

Effects of Antidiuretic Hormone upon Electrical Potential and Resistance of Apical and Basolateral Membranes of Frog Skin

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Received 3 October 1977; revised 16 January 1978; revised again 23 March 1978

Summary. The effect of ADH upon the intracellular potential and the resistance of inner and outer borders of the transport pathway was investigated on isolated skins of *Rana temporaria*. Within 40 min after ADH (100–300 mU/ml), the intracellular potential under short-circuit conditions decreased to about 40% of the control value (-79 ± 4 mV), concomitant with an increase in the short-circuit current to about 160% of the control value. Amiloride, applied when steady values under ADH had been reached, caused an immediate rise of the intracellular potential to values typical for control conditions. This confirms (i) the intracellular location of the microelectrode and the absence of impalement artifacts, and (ii) the ineffectiveness of ADH upon the electromotive forces of the inner border. ADH had no effect upon the intracellular potential after blockage of the Na entry by Amiloride. The equilibrium potential of the outer border was estimated to be about +20 mV under the influence of ADH. As this value is considerably less positive than might be expected for the chemical potential of Na, a significant contribution of ions other than Na to the outer border conductance and equilibrium potential is implicated. The resistance of the outer border was more significantly decreased than that of the active transcellular pathway after ADH due to an increase in the inner border resistance, which exceeded that of the outer border after ADH. The effect of ADH upon the outer membrane characteristics would be underestimated by a factor of two, if the alterations of the electrical potential difference were not taken into consideration.

Key words: Frog skin, Na transport, ADH, outer border effective emf, transcellular resistance.

The stimulation by antidiuretic hormone (ADH) of transepithelial Na transport is generally thought to result from an increase of the permeability of the outer border of the epithelial cells (for reference, see the recent review of Andreoli & Schafer, 1976) which enhances Na entry into the cellular compartment and, as a consequence, accelerates Na pumping across the basolateral border of the cells. Additional effects of ADH upon the Na pump itself seem, if existing, to be of minor importance for

the quantitative response of the epithelium. However, despite the numerous investigations describing the stimulative effect of ADH upon the Na transport by means of Na fluxes across the entire epithelium or across the outer border of the epithelial cell layer, quantitative estimates of the ADH-induced permeability changes of the outer border could not be made due to lack of information about chemical and electrical gradients under control conditions and after ADH. Reliable measurements of the electrical PD between the outer bathing solution and the intracellular space of the frog skin epithelium were only recently obtained by Nagel (1976) and Helman and Fisher (1977). Previous measurements of the intracellular potential of the toad bladder and conclusions regarding the effect of ADH upon the membrane resistance (Civan & Frazier, 1968) must be questioned in view of recent measurements of intracellular potential from the urinary bladders of *Necturus* (Higgins, Gebler & Frömter, 1977) and toad (Sudou & Hoshi, 1977).

The present investigation, using recently developed methods to obtain intracellular potentials of the frog skin epithelium (Nagel, 1976; Helman & Fisher, 1977), demonstrates that large changes of the electrical gradients occur after stimulation of Na transport with ADH. The data allowed quantitative estimation of the changes in resistance of the inner and outer membranes. It was observed that the outer border resistance decreased considerably more upon addition of ADH than would be expected from transepithelially obtained data, and that, surprisingly, the resistance of the basolateral border increased after ADH. The findings suggest that the resistance of the outer membrane of the frog skin, which is rate limiting for transepithelial Na transport under normal conditions, may be exceeded by the resistance of the basolateral membranes under conditions of increased Na permeability of the outer border. The electrochemical gradient across the outer border may then significantly influence the effective response of the epithelium.

Materials and Methods

List of Symbols

- V_t = Transepithelial potential difference
 V_o, V_i = Potential difference across the outer and inner membrane, respectively.
 V_{sc} = Intracellular potential under short-circuit conditions
 I_t = Transepithelially applied current
 I_{sc} = Short-circuit current

- E_1 = Break point of the $I-V$ relationship in the hyperpolarizing region according to Helman & Miller (1971)
 E'_1 = Transepithelial clamping potential to establish $V_0 = 0$ mV
 $F(R_o)$ = Fractional resistance of the outer border, $R_o/(R_o + R_i)$
 R_{Na} = Resistance of the active transcellular pathway
 R_o, R_i = Resistance of the outer and inner membrane, respectively
 E_{Na} = Effective driving force for transepithelial Na transport according to Ussing & Zerahn (1950)
 E_o, E_i = Equilibrium potential of the outer and inner membrane, respectively

Methods

Isolated abdominal skins of *Rana temporaria* were mounted with the epithelial side upward in a special chamber which was described previously (Nagel, 1976, 1977). The exposed area of the skin was 0.4 cm². The corial side was supported by a stainless steel grid. Heavy silicone grease (No. 7922, Merck) was used to prevent edge damage. Both sides of the skin were perfused continuously with Ringer's solution (110 mM NaCl, 2.4 mM KHCO₃, 1 mM CaCl₂, 5 mM Glucose, pH 8.2). A hydrostatic negative pressure of 10–30 cm H₂O was applied to the corial side which served (i) to provide a driving force for the perfusion (~5 ml/min) and (ii) to attach the skin to the supporting grid. Resulting from the dead space in the tubings and the chamber (~1.5 ml) and from the unstirred layer of the grid (~200 μm), substances added to the corial fluid reservoir come into contact with the skin only after a delay period of 45–60 sec. The epithelial side of the skin was perfused at a rate of ~15 ml/min. Change of the perfusion fluid was effective within a few sec resulting from the small chamber volume (0.3 ml) and the absence of unstirred layers at this side.

The transepithelial PD (V_t) was measured using calomel electrodes and Ringer's-filled bridges which ended 0.5 mm apart from the epithelial or corial surface of the skin. Silver plates, 4 mm apart from the skin surfaces and coated with silver chloride, served to apply current through the epithelium (I_t) for measurement of the short-circuit current (I_{sc}) or the current-voltage (I/V) relationship according to the method of Helman & Miller (1971).

Intracellular potentials were recorded with microelectrodes, prepared from micro-fiber glass capillaries (No. 30-32-1, Frederick Haer & Co.) of 1.5 mm OD and 1 mm inside diameter (ID) immediately before use on a two stage microelectrode puller. After filling with 3 M KCl from behind, the microelectrode input resistance was between 15 and 25 MΩ and the tip potential generally below 3 mV. The frog skin was impaled from the epithelial side perpendicular to the surface. A micromanipulator with stepping motor drive (No. ME-71, Narishige) served for movement of the microelectrodes. The microelectrode potentials were recorded with silver/silver chloride electrodes and with reference to the epithelial bathing solution. The criteria for intracellular location of the microelectrode tip and absence of cell injury caused by the impalement were described previously (Nagel, 1976).

Continuously recorded on one channel of a strip chart recorder was the intracellular potential under short circuit conditions (V_{sc}), while the I_{sc} was recorded on a second channel. In addition, the transepithelial PD (V_t), the transepithelially applied current (I_t), and the PD across the outer border (V_o) were displayed on digital panel meters to an accuracy of 0.1 μA and 0.1 mV, respectively. The digital output of the meters could be registered by use of a line printer. As described by Helman & Fisher (1977), the transepithelial clamping voltage was varied to values between -40 and +200 mV in steps of 20 mV. At pulse durations of 600 msec, I_t and V_o were found to reach steady-state

values well within this time, which was documented in part of the experiments by use of a Tektronix storage oscilloscope.

The steady-state values of V_t , I_t , and V_o (580 msec after onset of the pulse) were used (i) to estimate from the relationship between I_t and V_t the break point in the hyperpolarizing region, E_1 , (ii) to calculate E'_1 , the value of V_t which corresponds to $V_o = 0$ mV, and (iii) to compute the fractional resistance of the outer border, $F(R_o) \equiv R_o / (R_o + R_i) = \Delta V_o / \Delta V_t$ and the ratio of inner to outer border resistance, $R_o / R_i = \Delta V_o / \Delta V_i$. The resistance of the active transepithelial pathway, R_{Na} , was calculated using the definition $R_{Na} = E_{Na} / I_{sc}$ (Ussing & Zerahn, 1950). It was assumed that E'_1 after amiloride provides a measure of E_{Na} . Specific resistances of outer and inner membranes were calculated from R_{Na} using the $F(R_o)$.

