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Hydroosmotic and Water Balance Activities of Seritocin ([Ser^5, Ile^8]-oxytocin) on Toad *Sclerophrys regularis* (Reuss, 1833)

Dabiré Anankpètinan Prosper[1,2], Somé Andouormwine Abel[2], Sawadogo Stanislas[2]

[1] *Ecole Normale Supérieure, 01 BP 1757 Ouagadougou 01, Burkina Faso.*
[2] *Laboratory of animal physiology, Department of Animal Biology and Physiology, University Joseph KI-ZERBO, 03 BP 7021 Ouagadougou 03 Burkina Faso.*
Correspondence should be addressed to Prosper A. Dabiré; prosper.dabire@yahoo.fr

Abstract:
Background: *The neurohypophysis of toad Sclerophrys regularis contains at least three types of neuropeptides: vasotocin, hydrin-2 and seritocin. Vasotocin (AVT) and hydrin 2 are widely studied, but the effects of seritocin on the organs of osmoregulation such as skin, kidneys and bladder are not sufficiently documented. The aim of this work was to determine the effect of seritocin on the pelvic skin of the toad Sclerophrys regularis, the total water intake (TWI) and water balance (WB).*
Materials and Methods: *We used the Ussing chamber on which the pelvic skin pre-incubated with neuropeptides was mounted. A graduated pipette was used to monitor the water level on the serosal side of the Ussing chamber. Then, neuropeptides was administered subcutaneously to observe their effects on TWI and WB.* ***Results:*** *Our results indicate that AVT and hydrin 2 increase the water permeability of the skin. They stimulate TWI and WB. Seritocin does not promote water permeability of the pelvic skin of the toad Sclerophrys regularis. It does not stimulate TWI or WB in particular. Seritocin appears to be an antagonistic hormone to AVT and hydrin-2.*
Conclusion: *These results suggest that seritocin, contrary to hydrin 2 and AVT, does not stimulate water uptake in the pelvic skin nor its reabsorption in the bladder and kidneys.*
Key-words: *Sclerophrys regularis, seritocin, Hydroosmotic activity, water balance.*

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I. Introduction

Sclerophrys regularis, formerly called *Amietophrynus regularis* or *Bufo regularis*, is a widely distributed toad in Burkina Faso. This toad is found around sources of water, in open areas, under grasses or shrubs, in primitive areas as well as in human habitats. It occurs in dry and wet savannahs throughout West Africa and even penetrates disturbed tropical forest habitats (1,2). The wide distribution of this toad may be indicative of an adaptation to the need for water in its different living environments. It has been shown that amphibians in more arid environments have a higher water requirement than in aquatic environments. Thus, the bladder of amphibians in aquatic environments has a low storage capacity compared to those in terrestrial environments. Adaptations can also concern water regulation peptides through numerous gene mutations (3).

It has also been shown that for the physiology of water exchange control, five main hormones are involved in the regulation of WB in amphibians: arginine vasotocin, hydrins, angiotensin II, aldosterone and atrial natriuretic peptide. These peptides are involved in short-term fluid regulation. Other hormones exist but their roles are often poorly understood or they are involved in long-term water regulation in amphibians (4). Hydrins are known to present hydroosmotic activities on the frog urinary bladder and water balance on the skin (5). Similarly, AVT has the same effect in addition to the antidiuretic effect on frog kidney (6).

In the Bufonidae family, scientific research first highlighted neuropeptides such as vasotocin and hydrin 2 (5). Secondary, in the phylogenetic study of neuropeptides, a new neuropeptide called seritocin, which is related to oxytocin [Ser^5, I1e^8]-oxytocin, was found in *Sclerophrys regularis* in particular instead of mesotocin (3). This hormone was considered as a mesotocin-like. Mesotocin is known to be a neurohypophyseal hormone that acts as a diuretic in a dose-dependent manner (7,8). As for seritocin its diuretic or antidiuretic effect in the toad *Sclerophrys regularis* is not yet known. In view of the similarity of this hormone to oxytocin,

tests on the rat uterus have shown weak uterotonic activities compared to oxytocin and mesotocin (Chauvet et al. 1995).

This study aims to evaluate the effect of seritocin on the pelvic skin of the toad *Sclerophrys regularis* and to possibly determine its diuretic or antidiuretic effect through the total water intake (TWI) and the water balance (WB).

