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Effect of Acid Stress on Sodium Transport by Isolated Skins and on Osmotic Permeability of Intact Frogs

Paul O. Fromm

Department of Physiology, Michigan State University, East Lansing, MI 48824

Acidification of our surface waters, which results from atmospheric emissions of sulphur and nitrogen oxides, is currently one of the most serious problems of environmental pollution. It has been shown (PACKER & DUNSON 1970; LEIVESTAD & MUNIZ 1976; PACKER 1979; McDONALD et al. 1980) that acid stress upsets electrolyte balance in fish to the extent that one of the most significant physiological changes seen in adult fish dying from acid stress is iono-regulatory failure. How this is related to gill ion transport mechanisms is not completely understood (FROMM 1980).

It is known that ion uptake mechanisms exist in a large variety of freshwater animals; KROGH (1937) showed that salt depleted frogs can take up salt through the skin from very dilute solutions. The classic experiments of USSING & ZERAHN (1951) demonstrated that active ion transport is the source of electric current generated by the short-circuited isolated frog skin. Their technique of recording short-circuit current (SCC) provides us with a very convenient means of monitoring sodium transport in frog skins and other tissues. Measurements with microelectrodes are consistent with the idea that there is active transport of sodium from cells across the inward facing (baso-lateral or serosal) membrane of isolated skins and a passive leakage of Na^+ into cells from the solution bathing the outer surface (LINDEMANN & VAN DREISCHE 1978). With Ringer solution bathing both sides of an isolated frog skin the transport of sodium stays slightly ahead of the passive inward movement of chloride ions, thus charge separation is maintained. When anion flux is decreased by replacing chloride ions with impermeant sulfate ions the potential difference (PD) across the skin is increased. The addition of copper ions to the outside solution also decreases chloride permeability and causes an increase in PD.

With respect to the effect of pH on ion transport, USSING (1949) noted that aeration of the Ringer solutions bathing either side of isolated frog skins with gases having a CO_2 content as low as 1 to 2% caused a severe depression of the recorded potential difference and SCC. When phosphoric acid was used to lower the pH of the outside or inside solutions, i.e., solutions bathing the outer and inner surfaces of skins respectively, it was found (USSING & ZERAHN 1951) that as long as the inside pH remained around 8.0 the outside hydrogen ion concentration has little effect on sodium influx until the pH is about

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5 but below pH 5.0 ion flux drops to a very low value. SCHOFFENEILS (1955) also altered the pH of the bathing solutions with phosphoric acid and found that if the pH of both solutions was lowered the results obtained (PD, SCC and transkin resistance, R) could be superimposed on the results for lowering the pH of the inside solution alone. If the inside pH remained above 5.5 the effects of acid on SCC were reversible except when the outside pH dropped to 3.5 and was maintained for some time (duration of exposure not reported).

The experiments reported here were designed to determine the effects of increased external hydrogen ion concentrations on the ion transport capability of isolated frog skins measured as short-circuit current and to determine the nature of the interaction of hydrogen ions to sodium transport. Results from a study of the effects of acid exposure on the osmotic permeability of intact frogs are also reported.

MATERIALS AND METHODS

Adult frogs, Rana pipiens and Xenopus laevis, of both sexes were obtained from Mogul Ed, Oshkosh, WI. R. pipiens were kept in a cold room at 14°C prior to use and Xenopus were maintained in an aquarium at room temperature (20°C). After double pithing, a large portion of the abdominal skin was removed and mounted between the two chambers of a 'Ussing apparatus' for measurement of potential difference and short-circuit current. The apparatus used was essentially that as described by OAKLEY and SCHAFER (1978). The surface area across which measurements were made was 1.9 cm^2 and the pretreatment saline solution (35 mL) in each chamber was a Krebs-Ringer-Tris solution with the following composition: 154 meq/L NaCl; 4.75 meq/L KCl; 5.07 meq/L $CaCl_2$; 2.39 meq/L $MgSO_4 \cdot 7H_2O$; 154 meq/L Tris buffer and 5.5 meq/L glucose. The pH of this pretreatment saline solution was 7.4 at 20°C. All pH measurements were made with a glass electrode and a Markson ElectroMark Analyzer.

Aliquots of 0.1N H_2SO_4 were added to the outside solution to lower pH (Acid 1, 2 and 3). In other experiments 0.5 mL aliquots of 5.46 mM/L adrenalin chloride (Parke-Davis) or 0.5 mL of 10^{-4}M ouabain octahydrate (Sigma) were added to the inner solution.

HELMAN & MILLER (1973) have reported that edge damage can affect the electrical measurements and lead to a significant underestimate of both PD and resistance of isolated frog skins. While it is impossible to set up the isolated skin preparation without incurring some edge damage care was taken to keep it minimal. With skins properly mounted the PD across the skin and the SCC were determined at 5 min intervals using a Grass polygraph. Each experimental period lasted from 30 to 45 min and during the intervals between recordings the circuit remained open, i.e., SCC was not recorded continuously. Data for PD and SCC were transcribed from the permanent records and values for resistance were calculated.

