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Suppression of sex behavior by kappa opiates and stress steroids occurs via independent neuroendocrine pathways

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ABSTRACT

Endocannabinoids and their receptors are found throughout the brain of all vertebrates. By virtue of their wide distribution, endocannabinoids have the potential to affect many behaviors. Prior research has shown that cannabinoids inhibit courtship-clasping and mediate behavioral responses to stress in male rough-skinned newts, *Taricha granulosa*, and cannabinoid signaling is initiated by rapid actions of the steroid corticosterone (CORT) at its specific membrane receptor (mCR). This same mCR also recognizes κ-opioid receptor agonists and antagonists. Prior behavioral studies show that κ-opioid agonists suppress clasping behavior in a dose dependent manner. Combined, these studies suggest that κ-opioid agonists might suppress clasping behavior via the same pathway initiated by CORT: up-regulation of endocannabinoid signaling. We examined whether pretreatment with a CB_1 antagonist, AM281, would block κ-opioid-mediated suppression of clasping. We found that the CB_1 antagonist did not reverse κ-opioid-induced suppression of clasping, revealing that while endocannabinoids mediate CORT-induced suppression of clasping, endocannabinoids do not mediate the κ-opioid-induced suppression of clasping.

1. Introduction

The ability of an animal to respond appropriately when faced with an immediate threat is critical to survival. Behavioral responses to acute stressors have been identified in all major groups of vertebrates (Breuner et al., 1998; Burmeister et al., 2001; Lumley et al., 1999; Mitra and Sapolsky, 2008; Sandi et al., 1996). While glucocorticoids (stress-steroids) are instrumental in the chronic disease states developed after experiencing prolonged stress, they also initiate rapid behavioral responses to direct threats or acutely stressful situations (Breuner et al., 1998; Coddington et al., 2007; Moore and Miller, 1984). A majority of the neuroendocrine research into stress-steroid effects has focused on long-term genomic effects (Akama and McEwen, 2005; Carrasco and Van de Kar, 2003; Ferguson et al., 2008; Ferguson and Sapolsky, 2007; Kaufer et al., 2004; Sapolsky, 2000). However, the neuroendocrine regulation of rapid behavioral/neuronal responses to immediate threats is not yet well understood. Examining neural mechanisms of hormone regulation of behavior in the rough-skinned newt, *Taricha granulosa*, provides a unique perspective for three main reasons. First, *Taricha* offers the opportunity to examine the effects of acute stress on a well-characterized behavior, male courtship clasping (see below) (Propper, 1991; Rose and Moore, 1999). Second, the functional properties of the key medullary neurons that regulate clasping behavior have been well characterized using single-unit extracellular recordings (Rose et al., 1993, 1995, 1998; Rose and Moore, 1999). Third, the stress-induced neuroendocrine cascade and behavioral impact of corticosterone (CORT) on clasping; (Moore and Miller, 1984; Moore and Orchinik, 1994; Orchinik et al., 1991, 1994), κ-opiates (Deviche and Moore, 1987; Evans et al., 2000), and endocannabinoids (Coddington et al., 2007) have been well characterized in this species.

The behavior we have focused on in this study is courtship clasping, a robust behavior that is rapidly suppressed by acute stress. The courtship clasping behavior engaged by male *Taricha* is both consistent and predictable: male *Taricha* grasp sexually attractive females with their hind and forelimbs in an ardent amplectic clasp (Arnold, 1977; Propper, 1991). The essential element of clasping is a synchronized tightening of male hind legs, which is initiated and maintained in response to somatosensory stimulation of the male's cloacal region on the ventrum between the hind limbs. Previous studies have shown that when male *Taricha* are first subjected to an acute stressor and then presented with a female, they consistently fail to clasp (Coddington et al., 2007; Coddington and Moore, 2003; Moore and Miller, 1984).

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In *Taricha*, three neuromodulators have been shown to rapidly suppress clasping: CORT (Moore and Miller, 1984), κ-opioid receptor agonists (Deviche and Moore, 1987), and cannabinoids (Coddington et al., 2007; Soderstrom et al., 2000). Corticosterone administration, or exposure of males to stressors that naturally stimulate CORT secretion, rapidly suppresses clasping without affecting locomotor behavior (Moore and Miller, 1984). The behavioral response to CORT occurs within minutes of experiencing an acute stressor or administration of CORT, and is too rapid to be mediated by traditional genomic mechanisms; instead, ligand-binding assays using *Taricha* neuronal membranes revealed that CORT most likely binds to a membrane-associated G-protein coupled receptor (mCR) (Orchinik et al., 1991). This membrane-bound receptor gives an animal the advantage of rapid responses to hormonal changes.