II. Materials And Methods

Animals

For this work we used the species *Sclerophrys regularis*. This toad belonged to the genus *Amietophrynus* created in 2006 (9). Nowadays it belongs to the genus *Sclerophrys* which contain 45 species distributed essentially in sub-Saharan Africa, north and west of the Sahara: its type species is *Sclerophrys regularis* (10). This species is distinguished from other species by its size: snout-anus length (SLV) = 56 - 90 mm for the male and 70 - 130 mm for the female (2). It has in addition, a large head and a very obtuse snout, well developed parotid glands (smooth but porous), a relatively large tympanum whose diameter is greater than 2 / 3 of that of the eye, large and round warts on the whole back, five pairs of dorsal and well delimited dark spots, fused and strongly developed rictal glands, low and fused warts on the lower face of the forearms, transverse dark bands on the hind legs. Males have well-developed nuptial callosities on the first three digits, dark skin under the chin, very spiny warts, and a vocal sac with two openings (11). In addition to these criteria of distinction we relied on three keys of identification (2,12,13).

In this study, males and females were used. The males have a weight between 43g and 56.2g while females have a weight between 89.8g and 132.6g. The amphibians are caught at night in their natural environment at the University Joseph KI-ZERBO.

Reagents

The chemicals we used were essentially, Hydrin 2 (purity ~95% by HPLC), Seritocin (purity ~95% by HPLC) both acquired from Euromedex and Arginine Vasotocin (purity ~99%) from Sigma laboratories. The anesthetic used was methane tricaine sulfonate (MS222). At the concentration of 1.5g/L the amphibians lost their sensitivity within about 5 min of immersion.

The physiological solution was prepared according to Ogushi et al., (2010) with a little modification of NaCl value to obtain a tonicity of 220 mosmol^{-1} (in mmol^{-1}): 113.01 sodium chloride (NaCl), 1.877 potassium chloride (KCl), 1.081 calcium chloride ($CaCl_2$), 0.064 Monosodium phosphate (NaH_2PO_4) and 1.429 sodium bicarbonate ($NaHCO_3$).

***In vitro* hydro-osmotic activity on the pelvic skin**

This experiment was performed essentially as described by Ogushi et al., (2010). The toad is stabilized for 30 min in distilled water up to two thirds of its ventral skin. Then the animal is anesthetized (with MS222) and the pelvic skin is removed (about 4 cm^2) using fine scissors and a fine forceps. The sample, free of its various adhesions, is rinsed in a physiological solution for batrachians. It is then incubated for 30 min, either in physiological solution (for the control) or in a physiological solution containing the neuropeptide to be tested.

After incubation, the pelvic skin is mounted on the modified Ussing chamber device: the mucosal side is filled with distilled water while the serosal side is filled with one of the solutions to be tested (Physiological solution plus neuropeptide). The monitoring of the hydro-osmotic activity is done every 5 min with a micropipette mounted on the serosal chamber and inside which the liquid level rises if there is a passage of water from the mucosal chamber to the serosal chamber. For the study of interactions between ST, AVT and H2, the sample was incubated in a solution of ST (10^{-8}M) for 15 min, then in the solution of AVT or H2 (10^{-8}M), also for 15 min, before being mounted in the Ussing chamber.

***In vivo* hydro-osmotic activity**

The *in vivo* hydro-osmotic activity tests were achieved adult male (32.2g - 65.3g body weight) and female (77.8g - 103g body weight) of the toad *Sclerophrys regularis*. The animals were divided into groups of 7 for each manipulation phase, each of which lasted 90 min according to the approach of Ouédraogo, (1992) for the effect of the peptide or the control (0.65% NaCl). A distinction was made between the total body mass gain or total water intake (TWI) and the water balance (WB), in accordance with the method of Bentley, (1957).

The animals were bathed to above two-thirds of their ventral skin in distilled water and placed in the dark. Furthermore, the neuropeptides (AVT, hydrin 2 and seritocin) was diluted in 0.65% NaCl and a volume of 1 mL per 100 g of body mass was injected into the dorsal lymphatic sac at any desired dose for 90 min. At the end of 90 min, the animal was weighed after emptying the bladder (mass M1). After injection of the neuropeptides or control, the toads are placed back in the dark for 90 min against. The animals was then weighed without emptying the bladder (mass M2) and after emptying the bladder (mass M3). Then, we

calculated the total mass gain, which corresponds to the skin water intake (TWI) and the Water Balance (WB), which corresponds to the water gain.

