*Biochimica et Biophysica Acta*, 330 (1973) 102–107
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BBA 76493

# CHLORIDE FLUX ACROSS FROG SKINS OF LOW POTENTIAL DIFFERENCE

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(Received August 20th, 1973)

## SUMMARY

In a series of unselected isolated skins of *Rana pipiens* in summer, with a mean potential difference (PD) of 13 mV (5–30 mV), the short-circuit current equivalent was significantly less than net $Na^+$ flux. No discrepancy was found in absence of chloride (skins bathed in sulfate Ringers'). Net $Cl^-$ influx was demonstrated in $Cl^-$-containing Ringers' and was of appropriate magnitude to explain the discrepancy. The active transport of Na was quantitatively similar to values seen in skins of higher PD. Comparison of $Cl^-$ flux in this study with mean $Cl^-$ flux and PD values from other series suggests an inverse seasonal variation of $Cl^-$ flux and PD.

## INTRODUCTION

Ussing and co-workers[1,2,4] and Linderholm[3] first presented evidence in isolated frog skin of *Rana temporaria* and *Rana esculenta* that $Na^+$ is actively transported and $Cl^-$ transported passively. The following findings were presented as proof of passive transport of chloride: (1) The flux ratio of chloride was that expected for a given electrochemical gradient across the skin; (2) The short-circuit current, *i.e.* the electrical current across the skin in the absence of an electrical gradient and with equal concentrations of ions on both sides of the skin, was equivalent to the net $Na^+$ flux or $Na^+$ current. It has generally been assumed in the species *Rana pipiens* that chloride transport is also passive since equivalence between short-circuit current (s.c.c.) and net $Na^+$ flux has been repeatedly demonstrated in isolated skin.

Jorgensen *et al.*[5] demonstrated net inward transport of chloride from an external solution of low concentration of the ion, against an electrochemical gradient across the skin of several intact amphibian species including *R. temporaria* and *R. esculenta*[5]. Experiments in this laboratory, in living anesthetized *R. pipiens* with a wide range of spontaneous open-circuit potential difference (PD), demonstrated that the short-circuit current was less than net $Na^+$ flux in skins with PD values below 20–30 mV[6]. Active inward chloride transport across the skins of low PD was postulated to explain this discrepancy. Subsequently, Martin and Curran[7] found, in both *R. pipiens* and *R. esculenta*, net chloride transport inward under short-circuit

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Abbreviations: PD, potential difference; s.c.c., short-circuit current.

conditions but only when the concentration of chloride in the media on either side of the skin was very low (2 mM). Kristensen[8] confirmed these findings in *R. temporaria*.

In this laboratory, and probably in many others, isolated frog skins with PD values below 20 mV are not generally used for experimentation. They are considered "poor" skins. It seemed possible that significant active chloride transport or net chloride transport did exist at these low potential differences in isolated skin of *R. pipiens* even with bathing solutions of the NaCl concentrations usually used experimentally (100–120 mM). This seemed particularly likely on the basis of experiments in the living anesthetized frog since a NaCl concentration of 113 mM was used on both sides of the skin. The present study demonstrates net $Cl^-$ flux inward under short-circuit conditions at a $Cl^-$ concentration of 115 mequiv/l in a series of isolated skins of low PD. Despite the low PD the net $Na^+$ flux was of the magnitude usually seen in skins of higher PD.

## METHODS

Unfed 40–60 g male and female frogs (*Rana pipiens*) that were kept in large bins with running tap water were used. Abdominal skin was dissected from decapitated frogs and mounted between Lucite hemichambers. The volume of the compartments on each side of the skin was 8 ml and the skin area was 1.33 cm$^2$. The general design and size of the chambers was similar to that of Gonzalez *et al.*[9]. In each hemicell a shallow working chamber is connected, by inlet and outlet channels of 1 mm diameter orifices, to a main reservoir. The center of the orifices is 1 mm from the skin. Air lift circulation with moisturized air is used in one of the channels. Rapid circulation and oxygenation is thus obtained. However, all the components were machined into a single Lucite block to avoid leakage problems and assembly delays. The electrolyte solution bathing the skin, a modified Ringers' solution, had the following composition in mM/l: NaCl, 110; KCl, 5; $NaH_2PO_4$, 1.3; $NaHCO_3$, 4.0. The solution was adjusted to pH 7.5. In sulfate Ringers' NaCl and KCl were replaced with 55 mM $Na_2SO_4$ and 2.5 mM $K_2SO_4$.

