The Journal of Experimental Biology 206,1017-1023

doi:10.1242/jeb.00227

Active NaCl absorption across posterior gills of hyperosmoregulating *Chasmagnathus granulatus*

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Accepted 10 January 2003

Summary

Split lamellae of posterior gills of *Chasmagnathus granulatus* adapted to 2.5‰ salinity were mounted in a modified Ussing chamber. With NaCl-saline on both sides of the preparation a transepithelial voltage (V_{te}) of 4.1±0.5 mV (outside positive) was measured. After voltage-clamping, the negative short-circuit current (I_{sc}) amounted to –142±21 μA cm^{-2} at a conductance (G_{te}) of 44±5 mS cm^{-2}. Substitution of either chloride (by nitrate) or sodium (by choline) on both sides of split gill lamellae significantly reduced I_{sc} (by 70–80%) and G_{te} (by 30–50%). External CsCl (but not $BaCl_2$ or furosemide) inhibited the negative I_{sc} without affecting G_{te}. Addition of ouabain, $BaCl_2$ or diphenylamine-2-carboxylate to the internal bath inhibited I_{sc} at unchanged G_{te}. Internal acetazolamide did not affect I_{sc} or G_{te} across split gill lamellae. Unidirectional Na^+ influx across isolated and perfused posterior gills, however, was reduced by internal acetazolamide by approximately 20% at constant V_{te}. The results suggest that posterior gills of hyperosmoregulating *C. granulatus* display a high conductance epithelium that actively absorbs NaCl in a coupled way by an electrogenic mechanism similar to that seen in the thick ascending limb of Henle's loop and, to a minor degree, by an electroneutral mechanism, presumably *via* apical Na^+/H^+- and Cl^-/HCO_3^--antiports.

Key words: *Chasmagnathus granulatus*, Crustacea, crab gill, flux measurement, ion transport, osmoregulation, short-circuit current, transepithelial conductance, ion substitution, inhibitor, Ussing chamber.

Introduction

To maintain an outward-directed osmotic gradient, hyperosmoregulating Crustacea compensate their passive salt loss by active NaCl absorption across the gills (for a review, see Mantel and Farmer, 1983). It has been shown for numerous crabs that only the posterior gills are responsible for active NaCl absorption, whereas the anterior gills are specialised for respiration (for reviews, see Péqueux et al., 1988; Péqueux, 1995). Mainly based on measurements with whole, perfused crab gills, it has been proposed that active NaCl absorption proceeds *via* apical Na^+/H^+- and Cl^-/HCO_3^- antiports (Gilles and Péqueux, 1986; Péqueux et al., 1988; Lucu, 1990), although this hardly explains the transbranchial voltage measured in various crab species.

After refining the study of ion transport across crab gills using split gill lamellae mounted in modified Ussing chambers (Schwarz and Graszynski, 1989), the basic epithelial properties and transport mechanisms could be analysed more unambiguously. Posterior gills of crabs from freshwater display a tight epithelium and a mechanism of NaCl absorption similar to that of other freshwater animals such as fish and amphibia (Goss et al., 1992; Larsen, 1988, 1991). For Chinese crabs *Eriocheir sinensis* adapted to freshwater, Na^+ absorption was shown to proceed *via* apical Na^+ channels and the basolateral Na^+/K^+-ATPase, generating a positive short-circuit current in the absence of external Cl^- (Zeiske et al., 1992). The negative short-circuit current in the presence of external Cl^- was analysed to reflect Na^+-independent Cl^- absorption *via* apical Cl^-/HCO_3^- antiport and basolateral Cl^- channels, driven by an apical V-type H^+ pump (Onken et al., 1991; Onken and Putzenlechner, 1995). With *in vivo*-like, low external NaCl, the positive and negative currents short-circuit each other and enable transcellular NaCl absorption (Onken, 1999). The posterior gills of the hololimnetic *Dilocarcinus pagei* yielded similar results to Chinese crab posterior gills (Onken and McNamara, 2002). On the other hand, posterior gills of the shore crab *Carcinus maenas*, which is a strong hyperosmoregulator but unable to survive in freshwater, were found to be much more leaky (Onken and Siebers, 1992). Under short-circuit conditions a tight 1:2 coupling between Na^+ and Cl^- absorption was observed (Riestenpatt et al., 1996). In these animals NaCl absorption is exclusively driven by the Na^+/K^+-ATPase, and

