SHORT COMMUNICATIONS

BBA 73053

Active sodium transport in the isolated bullfrog cornea

The importance of ion transport for the control of cornea hydration and thickness has been recently stressed[1]. The existence of active transport of ions has been described in the isolated cornea of the rabbit[2], and in the North American bullfrog *Rana catesbiana*[3-5]. Na^+ was found to be the only ion actively transported across the rabbit cornea. Active transport of Cl^- from endothelium to epithelium was reported as being totally responsible for the transcorneal potential difference (p.d.) and short-circuit current (s.c.c.) in the isolated bullfrog cornea[3,4]. However, p.d. and s.c.c. do not decline to zero values when Cl^- is removed from the solutions bathing the frog cornea as would be expected from a pure-Cl^--transporting system. The p.d. decreases to values between zero and 2 mV only when the cornea is bathed in Ringer's solutions free of both Na^+ and Cl^- (replaced by choline sulfate). Furthermore, active transport of Cl^-, and the p.d. and s.c.c. resulting from it, are highly dependent of the presence of Na^+ in at least one of the solutions bathing the isolated cornea.

Removal of Na^+ from both bathing solutions produces a decline of the p.d. to zero, that is restored by addition of Na^+. Consistent with the importance of sodium for the maintenance of the electrical properties of the bullfrog cornea, this article presents direct evidence indicating that this isolated membrane actively transports Na^+. Also, the effect of pH on Na^+ and Cl^- potentials is reported.

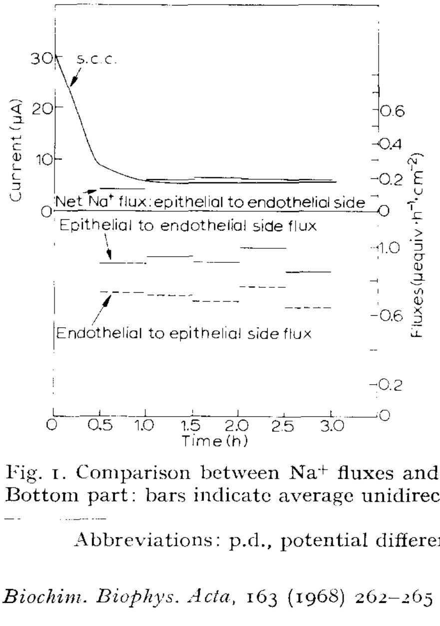

Fig. 1. Comparison between Na^+ fluxes and s.c.c. in Cl^--free Ringer's soln. Typical experiment. Bottom part: bars indicate average unidirectional flux for 30-min periods.

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

The cornea of the frog *Rana catesbiana* was dissected and mounted in a modified Ussing's-type chamber using the same technique as described by ZADUNAISKY[4].

Simultaneous unidirectional Na^+ fluxes were measured with ^{24}Na as tracer in one direction and ^{22}Na as tracer in the other direction. Samples representing one-sixth of the 3-ml hemi-chamber volume were taken from each side simultaneously every 30 min after allowing 30 min for the system to reach a steady state. Specific activity was kept constant by replacement of samples with radioactive Ringer's soln. Radioactivity in the less-radioactive side was 1 % or less of the activity in the more-radioactive side for each tracer throughout the experiments. Decay, geometry, resolving time and efficiency of the γ-detector were considered in the computations of the fluxes. The surface of cornea exposed to the solutions was 0.30 cm^2. Each experiment consisted of 5 or more periods of 30 min (Fig. 1). The corneas were maintained in an s.c.c. condition and the amount of current required was recorded continuously with an automatic voltage clamp recorder[6]. Potential drop due to solution resistance was also corrected. Identical solutions were always used in both hemi-chambers. The pH was also the same in both sides regardless of the pH level, and it was monitored by immersing pH electrodes in the chamber.