TWI= ((M2-M1)/M1) × 100 and **WB** = ((M3-M1) / M1) × 100

Statistical analysis

Our results were presented as the mean ± SEM. Multiple comparisons were made by Tukey's test since the variances were homogeneous. Statistical significance was set at *P < 0.05, **p<0.01.

III. Results

***In vitro* experiment result**

Our results show that the control solution (Physiological solution) does not increase the water level in the serosal side of the Ussing chamber. We observe an average decrease in the water level in the chamber on the serous side after 30 min of the experiment. In the presence of AVT and H2 the water volume increases significantly (p<0.01) compared to the control. This time-dependent increase in water on the serous side of the Ussing chamber is greater for AVT than for H2. After 30 min of the experiment, it is three times greater for AVT (132 ± 12 µL/cm²) than for H2 (43 ± 6 µL/cm²) (figure 1A).

Seritocin does not increase the water level in the serous side of the Ussing chamber. At concentrations 10^{-8}M, 2.10^{-8}M and 3.10^{-8}M seritocin cause a decrease in the water level during 30 min. This decrease tends to intensify in a concentration-dependent manner (figure 1B).

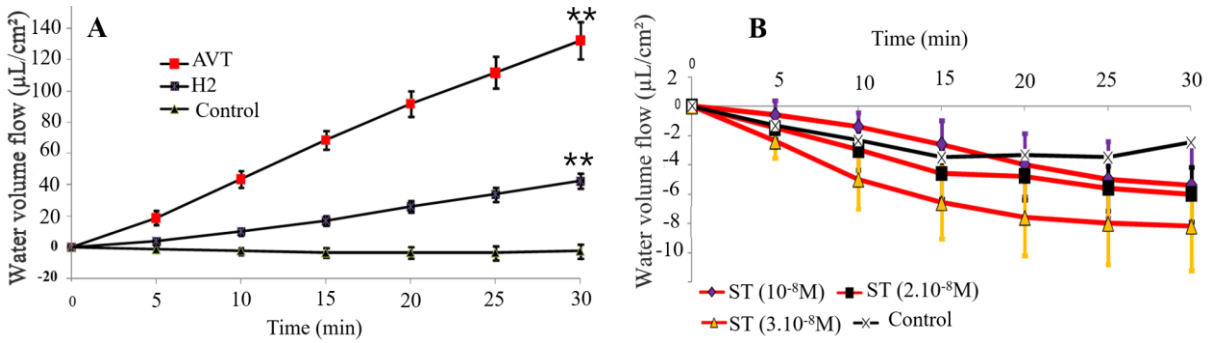

Figure 1: A: Effect of vasotocin (10^{-8}M) and hydrin 2 (10^{-8}M); B: Effect of seritocin (10^{-8} M, 2.10^{-8} M and 3.10^{-8} M) on *Sclerophrys regularis* pelvic skin; **P<0.01 vs control. H2=hydrin 2; AVT=arginine vasotocine; ST=seritocin; n=7.

When the pelvic skin of the toad is incubated in a solution of seritocin for 15 min and then in a solution of AVT or H2 for another 15 min, there is a decrease in the water volume flow through the mucosal side to the serosal (figure 2). This decrease is more important for H2 than for AVT. It is about 110 % for H2 and 70 % for AVT. The ST+AVT curve shows an increase in water level compared with the control (figure 2B). This increase is significant (p<0.05) after 30 min of experimentation.

