Gill lipid metabolism and unidirectional Na⁺ flux in the European eel (Anguilla anguilla) after transfer to dilute media: the formation of wax alcohols as a primary response

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Accepted 1 October 1998
Gill lipid metabolism and unidirectional Na⁺ flux in the European eel (Anguilla anguilla) after transfer to dilute media: the formation of wax alcohols as a primary response

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Abstract

The present study investigated the mechanism involved when fish tighten their gill membrane after transfer to dilute media. Ten individually assayed eels adapted to fresh water (FW) and labelled with radioactive 22Na in the plasma showed mean exchange rates in equilibrium with ambient radioactive Na⁺ in FW at 70 µmol kg⁻¹ h⁻¹. Just after transfer to demineralized water (DW), the mean Na⁺ release rate went up to 120 µmol kg⁻¹ h⁻¹; after 24 h in DW it came down again and levelled off at 40 µmol kg⁻¹ h⁻¹ during a period of up to 2 weeks. During the same period the mean Na⁺ uptake rate was 12 µmol kg⁻¹ h⁻¹, i.e., the general picture in DW was a net Na⁺ loss. In another similar experiment, groups of three FW-adapted eels were incubated in vivo for up to 24 h with (14C) acetate added as lipid precursor to the ambient water. Incubation in FW showed about 20% of the total (14C)-activity incorporated into gill lipids as (14C) wax alcohols (WA, octadecanol and elcosanol). This percentage went up to 50% shortly after transfer to DW and came down again to about 20% after 2 weeks in DW. Single eels labelled with 22Na in the plasma showed a statistically significant positive linear correlation of percentage (14C) wax alcohols with log [22Na eflux]. Based on the observed parallel between Na⁺ flux and gill lipid metabolism, it is suggested that the eel reacts at first to a loss of Na⁺ by synthesizing wax alcohols that can tighten the gill membrane. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Eels; Elasmobranchs; Gill; Lipid metabolism; Octadecanol; Osmoregulation; Sodium flux

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PI: S0044-8486(99)00091-5
1. Introduction

Euryhaline fishes can survive in aquatic habitats of widely varying salinities. Entrance into fresh water (FW) from brackish or sea water, or any reduction in ambient salinity in a hypoosmotic medium, will result in a reduction of fish gill ionic permeability to decrease the effects of osmotic gradients (Evans, 1975). Since no fish is absolutely ion impermeable in FW, some means of NaCl uptake from the environment must also be present to ensure FW survival in the long run. The relationship between net diffusional ion loss and active uptake determines the degree of osmoregulatory success (Perry and Laurent, 1989; Perry et al., 1992).

Not much is known about the actual mechanism involved in membrane tightening. Pickford et al. (1966), Marshall (1978), Wendelaar Bonga and Meis (1981) and Perry and Laurent (1989) all point to a prolactin-induced proliferation of mucus cells as a possible way of limiting ion efflux. However, early work on the eel (Krogh, 1939) had already shown that the production of mucus as such was apparently not enough to act as an effective barrier to the diffusion of ions; the movement of ions and sugars through mucus was almost as rapid as through water. Similar considerations by Marshall (1978) regarding not only the eel but a series of fish also concluded that “dilute mucous solutions could not impede the passive movement of ions sufficient to explain the sodium retaining effect of prolactin”.

Hadley (1985) points to the biological function of surface waxes as means to combat potential dehydration of land amphibians by tightening the skin. Schmid and Barden (1965) have found that the skin of the aquatic frog *Rana sphenecronalis* was less permeable to water and had a higher lipid content than that of the terrestrial toad *Bufo hemiophrys*. The authors concluded that the lipid was likely an important factor in preventing body fluid dilution by acting as a barrier to the inward flux of water. It seems reasonable to suggest that gill surface lipids could likewise be involved in a tightening of the gill membrane against both an inward flux of water and a passive ion loss to a hypoosmotic ambient medium.

The present investigation regards the further reduction of ambient salinity in an already hypoosmotic environment. Eels were adapted to natural FW with $[\text{Na}^+] = 0.5$ mmol $l^{-1}$ and then transferred to artificial demineralized water (DW) with $[\text{Na}^+] = 0.03$ mmol $l^{-1}$. Unidirectional Na$^+$ flux based on $^{22}\text{Na}/^{23}\text{Na}$ exchange was measured. Furthermore, by applying the radioactive lipid precursor (1-$^4$C) acetate in vivo, it was possible to study the current formation of even minute amounts of any biologically active gill lipids. It turned out that there was a significant positive correlation between $^{22}\text{Na}$ efflux and the percentage incorporation of (1-$^4$C) acetate into gill wax alcohols (WA).

