0013-7227/07/$15.00/0
Printed in U.S.A.

Endocrinology 148(2):490–492

doi: 10.1210/en.2006-1522

Endocannabinoids Link Rapid, Membrane-Mediated Corticosteroid Actions to Behavior

The rapid actions of steroid hormones, although recognized for over half a century, until recently were largely overshadowed by the study of genomic actions mediated by nuclear hormone receptors. Many steroid hormones are now known to exert physiologically and behaviorally important actions within seconds to minutes, a time frame far too rapid to be mediated by genomic mechanisms. The recent isolation of several G protein-coupled receptors (GPCRs) that mediate the actions of sex steroids and 1,25 dihydroxyvitamin D_3 has brought the rapid, membrane-mediated steroid signaling pathways to the forefront of neuroscience, endocrinology, and cell biology (1–5).

In a recent review, Mary Dallman (6) speculated that membrane steroid receptors represent a primitive form of signaling by steroidal compounds that arose before the appearance and radiation of the nuclear hormone receptor superfamily. Comparative studies of steroid hormone signaling highlight the importance of nontraditional, animal model systems for elucidating mechanisms of hormone action and their evolutionary history. The first definitive report of membrane-mediated action of a steroid hormone was described in oocytes of the frog *Xenopus laevis* (7). Recently, a GPCR that mediates membrane signaling by progestins was isolated from the spotted seatrout (3) and subsequently in mammals including humans (4).

Rapid, negative feedback actions of corticosteroids on the activity of the hypothalamo-pituitary-adrenal axis have been recognized for over half a century (6, 8). Other studies have reported fast actions of corticosteroids on neurophysiology and behavior (8). One of the earliest discoveries of a rapid effect of corticosteroids on behavior came from work done with the rough-skinned newt, *Taricha granulosa* by Frank Moore and colleagues at Oregon State University in Corvallis (9, 10) (see Fig. 1). In many amphibian species including newts, amplectic clasping behaviors are used during courtship and mating (11). Males embrace the female with their fore and hind legs, gripping her firmly for periods that can extend for many hours. Clasping is inhibited by exposure to stressors, or by injection of corticosterone (see Ref. 10 and references therein). The suppressive actions of corticosterone on clasping are rapid, occurring within minutes of injection. Moore's group went on to show that these rapid corticosteroid actions on behavior were mediated by a membrane corticosteroid receptor (9). The identity of this membrane corticosteroid receptor is still unknown, but several lines of evidence suggest that it is a GPCR (12–14). In collaboration with James Rose's group at the University of Wyoming, Moore and colleagues (15) went on to show that corticosterone causes rapid suppression of firing of medullary neurons that control the clasping behavior. These were the first experiments to link discrete neurophysiological responses to a steroid hormone with a specific behavioral output.

In this issue of *Endocrinology*, Emma Coddington *et al.* (16) extend these earlier findings by showing that the rapid actions of corticosterone on newt clasping behavior are mediated by endocannabinoid signaling. They show that blockade of the cannabinoid type 1 (CB1) receptor with a specific receptor antagonist inhibited the ability of corticosterone to suppress courtship clasping. Importantly, the CB1 receptor antagonist did not alter functioning of the hypothalamo-pituitary-adrenal axis, as measured by plasma corticosterone concentrations. Thus, the stress hormone corticosterone acts as a switch, allowing the animal to go from a reproductive behavioral mode (clasping) to one of vigilance or retreat, and therefore has immediate, adaptive value for the animal. Inhibition of corticosterone action via blockade of endogenous cannabinoid signaling results in the continuation of clasping behavior, which would be a maladaptive behavioral response should the animal be confronted with a predator.

