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INTRACELLULAR CHLORIDE ACTIVITY AND MEMBRANE POTENTIAL IN STRIPPED FROG SKIN (*RANA TEMPORARIA*)

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The regulation of cell chloride activity in frog skin was investigated using double barrelled Cl^--microelectrodes to measure cell membrane potentials and chloride activity in the isolated frog epidermis. Experiments were done under short-circuit conditions, impaling cells from the serosal side. The basic electrophysiological parameters of the isolated skin were similar to those reported in the literature for whole preparations. Intracellular chloride activity was on average 21.9 mM and membrane potential was about 57 mV, implying that chloride was distributed away from its electrochemical equilibrium (i.e., concentrated inside the cells). Chloride activity decreased after removal of either Cl^- or Na^+ from the serosal bathing solution, with no change in membrane potential. The chloride permeability of the serosal membrane was calculated to be $2.6 \cdot 10^{-6}$ cm $\cdot s^{-1}$ which represents about 1/4 of the total conductance of the serosal membrane. We suggest that an electrically silent sodium-dependent uphill transport of chloride is present at the basolateral membrane of the frog skin, which accounts for the non-passive distribution of chloride.

Estimates of cell chloride concentration in frog skin indicate higher levels than those predicted by a passive distribution [1–5]. This implies an uphill transport of chloride into the cells as part of the mechanism responsible for the regulation of cell chloride. It is therefore an interesting problem to identify the location and properties of this process, which need not be related to the transepithelial transport of chloride. Ferreira and Ferreira [5] using chemical determination and tracer analysis, showed that the cell chloride content is dependent on serosal Cl^- and Na^+ concentrations and that the mucosal membrane permeability to chloride ions is low. They also showed that intracellular chloride does not change upon removal of this ion from the mucosal solution or when the net inwards transepithelial chloride transport is inhibited with acetazolamide. The suggestion is, therefore, that the process of chloride accumulation is located at the baso-lateral membrane and somehow dependent on the Na^+ gradient. A more direct way of investigating the existence of a basolateral chloride permeation pathway is to measure cell chloride activity (a^c_{Cl}) and membrane potential whilst altering the ionic composition of the serosal bathing solution. This has been attempted here by using double-barrelled Cl^--sensitive microelectrodes and the isolated frog skin epidermis. The use of stripped epithelium enables the impalements to be carried out from the serosa and to have ready access to the basolateral membrane, which is not possible in the whole preparation. The present report is concerned with the basic characterization of the preparation, the measurement of a^c_{Cl} in the steady-

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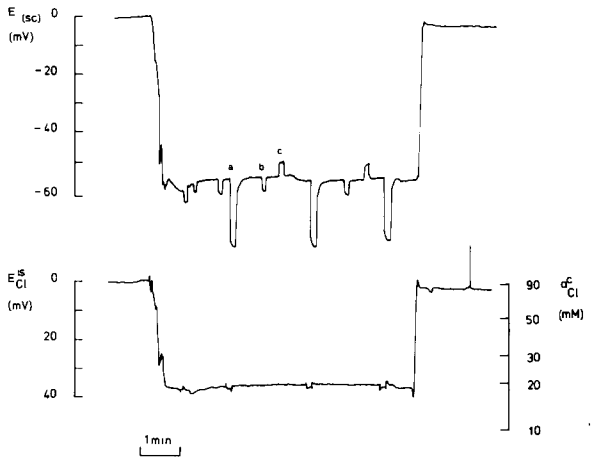

Fig. 1. Pen recording of a simultaneous measurement of intracellular potential and chloride activity with a double-barrelled Cl^--sensitive microelectrode in the isolated frog skin epidermis. The preparation was short-circuited and bathed with Frog-Ringer [5] in both sides. At (a) the skin was left in open circuited and at (b) and (c) voltage clamp pulses of ± 20 mV were applied. The scale at the right was drawn according to the electrode calibration curve. The resistance of the reference barrel was 45 MΩ and the slope of the Cl^--electrode was 54 mV/pCl^-. All potentials were referred to the serosal bath.

state and the study of the transients after serosal Cl^- or Na^+ removal.

