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The neurochemistry of the GnRH pulse generator

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Abstract. We review the crucial role of the two neurotransmitters norepinephrine (NE) and GABA in eliciting GnRH pulses. NE acts *via* an α1-receptor mechanism and also GABA acts at the α-subtype of the GABA receptor. The function of NE appears to be induction of phasic activation of GnRH neurons and GABA inhibits GnRH neurons tonically until they are all ready for phasic activation. By an unknown mechanism preoptic GABA release is dramatically reduced which causes simultaneous desinhibition of the GnRH neurons. Hence they release their product into the portal vessels simultaneously which is the appropriate signal for the pituitary ganodotrophs. The action of norepinephrine and GABA is most likely exerted at the perikarya level of the GnRH neurons since the α1-adreno receptor blocker doxazosin and GABA inhibit GnRH secretion only when applied into the medial preoptic/anterior hypothalamic area (where in the rat brain the GnRH perikarya are located). Utilizing a quantitative reverse transcription polymerase chain reaction, we demonstrate furthermore that GnRH receptors are present in the mediobasal hypothalamus as well as in the preoptic area of rats. Their function appears to serve autoinhibitory purposes since Buserelin added to medium significantly decreased GnRH release. Simultaneously, the release of GABA was increased and that of glutamate decreased. We conclude from these experiments that GABAergic and glutamatergic neurons in the hypothalamus may also be GnRH-receptive.

Key words: GnRH pulse generator, GABA, norepinephrine, GnRH-receptors, preoptic area, mediobasal hypothalamus

In all mammalian species studied so far, pituitary gonadotropin release is crucially dependent on pulsatile GnRH secretion from hypothalamic neurons (Knobil 1980). A prerequisite for GnRH neurons in the hypothalamus to release their peptide simultaneously is phasic and synchronous activation (Wilson et al. 1984). The location of the neurons and their neurotransmitter qualities involved in the induction of phasic and synchronous activation of GnRH neurons is largely unknown. In the rat, the perikarya of GnRH neurons are located in the rostral hypothalamus and these neurons accumulate in the medial preoptic/anterior hypothalamic area (MPO/AH) (Witkin et al. 1982, Jennes and Conn 1994). The axons of these neurons travel few mm into the median eminence where they terminate at portal vessels. Utilizing *in vivo* techniques with push-pull cannulae implanted either in the MPO/AH or in the mediobasal hypothalamic median eminence (MBH) complex, the release rates of a variety of neurotransmitters can be measured and correlated with the occurrence of LH pulses in the blood of each individual animal (Ondo et al. 1982, Jarry et al. 1988, 1991, 1992). The experimental paradigm is that each LH pulse in the blood is the result of a GnRH pulse released from the GnRH neuronal axon terminals in the median eminence (Levine and Ramirez 1980) and that this is the result of neurotransmitters acting either at the perikaryal and/or the terminal levels of the GnRH neurons (Jarry et al. 1995).

In animals with intact gonads, the GnRH pulse generator is influenced by gonadal steroid hormones feeding back into the hypothalamus and a variety of neurons and their transmitter qualities have been identified to be steroid-receptive. The first neurotransmitter identified in preoptic and hypothalamic estrogen-receptive neurons was the inhibitory amino acid neurotransmitter gamma-amino butyric acid (GABA). In these experiments it was clearly demonstrated that many glutamic acid decarboxylase (GAD) positive neurons are estrogen-receptive. (Flügge et al. 1986). Since GAD is the enzyme converting glutamate into GABA, it was concluded that a large number of preoptic and hypothalamic estrogen-receptive neurons utilize GABA as a neurotransmitter. Consequently, we studied in the past the *in vivo* release rates of a variety of amino acid neurotransmitters, including GABA, in the MPO/AH (the location of the GnRH perikarya) and compared it with the respective release rates in the MBH (Jarry et al. 1988, 1991). To avoid the confounding effects of gonadal steroids, the basal function of the GnRH pulse generator was studied in ovariectomized (ovx) rats. In the absence of estrogens and progestins pituitary LH secretion is high and in sequentially withdrawn blood samples (sampling interval 5 min) LH pulses with high amplitude which occur every 20-40 min, can easily be identified (Examples see Figs. 1-5).

