

The role of GABA_A and GABA_B receptors in the control of GnRH release in anestrus ewes

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Received: 22 September 2005; accepted: 15 December 2005

SUMMARY

The paper reviews data concerning the involvement of GABA_A and GABA_B receptors in the control of GnRH secretion in anestrus ewes. Generally, GABA influences the GnRH release through GABA_A and GABA_B receptors located on perikaria of the GnRH neurons in the preoptic area (MPOA) or through the influence on β -endorphinergic and catecholaminergic systems activity in MPOA and in ventromedial-infundibular region of the hypothalamus (VEN/NI). Stimulation of GABA_A receptors in VEN/NI and MPOA attenuates GnRH release, while activation of GABA_B receptors in MPOA decreases GnRH secretion, and in VEN/NI increases concentration of GnRH. The different neural mechanisms could be involved in this process: direct ligand action on the GABA_A and GABA_B receptors located on GnRH cells and axon terminals or indirect effect through the changes in the β -endorphinergic and catecholaminergic systems activity in these structures of the brain. *Reproductive Biology* 2006 6 (Suppl.2):3–12.

Key words: γ -aminobutyric acid, gonadotropin-releasing hormone, β -endorphin, catecholamines, ewe

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INTRODUCTION

Despite extensive studies in several species, we still do not understand the neural mechanisms that control gonadoliberein (GnRH) secretion. The regulation of GnRH secretion is associated with a complex interplay among various excitatory and inhibitory neurotransmitters and neurohormones within the hypothalamus. Among these trans-synaptic regulatory systems, γ -aminobutyric acid (GABA), a major inhibitory neurotransmitter in the hypothalamus [5] and preoptic area [16] affects GnRH secretion by two different classes of membrane receptors: GABA_A [19] and GABA_B [15] receptors.

Numerous experiments indicate that GABA_A receptors in hypothalamus are implicated in both stimulatory [6] and inhibitory [23] effects of GABA on GnRH release depending upon physiological state of animals [17] and the site of action [4]. The role of GABA_B receptors in the regulation of GnRH secretion is less known. It has been documented that in rodents GABA acts *via* GABA_B receptors to reduce GnRH secretory activity [2]. By contrast, in ovariectomized estrogen-treated ewes [17] and castrated or castrated testosterone-primed rams during non breeding season [7,13], activation of GABA_B receptors in the preoptic area or mediobasal hypothalamus greatly increases GnRH/LH secretion. On the other hand, neither activation nor blockade of GABA_B receptors in ovariectomized estrogen-treated ewes during breeding season affects GnRH/LH release [17, 18]. This dual inhibitory-stimulatory action of GABA on GnRH release results, at least in part, from the indirect action of GABA through the other neuronal systems involved in the control of this hormone release.

The studies outlined in this review were designed to analyze the role of the GABA_A and GABA_B receptor mediating systems in the control of GnRH release from the ventromedial-infundibular region (VEN/NI) of the hypothalamus and from the preoptic area (MPOA) of anestrous ewes.

THE INVOLVEMENT OF GABA_A RECEPTORS IN THE CONTROL OF GnRH RELEASE IN THE VEN/NI AND MPOA OF ANESTROUS EWES

During the anestrous period, stimulation of GABA_A receptors with muscimol in VEN/NI significantly attenuated GnRH release with concomitant increase of β -endorphin and dopamine (DA) concentration, but the blockade of these receptors did not affect GnRH outflow and reduced β -endorphin and DA output (tab.1; [21]).