Evidence from *in vitro* studies suggests that κ-opioid agonists might suppress clasping by binding to the same site on the same membrane receptor used by CORT, the mCR (Evans et al., 2000). U50488 binds to both the mCR and it's own native κOR in *in vitro* preparations of *Taricha* brains; binding to the mCR with the same affinity as CORT (K_i = 250 nM; (Evans et al., 2000)), and binding its own receptor (κOR) with a higher affinity (K_i = 3.4 nM; (Bradford et al., 2005). Furthermore, the mCR has a single CORT binding site that has very high specificity and affinity for the CORT and κ-opiate ligands, and very low affinity for mineralocorticoids or classical intracellular glucocorticoid receptor antagonists (Orchinik et al., 1991; Evans et al., 2000). Given that the κ-opioid agonist U50488 binds to the mCR *in vitro*, it is reasonable to hypothesize that κ-opioids might suppress clasping via the same mCR and neuroendocrine pathway used by CORT (H_0 in Fig. 1). Because the molecular identity of the mCR has yet to be determined, an arsenal of specific/selective antagonists and agonists is not yet available. We do have the capacity to test this hypothesis using behavioral pharmacology. If CORT and κ-opioids share the same mCR, then κ-opioids, like CORT, should also suppress courtship behaviors in *Taricha*. Indeed, studies examining courtship behavior of *Taricha* (Deviche and Moore, 1987) and other animals (Agmo et al., 1994) supports the notion that κ-opioids suppress courtship behaviors, but the mechanism remains unknown.

κ-opioid agonists also have discrete behavioral effects via their own receptors, such as suppression of locomotor activity (Deviche et al., 1989). Studies in *Taricha* (Deviche et al., 1989) and mice (Ukai and Kameyama, 1985) have revealed that high doses of κ-opioid agonists will depress locomotor activity, and that this effect is reversed by the general opiate antagonist naloxone. Therefore, it is also possible that κ-opioids might suppress clasping indirectly through binding to κ-opioid receptors.

Behavioral, molecular, and binding data suggest that CORT and κ-opioids might share a common mechanism, binding the mCR, by which they suppress courtship behaviors and mediate stress-induced behavioral responses (Fig. 1). Behavioral and electrophysiological data show that CORT inhibits clasping by up-regulating the signaling of endogenous cannabinoids (Coddington et al., 2007). This is likely to be a conserved mechanism by which CORT exerts its rapid effects in the central nervous system of vertebrates, as this neuroendocrine cascade has also been demonstrated in the hypothalamic magnocellular neurons of rodents (Di et al., 2003, 2005a,b). If CORT and κ-opioid agonists are working through the mCR, then κ-opioid agonists should elicit the same phenotype as CORT, which includes both CB signaling and specific suppressive effects on clasping. If so, the κ-opioid agonist U50488 would suppress clasping without suppressing locomotor activity, and this effect would be blocked by pretreatment with a cannabinoid antagonist as we have seen with CORT. Alternatively, if κ-opioid effects are elicited through its own receptor, then blocking cannabinoid signaling should have no effect on κ-opioid-mediated suppression of clasping, and doses of κ-opioid agonist that suppress clasping would also suppress locomotor activity.

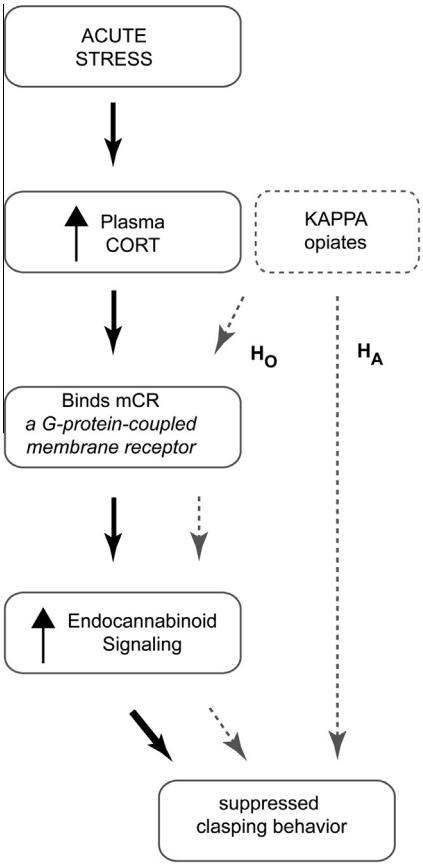

Fig. 1. Schematic outline of hypotheses. The neuroendocrine pathway by which acute stress leads to suppression of clasping behavior has been well established (indicated by solid boxes and arrows). Dotted boxes and lines outline the hypotheses tested in the current study (null hypothesis = H_o, alternative hypothesis = H_a). Based on several lines of evidence from behavioral, molecular, and binding studies we predicted that κ-opioid agonists suppress clasping by their action on membrane CORT receptors, mCR. If this is correct, then blocking endocannabinoid signaling should block κ-opioid-mediated suppression of clasping and blocking κ-opioid receptors should not block κ-opioid-mediated suppression of clasping.

2. Methods

2.1. Animals

Animals were collected from January Pond in Lincoln County, OR (Latitude 44.6, Longitude 123.6). Sexually mature and active adult male rough skinned newts (*T. granulosa*, 15 ± 1 g) were collected during the active breeding season (March 2007 and 2010). Sexually mature and active males are identified by the following criteria: smooth epidermis, thickened neck and dermis, enlarged tail height, mate-seeking behavior, and >13 g. Males were held in community tanks (10 Gallon glass aquaria: 20 × 10 × 12 inches) maintained with running, dechlorinated water (20 °C, *depth* 30 cm). Each tank held up to 30 male newts and had Styrofoam floats for the animals to rest on. Four chopped earthworms were fed to each community tank daily. Sexually mature and receptive females were captured en route to their natural breeding ponds and were housed in 10-gallon aquaria complete with mosses, ferns and dechlorinated water (20 °C). Females were injected intraperitoneally (i.p.) every 48 h with 5 I.U./ml prolactin (Sigma–Aldrich, USA) to maintain their sexual receptivity and attractiveness to