J. exp. Biol. 122, 25–35 (1986)
Printed in Great Britain © *The Company of Biologists Limited 1986*

AMILORIDE-SENSITIVE SODIUM FLUX AND POTENTIALS IN PERFUSED *CARCINUS* GILL PREPARATIONS

BY ČEDOMIL LUCU

Centre for Marine Research, Institute 'Rudjer Bošković', 52210 Rovinj, Yugoslavia

AND DIETRICH SIEBERS

Biologische Anstalt Helgoland, Notkestrasse 31, 2000 Hamburg 52, Federal Republic of Germany

Accepted 11 November 1985

SUMMARY

Sodium and chloride fluxes, as well as transbranchial potentials (TBP) were studied in isolated perfused gill filaments of the crab *Carcinus mediterraneus*. Experiments were carried out in media that were either hyposmotic to the perfusion solution (asymmetrical conditions) or isosmotic (symmetrical conditions).

Fluxes were found to be diffusional in gills under asymmetrical conditions; amiloride induced an inhibitory effect on influxes, without affecting TBP.

Under symmetrical conditions, TBP was $-7{\cdot}6 \pm 2{\cdot}3$ mV, suggesting that the electrogenic ion pump contributes significantly to the development of TBP. Immediately after addition of $2{\cdot}5 \times 10^{-4}$ mol l^{-1} amiloride to the external solution, sodium influxes were reduced to 31% of those in the control group, and TBP was significantly hyperpolarized from $-7{\cdot}6$ to $-14{\cdot}8$ mV. The absence of Ca^{2+} under symmetrical conditions diminished TBP hyperpolarization.

Half-maximal inhibition of sodium influxes by amiloride was at 7×10^{-5} mol l^{-1}. This low amiloride affinity is typical of low resistance leaky epithelia. Sodium transport is discussed as an amiloride-affected influx, probably as a Na/H antiport.

INTRODUCTION

The gills of marine and brackish-water organisms play a key role in the exchange of osmotically active substances between the environment and the internal '*milieu*'. The large surface area and well developed blood supply enable diffusion of the major ions and small organic molecules, as well as providing active ionic transport. Sodium transport mechanisms in the gills of Crustacea include diffusion, carrier-mediated exchange diffusion and active transport (Shaw, 1961; Evans, Cooper & Bogan, 1976; Lucu, 1977). The exchange diffusion mechanisms proposed for Na/Na, Na/H or Na/NH_4 exchanges are, however, still the subject of controversy.

Key words: sodium fluxes, amiloride inhibition, transbranchial potentials, perfused gills, Crustacea.

Isolated gill preparations seem to be promising experimental models for the elucidation of sodium and chloride transport mechanisms. Koch (1965) has already demonstrated active Na and Cl absorption in the ligatured posterior gills of the euryhaline crab *Eriocheir sinensis*. The posterior gills have numerous large mitochondria located in the folded proximal cell membranes (Dehnel, 1974). Sodium fluxes in perfused isolated crustacean gill preparations were experimentally determined by Croghan, Curra & Lockwood (1965). Mantel (1967), King & Schoffeniels (1969) and Pequeux & Gilles (1978, 1981).

Sodium and chloride transport have been intensively studied in numerous tight epithelia such as frog skin (Moreno *et al.* 1973; Benos, Simon, Mandel & Cala, 1976) and colon (Bentley & Smith, 1975) and low resistance leaky epithelia such as rabbit gall bladder (Frederiksen, 1983) and toad urinary bladder (Bentley, 1968; Palmer, 1984).

Evidence from several isolated tissues indicates that amiloride has profound effects on sodium transport through conductive pathways of tight epithelia at concentrations less than 10^{-6} mol l^{-1} (Eigler, Ketler & Renner, 1967; Nagel & Dörge, 1970). However, in low resistance leaky epithelia, amiloride affects sodium–proton exchangers at concentrations higher than 10^{-6} mol l^{-1} at high external sodium concentrations.

