

# GABA and Gonadotropin Secretion: Evidence From *In Vitro* Studies on Regulation of LHRH Secretion

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Over the last several years our knowledge of  $\gamma$ -aminobutyric acid (GABA) functions in the central nervous system has grown steadily. In particular, increasing evidence has been accumulated suggesting that GABA may play a fundamental role at the hypothalamic-pituitary level in the control of pituitary hormone secretion (11). In this chapter, we will present a summary of our own results and those of others on the involvement of GABA in the regulation of gonadotropin secretion.

The role of GABA and its analogs in the control of gonadotropin secretion has not yet been fully clarified, with some studies suggesting a stimulatory effect of GABA on gonadotropins, in particular LH, while others favoring an inhibitory effect. In terms of the site of action at which GABA may influence gonadotropin secretion one of the early studies seems to suggest a hypothalamic site more than a pituitary one (15).

In that study the injection of GABA directly into the anterior pituitary of anesthetized male rats did not significantly change LH plasma levels, while the intraventricular (IVT) injection induced a dose-dependent increase in LH but not FSH. A few years later the same author, using in this case conscious male rats, was unable to show an increase in LH levels after IVT injection of GABA (16). The different results obtained in anesthetized and conscious rats may be due to the ability of the anesthetic used to enhance GABAergic neurotransmission (3). However, in ovariectomized and ovariectomized estrogen-progesterone primed conscious rats the IVT injection of GABA was able to increase plasma LH levels and this action seems to be specific, since it can be completely blocked by bicuculline (19). In a subsequent study using chronically ovariectomized rats it was shown that the IVT injection of 4  $\mu$ moles of GABA, but

not 0.1  $\mu$ mol, increased LH levels and this increase was associated with an elevation in LHRH content in suprachiasmatic-medial preoptic area while the LHRH content in the ME remained unaltered (13). In that study the possibility that the GABA-induced activation of DA release at the median eminence (ME) may also lead to enhanced LHRH release and consequently increased LH secretion was also considered. However, since GABA-induced LH release is not altered by the specific dopaminoergic blocker, pimozide (20), any role of DA in the GABA-induced LH release seems to be unlikely. In disagreement with the above results, prevalently supporting a stimulatory effect of GABA on LH secretion, other reports suggest primarily an inhibitory action of this neurotransmitter on gonadotropin secretion. In fact, either the administration of gamma-acetylenic GABA (GAG), a GABA transaminase inhibitor which increases brain GABA levels (8), or the IVT injection of GABA are able to block pregnant mare serum (PMS) induced ovulation in immature rats (2). Moreover, GAG is also able to prevent the proestrus LH surge and ovulation in freely-moving adult female rats (6).

In order to better understand the involvement of GABA in the control of gonadotropin secretion, and therefore reconcile the contrasting results reported in the literature, we performed experiments to evaluate the direct effect of GABA and its specific agonist, muscimol, on LHRH release in vitro. We used adult intact male and one week orchidectomized (ORDX) Sprague-Dawley rats as tissue donors. A hypothalamic fragment consisting of the intact arcuate nucleus-median eminence (ARC-ME) region and surrounding structures was quickly microdissected and incubated in vitro in a Krebs buffer. LHRH release was evaluated by radioimmunoassay during a 30 min incubation period following a 30 min preincubation. As shown in Fig. 1, GABA and muscimol are able to increase LHRH release from ARC-ME fragments. The higher specificity of muscimol toward GABA receptors in relation to GABA itself might explain the more pronounced and dose-dependent effect of this drug on LHRH release. To evaluate the specificity of muscimol in LHRH release, vasopressin and oxytocin release were also measured. The results reported in Table 1 show that LHRH release increases after incubation with muscimol whereas vasopressin and oxytocin secretion do not change. The effect of muscimol seems to be mediated by the GABA-A receptor, since the effect of this agonist is completely prevented by bicuculline, a specific GABA-A receptor antagonist (4). Moreover, Baclofen, the most specific GABA-B receptor agonist (4), does not change basal LHRH release (Table 2) indicating that the GABA-B receptors are not likely involved in the stimulatory effects of GABA on LHRH release.

