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THE EFFECT OF EXTERNAL POTASSIUM IONS ON THE ELECTRICAL POTENTIAL MEASURED ACROSS THE GILLS OF THE TELEOST, *DORMITATOR MACULATUS*

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SUMMARY

1. A technique has been developed for the measurement of electrical potentials (TGP's) across the gills of free-swimming, *Dormitator maculatus*.

2. Transfer of fish to various KCl solutions is correlated with changes in the TGP, which are not of sufficient magnitude to account for the known potassium stimulation of sodium efflux from this species.

3. Transfer to potassium-free sea water results in little or no change in TGP while previous results have shown that such a transfer is correlated with a 22% reduction of sodium efflux.

4. Transfer to fresh water results in a reduction of TGP from +17 mV (inside positive) to −36 mV which is sufficient to account for the instantaneous reduction in sodium efflux previously shown for this species.

5. It is concluded that while changes in TGP can account for the 'Na-free effect' in *D. maculatus* they cannot account for the potassium effects on sodium extrusion. This supports the previous conclusion that sodium efflux and potassium influx are chemically linked in this species.

INTRODUCTION

During the past 8 years a working model for sodium extrusion by the sea water-acclimated teleost fish has emerged from both biochemical and physiological studies. It has been shown, by many investigators, that extracts from the gill tissue of either marine fish or euryhaline fish acclimated to sea water possess appreciably greater activities of the enzyme Na-K activated ATPase than gill tissue from fresh water fish or euryhaline fish acclimated to fresh water (Epstein, Katz & Pickford, 1967; Kamiya & Utida, 1969; Jampol & Epstein, 1970; Evans, Mallery & Kravitz, 1973). In addition, physiological studies have shown that changes in the potassium concentration of the medium surrounding a fish have marked effects on the efflux of sodium ions (Maetz, 1969; Motais & Isaia, 1972; Evans, *et al.* 1973). We have recently shown that in one species of teleost, *Dormitator maculatus* (the fat sleeper), the K_m of potassium stimulation of sodium efflux is identical to the K_m of potassium stimulation of the Na-K activated ATPase extracted from gill tissue of this species (Evans *et al.* 1973). Unpublished observations from our laboratory,* as well as data from other

* Note added in proof; Evans, D. H., Mallery, C. H. (1974). *J. Comp. Physiol.* (In press.)

workers, show that the time course of the increase in the effectiveness of potassium stimulation of sodium efflux and the increase in the activity of the Na-K activated ATPase are similar in at least two species of euryhaline fish after transfer from fresh water to sea water (Bornancin & de Renzis, 1972). All of these data indicate rather strongly that net sodium extrusion by teleost fish in sea water is coupled with potassium uptake and mediated via the enzyme Na-K activated ATPase.

However, an alternative explanation of the present data is possible. The enzyme, Na-K activated ATPase may be solely involved in the intracellular osmoregulation of gill cells – a parameter which certainly may change with external salinity. In addition, the physiological coupling of external potassium ions and sodium efflux may be not a matter of chemical exchange but of electrical potential changes. If one makes these two assumptions, then an alternative to the presently accepted model of parallel, but uncoupled, extrusion of sodium and chloride (Maetz, 1971) is possible. It is theoretically possible that the extrusion of chloride ions is at least slightly electrogenic and that the resulting internal positivity (relative to the outside medium) drives sodium ions out of the fish. In addition, if the gill membrane were appreciably more permeable to sodium and potassium than chloride ions, changes in the external concentration of either of these ions would affect the trans gill potential (TGP) and, concomitantly, the potential driven sodium efflux. Such a system has been shown to account for much of the sodium efflux from the marine crustacean, *Artemia salina* (Smith, 1969).

There is some evidence to support this alternative model for sodium efflux from marine fish. Measurements of the TGP of seawater acclimated fish indicate a potential of the order of 20 mV, inside positive (House, 1963; Maetz & Campanini, 1966; Evans, 1969). The Nernst Potential for sodium and chloride across the typical marine teleost gill is approximately 27 mV, inside positive for sodium and inside negative for chloride (House, 1963; Evans, 1969). If one uses the criteria of deviation from the Nernst Potential to indicate active transport (Gutknecht, 1970) then it seems evident, that while chloride ions are obviously out of electrochemical equilibrium and therefore actively extruded from the fish, sodium ions are nearly in equilibrium. Thus, a statement with regard to active sodium extrusion is not warranted from equilibrium potential data (House, 1963; Evans, 1969).

