



EFFECTS OF ARGININE VASOTOCIN ON THE HYPOTHALAMIC-PITUITARY-GONADS AXIS: A BEHAVIOURAL APPROACH

Ramallo M.¹, Grober M.², Pandolfi M.¹

¹Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina. Ciudad Universitaria. Pabellón II. CABA (C1428EHA). pandolfi@bg.fcen.uba.ar.

²Department of Biology, Georgia State University, USA.

Introduction:

The neuropeptide arginine vasotocin (AVT) has been linked to reproductive plasticity in a variety of teleosts with distinctive types of alternative phenotypes. The manipulation of AVT levels affects behaviours that are exhibited predominantly by animals of one phenotype, such as courtship and aggression [1, 2]. AVT is differentially expressed as a function of alternative phenotypes modulating social behaviour in many fish species [3]. *Cichlasoma dimerus* is a cichlid species in which males are found in one of two basic alternative phenotypes that are linked to both social and reproductive status. Non territorial (NT) males have dark ground coloration and lack immediate access to reproduction when compared with territorial (T) brightly colored males, that actively defend a territory. The aim of this study was to characterize the AVT system in males of *C. dimerus* in relation to social status and aggressive behaviour, with particular emphasis on the various putative sites of action of AVT across the hypothalamic-pituitary-gonads (HPG) axis, and its effects on reproductive and social physiology.

Methods:

The location and distribution of AVT neurons within *C. dimerus*' brain was studied by immunohistochemistry. Each AVT-ir soma was assigned to either the parvocellular (pPOA), magnocellular (mPOA) or gigantocellular (gPOA) preoptic area (POA) subpopulation based on neuroanatomical position, somata morphology and size. To assess differences in neural phenotype associated to social status, the size and number of AVT-ir somata, and the size of AVT-ir nucleus were measured in coronal sections. Cell numbers from each cell group were determined by duplicate by naked-eye observation. Cell and nucleus profile area were computed from digital images by tracing the cell somata's/nuclei's profile with a digitizing pen. To study the effect of AVT on pituitary LH and FSH secretion, pituitaries were incubated for 24 h with AVT (control, 0.1, 1 and 10 μ M). Beta-FSH and beta-LH presence in the culture media was semiquantified by immunoblotting. To investigate the effect of AVT on testicular androgen release testicular fragments were incubated with or without different AVT doses (control,

0.1, 1 and 50 mM). Androgen levels were measured by RIA. So as to study AVT gene expression in different organs, total RNA was isolated from the POA, head kidney, trunk kidney, intestine, muscle, testes and liver. RT-PCR was performed using specific primers for *C. dimerus* AVT preprohormone.

Results and Discussion:

AVT-ir neurons were localized exclusively within the POA. AVT-ir cells extended from behind the anterior commissure to the posterior POA, above the optic chiasm. Immunoreactivity was observed in all 3 POA cell subpopulations: pPOA, mPOA and gPOA, lining the border of the 3rd ventricle. Axons from pPOA cells, targeted ventrally and away from the ventricle. The greatest concentration of AVT-ir axons occurred within the POA where fibers from mPOA and gPOA cells, formed a dense preoptic-hypophyseal (PO-H) tract that entered the pituitary through the anterior and posterior infundibular stalks. Within the pituitary, immunostaining was observed mainly in the *pars nervosa*, proximal *pars distalis* and *pars intermedia*. Some AVT-ir axons from mPOA and gPOA cells targeted other brain regions. AVT-ir fibers were observed in the dorsal sac of the pineal complex, where AVT is believed to play a role in the coordination between photoperiodic cues and reproductive events. Thus, in *C. dimerus* mPOA cells might be involved in the synthesis of AVT which mainly acts as a neurohormone. In turn, the association between AVT-ir nerve terminals with the adenohypophysis, suggests a possible role as a moderating factor for hormone release. Nonetheless, not all AVT-ir fibers from the mPOA incorporated to the PO-H tract which suggests that AVT may act as a neuromodulator, possibly affecting sensory association pathways with social relevance. T individuals had mPOA neurons 11% larger compared with NT males. There were no differences in cells/section number and nuclear area between T and NT males for any of the 3 subpopulations analyzed. In *C. dimerus*, AVT significantly increased pituitary's gonadotropin release and testicular androgen synthesis *in vitro*. Beta-LH showed a triphasic response to AVT increasing doses, while beta-FSH and androgen responses were directly proportional. Androgens would modify, reinforce and/or stabilize the neural circuits



associated with behaviour typical of T males. Expression and peptide synthesis was found in small cells from the interstitial compartment of the testes, presumed to be Leydig cells. Though many studies have informed AVT expression on the testes of various vertebrates [4, 5], to our knowledge this is the first report of AVT detection at a cellular level in a teleost.

Conclusion:

In *C. dimerus*, the AVT system is highly complex with multiple sites of action along the HPG axis. AVT expression levels in mPOA cells are differentially associated to the different social phenotypes in which increased AVT expression, triggers behavioural and physiological changes typical of T males, ensuring access to territory and thus the possibility to reproduce.

References:

- [1]SEMSAR K., KANDEL F. L., GODWIN J., 2001. Manipulations of the AVT system shift social status and related courtship and aggressive behaviour in the bluehead wrasse. *Horm. Behav.*, 40(1):21-31.
- [2]SANTANGELO N., BASS A. H., 2006. New insights into neuropeptide modulation of aggression: field studies of AVT in a territorial tropical damselfish. *Proc. R. Soc., B* 273, 3085–3092.
- [3]GREENWOOD A. K., WARK A. R., FERNALD R. D., HOFMANN H. A., 2008. Expression of AVT in distinct preoptic regions is associated with dominant and subordinate behaviour in an African cichlid fish. *Proc. Biol. Sci.*, 275(1649):2393-402.
- [4]FILLION C., MALASSINE A., TAHRI-JOUTEI A., ALLEVARD A. M., BEDIN M., GHARIB C., HUGUES J. N., POINTIS G., 1993. Immunoreactive AVP in the testis: immunocytochemical localization and testicular content in normal and in experimental cryptorchid mouse. *Biol. Reprod.*, 48(4):786-792.
- [5]SINGH AND JOY K.P., 2008. Immunocytochemical localization, HPLC characterization, and seasonal dynamics of vasotocin in the brain, blood plasma and gonads of the catfish *Heteropneustes fossilis*, *Gen. Comp. Endocrinol.*, 159: 214–225.