REVISTA ESPAÑOLA DE FISIOLOGIA, 40, 117-122. 1984

Transmural Potential Differences and Short-circuit Current Intensity in the Posterior Intestine of *Blennius parvicornis*

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(Received on June 17, 1983)

A. BOLAÑOS and A. LORENZO. *Transmural Potential Differences and Short-circuit Current Intensity in the Posterior Intestine of* Blennius parvicornis. Rev. es., **40**, 117-122 1984.

Simultaneous measurements of the transmural potential difference (PD) and the short-circuit current intensity (Isc) in the posterior intestine of the fish *Blennius parvicornis* were made in normal Ringer and in solutions of different ionic composition. The ouabain effects on these two parameters were also tested in normal Ringer solution.

The absence of K^+ from the Ringer solution on both the mucosal and serosal sides has no apparent effect on the PD and Isc within the first 15 min, but it makes them null after 30 min.

When Na^+ is substituted in both compartments, using Tris as substitute, a serosal negativity increase is initially observed, but it gradually decreases to zero after 30 min of experimentation. Similarly the PD and Isc drop to zero in the absence of Cl^- (sulfate as substitute).

Ouabain diminishes the serosa negative potential difference to zero after 30 min presenting a lineal relation to the Isc.

A likely transport mechanism for Cl^- dependent on the Na^+ - K^+ pump, is discussed.

Key words: *Blennius parvicornis*, Intestine, Transmural potential, Short-circuit current.

Marine teleosts, in contrast to other vertebrates, have been shown to possess an electrically negative serosal side in relation to mucosa, and a short-circuit intensity (Isc) approximately equal to the difference between the net absorption of chloride and sodium in the many studied species; winter sole (11), eel (1) and European sole (15). The ion absorption seems, from such evidence, to be dominated by an electrogenic chloride, sodium-independent, transport. BADIA and LORENZO (4) argue that the serosa negativity in *Gobius pagenellus* is due to the active transport of chloride. Nevertheless, HOUSE and GREEN (10) in *Cottus scorpius* and FIELD (6) in sole show that the chloride and sodium transport can be coupled.

A model of a neutral coupled transport towards the lateral spaces of the mucosa cells has been proposed (7). The apparent transport of electrogenic chloride is obtained through the permiselective properties of the membrane at the unions or paracellular joints. The sodium which is transported toward the lateral spaces diffuses toward the mucosal solution across the cation selective joints of the mucosal cells, while chlorine diffuses preferably toward the serosal fluid bringing about the resulting serosal negative potential.

MACKAY and LAHLOU (12) show that at least one part of the chloride transport cannot be explained by the selective permeability properties of a paracellular path.

The present work offers data on the electric properties of the intestine in *Biennius parvicornis* and to shows that the chloride transport in this teleost is sodium transport dependent.

Materials and Methods

Fisch, *Blennius parvicornis*, 9 to 12 cm in length, captured at Punta del Hidalgo (Tenerife, Spain) and kept in a laboratory aquarium, were used. After they had been sacrificed, a portion of the posterior intestine was extracted, rinsed with standard glucosed Ringer solution, everted and mounted on a U-shaped glass cannula, similar to the one used by HERRERA (8), in order to measure the potential difference and the short-circuit current after the technique described by USSING and ZERAHN (16).

The transmural potential difference was measured across Ringer agar bridges at 3 % and a pair of calomel electrodes connected to a Keithley mod. 600B electrometer of high entrance impedance. The tissue was shortcircuited every five minutes by another pair of electrodes connected to a microamperimeter with the energy required to null the PD while the intensity of the short-circuit current was measured.

All the experiments were kept at the same temperature with circulating water at 18° C. The composition of the Ringer solution was as follows in mM/l: NaCl, 127.27; KCl, 5.09; $CaCl_2$, 2.72; KPO_4H_2, 1.27; $MgSO_4$, 1.27; HCl, 4.1; Tris. 4.9; and glucose, 5.0 mM (pH, 7.4). Oxygenation was kept constant with a mixture of 95 % O_2 and 5 % CO_2.