2. Materials and methods

2.1. Fish

Eels (*Anguilla anguilla*) weighing 50–80 g were caught in fixed gear in brackish water (Roskilde Fjord). They were predominantly in the yellow stage. They were
adapted to FW ([Na⁺] = 0.52, [Ca²⁺] = 2.2, [Mg²⁺] = 0.9, [K⁺] = 0.1, [HCO₃⁻] = 4.9, [Cl⁻] = 0.6, [SO₄²⁻] = 0.8 mmol l⁻¹) in two groups, for at least 1200 and 3000 h, respectively, in a 300 l aerated tank, max. 80 fish at a time, with separate recirculating systems (25 l min⁻¹, 400 l biological filter) at about 18°C. About half the water was renewed every 2 weeks. Fish were fed at unequal intervals, to maintain a constant body weight.

2.2. Whole-body fluxes

Uptake of ²⁴Na was studied in eels previously labelled with ²²Na. Ten eels (adapted to FW > 1200 h) were incubated with ²²NaCl (10 MBq, Amersham) in 50 l aerated FW for 2 weeks (with biofilter) and 0.1 ml plasma samples were taken from each. They were transferred to ten 0.6 l flux chambers (16°C), at first with running FW and then with running DW ([Na⁺] = 0.034, [Ca²⁺] = 0.12, [Mg²⁺] = 0.12, [K⁺] = 0.00, [HCO₃⁻] < 0.3, [Cl⁻] = 0.06, [SO₄²⁻] = 0.06 mmol l⁻¹). Water flow to the chambers was stopped (with continued aeration) for 5 × 2.3 h periods (FW, and after 7, 23, 119 and 335 h in DW) and for 4 h just after a change to DW. To each chamber was added 0.5 MBq ²⁴NaCl (30 MBq(mg Na)⁻¹, Risø National Lab.). Water samples (5 ml) were removed after a 15 min mixing period and then 1 and 2 h later, except during the 4 h stop where they were removed at 0.5 h intervals. After the 2 week experimental period, 0.1 ml plasma samples were again taken from each eel.

2.3. Formation of (¹⁴C) gill lipids

(¹⁴C) acetate was used as lipid precursor (Hansen and Abraham, 1979). It was added to the water in the incubation tank in order to study the formation of (¹⁴C) gill lipids in vivo as a function of time in DW. Twenty one eels (adapted to FW > 3000 h) were incubated in portions of three in 10 l water, added 130 MBq (¹⁴C) acetate (2 MBq µmol⁻¹, Amersham), as follows: FW (24 h), DW (5, 6, 12 and 24 h) and then 24 h in DW after pretreatment in running DW for 48 and 337 h, respectively.

In another similar experiment, eight eels were first labelled with ²²NaCl and then incubated with (¹⁴C) acetate under various conditions in DW as above. Water samples (5 ml) were removed after the incubations to assay ²²Na efflux.

2.4. Analytical techniques

²²Na and ²⁴Na were counted at 1275 and 1370 keV, respectively, with a Ge–Li gamma ray detector. Efflux measurements of ²²Na in the water were in each case derived from the radioactivity measurements by relating these to the corresponding mean plasma radioactivity observed in 15 µmol Na from 0.1 ml eel plasma. Uptake measurements were calculated by expressing the mean relative loss of ²⁴Na radioactivity in the water during 1.5 or 1.0 h incubation periods as percentage of the total 0.6 × 34 = 20 µmol Na present in the flux chambers with DW, and correspondingly, 0.6 × 520 = 312 µmol in the flux chambers with FW. Results were expressed relative to body weight as µmol kg⁻¹ h⁻¹.
Fish were killed with an overdose of urethane. Gill lipids were isolated and assayed by thin layer chromatography as before (Hansen and Abraham, 1979; Bolitius et al., 1991). $^{14}$C-labelled wax alcohols were first isolated and assayed as a group among other, neutral lipids. They were then identified individually, within the unsaponifiable fraction, after saponification of the corresponding spot on the thin layer foil. This was done by paper chromatography, making use of the fact that fatty (wax) alcohols run somewhat ahead of their two carbon atoms shorter corresponding fatty acid—i.e., octadecanol ahead of hexadecanoic acid (Hansen, 1969).

