TRANSEPITHELIAL POTENTIAL DIFFERENCE OF THE INTESTINE AND GALLBLADDER OF *Hoplias malabaricus*, A FRESHWATER TELEOST. EFFECT OF UROTENSINS I AND II

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RESUMO

Este trabalho analisou o efeito da injeção da urotensina I (UI) e da urotensina II (UII) na estabilização da diferença de potencial transepitelial (DPT) do intestino médio, reto e vesícula biliar de *Hoplias malabaricus,* para verificar se o transporte de íons nestes órgãos é afetado "in vivo" por estes neuro-hormônios. A DPT do intestino médio, reto e vesícula biliar é serosa positiva, e seu valor permaneceu estável desde a primeira medida. A injeção de ambas urotensinas não modificou a estabilização da DPT do intestino médio e reto quando comparada com o grupo injetado com salina. A injeção de UI aumentou a DPT da vesícula biliar no início (0-10 min) do período de estabilização e no intervalo de 20-30 min do período de estabilização quando os peixes foram sacrificados 2 h e 4 h após a injeção, respectivamente, em relação ao grupo injetado com salina. A injeção de UII aumentou a DPT da vesícula biliar somente no início (tempo 0) do período de estabilização em relação ao grupo tratado com salina sacrificado 2h após a injeção. Não houve alterações na DPT dos órgãos estudados quando os peixes foram sacrificados 4 h após a injeção da UII. Este estudo confirma a hipótese de que a UI e a UII poderiam participar na regulação da composição da bile dos peixes, uma vez que a injeção de ambos neuro-hormônios alterou a DPT da vesícula biliar de *H. malabaricus*.

SUMMARY

This study analyzed the effect of the injection of urotensin I (UI) and urotensin II (UII) on the stabilization of the transepithelial potential difference (TPD) of the medium intestine, rectum, and gallbladder of *Hoplias malabaricus* to investigate if the transport of ions in these organs is affected "in vivo" by these neurohormones. The TPD of the medium intestine, rectum and gallbladder was serosa positive, and remained constant since the first measurement. The injection of both urotensins did not alter the stabilization of the TPD of the medium intestine and rectum when compared with the saline-injected group. The injection of UI increased the TPD of the gallbladder in the beginning (0-10 min) of the stabilization period and in the interval of 20 - 30 min of the stabilization period when fishes were killed 2h and 4h after the injection, respectively, in relation to saline-injected group. The UII injection increased the TPD of the gallbladder only in the beginning (time 0) of the stabilization period in relation to saline when fishes were killed 2h after the injection. No changes in the TPD of the studied organs were detected when fishes were killed 4h after the injection of UII. This study confirms the hypothesis that UI and UII can participate in the regulation of the composition of the bile of fishes, since the injection of both hormones altered the TPD of the gallbladder of *H. malabaricus*.

INTRODUCTION

The caudal neurosecretory system is composed of spinal-cords neurons whose axons end in a neurohemal area, the urophysis, and produces two neurohormones: urotensins I (UI) and II (UII) (Larson and Bern, 1987). Since the organization of this system is comparable to the hypothalamo-neurohypophysial neurosecretory system, a relationship with osmoregulation of teleosts has been repeatedly suggested (Bern, 1985). Injection of urophysial extracts increased the ionic concentration of the plasma of seawater-adapted *Gillichthys mirabilis* (Bern and Nishioka, 1979) and freshwater-adapted *Ophiocephalus maculatus* (Woo *et al.*, 1980). Besides, experiments "in vitro" demonstrated that UI or UII altered the flow of water and ions in the intestine of freshwater-adapted *Oreochromis mossambicus* (Mainoya and Bern, 1982), 5% seawater-adapted *G. mirabilis* (Loretz et al., 1983), and *Anguilla anguilla* (Baldisserotto and Mimura, 1996).

Recent experiments with the freshwater teleost *Hoplias malabaricus* demonstrated that UI increases the flow of water of the gallbladder "in vitro" and its injection increased the levels of Na^+ of the plasma and gallbladder bile (Baldisserotto *et al.*, 1996-a). In the same species UII changed the flow of water and/or ions in the medium intestine, gallbladder, and urinary bladder "in vitro" (Baldisserotto et al., 1996-b). Consequently, this study analyzed the effect of the injection of UI and UII on the stabilization of the transepithelial potential difference (TPD) of the medium intestine, rectum, and gallbladder of *H. malabaricus* to investigate if the transport of ions in these organs is affected "in vivo" by these neurohormones.