Table 1. The influence of GABA_A receptor stimulation (GABA_A+) or blockade (GABA_A-) on the GnRH and β -endorphin release and catecholaminergic activity in VEN/NI and MPOA of anestrous ewes

| Compound | VEN/NI | | MPOA | |
|----------|---------------------|---------------------|---------------------|---------------------|
| | GABA _A + | GABA _A - | GABA _A + | GABA _A - |
| GnRH | ↓ | 0 | ↓ | 0 |
| B-END | ↑ | ↓ | 0 | 0 |
| NE | 0 | 0 | ↑ | 0 |
| MHPG | ↑ | 0 | ↑ | 0 |
| DA | ↑ | ↓ | ↓ | 0 |
| DOPAC | ↑ | ↓ | ↓ | 0 |

VEN/NI: ventromedial-infundibular region of the hypothalamus;
MPOA: preoptic area; B-END: β -endorphin; NE: norepinephrine;
MHPG: the main metabolite of norepinephrine; DA: dopamine;
DOPAC: the main metabolite of dopamine.
↑ increase, ↓ decrease, 0: lack of changes

These results indicate that regulation of GnRH release by GABA may involve at least serially-arranged interactions with GnRH-ergic, β -endorphinergic and dopaminergic neural systems in the VEN/NI. They support the view that the arcuate region of the hypothalamus is an important regulatory site, where activation of GABA_A receptors decreases GnRH/LH secretion, which was documented in other *in vivo* and *in vitro* studies on sheep [7] and rats [4]. There are at least two possible mechanisms to explain the action of GABA_A receptor agonist, muscimol, in these processes. First, muscimol may act directly by activating GABA_A receptors on the GnRH axon terminals in the VEN/NI. The second possibility is that

pharmacological suppression of GnRH release results from the activation of inhibitory neuronal systems or inhibition of excitatory neurons with respect to GnRH secretion. Our results suggest that GABA can, at least in part, suppress GnRH release in the VEN/NI through activation of β -endorphinergic and dopaminergic systems [21]. The concept that GABA may alter GnRH release by acting on β -endorphinergic and dopaminergic neuronal systems in VEN/NI is supported further by the observation that in rats, GABA-ergic neurons synapse with arcuate β -endorphinergic and dopaminergic neurons [11, 12] and in anestrus ewes the suppression of LH release results, to a large degree, from the inhibitory action of opioids and dopamine on hypothalamic GnRH neurons [8].

The blocking of GABA_A receptors in the VEN/NI had no evident effect on GnRH secretion in anestrus ewes. Similar paradoxical effects of GABA_A receptor agonist and antagonist were found in sheep and rats. Both muscimol and bicuculline elevated LH release when injected in the MPOA of ovariectomized estrogen-treated ewes, but both suppressed it when introduced into ovariectomized animals [17]. A similar injection of either GABA or bicuculline into the MPOA of ovariectomized rats reduced the circulating concentration of LH [10]. Thus, pharmacological manipulation of GABA_A receptors indicates, that these receptors are able to mediate both stimulatory and inhibitory effects of GABA on GnRH /LH release and that the effect to a large degree depends on the gonadal steroid milieu.

In another investigated structure, MPOA, stimulation of GABA_A receptors with muscimol in anestrus ewes, attenuated GnRH release, decreased dopaminergic and increased noradrenergic system activity without any evident changes in β -endorphin concentration. The blockade of GABA_A receptors had no visible effect on the activity of any of these systems (tab.1; [22]).

Activation of GABA_A receptors in MPOA of anestrus ewes leads to complex changes in GnRH release, noradrenergic and dopaminergic activity. The presented data are consistent with other *in vivo* and *in vitro* studies on rats [10, 14] and sheep [7, 17], which documented that MPOA is a major regulatory site where the activation of GABA_A receptors decreases GnRH/LH secretion. There are a few possible modes of action explaining the suppression of GnRH release by activation of GABA_A receptors. Muscimol may activate GABA_A

