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Effects of Adrenal Steroids on Na Transport in the Lower Intestine (Coprodeum) of the Hen

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Summary. The influence of adrenal steroids on sodium transport in hen coprodeum was investigated by electrophysiological methods. Laying hens were maintained on low-NaCl diet (LS), or on high-NaCl diet (HS). HS hens were pretreated with aldosterone (128 μg/kg) or dexamethasone (1 mg/kg) before experiment. A group of LS hens received spironolactone (70 or 160 mg/kg, for three days). The effects of these dietary and hormonal manipulations on the amiloride-sensitive part of the short-circuit current were examined. This part is in excellent agreement with the net Na flux, and therefore a direct electrical measurement for Na transport. After depolarizing the basolateral membrane potential with a high K concentration, the apical Na permeability and the intracellular Na activity were investigated by current-voltage relations for the different experimental conditions.

Plasma aldosterone concentrations (PA) were low in HS hens, dexamethasone-treated HS hens and spironolactone-treated LS hens (<70 pM). In contrast LS hens and aldosterone-treated HS hens had a PA concentration of 596 ± 70 and 583 ± 172 pM, respectively. LS diet (chronic stimulation) had the largest stimulatory effect on Na transport and apical Na permeability. Hormone-treated animals had three- to fourfold lower values. Spironolactone supply in LS hens decreased Na transport and apical Na permeability about 50%.

The results provide evidence that both mineralo- and glucocorticoids stimulate Na transport in this tissue by increasing the apical Na permeability. Quantitative differences between acute and chronic stimulation reveal a secondary slower adaptation in apical membrane properties.

Key Words hen coprodeum · Na transport · current-voltage relations · aldosterone · dexamethasone · spironolactone

Introduction

Mineralocorticoids (aldosterone) as well as glucocorticoids (dexamethasone) are potent stimulators of colonic electrolyte transport. These hormones showed multiple effects on mammalian colon (Binder, 1978; Bastl, Binder & Hayslett, 1980; Marver, 1984). There is growing evidence that these hormones play an important role in the diurnal regulation of large intestinal Na transport (Clauss, 1984). The current models of the hormonal action include the regulation of the apical Na permeability as the rate-limiting step of Na entry into the cells (Schultz, 1984) and direct stimulation on the basolateral NaK-ATPase (Geering et al., 1982). The specific action of aldosterone on the apical membrane properties and Na transport has been investigated in rabbit colon (Frizzell & Schultz, 1978; Clauss et al., 1985*b*) and several amphibian epithelia (Palmer et al., 1982). These epithelia showed under the influence of aldosterone an augmented Na current correlated with higher Na permeabilities. The most potent stimulation of Na transport due to adrenal steroids has been obtained, however, in an avian epithelium with a Na-deficient diet (Choshniak, Munck & Skadhauge, 1977; Bindslev, 1979; Thomas & Skadhauge, 1979; Rice & Skadhauge, 1982). The hen coprodeum is by far the most aldosterone-sensitive epithelium known to date (Skadhauge, 1984), and varies Na transport between zero and 14 μeq/cm^2 · hr. Christensen and Bindslev (1982) have shown with current fluctuation analysis, that Na entry in this epithelium occurs, as in the amphibian and mammalian epithelia, by diffusion through Na channels in the apical membrane. Aldosterone induces new transport sites (Bindslev, 1979; Eldrup, Mollgard & Bindslev, 1980) and they seem to originate from preformed cytoplasmic vesicles below the apical cell surface (Cuthbert et al., 1982). Recent investigators compared the different time-course of aldosterone injections (Thomas et al., 1980; Clauss et al., 1984) with the time-course of NaCl depletion or restoration (Thomas & Skadhauge, 1979, 1982; Skadhauge et al., 1983; Clauss et al., 1984). All these studies indicated a time-dependent dietary stimulation of Na transport, and that in spite of already augmented plasma aldosterone levels after two days (Arnason et al., 1986) it takes several days for the epithelium to achieve its maximal transport rate. Acute aldosterone injections do not stimulate Na transport to

