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Minireview

Gonadotropin-inhibitory hormone inhibits aggressive behavior of male quail by increasing neuroestrogen synthesis in the brain beyond its optimum concentration

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ABSTRACT

The action of testosterone on male socio-sexual behaviors, such as aggressive and sexual behaviors, requires its aromatization into estrogen (neuroestrogen) in the brain. Gonadotropin-inhibitory hormone (GnIH) is a hypothalamic neuropeptide that inhibits gonadotropin secretion from the pituitary. On the other hand, wide distribution of GnIH-immunoreactive (ir) neuronal fibers in the brain suggested their roles in the regulation of behavior. Our recent studies have shown that GnIH indeed inhibits aggressive and sexual behaviors. Accordingly, we further investigated the effect of GnIH on aromatase activity and estrogen synthesis in the brain. Abundant GnIH-ir neuronal fibers were observed in the vicinity of aromatase-ir cells in the brain, such as in the preoptic area (POA) that is thought to be the most critical site of aromatization action and neuroestrogen action for the regulation of socio-sexual behavior. GnIH receptor (GPR147) mRNA was also expressed in aromatase-ir cells in the POA. GnIH stimulated the activity of aromatase and increased neuroestrogen synthesis in the POA through GPR147. The increase in neuroestrogen concentration in the POA was associated with a significant decrease in aggressive behavior. Finally, centrally administered 17β-estradiol at higher doses inhibited aggressive behavior. These findings indicated that GnIH inhibits aggressive behavior by directly activating aromatase and increasing neuroestrogen synthesis in the brain beyond its optimum concentration for the expression of aggressive behavior. This review highlights recent findings of the role of GnIH in the regulation of neuroestrogen synthesis and its possible function in the regulation of socio-sexual behaviors.

1. Introduction

Gonadotropin-inhibitory hormone (GnIH) is a hypothalamic neuropeptide that was shown to inhibit gonadotropin secretion from the pituitary of the Japanese quail *Coturnix japonica* (Tsutsui et al., 2000; Ubuka et al., 2006; for reviews, see Tsutsui, 2009; Tsutsui et al., 2010; Tsutsui and Ubuka, 2013; Ubuka et al., 2013c; Ubuka and Bentley, 2010). In birds, GnIH is synthesized in neurons of the paraventricular nucleus (PVN) (Tsutsui et al., 2000; Ukena et al., 2003; Ubuka et al., 2003). GnIH neurons project to the median eminence to control anterior pituitary hormone secretion (Tsutsui et al., 2000; Ukena et al., 2003; Ubuka et al., 2003). On the other hand, abundant GnIH-immunoreactive (ir) fibers are observed in other brain areas, such as the preoptic area (POA) and the periaqueductal central gray (PAG) (Ubuka et al., 2008). The mRNA of the cognate G protein-coupled receptor (GPR147) for GnIH is also expressed in the POA and PAG (Yin et al., 2005; Ubuka et al., 2008). POA and PAG are the brain areas that regulate socio-sexual behaviors, such as aggressive and sexual behaviors (Cornil et al., 2012; Absil et al., 2001). Accordingly, GnIH released in these brain areas has a potential to modify aggressive and sexual behaviors.

To understand the role of GnIH in the regulation of behavior, Ubuka et al. (2012b) investigated the effect of RNA interference (RNAi) of the GnIH gene on the behavior of white-crowned sparrows *Zonotrichia leucophrys gambelii*, which is a highly social songbird species. Intracerebroventricular (ICV) administration of small interfering RNA (siRNA) against GnIH precursor mRNA to male and female birds increased locomotor activity and stimulated agonistic vocalizations. GnIH RNAi further enhanced territorial song of male birds when the birds were challenged by novel male songs. The overall results suggested that GnIH gene silencing induces

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socio-sexual arousal. It was thus hypothesized from this study that GnIH decreases socio-sexual arousal resulting in the inhibition of socio-sexual behaviors (Ubuka et al., 2012b,c, 2013b). It was also shown that ICV administration of GnIH to female white-crowned sparrows inhibited copulation solicitation, which is analogous to mammalian lordosis (Bentley et al., 2006).

Japanese quail is a commonly used laboratory species to study socio-sexual behaviors and its neurophysiological and neuroendocrine bases (Mills et al., 1997). Sexually mature male quail frequently fight with intense aggressiveness, by using series of stereotyped actions (Mills et al., 1997; Selinger and Bermant, 1967). Aggressive behavior of male quail is thought to be androgen dependent because it is reduced by castration and restored by androgen treatment (Mills et al., 1997; Selinger and Bermant, 1967), although there is generally no correlation between the order of aggressiveness and peripheral testosterone concentration (Tsutsui and Ishii, 1981). It is thought that full expression of testosterone action in the brain requires its aromatization into 17β-estradiol (E2), because aggression of inactive males is only activated by aromatizable androgen such as testosterone and androstenedione or E2, but not by non-aromatizable androgen such as dihydrotestosterone, and co-administration of aromatase inhibitors blocks testosterone-induced aggression (Tsutsui and Ishii, 1981; Schlinger and Callard, 1990). Although it is thought that the action of testosterone on male socio-sexual behaviors requires its aromatization into estrogen (neuroestrogen) in the brain (the aromatization hypothesis), the precise mechanism remains unclear (Yahr, 1979; Balthazart et al., 2009, 2011).

