The Role of Arginine Vasotocin in the Reproductive Behavior of the Brook Stickleback, *Culaea inconstans*

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Abstract

The neuropeptide arginine vasotocin (AVT) has diverse effects on vertebrates including but not limited to osmoregulation, cardiac output, suppression of motor neuron transmission, and reproductive behavior. Intraperitoneal injection of AVT has been shown to influence reproductive behavior in killifish, *Fundulus heteroclitis*, by eliciting a spawning reflex, but little additional behavioral work concerning reproductive behavior in fishes has been done.

I studied the response the brook stickleback, *Culaea inconstans*, to intraperitoneal injection of AVT. Spawning reflex and nest-building responses were assayed, however other responses were also noted. Amount of AVT injected ranged from 50-150 μg and was administered in .05 ml physiological saline.

No increase in spawning behavior was noted after injection, however there was a distinct difference between the AVT treated fish and the saline treated fish. In both assays the AVT treated fish had erect spines for a considerably shorter time than the saline treated fish. In the nesting assay, only the fish treated with AVT resumed nest building and fanning behaviors that corresponded to the baseline observation numbers while the saline treated fish ceased all nest building and fanning activity. These observations lead to the conclusion that AVT plays some physiological role in reducing the stress brought on by handling and injection. The exact mechanism for this is not known, but previous experimentation on other teleost fishes suggest that the response may lie in the pressor activity of AVT.

Introduction

The neuropeptide arginine vasotocin (AVT) has been found to be highly conserved among vertebrates. It has been shown to exist in nearly every class of vertebrates from primitive fishes to mammals and has been shown to influence such processes as behavior (Moore, 1987), osmoregulation (Amer and Brown, 1995; Hontela et. al, 1993) and suppression of motor neuron synaptic transmission (Goldberg et. al, 1987). More specifically, AVT has been shown to influence reproductive behaviors in lower vertebrates such as bullfrogs (Boyd, 1994), rough-skinned newts (Moore, 1987) and killifish (Pickford and Strecker, 1977).

Among the organisms studied for the effects of AVT on reproductive behavior fishes have been studied very little. Pickford and Strecker (1977) demonstrated that both natural and synthetic AVT produced a strong spawning reflex response in killifish. Beyond this experiment, though, little experimentation has been done concerning the effects of AVT on the reproductive behavior of fish.

As in the case of the killifish, I propose that AVT will affect the behaviors associated with reproduction in the brook stickleback, *Culaea inconstans*. The brook stickleback is a small fish of about 50 - 65 cm in length that is common to the brackish waters of north-central North America. It has a well-documented reproductive behavior that begins in early spring when the fish move into shallow water to spawn and lasts until water temperature reaches about 20° C (Wootton, 1976). The male builds a tube-shaped nest out of pieces of vegetation that are most commonly glued to stalks of vegetation by a mucus produced in the kidney. Upon completion of the nest the male courts a female and leads her to the nest where she deposits the eggs in the tunnel. The male then fertilizes the eggs after the female leaves the nest. In this experiment, I studied the effects of AVT on the spawning reflex of male and female sticklebacks and the role AVT plays in the nesting behaviors of male sticklebacks.

Materials and Methods

Fish collection and maintenance. Fish used in the spawning reflex assay were taken from their natural habitat immediately before use. All fish were collected in minnow traps from either Bog Pot or Tuesday Lake. They were placed in ten gallon aquaria in water collected at the time of capture from their native lake. No males with spawning coloration were found after the water temperature rose above 20° C as this indicated the end of the spawning season.

Males used in the nesting assay were maintained in an aquarium environment as close as possible to that of their natural habitat. Water, bottom substrate and vegetation were taken from the site of collection. Each tank from the same environment was identical with respect to bottom substrate and type and amount of vegetation. Water temperature was maintained between 15°C-19°C. Fish were kept under the same photoperiod as the natural environment and were fed daily a diet of zooplankton and invertebrates from their native lake.

Arginine vasotocin. The peptide used was received as a solid (Sigma Chemical Co.) and diluted to a stock solution of 5μg/μl. All dilutions for injection were prepared immediately before use. Stock solution was diluted with physiological saline (0.65g NaCl, 0.042g KCl, 0.025g $CaCl_2$ in 100 ml H_2O). Amount of peptide injected ranged from 50μg to 150μg and total injection amount of AVT solution or saline was 0.05 ml.

Spawning reflex assay. Each fish was placed in a ten gallon aquarium and allowed a 30 min. acclimation period. The fish was then given an intraperitoneal injection of either AVT solution or physiological saline and observed for 60 min.

Nesting assay. Male fish were placed in an aquarium in an environment constructed with material from their natural environment. The fish were allowed three days to acclimate to the new environment and begin construction of the nest. The assay was done after the fish began to tunnel into the nest. A baseline was determined via a 30 min. observation period immediately prior to injection. Following intraperitoneal injection with either 150 μg AVT in 0.05 ml physiological saline or 0.05 ml physiological

saline, the fish was then observed for 60 min. Nest building, behaviors associated with mating such as nest fanning and dancing, and physical traits were recorded.

