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Relationships Among Sodium Current, Permeability, and Na Activities in Control and Glucocorticoid-Stimulated Rabbit Descending Colon

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Summary. Effects of a potent synthetic glucocorticoid, methylprednisolone (MP), on transepithelial Na transport were examined in rabbit descending colon. Current-voltage (*I-V*) relations of the amiloride-sensitive apical Na entry pathway were measured in colonic tissues of control and MP-treated (40 mg im for 2 days) animals. Tissues were bathed mucosally by solutions of various Na activities, $(Na)_m$, ranging from 6.2 to 75.6 mM, and serosally by a high K solution. These *I-V* relations conformed to the "constant field" flux equation permitting determination of the permeability of the apical membrane to Na, P^m_{Na}, and the intracellular Na activity, $(Na)_c$. The following empirical relations were observed for both control and MP-treated tissues: (i) Na transport increases hyperbolically with increasing $(Na)_m$ obeying simple Michaelis-Mentin kinetics; (ii) P^m_{Na} decreased hyperbolically with increasing $(Na)_m$, but was unrelated to individual variations in $(Na)_c$; (iii) $(Na)_c$ increased hyperbolically with $(Na)_m$; (iv) both spontaneous and steroid-stimulated variations in Na entry rate could be attributed entirely to parallel variations in P^m_{Na} at each mucosal Na activity. Comparison of these empirical, kinetic relations between control and MP-treated tissues revealed: (i) maximal Na current and P^m_{Na} were greater in MP tissues, but the $(Na)_m$'s at which current and P^m_{Na} were half-maximal were markedly reduced; (ii) $(Na)_c$ was significantly increased in MP tissues at each $(Na)_m$ while the $(Na)_m$ at half-maximal $(Na)_c$ was unchanged. These results provide direct evidence that glucocorticoids cause marked stimulation of Na absorption across rabbit colon primarily by increasing the Na permeability of the apical membrane. While the mechanism for the increased permeability remains to be determined, the altered relation between P^m_{Na} and $(Na)_m$ suggests possible differences in the conformation or environment of the Na channel in MP-treated tissues.

Key Words glucocorticoids · methylprednisolone · rabbit descending colon · sodium transport · current-voltage relations · permeability · cell Na activity · electrophysiology

Introduction

Both mineralocorticosteroid and glucocorticosteroid hormones are potent stimulators of electrolyte and fluid transport in a variety of epithelial tissues including the mammalial colon. The mechanisms for mineralocorticoid stimulation of Na absorption have been studied extensively. In tight epithelia mineralocorticoids increase both the permeability of the apical membrane to Na (Palmer et al., 1982) and the Na,K-ATPase activity in the basolateral membrane (Will et al., 1981; O'Neil & Hayhurst, 1985). The interrelation between these two effects has been controversial and remains the subject of much investigative effort; however, recent studies suggest that the primary effect of mineralocorticoids is to increase the apical membrane permeability.

Both glucocorticoids and mineralocorticoids enhance Na absorption in rabbit distal colon (Frizzell & Schultz, 1978; Sellin & Desoigne, 1985), a moderately tight epithelium with amiloride-inhibitable Na channels. While it has been previously considered that glucocorticoids may stimulate transport by cross-reacting with mineralocorticoid receptors, recent evidence demonstrates the involvement of distinctly different cytosolic receptors for these two classes of steroid in gastrointestinal epithelia (Bastl et al., 1984; Marver, 1984). Moreover, in some intestinal segments (e.g., small bowel), glucocorticoids serve as potent stimulators of ion transport whereas mineralocorticoids have little or no effect (Charney et al., 1975; Will et al., 1981; Sellin & Desoigne, 1983). The mechanisms of glucocorticoid stimulation of Na transport by distal colon have not been extensively studied. However, previous studies have demonstrated a glucocorticoid-induced increase in Na,K-ATPase activity (Charney et al., 1975; Will et al., 1981), basal rates of Na absorption (Bastl et al., 1980), and Na absorptive capacity (Sellin & Desoigne, 1985).

Glucocorticoid effects on the apical Na permeability have only recently been investigated (Thompson & Sellin, 1984; Clauss et al., 1985). In order to more clearly define the relations among

increased transepithelial Na transport, apical Na permeability and intracellular Na activity, we measured instantaneous current-voltage relations of the Na entry pathway at various mucosal Na activities in both control and glucocorticoid-treated, K-depolarized rabbit distal colon.

Our studies demonstrate that treatment with glucocorticoids results in large increases in the apical membrane Na permeability accompanied by small, but significant, increases in cell Na. This result is consistent with a primary effect of glucocorticoids on the apical entry step. At lower values of mucosal Na, steroid-treated tissues yielded proportionally greater increases in Na permeability than controls indicating that the "self-inhibitory" effect of mucosal Na on Na entry (Fuchs et al., 1977) is intact, but has been altered. This result can be interpreted in terms of a steroid-induced change in the properties of the apical Na channel.

Materials and Methods

New Zealand white rabbits (3 to 4 kg) were maintained "in house" on standard Purina rabbit chow and tap water *ad libitum* for at least four days prior to the experiment. Control animals were untreated. Experimentals were litter mates of controls and were injected with the glucocorticoid methylprednisolone (40 mg im × 2 days) [MP tissues]. Animals were sacrificed by cervical dislocation, the distal colon removed, opened along the mesenteric border to form a flat sheet and rinsed free of intestinal contents. The outer muscle layers were removed by blunt dissection prior to mounting in Ussing-type chambers (Frizzell et al., 1976).

Four tissues were initially bathed on both sides by one of four sulfate-containing salines having different Na activities. The standard electrolyte solution contained (mM): 140 Na, 5.4 K, 1.2 Ca, 1.2 Mg, 25 Cl, 48.9 SO_4, 22 HCO_3, 2.4 HPO_4, 0.6 H_2PO_4, 49 mannitol, and 10 dextrose. The solution osmolarity was 275 mOsm and pH was 7.4 when gassed with 95% O_2 and 5% CO_2 at 37°C. Solutions having lower Na concentrations were made by proportional mixing with a Na-free saline which was made as above except that choline bicarbonate (22 mM), TMA-Cl (20.2 mM) and TMA-OH (97.4 mM) replaced $NaHCO_3$, NaCl and Na_2SO_4, respectively, and the resulting solution, equilibrated with 95/5 gas, was titrated to pH = 7.4 with 1 N H_2SO_4. Final Na concentrations used were 11.5, 23.0, 46.0 and 140 mM. Using an activity coefficient for Na_2SO_4 of 0.54 (Fuchs et al., 1977) the calculated Na activities (Na) were 6.2, 12.4, 24.9 and 75.6 mM.

Approximately 50 min after mounting the tissue the serosal solution was exchanged for one containing (mM): 140 K, 1.2 Ca, 1.2 Mg, 25 Cl, 46.2 SO_4, 22 HCO_3, 2.4 HPO_4, 0.6 H_2PO_4, 57.1 mannitol, and 10 dextrose. Because the activity coefficient for K in this solution is approximately 0.54 (Fuchs et al., 1977; Lewis et al., 1978), the potassium activity of the serosal solution was equal to that previously reported for intracellular K activity in this tissue (76 mM, Wills et al., 1979).

