Effects of diphenylamine-2-carboxylate on the transepithelial potential difference and Cl^- fluxes in gills of the Chinese crab *Eriocheir sinensis*

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SUMMARY: The effects of DPC (diphenylamine-2-carboxylate-10^{-4} M) have been studied on the transepithelial potential difference of anterior and posterior gills and on the Cl^- fluxes in posterior gills of the Chinese crab *Eriocheir sinensis* acclimated to fresh water. DPC is effective only on the serosal side of the posterior gills. It induces a marked depolarization of the epithelium and largely reduces the Cl^- uptake which takes place in these gills. The observed effects of DPC are consistent with a role of serosal Cl^- channels in the transepithelial transport of Cl^- taking place in the posterior gills of *Eriocheir sinensis*.

Key words: DPC, potential difference, Cl^- transport, crab, *Eriocheir sinensis*.

INTRODUCTION

Recently, models have been proposed accounting for Na^+ and Cl^- movements in isolated, perfused anterior and posterior gills of the euryhaline Chinese crab *Eriocheir sinensis* (GILLES & PÉQUEUX, 1986). These models derive from results of studies on ultrastructural, biochemical and physiological aspects of NaCl movements across the gills (GILLES & PÉQUEUX, 1981, 1983, 1985; PÉQUEUX & GILLES, 1981; BARRA *et al.*, 1983; PÉQUEUX *et al.*, 1984; GOCHA *et al.*, 1987). Accordingly, there is no transepithelial active transport of Na^+ or Cl^- in the anterior gills. These gills are largely permeable to Na^+ while showing only extremely low permeability to Cl^-. They further show a classical Na^+/K^+ pump and leak system at the serosal side. On the contrary, the posterior gills transport actively both the Na^+ and Cl^-. A Na^+/H^+ antiporter is implicated in the inward active Na^+ movement at the apical side of the epithelium. At the serosal side, Na^+ is moved out by an Na^+/K^+ exchanger. Cl^- is moved in by an apical Cl^-/HCO_3^- antiporter. It could be moved out by a SITS insensitive active transport process and/or through Cl^- channels located at the serosal side. In this case, Cl^- would follow the K^+ diffusion potential generated by the activity of the serosal Na^+/K^+ pump and leak system.

Diphenylamine-2-carboxylate (DPC) has been reported as a potent Cl^- channel blocker (DI STEFANO *et al.*, 1985; WANGEMANN *et al.*, 1986). To test the possible role of Cl^- channels in the Cl^- transepithelial movements, we have studied the effect of that compound on both the transepithelial potential difference (PD) and the Cl^- fluxes in the posterior gills which are know to transport actively Na^+ and Cl^-. DPC has also been tested on the PD of the anterior gills which do not show transepithelial uphill transport of NaCl.

MATERIAL AND METHODS

The experiments were performed on perfused anterior and posterior gills isolated from the euryhaline Chinese crab *Eriocheir sinensis* (Milne-Edwards) (Crustacea, Decapoda, Grapsidae). The crabs were captured in freshwater lakes near Emden (West Germany), transferred to the laboratory and kept in tanks of circulating tap water.

Gills were perfused and the transepithelial po-

tential difference (PD) was measured following methods described by PÉQUEUX & GILLES (1978). The so-called "anterior" and "posterior" gills correspond respectively to the arthrobranchiae III, IV and V and to the three most posteriorly located pairs of gills, i.e. the arthrobranchia VI and the pleurobranchiae I and II.

Gills were perfused with and incubated in a "freshwater saline" containing, in mM: NaCl, 240; KCl, 5; $MgCl_2$, 5; $CaCl_2$, 12.5 and H_3BO_3, 8.8. The pH was adjusted to 7.6 with Tris-base. The ion composition of this solution corresponded to the blood ion composition of crabs acclimated to freshwater. Diphenylamine-2-carboxylate (DPC) was used at a concentration of 10^{-4} M. It was previously dissolved in dimethylsulfoxide (DMSO) 0.1 %. Preliminary experiments showed that DMSO at that concentration had no significant effect on the transepithelial potential difference of the gills.

In some experiments, large Cl^- gradients were applied to the posterior gills, substituting that ion by gluconate in either the incubation or perfusion saline.

Cl^- fluxes were estimated using the radioactive tracer $^{36}Cl^-$ (0.25 μCi ml^{-1}). It was added either to the incubation medium or to the perfusion saline and its appearance on the opposite side measured in 1 ml aliquots of samples collected every 10 min. Sample radioactivity was measured with a liquid scintillator Tricarb Packard 3255, using Pico Fluor TM30 (Packard) as scintillation cocktail.