The possibility that gonadal steroids may modulate muscimol-stimulated LHRH release is suggested by our studies comparing the effect of this GABAergic agonist in intact and 1 week ORDX rats (Fig. 2). Muscimol enhances LHRH release in vitro both in

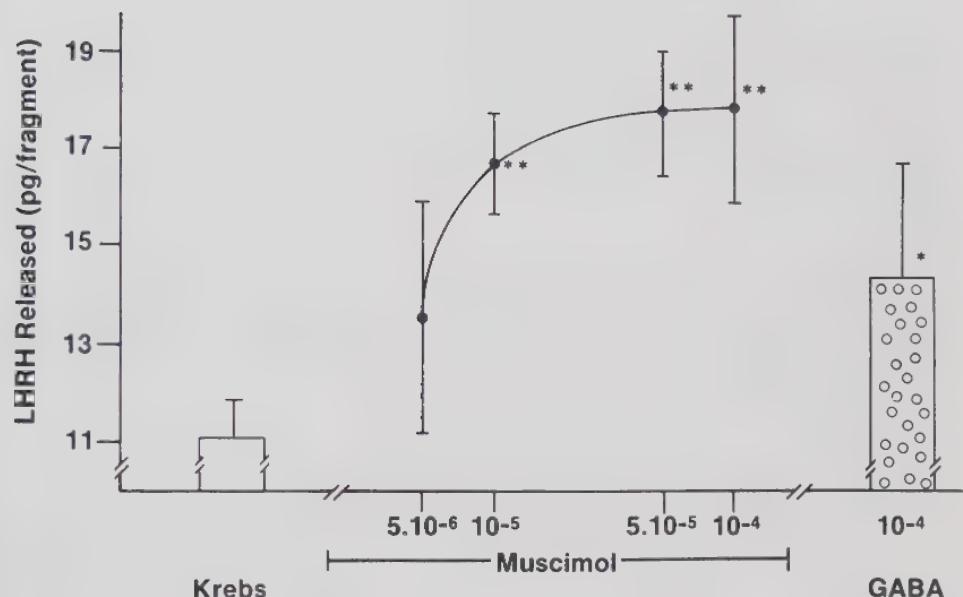


Figure 1. Effect of different concentrations of muscimol and  $\gamma$ -aminobutyric acid (GABA) on LHRH release from arcuate nucleus-median eminence fragments in vitro. \*P < 0.05 vs. Krebs. \*\*P < 0.01 vs. Krebs.

TABLE 1. Effect of muscimol on LHRH, arginine vasopressin (AVP), and oxytocin (OXY) release from arcuate nucleus-median eminence fragments incubated in vitro

Treatment	n	LHRH			AVP pg released/fragment	OXY
		10 <sup>-6</sup>	10 <sup>-5</sup>	10 <sup>-4</sup>		
Krebs	9	12.1 ± 0.5			101.3 ± 15.3	56.7 ± 9.1
Muscimol (10 <sup>-5</sup> M)	9		17.9 ± 0.9*		84.8 ± 11.2	68.8 ± 8.5

Values are mean ± S.E.M.; n = number of flasks per group.

\*P < 0.01 versus Krebs control buffer.

TABLE 2. Effect of different drugs on LHRH release from arcuate nucleus-median eminence fragments incubated in vitro.

	Control	Muscimol	Bicuculline	Muscimol + Bicuculline	Baclofen
LHRH released (pg/ fragment)	10.5 ± 1.8	14.2* ± 1.4	10.5 ± 1.3	9.6 ± 0.7	11.4 ± 0.9

Values are the mean ± S.E.M. of at least 7 flasks per group.

All drugs were used at the concentration  $10^{-4}$  M.

\*P < 0.05 versus control.

