THE BICARBONATE SENSITIVITY OF CHLORIDE SECRETION ACROSS THE OPERCULAR EPITHELIUM

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Previous investigations with isolated opercular epithelia noted that the bilateral titration of $NaHCO_3$ to the Ringer solutions produced a rapid and sustained stimulation of the short-circuit current, which was apparently independent of the changes in either the Na^+ concentration or the pH (Karnaky et al., Science 195:203, 1977; Degnan et al., J. Physiol. 271:155, 1977). In Cl^- absorbing epithelia, such as the flounder (Field et al., J. Memb. Biol. 41:265, 1978) and amphiuma (White, J. Memb. Biol. 53:95, 1980) intestine, the presence of HCO_3^- in the bathing media is known to stimulate Cl^- transport. It has been argued that this stimulatory influence of HCO_3^- results from its role as a precursor of carbamyl phosphate, which serves as a source of energy (Martin & Murphy, J. Memb. Biol. 18:231, 1974), or possibly from an electrically neutral Cl^-/HCO_3^- exchange across the serosal membrane, serving as a Cl^- exit step from the cell (Duffey et al., J. Memb. Biol. 42:229, 1978; White, J. Memb. Biol. 53:95, 1980). Investigations were therefore undertaken to study the HCO_3^- influences on the Cl^- secretion across the opercular epithelium.

Killifish, Fundulus heteroclitus, were kept in running seawater tanks. Opercular epithelia were dissected and mounted in lucite chambers by procedures reported previously (Degnan & Zadunaisky, Am. J. Physiol. 238:R231, 1980). The tissues were initially bathed bilaterally with the usual Ringer, containing 16 mM HCO_3^- and gassed with 95% O_2/5% CO_2 (pH 7.2). Steady-state, short-circuited $^{36}Cl^-$ or $^{22}Na^+$ fluxes were measured as previously described (Degnan et al., J. Physiol. 271:155, 1977) to obtain control flux values. The Ringer bathing one side of the tissue was then replaced with HCO_3^--free Ringer (phosphate buffers, gassed with air, pH 7.2) and flux measurements determined after the establishment of new steady-state conditions.

The effects of unilateral HCO_3^- substitutions on the Cl^- efflux ($J_{sm}^{Cl^-}$) and tissue conductance (G_T) of the opercular epithelium are summarized in Table 1. Serosal HCO_3^- substitution resulted in a significant 40.7% decrease in the $J_{sm}^{Cl^-}$,

Table 1.--The Effect of HCO_3^- Substitution on the Cl^- Efflux and Ionic Conductance of the opercular Epithelium

	$J_{sm}^{Cl^-}$ $\mu equiv \cdot cm^{-2} \cdot hr^{-1}$	G_T $mmho \cdot cm^{-2}$
Control (8)	7.521 ± 1.003	6.3 ± 1.0
HCO_3^--FREE, SEROSA	4.455 ± 0.289	6.3 ± 1.0
PERCENT CHANGE	40.7	0.0
P	< 0.01	> 0.90
Control (8)	6.552 ± 0.367	7.1 ± 0.5
HCO_3^--FREE, MUCOSA	5.632 ± 0.746	7.2 ± 0.6
PERCENT CHANGE	14.0	1.4
P	> 0.05	> 0.80

Data Expressed as Mean ± S.E.M.

Number of experiments in parentheses

while no significant effects of mucosal HCO_3^- substitution were observed. Neither substitution had any effect on the G_T.

The efflux experiments involving serosal HCO_3^- substitution could be arbitrarily divided into two groups of four experiments, representing those with control fluxes above (high) and below (low) 6.000 μequiv$\cdot$cm$^{-2}\cdot$hr^{-1}. A comparison of the effect of HCO_3^- substitution between these two groups, revealed a 52.5% ($P < 0.001$) reduction in the $J_{sm}^{Cl^-}$ for the high efflux tissues (10.075 $\pm$ 0.324 to 4.786 $\pm$ 0.465 μequiv$\cdot$cm$^{-2}\cdot$hr^{-1}), and a 16.7% ($P > 0.02$) reduction in the $J_{sm}^{Cl^-}$ for the low efflux tissues (4.949 $\pm$ 0.455 to 4.125 $\pm$ 0.316 μequiv$\cdot$cm$^{-2}\cdot$hr^{-1}). No such differences were observed with mucosal HCO_3^- substitutions. In three additional experiments, serosal HCO_3^- substitution had no significant ($P > 0.30$) effect on the Cl^- influx. Table 2 summarizes the results of HCO_3^- substitutions on the Na^+ fluxes across the opercular epithelium. No significant effects were observed.

Table 2.--The Effect of HCO_3^- Substitution on the Na^+ Fluxes and Ionic Conductance of the Opercular Epithelium

	FLUX μequiv$\cdot$cm$^{-2}\cdot$hr^{-1}	G_T mmho$\cdot$cm^{-2}
CONTROL $J_{sm}^{Na^+}$ (4)	3.549 $\pm$ 0.170	6.8 $\pm$ 0.5
HCO_3^--FREE, MUCOSA	3.482 $\pm$ 0.288	7.0 $\pm$ 1.1
PERCENT CHANGE	3.1	2.9
P	> 0.50	> 0.80
CONTROL $J_{ms}^{Na^+}$ (4)	4.994 $\pm$ 0.472	8.8 $\pm$ 1.2
HCO_3^--FREE, SEROSA	5.225 $\pm$ 0.378	9.7 $\pm$ 0.5
PERCENT CHANGE	4.6	10.2
P	> 0.25	> 0.30

Data expressed as Mean $\pm$ S.E.M.

