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FURTHER EVIDENCE FOR Na/NH_4 EXCHANGE IN MARINE TELEOST FISH

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SUMMARY

1. Four species of marine teleosts were shown to possess an external-NH_4-inhibited Na uptake from 1 mM-NaCl solutions. The inhibition was not due to changes in the transepithelial potential.

2. Injection of 2 μM-NH_4/g fish stimulated Na uptake by *Opsanus beta* and also stimulated ammonia efflux, 50% of which was dependent upon external Na.

3. The ammonia efflux from three species was partially dependent upon external Na.

4. Na/NH_4 exchange in *O. beta* could be reversed so that ^{22}Na efflux could be stimulated by the addition of 200 mM-NH_4 to the external solution.

5. These studies show clearly that marine teleosts possess an Na/NH_4 exchange system in sea water which results in a net influx of Na into the fish.

INTRODUCTION

It has recently been proposed (Evans, 1975*a*) that marine teleost fish extract Na from sea water in order to drive a Na/NH_4 and Na/H ionic exchange system which functions in nitrogenous waste excretion and acid-base regulation. This suggestion is supported by the finding that both the seawater-acclimated molly *Poecilia latipinna* and the marine pinfish *Lagodon rhomboides* displayed a saturable, NH_4-, H-, and amiloride-inhibited Na uptake when rapidly transferred to solutions of low NaCl concentration (Evans, 1973, 1975*b*; Carrier & Evans, 1976). Na/NH_4 and Na/H exchange may also be taking place in elasmobranchs. Payan & Maetz (1973) found that injection of either NH_4 or H into the dogfish shark (*Scyliorhinus canicula*) stimulated Na influx from sea water. In addition, Bentley, Maetz & Payan (1976) have recently shown that a fall in sea-water pH was followed by a decline in the ^{24}Na influx into *S. canicula*.

If Na/NH_4 exchange is indeed taking place across the marine teleost epithelium (presumably branchial) then an external-NH_4-inhibited Na uptake should be found in species other than the above, and this Na uptake should be stimulated by injection of NH_4 into the fish. These contrasting effects of external and internal NH_4 have frequently been used to delineate Na/NH_4 exchange in freshwater-acclimated fish (Maetz & Garcia Romeu, 1964; Garcia Romeu & Motais, 1966; Payan, Matty & Maetz, 1975). In addition, the efflux of NH_4 should be external-Na-dependent, i.e. removal of Na from seawater solutions should lead to a decline of NH_4 efflux. Finally, it should be possible to reverse the direction of Na/NH_4 exchange in a manner

analogous to the reversal of Na/K exchange in erythrocytes (Glynn & Karlish, 1975). Thus, enhancement of external NH_4 concentration should lead to a stimulation of isotopically-measured Na efflux from marine fish.

In this study, one or more of these criteria were found to be met for four species of marine teleost fish.

MATERIALS AND METHODS

The fish used in the present experiments were collected, using beamtrawls, by commercial bait-shrimp fishermen in Biscayne Bay, Florida. They comprised the gulf toadfish *Opsanus beta*; the lined seahorse *Hippocampus erectus*; the lined sole *Archirus lineatus*; and the striped burrfish *Chilomycterus schoepfi*. The fish were kept in sea water (500 mM-Na) in 30 gallon (136 litre) glass aquaria at 23 ± 1 °C and were fed either Tetramin, cut-up shrimp or live brine shrimp until 48 h before an experiment. The water was obtained by adding either distilled water or Instant Ocean sea salts to filtered Biscayne Bay water. Salinity was maintained by adding either more water or more sea salts.

Na uptake from 1 mM-NaCl solutions was measured as previously described (Evans, 1973, 1975*b*). The NH_4-enriched uptake solution contained 10 mM-NH_4 (as sulphate, pH = 6·8) as well as 1 mM-NaCl.

To test the effect of NH_4-loading on the rate of Na uptake from 1 mM-NaCl, fish were anaesthetized in 0·1 % MS-222 and injected intraperitoneally with 2 μM-NH_4/g fish (1 μl/g of 1 M-$(NH_4)_2SO_4$). Within 10 min of the injection the rate of Na uptake was determined as previously described. The rate of Na uptake of these NH_4-injected fish was compared with that of uninjected control fish as well as fish which had been injected with fish Ringer's solution (1 μl/g; Karnaky, Degnan & Zadunaisky, 1977).