The gills of decapods consist of a central stem which supports several flattened lamellae. Each lamella consists of sheets of epithelial cells covered by cuticle and separated by a blood space. Epithelia contain a transcellular route by which ions penetrate the apical and basal membranes, and paracellular pathways consisting mainly of tight junctions in series with the lateral spaces between epithelial cells (Cioffi, 1984).

Isolated perfused gill preparations should be investigated as a model for studies of ionic mechanisms. The current study was undertaken to investigate the effects of media hyposmotic and isosmotic to the perfusion solution on sodium fluxes and changes in TBP (transbranchial potentials) of isolated *Carcinus* gill epithelia. The effects of the specific Na transport inhibitor amiloride on sodium fluxes was examined.

MATERIALS AND METHODS

Specimens of *Carcinus mediterraneus* Csrn. were collected in the Adriatic Sea on the west Istrian coast near Rovinj (Yugoslavia). Crabs for experiments in which the gills were under symmetrical conditions were previously acclimated for a few weeks in plastic aquaria containing 50 l of sea water diluted with distilled water (14·5‰ salinity). Another group of crabs for experiments under asymmetrical conditions was kept in running seawater aquaria (38‰ salinity). Crabs were fed twice a week in a system with an internal aeration and filtration.

The posterior branchial gill pairs (gills no. 7 and 8) were severed from crabs of comparable sizes (carapace width $4{\cdot}9 \pm 0{\cdot}3$ cm) for these experiments. Immediately after dissection, the gills were immersed in the appropriate solutions for flushing

away the blood from the circulatory system. Perfusion solution was delivered by a perfusion pump (Orion Co., USA) through the afferent blood vessel and collected in a flask from the efferent blood vessel by polyethylene capillary tubes. Before the start of the experiments, gills were fitted with capillary tubes and fixed by a clamp constructed from Plexiglas and covered by neoprene material. The preparation was immersed in 40 ml of bathing solution (constant aeration at 20°C). Sodium and chloride fluxes were measured in successive periods of 10 min. After influx measurements the preparations were washed for 10 min in non-radioactive medium and afterwards the effluxes were measured continuously. The branchial preparations were kept at least 15 min in each medium for equilibration. The flow rate of perfusate was 0·15 ml min^{-1}. For experiments in which gills were under asymmetrical conditions, the bathing solution was ordinary sea water (496 mmol Na l^{-1}; pH 8·1) or diluted sea water (DSW; 102 mmol Na l^{-1}; pH 7·9) and the perfusion solution consisted of (in mmol l^{-1}): Na, 506; K, 10·6; Ca, 11·0; Mg, 57·3: HCO_3, 2·8; Cl, 589·8; SO_4, 30·3. In the experiments under symmetrical conditions, the medium on both sides of the gills was dilute ordinary sea water (450 mosmol l^{-1}; 201 mmol Na l^{-1}; pH 7·9). A Ca-free solution contained (in mmol l^{-1}) Na, 201·8; K, 4·2; Mg, 22·8; HCO_3, 1·1; Cl, 234·0; choline, 4·4.

Transbranchial potential (TBP) measurements were made with a Keithley Instruments 601 Electrometer, connected through Ag–AgCl reference electrodes to agar bridges (3% agar in 3 mol l^{-1} KCl; 3 mm diameter).

As a criterion for sodium and chloride fluxes under symmetrical conditions, we selected those preparations which showed a TBP more negative than −4 mV with respect to the bathing medium.

Sodium and chloride fluxes (f) were calculated from the following equation:

$$f = \frac{^{22}Na \text{ or } ^{36}Cl \times 6}{SRA \times W}, \quad (1)$$

where ^{22}Na (carrier free, produced by Institute 'R. Bošković', Zagreb) or ^{36}Cl (NEN, England) is the radioactivity collected during the 10-min flux periods; SRA is the specific radioactivity of the bathing or perfusion solution, and W the fresh weight of the gills in grams. Fluxes are expressed in μmol g^{-1} h^{-1}.

