Role of endothelin on the intestinal transport of seawater adapted Eel, *Anguilla anguilla*

Francesca Trischitta, Agata Torre, Grazia Fedele & Caterina Faggio

Dipartimento di Scienze della vita "M. Malpighi", Università di Messina - Viale F. Stagno d'Alcontres, 31 - 98166 Sant'Agata - Messina, Italy; *ftrischi@unime.it*

Abstract. Endothelin (ET), originally identified as a potent vasoconstrictor, is now known as a modulator of a wide variety of non-vascular biological activities, including ion transport in tissues of osmoregulatory organs. In this study the effect of ET on transepithelial ion transport was tested in the isolated intestine of the seawater adapted *Anguilla anguilla*. The isolated intestines were mounted in Ussing chambers and electrophysiological techniques were employed. Dose-response experiments showed that the serosal addition of ET-1, an agonist of both ETA and ETB receptors, at lower concentrations (10^{-10} M; 10^{-9} M) increased the serosa negative short circuit current (Isc), while at higher concentrations (10^{-8} M; 10^{-7} M) decreased it. Pre-treatment with phlorizin or omission of glucose from the luminal solution abolished the increase in I_{sc} produced by the lower concentrations of the peptide. In addition, ET was ineffective in tissues pre-treated with the loop diuretic bumetanide. The effect of the peptide on I_{sc} was inhibited in tissues pre-incubated with 10^{-4} M indomethacin. In addition PGE1 (10^{-5} M) decreased Isc while the following addition of ET-1 was ineffective and vice versa. Our results suggest that endothelin modulates both glucose and ion transport; this modulatory role probably depends on the production of prostaglandins.

Keywords: endothelin, fish intestine, ion transport, phlorizin, prostaglandin, Pisces.

INTRODUCTION

Endothelin (ET), a paracrine signalling peptide, originally identified as a potent vasocostrictor (Yanagisawa et al., 1988), is now know as a modulator of a wide variety of non-vascular biological activities (Kedziersky & Yanagisawa, 2001). The effects of ET are mediated by two distinct receptors termed ET_A and ET_B (Lee et al., 1998). Recent studies suggest that ET modulates ion transport in tissues of osmoregulatory organs. A modulatory role on kidney epithelium of mammals was observed by Plato et al. (2000) and Tomita et al. (1993). Evans et al. (2004) studied the effect of ET on opercular epithelium of *Fundulus heteroclitus*, a fish that experiences daily fluctuations in environmental salinity. In this fish endothelin and endothelin converting enzyme-1 were found in the gills and it was suggested that the peptide controls cell survival during osmotic stress and/or alters ion transport by the mitochondrion rich cells (Hyndman & Evans, 2007), the large acidophilic cells eliminating excess Na^+ and Cl^- in the seawater teleosts (Karnaky, 1980, 1986; Keys and Willmer, 1932). In the elasmobranch, endothelin produces constriction in gill blood vessels (Evans & Gunderson, 1999) and modulates the contractile properties of rectal gland, an important osmoregulatory organ in these fishes (Evans & Piermarini, 2001).

In view of these observations it should be interesting to know whether endothelin affects ion transport in the intestine of marine fish, an important osmoregulatory organ.

It is known that the diffusive loss of water across the gills, due to the large osmotic gradients experienced by teleosts in seawater, is counterbalanced by the intestinal active uptake of salt and hence of water. It is known that seawater-adapted teleost fish drink water to compensate for the osmotical loss from the body (Smith, 1930, 1932). Seawater is desalinated in the oesophagus because the passive absorption of Na^+ and Cl^- is not followed by a water flux into the blood since the osmotic water permeability of the oesophagus is very low (Hirano & Mayer-Gostan, 1976; Kirsch, 1978; Parmalee & Renfro, 1983; Nagashima & Ando, 1994). Once desalination is complete, the intestine actively absorbs Na^+ and Cl^-, which drives a water flux from lumen to blood following water potential gradient. The basolateral Na^+/K^+ pump provides the driving force for the Na^+-coupled transporters (mainly the $Na^+/K^+/2Cl^-$ co-transport) operating on the apical membrane of the enterocytes and extrudes Na^+ into the blood. Cl^- leaves the cell across the basolateral membrane by anion channels and/or Cl^-/HCO_3^- antiporter and K^+/Cl^- co-transport (Loretz, 1995).

