INFLUENCE OF UROTENSIN II ON PLASMATIC AND BILIAR IONIC AND OSMOTIC CONCENTRATION OF *Prochilodus scrofa* Steindachner, 1881.

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ABSTRACT

The present work focuses on the action of Urotensin II at two concentrations, 10^{-7} M and 10^{-8} M, on the ionic gall bladder and plasma and osmotic contents, of the freshwater teleost fish *Prochilodus scrofa*. Animals collected at Cachoeira de Emas (Pirassununga-S.P.) were fasted for 3 days before experiments. The results show: 1) Na^+, K^+, Ca^{++} and Mg^{++} contents were higher in bile than in plasma. Cl^- content was higher in plasma than in bile. These relations are usually seen in teleosts; 2) UII at both concentrations decreased Na^+ and K^+ biliar content; 3) UII injections caused no alteration in biliar Ca^{++}, Mg^{++}, Cl^- and in osmotic concentration; 4) 10^{-8} M UII induced decrease in Mg^{++} and Cl^- plasma contents; 5) UII injections caused no alterations in plasma Na^+, K^+ and Ca^{++} contents or in osmotic concentration; 6) Control females have biliar Na^+ higher than experimental ones and males injected with UII 10^{-7} M. Control females have K^+ higher than experimental ones; 7) These results suggest that UII may play an important role in *P. scrofa* osmoregulation and that it's gall bladder probably possesses receptors for this peptide.

Key-words: bile, ionic, osmotic, plasma, *Prochilodus scrofa*, teleost, urotensin II

RESUMO

Influência de concentração de urotensina ii nos teores ionos-osmóticos e biliateres de *Prochilodus scrofa* Steindachner, 1881

No presente trabalho procurou-se determinar a ação da Urotensina II em duas concentrações, 10^{-7}M e 10^{-8}M, nos teores iono-osmóticos plasmáticos e biliares do peixe dulciaquícola *Prochilodus scrofa*. Os animais coletados na Cachoeira de Emas (Pirassununga-SP) foram mantidos em jejum por 3 dias antes do início dos experimentos. Destes estudos foi visto que: 1) Os teores de Na^+, K^+, Ca^{++} e Mg^{++} são mais elevados na bile que no plasma e o teor de Cl^- plasmático é mais elevado que na bile. Estas relações são geralmente observadas na maioria dos teleósteos estudados; 2) A UII nas duas concentrações utilizadas causou diminuição dos níveis biliares de Na^+ e K^+; 3) Administração de UII não causou alterações nos níveis de Ca^{++}, Mg^{++}, Cl^- e concentração osmótica na bile.; 4) UII na concentração de 10^{-8}M causou queda nos níveis plasmáticos de Mg^{++} e Cl^-; 5) Administração de UII não causou

alterações nos teores de Na^+, K^+ e Ca^{++} plasmáticos, nem mesmo da concentração osmótica; 6) Fêmeas do grupo controle tem teor de Na^+ biliar mais elevado que aquelas dos grupos experimentais e machos injetados com UII na concentração de 10^{-7}M. Fêmeas do grupo controle tem níveis de K^+ mais elevados que aquelas dos grupos experimentais; 7) Estes resultados sugerem que a UII seja um hormônio importante na osmorregulação da espécie estudada e que a parede da vesícula biliar apresente receptores para este peptídeo.

Palavras-Chave: bile, iônica, osmótica, plasma, *Prochilodus scrofa,* teleósteo, urotensina II.

INTRODUCTION

Freshwater teleosts have blood osmotic concentration higher than surrounding water. These animals are therefore hyperosmotic in relation to the water in which they live, tending to an osmotic influx of water and efflux of ion, by diffusion, mainly across the opercular membrane.

In fishes, several hormones control osmoregulation, including urotensins. Urotensins I and II (UI and UII) are synthethyzed at caudal neurosecretory system and have influence on osmoregulatory organs. The peptides action may be different in each one, in most cases is still unknown.

Caudal neurosecretory system synthesize and release the two peptides. It's located at the posterior spinal cord, and are only found in teleosts. It consists of secretory neurons (Dahlgren-Speidel cells) located at the end of spinal cord, whose axons end at a neuro-hemal organ, the urophysis, located in a depression (urophysial fossa), of the last caudal vertebra.