the negative short-circuit current seems to reflect NaCl absorption, as in the thick ascending limb of Henle's loop of the mammalian nephron (Greger and Kunzelmann, 1990) *via* apical $Na^+/K^+/2Cl^-$ symport with an apical K^+ recycling *via* K^+ channels (Riestenpatt et al., 1996).

Chasmagnathus granulatus is a strong hyper- and hypo-osmoregulating crab that inhabits intertidal, estuarine coasts of Brazil, Uruguay and Argentina (Mougabure Cueto, 1998; Charmantier et al., 2002). The thickness of the posterior gill epithelium increases when the animals are adapted to either low or high salinities, suggesting the involvement of the gills in salt absorption of hyperosmoregulating crabs and in salt secretion in hypo-osmoregulating animals (Genovese et al., 2000). In a recent study with perfused posterior gills, the respective active transbranchial absorption and secretion of Na^+ have been directly demonstrated (Luquet et al., 2002); however, a satisfying analysis of the transport mechanisms is still lacking.

In the present study, the posterior gills of the euryhaline crab *C. granulatus* adapted to low salinity were investigated in order to obtain more detailed information about the transport mechanism responsible for active, transbranchial NaCl absorption in this species. The results indicate that at least the major part of NaCl is absorbed by an electrogenic mechanism that most likely resembles NaCl absorption across the gills of the shore crab *C. maenas*.

Materials and methods

Animals

Chasmagnathus granulatus Dana 1851 were collected at Faro San Antonio beach (36°18′S 56°48′W) at Samborombón Bay near the southern edge of the Rio de la Plata estuary, Argentina. In the laboratory, the intermolt crabs (28–32 mm carapace width) were kept at 20±2°C with a natural light:dark photoperiod. The animals were fed twice weekly with commercially available pellets of rabbit food. Prior to their use in the experiments, the crabs were adapted for at least 2 weeks to 2.5‰ salinity (artificial salt mixture 'Marinemix', Marine Enterprises International Inc., USA).

Preparations

After killing the animals by rapidly destroying the ventral and dorsal ganglia, the carapace was lifted and the posterior gills removed.

For the Ussing chamber experiments, single posterior gill lamellae were isolated and split according to Schwarz and Graszynski (1989). The split gill lamellae thus obtained were mounted in an Ussing chamber modified after De Wolf and Van Driessche (1986). Preparation and mounting were conducted under microscopic control. Silicon grease (Bayer, Germany) was used to minimise edge damage. An epithelial area of 0.002 cm^2 was exposed to the chamber compartments, bathing the internal and external sides of the tissue. Continuous perfusion of both chamber compartments with aerated saline was achieved by gravity flow (approximately 2 $ml\,min^{-1}$).

For the gill perfusion experiments, the afferent and efferent vessels of a posterior gill were connected *via* fine polyethylene tubings to a peristaltic pump (afferent) and to a glass tube (efferent). The tubings were fixed in position with a small Lucite clamp covered with smooth neoprene to avoid gill damage and to isolate the gill interior from the bathing medium. The gill was bathed in a beaker containing approximately 50 ml of aerated saline and was perfused at a rate of 0.1 $ml\,min^{-1}$, the perfusate being collected in a second beaker.