The sodium concentration was measured by conventional flame photometry, and the chlorides were measured by chloride titrator. Osmoconcentrations were measured using the Knauer vapour pressure osmometer modified for microsampling.

Amiloride was obtained from Merck, Sharp & Dohme, USA.

RESULTS

Sodium influxes and transbranchial potentials (TBP) of the isolated perfused gill preparation of *Carcinus mediterraneus* in sea water (SW; $c_{in}/c_{out} = 0{\cdot}98$) and diluted sea water (DSW; $c_{in}/c_{out} = 0{\cdot}20$) were measured. The effect of amiloride on the sodium influx in DSW was also examined (Table 1). Sodium influxes were

Table 1. *Sodium fluxes and TBP in* Carcinus *gill preparations perfused by saline and immersed in sea water and diluted sea water*

C_{out}/C_{in}	External [sodium] (mmol l^{-1})	Fluxes (μmol Na g^{-1} h^{-1})		TBP (mV)	J_{in}^{Na}/J_{out}^{Na}		
		Influx	Efflux		Predicted		Observed
0·98	496	3835 ± 1813 (7)	4493 ± 1545 (6)	−0·9 ± 0·7 (9)	1·01 ± 0·05 (6)	NS	1·44 ± 0·96 (6)
0·20	102	2199 ± 264 (6)	2785 ± 430 (6)	−27·0 ± 4·7 (8)	0·58 ± 0·13 (6)	NS	0·80 ± 0·15 (6)
0·20	102 + Amiloride ($2·5\times10^{-4}$ mol l^{-1})	1388 ± 209 (6)	2732 ± 488 (7)	−24·7 ± 4·9 (7)	0·53 ± 0·12 (6)	NS	0·50 ± 0·08 (6)

Effects of amiloride on fluxes and TBP are presented.
The values are given as the means ± S.E., with the number of observations in parentheses.
C_{out}/C_{in} are ratios of the external and perfusion saline sodium concentrations.
J_{in}/J_{out} are ratios of calculated and measured sodium fluxes (NS = not significantly different).
Calculated values of flux ratios were obtained according to the relationship:

$$J_{in}/J_{out} = C_{out}/C_{in}\, e^{-\frac{zF}{RT}E} \text{ (Ussing, 1949).}$$

reduced by 37% when the gills were transferred from sea water to diluted sea water. Reduction of the sodium influx following dilution of the external medium has also been described in the isolated gill preparation of *Carcinus maenas* (King & Schoffeniels, 1969). In the present experiments, the transfer to diluted sea water was observed to produce a change in TBP from −0·9 mV to −27·0 mV.

Since the conductance for Na and Cl is a function of the membrane permeability and the concentrations of these ions on the inner and outer sides of the membrane, the relative permeabilities of chloride and sodium (P_{Cl} and P_{Na}) can be measured by the following approximation:

$$P_{Cl}/P_{Na} = \frac{E_{Na} - TBP}{TBP - E_{Cl}}, \tag{2}$$

where TBP is the measured potential and E_{Na} and E_{Cl} are the equilibrium potentials calculated by the conventional Nernst equation $E = RT/zF \ln C_o/C_i$.

The relative ion permeabilities of isolated perfused gills of *C. mediterraneus* immersed in SW are

$$P_{Na} : P_{Cl} = 1 : 0{\cdot}34. \tag{3}$$

These results suggest that the gills are much more permeable to Na than to Cl. By substitution of the relative ion permeability for the SW coefficients in the Goldman, Hodgkin and Katz equation (Hodgkin & Katz, 1949), where Na_o, Cl_o, Na_i and Cl_i are external (o) and perfusate (i) ion concentrations of the DSW, we may predict diffusional potential (v) for DSW by

$$v = \frac{RT}{F} \ln \frac{Na_o + 0{\cdot}34Cl_i}{Na_i + 0{\cdot}34Cl_o}. \tag{4}$$

