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Response of Circulating Arginine Vasotocin and Isotocin to Rapid Osmotic Challenge in Rainbow Trout

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ABSTRACT. The plasma levels of arginine vasotocin (AVT) and isotocin (IT) in rainbow trout (*Oncorhynchus mykiss*) were studied to demonstrate changes in neurohypophysial nonapeptides following rapid osmotic challenge. Freshwater-adapted fish were transferred to brackish Baltic water (BW) and brackishwater-adapted fish were transferred to fresh water (FW). In both experiments blood samples for AVT, IT and osmolality were taken 30 min, 1, 2, 24 hr, and 10 days after transfer. Hormones were determined by gradient high-performance liquid chromatography preceded by solid-phase extraction. The pattern of AVT and IT response to external salinity changes was similar during the first hours and showed transient increase or decrease after transfer to brackish or fresh water, respectively. The AVT plasma concentration after 10 days in FW was significantly higher than that in BW, whereas the IT plasma concentrations in both salinities did not differ. These data suggest that the synthesis and or release of AVT and IT are controlled independently, and the roles of hormones in long-term osmotic adaptation are different. COMP BIOCHEM PHYSIOL 118A;3:773–778, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. Arginine vasotocin, isotocin, neurohormones, neurohypophysis, plasma osmolality, osmoregulation, rainbow trout, fish

INTRODUCTION

The teleost fish neurohypophysial peptide hormones, arginine vasotocin (AVT) and isotocin (IT), are synthesized by separate hypothalamic neurosecretory neurons. The axons of these neurons terminate in the neurohypophysis, where AVT and IT are released into circulation. AVT is believed to play a role in the maintenance of salt and fluid balance, cardiovascular activity, endocrine secretion, reproduction, and probably in neurotransmission and neuromodulation processes in the central nervous system in teleosts (1,4–7,10,11,19,22,23,26,27). While AVT displays antidiuretic activity in birds and amphibia, the results for fish are inconsistent (2,21). Arginine vasotocin was reported to be diuretic or antidiuretic depending on the dose (3,4,14). Athough it is known that synthesis and secretion of AVT is sensitive to environmental stimuli, the precise role of AVT in fish osmotic adaptation mechanisms remains unclear. There are a few studies reporting variations in plasma AVT levels with respect to salinity (6,17,24,25). Moreover, changes in the pituitary AVT content during osmotic stress has been described (13,24). Furthermore, the significant differences in pro-vasotocin mRNA levels in rainbow trout adapted to various salinities were detected (15). The RIA study showed that although there was no difference in plasma AVT concentration between freshwater- and seawater-adapted trout, there was one observed in flounder (29). Much less is known about the physiological significance of IT.

The purpose of the present study is to demonstrate changes in AVT and IT plasma levels following rapid osmotic challenge in rainbow trout.

MATERIALS AND METHODS

Animals and Blood Sampling

Rainbow trout *Onchorhynchus mykiss* (250–400 g) of mixed sex were obtained from a hatchery (Institute of Inland Fisheries in Rutki, Poland). The fish were progeny of single parent spawning. In January and February the animals were kept in tanks at 10–14°C on a commercial trout diet under natural photoperiod (the dark period occurred between 16:00 and 8:00). Freshwater-adapted fish (FW) were transferred to brackish water (BW: 166–170 mOsm/kg H_2O) and BW-adapted fish were transferred to FW. Fish were acclimated to brackish water for 2 weeks. The long-term study on rainbow trout adapted to FW and BW did not show any

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Abbreviations–AVT, arginine vasotocin; IT, isotocin; FW, fresh water; BW, brackish Baltic water.

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significant differences in neurohypophysial hormones levels in fish kept in unaltered salinity for over 2 weeks (17). In both experiments, blood samples for AVT, IT, and osmolality were taken 30 min, 1, 2, 24 hr and 10 days after transfer. Sampling started at 16:00 in FW-BW transfer experiment and at 11:00 in BW-FW experiment. Control samples were taken just before the transfer procedure. Blood was collected from the dorsal aorta of decapitated, unanesthetized fish. All blood samples were centrifuged at 1000 *g* for 5 min and stored at −70°C prior to analysis. Plasma osmolality was measured immediately after experiments using a 5500 Vapor Pressure Osmometer (Wescor Inc., Logan, U.S.A.).