RESULTS

Diphenylamine-2-carboxylate (DPC 10^{-4} M) has no significant effect on the transepithelial potential difference (PD) of the anterior gills (Fig. 1). Similarly, it remains without any significant effect on the

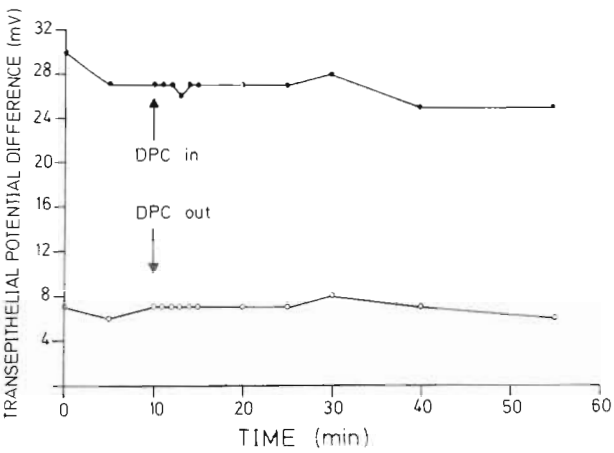

FIG. 1. — Effect of addition of DPC either to the incubation medium (out) or to the perfusion saline (in) on the transepithelial potential difference of isolated, perfused anterior gills of the Chinese crab *Eriocheir sinensis*.

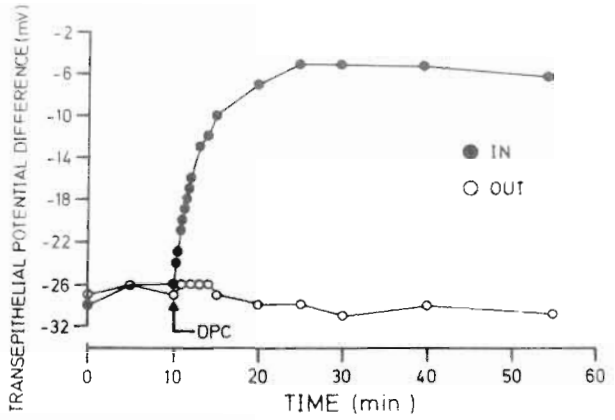

FIG. 2. — Effect of addition of DPC either to the incubation medium (out) or to the perfusion saline (in) on the transepithelial potential difference of isolated, perfused posterior gills of the Chinese crab *E. sinensis*.

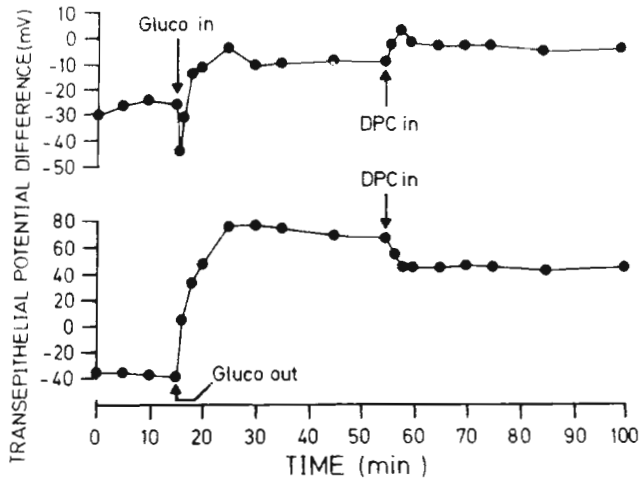

FIG. 3. — Effect of addition of DPC to the perfusion saline (in) on the transepithelial potential difference of isolated, perfused posterior gills of the Chinese crab *E. sinensis* when Cl^- is replaced by gluconate either in incubation medium (out) or in perfusion saline (in).

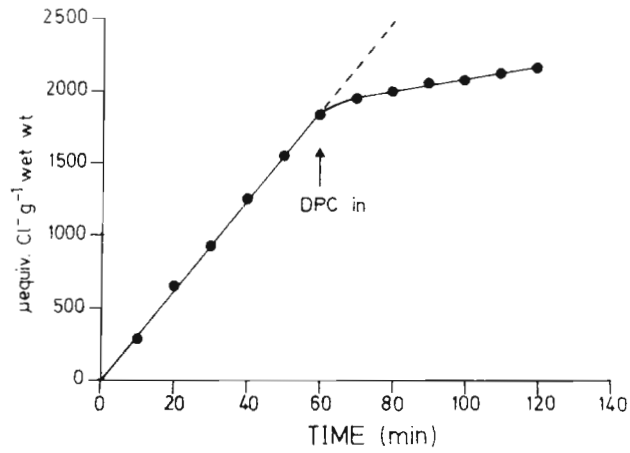

FIG. 4. — Effect of addition of DPC to the perfusion saline (in) on the Cl^- influx in isolated, perfused posterior gills of the Chinese crab *E. sinensis*.