Frog skin (*Rana temporaria*) were isolated according to the method described by Ferreira [6]. The epidermis was mounted, serosa upwards, on a modified Ussing chamber suitable for microelectrode work based on a design of Fromter [7]. The exposed area of skin was 0.2 cm. Impalements with microelectrodes were done from the serosal side. Double-barrelled Cl^--sensitive microelectrodes were constructed according to the method described by Zeuthen [8], using a liquid Cl^--ion exchanger (Corning code 477315). The procedure for calibration and the electrical arrangements have been described in detail elsewhere [9]. Experiments were done under short-circuit conditions except for short periods during which the preparation was either clamped at ± 20 mV or left in open circuit to measure the spontaneous transepithelial potential (E^T_{OC}).

The intracellular chloride activity (a^c_{Cl}) was measured as the electrical differences between the two barrels of the electrode. The technique is illustrated in Fig. 1 which shows the electrical potential and the differential signal (proportional to a^c_{Cl}) recorded by a double-barrelled Cl^--sensitive microelectrode during a cell impalement. It can be seen that cell membrane potential ($E_{(SC)}$) and a^c_{Cl} are stable for a reasonably long period and that both traces return to the original baseline upon withdrawing the microelectrode from the cell. A further test of the validity of the measurement is given by the constancy of the amplitude of the deflections produced by clamp pulses of ± 20 mV (b, c) and by leaving the preparation in open circuit (a). Moreover, the changes in $E_{(SC)}$ do not affect the differential recording, demonstrating a correct subtraction of the electrical component.

The results of experiments done in the steady state, with both sides of the epithelium bathed in frog-Ringer [5] are summarized in Table I. The general electrophysiological parameters are in agreement with previous reports on whole preparations [10–12]. The value of cell membrane potential and voltage divider ratio ($\alpha = \Delta E^m/\Delta E^s$) were remarkably constant from cell to cell within the same skin and for periods up to three hours. The conductance of the cellular pathway (G^c) was 0.35

TABLE I

ELECTROPHYSIOLOGICAL PARAMETERS OF THE STRIPPED FROG SKIN PREPARATION

Membrane potentials and cell chloride activity were measured with double-barrelled Cl^--sensitive microelectrode, making the impalements from the serosal side. $E^T_{(OC)}$ = transepithelial potential; I_{SC} = short-circuit current; $E_{(SC)}$ = cell membrane potential under short-circuit conditions; G^t = total conductance; α = voltage divider ratio. All potentials are refered to the serosal bath. Numbers are given as mean $\pm$ S.E. of five experiments.

$E^T_{(OC)}$ (mV)	I_{SC} ($\mu A \cdot cm^{-2}$)	$E_{(SC)}$ (mv)	G^t ($mS \cdot cm^{-2}$)	α	a^c_{Cl} (mM)
-64.5 ± 5.9	33.8 ± 5.1	-57.1 ± 4.7	0.59 ± 0.08	3.9 ± 0.9	21.9 ± 1.5

$mS \cdot cm^{-2}$. This value was computed from the short-circuit current (I_{SC}) and the Na^+ electromotive force (E_{Na}), the latter value being obtained by measuring the change in I_{SC} and G^t after amiloride (10^{-4} M) [13]. For a voltage divider ratio of 3.9 (see Table I) the corresponding value of the serosal conductance (G^s) is about 1.7 $mS \cdot cm^{-2}$.

The results of Table I (2nd and 6th columns) show that chloride is concentrated inside the cells of the isolated frog skin. The activity predicted by a passive distribution would be 9.8 mM instead of the observed value of 21.9 mM, revealing that E_{Cl} is positive to $E_{(SC)}$ by about 20 mV. The mean value of a^c_{Cl} agrees well with recent measurements with single-barrelled microelectrodes in whole preparations [4]. The main implication of this result is that chloride distribution cannot be accounted for by pure passive mechanisms.

