MECHANISMS OF NH_4^+ TRANSPORT ACROSS THE DOGFISH PUP (SQUALUS ACANTHIAS) GILL

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Recent studies in our laboratory have demonstrated that ammonia can traverse the teleost and hagfish gill as NH_4^+, both by diffusion and via basolateral Na^+/NH_4^+ exchange (Goldstein et al., J. Exp. Zool. 219, 395-397, 1982; Claiborne et al., J. Exp. Biol. 96, 431-434, 1982; Evans & Robbins, Bull. MDIBL 84, 52-53, 1984). The present study was undertaken to determine the modes of NH_4^+ transport across the shark gill epithelium, and the relative permeability to NH_4^+ of that epithelium.

Heads of dogfish pups were perfused as described previously (Evans & Claiborne, J. Exp. Biol. 105, 363-371, 1983), except that perfusion and irrigation flows were set at 650 µl/min and 10 ml/min respectively, and the perfusate did not contain epinephrine and was aerated with 1% CO_2 in air. The head was encased in a plastic chamber cooled to 10-15°C by iced tap water circulating through a water jacket, and the irrigation fluid was maintained in the same temperature range by a cooling plate. To test for a role of basolateral Na^+/NH_4^+ exchange in baseline total ammonia (NH_3 + NH_4^+ - denoted as T_{amm}) efflux, the head was perfused for 20 minutes with elasmobranch Ringer's solution (adjusted to pH ca. 7.8 by the addition of 20 mM $NaHCO_3$) containing ca. 500 µM T_{amm}, then for a second 20 minute period with the same perfusate containing 10^{-4} M ouabain. In a second series of experiments, the effect of specific increases in perfusate NH_4^+ concentration on T_{amm} efflux was examined by perfusing the head during the first period with perfusate whose T_{amm} concentration had been adjusted to ca. 1 mM (pH ca. 7.8) and during the second period with perfusate whose T_{amm} concentration had been adjusted to ca. 9 mM and pH lowered to ca. 6.8 by lowering the bicarbonate concentration to 2 mM, in order to maintain perfusate NH_3 concentration relatively constant. In these experiments, the head was perfused in a third, 20-minute period with either 10^{-4} M ouabain in the perfusate or Na^+-free artificial sea water for the irrigation solution, in order to test for a role of either basolateral Na^+/NH_4^+ exchange or apical Na^+/NH_4^+ exchange in the NH_4-stimulated T_{amm} efflux. In all experiments, pH and T_{amm} of perfusate were monitored at T-10 (10 min after the start of the experiment) of a given experimental time period and pH and T_{amm} of irrigate were monitored at T-0 and T-20 of each experimental time period. In this way actual NH_3 and NH_4^+ gradients were monitored in each experiment. Since subsequent experiments have shown a relatively high NH_3 permeability of shark gill epithelium (Evans, More, Robbins, unpublished), changes in T_{amm} efflux produced by inadvertent changes in the NH_3 gradient were factored out for each experiment. To test for structural leaks during perfusion, some heads were perfused with perfusate containing 15mg/ml of erioglaucine (Acid blue 9) for a final 20 minute period. T-0 and T-20 samples were monitored spectrophotometrically (632 nm) and compared with standard concentration vs. color curves for the dye.

Addition of 10^{-4} ouabain to the perfusate reduced the T_{amm} efflux from 27 to 13 and 35 to 15 $\mu mol \cdot 100 \cdot g^{-1} \cdot hr^{-1}$ from two heads perfused with 730 and 244 µM T_{amm}, respectively. Ouabain did increase the afferent pressure (proportional to gill resistance) by some 12 and 15 torr, respectively, but other experiments (Evans, More, Robbins, unpublished results) have