Electrophysiology

In the Ussing chamber experiments the transepithelial voltage (V_{te}) was measured using Ag/AgCl electrodes connected to both sides of the preparation (distance <1 mm) by agar bridges (3% agar in 3 $mol\,l^{-1}$ KCl). The reference electrode was in the internal bath. Silver wires coated with AgCl served as electrodes to apply current for short-circuiting V_{te} (measurement of the short-circuit current, I_{sc}*) from an automatic clamping device (VCC 600, Physiologic Instruments, USA). The area-specific resistance between the tips of the voltage electrodes (R_{tot}) was calculated from imposed voltage pulses (ΔV_{te}) and the resulting current deflections (ΔI). R_{tot} is the sum of the serial resistances of the solutions (R_s) and the tissue (R_{te}). Because of the low values of R_{tot}, it was necessary to correct the R_{tot} and I_{sc}* data to obtain values directly related to the preparations (R_{te}, I_{sc}). R_s was measured in the absence of a preparation separating the chamber compartments and was found to be 5.5 $\Omega\,cm^2$ for NaCl saline, 7 $\Omega\,cm^2$ for Na^+ saline, and 8 $\Omega\,cm^2$ for Cl^- saline. The corrected values for R_{te} are obtained by substracting R_s from R_{tot}, while the correction of I_{sc}* followed Ohm's law (see Riestenpatt et al., 1996). In the Results, only the corrected values of I_{sc} and G_{te} ($1/R_{te}$) are given. In the figures displaying time courses, the original I_{sc}* is shown, and the current deflections reflect the uncorrected conductance between the tips of the voltage electrodes.

During flux measurements with isolated and perfused whole gills, the transbranchial voltage was controlled with a millivoltmeter (Metrix, France) by connecting Ag/AgCl electrodes *via* agar bridges to the external bath and to the glass tube collecting the perfusate (internal side).

Na^+ influx measurements

Na^+ influxes across isolated and perfused posterior gills were measured by applying ^{22}Na [final activity 9.25×10^3 Bq (0.25 μCi) ml^{-1}] to the external bath. After 15 min of stabilization, the radioactivity appearing in the perfusate was measured at 10 min intervals using a gamma scintillation counter (Canberra series 35 plus, Canberra, USA). Sodium influx was calculated according to Lucu and Siebers (1986) and related to the fresh mass of the gill used.

Salines and chemicals

The basic NaCl saline contained (in $mmol\,l^{-1}$): 468 NaCl, 9.5 KCl, 7.5 $MgCl_2$, 12.5 $CaCl_2$, 5 Hepes, 2.5 $NaHCO_3$ and 5 glucose. In Na^+-free saline NaCl was replaced by choline chloride and $NaHCO_3$ by $KHCO_3$. KCl was reduced to

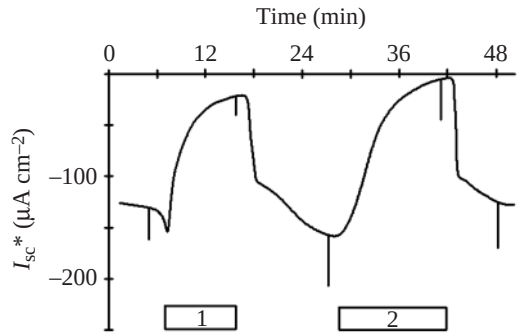

Fig. 1. Representative time course of measured I_{sc} (I_{sc}*) across split lamellae of posterior gills of *C. granulatus*, showing the effects of replacing Cl^- (1, by nitrates) or Na^+ (2, by choline) on both sides of the tissue. The vertical current deflections are due to 1 mV voltage pulses and reflect the uncorrected conductance across the preparation.

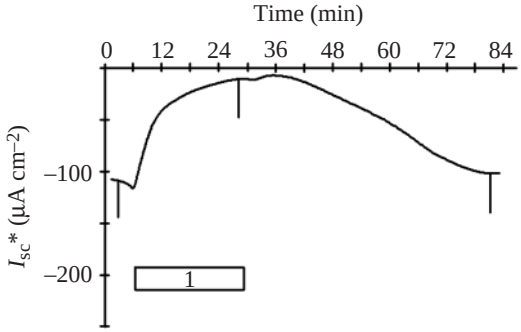

Fig. 2. Representative time course of I_{sc}* across split lamellae of posterior gills of *C. granulatus*, showing the influence of internal addition of 5 mmol l^{-1} ouabain (1). The vertical current deflections are due to 1 mV voltage pulses and reflect the uncorrected conductance across the preparation.

