A Low-Salt Diet Facilitates Cl Secretion in Hen Lower Intestine

Wolfgang Clauss†, Vibeke Dantzer‡, and Erik Skadhauge§
Institut für Veterinär-Physiologie†, Freie Universität Berlin, D-1000 Berlin 33, Federal Republic of Germany and Departments of Animal Physiology and Biochemistry§ and of Anatomy‡, The Royal Veterinary and Agricultural University, DK-1870 Frederiksberg C, Denmark

Summary. The regulation of sodium and chloride transport in hen coprodeum by mineralocorticoids was investigated with isolated epithelia under short-circuit conditions. Unidirectional fluxes of Na and Cl were measured by isotopes and modulated by amiloride, theophylline and bumetanide. Hens were maintained either on low-NaCl diet (LS) or on high-NaCl diet (HS). Plasma aldosterone (PA) levels of these groups were measured with radioimmunoassay. A group of HS hens received injections of aldosterone on a 6-hr schedule before experiments. Another group of LS hens was resalinated, and experiments carried out on a 24-hr interval.

Salt deprivation stimulated PA levels ninefold, compared to HS hens. Na absorption was stimulated according to previous reports. Electrogenic Cl secretion was elicited by theophylline and partially inhibited by bumetanide. Modulation of PA levels by diet, resalination or aldosterone injection changed the magnitude of electrogenic Cl secretion in parallel between 0.5 μeq/cm² · hr (HS) and 4 μeq/cm² · hr (LS), with pronounced alteration in tissue resistance.

The results demonstrate a new action of aldosterone which besides stimulating Na absorption also directly or indirectly elicits Cl secretion. Evidence is presented for a hormonal adaptation of chloride transport in this epithelium. There was a morphological change of the apical plasma membrane and further experiments will have to clarify the exact cellular nature of this process.

Key Words Aldosterone · hen · large intestine · chloride · electrogenic secretion · in vitro · adaptation

Introduction

The modulation of colonic electrolyte transport by aldosterone has been investigated mainly in mammalian tissues (cf. Fromm & Hegel, 1978; Lückhoff & Horster, 1984; Binder, Foster & Hayslett, 1985). It was found that aldosterone stimulated sodium absorption and potassium secretion. Effects on chloride transport have not been reported. In recent years Skadhauge and co-workers (cf. Skadhauge et al., 1985) have conducted a number of studies on the regulation of colonic electrolyte transport in the avian lower intestine. This organ showed a pronounced dependence of sodium absorption on dietary salt intake, mediated by adaptation in plasma aldosterone concentration (Thomas & Skadhauge, 1982). Hen lower intestine is segmented into colon and coprodeum (cloaca). These segments have different modes of electrolyte transport and undergo different modulation by aldosterone. Hen coprodeum is known as one of the most aldosterone-sensitive epithelia in the vertebrate phylum (Skadhauge, 1984). NaCl deprivation or aldosterone injections modulate sodium transport across this epithelium between zero and 14 μeq/cm² · hr. The effect of external aldosterone decayed substantially over five days when the dietary intake was switched from a low to a high level of NaCl (Clauss et al., 1984).

The regulation of colonic chloride transport is less well known. Similarly to Na transport, most of the knowledge about Cl transport has been obtained from studies on mammalian intestine (Frizzell, Field & Schultz, 1979). It is generally agreed that chloride absorption occurs electroneutral in exchange with bicarbonate, and that chloride secretion occurs electrogendly by distinct cells, located in the colonic crypts (Welsh et al., 1982; Halm & Frizzell, 1986). Such an electrogenic Cl secretion has also been found in hen colon (Loennroth & Munck, 1980; Andersen, Munck & Skadhauge, 1982; Voldsgaard & Bindslev, 1982; Munck, Andersen & Voldsgaard, 1984), but has not yet been demonstrated in hen coprodeum. Choshniak, Munck and Skadhauge (1977) have investigated NaCl transport across this epithelium and found almost unity of the unidirectional Cl fluxes, but have not probed on eliciting electrogenic Cl secretion.

Our study was designed to measure the regulation of Na- and Cl-transport in hen coprodeum under various dietary and hormonal states. We were
able to confirm previous findings about the hormonal regulation of Na transport (Thomas & Skadhauge, 1982; Clauss et al., 1984), and extended our investigation to the hormonal regulation of Cl transport across this tissue. Our aim was first to probe whether electrogenic Cl secretion can be elicited in hen coprodeum. Secondly, we aimed at investigating the dietary and hormonal influences on the regulation of coprodeum Cl transport. Thirdly, after having found profound alterations in Cl secretion, we aimed at tracing the epithelial and cellular site of Cl secretion, e.g., to tackle the question if distinct cells are involved in either Na- or Cl-transport (Langridge-Smith, 1985, 1986).

