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Bioelectric Parameters and Sodium and Chloride Fluxes Across the Intestine of *Blennius parvicornis*

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Simultaneous measurements of the electric potential difference and the short-circuit current intensity were taken, as well as measurements of unidirectional fluxes of sodium and chloride in the posterior intestine of *Blennius parvicornis* fish.

On incubating the tissue in standard Ringer solution, a potential difference of —0.9 mV, a mean net flux of sodium of 2.49 μEq/h × cm^2 and of chloride of 2.96 μEq/h × cm^2 with an Isc intensity of —0.37 μEq/h × cm^2 were obtained.

In the absence of sodium (choline as substitute), the net chloride flux and the Isc were completely blocked. In the absence of chloride (isocyanate as substitute) the net sodium flux and the Isc were equally blocked.

Ouabain lessened the sodium and chloride fluxes in the mucosa-serosa direction to the point of nulling the net fluxes of both ions. The values of the bioelectric parameters were also blocked in the presence of this inhibitor.

Acetazolamide did not significantly affect the values of either the unidirectional fluxes or the net fluxes of sodium and chloride. Nor did it affect the values of the bioelectric parameters.

A mechanism for the absorption of sodium and chloride is proposed in which these ions enter the cell in a coupled form and in 1:1 proportion, the transport being independent of the bicarbonate ion.

Key words: Bioelectric parameters, Na^+ and Cl^- fluxes, Intestine, *Blennius parvicornis*.

In contrast to mammals, marine teleosts present a negative serosa potential difference and absorb chloride in excess of sodium across their intestine when it is mounted in an Ussing chamber under short-circuit conditions. These evidences have shown that sodium and chloride transports are independent from each other and that chloride transport is furthermore electrogenic.

HOUSE and GREEN in *Cottus scorpius* (8) and FIELD *et al.* (5) in *Pseu-*

dopleuronectes americanus sole propound that the sodium and chloride transport in these teleosts takes place in the form of a neutral coupling. According to FIELD, the apparent electrogenic choride transport stems from the permiselective properties of paracellular unions.

Recently MACKAY and LAHLOU have indicated that in *Platichthys flesus*, at least part of the chloride transport is not coupled to the sodium transport (11). On the other hand ZUIDEMA *et al.* state that the chloride transport in gold fish is bound to the bicarbonate ion (19).

The present work offers new data on the sodium and chloride transports across the intestine of the marine teleost *Blennius parvicornis*.

Materials and Methods

Experimental procedure. The fish were captured at Punta del Hidalgo (Tenerife) and kept in an aquarium until use. After being sacrificed by decapitation, they had their abdomens open and their posterior portion of the intestine removed, which was then placed in Ringer solution at 0° C and gassed with O_2 at 95 %. Once rinsed, the intestine was cut longitudinally and placed between the two halves of an Ussing chamber with an exposure surface of 0.21 cm^2. A continuous flow of water thermostated at 18° C circled through the chamber. The tissue was bathed on both sides with 4 ml of Ringer solution continuously gassed with O_2 at 95 %. The standard Ringer solution used had the following composition in mmoles/l: NaCl, 107; $NaHCO_3$, 25; NaH_2PO_4, 0.2; Na_2HPO_4, 1.8; KCl, 4.5; $CaCl_2$, 1.25; $MgSO_4$, 1 and glucose, 5 (pH 7.2). In the experiments with ion omission, chloride was substituted by isocyanate and sodium by choline.

Electric measurements. The transmural potential difference (PD) was measured by means of Ringer-agar bridges at 3 % placed on each side of the tissue surface and connected via calomel electrodes to an electrometre Keithley mod. 600 B of high input impedance. The membrane was shortcircuited and a current high enough to reduce the PD to zero was passed through. The current reached the tissue across calomel electrodes and Ringer-agar bridges. The tissue was continuously shortcircuited except for the time when the PD readings were taken. The short-circuit current (Isc) was measured in a microamperimetre. Tissue conductance (G) was estimated from the PD and Isc.

Determination of unidirectional fluxes. Approximately 30 min after the tissue had been mounted in the chamber and the PD values had been verified as stable, the tissue was shortcircuited and thereafter the $^{22}Na^+$ or $^{36}Cl^-$ isotope was added to the solution on one of the sides of the tissue. After an additional 20 min period, samples from the marked side were collected, this time being reckoned as zero time (T_0). Samples of 0.2 ml from the non-marked reservoirs, were collected at zero time and at regular 20 min intervals during 1 h. Samples were taken in duplicate to avoid errors, the same volume being replaced with unmarked Ringer solution.