It is known for decades that catecholamines, particularly norepinephrine (NE), are of crucial importance for the normal function of the GnRH pulse generator and thus for normal pituitary LH release. Implantation or infusion of α1-adreno receptor blockers, such as prazosin or doxazosin, into the MPO/AH but not in the MBH disrupted promptly pulstatile LH secretion indicating that norepinephrine acts in the MPO/AH via an α1-receptive

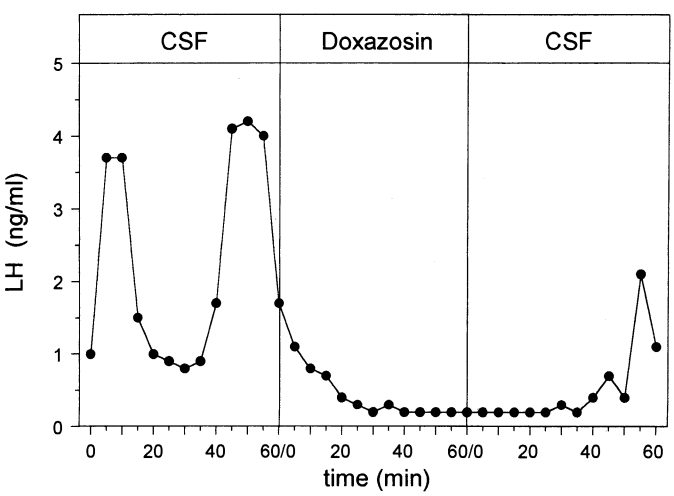

Fig. 1. Perfusion of the MPO/AH with artificial CSF did not disrupt LH pulsatility in the blood. When doxazosin, a water-soluble α1-adreno receptor blocker (2 μg/ml, flow rate of perfusion medium: 20 μl/min), was added to the perfusion medium, this resulted in an immediate cessation of LH pulsatility. When the doxazosin-containing artificial CSF was replaced by pure CSF, LH pulsatility reoccurred within the next hour. $*P < 0.05$ vs. pretreatment values.

mechanism (Jarry et al. 1990). A typical example of such experiment is shown in Fig. 1.

However, when norepinephrine release by the MPO/AH of ovx animals was studied, no correlation between NE release and the occurrence of LH pulses was demonstrable. Instead, NE secretion (and also the release of dopamine and epinephrine) occurred in pulses and these pulses appear to occur at random (Fig. 2). Nevertheless, as evidenced above, NE is indispensible for the proper function of the GnRH pulse generator. In earlier experiments, Condon et al. (1989) demonstrated in identified GnRH neurons of the guinea pig hypothalamus that activation of α1-receptors causes irregular phasic activation of the neurons. On the basis of these and the above described results we concluded that norepinephrine acts as part of the GnRH pulse generator to induce phasic activation of GnRH neurons.

As also elaborated above, not only phasic but also synchronous activation of GnRH neurons is a prerequisite for normal, highly sensitive function of the GnRH receptors in the pituitary gonadotrophs. The question must then be asked what causes synchronization of the norepinephrine-induced phasic activation of GnRH neurons? In a number of experiments (Ondo et al. 1982, Jarry et al. 1988, 1991, Seong et al. 1995) it was suggested that the amino acid neurotransmitter GABA has a tonic inhibitory action on hypothalamic GnRH neurons because preoptic GABA release rates - as measured by push-pull cannula techniques - were significantly lower in ovx animals as compared to ovx estrogen-treated or intact diestrous rats, and this is shown in Fig. 3.

Later, we were able to demonstrate that GABA release in the MPO/AH correlated inversely with pituitary LH release. Utilizing the *in vivo* push-pull cannula technique, Jarry et al. (1988) demonstrated a dramatic reduction of preoptic GABA release rates prior to the occurrence of LH pulses in the blood. They concluded that GABA tonically inhibits GnRH neurons and that this tonic inhibition is acutely disrupted such that all GnRH neurons are simultaneously desinhibited and thereby synchronized. Results of these experiments are shown in Fig. 4 for an individual representative animal.

