Physiology & Behavior 141 (2015) 154–163

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Oxytocin mechanisms of stress response and aggression in a territorial finch

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HIGHLIGHTS

- Oxytocin receptor antagonism reduces aggression in a territorial finch.
- Hypothalamic oxytocin cell groups respond to stressors (subjugation and pursuit).
- Oxytocin inhibition of the HPA axis may be permissive for aggression.

ARTICLE INFO

Article history:
Received 16 July 2014
Received in revised form 12 January 2015
Accepted 13 January 2015
Available online 14 January 2015

Keywords:
Oxytocin
Mesotocin
Aggression
Corticosterone
Stress Coping
Defense
Songbird
Violet-eared waxbill

ABSTRACT

All jawed vertebrates produce a form of oxytocin (OT), and in birds, mammals and fish, OT is strongly associated with affiliation. However, remarkably few data are available on the roles of OT and OT receptors (OTRs) in aggression. Because OT and OTRs exert anxiolytic effects in mammals (although context-specific) and modulate stress coping, we hypothesized that OTR activation is at least permissive for territorial aggression. Indeed, we find that peripheral injections of an OTR antagonist significantly reduce male–male and female–female aggression in a highly territorial finch. This finding suggests the hypothesis that aggression is accompanied by an increase in transcriptional (Fos) activity of OT neurons, but contrary to this hypothesis, we find that dominant male residents do not elevate OT-Fos colocalization following an aggressive encounter and that OT-Fos colocalization in the preoptic area and hypothalamus correlates negatively with aggression. Furthermore, OT-Fos colocalization increases dramatically in males that were aggressively subjugated or pursued by a human hand, likely reflecting OT modulation of stress response. Because OT inhibits the hypothalamo–pituitary–adrenal axis, the antagonist effects may reflect the fact that aggressive birds and mammals tend to be hyporesponsive to stress. If this is correct, then 1) the observed effects of OTR antagonism may reflect alterations in corticosterone feedback to the brain rather than centrally mediated OTR effects, and 2) the negative correlation between OT-Fos colocalization and aggression may reflect the fact that more aggressive, stress hyporesponsive males require less inhibition of the hypothalamo–pituitary–adrenal axis than do less aggressive males, despite the requirement of that inhibition for the normal display of aggression.

1. Introduction

The modulation of social cognition, affiliation and anxiety by the neuropeptides oxytocin (OT)[1] and vasopressin (VP) has become a major field of inquiry, with more than 100 relevant papers being published annually [7]. This literature supports the general view that OT reduces anxiety and promotes many aspects of affiliation in mammals [8–10]. OT also facilitates social approach in goldfish (*Carassius auratus*) [11], and similarly, we have found that flocking behavior in zebra finches (*Taeniopygia guttata*) is promoted by both central OTR activation [12] and OT production in the paraventricular nucleus of the hypothalamus (PVN; a female-specific effect) [4], suggesting an evolutionarily deep history of OT effects on sociality. OT immunoreactivity fluctuates in relation to seasonal flocking in sparrows [13], consistent with these findings in finches. However, OT and OT receptors (OTRs) also influence many other aspects of physiology and behavior, including appetite, immune function, glucocorticoid secretion and thermoregulation [14–17].

Of particular relevance here, OT promotes maternal aggression in selected rat lines ([18]; but see [19]), although it is not clear that OT effects

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[1] Vertebrates express a variety of OT forms, and although the canonical form in mammals is Leu^8-OT, some mammals also express Pro^8-OT or Ile^8-OT (mesotocin) [1–3]. This latter form is expressed in birds and other non-mammalian tetrapods. Similarly, multiple forms of VP have been identified, including the canonical Arg^8-VP form found in most (but not all) mammals, and Ile^3-VP (vasotocin), which is found in non-mammalian vertebrates [1,3]. Thus for clarity, we will simply refer to all OT forms as "OT" and all VP forms as "VP" (following other recent precedents [3,4]). We will also refer to homologous oxytocic receptors (of which one appears to be present in all vertebrates) as OTRs, following the nomenclature of Ocampo Daza et al. [5], and also Yamaguchi et al. [6].

http://dx.doi.org/10.1016/j.physbeh.2015.01.016
0031-9384/

in this context will be observed in other contexts of aggression. In fact, exogenous OT is capable of reducing aggression in male rats, despite the fact that OTR antagonism is largely without effects [20,21]. However, it must be considered that endogenous OT may modulate behavior via heterologous binding to V1a receptors (V1aRs), which are known to mediate at least some of OT's effects (e.g., [22,23]).

In contrast to the voluminous data on the affiliation effects of OT, relatively little is known about the role(s) that endogenous OT plays in same-sex offensive aggression. OT effects on intrasexual aggression have been primarily examined within a developmental framework (e.g., using neonatal OT injections in prairie voles, *Microtus ochrogaster*, and Mandarin voles, *Lasiopodomys mandarinus* [24,25] and intranasal OT administrations in pigs [26]) or using knockout mice that lack OT or the OTR [22,27]. The data obtained using these methods has provided consistent evidence that OT facilitates aggression, contrary to the popular conception of OT as a "prosocial peptide" [28–30]. Regardless, such manipulations do not directly address the influence of OT in adult animals, since these developmental manipulations likely act neonatally to organize brain and behavior [24,25].