Plasma AVT and IT Determination

AVT and IT were extracted from plasma by solid phase extraction using C_{18} Bakerbond SPE cartridges (J. T. Baker, Phillipsburg, NJ, U.S.A.). The acidified plasma sample was aspirated through the column, washed with HPLC-grade water followed by glacial acetic acid-HPLC-grade water (4:96). The sample was eluted twice with 6 M HCl-absolute ethanol (1:2000). The eluate was collected, dried under air, and held at −20°C prior to HPLC analysis. HPLC was performed with a Beckman modular system (Beckman Instruments, San Ramon, CA, U.S.A.) with UV detector. Data were digitised by a Beckman 406 analog interface and processed by a Beckman analytical series System Gold data acquisition software on an IBM compatible computer Chromatographic separations were carried out on an Ultrasphere C_{18} column (250 × 4.6 mm I.D., 5 μm particle diameter, 80Å pore size) connected to a precolumn (45 × 4.6 mm I.D.) filled with the same material, both obtained from Beckman Instruments. The system was run at a flow rate of 1.0 ml/min and the eluate was monitored at 215 nm. Linear gradient elution from 20 to 40% in 20 min was carried out with 0.1% trifluoroacetic acid (TFA) in water and 0.1% TFA in acetonitrile-water (3:1). Plasma AVT and IT were identified by their retention times compared with those of standards. The retention times of AVT and IT were 8.5 and 15.0 min, respectively. The difference in times of elution protects against the co-elution of these peptides. Quantitative determination of AVT and IT was performed on the basis of a standard curve. The linearity of signal responses was observed in the range of 10–500 pmol/ml. To assess the precision of the assay, the retention times of plasma AVT and IT and corresponding synthetic nonapeptides in the different gradient systems and column temperatures were studied. Samples were assayed five times in the same set of experiments and in three different series. The inter- and intra-assay coefficients of variation were 17 and 10% for AVT and 15 and 12% for IT, respectively. The detection limit for both peptides was 10 pmol/ml (injected volume 10 μl). When low concentrations were being measured, 10 ml of plasma sample was required. The method has been described in detail (16).

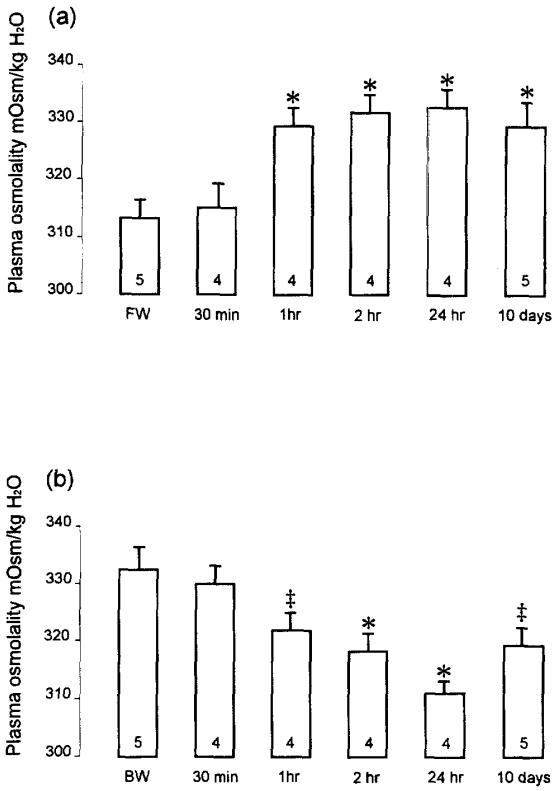

FIG. 1. Changes in plasma osmolality in rainbow trout after transfer from fresh to brackish water (a) and from brackish to fresh water (b). Each bar represents the mean ± SEM. Numbers of animals used are shown in the bars. Significantly different vs initial values: *$P < 0.01$ (a, b); and ‡$P < 0.05$ (b).

Chemicals

HPLC-grade acetonitrile, water, methanol and TFA were purchased from J.T. Baker (Deventer, The Netherlands). Glacial acetic acid and hydrochloric acid were supplied by E. Merck (Darmstadt, Germany). Synthetic AVT and IT and ethanol were obtained from Sigma (St Louis, MO, U.S.A.).

Statistics

Values are presented as means ± standard error of the mean (SEM). The statistical analysis of the data was performed using repeat measurement analysis of variance followed by Student's *t* statistics.