The rate of ammonia efflux ($NH_4 + NH_3$) and its dependency upon external Na was determined by transferring animals to 125 ml sea water for 30 min, and thence into the same volume of Na- and K-free sea water (after a 5 min wash in the latter solution to remove adhering salts from the seawater bath) for an additional 30 min and finally back into 125 ml of sea water for 30 min. 5 ml samples of each bath were removed and assayed for ammonia concentration by the method of Harwood & Kuhn (1970). The fish were weighed to the nearest 0·1 g on a Mettler Model P1200 balance and the rate of efflux calculated. The Na- and K-free sea water in these experiments was of the same formula as that previously used (Evans & Cooper, 1976) except that, to provide for a completely Na-free solution, $NaHCO_3$ was not included.

The effect of high concentrations of external NH_4 on the Na efflux was determined using a rapid-transfer technique similar to that described for investigation of Na/Na and Na/K exchange (Evans, Mallery & Kravitz, 1973; Evans & Cooper, 1976). In these experiments the fish were transferred (in 5–10 min intervals) from sea water to 'Na- and K-free sea water' (same formulation as Evans & Cooper, 1976, i.e. actually 2·5 mM-Na) and thence into 200 mM-NH_4, Na- and K-free sea water. The NH_4 was added as the chloride salt and replaced an equivalent amount of choline chloride. The rate of appearance of ^{22}Na was monitored using a flow system running through a Packard 'Armac' Model 443 scintillation detector attached to a Packard Tri-Carb Model 2001 scintillation spectrometer and Model 380 recording ratemeter.

Since the movements of either Na or NH_4 will be affected by electrical potential

Table 1. *Effect of external NH_4 on Na uptake by seawater-acclimated fish*

Species	Uptake in 1 mM-Na	Uptake in 1 mM-Na + 10 mM-NH_4
Opsanus beta (Gulf toadfish	45·1 ± 6·7 (9)†	21·2 ± 2·2 (7)*
Achirus lineatus (Lined sole)	55·9 ± 5·7 (21)	24·9 ± 2·0 (9)*
Hippocampus erectus (Lined seahorse)	113 ± 13·1 (16)	62·9 ± 21·3 (12)*
Chilomycterus schoepfi (Striped burrfish)	205 ± 9·5 (9)	86·7 ± 9·4 (9)*

* $P \ll 0.01$ compared to controls.
† $\mu m.100\ g^{-1}.h^{-1}$.

gradients the transepithelial potential (T.E.P.) was monitored under most of the experimental conditions described above using techniques modified from those which have already been described (Evans *et al.* 1973; Evans, Cooper & Bogan, 1976; Evans & Cooper, 1976). The major modification was the use of a Keithley Model 616 multimeter ($2 \times 10^{14}\ \Omega$ input impedance) connected to a Bausch and Lomb VOM recording multimeter ($10^6\ \Omega$ input impedance) to measure and record the T.E.P., respectively. Tip asymmetry between the 3 M-KCl, 2% agar-filled polyethylene bridges was always less than 0·5 mV and was recorded before and after a given experiment.

All data are expressed as mean ± standard error (no. of animals).

RESULTS AND DISCUSSION

Table 1 shows that all four species of marine teleost tested possess an external-NH_4-inhibited Na uptake from 1 mM-NaCl solutions. Maetz, Payan & de Renzis (1976) have proposed that NH_4 inhibition may be secondary to changes in the transepithelial potential (T.E.P.). This possibility was investigated in three of our four species by monitoring the T.E.P. while transferring them from sea water to 1 mM-NaCl, back to sea water and finally into 1 mM-NaCl plus 5 mM-$(NH_4)_2SO_4$ (5 min periods). T.E.P. was not measured in *C. schoepfi* because it was too difficult. *A. lineatus* and *O. beta* were unanaesthetized during these experiments but *H. erectus* was anaesthetized with 0·1% MS-222 before the determinations, and the experimental baths for the seahorse contained 0·003% MS-222 and 1 mM Imidazole to maintain pH between 7·5 and 8·0. The addition of 5 mM-$(NH_4)_2SO_4$ had no effect on the T.E.P. measured for these three species immediately after transfer to 1 mM-NaCl (Table 2); we therefore must ascribe the inhibition to chemical competition between the Na and NH_4 rather than electrical potential effects. It is interesting to note that all three species maintained a blood-negative T.E.P. in sea water. This is to be contrasted with other marine teleosts (see Evans, 1977, for a review) and confirms earlier data for these species (Evans & Cooper, 1976).

