ACTIVE ABSORPTION OF Cl^{-} AND Na^{+} IN POSTERIOR GILLS OF CHINESE CRAB Eriocheir sinensis: MODULATION BY DOPAMINE AND cAMP

Ji Ling Mo, Pierre Devos, and Gérard Trausch

Laboratoire de Biochimie et Physiologie Comparées, Département de Biologie, Facultés Universitaires Notre-Dame de la Paix, Namur-5000, Belgium (JLM, correspondence) present address, Krasnow Institute for Advanced Study, Mail Stop 2A1 George Mason University, Fairfax, Virginia 22030-4444, U.S.A. (jmo@gmu.edu)

ABSTRACT

Isolated posterior gills from the Chinese crab Eriocheir sinensis acclimated to freshwater (FW) were used for perfusion. Dopamine and dibutyryl cAMP (db-cAMP) induced a significant increase of 36Cl^{-} influx. When amiloride, a specific sodium selective channels blocker, was added to the incubation saline, it induced a dramatic decrease of 22Na^{+} influx, and such an effect could be reversed by dopamine or db-cAMP. In the same experimental condition, we observed that ouabain added to the serosal side induced a marked decrease of the stimulating effect of db-cAMP on 22Na^{+} uptake across the epithelium to the hemolymph, suggesting that dopamine stimulates Na^{+} and Cl^{-} influxes via cAMP and Na^{+}/K^{+}-ATPase pathways.

The euryhaline Chinese crab Eriocheir sinensis is a strong hyperregulator in dilute media (Krogh, 1939; Péqueux and Gilles, 1978; Péqueux and Gilles, 1988; Trausch et al., 1989; Onken, 1996; Onken and Riestenpatt, 1998). Hyperregulation is accomplished by activation of salt uptake and restriction of water permeability of the gill tissue (Péqueux, 1995). The mechanisms of Na^{+} and Cl^{-} uptake have been established using isolated gill and split gill lamellae preparations (Gocha et al., 1987; Péqueux and Gilles, 1988; Onken and Putzenlechner, 1995). Active electrogenic Na^{+} absorption proceeds mainly via apical Na^{+} channels and basolateral Na^{+}/K^{+}-pumps, whereas electrogenic Cl^{-} uptake proceeds via apical Cl^{-}/HCO_{3}^{-} antiporters and basolateral Cl^{-} channels (Péqueux and Gilles, 1988; Onken et al., 1991) and is driven by an apical V-type H^{+} pump (Putzenlechner et al., 1992; Riestenpatt et al., 1995).

The gills of crustaceans have been shown to be the most important osmoregulatory site, and they are relatively simple organs compared with the osmoregulatory systems found in mammals. However, the structures and the mediators of ion transport in the gills are similar in some respects to those of mammalian kidneys. Our experimental model, the Chinese crab, Eriocheir sinensis, is known as an euryhaline hyper-osmoregulator organism which spends the greatest proportion of its life in freshwater (FW) and, as an adult, is able to tolerate the entire range of salinity from seawater (SW) to FW (Peters and Panning, 1933). Moreover, it has been well established that the posterior gills of Eriocheir sinensis play a major role in ion transport (Péqueux and Gilles, 1988).

In recent decades, evidence has accumulated that osmoregulation is controlled by neuroendocrine factors, such as peptides and bioamines, that activate ion transport by the crustacean gill (Trausch et al., 1989; Kamemoto, 1991; Onken et al., 2000). Recently, dopamine has been proposed as a major neurohormonal factor involved via cellular surface receptors in gill epithelium where it acts as an important first messenger to control osmoregulation in crustaceans (Trausch et al., 1989; Mo et al., 1998). In early studies, it has been demonstrated that pericardial organ (PO) extracts, dopamine and octopamine, cause dramatic increases in cAMP levels and the uptake of Na^{+} in the isolated gills of Callinectes sapidus (see Kamemoto and Oyama, 1985). Such an effect can also be generated by a membrane-permeant cyclic AMP derivative, dibutyryl cyclic AMP (db-cAMP) (Lohrmann and Kakemoto, 1987). Sommer and Mantel (1988) determined that injection of PO extracts, dopamine, and db-cAMP into animals increases 22Na^{+} influx uptake in Carcinus maenas.
Subsequently, several studies have been published on the regulation effects of neuroendocrine factors via the intracellular second messenger, cAMP of the activation of Na\(^+\) and Cl\(^-\) uptake through the posterior gills of Chinese crab, Eriocheir sinensis, acclimated to freshwater (FW). Bianchini and Gilles (1990) have shown that db-cAMP increased \(^{22}\)Na\(^+\) and \(^{36}\)Cl\(^-\) influx in isolated posterior gills from Eriocheir sinensis. It has been observed that dopamine and db-cAMP increased the potential difference (PD) across isolated gills from Eriocheir sinensis (Detaille et al., 1992). Ristenpatt et al. (1994) reported that db-cAMP stimulated of electrogenic uptake of Cl\(^-\) across posterior gills of Eriocheir sinensis. More recently, Mo et al. (1998) found that dopamine and db-cAMP stimulated \(^{22}\)Na\(^+\) absorption and increased cAMP concentrations in posterior gills of Eriocheir sinensis. These observations indicated that cAMP acts as a second messenger, mediating the effects of neuroendocrine factors, such as dopamine, and is involved in the stimulation of NaCl uptake in posterior gills of Eriocheir sinensis.