UI is a 44 aminoacid peptide, and its aminoacid sequence is similar to that of CRH (corticotropin-releasing hormone) of mamiferous and was isolated in *Catostomus commersoni* (Lederis *et al.*, 1982), *Cyprinus carpio* (Ichikawa *et al.*, 1982) and *Hypoglossoides elassodon* (McMaster *et al.*, 1987).

UII is a dodecapeptide determined in *Gillichthys mirabilis* (Pearson *et al.*, 1979, 1980); *Catostomus commersoni,* where two forms of this peptide were identified (Lederis *et al.*, 1981; McMaster & Lederis, 1983) three forms in *Cyprinus carpio* (Ichikawa *et al.*, 1984). It was also identified in *Acypenser transmontanus* (Oka *et al.*, 1989), *Platichthys flesus* (Conlon *et al.*,1990), *Acypenser ruthenus* (McMaster *et al.*, 1992), *Scyliorhinus canicula* (Conlon *et al.*, 1992), *Oncorhynchus mykiss* e *Raja rhina* (Waugh & Conlon, 1993). However, the physiological function of these various forms is unknown, as already observed by Bern (1985).

UII has an amino acid sequence similar to that of somatostatin and both inhibit prolactin release "in vitro" in tilapia (Grau *et al.*, 1982). This peptide has a vasopressor action in *Anguilla rostrata* (Chan, 1975) and contract smooth muscles, like the urinary bladder in *Salmo gairdnerii* (now *Oncorhynchus mykiss*) (Lederis, 1969,1970a) and *Gillichthys mirabilis* (Loretz & Bern, 1981), of oviduct and the ovary in *Lebistes reticulatus* (Lederis, 1970b), of rectum in *Gillichthys mirabilis* (Lederis *et al.*, 1971) and the spermatic duct in *Gillichthys mirabilis* (Berlind, 1972).

Urophysectomy decreases urinary flux in *Carassius auratus* (Turtle. 1974 apud Bern & Nishioka, 1979) and in the eel decreases potassium, calcium and magnesium excretion (Chester Jones *et al.*, 1969b).

Mimura (1988) observed an increase in the plasmatic chloride level in *Rhamdia sebae* urophysectomized, while in control fishes and urophysectomized that were injected with urophysary extract, the chloride level decreases.

Urophysary extract can cause a lot of alterations in animals, such Na^+ influx at opercular membrane of *Caurassius auratus* (Maetz *et al.*, 1964) and natriuresis in eels (Bern *et al.*, 1967). In *Anguilla japonica*, extracts cause glomerular diuresis and increase chloride and sodium level, in the same way that plasmatic osmolality in freshwater acclimated animals, and can cause decrease of Cl^-, Na^+ and K^+ level, when those animals are acclimated to seawater at 40‰ (Woo *et al.*, 1980).

There are a lot of organs and hormones involved in osmoregulation in fishes. Recently, a lot of attention was turned to gallbladder role in this process. Diamond (1962a,b,c) studied water and ions transport across gallbladder of *Rutilus rutilus*, a freshwater teleost, and in his studies he emphasized the importance of this organ in osmoregulation.

Hirano & Bern (1972) showed that there is no difference in the absorption rate of water and ions in the isolated gallbladder of freshwater and seawater fishes. They observed that the absorption capacity is not altered by cortisol or prolactin treatment.

Concerning influences of urophysary hormones in the gallbladder transport, little information is available so far. Mimura & Baldisserotto (1989) observed increase in mucosal-serosal potassium and magnesium transport; water serosal-mucosal, and inhibition of calcium transport in the gallbladder epithelium, influenced by urophysary extracts, in *Synbranchus marmoratus*. In *Hoplias malabaricus*, UII increases water influx in gallbladder and intestine (Baldisserotto *et al.*, 1990a).

The present work focuses on the action of synthetic urotensin II, at osmotic and ionic concentration in the plasma and bile of a native, tropical teleost specie, collected in natural habitat, the "curimbata", and try to make relations between these parameters and sex of the animals.