The effect of NH_4 loading was examined in *O. beta*. Injection of 2 μM-NH_4/g fish was followed by an 84% increase in Na uptake when compared with control animals injected with the same volume (1 μl/g fish) of fish Ringer's solution (Table 3). It is interesting to note that the injected controls had the same rate of Na uptake from 1 mM-NaCl solutions as uninjected controls in these and earlier experiments (Table 1). The T.E.P. of NH_4-injected fish placed into 1 mM-NaCl was $+2{\cdot}6 \pm 0{\cdot}7$ (6) mV which was the same ($0{\cdot}10 > P > 0{\cdot}05$) as Ringer's injected controls ($+1{\cdot}40 \pm 0{\cdot}2$ (6)) or

Table 2. *Effect of NH_4 on the* T.E.P. *across three species of marine fish*

Species	S.W.	1 mM-NaCl	S.W.	1 mM-NaCl +5 mM-$(NH_4)_2SO_4$
O. beta (6)	−6±2†	+2±3	−4±1	+2±2
A. lineatus (7)	−5±1	+4±1	−2±2	+6±1
H. erectus (7)	−4±1	+6±1	−5±1	+7±1

† mV, blood relative to medium.

Table 3. *Effect of NH_4 injection on Na uptake by* O. beta

Uninjected controls	Injected controls	NH_4 injected
48.2±7.0(5)†	50.9±2.7(10)*	93.5±5.9(13)**

* Not significantly different from uninjected controls.
** $P \ll 0.01$ when compared with either injected or uninjected controls.
† μM.100g^{-1}.h^{-1}

Table 4. *Effect of external Na on NH_4 efflux*

	NH_4 efflux†		
Species	Sea water	Na- and K-free sea water	Sea water
O. beta (18)	14·9±0·9	6·0±0·9*	14·1±0·9**
A. lineatus (15)	29·5±4·1	24·1±3·4*	28·4±3·6**
H. erectus (10)	36·7±3·0	25·9±3·3*	29·8±3·5**

* $P \ll 0.01$ when compared with initial efflux in sea water (paired data).
** $P \ll 0.01$ when compared with efflux in Na- and K-free sea water (paired data).
† μm.100 g^{-1}.h^{-1}.

uninjected controls (Table 2). Thus, injection of an NH_4 load into a marine teleost stimulated Na uptake exclusive of changes in the T.E.P. Injection of larger NH_4 loads into the goldfish *Carassius auratus* (Maetz & Garcia Romeu, 1964; Maetz, 1972) and *S. canicula* (Payan & Maetz, 1973) also stimulated Na influx in these species, but in these experiments the T.E.P. was not monitored. It should be noted that the influxes recorded here were measured immediately (5 min) after fish were transferred to 1 mM-NaCl. We thereby avoid potential interference by Na/Na exchange diffusion or simple diffusional influx, both of which are probably minimal at this low salinity.

The external-Na-dependency of NH_4 efflux was examined in *O. beta*, *A. lineatus* and *H. erectus*. Table 4 shows that, while the three species varied in their sensitivity, all displayed a reduced ammonia efflux (NH_3 and NH_4) in Na- and K-free sea water which was at least partially reversible. The rates of ammonia efflux displayed by these three species are similar to the only other published determination of ammonia efflux for a marine teleost (Goldstein & Forster, 1961). If we assume that the Na-dependent ammonia efflux is in the ionic form (NH_4) then we must conclude that either something more than 40% of the ammonia efflux is via the uncharged base (NH_3), or there is a finite permeability to NH_4, or there is sufficient Na left adhering to the gill epithelial membranes after a total of 35 min in Na- and K-free sea water to allow at least some Na/NH_4 exchange to continue. The latter is unlikely since with *O. beta* addition of 100 mM-Na to Na- and K-free sea water resulted in only an approximately

Table 5. *Effect of external Na on ammonia efflux from NH_4-injected* O. beta

Ammonia efflux†	
s.w.	−K, −Na s.w.
194 ± 17·7 (25)	108 ± 10·9 (23)*

* $P \ll 0{\cdot}01$ compared to efflux in s.w.
† μM.100 $g^{-1}h^{-1}$.