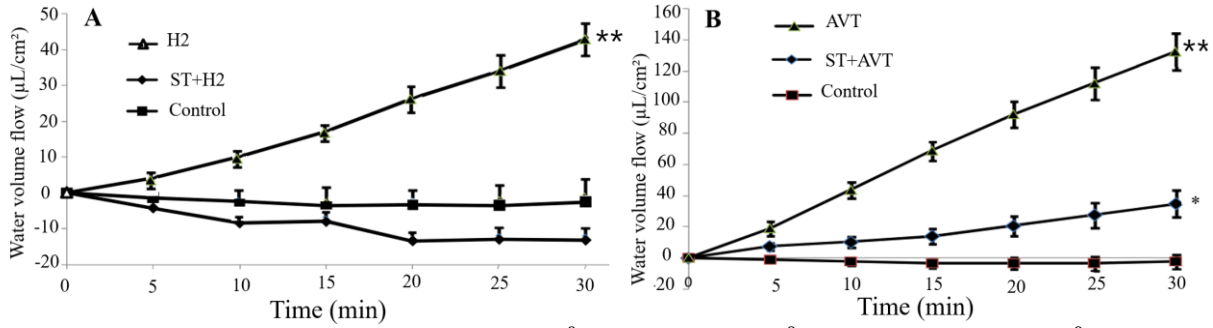

Figure 2: *In vitro* inhibitory effect of seritocin (10^{-8}M) on hydrin 2 (10^{-8}M) (A) and vasotocin (10^{-8}M) (B); **p<0.01, *p<0.05 *vs* control. H2=hydrin 2; AVT=arginine vasotocine; ST=seritocin, n=7.

***In vivo* experiment result**

At all doses of AVT used (25, 50 and 100 pmol/100g) there is a non-significant increase in TWI compared to the control. At 50 and 100pmol/100g, the TWI is higher than that observed at 25pmol/100g. Moreover at the dose 100pmol/100g we note a slight decrease of TWI compared to the dose 50pmol/100. For WB, the results show a significant increase ($p<0.01$) between the AVT doses used and the control. However there is no significant difference between the doses 25, 50 and 100pmol/100g compared to each other. At dose of 100pmol/100g there is no increase in TWI than that of dose 50pmol/100g (figure 3).

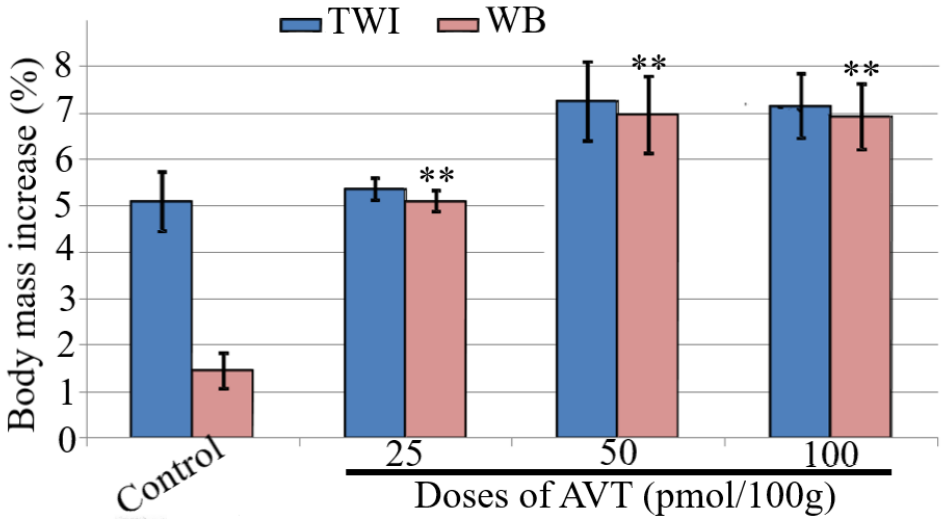

Figure 3: Effect of AVT (25, 50 and 100pmol/100g) on TWI and WB; **P<0.01 *vs* control, n=7.

Hydrin 2 used at different doses (25, 50 and 100 pmol/100g) induce a non-significant increase in TWI compared to the control. At 50 and 100 pmol/100g, the TWI is higher than that observed at 25 pmol/100g. Moreover at the dose 100 pmol/100g we note a slight decrease of TWI compared to the dose 50 pmol/100 which represent the maximum effect. For WB, we observe a significant increase ($p<0.05$) between the H2 doses used and the control. However there is no significant difference between the 25, 50 and 100 pmol/100g doses compared to each other. At the dose of 100 pmol/100g there is a slight decrease in WB compared to that of 50 pmol/100g which represents the maximum effect (Figure 4).