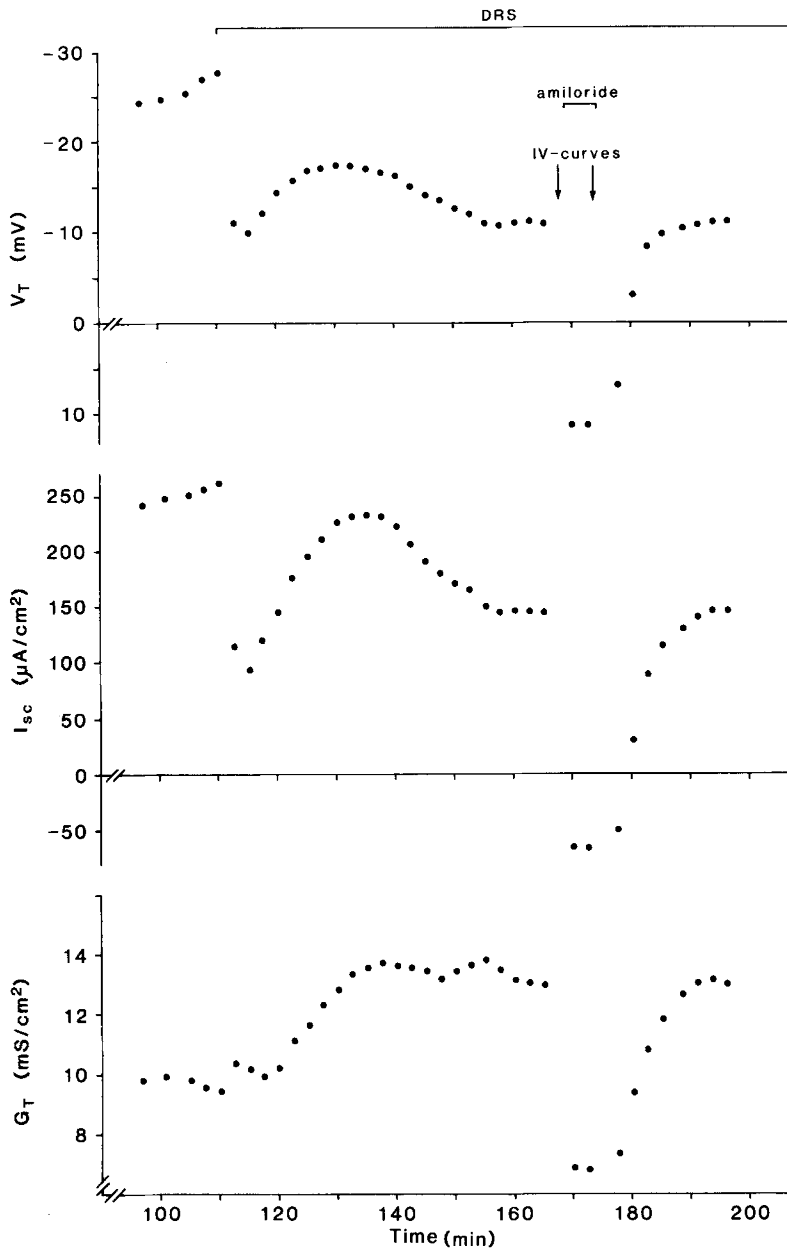

Fig. 1. Time-course of transepithelial voltage (V_T), short-circuit current (I_{sc}) and tissue conductance (G_T) of a representative experiment under LS conditions. The tissue has equilibrated under normal Ringer's solution, when at time = 110 min the serosal solution is changed to the depolarizing Ringer's solution (DRS). The tissue depolarizes to a new steady state within 60 min. Note the significant increase in G_T. Current-voltage relations are determined before and after the addition of amiloride (as indicated by the arrows). Note the complete restoration of the electrical parameters after the washout of amiloride

maximal rates in a few hours, as seen in mammalian colon (Frizzell & Schultz, 1978; Clauss et al., 1985*b*). It seemed therefore interesting to investigate at which part of the cellular Na-transporting system is the rate-limiting step, and if this secondary slow adaptation already involves the Na permeability of the apical cell membrane.

In addition, we investigated the possibility that other factors like glucocorticoids may influence the Na-transport system and modulate it differently or stimulate it to a higher degree. In hen lower intestine, as well as in rabbit distal colon, two classes of corticoid binding sites have been found (Marver, 1984; Sandor et al., 1986). One, the type I receptor is more specific for mineralocorticoids like aldosterone, whereas the type II receptor is more specific for glucocorticoids like dexamethasone. No data about the influence of glucocorticoids on coprodeal ion transport are known presently. Our study therefore aimed to investigate and compare mineralo- and glucocorticoid effects on Na transport across this tissue, and specifically addresses the hormonal modulation at the apical cell membrane, with regard to Na permeability and intracellular Na activity. We used spironolactone, a specific inhibitor of the type I receptor (Sakauye & Feldman, 1976) to evaluate the type I-mediated effect.

Our study demonstrates that both hormonal

classes profoundly affect Na transport at the apical cell membrane, and alter Na permeability. But acute injections do not stimulate apical Na permeability to the same degree as in chronic adapted epithelia. The slow adaptive component seems to be already located in the apical membrane. In addition we demonstrate the autoregulation of the cellular Na-transporting system (Schultz, 1981) and note the differential effects of aldosterone and dexamethasone on intracellular Na activity.

Materials and Methods

Animals

Forty adult white leghorn laying hens from the same batch [mean weight 1.5 ± 0.1 kg (SE)] were kept in individual cages in the animal house with a natural photoperoid (May–October). Nineteen birds were given tap water, crushed wheat and barley *ad libitum* (low-Na diet, LS) for at least 10 days prior to investigation. Twenty-one birds (high-Na diet, HS) obtained a composed commercial poultry food and 0.5% NaCl in tap water *ad libitum*. All HS hens were maintained on this diet for at least seven days prior to experiments.

Hormonal Treatment and Assay

Sixteen HS birds received a total dose of 128 μg/kg body weight D-aldosterone (Aldocorten, CIBA-GEIGY) split into three doses. Injections were given i.m. 20, 12 and 4 hr before decapitation. This injection schedule continuously elevates plasma aldosterone values as shown by Clauss et al. (1984). Maximal stimulatory effects on I_{sc} have been shown 4 to 8 hr after a single aldosterone injection (Clauss et al., 1984). Dexamethasone (1 mg/kg body weight · day; Serva, Heidelberg) was diluted in 1 ml 0.9% NaCl and given to a group of HS animals. The injections were given 12 and 4 hr prior to slaughtering. Nine LS birds received orally spironolactone (Boehringer, Mannheim) in a concentration of 70 or 160 mg/kg body weight and day, given at three successive days before experiment.

Carotid blood was sampled after decapitation into a heparinized beaker, centrifuged immediately and stored at −24°C. Plasma aldosterone was analyzed with radioimmunoassay ^{125}J (MAIA-technic, Serono) at the medical center in Stuttgart (Katharinenhospital).

Tissue Preparation and Mounting

After decapitation, the abdomen was opened and colon, coprodeum and urodeum were removed and opened longitudinally. The contents were washed out with warm normal Ringer's solution. The colon was then cut off and urodeum and the outer mucosal layer of the coprodeum was dissected with optical tweezers and fine scissors.

Four epithelial sheets of hen coprodeum were mounted in Ussing-type chambers with an area of 0.62 cm^2. On the mucosal side a silicone seal prevented edge damage. Both sides were perfused with 12 ml of a warmed (37°C) solution, which was recirculated by a pure oxygen gas lift. The normal Ringer's solution (NRS) contained (in mM): 137 Na, 141 Cl, 8 PO_4, 8 K, 2 Ca, 2 Mg, 10 glucose. The serosal depolarizing Ringer's solution (DRS) contained (in mM): 147 K, 141 Cl, 8 PO_4, 2 Ca, 2 Mg, 10 glucose. Both solutions had an osmolarity of 280 mOsm/kg and a pH of 7.3.

The serosal DRS solution depolarized the basolateral membrane potential and decreased the basolateral membrane resistance. This was indicated by a rapid decrease in transepithelial potential (V_T) and an immediate increase in transepithelial conductance (G_T). Figure 1 shows a tracing of the time-course of a representative experiment. Under these conditions a pulse of transepithelial voltage will produce a current step predominantly at the apical membrane. Therefore the transepithelial electrical potential difference under these conditions is assumed to reflect mainly the electrical potential difference across the apical membrane (Fuchs et al., 1977; Palmer, 1984).