Remarkably, only one study has clearly demonstrated that endogenous OT modulates resident-intruder aggression in adults. This experiment shows that OT infusions into the preoptic area-anterior hypothalamus (POA-AH) decrease resident-intruder aggression in female Syrian hamsters (*Mesocricetus auratus*), and more importantly, that OTR antagonism facilitates aggression [31]. However, broad generalization from this finding to males, and to other brain areas and species is difficult, because 1) AH infusions of VP exert opposing effects on aggression in male and female hamsters ([32]; and thus OT may likewise exert sex-specific effects, particularly given that OT-VP receptors tend to be promiscuous; e.g., [33–35]); 2) OT effects on maternal aggression are mediated in areas of the brain outside of the POA-AH, including the amygdala [18,19]; and 3) the neural distribution of OTRs is species-specific [12,33,36–39].

Importantly, because OT is important for anxiolysis, fear reduction and stress coping [40–42], and because aggressive birds, fish, and some rodents tend to be hyporesponsive to stress (defined primarily in terms of glucocorticoid secretion [43–47]), we hypothesized that OTR activation is at a minimum permissive for territorial aggression, even if it does not actively promote it (but note that stress reactivity likely interacts with coping style to modulate aggression, rather than having unitary effects; [48,49]).

One caveat is that endogenous OT does not exert anxiolytic effects across all contexts, but rather in specific contexts that are otherwise associated with OT release, such as lactation and parturition [50]. In fact, recent antisense experiments in zebra finches (conducted after the completion of the present experiments) demonstrate that OT neurons of the paraventricular hypothalamus (PVN) do not modulate anxiety when subjects are tested in a nonsocial context, and actually promote passive coping behavior [4] (note that although these more recent data could not inform our hypotheses for the experiments presented here, they are nonetheless important to consider in relation to the results, and will be revisited in the Discussion section). A second consideration is that OT inhibits basal and stress-induced activity of the hypothalamo–pituitary–adrenal (HPA) axis in a manner that appears to be uncoupled from the central modulation of anxiety [51], at least in rodents.

The species under investigation here, the violet-eared waxbill (Estrildidae: *Uraeginthus granatina*) is exceptionally territorial [52,53], and thus we hypothesized that aggressive interactions in this species will drive endogenous release of OT, which could thus modulate both the HPA axis and central anxiety processes (presuming socially- and/or stress-mediated OT release; see [50] for a consideration of context-specific effects on anxiety), thereby facilitating aggression. Other known effects of OT may also serve to facilitate aggressive response, such as enhanced sensory processing of social stimuli, and modulation of autonomic function (reviews: [7,54]).

Despite the potential for the independent modulation of central and peripheral processes [55], it is also possible that the central and peripheral effects of OT (e.g., sensory and autonomic effects) are coupled to some extent. This is because the parvocellular OT population of the PVN that projects to the anterior pituitary also projects centrally, including the autonomic brainstem; and magnocellular OT populations that project to the posterior pituitary also release peptide centrally from axons, soma and dendrites (reviews: [7,54,56]).

Thus, in order to examine the combined central and peripheral effect of oxytocic signaling on aggression, we here quantified resident-intruder aggression following peripheral injections of an OTR antagonist or vehicle in male and female violet-eared waxbills. In a second experiment we then quantified the Fos responses of OT neurons to handling (control), pursuit by a human hand, aggressive subjugation, or aggressive domination. OT-Fos colocalization was quantified for the two largest hypothalamic populations, which lie in the PVN and supraoptic nucleus (SON), as well as within the smaller OT populations of the medial preoptic nucleus (POM; magnocellular) and AH (embedded in the hypothalamo-pituitary tract; parvocellular).

2. Materials and methods

2.1. Subjects

Captive-bred violet-eared waxbills ($n = 9$ females and 24 males) were individually housed in cages 61 cm W × 36 cm D × 43 cm H on a 14L:10D photoperiod and provided finch mix and water ad libitum. Experiments were conducted under non-breeding conditions and subjects were separated from opposite-sex partners for a minimum of two weeks prior to testing. Violet-eared waxbills are generally encountered as singletons or as pairs in the wild (unless dependent young are present) ([53]; J.L. Goodson, pers. obs.), and thus the period of isolation is not expected to produce behavioral abnormalities. Violet-eared waxbills are aggressive year-round [52]. Experiments were performed in compliance with federal and institutional guidelines and were approved by the Institutional Animal Care and Use Committee of Indiana University.

2.2. OTR antagonist experiment

2.2.1. Behavioral screenings

Same-sex pairs of violet-eared waxbills were screened for aggression in short resident-intruder assays (most less than 1 min; none more than 2 min; terminated as soon as an obvious dominance relationship appeared) in order to select subordinate (intruder) and dominant (resident) subjects. Due to the limited number of birds available, most birds were used as both stimuli and subjects, with at least 1 week between tests. This was possible because most birds could dominate an intruder in their home cage, but were subordinated as intruders. Only subjects who dominated same-sex intruders in their own home cage were used as subjects.

2.2.2. Injections

Thirty minutes prior to testing, subjects (9 females and 10 males) were injected into the inguinal leg fold with either 0.05 cm^3 saline vehicle or vehicle containing 5 μg OTR antagonist (desGly-NH_2,d(CH_2)$_5$[Tyr(Me)^2,Thr^4] ornithine vasotocin) in a counterbalanced, repeated-measures design with 2 days allowed between tests. This dose has been used in previous studies and effects have been replicated using a much lower dose centrally [12]. Importantly, effects of systemic administration of the OTR antagonist do not produce general behavioral alterations. For instance, subjects do not decrease approach to conspecifics [12], and despite potent effects on nest-building behavior in female zebra finches, the behavior of males is not altered [57], suggesting that effects are highly specific. Another important consideration is that an iodinated form of this same