25–50% stimulation of ammonia efflux (unpublished observations). Thus it appears that Na/NH_4 exchange in seawater-acclimated *O. beta* has relatively high K_m and would therefore be unaffected by extremely low levels of external Na. It would be interesting to know if this residual ammonia efflux would continue if the external ammonia concentration were raised sufficiently to provide an inwardly-directed diffusion gradient for ammonia. Maetz (1973) has shown that the ammonia efflux from *C. auratus* continued unabated when this species was placed into ammonia-enriched fresh water but this was not tested for the species in the present study.

One might propose that the reduction of NH_4 efflux observed in Na- and K-free sea water was due to an increase in a blood-negative T.E.P. In fact, the T.E.P. did become more negative in all three species when they were placed into 2·5 mM-Na, K-free sea water (Evans & Cooper, 1976) but only by 6–8 mV; far below that necessary to account for the observed reduction of NH_4 efflux.

The external-Na sensitivity of the increased ammonia efflux from NH_4-injected fish was also investigated. Fish were injected with the same NH_4 load as before (i.e. 2 μM/g fish) and 5 min after the injection were placed into either 125 ml of sea water or the same volume of Na- and K-free sea water for 15 min. The net ammonia excretion rate was determined as above. Comparison of Table 4 and 5 shows that the injection resulted in a 13-fold increase in the efflux of ammonia during the first 20 min after the injection, and that nearly 50% of this increased efflux was dependent upon external Na. It appears that the Na-sensitive and non-Na-sensitive components of the ammonia efflux were affected equally by the injection of an NH_4 load since the Na-sensitive component was approximately 50% of the total efflux, and it was 40% of the total in the normal fish (Table 4). The observations that an NH_4 load resulted in a stimulation of Na uptake (Table 3) and a stimulation of Na-sensitive NH_4 efflux (Table 5) show clearly that the load prompted a stimulation of Na/NH_4 exchange in *O. beta*.

While Na/NH_4 exchange is generally accepted for a wide variety of freshwater animals (Maetz *et al.* 1976) it has often been difficult to demonstrate an external-Na-dependent ammonia efflux from fish. Kerstetter, Kirschner & Rafuse (1970) found that the ammonia efflux from the rainbow trout *Salmo gairdneri* did not vary when the external Na concentration was varied and deVooys (1968) had shown even earlier that ammonia efflux from the carp *Cyprinus carpio* continued in Na-free fresh water. In addition, Maetz (1973) found no correlation between the rate of ammonia excretion and net sodium uptake in *C. auratus*. However, he did find a significant correlation between net Na uptake and the sum of ammonia and acid excretion. Payan *et al.* (1975) have recently shown that the isolated perfused head of *S. gairdneri* displays a good

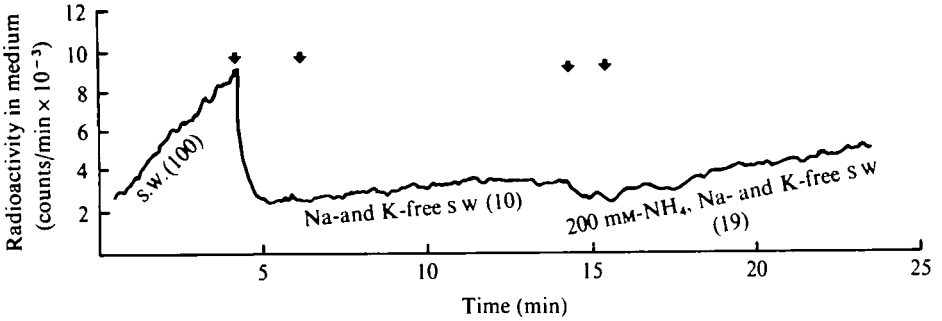

Fig. 1. Effect of Na- and K-free S.W. and 200 mM-NH_4, Na- and K-free S.W. on the efflux of Na from a single *O. beta*. External medium was changed during the time period delineated by the arrows. Numbers in parentheses are relative fluxes.

