SODIUM AND CHLORIDE TRANSPORT ACROSS THE ISOLATED OPERCULAR EPITHELIUM OF *Fundulus heteroclitus*

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The opercular epithelium lining the gill chamber of the killifish, *Fundulus heteroclitus*, adapted to 100% artificial seawater contains a preponderance of chloride cells (Karnaky and Kinter, J. Exptl. Zool., 1977 in press) which are identical to those described in the gill epithelium (Keys and Willmer, J. Physiol., 76:368, 1932). These chloride cells have been assumed to be responsible for the majority of the extrarenal osmoregulatory Na^+ and Cl^- secretion in seawater teleosts. Recently, Karnaky, Degnan and Zadunaisky (Science, 1977 in press) demonstrated that the isolated *in vitro* opercular epithelium of 100% seawater-adapted *Fundulus heteroclitus* actively secreted Cl^-. This isolated preparation offered an advantage over other preparations used in the study of teleost regulation in that the short-circuit current technique (Ussing and Zerahn, Acta Physiol. Scand. 23:110, 1951) can be readily applied and the isotope fluxes accurately measured. This report presents the findings on the isolated opercular epithelium of 100% seawater-adapted (1XSW) *Fundulus heteroclitus* with some preliminary findings on the epithelium of freshwater-adapted (FW) and 200% seawater-adapted (2XSW) *Fundulus*.

The epithelium lining the gill chamber of the fish was dissected free of its bony operculum, mounted as a flat sheet in a lucite chamber (Zadunaisky and Degnan, Exptl. Eye Res. 23:191, 1976), bathed on both sides with a modified Forster's solution (Forster, Science 108:65, 1948) containing 16 mM HCO_3^- and gassed with either 100% O_2 (pH 8.25) or 95% O_2:5% CO_2 (pH 7.3). The preparations were kept short-circuited and the current (I_{sc}) continuously recorded on a chart recorder. They were open-circuited every 30 minutes to record the transepithelial potential difference (p.d.) and to calculate the d.c. resistance (Ω-cm^2). The isotope flux studies were performed on paired pieces of epithelia obtained from the same fish and mounted in matching chambers. The average net flux was calculated as the difference between the average outflux (blood to seawater flux) and the average influx (seawater to blood flux) across these paired preparations. The influence of certain pharmacological agents on the electrical properties of the isolated epithelia were tested by introducing aliquots of the drug to both sides of the chamber simultaneously which, when diluted into the volume of the chamber half, gave the desired concentration.

In 60 experiments, the isolated opercular epithelia of 1XSW-adapted *Fundulus* gassed with 100% O_2 exhibited a mean I_{sc} of 136.5 ± 11.1 μA/cm^2, a mean p.d. of 18.7 ± 1.2 mV (seawater side negative), and a mean d.c. resistance of 173.7 ± 12.1 Ω-cm^2. The transepithelial p.d. was a linear function of the log of the $[Cl^-]$ with a Nernst slope of 28.3 ± 2.1 mV/10-fold concentration change and a more complex nonlinear function of the $[Na^+]$. The I_{sc} across these epithelia was dependent on the presence of Na^+ in the bathing solutions but increased linearly with increasing $[Cl^-]$ within the range of 0 to 160 m-equiv/l with no evidence of saturation. As presented in Table 1, these electrical parameters were influenced by a number of agents known to affect Cl^- transport. The substitution of the Cl^- with methylsulphate anions (chloride-free) in the Ringer bathing both sides of the tissue and nitrogen-induced anoxia (N_2) produced significant ($P < 0.001$) decreases in the I_{sc} and p.d. which were readily reversed with the reintroduction of the normal Cl^- concentration (chloride-rich) and oxygenation (O_2). Ouabain, furosemide, and thiocyanate also produced significant ($P < 0.01$) decreases in the I_{sc} and p.d. while theophylline produced variable but significant ($P < 0.001$) stimulations. Amiloride (10^{-5} M) and amphotericin B (10^{-5} M) had very slight or no effects on these electrical properties.

TABLE 1

The Effect of Cl^--Free Ringer's, Anoxia, and a Few Pharmacological Agents on the Electrical Properties of the Isolated Opercular Epithelium of 100% Seawater-Adapted *Fundulus heteroclitus*