RESULTS

Changes in plasma osmolality during the transfer protocols are presented in Fig. 1a and b. Plasma osmolality increased significantly 1 hr after transfer of FW-adapted fish to BW and remained higher compared to initial value for the next

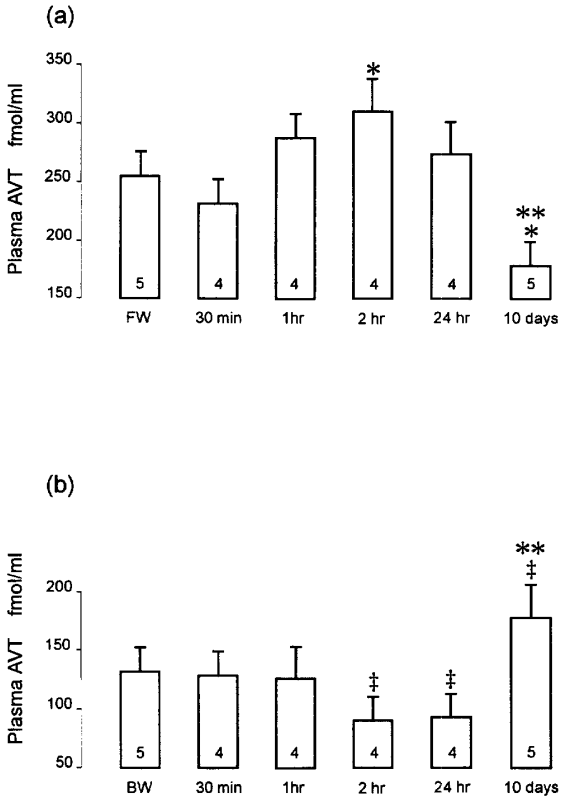

FIG. 2. Changes in arginine vasotocin plasma concentration in rainbow trout after transfer from fresh to brackish water (a) and from brackish to fresh water (b). Each bar represents the mean ± SEM. Numbers of animals used are shown in the bars. Significantly different vs initial and 30-min values: *$P < 0.01$ (a); significantly different vs 1-, 2-, and 24-hr values: **$P < 0.001$ (a); significantly different vs initial, 30-min and 1-hr values: ‡$P < 0.05$ (b); and significantly different vs 2-hr and 24-hr values: **$P < 0.001$ (b).

10 days (Fig. 1a). A significant decrease in plasma osmolality was observed 1 hr after transfer of BW-adapted fish to FW and maintained for 10 days (Fig. 1b).

Figure 2a and b illustrates the changes in AVT levels in BW- and FW-transferred fish. The corresponding results of plasma IT are shown in Fig. 3a and b. The AVT levels in fish transferred from FW to BW increased reaching the significantly higher value 2 hr after transfer (Fig. 2a). One day after transfer the level dropped to approximately the initial value. Plasma AVT concentration decreased significantly below the control value on day 10 after transfer. In FW-transferred fish, the AVT level decreased steadily achieving the lowest values 2 and 24 hr after transfer (Fig. 2b). A significant increase in AVT concentration above the control value was detected on day 10.

Plasma isotocin concentration in fish transferred from FW to BW was significantly higher 24 hr after transfer compared with initial value (Fig. 3a). After this temporary increase, IT concentration returned to the control value on day 10. A significant decrease in plasma isotocin level was observed 2 or 24 hr after transfer to FW (Fig. 3b). IT concentration recovered to approximately initial level 10 days after transfer.

Plasma arginine vasotocin concentrations were plotted against corresponding plasma osmolality values (Fig. 4). Two separate regression lines were drawn for FW-BW and BW-FW transfer experiments (the 10 days' values were excluded). Relationships are given by equations: $y = 2.05x - 386.09$ (correlation coefficient of $r = 0.43$, $P < 0.05$), and $y = 2.68x - 752.30$ (correlation coefficient of $r = 0.76$, $P < 0.001$), respectively. No similar relationship between plasma isotocin concentration and plasma osmolality was evident.

DISCUSSION

The rainbow trout is a euryhaline freshwater fish that can survive in brackish Baltic water after even rapid transfer.