In crustaceans, Na\(^+\)/K\(^+\)-ATPase is known to play a main role in hyper-osmoregulation (Towle et al., 1976; Holliday, 1985). Moreover, effects of neuroendocrine factors, such as dopamine, on ion transport seem to be mediated by modulation of the activity of the Na\(^+\)/K\(^+\)-ATPase (Trausch et al., 1989; Sommer and Mantel, 1991). The effect of neurohormones on the activity of Na\(^+\)/K\(^+\)-ATPase and ion transport is believed to be mediated by a second messenger, cAMP (Trausch et al., 1989; Morris and Edwards, 1995). On the other hand, several studies have implicated other intracellular cascade systems, and protein kinase C (Asselbourg et al., 1991) as well as calmodulin (Péqueux and Gilles, 1992) have also been shown to be involved in NaCl uptake in the gill of Eriocheir sinensis. However, the detailed mechanism of bioamine stimulation of NaCl uptake via activation of intracellular signal pathways has still to be elucidated.

Amiloride is a diuretic that blocks Na\(^+\) reabsorption through apical membrane channels in tight epithelia (Benos, 1982). When amiloride is added to the medium containing gills in which the Cl\(^-\) transport has been blocked by substitution of Cl\(^-\) with gluconate, the PD falls rapidly to very low values (Gilles and Péqueux, 1985). This indicates that the major part of the inward movement of Na\(^+\) across the apical membrane of the gill epithelium occurs via an amiloride sensitive Na\(^+\)/H\(^+\) antiporters. In addition, Zeiske et al. (1992) reported that a positive, Na\(^+\)-dependent short-circuit current was measured in external Cl\(^-\)-free saline, which seems to reflect an electrogenic Na\(^+\) uptake via apical Na\(^+\) channels and basolateral Na\(^+\)/K\(^+\)-ATPase.

The objective of the present investigation was to determine the effect of dopamine on Na\(^+\) and Cl\(^-\) absorption across the gills of Eriocheir sinensis acclimated to FW using a perfused isolated preparation of posterior gills. The primary aim of this investigation was to prove and explain some of the regulatory mechanisms modulated by dopamine and intracellular cascade on ion transport in the gills from Eriocheir sinensis. Our results suggest that dopamine stimulates active NaCl absorption in the gill of Eriocheir sinensis via the second messenger, cAMP.

**Materials and Methods**

**Animals.—**Experiments were performed using gills isolated from Eriocheir sinensis acclimated to FW. These crabs were captured in FW lakes near Emden (North Germany) and kept in tanks filled with circulating, aerated tap water (FW) at \(\pm 15^\circ\)C for at least two weeks before used in experiments. Measurements were conducted between December 1996 and June 1998.

**Methods—**Isolated gills were bathed on both sides with “FW saline” to measure potential difference (PD). The FW saline contains (in mmol L\(^{-1}\)): NaCl, 240; KCl, 5; MgCl\(_2\), 5; CaCl\(_2\), 12.5; and H\(_2\)BO\(_3\), 8.8. The pH was adjusted to 7.6 with Tris-Base. In substitution experiments, gluconate was used to replace Cl\(^-\) of the perfusion (inside) and incubation (outside) saline. The transepithelial PD was measured by means of calomel electrodes dipped into the incubation medium and the collecting beakers connected to the perfusion system. In this case, both of the epithelia were bathed with the same Ringer saline (Péqueux and Gilles, 1978). Preliminary experiments have shown that after a 30 min period of stabilization, the transepithelial PD remains stable for at least 6 h.