7 mmol l^{-1}. In Cl^--free saline the chlorides were replaced by the nitrates of the respective cations. Immediately before use, the pH of all salines was adjusted to 7.75.

All chemicals and reagents not mentioned separately below were purchased from Merck (Argentina). $NaHCO_3$ was obtained from Mallinckrodt (USA). $CaCl_2$ and Hepes were from J. T. Baker (USA). Ouabain and acetazolamide were purchased from Sigma (Germany). Diphenylamine-2-carboxylate was obtained from Fluka (Germany) and ^{22}Na (as chloride) was from Amersham Pharmacia Biotech. (USA).

Statistics

All values are means ± S.E.M. Differences between groups were tested using the paired Student's *t*-test. Significance was assumed at $P<0.05$.

Results

When split lamellae of posterior gills of *Chasmagnathus granulatus* adapted to 2.5‰ salinity were mounted in the Ussing chamber, the preparations spontaneously generated a positive transepithelial voltage (V_{te}) of 4.1±0.5 mV (N=7). After short-circuiting the voltage, a negative short-circuit current (I_{sc}) of –142±21 μA cm^{-2} and a conductance (G_{te}) of 44±5 mS cm^{-2} (N=7) were measured.

To analyse the ionic nature of the short-circuit current, ion substitution experiments were performed. On replacing Cl^- on both sides of the preparation by nitrate, the negative I_{sc} significantly decreased by approximately 75% from –124±16 to –32±5 μA cm^{-2} (N=5). At the same time the conductance of the preparation also decreased from 45±3 to 22±2 mS cm^{-2} (N=5). After readministering Cl^- on both sides of the tissue, I_{sc} was found to be completely reversible (–149±29 μA cm^{-2}; N=5). In most cases the new control current was even more negative than before Cl^- substitution. The conductance of the split gill lamellae increased to 63±3 mS cm^{-2} (N=5) after readministering Cl^-. A decrease in negative I_{sc} and G_{te} similar to that obtained after substitution of Cl^- was also observed when Na^+ was replaced by choline. In this case the negative I_{sc} decreased from –132±34 to –23±5 μA cm^{-2} (N=5) and the conductance of the split gill lamellae decreased from 61±8 to 45±8 mS cm^{-2} (N=5). After returning to NaCl salines, current and conductance returned to the control values seen before substitution of Na^+. A representative example of the above-described ion substitution experiments is shown in Fig. 1.

Thus far, the polarity of V_{te} and I_{sc}, and the current reductions after substitution of Na^+ or Cl^- indicate that the negative I_{sc} reflects Na^+-coupled Cl^- absorption. To analyse the transport mechanism in more detail, several inhibitors of transport proteins were used. Applying 5 mmol l^{-1} ouabain, a specific inhibitor of the Na^+/K^+-ATPase (Skou, 1965), to the internal perfusion medium, resulted in a significant decrease of the negative I_{sc} by approximately 80% from –110±16 to –22±8 μA cm^{-2} (N=6). The effect of ouabain on I_{sc} was slowly reversible. The conductance of split gill lamellae was not affected by internal ouabain. A representative time course of an experiment with ouabain is shown in Fig. 2. Internal addition of 2 mmol l^{-1} diphenylamine-2-carboxylate (DPC), an inhibitor of Cl^- channels (Di Stefano et al., 1985), significantly reduced the negative I_{sc} (from –92±11 to –25±6 μA cm^{-2}; N=5) at constant G_{te}. The effect of DPC on I_{sc} was slowly but completely reversible. The primary solvent for DPC, dimethylsulfoxide, was always applied alone and at the same concentration before DPC. The minor current decreases (see Fig. 3) were taken into consideration when the DPC effects were quantified. Internal $BaCl_2$ (10 mmol l^{-1}), a blocker of K^+ channels (Van Driessche and Zeiske, 1985), decreased the negative I_{sc} from –135±13 to –31±7 μA cm^{-2} (N=4) at unchanged G_{te}. To avoid influences of Cl^- diffusion currents, $BaCl_2$ was first applied to the external bath (where it had no effect on current or conductance), and afterwards added to the internal perfusion medium. A representative experiment showing the influences of $BaCl_2$ and DPC is shown in Fig. 3.

