Serum Regulates Na+/H+ Exchange in Caco-2 Cells by a Mechanism Which Is Dependent on F-actin*

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The regulation of Na+/H+ exchange has been studied extensively in a wide variety of epithelial and nonepithelial cells. Broadly, two patterns of regulation have emerged (1, 2). First, activation or inactivation of Na+/H+ exchange can be rapid in onset (3–8). This rapid regulation is believed to be mediated by phosphorylation of the Na+/H+ exchanger or a closely associated molecule (9). Activation of the transporter either by serum, α-thrombin, epidermal growth factor, or osmotic shrinkage (4–7) is thought to shift the intracellular pH sensitivity of the transporter to more alkaline values and decrease the affinity for internal Na+ (without changes in the affinity of the exchanger for external Na+ or H+ or change in the Vmax) (1, 2, 10). A second class of regulation requires hours or days to develop. Although less studied, this type of regulation occurs in the kidney proximal tubule in response to thyroxine, glucocorticoids, or chronic acidosis (11–13). In the cases studied, there is a change in the transport Vmax, but no change in the affinity for external Na+, the Hill coefficient, or the intracellular pH sensitivity (2). In epithelial cells, studies of the regulation of Na+/H+ exchange can be complicated by the presence of multiple Na+/H+ exchange proteins. In some epithelial cells (notably ileal villus cells and the pig kidney cell line PKE20) separate apical and basolateral Na+/H+ exchange have been identified (14–16). Apical and basolateral Na+/H+ exchangers can be different in amiloride inhibition kinetics and regulation by second messengers (15–17). These differences are consistent with the different physiologic functions which are likely to be mediated by apical exchangers (transepithelial Na+ absorption) versus basolateral exchangers (cell pH homeostasis).

Previously, we have characterized Na+/H+ exchange in Caco-2 cells as a potential model for regulation of the human intestinal basolateral exchanger (18). These cells have a basolateral membrane Na+/H+ exchanger which is highly sensitive to amiloride (Kᵢ, 3.2 μM), and which has a cytoplasmic H+ modifier site. The transporter is not regulated by addition of phorbol dibutyrate or changes in intracellular cyclic AMP, cyclic GMP, Ca2+, or cell volume (18). These properties can be contrasted with those of intestinal apical membrane Na+/H+ exchange which is less sensitive to amiloride (Kᵢ, 100–200 μM) (19, 20) and is inhibited by Ca2+, Ca2+/calmodulin kinase II, and protein kinase C (21–23). In the present study, the long-term regulation of Caco-2 Na+/H+ exchange by fetal bovine serum (FBS) is described. We observe that FBS deprivation reversibly decreases the Vmax of Na+/H+ exchange by a process which requires hours to occur and is dependent on actin polymerization. This regulation of Na+/H+ exchange in Caco-2 cells by serum is distinct from the rapid effects of serum and growth factors observed in other cell types (1, 4, 5).

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1 The abbreviations used are: FBS, fetal bovine serum; HEPES, N-2-hydroxyethylpiperazine-N'-3-ethanesulfonic acid; MES, 2-(N-morpholino)ethanesulfonic acid; BCGF, 2',7'-bis(2-carboxyethyl)-5,6-carboxyfluorescein; TMA, tetramethylammonium.
Experimental Procedures

Chemicals and Solutions—Amiloride was obtained from Merck, Sharp and Dohme Inc., (Rahway, NJ), digitonin from Fluka Chemical Co. (Ronkonkoma, NY), 2',7'-bis(2-carboxyethyl)-5',6'-carboxyfluorescein, acetoxyethyl ester, and nigericin from Molecular Probes Inc., (Junction City, OR), cytochalasin D from Sigma. All other chemicals were obtained from Sigma or Fluka unless otherwise noted.