Coddington and colleagues also found that the neurophysiological consequence of blockade of the CB1 receptor was the inhibition of corticosterone's ability to suppress firing of medullary neurons known to control the clasping behavior. Courtship clasping behavior is a reflex initiated by somatosensory stimulation of the cloaca that results in the bilateral gripping of the female by the male's hind limbs. The initiation, maintenance, and cessation of the behavior are directly controlled by hindbrain medullary inputs (15). The actions of endocannabinoids within vertebrate motor systems have recently begun to be investigated (17). Hill *et al.* (18) recently published results that support a role for endocannabinoids in modulating stress-induced behavior in rats, suggesting that such mechanisms are evolutionarily conserved. The power and novelty of the study by Coddington and colleagues is that they have linked rapid, neurophysiological responses to corticosteroids dependent upon endocannibinoid signaling with a discrete behavioral output.

Endocannabinoids are emerging as important signaling molecules functioning in most if not all major endocrine axes (19). They are derived from membrane phospholipids (*e.g.* arachidonic acid) and thus are not stored in vesicles and released upon a stimulus. Instead, endocannabinoid signaling is dependent upon the regulation of synthesis, uptake, and degradation (20). Endocannabinoids function in a paracrine manner as neuromodulators and have been shown to mediate several forms of neural plasticity, *e.g.* short-term removal of excitatory input (depolarization-induced suppression of excitation) or inhibitory input [depolarization-

Abbreviations: AVT, Arginine vasotocin; CB1, cannabinoid type 1; GABA, γ aminobutyric acid; GPCR, G protein-coupled receptor.

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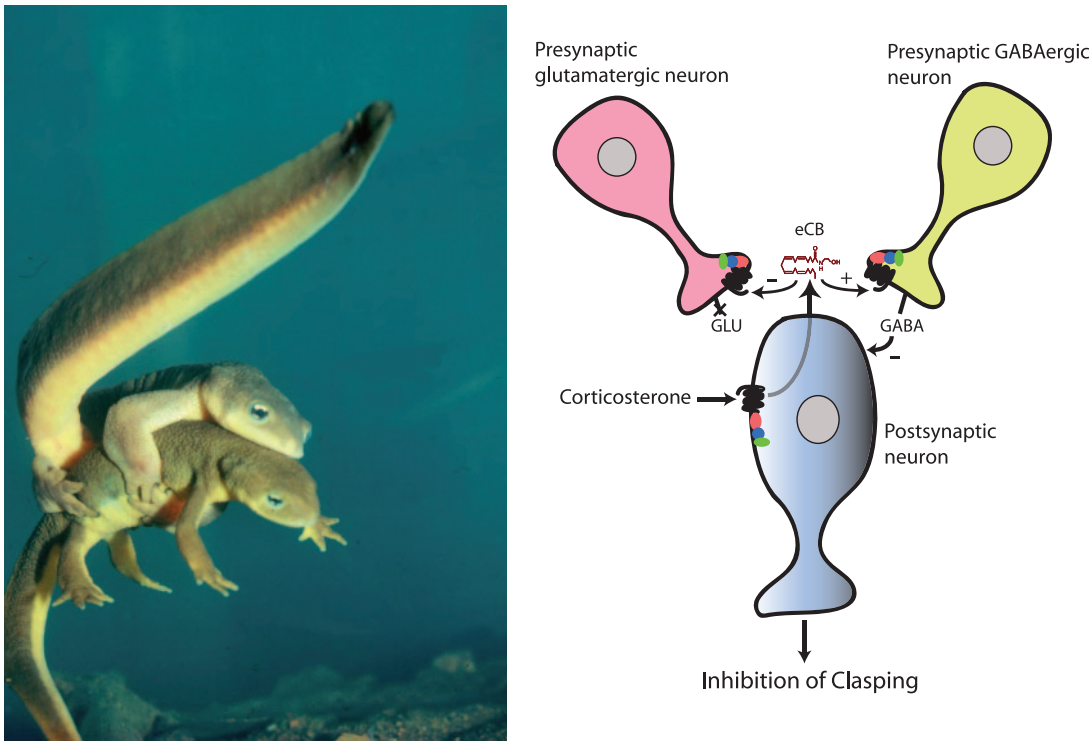