The \(^{22}\)Na\(^+\) influx was measured with the “FW saline” or 240 mmol L\(^{-1}\) Na-gluconate inside saline and 10 mmol L\(^{-1}\) Na-gluconate in the incubation medium. According to the experimental scheme, 10 mmol L\(^{-1}\) NaCl or 10 mmol L\(^{-1}\) Na-gluconate were added to the medium bathing the apical face of the gill (out), and undiluted “FW saline” or Na-gluconate saline was used for the perfusion medium (in). The \(^{36}\)Cl\(^-\) influx was measured with the NaCl inside 280 mmol L\(^{-1}\) and outside 25 mmol L\(^{-1}\).

Dopamine and db-cAMP were dissolved with perfusion saline. Amiloride was dissolved in the incubation medium at a concentration of 0.1 mmol L\(^{-1}\) or 1 mmol L\(^{-1}\).

**Chemicals.—**Dopamine hydrochloride (kept in the dark), amiloride hydrochloride, dibutyryl cyclic AMP, and Na-gluconate were obtained from Sigma Chemical Company (St. Louis, Missouri, U.S.A.). The CaCl\(_2\), H\(_2\)BO\(_3\), Tris aminomethane, K-gluconate, Mg-gluconate, and...
Ca-glucuronate were obtained from Fluka Chemical Company (Buchs, Switzerland). All other chemical compounds were obtained from Janssen Chemical Company (Beerse, Belgium). The $^{22}\text{Na}^+$ (sodium chloride in water) and $^{36}\text{Cl}^-$ (hydrochloric acid) were purchased from Amersham Company (Slough, England).

Perfusion.—The crabs were killed by removal of the dorsal part of their cephalothorax after destruction of the ventral ganglia. The posterior gills were cut off at their bases, rinsed immediately, using an internal flush of cold “perfusion saline” in order to avoid hemolymph coagulation. Polyethylene catheters were introduced into the afferent and efferent blood vessels of the gill and gently fastened by means of a neoprene-plexiglass clamp. The afferent catheters were then connected to the perfusion reservoir and the efferent catheters to the collecting beaker. The gill was placed in 20 mL of the incubation medium. The perfusion saline ran from the afferent vessel across the lamellae to the efferent vessel, mimicking normal hemolymph circulation, and was collected for analysis. The difference height between the perfusion reservoir and the collecting beaker was kept around 25 cm and adjusted to maintain a constant flow of the saline. This gives a pressure inside the gill quite similar to the pressure that drives blood in the open circulatory system of crustaceans, in agreement with data given by Belman (1976). The incubation medium was continuously aerated. This procedure is similar to the one described by Péqueux and Gilles (1978).

The inward movements of Na$^+$ or Cl$^-$ were estimated using radioactive tracers $^{22}\text{Na}^+$ or $^{36}\text{Cl}^-$. In each experimental condition, 5 Ci of $^{22}\text{Na}^+$ or $^{36}\text{Cl}^-$ (19375 MBq/mg Na or 604 MBq/g Cl) were added into the incubation medium (20 mL), and their appearance was measured on the other side. Samples were collected at 10 min intervals for 120–140 min. Dophone 0.2 mmol L$^{-1}$ (a nonphysiological concentration) was used; it is indeed known to become rapidly autooxidized at physiological pH. As it is also photosensitive, the perfusion bottle was covered with aluminium foil. The db-cAMP, 0.15 mmol L$^{-1}$, was added into perfusion saline to measure $^{22}\text{Na}^+$ or $^{36}\text{Cl}^-$ influxes. Amiloride 0.1 mmol L$^{-1}$ and amiloride 0.1 mmol L$^{-1}$ were added to the perfusion saline for 60–80 min. Finally, the inside medium was replaced with perfusion FW saline for 60 min in order to determine the reversibility of the effect (Fig. 1).