Results

Spawning reflex assay. Results obtained in the spawning reflex assay are inconclusive with respect to spawning behavior. While no spawning reflexes were observed, a variety of observations were made based on physiological changes of the fish, namely spine position. The average length of time that the fish spent with raised spines for both the male and female fish was considerably shorter for the AVT-treated fish than for the saline-treated fish (Table 1). Dosage of AVT also appeared to have an inverse effect between male and female fish on the length of time that the spines were raised. The length of time that female fish raised their spines increased with higher dosage while the length of time that male fish raised their spines decreased with higher dosage (Table 1). This trend seems to be reflected in the saline injection and therefore may be the result of stress due to injection (males exhibited raised spines for an average of 32.9 ± 7.5 min. while females had spines raised for an average of 41.8 ± 8.0 min. after injection with saline).

Nesting assay. The results of the nest building assay paralleled those of the spawning reflex assay. There was no marked increase in nest building activity or mating behaviors over the 30 min. baseline number, however only the AVT-injected fish continued to exhibit any nest building or fanning behaviors after injection. The AVT-treated fish continued nest building at an average frequency of 2.2 ± 0.5 behaviors and fanning at an average frequency of 0.6 ± 0.4 behaviors per fish over the hour observation period ($P < 0.05$, Mann-Whitney U test)(Table 2). All the AVT-treated fish resumed normal activity levels within 30 min. of injection while all of the saline-treated fish remained fairly sedentary and ceased all nest building and fanning behaviors

As in the case of the spawning reflex assay there was a distinct difference between the AVT-treated fish

TABLE 1- Spawning Reflex Assay
Response of Male and Female Brook Sticklebacks to
Intraperitoneal Injection of AVT

	Female AVT	Female Saline	Male AVT	Male Saline
50 µg AVT Injection	N=3	N=12	N=5	N=15
Spine Response (min. raised)	2.3±2.3	41.8±8.0	12±12	32.9±7.5
Surfacing (# behaviors)				
0-30 min.	5.7±3.1	2.8±2.1	1.8±0.7	1.1±0.5
31-60 min.	6.7±3.1	23.3±8.4	1.6±1.6	10.7±7.5
Total	12.3±7.6	26.0±9.5	3.4±2.0	11.8±7.7
Tunneling (# behaviors)				
0-30 min.	1.6±0.9	0.5±0.3	0.4±0.4	0.2±0.1
31-60 min.	1.0±1.0	2.0±0.8	0.6±0.6	2.7±2.1
Total	2.6±1.5	2.5±0.9	1.0±0.6	2.9±2.2
100 µg AVT Injection	N=4	N=12	N=5	N=15
Spine Response (min. raised)	8.5±1.4	41.8±8.0	8.8±7.2	32.9±7.5
Surfacing (# behaviors)				
0-30 min.	5.7±3.1	2.8±2.1	0.2±0.2	1.1±0.5
31-60 min.	6.7±4.4	23.3±8.4	9.0±9.0	10.7±7.5
Total	12.3±7.6	26.0±9.5	9.2±9.0	11.8±7.7
Tunneling (# behaviors)				
0-30 min.	1.6±0.9	0.5±0.3	0±0	0.2±0.1
31-60 min.	1.0±1.0	2.0±0.8	0±0	2.7±2.1
Total	2.6±1.5	2.5±0.9	0±0	2.9±2.2
150 µg AVT Injection	N=8	N=12	N=5	N=15
Spine Response (min. raised)	14.6±7.1	41.8±8.0	0±0	32.9±7.5
Surfacing (# behaviors)				
0-30 min.	12.6±4.6	2.8±2.1	1.6±1.2	1.1±0.5
31-60 min.	24.5±7.1	23.3±8.4	1.2±1.2	10.7±7.5
Total	37.1±10.3	26.0±9.5	2.8±2.3	11.8±7.7
Tunneling (# behaviors)				
0-30 min.	1.6±0.9	0.5±0.3	0±0	0.2±0.1
31-60 min.	5.8±3.8	2.0±0.8	0±0	2.7±2.1
Total	7.4±3.7	2.5±0.9	0±0	2.9±2.2

TABLE 2 - Nesting Assay

Effect of AVT (150 μg ip) or Saline on Nesting Behavior in Brook Sticklebacks

	Saline Injection (N=4)		AVT Injection (N=5)	
	Behavior	# Fish	Behavior	# Fish
Spine Response (min. raised)	60.0 ± 0.0	4	2.8 ± 1.7[A]	2
Nest Building Behaviors (Number of Behaviors)				
Baseline	0.5 ± 0.3	2	1.0 ± 0.3[A]	4[B]
0-30 min.	0.0 ± 0.0	0	0.8 ± 0.2[A]	4[B]
31-60 min.	0.0 ± 0.0	0	1.4 ± 0.5[A]	4[B]
Total (0-60 min.)	0.0 ± 0.0	0	2.2 ± 0.5[A]	5[B]
Fanning Behaviors				
Baseline (Number of Behaviors)	0.5 ± 0.3	2	0.4 ± 0.2	2
Baseline Duration (sec)	2.3 ± 1.3		2.4 ± 1.3	
Post-injection (Number of Behaviors)	0.0 ± 0.0	0	0.6 ± 0.4	2
Post-injection Duration (sec)	0.0 ± 0.0		2.1 ± 1.4	