Ubuka et al. (2013a) hypothesized that GnIH may inhibit aggressive behavior of male quail by regulating aromatase activity and neuroestrogen synthesis in the brain. Ubuka et al. (2013a) first measured daily changes in the frequency of aggressive behavior of male quail, and tested the effect of ICV administration of GnIH on aggressive behavior when its natural expression in high. Ubuka et al. (2013a) also investigated the effect of GnIH RNAi and concomitant ICV administration of GnIH on aggressive behavior of male birds. Because POA is thought to be the most critical site of aromatization and neuroestrogen action for the activation of socio-sexual behavior of male quail (Balthazart and Surlemont, 1990; Panzica et al., 1996), Ubuka et al. (2013a) investigated if GnIH neuronal fibers innervated aromatase cells and aromatase cells expressed GnIH receptor (GPR147) mRNA in the POA. Ubuka et al. (2013a) also measured daily changes in GnIH content and release, GPR147 mRNA expression, aromatase activity, E2 content and release in the brain blocks including the POA. Ubuka et al. (2013a) then investigated the effect of GnIH administration on aromatase activity and E2 synthesis in the POA *in vitro* and *in vivo*. Ubuka et al. (2013a) found that GnIH stimulates the activity of aromatase and increases E2 synthesis in the brain *in vitro* and *in vivo*. Finally, Ubuka et al. (2013a) tested the effect of central administration of E2 on aggressive behavior of male quail and showed that higher doses of E2 inhibit aggressive behavior. The overall results suggested that GnIH directly activates aromatase and increases neuroestrogen synthesis in the brain beyond its optimum concentration for the expression of aggressive behavior of male birds (Ubuka et al., 2013a).

2. Aggressive behavior is intense in the morning and gradually decreases until the evening

When sexually active male quail are paired in a relatively small cage they fight using sequential stereotypic aggressive actions. They often threaten the opponent by stretching the neck and walking around (Strutting behavior), approach and chase, peck the opponent (Pecking behavior), grab the back of the opponent's head or neck with their beak (Grabbing behavior), attempt to ride on the back of the opponent (Mounting behavior), ride on the back of the opponent and lower their cloaca close to the opponent's cloaca [Cloacal contact (CC)-like actions]. The frequency of these actions represents the activity of aggressive behavior of male quail (Mills et al., 1997; Selinger and Bermant, 1967; Tsutsui and Ishii, 1981; Schlinger and Callard, 1990).

All male quail used in the experiment were kept under long day photoperiods (16 h light, 8 h dark) to keep them sexually active. Ubuka et al. (2013a) counted the number of Strutting, Pecking, Grabbing, and Mounting actions, and CC-like action in 5 min during the light hours around zietgeiber time (ZT) 3, 6, 9, and 12 h. The frequency of Strutting, Pecking, and Grabbing actions was significantly high in the morning (ZT 3 h) and decreased in the afternoon (ZT 9 h) and the evening (ZT 12 h). The frequency of Mounting and CC-like actions tended to decrease until the evening.

3. Central administration of GnIH inhibits aggressive behavior independent of peripheral testosterone concentration

Ubuka et al. (2013a) then tested the effect of ICV administration of GnIH (100 pmol) on the frequency of aggressive behavior of male quail in the morning (ZT 3 h) when their natural expression is high. ICV administration of GnIH inhibited the number of Strutting and Pecking actions in 5 min, quantified 30 min after administration. Similar inhibitory trends in the number of Grabbing, Mounting, and CC-like actions by GnIH administration were observed.

To investigate the relationships between the frequencies of aggressive behaviors and peripheral testosterone concentration, Ubuka et al. (2013a) took blood samples immediately after the behavioral tests and measured testosterone concentration in the serum. However, there was no correlation between testosterone concentration in the serum and the number of Strutting, Pecking, Grabbing, and Mounting actions, or CC-like action in all birds tested.

4. GnIH may decrease socio-sexual arousal resulting in the inhibition of aggressive behavior

Ubuka et al. (2013a) also tested the effect of GnIH gene silencing and ICV administration of GnIH on aggressive behavior of male quail. The number of five stereotypic actions of aggressive behavior was compared among the birds that were administered with control RNA or GnIH siRNA (each 2 nmol) the day before the behavioral test, because one day treatment of GnIH siRNA most significantly suppressed the expression of GnIH mRNA and GnIH levels. The number of aggressive actions was compared in the evening when their natural expression was low. GnIH or vehicle was administered 35 min before the behavioral test. GnIH RNAi significantly increased the number of Strutting action, and ICV administration of GnIH decreased the number of Strutting action induced by GnIH RNAi treatment. The number of Pecking, Grabbing, and Mounting actions, and CC-like action showed similar trends by GnIH RNAi and GnIH administration.

Locomotor activity was also compared among the birds that were administered with control RNA or GnIH siRNA the day before and administered with vehicle or GnIH 30 min before the behavioral test as a measurement of socio-sexual arousal (Ubuka et al., 2012b; Pfaff, 2006; Pfaff et al., 2008). GnIH RNAi significantly increased locomotor activity and ICV administration of GnIH decreased locomotor activity induced by GnIH RNAi treatment. On the contrary, no effect of GnIH RNAi or GnIH administration was observed on food intake in 3 h that was measured immediately after the behavioral tests.

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