TABLE I

SIMULTANEOUS UNIDIRECTIONAL Na^+ FLUXES, NET Na^+ FLUX AND S.C.C. IN NaCl SOLUTIONS

Mean values from 15 isolated frog corneas. Paired comparison was made between epithelial to endothelial and endothelial to epithelial fluxes, H_0: (1) = (2), H_A: (1) > (2), the difference was statistically significant, H_0 rejected.

	Na^+ fluxes and s.c.c. in μequiv·h^{-1}·cm^{-2}			
	Epithelial to endothelial side (1)	*Endothelial to epithelial side (2)*	*Net (1)−(2)*	*s.c.c.*
Mean	0.636	0.540	0.095	1.100
Standard error			0.021	
			$0.005 > P$	

Table I shows a total of 15 experiments composed of 75 periods performed in NaCl Ringer's solution ($[Cl^-] = 75$ mM, $[Na^+] = 104$ mM). Air was used to aerate the solutions. Despite the high variability of the unidirectional fluxes, Na^+ flux from epithelium to endothelium was consistently larger than its respective paired flux from endothelium to epithelium. The mean of the differences (epithelium to endothelium *minus* endothelium to epithelium) was equal to a net Na^+ flux from epithelium to endothelium of 0.095 μequiv·h^{-1}·cm^{-2}. The mean of the differences was statistically different from zero.

In addition to this, 17 experiments (shown in Table II) composed of 85 periods were performed in Cl^--free (Na_2SO_4) Ringer's solution. An O_2–CO_2 (95:5, v/v) mixture was used to aerate the solutions. Two Na^+ concentrations were used in this series: 31 mM and 104 mM. The unidirectional Na^+ fluxes and the net, in less degree, were smaller in the lower concentration. Also in this condition the mean of the differences (epithelium to endothelium *minus* endothelium to epithelium) was equal to a net

TABLE II

SIMULTANEOUS UNIDIRECTIONAL Na^+ FLUXES, NET Na^+ FLUX AND S.C.C. IN Cl^--FREE SOLUTIONS

Mean values from 17 isolated frog corneas. Paired comparison was made: (a) between epithelial to endothelial and endothelial to epithelial fluxes, H_0: (1) = (2), H_A: (1) > (2), the difference was statistically significant, H_0 rejected; (b) between net Na^+ flux and s.c.c., H_0: (1) − (2) = (3), H_A: (1) − (2) ≠ (3), the difference was not statistically significant, H_0 can not be rejected.

	Na^+ fluxes and s.c.c. in μequiv·h⁻¹·cm⁻²				
	Epithelial to endothelial side (1)	*Endothelial to epithelial side* (2)	*Net* (1) − (2)	*s.c.c.* (3)	*Net minus s.c.c.* (1) − (2) − (3)
Mean	0.624	0.481	0.143	0.162	−0.019
Standard error			0.043		0.049
			$0.005 > P > 0.0005$		$0.8 > P > 0.6$

Na^+ flux from epithelium to endothelium of 0.143 μequiv·h⁻¹·cm⁻². Comparison between the net Na^+ flux and s.c.c. shows a small difference between them. This difference can not be proved to be statistically significant.

Net Na^+ flux was 0.048 μequiv·h⁻¹·cm⁻² higher in Cl^--free than in Cl^--rich Ringer's soln., although not statistically significant.

It should be noted that the pH of the NaCl Ringer's soln. was 8.6 and the pH of the Na_2SO_4 Ringer's soln. was 7.2 during the determination of the Na^+ fluxes.

The type of gas used to aerate and circulate the solutions bathing the cornea (air or an O_2–CO_2 (95:5, v/v) mixture) significantly affected the pH of the solution and the transcorneal p.d. Air bubbling stabilized the pH of the NaCl Ringer's soln. at about 8.6 and the pH of the Na_2SO_4 (Cl^--free) Ringer's soln. at about 8.9. Bubbling with the O_2–CO_2 mixture maintained a stable pH of about 7.2 in both types of Ringer's solution. p.d. and s.c.c. were greater when Na_2SO_4 Ringer's soln. was bubbled with the O_2–CO_2 mixture than bubbled with air. p.d. was lower when the NaCl Ringer's soln. was bubbled with the O_2–CO_2 mixture than when bubbled with air. This is shown in Fig. 2.