Tissues were short-circuited using automatic voltage clamps (Physiologic Instruments, model VCC600, Houston, TX) interfaced to a microcomputer (IBM XT) as illustrated in Fig. 1. Just prior to measurement of current-voltage (*I-V*) relations the voltage clamps were adjusted to automatically compensate for resistances in series with the capacitance of the surface epithelium by passing a 50-μA, 4-kHz sinusoidal current across the tissue and nulling the transepithelial voltage deviation being displayed on an oscilloscope (S.M. Thompson, *submitted*). From the computer keyboard any of four voltage clamps could be selected and transepithelial current-voltage relations could be measured by sending a computer-generated sequence of voltages to the clamping device. The voltage pulse train consisted of 40 alternating polarity pulses so that ψ^{ms} was clamped over the range ±100 mV in 5-mV steps (Fig. 1). Both the duration of and interval between each pulse were 30 msec so that the entire *I-V* relation was obtained in less than 2.5 sec. All reported values for voltage and current were obtained 30 msec after perturbing ψ^{ms} (i.e., just prior to returning ψ^{ms} to base line).

The experimental protocol was as follows: Four tissues from each rabbit were mounted and bathed on both sides by one of four salines of different Na concentration. After 20 min the tissues were short-circuited and the I_{sc} and G_t were monitored on strip chart recorders (Kipp-Zonen, BD41) for 30 min. The serosal bath was then exchanged for the high-K depolarizing solution resulting in a sudden decrease and secondary rise in I_{sc} and R_t (Fig. 2*A*). When I_{sc} plateaued (approximately 10 to 20 min following depolarization) transepithelial current-voltage relations were obtained before and after addition of 10^{-5} M amiloride to the mucosal bath.

The *I-V* relations for the amiloride-sensitive Na entry pathway were evaluated by constraining the Goldman (1943), Hodgkin and Katz (1949) (GHK) flux equation for a single permeant ion

$$I^m_{Na} = -\frac{P^m_{Na}F^2\psi^{mc}}{RT}\frac{(Na)_m - (Na)_c \exp(F\psi^{mc}/RT)}{(1 - \exp(F\psi^{mc}/RT))} \quad (1)$$

to pass through two data points, one near the point of current reversal (E^m_{Na}) and the other at approximately −50 mV. These two points were varied to provide the "best" fit by eye over this voltage range. From these two data points and the above equation values for the apical membrane Na permeability, P^m_{Na}, and intracellular Na activity, $(Na)_c$, were determined.

Results

Replacing the solution bathing the serosal surface of rabbit colon with a solution high in potassium has been demonstrated to reduce the voltage across the basolateral membrane to near zero and to markedly reduce the resistance of that membrane (Thompson et al., 1982*a*; *also see* Fuchs et al., 1977, and Palmer, 1984).[1] Therefore, under these experimental condi-

[1] Direct measurements of the extent of depolarization by elevated serosal K in tissues other than rabbit colon have also been reported. In frog skin basolateral membrane potentials range from −2 to −35 mV following depolarization using either KCl or K_2SO_4 salines (Nagle, 1977; Tang et al., 1985; DeLong & Civan, 1984; Klemperer et al., 1984). In rabbit urinary bladder basolateral membrane potentials were −10 to −15 mV following K-depolarization (Lewis et al., 1978). In toad urinary bladder indirect arguments have been made for near complete depolarization of the basolateral membrane (Palmer et al., 1980). This subject has been recently reviewed by Palmer (1984).

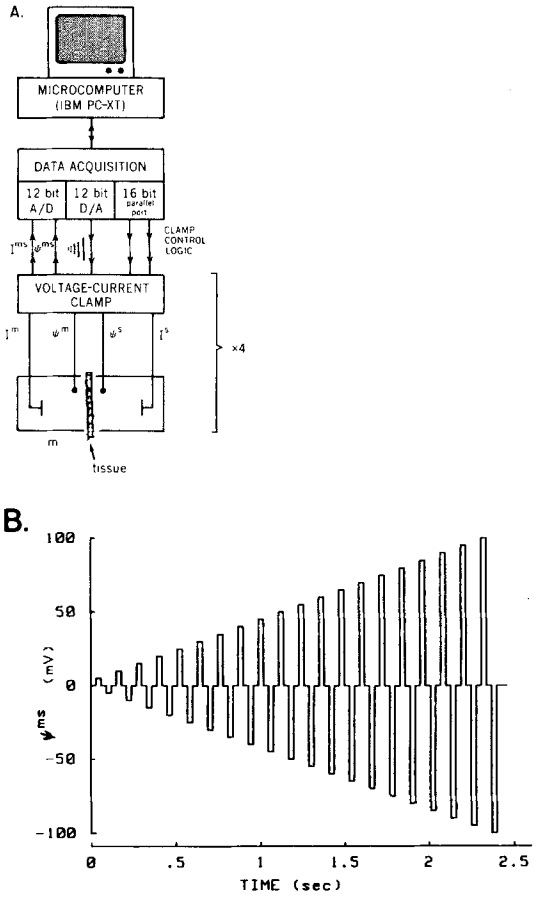

Fig. 1. (*A*) The experimental apparatus consisted of a microcomputer system (IBM PC-XT) interfaced to four voltage-clamp devices (Physiologic Instruments VCC600) via an interface card which contained the requisite A/D and D/A converters and parallel logic port (Data Translation DT2801). (*B*) Computer-generated pulse train used to measure transepithelial *I-V* relations

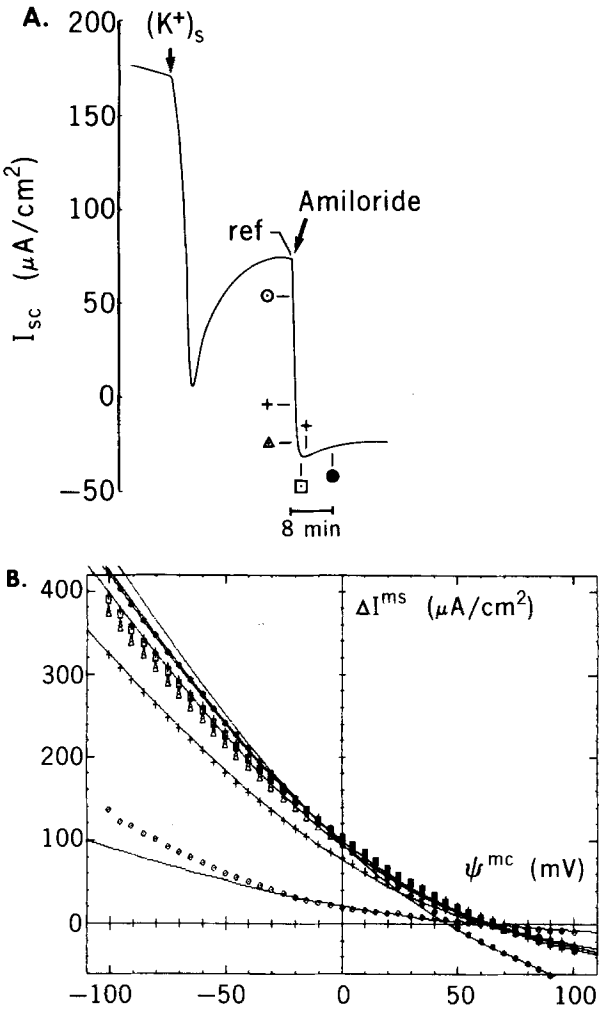

Fig. 2. (*A*) The protocol used to obtain the *I-V* relations for Na entry is illustrated. The tissue was first depolarized by elevating serosal K resulting in a rapid decrease and subsequent rise in I_{sc}. When I_{sc} plateaued a reference transepithelial *I-V* relation was recorded. Amiloride (10^{-5} M) was added to the luminal bath and six transepithelial *I-V* relations were obtained during the blockade of Na entry by amiloride. (*B*) *I-V* relations for the component of Na entry blocked by amiloride are given by the differences between the reference *I-V* relation and those obtained after addition of amiloride. ψ^{mc} is the clamped transapical membrane voltage. The symbols correspond to the post-amiloride *I-V* relations indicated in panel *A*

tions, the transapical membrane voltage is closely approximated by the clamped transepithelial voltage, ψ^{ms}, so that the current through the amiloride blockable pathway at any ψ^{ms} may be calculated as the difference between the current obtained before (I^{ms}) and after ($I^{ms'}$) blocking Na entry with amiloride (i.e., $I^m_{Na} = I^{ms} - I^{ms'}$) even in the presence of conductive pathways to ions other than Na in the apical membrane of rabbit descending colon (Wills et al., 1979; Thompson et al., 1982*a*,*b*). An underlying assumption of this calculation is that amiloride blocks only Na entry across the apical membrane and does not directly, or secondarily, alter any other transport property of the epithelium. In terms of an electrical equivalent circuit model for this epithelium (Thompson et al., 1982*a*), this assumes that amiloride increases the resistance of the apical membrane to Na ions, R^m_{Na}, and does not alter any other resistance or electromotive force.