Electronic Equipment and Data Acquisition

The transepithelial potential is given as the potential in the external (mucosal) bath with reference to the internal bath (serosa = ground). Inward currents are taken as positive. A computer-controlled digital voltage clamp (AC Copy, Aachen) superimposed bipolar pulses of 100-μA amplitude and 500-msec duration on the short-circuit current (I_{sc}). Tissue conductance was calculated from the voltage deflections produced by these pulses. This voltage clamp controlled four chambers in parallel. For measuring near-instantaneous *I-V* relationships each chamber was periodically connected to a fast analog voltage clamp (Nagel Biomed. Instruments, Munich) and *I-V* curves were recorded before and after exposing the tissue to 50 μM amiloride (*see* Fig. 1). After this procedure the chamber was reconnected to the four-channel clamp and the next chamber was hooked to the fast clamp. Each chamber was connected for about 15 min to the fast voltage clamp and by the sequential analysis of one tissue after the other the measurements were completed in about 1 hr. As three of the chambers were continuously monitored (as control) by the digital voltage clamp in parallel, we could ensure that no change in basic electrical properties or in ion transport occurred during that time.

The frequency response of the fast voltage clamp was adjusted for each tissue by changing the capacitance in the feedback amplifier. For that purpose the voltage- and current transients in response to a +20-mV clamp pulse of 200-msec duration were inspected on a storage oscilloscope (Tektronix 511A). We attempted to optimize the voltage response time to a rectangular shape and to avoid overshoots. Thereafter the time for charging tissue capacitance was estimated by the voltage response of the tissue to a constant current pulse of 500-msec duration. Current- and voltage transients were recorded on a digital storage oscilloscope (Nicolet 2090). Figure 2 shows an example of such a measurement and reveals that capacitive transients were terminated after at least 20 msec.

The analog voltage clamp was connected via a microprocessor-based analog-digital and digital-analog interface subsystem (AC Copy, Aachen) to a microcomputer (Apple II). A series of programs (written in assembly language) allowed a fast control and a precise multichannel data acquisition from the clamp (2 kHz bandwidth, 12 bit conversion). By this system, a bipolar train of clamp command voltages (±200 mV in 5-mV increments, beginning from 0 mV) was generated by the computer, and the corresponding current responses were recorded automatically (Fig. 3). In order to avoid systematic ion redistributions which

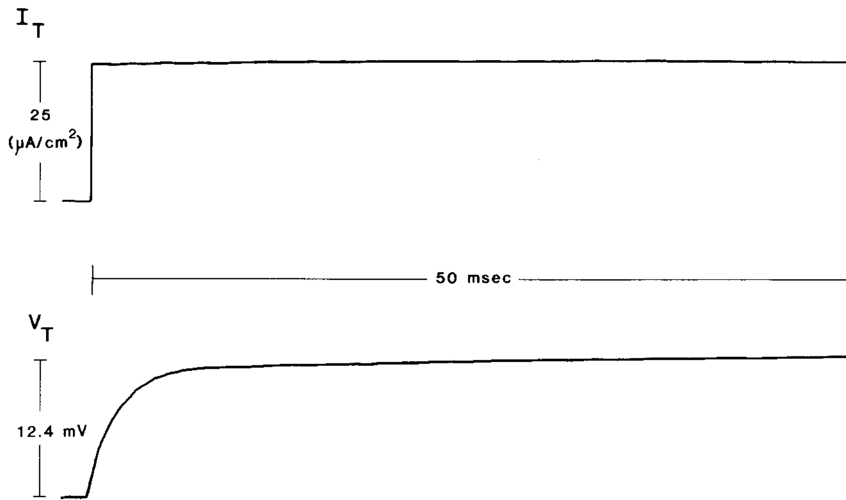

Fig. 2. Voltage displacement of a HS tissue in response to a constant current step of +25 μA/cm^2. The capacitive transient was terminated after about 25 msec. Arrow indicates time of data sampling for the current-voltage relations

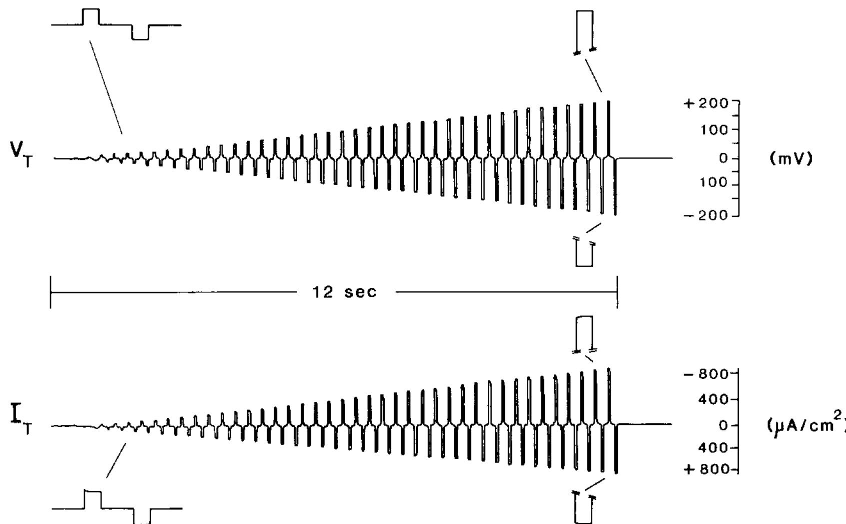

Fig. 3. Original recording of a transapical current-voltage experiment of a HS tissue. Upper tracing shows the bipolar train of command voltages, generated from the computer. Lower tracing shows the correspondent current responses. Traces are enlarged for close inspection of the transients at the beginning and at the end

could occur by the increasing voltage displacements, the program was written to return the command voltage to zero (short-circuit state) between the pulses. In addition alternating command steps were applied (0, +5, 0, −5, 0, +10, 0, −10, 0, . . . +200, 0, −200 mV). The duration of the clamp steps and the zero interval was varied between 20 and 500 msec in order to investigate timing effects on the experiment. Voltage and current were sampled in the last 2 msec before the end of the command step. For the analysis of the data with the constant-field equation we used experiments with a pulse duration of 50 msec and an interval length of 100 msec. The influence of capacitive transients or ion redistributions were minimized by this way, and our measurements therefore done under "near-instantaneous conditions" as defined by Schultz (1979) and Schultz, Thompson and Suzuki (1981).

