Biochimica et Biophysica Acta, 1111 (1992) 159–164
© 1992 Elsevier Science Publishers B.V. All rights reserved 0005-2736/92/$05.00

BBAMEM 75798

Expression of amiloride-sensitive Na^+ channels of hen lower intestine in *Xenopus* oocytes: electrophysiological studies on the dependence of varying NaCl intake

Wolf-Michael Weber [a], Carol Asher [b], Haim Garty [b] and Wolfgang Clauss [a]

[a] *Institut für Tierphysiologie, Justus-Liebig-Universität Giessen, Giessen (Germany) and* [b] *Weizmann Institute of Science, Rehovot (Israel)*

(Received 12 June 1992)

Key words: Oocyte; Sodium ion channel; Amiloride; Expression; Current-voltage relationship; (Hen intestine); (Colon); (Coprodeum)

Epithelial Na^+ channels were incorporated into the plasma membrane of *Xenopus laevis* oocytes after micro-injection of RNA from hen lower intestinal epithelium (colon and coprodeum). The animals were fed either a normal poultry food which contained NaCl (HS), or a similar food devoid of NaCl (LS). Oocytes were monitored for the expression of amiloride-sensitive sodium channels by measuring membrane potentials and currents. Oocytes injected with poly(A)$^+$RNA prepared from HS animals or non-injected control oocytes showed no detectable sodium currents, whereas oocytes injected with LS-poly(A)$^+$RNA had large amiloride-blockable sodium currents. These currents were almost completely saturated by sodium concentrations of 20 mM with a K_m of about 2.6 mM sodium. Amiloride (10 μM) inhibits the expressed sodium channels entirely and examination of dose response relationships yielded a half-maximal inhibition concentration (K_i) of 120 nM amiloride. *I–V* difference curves in the presence or absence of sodium or amiloride (10 μM) indicate a potential dependence of the sodium transport which can be described by the Goldman equation. When Na^+ is replaced by K^+, no amiloride response was detected indicating a high selectivity for Na^+ over K^+. These results provide strong evidence that intestinal Na^+ channels are regulated by dietary salt intake on the RNA level.

Introduction

One of the characteristics of tight epithelia is the presence of amiloride-sensitive sodium channels in the apical membrane. These channels are present in tight epithelia from a wide variety of mammalian, amphibian and even invertebrate species (for review see Refs. 1 and 2). They have been well characterized in organs such as colon [3], lung [4], urinary bladder [5] and skin of frogs [6]. They could also be found in rat collecting tubules [7], in trachea of mammals [8], and moreover in invertebrate tissues like integument of leeches [9] and crab gills [10]. It has been shown that this typical epithelial Na^+ conductance could be modulated by mineralcorticoids such as aldosterone [11–14] via an increase in the number of active channels in hen lower intestine. Basic studies of Choshniak et al. [15] revealed evidence for regulation mechanisms that are dependent on dietary sodium uptake. The apical sodium permeability of hen lower intestine varies with the amount of NaCl intake [16]. Animals fed with a normal or high NaCl diet express nearly no amiloride-sensitive sodium channels. Only tissue derived from hens raised on a low Na^+ diet exhibits a high amiloride-blockable sodium permeability.

Asher et al. [17] used these findings to differentially express Na^+ channel activity in *Xenopus* oocytes. In a first step they isolated poly(A)$^+$RNA from hen lower intestine and coprodeum after feeding the animals with different salt diets. The oocytes used were from the South-African clawed toad *Xenopus laevis* which have been well established as an expression system for foreign RNAs (for review see Ref. 18). These giant cells proved also suitable for the expression of amiloride-blockable Na^+ channels from different epithelia as reported recently [8,19–21]. After expression in the oocytes Asher et al. [17] showed that low-salt diet increases the rate of sodium permeability via amiloride sensitive channels by measuring the uptake of ^{22}Na into the oocytes. We now contribute some new electrophysiological data to their findings using two electrode voltage-clamp.

Correspondence to: W.-M. Weber, Institut für Tierphysiologie, Justus-Liebig-Universität Giessen, Wartweg 95, D-6300 Giessen, Germany.

Preliminary accounts of this report have been given at the fall meeting of the German Physiological Society in Freiburg, Germany [22].

