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Potential roles of arginine-vasotocin in the regulation of aggressive behavior in the mudskipper (*Periophthalmus modestus*)

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ABSTRACT

The hypothalamic hormones, arginine-vasotocin (VT) and isotocin (IT), play central roles in osmoregulation and in the regulation of social behaviors including aggressive behavior in many vertebrates including fish. Here, we examined whether these hormones are associated with aggressive behavior in the mudskipper (*Periophthalmus modestus*). The mudskipper is an amphibious fish, which lives in the brackish water of river mouths and displays unique aggressive behavior. Upon introduction to each other in an experimental tank with aquatic and terrestrial areas, a pair of males can be classified as aggressive dominant or submissive subordinate based on the frequency of their aggressive acts, which is significantly higher in dominant male. Additionally, the length of stay in terrestrial area of dominant was longer than that of the subordinate. The latter remained in aquatic area almost throughout the period of behavioral observation. The expression of brain VT mRNA was significantly higher in subordinate than in dominant, whereas neither IT mRNA expression nor plasma cortisol level differed between subordinate and dominant male. On the other hand, an intracerebroventricular injection of VT increased aggressive behaviors in mudskippers. In addition to known roles of VT in mediation of aggressive behavior, these results may shed light on the role of endogenous VT toward water migration in submissive mudskippers. The amphibious fish is a valuable experimental model to observe the relationship between effects of central VT on the osmoregulation and social behavioral regulation in vertebrates.

1. Introduction

The neuropeptides, arginine-vasotocin (VT) and isotocin (IT), are the teleost homologues of the mammalian neurohypophysial hormones, arginine-vasopressin (VP) and oxytocin (OT), respectively. These peptides are produced by neurons localized in the hypothalamic regions, which project to multiple extrahypothalamic regions including the hypophysis (Goodson and Bass, 2001; Goodson et al., 2004; Holmqvist and Ekström, 1995; Semsar et al., 2001) and are known to play pivotal roles not only in osmoregulation, the cardiovascular and stress response, but also in mediation of reproductive and social behaviors in vertebrates (Goodson and Bass, 2000; Hyodo and Urano, 1991; Kleszczyńska et al., 2006, 2007; Mancera et al., 2008; Motohashi et al., 2009; Semsar et al., 2001; Warne, 2002). In particular, the association of VT with aggressive behavior and social status has been reported in several teleosts. For example, VT expression is increased in aggressive dominant males compared with subordinates in the zebrafish, medaka as well as the African cichlid (Greenwood et al., 2008; Kagawa, 2013; Larson et al., 2006). Furthermore, central administration of VT increases aggressive and/or courtship behaviors in the rainbow trout and bluehead wrasse (Backstrom and Winberg, 2009; Semsar et al., 2001). These reports focused on behavioral roles induced by central VT in sedentary fish in osmotically stable seawater or fresh water, whereas much less is known about species living in the mouths of rivers. These brackish species are affected considerably by salinity oscillations between hypotonic river water at ebb tide and hypertonic seawater at rising tide (Sakamoto et al., 2011; Sakamoto and Ando, 2002). In euryhaline fish, VT and IT expression depend on changes in environmental salinity (Hiraoka et al., 1996; Hyodo et al., 1991). Taken together, these facts have led us to hypothesize that VT regulation is related to aggressive behavior in brackish fish and may also be associated with the osmotic conditions. If so, it may provide new insight into the role of VT in mediating dehydration caused by amphibious behavior as well as in regulation of aggressive behavior.

The mudskipper (*Periophthalmus modestus*) lives in the mouths of the river and have an ability to maintain its osmotic balance in a wide range of water osmolality. Furthermore, they are amphibious

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and spend the greater part of their lives out of water to feed and to avoid capture by aquatic predators. Therefore, they have behavioral and physiological specializations adapted to amphibious life (Clayton, 1993; Graham, 1997; Sakamoto et al., 2002, 2011; Sakamoto and Ando, 2002). In association with the role of VT in amphibious life, its role in the mediation of aggressive behavior are highly intriguing in this species. However, previous studies have not clarified how VT regulates the aggressive behavior in the mudskipper. Thus, the mudskipper is a valuable experimental model to elucidate the interacting effects of VT in regulating both osmoregulation and behavior. Here, we performed behavioral testing for aggression in mudskipper males and examined VT and IT expression in the brain of dominant (*i.e.*, aggressive) and subordinate (*i.e.*, submissive) males, by real-time polymerase chain reaction (PCR) assays for mudskipper VT and IT mRNA. In addition, the concentration of cortisol in plasma was compared between dominant and subordinate fish. Furthermore, we used an intracerebroventricular (ICV) injection of VT to examine whether VT induces aggression in these fish.