For PD measurements, matched Corning calomel electrodes were placed in the reservoir. The circulatory channels containing the Ringers' solutions served as Ringer bridges to the skin. Carbon electrodes, connected by Ringer agar bridges, were used to short-circuit the skin[4]. The skin was continuously short-circuited by an automatic device which consists of a simple circuit, using three operational amplifiers and a power supply. This circuitry is to be described elsewhere. The short-circuit current and PD were recorded on an Esterline-Angus recorder (model E1124E). This is a multi-channel dot recorder (set for six channels) which contains a custom-made automatic switching device connected to relays in the short-circuiting system so that the open-circuit potential difference can be recorded 30 s of every 6 min. Thus, up to six pieces of skin can be studied at one time.

At the beginning of each experiment, $^{36}Cl$ or $^{22}Na$ was added to the inside or outside solutions. Influx and outflux measurements were begun at the end of 1 h. The measurements were made in the conventional manner by removing samples from the two fluid compartments. $^{36}Cl$ was counted in a liquid scintillation spectrometer using a liquid scintillator in naphthalene–dioxane. $^{22}$Na was counted in an auto-gamma spectrometer.

## RESULTS

The studies were performed between May 15 and August 15, 1971. With rare exception, the isolated skin of the animals received in the several different shipments from Steinhilber Inc. of Oshkosh, Wisconsin, had potentials below 25 mV. The range in skins used in this study was 5–30 mV. This is a value taken 1.5 h after mounting the skin.

$^{22}$Na unidirectional influx and outflux measurements were performed on paired pieces of skin from the same animals (Table IA). In each pair net flux was calculated as well as the short-circuit current equivalent on the influx experiments, for the 1-h flux period. The skins in chloride Ringers' had a net flux which was 0.67 $\mu$equiv/cm$^2$ per h greater than the s.c.c. equivalent. When treated as paired data this difference was statistically significant ($P<0.02$). In the absence of Cl$^-$ (sulfate Ringers') there was no discrepancy between the net Na$^+$ flux and s.c.c. equivalent.

This lack of equivalence is not explained by the error inherent in short-circuiting low resistance membranes. The lowest and mean d.c. resistances in the series in Table IA were 114 and 319 $\Omega$/cm$^2$, respectively. This figure is calculated from the short-circuit current and PD at the mid-point of the flux period. Assuming a specific resistance of the layers of the Ringers' solution between the potential probes equal to that of 100 mM NaCl and to be 2 mm thick (see Methods) it can be shown that the approximated greatest and mean errors in the s.c.c. equivalent were −18 and −6%, respectively[10,11]. Yet, the mean s.c.c. equivalent was actually 30% lower than the net Na$^+$ flux.

In Table IB are the Cl$^-$ flux values. In the first series of 18 influx and outflux experiments, which were performed during the same period as the Na$^+$ flux studies

TABLE I

Na$^+$ AND Cl$^-$ FLUX IN FROG SKINS OF LOW POTENTIAL DIFFERENCE

All values are expressed as $\mu$equiv/cm$^2$ per h and represent the mean ±S.E. for the number ($N$) indicated. $P$ refers to student $t$ test comparing Cl$^-$ influx and outflux. Values greater than 0.05 are considered not significant (N.S.).

| | *N* | *Influx* | *N* | *Outflux* | *Net flux* | *s.c.c. equivalent* | *P* |
|---|---|---|---|---|---|---|---|
| (A) *Sodium flux* | | | | | | | |
| Chloride Ringers | 16 | 2.85 ± 0.29 | - | 0.57 ± 0.14 | 2.28 ± 0.29 | 1.59 ± 0.12 | - |
| Sulfate Ringers | 9 | 1.85 ± 0.25 | - | 0.55 ± 0.10 | 1.30 ± 0.20 | 1.29 ± 0.20 | - |
| (B) *Chloride flux** | | | | | | | |
| Chloride Ringers | 18 | 4.24 ± 0.61 | 18 | 3.48 ± 0.67 | 0.75 | - | N |
| Chloride Ringers | 60 | 4.71 ± 0.31 | 61 | 3.75 ± 0.30 | 0.96 | - | <0 |

* The series of 18 Cl$^-$ influx and 18 outflux were performed concomitantly with the Na$^+$ flux studies in Series A. The series of 60 influx and 61 outflux pools the first series of 18 influx and outflux, respectively, with all other studies performed between May 15 and August 15, 1971.

in Table IA, there was a mean difference of 0.75 $\mu$equiv/cm$^2$ per h. The Cl$^-$ influx and outflux difference was not statistically significant but was quite close to the value of the discrepancy between s.c.c. equivalent and net Na$^+$ flux.

The second series consists of all Cl$^-$ flux studies performed during the 3-month period mentioned above including the 18 influx and outflux studies done at the time of the Na$^+$ flux measurements. The pooled results yield a net influx of 0.96 $\mu$equiv/cm$^2$ per h. There is a significant difference between the Cl$^-$ influx and outflux in this case.

