Sodium Transport and Electrical Properties of the Chick Chorioallantoic Membrane

J. PÁCHA, E. UJEC, M. POPP and K. ČAPEK

Institute of Physiology, Czechoslovak Academy of Sciences, Vídeňská 1083, 142 20 Prague, Czechoslovakia

Abstract. Transport and electrical properties of the chick chorioallantoic membrane (CAM) were studied in order to find the osmoregulatory organ which helps to compensate the renal filtration-reabsorption disbalance of chick embryos. It could be shown that CAM resembles Na^+ transporting epithelia in that active Na^+ absorption is responsible for the potential difference and short circuit current, which could be abolished by ouabain on the ectodermal and amiloride on the endodermal side. The transepithelial conductance rose with increasing sodium concentration in accordance with the Michaelis-Menten kinetics. The allantoic sac thus plays a role similar to the toad urinary bladder despite the low potential difference and resistance which indicate that CAM is a leaky epithelium. CAM is therefore not only a respiratory but also an osmoregulatory organ.

Key words: Chorioallantoic membrane — Sodium transport — Electrical properties — Chick embryo

Introduction

During embryogenesis, the chick embryo loses salt and water because of a renal filtration-reabsorption disbalance. The glomerular filtration rate is higher and the tubular reabsorption lower than in the adult kidney (Pácha et al. 1982). The excreted hypotonic urine is accumulated in the allantoic sac up to a maximal volume of 7—8 ml on the 13th day of incubation (Freeman and Vince 1974). Thereafter, water is removed from the allantoic fluid down an osmotic gradient (Stewart and Terepka 1969) as it is the case in the toad urinary bladder. The hypotonicity of urine is maintained by the removal of sodium and chloride from the allantoic fluid (Stewart and Terepka 1969; Doneen and Smith 1982). In addition to these similarities between the bladder and the allantoic sac, Hoyt (1979) observed that the chick embryo *in ovo* can protect itself against excessive water loss by shifting fluid from the allantois to the embryo, which may be caused by

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arginine vasotocine (Murphy et al. 1981). Coleman and Terepka (1972) also found a striking histological resemblance between the allantoic epithelium and the toad bladder.

These functional and microscopic studies indicate that the allantoic sac possesses similar transport characteristics as the urinary bladder, colon and cloaca, i. e. sodium transporting organs developed from the same germ layer (hindgut endoderm). The present study was therefore undertaken in order to explore the electrical and transport properties of the chorioallantoic membrane (CAM)[1] which is formed by the fusion of the chorion and allantois.

Materials and Methods

Eggs from White Leghorn hens were used in all experiments. The eggs were incubated in an incubator at 37.5 °C, 60—70% relative humidity, and were turned twice daily. The CAMs used in this study were isolated from eggs between the 13th to 18th day of incubation cutting out a circular segment of CAM under the air space together with acellular shell membrane. The segments were then mounted into an Ussing chamber. To prevent edge damage and to obtain good electrical sealing the inside faces of both half chambers were covered with a layer of silicone grease and gently pressed. The exposed surface area of CAM was 0.78 cm^2 or 0.38 cm^2. The Ringer solution used had the following composition (in mmol/l): NaCl 106; $NaHCO_3$ 25; KCl 6.0; $MgSO_4$ 1.2; $CaCl_2$ 1.0; Na_2HPO_4 1.0; glucose 10; it was bubbled with 95% O_2 and 5% CO_2. Ouabain (Sigma), amiloride (Merck, Sharp, Dohme) and potassium cyanide (Labora) were dissolved in Ringer solution and added to the ectodermal or endodermal media to final concentrations of 10^{-4} or 10^{-3} mol/l. All experiments were performed at 37 °C.

The transepithelial potential difference (*PD*, ectoderm grounded) and the short circuit current (*SCC*) were measured with a voltage clamp device (Fig. 1) using saturated calomel half cells, connected to the fluid compartments through agar bridges and Ag/AgCl electrodes immersed directly into the bathing solution. The electrical resistance was calculated from the slope of current-voltage relationship (*I—V* curves) generated by stepwise voltage clamping and measuring the corresponding current or from changes in *SCC* during square wave voltage pulses of 1 mV and 10 s duration. In the former experiments the CAM was under open circuit conditions till measuring *I—V* relationship, in the latter the CAM was continually short-circuited. The transepithelial resistance was estimated by subtracting the solution resistance between agar bridges from the total resistance (R_m, Fig. 1) measured.

The capacitance was determined by the application of repetitive constant current pulses (10 μA, 4 ms) across the epithelium. The current pulse and time-dependent voltage trace were displayed on a Tektronix D 12 oscilloscope for measurement. The capacitance was computed according to the equation $C_t = \tau / R_t$, where C_t is the capacitance in $\mu F/cm^2$, τ is a constant calculated as the time in ms to achieve $100 \cdot (1 - e^{-1}) = 63$ % of the steady-state voltage, and R_t the resistance in kΩ.