In order to examine the assumption that amiloride only increases R^m_{Na} in depolarized rabbit colon, we performed the experiment illustrated in Fig. 2*A* on each tissue studied. The protocol followed was to measure a reference transepithelial *I-V* relation just prior to adding amiloride (10^{-5} M) to the mucosal bath. Subsequently, when the I_{sc} had decreased by 5 to 20 μA/cm^2 a second *I-V* relation was recorded, and thereafter *I-V* relations were obtained sequentially throughout the time course for

action of amiloride. The rationale for this procedure was as follows: If the effect of amiloride is to simply block Na entry, then subtraction of a post-amiloride *I-V* curve from the pre-amiloride curve will yield the *I-V* curve for that component of the total Na influx that has been blocked by amiloride. Under these circumstances, curves for I^m_{Na} *vs.* ψ^{mc} determined early after the addition of amiloride, when only a portion of Na entry has been blocked, will have a low apparent permeability. Those determined later will have a higher apparent P^m_{Na}. On the other hand, the reversal potential which graphically represents E^m_{Na}, the chemical driving force for Na entry, provides a measure of the pre-amiloride cell Na activity, and therefore, should be the same for each set of data.

The reversal potential for these derived curves is the voltage at which the pre- and post-amiloride transepithelial curves intersect, where

$$I^{ms} = I^m_{Na} + I_L = G^m_{Na}(E^m_{Na} - \psi^{mc}) + G_L(E_L - \psi^{mc}) \quad (2a)$$

and

$$I^{ms'} = I^{m'}_{Na} + I'_L = G^{m'}_{Na}(E^{m'}_{Na} - \psi^{mc}) + G'_L(E'_L - \psi^{mc}). \quad (2b)$$

The primes (′) indicate values at any time after addition of amiloride and G_L and E_L are the equivalent chord conductance and electromotive force for all transepithelial current pathways, cellular and paracellular, other than the apical membrane Na-entry pathway (Wills et al., 1979; Thompson et al., 1982*a*,*b*). From these equations it can be seen that the reversal potential for the difference current, or *apparent* E^m_{Na}, is given by

$$(E^m_{Na})_{apparent} = \frac{(G^m_{Na}E^m_{Na} - G^{m'}_{Na}E^{m'}_{Na}) + (G_LE_L - G'_L - E'_L)}{(G^m_{Na} - G^{m'}_{Na}) + (G_L - G'_L)}. \quad (2c)$$

In general, therefore, the apparent reversal potential will equal E^m_{Na} and $I^{ms} - I^{ms'}$ will be the amiloride-blocked Na current at any ψ^{mc} and any specified time only if $E^{m'}_{Na}$, G'_L and E'_L are not different from their pre-amiloride values. For example, if $(Na)_c$ were to decrease during the action of amiloride, then $G^{m'}_{Na}$ would be further reduced due to its dependence on $(Na)_c$ (*cf.* Thompson, 1986*a*), but $E^{m'}_{Na}$ would be increased. The net effect upon subtraction of the two transepithelial *I-V* curves would be a decrease in the apparent reversal potential. Alternatively, a decrease in G_L subsequent to addition of amiloride would also result in a decreased apparent reversal potential. Therefore, because E^m_{Na} derived from the intersection of I_{ms} and $I_{ms'}$ may be interpreted to be a measure of the $(Na)_c$ just prior to the addition of amiloride, a change in the apparent reversal potential can be used to determine when electrical circuit parameters, other than the resistance to Na entry, begin to change during blockade of Na entry with amiloride.

Table 1. Relative effects of methylprednisolone[a]

$(Na)_m$	${}_0I^m_{Na}$	P^m_{Na}	$(Na)_c$	E^m_{Na}	${}_0G^m_{Na}$	Time
6.2	4.25	5.13	1.44	0.70	6.25	0.75
12.4	4.26	5.25	1.40	0.72	6.31	0.75
24.9	2.99	3.53	1.52	0.79	4.13	0.84
75.6	2.25	2.41	1.44	0.87	2.73	0.91

[a] Values are expressed as MP/control. Time is the relative delay between addition of amiloride and attainment of the I_{sc} minimum.

Figure 2*B* shows plots of the difference current, $I^{ms} - I^{ms'}$, calculated from the transepithelial *I-V* curves generated in Fig. 2*A*. Successive *I-V* curves for Na entry are associated with an increased slope and an apparent reversal potential that is essentially constant, significantly changing only for the *I-V* curve that corresponds to the last post-amiloride transepithelial *I-V* measurement in Fig. 2 (i.e. 8.4 min after addition of amiloride). In most cases, shifts in the apparent reversal potential did not occur before the I_{sc} had decreased to near its minimal value. For curves derived from post-amiloride *I-V* data obtained after the I_{sc} nadir, the reversal potential was generally shifted towards zero yielding progressively higher apparent values for $(Na)_c$. Because during this time ($t > 5$ min post-amiloride) the effect of amiloride on Na influx was likely complete, yet both the I_{sc} and tissue resistance continued to increase slightly, the shift in the apparent E^m_{Na} was likely due to change in a resistive element in parallel, or in series, with ${}_0G^m_{Na}$. The post-amiloride times which resulted in a maximal P^m_{Na}, but without a shift in the apparent reversal potential, are given in Table 1. For both control and MP-treated tissues the mean time for measurement is essentially independent of $(Na)_m$. However, as indi-

Table 2. Effect of methylprednisolone on electrical parameters[a]

$(Na)_m$ =	6.2 mM		12.4 mM		24.9 mM		75.6 mM	
	Control	MP	Control	MP	Control	MP	Control	MP
${}_0I^m_{Na}$ (μA/cm^2)	11.0 ±1.6	46.7[b] ±5.8	13.8 ±1.6	58.6[b] ±6.2	23.6 ±6.0	70.6[b] ±6.7	39.7 ±8.2	89.5[c] ±10.2
P^m_{Na} (10^{-6} cm/sec)	25.9 ±3.9	132.5[b] ±16.8	16.1 ±1.5	84.6[b] ±8.9	12.4 ±3.4	43.8[b] ±5.6	5.7 ±1.2	13.7[b] ±1.3
$(Na)_c$ (mM)[c]	1.7 ±0.2	2.4[c] ±0.1	3.4 ±0.3	4.8[c] ±0.3	4.4 ±0.3	6.6[c] ±0.8	6.2 ±0.9	8.9 ±1.9
E^m_{Na} (mV)	37.0 ±2.8	26.1[c] ±1.6	35.8 ±2.4	25.8[c] ±1.9	47.4 ±2.5	37.3[c] ±2.9	70.1 ±4.2	60.8 ±4.7
${}_0G^m_{Na}$ (mS/cm^2)	0.32 ±0.05	2.00[b] ±0.26	0.39 ±0.03	2.46[b] ±0.29	0.55 ±0.16	2.27[b] ±0.40	0.56 ±0.13	1.53[b] ±0.19
time (min)	3.3 ±0.3	2.5 ±0.2	3.3 ±0.3	2.5[d] ±0.3	3.0 ±0.4	2.6 ±0.2	3.5 ±0.3	3.2 ±0.5
n	14	10	13	10	10	13	12	9

[a] Values given are ± standard error of the mean.
[b] $P < 0.001$.
[c] $P < 0.01$.
[d] $P < 0.05$.

cated in Table 2, the MP tissues responded approximately 20% faster to amiloride.[2]

Of importance to these experiments is that, by using this protocol, it is possible to distinguish *when* after addition of amiloride *I-V* curves for Na entry become affected by changes in transport properties other than decreased P^m_{Na}. In most instances the *I-V* relations appeared valid until the I_{sc} had decreased to within 90% of the maximal response. Results on tissues for which this was not true were rejected.