The depressive effect of the pH on Cl^- potential could not be tested in a Na^+-free Ringer's soln. since Cl^- transport is Na^+ dependent. Nevertheless, it can be seen that the corneal p.d. is about the same in NaCl as in Na_2SO_4 (Cl^--free) Ringer's soln. at pH 7.2 (Fig. 2).

Reduction of the pH in air-aerated solutions by the addition of H_2SO_4 also increased the p.d. in corneas bathed in Na_2SO_4 solutions.

It should be pointed out that unidirectional Na^+ fluxes reported by both ZADUNAISKY[4], and PLOTH AND HOGBEN[5] were about the same even though the former used air and the latter O_2–CO_2 for aeration of solutions.

The corresponding unidirectional Na^+ fluxes can be very different in two electrically well-matched corneas obtained from the same frog. We regard this fact as responsible for the failure of ZADUNAISKY, and PLOTH AND HOGBEN to prove a net Na^+ movement when group comparison is used.

Analysis of the flux data suggests that free and exchange diffusion account for a large fraction of the unidirectional Na^+ fluxes and may explain the large variation in unidirectional fluxes between different corneas[7,8]. However, such processes could not lead to a net transfer of Na^+ under s.c.c. condition with identical bathing fluids.

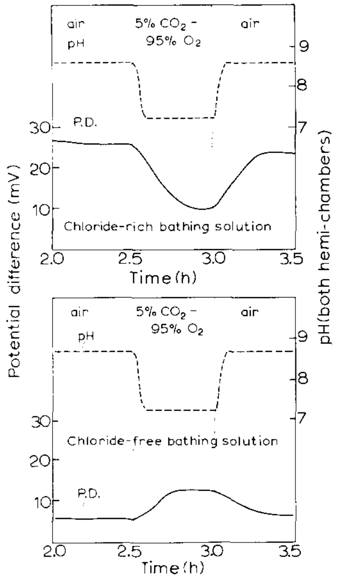

Fig. 2. Effect of gas bubbled on pH of Ringer's solutions and p.d. in the isolated frog cornea. Bicarbonate concentration in both, Cl^--rich and Cl^--free bathing solutions, was 25.12 mM.

The mechanism for the effect of pH on Na^+ and Cl^- potentials although unexplained at the present time, could be an important factor for further characterization of the ion transport in this complex membrane.

The existence of both an active transport of Na^+ and an Na^+-dependent Cl^- transport in the bullfrog cornea suggests that Na^+ could play a fundamental role in the control of the hydration of the frog cornea similar to its function evidenced in the rabbit cornea.

This investigation was supported by grants from U.S. National Institutes of Health GM-13953 and NB-05516.

Department of Ophthalmology,
School of Medicine, University of Louisville,
Louisville, Ky. 40208 (U.S.A.)

OSCAR A. CANDIA*
WALLACE A. ASKEW

1 K. GREEN, *Nature*, 217 (1968) 1074.
2 A. DONN, D. M. MAURICE AND N. L. MILLS, *A.M.A. Arch. Ophthalmol.*, 62 (1959) 741.
3 J. A. ZADUNAISKY, *Nature*, 209 (1966) 1136.
4 J. A. ZADUNAISKY, *Am. J. Physiol.*, 211 (1966) 506.
5 D. W. PLOTH AND C. A. M. HOGBEN, *Invest. Ophthalmol.*, 6 (1967) 340.
6 O. A. CANDIA, *IEEE Trans. Bio-Med. Engineering*, 13 (1966) 189.
7 A. ESSIG, *Biophys. J.*, 8 (1968) 53.
8 M. P. SHAPIRO AND O. A. CANDIA, *Biophys. J. Soc. Abstr.*, 8 (1968) A-33.

Received May 27th, 1968

---

* Present address: Department of Ophthalmology, Mount Sinai School of Medicine of the City University of New York, N.Y. 10029, U.S.A.