J. exp. Biol. (1982), **96**, 431–434
Printed in Great Britain

FISH BRANCHIAL Na^+/NH_4^+ EXCHANGE IS VIA BASOLATERAL Na^+–K^+-ACTIVATED ATPase

By J. B. CLAIBORNE,[1] DAVID H. EVANS[1,3] AND LEON GOLDSTEIN[2]

[1] *Department of Biology, University of Miami, Coral Gables, FL* 33124
[2] *Division of Biology and Medicine, Brown University, Providence, RI* 02912
[3] *Present address: Department of Zoology, University of Florida, Gainesville, FL* 32611

Krogh (1939) first proposed that freshwater organisms extract needed Na^+ in exchange for NH_4^+ to maintain near-electroneutrality across the skin or gills. Maetz & Garcia-Romeu (1964) provided an indirect demonstration of Na^+/NH_4^+ exchange in the goldfish (*Carassius auratus*) by showing that injected NH_4Cl stimulated Na^+ uptake, but addition of NH_4Cl to the freshwater inhibited Na^+ uptake. They proposed that Na^+/NH_4^+ exchange was apical (on the mucosal surface of the transporting epithelium, facing the fresh water) and that blood NH_3 entered the basolateral surface of the cell, combined with a proton generated by the carbonic anhydrase hydration of CO_2 (and the subsequent dissociation of carbonic acid), and left the cell in exchange for Na^+. The role for carbonic anhydrase was indicated by their finding that injection of acetazolamide inhibited Na^+ uptake. Subsequently, Kerstetter, Kirschner & Rafuse (1970) demonstrated that acetazolamide injection inhibited the influx of Na^+ into the irrigated gills of the trout (*Salmo gairdneri*). However, ammonia efflux was not significantly inhibited, while acid efflux was. They concluded that Na^+/H^+, rather than Na^+/NH_4^+ exchange, occured at the apical surface. More recent evidence supporting the model for basolateral uptake of NH_3 and apical Na^+/NH_4^+ exchange was presented by Payan (1978). He used the isolated, perfused, head of the trout (*Salmo gairdneri*) and showed that: (1) addition of amiloride to the solution irrigating the gills inhibited both Na^+ influx and ammonia efflux, (2) acetazolamide added to the perfusate inhibited ammonia efflux, and (3) reduction in the NH_3 concentration of the perfusate (by lowering pH) inhibited ammonia efflux as well as Na^+ influx. Furthermore, addition of ouabain to the perfusate inhibited ammonia efflux, presumably because Na^+-K^+-activated ATPase at the basolateral surface mediates the final transfer of Na^+ into the serosal medium, and is ultimately limiting in the regulation of apical Na^+/NH_4^+ exchange.

However, some data have been published which support the proposition that Na^+/NH_4^+ exchange may be basolateral rather than apical. Kerstetter & Keeler (1976) found that Na^+ influx into isolated gills of the trout (*S. gairdneri*) was not affected by a unit change in the pH of the perfusion fluid. Since the NH_3 concentration of the perfusate would have changed by a factor of 10 in these circumstances, if basolateral NH_3 uptake was limiting apical Na^+/NH_4^+ exchange, the Na influx

Table 1. *The effect of ouabain* (2×10^{-4} M) *on ammonia efflux from the perfused head of* O. beta

	Ammonia efflux (μM . 100 g^{-1} . h^{-1})
Control	$16{\cdot}04 \pm 1{\cdot}85$ (7)
Ouabain	$7{\cdot}82 \pm 1{\cdot}18$* (7)

$X \pm$ S.E. (N); *$P < 0{\cdot}02$ using paired data, Student's t test.

Table 2. *The effect of perfusate* K^+ *on ammonia efflux from the perfused head of* O. beta

	Ammonia efflux (μM . 100 g^{-1} . h^{-1})
K^+-free Ringer's	$19{\cdot}62 \pm 1{\cdot}11$ (6)
2·6 mM K-Ringer's	$15{\cdot}01 \pm 1{\cdot}81$ (6)*

$X \pm$ S.E. (N); *$P < 0{\cdot}05$ using paired data, Student's t test.