receptors on GnRH perikaria in the MPOA, as has been suggested for GABA's effect in rats [9]. On the other hand, the suppression of GnRH release may result, at least in part, from a serial interaction of muscimol with GABA_A receptors on catecholaminergic and opioidergic neuronal systems in MPOA. On the basis of all of the presented results it is still not possible to evaluate to what extent the inhibitory effect of muscimol on GnRH release is exerted directly through GABA_A receptor mechanism on GnRH cells or indirectly through neurochemical processes modulating catecholaminergic system activity. First of all, it remains to be clarified why blocking of GABA_A receptors in the MPOA of intact anestrous ewes does not cause any changes in GnRH/LH release and catecholaminergic system activity. In light of recent data it is not clear why stimulation and inhibition of GABA_A receptors in the MPOA in the same physiological state (anestrous ewes) act in a similar fashion on GnRH/LH release [17]. Perhaps the most reasonable explanation for these contradictory data sets may be the assumption that GABA_A receptors are located on numerous stimulatory and inhibitory neuronal systems with respect to GnRH secretion. Finally, results clearly demonstrated that neither muscimol nor bicuculline had an evident effect on extracellular β -endorphin concentration [22], suggesting that the GABA_A receptor mechanism modulating GnRH/LH secretion does not act *via* the β -endorphin neuronal system in the MPOA of anestrous ewes.

In conclusion, the activation of GABA_A receptors suppresses GnRH release. It is suggested that GABA may operate in this process directly by GABA_A receptors located on GnRH axon terminals in the VEN/NI or on GnRH cells in MPOA, or indirectly through the GABA_A receptor mechanism on β -endorphinergic and catecholaminergic neurons in VEN/NI and on catecholaminergic systems in MPOA. These results and data from literature failed to provide a basis for distinguishing between these two processes.

EFFECTS OF GABA_B RECEPTOR MODULATION ON GnRH RELEASE IN VEN/NI AND MPOA OF ANESTROUS EWES

Stimulation of GABA_B receptors with baclofen in VEN/NI during the anestrous period, led to complex changes in GnRH-ergic, β -endorphinergic

and noradrenergic systems activity. It induced an increase in GnRH release, decrease in β -endorphin secretion and noradrenergic system activity without any effect on the content of DA and DOPAC, the main metabolite of dopamine. A blockade of GABA_B receptors with phaclofen in this structure had no effect on GnRH release and catecholaminergic activity but increased β -endorphin concentration (tab. 2; [20]).

Table 2. The influence of GABA_B receptor stimulation (GABA_B +) or blockade (GABA_B -) on the GnRH and β -endorphin release and catecholaminergic activity in VEN/NI and MPOA of anestrus ewes

| Compound | VEN/NI | | MPOA | |
|----------|---------------------|---------------------|---------------------|---------------------|
| | GABA _B + | GABA _B - | GABA _B + | GABA _B - |
| GnRH | ↑ | 0 | ↓ | 0 |
| B-END | ↓ | ↑ | 0 | ↓ |
| NE | ↓ | 0 | ↑ | 0 |
| MHPG | ↓ | 0 | ↑ | 0 |
| DA | 0 | 0 | ↑ | 0 |
| DOPAC | 0 | 0 | ↑ | 0 |

VEN/NI: ventromedial-infundibular region of the hypothalamus;
MPOA: preoptic area; B-END: β -endorphin; NE: norepinephrine;
MHPG: the main metabolite of norepinephrine; DA: dopamine;
DOPAC: the main metabolite of dopamine.
↑ increase, ↓ decrease, 0: lack of changes

The stimulatory influence of baclofen on GnRH release in anestrus ewes is consistent with data obtained in other studies on ovariectomized estrogen-treated ewes [18] and castrated or castrated testosterone-primed rams [7,13]. By contrast, similar treatment of ewes during the breeding season has no evident effect on gonadotropin secretion [17,18]. The obtained results suggest that the stimulatory effect of baclofen on GnRH release in the VEN/NI may be considered the net result of at least the three following possible modes of action: (i) the direct influence of this ligand on GABA_B receptors on GnRH nerve terminals, (ii) the indirect action through GABA_B receptor processes on the noradrenergic system, (iii) action on β -endorphinergic neurons in this structure.