Effects of MP on Electrical Parameters

Distal colons from MP-treated rabbits displayed higher short-circuit currents and conductances when bathed symmetrically with 140 mM Na than did control tissues, in accordance with previously observed stimulatory effects of glulcocorticoids on Na absorption in this tissue (Marver, 1984; Clauss et al., 1985; Sellin & Desoigne, 1985). Upon abolition of the electrical driving force for Na entry by elevating serosal K, the amiloride-sensitive current in MP tissues remained more than 100% increased over controls, 89.5 ± 10.2 *vs.* 39.7 ± 8.2 μA/cm^2 (Table 1). Figure 3 compares the kinetics of the transapical Na current, ${}_0I^m_{Na}$, as a function of the Na activity in the luminal bath, $(Na)_m$, between control and MP-treated tissues. In both control and MP tissues ${}_0I^m_{Na}$ increases hyperbolically with increasing

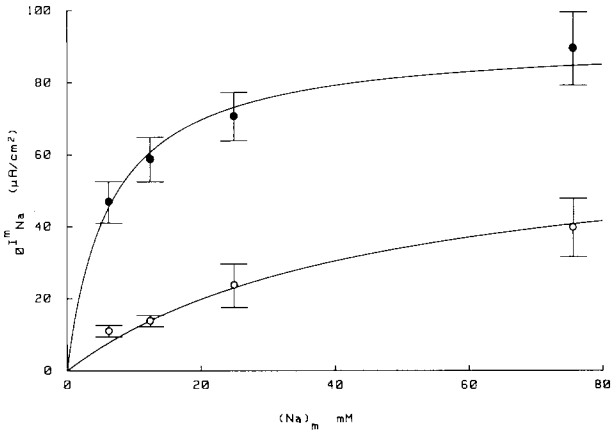

Fig. 3. Mean values of the steady-state rate of Na entry under short-circuit conditions are plotted versus the luminal Na activity in control (○) and MP-treated (●) tissues. The solid lines are according to the Michaelis-Menten equation (*see* text)

[2] A more rapid response of MP tissues to amiloride could reflect a structural alteration of the Na channel. Alternatively, if the rate of change in a resistive pathway that either parallels, or is in series with, G^m_{Na} is affected directly by glucocorticoids or by the magnitude of the change in Na transport, then the minimum value of I_{sc} may be reached before complete block of Na transport has occurred. Under these circumstances the values for P^m_{Na} given herein may be underestimated for the MP tissues. General conclusions drawn from these data, however, will not be affected.

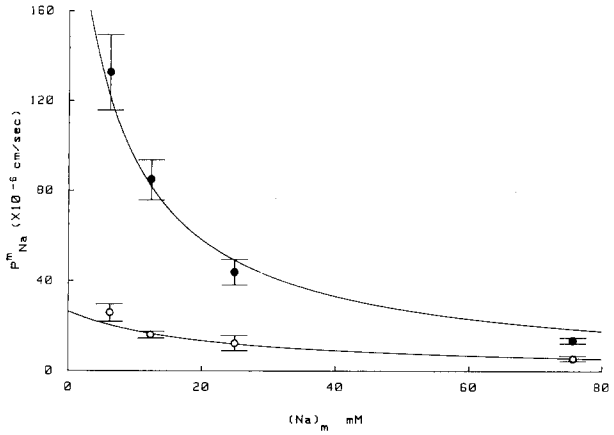

Fig. 4. Comparison of the functional dependence of the mean apical membrane Na permeability on mucosal Na between control (○) and MP-treated (●) tissues. The solid lines are drawn according to Eq. (3) (*see* text)

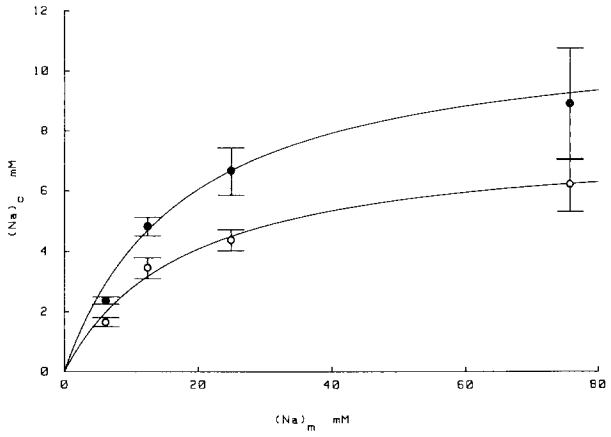

Fig. 5. Dependence of cell Na activity on mucosal Na in control (○) and MP-treated (●) tissues. The solid lines are drawn according to Eq. (5) (*see* text)

$(Na)_m$. The solid lines are the derived Michaelis-Menten curves (Tunheim et al., 1983). I_{max} is increased by MP (92 *vs.* 66 $\mu A/cm^2$ in controls) while the value of $(Na)_m$ at which current is half maximal K_m is markedly decreased (6.4 *vs.* 46 mM in controls). In principle, steroid-induced increases in I_{max} under depolarized conditions could result from an increased permeability of the apical membrane to Na, an increased Na activity gradient across the membrane, or both.

Alterations in Apical Na Permeability

Distal colon and other tight epithelia exhibit an adaptive response to changes in $(Na)_m$ such that a lowering of $(Na)_m$ results in a reciprocal increase in P^m_{Na} (Fuchs et al., 1977; Palmer et al., 1982; Thomas et al., 1983; Turnheim et al., 1983). Comparisons of the dependence of P^m_{Na} on $(Na)_m$ between control and MP-treated tissues (Fig. 4) reveals that this adaptation response is clearly also present in MP-treated tissues and, moreover, that it appears to be augmented. For example, in Table 1, MP-treated tissues exhibit a 2.4-fold increase in P^m_{Na} over controls when mucosal Na activity is high (75.6 mM), but a fivefold greater P^m_{Na} when $(Na)_m$ is low (6.2 mM). For both controls and MP tissues the curvilinear relation between P^m_{Na} and $(Na)_m$ may be described *empirically* by the expression (Fuchs et al., 1977; Turnheim et al., 1983):

$$P^m_{Na} = \frac{P^m_{Na(max)} \cdot (Na)_{m(0.5)}}{(Na)_m + (Na)_{m(0.5)}}. \quad (3)$$

The solid curves in Fig. 4 are drawn according to Eq. (3) using $P^m_{Na(max)} = 26.6 \times 10^{-6}$ cm/sec and $(Na)_{m(0.5)} = 20.6$ mM for controls while for the MP-treated tissues these values are 240×10^{-6} cm/sec and 6.4 mM, respectively, demonstrating the altered kinetics for inhibition by mucosal Na.

