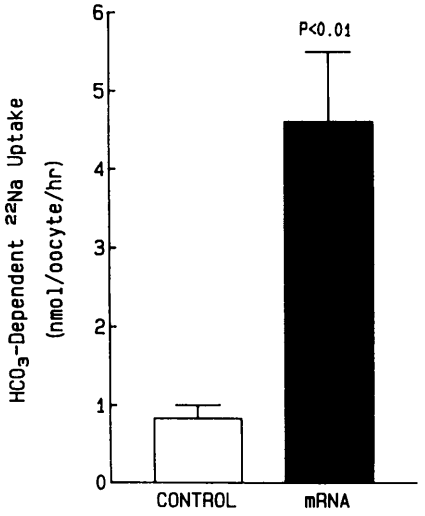

Figure 1. HCO_3-dependent ^{22}Na uptake (^{22}Na uptake in presence of HCO_3 minus uptake in presence of gluconate) in vehicle- and mRNA-injected oocytes. Oocytes were injected with 50 nl of 0.2 mg/ml poly (A^+) RNA or 50 nl of water. ^{22}Na uptake was then assayed after 3–5 days postinjection as described in Materials and Methods. Each bar represents mean uptake ± SEM from ten batches of oocytes. Each experiment was done in triplicates to quadruplicates.

than that of oocytes injected with vehicle as shown in Figure 2.

Figure 3 shows that HCO_3-dependent ^{22}Na uptake is concentration dependent, reaching maximum at 40 mM Na. The half maximal uptake occurred at 20 mM Na concentration, a value similar to the K_m reported for the native Na-HCO_3 cotransporter of basolateral membranes (6). The expressed Na-HCO_3 cotransporter was inhibited 33% ± 9% by 1 mM disulfonic stilbene, SITS (4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid) (n = 4), and 100% by 1 mM DNDS (disodium 4,4′-dinitrostilbene-2,2′-sulfonate) (n = 5). It was electrogenic as evidenced by 218% ± 54% enhancement in activity by a positive potential inside the oocyte generated by an inwardly directed K gradient (150 mM outside/100 mM inside) and 7 μM valinomycin. Amiloride, 10^{-3} M, did not affect ^{22}Na uptake in the presence of HCO_3 or gluconate.

To test for the anion sensitivity of the expressed Na-HCO_3 cotransporter, ^{22}Na uptake was measured in mRNA-injected oocytes in presence of HCO_3 and other anions which were used to replace HCO_3. It can be seen from Figure 4 upper panel, that the only anion that enhanced ^{22}Na uptake compared to gluconate was HCO_3. In contrast, in presence of HCO_3, ^{22}Na uptake was enhanced by CO_3^{2-} and SO_3^{2-} over HCO_3 (Fig. 4, lower panel). This finding is very similar to that described for the native Na-HCO_3 cotransporter, and this finding has been used to postulate the existence of distinct sites for HCO_3 and CO_3^{2-} on the transporter (24).

To identify the mRNA fraction encoding the Na-HCO_3 cotransporter, total mRNA was separated into 20 fractions on a 5%–25% linear sucrose gradient and the fractions were injected into oocytes. Figure 5 shows that HCO_3-dependent ^{22}Na uptake was enhanced 44-fold in mRNA-injected oocytes over the vehicle-injected oocytes. This greater enhancement of expression of the Na-HCO_3 cotransporter activity compared with data of Fig. 1 is partially explained by the fact that in these experiments, the Na-HCO_3 cotransporter in uninjected oocytes was lower (average 0.12 nmol/oocyte/hr). Nevertheless, fractions 2 and 3 consistently increased HCO_3-dependent ^{22}Na uptake 2- and 3-fold respectively over that present in oocytes injected with total mRNA. Fraction 3 had the highest activity increasing HCO_3-dependent ^{22}Na uptake 105-fold over the vehicle and 3-fold over mRNA-injected oocytes. This fraction corresponds to a mRNA size-class within the 18S subfraction between 1.50 and 3.48 kb.