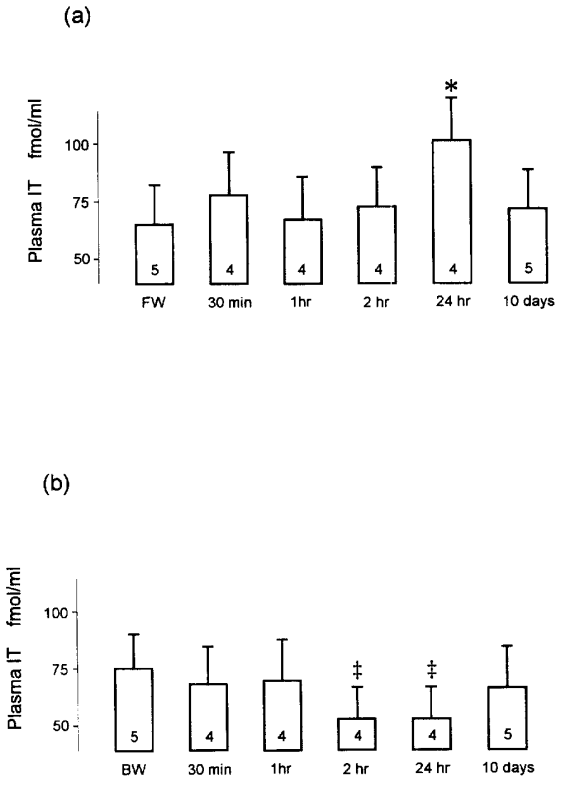

FIG. 3. Changes in isotocin plasma concentration in rainbow trout after transfer from fresh to brackish water (a) and from brackish to fresh water (b). Each bar represents the mean ± SEM. Numbers of animals used are shown in the bars. Significantly different vs the other values: *$P < 0.01$ (a); and significantly different vs the other values: ‡$P < 0.05$ (b).

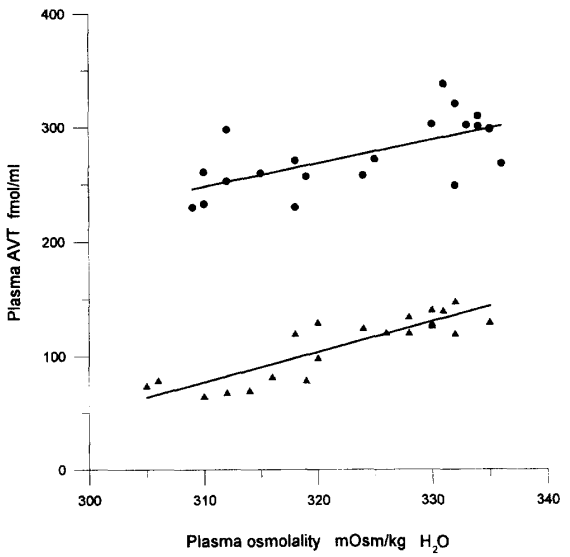

FIG. 4. Relationship between plasma AVT concentrations and corresponding plasma osmolalities in samples taken according to transfer protocols (10 days' values were excluded). A regression line for FW-BW experiment (circles) was drawn according to the equation: $y = 2.05x - 386.09$ ($r = 0.43$, $P < 0.05$). A regression line for BW-FW experiment (triangles) was drawn according to the equation: $y = 2.68x - 752.30$ ($r = 0.76$, $P < 0.001$).

Plasma osmolality increased steadily in rainbow trout transferred directly from FW to BW and remained elevated at all samplings in BW (Fig. 1a). Such a response is consistent with those presented elsewhere (8,15,20,24,28). It should be noted that the brackish water was still hipoosmolal to fish blood. A rapid transfer of BW-adapted rainbow trout to FW resulted in a decrease of plasma osmolality (Fig. 1b) comparable with that reported elsewhere (15).

A question arises—if the change in plasma osmolality is the factor responsible for the change in circulating neurohypophysial hormones. A consistent relationship between plasma AVT concentration and plasma Na^+, Cl^-, and osmolality was demonstrated in sea-water-adapted flounder (30). The plasma vasotocin concentration in the current study also correlated with plasma osmolality (Fig. 4). No such relationship was observed for plasma isotocin concentration. Two separate regression lines shown in Fig. 4 may represent the differing roles for AVT during FW-BW and BW-FW fish transfers. A distinct difference in P values calculated for two experimental groups is worth underlining. It should be noted that the plasma AVT level was elevated in FW-BW transferred fish within first 24 hr of adaptation. Therefore, the presumable effect of increasing plasma osmolality on circulating AVT in fish transferred to BW might not be so pronounced. On the other hand, the apparently tight osmotic control was exerted over the lower plasma concentration in BW-FW transferred fish. This phenomenon was presented earlier for sea-water- and freshwater-acclimated flounder (24). Similarly, the plasma AVT concentration in seawater-adapted flounder was correlated with plasma Na^+ concentration though no such relationship was evident in FW-adapted fish (7). It is worth mentioning that the correlation between plasma osmolality and plasma AVT concentration was more remarkable during the dynamic adaptation processes than in steady state of fully adapted fish: No significant correlation was observed after 10 days of adaptation. These values were excluded from the regression lines analysis presented in Fig. 4.