Our study shows that aldosterone and dietary manipulations modulate sodium absorption and electrogenic Cl secretion profoundly. This is correlated to changes in the apical plasma membrane, and the occurrence of special “Cl cells.” Therefore adaptational processes may be involved in the hormonal regulation of NaCl transport across this epithelium.

**Materials and Methods**

**ANIMALS**

White Plymouth Rock laying hens, weighing 3.3 to 4.5 kg, all from the same batch were kept in two groups on either a low-NaCl diet (LS) or on a high-NaCl diet (HS) ad libitum. The LS diet consisted of a low NaCl-balanced ration (wheat, barley and soya with added vitamins and minerals) and demineralized water. The HS diet consisted of a high NaCl-balanced ration (low-NaCl ration with 1% NaCl wt/wt added) and 0.5% NaCl (wt/vol) drink. The detailed compositions of the rations are given by Skadhauge et al. (1983). The animals were adapted to these diets at least three weeks prior to the experiment. For resalination, hens which had been on LS diet received an initial oral load of 10 mL 0.75 M NaCl/kg body weight, and were then continued on the HS diet.

**ALDOSTERONE INJECTIONS AND BLOOD SAMPLING**

d-aldosterone (a kind gift of Ciba Geigy, Basle, Switzerland) was used in a dosage of 128 μg/kg body weight. Single injections were given at 6-hr intervals for the last 24 hr, with the last injection exactly 4 hr prior to the experiment. All injections were given intramuscularly and the hens remained in their cages until experiment. Blood samples were obtained by heart puncture immediately before decapitation, and plasma aldosterone was determined by radioimmunoassay as described by Arnason et al. (1986).

**ELECTRICAL MEASUREMENTS**

For the experiment the hens were killed by decapitation. The coprodeum was taken out from the abdomen and a stripped preparation was obtained by dissecting the muscular layers with fine forceps and tweezers. This method of preparation has been established in several previous studies (Choshniak et al., 1977; Thomas & Skadhauge, 1982; Clauss et al., 1984). Up to six coprodeal preparations were mounted in Ussing chambers and incubated simultaneously in a circulating standard Krebs-phosphate buffer (values in mmol/liter): 140 Na, 8 K, 2.6 Ca, 1 Mg, 139 Cl, 1 SO4, 8 phosphate, 15 glucose, with a pH of 7.3 aerated with pure O2, and maintained at 38°C. The Ussing chambers had an opening area of 0.62 cm2 and a soft O-ring on the mucosal side to minimize edge damage. For the electrical measurements 3 m KCl-agar bridges were connected to a multichannel, computer-controlled voltage clamp. The 3 m KCl did not significantly increase the bath K concentration after a 2 to 3-hour incubation period. The tissues were continuously short-circuited with current passed through Ag/AgCl electrodes. The computer (IBM PC) was relayed to the voltage clamp by an analog-digital and a digital-analog interface with a 12-bit resolution (IBM DACA). A computer software was developed which permitted a time-sharing on-line control and data acquisition of the six voltage clamps. The computer measured the short-circuit current (Isc). The Isc was reckoned positive when current flowed from the mucosal to the serosal side. For the determination of the tissue resistance (Rr) the computer posed bipolar voltage command steps (usually ±10 mV amplitude and 300 msec duration) to the voltage clamp. Resulting clamp voltage (ΔV command) and current deflections (ΔIsc) were sampled 10 msec before offset of the pulses. The current transients were regularly inspected on an oscilloscope to avoid interference with membrane time constants or secondary ion rearrangements. Tissue resistance was calculated according to

\[ R_r = \Delta V \text{ command/} \Delta I_{sc} (\Omega \cdot \text{cm}^2). \] (1)

In order to eliminate any effects of a possible rectification, the positive and negative current deflections were pooled, and the mean deflection was used for the calculation. The theoretical open-circuit potential difference (Vr) was then calculated according to Ohm’s law:

\[ V_r = R_r \cdot I_{sc}(\text{mV}). \] (2)

Vr was calculated with the serosal side as reference. Hence it was negative when Isc was positive and current (Na absorption) flowed from the mucosal to the serosal side. The computer controlled the six chambers independently in a time-sharing mode and corrected the values for series resistance, which was measured before mounting of the tissue. Vr, Isc and Rr were then tested on a two-channel chart recorder (Kipp and Zonen BD 9).

**EXPERIMENTAL PROTOCOL**

After an initial period of stabilization of the short-circuit current, 0.1 mM amiloride (Merck, Sharp & Dohme) was added to the mucosal side, to block Na absorption and Isc totally. After a short time period of about 4 min, 7 mM theophylline (Sigma) was added to both sides, with amiloride still present. Theophylline is known to stimulate electrogenic Cl secretion and therefore to increase the Isc (Frizzell et al., 1979). After onset of stimulation and a stabilizing period of about 16 min, 0.1 mM bumetanide (Leo Pharmaceuticals) was added to the serosal side with the other drugs...