MATERIALS AND METHODS

The animals used were freshwater teleost fish, *Prochilodus scrofa* Steindachner, 1881 (Osteichthyes, Characiformes, Prochiolodontidae). There were nine captures, between november/92 and december/93. They were captured, in Mogi-Guaçu river, at Cachoeira de Emas (Pirassununga/SP) (21°58'S - 47°26'W), S.P. by employees of CEPTA/IBAMA.

They were fasted for 3 days before experiments in 3000l fiberglass tanks, with running water, in a laboratory of CEPTA/IBAMA.

In each collect, 18 animals were obtained, classified and had their total weight, total length and G.S.I. (gonadosomatic index) determined. They were separated in 3 groups: 2 experimental (E1 and E2) and 1 control (C).

There were a total of 144 animals (84 females and 60 males) at 4 maturation stages: resting, maturation, mature and regression.

Experimental groups received injection of UII (SIGMA BIOCHEM Co), intraperitonially, in only one dosis (200ul of UII 10^{-7}M/100g of total weight - E1; or UII 10^{-6} M/100g of total weight - E2). As in the case of works of others researchers (Baldisserotto, 1986; Sheridan *et al.*, 1987; Arnold-Reed & Balment, 1989), in the control group (C) a physiological saline solution for fish was injected (Prochilodontidae) (Baldisserotto & Mimura, 1988) in the same volume/weight relation.

The weight range was 210 to 2980g; their total length from 22,9 to 60,5cm and gonadosomatic index from 0,188 to 22,43 for females and 0,0074 to 1,26 for males.

Blood samples were collected in the caudal vasculature 3 hours after they had received injections of UII and physiological saline solution. This period of 3 hours was observed because according to some authors, this is when the effects of hormone action is more evident (Fryer *et al.*, 1978; Woo *et al.*, 1980; Loretz & Bern, 1981; Mimura, 1988). No anesthetic was used before collecting blood sample, to avoid they can altering ionic plasmatic concentration. Blood was centrifuged at 3.000 rpm for 5 minutes and plasma was frozen at -18°C for the ionic and osmotic analysis.

Terminal spinal cord and urophysis were excised; and the abdominal cavity was open to obtain gallbladder. Bile was collected and frozen at -18°C.

Sexes were determined by the observation of gonads. They were excised and weighed, for the calculation of G.S.I. (gonadosomatic index).

Ionic concentration was determined by flame spectophotometry (Zeiss Pm Q1-11), by emission (Na^+ and K^+) and atomic absorption (Ca^{++} and Mg^{++}) and by microtitulometry (Cl^-), according to Schales & Schales (1941). Osmotic plasmatic and biliar concentrations were determined by a vapor microsmometer (Wescor, Vapor Pressure Osmometer 5500), using 10ul samples. Bile-plasma relation (gallbladder concentration index) was also analyzed for each ion, so the gallbladder concentration capacity, was known for the different ions.

The statistical analysis used was: Variance Analysis (ANOVA) and the Student Newman-Keuls (SNK), and sometimes t-student, through the software Primer of Biosthathystic by Copyright © 1988 by McGraw-Hill. They were used to compare UII at the two treatments, sex and, in some cases, maturation stage.

RESULTS

Mg^{++} and Cl^- plasmatic concentrations decreased after injection of UII 10^{-8}M ($p<0,05$), but Ca^{++}, Na^+ and K^+ were not affected.

Urotensin II at both concentrations decreased gallbladder bile Na^+ and K^+, but it did not affect Ca^{++}, Mg^{++} and Cl^-.

Table I shows data on plasmatic and biliar ionic concentrations.

There were differences in the ionic biliar content after UII injections. In females, UII at 10^{-8}M and 10^{-7}M decreased biliar Na^+ and in males there was a UII 10^{-7} M concentration (TableII)

For sexes, UII in both concentrations decreased biliar K^+ (Table 3). Mg^{++}, Ca^{++} and Cl^- were not affected (Tables III and IV).

Plasmatic Na^+, K^+, Mg^{++}, Ca^{++} and Cl^- were not statiscally different between males and females (Tables II to IV).

Using bile-plasma relation it was shown that instead Cl^-, all ions have higher concentration in bile than in plasma (Table V). This ratio was altered Na^+ and K^+ in both treatments (E1 and E2). K^+ and Cl^- were altered in UII 10^{-8}M. For Mg^{++} and Ca^{++} this ratio was not modified.