Discussion

The present studies were aimed at identifying the mRNA encoding the renal basolateral Na-HCO_3 cotransporter, the main transporter responsible for

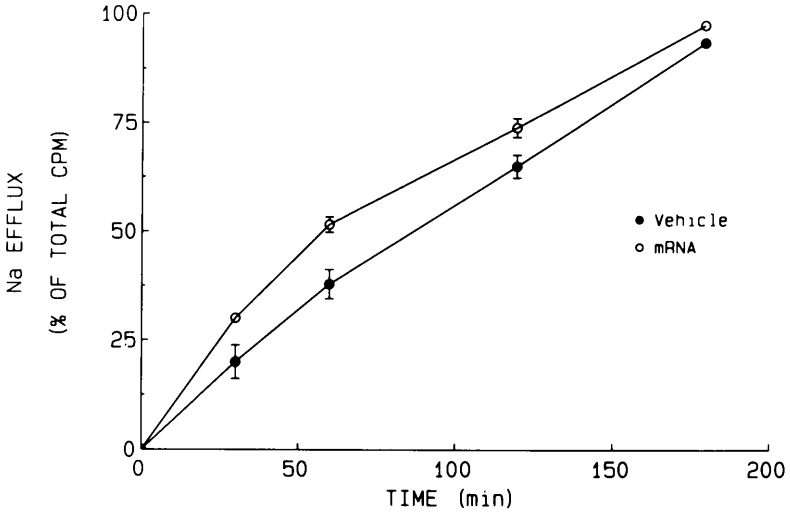

Figure 2. ^{22}Na efflux in vehicle- and mRNA-injected oocytes. Oocytes injected with 50 nl of 0.2 mg/ml mRNA or 50 nl of vehicle were incubated with ^{22}Na and HCO_3 for 1 hr, washed, and transferred to a Na-free medium; ^{22}Na was allowed to efflux and radioactivity in the solution measured at different time points. Each point represents the rate of cumulative Na efflux in Na- and HCO_3-free medium at different time points expressed as a percentage of total cpm in the oocytes, mean ± SEM, $n = 5$. P values for vehicle versus mRNA-injected oocytes at 30, 60, 120, and 180 min: $P < 0.05$, $P < 0.01$, $P < 0.05$, and $P < 0.02$, respectively.

HCO_3 transport from the cell into the blood. For sake of simplifying the experimental protocol, the experiments were designed to measure ^{22}Na uptake in the presence of HCO_3 or gluconate and the difference was taken as Na-HCO_3 cotransporter activity. The ratio of extracellular/intracellular concentration of Na (outside, 40 m*M;* inside, only 10m*M*), and HCO_3 (outside, 25 m*M;* and inside, approximately 25 m*M* if one assumes an equilibrium pCO_2 of around 10 mm Hg and intracellular pH of 7.50–7.60) and membrane potential (−30 mV) were such to favor net influx of Na and HCO_3 (25). Unpublished results from our laboratory showed an average membrane potential of −30 mV (inside negative). For the uptake experiments, the membrane potential was set at approximately 0 mV. Calculation of the membrane potential using published permeabilities for Na and K (26) indicate that the membrane potential was close to zero (− 1–2 mV). Under these conditions, the direction of Na uptake is determined by the ratio Na_i/N_o ratio = $(HCO_{3o}/HCO_{3i})^3$. Since the HCO_3 was approximately equal, a Na_i/Na_o ratio less than 1 would favor net ^{22}Na uptake (27). It is of interest to note however, that although ^{22}Na uptake in presence of gluconate was variable, we observed that in paired experiments, ^{22}Na uptake in the presence of HCO_3 was always higher than in the presence of gluconate. This endogenous Na-HCO_3 cotransporter activity was of small magnitude (<1 nmol/oocyte/hr). This finding clearly indicates that frog oocytes contain a HCO_3-dependent ^{22}Na uptake, which can be expressed by injection of renal cortical mRNA. It is of interest to note that ^{22}Na uptake in presence of gluconate (diffusive ^{22}Na uptake) was not enhanced by mRNA injection suggesting that ^{22}Na uptake is not mediated by expression of Na channel. Further evidence against the expression of a Na channel is the fact that amiloride did not inhibit ^{22}Na uptake in presence of gluconate or HCO_3. These studies are in agreement with studies of other investigators showing absence of Na channel activity in uninjected oocytes (13).