[1] Abbreviations used: CAM chorioallantoic membrane, *SCC* short circuit current, *PD* potential difference

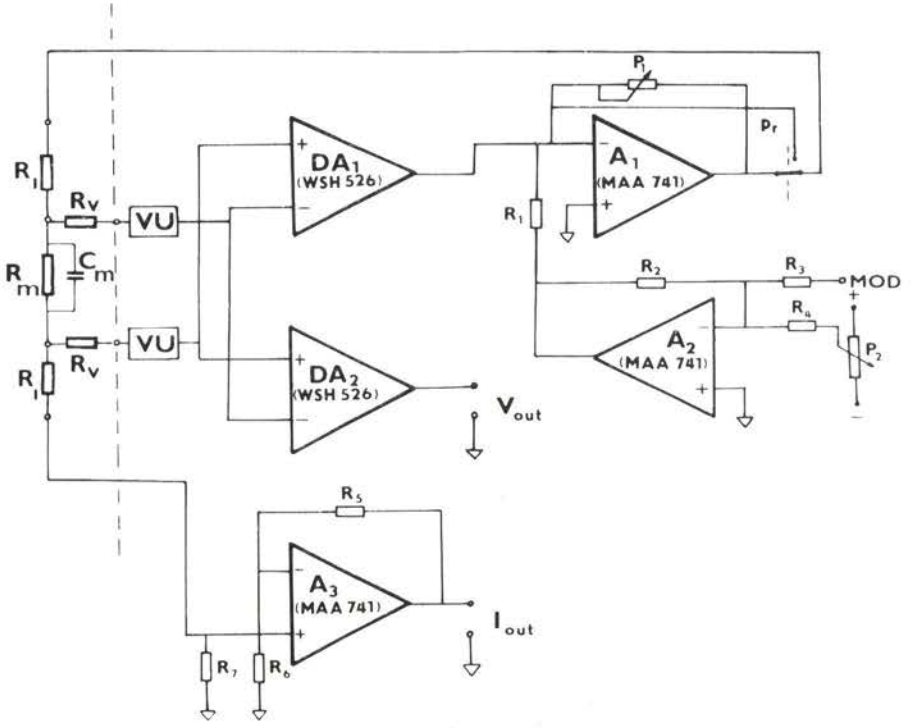

Fig. 1. The principal layout of a four-electrode voltage-clamp circuit. Hybrid and integrated circuits (Tesla) were used. Their type numbers are given in parentheses. The circuits DA_1 and DA_2 serve as input differential amplifiers with high input resistances. Voltage course of transepithelial voltage difference (*PD*) can be recorded on DA_2 output. Circuit A_1 is used as a feed-back amplifier with adjustable amplification (P_1) in connection with DA_1, and with A_2 as an amplifier for holding potential control. The parameters of the short circuit current (*SCC*) are recorded by the amplifier A_3. Two flowing voltage supply units (VU) serve to eliminate potentials of the voltage electrodes e. g. for adjusting the basic level. The circuits left to the broken line diagrammatically show the principal arrangement of the inputs of the four-electrode voltage-clamp; R_e represents the resistances of current electrodes; R_v are the resistances of voltage-recording electrodes. The epithelial membrane is represented by the equivalent RC circuit (R_mC_m).

The above set-up was used to measure *PD* and *SCC* and to estimate R_t and C_t (for details see text).

Results

When mounted as described above and exposed on both sides to the same Ringer solution, CAM generated an endoderm-negative *PD*. The values varied between

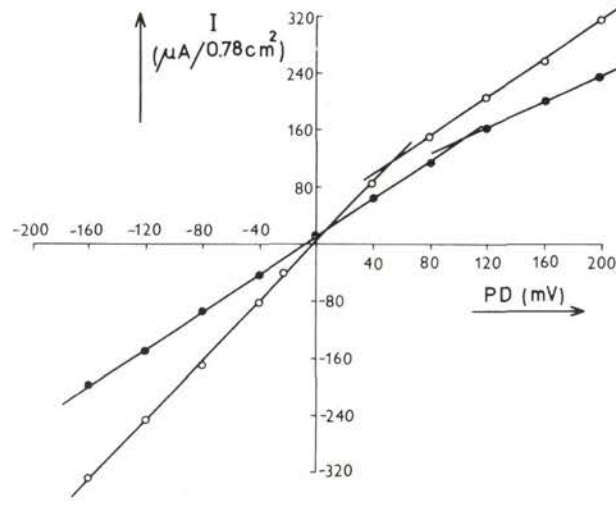

Fig. 2. A typical current-voltage relationship for two chorioallantoic membranes. Data were obtained by stepwise voltage clamping (5s) and measuring the corresponding current. Deviation from linearity at higher voltages indicates increased resistance.