For most experiments, cells were plated in a medium resembling culture medium in ionic content ("Na+ medium") containing (in mM): 130 NaCl, 5 KCl, 2 CaCl2, 1 MgSO4, 0.75 Na2HPO4, 0.25 NaH2PO4, 25 glucose, 20 HEPES, titrated to pH 7.30 at 33 °C with NaOH. In some experiments, a medium with tetratetramethylammonium replacing Na+ ("TMA medium") was used. A pH clamp medium was also used for calibration of 2',7'-bis(2-carboxyethyl)-5',6'-carboxyfluorescein with 75 KCl, 35 mM sodium formate, 15 sodium gluconate, 1 CaCl2, 1 MgSO4, 20 HEPES, and 20 MES titrated to pH 7.30 at 33 °C with TMA-OH (18).

Cell Culture—Caco-2 cells were purchased from American Type Culture Collection (Rockville, MD), and experiments were performed using cells between passages 27 and 37. Cells were grown in a 5% CO2/95% air incubator at 33 °C in Dulbecco's modified Eagle medium (Hazleton Biologics Inc., St. Lenexa, KS) supplemented with 25 mM NaHCO3, 10 mM HEPES, 50 IU/ml penicillin, 50 μg/ml streptomycin, 1% nonessential amino acids, and 10% fetal bovine serum. In some experiments, fetal bovine serum (FBS) was removed from some passages (28-30), a cloned cell line of Caco-2 cells enriched in villin, were used. These cells were a gift from Dr. Mark Mooseker, Yale University. No qualitative or quantitative difference in results was noted between the two cell types. Caco-2/C11 cells were grown in the same conditions as uncloned Caco-2 cells except that 10 μg/ml transferrin was added to the growth medium. The cloned Chinese hamster fibroblast line P3122 (a gift from Dr. Jacques Pouyssegur, Nice, France) is derived from the CCL39 line and was maintained in Dulbecco's modified Eagle medium (GIBCO) supplemented with 10% fetal bovine serum, penicillin (50 IU/ml), and streptomycin (50 μg/ml) in an atmosphere of 5% CO2/95% air at 33 °C.

For fluorometry, filters were glued to plastic supports with silicone rubber adhesive (General Electric, Waterford, NY) or acetalone, and coated with rat tail collagen (18, 27). Cells were seeded onto filters and studied 10-24 days after seeding (7-21 days postconfluency).

Intracellular pH Measurement and Calibration—Intracellular pH (pHi) was measured with BCECF as described previously (18, 24). Briefly, cells on filters were loaded with BCECF by exposure for 60-90 min to 6.25 μM of the acetoxyethyl ester at room temperature in Na+ medium. The cells to be studied in the microscope were mounted and perfused as described previously (18, 27). For study in a standard fluorometer, cells were perfused three times with TMA medium to remove extracellular dye and then mounted at 45° in a glass cuvette. In experiments where fetal bovine serum (FBS) was removed, the cells were washed in Hanks' medium (GIBCO) supplemented with 10% fetal bovine serum, penicillin (50 IU/ml), and streptomycin (50 μg/ml) in an atmosphere of 5% CO2/95% air at 33 °C.

As described previously (18), BCECF fluorescence was measured by dual excitation (500/440 nm) rationing in an SLM spectrofluorometer (SPF-600C, SLM, Urbana, IL) equipped with a stirred cuvette, and monitored at 33 °C. The protocol used to calibrate intracellular BCECF is based on the method of Thomas et al. (18, 25) employing nigericin.

A calibration curve of intracellular pH was constructed by equilibrating cells in pH-clamp medium with 10 μM nigericin, and titrating pH with additions of HNO3. Individual experiments were calibrated as follows. In the case of perfused experiments, the cells were equilibrated in pH-clamp medium (titrated to pH 7.30) plus 10 μM nigericin and a single point calibration used to normalize data to the calibration curve. For nonperfused experiments in the fluorometer, the dye was released with 100 μM digitonin and single point calibration used to normalize data to the calibration curve. We have previously shown that these two calibration methods are equivalent in Caco-2 cells (18).

Measurement of Cellular Buffering Capacity for Hydrogen Ions—Buffering capacity (β) was determined by exposure of the cells loaded with BCECF to NH4Cl as described previously (18). Briefly, cell pHi, was first set by preincubation of 30 mM NH4Cl in culture medium in ionic content ("Na+ medium") containing (in mM): 130 NaCl, 5 KCl, 2 CaCl2, 1 MgSO4, 0.75 Na2HPO4, 0.25 NaH2PO4, 25 glucose, 20 HEPES, titrated to pH 7.30 with NH4OH at 23 °C with TMA-OH (18).