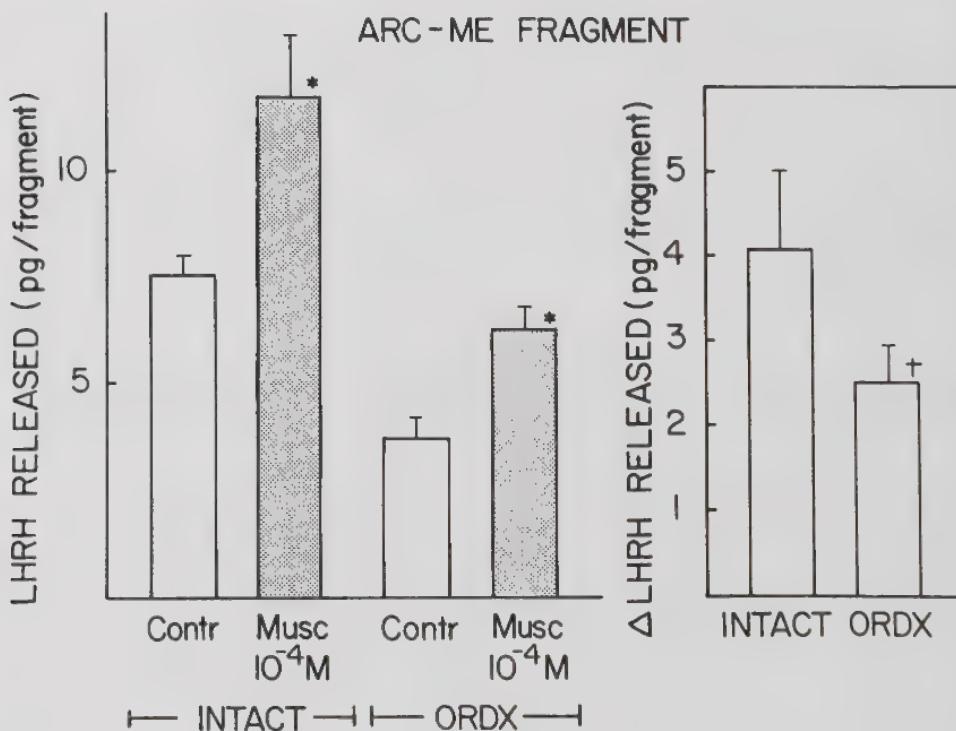


Figure 2. Influence of orchidectomy on muscimol-induced release of LHRH from arcuate nucleus-median eminence in vitro. \*P < 0.05 vs. respective control. +P < 0.05 vs. intact animals.

intact and in ORDX rats; however in terms of  $\Delta$  LHRH release the effect of the drug is more pronounced in intact than in ORDX animals. This last result suggests that in our experimental model the presence of gonadal steroids may modulate GABAergic action on LHRH release. The possible modulatory action of gonadal steroids should be carefully evaluated. Results reported in the literature indicate that the physiological state of the animal used in the experiment may influence the stimulatory or inhibitory action of GABA on gonadotropin secretion. In fact, stimulatory effects were seen during tonic gonadotropin secretory conditions (4, 19), whereas inhibitory effects can prevalently be seen, as reported by Donoso and Banzan (6), in phasic secretory situations such as the preovulatory LH surge in female rats.

Another issue that may be important to explain the different effects of GABA on gonadotropin secretion is the site of action. As already mentioned, a direct action of GABA on anterior pituitary gonadotropes seems unlikely (15), even if the presence of GABAergic receptors has been shown in the anterior pituitary gland. However, these receptors seem to be primarily involved in the control of PRL secretion (1, 17). Most likely GABAergic effects on gonadotropin secretion take place at the hypothalamic level. At this level, the existence of an intrinsic hypothalamic pathway with cell bodies in the arcuate nucleus and terminals in the ME and perhaps in other hypothalamic and extra-hypothalamic areas has been suggested (11, 18). Further observations from more recent work using double-label immunocytochemical techniques have also shown interconnections between GABAergic, catecholaminergic and LHRH neurons both in the arcuate nucleus and in the medial preoptic area (10). In these areas it is also well known that the opioid peptides have a tonic inhibitory action on gonadotropin secretion (9, 12). The results from our ARC-ME fragment incubation indicate that the stimulatory action of GABA on gonadotropin secretion is due to an increased release of LHRH from the ME (Fig. 3) and that the ARC-ME region may be the place where the stimulatory action of GABA actually takes place. The mechanism of this action on LHRH is still unknown. Since the neurotransmitter GABA plays mainly an inhibitory role (14) a direct stimulatory influence of GABAergic neurons on LHRH terminals, as hypothesized in Fig. 3, seems to be unlikely. However, we can not at the moment exclude this possibility. Another possibility is that GABA exerts its stimulatory action on LHRH release by inhibiting some system that has an inhibitory influence on LHRH, such as the opioid system. *In vitro* incubation of the ARC-ME fragment with naloxone has shown the presence of a tonic inhibitory influence exerted by the opioid system on LHRH release (12). Further studies are being carried out to investigate possible interactions between GABAergic and opioid systems at the ARC-ME level.