Because permeability is increased by MP at all mucosal Na, this effect accounts, at least in part, for the MP-induced increase in Na transport.

MP Effect on Intracellular Na

An additional mechanism by which steroids might alter the rate of Na entry, at least over a limited range, is by changing intracellular Na activity, $(Na)_c$, because under depolarized conditions I^m_{Na} is directly dependent on the activity difference across the apical membrane, i.e.

$$_0I^m_{Na} = F \cdot P^m_{Na} \cdot [(Na)_m - (Na)_c]. \quad (4)$$

Accordingly, a decrease in $(Na)_c$ would be required under these conditions for a steroid-induced change in driving force to cause an increase in $_0I^m_{Na}$. More importantly, however, changes in $(Na)_c$ may provide a clue as to the primary effect of steroids on transport. If, for example, $(Na)_c$ were consistently lowered following glucocorticoid treatment, this would suggest a primary stimulation of the basolateral Na pump. Alternatively, if $(Na)_c$ were higher, this would be consistent with steroids having their primary effect on the apical membrane. Figure 5 compares the dependence of $(Na)_c$ on $(Na)_m$ for control and MP-treated tissues. Interestingly, in spite of marked increases in the rate of Na transport in MP tissues, the dependence of cell Na on mucosal Na parallels that of control tissues. For both groups this dependence on mucosal Na is described *empirically* by

Table 3. Kinetic relations—Summary

Equation	Parameter	Control	MP	Units
${}_0I^m_{Na} = \frac{{}_0I^m_{Na(max)} \cdot (Na)_m}{K_m + (Na)_m}$	${}_0I^m_{Na(max)}$	66	92	$\mu A/cm^2$
	K_m	6	6.4	mM
(3) $P^m_{Na} = \frac{P^m_{Na(max)} \cdot (Na)_{m(0.5)}}{(Na)_m + (Na)_{m(0.5)}}$	$P^m_{Na(max)}$	26.6	240	$\times 10^{-6}$ cm/sec
	$(Na)_{m(0.5)}$	20.6	6.4	mM
(5) $(Na)_c = \frac{(Na)_m \cdot (Na)_{c(max)}}{K + (Na)_m}$	$(Na)_{c(max)}$	7.8	11.5	mM
	K	18	18	mM

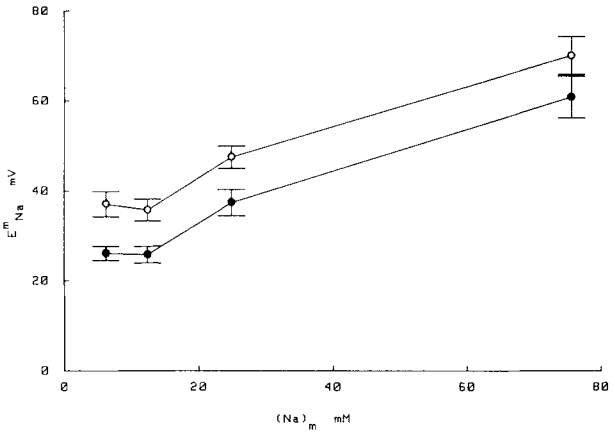

Fig. 6. Dependence of the reversal potential for Na entry on the luminal Na activity in control (○) and MP-treated (●) tissues

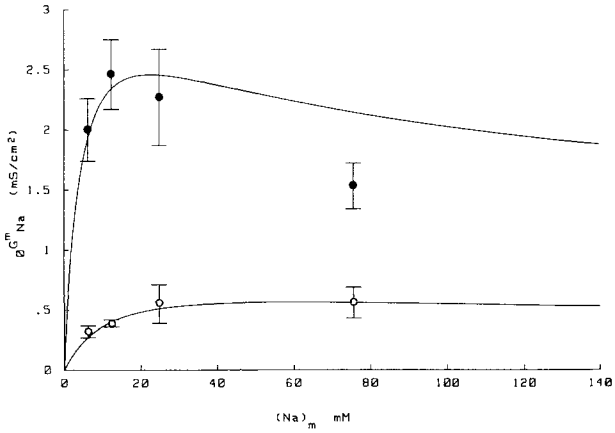

Fig. 7. Chord conductance of the apical membrane is plotted versus mucosal Na in control (○) and MP-treated (●) tissues. The solid lines are drawn according to Eq. (6) (*see* text)

$$(Na)_c = \frac{(Na)_m \cdot (Na)_{c(max)}}{K + (Na)_m} \quad (5)$$

where K is the value $(Na)_m$ at which $(Na)_c$ is half-maximal. For control tissues $(Na)_{c(max)}$ = 7.8 mM and K = 18 mM while for MP-treated tissues these values were 11.5 and 18 mM, respectively. These kinetic relations are summarized in Table 3.

Because, at each $(Na)_m$ the $(Na)_c$ of MP-treated tissues is increased over control levels, the chemical driving force is altered, but in a direction that, if occurring alone, would decrease I^m_{Na}. Therefore, MP-induced increases in P^m_{Na} are greater than would be indicated from current measurement alone. Moreover, the finding that $(Na)_c$ is increased implies that the primary stimulatory effect of the steroid is on the apical entry step (P^m_{Na}) rather than the basolateral pump.

Dependence of E^m_{Na} on $(Na)_m$

Under depolarized conditions the net thermodynamic driving force for Na entry is essentially the chemical potential difference across the apical membrane, E^m_{Na}. Figure 6 compares values for E^m_{Na} between control and MP tissues when mucosal Na is varied. As described previously for this preparation (Turnheim et al., 1983), E^m_{Na} of control tissues is nearly constant at low $(Na)_m$ and increases with mucosal Na. The E^m_{Na} of MP-treated tissues parallels this dependence on $(Na)_m$, but is uniformly displaced by approximately 10 mV. Because the transapical membrane voltage is nearly zero under short-circuit conditions, ${}_0I^m_{Na}$ is directly related to E^m_{Na} by the chord conductance of the apical membrane to Na, ${}_0G^m_{Na}$. Thus, the continuous rise in I^m_{Na} observed with increasing $(Na)_m$, combined with an essentially constant E^m_{Na} at low $(Na)_m$, suggests that ${}_0G^m_{Na}$ is not a simple monotonic function of $(Na)_m$ as would be expected if $(Na)_c$ were constant (Thompson, 1986*a*). Rather, it rises steeply at low $(Na)_m$, reaches a maximal value, and then declines due to the inhibitory effects of mucosal Na on P^m_{Na} (Fig. 7) with the inhibitory effect being more pronounced in the steroid-treated tissues. This dependence of conductance on mucosal Na may be predicted by inserting the empirical relations for P^m_{Na} and $(Na)_c$

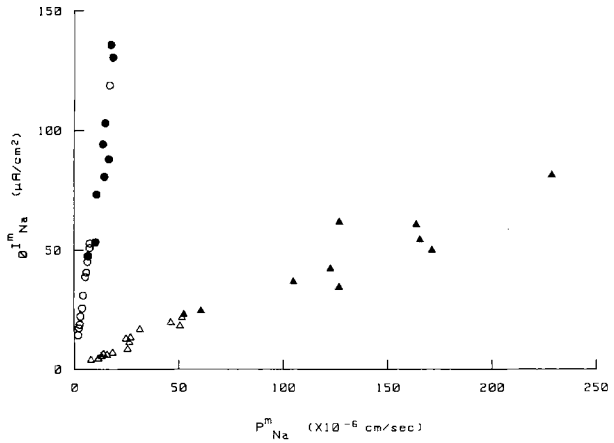

Fig. 8. Values for $_0I^m_{Na}$ are plotted versus P^m_{Na} for individual tissues bathed by 6.2 mM (△,▲) or 75.6 mM (○,●) mucosal Na. Controls (open symbols); MP-treated tissues (solid symbols)