Our rationale for such a careful exploration of the electrophysiological condition was a preliminary study of our laboratory (Skadhauge et al., 1985). In those experiments we employed a staircase clamp sequence with longer clamp steps (6 sec) where quasi steady state was reached in about 2 sec. Under these conditions we found strong evidence for ion redistribution and artificial high intracellular Na activities.

Experimental Protocol

The experimental protocol was the same for LS, LS-spironolactone, HS, and hormone-treated HS birds. The tissues had a stabilization period with NRS on both sides of approximately 1 hr. Then the serosal side was depolarized with the high-K solution in order to obtain the transapical conditions. After a complete depolarization, which was seen by a stabilization of G_T, I_{sc} and V_T at new levels, each chamber was connected to the fast voltage clamp, the frequency response adjusted and the capacitive transient determined. Then I-V recordings were performed for control conditions, and subsequently repeated after the addition of 50 μM amiloride to the mucosal bath. This amiloride concentration has been shown to block Na transport in this tissue completely (Bindslev et al., 1982) and the I-V recordings under this

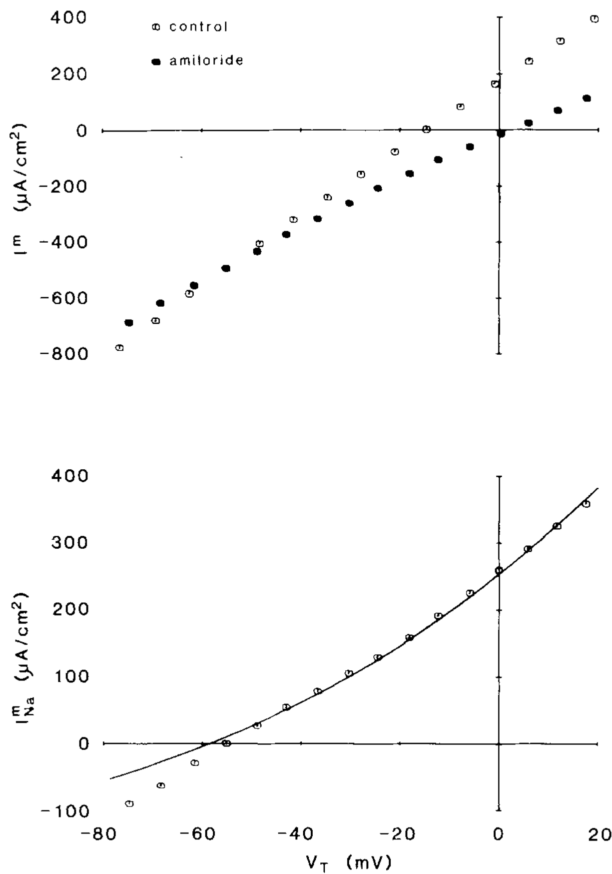

Fig. 4. Example of an apical current-voltage relationship of serosal-depolarized LS-hen coprodeum. Upper curves are *I-V* relations before (○) and after (●) addition of amiloride. Symbols in the lower graph are derived by differences of two upper curves and represent apical Na current. Solid line in lower graph is curve fitted by GHK equation. I^m, current across apical membrane; I^m_{Na}, Na current across apical membrane; V_T, transepithelial voltage under depolarized conditions (assumed to be close to the apical membrane voltage)

condition represented the leak current, caused by other ionic conductances than the Na channels. With our usual command setting (pulse = 50 msec, interval = 100 msec) a complete *I-V* sweep with sampling of 240 values was completed in 12 sec.

Data Analysis

The applied clamp voltages and the corresponding currents were stored on floppy disks. The whole analysis procedure was performed by a series of programs on the computer. First the current values were corrected to a nominal area of 1 cm² and displayed on screen. They were inspected in order to disclose any shift of the zero position (short-circuit state), and thereafter zero values were eliminated, the data rearranged from minimal to maximal voltages and overflows (saturation of clamp level) deleted. Then corresponding *I-V* curves (same pulse setting, recorded before and after amiloride) were paired and stored on disk. The amiloride-sensitive current (= Na current) was computed by subtraction of the corresponding currents at each voltage value. The resulting values represented the *I-V* curve of the ensemble of Na channels in the apical membrane. Figure 4 shows an example of this procedure. This *I-V* curve could be fitted with a nonlinear regression with the Goldman-Hodgkin-Katz constant field equation (Goldman, 1943):

$$I^m_{Na} = \left[\frac{P^m_{Na} \cdot F^2 \cdot V}{R \cdot T}\right]\left[\frac{Na_m - Na_c \cdot \exp(F \cdot V/R \cdot T)}{1 - \exp(F \cdot V/R \cdot T)}\right] \quad (1)$$

and P^m_{Na} and $(Na)_c$ were calculated. A quite excellent fit was obtained in the range between the E^m_{Na} and the intercept of the *y*-axis ($^oI^m_{Na}$). The fitting procedure was displayed on screen and plotted, and the values were stored on disk.

Statistical evaluations of the data were based on Student's *t*-test with a significance level of $P \leq 0.05$. All data are given as mean ± standard error of the mean (SEM), number of animals (*N*) and of tissues (*n*) in brackets, respectively.

Table 1. Mean plasma aldosterone concentrations for the various experimental groups[a]

Treatment	N	Aldosterone (pM)
LS (wheat and barley)	6	596 ± 69
LS spironolactone (70 mg/kg)	4	288 ± 86
LS spironolactone (160 mg/kg)	3	<70
HS	6	<70
HS aldosterone	14	583 ± 172
HS dexamethasone	9	<70

[a] Values >70 pM represent mean ± SEM; values <70 pM were below the limit of detection; *N* = number of animals.