As described previously (18), buffering capacity was calculated using the medium pH and the pHi, before and after addition of NH4Cl, according to the formula: β = Δ(pHi)/ΔpH. Measurement of Na+/H+ Exchange—Na+/H+ exchange activity was measured after the cells had been acclimated by transient exposure to 30 mM NH4Cl. Acidified cells in a Na+-free (TMA medium) had a stable pH. When the TMA medium was replaced with Na+ medium there was a prompt alkalization (pHi recovery) due to the basolateral Na+/H+ exchanger (26, 27). Na+/H+ exchange rates were determined by multiplication of the initial rate of alkalization over approximately 30-60 s (ΔpHi/min) by the buffering capacity of the cells at the initial pH, from which the alkalization started.

Cell Ion Content and Cell Volume Measurements—Total cell Na+ and K+ of cell monolayers were determined by flame photometry as described previously (26, 27) and expressed as nanomoles per mg of protein. Cells were suspended by exposure to 0.1% trypsin in Hanks' medium (GIBCO). Cell volume was measured in a Coulter Counter (model ZM) coupled to a computerized pulse-height analyzer (The Nucleus, Oakridge, TN), which was calibrated using latex beads (Coulter) of defined size (28). The median single cell volume of suspended cells was determined automatically by computer.

Measurement of [3H]Leucine Uptake and Incorporation into Protein—Caco-2 cells grown in 24-well plates were incubated with 500 μl of growth media containing 1 μCi/ml [3H]leucine (Du Pont-New England Nuclear Research Products, MA) plus varying concentrations of cycloheximide. After the incubation period, the medium was aspirated and the cells washed three times with Hanks' medium and then incubated with 500 μl of 10% trichloroacetic acid for 1 h at 4 °C. The trichloroacetic acid was then aspirated and cells incubated in 2 ml NaOH overnight at 23 °C. The NaOH was collected, neutralized with concentrated HCl, and counted in a scintillation counter.

Statistical Analysis—Where applicable the data are presented as the mean ± S.E. of the mean unless indicated. Comparison of means was performed by Student's unpaired t test. A probability of <0.05 was considered significant.

Results

Effect of Serum Removal on pHi, Recovery from an Acid Load—Caco-2 cells recover from an acid load by activation of a basolateral Na+/H+ exchanger (18). Previously, we have demonstrated a number of second messenger pathways do not rapidly alter Na+/H+ exchange rates in Caco-2 cells despite the ability of these pathways to regulate CI- secretion (18). Since serum addition rapidly stimulates Na+/H+ exchange in several other cell types (1, 4), the effect of serum addition was evaluated in Caco-2 cells. Prior to exposure to 10% FBS, cells were FBS-deprived for 4 h and allowed to reach a steady-state pHi, after recovery from an NH4Cl-induced acid load. Using fibroblasts under these experimental conditions as positive controls, a Na+-dependent and amiloride-sensitive alkalization was consistently observed over a 10-min time course, indicative of Na+/H+ exchange activation (Fig. 1A).

In contrast, addition of serum to Caco-2 cells under these experimental conditions caused a slight acidification [potentially due to an increase in metabolic acid production (29)], but did not demonstrate an alkalization above resting pHi, (Fig. 1B). These results suggest that Caco-2 Na+/H+ exchange is not rapidly activated by serum readdition.

Despite the lack of rapid stimulation of Na+/H+ exchange by serum readdition, removal of FBS from Caco-2 cells for 4 h reduced the rate of pH, recovery from an acid load (Fig. 2). As this reduction in alkalization rate could have been due to an increase in cellular buffering capacity without a change in transport by the Na+/H+ exchanger, the buffering capacity of FBS-starved cells was compared with the buffering capacity of normal cells. As shown in Fig. 3, 4-h serum removal did not change buffering capacity; although the buffering capacity was decreased at acidic pHi, values after cells had been FBS-starved for 24 h. Since an increase in buffering capacity would

[2] A. J. M. Watson, S. Levine, M. Donowitz, and M. H. Montrose, unpublished observations.
be required to explain the observed decrease in the alkalization rate, the results imply that a decrease in Na+/H+ exchange activity is responsible for the reduced rate of pHi recovery.