The experiments started with a control period of at least 60 min, during which the skin was short circuited and the I_{sc} was allowed to reach a steady value. At the end of this period, several control impalements were made to determine accurately the value of the V_{sc} . The data were remarkably consistent within a single skin, differing usually by no more than a few mV, as reported previously (Helman & Fisher, 1977). In most punctures, however, a drop of the V_{sc} indicated progressive injury and eventually complete damage of the impaled cell after several seconds or minutes. In the present study, stability of a single intracellular recording for more than 30–40 min was desired, i.e. the time necessary to reach steady values under ADH. Long term stability was found mostly to occur when the intracellular recordings were stable for more than 10 min under control conditions (Nagel, Helman & Fisher, *unpublished observations*). During this time, several measurements of the I/V relationship were performed in intervals of 1–3 min.

ADH (Arginin-Vasopressin, Ferring) was added to the corial perfusion solution to give final concentrations between 100 and 200 μ M/ml. The measurement of the electrical parameters was continued until steady values of I_{sc} and V_{sc} after ADH were reached. Then, $2\text{--}10 \times 10^{-5}$ M Amiloride (Merck, Sharp & Dohme) was applied to the epithelial side as a control for the intactness of the impaled cell. In some experiments, the intracellular recording became unstable before the effect of ADH was complete, detectable as a breakdown (Nagel, 1976). When another cell could be punctured soon after, the electrical parameters were found to be very similar to those obtained from the previously punctured cell before the breakdown. The time course of the effect of ADH upon the intracellular potentials was the same whether the values were obtained by continuous recording from a single cell or were collected by impalement of different cells. This excludes that time-dependent alterations of the cell caused by the microelectrode were responsible for the results.

Part of the experiments were done under nontransporting conditions achieved by addition of Amiloride (10^{-4} M) to the epithelial side. At least 30 min were allowed to insure full effect of Amiloride. Then, a cell was impaled and measurements done as described above.

Mean values are given \pm SEM.

Results

The effect of ADH upon the electrical parameters was investigated in 11 Na transporting skins. Among these, it was possible in 7 experiments to follow the time course of the hormone action in a single cell. The remaining experiments required impalement of several cells. The control

Table 1. Control data of Na-transporting frog skins ($n = 11$)

| | I_{sc} ($\mu\text{A}/\text{cm}^2$) | V_{sc} (mV) | E'_1 (mV) | $\%R_o$ | E'_1/E_1^a |
|-----------|----------------------------------------|---------------|-------------|-------------|--------------|
| \bar{x} | 39.6 | -79.2 | 108.1 | 0.768 | 0.945 |
| \pm SEM | ± 4.3 | ± 3.7 | ± 2.8 | ± 0.037 | ± 0.052 |
| Range | 21.8-61.3 | 57.0-95.0 | 96.0-123.6 | 0.493-0.887 | 0.833-1.051 |

^a $n = 5$

data observed immediately before addition of ADH are presented in Table 1. The values of V_{sc} and $F(R_o)$ were similar to previously reported values (Nagel, 1976; Helman & Fisher, 1977), while the values of E'_1 were slightly less than those obtained in *R. pipiens* (Helman & Fisher, 1977; Nagel & Helman, 1977). Simultaneous measurements of the I/V relationships of the entire skin and of the E'_1 could be made in 5 experiments. Figure 1 shows typical I/V plots obtained from the same experiment under control conditions, during incubation with ADH and after application of Amiloride in the ADH-stimulated state. All plots yield the usual break points as described by Helman & Miller (1971). Under control conditions, the break point E_1 in the hyperpolarizing region was 116 mV. The value of V_t corresponding to $V_o = 0$ mV, i.e., E'_1 ,

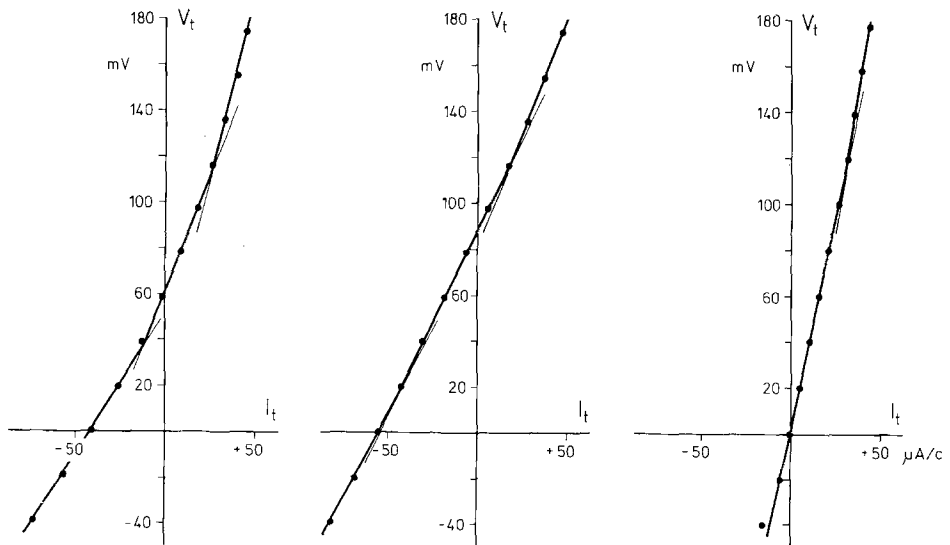


Fig. 1. Plots of the $I-V$ relationship of a frog skin under control conditions (left), during maximal stimulation of Na transport by ADH (middle) and after addition of Amiloride (10^{-4} M) in the ADH-stimulated state (right). Two break points, E_1 in the hyperpolarizing region and E_2 in the depolarizing region, exist as described by Helman & Miller (1971) under control conditions and during ADH. Only break point E_1 is detectable after Amiloride

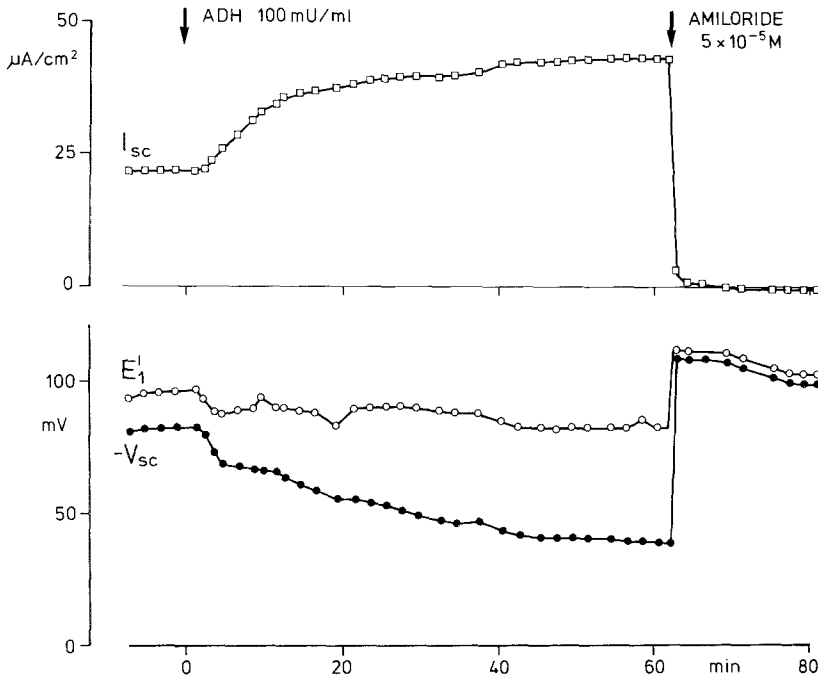


Fig. 2. Record of a typical experiment, showing the effect of ADH (100 mU/ml at the corial side) upon I_{sc} , V_{sc} and E'_1 . ADH was applied at 0 min. Amiloride, added to the epithelial bathing solution, caused immediate reversal of the effect of ADH. The microelectrode position was not altered during the entire period

was 111 mV and not much different from E_1 in this particular experiment. On the average, however, the ratio of E'_1/E_1 was found to be clearly less than unity (Table 1), which is different from the observations in *R. pipiens* (Helman & Fisher, 1977).¹

Figure 2 shows the typical responses of I_{sc} , V_{sc} and E'_1 upon addition of ADH. The I_{sc} increased within 40 min after ADH (100 mU/ml) from 21.8 $\mu\text{A}/\text{cm}^2$ to 41.0 $\mu\text{A}/\text{cm}^2$. This increase to about 190% of the control value was accompanied by a drop of the V_{sc} from -82 mV to -40 mV, i.e., to less than 50% of the control value, and a decrease of the E'_1 from

¹ This could reflect a systematic difference between *R. pipiens berliendieri* and the European frogs, *R. temporaria* and *R. esculenta*. In the latter species, E'_1 is generally and independently of seasonal variations found to be less than E_1 and also smaller in the Na transporting state than under nontransporting conditions (Amiloride, low epithelial $[\text{Na}]$) (Nagel, unpublished results). For *R. pipiens*, however, Helman & Fisher (1977) reported that E'_1 is equal to E_1 and unaffected by the I_{sc} . In accordance with the observations from ADH-stimulated skins, which will be presented below, it appears reasonable to speculate that differences in fractional resistance of the outer border between the species might explain this variation.