In this paper the effect of the peptide endothelin on the short circuit current, I_{sc}, a measure of transepithelial ion transport across a variety of epithelial membranes including intestinal mucosa (Gonzalez Bosc et al., 2001; Evans et al., 2004; Karaki & Kuwahara, 2004), was studied on the isolated intestine of the eel, *Anguilla anguilla.* This study was undertaken with the aim to know if the peptide affects ion transport in fish intestine of an euryhaline fish and if the effect of the peptide involves NO and prostaglandins, as already suggested for the opercular epithelium of *F. heteroclitus* (Evans et al., 2004).

MATERIALS AND METHODS

For this study 33 eels (*Anguilla anguilla)* were used (mean weight ±SD=141.1±7.25g). They were obtained from a local fish farm and kept in large tanks with flowing natural seawater (37.5 PSU). The water temperature was 20±2° C. Fish were acclimated for at least 3 weeks prior to experimentation. They were fed minced crustacean and fish, but food was withheld a 1 week prior to the experiments. Fish were killed by overdose of tricaine methanesulfonate (MS 222; 0.5 gl^{-1}) and the entire intestine was obtained by dissection. For each experiment a different animal was used.

The middle intestine (the region anterior to the muscular sphincter separating it from the posterior intestine) was isolated and stripped of longitudinal and circular layers using two pairs of fine forceps. The mucosa minus underlying muscularis was mounted vertically in a modified Ussing chamber (CHM6, World Precision Instruments, Berlin, Germany; membrane area: 0.13 cm^2). 6 ml of Ringer solution were added to circulation reservoirs, with jacketed chambers for temperature control, connected to each side of the chamber. The solution was gassed with a mixture of 1% CO_2 and 99% O_2 to yield a pH of 8.0 and to provide oxygenation and good mixing by gas. The Ringer solution composition was: NaCl 133 mM, KCl 3.2 mM, $NaHCO_3$ 20 mM, $MgCl_2$ 1.4 mM, $CaCl_2$ 2.5 mM, KH_2PO_4 0.8 mM, glucose 20 mM (osmolarity: 315 mOsm kg^{-1}). The temperature of the bathing Ringer solution was kept constant at about 18°C.

Tissues were connected to an automatic short-circuit current device (DVC-1000, World Precision Instruments) by four Ag/AgCl electrodes (two voltage electrodes and two current

electrodes- EKVC-World Precision Instruments) that made contact with the bathing solutions via cartridges filled with Ringer solution in which 4% weight agar was dissolved.

The short-circuit current (I_{sc}) was measured by passage of sufficient current through Ag/AgCl electrodes to reduce the spontaneous transepithelial voltage (V_t) to zero (resistance of the chamber fluid was subtracted automatically). V_t was measured with respect to mucosal side (grounded). The preparations were kept open circuited during the experiments, except for a few seconds every 5 min for recording I_{sc}. The negative sign of I_{sc} indicates current flowing from the apical to the basolateral side; transepithelial conductance, g_t, was calculated according to Ohm's law (g_t =I/V). The transepithelial parameters were allowed to stabilize (about 30 min) before starting any experimental procedure.

All the drugs used were obtained from SIGMA (St Louis, MO). They were prepared as stock solutions in the appropriate solvent, according to manufacturer's instruction, and diluted in the experimental chamber in order to obtain the desired concentration. The amount of stock solution added ranged from 0.016 to 1.6 of the volume of the solution in the chamber. ET-1 (endothelin-1) was dissolved in distilled water. However, in reason of its limited lifetime in solution, long term storage was avoided. SNP (sodium nitroprusside), SIN (3-morpholinosydnonimine), L-NAME (Nω-nitro-L-arginine methyl ester) and phlorizin were dissolved in physiological saline, indomethacin and PGE_1 (prostaglandin E_1) in ethanol, bumetanide in DMSO. The final concentration of both ethanol and DMSO in the chamber was 0.1 %; preliminary experiments showed that it did not alter the control electrophysiological parameters. Endothelin was added to serosal or mucosal side (see Results section), bumetanide and phlorizin to the luminal side, the other drugs were added bilaterally. Except for endothelin, for which dose-response experiments were performed, the drug concentrations we used were those able to produce maximal effects of I_{sc} in our previous studies (Trischitta et al., 1992, 1996, 2007).