Equation 2 provides a good fit for DSW, on the assumption that the fluxes represent passive diffusion through the membranes. The value obtained theoretically (−21·6 mV) is close to the experimentally obtained TBP (−27·0 ± 4·7 mV). Strictly, this equation should not be applied to such a system which has more than two membrane barriers, and also because the permeability constants vary with temperature, ionic concentration changes and independent ion movement. In further attempts to characterize sodium fluxes from the apical membrane side, we compared experimentally obtained values J_{in}/J_{out} and values calculated from Ussing's criterion (Ussing, 1949). In the control and amiloride-treated groups, experimentally obtained and calculated flux ratios were not significantly different, indicating diffusion rather than active sodium transport processes (Table 1). Amiloride ($2{\cdot}5 \times 10^{-4}$ mol l^{-1}) applied from the apical side significantly inhibited sodium influxes in DSW, whereas it had no significant effect on diffusional TBP.

In parallel, we investigated Na and Cl fluxes and TBP, in an external medium isosmotic to that being perfused through the gills (symmetrical conditions). As shown in Table 2, a sodium influx of 758 μmol g^{-1} h^{-1} and TBP of −7·6 ± 2·3 mV (inside negative polarity with respect to the outside medium) did not appear to arise solely from passive physico-chemical forces, because in the absence of any chemical

Table 2. *Sodium and chloride fluxes, TBP in isolated perfused* Carcinus *gills with identical media on both sides of the gills (201·8 mmol Na l^{-1}; 234·0 mmol Cl l^{-1})*

	Influx (μmol g^{-1} h^{-1})			Efflux (μmol g^{-1} h^{-1})			J_{in}/J_{out}		
	Na	Cl	TBP (mV)	Na	Cl	TBP (mV)	Observed		Predicted
Control	758 ± 124 (6)	726 ± 249 (4)	−7·6 ± 2·3 (6)	512 ± 106 (6)	536 ± 198 (4)	−7·2 ± 2·1 (6)	1·92 ± 0·38 (6)	Sodium $P<0·01$	1·30 ± 0·10 (6)
	$P<0·001$	NS	$P<0·05$	$P<0·001$	NS	$P<0·01$	2·84 ± 0·61 (4)	Chloride $P<0·01$	0·70 ± 0·03 (4)
Amiloride ($2·5\times10^{-4}$ mol l^{-1})	232 ± 60 (6)	693 ± 262 (4)	−14·8 ± 6·4 (7)	145 ± 47 (7)	470 ± 51 (4)	−15·8 ± 5·7 (9)	1·50 ± 0·30 (4)	Sodium NS	1·90 ± 0·40 (4)

The values are given as means ± S.E., with number of observations in parentheses.
The statistical significance of differences between amiloride-treated and control groups (NS, not significant; Student's *t*-test) was examined.
J_{in}/J_{out} are ratios of the calculated and measured fluxes, with the level of statistical significance.

gradient between the apical and basal side of the tissues it could be maintained for 6 h or more. The addition of $2{\cdot}5\times10^{-4}$ mol l^{-1} amiloride to the external medium caused a rapid decrease in sodium influx from 758 to 232 μmol g^{-1} h^{-1} and TBP from $-7{\cdot}6$ to $-14{\cdot}8$ mV. Also, reduction of sodium effluxes were observed. However, chloride fluxes were not affected by amiloride. A tendency to partial restoration of the TBP (Fig. 1) was shown upon removal of amiloride.

DISCUSSION

Isolated perfused gill preparations showed reduced sodium influx after transfer from SW (slightly hyposmotic to the perfusion solution) into DSW (20% diluted sea water). In DSW the sodium influx was affected by externally applied amiloride, but the TBP, which is diffusional in origin, was unaffected (Table 1).

An active transport component could be masked by predominantly diffusive fluxes in these media which are hyposmotic to the perfusion solution.