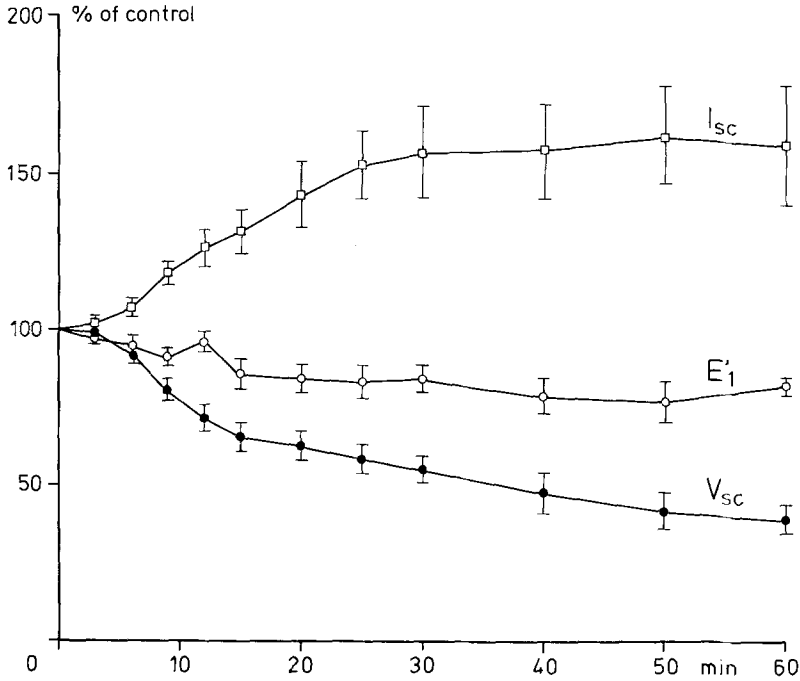


Fig. 3. Time course of the effect of ADH upon I_{sc} , V_{sc} and E'_1 , expressed in % of the respective control values. The fractional changes of the data were calculated for the individual experiments and averaged

97 mV to 83 mV (=86% of the control value). To exclude that these results were due to injury of the impaled cell, Amiloride (5×10^{-5} M) was added to the epithelial bathing solution after the effect of ADH appeared to be maximal. Within seconds and not different in velocity from the response of the I_{sc} , the intracellular potential V_{sc} increased to -106.5 mV. E'_1 measured 50 sec after application of Amiloride was 111.2 mV, and the fractional resistance of the outer border, $F(R_o)$, was 0.97 at that time. Since such values of V_{sc} , E'_1 and $F(R_o)$ are typically and only obtained when impalement artifacts or improper sealing of the cell around the microelectrode are absent, it was concluded that the intracellular potentials observed before addition of Amiloride were also recorded under proper conditions.

Figure 3 presents the time course of the changes of I_{sc} , V_{sc} and E'_1 upon addition of ADH, observed in all 11 experiments. The effect of ADH was maximal within 40 min after addition of the hormone. At that time, the I_{sc} was increased to $157 \pm 14\%$, while the V_{sc} and the E'_1 were reduced to $42 \pm 6\%$ and $79 \pm 6\%$, respectively. Although E'_1 decreased

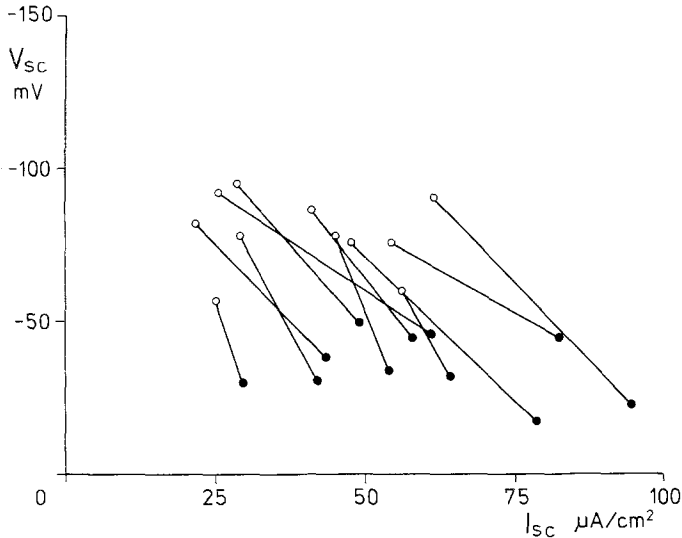


Fig. 4. Summary of the dependency between V_{sc} and I_{sc} . Steady values before and after ADH are indicated by \circ and \bullet , respectively. Lines are drawn between points obtained from individual experiments

only moderately compared to the drop of the V_{sc} , both changes were highly significant ($2P < 0.01$).

The dependency between the steady values of the I_{sc} and the V_{sc} before and after ADH obtained in the individual experiments is shown in Fig. 4. Obviously, I_{sc} and V_{sc} changed in a reciprocal manner, the slope of $\Delta V_{sc}/\Delta I_{sc}$ being remarkably similar for all experiments and independent of the initial values of the I_{sc} or the V_{sc} . Upon addition of Amiloride to the epithelial solution after the effect of ADH appeared to be maximal, the following values were recorded: $I_{sc} = 0.4 \pm 0.1 \mu\text{A}/\text{cm}^2$; $V_{sc} = -117 \pm 2 \text{mV}$; $E'_1 = 124 \pm 3 \text{mV}$. It is interesting to note that V_{sc} and E'_1 remained constant for several minutes after the addition of Amiloride. Then, however, they started to decline reaching significantly smaller steady values after periods of 15–30 min. Similar results were obtained in control experiments (W. Nagel, *unpublished*).

Essentially no effect of ADH upon the intracellular potentials could be detected when the hormone was added after application of Amiloride to increase the outer membrane resistance to high values. Figure 5 demonstrates the result of a typical experiment. As the I_{sc} , neither V_{sc} nor E'_1 showed any significant variation upon addition of 300 mU/ml ADH. Thus, the effectiveness of ADH depends upon the possibility of

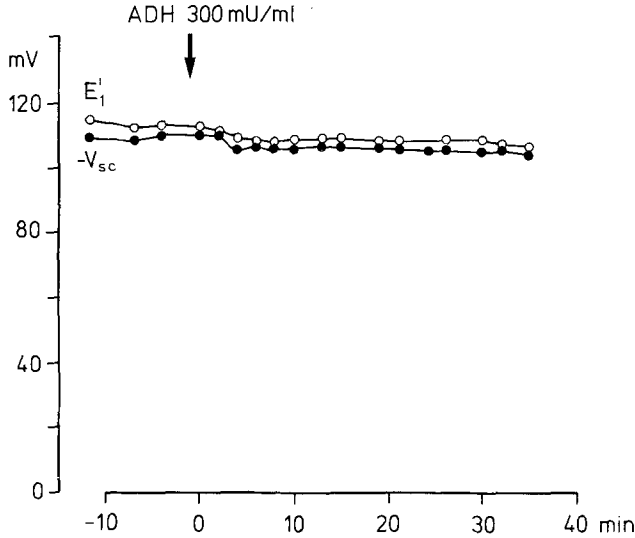


Fig. 5. Record from a typical experiment showing the ineffectiveness of ADH (300 mU/ml at the corial side) upon V_{sc} and E'_1 when Amiloride (10^{-4} M) was present in the epithelial bathing solution

outer membrane resistance changes. Similar results were obtained in 5 experiments. 40 min after addition of ADH, V_{sc} and E'_1 were -102.4 ± 4.2 mV and 104.7 ± 2.3 mV, respectively. These values are statistically not different from the control values before ADH, which were: $V_{sc} = -105.7 \pm 6.3$ mV and $E'_1 = 109.2 \pm 6.1$ mV.

