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Modulation of rat distal colonic brush-border membrane Na^+–H^+ exchange by dexamethasone: role of lipid fluidity

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Earlier studies by our laboratory have suggested a relationship between an amiloride-sensitive Na^+–H^+ exchange process and the physical state of the lipids of rat colonic brush-border membrane vesicles. To further assess this possible relationship, a series of experiments were performed to examine the effect of dexamethasone administration (100 μg/100 g body wt. per day) subcutaneously for 4 days on Na^+–H^+ exchange, lipid composition and lipid fluidity of rat distal colonic brush-border membrane vesicles. The results of these studies demonstrate that dexamethasone treatment significantly: (1) increased the V_{max} of the Na^+–H^+ exchange without altering the K_m for sodium of this exchange process, utilizing the fluorescent pH-sensitive dye, acridine orange. ^{22}Na flux experiments also demonstrated an increase in amiloride-sensitive proton-stimulated sodium influx across dexamethasone-treated brush-border membrane vesicles; (2) increased the lipid fluidity of treated-membrane vesicles compared to their control counterparts, as assessed by steady-state fluorescence polarization techniques using three different lipid-soluble fluorophores; and (3) increased the phospholipid content of treated-membrane vesicles thereby, decreasing the cholesterol/phospholipid molar ratio of treated compared to control preparations. This data, therefore, demonstrates that dexamethasone administration can modulate amiloride-sensitive Na^+–H^+ exchange in rat colonic distal brush-border membrane vesicles. Moreover, it adds support to the contention that a direct relationship exists between Na^+–H^+ exchange activity and the physical state of the lipids of rat colonic apical plasma membranes.

* Data supplementary to this article are deposited with, and can be obtained from Elsevier Science Publishers BV (Biomedical Division), BBA Data Deposition, P.O. Box 1345, 1000 BH Amsterdam, The Netherlands. Reference should be made to No.: BBA/DD/381/73792/905 (1987) 485. The supplementary information includes: Two tables of the compositional analysis of neutral phospholipids as well as the total fatty acids of distal colonic brush-border membranes of control and dexamethasone-treated rats.

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Introduction

The mammalian large intestine is important in the maintenance of normal electrolyte and water balance [1]. In vivo, the colon absorbs sodium, chloride and water and secretes potassium and bicarbonate [1]. While the action of mineralocorticoids on electrolyte transport in the large intestine has been well established [2–4], until recently, glucocorticoids were not considered to have an effect on colonic sodium absorption. Recent

studies, however, have demonstrated that these latter steroids are also important in mediating cation transport in this organ [5–7]. For example, pharmacological doses of dexamethasone and/or methylprednisolone have been shown to increase the net uptake of sodium and water as well as influence the secretion of potassium in the colon [5–7]. In these studies, glucocorticoids stimulated electrogenic sodium absorption, as increases in sodium absorption were accompanied by a rise in transmural potential differences [6,7].

In the rat colon, although electrogenic sodium absorption is present, the predominant sodium absorptive process appears to be electroneutral sodium transport [7]. Recent studies from our laboratory [8–11] and others [12] have, in fact, demonstrated the presence of an amiloride-sensitive, electroneutral Na^+–H^+ exchange process in rat colonic brush-border membrane vesicles. This transport system possesses a number of characteristics similar to Na^+–H^+ exchangers previously described in other plasma membranes [13,14]. In this regard, dexamethasone has been shown to modulate Na^+–H^+ exchange activity in rat kidney cortex brush-border membrane vesicles [15,16]. To date, similar studies of the effect of dexamethasone on Na^+–H^+ exchange in the mammalian colon have not been undertaken.

Prior studies from our laboratory have also suggested a direct correlation between lipid fluidity * and the Na^+–H^+ exchange process in rat colonic brush-border membrane vesicles [9–11]. Since dexamethasone has previously been shown to influence membrane lipid fluidity in rat small intestinal and hepatic membranes [17,18], it was of interest to examine the effect of this steroid not only on Na^+–H^+ exchange process but also on the lipid fluidity of rat colonic brush-border membrane vesicles.

