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The effects of salinity adaptation on intracellular chloride accumulation in the european flounder

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In the small intestines of flounders adapted to sea-water or to fresh water, intracellular chloride activity is maintained above equilibrium activity. In sea-water adapted animals this accumulation is inhibited by piretanide whereas fresh-water adapted animals are insensitive to the drug. This indicates different chloride accumulation mechanisms.

The question of how a euryhaline teleost fish such as the European flounder (*Platichthys flesus*) accomplishes its adaption to waters of differing salinities remains largely unresolved. In the intestine of the flounder, a major site of adaptation [1], previous work has shown that a net flux of chloride in the mucosa-to-serosa direction, equivalent to the short-circuit current, is maintained both by flounders adapted to sea-water (SW) and by flounders adapted to fresh water (FW) [2,3]. The net flux maintained in fresh-water adapted fishes is, however, much lower than the net flux observed for fishes adapted to sea-water. Transepithelial unidirectional fluxes show that after sea-water fishes have been adapted to fresh water, the chloride fluxes are reduced in both directions, but that the reduction in net flux is due to a greater decrease in the undirectional mucosa-to-serosa flux [2,3].

Microelectrode and tracer studies have shown that chloride transport in the intestines of fishes adapted to the marine environment [5–9] is maintained by a Na^+-Cl^- coupled transport process at the mucosal membrane of the intestinal cell. This process is inhibited by the loop diuretics furosemide and piretanide.

On this evidence, the adaptive process from sea-water to fresh water in the intestine would seem to involve both a general decrease of the permeability of the tissue to chloride, and an inhibition of the active absorption of chloride across the tisue; the latter presumably by inhibition of the coupled entry step at the mucosal border. However, mucosal uptake studies [2,4] have been unable to show any effect of adaptation from sea-water to fresh water on the mucosal uptake of chloride which points to a serosally based change. This report presents the results of a study undertaken to probe the sites of the adaptive changes taking place in the fish small intestine as a result of adaptation from sea-water to fresh water.

Double-barrelled and single-barrelled chloride selective microelectrodes were used to measure the intracellular chloride activities in the intestines of European flounders adapted to either sea-water or fresh water. The properties of, and results obtained with the two types of microelectrodes did not differ significantly and so have been combined together. The microelectrodes were prepared according to the method described by Zeuthen et al. [10]. The electrodes had a mean slope of −57.6

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$\pm$ 1.4 mV (S.E., $n = 11$) and a selectivity coefficient in the presence of 25 mM HCO_3^- (K_{ij}) of $3 \cdot 10^{-2}$ (fixed interference method [11]). Both these parameters compare favourably with values reported in the literature for these types of electrodes.

For each experiment an intestine was removed from a previously adapted animal, opened out into a flat sheet and its serosal musculature removed. A segment was then mounted between perspex half-chambers. Each half-chamber was then perfused with a control saline of composition (in mM): 130 NaCl; 2.5 $CaCl_2$; 10 CH_3 COOK; 1.1 $MgSO_4$; 2 alanine; 10 glucose; 25 choline bicarbonate and gassed with 95% O_2/5% CO_2 with pH 7.2–7.4. After intracellular chloride activities were obtained in the control saline, the mucosal control saline was replaced by one containing 10^{-3}–10^{-4} M piretanide and the intracellular chloride activity was again measured.

Table I presents the data collected from flounders adapted to fresh water or to sea water. The mean mucosal membrane potential ($\bar{V}_{MC}$) in control conditions is not significantly different between the two differently adapted groups of fishes (sea-water -44 ± 3 mV (7), fresh-water -47 ± 3 mV (7)); moreover, the control values for the mean intracellular chloride activity ($\bar{a}_i$(Cl)) are the same at 23 ± 3 mM (7) for both fresh-water and sea-water fishes. Thus, fresh-water adapted flounders maintain $\bar{a}_i$(Cl) well above the expected equilibrium activity (obs/eq is 1.51 ± 0.14 (7)) and there is no significant difference between the obs/eq of the two groups of fishes indicating that, fresh-water fishes actively accumulate chloride to the same extent as sea-water fishes. When the loop diuretic piretanide is added to the mucosal solution of these intestines, a_i(Cl) is reduced to the equilibrium value in the steady state in sea-water adapted fishes as would be expected from a previous report [7]. In fresh-water adapted fishes, however, piretanide has no effect and there is no significant difference between control values and experimental values (Fig. 1).

Quite clearly, the elevated intracellular chloride activities observed for fresh-water adapted flounders reflects an active chloride uptake and this is consistent with the observations of unchanged mucosal tracer uptake in fishes adapted to fresh water from sea-water made by Smith et al. [2] and Lahlou and MacFarlane [4]. However, the mucosal process maintaining the elevated intracellular chloride activity in fresh-water fishes is different from that operating in sea-water adapted fishes

TABLE I

Flounders were stored in tanks circulated with sea-water (SW) until required either for adaptation to fresh water (FW) or for experiment. Sea-water fishes were adapted to fresh water by directly transferring them to tanks containing aerated domestic tap water. They were kept in fresh water for at least a week before use. Both sea-water and fresh-water tanks were maintained at 12°C. Data were collected in the steady state by sampling each tissue several times. The means from each tissue were treated as single observations and statistical evaluations were carried out over the tissue means (N = number of tissue means). Unpaired t-tests were used to evaluate the data. Criteria for successful impalements were: a sudden deflection on entering the cell; maintenance of initial value with less than 10% variation for 30 s; return to base-line on leaving the cell. P_{diff} is the probability that the values between the two groups of fishes are not different; $P < 0.05$ is regarded as significant. *$P < 0.05$ compared with control value in the same group of fishes. $\bar{V}_{MC}$ is the mean mucosal membrane potential. $\bar{a}_i$(Cl) is the mean intracellular chloride activity. $\overline{obs/eq}$ is the mean ratio between the observed $\bar{a}_i$Cl and the expected equilibrium $\bar{a}_i$(Cl). S.E. is the standard error of mean. n.s., not significant.