Data were obtained from individual skins during six sequential experimental periods. After the Pretreatment period the pH of the outside solution was lowered step-wise (Acid 1 and Acid 2)

with H_2SO_4. At the end of the Acid 2 period the solutions bathing both sides of the skin were replaced with fresh saline (Post-Acid). Following the Post-Acid period skins were treated with either adrenalin or ouabain and for the final period the pH of the outside solution was lowered by addition of H_2SO_4 (Acid 3).

Values for pH were converted to hydrogen ion concentrations, averaged and this average reconverted to pH. The range in pH values for solutions whose mean pH was above 7.0 was typically less than 0.2 pH units, whereas, that for the acidic solutions was higher but rarely above 0.4 pH units. The other experimental data were subjected to an analysis of variance and the Student-Newman-Keuls test (SOKAL & ROHLF 1969) was used for comparison of treatment means. Those mean values in Tables 1-3 that are not statistically different ($p \geq 0.05$) are underlined.

RESULTS

The generalized responses of isolated skins of both R. pipiens and Xenopus were similar to what has been previously reported, namely, they are quite impermeable to protons (hydrogen ions), stimulation with adrenalin caused an increase in SCC accompanied by a decrease in PD, and treatment with ouabain reduced both SCC and PD.

Table 1. Comparison of the effect of acid stress on isolated skins of R. pipiens and Xenopus laevis

R. pipiens (N = 10)	Pretreatment	Acid 1	Acid 2	Post-Acid
PD in mV	44.5	23.1	23.4	24.2
SCC in mCoul/h cm^2	109.0	36.9	25.8	50.6
R in Ω/cm^2	1470	2253	3265	1721
Mean pH outside	7.45	4.62	3.14	7.41
Mean pH inside	7.35	7.39	7.35	7.38
Xenopus (N = 9)				
PD in mV	27.2	19.4	9.6	9.0
SCC in mCoul/h cm^2	38.8	26.3	18.8	10.0
R in Ω/cm^2	2523	2656	1838	2851
Mean pH outside	7.40	4.53	3.23	7.37
Mean pH inside	7.40	7.22	7.21	7.38

In experiments with R. pipiens data in Table 1 indicate that a stepwise lowering of the pH of the solution bathing the outside surface of isolated skins to 4.62 and 3.14 caused a 66 and 76% reduction in SCC respectively. The PD was some 48% below pretreatment values in both cases. Restoring the outside pH to pretreatment levels following exposure (1.5 h maximum duration) to acid led to some slight recovery of the ability of the skins to transport sodium but SCC still remained an average of 54% below the pretreatment level.

When skins of Rana were stimulated with adrenalin following

the post-acid recovery period the SCC produced by these skins was increased some 61% and the resultant mean value was not statistically different from the pretreatment mean. Again, exposure to acid reduced the SCC (Acid 3 Table 2). Ouabain, which blocks the sodium pump, reduced SCC below post-acid recovery values and subsequent acid exposure had no statistically significant effect.

Table 2. Effect of acid on adrenalin and ouabain treated skins of R. pipiens

R. pipiens (N = 6)	Pretreatment	Post-acid	Adrenalin	Acid 3
PD in mV	43.2	25.5	22.7	10.5
SCC in mCoul/h cm^2	104.5	55.0	88.5	40.3
R in Ω/cm^2	1488	1669	923	937
Mean pH[a] outside	7.51	7.46	7.53	3.54
Mean pH[a] inside	7.43	7.39	7.35	7.38
R. pipiens (N = 6)				
PD in mV	46.2	22.8	8.4	10.6
SCC in mCoul/h cm^2	114.4	45.4	19.0	13.0
R in Ω/cm^2	1453	1808	1591	2935
Mean pH outside	7.51	7.46	7.46	3.19
Mean pH inside	7.43	7.39	7.34	7.30

[a]based on N = 5

Table 3. Effect of acid on adrenalin and ouabain treated skins of Xenopus

Xenopus (N = 5)	Pretreatment	Post-acid	Adrenalin	Acid 3
PD in mV	38.8	10.2	29.2	17.2
SCC in mCoul/h cm^2	55.4	13.6	90.6	52.0
R in Ω/cm^2	2521	2700	1160	1191
Mean pH outside	7.40	7.37	7.33	3.41
Mean pH inside	7.40	7.37	7.34	7.27
Xenopus (N = 4)				
PD in mV	12.8	5.3	2.8	2.8
SCC in mCoul/h cm^2	18.0	5.8	3.0	5.3
R in Ω/cm^2	2560	3289	3360	1902
Mean pH outside	7.40	7.38	7.31	3.36
Mean pH inside	7.40	7.37	7.35	7.29

Pretreatment values for PD and SCC of Xenopus skins were lower than comparable values for Rana but the response of these skins to acid was essentially the same except that Xenopus skins showed a decrease in SCC during the post-acid recovery period. Apparently the skin of the predominantly aquatic

Xenopus is more sensitive to acid stress than that from the more amphibious Rana.