The results described below demonstrate that dexamethasone increased the Na^+–H^+ exchange activity and the lipid fluidity of rat distal colonic brush-border membrane vesicles. The increase in lipid fluidity was due, at least in part, to a decrease in the cholesterol/phospholipid molar ratio, secondary to a higher phospholipid content in membranes prepared from dexamethasone-treated rats. These findings serve as a basis for the present report.

Experimental procedures

Materials

Dexamethasone was obtained from Sigma Chemical Company. DL-2-(9-anthroyl)stearic acid and DL-12-(9-anthroyl)stearic acid were purchased from Molecular Probes (Eugene, OR). 1,6-Diphenyl-1,3,5-hexatriene was obtained from Aldrich Chemical Company (Milwaukee, WI). Fatty acid methyl esters, GLC columns and lipid standards were all purchased from Applied Science Corp. (State College, PA) and/or Supelco (Bellefonte, PA). All other chemicals were obtained from either Fisher Chemical Company (Fairlawn, NJ) or Sigma Chemical Company (St. Louis, MO), unless otherwise indicated.

Animals and steroid administration

Male Sprague-Dawley rats (250–300 g) were fed Purina rat chow pellets ad libitum. The experimental and control groups received daily subcutaneous (s.c.) injections (100 μg/100 g body weight) of dexamethasone in 0.9% saline or diluent, respectively, for 4 days. The final injection was given to rats 2 h before killing on the fourth day.

Preparation of colonic brush-border membrane vesicles

Rats were fasted 18 h with water ad libitum before being killed. The distal halves of the colons were excised and epithelial cells, relatively devoid of goblet cells, were then obtained using a technique which combined chelation of divalent cations with mild mechanical dissociation [19]. Brush-border membrane vesicles were then prepared from these cells as described by Brasitus

* The term 'lipid fluidity' as applied to anisotropic bilayer membranes is used to represent the relative motional freedom of the lipid molecules or substituents thereof. A more detailed description of the sense in which the term is used is given in Ref. 33. Briefly, as evaluated by steady-state fluorescence polarization of lipid fluorophores, fluidity is assessed via the parameters of the modified Perrin equation described under Experimental Procedures. An increase in fluidity corresponds to a decrease in either the correlation time, T_c, or the hindered anisotropy, r_∞, of the fluorophore. Hence, the term combines the concepts of the 'dynamic' and 'static' (lipid order) components of fluidity.

and Keresztes [19]. The purity of the membrane suspensions and the degree of contamination with intracellular organelles were assessed by appropriate marker enzymes. The specific activity ratios [(purified apical membrane)/(crude homogenate)] for the brush-border enzyme markers, total alkaline phosphatase (*p*-nitrophenylphosphatase) and cysteine-sensitive alkaline phosphatase, ranged from 15 to 20 in all membrane preparations. Moreover, the specific activity value for these brush-border membrane markers in the homogenates and final pellets were not found to be significantly different in treated and control preparations (not shown). The corresponding values for succinate dehydrogenase, NADPH–cytochrome-*c* reductase and sodium-potassium-dependent adenosine triphosphatase, marker enzymes for mitochondrial, microsomal and basolateral membranes, respectively, ranged from 0.50 to 1.50 in each of these membrane preparations [19]. Protein was estimated by the method of Lowry et al. [20]. Liposomes were prepared from the extracted lipids of each membrane as previously described [21].

Na^+–H^+ exchange studies

Fluorescence dye experiments. Amiloride-sensitive-sodium-stimulated proton efflux was measured in colonic brush-border membrane vesicles, utilizing the pH-sensitive dye, acridine orange, as previously described by our laboratory [8–11] and others [22–24]. A Perkin-Elmer 650-40 spectrofluorometer (Perkin Elmer Corp., Norwalk, CT) (excitation 493 nm, emission 530 nm) equipped with a thermostated cuvette, stirring system and adding port was used in all experiments.