[A,B]AVT-treated groups differ significantly from Saline-treated groups [A](Mann-Whitney U test; $P < 0.05$), [B](Fisher Exact Test; $P < 0.05$). Data presented are mean ± SEM.

and the saline-treated fish with respect to spine position. The spines on all of the AVT-treated fish either remained lowered or were lowered shortly after injection while the spines on all of the saline-treated fish remained raised for the duration of the observation period (spines raised for an average of 2.8 ± 1.7 min. for AVT-treated fish while spines raised for an average of 60.0 ± 0.0 min. for saline-treated fish; $P < 0.01$, Mann-Whitney U test).

Discussion

The results show that AVT has some influence on the behavior of the brook stickleback, but the exact mechanisms and the extent of the influence of AVT is not known. The major role that AVT seemed to play was in the reduction of stress brought on by handling and injection. In the nesting assay all of the AVT treated fish either lowered their spines shortly after injection or kept them lowered for the duration of the observation period while all of the saline treated fish kept their spines erect for the whole hour. This observation was supported by the spawning reflex assay. The amount of time that the AVT treated fish had erect spines was considerably shorter than the fish treated with saline.

The raised spine response has been shown to be an indicator of stress brought on by territory defense and courtship (Wootton, 1976) as well as injury incurred during trapping and handling (previous observation). At the onset of an aggressive encounter between two males both fish erect their dorsal spines and the aggressor faces the broadside of the opponent (Wootton, 1976). At the end of the encounter, the spines of both fish are usually still raised. The erect spine response is most evident during the aggressive component of courtship behavior. As a male approaches a gravid female, he erects all his spines and lunges toward her, striking her head or upper body (McLennan, 1995). This "pummeling" may or may not bring about a nuptually receptive response in the female, but no spine response in the female has been recorded .

The fact that only AVT treated fish resumed normal nest building and fanning activity also supports the conclusion that AVT reduces the stress caused by handling and injection. The number of behaviors or the duration of the behaviors was not significantly increased after injection with AVT, but the AVT fish did return to the baseline activity levels while the saline treated fish ceased all nest building and fanning.

The physiological mechanism behind the response of the fish to AVT is open to speculation. Populations of cells that are immunoreactive for AVT have been found in the brain of the bullfrog (Boyd, 1992). This may indicate that AVT plays a neurological role in the regulation of behavior in the stickleback. Also, concentration of AVT in the brains of brook trout has been used as an indicator of stress induced by low pH in lakes subject to inflow of acid rain (Hontela et. al, 1993). In this case AVT acts to regulate osmo-ionic homeostasis in order to counteract ion loss and dilution of body fluids as a result of exposure to low pH. In other teleost fish AVT has been shown to have either diuretic or antiduretic effects on freshwater rainbow trout and saltwater flounder (Amer and Brown, 1995; Warne and Balment, 1995) as well as increasing cardiac output in freshwater eels (Oudit and Butler, 1995). These studies suggest that AVT may play a role as a pressor or depressor as the mechanism that reduces stress. The paper by Pickford and Strecker (1977) implicates the pressor activity of AVT as playing a key role in the spawning reflex response of killifish but the exact mechanism was not specified. The findings of other studies may or may not be directly applicable to the role that AVT plays in regulating stickleback behavior as the varied effects that AVT has on other teleost species make it difficult to conclusively say how AVT affects another species.

As far as dose dependence is concerned, there does not seem to be any correlation between higher doses of AVT and an increase or decrease in activity. A dose-response curve may be made possible by dramatically lowering the doses given. Pickford and Strecker (1977) demonstrated that spawning reflex responses were given by 40-60% of male killifish at a dose of 7.5-13.6

Fundulus units, or roughly 47-62.5 ng AVT. Small increases in 50 ng increments up to 180 ng elicit a response in 100% of the test fish. Any increase over this amount brings about the same response. Other work on the pressive action of AVT on other teleost fishes was done using doses on the order of 100-200 ng AVT/kg body mass of fish. Because much smaller doses elicited definite responses in other species, it may be likely that all the doses used in this assay were sufficiently large enough to produce the same response. It may be beneficial for future work to start with a much reduced dose and continue decreasing the dose until a threshold where a noticeable decrease in response is found.

While the exact function of AVT in the brook stickleback is still unclear, it does play some homeostatic or regulatory role. While my results did not parallel those of Pickford and Strecker with killifish, I cannot discount AVT as a regulator of reproductive behavior. Even though this work was done during the breeding season, there may be other time constraints in the breeding cycle of the stickleback that trigger other responses to AVT. For example, AVT may play a role earlier in the season that causes physiological changes associated with the onset of the breeding season such as coloration or hormone release. Also, AVT may act with other hormones in regulating behavior. In addition, AVT may play a role as a diuretic or antidiuretic in regulation of blood pressure or osmoregulation as none of these properties were assayed.

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