Sodium and chloride fluxes and effects of amiloride on these fluxes were studied in detail under symmetrical conditions, i.e. with identical media on both sides of the gills. Under these conditions potentials are not diffusional and are produced by the activity of ion pumps in the branchial epithelia. According to our unpublished results, ouabain-sensitive sodium fluxes in *Carcinus maenas* gill preparations are located on the basal and not apical membrane side (D. Siebers, Č. Lucu &

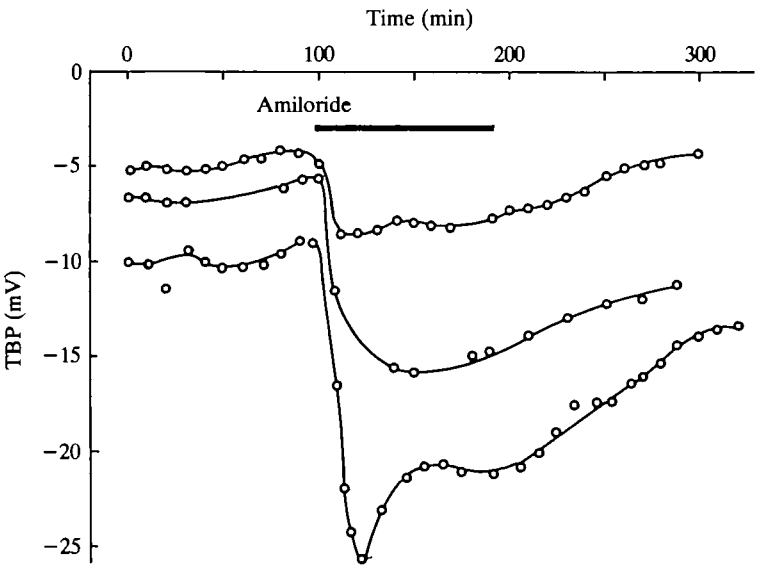

Fig. 1. Effect of amiloride ($2{\cdot}5\times10^{-4}$ mol l^{-1}) on transbranchial potential (TBP) of the *Carcinus* gill preparation in symmetrical solutions. Results are presented in three separate experiments. During the first 100 min TBP values were recorded under control conditions. The horizontal bar represents the presence of amiloride in the external solution followed by restoration of TBP in the solution without amiloride.

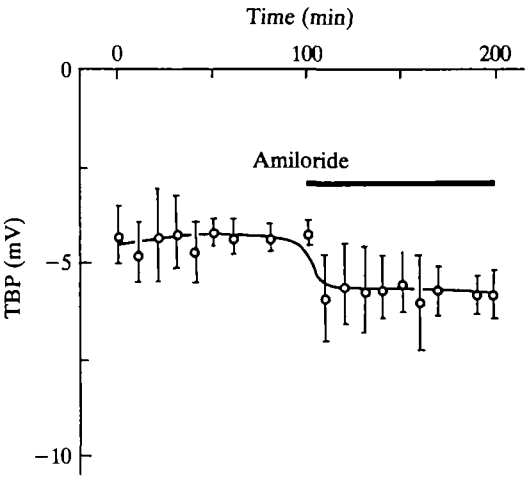

Fig. 2. Effect of amiloride ($2{\cdot}5\times10^{-4}$ mol l^{-1}) on transbranchial potential (TBP) of *Carcinus* perfused gills in calcium-free symmetrical solutions. During the first 100 min TBP values were measured in Ca^{2+}-free isosmotic solutions. The horizontal bar represents the external application of $2{\cdot}5\times10^{-4}$ mol l^{-1} amiloride. Results are means ± S.E. of five separate observations.