As the apparent permeability of the mucosal barrier to Cl^- is very low [5] it is interesting to focus the problem on the properties of the basolateral membrane. Some information about the size of the movements of Cl^- and their dependence on the ionic composition of the bathing solution can be obtained by analyzing the transient changes of a^c_{Cl} after altering the composition of the serosal solution. Fig. 2 shows the effect of serosal Cl^--removal on $E_{(SC)}$ and a^c_{Cl}. Immediately after the removal of Cl^-, a^c_{Cl} starts to decrease with virtually no change $E_{(SC)}$. The decrease in a^c_{Cl} followed an exponential time-course that could be reasonably fitted by a single rate constant. The results of several experiments like the one in Fig. 2 are summarized in Fig. 3 (filled circles). The initial rate of change in a^c_{Cl} after 0-Cl^- ($(da^c_{Cl}/dt)_{t=0}$) was 0.072 $mM \cdot s^{-1}$. Assuming that the observed change in a^c_{Cl} is only due to transmembrane fluxes of chloride, the net Cl^--efflux from the cells in 0-Cl^- can be computed from the rate of decrease in a^c_{Cl} and the volume to surface ratio. For an activity coefficient of 0.78 [14] and a volume to surface ratio of 20 μm [15], the initial Cl^--efflux is 184 $pmol \cdot cm^{-2} \cdot s^{-1}$. This value represents the net outward movement of chloride across the basolateral membrane as the driving forces across the mucosal barrier were virtually unaltered at $t = 0$.

As a first approximation, it can be considered that the observed Cl^--efflux is due to the electrodiffusional movement of Cl^- down its electrochemical gradient, in which case the Cl^--permeability of the serosal membrane (P^s_{Cl}) can be computed using the constant field theory [16]. For a net Cl^--efflux of 184 $pmol \cdot cm^{-2} \cdot s^{-1}$ and the values shown in Table I, the calculated P^s_{Cl} is $2.6 \cdot 10^{-6} \cdot s^{-1}$. This corresponds to a slope con-

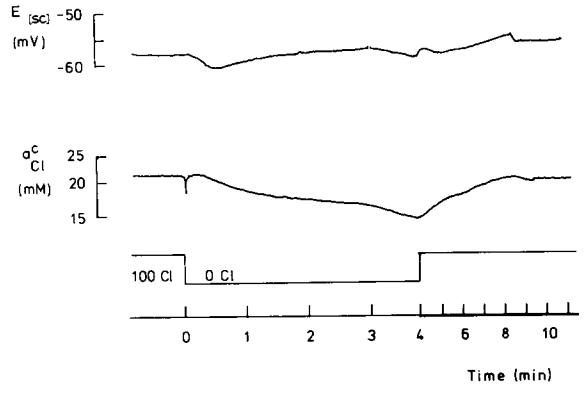

Fig. 2. Effect of serosal Cl^--removal on a^c_{Cl} and $E_{(SC)}$. The recording starts with the Cl^--sensitive electrode inside the cell. The upper trace shows $E_{(SC)}$ and the lower shows the differential recording, proportional to a^c_{Cl}. For the period indicated at the bottom the serosal bathing solution was changed from frog-Ringer to Cl^--free (0-Cl^-) Ringer (gluconate-substituted [5]).