Plasmatic osmolarity is higher than biliar, but they don't differ in the UII groups and control.

For females, UII injected, osmotic biliar concentration decreased when compared to males. Plasmatic osmotic concentration was not affected by UII treatments, when sexes were related (TableVI).

DISCUSSION AND CONCLUSIONS

Table II shows that Na^+ and K^+ biliar concentration were modified after UII administration and this may have be the result of variation of gallbladder permeability to ions and/or water. This occurred in the same way when comparing both sexes.

Based on table data, it's seems that UII alters gallbladder concentration capacity only for Na^+ and K^+.

This work focused only in treatments with UII injections, and additional relations between alterations and sexes.

According to bile-plasma relation, instead of Cl^- (index < 1,0) all ions are more concentrated in bile than plasma (Table III).

Considering *Prochilodus scrofa* plasma, the data suggested that Cl^- and Mg^{++} were altered by UII, and Ca^{++}, but Na^+ and K^+ was not.

In the 70's the utilization of purified urotensin started (UI and UII) and some works related plasmatic ionic and osmotic alterations were influenced by these hormones (Marshall & Bern, 1979; 1981; Loretz & Bern, 1981; Foskett & Hubbard, 1981 and Baldisserotto & Mimura, 1992).

Gillichthys mirabilis purified UII increases plasmatic Mg^{++} but has no effect in Na^+ and Cl^- in the same specie. On the other hand, in this same specie, *Catostomus commersoni* UI increased plasmatic Na^+, Cl^- and Mg^{++}, also in *Gillichthys mirabilis* (Bern & Nishioka, 1979).

Marshall & Bern (1979, 1981) verified that urophysial hormones are antagonic when they are controlling plasmatic Cl^-, because UI increases Cl^- secretion in the opercular membrane of *Gillichthys mirabilis* seawater acclimated, and UII decreases this ion secretion.

On the other hand, Foskett & Hubbard (1981) verified that both, UI and UII decrease Cl^- efflux in *Oreochromis mossanbicus* opercular membrane, and this may increase the plasmatic level of this ion.

Plasmatic Mg^{++} in *Prochilodus scrofa* decreases after UII 10^{-8}M injections. So, it seems that urophysial hormones tend to decrease the plasmatic concentration of these ions.

Biliar Na^+ and K^+ decrease may affect the amount of some biliary salts. On the other hand, it can be a water influx. These two aspects may be the reason of biliar osmotic concentration decrease.

Biliar osmotic concentration was modified in both treatments (E1 and E2). They decreased substantially their values, when comparing males and females (Table VI), but taking into account all the animals; plasma and bile

suffer no variation (Table VII).

In spite of the results obtained using neurosecretor caudal peptides, their mechanism of action was not yet determined. There are only speculations about it.

When using fosfodiesterase inhibitors, Marshall & Bern (1979) believe that UII mechanism of action is not AMPc mediated, suggesting that these hormone may act trough another intracellular messenger, perhaps GMPc.

Loretz (1985) suggested a UII mechanism of action in the target organ; the accoupling to the receptor inhibits adenylate cyclase, decreasing AMPc. These mechanisms are not in accordance with the one suggested by Woo *et al.* (1980), in which, urotensins action was related with prostaglandin synthesis. But this study was based in plasmatic ionic alteration using urophysary extract, where there are both, UI and UII, instead of a lot of unknown substances.

As it was mentioned before, on account of a possible structured similarity to somatostatin UII inhibits prolactin release.

Structural similarity between somatostatin and *Gillichthys mirabilis* UII, is only at 1-2 position (Ala-Gly) and 7-9 (Phe-Trp-Lys) (Pearson *et al.*, 1980). Rivier *et al.* (1975) related that 1-2 positions are not important to somatostatin activity. On the other hand, UII forms of *Catostomus commersoni* (McMaster & Lederis, 1983), *Cyprinus carpio* (Ichikawa *et al.*, 1984) and *Acypenser ruthenus* (McMaster *et al.*, 1992) 1-2 positions are not occupied by these aminoacid (Ala-Gly). UII forms of *Catostomus commersoni,* that have same 7-9 positions of somatostatin, do not have crossed reaction in radioimmunoassay, in sufficient concentrations to detect somatostatin. It's possible that Phe-Trp-Lys sequence, that is important to somatostatin activity, and probably to UII, may be a distant interligation in these peptides evolution and the neurosecretory systems involved in their synthesis and release (McMaster & Lederis, 1983).