MATERIAL AND METHODS

Specimens of *Hoplias malabaricus* (Erythrinidae) (200 - 500 g) were captured with nets placed in ponds situated on the campus of the Federal University of Santa Maria (UFSM), Santa Maria, Southern Brazil. Fishes were maintained in fasting during 3 days, according to Baldisserotto *et al.* (1990) prior to experiments. Fishes were divided in seven groups: control, injected with saline, 200 mU/kg UI (500 ng/kg, Sigma) or 200 mU/kg UII (272 ng/kg, Sigma) sacrificed 2 or 4 h after the treatments. The abdominal cavity was opened to expose the medium intestine, rectum (names of these portions of the intestine are according to description of Menin, 1988) and gallbladder. These organs were separated and cleaned with a Ringer-bicarbonate solution that contained (in mM): NaCl 120.0; KCl 5.5; $MgSO_4.7H_2O$ 1.45; $CaCl_2.2H_2O$ 3.0; $NaHCO_3$ 10.0; glucose 2.5; adjusted to pH 7.0 with HCl 10 M. Uneverted sacs of each segment were prepared, and 0.3-0.5 ml of the Ringer-bicarbonate solution and a 3% agar-saline bridge were introduced in each sac. The sacs were immersed in the same Ringer-bicarbonate solution. Stabilization of the TPD (expressed in mV with the polarity of the serosal relative to the mucosal side) was measured during 90 min with a multimeter plugged to a pair of calomel electrodes connected to the sacs by the agar-saline bridges.

All values were expressed as the mean ± SE. Comparisons of the TPD of the different groups were assessed using the one-way analysis of variance

(Microstat program, Ecosof Inc.) and Student-Newman-Keuls test. The minimum significant level was $p < 0.05$.

RESULTS

The TPD of the medium intestine, rectum and gallbladder was serosa positive, and a regular state was observed since the first measurement. After 50 min the TPD of the medium intestine was reduced until the last measurement (90 min), and since no additional measurements were made, it was not possible to determine if a new regular state would be obtained (figure 1). The TPD of the rectum presented a cyclic variation ranging from approximately 2.0 to 3.0 mV within 50 min (figure 2).

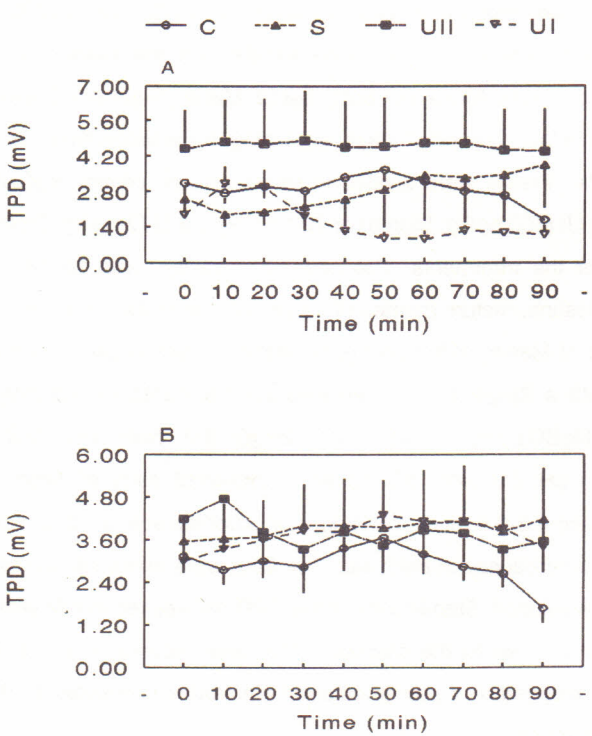

Figure 1 - Stabilization of TPD of the medium intestine of *Hoplias malabaricus*. A - fishes sacrificed 2 h after injection; B - fishes sacrificed 4 h after injection. C - control; S - saline-injected; UII - UII-injected; UI - UI-injected.

The injection of both urotensins and saline did not change the stabilization of the TPD of the medium intestine when compared with that of the control group (figure 1). An increase of the TPD of the rectum in relation to the control group occurred only in the first measurement (time 0) of fishes killed 2 and 4 h after the injection of UI and saline. UII increased the TPD of the rectum in the first measurement of fishes killed 2 h after the injection, in relation to the control group. However, this neurohormone did not change the TPD of fishes killed 4h after injection or to the saline-injected group (killed 2 or 4 h after injection) in relation to the control group (figure 2).