should have been altered. Unfortunately these experiments did not measure ammonia effluxes. We have recently utilized the isolated, perfused head technique to examine the mechanisms of ammonia transfer across the gill epithelium of two marine teleost fishes (Goldstein, Claiborne & Evans, 1981). Alteration of the pH of the perfusate by 1 pH unit did not alter the efflux of ammonia from either *Opsanus beta* or *Myoxocephalus octodecimspinosus*. However, increasing the NH_4^+ concentration of the perfusate (with constant NH_3 concentrations) stimulated ammonia efflux. This indicates that ammonia crosses these gills as NH_4^+ (via both Na^+/NH_4^+ exchange and diffusion of NH_4^+), that non-ionic diffusion of NH_3 does not occur, and possibly that Na^+/NH_4^+ exchange is basolateral, rather than apical. As Kerstetter & Keeler (1976) proposed, if Na^+/NH_4^+ is apical, and limited by basolateral NH_3 entry, then alteration in the NH_3 concentration of the perfusate should have produced a change in sodium influx. Such an effect was not observed. However, NH_4^+ could cross the basolateral membranes, traverse the cytoplasm and exchange for Na_4^+ at the apical membrane.

To test further the hypothesis of basolateral Na^+/NH_4^+ exchange we examined the effect of adding ouabain or K^+ to the perfusate on the efflux of ammonia from the isolated, perfused head of the marine teleost fish *Opsanus beta*, using the procedure of Claiborne & Evans (1980) and Goldstein, Claiborne, & Evans, (1981). The heads were perfused for 30 min, with Ringer's solution containing either 2×10^{-4} M ouabain (after an initial control period) or 2·6 mM K^+ (after an initial control period of K^+-free Ringer's solution). Our data indicate that ouabain inhibits approximately 50% (Table 1) and addition of K^+ 25% of ammonia efflux (Table 2). Measurements of afferent perfusion pressures showed that addition of ouabain and K^+ was followed by slight increases in gill resistance (26% and 40%, respectively), at the end of the experiments. The increases were, however, not significant when the pressures at the mid-point of the experimental period were compared with that of the controls (Table 3). Thus, it is unlikely that a significant portion of the ouabain or K^+ inhibition of ammonia efflux can be secondary to alterations in either pressure or pattern of branchial perfusion.

Table 3. *The effect of ouabain or K^+ on afferent perfusion pressure*

	Control	Midpoint	Final
Ouabain (6)	31·5 ± 3·5	38·0 ± 4·6*	39·5 ± 4·5**
K^+ (6)	21·2 ± 3·1	24·8 ± 3·5*	29·0 ± 4·7**

$\bar{X} \pm$ S.E. (N). Pressures are in Torr. Midpoint and Final refer to time of measurement during the 30 min experiment period subsequent to perfusing the heads with either ouabain (2×10^{-4} M) or K^+-containing Ringer's solution. Control pressures were measured immediately preceding the change of Ringer's perfusate.

*, not significant, using Student's t test for paired data, **$P < 0.05$.

The inhibition of ammonia efflux by ouabain has been ascribed (Payan, 1978) to inhibition of basolateral Na^+–K^+-activated ATPase which limits apical Na^+/NH_4^+ exchange, presumably after an increase in intracellular Na^+ concentration. Increased intracellular Na^+ would thus compete for the cytoplasmic side (NH_4^+) site of the ionic exchanger, which normally extrudes NH_4^+ in exchange for external Na^+. However, one could just as easily propose that the ouabain effect is a direct one on a basolateral Na^+–K^+-activated ATPase which also has some affinity for serosal NH_4^+. Indeed, it has recently been shown that the Na^+–K^+-activated ATPase extracted from *O. beta* is even more sensitive to NH_4^+ than it is to K^+, and NH_4^+ stimulation of activity is inhibited by ouabain (Mallery, 1979). While the ouabain sensitivity of ammonia efflux supports either an apical or basolateral placement for the ionic exchange, the effect of K^+ enables us to delineate the position more unequivocally. If the Na^+/NH_4^+ ionic exchange is apical, and is ultimately limited by basolateral Na^+/K^+ exchange, removal of K^+ from the perfusate should inhibit, and addition should stimulate ammonia efflux, secondarily to alterations in cytoplasmic Na concentrations (see above). That the reverse was observed (Table 1) indicates quite strongly that K^+ is competing at a basolateral site with NH_4^+ for a site on an ionic exchanger. Thus, Na^+/NH_4^+ exchange in this species must be basolateral.