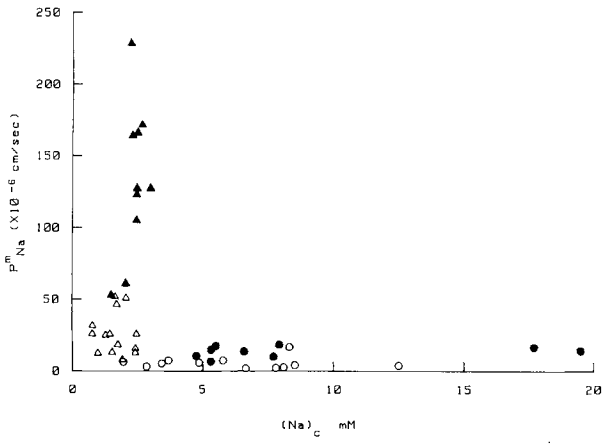

Fig. 9. Absence of a dependence of permeability on intracellular Na activity is demonstrated for individual tissues bathed by 6.2 mM (△,▲) or 75.6 mM (○,●) mucosal Na. Controls (open symbols); MP-treated tissues (solid symbols)

(Eqs. 2 and 3) and their respective kinetic constants into

$$_0G^m_{Na} = \frac{zFP\{(Na)_m - (Na)_c\}}{E^m_{Na}}. \quad (6)$$

The solid curves in Fig. 7 are derived from Eq. (6). Deviation of this curve from the MP data at high $(Na)_m$ reflects the fit of Eq. (3) to the data in Fig. 4.

Relation Between $_0I^m_{Na}$ and P^m_{Na}

Equation (4) shows that, in the K-depolarized preparation, $_0I^m_{Na}$ is dependent on both the permeability of the apical membrane and the Na activity difference across that apical membrane. Therefore, if the principal regulator of the rate of Na transport is permeability of the apical membrane, then a plot of individual values of $_0I^m_{Na}$ versus P^m_{Na} should be linear having a slope proportional to the difference in Na activities. Turnheim et al. (1983) demonstrated such a relation in untreated, depolarized rabbit colon for $(Na)_m$ = 99.4 mM. Under those circumstances transport varied over a narrower range and the activity difference was dominated by $(Na)_m$.

In Fig. 8 we plot both the spontaneous variations in $_0I^m_{Na}$ observed among control and MP-treated tissues at fixed mucosal Na activities of 6.2 and 75.6 mM. Similar plots are obtained for 12.4 and 24.9 mM mucosal Na. Clearly, at each $(Na)_m$, the individual data points for each group, control and MP, display a linear dependence of $_0I^m_{Na}$ on P^m_{Na}. Moreover, the MP data points fall essentially "in line" with the controls in spite of markedly increased rates of transport. Palmer et al. (1982) have reported similar results for aldosterone-induced Na transport by toad bladder at 20 mM $(Na)_m$. Thus, at each $(Na)_m$, the driving force for entry is nearly constant so that spontaneous variations, as well as increases in $_0I^m_{Na}$ due to MP treatment, result primarily from changes in permeability rather than driving force.

Independence of P^m_{Na} and $(Na)_c$

The preceding observation that wide variations in I^m_{Na} occur even though the driving force is nearly constant implies that $(Na)_c$ is neither a significant regulator of P^m_{Na} nor I^m_{Na} in the K-depolarized preparation. This finding is demonstrated in Fig. 9 by plotting P^m_{Na} *vs.* $(Na)_c$ for both control and MP tissues at two mucosal Na activities, 6.2 and 75.6 mM. Analogous results are obtained at 12.4 and 24.9 mM $(Na)_m$ and also in plots of $_0I^m_{Na}$ *vs.* $(Na)_c$ at each $(Na)_m$ (*data not shown*). For $(Na)_m$ = 6.2 mM, P^m_{Na} ranges between 7.9 and 51.7 × 10^{-6} cm/sec in controls (mean: 25.9 ± 3.9 × 10^{-6} cm/sec) and 52.6 to 229 × 10^{-6} cm/sec for MP tissues (mean: 132.5 ± 16.8 × 10^{-6} cm/sec). For both groups, however, the $(Na)_c$ is tightly clustered near 2 mM (means: 1.7 ± 0.15 and 2.4 ± 0.12 mM for control and MP tissues, respectively). On the other hand, at high mucosal Na the converse is observed; namely, for both control and MP tissues the permeability is lower and displays relatively little variation (mean values: 5.7 ± 1.2 × 10^{-6} and 13.7 ± 1.3 × 10^{-6} cm/sec for control and MP tissues, respectively), while $(Na)_c$ is higher

and displays considerably more variation (mean values: 6.2 ± 0.9 and 8.9 ± 1.9 mM).

Discussion

Glucocorticoids, like mineralocorticoids, are potent stimulators of electrogenic Na transport by gastrointestinal epithelia (Charney et al., 1975). Early studies attributed stimulation of Na transport by glucocorticoids to crossover binding to the mineralocorticoid (aldosterone) receptor. In support of this view were the observed similarities among the effects of the two classes of steroid hormone on Na transport. For example, treatment with either class of steroid results in an increased Na,K-ATPase activity in the basolateral membrane (Charney et al., 1975; Will et al., 1981). However, recent studies offer compelling evidence that glucocorticoids bind to distinctly different cytosolic receptors than do the mineralocorticoids (Bastl et al., 1984; Marver, 1984). In view of biological receptor-function specificity, this finding suggests that glucocorticoids may have regulatory functions and mechanisms of action that are distinct from those of mineralocorticoids. In these regards, Bastl et al. (1980) presented evidence that glucocorticoids, but not mineralocorticoids, may control basal rates of colonic Na absorption. Will et al. (1981) demonstrated that the rise in Na, K-ATPase activity in the basolateral membrane of rat intestine appears 2 to 3 times faster following glucocorticoid treatment than with aldosterone treatment.[3]

In order to examine the mechanism(s) by which the glucocorticoid methylprednisolone (MP) stimulates transepithelial Na absorption, we measured properties of the Na entry pathway as a function of mucosal Na activity in distal colonic epithelia from normal rabbits and from rabbits pretreated with MP. Values for apical membrane Na permeability, conductance, and intracellular Na were derived from measurements of the instantaneous transepithelial *I-V* relations of K-depolarized colon (Fuchs et al., 1977) made before and after blockade of Na entry by amiloride. The adequacy of this procedure in this tissue has been addressed previously by Thompson et al. (1982*a*).

Measurement of the I^m_{Na} *vs.* ψ^{mc} Relation

As detailed in Results, an assumption required to derive the *I-V* relation for Na entry across the apical membrane is that amiloride affects *only* the resistance of the apical membrane to the entry of Na ions during the time required to completely abolish Na entry. Resistances of all other ionic pathways across the epithelium as well as intracellular activities for each permeant ion must remain constant. The validity of this assumption in rabbit colon has not previously been examined rigorously. However, effects of amiloride on the resistance of the paracellular pathway have been reported for frog skin (Nagle et al., 1983) and cortical collecting duct (Sansom & O'Neil, 1985). Increases in the basolateral membrane resistance have been observed in nondepolarized toad and frog bladder (Davis & Finn, 1982), and rabbit colon (K. Turnheim, *personal communication*) following blockade of Na absorption by amiloride. The presence of an effect of amiloride on either the resistance of a pathway other than the Na entry pathway or an electromotive force across the apical membrane of K-depolarized rabbit colon can be deduced from the data illustrated in Fig. 2.