The time course and reversibility of the FBS deprivation effects on Na+/H+ exchange were determined (Fig. 4, Table I). Serum was removed from Caco-2 cells for 2, 4, or 24 h before Na+/H+ exchange rate was determined. Since the rate of Na+/H+ exchange is sensitive to pH, data are presented as a scatter plot of individual measurements of Na+/H+ exchange activity versus the starting pH, values (Fig. 4) and compiled data are categorized by the pH, range under study (Table I). 4-h FBS deprivation was sufficient to obtain a maximal inhibition of Na+/H+ exchange, as no further reduction in Na+/H+ exchange activity was obtained after 24-h FBS starvation (Fig. 4). FBS deprivation for 2 h inhibited Na+/H+ exchange, but produced a submaximal reduction in Na+/H+ exchanger rate (Table I).

After 4-h FBS deprivation, readddition of 10% FBS for 4 h was sufficient to restore Na+/H+ activity to control levels (Fig. 4), but readddition of 10% FBS for 1 h caused only a partial recovery (data not shown). When Caco-2 cells were FBS-deprived for 24 h, full Na+/H+ activity could be restored by readddition of 10% FBS for a further 24 h (Fig. 4).
Reduction of Na+/H+ exchange after 2- and 4-h FBS deprivation based on the degree of initial acidification.

| Initial pH range | Control rate of Na+/H+ exchange | Rate of Na+/H+ exchange after 4-h serum deprivation | Reduction in rate after 4-h serum deprivation |
|------------------|---------------------------------|-----------------------------------------------|---------------------------------|
| pH               | µM H+ /s                         | µM H+ /s                                      | µM H+ /s (%)                     |
| 6.61-6.70        | 114 (2)                         | 83 (2)                                        | 41 (1) 64                       |
| 6.71-6.80        | 75 ± 21 (5)                     | 41 (1)                                        | 28 ± 13 (4) 63                  |
| 6.81-6.90        | 61 ± 12 (6)                     | 81 (2)                                        | 22 ± 6 (4) 64                   |
| 6.91-7.00        | 47 ± 12 (8)                     | 37 (3)                                        | 21 ± 1 (4) 55                   |
| 7.01-7.10        | 43 ± 12 (4)                     | 23 (2)                                        | 12 ± 2 (4) 72                   |

Table II

Change in intracellular ion content after 24-h FBS-deprivation or exposure to ouabain

Cells were FBS starved for 24 h to maximize the effects of serum deprivation. Intracellular Na+ and K+ content was measured by flame photometry. As indicated, some cells were exposed to 100 µM ouabain for 4 h, as a positive control. Results were normalized to milligrams of cellular protein. Cell volume was measured using a Coulter Counter, and results are presented in femtoliters (fl) as the mean volume of single cells in the population. Means ± S.E. of at least four separate determinations are presented.

| Na+  | K+  | Cell volume |
|------|-----|-------------|
|      |     | fl          |
| Control | 112 ± 17 | 416 ± 26 | 1371 ± 18 |
| FBS-starved | 96 ± 15 | 340 ± 32 | 1320 ± 8 |
| Ouabain | 470 ± 60* | 255 ± 30* | ND        |

* Indicates statistical significance versus control (p < 0.05). ND, not determined.

cating that Na+/H+ exchange was inactive at the steady-state pH (data not shown).

Effect of FBS Deprivation on Intracellular Ion and Osmolyte Content—The reduction of Na+/H+ exchange rate in FBS-deprived cells could be due to a decrease in the driving force for Na+/H+ exchange caused by a rise in intracellular [Na+]. However, as shown in Table II, FBS deprivation for 24 h caused no increase in cellular Na+ content. Direct inhibition of the Na+,K+-ATPase with ouabain shows that changes in Na+ and K+ content are detectable with this technique. FBS deprivation caused no change in total cellular protein on the 35-mm plates (data not shown), but caused a small (4%) reduction in cell volume (Table II). When combined with the measured ion content of the cells, this change in volume predicts that intracellular ion concentration may have dropped by 10%. Thus the reduction in Na+/H+ exchange activity cannot be accounted for by a decrease in the driving force, as this would have required an increase in cellular [Na+].