Abbreviations and Definitions

PA—Plasma aldosterone concentration
I_{sc}—Short circuit current (i.e., at $V_T = 0$)
V_T—Transepithelial voltage
G_T—Transepithelial conductance
P^m_{Na}—Apical Na permeability
$^oI^m_{Na}$—Apical Na current ($V_T = 0$)
Na_c—Intracellular Na activity
E^m_{Na}—Apical reversal potential

Results

Plasma Aldosterone Levels

As shown in Table 1 the birds fed with crushed wheat and barley (LS) had significantly higher plasma aldosterone (PA) levels compared with the other treatments except aldosterone. Thomas et al. (1980) showed similar effects. With wheat and barley diet (LS) the PA level in the study from Thomas et al. (1980) reached 265 ± 17 pM. With our dietary

Table 2. Transepithelial potential difference (V_T), short-circuit current (I_{sc}) and conductance (G_T) of isolated hen coprodeum in depolarized Ringer's solution under low NaCl conditions (LS) alone and with spironolactone treatment, and under high NaCl conditions (HS) with and without treatment of aldosterone and dexamethasone[a]

	LS	LS-spironolactone	HS-aldosterone	HS-dexamethasone	HS
n	29	17	18	19	6
V_T:					
NRS	−37.4 ± 2	−33.6 ± 2	−22.7 ± 3[b,c]	−24.2 ± 3[b,c]	0.3 ± 2[b,c,d,e]
DRS	−15.4 ± 1.3[f]	−10.3 ± 0.9[b,f]	−8.2 ± 1.1[b,f]	−7.8 ± 1.3[b,f]	
amiloride	1.2 ± 0.5[g]	1.6 ± 0.3[g]	2.3 ± 0.3[b,g]	1.1 ± 0.5[d,g]	0.4 ± 2
I_{sc}:					
NRS	336 ± 25	284 ± 16	105 ± 12[b,c]	105 ± 12[b,c]	0.0 ± 1[b,c,d,e]
DRS	169 ± 16[f]	89 ± 8[b,f]	43 ± 8[b,c,f]	21 ± 4[b,c,d,f]	
amiloride	−7 ± 3[g]	−9 ± 2[g]	−9 ± 2[g]	−4 ± 2[g]	0.0 ± 1[b,c,d,e]
G_T:					
NRS	8.8 ± 0.4	8.6 ± 0.5	5.0 ± 1.6[b,c]	4.5 ± 0.4[b,c]	2.4 ± 1[b,c,d,e]
DRS	11.3 ± 0.4[f]	9.0 ± 0.5[b]	6.0 ± 0.8[b,c]	4.2 ± 0.3[b,c,d]	
amiloride	7.6 ± 0.3[g]	6.9 ± 0.4[g]	4.8 ± 0.6[b]	3.5 ± 0.2[b]	2.4 ± 1[b,c,d,e]

[a] All values represent mean ± SEM; n = number of tissues. Units are as follows: V_T (mV), I_{sc} (μA/cm^2), G_T (mS/cm^2); NRS = normal Ringer's solution; DRS = depolarizing Ringer's solution; amiloride = NRS with 0.05 mM amiloride in the mucosal solution.
[b] Different from LS.
[c] Different from spironolactone.
[d] Different from aldosterone.
[e] Different from dexamethasone.
[f] Different from NRS.
[g] Different from DRS; $P \leq 0.05$.

regime we obtained a twofold higher PA level. Commercial diet in the study from Thomas et al. (1980) decreased PA levels to 160 ± 37 pM. The commercial diet in their study is comparable with our HS diet, but the concentration of circulating aldosterone was also under the limit of detection in hens with HS diet in our observations. The PA level in hens with dexamethasone injections was under the detection limit. Spironolactone treatment in LS hens resulted in significantly lower PA levels than LS diet alone, whereby in three hens the PA level was so low that no exact data could be obtained. HS birds with aldosterone treatment reached the same PA levels as LS hens.

Transepithelial Properties

LS birds and those pretreated with spironolactone had high potential differences in the nondepolarized state (Table 2). Spironolactone-treated LS birds had lower I_{sc} and G_T than untreated LS hens. There were no remarkable differences in electrical parameters of the two spironolactone-treated groups with high or low PA level. Three injections of aldosterone caused a significant augmented V_T, I_{sc} and G_T in birds fed with HS diet. Results from Thomas et al. (1980) showed a similar increase of electrical properties under aldosterone injections (128 μg aldosterone/kg body weight). Application of aldosterone and dexamethasone in HS birds showed no significant differences in V_T, I_{sc} and G_T between these two groups, but all the electrical properties were significantly lower than the parameters in the LS group. This indicates that the rate of Na transport is highest during endogenous elevated hormone levels. A completely different situation is seen in HS birds without hormonal pretreatment (Table 2). Both potential, short-circuit current, and net Na transport are near zero, as Choshniak et al. (1977) pointed out earlier.

Depolarization

As microelectrode impalements of hen coprodeum mucosal cells have so far been unsuccessful, the approach of the depolarizing technique was at the moment the only possible way to get information of the transapical Na-transporting system of these cells.

Adding the depolarizing solution (DRS) to the serosal side caused a significant decrease in V_T, a significant fall of I_{sc} and a remarkable increase in G_T

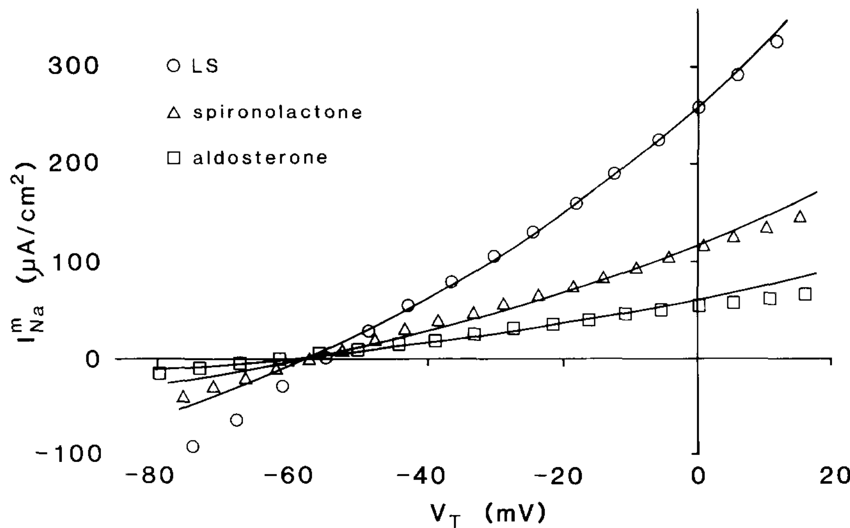

Fig. 5. Transapical current-voltage relations of three representative experiments under LS, LS-spironolactone, and HS-aldosterone conditions. Symbols represent experimentally derived values, and solid lines represent fits of the corresponding *I-V* curves with the GHK equation. Note the excellent fit over the range between the intercepts

in most groups (Fig. 1 and Table 2). The DRS solution seemed to decrease the basolateral membrane resistance as seen by the increase in G_T. This increase is highest in the LS group, smaller in the LS-spironolactone and HS-aldosterone group, and there is even a slight decrease in the HS-dexamethasone group. Possible reasons for this will be discussed later. LS- and spironolactone-treated LS hens even in the depolarized state had higher levels of V_T, I_{sc} and G_T compared to hormone-treated birds.