Materials and Methods

RNA preparation

The procedures of animal feeding and poly(A)$^+$ RNA preparation were described earlier [17]. Briefly, poly(A)$^+$RNA was prepared from lower intestine (colon and coprodeum) from hens (Cobb broilers). They were fed either a normal poultry food (NRC recommended ration) which contained 0.3% (w/w) NaCl (high-sodium diet, HS), or a similar food which was devoid of NaCl (low-sodium diet, LS). Four weeks after hatching the animals were killed by cervical dislocation. Colon and coprodeum were removed immediately, cut open, and rinsed in a phosphate-buffered saline solution on ice. Parts of the tissue were quickly lysed in guanidinium salt solution and treated as described previously (see above). For RNA preparation we used either a guanidinium chloride [23], or a guanidinium thiocyanate procedure [24] which both gave comparable results. After two passes of the total RNA through an oligo dT column the poyly(A)$^+$RNA was stored at -70 C°.

Oocytes

The following methods to obtain defolliculated oocytes were identical to those described in more detail previously [25]. Females of the clawed toad *Xenopus laevis* (purchased by Kähler, Hamburg, Germany) were decapitated and small pieces of ovary were incubated in oocyte Ringer solution (ORi, see below) containing collagenase (1.5 U/ml, Serva, Germany) while shaking gently. After 7 h oocytes were removed and washed 10 min in Ca^{2+} free ORi to remove the surrounding follicular cell layer. Healthy looking full grown oocytes (Type V or VI [26]) were injected with 50-nl aliquots containing either 12.5 ng HS-poly(A)$^+$RNA or LS-poly(A)$^+$RNA using a nanoliter pump (Baco fer, Reutlingen, Germany). Non-injected control oocytes and injected oocytes were incubated separately for several days at 22°C in ORi containing penicillin (0.08 mM) and streptomycin (0.03 mM). Three days after injection surviving oocytes were selected and used for further experiments (for details concerning the injection procedures see Ref. 27). Since no differences between water injected and non-injected oocytes could be seen in pilot experiments, for the present study only non-injected oocytes were used as controls.

Electrophysiological measurements

For the electrophysiological measurements single oocytes were placed in a small plexiglass chamber (0.5 ml volume) and were superfused constantly. Voltage-clamp was performed by conventional two-microelectrode techniques [28] bathing the oocytes in ORi (see below) which contained 20 mM tetraethylammonium (TEA) as well as 5 mM $BaCl_2$, and 10 μM ouabain to block leak currents and the Na^+/K^+-ATPase, respectively [29]. For determination of current-voltage relationships, steady state current was measured during the last 100 ms of 500 ms rectangular voltage pulses to different potentials. These pulses were applied from the holding potential at a frequency of 0.25 Hz using a voltage-clamp amplifier (NPI TurboTec, Tamm, Germany) controlled by a personal computer with a CED 1401 (CED, Cambridge, UK). The software used for the performance of current-voltage curves and data acquisition was kindly given to us by Dr. W. Schwarz (Max-Planck-Institute for Biophysics, Frankfurt, Germany).

Solutions

The composition of the oocyte Ringer solution (ORi) was (in mM): 110 NaCL, 3 KCl, 2 $CaCl_2$ and 5 *N*-2-hydroxyethylpiperazine-*N'*-ethanesulfonic acid (Hepes, adjusted to pH 7.6). If lower Na^+ concentrations were used, the Na^+ was replaced by tetramethylammonium (TMA). All experiments were performed at room temperature (22°C to 24°C).

Statistics

Results, when not stated otherwise, are expressed as means ± S.E.

Results

Xenopus oocytes have only very small endogenous Na^+ currents as depicted in Fig. 1A. When sodium is removed from the bath solution the holding current decreased only by about 10 nA. In initial experiments we evaluated the amount of RNA that was sufficient for maximal expression of amiloride-blockable Na^+ conductances. We found that injection of 12.5 ng RNA per oocyte yielded maximal results. The experiments were performed three to six days after injection. Under our experimental conditions control and non-injected oocytes showed resting potentials of about -40 to -50 mV. LS-RNA injected oocytes had even lower resting potentials near -30 mV. Therefore all oocytes were clamped to a holding potential of -70 mV.