All data are expressed as mean ± SE. Data were normally distributed ($p<0.05$, Kolmogorov-Smirnov test). In each set of experiments each tissue served as its own control. Statistical analyses were performed using GraphPad Prism 5. The effect of the different treatments on control I_{sc} was evaluated by one way ANOVA. Dunnett 's multiple comparison test was applied for post hoc comparison. Differences were considered significant at $p<0.05$.

RESULTS

First of all dose-response experiments were performed in order to know the concentration of ET-1, an agonist of both ET_A and ET_B receptors, able to produce the maximal effect on I_{sc}. For this purpose progressively larger amounts of the substance were added to the serosal side. Following each addition I_{sc} reached a new steady state within about 20 min, thereafter the next higher concentration was added. A small increase of serosa negative I_{sc} was produced by the lower concentrations of the peptide (10^{-10} M and 10^{-9} M) but a decrease of serosa negative I_{sc} was produced by the higher concentrations (10^{-8} M and 10^{-7} M; Fig. 1). ANOVA showed a statistically significant effect of all ET-1 concentrations on I_{sc} ($P < 0.0001$; $F_{(4;20)} = 89.68$).

In another series of experiments the tissues were perfused with standard Ringer on both sides, when the transepithelial parameters had reached stable values (almost 30 minutes), glucose was omitted from the luminal solution and substituted with mannitol in order to not

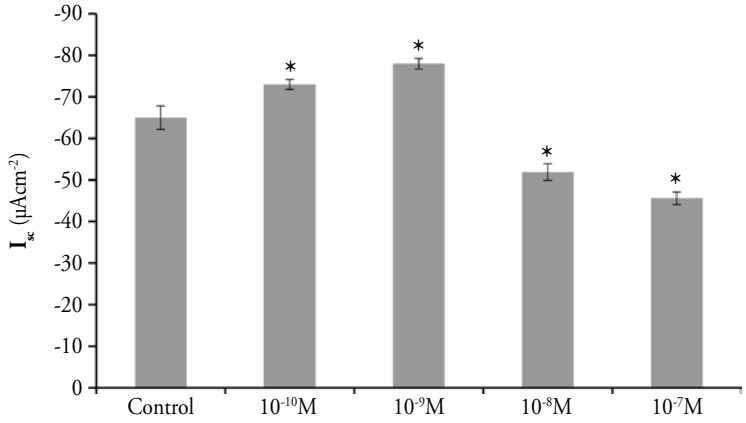

Fig. 1 – The effect of serosal endothelin (ET-1) on I_{sc} in a dose dependent fashion. The short circuit current (I_{sc}) is plotted against concentrations (in M) of ET-1. The values are means ± SE; number of experiments = 6; *p<0.05 vs control I_{sc}.

modify the osmotic pressure, then the dose response experiments were repeated. In the 3 experiments performed, the omission of glucose increased the serosa negative I_{sc} by 10 ± 3 μA (p<0.05), but did not modify the response of I_{sc} to the different concentrations of ET-1. As some glucose could remain in the unstirred layers of the epithelium (Barry & Diamond 1984) the experiments were repeated by omitting luminal glucose from the beginning of the experiments. In these experimental conditions neither 10^{-10} M nor 10^{-9} M ET-1 increased the serosa

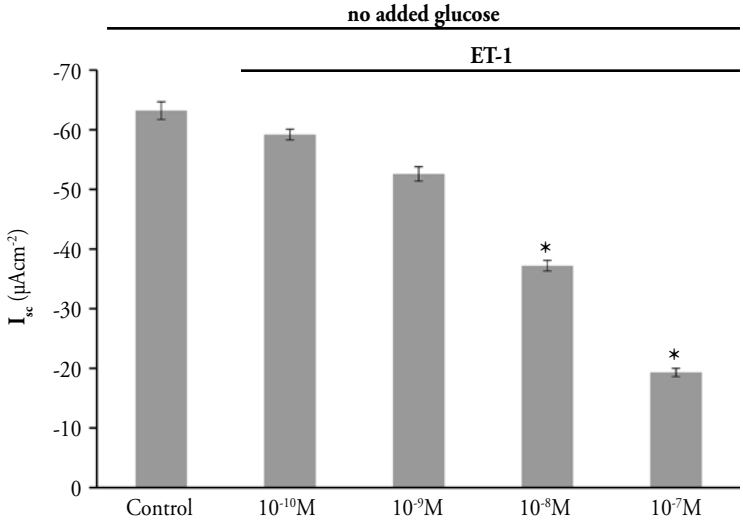

Fig. 2 – Effect on I_{sc} of ET-1, added at different concentrations, in the absence of luminal glucose (no added glucose). The value are means ± SE, number of experiments = 5 *p<0.05 vs control I_{sc}.