We attempted to characterize the expressed pro-

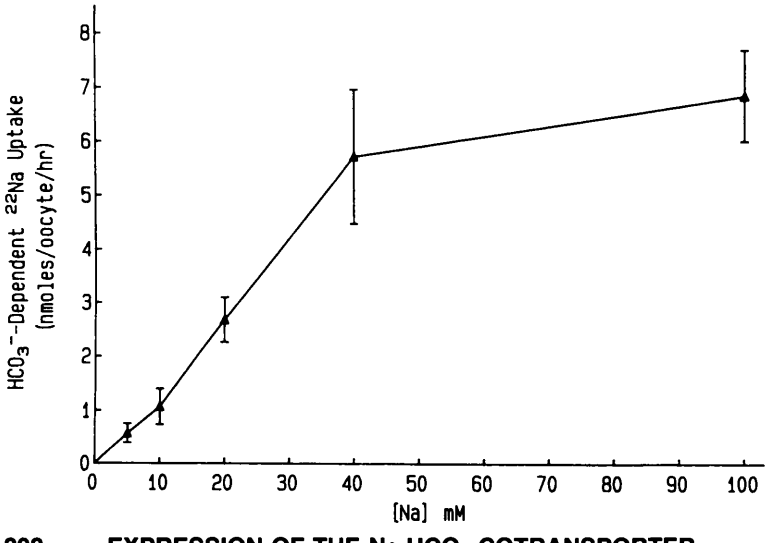

Figure 3. HCO_3-dependent ^{22}Na uptake in presence of different Na concentrations. ^{22}Na uptake assayed in mRNA-injected oocytes was done in presence of 5 to 100 m*M* Na in the uptake medium. Each point represents mean uptake ± SEM of five different oocyte preparations with each experiment done in triplicate.