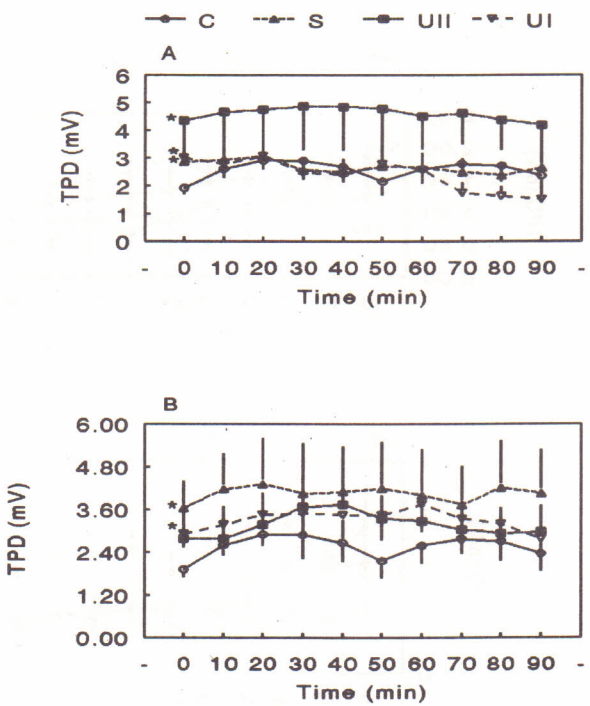

Figure 2 - Stabilization of TPD of the rectum of *Hoplias malabaricus*. A - fishes sacrificed 2 h after injection; B - fishes sacrificed 4 h after injection. C - control; S - saline-injected; UII - UII-injected; UI - UI-injected.
significantly different from control * $p < 0.05$

Injection of UI increased the TPD of the gallbladder at the beginning (0-10 min) of the stabilization period when compared to the control and saline-injected groups killed 2 h after injection. Four hours after application of UI there was an increase of TPD during the interval of 10 - 30 min of the stabilization period with relation to that of the control, and an increase during the interval of 20 - 30 min when compared to the saline-injected group. Saline injection decreased the TPD in relation to control group in the first measurement of the stabilization period of fishes killed 2 h after injection, and increased the TPD during the first 10 min of the stabilization period of fishes killed 4 h after injection. UII injection increased the TPD of the gallbladder only at the beginning (time 0) of the stabilization period in relation to saline of fishes killed 2 h after injection. No changes in the TPD of the gallbladder were detected in fishes killed 4 h after injection of UII (figure 3).

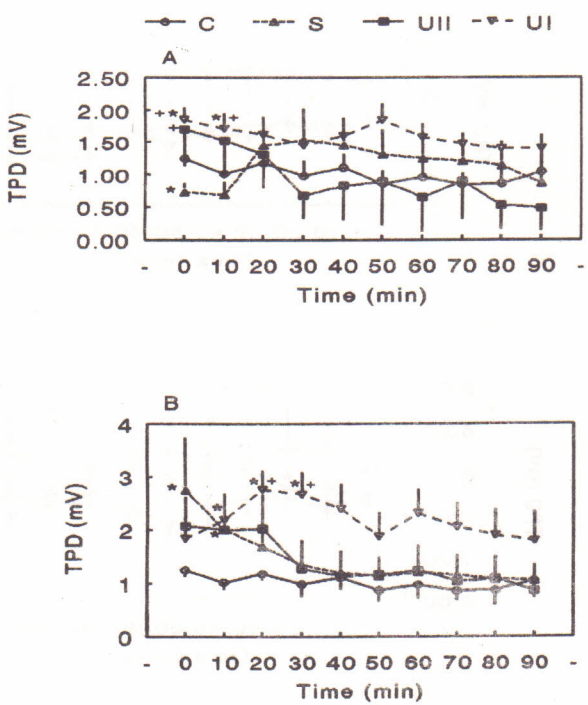

Figure 3 - Stabilization of TPD of the gallbladder of *Hoplias malabaricus*. A - fishes sacrificed 2 h after injection; B - fishes sacrificed 4 h after injection. C - control; S - saline-injected; UII - UII-injected; UI - UI-injected.
significantly different from control * $p < 0.05$
significantly different from saline-injected + $p < 0.05$