A. Winkler, in preparation). In the control (non-amiloride treated) group, experimentally obtained and calculated flux ratios (J_{in}/J_{out}) for Na and Cl are statistically different suggesting active transport processes (Table 2). However, under symmetrical conditions where amiloride ($2{\cdot}5\times10^{-4}$ mol l^{-1}) was applied apically, the sodium influx fell and TBP was hyperpolarized. In the Ca^{2+}-free medium, TBP was less hyperpolarized by amiloride (from $-4{\cdot}4\pm0{\cdot}6$ to $-5{\cdot}7\pm0{\cdot}8$ mV) than in the presence of calcium (Fig. 2). Study of toad preparations (Benos, Mandel & Balaban, 1979) has also indicated that the sensitivity of the amiloride response to the sodium influx depends on the presence of external calcium. Although the mechanism whereby calcium affects TBP is still unclear, Ca^{2+} seems to play an important role in ion and water transport across the gills of aquatic organisms (Ando, 1980).

The effect of amiloride on sodium influxes was a concentration-dependent process, with incomplete inhibition at the highest concentration used ($2{\cdot}5\times10^{-4}$ mol l^{-1}). From the variation of inhibition under the various amiloride concentrations in the 201·8 mmol Na l^{-1} external medium, identical to the perfusion solution, the half-maximal inhibition (ID_{50}) of the sodium influxes by amiloride was approximately at 7×10^{-5} mol l^{-1} (Fig. 3). Since the amiloride concentration required for inhibition of sodium influxes on the apical membrane side of gills was rather higher than 1 μmol l^{-1} it seems likely that the molecule responsible for this transport is capable of Na–proton counter transport (Na/H antiport) rather than conductive Na movement through the sodium channels (Kinsella & Aronson, 1981; Erikson & Spring, 1982). Inhibition of the electrogenic system described as transport through the sodium channels requires amiloride concentrations that are at least 1 or 2 orders of magnitude lower. The inhibitory effect of amiloride is exerted at separate and distinct regions of

the transporting sites depending on the ionic composition of the external solution (Benos *et al.* 1976). It appears that in our case sodium transport mechanisms showed a low amiloride affinity, typical of low resistance, leaky epithelia.

In the presence of $2{\cdot}5\times10^{-4}$ mol l^{-1} amiloride a large hyperpolarization of TBP occurred (basal side electronegative to the apical side). Furthermore, as shown in Table 2, amiloride inhibits only sodium fluxes and has no effect on chloride fluxes. A similar effect has been observed by Bentley (1968) and Kirschner, Greenwald & Kerstetter (1973). The amiloride-sensitive protein responsible for the Na^+ exchange mechanism had no effect on Cl^- fluxes. This phenomenon might be explained at least partially by separate paths for chloride and sodium through the branchial membrane, or by the passage of chloride by the same carrier without any amiloride interaction.

In the case of electrogenic sodium influxes, the internally more negative TBP which developed after amiloride application would stimulate sodium influxes. On the contrary, sodium influxes were markedly decreased. Therefore, it may be suggested that the effect of amiloride upon the Na influx was not due to the effect upon the TBP. Assumed Na/H exchange appears to be an electroneutral process. In freshwater animals hydrogen ion excretion is coupled with Na exchange through the gill epithelia, to maintain electrical neutrality (Kirschner *et al.* 1973). Amiloride could inhibit acidification (H^+ secretion) and hence oppose the movement of protons from the basal side of the membrane (Benos, 1981). A direct interaction of amiloride with proteins responsible for the Na/H exchange was also suggested, by La Belle & Eaton and Paris & Pouyssegur (1983). Salako & Smith (1970), Cuthbert & Shum (1974) and Cuthbert (1981) have reported an effect of amiloride on Na transport in the abdominal skin of *Rana temporaria*.