Concomitant with the increase of the I_{sc} and the decrease of the intracellular voltages V_{sc} and E'_1 , considerable changes were observed in the fractional resistance of the outer border of the cellular pathway, $F(R_o)$. Figure 6 demonstrates this result in terms of the mean values of the 11 experiments. Expressed in % of the control values, $F(R_o)$ decreased to about 55% of the control values within 50 min after ADH. The changes are more pronounced, if the ratio of outer to inner border resistance R_o/R_i is calculated from the data. R_o/R_i decreased from 3.62 ± 0.60 in the control to about 0.75 after ADH, i.e., to approximately 20% of the control values. Thus the inner border had a larger resistance after ADH than the outer border, in contrast to the distribution found under control conditions.

To find out whether this result was due to alterations of the outer and/or inner border resistances under the influence of ADH, an attempt was made to estimate the specific resistances R_o and R_i of the both

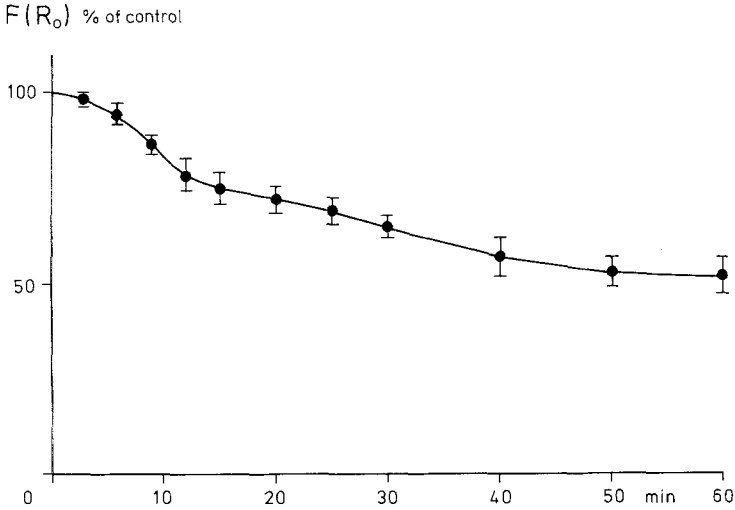


Fig. 6. Time course of the effect of ADH upon the $F(R_o)$, expressed in % of the control value. The control value of $F(R_o)$ was 0.77 ± 0.04

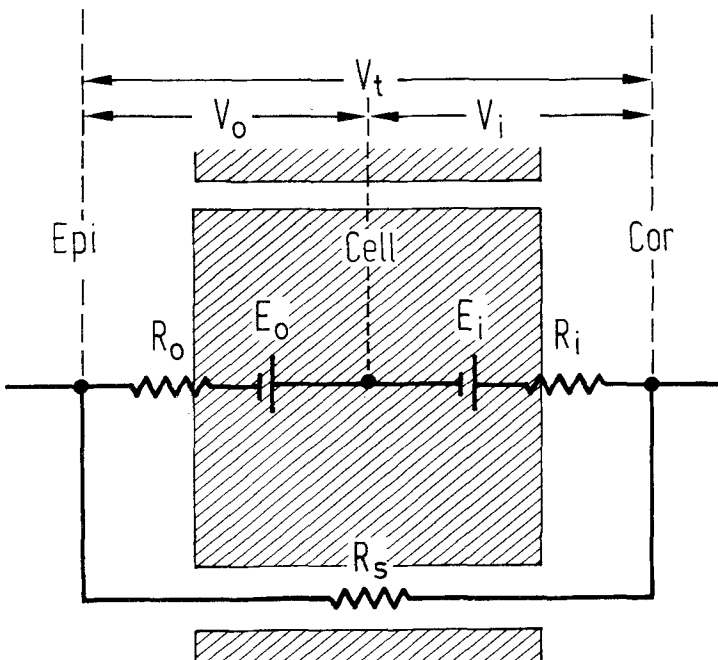


Fig. 7. Electrical equivalent circuit model of frog skin epithelium representing the effective (net) processes at both membranes by electrical circuit parameters. E_o ; E_i = equilibrium potential at outer and inner border, respectively. R_o ; R_i = resistance of outer and inner border, respectively. The resistance of the transcellular pathway, R_{Na} , is the sum of R_o and R_i . R_s = resistance of paracellular shunt pathways. V_o ; V_i = actually measured potential differences between intracellular space and bathing solutions at outer and inner surface, respectively. V_t = transepithelial potential difference

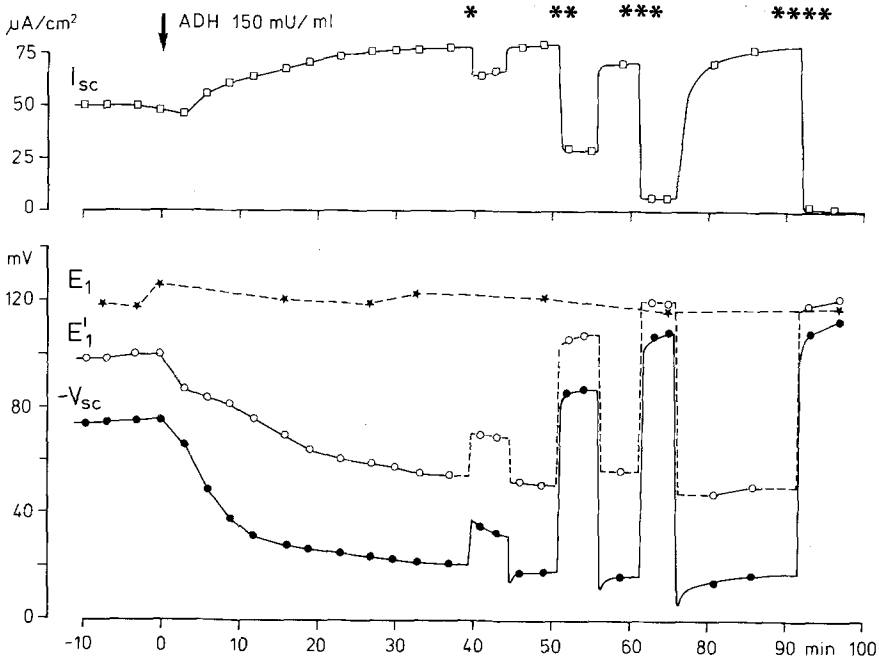


Fig. 8. Record showing the influence of ADH upon I_{sc} , V_{sc} , E'_1 , and E_1 and the effect of subsequent application of Amiloride upon these parameters. Addition and concentration of Amiloride is indicated by: $*$ = 2×10^{-7} M; $**$ = 10^{-6} M; $***$ = 5×10^{-6} M; $****$ = 2×10^{-5} M. The microelectrode position in the same cell was not altered during the entire period

membranes. Based upon the equivalent circuit shown in Fig. 7, R_o and R_i could be calculated if the resistance of the active transcellular pathway, R_{Na} , were known. Estimation of R_{Na} would be possible using the definition $R_{Na} = E_{Na}/I_{sc}$ (Ussing & Zerahn, 1950), provided the value of E_{Na} could be obtained. For skins of *R. pipiens* under control conditions, evidence was presented (Helman & Fisher, 1977) that the value of E'_1 represents a close measure of the E_{Na} .

