Endocrine, *vol. 28, no. 3, 295–301, December 2005* 0969–711X/05/28:295–301/$30.00

Role of Excitatory Amino Acids in the Control of Growth Hormone Secretion

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Excitatory amino acids (EEAs), such as glutamate, are pivotal elements in the hypothalamic circuitry involved in the control of pituitary function. The actions of EEAs are mediated by different postsynaptic receptor subtypes, which include the ionotropic receptors *N*-methyl-D-aspartate (NMDA), kainate (KA), 2-amino-3-hydroxy-5 methyl-4-isoxazol propionic acid (AMPA), as well as metabotropic receptors. In this review, we summarize the experimental work on the role of EAA neurotransmission in the control of GH secretion in the rat. Detailed characterization of the effects of agonists and antagonists of glutamate receptors on GH release revealed that activation of NMDA, KA, and AMPA receptors at different age-points resulted in clear-cut stimulation of GH secretion, although age- and sex-dependent differences were detected in the pattern of response to the different agonists. This stimulatory action was proven nitric oxide (NO)–dependent and not exerted at the pituitary level. Moreover, the effects of NMDA on GH release likely involve additional mediators other than hypothalamic GH-releasing hormone (GHRH). In contrast, the role of metabotropic receptors seems to be marginal, and only inhibitory actions were observed after activation of different receptor subtypes. Furthermore, evidence was obtained on the modulation of the EEA system by gonadal factors in the control of GH secretion, and on the physiological relevance of EEA pathways in the regulation of pulsatile GH release. The analysis of interactions between EAA receptors and other neuronal pathways evidenced the close interactions between different systems involved in the control of GH secretion. Blockade of glutamate receptors abolished the stimulatory effect of GABA and ghrelin on GH secretion and, inversely, blockade of ghrelin or GABA receptors abolished the stimulatory effect of EAAs. In conclusion, our data using the rat as animal model provide evidence for a pivotal role of glutamate pathways in the regulation of GH secretion throughout the lifespan.

Received July 13, 2005; Accepted July 13, 2005.

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Key Words: Growth hormone; glutamate; excitatory amino acids; inhibitory amino acids; rat.

Introduction

L-glutamic and L-aspartic acid are the main excitatory amino acids (EAAs) in the central nervous system *(1,2)*. The initial demonstration of a role of glutamate on neuroendocrine function was obtained after observations that neonatal administration of monosodium glutamate (MSG) was followed by brain lesions and obesity *(3)*. Experiments carried out thereafter showed that MSG administered between d 5 and 10 postnatally induced an 80% decrease in the number of perikarya in the arcuate nucleus *(4)*. The arcuate lesion resulted in endocrine deficits; reproductive capacity was reduced, animals were smaller in stature and obese, and the weights of the anterior pituitary, ovaries, and testes were significantly decreased while the adrenals were unaffected *(5)*. Light microscopic studies revealed no significant changes in thickness or general histological appearance of the median eminence. These initial observations were followed by more refined studies showing that adult animals had a significant increase in body fat without an increase in weight and that serum growth hormone (GH) was markedly reduced in both sexes *(6,7)*. In 1983, Mason and co-workers *(8)* described the effects of administration of four excitatory amino acids on pituitary hormone secretion in the rat, thus opening a very interesting field in neuroendocrinology *(9)*. The aim of this review is to describe our current knowledge of the physiological role of different subtypes of glutamate receptors in the control of GH secretion, using the rat as the experimental animal model.

GH release is mainly controlled by the interaction between two hypothalamic signals: GH-releasing hormone (GHRH) and somatostatin. In turn, GHRH and somatostatin secretion is under the influence of a complex neural network involving multiple neurotransmitters *(10)* and peripheral signals such as leptin and ghrelin *(11,12)*. The latter has recently emerged as the natural ligand of GH secretagogue receptor (GHS-R), whose contribution to the physiological control of GH secretion is yet to be fully determined. Likewise, although circulating ghrelin originating from the stomach appears to be the most abundant of the peptide, the possibil-

ity that locally (intra-hypothalamic) produced ghrelin may conduct important neuroendocrine functions (including control of GH secretion) has been debated extensively.