Skins of Xenopus previously exposed to acid showed a greater response to adrenalin than similarly treated skins from Rana. As with Rana, subsequent exposure of adrenalin stimulated skins to acid caused a reduction in SCC. Also ouabain reduced SCC of Xenopus skins from the post-acid recovery levels and subsequent acid exposure had no significant effect.

Table 4. Effect of lowered environmental pH on osmotic permeability of intact frogs, R. pipiens

Initial pH of bath	N	Osmotic permeability (L_p)[a]
6.3	13	0.88 ± 0.20 (mean ± S.D.)
4.0	7	0.71 ± 0.21
3.0	14	1.25 ± 0.44

[a] $g \cdot h^{-1} \cdot 100\ cm^{-2} \cdot 100\ mOsm^{-1}$

Except for the adrenalin stimulated skins, when the SCC of R. pipiens skins was reduced by acid stress there was an accompanying decrease in PD such that the transkin resistance (Table 3) increased, i.e., during acid exposure the relative decrease in PD was less than that for SCC. The mean pretreatment value for resistance (R) of Xenopus skins was some 72% above that for Rana and although exposure of these skins to acid generally caused decreases in both SCC and PD the transkin resistance was not affected except in the case of Acid 2 skins (Table 1) where, unexplainably, R decreased rather than increased.

Experiments were conducted to measure the effect of acid exposure on frog skin osmotic permeability using the method described by PARSONS & LAU (1976). Cloacal ligatures were applied to intact R. pipiens and they were exposed to 1% saline solutions whose pH was adjusted by addition of sulphuric acid. The data (Table 4)were quite variable and no statistically significant change in osmotic permeability was noted with exposure to lowered pH. Based on visual observations the activity of frogs in the most acidic solutions was greater which may have given rise to a higher internal osmolarity in these animals. However, the manner of calculating L_p normalized the data with respect to any variation in osmotic gradient across the skin.

DISCUSSION

The data presented for isolated frog skins are from experiments designed to mimic exposure of frogs to acidic waters. The results on R. pipiens and Xenopus confirm the earlier information published by USSING & ZERAHN (1951) and SCHOFFENEILS (1955) for R. temporaria, that is, exposure of isolated skins to lowered pH (regardless of whether the lowering resulted from addition of CO_2, phosphoric acid or sulphuric acid) causes a decrease in the short-circuit current generated by the skins.

The pretreatment values for PD and SCC for R. pipiens skins were significantly higher and the resistance lower than comparable values for Xenopus (Table 1). These differences may be an indication that Xenopus, a freshwater aquatic species, has developed a highly ion impermeable skin to aid in its osmotic and ionic regulation. Xenopus skin is heavily coated with mucus and it also appears to be slightly thicker than that of Rana. It was also noted that when copper ions were added to the outside solutions the PD across skins from Rana increased some 67% indicating a significant decrease in chloride permeability, whereas, skins of Xenopus showed no effect, possibly an indication of a characteristic low permeability of Xenopus skins.

R. pipiens skins exposed to severe acid stress (pH = 3.1) exhibited a very significant decrease (76%) in SCC and little recovery was noted when the acid stress was removed (e.g., Post-acid values, Table 1). Since the level of SCC produced in skins which had been exposed to acid conditions was increased by adrenalin stimulation and reduced by ouabain it is obvious that acid exposure did not destroy sodium pump activity per se.

The first step of sodium uptake by frog skin is the entrance of sodium ions from the subcorneal space into the cytosol of the outer stratum granulosum. This transport is rate limiting for the overall uptake of sodium and is controlled by several hormones. The epithelial cells of frog skin appear to possess pores in their apical or outer membranes through which sodium passes in the direction determined by the electrochemical gradient (LINDEMANN & VAN DREISSCHE 1978). These pores apparently open and close randomly and the frequency of closure increases with the outer sodium concentration. Titration or exposure of the outer membrane with protons may in some way cause a protonation of the Na-receptor groups associated with the pores and cause a decrease in the Na-permeability of the outer membrane. The data presented in this paper is consistent with this hypothesis, viz., that exposure of the outer surface of frog skin to increased acidity decreases the flux of sodium ions into the skins and thereby decreases overall sodium transport.

The effects of adrenalin on SCC tends to argue against any postulation that exposure to increased acid causes an irreversible inhibition of the cellular metabolic machinery needed for sodium pump activity. Perhaps acid exposure did interfere with the role of ATPase in sodium transport, however, if this was the case the inhibition was not complete since SCC was reduced by ouabain in skins previously exposed to acid. With all factors considered, it appears that in the acidic environment frogs, like fish, may suffer iono-regulatory failure and, based on experiments reported here, this loss of regulation would be associated with decreased uptake rather than excessive ion loss.

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