To test the alternative model (that sodium is in electrochemical equilibrium across the fish gill and that the effects of potassium on sodium efflux are due to electrical potential changes rather than chemical coupling) we have examined the TGP of the intact teleost, *Dormitator maculatus* in sea water, potassium-free sea water and immediately after transfer to fresh water or various potassium chloride solutions.

MATERIALS AND METHODS

Mature individuals (5–15 g) of *D. maculatus* were captured and kept in the laboratory as described previously (Evans *et al.* 1973), and were acclimated to sea water (500 mM-Na/l) for one week before use. Experimental animals were anaesthetized with MS-222 (0·01 % ethyl-m-aminobenzoate methane sulfonic acid, Sigma Chemical Co.) and a 20 cm length of PE 10 tubing (Clay Adams) filled with 3 M-KCl in 2 % agar was inserted through the lateral musculature into the peritoneal cavity. For the insertion, the salt bridge was threaded through a 19-gauge needle which was then withdrawn.

The proximal end of the implanted bridge was inserted into a PE100, 3 M-KCl agar filled bridge which terminated in a 3 M-KCl solution. A second 3 M-KCl solution contained the termination of a similar bridge (PE 10 plus PE 100 tubing) which was placed in the solution surrounding the experimental fish. The two KCl solutions were then connected to a Bausch and Lomb VOM 7 potentiometric recorder (10 megohms input impedance) via a miniature calomel electrode (Corning) immersed in each solution. In order to zero the electrodes, a removable 3 M-KCl-agar filled length of PE 100 tubing connected the two KCl solutions. Asymmetry between the two bridges and their electrodes was examined before and after each experiment by placing both bridges in the same sea water bath and comparing this 'zero potential' with the 'zero potential' recorded by placing a bridge between the calomel electrodes. The maximal asymmetry was 2 mV and no significant drift was noted during the course of an individual experiment. The precision and response time of the B & L potentiometric recorder was checked by attaching input leads from this instrument directly to a calibrated, variable DC power source. Response to changes in the input potential over the range of -100 mV to $+100$ mV was less than 1 sec and its precision was $\pm 2\%$ over the same range of potentials.

After implantation of the salt bridge, the experimental animal was placed in 400 ml of sea water to allow for recovery from the anaesthetic. Potentials were recorded during this rest period and were found to drift from an initial internal negativity to a stable internal-positive potential after approximately 15 min. The potential recorded during this period of stability was taken as the control TGP for *D. maculatus* in sea water.

To test for the possibility of short-circuiting around the site of bridge implantation individuals were lightly anaesthetized and implanted with the bridge in the normal way. The site of implantation was then rinsed off with distilled water and blotted dry. The fish was then held with only its head and gills submerged so that the site of implantation was above the water. TGP's recorded in this manner were compared with those recorded in the same fish immediately after it was totally submerged in the bath. No difference was noted.

The assumption that the measured potentials are essentially those existing across the gill membranes and not the body wall is supported by our finding that, if the experimental fish was disturbed manually during the course of the experiment, opercular movements ceased for a few seconds with a concomitant reduction in measured potential toward zero. As soon as the operculum was opened again the potentials rose to the level displayed by the undisturbed fish. If the body wall presented a significant electrical shunt during the course of these experiments, opercular closing would have had little effect on the measured potential.

After a stable TGP had been recorded in sea water the fish was transferred:

(1) Through a series of baths containing pure KCl solutions of increasing concentration (1 mM, 4 mM, 10 mM and 50 mM/l), or

(2) through a series of baths containing pure KCl solutions of decreasing concentration (reverse of above order). Transfers from one solution to the next were made only after a stable potential had been reached (in most cases within 10 minutes after transfer), or

(3) to potassium-free sea water or fresh water.