![Diagram](image)

**Fig. 1.** The effect of acclimation to denitrified artificial fresh water (DFW) on whole-body sodium fluxes in the same group of eels, *A. anguilla* (n = 10), determined as means of results from seven successive 0.5 h flux periods during the first 4 h in DW and since as means of two successive 1 h flux periods. Each eel was assayed separately. Open bars indicate unidirectional Na$^{+}$ fluxes (± SEM) and shaded areas, net Na$^{+}$ fluxes (± SEM).
3. Results

3.1. Unidirectional Na\(^+\) flux

The effect of FW → DW transfer is presented in Fig. 1. Originally, the cells were in osmotic equilibrium at a mean value of about 70 \(\mu\)mol kg\(^{-1}\) h\(^{-1}\) for Na\(^+\) uptake as well as efflux. Just after transfer to DW Na\(^+\) uptake stopped and Na\(^+\) efflux was enhanced to 120 \(\mu\)mol kg\(^{-1}\) h\(^{-1}\). During the following 4 h there was still no Na\(^+\) uptake and Na\(^+\) efflux came down to 50 \(\mu\)mol kg\(^{-1}\) h\(^{-1}\) at which value it started to level off reaching 40 \(\mu\)mol kg\(^{-1}\) h\(^{-1}\) after 2 weeks, i.e., an adapted efflux somewhat lower than the equilibrium value in FW. Even though Na\(^+\) uptake began again after 8 h

\[
\text{FW} \rightarrow \text{DW}
\]

\[\text{EEL GILLS}\]

\[\text{\(^{14}\)C-wax alcohol (\%)}\]

**Fig. 2.** The incorporation in vivo into gill wax alcohols of (\(^{14}\)C) acetate, added to the water in the incubation tank, expressed as percentage of total (\(^{14}\)C) lipids. (A) Presented as a function of time in DW, similar to Fig. 1: means ± SEM of groups of three individually assayed eels. (B) Presented as a function of Na\(^+\) efflux; single eels.
in DW it did not at any time achieve equilibrium with Na⁺ efflux; i.e., the general picture in DW was a net Na⁺ loss.

3.2. (¹⁴C) wax alcohols in the gills

Fig. 2 presents the incorporation of (¹⁴C) acetate into wax alcohols corresponding to the flux values presented in Fig. 1. The percentages refer to all ¹⁴C-labelled gill lipids, neutral lipids as well as phospholipids. The wax alcohols have been identified as mixtures of mainly (¹⁴C) octadecanol and (¹⁴C) eicosanol.

There is an apparent parallel between the pattern of Na⁺ efflux in Fig. 1 and (¹⁴C) WA% as a function of time in DW in Fig. 2. Just after transfer to DW gill (¹⁴C) WA% went up, to come down again 2 weeks later (the mean value in Fig. 2 of initial (FW) and final (361 h in DW) is 24 ± 2 (¹⁴C) WA%, f = 5; after 3 and 6 h in DW it is 52 ± 3 (¹⁴C) WA%, f = 5; the difference is significant at P < 0.001). The parallel was confirmed in the next experiment when gill (¹⁴C) WA% was expressed as a function of ³²Na efflux. We found (Fig. 2) a linear dependence: (¹⁴C) WA% = -23 + 26 log (μmol kg⁻¹ h⁻¹) with R² = 0.64, P < 0.03 that the slope = 0.

4. Discussion

The results clearly indicate that during the first 2 weeks after transfer from FW to DW, the eel reacts above all by reducing an enhanced Na⁺ efflux rather than by enhancing the corresponding Na⁺ uptake. This is much in agreement with the results of Perry and Laurent (1989) regarding Cl⁻ efflux from rainbow trout in DW, but not to the same degree regarding the corresponding Na⁺ efflux; in the latter case the trout is apparently also able to regulate its net Na⁺ balance by enhancing Na⁺ uptake shortly after transfer to DW. Perry et al. (1992) have furthermore shown in general that the rainbow trout is better able to take up Na⁺ from its ambient medium than the European eel. The present results are in agreement with other investigations which indicate that the eel, to a greater extent than other fish, relies on a low overall permeability in its osmoregulation. In agreement with the observed net Na⁺ loss (Fig. 1), Krogh (1959) mentions that he has seen eels die after 1 month in running DW.

The correlation between gill (¹⁴C) WA% and sodium efflux indicates that the synthesis of WA could be one of the means by which the eel is able to tighten the gill membrane. Further studies are needed, especially regarding the influence of prolactin, to clarify this aspect, but it seems reasonable to suggest that it is the mucocytes that are involved in the process (Wendelaar Bonga and Mcis, 1981). Wax alcohols are most likely formed in only small amounts in close connection with the gill surface and are thus difficult to assay by other means than the incorporation of radioactive markers.

References


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Printed in The Netherlands
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