The first mode of action, (i.e. that baclofen activates GnRH release from the nerve terminals in the VEN/NI) appears to be unlikely. All re-

ports, at least in rodents, have implicated GABA_B receptors as mediating a suppressive effect of GABA on GnRH release [2]. The indirect mode of action – through the noradrenergic and β -endorphinergic systems appears to be more likely. The most suggestive evidence for this point of view is the fact that GABA has a stimulatory effect on GnRH in ovariectomized estrogen-treated ewes during the non-breeding season but has no effect in the breeding period [17,18]. The seasonal-dependent role of norepinephrine and β -endorphin in the regulation of GnRH secretion in ewes is well recognized. The most probable hypothesis is that the increase in GnRH release during baclofen perfusion might be accounted, at least in part, by a GABA_B receptor mechanism on the noradrenergic system in the VEN/NI. Stimulation of GABA_B receptors decreased norepinephrine output and hence its inhibitory effect on GnRH secretion in anestrus ewes. On the basis of these results [18], the lack of any changes in catecholaminergic activity and GnRH release in the VEN/NI during the blockade of GABA_B receptors is rather difficult to interpret. The increased activity of the β -endorphinergic system during phaclofen treatment does not affect GnRH release supporting the view that GABA does not play a significant role in the control of GnRH secretion through β -endorphinergic neurons.

In the MPOA, during the anestrus period, the stimulation of GABA_B receptors with baclofen significantly attenuated GnRH release, with a concomitant increase in dopaminergic and noradrenergic system activity. Blocking GABA_B receptors with phaclofen did not affect GnRH secretion and catecholaminergic system activity. The only observed effect was a decrease in the β -endorphin concentration (tab. 2; [Tomaszewska-Zaremba, unpublished]).

These results are consistent with other reports stating that, at least in rodents, GABA_B receptors are involved in mediating a suppressive effect of GABA on GnRH release. In rats, GABA affects GnRH release *via* GABA_B receptors, reducing GnRH release, as pharmacological stimulation of these receptors inhibits both the preovulatory LH surge [1] as well as neurotransmitter-mediated activation of GnRH/LH secretion [3]. The complex changes in GnRH release, noradrenergic and dopaminergic system activities during the stimulation of GABA_B receptors in the MPOA of anestrus ewes suggest that inhibition of GnRH secretion in this structure

involves direct action of GABA on GnRH cells in the MPOA as well the indirect GABA_B receptor mechanism on catecholaminergic systems.

In conclusion, the activation of GABA_B receptors in the VEN/NI of anestrus ewes increased GnRH release while in the MPOA attenuated the secretion of this hormone. It may be suggested, that the activation of GnRH secretion in the VEN/NI of anestrus ewes results primarily from a decrease in norepinephrine output hence the inhibitory effect on GnRH release from GnRH nerve terminals in this structure. Our results indicate that activation of GABA_B receptors suppresses GnRH release in the MPOA during anestrus, suggesting that GABA may operate in this process directly by GABA_B receptors located on GnRH cells in this structure or indirectly through GABA_B receptor mechanism on catecholaminergic neurons.

CONCLUSIONS

In summary, GABA is one of the most important inhibitory neurotransmitters in the regulation of GnRH secretion. Our results show that GABA influences the GnRH secretion in anestrus ewes through GABA_A and GABA_B receptors located on the perikaria of the GnRH neurons in the MPOA or through influence on the activity of β -endorphinergic and catecholaminergic systems in the MPOA and VEN/NI. Stimulation of GABA_A receptors in the VEN/NI and MPOA attenuates GnRH release and modulates in a specific manner the activity of β -endorphinergic and catecholaminergic systems. Stimulation of GABA_B receptors in the MPOA decreases GnRH secretion while in the VEN/NI increases the concentration of GnRH. Activation of GnRH secretion in the VEN/NI results primarily from a decrease in norepinephrine output. Different neural mechanisms could be involved in the suppressive influence of muscimol on GnRH release in the VEN/NI and MPOA, and suppressive influence of baclofen in the MPOA of anestrus ewes, either directly through the GABA_A and GABA_B receptors located on GnRH cells and axon terminals or indirectly through changes in the activity of β -endorphinergic and catecholaminergic systems in these brain structures.

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