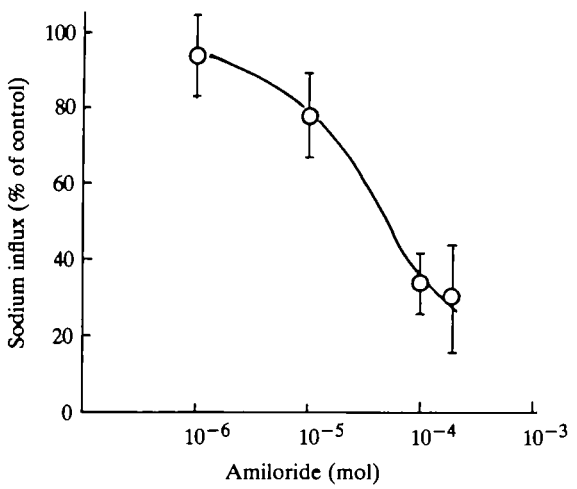

Fig. 3. Amiloride inhibition of apical sodium influx (symmetrical solutions) into *Carcinus* gill epithelia. The points are means of the five values, with the bars indicating S.E. Data are presented as a percentage of influxes observed in the control group without amiloride.

In the isolated gill epithelia, amiloride might produce the hyperpolarization of the TBP by reducing Na fluxes with no effect upon Cl flux. If an Na/H exchanger was blocked, intracellular acidification would occur, and pH would decrease (Benos, 1981). However, if the H^+ permeabilities across the paracellular pathways exceed that for Na, this could also explain the hyperpolarization of the TBP. These hypotheses should be experimentally tested in the future.

The authors are indebted to the Self Management Community of Interest for Scientific Research in Croatia for financial support. The research is also part of programme of the German–Yugoslav scientific cooperation which help is greatly appreciated. We are grateful to Drs R. S. Fisher and S. Lewis for critical reading of the manuscript, Mrs M. Marečić and Mr B. Jagić for excellent technical assistance.

REFERENCES

ANDO, M. (1980). Chloride-dependent sodium and water transport in the seawater eel intestine. *J. comp. Physiol.* **138**, 87–91.

BENOS, D. J. (1981). Acidification and sodium entry in frog skin epithelium. *Biochim. biophys. Acta* **647**, 40–48.

BENOS, D. J., MANDEL, L. J. & BALABAN, R. S. (1979). On the mechanism of the amiloride–sodium entry site interaction in anuran skin epithelia. *J. gen. Physiol.* **73**, 311–326.

BENOS, D. J., SIMON, S. A., MANDEL, L. J. & CALA, P. M. (1976). Effect of amiloride and some of its analogues on cation transport in isolated frog skin and thin lipid membrane. *J. gen. Physiol.* **68**, 43–63.

BENTLEY, P. J. (1968). Amiloride: a potent inhibitor of Na transport across the toad bladder. *J. Physiol., Lond.* **195**, 317–336.

BENTLEY, P. J. & SMITH, M. W. (1975). Transport of electrolytes across the helicoidal colon of the new-born pig. *J. Physiol., Lond.* **249**, 103–117.

CIOFFI, M. (1984). Comparative ultrastructure of arthropod transporting epithelia. *Am. Zool.* **24**, 139–156.

CROGHAN, P. C., CURRA, R. A. & LOCKWOOD, A. P. M. (1965). The electrical potential difference across the epithelium of isolated gills of the crayfish *Austropotamobius pallipes* (Lereboullet). *J. exp. Biol.* **42**, 463–474.

CUTHBERT, A. W. (1981). Sodium entry step in transporting epithelia: results in ligand-binding studies. In *Transport by Epithelia* (ed. S. B. Schultz), pp. 181–195. New York: Raven Press.

CUTHBERT, A. W. & SHUM, W. K. (1974). Amiloride and the sodium channel. *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **281**, 261–269.

DEHNEL, P. A. (1974). Gill-tissue respiration in the crab *Eriocheir sinensis*. *Can. J. Zool.* **52**, 923–937.

EIGLER, J., KETLER, J. & RENNER, E. (1967). Wirkungs-charakteristik eines neuen acylguanidins amiloride-HCl (MK 870) an der isolirten haut von Amphibien. *Klin. Wschr.* **45**, 737–738.