The assay solution contained 6 μM acridine orange, 250 mM sucrose, 100 mM *N*-methylglucamine gluconate and 10 mM Tris-Hepes, (pH 7.5). After 2 ml of this buffer reached steady-state fluorescence (approx. 90 s), 50 μl of colonic brush-border membrane vesicles (100–150 μg protein), preloaded with 250 mM *N*-methylglucamine gluconate and 10 mM Tris-Hepes (pH 6.0) were added. As previously reported [22–24], there was a 30–40% quenching in acridine orange fluorescence signal which reached equilibrium within 2 min. Sufficient amounts of sodium gluconate solution were then added with constant stirring to achieve a final concentration of 5–50 mM in the assay medium. The addition of sodium resulted in a collapse of the outwardly directed H^+ gradient and a reappearance of acridine orange fluorescence [22]. The increase in fluorescence was linear for at least 2 s and the initial rate of acridine orange fluorescence reappearance was measured as the initial slope. After 300 s the pH gradient was dissipated with 150 mM potassium gluconate and 10 μg nigericin as described [10]. The small fluorescence quenching still remaining after nigericin addition was due to binding of the dye to the membranes [11]. Appropriate corrections were made for this binding as described by Burnham et al. [25]. All the experiments were performed at 26 °C. Care was exercised to maintain a constant temperature and pH which are known to influence the reproducibility of these results [22].

^{22}Na flux experiments. Brush-border membrane vesicles were prepared as described above and the final pellet was resuspended and washed twice according to the method of Freiberg et al. [15]. All preparations were initially washed in 300 mM mannitol, 5 mM Tris-Hepes (pH 7.5). In studies where the pH of the intracellular medium was 7.5, a second washing with 144 mM KCl, 5 mM Mes, 13 mM Tris, 13 mM Hepes (pH 7.5) was used and the final pellet brought up in the same buffer. In experiments where the pH of the intravesicular medium was pH 5.5, the second washing solution containing 150 mM KCl, 25 mM Mes, 4.6 mM Tris (pH 5.5).

Uptake of ^{22}Na was measured at 26 °C by a Millipore filter technique as described by Murer et al. [26]. The incubation medium contained 144 mM KCl, 5 mM Mes, 13 mM Hepes, 13 mM Tris, 1 mM NaCl (pH 7.5), ±1 mM amiloride. The experiment was started by the addition of 160 μl of the incubation media containing 1–2 μCi of ^{22}Na to 40 μl of the membrane suspension. After designated periods of time (1 min and 180 min), the reaction was terminated by the addition of 5 ml of ice-cold stop solution containing 150 mM LiCl, 16 mM Hepes, 10 mM Tris (pH 7.5). The diluted sample was immediately filtered through a 0.45 μm Millipore filter (HAWP), and the filter was washed three times with 5 ml of cold stopping solution. Filters were dissolved in scintillation fluid, and the radioactivity was measured in a

Beckman LS-6800 scintillation counter [9]. Each experiment was performed in triplicate on three separate preparations.

Fluorescence polarization studies

Three different fluorophores were used: 1,6-diphenyl-1,3,5-hexatriene (DPH), DL-2-(9-anthroyl) stearic acid (2-AS), and DL-12-(9-anthroyl)stearic acid (12-AS). Steady-state fluorescence polarization studies were performed with a Perkin-Elmer 650-40 spectrofluorometer adapted for fluorescence polarization. The methods used to load the membranes and liposomes and the quantification of the polarization of fluorescence have been described [21,27,28]. The content of each fluorophore in the membranes and liposomes was determined fluorometrically as described by Cogan and Schachter [29]. Final molar ratios of probe/lipid ranged from 0.002 to 0.003 and the anisotropy differences observed in these studies could not be ascribed to differences in probe concentrations in the membranes and/or liposomes. Correction for light scattering was also routinely made as previously described [21,27,28].