Of importance in this figure are the consistent findings that the I_{sc} fell to a minimal value following application of amiloride and then generally rose again. The mechanism for this secondary rise in current is unknown. However, associated with this secondary rise in I_{sc} was an increase in the tissue resistance indicating that the resistance of some ionic pathway in parallel, and perhaps also one in series, with the apical Na entry pathway must increase.

Analysis of *I-V* relations as a function of time following addition of amiloride also leads to the above conclusion. If amiloride were to only increase R^m_{Na}, or alternatively, to decrease P^m_{Na}, then successive plots of I^m_{Na} *vs.* ψ^{mc} following application of amiloride would differ only in slope and have identical intercepts on the voltage axis (E^m_{Na}). The finding that the apparent reversal potential for Na entry changes for times greater than that required for I_{sc} to reach its nadir (Fig. 2*B*), indicates that an equivalent circuit component in addition to R^m_{Na} must be affected. Details of this effect of amiloride will require further investigation.

[3] Cross reactivity of MP with mineralocorticoid receptors has not been assessed in this study. However, in more recent studies we have observed similar increases in P^m_{Na} using much lower doses of MP (2 mg/kg IM for 24 hr). Moreover, we have determined that aldosterone-induced increases in P^m_{Na} are abolished by systemic treatment with amiloride whereas those induced by MP are not diminished by this diuretic (Thompson, 1986*b*). Similarly, increases in Na,K-ATPase activity in rat colon subsequent to aldosterone, but not to MP, are abolished by spironolactone (Charney et al., 1981).

Mechanism of Stimulation by MP

In the steady state the rate of Na entry across the apical membrane must exactly match the rate of exit across the basolateral membrane to maintain cellular homeostasis. Therefore, stimulation of either process alone, entry or exit, requires a compensatory increase in the other in order that a new steady-state rate of transport be attained. Stimulation of Na transport by glucocorticoids may, for example, result from a primary effect on Na exit (increased amount or turnover of the Na,K-ATPase pump) which invokes a subsequent, homeostatic compensatory increase in Na entry (increased permeability or driving force). Such a mechanism was suggested to account for aldosterone-induced stimulation of Na absorption by rabbit urinary bladder (Lewis et al., 1976) and distal colon (Frizzell & Schultz, 1978). For distal colon the compensatory increase in P_{Na}^m was proposed to be due to a release from a negative feedback mechanism when $(Na)_c$ is lowered following stimulation of the pump (Turnheim et al., 1978). Alternatively, the primary effect of the steroid may be on the entry step. A primary increase in apical permeability should increase the rate at which Na enters the cell and thereby give rise to at least a transient increase in $(Na)_c$. Increased exit at the basolateral membrane could then result from a steep dependence of the pump on $(Na)_c$ (Eaton, 1981; Lewis & Wills, 1983), a change in the kinetics of individual pump units [e.g. stoichiometry for the Na,K-ATPase (S.I. Helman in Reuss et al., 1984; Sansom & O'Neil, 1986) or pump turnover rate], or an increase in the total number of pump units.

Our findings that both P_{Na}^m and $(Na)_c$ are increased following *in vivo* treatment with MP are consistent with a principal effect of MP being on the apical membrane entry mechanism and that effects on the basolateral Na exit are secondary. Similar results were reported for *in vitro* stimulation of Na transport by aldosterone in K-depolarized toad urinary bladder by Palmer et al. (1982) and rabbit cortical collecting tubules (Petty et al., 1981).

Regulation of P_{Na}^m

The regulation of apical Na permeability in so-called "tight" epithelia involves many different factors which include mucosal Na, intracellular Na and/or Ca^{2+} and steroid hormones.

Direct effects of mucosal Na on P_{Na}^m were demonstrated by Fuchs et al. (1977). They suggested that this negative effector action may result from Na ions binding to a modifying site in the vicinity of the channel and thereby cause a conformational change to partly or completely close the channel. Decreases in Na channel density with increasing $(Na)_m$ have been confirmed in frog skin using noise analysis (VanDriessche & Lindemann, 1979) and subsequently in other tissues (*cf.* Lewis et al., 1984). Results presented in Fig. 4 for the control tissues are consistent with this inverse dependence of macroscopic P_{Na}^m on $(Na)_m$.

The mechanism(s) by which glucocorticoids increase P_{Na}^m are largely unknown. If MP were simply to reduce the self-inhibitory effect of $(Na)_m$ on P_{Na}^m, then an increased P_{Na}^m at high $(Na)_m$ but no change in P_{Na}^m at low $(Na)_m$ would be expected (Palmer et al., 1982). This, however, is not the case for rabbit colon where the inhibitory effect of $(Na)_m$ on P_{Na}^m is actually augmented in MP tissues (Fig. 4). Alternatively, macroscopic permeability may be increased by altering either, or both, the single channel permeability or functional channel density. Studies using aldosterone on toad urinary bladder suggest that this mineralocorticoid increases P_{Na}^m by increasing the number of conducting channels rather than increasing the conductance of individual channels (Palmer et al., 1982) and that this is accomplished by activating normally quiescent channels already present in the membrane (at least within the first 5 hr following exposure to the hormone) rather than causing the insertion of "new" channels into the membrane (Palmer & Edelman, 1981; Garty & Edelman, 1983).

Whether glucocorticoids act in rabbit colon by increasing the permeability of individual channels, activate identical channels that pre-exist within the membrane, or cause insertion of new channels cannot be discerned from our experiments. However, the increased sensitivity of P_{Na}^m to $(Na)_m$ in MP-treated tissues suggests that the apical Na channel, at least with respect to its Na-inhibitory site, is altered compared to controls. This result differs from that of Palmer et al. (1982) who found no significant change in the relationship between P_{Na}^m and $(Na)_m$ following addition of aldosterone to toad urinary bladder. This either may reflect actual differences in the actions of these two classes of steroid, or simply differences in time of exposure to the respective steroids, approximately 5 hr for aldosterone versus two days for MP. In the latter case, the short-term steroid-induced increase in P_{Na}^m may result from activation of already existing channels, while long-term stimulation may cause the insertion of "new" channels from intracellular stores (Lewis, 1983). Evidence suggesting cytoplasmic stores for amiloride-sensitive channels has been reported for the hen coprodaeum (Cuthbert et al., 1982) and rabbit urinary bladder (Lewis & de Moura, 1984). Differ-

ences in the kinetics of the MP-induced increase in macroscopic P^m_{Na} from those for the control tissues may simply reflect the average "age" of the Na channels (Loo et al., 1983), where the average channel in MP tissues would be "young" compared to controls. Aging of Na channels in rabbit urinary bladder has been attributed to enzymatic degradation of the channel (Loo et al., 1983).

Intracellular Na activity also has been implicated as being a regulator of P^m_{Na} acting either directly or via changes in intracellular Ca^{2+} (*cf.* Taylor, 1981; Schultz, 1985). In K-depolarized epithelia, however, no direct correlation has been observed between $(Na)_c$ and P^m_{Na} (Turnheim et al., 1983; and Fig. 9). Indeed, several investigators have observed that large increases in $(Na)_c$ caused by metabolic inhibitors are associated with relatively small decreases in P^m_{Na} (Palmer et al., 1980; Palmer, 1985; S.M. Thompson, *unpublished*). Thus, it may be that the mechanisms for "negative feedback" inhibition of P^m_{Na} by $(Na)_c$ are either aborted in the depolarized preparation (Turnheim et al., 1983) or that this mechanism is not active at the low levels of $(Na)_c$ normally observed.