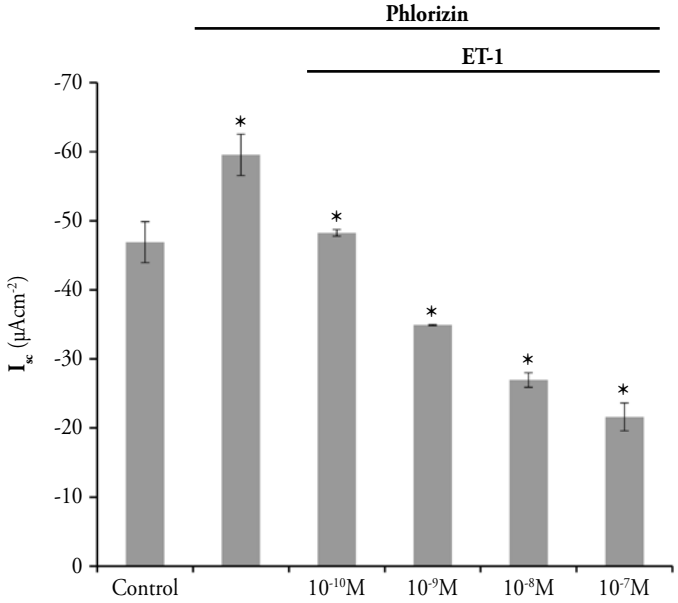

Fig. 3 – Effect on I_{sc} of ET-1, added at different concentrations, in tissues perfused with Ringer containing glucose and preincubated with luminal phlorizin (10^{-3} M). The values are means ± SE; number of experiments = 4 *p<0.05 vs control I_{sc}.

negative I_{sc}, indeed a small but decrease was observed (Fig. 2). ANOVA showed a statistically significant effect of ET-1 on I_{sc} ($P < 0.0001$; $F_{(4;20)} = 318.2$).

Time-matched control experiments (not shown), performed to verify whether the luminal absence of glucose produced per se an inhibition of I_{sc}, showed that the electrical parameter remained stable for the duration of the experiment (100 minutes). Dose-response experiments were repeated in tissues pre-incubated for 20 min with luminal phlorizin (10^{-3} M), a glycoside which specifically inhibits the activity of the Na^+/glucose transporter (Ehrenkranz et al., 2005), and perfused with standard Ringer (containing glucose). The inhibitor increased the basal serosa negative I_{sc} and prevented the increase in the serosa negative I_{sc} when 10^{-10} M and 10^{-9} M endothelin was added to the serosal bath (Fig. 3). ANOVA showed a statistically significant effect of ET-1 on I_{sc} ($P < 0.0001$; $F_{(5;25)} = 87.10$).

In the presence of phlorizin these peptide concentrations produced a significant decrease of the serosa negative I_{sc}, as observed in the absence of luminal glucose (Fig. 2).

ET-1 (10^{-7} M) was ineffective when added to the mucosal side, while the addition of 10^{-7} M of ET-1 to the serosal side produced an inhibition of I_{sc}, as observed in the cumulative dose-response experiments, without altering g_t (Tab. 1). However no concentration was able to affect the tissue conductance (not shown).

The luminal addition of 10^{-5} M bumetanide strongly inhibited I_{sc} (from 56.2±3.1 to 8.1± 0.5 μA; n=3; p<0.001), while the following addition of ET-1 (10^{-7} M) was ineffective (Fig. 4).