The results were obtained according to modified Perrin relationship [30,31]:

$$r = r_\infty + (r_0 - r_\infty)[T_c/(T_c + T_f)]$$

where r is the fluorescence anisotropy; r_0 is the maximal limiting anisotropy taken as 0.365 for DPH [32] and 0.285 for the anthroyloxy probes [33]; r_∞ is the limiting hindered anisotropy; T_c is the correlation time; and T_f is the mean lifetime of the excited-state. The lifetime, T_f, was estimated by phase fluorimetry at 30 MHz [34,35] in an SLM 4800 subnanosecond polarization spectrophotometer (SLM-Aminco, Urbana, IL) as described [36,37]. Values of r_∞ for DPH were calculated from r values as previously described by Van Blitterswijk et al. [38]. The 'static component' of membrane fluidity was assessed by an order parameter, S, where $S = (r_\infty/r_0)^{1/2}$, as described previously [30,31,38].

Lipid composition studies

Total lipids from colonic brush-border membranes were extracted by the method of Folch et al. [39]. Cholesterol [40] and phospholipids [41] were measured as previously described. The neutral and phospholipid compositions of the extracts were further analyzed by thin-layer chromatography as described by Katz et al. [42] and Schwarz et al. [43], respectively. Fatty acids of the total lipid extracts were derivatized and the fatty acid methyl esters analyzed on a Hewlett-Packard 5790A gas-liquid chromatography equipped with a flame ionization detector as previously described by Gartner and Vahouny [44], using authentic fatty acid methyl ester standards to identify retention times.

Statistical methods

Values were expressed as means ± S.E. Paired or unpaired t-tests were used for all statistical analysis. A P value < 0.05 was considered significant.

Results

Effect of dexamethasone treatment on Na^+–H^+ exchange

Using an acridine orange fluorescence technique, sodium-stimulated proton efflux was measured in colonic brush-border membrane vesicles prepared from control and dexamethasone-treated rats. Kinetic studies using various concentrations of sodium demonstrated saturation kinetics in both control and treated preparations. This exchange process was inhibited by approx. 85% by amiloride (1 mM) (not shown). As assessed by alterations in the initial slope of fluorescence, dexamethasone treatment significantly increased sodium-stimulated proton efflux at all concentrations of sodium tested (5.0–50 mM) (not shown). The values for V_{max} (expressed in arbitrary fluorescence units) and K_m for sodium were then obtained from Hofstee plots [45] (Fig. 1), and this data is presented in Table I. Dexamethasone treatment significantly increased the V_{max} of this process (approx. 30%). Dexamethasone treatment, however, did not significantly alter the appearance affinity (K_m) for sodium of this exchange process (Table I).

In agreement with the acridine orange studies noted above, amiloride-sensitive, pH-stimulated sodium influx using ^{22}Na at 1 min was significantly higher in the dexamethasone-treated rat

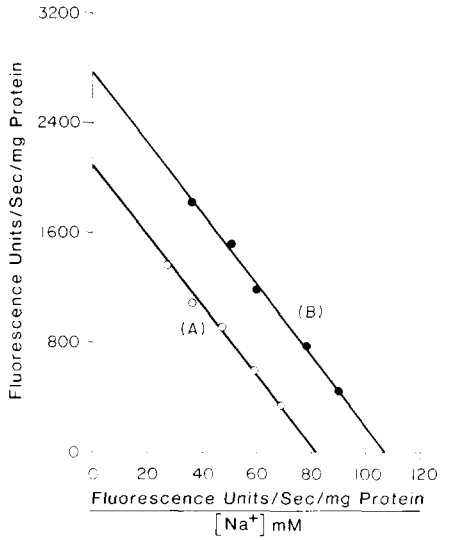

fig. 1. Amiloride-sensitive sodium-stimulated proton efflux was measured in rat colonic brush-border membrane vesicles, using the pH-sensitive fluorescent dye, acridine orange, as described under Experimental Procedures. Representative Hofstee plots of six separate experiments, using colonic membranes prepared from control (A) and dexamethasone-treated (B) animals, are shown.