As described above, HS birds without hormonal supplement showed no amiloride-sensitive Na current. Therefore no depolarization and no investigation of apical *I-V* relations was conducted in this group. In all other groups amiloride caused a rapid decrease of V_T, and I_{sc}, with an evident increase of tissue resistance. This indicates a complete inhibition of the Na current.

Transapical Current-Voltage Relations

As described above the apical *I-V* relation is derived in the depolarized state from the difference between the two current values at each voltage step obtained before and after adding amiloride to the mucosal solution. Figure 4 shows a representative example from one experiment. The two corresponding curves in the upper panel are recorded before (○) and after (●) inhibition with amiloride. The curve in the lower panel was obtained by subtraction of the corresponding values of the two upper curves. In this example E^m_{Na} is 58 mV and ${}^oI^m_{Na}$ is 256 μA/cm². Figure 5 shows the voltage dependence of I^m_{Na} of three representative experiments in the LS, LS-spironolactone and HS-aldosterone groups. Assuming that the apical Na channels in hen coprodeum are highly Na selective, a reasonable good fit is the condition for the estimation of P_{Na} and Na_c. The continuous lines in Fig. 5 are the constant field curves fitted with the GHK equation to the experimental data which yielded values for P^m_{Na} and $(Na)_c$. Mean values are shown in Table 3. The Na reversal potential for LS hens, spironolactone-treated LS hens and aldosterone-treated HS hens were all in the range of 58 to 67 mV without significant differences between these groups but were significantly lower than the E^m_{Na} of dexamethasone-treated HS hens. In some LS experiments we noted a deviation of the actual reversal potential from the GHK fit (*see* Fig. 4). Such a shift of about 4 mV would increase $(Na)_c$ only about 2 mM, and would therefore be in the range of the standard error, and would not significantly influence our finding of a similar $(Na)_c$ in the LS, LS-spironolactone, and HS-aldosterone group (Table 3). The intracellular Na activity was, however, significantly lower in dexamethasone-treated HS hens than in all other groups. In LS birds we obtained the highest ${}^oI^m_{Na}$ values. Aldosterone- and dexamethasone-treated HS hens showed no significant difference in ${}^oI^m_{Na}$. It should be noted that these two groups had significantly lower values than LS spironolactone. The high Na current was correlated with high apical Na permeabilities, almost twice as high in LS birds than in LS spironolactone and three- to fourfold higher than in HS aldosterone or HS dexamethasone (Table 3, Fig. 5). Figure 6 shows a strong linear correlation of Na currents and Na permeabilities throughout the various dietary states and hormonal exposures. A similar linear correlation was found for all groups between the initial I_{sc} and the Na permeability (*data not shown*). This indicates that the relation of Na

Table 3. Electromotive force for Na entry (E^m_{Na}), apical Na current ($^oI^m_{Na}$), intracellular Na activity ($(Na)_c$) and apical Na permeability (P^m_{Na}) in low-NaCl hens (LS) alone and LS spironolactone, and in hens which were fed with a high-NaCl diet (HS), and which were treated with aldosterone or dexamethasone[a]

	n	E^m_{Na} (mV)	$^oI^m_{Na}$ ($\mu A/cm^2$)	$(Na)_c$ (mM)	$P^m_{Na} \cdot 10^{-2}$ (cm/hr)
LS	29	67 ± 4	182 ± 18	10.2 ± 1.3	7.25 ± 0.6
LS spironolactone	17	58 ± 5	99 ± 9[b]	14.6 ± 2.2	4.50 ± 0.4[b]
HS aldosterone	18	66 ± 5	56 ± 10[b,c]	10.7 ± 2.0	2.25 ± 0.4[b,c]
HS dexamethasone	19	86 ± 4[b,c,d]	44 ± 7[b,c]	4.6 ± 0.6[b,c,d]	1.71 ± 0.3[b,c]

[a] All values represent mean ± SEM; n = number of tissues.
[b] Different from LS.
[c] Different from LS spironolactone.
[d] Different from HS aldosterone; $P \leq 0.05$.

transport between the groups was not changed after depolarization.

The exogenous administered hormones (aldosterone and dexamethasone) therefore gave qualitatively the same effects on the apical membrane properties, but the endogenous stimulation, caused by the low-NaCl diet, had by far the highest stimulatory effect on apical membrane properties. Spironolactone decreased P^m_{Na} partially. The intracellular Na activity was similar in all groups, except the dexamethasone group, where it was significantly lowered.

Because coprodeal epithelia under HS conditions exhibit no Na current and are poorly amiloride-sensitive (Thomas et al., 1980) it was not possible to apply our method to this dietary condition and to obtain permeability measurements for low Na-transporting coprodeal epithelia (Bindslev, 1979).

Discussion

Endogenous Aldosterone Level

As discussed previously (Thomas et al., 1980; Skadhauge et al., 1983; Arnason et al., 1986), the concentration of plasma aldosterone (PA) is high in low-NaCl fed birds and decreased by resalination. The spironolactone dose in our study lowered PA level in one group of LS hens to about half the control values; in the second group with the higher spironolactone dosage, the PA level was below the limit of detection. This indicates that high doses of spironolactone may have had pharmacological effects and may have partially or totally inhibited aldosterone synthesis in the adrenal gland, as found by Conn and Hinerman (1977).