The important question of the ubiquity of basolateral Na^+/NH_4^+ exchange remains unanswered. The fact that amiloride inhibits both Na^+ influx and NH_4^+ efflux (Kirschner, Greenwald & Kerstetter, 1973; Payan, 1978) supports an apical position for Na^+/NH_4^+ exchange (because amiloride is generally considered to block the mucosal entry step for Na in a wide variety of epithelia (Cuthbert, Fanelli & Scriabine, 1979)); however, the inhibition of ammonia efflux could merely be secondary to a decline in cell Na^+, produced by a fall in apical uptake, which leads to a fall in basolateral Na^+/NH_4^+ exchange. The inhibitory effect of acetazolamide on both Na^+ influx (Maetz & Garcia-Romeu, 1964; Kerstetter *et al.* 1970) and ammonia extrusion (Payan, 1978) is difficult to reconcile with a basolateral Na^+/NH_4^+ exchange. One might propose that carbonic anhydrase is sequestered in the basolateral infoldings of chloride cells (which may not even transport ammonia, Girard & Payan, 1980), much as it may be sequestered in the brush-border of proximal renal tubules (Malnic & Giebisch, 1979), but we have no evidence for this idea. It is obvious that more species need to be investigated.

The fact that ouabain only inhibits approximately 50% of the ammonia efflux indicates that the other 50% must be traversing the branchial epithelium via a pathway other than through the Na^+–K^+-activated ATPase. Interestingly, various

studies have shown that 50% or less of the ammonia efflux is sensitive to external Na^+ concentrations (Evans, 1977; Payan, 1978) and therefore running through Na^+/NH_4^+ exchange. We have recently shown that in at least two marine teleosts (*O. beta* and *Myoxocephalus decimspinosus*) the remainder of ammonia efflux is probably via free diffusion of NH_4^+ through either transcellular or paracellular pathways (Goldstein, Claiborne & Evans, 1981).

This study was partially supported by NSF grants PCM 80-03866 to D.H.E. and PCM 79-21476 to L.G.

REFERENCES

CLAIBORNE, J. B. & EVANS, D. H. (1980). The isolated, perfused head of the marine teleost fish, *Myoxocephalus octodecimspinosus*: hemodynamic effects of epinephrine. *J. comp. Physiol.* **138**, 79–85.

CUTHBERT, A. W., FANELLI, G. M., JR & SCRIABINE, A. (eds) (1979). *Amiloride and Epithelial Sodium Transport*. Baltimore: Urban and Schwarzenberg.

EVANS, D. H. (1977). Further evidence for Na/NH_4 exchange in marine teleost fish. *J. exp. Biol.* **70**, 213–220.

GIRARD, J. P. & PAYAN, P. (1980). Ion exchanges through respiratory and chloride cells in freshwater- and seawater-adapted teleosteans. *Am. J. Physiol.* **238**, R260–R268.

GOLDSTEIN, L., CLAIBORNE, J. B. & EVANS, D. H. (1981). Ammonia excretion by the gills of two marine teleost fish: an important role for ionic diffusion. *J. exp. Zool.* (In the press.)

KERSTETTER, T. H. & KEELER, M. (1976). On the interaction of NH_4^+ and Na^+ fluxes in isolated trout gill. *J. exp. Biol.* **64**, 517–527.

KERSTETTER, T. H., KIRSCHNER, L. B. & RAFUSE, D. D. (1970). On the mechanisms of sodium ion transport by the irrigated gills of rainbow trout (*Salmo gairdneri*). *J. gen. Physiol.* **56**, 342–359.

KIRSCHNER, L. B., GREENWALD, L. & KERSTETTER, T. H. (1973). Effect of amiloride on sodium transfer across body surfaces of fresh water animals. *Am. J. Physiol.* **224**, 832–837.

KROGH, A. (1939). *Osmotic Regulation in Aquatic Animals*. Cambridge: University Press.

MAETZ, J. & GARCIA ROMEU, F. (1964). The mechanisms of sodium and chloride uptake by the gills of a fresh water fish, *Carassius auratus*. II. Evidence for NH_4/Na^+ and HCO_3/Cl exchanges. *J. gen. Physiol.* **47**, 1209–1227.

MALLERY, C. H. (1979). Ammonium stimulated properties of K-dependent ATPase in *Opsanus beta*, a teleost with an NH_4/Na exchange pump. *Am. Zool.* **19**, 944 (abstract).

MALNIC, G. & GIEBISCH (1979). Cellular aspects of renal tubular acidification. In *Membrane Transport in Biology*, vol. IV A, (ed. G. Giebisch, D. C. Tosteson and H. H. Ussing), pp. 299–355. Berlin: Springer-Verlag.

PAYAN, P. (1978). A study of the Na^+/NH_4^+ exchange across the gill of the perfused head of the trout (*Salmo gairdneri*). *J. comp. Physiol.* **124**, 181–188.