Effect of FBS Deprivation on Na+/H+ Exchange Kinetics and Amiloride Sensitivity—Another potential explanation for the reduction in Na+/H+ exchange is that FBS deprivation might have altered the Na+ activation kinetics of Na+/H+ exchange. To address this possibility, the initial rate of Na+-dependent H+ efflux was measured at external Na+ concentrations between 2 and 140 mM (actual Na+ concentrations were determined by flame photometry). These measurements were made at similar initial pH values: 6.80 ± 0.09 (n = 11) for control cells, and 6.66 ± 0.10 (n = 13) for FBS-deprived cells. To control for variability between preparations, a protocol was employed in which H+ efflux rates at low [Na+], were normalized to the efflux rate at 138 mM Na+ in the same preparation as described previously (18, 27, 30). As shown qualitatively in Fig. 5, A and B, and quantitatively in Fig. 5C, FBS removal changed the external Na+ activation kinetics of Na+/H+ exchange. The Kt (Na+) was lower in FBS-deprived cells than control cells (6 versus 21 mM, respectively), but this decrease in Kt (Na+) cannot explain the observed reduction in Na+/H+ exchange after serum deprivation. Note that the Vmax in absolute H+ efflux units cannot be determined directly from the plot in Fig. 5C because the data is normalized to the alkalization rate at 138 mM Na+. However, because the rate of Na+/H+ exchange at 138 mM Na+ is known from Fig. 4, the Vmax in FBS-deprived cells can be calculated to be 43% of that in control cells (0.0013 pH units/s and 0.003 pH units/s, respectively). The reduction in Vmax is the kinetic observation which explains the effect of FBS deprivation.

The reduction in Kt (Na+) when Caco-2 cells are deprived of FBS could be explained by the presence of multiple iso-
forms of Na+/H+ exchanger molecules which alter their relative functional importance when cells are FBS-deprived. Since Na+/H+ exchangers with higher (15) or lower affinity (20) for amiloride have been identified, the amiloride IC50 of Caco-2 Na+/H+ exchange was measured after FBS deprivation (18). As shown in Fig. 6, the IC50 was 50 μM in FBS-deprived cells, similar to the value of 28 μM previously measured in cells which had not been FBS-deprived (18). Based on these results there is no evidence to suggest the appearance of a new population of Na+/H+ exchangers with different sensitivity to amiloride.

In a further attempt to resolve heterogeneity of Na+/H+ exchange activity in Caco-2 cells, it was hypothesized that there could be different isoforms of Na+/H+ exchange in separate cell subpopulations after FBS deprivation, and/or that only some cells have reduced Na+/H+ exchange in response to FBS deprivation. To evaluate heterogeneity in cellular expression of Na+/H+ exchange after FBS deprivation, pH recovery of FBS-deprived cells was determined using a microscope-based image analysis system, as described (18). A 34,000-μm2 field of Caco-2 cells (approximately 600 cells) was studied. Following NH4Cl acid loading, the mean pH, over the field was 6.84 with a coefficient of variation of 53%. After allowing partial pH recovery (9-min exposure to 138 mM NaCl) there was no significant change in the broadness of the population distribution (mean pH, = 7.04 with a coefficient of variation of 50%), implying that all cells recovered at the same rate. If cells had varying numbers (or types) of Na+/H+ exchangers, it is predicted that the broadness of the pH, distribution should widen in the total population (31). The constancy in the coefficient of variation suggests that the Na+/H+ exchange activity is homogenous in FBS-deprived Caco-2 cells with no evidence of a subpopulation of cells with different transport rates.