To verify the involvement of prostaglandins in the effect of ET-1, three series of experiments were performed. In the first series ET-1 was tested in the presence of indomethacin (10^{-4}

Tab. 1 – Effect on the transepithelial electrical parameters of endothelin (10^{-7} M) added to the luminal and serosal bath. Values are means ± SE. $\mathbf{V_t}$ = transepithelial voltage; $\mathbf{I_{sc}}$ = short circuit current; $\mathbf{g_t}$ = transepithelial conductance; **n** = number of tissue; **S** = serosal side; **M** = luminal side. The – sign of $\mathbf{V_t}$ indicates that the serosal side is negative when referred to the mucosal (grounded) side. The – sign of $\mathbf{I_{sc}}$ indicates current flowing from the mucosal to the serosal side. * p<0.05.

Experimental conditions	$\mathbf{V_t}$ (mV)	$\mathbf{I_{sc}}$ ($mAcm^{-2}$)	$\mathbf{g_t}$ ($mScm^2$)	n
Control	-5.7 ± 0.2	-58.3 ± 2.5	10.1 ± 0.2	6
Endothelin (M)	-5.5 ± 0.2	-56.6 ± 2.3	10.2 ± 0.3	
Endothelin (S)	-3.5 ± 0.2	-35.3 ± 1.2*	10 ± 0.5	

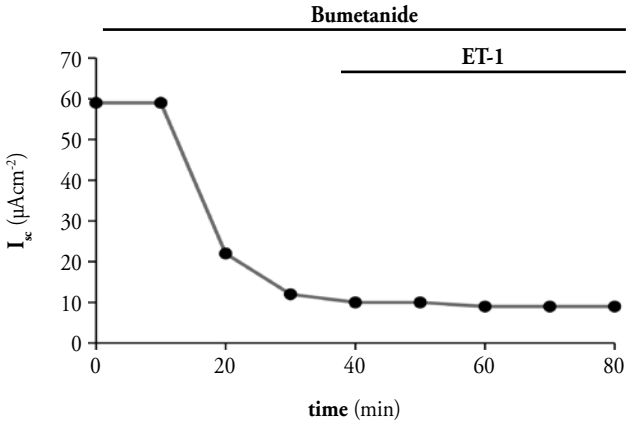

Fig. 4 – Time course of the effect of luminal bumetanide (10^{-5} M) and of the following addition of ET-1 (10^{-7} M). A typical experiment representative of 3.

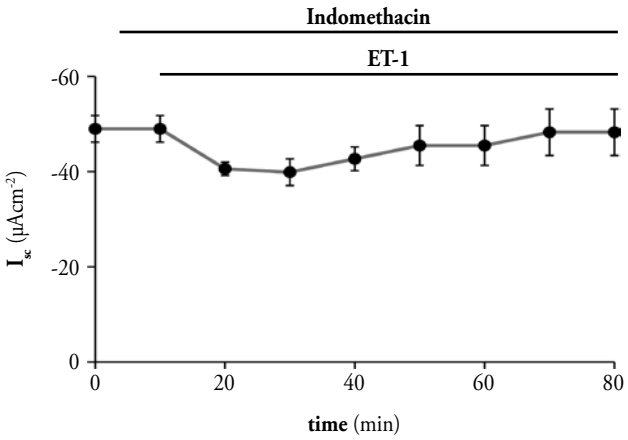

Fig. 5 – Time course of the effect of ET-1 (10^{-7} M) in tissues preincubated with bilateral indomethacin (10^{-4} M). The data are means ± SE of 4 experiments.