## DISCUSSION

These experiments show a lack of equivalence between short-circuit current and net Na$^+$ flux in a series of isolated skins of *R. pipiens* with low PD values (generally below 25 mV). This is similar to previous findings in living anesthetized *R. pipiens*[6]. The discrepancy is not present in the absence of Cl$^-$ (sulfate Ringer's). It is reasonably well explained by the demonstration of net chloride transport inward of appropriate magnitude, as originally postulated for the living frogs.

As mentioned, in this laboratory, skins of low PD are often considered "poor" and are not usually used for isolated skin experiments. However, the magnitude of net Na$^+$ flux in these experiments with low PD skins is comparable to values generally found for skins of higher PD[12,13] so that they certainly cannot be considered "poor" in terms of sodium transport. Selection of frog skins with higher PD for experimentation probably explains why this phenomenon of net Cl$^-$ flux inward under these conditions has not previously been reported.

Table II shows mean values of Cl$^-$ flux consisting of pooled influx and outflux measurements in each of three other series performed earlier in this laboratory at other times of the year. These mean values and the influx and outflux means, respectively, from Table IB are presented in Fig. 1 in relation to season. The mean PD was lowest in summer, rose in fall to a peak in winter and fell again in the spring. This is approximately the same seasonal relationship reported by Huf *et al.*[15] for two sequential years (1955 and 1956). The Cl$^-$ flux–seasonal relationship is inverse to the PD.

In a small number of experiments by Koefoed-Johnson *et al.*[1] both unidirectional Cl$^-$ influx and outflux, respectively, in the open-circuit state seemed to approximate an inverse correlation with the skin PD. This relationship was explained by the equivalent circuit model for frog skin assuming that Cl$^-$ flux is predominantly

TABLE II

MEAN Cl⁻ FLUX AND PD IN THREE OTHER SERIES IN ISOLATED FROG SKIN

Each series includes both influx and outflux experiments in approximately equal numbers.

| *Source* | *Inclusive dates* | *N* | *Cl⁻ flux (μequiv/cm² per h)* | *PD (mV)* |
|---|---|---|---|---|
| Unpublished | Oct.–Nov. '69 | 20 | 0.73 ± 0.10 | 24 ± 1.7 |
| Unpublished | Jan.–Apr. '69 | 23 | 0 37 ± 0.09 | 32 ± 2.6 |
| Ref. 14 | Nov. '67–Feb. '68 | 19 | 0.33 ± 0.06 | 50 ± 4.3 |

passive. Increased shunting of the electromotive force associated with active sodium transport by chloride, the major passive ion present, produces a decreased skin PD. The study reported here differs in that all fluxes were performed at zero electrochemical potential gradients, except for brief intervals of open-circuit conditions for the PD measurements. Thus, the $Cl^-$ flux in either direction (except for the relatively small portion of "active" $Cl^-$ influx such as shown in this study) is a measure of passive partial ionic conductance of $Cl^-$ (ref. 3). The data in Fig. 2 suggests that the passive $Cl^-$ conductance is high in summer, decreases in fall and winter and increases again in spring. It is possible that active chloride transport exists at all $Cl^-$ conductance levels but at higher PD values is too small to be easily detected by the short-circuit current technique. For instance, if the mean $Cl^-$ flux ratio in this study (1.24) existed when the mean $Cl^-$ influx is, *e.g.* 0.25 μequiv/cm² per h, the net $Cl^-$ flux would be 0.05 μequiv. This net flux or ion current would be only 2.5% of a representative active sodium transport or short-circuit current value of 2.0 μequiv/cm² per h and easily be within methodologic error.

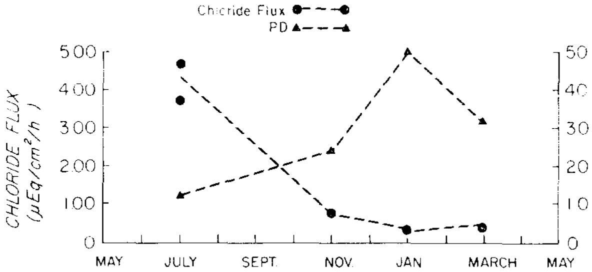

Fig. 1. Relationship of $Cl^-$ flux and PD to season in four series of isolated frog skins. The mid points of the time interval of each series are used. Mean $Cl^-$ influx and outflux are plotted separately for data from Table IB. The three series from Table II are mean values for influx and outflux, pooled.

The magnitude of unidirectional $Cl^-$ flux and net flux in this study was similar to the findings in the South American frog, *Leptodactylus ocellatus*[16], as was the PD range (5–30 and 10–40 mV, respectively). Whether this chloride transport system is similar to that in the South American frog and/or the system described by Martin and Curran[7] in skins of *R. pipiens* exposed to low concentrations of $Cl^-$ remains to be investigated.

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