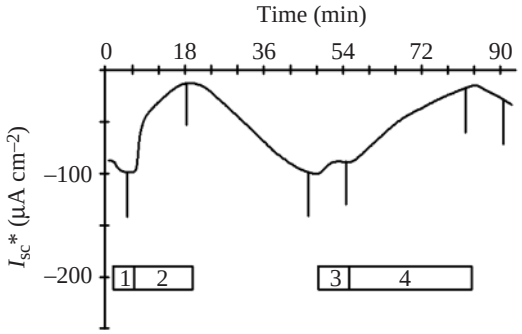

Fig. 3. Representative time course of I_{sc}* across split lamellae of posterior gills of *C. granulatus*, showing the effects of internal addition of 10 mmol l^{-1} $BaCl_2$ (2) and 2 mmol l^{-1} diphenylamine-2-carboxylate (4, DPC as free acid). At (1) $BaCl_2$ was added to the external bath as a control for diffusive movements of Ba^{2+} and Cl^-. At (3) dimethylsulfoxide, the primary solvent for DPC, was added to the internal solution. The vertical current deflections are due to 1 mV voltage pulses and reflect the uncorrected conductance across the preparation.

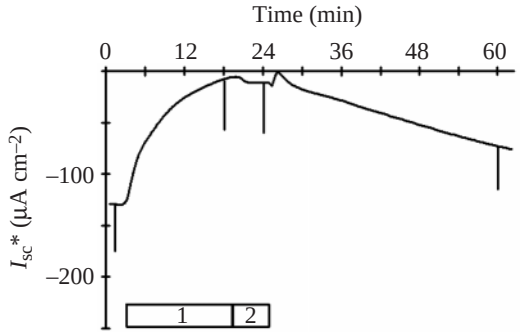

Fig. 4. Representative time course of I_{sc}* across split lamellae of posterior gills of *C. granulatus*, showing the influence of external addition of 50 mmol l^{-1} CsCl (1). At (2) CsCl was also added to the internal bath as a control for diffusive movements of Cs^+ and Cl^-. The vertical current deflections are due to 1 mV voltage pulses and reflect the uncorrected conductance across the preparation.

Internal addition of acetazolamide (0.1 mmol l^{-1}), an inhibitor of carbonic anhydrases (Maren, 1967), had no effect on I_{sc} or G_{te} (N=4; not shown).

Neither $BaCl_2$ (10 mmol l^{-1}; N=4, see above and Fig. 3) nor furosemide (N=4; not shown), a blocker of $Na^+/K^+/2Cl^-$ symporters (Greger and Kunzelmann, 1990), showed any effects on current and conductance across *C. granulatus* split gill lamellae when applied to the external bathing medium. External Cs^+ ions (50 mmol l^{-1} CsCl), another inhibitor of K^+ channels (cf. Van Driessche and Zeiske, 1985), however, reduced the negative I_{sc} from –135±18 to –29±10 μA cm^{-2} (N=3) when applied to the external bath. The conductance of the tissue remained unchanged in the presence of CsCl. To reveal a possible contribution of inward-diffusing Cs^+ ions to the reduction of the negative I_{sc}, the above values were also determined after adding the same amount of CsCl to the internal bath. This, however, resulted only in minimal recoveries of the negative current, showing that Cs^+ diffusion is very low or almost equal to Cl^- diffusion. The effect of external Cs^+ on the current was reversible. An example of the experiments with CsCl is shown in Fig. 4.