	I_{sc} ($\mu A/cm^2$)	p.d. (mV)	R (Ω-cm^2)
Control	234.4 ± 30.5	26.1 ± 4.0	122.7 ± 20.5
Chloride-free	8.8 ± 3.9	1.9 ± 0.6	58.5 ± 40.0
Percent change	96.3%	92.7%	52.3%
P	< 0.001	< 0.001	< 0.025
Chloride-rich	224.5 ± 27.8	23.5 ± 2.5	121.3 ± 28.9
Control (4)	236.6 ± 4.7	26.3 ± 2.5	110.7 ± 9.8
Anoxia, N_2	39.6 ± 10.4	8.6 ± 1.7	269.2 ± 74.7
Percent change	83.3%	67.3%	143.2%
P	< 0.001	< 0.001	< 0.025
Oxygenation	233.0 ± 14.7	30.8 ± 2.6	131.9 ± 5.3
Control (5)	130.3 ± 33.6	19.6 ± 3.6	164.8 ± 30.1
Ouabain, 10^{-5} M	12.7 ± 8.4	1.2 ± 0.6	79.4 ± 8.4
Percent change	90.3%	93.9%	51.8%
P	< 0.01	< 0.005	< 0.02
Control (6)	224.3 ± 39.9	28.1 ± 3.3	149.6 ± 38.2
Furosemide, 10^{-3} M	33.7 ± 11.7	3.7 ± 0.9	157.5 ± 47.5
Percent change	85.0%	86.8%	5.3%
P	< 0.005	< 0.001	< 0.50
Control (4)	110.9 ± 5.3	19.7 ± 3.2	125.8 ± 23.6
Thiocyanate, 10^{-2} M	54.3 ± 4.2	10.0 ± 1.7	133.4 ± 29.1
Percent change	51.0%	49.2%	6.0%
P	< 0.001	< 0.01	< 0.25
Control (12)	79.9 ± 25.8	9.6 ± 1.2	198.0 ± 29.6
Theophylline, 10^{-4} M	123.8 ± 27.6	16.8 ± 1.8	162.6 ± 22.4
Percent change	54.9%	75.0%	17.9%
P	< 0.001	< 0.001	< 0.001

Mean ± s.e.m.; Number of experiments in parentheses.

The effects of the pharmacological agents were studied by adding aliquots of the drug stock to both sides of the tissue simultaneously. Chloride-free refers to Ringer in which all the Cl^- was replaced with equimolar amounts of methylsulphate anions. Chloride-rich refers to the Ringer normally used in these studies which contained 142.5 m-equiv/l. Cl^-. Statistical significance was taken at $P < 0.025$.

The I_{sc} and p.d. across the opercular epithelium of 1XSW-adapted *Fundulus* were sensitive to the bicarbonate concentration in the bathing solutions. The stepwise titration of bicarbonate into the bathing media (0 to 30 mM in 10 mM increments) produced corresponding increases in the I_{sc} and p.d. which were independent of any pH effects. These bicarbonate titrations produced slight increases in the pH of the bathing solutions but similar pH increases produced by tirating NaOH into the bathing solutions had no stimulatory effects.

Table 2 lists the results of the paired $^{36}Cl^-$ and $^{22}Na^+$ fluxes across the epithelia of 1XSW-adapted *Fundulus heteroclitus*. When gassed with 100% O_2 the $^{36}Cl^-$ outflux was 4.3 times greater than

TABLE 2

$^{36}Cl^-$ and $^{22}Na^+$ Fluxes Across the Isolated Opercular Epithelium of 100% Seawater-adapted *Fundulus heteroclitus*

Isotope	Outflux μA/cm²	Influx μA/cm²	Net Flux μA/cm²	Mean I_{sc} μA/cm²	$[HCO_3]$ mM	Gassing
$^{36}Cl^-$	211.7 ± 27.1 (10)	48.9 ± 10.0 (10)	162.8 ± 30.9	158.6 ± 16.3 (10)	16	100% O_2
$^{22}Na^+$	32.2 ± 3.3 (10)	34.8 ± 4.1 (10)	-2.6 ± 4.6	56.9 ± 8.0 (20)	16	100% O_2
$^{36}Cl^-$	196.3 ± 57.2 (8)	76.6 ± 30.2 (8)	119.6 ± 29.3	119.2 ± 22.9 (16)	16	95% O_2:5% CO_2
$^{22}Na^+$	70.4 ± 12.0 (8)	79.0 ± 7.0 (8)	-8.6 ± 16.5	74.4 ± 10.3 (16)	16	95% O_2:5% CO_2

Mean ± s.e.m.; Number of experiments in parentheses

The fluxes are presented as μA/cm² for comparison to the I_{sc}. For preparations gassed with 100% O_2 the mean unidireftional $^{36}Cl^-$ fluxes were statistically different ($P < 0.001$) while the mean unidirectional $^{22}Na^+$ fluxes were not ($P < 0.25$). For preparations gassed with 95% O_2:5% CO_2 the mean unidirectional $^{36}Cl^-$ fluxes were statistically different ($P < 0.025$) while the mean unidirectional $^{22}Na^+$ fluxes were not ($P < 0.40$). In both preparations the net $^{36}Cl^-$ flux was not statistically different from the mean I_{sc} with P values of < 0.80 and < 0.90 respectively.