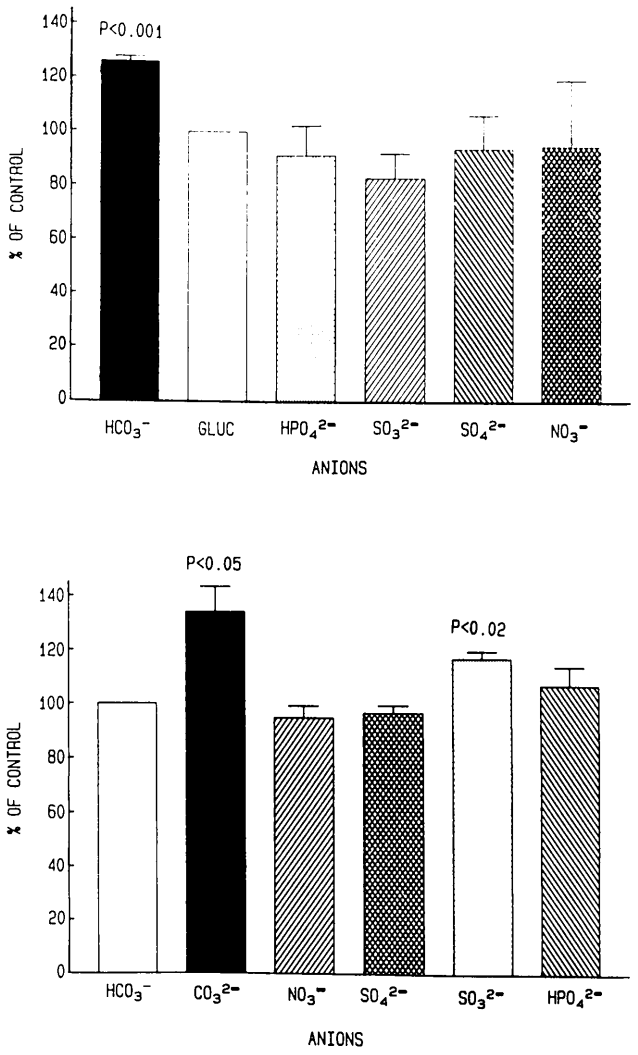

Figure 4. Effect of different anions on HCO_3-dependent ^{22}Na uptake. In the upper panel, with gluconate taken as control, ^{22}Na uptake was assayed in mRNA-injected oocytes pre-equilibrated in a Cl-free solution in presence of 40 m*M* Na gluconate, 1 m*M* Mg gluconate, 0.25 μCi ^{22}Na and 45 m*M* concentration of each of the anions added as potassium salt. In the lower panel, with bicarbonate taken as control, ^{22}Na uptake was measured in presence of 25 m*M* $KHCO_3$ plus 45 m*M* of each of the anions added as potassium salt. Each bar represents mean uptake ± SEM of three different oocyte preparations.

tein to provide evidence that it is indeed the Na-HCO_3 cotransporter and not another protein, particularly the Na-dependent Cl-HCO_3 exchanger. To exclude the possibility that the protein expressed was the Na-dependent Cl-HCO_3 exchanger, we depleted the oocytes of chloride by exposing them to chloride-free solution for more than 60 min, a maneuver that has been used successfully to deplete chloride in other cells (28). Thus, our experiments performed in the presence of chloride depletion and chloride-free medium indicate that the expressed protein represents the Na-HCO_3 cotransporter and not the Na-dependent Cl-HCO_3 exchanger. In addition, this latter transporter should stimulate Na uptake in the presence of nitrate or sulfite and, as can be seen in Figure 4 (upper panel), nitrate or sulfite could not replace HCO_3, arguing strongly against the presence of Na-dependent Cl-HCO_3 exchanger. Regarding the ability of anions to stimulate Na uptake, it is of interest to note that in mRNA-injected oocytes, the only anion that enhanced ^{22}Na uptake over the values observed in presence of gluconate was HCO_3. In addition, in presence of HCO_3, only CO_3^{2-} and SO_3^{2-} were able to enhance ^{22}Na uptake over that observed in presence of HCO_3. These findings are qualitatively very similar to those described for the native renal Na-HCO_3 cotransporter (24) and are compatible with the existence of distinct sites for HCO_3 and CO_3^{2-} on the cotransporter.

The expressed Na-HCO_3 cotransporter has a K_m for Na of 20 m*M* and this in the range of K_m (10–20 m*M*) for the Na-HCO_3 cotransporter measured in our laboratory (6). In addition, the expressed Na-HCO_3 cotransporter, like the native cotransporter, is electrogenic and is sensitive to SITS. The inhibitory effect of SITS, however, was less in the oocytes than in basolateral membranes. Differences in stilbene sensitivity of Na-HCO_3 cotransporter have been observed between epithelial and nonepithelial tissues (3). This difference has been attributed to post-translational modifications of the cotransporter or to alterations in the structure of the cotransporter. In contrast to SITS, the