In Ringer solutions free of sodium, the sodium ion was substituted by Tris and the solution was kept at the same pH and osmolarity. Chloride was similarly substituted by sulfate and mannitol.

Ouabain at 10^{-3} M was added to the serosal side 30 min after initiating the experiment, when the PD and Isc had reached stable levels.

Results

The potential difference values at the posterior intestine show a gradual increment with time until they stabilize at —6.2 mv (serosa negative) after 30 min of experimentation. The short-circuit current intensity also increases to about 500 A/cm^2 (fig. 1).

Effects of sodium absence from both compartments. When the PD and Isc had reached a stable level (approximately 30 min from the beginning of the experiment) the Ringer solution of the medium was substituted for 15 min by another one containing Tris as substitute, which pro-

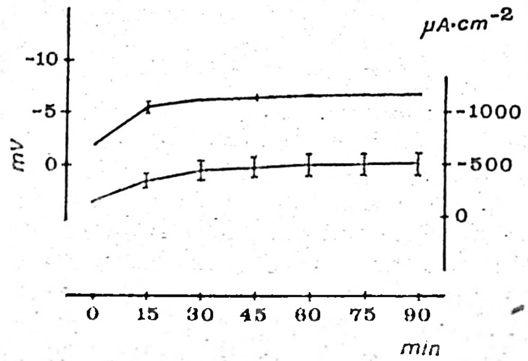

Fig. 1. *Transmural potential difference (PD) and short-circuit current (Isc) across the posterior intestine of* Blennius parvicornis.
Control values. ▬▬ PD, ——— Isc.

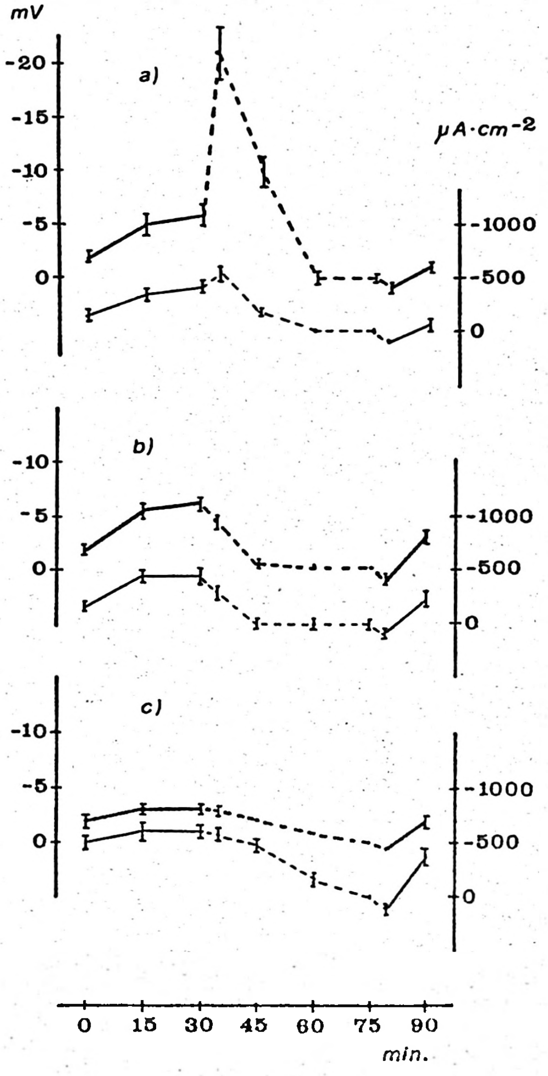

Fig. 2. *Effects of the bicompartimental substitution of Na^+, Cl^- and K^+ ions on the transmural potential difference ——— PD and short-circuit current intensity ——— Isc.*
a) Na^+, b) Cl^- and c) K^+. Omitted ion ----.

duced a sharp increase in serosa negativity (—21 mV). The Isc likewise increased but less markedly (table I). If the absence of the sodium ion was extended, the PD and Isc values decreased to zero or near zero 30 min after omitting the ion from both compartments. Restoring the Na^+ ion to the medium led to the recovery of the standard values for PD and Isc (table I and fig. 2a) in both cases.