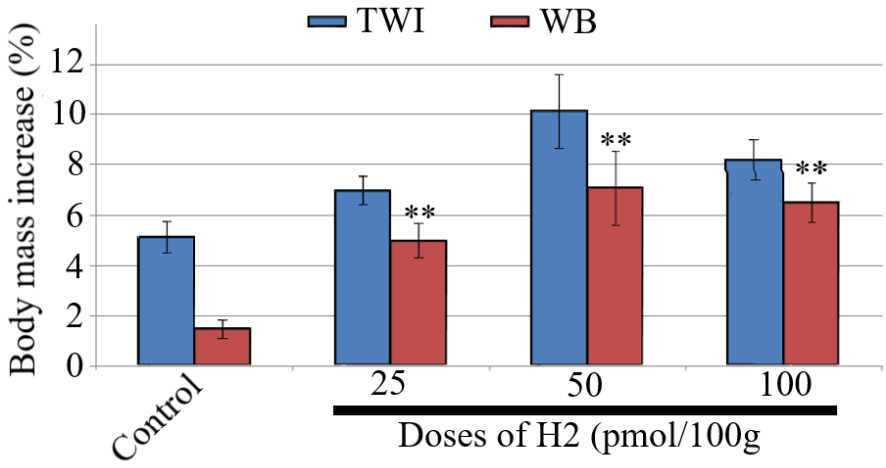

Figure 4: Effect of H2 (25, 50 and 100pmol/100g) on TWI and WB; **P<0.01 *vs* control, n=7.

The used of ST (25, 50 and 100 pmol/100g) showed that there is a non-significant increase in TWI compared to the control. At the dose of 50 pmol/100g, we observe the minimum of TWI. At the dose of 100 pmol/100g we notice the maximum TWI. In contrast, at the dose 50 pmol/100g we found the lowest TWI. About the WB, we observe a non-significant decrease ($p>0.05$) between the ST doses used and the control. However the decrease in WB at the doses of 50 and 100 pmol/100g are higher that of 25 pmol/100g. (figure 5).

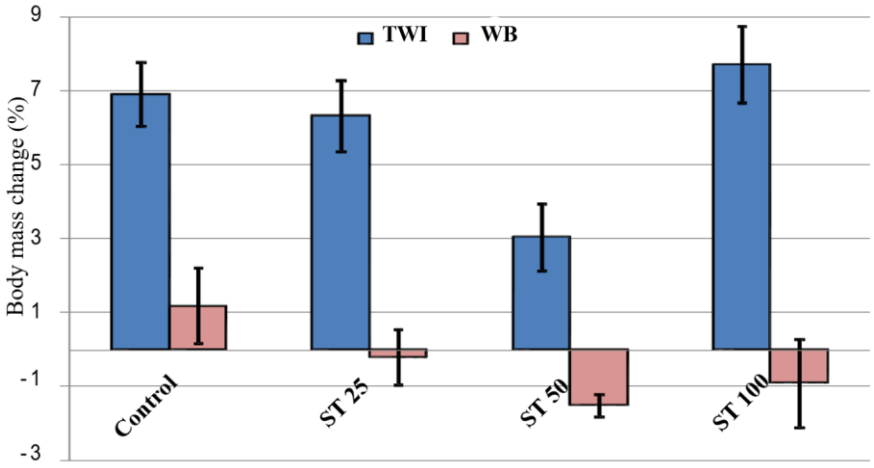

Figure 5: Effect of ST (25, 50 and 100 pmol/100g) on TWI and WB; **P<0.01 *vs* control, n=7.

The interactions, seritocin vs hydrin 2 and seritocin vs AVT shows a nonsignificant increase in TWI compared with seritocin alone or vasotocin alone. In addition, these interactions cause a nonsignificant decrease in WB to values lower than those of control (figure 6).

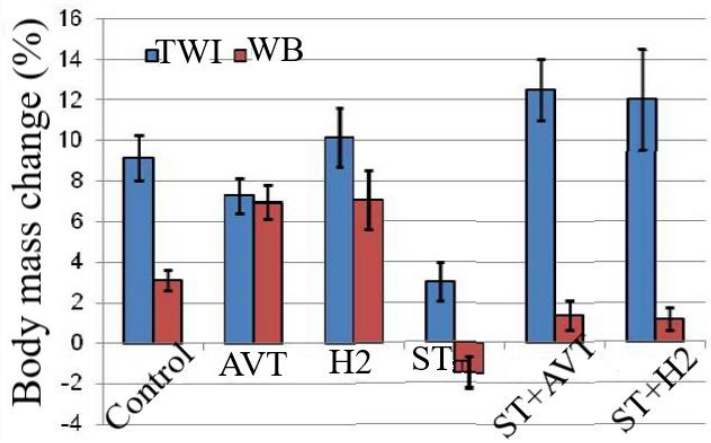

Figure 6: Interactions between ST (50 pmol/100g), AVT (50 pmol/100g) and H2 (50 pmol/100g) on TWI and WB; **P<0.01 *vs* control, n=7.