HS-RNA injected oocytes did not express any additional sodium conductance and the current trace in response to removal of sodium was the same as that of non-injected controls. Two different preparations of HS-RNA were tested in oocytes from five animals with the same negative results. Injection of LS-RNA resulted in the expression of functional Na^+ channels in the oocyte plasma membrane. After removal of sodium the holding current decreased drastically (Fig. 1B).

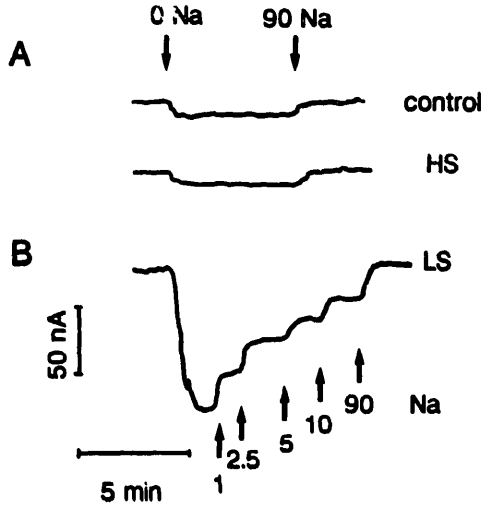

Fig. 1. Voltage-clamp recordings from control and RNA-injected oocytes. Oocytes were voltage-clamped to −70 mV and the resulting inward currents registered. After removal of Na^+ in the bath solution the currents decreased reversibly by about 10 nA in control and in HS-RNA-injected oocytes (Fig. 1A). Holding current of these oocytes in the presence of Na^+ is about −30 nA. The current decrease, however, is too small to apply the same protocol as in Fig. 1B where, Na^+ was added successively. The oocyte was injected with LS-RNA and holding current was about −150 nA at −70 mV. Individual current traces from single oocytes of typical experiments are shown (five observations in oocytes from three donors).

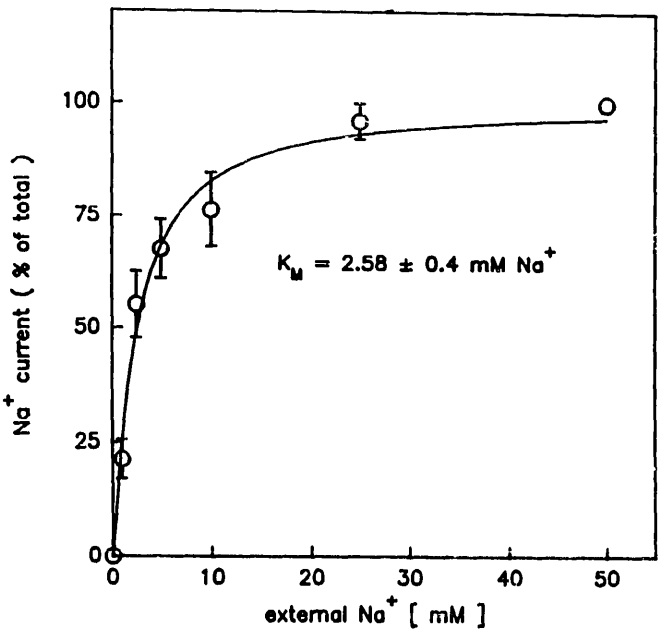

Fig. 2. Dependence on extracellular Na^+ concentration. LS-RNA injected oocytes were voltage-clamped to −70 mV and the resulting currents were measured in the absence and presence of varying sodium concentrations. Data represent averages from five oocytes of two donors, bars indicate S.E. The solid line was fitted to the Michaelis-Menten equation. Control oocytes or HS RNA injected oocytes show no detectable response in this experimental protocol.

Successive readditon of Na^+ led to the former holding current again (for details see figure legend). The Na^+ mediated current at a holding potential of −100 mV exceeded an average of −80 ± 10 nA in LS-poly(A)$^+$RNA injected oocytes. In a few batches of oocytes sodium mediated currents up to −250 nA at holding potentials of −70 mV could be recorded after injection of LS-poly(A)$^+$RNA.