The present results, which show that the plasma AVT level is lower in BW-adapted fish compared with FW levels at the same time of the day, are in harmony with previous study (17). According to those data, choosing the proper time to start the experiment is essential. Therefore, the FW-BW transfer experiment was started at the time of maximal plasma AVT level so that the observed increase would depend on plasma osmolality changes only (Fig. 2a). Similarly, the BW-FW transfer experiment was started before that time so that the decrease in plasma AVT would be associated with osmotic challenge (Fig. 2b). The significant decrease in plasma AVT concentration observed in fish after 10 days in BW was consistent with the data for rainbow trout adapted to 25% seawater (25).

It should be noted that the mean plasma AVT levels reported in rainbow trout show great variation (6,24–26,29). Plasma AVT concentrations measured by Perrott *et al.* (24) in FW- and SW-adapted rainbow trout were in the 10^{-10} M range, comparable with the present study. More recently, however, plasma AVT levels in rainbow trout have been measured at 10^{-12} M (6,29). Conversely, Pierson *et al.* (25,26) reported plasma AVT levels in the 10^{-8} M range. These differences may arise from many factors including techniques used, animal strains, or their physiological state. The plasma AVT and IT levels found in the present study are in accordance with doses producing maximum inhibition of adenylate cyclase activity in rainbow trout gills–the important osmoregulatory organ in fish (12).

It was shown that the AVT content in the pituitary of FW-adapted rainbow trout temporarily decreased acutely, upon transfer of fish to sea-water, but eventually recovered to freshwater-adapted level (9,18). A similar pattern of change was found in the medaka (*Oryzias latipes*) during osmotic stress (13). The higher pituitary AVT content in FW-acclimated flounder, compared with that in sea-water, was reported (24). In agreement with some of the above results, the higher plasma AVT in fish transferred to BW in this study, may reflect the intense release of AVT in the first 2 hr after transfer (Fig. 2a). The decreased AVT plasma concentration observed in fish after 1 and 10 days in BW may reflect the inhibition of AVT synthesis and/or release during BW adaptation. These results conform to the decrease of pro-vasotocin mRNA amount in sea-water-

adapted rainbow trout (15). On the other hand, in fish transferred to FW the plasma AVT level decreased significantly within a few hours (Fig. 2b). This effect may indicate that the AVT release was temporarily acutely decreased. It is supported by the increase of pituitary AVT content during the first 2 hr of readaptation to FW (13). The increase of pro-vasotocin mRNA amount in fresh-water readapted rainbow trout (15), indicating possible AVT synthesis stimulation, may explain the significant rise of plasma AVT level observed on day 10 (Fig. 2b).

The plasma isotocin concentrations were significantly lower than those of AVT (Fig. 3a and b). These results are consistent with those reported previously (17,25). The plasma IT level in BW transferred fish was transiently higher 1 day after transfer (Fig. 3a), which indicated an intense release of IT. The following decrease may be a result of the inhibition in IT synthesis, taking into consideration a significant decrease in pro-isotocin mRNA levels in sea-water rainbow trout on day 1 after transfer (15). In the FW-transfer experiment the significantly lower isotocin levels indicated the transient inhibition of the storage and/or release. The similar FW and BW isotocin concentrations, presented in this study and previously (17), agree with the lack of changes in proIT mRNA levels observed in freshwater- and sea-water-adapted rainbow trout (15).

All of these observations together support a role for both neurohypophysial hormones in teleost fish osmoregulation, especially in quick adaptation mechanisms. Control of their releases appears differentially sensitive to associated changes in plasma osmolality. The pattern of AVT and IT response to external salinity changes is similar during the first hours, with one exception: a delay in IT release in BW transferred fish. However, on day 10 after transfer, the plasma IT levels return to initial levels, but the plasma AVT levels vary significantly versus the initial values. The physiological significance of this observation is difficult to assess at present, but may suggest the different moles of hormones in long-term osmotic adaptation mechanisms. Moreover, the synthesis and/or release of arginine vasotocin and isotocin probably are controlled independently, as was suggested previously (17).

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