Number of experiments in parentheses

The data demonstrate that the Cl^- secretion across the opercular epithelium is HCO_3^- (or CO_2) sensitive on the serosal side only, while the unidirectional Na^+ fluxes and Cl^- influx are insensitive to "trans" HCO_3^- (or CO_2) substitutions. This HCO_3^- sensitivity also appears to be most evident in those epithelia with relatively high Cl^- transport rates. These observations eliminate a possible mucosal Cl^-/HCO_3^- serving as a Cl^- exit step, and suggest a possible serosal Cl^-/HCO_3^- exchange serving as a Cl^- entry step. The lack of any concomitant conductance changes with reductions in the $J_{sm}^{Cl^-}$ in response to serosal HCO_3^- substitution, suggests an electrically silent exchange mechanism. However, this could also be explained if the active Cl^- pathway contributed relatively little to the conductance measurement, or if serosal HCO_3^- (or CO_2) substitution affected the Cl^- transport in some other fashion, such as through intracellular pH changes or metabolic substrate depletion. Previous investigations involving Cl^- substitutions revealed inconsistant effects on the G_T, resulting in no significant change (Degnan & Zadunaisky, Am. J. Physiol. 238:R231, 1980), and suggesting that the transepithelial Cl^- pathways (active and passive) may not contribute much to the G_T.

In order for a Cl^-/HCO_3^- exchange to contribute to the Cl^- secretion, HCO_3^- would first have to gain access to the cell interior and then be exchanged for external Cl^- in the serosal bathing medium. This could be accomplished by CO_2 diffusion across the basolateral membranes and its subsequent hydration and dissociation to H^+ and HCO_3^- in the cell interior. Carbonic anhydrase would not contribute significantly to this sequence of events, since its inhibition by acetazolamide (Degnan et al., J. Physiol. 271:155, 1977) or methazolamide (unpublished observations) has no inhibitory effect on the Cl^- secretion across the opercular epithelium. The fact that only part of the $J_{sm}^{Cl^-}$ is sensitive to serosal HCO_3^- (or CO_2), suggests either another Cl^- entry step, such as a Na^+-coupled Cl^- transport (Silva et al., Am. J. Physiol. 233:F298, 1977), or that the intracellular CO_2 generation is sufficient to maintain a Cl^-/HCO_3^- exchange at a significant level. The known presence of a HCO_3^--ATPase in gill epithelia (Kerstetter & Kirschner, Comp. Biochem. Physiol. 48:581, 1974), and the recent demonstrations of the presence of this enzyme in uncontaminated microsomal fractions (Kinne-Saffran & Kinne, J. Memb. Biol. 49:235, 1979; Bornancin et al., Am. J. Physiol. 238:R251, 1980), makes the possible involvement of this enzyme in Cl^- transport more plausible than previously believed. This work was supported by NIH grants GM25002 and EY 01340 and the Commissariat a l'Energie Atomique, France.

THE EFFECT OF PERFUSION AND IRRIGATION FLOW RATE VARIATIONS ON NaCl EFFLUX FROM THE ISOLATED, PERFUSED HEAD OF MYOXOCEPHALUS OCTODECIMSPINOSUS

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Previous investigations of the isolated, perfused head of the long-horn sculpin, Myoxocephalus octodecimspinosus, have demonstrated that it displays prolonged viability when compared to the perfused head of the trout (Payan and Girard, Am. J. Physiol. 232:H18-H23, 1977) and, like the trout, responds to epinephrine with a transitory, alpha-mediated vasoconstriction and longer-term, betamediated vasodilation (Claiborne and Evans, Bull. MDIBL 19:96-101, 1979, J. Comp. Physiol. 138-79-85, 1980). The present study examines the effect of variation of either perfusion or irrigation flow rates on Na and Cl effluxes, determines in vivo afferent blood pressures and compares in vivo Na and Cl effluxes with those from the isolated, perfused head.

The isolated, perfused head of M. octodecimspinosus was prepared as described previously (Claiborne and Evans, ibid.). To determine the unidirectional effluxes, either ^{22}Na or ^{36}Cl was added to the afferent perfusate at approximately 3 uCi/500 mls. Effluxes were monitored by taking 5 ml samples of the irrigation bath at various time intervals, mixing the sample with 5 mls of Aquasol-2 and counting in a Packard Tricarb Liquid Scintillation System. Quenching was corrected by internal standardization. The rate of efflux ($uM \cdot 100g^{-1} \cdot hr^{-1}$) was calculated by dividing the appearance of radioactivity per unit time in the irrigation solution by the specific activity of the perfusate and correcting for the weight of the animal. To monitor the effect of alterations of perfusion (and pressure) on the ^{22}Na or ^{36}Cl efflux, the efflux was measured during an initial control rate period (afferent pressure approximately 30 torr), followed by one period (^{22}Na) or two periods (^{36}Cl) at reduced afferent flow rates. ^{22}Na efflux periods were 20 minutes long while ^{36}Cl efflux periods were 15 minutes in length. In all cases an equilibration period (at the new pressure) of the same length was interspersed between the experimental periods. The effect of variations of the irrigation flow rates was determined for either isotope during 4 successive, 15 minute efflux periods during which the irrigation rate was varied over the range of 138 to 980 mls/min. The sequence of irrigation rate changes was different for each perfused head. In these experiments the afferent perfusion pressure was maintained at approximately 25 torr. In vivo ^{22}Na and ^{36}Cl efflux rates were determined by injecting approximately