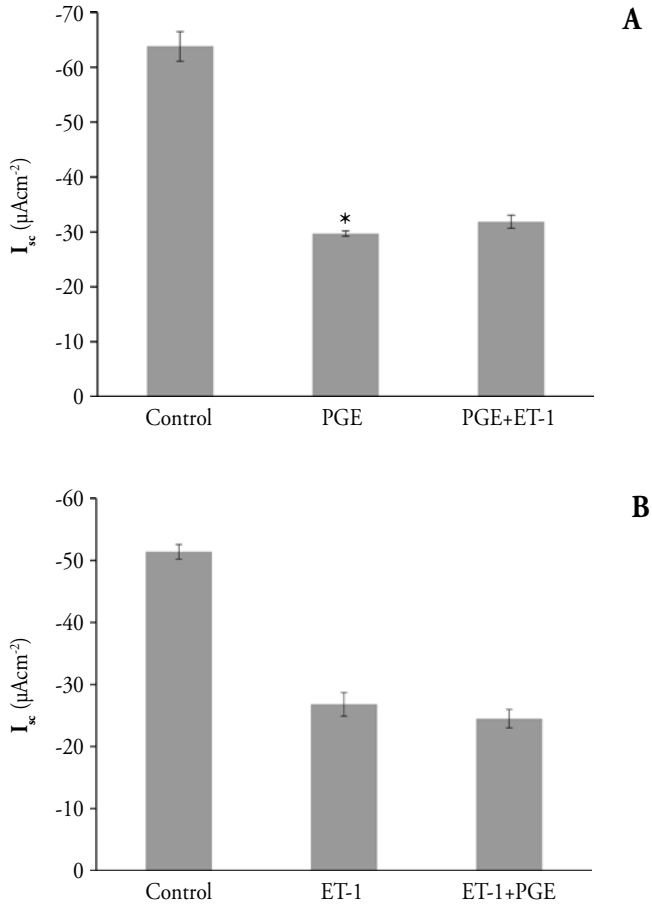

Fig. 6 – A) Effect on I_{sc} of PGE_1 (10^{-5} M) and of the following addition of ET-1(10^{-7} M). The values are means ± SE, number of experiments = 5 *p<0.05 vs control I_{sc}; B) Effect on I_{sc} of ET-1 (10^{-4} M) and of the following addition of PGE_1 (10^{-5} M). The values are means ± SE, number of experiments = 5 *p<0.05 vs control I_{sc}.

M), a cyclo-oxygenase inhibitor. When the control I_{sc} had reached stable values, the drug was added to both the luminal and the serosal bath. After 20 min of incubation (during which I_{sc} was not modified, not shown) ET-1 (10^{-7} M) was added to the serosal bath. As Fig. 5 shows, in these experimental conditions, ET-1 produced a transitory decrease of I_{sc} followed by a return to the baseline. In the second series of experiments, ET-1 was tested in the serosal presence of PGE_1 (10^{-5} M). As Fig. 6A shows the prostanoid reduced I_{sc} by more than 50%. ANOVA showed a statistically significant effect of PGE_1 on I_{sc} ($P < 0.0001$; $F_{(2;10)} = 261.0$). The following addition of 10^{-7} M ET-1 was ineffective. In the third series of experiments, PGE_1 was added to the serosal side after ET-1 had elicited its maximal reduction of I_{sc}. ANOVA showed a statistically significant effect of ET-1 on I_{sc} ($P < 0.0001$; $F_{(2;10)} = 148.6$). It is evident that in these experimental conditions PGE_1 produced no significant effect on I_{sc} (Fig. 6B). Both PGE_1 and ET-1 produced their maximal effect on I_{sc} after 20 minutes.

DISCUSSION

To our knowledge this is the first paper suggesting a role of endothelin in the modulation of ion transport in the intestine of a sea-water adapted fish. We showed that ET-1 exerts a dual effect on the short circuit current in the isolated middle intestine of the eel, *A. anguilla*. In fact, we observed a small but significant increase of the serosa negative I_{sc} when the peptide was added to the serosal bath at concentrations as low as 10^{-10} M and 10^{-9} M, while a decrease of the electrophysiological parameter was observed when the peptide was added at higher concentrations (Fig. 1). We hypothesized that the lower concentrations of ET-1 inhibited the electrogenic Na^+ dependent-glucose transport. This hypothesis is reasonable because the inhibition of the electrogenic transporter would produce an hyperpolarization of the cell, thus increasing the driving force for Cl^- exit through Cl^- conductive pathways, already demonstrated in the *A. anguilla* enterocyte (Trischitta et al., 1992). This would produce an increase of the serosa negative I_{sc}, that in the control condition is due to the net Cl^- absorption (Trischitta et al., 1992). An inhibitory effect on Na^+-glucose co-transport has been already observed in *in vitro* experiments performed with ET-1 and ET-3 in human and rat intestine respectively (Kuhn et al., 1997; Gonzalez Bosc et al., 2001). The observation that ET-1 (10^{-10} M and 10^{-9} M) was not able to increase I_{sc} in the absence of luminal glucose (Fig. 2), supported our original hypothesis. It was strengthened by the experiments with phlorizin, depicted in Fig. 3. In fact the preincubation of the intestine with the glycoside in the luminal bath increased I_{sc}, as predicted by the inhibition of the electrogenic Na^+-glucose co-transport, while the following addition of the lower concentrations of ET-1 did not produce the increase of I_{sc} observed in the control conditions. However, flux studies should be necessary to confirm both the luminal presence of Na^+-glucose co-transport and the effect of endothelin on this transporter.