On the basis of these findings it was postulated that continuous GABA infusion in the MPO/AH should result in maintenance of tonic inhibition of GnRH neurons. This could indeed be demonstrated (Fig. 5) in that infusion of physiologic concentrations of GABA in the MPO/AH inhibited pulsatile pituitary LH secretion (Leonhardt et al. 1995), and these authors were able to demonstrate that $GABA_A$

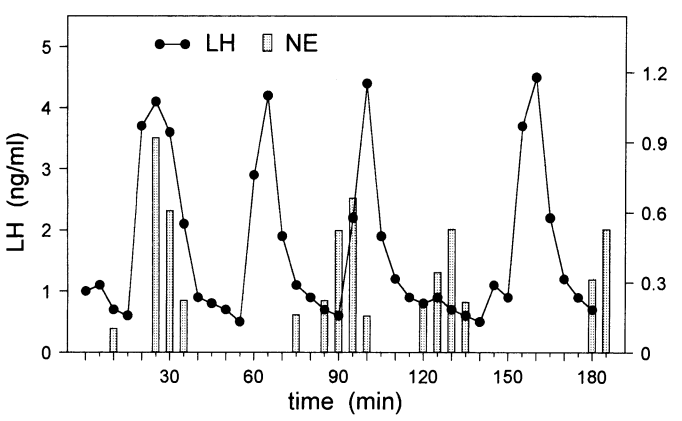

Fig. 2. Measurement of LH in the serum of an ovariectomized rat clearly indicates that pituitary LH release occurs in pulses. In the same animal a push-pull cannula was implanted into the medial preoptic/anterior hypothalamic area and norepinephrine (NE) was measured in the push-pull perfusates. There was no correlation between LH pulses in the blood and NE release rates in the MPO/AH although NE release did occur in pulses.

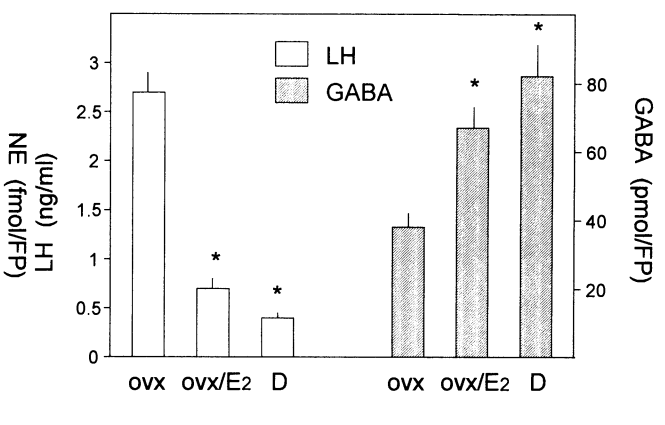

Fig. 3. Mean LH levels in ovx rats are high and significantly lower following estradiol treatment. Lowest levels are present in intact diestrous rats. Mean GABA release rates in the MPO/AH (as measured by the push-pull cannula technique) are lowest in ovariectomized rats, significantly higher in the estrogen-treated animals and highest in intact diestrous rats.

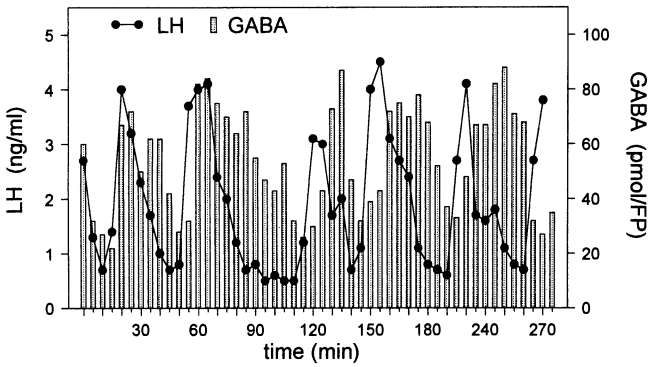

Fig. 4. Preoptic GABA release as measured by the push-pull cannula technique correlates inversely with serum LH levels. Prior to the occurrence of the LH episode, preoptic GABA release is dramatically reduced to increase again when LH levels are high.

but not $GABA_B$ receptors mediate the effects of GABA. The demonstration of GABAergic axon terminals on GnRH perikarya (Leranth et al. 1985) makes it highly likely that the inhibitory effects of GABA on GnRH release are directly exerted at the perikaryal levels of the GnRH neurons. It appears also that NE acts at the level of the GnRH cell bodies rather than at the axon terminals because attempts to modulate pulsatile LH release in ovx rats by manipulating noradrenergic or GABAergic mechanisms residing in the MBH failed: neither infusion of doxazosin (a specific water-soluble α1-receptor blocker) nor infusion of GABA or muscimol into the MBH had effects on pulsatile LH secretion. These observations suggest that the crucial noradrenergic and GABAergic mechanisms for the generation of phasic and synchronous activation of GnRH neurons reside in the MPO/AH. Hence, NE and GABA most likely act at the perikaryal levels of GnRH neurons. Their function appears to be induction of phasic activation of GnRH neurons by NE through an α1-receptive mechanism. This phasic activation is tonically inhibited by GABAergic neurons. These GABAergic neurons seize acutely to release their inhibitory neurotransmitter and this results in synchronous activation of GnRH neurons.

