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Preliminary Studies on Transmural Potential and Intensity of the Short-Circuit Current in Intestine of *Gobius maderensis*

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The electrical difference (PD) and short-circuit current (Isc) across anterior intestine have been studied *in vitro* with solutions of different ionic composition in *Gobius maderensis*. 30 minutes after the beginning of the experiment a PD of about 2 mv negative serosa and an Isc of approximately 200 $\mu A/cm^2$ were recorded.

Omission of sodium (Tris as substitute) from the luminal side or from both sides leads to an increment of 800 % in PD more negative serosa together with an increase of 969 % in Isc.

In the absence of chloride (sulphate as substitute) the PD and Isc decreased and attained constant positive values.

When the potassium was omitted from the luminal side or from both sides neither the PD nor the Isc changed.

These findings corroborate the assumption that active transport of chloride ions from mucosa to serosa is the major factor for transmural PD and account for the short-circuit current in the anterior intestine of *Gobius maderensis*.

The ion transport in teleost fish that live in both fresh and sea water has so far been carried out with techniques to measure transmural potential difference (PD) and short-circuit current intensity (6-9, 11). In marine teleosts PD was found to be serosa negative in most of the animals studied (1-4) whereas in fresh water teleosts both positive and negative potential differences have been recorded.

The euryhaline teleosts have been more intensively studied and differences in PD and Isc have been observed when the animals are adapted to different environments. ANDO and UTIDA (2) have found that when eels transferred from fresh to sea water, unidirectional fluxes of sodium and chloride ions increase considerably in both directions as compared with those of fresh water eels. The net influx of chloride ions is greater in sea than in fresh water eels, while that of Na^+ ions shows no appreciable change, producing a negative serosa PD. The development of this pump

together with a serosa negative potential, seems to be an important functional change of eel intestine during sea water adaptation.

The experiments reported in this paper offer new data on the relation between some ions and the electrical difference potential and short-circuit current (Isc) developed in the intestinal wall of *Gobius maderensis*.

Materials and Methods

Fish specimens of *Gobius maderensis* measuring from 10 to 12 cm, captured in their natural environment (Punta del Hidalgo, Tenerife) have been used. They were killed by decapitation and after opening the abdomen, the entire intestine was removed and washed by flushing inside and outside with Ringer solution. With the help of a thin glass rod, the anterior intestine was everted before determining its potential difference and short-circuit current after the method of USSING and ZERAHN (10) modified by HERRERA (5).

Measurement of PD across the intestinal membrane was made with 3 % Ringer-agar bridges, the tip of which was placed within 1 mm from each surface of the membrane. The PD between these bridges was measured by a pair of calomed electrodes connected to a 600 B amplifier electrometer of high imput impedance. The PD across the membrane was expresed in terms of the potential on the serosa side with respect to the mucosal side. The membrane was short-circuited by passing just enough voltage to reduce the PD to zero through a pair of calomed electrodes and Ringer-agar birdges.

The standard solution used for control experiments had the following composition: NaCl, 127.27 mM; KCl, 5.09 mM; $CaCl_2$, 2.72 mM; KH_2PO_4, 1.27 mM; $MgSO_4$, 1.27 mM; HCl, 4.1 mM; Tris, 4.9 mM, and 5 mM glucose (pH 7.2). Other solutions of equal pH and osmolarity but lacking sodium, potassium or chloride were used by replacing the cations with Tris and the anion with sulphate. All experiments were carried out in an outer box were 30° C water was circulated.

Results

PD, Isc and tissue resistance in control solution. In *Gobius maderensis*, PD and Isc across the anterior intestine bathed on both sides with identical normal Ringer-Tris solution, were found to be —0.85 mv and —2.08 $\mu A/cm^2$ respectively with $t = 0$ (fig. 1 *a*). These values kept increasing during the first 30 minutes, after which they reached a steady level with a rate of —2 mv and —258 $\mu A/cm^2$, respectively, until the end of the experiment.

Tissue resistance did not change significantly during this period, having a slight variation from 5.15 Oh $\times$ cm^2 ($t = 0$) to 7 Oh $\times$ cm^2 ($t = 60$ minutes).