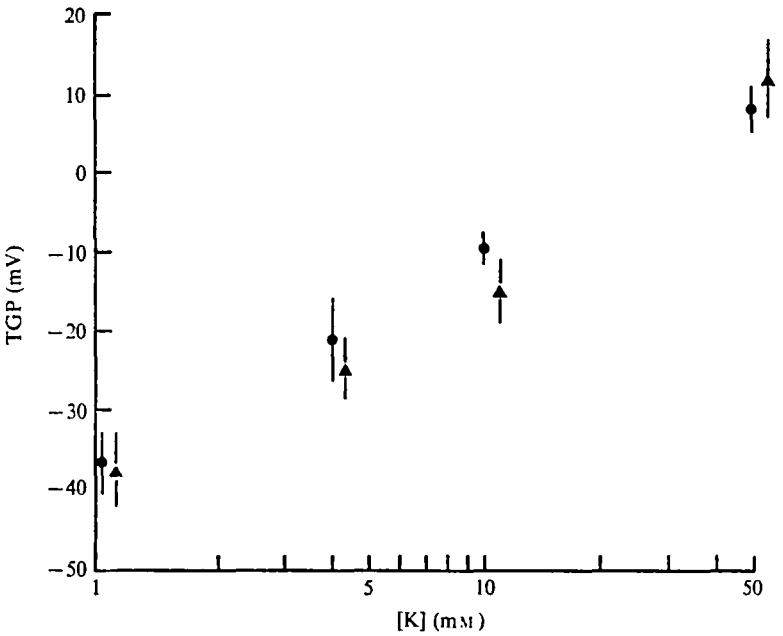

Fig. 1. Trans gill electrical potential (TGP) as a function of external potassium chloride concentration. Each point represents the mean (± standard deviation) for four fish. Fish were acclimated to sea water, implanted with KCl/agar bridge and transferred to various KCl solutions after a stable TGP had been recorded in sea water. Circles, data from fish transferred in the sequence 1 to 50 mM-KCl, triangles, data from fish transferred in the sequence 50–1 mM-KCl (shifted to right for graphical clarity). See text for experimental details.

RESULTS AND DISCUSSION

1. *The effect of external potassium ions*

Fig. 1 shows the results of these experiments. To avoid the effects of gill permeability changes during the course of the transfers, four fish were transferred up the series of concentrations and four fish were transferred in the reverse order. While differences between the TGP's measured in the two experiments are present they are not of sufficient magnitude or consistency to warrant separation of the two groups of data. In addition, separation would not substantially affect the subsequent calculations; therefore the data have been combined. While the data in Fig. 1 are approximately linear the slope is far removed from that expected of a 'potassium electrode' whose potential gradient is defined solely by the Nernst equation for different potassium gradients. Data from such an electrode would display a slope of 58 mV for each 10-fold change in potassium concentration. It appears therefore that the permeability to ions besides potassium plays a finite role in the production of the potential across the gill of *Dormitator maculatus*.

It has been shown by various authors (Hodgkin & Katz, 1949; House, 1963; Potts & Parry, 1964) that the flux of an ion species that is passively distributed across a membrane may be related to the electrical potential across that membrane by equation 1.

$$\mathcal{J} = \frac{P(zFE/RT)\cdot C\cdot \exp(zFE/RT)}{1-\exp(zFE/RT)}, \qquad (1)$$

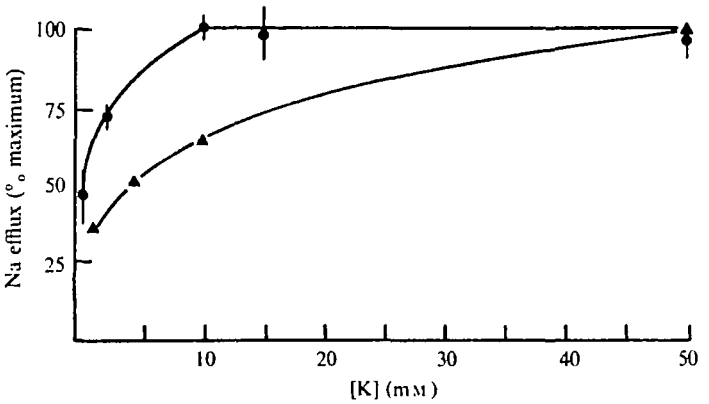

Fig. 2. The sodium efflux as a function of external KCl concentrations. Circles, data published previously (Evans *et al.* 1973) on the actual stimulation of sodium efflux by external potassium. Each point is the mean (±S.D.) for from 3 to 25 animals and the efflux in KCl solutions above 10 mM/l was taken as the maximum efflux. Triangles, theoretical relative sodium efflux in the same KCl solutions calculated from the TGP data in Fig. 1 and equation (1). The lack of correspondence between the two curves indicates that at least some of the potassium stimulation of sodium efflux is due to chemical, not electrical, coupling.