During the progress of this study, however, it became clear that, under the influence of ADH, E'_1 could not reasonably be assumed to estimate E_{Na} . This is demonstrated in Fig. 8 by a typical experiment showing the changes of I_{sc} , V_{sc} , E'_1 and E_1 upon addition of ADH and subsequent application of Amiloride at increasing concentrations. In accordance with results of O'Neill & Helman (1976), essentially no changes of E_1 from the value of 120 mV in the control period were observed under the influence of ADH or Amiloride in addition to ADH. In contrast, E'_1 decreased from the control value of 99 mV until values of

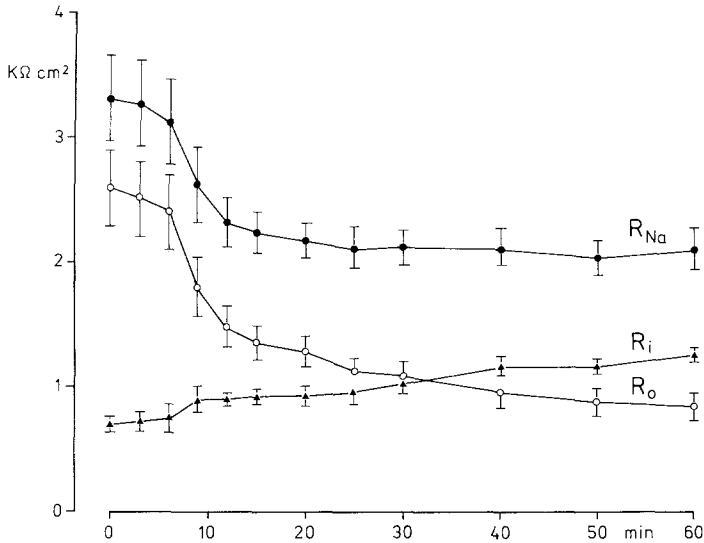


Fig. 9. Summary of the effect of ADH upon the resistance of the transcellular pathway, R_{Na} , and the resistance of the outer and inner border, R_o and R_i

~ 60 mV, concomitant with the increase of the I_{sc} . This change of E'_1 , however, is exclusively dependent upon the alteration of the outer membrane resistance: E'_1 , measured immediately after addition of Amiloride at increasing concentrations, increased stepwise as the I_{sc} was reduced by virtue of increasing the outer membrane resistance. When the I_{sc} approached zero, the values of E'_1 and E_1 became identical.

Similar results were obtained in all experiments which allowed simultaneous measurements of the E'_1 and the E_1 . Thus, the value of E'_1 does not provide a direct estimate of the E_{Na} . If, on the other hand, the values of E'_1 after Amiloride were assumed to represent the E_{Na} in that state of transport and if they are furthermore considered to yield a measure of the E_{Na} before Amiloride, i.e., during the action of ADH, this might allow us to approximately calculate the resistance of the active pathway. Further reasons which support the applicability of this method, will be presented later.

Mean values of R_{Na} , R_o , and R_i of all experiments are shown in Fig. 9. On the average, R_{Na} decreased within 30 min to some $2.2 \text{ k}\Omega\text{cm}^2$, which is about 65% of the control value of $3.3 \pm 0.3 \text{ k}\Omega\text{cm}^2$. More pronounced were the changes of the R_o , which decreased from $2.6 \pm 0.3 \text{ k}\Omega\text{cm}^2$ within

50 min to about $0.9 \text{ k}\Omega\text{cm}^2$ ($\sim 35\%$ of the control value). The opposite behavior, i.e., an increase under the influence of ADH, was observed for the inner border resistance, R_i increased from $0.71 \pm 0.10 \text{ k}\Omega\text{cm}^2$ within 50 min to about $1.2 \text{ k}\Omega\text{cm}^2$ or 170% of the control value. Thus, both membranes of the epithelial cells were affected by ADH, surprisingly, however, in opposite sense so that the inner membrane resistance exceeded the resistance of the outer membrane after ADH.

Discussion

The present investigation was done in an attempt to provide the data which must be considered if the effects of ADH upon electrolyte permeability of epithelial cell membranes are to be expressed in quantitative terms. Since epithelial structures consist of, at least, two functionally different membranes, separating the intracellular space from the outer and the inner bathing solutions, *intracellular* electrical potentials and electrolyte concentrations must be estimated in addition to unidirectional fluxes of ions across the individual membranes. In contrast to the many investigations dealing with the effect of ADH upon electrolyte concentrations and tracer fluxes in amphibian skins and bladders (for references, see Andreoli & Schafer, 1976), surprisingly little effort has been done to characterize the effect of ADH upon the electrical PD across the outer and inner borders.

Only two previous studies reporting measurements of ADH effects in amphibian epithelia were published (Civan & Frazier, 1968; Rawlins *et al.*, 1970) but neither present values of intracellular potentials under the influence of ADH. The investigations were restricted to the question of which membranes were responsible for the decrease in transepithelial resistance induced by ADH. The results could be interpreted to suggest that only the outer membranes of toad bladder (Civan & Frazier, 1968) or toad skin (Rawlins *et al.*, 1970) were affected by ADH. Irrespective of the validity of this conclusion, however, the experimental verification must be seriously questioned. Recent microelectrode investigations (Nagel, 1976; Helman & Fisher, 1977) demonstrated that previously reported intracellular potentials of frog skins were artifactual and, thus, the conclusions questionable. Similar doubts must be raised regarding the reliability of microelectrode data from toad bladders (Civan & Frazier, 1968). Using similar techniques, Frazier

(1962) reported intracellular potentials under short circuit conditions of about -5 mV. When the mucosal $[\text{Na}]$ was changed to <1 mM, which should reduce the I_{sc} considerably, the V_{sc} decreased to about -3.5 mV. In contrast, Schultz, Frizzell and Nellans (1977) demonstrated by use of an equivalent circuit for epithelial cells that the V_{sc} should increase when the I_{sc} is reduced. Data reported recently by Sudou and Hoshi (1977) indicate that this conclusion is valid for toad bladder. In addition, Frömter and Gebler (1977) pointed out on theoretical grounds that the PD across the serosal border under open circuit conditions must increase if the outer membrane resistance is elevated, and they were able to demonstrate this behavior in *Necturus* urinary bladder. The data of Civan and Frazier (1968), however, show opposite behavior of V_b , which always changed in proportion to the transepithelial PD. This and the low value of some -25 mV for the serosal membrane PD appears to be rather unique for cell membranes. It is interesting to note that artifactual inner membrane potentials of amphibian skins obtained in previous investigations (Whittembury, 1964; Ussing & Windhager, 1964; Cerejido & Curran, 1965; Biber, Chez & Curran, 1966) showed similar characteristics. Thus, it appears that potential measurements as well as estimates of outer and inner membrane resistance from toad bladders are similarly questionable as previous measurements from amphibian skins and require critical reinvestigation with improved techniques.

In the present study with frog skins, microelectrode techniques were applied which avoided injury of the impaled cells by the microelectrode (Nagel, 1976). Then, it could be observed, upon addition of ADH, that the intracellular potential under short-circuit conditions, V_{sc} , decreased considerably in all experiments to values between -20 and -50 mV at times when the effect of the hormone was maximal, as measured by the I_{sc} . Since this required recording intracellular potentials from a single cell for more than 30–40 min, it could be suspected that injuries of the impaled cell might explain all or part of the changes. Several observations, however, argue against this possibility.

- 1) In control experiments, it is often possible to record intracellular potentials up to 1–2 hr without indications of cell injury.

- 2) Changes of the V_{sc} during the entire course of a single experiment were closely related to alterations of the I_{sc} , suggesting a causative dependency between the changes of V_{sc} and I_{sc} .

- 3) The results obtained in experiments, in which different cells were punctured during the action of ADH, were, except for a slightly higher scatter, very similar to those which were recorded from a single cell.

4) After steady values of V_{sc} and I_{sc} under the influence of ADH were reached, Na entry across the outer border was blocked with Amiloride. In all punctures, which were accepted, this produced an immediate increase of the V_{sc} to values between -110 and -130 mV, averaging -117 ± 2 mV. Similar values were observed when Amiloride was applied under control conditions (Nagel, 1975; Helman & Fisher, 1977). Furthermore, the fractional resistance of the outer border increased to values near 1.0, indicating the absence of quantitatively important leaks at the outer border. In contrast, considerably smaller or even no changes in V_{sc} and values of $F(R_o)$ clearly less than 1.0 were recorded upon addition of Amiloride, both under control conditions and after ADH, when the cells were injured spontaneously or intentionally. Since it seems most unlikely that a previously lost or injured cell could be resealed by addition of Amiloride, fast and large changes of V_{sc} , together with increases to values of more than -110 mV and $F(R_o)$ close to 1.0 after Amiloride, were considered as proof for proper cellular origin of the values recorded under the influence of ADH. This test was applied in all experiments, and only those were accepted which showed the required response.

On the average, the V_{sc} decreased from -79 mV in the control period before ADH to -36 mV under the influence of ADH. This decrease to less than 50% of the control value may equally well be attributed to alterations of electromotive or conductive components at the outer or inner border of the epithelial cells. In an attempt to discriminate between the different possibilities, the experimental data were analyzed with the simple electrical equivalent circuit show in Fig. 7. The transport processes at the two membranes are each represented by a single electromotive force and a single resistance in series, which appropriately models any number of parallel transport pathways (Schultz *et al.*, 1977). The electromotive forces E_o and E_i refer to the equilibrium potentials of the outer and inner border, respectively. They represent the weighted sum of the electromotive forces of all ions contributing to the respective border conductance (Goldman, 1943):

$$E_o = \sum_x \frac{g_x}{g_o} \varepsilon_x \quad \text{and} \quad E_i = \sum_y \frac{g_y}{g_i} \varepsilon_y.$$

$g_o = \frac{1}{R_o}$; $g_i = \frac{1}{R_i}$: conductance of outer and inner border.

g_x ; g_y : partial conductance for ions X at outer and ions Y at inner border.