When the effect of the absence of an ion on the unidirectional fluxes was looked for, the chamber was emptied after the last control sample had been taken, the tissue being thereafter incubated with the new solution free of the ion. In the same way, when the potentials were stable, the tissue was shortcircuited and the radioactive isotope was added to one side of the chamber, samples being taken anew at 20 min intervals. The flux measure-

ments obtained on omitting the ion were compared with those obtained under standard conditions.

When the effect of a specific substance on the unidirectional fluxes was studied, it was added to the solution in the chamber after the last control sample had been taken (T_{60}), a prudential lapse of time was allowed to pass and then new samples were taken at equal intervals. The fluxes obtained before adding the substance were compared with those obtained after the addition.

The activities of $^{22}Na^+$ and $^{36}Cl^-$ were measured in a scintillating counter (Nuclear Chicago mod. Isocap/300).

The unidirectional fluxes of sodium and chloride were determined from the standard equations of SCHULTZ and ZALUSKY (15).

Results

EFFECTS OF A NON-PERMEABLE ION

On the bioelectric parameters. In the absence of the sodium ion from the incubation medium, choline as substitute, both the PD and the Isc were abolished 30 min after the omission. These parameters regained their control values when the tissue was again incubated in standard Ringer solution (fig. 1b).

A similar phenomenon took place when the chloride ion was substituted by isocyanate; both parameters, PD and Isc, were annulled, but recuperated their control values on restoring the chloride ion to the incubation medium (fig. 1a).

Tissue conductance decreased in the absence of the sodium ion, whereas it increased its mean values when the chloride ion was absent.

On the transmural fluxes of sodium and chloride. When the chloride ion was substituted by isocyanate, the net sodium flux decreased significantly from its control value of 2.49 μEq/h × cm² to near zero values due mainly to a drop of the sodium flux in the mucosa-serosa direction, from 12.02 to 10.34 μEq/h × cm². In the absence of the sodium ion (Ringer choline), the net chloride flux also dropped to close to zero values and a lessening of the chloride flux was equally observed in the mucosa-serosa direction from 14.29 to 11.79 μEq/h × cm².

In both cases the unidirectional fluxes regained the control values when the tissue was again incubated in standard Ringer solution (table I).

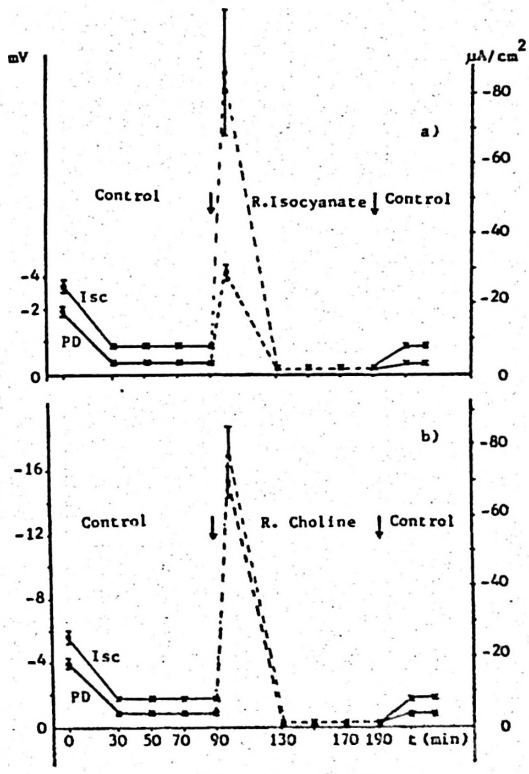

Fig. 1. *Effect of the bicompartimental substitution of chloride (a) and sodium (b) on the bioelectric parameters (PD and Isc).*

Table I. *Effect of the substitution of ions on the transmural fluxes (μEq/h × cm²).*
The results are the mean ± S.E.M. Number of experiments in parenthesis. *, ** and *** Statistics significance at 5, 1 and 0.1 % respectively from control values.

	J^{Na+}_{m-s}	J^{Na+}_{s-m}	J^{Na+}_{net}	PD (mV)	Isc (μEq/h × cm²)	G (mmhos × cm²)
Ringer ClNa (10)	12.02 ± 0.58	9.53 ± 0.45	2.49 ± 0.37	—0.83 ± 0.13	—0.37 ± 0.07	11.60 ± 1.2
R. Isocyanate	10.34 ± 0.24**	11.1 ± 0.26*	—0.82 ± 0.10*	—0.27 ± 0.15***	—0.08 ± 0.05***	14.47 ± 2.2***
Ringer ClNa	12.06 ± 0.57	9.61 ± 0.45	2.45 ± 0.36	—0.77 ± 0.11	—0.32 ± 0.04	11.4 ± 1.27
	J^{Cl-}_{m-s}	J^{Cl-}_{s-m}	J^{Cl-}_{net}	PD	Isc	G
Ringer ClNa (10)	14.29 ± 0.62	11.32 ± 0.55	2.96 ± 0.26	—0.97 ± 0.19	—0.35 ± 0.06	12.93 ± 1.47
R. Choline	11.79 ± 0.26***	11.46 ± 0.34	0.32 ± 0.15***	—0.17 ± 0.14***	—0.1 ± 0.04***	9.2 ± 1.1***
Ringer ClNa	14.32 ± 0.56	11.41 ± 0.53	2.90 ± 0.29	—0.71 ± 0.13	—0.28 ± 0.04	13.96 ± 1.05