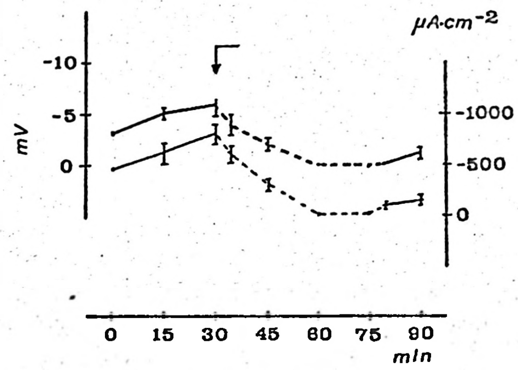

Fig. 3. *Effects of ouabain addition on the transmural potential difference ——— PD and short-circuit current intensity ——— Isc.*
Ouabain (10^{-3} M) was added on the serosal side 30 min after initiating the experiment.

Effects of Cl^- absence. In a Cl^- free medium (sulfate as substitute) both the PD and Isc dropped to zero whether the substitution was conducted for 15 or 30 min (table I and fig. 2b). When Cl^- was restored to the medium, the tissue tended to recover the standard values in both cases.

Effects of K^+ substitution. The K^+ omission in both compartments for a 15 min period did not affect the PD and Isc values (table I) significantly, but these values dropped to zero when the omission lasted more than 30 min (fig. 2c). The DP and Isc values were recovered when the ion was restored to the medium.

Effects of ouabain. The intestine was bathed in normal Ringer for half and hour until stable levels were reached and then 10^{-3} M ouabain was added to the serosal solution. Both PD and Isc decreased to zero 30 min after adding the

Table I. *Electrical potential difference (PD), short-circuit current (Isc) and tissue resistance (PD/Isc) across the posterior intestine of* Blennius parvicornis. *Effects of omission of ions from both sides.*

The results are the means ± S.E.M. Number of experiments in parentheses. a, b, Statistical significance at 5 and 0.5 per cent levels, respectively from 30 minutes.

Experimental condition ion omitted	Parameters	Time (minutes) and solutions					
		CONTROL		OMISSION		RESTITUTION	
		0	30	35	45	50	60
Na+ (8)	PD (mV)	−2.08 ± 0.59	−6.25 ± 0.93	−21.16 ± 2.91[a]	−10.66 ± 1.58	+1.23 ± 0.38	−0.9 ± 0.46
	Isc (μA/cm^2)	−163.3 ± 58.8	−408.6 ± 43.7	−521.6 ± 72.3	−181.6 ± 32.4	+70 ± 18.4	−40 ± 43.6
	PD/Isc (Ω.cm^2)	11.79 ± 2.78	15.87 ± 2.73	35.04 ± 5.85[b]	61.59 ± 4.95	10.71 ± 3.09	13.44 ± 2.46
Cl− (8)	PD	−1.73 ± 0.44	−11 ± 0.71	−4.77 ± 0.62	−0.21 ± 0.42	+1.25 ± 0.65	−3.4 ± 0.29
	Isc	−165 ± 31.6	−408.3 ± 75.5	−313.3 ± 53.1	0 ± 30.5	+81.6 ± 49	−236.6 ± 80.9
	PD/Isc	10.58 ± 1.77	17.88 ± 3.75	17.26 ± 3.41	18.23 ± 5.11	14.01 ± 4.45	14.96 ± 3.2
K+ (8)	PD	−2.03 ± 0.6	−3.86 ± 0.49	−4.62 ± 0.54	−2.08 ± 0.2	+0.43 ± 0.41	−2.03 ± 0.49
	Isc	−485 ± 163	−781.6 ± 134	−906.6 ± 179	−455 ± 96.4	+181.6 ± 84.1	−428.3 ± 87
	PD/Isc	5.09 ± 1.13	7.23 ± 2.4	6.7 ± 1.8	7.42 ± 2.04	7.5 ± 2.68	7.23 ± 2.09

ouabain. The tissue recovery was much slower than in the absence of an ion, preventing its showing in figure 3.

Discussion

The transmural potential difference and the short-circuit current have been studied in several species of marine Teleosts in solutions of different ionic composition.