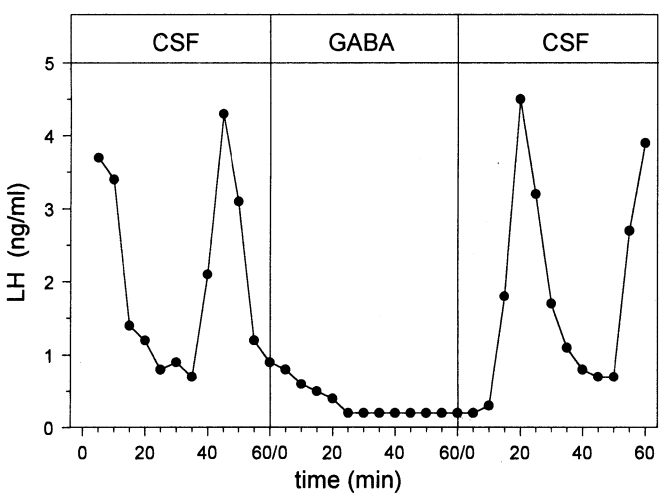

Fig. 5. When the MPO/AH is perfused with GABA (10^{-6} M), this blocks the LH pulsatility in the blood. When the GABA-containing CSF was replaced by pure CSF, an LH episode occurs immediately after a medium was changed.*P <0.05 vs. pretreatment values.

The above described mechanisms resulting in pulsatile GnRH release into the portal vessels are necessary for maintenance of high sensitivity of GnRH receptors in the pituitary. GnRH receptors, however, are also present in a number of CNS structures, particularly in the MPO/AH and in the MBH (Jennes and Conn 1994). The function of these GnRH receptors are largely unknown. It is also not known whether their proper sensitive function is also dependent on pulsatile exposure to their ligand. Recently, the rat GnRH receptor was cloned (Kaiser et al. 1992, 1993, Reinhart et al. 1992, Tsutsumi et al. 1992) and therefore specific primers for quantitative reverse transcription polymerase chain reaction (RTP-CR) are now available. This allows the study of GnRH receptor gene expression in the pituitary and in hypothalamic structures provided the GnRH receptors in pituitary and CNS structures are identical. Utilizing a quantitative RTP-CR (Kim et al. 1993, Seong et al. 1995), we demonstrated recently the identity of anterior pituitary and MPO/AH and MBH of the PCR products. We elaborated furthermore that treatment of ovx rats with muscimol (which like GABA blocks pulsatile pituitary LH release) blocks gene expression of the GnRH receptor within 2 h (Fig. 6). Hence, it appears that the preoptic as well as the mediobasal hypotha-

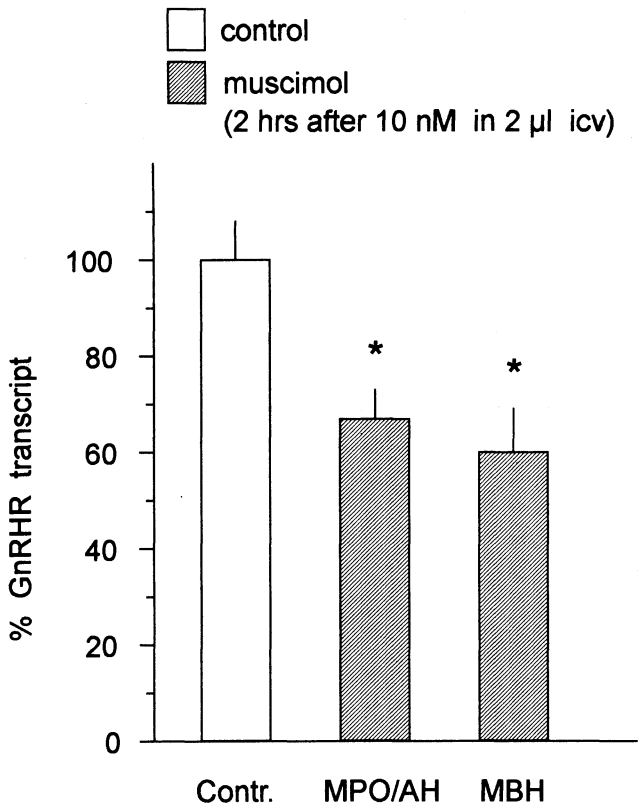

Fig. 6. Intracerebro ventricular injection of muscimol blocks pulsatile LH secretion in ovx rats. Two hours after this treatment, the GnRH receptor mRNA concentrations in the MPO/AH as well as in the MBH are significantly reduced ($P < 0.05$). This indicates that the capacity of the cells to synthesize the GnRH receptor protein is largely dependent on pulsatile exposure to GnRH.