These neuropeptides can act in three different forms: (1) Through vasopresor urotensins activity, controlling blood flux at various osmoregulatory surfaces, influencing ion and water absorption or secretion rate; (2) Through urotensins effect on hypophysis, specially important in euryhalin animals. CRH-like activity of UI that helps in hyperosmotic regulation and the somatostatin-like action of UII can be responsible for this hypophysiotropic effect; (3) Direct modulation in the transepithelial transport through a great number of osmoregulatory surfaces.

Prolactin does not alter water and ions transport in the isolated gallbladder, in seawater adapted *Anguilla japonica*. Analyzing results of administration of UII in *Prochilodus scrofa*, it was suggested at least two mechanism of action of this peptide in gallbladder: an indirect action, through inhibition of prolactin release and/or a direct action in the gallbladder epithelium.

Direct action in the gallbladder wall seems viable, because urophysial extracts and UII modify water and ions "in vitro" fluxes, in *Synbranchus marmoratus* (Mimura & Baldisserotto, 1989), *Prochilodus marggravii* and *Hoplias malabaricus* (Baldisserotto & Mimura, 1990a).

Thus, based in the results obtained in this work, it's possible to infer that UII is an important osmoregulatory hormone in *Prochilodus scrofa*, and that gallbladder wall in these specie have receptors for this peptide.

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TABLE I - Biliar ionic concentration (meq/l) of *P. scrofa* - experimental and control groups.

Groups	N	Na^{++}		N	K^{+}		N	Mg^{++}		N	Ca^{++}		N	Cl^{-}	
		bile	plasma		bile	plasma		bile	plasma		bile	plasma		bile	plasma
C	48	221,7± 11,4	131,2± 4,95	48	5,9±0, 30	2,5± 0,12	48	3,3± 0,26	1,7± 0,04	48	14,0± 0,58	4,2± 0,10	48	12,6± 0,76	93,0± 2,25
E_1	47	160.5± 8,4*	129,8± 4,63	47	5.1± 0,26*	2,6± 0,12	47	3,6± 0,25	1,6± 0,06	47	14.4± 0,58	3,9± 0,12	47	11,2± 0,61	90,5± 2,55
E_2	48	164.8± 7,8*	122,6± 4,08	48	4.8± 0,23*	2,6± 0,10	48	3.4± 0,25	1,5± 0,04*	48	13,3± 0,22	3,9± 0,10	48	13,5± 0,65	83,0±± 2,60*

C - Control (injected with physiological solution)
E_1 - Experimental 1 (injected with UII 10^{-7}M)
E_2 - Experimental 2 (injected with UII 10^{-8} M)
mean ±standard error
N = size of the sample
* Concentration significatively lower than Control group ($p<0,05$)

TABLE II - Plasmatic and biliar concentration of Na^{++} e K^{+} (meq/l) in males and females of *P. scrofa* control and experimental groups

Groups	Na^{++} - plasma		Na^{++} - bile		K^{+} - plasma		K^{+} - bile	
	Males	Females	Males	Females	Males	Females	Males	Females
C	N=21 129,43±7,70	N=27 132,56±6,58	N=21 2,42±0,19	N=27 2,56±0,16	N=21 5,25±0,36	N=27 6,42±0,44	N=21 219,45±19,91	N=27 223,50±13,58
E_1	N=19 135,84±8,47	N=29 125,83±5,63	N=19 2,52±0,21	N=29 2,62±0,13	N=18 5,44±0,43	N=29 * 4,95±0,33	N=18 * 164,60±13,02	N=29 * 158,01±11,18
E_2	N=20 120,86±5,68	N=27 123,91±5,81	N=20 2,45±0,16	N=27 2,76±0,15	N=20 5,07±0,37	N=28 * 4,56±0,28	N=20 181,30±12,53	N=28 * 153,01±9,65

C - Control (injection of physiological solution)
E_1 - Experimental 1 (injected with UII 10^{-7}M)
E_2 - Experimental 2 (injected with UII 10-8 M)
mean±standard error
N = size of the sample
*Concentration significatively lower than females of Control group ($p<0,05$)