Table II. *Effects of ouabain and acetazolamide on the transepithelial fluxes (μEq/h × cm²).*
The results are the mean ± S.E.M. Number of experiments in parenthesis. *, ** and *** Statistics significance at 5, 1 and 0.1 % respectively from control values.

J^{Na+}_{m-s}	J^{Na+}_{s-m}	J^{Na+}_{net}	J^{Cl-}_{m-s}	J^{Cl-}_{s-m}	J^{Cl-}_{net}	PD (mV)	Isc (μEq/h × cm²)	G (mmhos × cm²)
Control (8)								
13.43±0.51	11.25±0.70	2.18±0.52	14.6±1.07	11.8±0.36	2.85±1.28	—0.44±0.13	—0.28±0.1	19.28±0.3
Ouabain								
11.68±0.42*	11.38±0.6	0.3±0.36*	11.71±0.72*	11.67±0.39	0.07±0.48*	—0.05±0.02***	—0.03±0.15***	— 5.2±2.7***
Control (8)								
12.8 ±0.86	10.5 ±0.57	2.33±0.53	12.79±0.87	9.86±0.57	2.83±0.74	—0.44±0.09	—0.22±0.03	18.29±2.7
Acetazolamide								
12.18±0.4	10.3 ±0.48	1.87±0.35	12.91±0.45	9.95 ±0.57	2.87±0.64	—0.35±0.06	—0.21±0.03	18.7 ±2.5

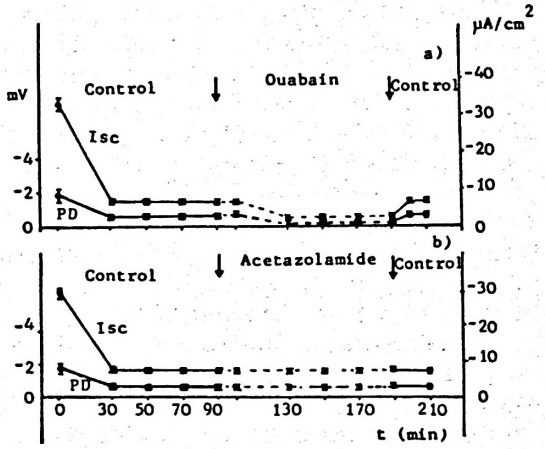

Fig. 2. *Effect of ouabain 10^{-3} M (a) and acetazolamide 10^{-3} M (b) on the bioelectric parameters (PD and Isc).*

EFFECT OF OUABAIN

On the bioelectric parameters. When 10^{-3} M ouabain was added to the incubation medium, both the PD and the Isc descended gradually to zero. Tissue conductance also decreased in a statistically significant way. On incubating again the tissue in control conditions the bioelectric parameters recovered the values of the initial control (fig. 2a).

On the transmural fluxes of sodium and chloride. Ouabain treatment caused the sodium and chloride fluxes in the mucosa-serosa direction to decrease from their control values of 13.43 to 11.68 and of 14.6 to 11.71 μEq/h × cm^2 respectively, without altering the fluxes in the opposite direction. The net fluxes of sodium and chloride were abolished (table II).

EFFECTS OF ACETAZOLAMIDE

On the bioelectric parameters. The addition of acetazolamide to a 10^{-3} M concentration did not provoke any significant change in the values of the bioelectric parameters obtained under standard conditions. The values obtained under these experimental conditions did not differ from the control values (fig 2b).

On the transmural fluxes of sodium and chloride. Acetazolamide did not affect the unidirectional fluxes of sodium and chloride in any direction; for this reason the mean net fluxes of these ions did not differ stastistically from the control values (table II).

Discussion

When the isolated intestine of teleost fish is incubated in an Ussing chamber, it develops a near zero PD, as in *Cottus scorpius* (8), or a positive serosa as in gold fish and other fresh water fish (1, 16, 19) or a negative serosa as in most marine euryhaline teleosts (2, 10, 13, 17).