colonic brush-border membrane vesicles (1.70 ± 0.08 nmol/mg protein, $N = 3$) than in control membrane vesicles (1.37 ± 0.04, $N = 3$) ($P < 0.05$). Accumulations of Na^+ after 180 min of incubation, at a time when both Na^+ and H^+ gradients were dissipated, were, however, similar in the vesicles of both groups (control, 0.86 ± 0.10; dexamethasone-treated, 0.66 ± 0.12 nmol/mg protein) ($P > 0.05$). As previously discussed [46], the latter finding indicates that the average intravesicular volumes of vesicles isolated from control and treated animals were comparable, suggesting that the difference in the rates of uptake seen using acridine orange and ^{22}Na techniques (see above) were not due to alterations in vesicle size. Since similar aliquots of membrane protein were used in the transport experiments, this also rules out changes in vesicular number as being responsible for the Na^+–H^+ exchange differences seen in the present studies between control and treated-preparations.

Fluorescence polarization studies

As shown in Table II and Table III, the lipid fluidity of colonic brush-border membranes of rats treated with dexamethasone for 4 days was significantly higher than control membranes, as assessed by steady-state fluorescence polarization techniques using all three fluorophores. As previously discussed [37], these fluorophores differ in a number of their characteristics. In biological and artificial membranes, the structural organization of the lipid bilayer appears to limit the extent of rotation of DPH; therefore, r_∞ values for this probe are high and largely determine r [38]. 2-AS and 12-AS yield relatively low values of r_∞ in bilayer membranes, and their r values reflect mainly T_c, i.e., speed of rotation [32,47].

In the present studies, both the 'static' and 'dynamic' component of membrane fluidity, as assessed by r_∞ and 'S' values of DPH (Table II) and r values of 2-AS and 12-AS (Table III), respectively, were found to be increased in the membranes prepared from dexamethasone-treated rats. Differences in fluidity were also detected in liposomes of colonic membranes prepared from dexamethasone-treated rats compared to controls using DPH (Table II).

In order to ascertain that the fluidity differences observed in the present studies were not secondary to differences in lifetimes of the excited state of the fluorophores, the mean lifetimes of the excited-state of DPH, 2-AS and 12-AS were de-

TABLE I

EFFECT OF DEXAMETHASONE ON KINETIC PARAMETERS OF AMILORIDE-SENSITIVE SODIUM-STIMULATED PROTON EFFLUX OF RAT DISTAL COLONIC BRUSH-BORDER MEMBRANES

Parameters	N	Control (mean)	Dexamethasone (mean)	Mean ± S.E. of differences	P
K_m	6	25.1	27.3	2.9 ± 1.6	> 0.05
V_{max} (fluorescence units/s per mg protein)	6	2045	2633	587 ± 82	< 0.001

TABLE II

EFFECT OF DEXAMETHASONE ON THE FLUIDITY OF RAT DISTAL COLONIC BRUSH-BORDER MEMBRANES AND THEIR LIPOSOMES USING DPH AT 25 °C

Preparations	Fluidity parameters	N	Control (mean)	Dexamethasone (mean)	Mean ± S.E. differences	P
Intact membranes	r	11	0.245	0.238	0.007 ± 0.002	< 0.05
	r_∞	11	0.227	0.218	0.009 ± 0.003	< 0.05
	S	11	0.792	0.771	0.016 ± 0.005	< 0.05
Liposomes	r	6	0.227	0.200	0.027 ± 0.008	< 0.05
	r_∞	6	0.202	0.166	0.036 ± 0.011	< 0.05
	S	6	0.744	0.672	0.072 ± 0.023	< 0.05

termined in all preparations. There were no significant differences observed in the lifetimes of these fluorophores in preparations prepared from control and dexamethasone-treated rats (data not shown).

TABLE III

EFFECT OF DEXAMETHASONE ON THE FLUIDITY OF RAT DISTAL COLONIC BRUSH-BORDER MEMBRANES USING 2-AS AND 12-AS

Values are means ± S.E. of 6–8 separate preparations.