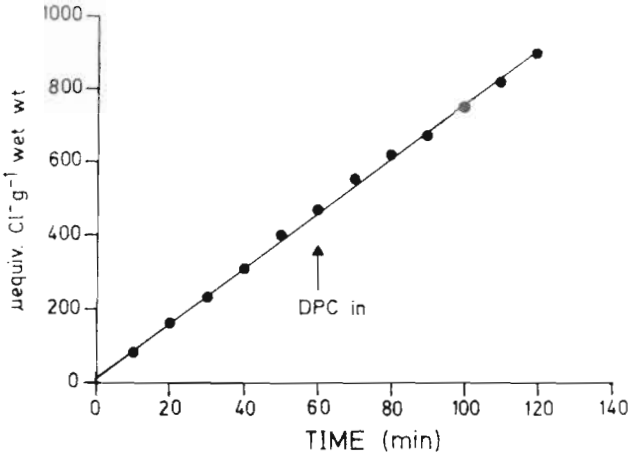

FIG. 5. — Effect of addition of DPC to the perfusion saline (in) on the Cl^- efflux in isolated, perfused posterior gills of the Chinese crab *E. sinensis*.

PD of the posterior gills when added to the incubation medium (Fig. 2). However, this compound induced a marked depolarization of these gills when added to the perfusion saline (Fig. 2).

Cl^- replacement by gluconate in the perfusion medium depolarizes the gill epithelium. A further depolarization is induced in this condition by DPC, when added to the perfusion saline (Fig. 3). Cl^- substitution in the incubation medium induces a reversal of the PD. In this case addition of DPC to the perfusion saline induces a depolarization of the epithelium (Fig. 3).

Results from Cl^- fluxes study show that addition of DPC to the perfusion saline markedly reduces the influx (Fig. 4; Table I), while it remains without any significant effect on the efflux (Fig. 5, Table I).

TABLE I. — Effect of addition of diphenylamine-2-carboxylate (DPC) to the perfusion saline on the Cl^- fluxes of isolated, perfused posterior gills of the Chinese crab *Eriocheir sinensis*. Results are expressed in $\mu eq\ g^{-1}$ wet wt and are means ± SD.

	Control	*Experimental*
Influx	1762 ± 267 (n = 4)	227 ± 14 (n = 4)
Efflux	392 ± 68 (n = 3)	357 ± 76 (n = 3)