Fig. 2 depicts the relationship between sodium mediated inward current and the sodium concentration in the bath solution surrounding the oocyte. The half-maximal sodium concentration (K_m) is 2.58 ± 0.4 mM. The sodium dose response curve also implies that the Na^+ conductances saturate with an outer Na^+ concentration of about 25 mM. Further increase of the sodium concentration in the bath solution caused no additional rise in current. Applying the same experimental protocol to non-injected control oocytes or to HS-poly(A)$^+$RNA injected oocytes produced no significant effects on clamp current. The responses on sodium free medium were too small to be measured. Nevertheless it should be mentioned again that removal of sodium in the bath solution resulted in a small current decrease in control oocytes while voltage-clamped to negative potentials (−70 mV). This decrease in current was independent of the addition of Ni^+ (5 mM) indicating that the Na^+/Ca^{2+}-exchanger is not responsible for the effect. Due to the presence of ouabain (20 μM) in all solutions the decrease in current cannot be mediated by the Na^+/K^+-ATPase. The resulting slight increase of the intracellular sodium concentration during the experiments caused no significant effects.

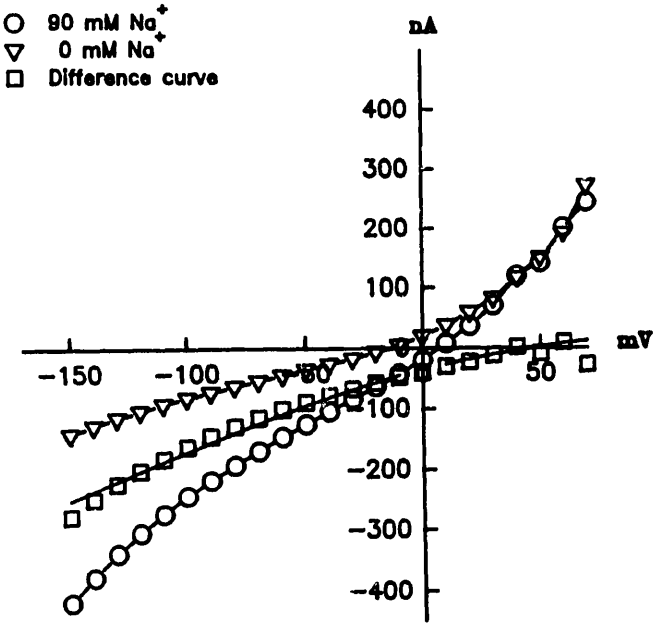

Fig. 3. Current-voltage relationship of the Na^+-mediated current The oocyte was injected with LS-RNA. I-V curves were performed in the presence (circles) and absence (triangles) of Na^+ (90 mM) in the bath solution. Squares show the calculated difference curve between the two curves. The solid line is a fit to the Goldman equation [30] (Na_o = 90 mM, Na_i = 15 mM, $P_{Na} = 4.8 \cdot 10^{-6}$ cm/s). A typical result out of a set of multiple observations is shown.

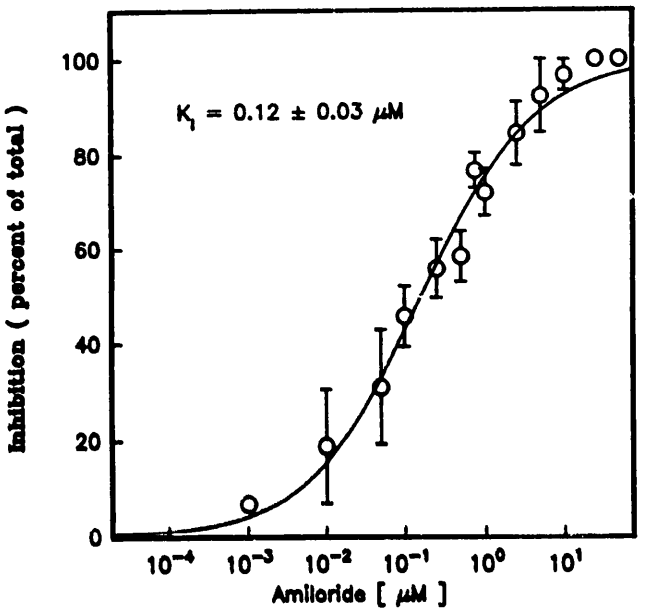

Fig. 4. Amiloride dose-response curve. Oocytes injected with 12.5 ng LS-RNA were voltage-clamped to different holding potentials (− 100 mV and −50 mV). Increasing concentrations of amiloride were added to the bath solution and resulting changes in current were measured. Data represent averages from 12 oocytes of five different donors, bars indicate S.E. The solid line was fitted to the equation:

$$k = K_{max} \cdot [S]^n / ((K_i)^n + [S]^n)$$

where k represents the rate of inhibition and [S] is the inhibitor concentration. The best fit was obtained with $n = 1$, $K_i = 120$ nM amiloride and $K_{max} = 100\%$. The different holding potentials showed no significant difference in the resulting dose-response curves. Control oocytes or HS-RNA-injected oocytes show no response to amiloride.

Current-voltage relationships acquired from LS-poly(A)$^+$RNA injected oocytes in the presence and absence of sodium in the bath solution showed clear differences in the resulting curves (Fig. 3). The difference between the curves in the presence of sodium and in the absence of sodium yielded the current mediated by the expressed sodium channels. A fit of the constant field equation [30] to the difference data with the indicated fit parameters (see figure legend) yielded a reversal potential of about +50 mV as expected for a sodium selective conductance. The current-voltage relationship for the sodium conductance showed slow inward rectification at negative membrane potentials. HS-poly(A)$^+$RNA injected and control oocytes exhibited no significant differences in their current-voltage relationships with or without sodium.

In order to evaluate the affinity for amiloride we applied different concentrations of this blocker (Fig. 4). Oocytes injected with LS-poly(A)$^+$RNA showed high affinity for amiloride with a half-maximal blocker concentration (K_i) of about 120 nM while with HS-poly(A)$^+$RNA and non-injected control oocytes no response to amiloride (up to 100 μM) could be detected.

Fig. 4 also depicts that 10 μM of the inhibitor in the bath solution was sufficient to block the expressed Na^+ channels completely. The K_i measured is consistent with the values measured in the intact epithelium [16]. This high affinity for amiloride indicates strongly that the Na^+-mediated current found in LS-poly(A)$^+$RNA injected oocytes is the Na^+ channel described for the native epithelium of hen coprodeum [11]. The rheogenic Na^+/Ca^{2+}-exchanger, the electroneutral Na^+/H^+-exchanger and some calcium channels are also effected by amiloride, but only when applied in higher concentrations of about 1 mM. These concentrations are two orders of magnitude higher than those we used for this study so blocker interactions with the above mentioned transport systems can be neglected. By comparing the K_i values measured at different holding potentials we found no potential dependence of the amiloride efficacy.