HS hens treated in vivo with aldosterone showed PA levels indistinguishable from birds with dietary Na depletion. Regarding the spironolactone effect at the receptor level, a comparison between LS hens and spironolactone-treated LS hens shows a reduced I_{sc}. This indicates that spironolactone may have partially blocked Na transport at the receptor level. It is, however, not possible to draw conclusions about the degree of inhibition, because this depends on the receptor occupancy which is unknown. Furthermore no spironolactone was added *in vitro* during the experiment to the bathing solutions. As spironolactone has a relatively poor affinity for type I or type II binding sites, compared to aldosterone and dexamethasone, it is quite possible that the antagonist effect has been partly washed away. Injection of dexamethasone had no effect on PA level, but showed the same increased V_T and I_{sc} values as in aldosterone-treated HS birds. The latter ones had undetectable low aldosterone concentration which is consistent with the low electrical parameters of V_T, I_{sc} and G_T. In conclusion, plasma aldosterone concentration is an indication of the hormonal status, but gives no exact information or prediction about the magnitude or mechanism of Na absorption in hen corprodeum under various experimental conditions.

Our findings could be explained by the existence of two different binding sites, one for glucocorticoids (dexamethasone) and one for mineralocorticoids (aldosterone), as has been shown for the rabbit colon (Marver, 1984), and for rat kidney (Funder, Feldmann & Edelman, 1973). Sandor et al. (1986) have recently established these two receptor systems in the avian intestine. There is, however, also the possibility of crossover binding of corticosteroids to the type I receptor, and thereby exerting their stimulatory effect indirectly through the aldosterone system. It is not possible and was not the aim of our electrophysiological study to clarify this point, as this would require receptor binding studies

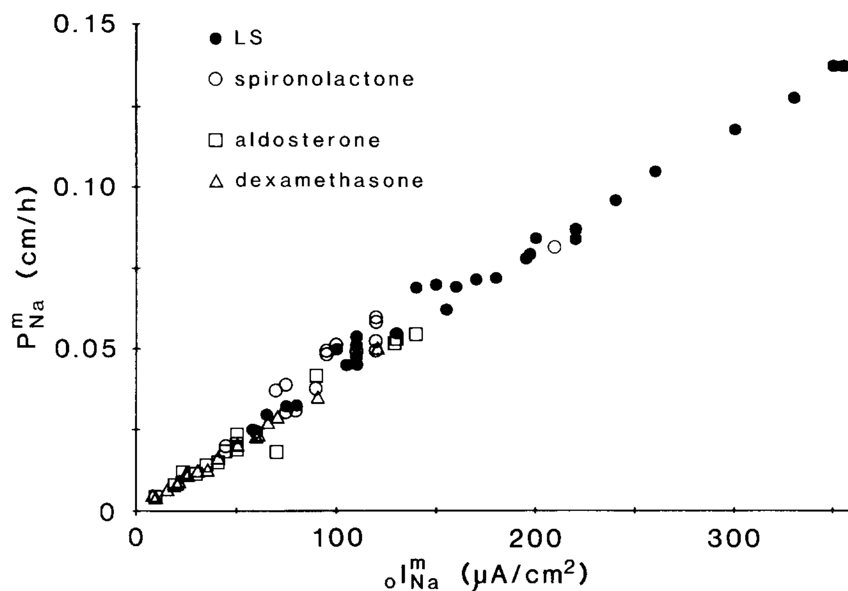

Fig. 6. Values for apical Na permeability (P^m_{Na}) are plotted versus apical sodium current (${}^oI^m_{Na}$) for the different experimental groups. Note that all groups fit into the same linear relationship

with the appropriate hormonal dosages. Recent studies, however, have clearly shown specific and differential effects of glucocorticoids on colonic membrane properties (Sellin & DeSoignie, 1985), transepithelial resistance (Clauss et al., 1985*b*), basolateral NaK-ATPase (Charney et al., 1975; Will et al., 1981), apical Na permeability (Thompson & Sellin, 1986), as well as evidence about a conformational change of the apical Na channels by glucocorticoids (Thompson & Sellin, 1986). Obviously, dexamethasone has an effect on sodium absorption itself, and in our study its action is different from that in rabbit colon, where in addition to the stimulation of apical Na permeability it mainly increases G_T (Dürr & Clauss, 1984; Marver, 1984; Clauss et al., 1985*b*).

Stimulation and Inhibition of Na Transport in Hen Coprodeum

In hen coprodeum the amiloride-sensitive short-circuit current is, as in rabbit distal colon (Frizzell & Turnheim, 1978), in excellent agreement with net sodium absorption. This was shown for LS and HS birds and hormone-treated HS birds (Choshniak et al., 1977; Skadhauge, 1983).

In HS birds without hormonal supplement the Na current was negligible. Under hormonal treatment, however, an augmented Na current emerged which was completely inhibited by amiloride. Thus the increases of V_T, I_{sc} and G_T were induced either with LS diet or the treatment with one of the two steroids, aldosterone or dexamethasone.

The effects of in vivo injections of aldosterone on Na absorption depend on the time-course of the application. Clauss et al. (1984) found a maximal stimulation of the I_{sc} after 4 hr p.i. It is well known that a change from HS to LS and vice versa is accompanied by an alteration in I_{sc} and plasma aldosterone concentration (PA) (Thomas et al., 1980; Thomas & Skadhauge, 1982; Skadhauge et al., 1983; Arnason et al., 1986). From LS to HS treatment, I_{sc} and PA decreased within 16 to 24 hr after resalination most likely by suppression of aldosterone release (Thomas & Skadhauge, 1982). In contrast NaCl depletion (LS) augmented the I_{sc} to maximal values only within eight days. Our attempt to reduce this maximal value of LS hens with the aldosterone antagonist spironolactone had no significant effect on the transepithelial parameters in the undepolarized tissue, although I_{sc} was conspicuously reduced. Our spironolactone dosage, however, was only 25% of the dosage at which Charney et al. (1981) found a preventive effect on DOCA treatment in rat colon. We note that spironolactone in our study obviously affects the apical membrane predominantly, because it is unmasked in the depolarized tissues. Under these experimental conditions, we clearly can see a significant reduction of I_{sc} and a decrease in G_T (Table 2), as well as significant reductions of ${}^oI^m_{Na}$ and P^m_{Na}.

Near-Instantaneous *I-V* Relations

The present study is, to the best of our knowledge, the first examination of *I-V* relations in hen coprodeum. Application, calculation and meanings of *I-V* relations have been extensively discussed elsewhere (Palmer, Edelman & Lindemann, 1980; Schultz et al., 1981; Palmer, 1984; Schultz, 1984).