In apparent contrast to the above conclusion, Turnheim et al. (1983) observed a highly linear correlation between *mean* values of P^m_{Na} and $(Na)_c$ obtained at each of several $(Na)_m$. Similarly, the data in Table 1 also yield linear inverse relations between mean values of P^m_{Na} and $(Na)_c$ in both control and MP-treated tissues. It is probable, however, that the correlation between the mean values of P^m_{Na} and $(Na)_c$ do not reflect a direct dependence of P^m_{Na} on $(Na)_c$, but rather the fact that both P^m_{Na} and $(Na)_c$ are dependent on mucosal Na (Figs. 4 and 5). By combining the empirical equations relating P^m_{Na} to $(Na)_m$ (Eq. 3) and $(Na)_c$ to $(Na)_m$ (Eq. 5), it can be shown that

$$P^m_{Na} = \frac{P^m_{Na(max)}[(Na)_{c(max)} - (Na)_c]}{(Na)_{c(max)} - [1 - K/(Na)_{m(0.5)}]Na_c}. \quad (7)$$

By inserting the empirical kinetic parameters used for Figs. 4 and 5 into Eq. (7), reasonable fits to the mean values of P^m_{Na} and $(Na)_c$ are obtained (Fig. 10).

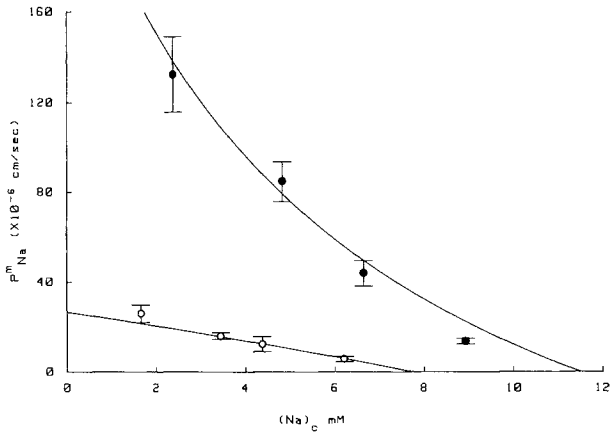

Fig. 10. Data points are mean values of permeability plotted versus mean values of $(Na)_c$. Means were obtained at four different luminal Na activities in control (○) and MP-treated (●) tissues. The solid lines are drawn according to Eq. (7) (*see* text)

Steady-State Current is Independent of $(Na)_c$

In the depolarized colon, the current through the basolateral pump under steady-state short-circuit conditions is proportional to the rate of Na entry across the apical membrane (Na is absent from the serosal bath). Based on enzyme kinetics, one expects the current through individual pump units to have a direct dependence on the substrate activity $(Na)_c$. Therefore, providing that the stoichiometry and number of pumps are constant among controls and among MP tissues, regardless of $(Na)_m$, a direct dependence of ${}_0I^m_{Na}$ on $(Na)_c$ should obtain for each tissue group. However, plots (*not shown*) of ${}_0I^m_{Na}$ versus $(Na)_c$ reveal no discernable relation for either control or MP tissues. This lack of a dependence of steady-state current on $(Na)_c$ can be seen in Figs. 8 and 9 where at each $(Na)_m$ the ${}_0I^m_{Na}$ is linearly dependent on P^m_{Na}, but P^m_{Na} is independent of $(Na)_c$ for both control and MP-treated tissues. This finding is in agreement with several recent studies on epithelia. For example, in *Necturus* urinary bladder Thomas et al. (1983) demonstrated that ${}_0I^m_{Na}$ could be increased fourfold by varying mucosal Na without significantly increasing $(Na)_c$. In *Necturus* small intestine transepithelial Na transport and Na pump activity could be markedly increased by addition of galactose to the mucosal bath, but measured values of $(Na)_c$ remained essentially unchanged (Hudson & Schultz, 1984). And, finally, in nondepolarized distal colon from normal and Na-deprived rabbits no direct relation was observed between the rate of Na transport which varied over wide ranges and the $(Na)_c$ determined using Na-selective microelectrodes (K. Turnheim and S.G. Schultz, *personal communication*).

In contrast, other studies have demonstrated an apparent sigmoidal dependence of total pump current on $(Na)_c$ and have suggested kinetic models based on either cooperative interaction among Na ions binding to the pump (Lewis & Wills, 1983; Turnheim et al., 1983) or on noncooperative interaction (Nielson, 1979; Eaton, 1981; Eaton et al.,

1982; Halm & Dawson, 1983). No consensus has been reached as to the appropriate kinetic model for the Na,K-ATPase in epithelia.

In a previous study using a similar approach to that of these experiments, Turnheim et al. (1983) reported a sigmoidal relation between the mean values of ${}_0I^m_{Na}$ and the mean $(Na)_c$ obtained at each $(Na)_m$ in rabbit colon. In those studies, as in these, both ${}_0I^m_{Na}$ and $(Na)_c$ are saturating functions of the mucosal Na activity. Therefore, because both parameters are functions of $(Na)_m$, the relationship between the mean values of ${}_0I^m_{Na}$ and $(Na)_c$ discussed above, presumably reflects more their dependencies on $(Na)_m$ than a direct dependence of total pump current on $(Na)_c$. As for the mean values for permeability above, the empirical equations (Eqs. 3 and 4) can be solved to predict a relationship between the mean values of ${}_0I^m_{Na}$ and $(Na)_c$ which provides a close fit to the mean values of current and $(Na)_c$ obtained in both control and MP tissues and for the experiments of Turnheim et al. (1983, Fig. 7*b*).

Finally, the lack of finding a definitive relationship between ${}_0I^m_{Na}$ and $(Na)_c$ among individual control or MP-tissue suggests that either 1) some signal other than $(Na)_c$ is responsible for increasing the turnover rate or the stoichiometry of a fixed number of operating pumps, or 2) there is an increase in the number of operating pumps in the membrane (Hudson & Schultz, 1984; Schultz, 1985). Evidence correlating increases in the rate of transport in the mammalian collecting duct with increases in basolateral membrane area suggests the possibility that additional "pump-leak membrane units" are incorporated into the basolateral membrane in response to increased transport requirements (O'Neil, 1986). If indeed the number of active pumps in the membrane is not constant, but rather varies with transport rate, then ${}_0I^m_{Na}$ would not be expected to correlate with $(Na)_c$. Moreover, relating total pump current and $(Na)_c$ using simple equilibrium binding would be invalid because such kinetic models require I_{max} to be constant, and I_{max} is proportional to the number of pump units (Segel, 1975).

Conclusion

The regulation of transepithelial Na transport has been widely investigated, but as yet is not fully understood. In this paper we have demonstrated that the glucocorticosteroid methyprednisolone stimulates transepithelial Na absorption in mammalian distal colon principally by increasing the Na permeability of the apical membrane which, in turn, leads to an increased $(Na)_c$. The mechanism for increased permeability remains to be determined; however, the altered relationship between P^m_{Na} and $(Na)_m$ suggests possible differences in either the conformation of the Na channel or the chemical environment surrounding the apical Na channel in tissues from MP-treated animals compared to controls. This finding is consistent with the observation by Sellin and Desoigne (1985) that the ionophore amphotericin B, which markedly increases apical permeability in control tissues, is ineffective in tissues from MP-treated rabbits.

Whatever the mechanism for the increased P^m_{Na} by MP, the apical Na permeability in both control and MP tissues is highly dependent on mucosal Na, but is rather independent of $(Na)_c$. Moreover, it appears that cell Na is also a function of mucosal Na, having similar kinetics in MP tissues as in controls, but with a somewhat elevated set point. The mechanism by which mucosal Na appears to determine the value of $(Na)_c$ will require further investigation.

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