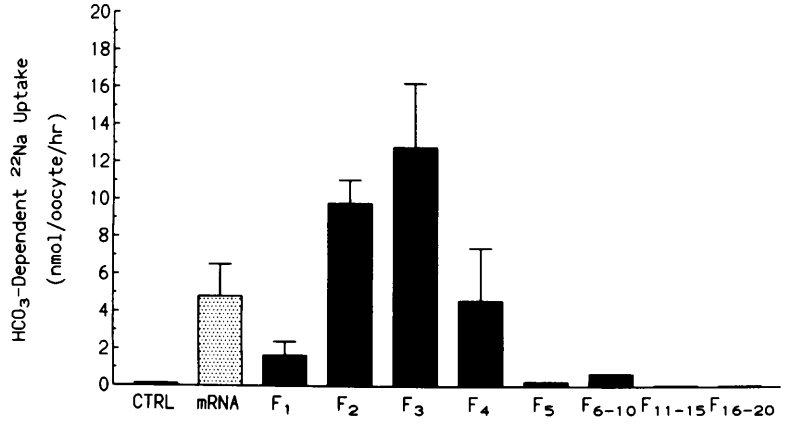

Figure 5. HCO_3-dependent ^{22}Na uptake in uninjected oocytes and in oocytes injected with total mRNA and size-fractionated mRNA. Approximately 200 μg of total mRNA was size-fractionated by sucrose gradient centrifugation. Oocytes were injected with 10 ng of either total or size-fractionated mRNA or with an equivalent volume of vehicle (control) and then ^{22}Na uptake assayed 3 days after. Fractions 6–20 were pooled to give adequate mRNA centrifugation. Each bar represents mean ± SEM of three to five different oocyte preparations.

disulfonic stilbene, DNDS, completely inhibited the Na-HCO_3 cotransporter activity in the oocyte. This finding is similar to that reported by Morgan *et al.* who also found that DNDS was a more potent inhibitor of the band 3 protein expressed in the oocyte than DIDS (29). Fractionation of a mRNA fraction by sucrose gradient centrifugation identified a mRNA fraction encoding the Na-HCO_3 cotransporter to be a mRNA class within the 18S subfraction between 1.50 and 3.48 kb.

This work was supported by the Merit Review Program from the Veterans Administration Central Office. The authors thank Clary Olichwier for assistance in the preparation of this manuscript. Part of this study has been published in abstract form (Clin Res **38**:874A, 1990; J Am Soc Nephrol **1**:657, 1990).