Addition of 0.2 mmol L$^{-1}$ dopamine to the perfusion saline dramatically increased $^{36}\text{Cl}^-$ influx, from 278.27 ± 18.89 to 473.08 ± 70.28 pmol g$^{-1}$ tissue h$^{-1}$ ($P < 0.05$) (Fig. 2). When 0.15 mmol L$^{-1}$ db-cAMP was added to the perfusion saline, it produced an increase in $^{36}\text{Cl}^-$ inward movement of similar magnitude from 248.33 ± 12.03 to 468.00 ± 123.49 pmol g$^{-1}$ tissue h$^{-1}$ ($P < 0.05$) (Fig. 2).

Amiloride Modifies $^{22}\text{Na}^+$ Influx

Figure 3 shows the effect of 0.1 mmol L$^{-1}$ amiloride in the incubation medium on $^{22}\text{Na}^+$ influx across the epithelium of posterior gills from Eriocheir sinensis acclimated to FW and perfused without Cl$^-$, Amiloride induces a rapid 80% decrease of $^{22}\text{Na}^+$ influx. Replacement of the incubation medium with Na-glucuronate saline free of amiloride resulted in a slow partial restoration of the $^{22}\text{Na}^+$ influx.

This preliminary experiment indicates that amiloride is an effective inhibitor of inward Na$^+$ movement across the epithelium in posterior gills of the Chinese crab. These results and conclusions are confirmatory of experiments conducted with normal Cl$^-$ containing saline.

Effect of Dopamine on $^{22}\text{Na}^+$ Influx in the Presence of Amiloride

In a further experiment, the effect of dopamine, added to the perfusion saline, on $^{22}\text{Na}^+$ influx was investigated in posterior gills in which the sodium inward movement was inhibited by amiloride. The addition of 0.1 mmol L$^{-1}$ amiloride outside induced a rapid and enormous decrease of $^{22}\text{Na}^+$ influx from 96.28 ± 47.69 to 6.67 ± 1.41 pmol g$^{-1}$ tissue h$^{-1}$ ($P < 0.001$) which remained stable for a 70 min period. When 0.2 mmol L$^{-1}$ dopamine was then added to the perfusion saline, a small but significant increase in $^{22}\text{Na}^+$ influx from 6.67 ± 1.41 up to 10.18 ± 1.25 pmol g$^{-1}$ tissue h$^{-1}$ ($P < 0.05$), was observed as shown in Table 1.

Effect of db-cAMP on $^{22}\text{Na}^+$ Influx in the Presence of Amiloride and/or Ouabain

Isolated posterior gills of Eriocheir sinensis acclimated to FW were perfused with 240 mmol L$^{-1}$ Na-glucuronate in perfusion saline and 10 mmol L$^{-1}$ Na-glucuronate in incubation medium to measure $^{22}\text{Na}^+$ influx. As shown in Fig. 4, the
addition of 1 mmol L\(^{-1}\) amiloride to the incubation saline induced a substantial decrease of influx and the subsequent addition of 0.15 mmol L\(^{-1}\) db-cAMP to the perfusion saline caused a significant rise in the Na\(^{+}\) influx which could be abolished with 1 mmol L\(^{-1}\) ouabain.

**DISCUSSION**

It is currently believed that neuroendocrine factors such as dopamine control ion transport in the epithelia of crustacean gills, especially in the posterior gills of Chinese crab, *Eriocheir sinensis*, which are important in osmoregulatory processes. Dopamine is a major catecholamine controlling the active branchial ion uptake. Such studies have raised the interest as to how cell surface receptors, such as dopamine receptors, are implicated in the regulation of Na\(^{+}\) and Cl\(^{-}\) movements via this pathway in crustacean gills (Kamemoto and Oyama, 1985; Trausch et al., 1989; Sommer and Mantel, 1991; Morris and Edwards, 1995; Mo et al., 1998). Transduction of the effect of dopamine on intracellular signal is widely accepted to be mediated through G-protein-coupled receptors (Dohlman et al., 1987). G-protein itself may interact with transport proteins such as ion pumps, channels and antiporters, or may modify the intracellular cAMP concentrations to increase phosphorylation of protein kinase A (Satoh et al., 1993).