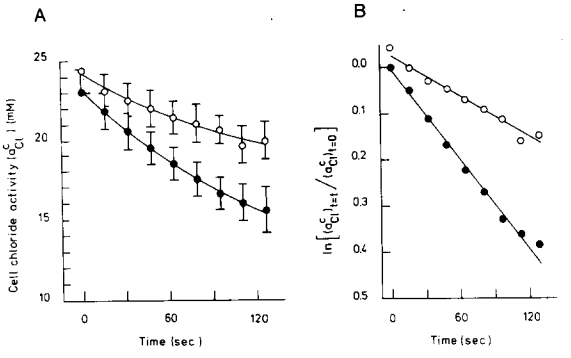

Fig. 3. Effect of serosal Cl^- or Na^+ removal on a^c_{Cl}. (A) Linear plot of the decrease in a^c_{Cl} after 0-Cl (●) and 0-Na^+ (○) against time. Cl^- was replaced by gluconate and Na^+ by choline [5]. Each point represents pooled data from continuous measurements in different experiments ($n = 5$ for 0-Cl^- and $n = 4$ for 0-Na^+). The bars indicate S.E. (B) Semilogarithmic plot of the same data. The slope of the straight lines (k) are $-3.1 \cdot 10^{-3}$ and $-1.6 \cdot 10^{-3}$ s^{-1} for 0-Cl^- (●) and 0-Na^+ (○), respectively. The initial rate of change in a^c_{Cl} was calculated from: $(da^c_{Cl}/dt)_{t=0} = k \cdot \Delta a^c_{Cl}$; $\Delta a^c_{Cl} = 23.1$ mM.

ductance for Cl^- of 0.44 $mS \cdot cm^{-1}$, that is about 1/4 the conductance of the serosal membrane given above. This value is consistent with the K^+-selectivity of the serosal membrane and with the results reported by Nagel (1979) on the residual basolateral conductance after blockade of K^+-channels with Ba^{2+} [12]. The serosal permeability calculated from this experiment would give a net efflux of chloride into normal Ringer of about 110 $pmol \cdot cm^{-2} \cdot s^{-1}$, which is comparable with the steady-state efflux measured with isotopes in the same preparation [5]. It has to be stressed however, that P^s_{Cl} can be overestimated in this type of experiment if part of the measured efflux occurs through a carrier mediated mechanism running 'backwards', i.e. from cell to serosa, and this mode of operation might well be exposed or activated by the removal of serosal chloride (Ref. 17, and Ferreira, K. unpublished data). We have no information on the transient changes in cell volume in the conditions of the experiment. The final change in cell volume after long exposure to Cl^--free solutions has been reported to be of about 20–30% [5]. If this change were to take place at a similar rate to that observed after drastic osmotic changes of the serosal bathing solution (about 0.02% s^{-1}, see Ref. 17) the initial Cl^--efflux would have a negligible error of about 10 $pmol \cdot cm^{-2} \cdot s^{-1}$.

Sodium removal from the serosal bath led also to a decrease in a^c_{Cl} without any immediate change in the intracellular potential. Preliminary experiments indicate however that the cells slowly depolarized after long exposures (1 h) to 0-Cl^- or 0-Na^+ solutions. The results of different experiments in which the transients of a^c_{Cl} after Na^+-removal from the serosal bathing solution were studied, are shown in Fig. 3 (open circles). The initial rate of change in a^c_{Cl} was 0.037 $mM \cdot s^{-1}$ and from this value a net Cl^--efflux of 94 $pmol \cdot cm^{-2} \cdot s^{-1}$ can be calculated using the same procedure as for the 0-Cl^- experiments. Again, if this efflux is postulated to be electrodiffusional, after stopping a Na^+-dependent inward movement of Cl^-, P^s_{Cl} would be about $2.2 \cdot 10^{-6}$ $cm \cdot s^{-1}$, but as mentioned above the exposure of a carrier mediated outward flux of Cl^- cannot be excluded.

In conclusion, the results show that the isolated frog skin can be used in conjunction with the double-barrelled microelectrode technique to study cell ionic activities and membrane potentials in both steady and non steady-state conditions. The measurements of a^c_{Cl} confirm previous indications that chloride ions are non-passively distributed between the cellular and the extracellular compartments. The effects of Cl^- and Na^+ removal on a^c_{Cl} and cell membrane potential suggest that the basolateral membrane is the site for the mechanism responsible of chloride accumulation and that it is electrically neutral and dependent on serosal Na^+.

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