In addition, we suggest a role of the peptide in the modulation of salt transport. This modulation has been already indicated for other osmoregulatory organs (Tomita et al., 1993; Evans & Gunderson, 1999; Plato et al., 2000; Evans et al., 2004; Hydman & Evans 2007, 2009). It is likely that the target of the peptide is the transcellular pathway. An effect on the paracellular pathway seems ruled out by the observation that ET-1 did not modify tissue conductance (Tab. 1), that in the intestine is dominated by the conductance of the tight junctions, because of the leaky nature of the epithelium (Powell, 1981).

There are many publications documenting that some ligands regulate epithelial functions by acting on receptors located on the luminal membrane (see Karnaky, 1998). The lack of effect of ET-1, when added to the luminal side (Tab. 1), suggests that the endothelin receptors are located only on the basolateral membrane of *A. anguilla* enterocyte.

It is conceivable that ET-1 exerts its modulatory effects of salt transport mainly stimulating the production of prostaglandins on the basis of the observations discussed below. When the synthesis of the prostanoid was inhibited by indomethacin, ET-1 produced only a transitory reduction of I_{sc}, that rapidly returned to the baseline (Fig. 5). In addition, the pretreatment of the tissues with PGE_1 decreased I_{sc}, while the following addition of ET-1 was ineffective (Fig. 6A). Vice versa PGE_1, when added in tissues in which I_{sc} was inhibited by ET-1, was without effect (Fig. 6B).

Our previous studies showed that nitric oxide modulates ionic transport in the isolated intestine of the *A. anguilla* (Trischitta et al., 2007). An endothelin to NO, prostanoid signal-

ling axis was demonstrated in the opercular epithelium of the euryhaline fish *F. heteroclitus* (Evans et al., 2004). In order to clarify whether ET-1 effects were mediated also by NO, we tested the peptide in the presence of NO-donors such as SNP (10^{-3} M) and SIN-1 (10^{-4} M) and in the presence of L-NAME (10^{-3} M) to inhibit the nitric oxide synthase. Unfortunately, we obtained contradictory results (not shown) in the experiments performed with different animals, even if they were in the same life stage (yellow) and were kept in identical laboratory conditions for at least 3 weeks for acclimation. At present we do not have rational explanations for this individual variability. Thus the involvement of NO in endothelin signalling pathways will be addressed in future studies.

We suppose that endothelin plays a role during acclimation to fresh water. In the intestine of the *A. anguilla*, like in other marine teleosts, absorption of NaCl is mediated by the luminal $Na^+/K^+/2Cl^-$ co-transport (Trischitta et al., 1992). It is known that salt absorption is the means by which marine fishes gain water to compensate the osmotic loss into the hyperosmotic external medium. Our previous data suggested that the basic transport properties of the luminal membrane of the intestinal cell of *A. anguilla* do not change during the adaptation from seawater (SW) to fresh water (FW) or viceversa. In fact the $Na^+/K^+/2Cl^-$ co-transport was operating in both SW and FW adapted fish (Trischitta et al., 1992). On the other hand, in other euryhaline fishes luminal $Na^+/K^+/2Cl$ is expressed only during SW adaptation (Lau, 1985; Aguenaou et al., 1989). The presence of the same transport mechanism in both SW and FW *A. anguilla* could explain the fact that these fish adapt quickly to abrupt changes of salinity. In other teleosts (e.g. trout), exhibiting changes of the transport properties of the luminal membrane of the enterocyte, the adaptive process requires time to occur. It was suggested (Trischitta et al., 1992) that the co-transport system undergoes a partial deactivations during FW adaptation of the *A. anguilla*. Indeed, a high activity of the $Na^+/K^+/2Cl^-$ co-transport that produces salt absorption followed by water absorption would be maladaptive during the transfer to FW. So it is conceivable that endothelin could act as a paracrine agent that reduces the activity of the triporter during the acute transfer to fresh water. This hypothesis is strengthened observing that endothelin was ineffective when the activity of the $Na^+/K^+/2Cl^-$ was blocked by luminal bumetanide (Fig. 4), a well known inhibitor of this transport mechanism. The following literature data are in support of our conclusion: 1) an inhibition of $Na^+/K^+/2Cl^-$ co-transport has been already showed in the thick ascending limb of mammalian kidney (Herrera et al., 2009), an epithelium exhibiting transport mechanisms similar to those operating in the intestine of marine teleosts; 2) a role of endothelin during acute acclimation was proposed by Hyndman and Evans (2007). It was shown that acute acclimation to FW increased the levels of endothelin and endothelin converting enzyme-1 in the fish gill of the euryhaline killifish, *F. heteroclitus*, and it was proposed that this increase could inhibit net chloride transport, helping the fish retain ions.