HUANG and CHEN (9) found in sole intestine that the transmural net flux of chloride persists when it is bathed in Ringer cholide solution, concluding that chloride and sodium must be actively and independently transported in this fish intestine. ANDO *et al.* (1) observed in fresh water eel that both PD and Isc increased markedly when sodium was replaced by choline, suggesting that chloride transport was maintained in the absence of the sodium ion. Subsequently FIELD *et al.* (5) found in intestine of the *Pseudopleuronectes americanus* sole a negative serosa PD; as when sodium is substituted by choline the negativity increases but immediately drops until it reaches zero values within 50 min time, they proposed a model in which sodium and chloride were transported in the form of a neutral coupling, this being masked by the permiselective properties of the paracellular unions. Sodium, which is transported towards the lateral spaces, diffuses once more towards the mucosa

across the tight junction, cation selective, whereas chloride diffuses preferably towards the serosal solution, the serosa negativity being thus explained.

MACKAY and LAHLOU obtained in intestine of the *Platichthys flesus* sole (11) different results from those obtained by Field. Since in this fish the absence of sodium does not cancel the net chloride transport, but inhibits it, and the absence of chloride blocks completely the net sodium transport, they postulated the existence of a coupled transport, about 30 % for chloride and totally coupled for sodium.

In the present work, the posterior segment of *Blennius parvicornis* intestine developed a near zero PD when placed in an Ussing chamber, which differed from previous results (4) where the PD reached —4 mV when the intestine was incubated as an everted sac. These differences might be due to the stretching process to which the tissue is subjected on being placed onto the chamber. Similarly GAZITÚA and ROBINSON (7) found differences between their results and those from BINDER and RAWLINS (3) in rat colon, attributing these differences to the tissue lesions and stretchings produced on placing the tissue in the chamber.

The net fluxes of sodium and chloride were similar, generating a close to zero PD, just like in *Cottus scorpius* (table I). The fact that in the absence of sodium from the incubation medium, the net chloride flux is canceled, and in the absence of chloride the net sodium flux is equally blocked, indicates that the coupling between the two ions is complete and electrically neutral, since under standard conditions the PD is almost zero and the net transport of sodium and chloride is similar. These observations agree with those described by FIELD and FRIZZEL (5, 6) in sole, but differ from those obtained by MACKAY and LAHLOU (11) who reported a partial coupling.

The addition of ouabain lessened the sodium and chloride fluxes in the mucosa-serosa direction blocking out the net fluxes of both ions, as well as the PD and Isc. This effect corroborates the importance of the sodium-potassium pump for the transport of both ions. This mechanism ejects sodium actively from the inner cell towards the intercellular spaces, being responsible for maintaining the low concentration of intracellular sodium and creating, therefore, an electrochemical gradient across the apical membrane for the influx of sodium into the cell, a gradient which energizes the influx of chloride across the said membrane.

Acetazolamide, a substance that on blocking carbonic anhydrase, inhibits the formation of endogenous bicarbonate, affected neither the bioelectric parameters nor the unidirectional fluxes of sodium and chloride, which indicates that the transport of the said ions is independent from both endogenous and exogenous bicarbonate, since the experiments in the presence of acetazolamide were conducted in the absence of bicarbonate from the incubation medium.

Summing up, we propose a model similar to that described by FIELD for sole (5), by STOEBEL and GOLDNER for cat colon (18) and by NELLANS *et al.* for rabbit ileum (12), according to which the mechanism responsible for the sodium-chloride coupling is located in the mucosal or apical membrane of the epithelial cell. The influx of chloride across the apical border is energized by the sodium gradient present across this membrane. The sodium gradient is established by the action of the sodium-potassium pump, sensitive to ouabain at the basolateral membrane.

Resumen

Se realizaron medidas simultáneas de la diferencia de potencial eléctrico e intensidad de

corriente de cortocircuito (Icc), así como de flujos unidireccionales de sodio y cloro en el intestino posterior del pez *Blennius parvicornis*.

Incubando el tejido en solución Ringer estándar se obtiene una diferencia de potencial de —0,9 mV, un flujo neto medio de sodio de 2,49 μEq/h × cm^2 y de cloro de 2,96 μEq / h × cm^2 con una intensidad de Icc de —0,37 μEq/h × cm^2.

En ausencia de sodio (colina como sustituto) se anula el flujo neto de cloro y la Icc, en ausencia de cloro (isocianato como sustituto), el flujo neto de sodio y la Icc.

La ouabaína produce una disminución de los flujos de Na^+ y Cl^- en sentido mucosa-serosa dando lugar a que se anulen los flujos netos de ambos iones, así como los valores de los parámetros bioeléctricos.

La acetazolamida no afecta de forma significativa los valores de los flujos unidireccionales ni los flujos netos de Na^+ y tampoco afecta a los valores de los parámetros bioeléctricos.

Se propone un mecanismo para la absorción de sodio y cloro en el que estos iones entran a la célula en forma acoplada y en proporción 1:1, siendo el transporte independiente del ion bicarbonato.

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