Effect of the bilateral substitution of ions. After bathing both sides of the intestinal membrane from *Gobius maderensis* with Ringer-Tris for 30 minutes, when the PD and the Isc had reached a steady level, the sodium ion was replaced by Tris, causing immediately a very high negative potential and Isc (table I, fig. 1 *b*). Tissue resistance changed significantly as compared with that in Ringer-Tris. By restoring the standard solution, the rate of the PD, Isc and tissue resistance were recovered (fig. 1 *b*). In just the absence of chloride (sulphate as substitute) the PD and the Isc reversed their values so that constant positive values were attained (table I, fig. 1 *c*). The negative potential and Isc were recovered by replacing the Na_2SO_4 mathing solution with ClNa-Ringer. The tissue resistance (fig. 3 a) in Na_2SO_4-Ringer did not change significantly as compared with that in NaCl-Ringer. When the potassium ion was omitted from both mucosal and serosal sides (table I, fig. 1 *d*) neither the PD nor the Isc changed

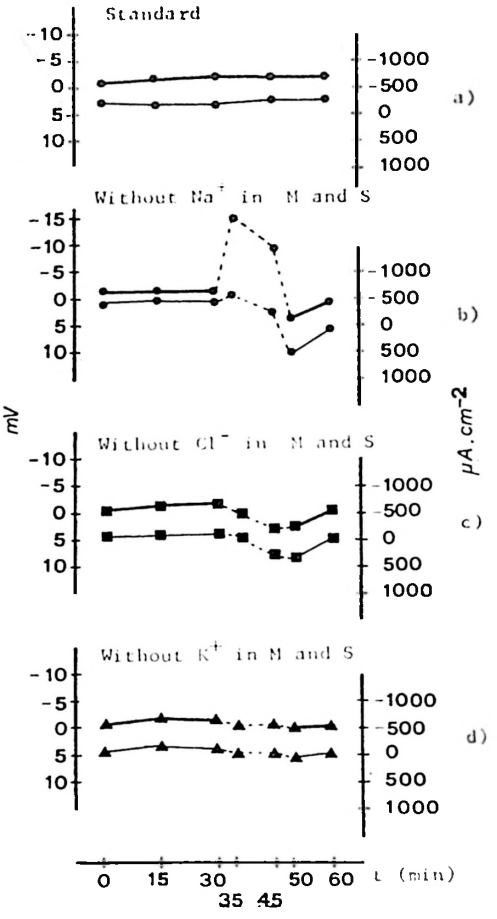

Fig. 1. *Electropotential difference (PD) and short-circuit current (I_{sc}) across the anterior intestine of* Gobius maderensis.
Effects of omission and restitution of ions on both mucosal (M) and serosal (S) sides. — PD, — I_{sc} control. ---- ion omitted. ● Na^+, ■ Cl^- ▲ K^+.

significantly, and tissue resistance remained constant as well.

Unilateral omission of ions. When after an incubation of 30 min, the standard solution in the mucosal compartment was changed by another solution without Na^+, Cl^-, or K^+ the results produced were similar in quality to those described when the ions were absent from both compartments (table II). After replacing sodium the PD and the Isc increased, the serosa being more negative, while tissue resistance increased about 19 Oh × cm². Val-

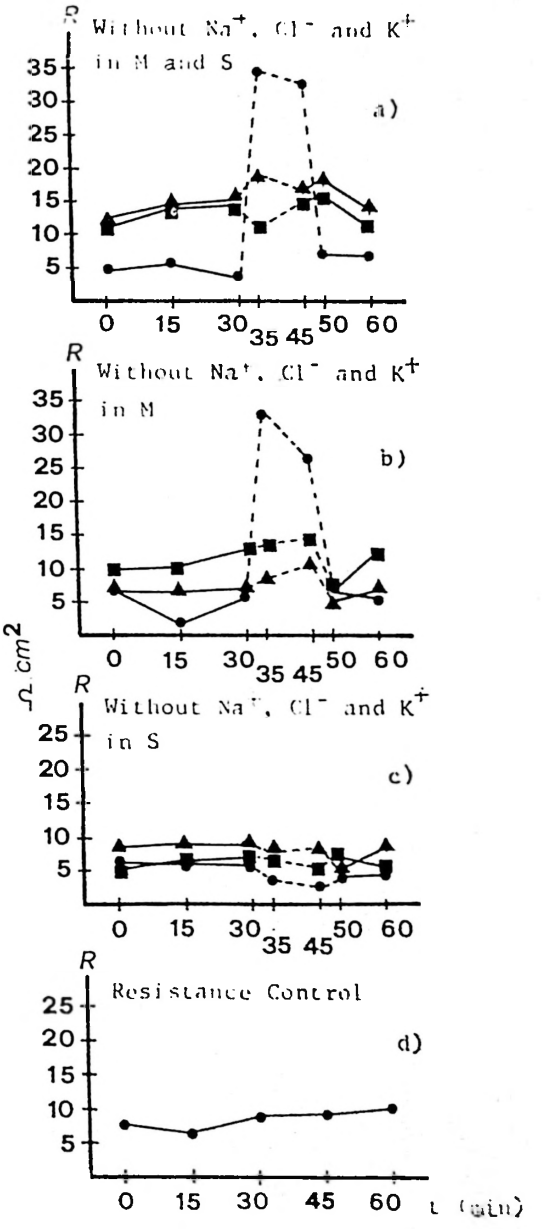

Fig. 2. *Tissue resistance (R) across the anterior intestine of* Gobius maderensis.
Effects of omission and restitution of ions. ● Na^+, ■ Cl^-, ▲ K^+.