Table 6. *The effect of external NH_4 on Na efflux from O.* beta

Sea water	'Na- and K-free sea water'	Na- and K-free sea water plus 200 mM-NH_4
100 (14)	9 ± 1 (14)*	19 ± 1 (14)**

* Fluxes expressed as percentage of initial flux in sea water.
** $P \ll 0.01$ compared to efflux in Na- and K-free sea water (paired data).

correlation between the rates of Na influx and ammonia excretion. The addition of acetazolamide ($10^{-5} - 10^{-4}$ M) to the perfusion fluid significantly reduced both Na influx and ammonia excretion.

The reversal of Na/NH_4 exchange was only studied in *O. beta* since this was the only species that seemed unaffected by a sojourn in 200 mM-NH_4. Fig. 1 is a tracing of a recording from a single, representative fish which had been injected with ^{22}Na and then rapidly transferred through a series of ion-substituted solutions. It is obvious that 200 mM-NH_4 significantly stimulated the rate of efflux of ^{22}Na from this fish. Table 6 presents a summary of 14 experiments. The T.E.P. was monitored in another, similar series of transfer experiments (9) and the T.E.P. was found to change from -8 ± 1 mV in sea water to $-14 + 1$ mV in 'Na- and K-free sea water' (actually 2·7 mM-Na) to $-8 + 1$ mV in 200 mM-NH_4, 'Na- and K-free sea water'. It should be noted that the T.E.P.s measured in the first two solutions were nearly identical to those described for *O. beta* in the same solutions in an earlier series of experiments (Evans & Cooper, 1976). The 6 mV decrease in internal negativity observed on transfer to the 200 mM-NH_4, 'Na- and K-free sea water' would stimulate cationic (such as NH_4) efflux by approximately 10% (see Evans *et al.* 1974 and Kirschner *et al.* 1974 for relevant formulae), far below that actually seen in Table 6. Thus, at least in *O. beta*, it is possible to drive Na from the fish by reversing the normal direction of the Na/NH_4 exchange system. That the 200 mM-NH_4 brought the T.E.P. back to the level found in sea water indicates that the epithelium of *O. beta* has a rather large relative permeability to NH_4 or that Na/NH_4 exchange was electrogenic. The magnitude of the effect on the T.E.P. (but of course not the sign) was the same as that produced by the removal of 500 mM-Na and 10 mM-K from the artificial sea water. Unpublished experiments in this laboratory indicate that addition of 300 mM-Na to Na- and K-fr[illegible] sea water depolarized the T.E.P. across *O. beta* from -16 mV to -9 mV. Thus the

permeance of NH_4 may be of the same order as that of Na. If, indeed, the epithelium is leaky to NH_4 this could account for some of the residual ammonia efflux in Na- and K-free sea water (Table 4).

The present studies have shown definitively that Na/NH_4 exchange is a component of the ion balance of marine teleosts. Including earlier work (Evans, 1973, 1975*b*; Carrier & Evans, 1976) we now have clear evidence that such a system is functional in six species of teleosts, each of which is a member of a different teleostean order. In fact, more criteria have been met supporting Na/NH_4 exchange in marine teleosts than supporting Na/NH_4 (and Na/H) exchange in freshwater teleosts (Maetz *et al.* 1976).

Since it is apparent that Na/NH_4 exchange is taking place in the marine fish epithelium (presumably branchial) it is relevant to ask how significant is the net Na influx generated by this exchange in terms of Na balance of the marine teleost.

No direct measurements have been made on the relative magnitude of Na influx via Na/NH_4 exchange when compared to the diffusional and oral influxes of Na which all marine teleosts face, but an estimation is possible. Unfortunately no unequivocal determination of the magnitude of net diffusional and oral influx of Na exists for a marine teleost. However, Potts, Fletcher & Eddy (1973) have estimated that the active output of Na from the flounder, *Platichthys flesus*, is 259 μM . 100 g^{-1} . h^{-1}. If we assume that this species has a rate of Na/NH_4 exchange of the same order as the animals of the present study, i.e. 5–10 μM . 100 g^{-1} . h^{-1}, it is apparent that only 2–4% of the net Na extrusion may be in response to the Na load presented by Na/NH_4 exchange in sea water. This must be regarded as a rather gross estimate because the rates of active Na extrusion may be very different for other species, and we have not taken into consideration the role of Na/H exchange which is probably also taking place (Evans, 1975*a*, *b*; Carrier & Evans, 1976).

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