ε_x ; ε_y : Nernst potential for ions X at outer and ions Y at inner border.

Based upon measurements of transepithelial potentials, it was concluded (Koefoed-Johnsen & Ussing, 1958), that the permeability for Na at the outer border of the frog skin exceeds that of other cations considerably. This implied that E_o would be more or less equivalent to the ε_{Na} , the Nernst potential for Na. Measurements of the transepithelial potentials in relation to the ionic composition of the epithelial bathing solution (Koefoed-Johnsen & Ussing, 1958; Lindley & Hoshiko, 1964; Leb, Hoshiko & Lindley, 1965) are hitherto the only experimental proofs of this hypothesis. On the other hand, it was suggested (Winn *et al.*, 1966; Finn, 1974) that this simple and comprehensive model might not provide a valid description of apical and basolateral membrane transport properties. Furthermore, direct measurements using microelectrodes (Helman & Fisher, 1977; Nagel, 1977) demonstrated that both presumptions of the Koefoed-Johnsen & Ussing model, i.e., $E_o \approx \varepsilon_{\text{Na}}$ and $E_i \approx \varepsilon_{\text{K}}$, are not fulfilled in frog skin (Nagel, 1977; Helman & Fisher, 1977; Nagel & Helman, 1977; Helman, Nagel & Fisher, *in preparation*). Irrespective of these restrictions, however, the electrical equivalent circuit of Fig. 7 provides a correct description of the effective electrical processes (net currents).

It was derived (Helman & Fisher, 1977; Schultz *et al.*, 1977) that

$$V_{\text{sc}} = E_o \cdot [1 - R_o/(R_o + R_i)] - E_i \cdot R_o/(R_o + R_i).$$

This equation can be used to estimate E_o and E_i from the directly measured values of V_{sc} and $R_o/(R_o + R_i) = F(R_o)$. For this purpose, the values of V_{sc} before and immediately after Amiloride were plotted against the corresponding values of $F(R_o)$. Amiloride was applied in each experiment, when the effect of ADH was maximal. Values from the same experiments (i.e., the same cell) are connected by lines. It was presumed that Amiloride affects only the outer border of the frog skin (Dörge & Nagel, 1970; Biber, 1971; Cuthbert, 1971; Rick, Dörge & Nagel, 1975) and that secondary influences upon the inner border are neglectable if steady values of V_{sc} and $F(R_o)$ can be obtained immediately after addition of the inhibitor. In the present study, the complete change of the V_{sc} after Amiloride could be recorded within 2–3 sec. Alterations of the intracellular electrolyte concentrations during this short period are most likely unimportant in quantitative respect. The V_{sc} remained essentially constant for several minutes thereafter, supporting the idea that eventually existing changes of intracellular electrolyte concentrations exert only minor effects upon the inner membrane. It is obvious from Fig. 10, that the results from the individual experiments were very similar. Thus,

the data of all experiments were pooled and used to calculate a linear regression line. Extrapolating above and below the measured values of $F(R_o)$, the values of V_{sc} at $F(R_o)=1$ and $F(R_o)=0$ were calculated as estimates of E_i and E_o , respectively. They were found to be: $E_i = -123 \pm 3$ mV, and $E_o = +19 \pm 5$ mV.

The value of E_i under the influence of ADH is not different from values observed under control conditions (Helman & Fisher, 1977; Nagel & Helman, 1977; Nagel, *unpublished results*).² Similar values of E_i are obtained by a formally different approach by using the same equivalent circuit of Fig. 7. The dependency between the V_{sc} and the I_{sc} follows the equation

$$V_{sc} = -(E_i - I_{sc} \cdot R_i)$$

(Helman & Fisher, 1977; Schultz *et al.*, 1977). Thus, the value of V_{sc} at $I_{sc} = 0$ provides a measure of E_i , while the slope of the correlation estimates R_i . As above, it is assumed that Amiloride affects only the outer membrane resistance. Fig. 11 shows the dependency between V_{sc} and I_{sc} obtained in a single characteristic experiment (impalement). A linear relationship fits to the values over the entire range. The intercept with the y -axis at $I_{sc} = 0$ yields $E_i = -118$ mV. Similar values are obtained under control conditions (Nagel, *unpublished observations*). No effect of ADH upon the intracellular potential could be detected, furthermore, if Amiloride had been applied previous to the hormone. From these results it appears most appropriate to suggest that ADH has no direct influence upon the inner border, i.e., the Na pump. However, the ineffectiveness of ADH upon the V_{sc} , if mucosal Na entry had been prevented, might be explained in a different way. Provided the Na influx across the basolateral border of the epithelium is negligibly small (Canessa, Labarca & Leaf, 1976; Dörge, Rick & Thurau, 1976), intracellular [Na] might be already close to zero in Amiloride-treated skins. Stimulation of the Na

² Values of $E_i > 100$ mV, which are generally obtained in frog skin, infer that potassium cannot be in equilibrium across the inner border. Reasonable values of the potassium equilibrium potential, ϵ_K , may not exceed 100 mV at 2.5 mM [K] in the inner bathing solution and intracellular [K] of 115 mM (Rick *et al.*, 1977). Regardless of the mechanism(s) that increase the inner border potential difference above the ϵ_K , this finding implies that equilibration of potassium between the inner bathing solution and the intracellular space includes components which either reduce the intercellular [K] below 2.5 mM, increase the intracellular [K] above 115 mM, or expel potassium actively out of the cell. As discussed in detail elsewhere (Helman, Nagel & Fisher, *manuscript submitted*), the latter possibility, i.e., active potassium extrusion at least under certain conditions, seems to be the most feasible explanation of the results. A comprehensive discussion of this problem, however, would be beyond the aim of this communication.

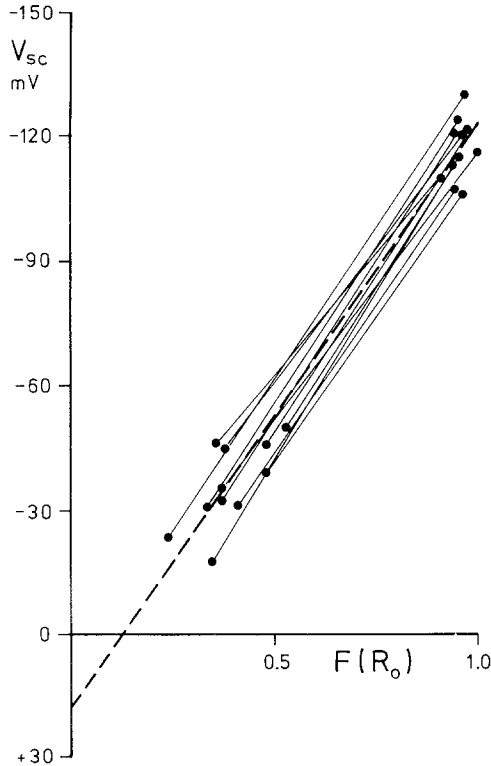


Fig. 10. Correlation between V_{sc} and $F(R_o)$ obtained before and after addition of Amiloride (10^{-5} M) at times when the effect of ADH upon the electrical parameters of frog skin was maximal. Lines connect values from individual experiments. The dashed line represents the regression line calculated for the pooled data using the method of least squares. The intercepts with the Y-axis represent E_o at $F(R_o)=0$ and E_i at $F(R_o)=1.0$

pump, even if ADH had this effect, would be electrically silent in lack of the substrate Na. Thus, stimulation of the Na pumping mechanism by ADH, particularly at elevated intracellular $[Na]$ (Aceves, 1977), cannot unequivocally be excluded.