Table I. *Electrical potential difference (PD) and short-circuit current (I_{sc}) in anterior intestine of fish* Gobius maderensis. Effects of omission of ions from both sides. Statistical significance (Student't test): a, $p < 0.001$; b, $p < 0.005$; c, not significant. Mean values ± S.E. Potential (PD) in mv. Short-circuit current (I_{sc}) in $\mu A/cm^2$. Number of experiments in parentheses. Changes % : +, increase; — decrease.

Experimental condition Ion omitted	Parameters	Time (minutes) and solutions						Changes %	
		Standard		Omission		Restitution			
		0	30	35	45	50	60	Omission	Restitution
Na^+ (4)	PD	—1.5± 0.8	—1.4± 0.5	—15.5± 1.7	—9.5± 1.3	+3.6± 0.6	+0.6± 0.4	+968.9[a]	—348.3[a]
	Isc	—484.8±228.9	—476.3±63.3	—543.5±133.5	—309.1± 49.1	+613.3±127.2	+72.9±57.6	+14.1[c]	—223.8[a]
Cl^- (5)	PD	—0.8± 0.3	—1.7± 0.4	—0.3± 0.6	+2.7± 0.4	+2.6± 0.5	—0.3± 0.5	—82.3[c]	—252.9[a]
	Isc	—96.4± 52.3	—147.5±27.3	—115.5± 72.9	+272.2±108	+309.4± 91.5	—2.8±32.4	—21.9[c]	—309.7[a]
K^+ (4)	PD	—0.9± 0.6	—1.6± 0.2	—0.9± 0.3	—0.8± 0.2	0.0± 0.3	—0.6± 0.3	—43.7[b]	—100.0[a]
	Isc	—110.2± 57.8	—127.4±30	—66.0± 22	—54.4± 18.3	—2.4+ 14.5	—53.3+26.9	—48.2[b]	—100.8[a]

Table II. *Electrical potential difference (PD) and short-circuit current (I_{sc}) in anterior intestine of fish* Gobius maderensis. Effects of omission of ions from only one side. Statistical significance (Student't test): a, $p < 0.001$; b, $p < 0.005$; c, $p < 0.01$; d, $p < 0.025$; not significant. Units and conditions as in table I.

Experimental condition Ion omitted	Parameters	Time (minutes) and solutions						Changes %	
		Standard		Omission		Restitution			
		0	30	35	45	50	60	Omission	Restitution
Na^+ - M	PD	+0.3±0.3	—1.2±0.5	—10.8±1.5	—5.9±1.6	+0.7±0.3	—0.5±0.3	+800.00[a]	—158.3[a]
(5)	Isc	+112.6±96.2	—406.1±201.8	—484.6±191.6	—306.2±100	+147.5±37.9	—229.6±112.3	—19.33[e]	—136.3[d]
Cl^- - M	PD	—0.7±0.05	—1.7±0.6	+5.4±0.7	+8.5±0.7	+1.1±0.6	—0.5±0.5	—417.64[a]	—164.7[b]
(4)	Isc	—89.7±18.3	—171.5±63.9	+612.6±192.6	+891.6±274.8	+191.5±73.2	—79.7±80.8	—457.20[d]	—211.6[c]
K^+ - M	PD	—1.1±0.5	—0.87±0.07	—0.7±0.3	—1.02±0.12	—0.05±0.15	—0.8±0.1	—17.24[e]	—94.2[c]
(4)	Isc	—207.6±82.1	—185.5±74.3	—116.4±43.4	—239±148.4	—32.3±32.6	—170.6±64.9	—37.25[e]	—82.6[e]
Na^+ - S	PD	—0.9±0.7	—2.7±0.79	—3.7±0.2	—2.65±0.55	+0.75±0.38	+0.1±0.5	—32.40[e]	—125.0[c]
(4)	Isc	—215.7±182.6	—561.1±196.8	—1142.9±179.9	—1166.8±322.2	+698.9±640.9	+21.3±318.9	+103.68[a]	—224.5[e]
Cl^- - S	PD	—0.42±0.002	—2.1±0.49	—3.9±0.46	—5.9±0.43	—0.7±0.4	—1.5±0.65	+90.47[e]	—66.6[e]
(4)	Isc	—123.08±34.8	—432.1±159.6	—1041.7±305.6	—1546.2±375.1	—155.3±63.73	—350.0±125.8	+141.07[d]	—70.9[e]
K^+ - S	PD	—1.04±0.26	—0.76±0.16	+0.02±0.26	—0.42±0.16	—0.18±0.11	—1.16±0.72	—97.36[a]	—76.3[c]
(5)	Isc	—127.5±32.2	—84.7±14.6	—13.4±34.9	—37.8±12.5	—19.3±10.3	—49.5±12	—84.14[c]	—77.2[c]

ues returned to normal when Na^+ was again in the medium.