DISCUSSION

The studied species (*H. malabaricus*) presented a TPD of the medium intestine and rectum similar to that of the intestine of typical freshwater fish, where the serosa is positive in relation to the mucosa (Ferraris and Ahearn, 1984). In addition, the stabilization period of the medium intestine and rectum of *H. malabaricus* is always positive (figures 1 and 2). Nevertheless, in the intestine of the freshwater-adapted euryhaline fish *Platichthys flesus* (Smith *et al.*, 1975) and *Anguilla anguilla* (Baldisserotto and Mimura, 1994) at the beginning of the experiment the serosa is slightly positive, and becomes negative with time. When *P. flesus* (Smith *et al.*, 1975) and *A. anguilla* (Simonneaux *et al.*, 1987; Trischitta *et al.*, 1992) are seawater-adapted, the pattern is different: the serosa is always negative during TPD stabilization period, but its negativity reduces with time. These data suggest that the pattern of stabilization of the TPD of the intestine of teleosts "in vitro" could be related to the capacity of adaptation of the fish to waters of different salinity (fresh and seawater): freshwater-adapted euryhaline fish would be serosa positive, and become negative when seawater-adapted. The alteration of the value of the TPD of the intestine during the stabilization period indicates that this organ quickly attain a great part of its adaptation to a new environment. On the other hand, stenohaline fish would only have small changes of TPD and the value would always be serosa positive during the stabilization period when obtained from freshwater and always serosa negative when obtained from seawater. The fact that *H. malabaricus* lives only in freshwater (Azevedo and Gomes, 1943) and that the TPD of the intestine of *Gobius maderensis* (Badia and Lorenzo, 1982) and *Blennius parvicornis* (Bolaños and Lorenzo, 1984) (stenohaline seawater fishes) is always serosa negative during stabilization period reinforces this hypothesis.

Experiments "in vitro" with the intestines of freshwater-adapted teleosts verified that UII stimulated absorption of Na^+ and Cl^- in *G. mirabilis* (Loretz *et al.*, 1983), and absorption of water and Mg^{++}, and inhibited the secretion of Ca^{++} in the medium intestine of *H. malabaricus* (Baldisserotto *et al.*, 1996-b). On the other hand, UI inhibited absorption of water, Na^+, and Cl^- in freshwater-adapted *O. mossambicus* (Mainoya and Bern, 1982). However, the

injection of both urotensins did not alter the stabilization of the TPD of the medium intestine and rectum of *H. malabaricus* in relation to saline injection. Probably the effect of UI and UII "in vivo" in the intestine of this species is supplanted by other hormones.

The inhibition of Ca^{++} secretion "in vitro" in the gallbladder of *H. malabaricus* by UII (Baldisserotto *et al.*, 1996-b) could explain the increase of the TPD of the stabilization period of this organ (time 0) observed when fishes were killed 2 h after injection of UII. The injection of UI increased the level of Na^{+} in the gallbladder bile of *H. malabaricus* (Baldisserotto *et al*, 1996-a). Consequently, the increase of the TPD of the stabilization period of the gallbladder detected when fishes were killed 2 (0-10 min) and 4 h (20-30 min) after injection of UI probably is not due to stimulation of Na^{+} absorption by this organ. Since there are not other studies of the effect of urotensins on the gallbladder, additional investigations must be done to determine which transport systems of this organ are altered by UI and UII.

Injection of saline altered the TPD at the beginning of the stabilization period of the rectum and gallbladder when fishes were killed 2 and 4 h after injection in relation to control. The stress of handling or injection could provoke the liberation of some substance that would alter the TPD of these organs, since injection of saline also led to increases in plasma ions in *G. mirabilis* (Fryer *et al.*, 1978), *O. maculatus* (Woo *et al.*, 1980), and *H. malabaricus* (Baldisserotto *et al*, 1996-a).

Based on the results obtained, it is possible that the increase of the level of Na+ in the plasma of *H. malabaricus* induced by injection of UI (Baldisserotto *et al*, 1996-a) is not due to alterations of the flow of ions in the medium intestine or rectum of this species. Probably this effect is due to some change in the functioning of the urinary bladder or the gills. This study also confirms the hypothesis that UI (Baldisserotto *et al*, 1996-a) and UII (Baldisserotto *et al*, in 1996-b) could participate in the regulation of the fish bile composition, since in *H. malabaricus* both neurohormones altered the stabilization of TPD of the gallbladder.

ACKNOWLEDGMENTS

The authors thanks Mr. Florindo Duarte (UFSM) for his technical assistance on fish collection and Dr. Mário de la Rue (UFSM) for the critical analysis of this article.

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