Fish group	Mucosal solution	$V_{MC} \pm$S.E. (N)	$\bar{a}_i$(Cl $\pm$S.E. (N)	$\overline{obs/eq} \pm$S.E. (N)
SW flounders	Control	-44 ± 3 (7)	23 ± 3 (7)	1.41 ± 0.19 (7)
	Control + 1–0.1 mM piretanide	-49 ± 1 (7)	15 ± 1 (7)*	1.10 ± 0.07 (7)
FW flounders	Control	-47 ± 3 (7)	23 ± 3 (7)	1.51 ± 0.14 (7)
	Control + 1–0.1 mM piretanide	-45 ± 4 (7)	25 ± 3 (7)	1.56 ± 0.17 (7)
P_{diff}	Control	n.s. (14)	n.s. (14)	n.s. (14)
	Control + 1–0.1 mM piretanide	n.s. (14)	< 0.01 (14)	< 0.05 (14)

insofar as the piretanide sensitivity of the two processes is different. Since fresh-water fishes show a markedly reduced net transepithelial chloride flux compared with sea-water fishes [2,3], the maintenance of a similar intracellular chloride activity must mean that not only has the nature of the mucosal entry step changed but that the serosal exit process has also changed. This implies that even if chloride exit across the serosal membrane is passively driven by the chloride gradient, the process is still maintained under close regulation by the cell. (Since the mucosal membrane potential does not change, the fall in the transepithelial potential consequent upon the reduction of net chloride transport must be reflected in the basolateral membrane potential. The fish intestine maintains a serosa negative transepithelial potential in sea-water [12] and therefore the basolateral membrane potential must increase in fresh-water and consequently the electrochemical gradient driving chloride out of the cell across that membrane must also increase). It follows from this that if the mucosal entry process functions at the same rate in fresh water as in sea-water then mucosal exit of chloride must be increased so that $\bar{a}_i(Cl)$ does not increase to higher levels. If this is not the case, then the rate of chloride entry must be decreased. Conductance measurements of the mucosal membrane in the two adapted states would clearly be helpful in determining which of the alternatives is the case. Whichever, adaptation from sea-water to fresh water can now be seen to involve substantial changes in chloride transport at both the mucosal and serosal borders of the intestinal cell.

Active salt transport is well established as the means by which the sea-water adapted fish absorbs water across the intestine [1,13,14] for the purpose of replacing osmotic water losses, and the elevated $\bar{a}_i(Cl)$ observed is a reflection of this process. Recent work indicates that the mucosal entry step consists of a K^+-Na^+-Cl^- neutral triporter [15] with basolateral exit mediated perhaps by conductive channels. Chloride transport in fresh-water fishes has not been correlated with net water flux [16] so that the question arises of what functional significance is the active accumulation of chloride in the intestinal cell? One possibility is that it may be related to the regulation of intracellular pH via a dual exchanger Na^+/H^+-Cl^-/HCO_3^-. This would explain the loss of piretanide sensitivity of the chloride accumulation process in the fresh-water fish intestine and has the attraction that the existence of such a dual exchanger is well-documented [17] although it has not been shown to be present in fish intestines. Exchange of one transport process for the other could be accomplished by the insertion into and removal from the mucosal membrane of the appropriate carriers as has been postulated for both channels and carriers in various epithelia [20,21]. A similar process could explain the reduction of chloride exit across the basolateral membrane. These are interesting and important questions that remain to be resolved.

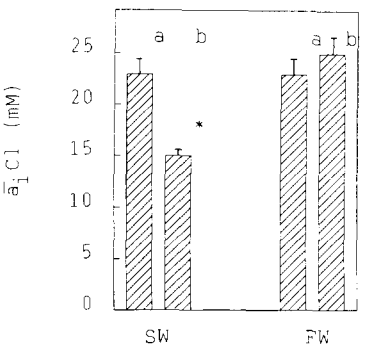

Fig. 1. The effect of piretanide on intracellular chloride activity. 1–0.1 mM piretanide was added to the mucosal surface of flounder small intestines. The flounders had been adapted to either sea-water (SW) or fresh water (FW). a, control; b, piretanide; *, significantly different from control.

The present work demonstrates that substantial changes occur at both the mucosal and serosal borders of the fish intestine during adaptation from sea-water to fresh water. This indicates that both borders are closely regulated by the cell and their concerted changes in response to salinity adaptation makes this an interesting example of 'cross-talk' between the two membranes [18,19]. Furthermore, the maintenance of elevated intracellular chloride activities in fresh-water fish intestines indicates that chloride uptake is a physiologically important process in both sea-water and fresh-water environments. Since, in the fresh-water state, water transport is not correlated with chloride transport intracellular pH regulation is suggested as a possible function for this process. Adaption from sea-water to fresh water is thus

seen as the exchange of one chloride uptake process (K^+-Na^+-Cl^- neutral triporter) for another (Na^+/H^+-Cl^-/HCO_3^- dual exchanger) reflecting a change in the physiological function of chloride transport.

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