the $^{36}Cl^-$ influx ($P < 0.001$) resulting in a net outflux of 162.8 μA/cm² which was not statistically different ($P < 0.80$) from the mean I_{sc} of 158.6 μA/cm² for all these flux studies. The unidirectional $^{22}Na^+$ fluxes were not statistically different from each other ($P < 0.25$) resulting in no net movement of this ion and suggested that the I_{sc} across these preparations could be accounted for by the net secretion of Cl^-. When gassed with 95% O_2:5% CO_2 the mean $^{36}Cl^-$ outflux was 2.8 times greater than the $^{36}Cl^-$ influx ($P < 0.025$) resulting in a net outflux of 119.6 μA/cm² which was not statistically different ($P < 0.90$) from the mean I_{sc} of 119.2 μA/cm² for these flux studies. The unidirectional $^{22}Na^+$ fluxes were not statistically different from each other ($P < 0.40$) resulting in no net movement of this ion. When gassed with either 100% O_2 or 95% O_2:5% CO_2 the mean net $^{36}Cl^-$ flux and the mean I_{sc} were equivalent which suggested that there was no neutral Cl^-/HCO_3^- transepithelial exchange mechanism operating across these tissues. However, the stimulatory effect of HCO_3^- on the Cl^- secretion (as reflexted in the I_{sc}) suggested a definite link between these two ions. Whether this is accomplished via a Cl^-/HCO_3^- exchange across one membrane resulting from an increased intracellular pCO_2 remains to be determined.

The preliminary data obtained from epithelia of FW- and 2XSW- adapted *Fundulus* are presented in Table 3 and compared to that obtained from 1XSW-adapted fish. All three of these differently adapted fish displayed sizable net secretions of ^{36}Cl. The epithelia from the 2XSW-adapted fish also displayed a net $^{22}Na^+$ secretion resulting partly from the reduced influx of this ion. This may have resulted from the relatively high (2-3 times) d.c. resistances across these epithelia as compared to those of the FW- and 1XSW-adapted fish, and may also explain the reduced influx of $^{36}Cl^-$ in these same epithelia. With epithelia from 2XSW-adapted fish the net secretion of both $^{36}Cl^-$ and $^{22}Na^+$ could account for the relatively low I_{sc} observed across these preparations. The epithelia from the FW-adapted fish had electrical properties similar to those of the 1XSW-adapted fish but appeared to show a small net $^{22}Na^+$ secretion. The difference between the net ion fluxes and the mean I_{sc} in these epithelia could be accounted for by the net outflux of some other anion or the net influx of some cation. Further $^{22}Na^+$ flux studies are required to determine whether there is any significant difference between the unidirectional fluxes across this tissue.

TABLE 3

$^{36}Cl^-$ and $^{22}Na^+$ Fluxes Across the Isolated Opercular Epithelium of *Fundulus heteroclitus*

Mean Flux ($\mu A/cm^2$)	100% Seawater	Freshwater	200% Seawater
$^{36}Cl^-$ outflux	211.7 ± 27.1 (10)	138.8 ± 22.9 (3)	122.5 ± 66.2 (3)
$^{36}Cl^-$ influx	48.9 ± 10.0 (10)	57.0 ± 5.2 (3)	7.3 ± 7.3 (3)
$^{36}Cl^-$ net flux	162.8 ± 30.9	81.8 ± 18.9	115.2 ± 67.7
Mean I_{sc}	158.6 ± 16.3 (20)	122.1 ± 8.7 (6)	40.2 ± 18.8 (6)
$^{22}Na^+$ outflux	32.2 ± 3.3 (10)	112.3 ± 28.7 (3)	74.4 ± 54.2 (2)
$^{22}Na^+$ influx	34.8 ± 4.1 (10)	86.0 ± 19.1 (3)	15.9 ± 6.9 (2)
$^{22}Na^+$ net flux	-2.6 ± 4.6	26.3 ± 11.4	58.5 ± 47.3
Mean I_{sc}	56.9 ± 8.0 (20)	98.1 ± 8.1 (6)	14.8 ± 3.7 (4)
Mean electrical properties for all flux studies			
I_{sc} ($\mu A/cm^2$)	107.7 ± 12.1 (40)	110.1 ± 6.7 (12)	30.0 ± 9.4 (10)
p.d. (mV)	15.7 ± 1.3 (40)	16.8 ± 2.7 (12)	8.8 ± 1.8 (10)
R (Ω-cm^2)	192.6 ± 16.4 (40)	151.2 ± 16.5 (12)	472.3 ± 81.9 (10)

Mean s.e.m.; Number of experiments in parentheses.

The fluxes are presented as $\mu A/cm^2$ for comparison to the I_{sc}. The preparations were bathed in a modified Forster's solution containing 16 mM HCO_3^- and gassed with 100% O_2. The mean electrical properties presented are those for these particular flux experiments.

Finally, it should be noted that dramatic seasonal variations in the electrical properties and active Cl^- secretion of epithelia from 1XSW-adapted fish were observed. During late Spring these epithelia displayed high I_{sc}'s and p.d.'s and somewhat lower d.c. resistances, whereas during the Summer they displayed low I_{sc}'s and p.d.'s and higher d.c. resitances. This appeared to reflect seasonal variations in the rate of the active $^{36}Cl^-$ secretion and not the appearance of some other ion transporting mechanism. In the Spring these tissues transported Cl^- at a rate 2-3 times greater than in the Summer but in both cases the mean net $^{36}Cl^-$ secretion and the I_{sc} were equivalent. The data were pooled regardless of the season but certain studies (i.e., theophylline effects and the $^{22}Na^+$ flux studies) were performed primarily during the Summer which one reflected in the much lower mean I_{sc}'4 and p.d.' s and higher mean d.c. resistances.

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