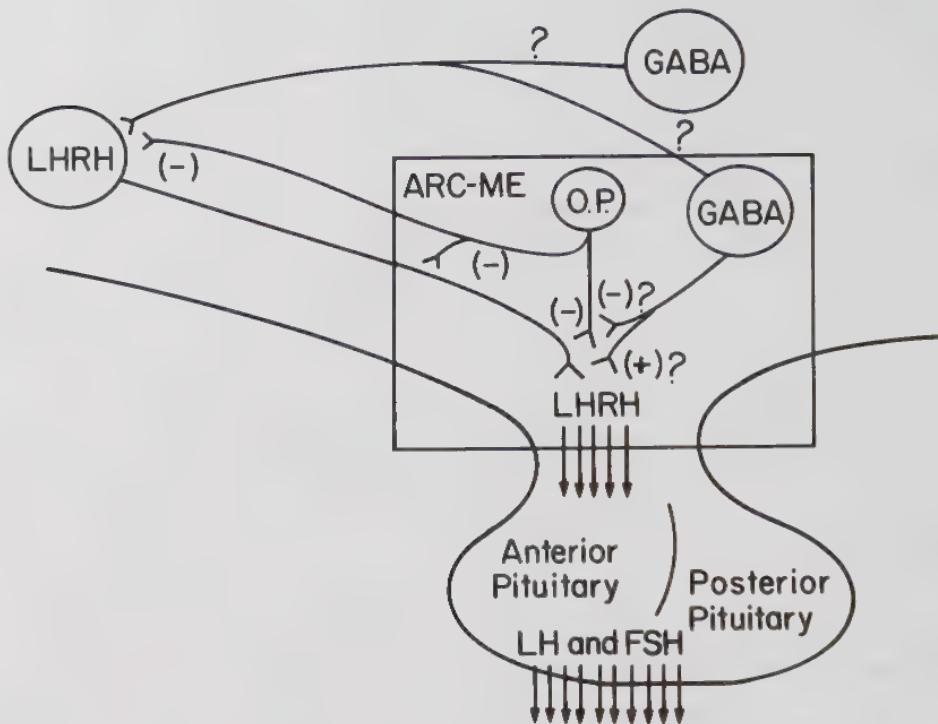


Figure 3. Schematic representation of possible connections in hypothalamic and extrahypothalamic areas known to be involved in the control of gonadotropin secretion. O.P.: opioid peptide neuron. ARC-ME: the rectangle depicts interactions that may occur within the arcuate-median eminence region.

The stimulatory effect of GABA observed in our ARC-ME fragment *in vitro* incubation is similar to experiments in which GABA is given IVT (see above references), another situation where high concentration of GABA will be obtained at the arcuate nucleus level. In contrast, inhibitory effects are known to occur after administration of GABA-T inhibitors which are able to increase GABA concentration in all brain areas indiscriminately (7). In this last situation the increased GABA content in the medial preoptic area, where typical inhibitory GABAergic synapsis on LHRH neurons are located (10), may overcome the stimulatory action of GABA on gonadotropin secretion and an inhibitory effect, in this way, may prevail. The hypothesis that the inhibitory effects of GABA on gonadotropin secretion may take place at the medial preoptic area level is supported by some results reported by Wuttke et al (21). In the medial preoptic area a population of estrogen-receptive GABAergic neurons are believed

to inhibit NE release by the mechanism of presynaptic inhibition. Since NE has been shown to have a stimulatory action on LHRH neurons these GABAergic neurons may indirectly modulate the activity of LHRH neurons (22). Implantation of estradiol into the medial preoptic area as well as implantation of muscimol in this area induces suppression of blood LH levels and this action seems to be mediated by the noradrenergic system (21). This last hypothesis has been recently supported by a study evaluating the preoptic catecholamine and GABA release utilizing the push-pull cannula technique (5). In this study, the authors show that the preoptic GABA release rates have generally an inverse pattern to NE and E release and consequently also to blood LH levels.

### CONCLUSION

In conclusion, our studies underline the involvement of the GABAergic system in the control of gonadotropin secretion and also suggest that this action may be mediated by other neurotransmitter and/or neuropeptide systems and modulated by the gonadal steroid milieu. Furthermore, our results focus on the possibility that different hypothalamic and extrahypothalamic site(s) of action of GABA on LHRH neurons may be the key to understand both the stimulatory and the inhibitory action of GABA on gonadotropin secretion.

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