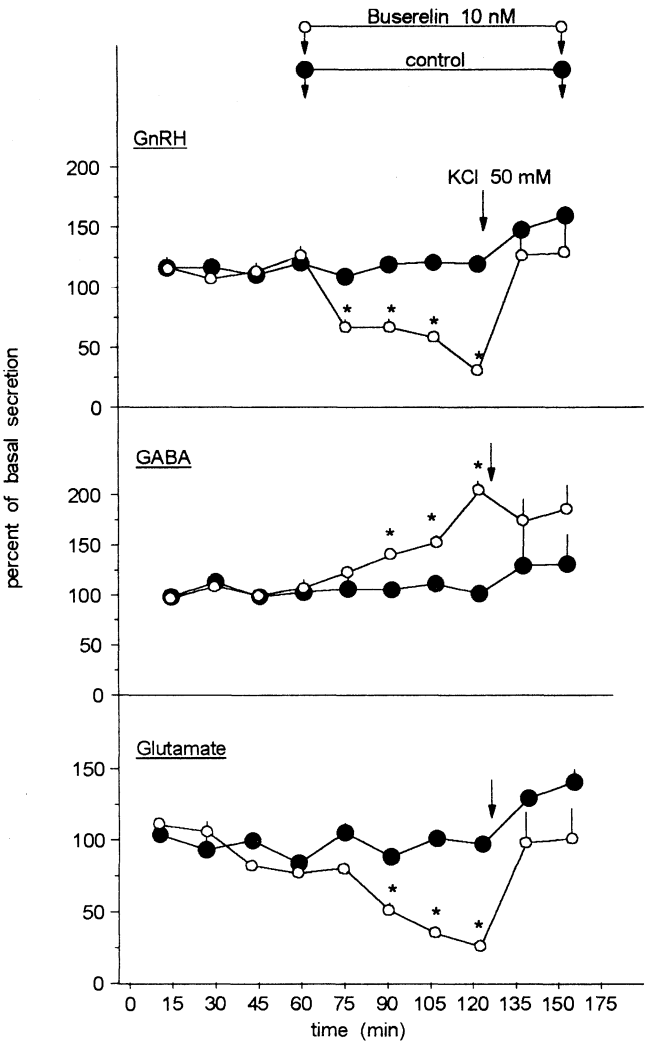

Fig. 7. Hypothalamic fragments (which include the MPO/AH and the MBH) were superfused with artificial CSF *in vitro*. GnRH, GABA and glutamate concentrations were measured in the superfusates. In response to Buserelin (a long-lasting GnRH superagonist at a concentration of 10^{-8} M) was added. This reduced GnRH and glutamate release significantly whereas the release of GABA was increased. In response to a K^+ (55 mM) the secretion of all 3 compounds can be increased. *$P < 0.05$ vs. controls or pretreatment values, respectively.

lamic expression of the GnRH receptor gene are crucially dependent on the regularly occurring LHRH pulses.

Little is known about the function of the preoptic and hypothalamic GnRH receptors. We studied therefore recently the effects of various GnRH analogues on GnRH release from explanted rat hypothalami kept *in vitro* under superfusion conditions. These fragments contained both, the MPO/AH and the MBH. Superfusion medium was collected either at 5 or 15 min intervals and GnRH, GABA and glutamate were measured in each sample. After a washout superfusion period of 60 min, basal GnRH release had stabilized (Fig. 7). When Buserelin (at a concentration of 10^{-8} M) was added to the superfusion medium, this resulted in reduced GnRH secretion within 15 min, maximal inhibition was achieved within 1 h and the release was inhibited by almost 80 %. Measurement of the inhibitory amino acid neurotransmitter GABA yielded the information that Buserelin stimulated the release of this transmitter whereas the hypothalamic secretion of the excitatory amino acid neurotransmitter glutamate was inhibited.