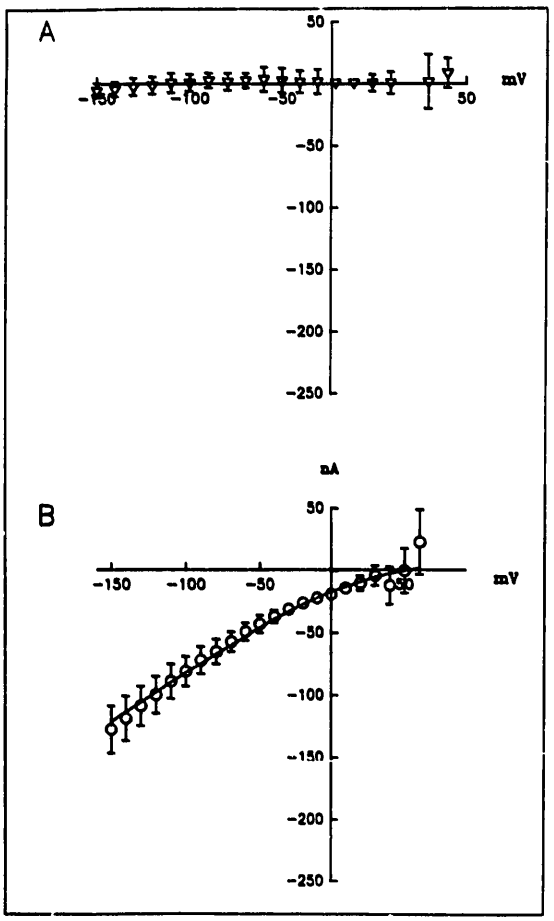

Fig. 5. Current-voltage relationships of the amiloride-sensitive Na^+ current. Injected oocytes were voltage-clamped from − 150 mV to 60 mV in steps of 10 mV for 500 ms and currents were measured during the last 100 ms of the pulse. Shown are the difference curves in the presence and absence of amiloride (10 μM) in the medium bathing the oocyte. Values are averages (±S.E.) of 5–8 oocytes of three different animals. (A) $I-V$ difference curve of oocytes injected with HS-RNA. (B) $I-V$ difference curve of oocytes injected with LS-RNA. The solid line represents a fit of the Goldman equation [30] (Na_o = 90 mM, Na_i = 15, $P_{Na} = 1.9 \cdot 10^{-6}$ cm/s).

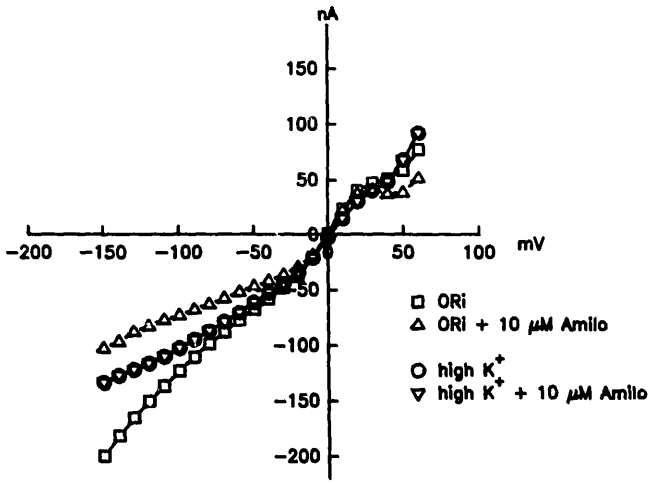

Fig. 6. Selectivity of the Na^+ transport. A LS-RNA injected oocyte was voltage-clamped as described above. Na^+ in the bath solution was replaced by K^+. Resulting I-V curves show no difference with and without amiloride (10 μM). Shown is a typical experiment with a single oocyte.

The amiloride-sensitive current could be resolved by the difference of current-voltage relationships recorded in the presence and absence of the blocker. HS-poly(A)$^+$RNA injected oocytes and control oocytes showed no response to amiloride (10 μM); the difference curve is close to zero (Fig. 5A). Fig. 5B shows curves in the presence and absence of amiloride (10 μM) from oocytes injected with LS-poly(A)$^+$RNA. The difference curve can also be fitted to the Goldman equation [30] yielding comparable results as mentioned above (see figure legend).

The ion selectivity of the expressed channel for sodium was evaluated by replacing sodium by potassium in the bath solution and testing the ability of amiloride to inhibit currents under both experimental conditions. When Na^+ was replaced by K^+ the amiloride induced change on the clamp current disappeared over the whole voltage range (Fig. 6). Current-voltage relationships with high K^+ concentrations in the bath solution showed no difference indicating high selectivity of the expressed conductance for Na^+ over K^+. Thus sodium, but not potassium can enter the oocyte through the amiloride-sensitive pathway expressed after injection of LS-RNA.

Discussion

High amiloride-sensitive sodium mediated currents can be detected in *Xenopus* oocytes two or three days after injection of RNA purified from colon and coprodeum of hen which were fed a low-salt diet. This result coincide with findings in the native epithelium from hen lower intestine [11–13,16]. Amiloride-blockable sodium permeabilities can be found only in tissue derived from hens raised on low-salt diet. When animals are fed a normal NaCl containing diet no amiloride-sensitive Na^+ permeabilities can be detected.