IV. Discussion

Toads are animals that can easily lose water from their bodies. To prevent this, they have efficient adaptation systems. For example through the pelvic skin toads can easily take up water. Also for another example, the nephron do not have a loop of Henle, which allows them to store diluted urine in the bladder for rehydration purposes (17). Most adult anuran amphibians, with the exception of aquatic species, absorb water through the ventral pelvic skin and reabsorb it from the urine into the urinary bladder. Many terrestrial and arboreal species utilize an area of the posterior or pelvic region of the ventral skin that is specialized for rapid rehydration from water sources or moist substrates. In the adult toad there are four main osmoregulatory organs: skin, bladder, kidney and intestine (18). According to the same author, the water permeability of the bladder, nephrons, and kidneys is controlled by neurohypophyseal peptides such as vasotocin and hydrins. In the toad *Sclerophrys regularis* three pituitary neuropeptides named vasotocin, hydrin 2 and seritocin have been identified (3,6). In this work our results showed that vasotocin and hydrin 2 cause an increase in the volume of water on the serosal side of the Ussing chamber. This result corroborates with the results of several authors who also showed the effect of these neurohypophyseal hormones on the skin of amphibians (18,19). Thus, on the skin of frog *Hyla japonica* the effect of vasotocin is greater than that of hydrin 2 (14). Currently, it is well known that these neuropeptides hormones act on toad's skin by increasing the expression of aquaporins on the first-reacting cell (FRC) layer (19).

Seritocin shows no effect on the pelvic skin of the toad. This means that seritocin does not induce the expression of aquaporins on the FRC layer in order to increase water flow through the membrane. However, it does decrease the effect of vasotocin and hydrin 2. This result suggests that seritocin could be an antagonist of

vasotocin and hydrin 2. These two hormones are agonists of the same receptor named V2 receptor on the skin which means that an antagonism for this receptor could inhibit the effect of vasotocin and hydrin 2.

For *in vivo* experiments, the distinction between TWI and WB allows to specify the roles of the different peptides, by dissociating the action of the skin from that of the bladder and kidneys. Indeed, the WB results from the activity of these three organs. Our results show that AVT and hydrin 2 have similar effects for TWI and WB. Vasotocin and hydrin 2 do not have a significant increase on TWI. However, WB increases with AVT and hydrin 2 effects significantly. The toad urinary bladder is a target for vasotocin and hydrin 2. The WB in this experiment is the result of water entry that occurs at the pelvic skin, body water storage in the extracellular and intracellular fluid, and water loss through the urinary bladder when we empty it. The increase in the body weight when the urinary bladder is emptied means that AVT and hydrin in addition to acting on the skin to increase water absorption, promotes its storage in extracellular and intracellular fluid by reabsorbing water from kidney and bladder. Concerning the bladder, it is known that AVT and hydrins act on V2 receptors to promote reabsorption. Their actions are not very different on isolated urinary bladder of the toad (20). Water reabsorption may also occur at the nephron in response to AVT but not hydrin 2 (18).

For seritocin, our *in vivo* results corroborate those obtained *in vitro*. The TWI decreases at doses 25 and 50 pmol/100g. The WB is lower than the control at all doses. These results suggest that seritocin could not stimulate water reabsorption through the bladder and kidney. Its interaction with AVT and hydrin 2 results in a decrease in WB. This can be explained in part by the fact that AVT and Hydrin 2 have the same V2 receptors in the bladder as in the skin. This means that an antagonism of seritocin on the V2 receptors would inhibit the effects of AVT and hydrin 2.

V. Conclusion

The results of this work allowed us to show that AVT and hydrin 2 have hydroosmotic activities on the skin of the toad *Sclerophrys regularis*. These neuropeptides stimulate the TWI and especially the WB. As for seritocin, it does not show any hydroosmotic activity on the pelvic skin of the toad *Sclerophrys regularis* and stimulates neither the TWI nor the WB. Rather, it seems to have an antagonistic effect towards AVT and hydrin 2. Furthermore, it would be important to demonstrate the expression of aquaporins in response to vasotocin, hydrin 2 and seritocin and the mechanisms of interactions ST *vs* AVT and ST *vs* hydrin 2 on the osmoregulatory organs such as skin, bladder and kidneys.

Consent for publication

All authors read and approved the final content of this manuscript for publication.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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