Our attempts to impale the coprodeal cells with a microelectrode have so far been unsuccessful. Therefore the approach of the depolarizing technique was the only possible way to gain some insight in the transcellular Na-transporting system of these cells. Because it was the first application of the method in this tissue, we must bear in mind the possibility of an incomplete depolarization. Although Fig. 1 shows a decrease of V_T and a new steady state, we note an intermediate rise which points to secondary effects. There is evidence, however, from other tissues, that the basolateral membrane is depolarized to a considerable extent (Lewis, Wills & Eaton, 1978; Thompson, Suzuki & Schultz, 1982; DeLong & Civan, 1984; Tang et al., 1985; Klemperer et al., 1986). The marked increase in tissue conductance in the LS group (Fig. 1) provides additional evidence for a marked reduction of the electrical properties of the basolateral membrane. It may be possible that the hormonal treatment also affected the conductance properties of the basolateral membrane, because of the smaller change in G_T after depolarization in these groups (Table 2).

In preliminary studies we evaluated steady-state *I-V* relations with long clamp steps of 6 sec per pulse and not in a bipolar manner. In those experiments we found similar values of $^{o}I^{m}_{Na}$ for LS hens (104.5 ± 12.9 μA/cm^2). Due to the long step duration and the starting point at the highest positive clamp voltage we observed high intracellular Na activity of 27 to 42 mM Na (Skadhauge et al., 1985). This indicates that Na can be accumulated in the cell subsequently to ionic redistribution by the increased electrical driving forces across the apical cell membrane. Too short pulse duration is also not appropriate, because it is necessary that the ionic profile in the membrane and the capacitive transients should be terminated (Garcia-Diaz & Essig, 1985; Schoen & Erlij, 1985). With out improved equipment applied in this study with short (50 msec) and bipolar pulses and under the control of a storage oscilloscope we evaluated $(Na)_c$ of 10.2 to 14.6 mM in LS hens, spironolactone-treated LS and aldosterone-treated HS hens. These values are in quite excellent agreement with studies on rabbit colon (Thompson et al., 1982), guinea pig colon (Clauss et al., 1985*a*) and several amphibian epithelia (Helman, O'Neil & Fisher, 1975; Fuchs et al., 1977). In contrast, dexamethasone-treated HS birds had a significantly lower intracellular Na activity. Studies on rabbit colon report a decreasing $(Na)_c$, when the apical Na permeability is stimulated by decreasing the luminal Na concentration (Turnheim, Thompson & Schultz, 1983). This negative feedback inhibition has been demonstrated for large intestinal epithelia under control conditions, but not yet for hormone-stimulated tissues. In contrast, Thompson and Sellin (1986) found in rabbit distal colon after stimulation with methylprednisolone an increased $(Na)_c$.

Apical Na-Entry Step

The Na current and Na permeability are strongly correlated (Fig. 6) for all experimental groups. This indicates that the Na current is not regulated at the basolateral membrane, but depends on the magnitude of conductance at the apical membrane, and that the Na current seems to be regulated in all groups by the same system. Similar linear correlations have been found by Palmer et al. (1982) and Thompson and Sellin (1986).

Studies in urinary bladder (Frömter, Higgins & Gebler, 1981; Palmer et al., 1982) described a multiple site effect of increased apical Na permeability in consonance with intensified pump activity at the basolateral membrane. This multisite mechanism allows a near constant $(Na)_c$ level throughout the dietary or hormonal treatment. Such a mechanism could also explain our findings of a constant $(Na)_c$ after aldosterone stimulation.

It is known from studies on amphibian epithelia (Li et al., 1982; Palmer et al., 1982) that NaCl-conserving hormones (oxytocin and aldosterone) increase the apical Na permeability by recruitment of Na channels from a pre-existing pool of electrically silent channels. Evidence for such a pre-existing pool of Na channels has been found for hen coprodeum by Cuthbert et al. (1982). Bindslev (1979) proposed, and Christensen and Bindslev (1982) showed that NaCl depletion as well as aldosterone injection in NaCl-loaded hens induced new transport sites at the luminal membrane. Eldrup, Mollgard and Bindslev (1980) found in an electron-microscopic study under dietary stimulation rod-shaped particles in the apical surface.

Due to the enhanced P^{m}_{Na} for hormone-treated HS hens we propose a direct effect of aldosterone and dexamethasone on apical Na channels. Spironolactone seems to be only a weak aldosterone antagonist in hen coprodeum. In contrast, other studies report an agonizing effect of spironolactone on aldosterone (Sakauye & Feldman, 1976). This presumably is the reason for the high potential values which are overlapping the results of untreated LS birds. Our values for apical Na permeability in hormone-treated HS birds are in good agreement with Na permeabilities observed by Dürr and Clauss (1984) in rabbit distal colon under aldosterone (0.02 ± 0.001 cm/hr) and dexamethasone stim-

ulation (0.03 ± 0.002 cm/hr). But as pointed out elsewhere (Thomas et al., 1980; Clauss et al., 1984) our data confirm that exogenous applied steroids could not fully mimic the large endogenous stimulatory effect of a chronic LS diet, and attribute this incomplete action to the apical membrane.

Conclusion

Aldosterone and dexamethasone therefore seem to be mediators rather than complete effectors of the stimulation of Na transport in hen coprodeum. The failure of these hormones to induce within 24 hr similar increases in apical Na permeability as in the chronic adapted LS tissue shows that there may be two phases of hormonal action: First an immediate increase in apical Na permeability of about 20%, and then in a slower secondary phase a further increase to the LS values. So far we cannot distinguish whether these two phases take place in one cell population, or if the secondary permeability increase results from a differentiation of additional cells. Regardless of the possibility of crossover binding, glucocorticoids cause compared to aldosterone, a lower transport rate, a smaller P^{m}_{Na}, and a lower intracellular Na activity. The suppression of PA level and the partial inhibition of Na transport by spironolactone, reveals antagonist effects on aldosterone biosynthesis and at the cellular receptor level. From these results we conclude on major regulatory effects of adrenal steroids on Na transport in this tissue. This study focussed on the apical membrane. It remains to be elucidated in further studies how the other properties of the coprodeal cells respond to the hormonal and dietary stimulus.

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