If the mucosal solution was made chloride-free by substituting the anion by sulphate, the PD and the Isc reversed and attained constant positive values (table II), while tissue resistance did not change significantly. By restoring Cl^- the PD, Isc and the tissue resistance recovered their control values.

The omission of the potassium ion in mucosa did not significantly affect the PD, Isc and tissue resistance (table II).

When Na^+ was omitted from the serosal solution, the PD and the Isc increased 32.14 % and 103 %, respectively, both PD and Isc returning to normal when the Na^+ was again present in the medium.

The absence of Cl^- in the serosal side (sulphate as substitute) increased the PD and the Isc 90 % and 141 %, respectively. When replacing the bathing fluid by the standard solution, the rate of the PD and Isc were recovered (table II). The tissue resistance did not change significantly.

The omission the K^+ in this compartment did not generate any changes in the PD, Isc and tissue resistance.

Discussion

When the intestine of *Gobius maderensis* is incubated in a control solution it creates a potential difference (serosa negative with respect to mucosa) which requires a short-circuit current to annul it. Both the potential difference and the short-circuit current increase during the first half hour of experimentation, so that —2 mv and —258 $\mu A/cm^2$ values are reached after that period (fig. 1 *a*), and remain unchanged parameters until the end of the experimental time (t = 60 min). The presence of this net ionic flux shows the existence of an active ionic electrogenical mechanism which is able to carry anions either from mucosa to serosa or cations from serosa to mucosa.

In other teleost fish a transmural potential difference (to those found by us) has also been recorded (1-4, 6-9, 11).

The omission of chloride and sodium ions in the incubation medium shows the existence of an active transport of sodium and chloride from mucosa to serosa, sodium transport being greater than chloride, which results in a potential difference of negative serosa with respect to mucosa.

The omission of chloride in both compartments (table I, fig. 1 *c*) reverses the PD and the Isc, which shows that under circumstances there continues to be an active transport of cations from mucosa to serosa. When the chloride is omitted in mucosa the active transport system of this ion is blocked and a decrease in the negativity of the serosa can be detected (table II). The values recorded for the PD and Isc become more and more positive each time due to the operation of the sodium-potassium pump. On the other hand if chloride is omitted in serosa (table II), the flux of the chloride ion from mucosa to serosa increases the negativity of serosa. The presence of the sodium ion in the medium stops the resistance of the tissue from increasing so that the flux in both sodium and chloride ions can be detected in the change of the Isc (table II).

The omission of sodium in both compartments or in the mucosal side alters the normal working of the Na^+-K^+ pump, which brings out the active transport of Cl^- and renders PD and Isc much more negative than under standard conditions. If the sodium is omitted in serosa, neither the chloride nor the sodium ions are altered when passing fron mucosa to serosa and the Isc value becomes very high (table II) with respect to control conditions. Although PD becomes more negative, it does not reach values as high as when sodium is substituted either in both compartments or in mucosa, since the passing of chloride into serosa at the same time as sodium renders the potential difference lower.

Another great change in the sodium bicompartmental substitution (fig. 2 *a*) is constituted by the increase in tissue resistance. However, when the substituted ions are those of chloride (sulphate as substitute) the tissue resistance remains constant. From these results a permeability increase of sodium ions is supposed to be mainly involved in the electrical resistance decrease. SKADHANGE (9) suggests that the permeability increase of sodium ions seems to play an important role in promoting water absorption from he intestine of sea water eels.

Resumen

Se estudia la diferencia de potencial transmural e intensidad de corriente de cortocircuito *in vitro* en el intestino anterior del pez *Gobius maderensis*, con soluciones de diferente composición iónica.

Después de 30 minutos de iniciado el experimento, el potencial es alrededor de 2 mv (la serosa negativa respecto a la mucosa) y la intensidad de corriente aproximada 200 $\mu A/cm^2$.

La omisión de sodio en el lado luminal, o en ambos lados, produce un incremento del potencial de un 969 %.

En ausencia de cloro, el potencial disminuyó hasta alcanzar valores positivos.

Estos hechos revelan que el transporte activo de iones de cloro de mucosa a serosa es el factor principal que contribuye a mantener una diferencia de potencial transmural negativa en el intestino anterior de *Gobius maderensis*.

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