Na^+/NH_4^+ co-transport in isolated perfused gills of the Chinese crab *Eriocheir sinensis* acclimated to fresh water

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Summary. In isolated perfused posterior gills of *E. sinensis* acclimated to fresh water, NH_4^+ may be used as a counter-ion for Na^+ active transport. This Na^+/NH_4^+ coupled transport can, however, only account for a small part of the Na^+ total active influx.

The main features of the blood sodium balance achieved by euryhaline crustaceans when in fresh water have been known for a long time and repeatedly reviewed[2-5]. Essentially, the salt lost in diluted media is counter-balanced by an active inward movement of Na^+, a major part of which occurs at the level of the branchial epithelium. Although the gills play a prominent part in this absorption process, information about the Na^+ transport phenomena occurring in this tissue has up to now remained scanty.

This report deals with the possible existence in isolated perfused gills of *E. sinensis* of the Na^+/NH_4^+ coupling postulated to occur in this tissue by several authors when working on whole crustaceans[4,6,7]. This study has been restricted to the 3 posterior pairs of gills of the chinese crab, since they have been reported to be the only ones involved in Na^+ active transport[4,8-12].

Material and methods. Experiments have been performed on isolated perfused 'posterior' gills from chinese crabs *Eriocheir sinensis* adapted to FW. Technical details concerning the dissection and the perfusion of the gills have already been given in another paper[10]. The perfusion medium contains 240 mM NaCl, 5 mM KCl, 5 mM $MgCl_2$, 12.5 mM $CaCl_2$, and is adjusted at pH 7.6 with a borate buffer (9 mM).

A so-called bathing medium is achieved by diluting the perfusion saline 250 times, while keeping the same pH value and buffer concentration. The Na^+ content of this medium is adjusted at the desired concentration by adding NaCl. Na^+ fluxes measurements are performed by means of radioactive $^{22}Na^+$ (0.25 μCi/ml) added either to the bathing medium or to the perfusion saline. Its appearance on the other side is measured as a function of the incuba-

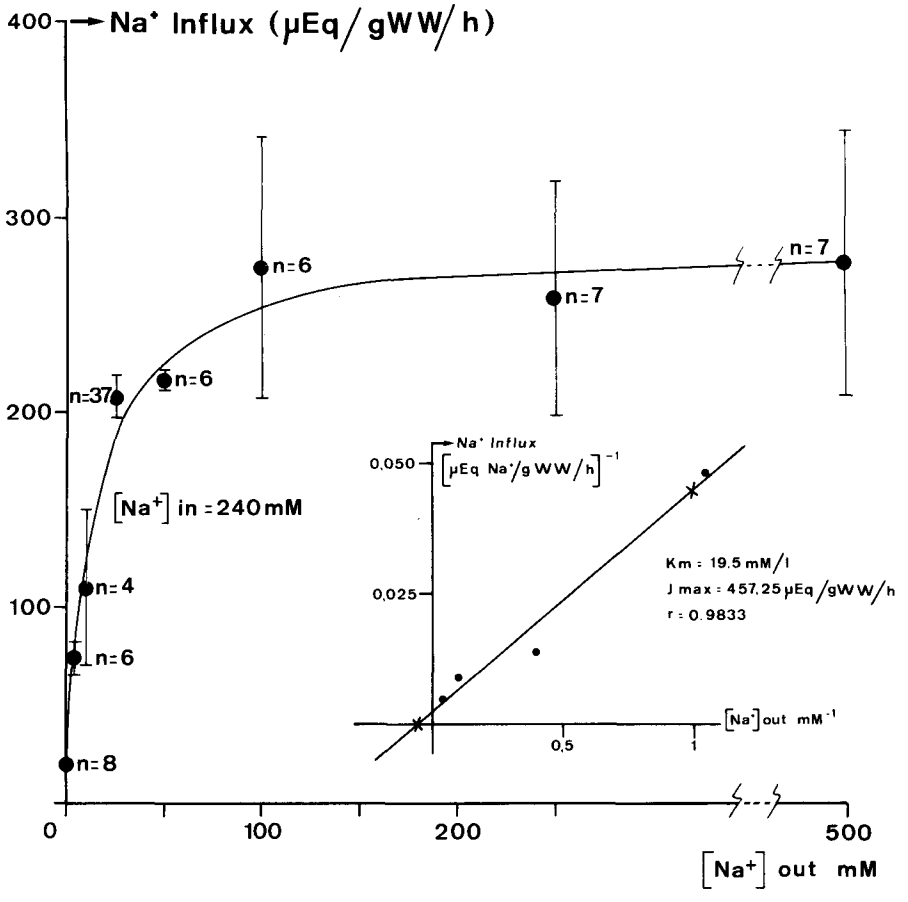

Fig. 1. Relation between external Na^+ concentration (mM, abscissa) and Na^+ influx (μEq/g WW/h, ordinate) in posterior perfused gills isolated from freshwater acclimated *E. sinensis*. Mean ± SEM. The small figure shows the calculation of the k_m value of the carrier for the external Na^+ (abscissa: reverse of external Na^+ concentration; ordinate: reverse of Na^+ influx measurements).

tion time with a γ scintillator in 0.25-ml samples. NH_4^+ outfluxes are measured by following the appearance of NH_4^+ in the outside bathing medium. NH_4^+ concentrations are determined by means of the sigma ammonia kit (sigma ammonia color reagent No. 14-2) and results are expressed as µEq NH_4^+/g WW/h.