Probe	Preparations	r at 25 °C
2-AS	control	0.168 ± 0.003
	dexamethasone	0.149 ± 0.009 *
12-AS	control	0.110 ± 0.003
	dexamethasone	0.094 ± 0.008 *

* $P < 0.05$ or less compared to controls.

Lipid composition studies

Prior studies in model bilayers and natural membranes have correlated a high lipid fluidity with lower molar ratios of cholesterol/phospholipids and sphingomyelin/phosphatidylcholine [33,48–50]. More unsaturated or shorter fatty acyl chains in phospholipids have also been associated with a higher membrane lipid fluidity [48]. It was, therefore, of interest to examine these parameters in control and treated-rats (Table IV). Membranes prepared from dexamethasone-treated animals were found to possess a higher phospholipid content (Table IV) and a lower molar ratio of cholesterol/phospholipid than membranes of control animals (Table IV). The other parameters including the saturation indices and the sphingomyelin/phosphatidylcholine molar ratios were not significantly different in these membranes (Table IV). No significant differences

TABLE IV

COMPOSITIONAL ANALYSIS OF LIPID EXTRACTS OF BRUSH-BORDER MEMBRANES OF RAT DISTAL COLONOCYTES FROM CONTROL AND DEXAMETHASONE-TREATED RATS

Values are mean ± S.E. of 4–5 separate preparations.

Parameter	Control	Dexamethasone
Cholesterol/phospholipid (mol/mol) [c]	0.92 ± 0.08	0.71 ± 0.07 *
Sphingomyelin/PC (mol/mol) [c]	0.39 ± 0.08	0.47 ± 0.05
Saturation index [a]	0.36 ± 0.02	0.37 ± 0.04
Double-bond index [b]	1.21 ± 0.04	1.20 ± 0.07
Cholesterol/protein (w/w)	0.15 ± 0.02	0.15 ± 0.02
Phospholipid/protein (w/w)	0.26 ± 0.03	0.36 ± 0.02 *

[a] Number of saturated acyl chains divided by sum of each unsaturated chain multiplied by the number of double bonds.
[b] Sum of each unsaturated chain multiplied by the number of double bonds/100.
[c] Calculated from raw data.

were observed in relative percentages of individual lipid species, as analyzed by TLC, in membranes of control and treated-animals (not shown) *. Additionally, compositional analysis of total fatty acids by GLC revealed no significant differences in treated-membranes compared to their control counterparts (not shown) *.

Discussion

In agreement with prior studies in rat kidney [15,16], the present studies demonstrate for the first time that dexamethasone administration increased the Na^+–H^+ exchange activity of rat distal colonic brush-border membrane vesicles. Concomitant with this increase in Na^+–H^+ exchange, the lipid fluidity of these membranes was also increased. Dexamethasone increased both the 'dynamic' as well as the 'static' components of membrane lipid fluidity. The increase in lipid fluidity of these treated membranes appeared to be due, at least partially, to an increased phospholipid content, resulting in a decreased cholesterol/phospholipid molar ratio.

Accumulated evidence [9–11,15,16,46,51–53] supports the existence of regulatory mechanism(s) for Na^+–H^+ exchange in several epithelia. For example, Na^+–H^+ exchange activity in rat and/or rabbit renal brush-border membrane vesicles has been shown to be modulated by such in vivo perturbations as: (1) hypo- and hyperthyroidism [46], (2) glucocorticoid administration [15,16,52] and (3) metabolic acidosis [52,53]. Studies in rat renal brush-border membrane vesicles have also demonstrated the presence of a nontransporting H^+ modifier site in its Na^+–H^+ exchanger [51]. In this regard, recent studies in our laboratory, using benzyl alcohol [9] and *S*-adenosyl-L-methionine-mediated transmethylation of phospholipids and/or proteins [10] to fluidize colonic brush-border membrane vesicles, have suggested a parallel relationship between membrane vesicular fluidity and Na^+–H^+ exchange activity in these membranes. Increases in fluidity were associated with increases in the V_{max} without influencing the apparent affinity (K_m) for sodium of this process [9,10]. Similarly, a decrease in colonic brush-border membrane lipid fluidity in estradiol-treated rats was associated with a decrease in V_{max} of Na^+–H^+ exchange without influencing the K_m for sodium of this process [11]. The present findings, therefore, further support the contention that membrane fluidity may also play a role in modulation of the Na^+–H^+ exchange process in these membranes.