FIG. 1. Corticosterone inhibits newt clasping behavior via an endocannabinoid-dependent pathway. The *left panel* shows a male rough-skinned newt (*Taricha granulosa*) clasping a female newt. This behavior is rapidly inhibited by exposure to a stressor or by injection of corticosterone. The *right panel* depicts a proposed model for endocannabinoid-mediated inhibition of newt clasping. Corticosterone binds to a GPCR expressed on membranes of medullary neurons, causing the release of an endocannibinoid (eCB). The eCB acts on presynaptic neurons in a retrograde manner to inhibit the release of glutamate (GLU) and/or to stimulate the release of GABA. This suppresses activity in the postsynaptic neuron leading to the inhibition of clasping. Model is based on Coddington *et al.* (16) and Di *et al.* (23, 25, 26). Photo courtesy of Frank Moore.

induced suppression of γ aminobutyric acid (GABA) inhibition; also, depolarization-induced potentiation of inhibition (21, 22)].

Endocannabinoids signal in a retrograde manner, being produced by the postsynaptic cell to influence the activity of the presynaptic cell (Ref. 21; and see Fig. 1). In the context of rapid actions of corticosteroids on the brain, recent work from Jeffrey Tasker's laboratory showed that the inhibitory actions of corticosteroids on the mammalian hypothalamus depend upon endocannabinoids acting in a retrograde manner (23–26). They showed that corticosteroids rapidly inhibited glutamate release onto identified parvocellular neurons in the paraventricular nucleus that included corticotropin-releasing factor neurons (26). Their findings are consistent with corticosteroids activating a GPCR to cause the release of an endocannabinoid by the postsynaptic cell, leading to retrograde inhibition of presynaptic glutamatergic neurons. They subsequently went on to show that endocannabinoids (in this case they studied the magnocellular neurons of the supraoptic and paraventricular nuclei producing oxytocin and vasopressin) not only suppress glutamate release, and but also facilitate GABA release by activating a putative membrane corticosteroid receptor (25). Thus, the postsynaptic cell regulates, via the production of endocannabinoids, inhibitory or excitatory input to itself. Interestingly, exposure to chronic stress is known to blunt responses to acute stress, and one mechanism for this may be the down-regulation of endocannabinoid signaling (27).

Corticosterone does not always suppress newt clasping behavior, but is dependent on the animal's prior physiological and behavioral state (28, 29). Arginine vasotocin [(AVT) or arginine vasopressin (AVP)] is known to promote clasping behavior in newts and other amphibians (30). Signaling via AVT neurons can influence responses of male newts to corticosterone. For example, injecting AVT or allowing a male to clasp a female for an extended period (*e.g.* up to 1 hr) before corticosterone injection abrogates the effect of corticosterone on clasping behavior (28). These results highlight the context-dependent nature of corticosterone effects on behavior and could reflect state-dependent endocannibinoid release and/or actions. Zhuang *et al.* (31) recently showed that endocannabinoid mediation of depolarization-induced suppression of GABA inhibition depends on temporal summation of converging synaptic inputs to allow calcium entry into the postsynaptic cell. Thus, suppression of inhibition may require that the recipient postsynaptic neuron (producing the endocannabinoid) receive multiple, coincident excitatory synaptic inputs. The possibility that context might be coded by the brain to determine endocannibinoid release, and thus produce contextually relevant behavioral responses to elevated stress hormones, is a plausible hypothesis that deserves investigation.

Taken together with work showing that endocannabinoids mediate rapid actions of corticosteroids on hypothalamic neurosecretory neurons, the work of Coddington and colleagues in the newt suggest that endocannabinoids partici-

pate at multiple levels to coordinate endocrine and behavioral responses to stressors. The survival of the individual, and ultimately the perpetuation of the species, depends upon the ability to respond adaptively to a threatening situation. Coddington and colleagues hypothesize that endocannabinoid mediation of rapid corticosteroid actions on behavior may be generalizable to other vertebrates, including mammals, given the deep evolutionary conservation of animal endocrine systems.

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Acknowledgments

Received November 14, 2006. Accepted November 22, 2006.

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Disclosure Statement: R.J.D. has nothing to declare.

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