Results and discussion. When posterior perfused gills are incubated in artificial FW containing 0.96 mEq/l Na^+, the Na^+ influx across the epithelium is 20.7±2.4 (n=8) µEqNa^+/g WW/h while there is concomitantly no significant Na^+ outflux in spite of the important concentration gradient. This results in a net entry of Na^+ from the diluted incubation medium toward the perfusate, the influx values being the exact reflection of the Na^+ pump activity.

The results quoted in figure 1 show that the magnitude of this net Na^+ influx increases with the Na^+ concentration of the outside medium, a maximum flux value of 250-300 µEqNa^+/g WW/h being already reached when the outside medium contents 100 mM NaCl. This leads to the calculation of a k_m value of the carrier for the external Na^+ of about 19.5 mM/l. Besides this dependency on the external Na^+, the active Na^+ influx can also be related to the Na^+ concentration in the perfusion saline. The fluxes data indeed fall to very low values whatever the Na^+ amount in the bathing medium, when the gills are perfused by a seawater saline containing 480 mM NaCl.

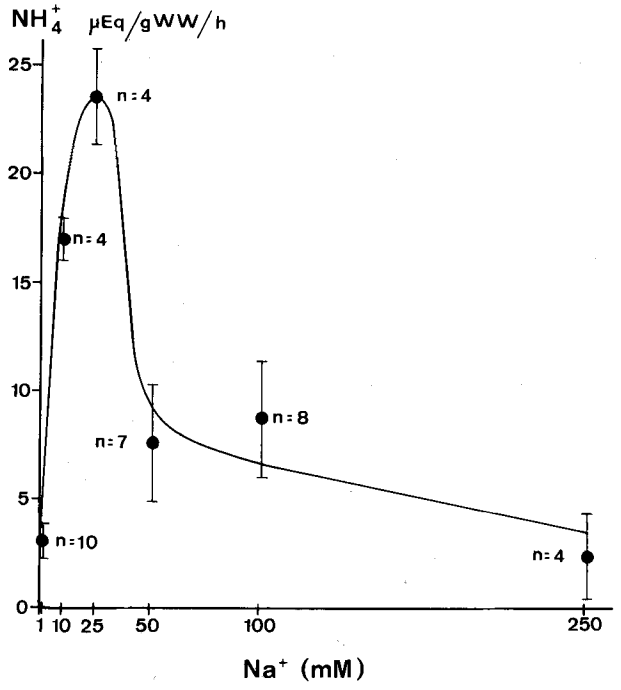

Fig. 2. Relation between external Na^+ concentration (mM, abscissa) and NH_4^+ outflux (µEq/g WW/h, ordinate) in posterior perfused gills isolated from freshwater acclimated *E. sinensis*. Mean±SEM.

The relation between the NH_4^+ outflux and the external Na^+ concentration is shown in figure 2. If the NH_4^+ outflux is particularly low in FW (it has indeed been observed to be lower in posterior than in anterior gills), it reaches a maximum value when the outside Na^+ content is 25 mM NaCl but rapidly falls down at higher concentrations. This dependency of the NH_4^+ efflux on the external Na^+ content is in agreement with the idea that NH_4^+ could be used as a counter-ion in driving Na^+ in. This conclusion further fits the facts that ouabaïn 10^{-3} M when added to the outside medium inhibits both the NH_4^+ outflux and the Na^+ influx, and that the membrane $(Na^+ + K^+)$ATPase supposedly bound to the Na^+ pump has been observed to utilize NH_4^+ as effectively as K^+[5,13]. Comparison of the data reported in figures 1 and 2, however, clearly show that an eventual coupling Na^+/NH_4^+ can only account for a small part of the total Na^+ active influx.

Furthermore, this relationship Na^+/NH_4^+ only appears to hold in the lower range of outside Na^+ concentrations used (maximum 25-50 mEq/l). At higher external Na^+ concentrations, there seems to be no relation whatsoever between the Na^+ influx and the NH_4^+ outflux. The same picture holds even when NH_4^+ is added to the perfusion FW saline at concentrations similar to or higher than those normally found in the blood (from $5 \cdot 10^{-4}$ to 10^{-3} M).

In the same way, addition of amino acids such as glutamate or proline (10^{-2} M) to the perfusion FW saline remains without significant effect on the Na^+ influx or on the NH_4^+ efflux, at least for Na^+ concentrations in the external bathing medium of 0.96 and 25 mM.

This evidence prompts to minimize the role of an eventual Na^+/NH_4^+ coupling in the regulation of the blood Na^+ balance of crustaceans. It thus seems that the increase in NH_4^+ output reported to occur in intact animals upon acclimation to diluted media, is effected through leakages (excretory system, possibly also anterior gills) and is better related to the increased amino-acid catabolism occurring during the isosmotic intracellular regulation process (for review[5]) than to the Na^+ active pumping at the gills level.

Unless the Na^+ pump works according to an electrogenic scheme, the existence of a counter-ion other than NH_4^+, possibly H^+, must be considered. Experiments are in progress in this laboratory in order to assess this hypothesis.

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