Non-injected oocytes or oocytes injected with RNA from animals on a normal diet show no significant sodium currents. The presence of amiloride, an inhibitor known to be highly specific for Na^+ channels in submicromolar doses evokes absolutely no response in those oocytes. LS-poly(A)$^+$RNA induces the expression of Na^+ conductances which are characterized by high sensitivity to amiloride. This fact is demonstrated by the half-maximal blocker concentration (K_i) of about 120 nM. The value is in accordance to the findings of Asher et al. [17] although their K_i for amiloride is slightly lower than 100 nM and 1 μM amiloride is sufficient for total inhibition of $^{22}Na^+$ uptake while in our experiments this blocker concentration inhibits only about 80 percent of total Na^+ current. Our results demonstrated that the amiloride block is voltage independent. Therefore these small discrepancies might be due to the lower Na^+ concentrations in the solutions Asher et al. [17] used. The amiloride inhibitable current induced in LS-RNA injected oocytes can be described by the constant field equation [30] as depicted in Fig. 5.

Our findings are also consistent with the results derived from native tissue of hen coprodeum [11] using a Ussing chamber to measure short-circuit currents and the inhibitory effect of amiloride on it. The affinity for extracellular Na^+ with half-maximal Na^+ concentration of 2.58 mM is also in good accordance with the values measured in native epithelia. Bindslev et al. [16] found in hen coprodeum that 5–7 mM sodium on the apical side is sufficient to half saturate short-circuit current.

Removal of extracellular sodium results in a small reduction of the holding current in voltage-clamped non-injected oocytes and in HS-poly(A)$^+$RNA injected oocytes. However this decrease of current is several orders of magnitude smaller than the decrease that can be observed in oocytes injected with LS-poly(A)$^+$RNA. To describe the entry of Na^+ through channels located in the apical membranes of a variety of epithelia one can use the constant field equation [30]. This equation considers electrodiffusional processes and was used to analyse our data from current-voltage relationships. Data from LS-RNA injected oocytes match very well with the predictions of the Goldman equation: They are non-linear and show small inward rectification at negative holding potentials, and the reversal potential lies close to +50 mV. The sodium mediated current is identical with the amiloride-sensitive current. This becomes obvious by comparing the results shown in Fig. 3 and Fig. 5. These results taken together confirm the expression of the amiloride-blockable Na^+ channel from hen lower intestinal epithelia.

These channels are also characterized by a high selectivity for Na^+ over K^+. In oocytes injected with LS-poly(A)$^+$RNA this selectivity is also detected. Potassium ions cannot enter the cell through the expressed amiloride-blockable channels as demonstrated when Na^+ is replaced by K^+ in the bath solution. When K^+ is the main ion in oocyte bathing solution amiloride fails to induce any detectable effect on the holding current. Difference curves in the presence of Na^+ and when Na^+ substituted by K^+ are close to zero indicating high selectivity for Na^+ over K^+.

RNA extracted from animals fed a low-salt diet seems to bear nucleotide sequences that are coding for the amiloride-sensitive Na^+ channel while RNA from animals on a high NaCl diet seems to lack this information. However two alternative explanations worth considering were that both RNA preparations, HS-RNA and LS-RNA, contain nucleotide sequences that are able to induce expression of functional Na^+ channels into the oocyte plasma membrane, but in addition, HS-RNA induces expression of a regulatory protein with inhibitory effects either on the translation or the function of the Na^+ channel. This hypothesis can be tested by co-injection of both RNA preparations into the same cell. After injection of a mixture of both RNA's functional Na^+ channels could be detected as reported by Asher et al. [17].

A second hypothesis which was also tested by Asher et al. [17] is that LS-RNA could bear the information for a gene regulatory protein which could activate an endogenous amiloride-sensitive Na^+ conductance in the oocyte. However, when they applied Actinomycin D after injection of LS-RNA in order to inhibit transcription of endogenous oocyte-DNA they found functional Na^+ channels.

Taken together all these findings provide strong evidence that a low-salt diet causes the formation of active Na^+ channels in hen colon and coprodeum and that the information for these channels is present in the RNA of these cells. Intestinal cells from hens raised with normal NaCl diets lack this information. They could have other ways for Na^+ uptake, e.g., sodium cotransport systems for amino acids or other substrates which will be a subject for our future studies.

Acknowledgements

We gratefully acknowledge the practical and theoretical help from Prof. Dr. Wolfgang Schwarz (Max-Planck-Institute for Biophysics, Frankfurt/Main, Germany). He wrote also the computing programs that we used to perform and sample the *I–V* curves. This work was supported by the Deutsche Forschungsgemeinschaft (CL 63/7-1) and by a grant from the American Cystic Fibrosis foundation to H.G.