Our results provide evidence that dopamine dramatically increases 36Cl\(^{-}\) influx in posterior gills of *Eriocheir sinensis* acclimated to FW (Fig. 2). Our findings support the idea that dopamine induces increased intracellular cAMP concentrations, which in turn stimulates Cl\(^{-}\) uptake by the epithelium of posterior gills of *Eriocheir sinensis* acclimated to FW. Our results are consistent with the data from Bianchini and Gilles (1990) and Riestenpatt et al. (1994), who showed db-cAMP activation of Cl\(^{-}\) uptake in posterior gills of *Eriocheir sinensis*. The most likely mechanism is as follows. Dopamine stimulates an increase in intracellular cAMP concentrations, which modulates the apical V-type H\(^{+}\)-ATPase that provides the driving force for active Cl\(^{-}\) uptake via
Table 1. Effect of 0.2 mmol L\(^{-1}\) dopamine inside and 0.1 mmol L\(^{-1}\) amiloride outside on \(^{22}\)Na\(^+\) influx with Cl\(^{-}\)-free saline in isolated posterior gills from *Eriocheir sinensis* acclimated to fresh water.

<table>
<thead>
<tr>
<th></th>
<th>(^{22})Na(^+) influx µmol g(^{-1}) tissue h(^{-1}) (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>96.28 ± 47.69</td>
</tr>
<tr>
<td>0.1 mmol L(^{-1}) amiloride (out)</td>
<td>6.67 ± 1.41</td>
</tr>
<tr>
<td>0.2 mmol L(^{-1}) dopamine (in)</td>
<td>10.18 ± 1.25</td>
</tr>
<tr>
<td>0.1 mmol L(^{-1}) amiloride (out)</td>
<td></td>
</tr>
</tbody>
</table>

apical Cl\(^{-}/\)HCO\(_3^-\) antiporters and basolateral Cl\(^{-}\) channels (Riestenpatt et al., 1994) (Fig. 5). Moreover, evidence from studies with a mammalian tissue has established that Cl\(^{-}\) channels were activated by the adenylyl cyclase-cAMP-protein kinase A (PKA) pathway that causes Cl\(^{-}\) channels phosphorylation (Hume et al., 2000).

That dopamine is involved in ion transport via intracellular second messengers like cAMP has been previously demonstrated in the gills of several crustaceans (Sommer and Mantel, 1988; Kame moto, 1991; Detai lle et al., 1992). From our current observations and those of previous investigators (Mo et al., 1998), it is clear that dopamine induces an increase in cAMP concentrations, which in turn stimulates both Na\(^+\) and Cl\(^{-}\) uptake by the epithelium of posterior gills of *Eriocheir sinensis* acclimated to FW. However, the question is still open as to whether the Na\(^+\)/K\(^+\) pump is a direct target of dopamine. In other words, is the dopamine-stimulated increase of Na\(^+\)/K\(^+\)-ATPase activity dependent on the density of Na\(^+\) channels or Na\(^+\)/H\(^+\) antiporters? To investigate this possibility, the effect of dopamine on the Na\(^+\) transport in the presence of amiloride was examined.

It is known that amiloride blocks the apical Na\(^+\) channels in epithelial cells and the Na\(^+\)/H\(^+\) antiporters in crustacean gills (Siebers et al., 1987; Péqueux and Gilles, 1988). Amiloride at low concentrations (0.1 mmol L\(^{-1}\)) blocks sodium channels and at a higher concentration (1 mmol L\(^{-1}\)), it blocks both Na\(^+\)/H\(^+\)-antiporters and sodium channels (Péqueux, 1995). Amiloride blocks Na\(^+\) reabsorption through apical channels in tight epithelia (Benos, 1982). Regarding the mechanism of inhibition of Na\(^+\) influx, amiloride is believed to prevent Na\(^+\) entry across the apical membrane rather than to inhibit directly energy requiring active transport step. Recently, cuticular amiloride-sensitive Na\(^+\) conductance has been reported in *Carcinus maenas* gills (Riestenpatt et al., 1994), suggesting that amiloride not only interacts with the gill epithelium but with the cuticular epithelium as well.

In the present study, we demonstrated (Fig. 3) that 0.1 mmol L\(^{-1}\) amiloride (a concentration known to block only the apical Na\(^+\) channels) induced an 80% decrease of \(^{22}\)Na\(^+\) inward movement in isolated posterior gills of FW *Eriocheir sinensis*. Under our experimental conditions, apical Na\(^+\)/H\(^+\) antiporters appeared to be less important than Na\(^+\) channels for the uptake of ions from the external to the intracellular medium.