Previous studies in a number of different cell types have shown that mineralocorticoids and glucocorticoids can influence the plasma membrane lipid composition of these cells [54–57]. In the present studies, dexamethasone administration produced an increased total phospholipid content in rat distal colonic brush-border membrane vesicles resulting in a decreased cholesterol/phospholipid molar ratio. In this regard, prior studies have shown that corticosteroid administration resulted in an increase in total phospholipid content of rat and rabbit lung surfactants [57–59]. In the latter studies, increases in phosphatidylcholine content in lung surfactant lipids appeared to be due to increases in the specific activity of choline-phosphate cytidyltransferase. In the present studies, this enzymatic activity was not examined since the relative percentage of phosphatidylcholine was not increased in dexamethasone-treated membranes. Further studies will, therefore, be required to elucidate the mechanisms involved in the production of the lipid alterations of rat colonic brush-border membranes (noted in animals). Regardless of the mechanism(s) involved in the latter membrane phenomenon, however, the present observations, taken together with our previous studies [9–11], suggest that dexamethasone administration increased membrane lipid fluidity which, in turn, may result in an increase in Na^+–H^+ exchange activity.

It should be noted, however, that the present studies do not rule out other possibilities. Dexamethasone could directly influence the Na^+–H^+ exchanger, increase the number of exchangers per unit membrane area, or alter the initial rate of the exchanger process by influencing sodium and/or proton permeability of these membrane vesicles. Alternatively, dexamethasone may also influence Na^+–H^+ exchange in colonic brush-border membranes by altering the sensitiv-

* See footnote on p. 485.

ity of a possible nontransporting H^+ modifier site. The existence of such a modifier site, however, has not yet been established in the Na^+–H^+ exchanger of colonic apical membranes.

Prior studies by Freiberg et al. [15] in rat renal proximal tubule brush-border membranes have demonstrated that the glucocorticoid dexamethasone, but not the mineralocorticoid aldosterone, increased amiloride-sensitive Na^+–H^+ exchange whereas, Na^+ uptake, independent of amiloride, was not changed. Moreover, dexamethasone was found to decrease Na^+ gradient-dependent phosphate uptake and not affect Na^+ gradient-dependent glucose uptake [15], a further indication of the specificity of glucocorticoid action.

In this regard, prior in vitro studies in the distal segment of the rat large intestine have also shown that i.p. administration of dexamethasone for 3 days at much higher doses (600 μg/100 g body wt. per day) than used in the present experiments, stimulated electrogenic sodium absorption but abolished potassium absorption [7]. Whether the latter alterations in transport can also be detected using the present regimen of dexamethasone administration (see Experimental Procedures) remains to be determined as does the possible role of fluidity in modulating any of these other colonic transport processes. In the present studies, however, dexamethasone treatment was not found to change the activities of either alkaline phosphatase or cysteine-sensitive alkaline phosphatase in distal colonic membrane preparations. This is of interest, since previous studies by our laboratory have shown that both of these brush-border membrane marker enzymes can be increased by increases in the lipid fluidity of these colonic plasma membrane [19]. This would indicate that the fluidity alterations induced by dexamethasone in the present studies may involve particular 'microdomains' of colonic membrane lipid. Evidence for such microdomains in membranes using fluorescent probes such as DPH have previously been published by our laboratory [60–62] and others [63,64]. Taken together, these observations suggest that the fluidity alterations seen in these experiments after treatment with dexamethasone may be specific for modulation of the Na^+–H^+ exchanger in rat distal colonic brush-border membranes. Further experiments will be necessary, however, to clarify this issue as well as to determine the mechanism(s) whereby alterations in fluidity modify Na^+–H^+ exchange in these colonic plasma membrane.

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