References

1 Garty, H. and Benos, D.J. (1988) Physiol. Rev. 68, 303–373.
2 Wills, N.K. and Zweifach, A. (1987) Biochim. Biophys. Acta 906, 1–31.
3 Krattenmacher, R., Fischer, H., Van-Driessche, W. and Clauss, W. (1988) Pflügers Arch. 412, 568–573.
4 Fischer, H., Van-Driessche, W. and Clauss, W. (1989) Am. J. Physiol. 256, C764–C771.
5 Li, J.H.-Y., Palmer, L.G., Edelmanand, I.S. and Lindemann, B. (1982) J. Membr. Biol. 64, 79–89.
6 Van-Driessche, W. and Lindemann, B. (1979) Nature 282, 519–520.
7 Frindt, G., Sackin, H. and Palmer, L.G. (1990) Am. J. Physiol. 258, F562-F567.
8 Kroll, B., Bautsch, W., Bremer, S., Wilke, M., Tümmler, B. and Frömter, E. (1989) Am. J. Physiol. 257, L284-L288.
9 Dannenmaier, B., Weber, W.-M. and Clauss, W. (1991) Pflügers Arch. 419 (Suppl.), R103.
10 Zeiske, W., Onken, H., Schwarz, H.J. and Graszynski, K. (1992) Biochim. Biophys. Acta 1105, 245–252.
11 Clauss, W., Dürr, J.E., Guth, D. and Skadhauge, E. (1987) J. Membr. Biol. 96, 141–152.
12 Clauss, W., Dantzer, V. and Skadhauge, E. (1988a) J. Membr. Biol. 102, 83–96.
13 Clauss, W. and Skadhauge, E. (1988b) Comp. Biochem. Physiol. 90A, 583–589.
14 Palmer, L.G., Corthesy-Theulaz, I., Gaeggeler, H.-P., Krachenbuhl, J.-P. and Rossier, B.C. (1990) J. Gen. Physiol. 96, 23–46.
15 Choshniak, I., Munck, B.G. and Skadhauge, E. (1977) J. Physiol. 271, 489–504.
16 Bindslev, N., Cuthbert, A.W., Edwardson, J.M. and Skadhauge, E. (1982) Pflügers Arch. 392, 340–346.
17 Asher, C., Singer, D., Eren, R., Dascal, N. and Garty, H. (1992) Am. J. Physiol. 262, G244-G248.
18 Dascal, N. (1987) CRC Crit. Rev. Biochem. 22, 317–387.
19 George, A.L. Jr, Staub, O., Geering, K., Rossier, B.C., Kleyman, T.R. and Kraehenbuhl, J.-P. (1989) Proc. Natl. Acad. Sci. USA 86, 7295–7298.
20 Hinton, C.F. and Eaton, D.C. (1989) Am. J. Physiol. 257, C825–C829.
21 Kroll, B., Bremer, S., Tümmler, B., Kottra, G. and Frömter, E. (1991) Pflügers Arch. 419, 101–107.
22 Weber, W.-M., Asher, C., Garty, H. and Clauss, W. (1991b) Pflügers Arch. 419 (Suppl.), R105.
23 MacDonal, R.J., Swift, G.H., Przybyla, A.E. and Chirgwin, J.M. (1987) Methods Enzymol. 152, 219–227.
24 Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
25 Weber, W.-M., Schwarz, W. and Passow, H. (1989) J. Membr. Biol. 111, 93–102.
26 Dumont, J.N. (1972) J. Morphol. 136, 153–180.
27 Weber, W.-M., Püschel, B., Steffgen, J., Koepsell, H. and Schwarz, W., (1991a) Biochim. Biophys. Acta 1063, 73–80.
28 Lafaire, A.V. and Schwarz, W. (1986) J. Membr. Biol. 91, 43–51.
29 Vasilets, L.A., Schmalzing, G., Mädefessel, K., Haase, W. and Schwarz, W. (1990) J. Membr. Biol. 118, 131–142.
30 Goldman, D.E. (1943) J. Gen. Physiol. 27, 37–60.