The Roles of Transcellular Transport Steps in Ammonia Extrusion by the Gill Epithelium of the Shark, Squalus acanthias

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Preliminary studies last year (Evans & Robbins, Bull. MDIBL 25:166-167, 1985) indicated that both non-ionic diffusion of NH_3 and ionic diffusion of NH_4^+ play measurable roles in the transport of ammonia across the shark gill. In addition, when ouabain (0.1 mM) was added to the perfusate in two experiments the ammonia efflux declined significantly, consistent with the idea that basolateral, ouabain-sensitive steps were involved. Apical Na^+/NH_4^+ exchange is also possible in this species, although other experiments last year found that irrigation of the perfused gill with Na-free artificial sea water did not reduce the total ammonia efflux, and earlier studies on another elasmobranch (Raja erinacea; Evans et al., J. Exp. Zool. 208:431-437, 1979) failed to demonstrate any reduction in ammonia efflux from intact skates when amiloride (0.1 mM) was added to the external sea water. However, our investigations of the mechanisms of net acid secretion by intact S. acanthias pups (Evans, J. Exp. Biol. 97:298-299, 1982) found that transfer of intact pups to Na-free artificial sea water abolished net acid secretion to produce net secretion of base. Such an alteration in net secretion of acid could have significant effects on the pH of the boundary layers of sea water irrigating the gill epithelium and thereby diffusional gradients for NH_3 (Evans & Cameron, J. Exp. Zool. 239:17-23, 1986). We therefore investigated the effects of the addition of bumetanide and ouabain to the perfusate (serosal surface) and the addition of amiloride to the irrigate (mucosal surface) on ammonia efflux.

The methods of perfusion of dogfish pup heads, and determination of net effluxes of erioglaucine (measurement of structural integrity) and total ammonia effluxes have been previously described (Evans & Claiborne, J. Exp. Biol. 105:363-371, 1983; Evans & Robbins, op. cit., 1985). Erioglaucine leaks in all experiments in this report were less than 1%, indicating that structural leaks could account for less than 2% of the measured ammonia efflux. Initial, control experiments (Table 1) demonstrated that during three, sequential 20-min efflux periods the total ammonia efflux (sum of both NH_3 and NH_4^+) declined by 17% during the second period ($p = 0.01$, one-tailed t-test) but remained stable during the third 20 min period.

Table 1. Total ammonia effluxes across the shark gill during successive 20 min periods (mean ± S.E., N = 9; fluxes in $\mu mol \cdot 100\ g^{-1} \cdot h^{-1}$).

Period 1	Period 2	Period 3
19.4 ± 2.6	16.2 ± 2.1	16.1 ± 2.1

Addition of bumetanide (kindly supplied by Rolf Kinne) to the perfusate during the second period in another series of experiments reduced the total ammonia efflux by 34%, twice ($p < 0.05$, unpaired t-test, N = 6) the control experiment. Subsequent addition of ouabain to the perfusate containing bumetanide did not reduce the ammonia efflux further (Table 2).

Table 2. The effect of bumetanide (5×10^{-5} M) and bumetanide plus ouabain (10^{-4} M) on total ammonia efflux across the shark gill.

Period 1 (Control)	Period 2 (Bumetanide)	Period 3 (Bumetanide + Ouabain)
19.06 ± 0.8	12.9 ± 1.3	10.7 ± 2.1

The lack of a direct role for a ouabain-sensitive, basolateral transport step in ammonia efflux is corroborated in the third experiment (Table 3) when addition of ouabain alone to the perfusate had no affect on the total ammonia efflux ($p > 0.10$, $N = 6$). Moreover, subsequent addition of even 1 mM amiloride to the irrigate (in the presence of ouabain) also had no effect ($p > 0.10$).

Table 3. The effect of ouabain (10^{-4}) and ouabain plus amiloride (10^{-3}) on the total ammonia efflux across the shark gill.

Period 1 (Control)	Period 2 (Ouabain)	Period 3 (Ouabain + Amiloride)
20.9 ± 4.9	21.2 + 3.6	21.5 ± 3.4

(It is important to note that this high concentration of amiloride in sea water inhibited the phenolhypochlorite reaction for ammonia determinations (Solorzano, Limnol. Oceanog. 14:799-801, 1969) by some 25%, resulting in an apparent amiloride inhibition of ammonia efflux, if uncorrected. In addition, preliminary experiments using perfusate to which bumetanide dissolved in DMSO (1 ppt) was added determined that the DMSO inhibited the phenolhypochlorite reaction also. In all experiments reported here, bumetanide was dissolved in a small amount of 1 N NaOH, added to the perfusate, and that solution then corrected to pH 7.8 by the addition of 1 N HCl.)

Our finding of a significant bumetanide sensitivity of ammonia transport across the shark gill is especially interesting because Kinne et al. (Pflugers Arch. 405 (Suppl. 1):S101-S105, 1985) have recently demonstrated that NH_4^+ is able to interact with the K (Rb) transport site on the NaCl/KCl cotransporter in membrane vesicles from shark rectal gland and mammalian, thick ascending loop of Henle. Our results therefore indicate that this transporter is present on the basolateral surface of the gill epithelium and mediates some 17% of the net efflux of ammonia. Subsequent experiments in Gainesville have shown that changes in the transepithelial electrical potential across the perfused head in the presence of bumetanide are less than 1 mV and therefore cannot account for any NH_4^+ efflux changes. Our data corroborate those of Good et al. (Am. J. Physiol. 247:F35-F44, 1984) who found a furosemide-sensitive reabsorption of NH_4^+ in the medullary TAL of the rat kidney. They, however, hypothesized that the driving force was changes in the PD, rather than direct interaction of the cotransporter with NH_4^+. Our data also bring up the extremely interesting possibility that a NaCl/KCl cotransporter could be involved in NaCl extrusion by the elasmobranch gill. Since there is only indirect evidence for branchial NaCl extrusion mechanisms in the shark gill (e.g. Evans et al., J. Exp. Biol. 101:295-305, 1982), a potential role for a bumetanide-sensitive extrusion deserves further investigation.

The insensitivity of ammonia efflux to either perfusate ouabain or irrigate amiloride indicates that neither basolateral nor apical Na^+/NH_4^+ exchange plays measurable roles in ammonia efflux across the shark gill. In

addition, our data demonstrate that diffusive efflux of NH_3 is not affected by the cessation of apical Na^+/H^+ exchange which was presumably produced by addition of 1 mM amiloride to the mucosal surface.

In summary, these data demonstrate that ammonia can cross the basolateral aspect of the branchial cell of the shark via a bumetanide-sensitive carrier, but that neither basolateral, ouabain-sensitive nor apical, amiloride-sensitive Na^+/NH_4^+ exchanges plays a role in ammonia efflux. (Supported by NSF PCM 8302621.)