2. Materials and methods

2.1. Animals

Mature male mudskippers (*P. modestus*) weighing 2–3 g were collected from the estuary of the Fujii River that flows into the Inland Sea of Seto, Japan. Fish were acclimated for more than one week in laboratory tanks (3 L). Since these fish were collected from brackish water, the water in all tanks was maintained as isotonic diluted seawater (10 ppt, 149 mM Na, 176 mM Cl, 3.8 mM Ca, 346 mOsml/kg). All specimens were maintained at a temperature of 22–25 °C under a daily photoperiod cycle of 12 h light/12 h dark (lights on at 7:00 a.m.) and were daily fed Tetrafin flakes (TetraWerke, Melle, Germany). Small plates were placed in each tank to allow mudskippers an opportunity to climb on them. All fish were handled, maintained, and used in accordance with the Guidelines for Animal Experimentation established by the Committee for Animal Research, Okayama University, in accordance with international standards on animal welfare and in compliance with national regulations. Before being handled, the fish were anesthetized with 0.01% tricaine methanesulfonate (Sigma, MO, USA), which was neutralized with sodium bicarbonate. The timing of lost equilibrium after anesthesia was consistent for each fish. Full recovery from anesthesia was accomplished within 1–2 min.

2.2. Experimental procedure

2.2.1. Experiment 1: behavioral testing under terrestrial and aquatic conditions

A size-matched pair of males was transferred from the stock tank to a contest tank (12 × 20 × 13 cm) with a land and a water area at 10:00 a.m. (Fig. 1). The land area was made of an opaque plastic box (5 × 7 × 4 cm). The water area contained 10 ppt seawater with 3 cm in depth. Immediately after transfer, the aggressive behavior of each fish was observed in the contest tank. Higher and lower aggression individuals in each pair of fish were classified as dominant and subordinate males, respectively, based on the frequency of aggressive behaviors observed in contests. In addition to aggressive behavior, the frequency of migration to the land from the water area and the length of time on land were recorded for each contestant. Immediately after contests, fish were quickly anaesthetized and their body weight and length measured. Blood samples were then quickly collected from the hemal arch in the region of the caudal peduncle and plasma was kept at −80 °C until plasma cortisol was analyzed. Following blood sample collection, fish were decapitated and the whole brain was quickly removed. Brains were immediately frozen in liquid nitrogen and stored at −80 °C until VT and IT expression was analyzed.

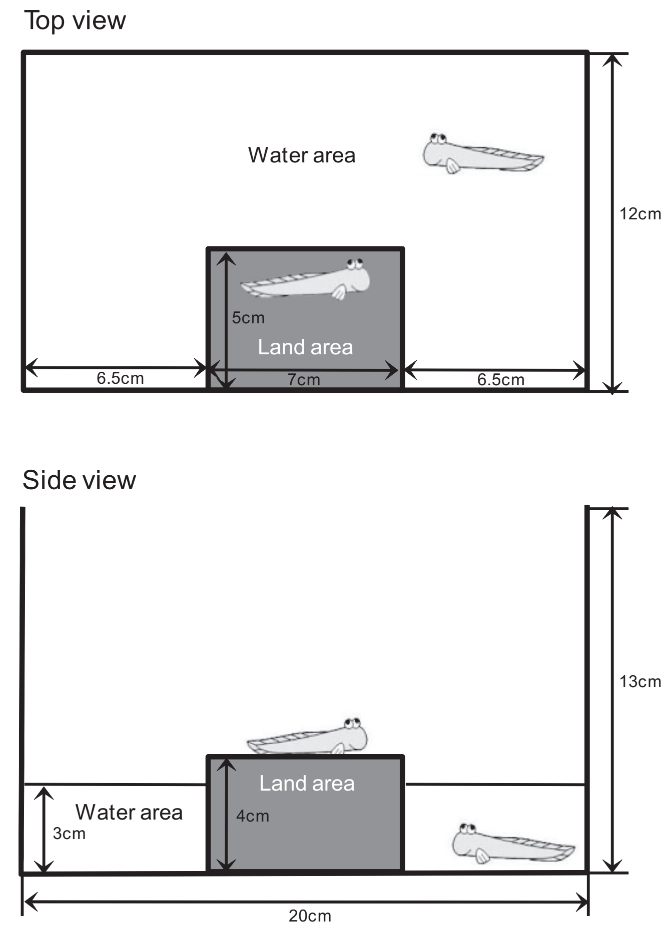

Fig. 1. Schematic diagram of the apparatus to observe aggressive and amphibious behavior in mudskippers. This apparatus was used in Experiment 1. The tank without the land area was used in Experiment 2.

2.2.2. Experiment 2: effects of VT on aggressive behavior under aquatic condition

In Experiment 1, VT expression differed between dominants and subordinates, but not IT (see Fig. 3). Therefore, we examined the effects of ICV VT injection on aggressive behavior in the mudskipper. The size-matched pair of males was chosen from the stock tank and each fish was intracerebroventricularly injected with VT or vehicle between 9:00 and 10:00 a.m. After the injection, the pair was recovered for 30 min in a contest tank. The pair did not show the aggressive behavior during the recovery period. Following the recovery, the aggressive behavior of each fish was observed in the contest tank without the land area (Fig. 1), because two male mudskippers showed higher frequencies of aggressive behavior in the water area compared with on the land area during behavioral testing in the tank with both the land and water areas in Experiment 1 (see Fig. 1).

2.3. Behavioral observation

Behavioral observations were conducted for 1 h. Six pairs of different males were applied for each Experiment 1 and 2. The frequency of aggressive behavior was determined as described by Grossman (1980) and Picciulin et al. (2006). Six types of aggressive