Although external furosemide had no effect on I_{sc}, the results so far collected suggest that the negative current reflects electrogenic, Na^+-coupled Cl^- absorption, as in the gills of *Carcinus maenas* (Riestenpatt et al., 1996). To evaluate a possible contribution of an electroneutral uptake *via* apical Na^+/H^+- and Cl^-/HCO_3^--antiports to overall NaCl absorption, we measured the influence of acetazolamide (0.1 mmol l^{-1}) on unidirectional Na^+ influx across isolated and perfused posterior gills of *C. granulatus*. In five experiments the drug significantly reduced the mean Na^+ influx from 1211±179 to 979±188 μmol h^{-1} g^{-1} (N=5; see Fig. 5) without affecting the transbranchial voltage.

Discussion

Methodical aspects

So far, split gill lamellae of three different crab species (*Eriocheir sinensis*, *Carcinus maenas* and *Dilocarcinus pagei*) have been successfully used to determine the basic characteristics of crab gill epithelia and the mechanisms of NaCl absorption (Onken and Riestenpatt, 1998; Onken and McNamara, 2002). However, *Chasmagnathus granulatus* and its gills are significantly smaller and it was necessary to use an Ussing chamber with a smaller epithelial surface (0.002 cm^2 instead of 0.01 or 0.02 cm^2). Obviously this decreases the precision of the determined current and conductance values, and the contribution of edge damage may increase. Moreover, the smaller connection between the internal and external bathing medium may result in a larger unstirred layer in front of each side of the tissue. This may explain the relatively slow responses after solution changes observed in the present study.

Nevertheless, despite the above mentioned disadvantages the results of the present study clearly show that measurements with split gill lamellae of *C. granulatus* can be successfully

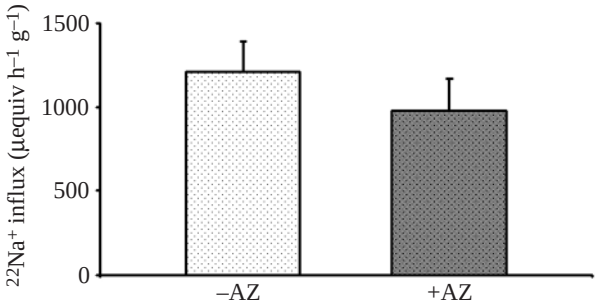

Fig. 5. Diagram showing the unidirectional influxes of Na^+ across isolated posterior gills of *C. granulatus* in the absence (white column) and presence (grey column) of 0.1 mmol l^{-1} acetazolamide (AZ). Analysis of the two groups by paired Student's *t*-test revealed that the difference was significant at P=0.0007. See Materials and methods for details.

performed and result in useful information about the characteristics of the epithelium. The voltage across split gill lamellae was in exactly the same range as that in a recent study of isolated and perfused gills (Luquet et al., 2002), clearly showing that the splitting hardly damages the epithelium, and that edge damage is not a serious problem.

Active NaCl absorption in C. granulatus *posterior gills*

In diluted media, *C. granulatus* maintains an outward-directed osmotic gradient (Mougabure Cueto, 1998; Charmantier et al., 2002). When the animals were adapted from seawater to brackish water, the thickness of the posterior gill epithelium increased (Genovese et al., 2000), indicating its involvement in osmoregulatory active NaCl absorption, as observed in a variety of Crustacea (Péqueux et al., 1988). Recently, a ouabain-sensitive net influx of Na^+ was determined with isolated and perfused posterior gills (Luquet et al., 2002). The magnitudes of the transepithelial voltage (V_{te}), short-circuit current (I_{sc}) and conductance (G_{te}) measured in the present study with NaCl salines on both sides of split lamellae of *C. granulatus* characterise the tissue as a leaky epithelium with a high conductance paracellular pathway. The negative I_{sc} was dependent on the presence of Na^+ and Cl^- (see Fig. 1), indicating that the current reflects active, electrogenic and Na^+-dependent Cl^- absorption.