**Effect of Protein Synthesis Inhibition on the Regulation of Na+/H+ Exchange Activity by FBS**—Cycloheximide was used to determine whether protein synthesis was required to observe the changes in Na+/H+ exchange caused by FBS deprivation or replacement. The efficacy of cycloheximide was determined by measuring the inhibition of [3H]leucine incorporation into protein (Fig. 7A). To determine the time course of cycloheximide action, [3H]leucine incorporation was measured after a 1.5-4 h exposure to 5 μM cycloheximide and compared with control cells which had not been exposed to cycloheximide. As shown in Fig. 7B, extrapolation of results from cycloheximide-treated cells back to an intersection with the control data gives an estimate for the time of onset of cycloheximide action of 7 min. These data demonstrate that exposure of Caco-2 cells to 5 μM cycloheximide rapidly inhibits protein synthesis by greater than 80%.

Exposure of Caco-2 cells to 5 μM cycloheximide for 4 h in the presence of 10% FBS had no effect on Na+/H+ exchange (Fig. 7C), nor did cycloheximide alter the buffering capacity of the cells (data not shown). Cycloheximide did not alter the reduction in Na+/H+ exchange activity which followed 4-h FBS deprivation, suggesting that the reduction in Na+/H+ exchange was not due to the synthesis of a protein inhibitor of Na+/H+ exchange. As shown in Fig. 7C, cycloheximide also did not inhibit the restoration of Na+/H+ exchange when Caco-2 cells are re-exposed to 10% FBS for 4 h. Similar results were obtained when 10 μg/ml actinomycin D was used (data not shown). These latter data suggest that the restoration of Na+/H+ exchange activity is not due to the synthesis of new proteins, including Na+/H+ exchanger molecules. The experiments with cycloheximide suggest that the effect of FBS on Na+/H+ exchange is at a posttranslational level.

**Effect of Low Temperature on the Regulation of Na+/H+ Exchange by FBS**—To test whether the effect of FBS requires intracellular transport of membrane proteins, the effect of 4-h FBS deprivation was studied at 13 °C, a temperature known to slow intracellular membrane traffic (32–34). When cells were FBS-deprived at 13 °C for 4 h and then Na+/H+ exchange measured at 37 °C, the reduction in Na+/H+ exchange
activity was prevented (Fig. 8). Furthermore, when the cells were 4-h FBS-deprived at 37 °C and then exposed to 10% FBS at 37 °C, Na+/H+ exchange was not restored but instead remained at levels found after 4-h FBS deprivation (Fig. 8). These data, together with the kinetic data that the \( V_{\text{max}} \) of the exchanger is reduced, suggest that the number of functional exchanger molecules in the plasma membrane may be reduced after FBS deprivation.

**Effect of Cytochalasin D and Colchicine on the Regulation of Na+/H+ Exchange by FBS**—To obtain further evidence that FBS deprivation might alter the number of functional Na+/H+ exchanger molecules in the plasma membrane, the effects of cytochalasin D and colchicine were studied. Cytochalasin D is known to disrupt actin microfilaments (35-37) and previously has been used to inhibit plasma membrane cycling events (38, 39). As shown in Fig. 9, 20 \( \mu \)M cytochalasin D blocked the reduction in Na+/H+ exchange activity normally caused by 4-h FBS deprivation, suggesting that polymerized F-actin is required for this process. Since nonselective effects of a high cytochalasin D concentration could also explain this observation, experiments were performed using lower concentrations of drug, and transport rates compared at a similar pH (pH = 6.70 ± 0.01, n = 16). Cells deprived of serum for 4 h in the presence of either 0.5 or 1.0 \( \mu \)M cytochalasin D had transport rates which were, respectively, 119 ± 22% (n = 5) and 88 ± 11% (n = 5) of the transport rates observed when cells were kept in FBS. Since FBS deprivation (without drug) was able to lower transport to 35 ± 5% (n = 3) of the same control in the same experimental series, it is clear that low concentrations of cytochalasin D block the effects of FBS deprivation.