TABLE III - Plasmatic and biliar concentration of Mg^{++} e Ca^{++} (meq/l) in males and females of *P. scrofa*-control and experimental groups

Groups	Mg^{++} - plasma		Mg^{+} - bile		Ca^{++} - plasma		Ca^{++} - bile	
	Males	Females	Males	Females	Males	Females	Males	Females
C	N=20 1,61±0,08	N=27 1,76±0,06	N=21 4,20±014	N=27 4,23±0,16	N=21 14,13±0,69	N=27 13,91±0,88	N=21 3,60±0,29	N=27 3,09±0,40
E_1	N=19 1,65±0,12	N=29 1,57±0,06	N=18 3,83±0,39	N=29 4,04±0,17	N=18 15,04±0,90	N=29 14,09±0,77	N=18 4,14±0,47	N=29 3,26±0,29
E_2	N=20 1,48±0,06	N=25 1,62±0,07	N=20 3,73±0,16	N=27 4,10±0,13	N=20 13,67±1,14	N=28 13,00±0,94	N=19 3,01±0,34	N=26 3,76±0,36

C - Control (injected with physiological solution)
E_1 - Experimental 1 (injected with UII 10^{-7}M)
E_2 - Experimental 2 (injected with UII 10^{-8} M)
mean±standard error
N = size of the sample

TABLE IV - Plasmatic and biliar concentration of Cl- (meq/l) in males and females of *P. scrofa* - control and experimental groups.

Groups	Cl^- Plasma		Cl^- - Bile	
	Males	Females	Males	Females
C	N=21 96,55±3,04	N=27 90,28±3,17	N=21 12,67±1,25	N=27 12,52±0,40
E_1	N=19 89,20±3,38	N=29 91,40±3,65	N=18 11,59±0,76	N=29 11,01±0,88
E_2	N=20 82,15±4,68	N=26 85,04±2,69	N=20 14,34±1,12	N=28 12,95±0,79

C - Control (injected with physiological solution)
E_1 - Experimental 1 (injected with UII 10^{-7} M)
E_2 - Experimental 2 (injected with UII 10-8 M)
mean ±standard error
N = size of the sample

TABLE V - Gallbladder concentration index of Na^+, K^+, Ca^{++}, Mg^{++}, and Cl^-, in *P. scrofa.*

Groups	N	Na^+	N	K^+	N	Mg^{++}	N	Ca^{++}	N	Cl^-
C	48	1,75±0,11	48	2,58±0,18	47	2,03±0,16	48	3,39±0,15	48	0,13±0,01
E_1	47	1,27±0,07*	47	2,19±0,17	47	2,41±0,19	46	3,86±0,22	47	0,13±0,01
E_2	47	1,37±0,07*	47	1,99±0,14*	46	2,28±0,19	47	3,42±0,20	47	0,16±0,01**

C - Control (injected with physiological solution)
E_1 - Experimental 1 (injected with UII 10^{-7}M)
E_2 - Experimental 2 (injected with UII 10^{-8} M)
mean±standard error
* Relation significatively lower than Control group
** Relation significatively higher than Control group

TABLE VI - Plasmatic and biliar osmotic concentration (mosmol/kg.H_2O) in all animals, and in males and females of *P. scrofa* - control and experimental groups.

Groups	N	Plasma	N	Bile	Plasma		Bile	
		Total		Total	Males	Females	Males	Females
C	48	226,77±6,22	47	209,06±13,95	N=21 219,76±10,52	N=27 231,29±8,12	N=21 178,95±19,86	N=27 228,14±18,51
E_1	48	231,20±7,34	47	176,76±08,48	N=19 236,73±13,36	N=29 227,58±8,57	N=18 187,61±12,82	N=29 * 170,03±11,19
E_2	47	231,87±5,62	48	177,33±11,34	N=20 227,85±10,16	N=27 235,37±6,44	N=20 191,95±15,20	N=28 * 166,89±16,06

C - Control (injected with physiological solution)
E_1 - Experimental 1 (injected with UII 10^{-7}M)
E_2 - Experimental 2 (injected with UII 10-8 M)
mean±standard error
N = size of the sample
* Osmotic biliar concentration significatively lower than Control females ($p<0,05$)