In the marine teleostean *Cottus scorpius* (10) the PD was close to zero, the Cl^- absorption was completely blocked when Na^+ was replaced by choline, and the Na^+ absorption was reduced by 80 % when most of the Cl^- (it remained at 13 mM) was replaced by sulfate. These results suggested a coupled transport of sodium and chloride across the mucosal border.

The observations in sole (11, 15), in eel (1, 2, 9) and in other species of marine teleosts (1) have yielded a different explanation for Cl^- absorption. As regards teleosts the existence of an electrogenic pump for chloride, independent of sodium, has been suggested. When the intestine of these teleosts was bathed on both sides with normal Ringer solution, the transmural potential difference was serosa negative. When sodium was replaced by choline, the serosa negativity persisted (11) or increased (3, 9), and when chloride was replaced by sulfate (1, 11) the potential difference became positive (serosa positive).

According to FIELD (7) the negative values of the potential difference and the short-circuit current intensity observed by ANDO (1) on substituting the sodium, are not to be taken as proof that the chloride transport is independent of sodium, since the serosa negativity on substituting sodium by choline does not remain stable, but decreases gradually to zero during a 50 min period (9). Such a difference in negative potential seems to be the result of diffusion potentials produced on changing the sodium in the medium.

In the present study, when the posterior intestine was incubated in normal Ringer solution, it presented a serosa negative potential difference in relation to the mucosa, which reached a stable value of —6 mV 30 min after initiating the experiment. The absence of sodium from both sides (Tris as substitute) for 15 min (table I) increased the serosal negativity, but if the omission was extended (fig. 2a) the negativity dropped to zero after 60 min. Therefore the negativity increase on the seroral side might be due to diffusion potentials produced by chloride on substituting the sodium in the medium rather than to an active mechanism. In sole intestine, an increase in the unidirectional flows of chloride in a sodium free Ringer solution or after treatment with ouabain was observed (7). The blocking of the sodium pump brought about an increase in the diffusion potentials of chloride in eel intestine (3).

When chloride was substituted on both the mucosal and serosal sides (sulfate as substitute), the PD and Isc also decreased to zero (table I and fig. 2b), indicating that an independent active transport of sodium could hardly have taken place, for in such circumstances the serosa ought to have reached a positive value, but this did not occur.

When the potassium ion was omitted from the bath solution for 15 min (table I), no apparent effect on the PD and Isc was observed, but if the substitution was extended, these values decreased to zero (fig. 2c). This shows that when potassium is absent from the medium for a prolonged period of time, the chloride transport is suppressed, since the serosa negativity is lost. The same phenomenon occurs when the sodium-potassium pump is blocked by ouabain (fig. 4), where the PD and Isc also drop to zero. Recently a chloride transport in various epithelia (5, 13, 14, 17), ouabain sensitive, has been described.

Summing up we may conclude that chloride transport in the posterior intestine of *Blennius parvicornis* is dependent on the sodium-potassium pump. The blocking of this pump by the omission of sodium or potassium, or by the presence of ouabain, brings about the nulling of both the PD and the Isc.

Resumen

Se miden la diferencia de potencial transmural (DP) y la intensidad de corriente de cortocircuito (Icc) en el intestino posterior del pez *Blennius parvicornis* en Ringer normal y en soluciones de diferente composición iónica. También se estudia el efecto de la ouabaina sobre estos dos parámetros en solución Ringer normal.

La ausencia de K^+ en la solución Ringer, en ambos lados mucosal y serosal, no tiene ningún efecto aparente sobre la DP e Icc durante los 15 primeros minutos aunque anula dichos parámetros a los 30 min.

Cuando se sustituye el Na^+ por Tris en los dos compartimientos se observa inicialmente un aumento de la negatividad serosal y posteriormente desciende hasta llegar a cero a los 30 min de iniciado el experimento. En ausencia de Cl^-, con sulfato como sustituto, tanto la DP como la Icc caen a cero.

La ouabaina reduce a cero la DP serosal negativa en 30 minutos, presentando una relación lineal con la Icc.

Se discute un posible mecanismo de transporte de Cl^- dependiente de la bomba de Na^+-K^+.

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