The inhibition of the luminal Na^+-glucose of *A. anguilla* intestine by endothelin, we suggested on the bases of our findings, could have the role to further reduce absorption of solutes and hence of water during the acute transfer to FW. Moreover, the enterocyte has a reduced requirement of metabolic substrate for the active Cl^- absorption.

In conclusion this paper suggests that during osmotic stress endothelin affects the transport properties of the isolated *A. anguilla* intestine by acting on basolateral located receptors by means of a mechanism that probably involves the release of prostaglandins. Indeed, the im-

portance of prostanoid in tissues facing osmotic stress has been already outlined in both mammalian (Hao et al.,1999; Hao et al., 2000) and fishes (Hyndman & Evans, 2007).

Our hypotheses could be confirmed determining whether endothelin is expressed in the *A. anguilla* intestine and whether the level of expression is regulated during the acute and chronic acclimation.

Further studies relating the expression of endothelin and its receptors will be necessary.

RIASSUNTO

Ruolo dell'endotelina sui trasporti intestinali dell'Anguilla *Anguilla anguilla* adattata all'acqua di mare

L'Endotelina (ET), un peptide di segnalazione paracrina, originariamente identificato come potente vasocostrittore, è oggi noto come un modulatore di una vasta gamma di attività biologiche non vascolari, tra cui il trasporto di ioni nei tessuti di organi osmoregolatori, quali l'epitelio renale dei mammiferi, l'epitelio opercolare dei teleostei, l'epitelio della ghiandola rettale degli elasmobranchi. Gli effetti di ET sono mediati da due distinti recettori denominati ET_A e ET_B. In questo studio è stato testato l'effetto di ET sul trasporto transepiteliale di ioni nell'intestino isolato di *Anguilla anguilla* adattata all'acqua di mare, perché l'intestino gioca un importante ruolo nella osmoregolazione dei teleostei marini. L'intestino isolato è stato montato nelle camerette di Ussing ed è stata applicata la tecnica della cortocircuitazione. Esperimenti dose-risposta dimostrano che l'aggiunta di ET-1 dal lato serosale, agonista sia dei recettori ET_A che ET_B, a basse concentrazioni (10^{-10} M; 10^{-9} M) determinava un aumento della corrente di cortocircuito negativa del lato serosale (I_{sc}), generata dall'assorbimento netto di Cl^- in condizioni di controllo. Concentrazioni più elevate (10^{-8} M; 10^{-7} M) producevano invece una riduzione di I_{sc}. Il pretrattamento con florizina, inibitore del trasportore Na^+-glucosio o l'assenza di glucosio nella soluzione luminale bloccava l'aumento della I_{sc} causato dalle basse concentrazioni del peptide. ET era inefficace in tessuti pretrattati con bumetanide aggiunta alla soluzione luminale, un diuretico dell'ansa che notoriamente inibisce il cotrasportatore $Na^+/K^+/Cl^-$. Tale trasportatore rappresenta il più importante meccanismo di assunzione dei sali operante sulla membrana apicale dell'enterocita dell'anguilla. L'effetto del peptide sulla I_{sc} era inibito in tessuti preincubati con indometacina, inibitore della ciclossigenasi, alla concentrazione di 10^{-4} M. Inoltre PGE_1 (10^{-5} M) determinava una diminuzione di I_{sc} mentre l'aggiunta successiva di ET-1 era inefficace e viceversa. I nostri risultati suggeriscono che nell'intestino di anguilla, l'endotelina modula sia il trasporto di glucosio che di ioni; tale ruolo dipende probabilmente dalla produzione di prostaglandine e potrebbe essere importante nell'acclimatizzazione dell'animale ad ambienti con diversa salinità.

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