In view of the fact that ADH primarily affects the Na entry step across the outer border, it was of particular interest to determine the effective driving force E_o at the outer border under these conditions. As discussed above, $E_o = +19$ mV was estimated from the correlation between V_{sc} and $F(R_o)$. In a different way, the value of E_o can be obtained from the definition of E_{Na} as the effective driving force of the transport pathway (Ussing & Zerahn, 1950), since $E_{Na} = E_o + E_i$. Using this definition, Helman & Fisher (1977) demonstrated recently that $E_{Na} \approx E_i$ under control conditions and had to conclude that E_o is not far different

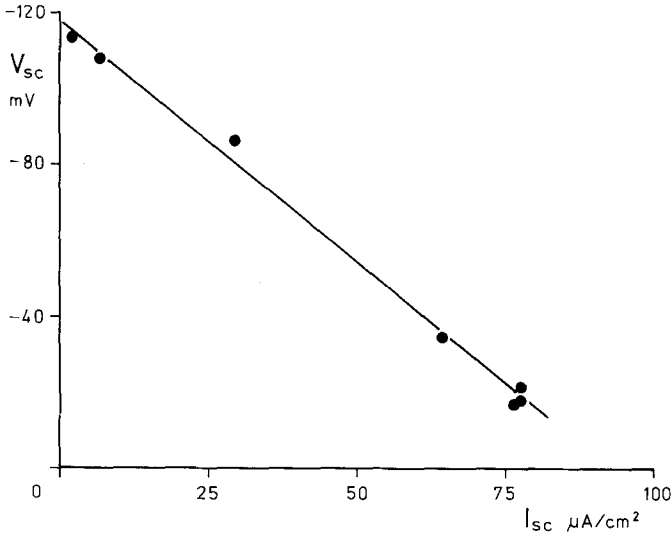


Fig. 11. Graph of the correlation between V_{sc} and I_{sc} from a single experiment. The values were obtained when the skin was maximally stimulated by ADH immediately after addition of Amiloride (2×10^{-7} to 2×10^{-5} M) under these conditions

from zero, i.e., that the net ion flux across the outer border must approach zero when the potential difference across this membrane is reduced to zero, irrespective of the still existing chemical potential gradient for Na. The present study suggests that skins under the influence of ADH might behave differently. As shown in Figs. 2, 3 and 8, E'_1 is reduced in proportion to the increase of the I_{sc} . These values of E'_1 are not estimates of the E_{Na} . If the skins were clamped to the value of E_{Na} at E'_1 , the current through the cellular pathway should have been abolished, and any change of the resistance would have no effect upon E'_1 . In contrast, applying Amiloride to ADH-treated skins resulted in a change of E'_1 by 41 ± 5 mV (range: 17–79 mV). Thus, the skins were not clamped to the value of E_{Na} at $V_o = 0$ mV.

To obtain a better approximation of the E_{Na} , the I/V -relationship was used to calculate those values of V_o and V_i , which fulfilled the requirement that the transcellular current was the same before and after Amiloride. This would be indicated by a constancy of V_i since R_i can be assumed to be unaffected by Amiloride. This condition obviously requires that the current had already been reduced to zero by voltage clamping the skin. Assuming that zero current flow is established by this at the outer and inner membrane, i.e., that net changes of intracellular

electrolytes are absent, the potential differences V_o and V_i across the outer and inner border, respectively, can be taken as direct estimates of the equilibrium potentials E_o and E_i of both membranes. From the data of the present investigation, $E_o = +24 \pm 2$ mV (range: +14 to +34 mV) was obtained for ADH-treated skins. This value is remarkably similar to the above estimate of +19 mV from V_{sc} and $F(R_o)$, using the same experimental data but different theoretical approach and different assumptions. Thus, it appears reasonable to accept a value of about +20 mV for the equilibrium potential of the outer border after ADH. Smaller values of E_o , averaging below 10 mV, were obtained under control conditions in skins of *R. temporaria* (Nagel, unpublished results).

These experimentally determined values of the equilibrium potential of the outer border should be compared with estimates of the chemical potential for Na of this membrane, since Na is presumed to be the most –if not the only– permeant cation. In skins incubated under control conditions and with 110 mM [Na] in the outer bathing solution, intracellular [Na] of about 7 mM was measured (Rick *et al.*, 1978). Only small increases were observed after ADH (Rick, personal communication). Accordingly, the ε_{Na} must be expected in the range of +50 to +70 mV. The values of E_o observed under control conditions and when the Na transfer across the outer border was stimulated by ADH, were always considerably less than these values predicted on the assumption that the apical membrane is impermeable to cations except Na. Thus, the present data would be incompatible with basic thermodynamic rules, if this presumption is correct. Experimental verification, except that of transepithelial measurements, is still lacking. Results from microelectrode investigations, which appeared to be a direct proof (Engbaek & Hoshiko, 1957; Cereijido & Curran, 1965; Biber *et al.*, 1966), were demonstrated to represent impalement artifacts (Nagel, 1976, 1977; Helman & Fisher, 1977). In contrast, results from transepithelial measurements (Winn *et al.*, 1966; Finn, 1974) and microelectrode investigations (Helman & Miller, 1971; Nagel, 1977; Frömter & Gebler, 1977; *present study*) raise doubts whether the Koefoed-Johnsen & Ussing model is valid without serious restrictions. One restriction might be that ions other than Na are significantly permeant at the outer border. Indeed, recent electrophysiological studies of *Necturus* urinary bladders (Frömter & Gebler, 1977) demonstrate that leak conductance for Cl^- and/or K^+ may decrease E_o below the ε_{Na} at high values of the outer border resistance. The present results and those of Helman and Fisher (1977), however, cannot be explained on this basis since the deviation between E_o and ε_{Na}

is also obtained at low values of R_o . Although the outer border of *R. temporaria* was found to be permeable to potassium (Hirschmann & Nagel, 1978), this appears to be significant only under certain conditions (no Na flux across the outer border, low intracellular [Na]) and should have only minor influence upon the E_o under conditions of large Na transport when more than 95% of the outer border conductance is due to the flux of Na. Similar considerations argue against quantitatively meaningful contributions of other ions (H^+ , Cl^- , HCO_3^-) to the outer border equilibrium potential. Thus, feasible explanations for the experimental observation that $E_o \neq \varepsilon_{Na}$ cannot be provided by presently available data and model considerations of the outer border transport mechanisms. It is interesting to note that more positive values of E_o are obtained after stimulation of Na transport by ADH than under control conditions, i.e., E_o is shifted in direction of the ε_{Na} at increasing g_{Na} . In view of the comparatively small change, however, it is not surprising that E_{Na} , the effective driving force for transepithelial Na transport, was found to be essentially unchanged under the influence of ADH (Ussing & Zerahn, 1950; Civan, Kedem & Leaf, 1966; Civan, 1970; Yonath & Civan, 1971; O'Neill & Helman, 1976). The present data are in agreement with these observations.

The equivalent circuit, shown in Fig. 7, describes the transport processes at the individual membranes of the epithelium in terms of electrical currents, irrespective of responsible ion fluxes and transport mechanisms. Thus, it provides a tool to estimate the total resistance of the transepithelial pathway, R_{Na} , and the resistances of the individual membranes, R_o and R_i . R_{Na} was calculated from the I_{sc} and the value of E_i after Amiloride, which appears to provide a feasible approximation of the E_{Na} (the transcellular current is zero when the skin is clamped to this potential). Assuming, in addition, that effects of ADH upon the E_{Na} are quantitatively unimportant, it was obtained that R_{Na} decreased to about 65% of the control value under the influence of ADH. This comparatively small change of the overall resistance of the epithelial cells was the result of opposite alterations of the outer and inner border resistances. The decrease of R_o to about 35% of the control value was considerably larger than the response of R_{Na} . This demonstrates that the effect of ADH upon the outer membrane, which has been described by previous investigators (for reference, see Andreoli & Schafer, 1976), is considerably larger than expected from transepithelial measurements. Unexpected and rather surprising was the observation that R_i increased to about 175% of the control values after ADH. This behavior, which is difficult to trace

back to systematic experimental errors or incorrect assumptions,³ cannot be feasibly explained and deserves further investigation.

The present investigation shows that the intracellular potential of the frog skin epithelial cells may be considerably influenced under hormonal stimulation of the transepithelial Na transport. The intracellular potential is the result of the electromotive forces and the fractional resistances of the two epithelial membranes. The depolarization of the intracellular potential after ADH was the consequence of changes in E_o to more positive values, on one side, and of opposite alterations of the resistances R_o and R_i , resulting in an increase of the fractional resistance at the inner border, on the other side. Since the intracellular negative potential provides a significant fraction of the driving force for Na entry across the outer border, this driving force is attenuated by the influence of ADH. The actual effect of the hormone upon the apical membrane conductance of Na must thus be underestimated if calculations were based upon the overall effects regardless of whether data from transepithelial (I_{sc} , Na net flux) or transmembranal (Na uptake) measurements were considered. If the changes of the electrical gradients were not accounted for, the effect of ADH upon the outer membrane of the frog skin would be underestimated by a factor of two. Whether additional alterations of the chemical gradients for Na exist, and whether this would require further corrections of the quantitative estimates of the effect of ADH, must be analyzed by appropriate methods.