In contrast, incubation of FBS-deprived cells with 10% FBS for 4 h in the presence of 20 \( \mu \)M cytochalasin D did not inhibit restoration of Na+/H+ exchange activity; in fact rates were slightly above control values (Fig. 9). The latter observation suggests a dynamic equilibrium between a cytochalasin D-sensitive process which reduces the number of Na+/H+ exchange proteins in the plasma membrane and a separate cytochalasin D-insensitive process which restores Na+/H+ exchange to the plasma membrane. To test whether such a dynamic equilibrium is present in cells which have not been FBS-deprived, Na+/H+ exchange activity was measured in normal cells which had been incubated with 20 \( \mu \)M cytochalasin D for 4 h. Under these conditions there was no change in Na+/H+ exchange activity (Fig. 9).

The effect of 20 \( \mu \)M colchicine (which disrupts microtubules) was also studied (40-43). Colchicine blocked neither the reduction of Na+/H+ exchange activity following FBS deprivation nor the restoration of Na+/H+ activity following 4-h FBS readdition (Fig. 9) suggesting that microtubules do not play a role in this regulation of Caco-2 Na+/H+ exchange.

**DISCUSSION**

This study demonstrates that FBS deprivation decreases Caco-2 Na+/H+ exchange, causing a 60% reduction in the \( V_{\text{max}} \) of the exchanger and a reduction of \( K_{i} \) (Na+) through a mechanism that takes hours to manifest or reverse. This process is distinct from the commonly observed stimulatory effects of serum on Na+/H+ exchange in both time course and kinetic characteristics (1, 4-6). The effect of serum on Caco-2 cells most closely follows a pattern of slow regulation previously found in renal proximal tubular cells in response to thyroxine, glucocorticoids, and acidosis (12-14). The response to these hormones requires hours and has been characterized by changes in the \( V_{\text{max}} \) without changes in the pH sensitivity or the \( K_{i} \) for external Na+ (12-14).

The observed decrease in \( K_{i} \) (Na+) in FBS-deprived cells could have been due to a change in the relative abundance of multiple subtypes of Na+/H+ exchanger proteins. Our studies provided no corroborating evidence for the presence of kinetically distinct isomers of Na+/H+ exchangers in Caco-2 cells. Evidence suggests that in epithelial cells significant differences exist between apical and basolateral Na+/H+ exchangers with respect to amiloride sensitivity and pH sensitivity (15, 16, 27, 30). However, no changes in these parameters were induced by FBS deprivation. Similarly, there was no measurable heterogeneity of transport among individual cells in Caco-2 monolayers as there was no change in the population distribution of Na+/H+ exchange rates. Alternative explanations for the alteration in \( K_{i} \) (Na+) include a change in the
function of remaining Na\(^+\)/H\(^+\) transporters after FBS removal, perhaps due to changes in membrane lipid composition or cytoskeletal structure (see below).

The process affected by FBS deprivation is unknown. However, the data are most consistent with a model in which the number of functional exchange proteins in plasma membrane is reduced when the cells are FBS-deprived. This is based on three lines of evidence. First, the changes in activity are due to changes in the $V_{max}$ of the exchanger, without requiring protein synthesis of either new Na\(^+\)/H\(^+\) exchange proteins or associated inhibitory molecules. Second, incubating the cells at 13 °C blocks the effects of FBS deprivation and FBS reintroduction (Fig. 8). Incubation of cells at low temperature (<20 °C) has been shown to inhibit a number of membrane fusion events including endocytosis (32), fusion of pinocytic vesicles with lysosomes, and exocytosis (33, 34). Third, the actin-disrupting agent, cytochalasin D (35–37), inhibits the reduction in Na\(^+\)/H\(^+\) exchange activity after FBS deprivation, but does not affect restoration of transport after readdition of FBS. Since colchicine had no effect during either FBS deprivation or reintroduction, the data implicates a role for F-actin in the regulation of Na\(^+\)/H\(^+\) exchange proteins which modulate the activity of Na\(^+\)/H\(^+\) exchange proteins in the plasma membrane in a regulatory compartment. The observation that cytochalasin D does not alter the restoration process (Fig. 9) argues against a modulation of function via reversible interaction of F-actin filaments with plasma membrane Na\(^+\)/H\(^+\) exchange proteins or associated molecules. The differential sensitivity of Na\(^+\)/H\(^+\) exchange reduction and restoration to disruption of actin microfilaments is more consistent with an F-actin-dependent activity.

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