EVIDENCE FOR A Cl^-/HCO_3^- EXCHANGER IN THE CHLORIDE CELLS OF FUNDULUS HETEROCLITUS

Jose A. Zadunaisky[1], Dawn M. Roberts [2]and Edmund Fisher[1]
[1]Department of Physiology and Biophysics
New York University Medical Center
New York, New York 10016
[2]Oglethorpe University
Atlanta, Georgia 30319

The opercular epithelium of the teleost, Fundulus heteroclitus, contains high concentrations of chloride cells that function in ion transport between the mucosa of the fish and the sea water environment that the fish is in (Zadunaisky 1984, Fish Physiology Vol. X). This opercular epithelium can be dissected off the gill flap and mounted in an Ussing chamber, where its net chloride transport can be recorded as short-circuit current. The chloride transport is affected by intracellular pH changes, and it is part of the rapid signal for adaptation to different salinities of euryhaline fish (Zadunaisky 1990, Proceedings of Gottingen conference, Germany).

Experiments where the operculum was placed in an HCO_3^- free environment showed a reduction of 90% in the short-circuit current, indicating that less Cl^-ions were being transported across the tissue. This drastic requirement of bicarbonate for the maintenance of potential difference and short-circuit current in past experiments was an indication that bicarbonate is involved in the process of secretion of chloride.

In order to determine the existence of this process, the effect of bicarbonate and carbon dioxide-free solutions on the short-circuit current was observed. The specific effects of DIDS and SITS, two stilbenes that affect the function of exchangers for bicarbonate, was also tested.

Male and female killifish, Fundulus heteroclitus, were collected locally near Salsbury Cove, ME during the Summer. The fish were fed bloodworms daily and maintained in running seawater aquariums in the natural light and temperatures of Salsbury Cove in late June, July and August.

After the fish were pithed, the opercular flap was removed. The isolated operculum was placed in teleost Ringer, and the epithelium that lines the operculum was gently teased away from the bone using microdissection. This inner operculum sheet was then placed over a plastic disk with a central circular aperture and secured by placing a small, flat, circular ring over the epithelium and pressing it into a circular indentation in the plastic disk. This "chip" was then placed between two lucite sheets for mounting in an Ussing perfusion chamber. This method prevented the folding back of the tissue upon itself and also minimized edge damage and holes in the tissue. This method supersides the old "pinning down" of the epithelium to Sylgard discs.

The HCO_3- buffered Ringer solution used to bathe the epithelium in the chamber consisted of (in mM): NaCl, 135.0; KCl, 2.5; $MgCl_2$, 1,0; $NaHCO_3$, 16.0; $CaCl_2$, 1.5; glucose, 5.5, and was gassed with 1% CO_2-95% O_2. The HCO_3- free Ringer solution has 25 mM HEPES substituted for $NaHCO_3$, and it was gassed with air (1% CO_2), Two stilbenes, DIDS and SITS, were also used separately in the Ringer solution of some experiments in concentrations of O.1 mM and lmM respectively.

After the tissue was mounted in the chamber and bathed in the Ringer solution on both sides, the potential difference (PD) across the tissue was measured using electrodes attached to a voltage clamp. After this PD stabilized, current was passed from the voltage clamp to the Ussing chamber through bridges made of teleost Ringer and agar. The current clamped the tissue's voltage to zero so that its short-circuit current (SCC) could be determined. Conductance was monitored throughout the experiment by sending a 1 mV pulse across the short-circuited tissue every 50 seconds. After the SCC stabilized, other solutions and gasses were substituted for the bicarbonate Ringer and 1% CO_2 at 99% O_2 gas to test for the Cl^-/HCO_3^- pump. The pH of all solutions was adjusted to 7.4 with O.lN HCl or O.lN NaOH.

To substitute a solution, the original solution on the basolateral side of the tissue was quickly suctioned out of the Ussing chamber, and an equal volume of the new solution was added to the chamber. This process was repeated three times in order to assure that the old solution that bathed the tissues was replaced almost completely by the new solution. Both the gas tank and the air pump hoses were fed into a common hose connected to each side of the chamber, and the gasses were substituted by clamping off one or the other hose. The air was pumped in at a rate that allowed a slow bubbling, enough to aerate the tissue without causing it stress from excessive air pressure. The DIDS and SITS were added to the substituting solution before it was added to the chamber.

After the SCC of the operculum mounted with bicarbonate RINGER and 1% CO_2 gas on both sides stabilized, the Ringer and the gas on the basolateral side of the tissue were replaced with bicarbonate-free Ringer and air. This caused the SCC to drop steadily. After this drop stabilized, the basolateral side of the tissue was again washed out and replaced with bicarbonate Ringer and 1% CO_2 at 99% O_2, causing the SCC to increase. The increase stabilized at almost the same level as it had been before the first substitution, showing that HCO_3 is a necessary component in the ion transport that causes the substantial short-circuit current in the chamber.

After the SCC of the operculum mounted in bicarbonate Ringer and 1% CO_2 on both sides stabilized, the Ringer and the gas on the basolateral side of the tissue were replaced with air and bicarbonate-free Ringer containing 1mM SITS. This again caused the SCC to drop steadily. After the SCC stabilized, the gas and solution on the basolateral side was again replaced, this time with 1% CO_2 and bicarbonate Ringer containing 1mMSITS. The SCC increased only slightly, not returning to its original level because SITS partially blocked the ion transport's recovery.

Because SITS did not completely block the SCC's recovery, it was replaced with 0.1mM DIDS and the same experiment conducted. When the bicarbonate-free Ringer with DIDS and air were replaced with bicarbonate Ringer with DIDS and 1% CO_2 gas, the SCC did not increase at all, showing that DIDS is a complete inhibitor of the Cl/HCO_3 exchanges. Reductions of 80% of SCC were found in bicarbonate-free media in 10 experiments. DIDS produced also an 80% reduction of the current in 11 experiments. The effects of DIDS were not reversible, however, partial recovery after SITS was found.

The lowering of the SCC and the action of the DIDS confirm that the system has as part of the secretory mechanisms the exchange of Cl^- for HCO_3^-. When HCO_3^- ions are unavailable for transport, the pumping of Cl^- ions and the SCC dropped drastically. When the HCO_3^- ions were restored to the basolateral side of the tissue, the SCC rose again and the Cl/HCO_3 exchanger began exchanging ions through the operculum again. DIDS blocked this exchanger effectively so that, even when the HCO_3^- ions were restored to the system, the ion transporting mechanism did not take place.

Supported by USPHS Grant EY 1340 and a Fellowship of the Pew Foundation to D. M. Roberts. Mr. Edmond Fisher is now a medical student at Cornell University Medical College, New York, New York.