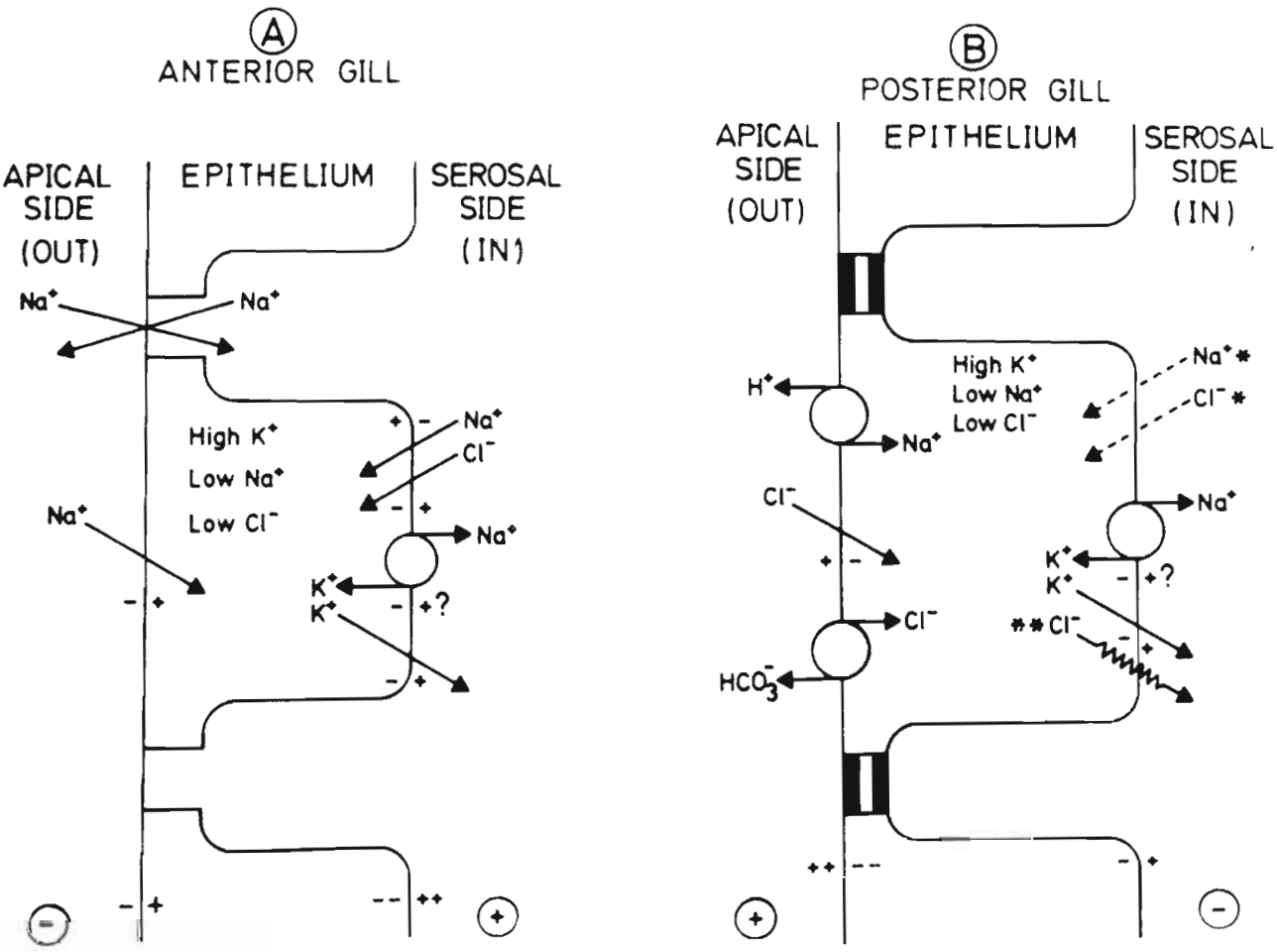

FIG. 6. — Models of Na^+ and Cl^- movements in isolated, perfused anterior (A) and posterior (B) gills of *E. sinensis* acclimated to freshwater. Gills are bathed with a same "freshwater saline" on both sides: the inward movements of Na^+ and Cl^- at the serosal side do not play a significant role in PD generation (Gilles *et al.*, 1988).

DISCUSSION

The present study essentially attempts to investigate the possible participation of Cl^- channels in the Cl^- movement in the gills of the Chinese crab *Eriocheir sinensis* acclimated to freshwater. The PD recorded in both anterior and posterior gills as well as the effects of Cl^- replacement by gluconate on the PD have been largely discussed in earlier studies (GILLES & PÉQUEUX, 1986) and will not be considered any further in this paper.

Diphenylamine-2-carboxylate (DPC) has no significant effect on the PD of the anterior gills, suggesting that Cl^- channels are not implicated in the formation of the electrical potential of these gills. On the other hand, DPC induces a marked depolarization of the posterior gills. In this case, it is only effective when added to the perfusion saline. Further, addition of this compound to the perfusion medium induces a very important decrease of the Cl^- influx, while the efflux remains unchanged. These results are in full agreement with the idea that Cl^- channels are implicated in the Cl^- movements at the serosal side of the gill epithelium. In this view, a block of the Cl^- channel would indeed inhibit the serosal outward movement of Cl^- which follows and short-circuits the K^+ diffusion potential. As shown in figure 6, the reduction of the short-circuit so obtained should lead to a depolarization. The DPC induced depolarization observed when Cl^- is replaced by gluconate can be explained in the same way. In this condition, the PD reversal observed upon substitution of Cl^- by gluconate in the incubation saline can be accounted for by a reversal of the Cl^- diffusion potential on the apical side (Fig. 6.).

The present results are thus strong arguments to consider that Cl^- channels play a prominent role in the Cl^- transepithelial transport occurring in the posterior gills of the Chinese crab *Eriocheir sinensis*. They are by no means excluding a possible role for other pathways. Notable among these are the co-transport systems $Na^+ - Cl^-$ or $Na^+ - K^+ - 2Cl^-$, which should deserve some attention.

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