AMMONIA IS TRANSPORTED ACROSS THE HAGFISH (Myxine glutinosa) GILL AS NH_4^+ ONLY

David H. Evans and Steven D. Robbins
Department of Zoology, University of Florida, Gainesville, FL

In recent years we have utilized the isolated, perfused fish head to investigate the modes of ammonia transport across fish gills, by measuring the effects on ammonia efflux (NH_3 + NH_4^+; hereafter referred to as T_{amm}) of specific alterations in the perfusate NH_3 and NH_4^+ concentrations. Our initial studies demonstrated that NH_4^+ crosses the branchial epithelium of the long-horned sculpin Myoxocephalus octodecimspinosus) and the Gulf toadfish (Opsanus beta) but indicated that NH_3 permeability in these species may approach zero (Goldstein et al., J. Exp. Zool. 219:395, 1982). More recent experiments using perfused Squalus acanthias pup heads have demonstrated that both ammonia species cross this branchial epithelium, and that T_{amm} efflux via NH_3 diffusion is about 3 times the T_{amm} efflux via NH_4^+ diffusion (Evans, in preparation). We have also found that the branchial epithelium of at least O. beta is indeed permeable to NH_3, so it is clear that the actual modes of T_{amm} transport, and the relative importance of each, may be species specific. We have recently demonstrated that T_{amm} efflux from the intact hagfish (Myxine glutinosa) is not via Na^+/NH_4^+ exchange (Evans, J. Exp. Biol., in press, 1984), so it is of interest to determine if the T_{amm} efflux from this species is via NH_4^+ or NH_3 diffusion.

Perfused hagfish heads were prepared by anesthetizing individuals in 0.02% MS-222, exposing the branchial heart, ligating and cutting the ventricle, and suturing a cannula composed of PE 50 tubing in place in the ventral aorta just before it gives off the afferent branchial vessels. Hagfish Ringer's solution was formulated from the analysis of Robertson ("Contemporary Studies in Marine Science", H. Barnes, ed., Allen & Unwin, London, p. 631, 1966), corrected to be isotonic to ambient sea water. There are no data on cardiac outputs in hagfishes, but our preliminary investigations showed that afferent pressures were extremely low, of the order of 3-10 torr (in the same range as those described by Johansen (Biol. Bull., 118:289, 1960)). We therefore perfused the gills at a flow of approximately 150 µl/min, which produced afferent pressures in this range. Other experiments using this preparation have demonstrated that it maintains consistent afferent pressures for hours, is sensitive to adrenergic stimulation at concentrations as low as 10^{-7} M (unpublished results), has completely cleared gills at the end of the experiment (visual examination), and has efferent perfusate flows which are equivalent to afferent perfusate inputs. After cannulation, a plastic irrigation tube is sewn into the mouth, the majority of body is removed, and the isolated head is placed into a V-shaped trough, with a drain below the paired gill openings, and the cut body-end at the edge of the trough, so that efferent irrigate can be separated from the efferent perfusate. The trough formed the top of a plexiglas box through which iced tap water was circulated in order to maintain the head at 12 ± 2°C. Irrigation flows were set at 10 ml/min. To test the effect of specific alterations in perfusate NH_4^+ or NH_3 concentrations on T_{amm} efflux we monitored irrigate T_{amm} concentrations during experimental periods when the perfusate concentrations of these solutes were altered (in the range of 0.1 to 9 mM T_{amm}), as described previously (Goldstein et al., 1982, Op. Cit.). Net gradients for each solute were calculated under each experimental condition since the perfusate and irrigate T_{amm} concentrations and pH were measured directly. The pK of NH_3/NH_4^+ was taken as 9.75, based upon the data of Cameron and Heisler (J. Exp. Biol., 105:107, 1983).

We found that an increase in perfusate NH_4^+ concentration stimulated T_{amm} efflux significantly, (slope = 0.004 $\mu mol \cdot 100g^{-1} \cdot hr^{-1} \cdot \mu M^{-1} \cdot l^{-1}$; n = 14)) but that substantial changes in the perfusate NH_3 concentrations were without a measureable effect. Since the NH_4^+-stimulation experiments actually involve a substantial increase in the perfusate T_{amm} concentration, while the NH_3-stimulation experiments merely involve changing the perfusate pH with no change in T_{amm} concentration, it is obvious that any morphological leak in the head would result in an apparent NH_4^+-stimulated efflux, even if the gills were relatively impermeable to that solute. To test for this possibility we perfused some heads with hagfish Ringer's solution containing 100 mg/l methylene blue, and tested for its appearance in the irrigate. Standard curves were prepared which indicated that a leak of as little as 1% of the perfusate during the experimental time period would be easily distinguished. In 8 heads so examined, the leak never approached 1%, even when the afferent pressures were approximately doubled (in 5 heads) by doubling the perfusion rate. At the highest perfusate NH_4^+ concentrations used (ca. 9 mM), a 1% leak would produce an apparent efflux of only 1.6 $\mu mol \cdot 100g^{-1} \cdot hr^{-1}$, only about 4% of the average measured T_{amm} efflux from the head at that perfusate T_{amm} concentration. Thus, it is clear that a morphological leak cannot account for our NH_4^+-stimulated T_{amm} efflux.

Our data are consistent with the proposition that the hagfish branchial epithelium is permeable to NH_4^+, but not to NH_3. This is especially surprising since an earlier study (Evans & Hooks, Bull. MDIBL 23:61, 1983) found that the efflux of Na^+ is extremely low in this species, indicating a relatively low cationic permeability. However, the slope of the NH_4^+-stimulated T_{amm} efflux in the present experiments is nearly identical to that found for *Squalus acanthias* gill (0.0035: Evans, in preparation), which also has a very low Na^+ permeability. It is clear that the relationship between cationic permeability and the relative importance of ionic vs. non-ionic diffusion of T_{amm} across the fish gill remains to be elucidated. (Supported by NSF PCM 83-02621 to DHE.)