−2.9 and −12.5 mV, the mean of 19 CAMs was -5.3 ± 0.5 mV. The corresponding value of SCC varied between 8.7 and 22.5 $\mu A/cm^2$, the mean value was 18.6 ± 1.0 $\mu A/cm^2$, i. e. an ion net flux of 0.69 $\mu mol/cm^2/h$. Both these parameters were always small at the beginning of an experiment, but increased steadily and reached the values as above. This initial rise may reflect "sealing" of the preparation because a concomitant increase of the electrical resistance between both compartments of the chamber was observed.

The transepithelial resistance of the short circuited CAM in the steady state, determined by measuring the current deflection caused by rapidly clamping the tissue from 0 mV to −1 mV, reached a mean value of 277 ± 28 Ω/cm^2 (conductance 3.6 mS/cm^2, $n = 19$). This procedure was justified as the current-voltage relationship was linear within the ranges of currents and voltages employed. This relationship of CAM in open circuit conditions (two representative ones are presented in Fig. 2) is quite linear over a significant range of *PD*, but then the slope changes to indicate a higher resistance. The mean value of the lower slope resistance determined from these *I—V* curves was 352 ± 33 Ω/cm^2 (conductance 2.8 mS/cm^2, $n = 16$), and differed significantly from the resistance measured in the short-circuited state ($P < 0.05$). The capacitance was calculated according to the equation $C_t = \tau/R_t$ (for details see Materials and Methods). The voltage time course following a square constant current pulse fits approximately a single exponential and corresponded to a tissue capacitance of 0.6 ± 0.2 $\mu F/cm^2$, ($n = 4$).

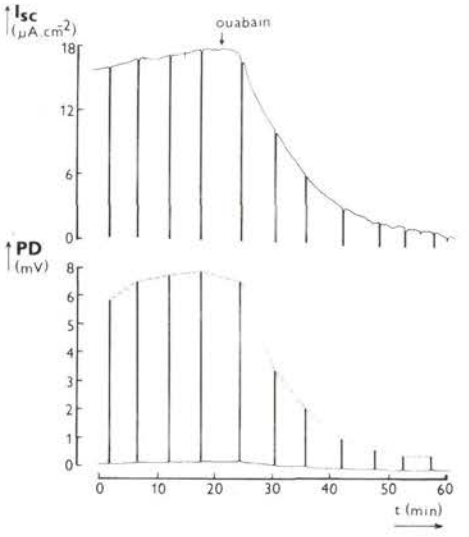

Fig. 3. The effect of ouabain on the short circuit current and potential difference of the chorioallantoic membrane (10^{-4} mol/l, ectodermal solution). A tracing of an original short circuit current recording is given. The membrane was continuously short circuited, except when the external circuit was opened in order to provide measurements of the instantaneous transepithelial potential difference.

To identify the ions responsible for *PD* and *SCC*, we studied four phenomena: the effects of potassium cyanide, ouabain, amiloride and ion substitution on *SCC*, *PD* and transepithelial conductance, respectively. After poisoning the metabolism with cyanide (10^{-3} mol/l in both compartments), *PD* and *SCC* dropped towards zero with a half-time ($t_{1/2}$) of 4.5 min. This experiment demonstrated the significance of the low PD and active ion current. The polarity of the active transport potential indicated that there was an active cation absorption from the allantois or anion secretion into it. The question of whether CAM is capable of transporting Na^+ ions actively was further examined by applying ouabain, a specific inhibitor of active Na^+ transport. The addition of ouabain into the ectodermal compartment (10^{-4} mol/l), resulted in acute depolarization and inhibition of *SCC* ($t_{1/2} = 15$ min). The original recording is reproduced in Fig. 3. Amiloride, a specific blocker of sodium pores and the Na^+—H^+ exchange system, affected CAM in the same manner as ouabain, but considerably more rapidly and only from the endodermal side. The effect of a high concentration of amiloride (10^{-4} mol/l) had a half-time $t_{1/2} < 1$ min and about 90% of *SCC* were inhibited.