The cuticle

In Crustacea, the gill epithelium is covered on the apical side by cuticle. On the one hand, the presence of this chitinous layer is of advantage for measurements with split gill lamellae, because it gives the tissue a mechanical stability essential for preparation and mounting. On the other hand, the cuticle acts as serial resistance in front of the epithelium and as a barrier for drugs. In the crab species studied so far, the cuticular resistance was found to be very small when compared to the resistance of the whole preparation. In *C. maenas*, for example, the resistance of split gill lamellae was approximately $25\,\Omega\,cm^2$ and the cuticular resistance was below $2\,\Omega\,cm^2$ (Riestenpatt et al., 1996; Onken and Riestenpatt, 2002). Thus, its influence as serial resistance seems of minor importance. With respect to the cuticular permeability to inhibitors of transport proteins it is of interest that in *C. maenas*, neither furosemide nor amiloride seem to pass the cuticle (Riestenpatt et al., 1996; Onken and Riestenpatt, 2002). With respect to furosemide, the same may be the case for the gill cuticle of *C. granulatus* (see below). In the present investigation, the gill cuticle of *C. granulatus* has not been studied separately, and a detailed investigation of this layer should be performed in the future.

The apical membrane

Ba^{2+} and Cs^+ ions are well-known blockers of K^+ channels (Van Driessche and Zeiske, 1985). With *C. maenas*, split gill lamellae, external Cs^+ inhibited the negative I_{sc} more effectively than Ba^{2+} (Riestenpatt et al., 1996), although Cs^+ is usually a less potent inhibitor than Ba^{2+} (Van Driessche and Zeiske, 1985). This result may reflect a different permeability of the gill cuticle to the two cations, resulting in a better accessibility of the apical membrane by Cs^+. Similarly, with *C. granulatus* split gill lamellae, external Ba^{2+} levels had no effect on the negative I_{sc} (see Fig. 3), whereas high concentrations of external Cs^+ inhibited the current (see Fig. 4). The conductance of the preparation was not affected by external Cs^+. However, a possible effect on the transcellular conductance might have been hidden by a conductance increase of the paracellular pathway due to the augmentation of the solution ionic strength after addition of CsCl. Besides their presence in tight NaCl absorbing epithelia, where they serve for K^+ secretion (Van Driessche and Zeiske, 1985), apical K^+ channels are often present in epithelia with K^+-dependent cotransporters in the apical membrane, as in the vertebrate gastric epthelium (K^+/H^+-ATPase; Wolosin and Forte, 1984) or the thick ascending limb (TAL) of Henle's loop in the mammalian nephron ($Na^+/K^+/2Cl^-$ symport; Greger, 1985). In the TAL, the function of these K^+ channels is not only to supply the K^+-dependent cotransporters with their substrate. They also contribute to the cell's negativity, supporting the movement of Cl^- ions from the cell to the internal medium across the basolateral membrane, and they are the basis for the electrogenicity of the overall absorption process, allowing cation absorption along the paracellular pathway under open-circuit conditions.

As seen with split gill lamellae of the shore crab *C. maenas*, furosemide had no effect on I_{sc} or G_{te} across split gill lamellae of *C. granulatus*. In the shore crab, however, a dependence of Cl^- absorption on potassium was demonstrated, and therefore it was concluded that NaCl absorption proceeds *via* apical $Na^+/K^+/2Cl^-$ symporters (Riestenpatt et al., 1996) in this species. So the presence of apical K^+ channels in an epithelium generating coupled NaCl absorption and a negative I_{sc} suggests the involvement of this symporter (see above, cf. Greger and Kunzelmann, 1990). Thus in *C. granulatus*, as in *C. maenas*, we propose that apical K^+ channels and $Na^+/K^+/2Cl^-$ symporters are the basis of electrogenic, coupled NaCl absorption (see Fig. 6).