demonstrated that, over a similar range of pressures, T_{amm} efflux is not correlated with pressure. So it is unlikely that the significant reduction in T_{amm} efflux is secondary to the hemodynamic effects of ouabain. Thus, it appears that a significant percentage (ca. 50%) of the "baseline" T_{amm} efflux runs through Na^+/NH_4^+ exchange via basolateral Na-K-activated ATPase. Similar results have been found with the teleost Opsanus beta (Claiborne et al., op. cit., 1982; Evans & More, unpublished). When the perfusate NH_4^+ concentration was raised from 1.3 ± 0.1 mM (SE) to 9.1 ± 0.3 mM (9) the T_{amm} efflux increased significantly with a slope of 2.2 ± 0.7 $\mu mol \cdot 100g \cdot hr^{-1} \cdot mM^{-1} \cdot 1^{-1}$. Subsequent addition of 10^{-4} ouabain to the perfusate in four of these experiments did not alter the T_{amm} efflux (+ 7.8 ± 8.1 $\mu mol \cdot 100g^{-1} \cdot hr^{-1}$), so it appears that enhanced T_{amm} efflux does not run through basolateral Na^+/NH_4^+ exchange. In these ouabain experiments, the increase in afferent pressure was only 6 ± 2 torr. Use of Na^+-free artificial seawater irrigation during the third period in five experiments actually stimulated the efflux by 32 ± 16 $\mu mol \cdot 100g^{-1} \cdot hr^{-1}$, rather than inhibit it as one would expect if apical Na^+/NH_4^+ exchange played a major role in NH_4-stimulated T_{amm} efflux. Perfusion with Acid Blue 9 demonstrated that the structural leak after some 90 minutes of perfusion was only 1.8 ± 0.7% (7), which could account for an apparent T_{amm} efflux of only some 0.5 $\mu mol \cdot 100g^{-1} \cdot hr^{-1}$, approximately 1% of that actually measured in these experiments. Therefore, structural leaks obviously cannot account for the T_{amm} effluxes from the perfused pup head. Thus, the slope of the NH_4^+-stimulated T_{amm} efflux is proportional to the gill epithelium's permeability to that cation.

If we assume that the functional branchial surface area of the pup is approximated by the structural surface area of the adult dogfish (3.7 cm^2/g; Hughes & Wright, Z. Zellforsch mikrosk. Anat. 104, 478-493, 1970), we can calculate that the apparent $P_{NH_4^+}$ is 1.7 x 10^{-6} $cm \cdot s^{-1}$, somewhat less than (but of the same order as) that described for the turtle bladder (4.5-4.9 x 10^{-6} $cm \cdot s^{-1}$; Arruda et al., Am. J. Physiol. 246, F635-F647, 1984; Schwartz & Tripolone, Am. J. Physiol. 245, F210-F216, 1983). Since it is likely that the functional surface area of the branchial epithelium is somewhat less than the structural surface area (and therefore the apparent $P_{NH_4^+}$ is somewhat underestimated) it is clear that the $P_{NH_4^+}$ of the shark pup gill is similar to that described for a model tight epithelium like the turtle bladder. The relatively low $P_{NH_4^+}$ of the shark gill does correlate with its relatively low permeability to Na^+ (Evans, in Osmotic and Ionic Regulation in Animals, ed. by G. M. O. Maloiy, Academic Press, pp. 305-390, 1979). Subsequent experiments in our laboratory in Gainesville have determined that the apparent P_{NH_3} of the shark pup gill is 4.2 x 10^{-4} $cm \cdot s^{-1}$, somewhat higher than that described for the turtle bladder (0.7 and 2.6 x 10^{-4} $cm \cdot s^{-1}$; Arruda et al., 1984, op. cit., and Schwartz & Tripolone, 1983, op. cit., respectively).

In summary, our data indicate that the branchial epithelium of the dogfish shark is approximately 250 times as permeable to NH_3 as to NH_4^+. However, basolateral Na^+/NH_4^+ exchange can account for approximately 50% of the total ammonia transport, and the fact that the pH of shark blood is 2 pH units below the pK for ammonia dictates that, of the remaining 50% of ammonia efflux, 14% is via ionic diffusion and 35% is via non-ionic diffusion.
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