CAM thus seems to transport sodium actively from the endoderm to the ectoderm and this was further supported by the finding of a close correlation between the transepithelial conductance and sodium concentration (Fig. 4). The CAM was initially left in 150 mmol/l NaCl plus substrate until the conductance had

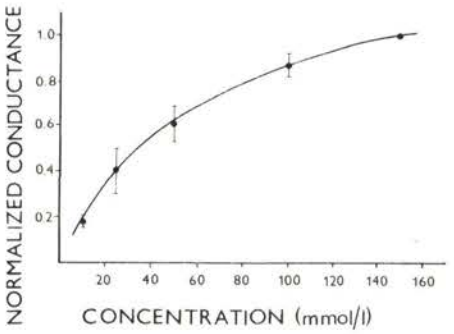

Fig. 4. Normalized conductance as a function of Na^+ concentration; Na^+ concentration was changed by symmetrical isoosmotic replacement with mannitol. For details see the text.

achieved a steady-state value. NaCl concentrations in both compartments were then lowered symmetrically and simultaneously by isoosmotic replacement with mannitol. Since the control conductance $(G_t)_c$ varied between individual preparations, the experimental conductance $(G_t)_e$ was adjusted to the control conductance by calculating a normalized value $(G_t)_e/(G_t)_c$. As shown in Fig. 4, the normalized conductance rose with the increasing sodium concentration in accordance with the Michaelis-Menten kinetics. In experiments in which the bathing solution contained 10^{-3} mol/l potassium cyanide, the dependence of the conductance on NaCl concentration was approximately linear.

Discussion

Even though numerous morphological and functional findings indicate a similarity between CAM and the toad bladder (Coleman and Terepka 1972; Hoyt 1979; Doneen and Smith 1982), our electrophysiological data show some differences according to the presently accepted criteria of Frömter and Diamond (1972). CAM seems to be a leaky epithelium, although not so leaky as the proximal tubule or the gallbladder. On the contrary, the toad bladder is typically tight. The low values of *PD* (−5.3 mV) recorded between symmetrical bathing solutions may thus arise from shunting CAM by low resistance junctions. As the epithelial cell membranes generally have resistances approaching several thousands of ohms (Frömter and Diamond 1972), the transepithelial resistance of CAM mainly represents the resistance of paracellular electrical shunts.

The capacitance of 0.6 $\mu F/cm^2$ is also similar to the value found in other leaky epithelia (Bindslev et al. 1974), but the non-linear current-voltage relationship seems analogous to that found in tight epithelia (Frömter and Diamond 1972). Our data points can be fitted into two regions of a linear slope resistance as in the frog skin (Helman and Fisher 1977) and the toad bladder (Finn and Rogenes 1980;

Palmer et al. 1980). According to Helman and Fisher (1977) the inflection of the $I—V$ curves is a property of the Na^+ pump (or a driving force E_{Na}) but other studies indicate that changes in resistance occur in the paracellular path (Finn and Rogenes 1980), persist in Na^+-free amiloride containing mucosal media and in the presence of serosal ouabain (Palmer et al. 1980).

The significantly different resistances in short- and open-circuited conditions seem to be caused by voltage clamping, since it has been shown in the frog skin and the toad bladder that current or voltage clamping decreased the transepithelial resistance and increased the transepithelial ion flux through the extracellular pathway (Finn and Rogenes 1980). Similar effects were observed in leaky epithelia by Bindslev et al. (1974).

Our experiments have also shown that CAM resembles the toad urinary bladder and other Na^+ transporting epithelia in that active Na^+ absorption 1. is responsible for *PD* and *SCC*, 2. gets abolished by amiloride in the endodermal solution, 3. gets abolished by ouabain in the ectodermal solution and 4. is a function of Na^+ concentration in the solution. The long half-time of ouabain was probably caused by diffusion from the ectodermal solution through the chorionic epithelium and mesenchyme to the allantoic epithelium, a site where Saleuddin et al. (1976) found $Na^+—K^+$ ATPase. If, as seems likely, ouabain at this high concentration inhibited all pump sites, the declining *SCC* after ouabain would also represent Na^+ inward current at the apical membrane and, most likely, K^+ outward current at the basolateral membrane leading to a 1:1 exchange of K^+ for Na^+ within the cells and to a loss of intracellular potassium. The fast inhibition of *SCC* by amiloride ($t_{1/2} < 1$ min) provided evidence for a blockade of this inward Na^+ current. Despite the fairly high amiloride concentration (10^{-4} mol/l) this drug probably inhibited the apical electrogenic Na^+ conductive pathway because the amiloride sensitive $Na^+—H^+$ exchange system is electroneutral (Benos 1982).

As the electrical potential profile of unperturbated CAM *in situ* reveals that the mesenchymal side of the allantoic epithelium is positive compared to the luminal side and represents the majority of the transepithelial *PD* (Graves et al. 1984) it is obvious that, *in ovo*, Na^+ is actively transferred across the endoderm (allantois) into the embryonic circulation, even though Moriarty and Hogben (1970) reported active *in vitro* Na^+ transport from the ectoderm to the endoderm. This controversial result was probably due to differences in the medium employed.

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