where J represents the unidirectional flux of the ion species, P the permeability of the membrane to that ion species, C the concentration of the ion in the compartment from which flux is being measured, and z, f, E, R, T have their usual meaning. The use of equation (1) involves two assumptions: (1) that the membrane presents a constant electrical field and (2) that the only driving forces for the ion species in question are concentration gradients and electrical potentials across the membrane. While it is obvious that a membrane as complex as a gill epithelium probably cannot be treated strictly as a constant field, use of this equation should give us a first approximation of the relationship between TGP and ionic fluxes until more detailed knowledge of the electrical properties of this epithelium are known. It has been shown (Hodgkin & Katz, 1949; House, 1963; Potts & Parry, 1964) that equation (1) can be used to calculate relative fluxes of the ion species under the conditions of different electrical potentials as long as one assumes that neither P nor C change appreciably during the course of changes in the electrical potential across the membrane. In the present experiments it seems reasonable to make this final assumption since the potentials measured in a given KCl concentration were little affected by the sequence of changes of the KCl solutions (see Fig. 1). We therefore used the average potential measured at 50 mM KCl/l as the control point and calculated the theoretical relative efflux of sodium ions from the fish under the conditions of the various TGP's measured across *D. maculatus* in the other KCl solutions. All calculations were performed on a Monroe Model 1775 programmable desk calculator.

The curve for theoretical relative sodium efflux in various KCl solutions is shown in Fig. 2. This curve will define the actual sodium efflux in various KCl solutions if potassium stimulation of sodium efflux is TGP mediated. For comparison we have also included in Fig. 2 the data on actual potassium stimulation of sodium efflux from *D. maculatus* which we published previously (Evans *et al.* 1973). The discrepancy between the two curves shows clearly that potassium stimulation of the sodium

efflux in this species is not totally TGP-mediated. These data support our previous suggestion that potassium stimulation of sodium efflux from *D. maculatus* is via a Na/K exchange system.

2. *The effect of removal of potassium from sea water*

When fish with implanted bridges were transferred to potassium-free sea water after a stable TGP had been monitored in sea water, the TGP changed little ($-0{\cdot}6 \pm 3{\cdot}5$ mV; $\bar{x} \pm$ S.D., seven fish). In terms of theoretical relative efflux (calculated from equation (1)), the fish in potassium-free sea water should have a sodium efflux that was $99 \pm 6\%$ of the control efflux in sea water. It has been shown previously that the sodium efflux from *D. maculatus* is reduced by 22% when it is rapidly transferred to potassium-free sea water. Thus, it appears that removal of potassium ions decreases sodium efflux by uncoupling of a Na/K exchange system and not by TGP changes.

3. *The 'Na-free effect'*

The TGP recorded for eight individuals in fresh water after transfer from sea water was -36 ± 11 mV. The TGP in sea water recorded in this experiment as well as previous experiments was $+17 \pm 4$ mV for 41 determinations. Using equation (1), the sodium efflux in fresh water should be 33% of the efflux in sea water if the 'Na-free effect' is TGP-mediated. The actual sodium efflux in fresh water immediately after transfer from sea water has been shown to be $30 \pm 7\%$ of the efflux in sea water for 25 fish (Evans *et al.* 1973). It therefore appears that the 'Na-free effect' in at least *D. maculatus* can be fully accounted for by instantaneous changes in the TGP. These data agree with those of Smith (1969) who showed that the 'Na-free effect' of *Artemia* was due to an instantaneous reduction in internal positivity with a concomitant reduction in TGP-driven sodium efflux rather than an uncoupling of a Na-for-Na exchange diffusion system which had been proposed earlier (Croghan, 1958; Thuet, Motais & Maetz, 1968).

In summary, the present experiments indicate, at least in *D. maculatus*, that while the 'Na-free effect' is TGP-mediated, TGP changes alone cannot account for the physiological coupling between external potassium ions and the extrusion of sodium ions from this species in sea water.

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