When isolated posterior gills were perfused with Na-gluconate salines on both sides (perfusion saline 240 mmol L\(^{-1}\) and incubation medium 10 mmol L\(^{-1}\)), the activity of both Na\(^+\) antiporters and channels were blocked. We assume that when amiloride was used to block Na\(^+\) entrance into the cell, dopamine was still able to produce a significant increase in \(^{22}\)Na\(^+\) influx (6.67 ± 1.41 up to 10.18 ± 1.25 µmol g\(^{-1}\) tissue h\(^{-1}\)) (\(P < 0.05\)) (see Table 1). Similarly, Na\(^+\) influx was reduced from 41.7 to 12.5 µmol g\(^{-1}\) tissue h\(^{-1}\) by using amiloride 1 mmol L\(^{-1}\). Moreover, if db-cAMP 0.15 mmol L\(^{-1}\) was added in the perfusion saline, there was a small rise in Na\(^+\) influx (12.5 to 16.7 µmol g\(^{-1}\) tissue h\(^{-1}\)), which was reduced (16.7 to 6.6 µmol g\(^{-1}\) tissue h\(^{-1}\)) following the addition of ouabain 1 mmol L\(^{-1}\) to the perfusion saline (Fig. 4). Because the paracellular flux characteristics of the gills seem not to be modified by db-cAMP (Riestenpatt et al., 1994), this study
further supports the idea that cAMP stimulates Na$^+$ uptake via effects serosal Na$^+$/K$^+$-pump even when apical channels and antiporters are blocked with amiloride.

It is therefore reasonable to assume that, in our system, dopamine stimulates Na$^+$ uptake via an increased intracellular cAMP concentrations which activate the Na$^+$/K$^+$-pump and that this effect appears even without Cl$^-$ in both sides (Fig. 5). It is interesting to point out the activity of cAMP-PKA–Na$^+$/K$^+$-pump pathway could result from change in the substrate concentration, exocytotic insertion of new pumps, activation of a latent pool, or modulation of the kinetics of the pumps already present in the membrane. Hildebrandt (1997) demonstrated that the activation of Na$^+$/K$^+$-ATPase by cAMP may occur by two mechanisms: the stabilization of existing and newly synthetic protein and, subsequently, the induction of additional protein synthesis.

In summary, our present results provide new evidence on the mechanism of inward movement of $^{22}$Na$^+$ and $^{36}$Cl$^-$ across isolated, perfused posterior gills of the euryhaline Chinese crab, *Eriocheir sinensis*. Data are consistent with the idea that dopamine is implicated in the control of ion transport: (1) dopamine elevates $^{36}$Cl$^-$ influx, and its effect appears to be mediated by an increase of intracellular cAMP concentrations (Bianchini and Gilles, 1990) which stimulate both apical V-ATPase and basolateral membrane Cl$^-$ channels (Riestenpatt et al., 1994); (2) dopamine stimulates Na$^+$ transport across the epithelium to the hemolymph in the absence of chloride ions present and when apical membrane Na$^+$ channels or Na$^+$/H$^+$-antiporters are blocked by amiloride.

Our db-cAMP studies suggest that dopamine stimulates Na$^+$ influx via cAMP and Na$^+$/K$^+$-pump pathways because in situations where amiloride is used to block apical Na$^+$ channels or Na$^+$/H$^+$-antiporters, the stimulating effect of db-cAMP can be eliminated by a Na$^+$/K$^+$-pump blocker, ouabain. We have yet to demonstrate that the stimulating effect of dopamine on the inward movement of Na$^+$ and Cl$^-$ are linked, in
the gill of *Eriocheir sinensis*, to the epithelial Dopamine D<sub>1</sub> receptor (Mo, 1999).

**ACKNOWLEDGEMENTS**

This work was partially supported by a research grant to Mo Ji Ling from the Facultés Universitaires Notre-Dame de la Paix (FUNDP), Namur 5000, Belgium. We would like to thank Professor P. Greenaway for comments and advice on this manuscript.

**LITERATURE CITED**


———, K. Graszyński, and W. Zeiske. 1991. Na<sup>+</sup>- and Cl<sup>−</sup>-independent electrogenic Cl<sup>−</sup> uptake across the posterior gills of the Chinese crab *Eriocheir sinensis*: voltage-clamp and microelectrode studies.—Journal of Comparative Physiology 161B: 293–301.


Ristenpatt, S., G. Petrausch, and D. Siebers. 1995. Cl<sup>−</sup> influx across posterior gills of the Chinese crab (*Eriocheir sinensis*): potential energization by a V-type


Received: 7 January 2002.

Accepted: 19 November 2002.