EVANS, D. H., COOPER, K. & BOGAN, M. B. (1976). Sodium extrusion by the sea water-acclimated fiddler crab *Uca pugilator*: comparison with other marine Crustacea and marine teleost fish. *J. exp. Biol.* **64**, 203–219.

ERIKSON, A. C. & SPRING, K. R. (1982). Volume regulation by *Necturus* gallbladder: apical Na–H and Cl–HCO_3 exchange. *Am. J. Physiol.* **243**, 146C–150C.

FREDERIKSEN, O. (1983). Effect of amiloride on sodium and water reabsorption in the rabbit gall-bladder. *J. Physiol., Lond.* **335**, 75–88.

HODGKIN, A. L. & KATZ, B. (1949). The effect of sodium ion on the electrical activity of the giant axon of the squid. *J. Physiol., Lond.* **108**, 37–77.

KING, E. N. & SCHOFFENIELS, E. (1969). In vitro preparation of crab gill for use in ion transport studies. *Archs int. Physiol. Biochim.* **77**, 105–111.

KINSELLA, J. L. & ARONSON, P. S. (1981). Interaction of NH_4 and Li with the renal microvillus membrane Na–H exchanger. *Am. J. Physiol.* **241**, 220C–226C.

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

KOCH, H. J. (1965). Transcellular active transport of mineral ions by epithelial membranes of Arthropods. *Archs Biol. Liège* **76**, 157–187.

LA BELLE, E. F. & EATON, D. C. (1983). Amiloride-inhibited Na^+ uptake into toad bladder microsomes is Na^+-H^+ exchange. *Biochim. biophys. Acta* **733**, 194–197.

LUCU, Č. (1977). Sodium kinetics in the shrimp *Palaemonetes pugio*. II. Sodium fluxes and electrochemical potentials. *J. comp. Physiol.* **115**, 207–214.

MANTEL, L. H. (1967). Asymmetry potentials metabolism and sodium fluxes in gills of the blue crab *Callinectes sapidus*. *Comp. Biochem. Physiol.* **20**, 743–753.

MORENO, J. H., REISEN, I. L., RODRIGUES BOULAN, E., ROTUNNO, C. A. & CEREIJIDO, M. (1973). Barriers to Na movement across frog skin. *J. Membrane Biol.* **11**, 99–115.

NAGEL, W. & DÖRGE, A. (1970). Effects of amiloride on sodium transport of frog skin. *Pflügers Arch. ges. Physiol.* **317**, 84–92.

PALMER, L. G. (1984). Voltage-dependent block by amiloride and other monovalent cations of apical Na channels in the toad urinary bladder. *J. Membrane Biol.* **80**, 153–165.

PARIS, S. & POUYSSEGUR, J. (1983). Biochemical characterization of the amiloride Na/H antiport in chinese hamster lung fibroblast. *J. biol. Chem.* **258**, 3503–3508.

PEQUEUX, A. & GILLES, R. (1978). Osmoregulation of the euryhaline chinese crab *Eriocheir sinensis*. Ionic transport across isolated perfused gills as related to the salinity of the environment. Proceedings of the 12th EMBS Stirling, Scotland, *Physiology and Behaviour of Marine Organisms*, pp. 105–111. Oxford, New York: Pergamon Press.

PEQUEUX, A. & GILLES, R. (1981). Na^+ fluxes across isolated perfused gills of the chinese crab *Eriocheir sinensis*. *J. exp. Biol.* **92**, 173–186.

SALAKO, L. A. & SMITH, A. J. (1970). Effects of amiloride on active sodium transport by the isolated frog skin: evidence concerning site of action. *J. Pharmac.* **38**, 702–718.

SHAW, J. (1961). Studies on ionic regulation in *Carcinus maenas* L. I. Sodium balance. *J. exp. Biol.* **33**, 135–152.

USSING, H. H. (1949). The distinction by means of tracers between active transport and diffusion. *Acta physiol. scand.* **19**, 43–56.