I am indebted to Dr. S.I. Helman for helpful suggestions and a generous loan of recording equipment for the I/V measurements. The technical assistance of Miss V. Hilka, the construction of the interfacing circuit for the line printer by Mr. Schickle, the expert drawing of the illustrations by Mrs. C. Kottmair, and the skillful preparation of the manuscript by Miss Ch. Jenke is gratefully acknowledged.

Amiloride was a gift of Merck, Sharp & Dohme, Munich, W. Germany. The work was supported by the Deutsche Forschungsgemeinschaft.

References

Aceves, J. 1977. Sodium pump stimulation by oxytocin and cyclic AMP in the isolated epithelium of the frog skin. *Pfluegers Arch.* **371**:211

³ R_i was calculated according to: $R_i = [1 - F(R_o)] \cdot R_{Na}$. It is obvious that the decrease of $F(R_o)$ was responsible for the increase of R_i despite a decrease of R_{Na} . Erroneous estimation of R_{Na} by more than 40% of its value after ADH (which would result in constancy of R_i) seems most unlikely. This would require that E_{Na} decrease by that amount after ADH. Too low estimates of the $F(R_o)$ would be obtained, if the apical membrane would not seal properly around the microelectrode tip. The fact that $F(R_o)$ increased to almost 1.0 upon addition of Amiloride in all accepted impalements excludes this possible experimental error.

- Andreoli, Th.E., Schafer, J.A. 1976. Mass transport across cell membranes: The effects of antidiuretic hormone on water and solute. *Annu. Rev. Physiol.* **38**:451
- Biber, T.U.L. 1971. Effect of changes in transepithelial transport on the uptake of sodium across the outer surface of the frog skin. *J. Gen. Physiol.* **58**:131
- Biber, T.U.L., Chez, R.A., Curran, P.F. 1966. Na transport across frog skin at low external Na concentrations. *J. Gen. Physiol.* **49**:1161
- Canessa, M., Labarca, P., Leaf, A. 1976. Metabolic evidence that serosal sodium does not recycle through the active transepithelial transport pathway of toad bladder. *J. Membrane Biol.* **30**:65
- Cerejido, M., Curran, P.F. 1965. Intracellular electrical potentials in frog skin. *J. Gen. Physiol.* **48**:543
- Civan, M.M. 1970. Effects of active sodium transport on current voltage relationship of toad bladder. *Am. J. Physiol.* **219**:234
- Civan, M.M., Frazier, H.S. 1968. The site of the stimulatory action of vasopressin on sodium transport in toad bladder. *J. Gen. Physiol.* **51**:589
- Civan, M.M., Kedem, O., Leaf, A. 1966. Effect of vasopressin on toad bladder under conditions of zero net sodium transport. *Am. J. Physiol.* **211**:569
- Cuthbert, A.W. 1971. Neurohypophyseal hormones and sodium transport. *Phil. Trans. R. Soc. London B.* **262**:103
- Dörge, A., Nagel, W. 1970. Effect of amiloride on sodium transport in frog skin. II. Sodium transport pool and unidirectional fluxes. *Pfluegers Arch.* **321**:91
- Dörge, A., Rick, R., Thurau, K. 1976. Characterization of the transport pool for sodium in frog skin by X-ray microanalysis. *J. Physiol.* **263**:202P
- Engbaek, L., Hoshiko, T. 1957. Electrical potential gradients through frog skin. *Acta Physiol. Scand.* **39**:348
- Finn, A.L. 1974. Transepithelial potential difference in toad urinary bladder is not due to ionic diffusion. *Nature (London)* **250**:495
- Frazier, H.S. 1962. The electrical potential profile of the isolated toad bladder. *J. Gen. Physiol.* **45**:515
- Frömter, E., Gebler, B. 1977. Electrical properties of amphibian urinary epithelia. III. The cell membrane resistances and the effect of amiloride. *Pfluegers Arch.* **371**:99
- Goldman, D.E. 1943. Potential, impedance, and rectification in membranes. *J. Gen. Physiol.* **27**:37
- Helman, S.I., Fisher, R.S. 1977. Microelectrode studies of the active Na transport pathway of frog skin. *J. Gen. Physiol.* **69**:571
- Helman, S.I., Miller, D.A. 1971. *In vitro* techniques for avoiding edge damage in studies of frog skin. *Science* **173**:146
- Higgins, J.T., Jr., Gebler, B., Frömter, E. 1977. Electrical properties of amphibian urinary bladder epithelia. II. The cell potential profile in *Necturus maculosus*. *Pfluegers Arch.* **371**:87
- Hirschmann, W., Nagel, W. 1978. The outer membrane of frog skin: Impermeable to K^+ ? *Pfluegers Arch.* **373**:R48
- Koefoed-Johnsen, V., Ussing, H.H. 1958. The nature of the frog skin potential. *Acta Physiol. Scand.* **42**:298
- Leb, D.E., Hoshiko, T., Lindley, B.D. 1965. Effects of alkali metal cations on the potential across toad and bullfrog urinary bladder. *J. Gen. Physiol.* **48**:527
- Lindley, B.D., Hoshiko, T. 1964. The effects of alkali metal cations and common anions on the frog skin potential. *J. Gen. Physiol.* **47**:749
- Macknight, A.D.C., Leaf, A., Civan, M.M. 1971. Effects of vasopressin on the water and ionic composition of toad bladder epithelial cells. *J. Membrane Biol.* **6**:127
- Nagel, W. 1975. Reinvestigation of intracellular PD of frog skin epithelium (*Abstr.*) 5th International Biophysics Congress, Copenhagen

- Nagel, W. 1976. The intracellular electrical potential profile of the frog skin epithelium. *Pfluegers Arch.* **365**:135
- Nagel, W. 1977. The dependence of the electrical potentials across the membranes of the frog skin upon the concentration of sodium in the mucosal solution. *J. Physiol. (London)* **269**:777
- Nagel, W., Helman, S.I. 1977. Evidence for electrogenic transport of Na in frog skin revealed in microelectrode studies using ouabain. *Pfluegers Arch.* **368**:R22
- O'Neill, R.G., Helman, S.I. 1976. Influence of vasopressin and amiloride on shunt pathways of frog skin. *Am. J. Physiol.* **231**:164
- Rawlins, F., Mateu, L., Fragachan, F., Whitttembury, G. 1970. Isolated toad skin epithelium: Transport characteristics. *Pfluegers Arch.* **316**:64
- Rick, R., Dörge, A., Arnim, E. von, Thurau, K. 1978. Electron microprobe analysis of frog skin epithelium: Evidence for a syncytial Na transport compartment. *J. Membrane Biol.* **39**:3/3
- Rick, R., Dörge, A., Nagel, W. 1975. Influx and efflux of sodium at the outer surface of frog skin. *J. Membrane Biol.* **22**:183
- Schultz, S.G., Frizzell, R.A., Nellans, H.N. 1977. An equivalent electrical circuit model for "sodium-transporting" epithelia in the steady-state. *J. Theoret. Biol.* **65**:215
- Sudou, K., Hoshi, T. 1977. Mode of action of amiloride in toad urinary bladder. An electrophysiological study of the drug action on sodium permeability of the mucosal border. *J. Membrane Biol.* **32**:115
- Ussing, H.H., Windhager, E.E. 1964. Nature of shunt path and active sodium transport path through frog skin epithelium. *Acta Physiol. Scand.* **61**:484
- Ussing, H.H., Zerahn, K. 1950. Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. *Acta Physiol. Scand.* **23**:110
- Whitttembury, G. 1964. Electrical potential profile of the toad skin epithelium. *J. Gen. Physiol.* **47**:795
- Winn, P.M., La Prade, N.S., Tolbert, W.R., Huf, E.G. 1966. On the nature of the resting frog skin potential. *MCVQ Med. Coll. Va Q.* **2(2)**:116
- Yonath, J., Civan, M.M. 1971. Determination of the driving force of the Na⁺ pump in toad bladder by means of vasopressin. *J. Membrane Biol.* **5**:366