As seen in the shore crab *C. maenas* (Onken and Siebers, 1992), acetazolamide, a blocker of the carbonic anhydrase (Maren, 1967), had no effect on I_{sc} or G_{te} across split gill lamellae of *C. granulatus*. However, approximately 20% of the Na^+ influx across isolated and perfused gills was inhibited by this drug (see Fig. 5) without affecting the transbranchial voltage. This finding suggests that at least a part of NaCl absorption depends on the rapid supply of H^+ and HCO_3^-. We therefore propose that electrogenic NaCl absorption as described above is accompanied by electroneutral NaCl absorption *via* apical Na^+/H^+- and Cl^-/HCO_3^--antiports (see Fig. 6), as has been described for the cortical TAL of the mouse and rat nephron (Greger, 1985). However, this proposal requires further investigation.

The basolateral membrane

The inhibition of I_{sc} by internal ouabain (see Fig. 2) is consistent with recent Na^+ flux measurements obtained with

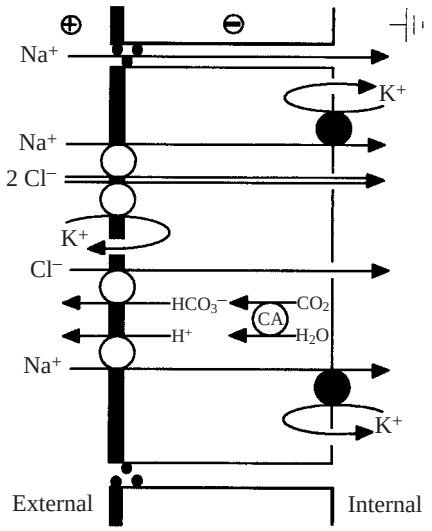

Fig. 6. Proposed functional model for active NaCl absorption across the gill epithelium of hyperosmoregulating *C. granulatus*.

isolated and perfused gills (Luquet et al., 2002) and shows that Na^+-dependent Cl^- absorption is driven by the basolateral Na^+/K^+-ATPase. The reduction in I_{sc} seen with the Cl^- channel blocker DPC (Di Stefano et al., 1985; see Fig. 3) indicates that Cl^- ions, which are most likely to be absorbed by a secondary active mechanism across the apical membrane (see above), exit from the cells *via* basolateral Cl^- channels. The effect of internal $BaCl_2$ (see Fig. 3), similarly already observed with isolated and perfused gills (Luquet et al., 2002), indicates the presence of K^+ channels in the basolateral membrane. Their involvement in NaCl absorption can be considered as the basis for a negative cellular potential, participating in the driving force for the inward movement of Cl^- ions through channels in the basolateral membrane. Na^+/K^+-ATPase, Cl^- channels and K^+ channels (see above) are the usual 'equipment' present in basolateral membranes of many NaCl absorbing epithelia (Greger and Kunzelmann, 1990), and have also been found in the other crab gill epithelia studied so far (Onken et al., 1991; Riestenpatt et al., 1996; Onken and McNamara, 2002).

Comparative aspects

The results obtained in the present study clearly indicate that the basic epithelial characteristics and the mode of active NaCl absorption are very similar in hyperosmoregulating *C. maenas* and *C. granulatus*. Both animals are strong hyperosmoregulators which, however, are not able to survive in freshwater. The epithelium of the posterior gills of both animals displays a high conductance and a directly coupled NaCl absorption, similar to the TAL. It might well be that these characteristics are general features of animals from brackish waters, contrasting with the patterns of the gills of freshwater crabs and the absorptive epithelia of freshwater vertebrates (low conductance and electrical coupling of NaCl absorption *via* different pathways; see Introduction). It is obvious, however, that this hypothesis requires further studies on more animals from brackish waters.

Financial support by CAPES (Brazil), UBACYT (Argentina) and DAAD (Germany) is gratefully acknowledged. We also acknowledge Dr R. Marqués for allowing us to use the Radiochemistry laboratory and equipment.

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