When intact male rats were treated twice daily with Buserelin for 4 days (30 μg/100 g bodyweight/injection) their blood LH levels were undetectable in most animals because the superanalogue down-regulated pituitary GnRH receptors. When hypothalamic fragments of such treated and of control animals were superfused *in vitro*, the hypothalami of the Buserelin-treated animals released significantly less GnRH and glutamate but more taurin and GABA (Fig. 8). When these hypothalami were under the continuous influence of Buserelin also under *in vitro* superfusion conditions, their GnRH release remained low whereas the hypothalamus of *in vivo* Buserelin-treated animals released increasingly more GnRH when the superfusion medium did not contain Buserelin. Continuous *in vivo* and *in vitro* exposure of hypothalami to Buserelin had also a continuous suppressive effect on glutamate and a stimulatory effect on GABA release. Those hypothalami which were down-regulated under *in vivo* conditions but superfused in the absence of Buserelin, released after the wash-out period significantly more glutamate but less GABA.

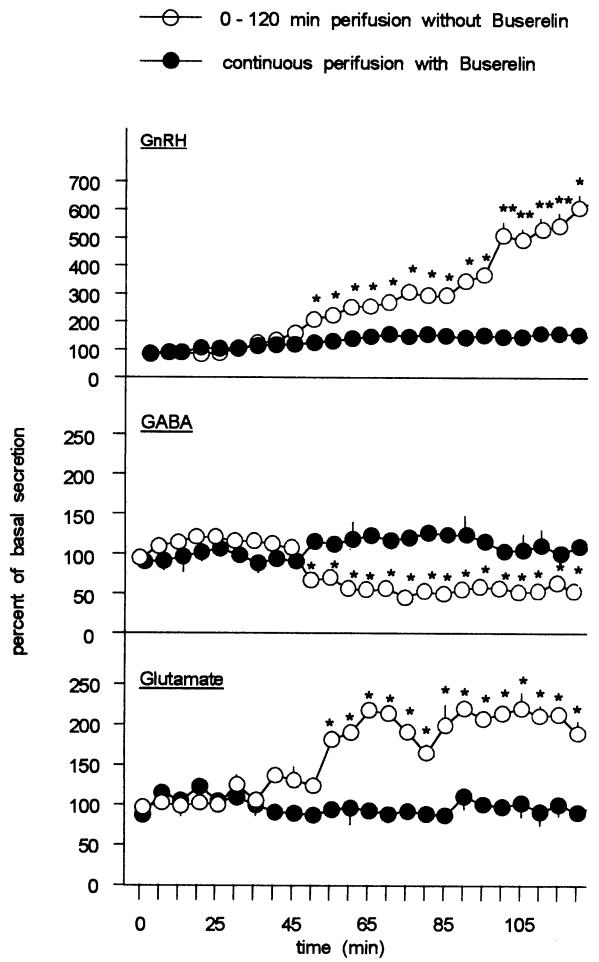

Fig. 8. Male rats were twice daily injected with Buserelin for 4 days. This resulted in downregulation of the pituitary GnRH receptors and therefore LH levels in the blood of most animals were undetectable. After 4 days the animals were sacrificed and their hypothalami superfused with artificial CSF either containing or not containing Buserelin. In the continuous presence of Buserelin, GnRH, GABA and glutamate secretion remained constant whereas in the absence of Buserelin GnRH and glutamate secretion increased significantly within the first hour of superfusion whereas the release of GABA decreased. *P <0.05 vs. control values.

From these results we conclude that the function of preoptic and/or hypothalamic GnRH receptors are manyfold: The synaptic contacts between GnRH axon terminals and GnRH perikarya in the MPO/AH may serve autoinhibitory effects. This can explain the chronic and acute effects of Buserelin on GnRH secretion. Since no GnRH perikarya are located in the mediobasal hypothalamus and since we have observed clear effects of Buserelin on amino acid neurotransmitter release, we postulate furthermore that the GnRH receptors described in the mediobasal hypothalamus of the rat are located on GABA and/or glutamate containing neurons. These neurons may in turn influence GnRH release by an action at the axon terminals.

In summary, we presented evidence that norepinephrine and GABA are indispensible neurotransmitters for the proper functioning of the GnRH pulse generator. Both appear to act at the GnRH perikaryal level in the MPO/AH. GnRH neurons communicate with each other since chronic exposure of the GnRH neuronal system, both *in vivo* and *in vitro* inhibit GnRH release and as a consequence, inhibit also expression of the GnRH receptor gene. Whether or not the GnRH receptors in the MBH are also part of the GnRH pulse generator, remains to be determined.

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