1. Akiba T, Alpern RJ, Eveloff J, Calamina J, Warnock DG. J Clin Invest **78**:1472–1478, 1986.
2. Alpern RJ. Cell mechanisms of proximal tubule acidification. Physiol Rev **70**:79–114, 1990.
3. Boron WF, Boulpaep E. The electrogenic Na/HCO_3 cotransporter. Kidney Int **36**:392–402, 1989.
4. Grassl SM, Aronson PJ. Na^+/HCO_3^- cotransport in basolateral membrane vesicles isolated from rabbit renal cortex. J Biol Chem **262**:8778–8783, 1986.
5. Akiba T, Rocco VK, Warnock DG. Parallel adaptation of the rabbit renal cortical sodium/proton antiporter and sodium/bicarbonate cotransporter in metabolic acidosis and alkalosis. J Clin Invest **80**:308–315, 1987.
6. Ruiz OS, Arruda JAL, Talor Z. Na-HCO_3 cotransport and Na-H antiporter in chronic respiratory acidosis and alkalosis. Am J Physiol **256**:F414–F420, 1989.
7. Renner EL, Lake JR, Scharschmidt BF, Zimmerli B, Meier PJ. Rat hepatocytes exhibit basolateral Na^+/HCO_3^- cotransport. J Clin Invest **83**:1225–1235, 1989.
8. Winterhager JM, Stewart CP, Heintze K, Petersen K. Electroneutral secretion of bicarbonate by guinea pig gallbladder epithelium. Am J Physiol **250**:C617–C628, 1986.
9. Ruiz OS, Arruda JAL. Regulation of the renal Na/HCO_3^- cotransporter by cyclic AMP and Ca-dependent protein kinases. Am J Physiol **31**:F560–F565, 1992.
10. Bernardo AA, Kear F, Ruiz OS, Arruda JAL. Renal cortical basolateral Na^+/HCO_3^- cotransporter: I. Partial purification and reconstitution. J Membrane Biol **140**:31–37, 1994.
11. Aoshima H, Tomita K, Sugio S. Expression of amino acid transport systems in Xenopus oocytes injected with mRNA of rat small intestine and kidney. Arch Biochem Biophys **265**:73–81, 1988.
12. Burnham CE, Kidd J, Palfrey HC. Expression of the Na^+-K^+-$2Cl^-$ cotransporter in Xenopus oocytes. Am J Physiol **259**(28):F383–F388, 1990.
13. George AL Jr., Staub O, Geering K, Rossier BC, Kleyman TR, Kraehenbuhl JP. Functional expression of the amiloride sensitive sodium channel in Xenopus oocytes. Proc Natl Acad Sci USA **86**:7295–7298, 1989.
14. Lu L, Montrose-Rafizadeh C, Guggino WB. Ca^{2+}-activated K^+ channels from rabbit kdiney medullary thick ascending limb cells expressed in Xenopus oocytes. J Biol Chem **265**:16190–16194, 1990.
15. Milovanovic S, Frindt G, Tate S, Windhager EE. Expression of renal Na^+-Ca^{2+} exchange activity in *Xenopus laevis* oocytes. Am J Physiol **261**:F207–F212, 1991.
16. Uchida S, Kwon HM, Preston AS, Handler JS. Expression of Madin-Darby canine kidney cell Na^+ and Cl^- dependent taurine transporter in *Xenopus laevis* oocytes. J Biol Chem **266**:9605–9609, 1991.
17. Werner A, Biber J, Fargo J, Palacin M, Murer H. Expression of renal transport systems for inorganic phosphate and sulfate in *Xenopus laevis* oocytes. J Biol Chem **265**:12331–12336, 1990.
18. Chomczynski PL, Sacchi N. Single-step method of RNA isolation by acid guanidnium thiocyanate-phenol-chloroform extraction. Anal Biochem **162**:156–159, 1987.
19. Sambrook J, Fritsch EF, Maniatis T. Selection of poly $(A)^+$ RNA. In: Nalom C, Ed. Molecular Cloning, A Laboratory Manual (2nd ed). Cold Springs Harbor, NY: Cold Spring Harbor Laboratory Press, Vol **7**: pp 26–29, 1989.
20. McGookin R. Messenger RNA fractionation on neutral sucrose gradients. In: Walker JM, Ed. Methods in Molecular Biology. Clifton, NJ: Humana Press, Vol **2**: pp 121–124, 1984.
21. Colman A. Translation of eukaryotic messenger RNA in Xenopus oocytes. In: Hames BD, Higgins SJ, Eds. Transcription and Translation: A Practical Approach. Oxford: pp 271–302, 1984.
22. Marcus-Sekura CJ, Hitchcock MJM. Preparation of oocytes for microinjection of RNA and DNA. In: Berger SL, Kimmel AR, Eds. Methods in Enzymology. San Diego, CA: Academic Press, Vol **152**: pp 284–288, 1987.
23. Dumont JN. Oogenesis in *Xenopus laevis* (Daudin). I. Stages of oocytes development in laboratory maintained animals. J Morphol **136**:153–180, 1972.
24. Soleimani M, Aronson PS. Ionic mechanism of Na^+-HCO_3^- cotransport in rabbit renal basolateral membrane vesicles. J Biol Chem **264**:18302–18306, 1989.
25. Lee SC, Steinhardt RA. pH changes associated with meiotic maturation in oocytes of *Xenopus laevis*. Devel Biol **85**:358–369, 1981.
26. Costa PF, Emilio MG, Fernandes PL, Ferriera HG, Ferriera KG. Determination of ionic permeability coefficients of the plasma membrane of *Xenopus laevis* oocytes under voltage clamping. J Physiol **413**:199–201, 1989.
27. Soleimani M, Lesoine GA, Bergman JA, McKinney TD. A pH modifier site regulates activity of the Na^+:HCO_3^- cotransporter in basolateral membranes of kidney proximal tubules. J Clin Invest **88**:1135–1140, 1991.
28. Boyarsky G, Ganz MB, Sterzel B, Boron WF. pH regulation in single glomerular mesangial cells. I. Acid extrusion in absence and presence of HCO_3. Am J Physiol **255**:C844–C856, 1988.
29. Morgan M, Hanke P, Grygorczyk R, Tintschl A, Fasold H, Passow H. Mediation of anion transport in oocytes of *Xenopus laevis* by biosynthetically inserted band 3 protein from mouse spleen erythroid cells. EMBO J **4**:1927–1931, 1985.