In this scenario, specific regulatory actions of EEAs in the neuroendocrine control of anterior pituitary hormone secretion, and specifically of GH, has been proposed. In general terms, the actions of EAAs are mediated through interaction with different postsynaptic receptors, which include ionotropic (iGlu) receptors, as *N*-methyl-D-aspartate (NMDA) receptors, kainate (KA) receptors, and 2-amino-3-hydroxy-5 methyl-4-isoxazol propionic acid (AMPA) receptors, as well as metabotropic receptors *(9,13,14)*. In recent years, the pivotal role of EAA pathways in the control of neuroendocrine function has been firmly established (for a review see ref. *9*). However, most of the research activity in the area has focused on the contribution of ionotropic receptor-mediated actions of EAAs.

Role of Ionotropic Receptors in the Control of GH Secretion: General Aspects

Compelling evidence indicated that the secretion of several anterior pituitary hormones is under the control of EEA pathways. In this sense, a crucial role for the EEA system in the regulation of gonadotropin secretion and reproductive function has been firmly established *(9)*. However, analysis of the involvement of this system in the control of secretion of pituitary hormones other than gonadotropins had received less attention. Yet, Nemeroff et al. *(15)* observed diminished serum GH levels after acute administration of MSG, and Mason et al. *(8)* showed that NMDA and KA increased GH secretion in adult male rats. We aimed to extend these observations, and characterize in detail the pattern of GH response to different agonists and antagonists of glutamate receptor subtypes, targeting as a first approach the ionotropic receptors *(16–18)*. Our experimental data showed that activation of NMDA and KA receptors increased GH secretion in neonatal, prepubertal, and adult male and female rats (see Fig. 1). Likewise, AMPA receptor activation induced robust GH secretory responses throughout the lifespan (Fig. 2). Interestingly, age- and sex-dependent differences were detected in the pattern of response to the different agonists *(19,20)*. In this sense, the ability of KA, but not of NMDA and AMPA, to stimulate GH release disappeared in adulthood, whereas in prepubertal rats, the relative potency of different agonists was proven sex-dependent: AMPA was more effective in prepubertal males, KA was more potent in prepubertal females, and NMDA was equally potent in both sexes. Furthermore, the physiological role of the EAA system in the control of GH secretion is sustained by the fact that blockade of endogenous NMDA and AMPA receptors by means of administration of specific antagonists (MK-801, antagonist of NMDA receptors; NBQX, antagonist of AMPA receptors) resulted in decreased serum GH levels

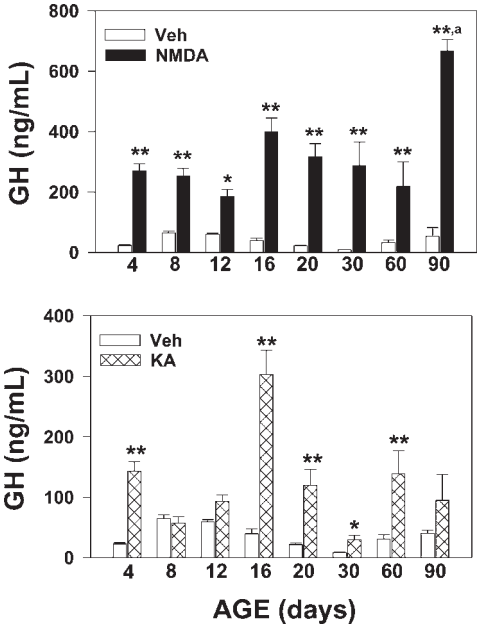

Fig. 1. Serum GH concentrations in male rats of different ages, 15 min after ip injection of vehicle, NMDA (15 mg/kg), or KA (2.5 mg/kg). Values are the mean ± SEM of 10 determinations per group. $*p < 0.05$, $**p < 0.01$ vs corresponding vehicle-injected controls; $^{a}p < 0.01$ vs other groups injected with NMDA (ANOVA followed by Student–Newman–Keuls multiple range test).

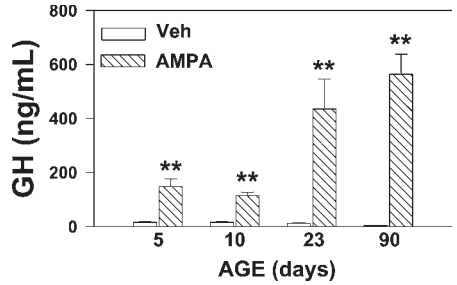

Fig. 2. Serum GH concentrations in male rats of different ages, 15 min after ip injection of vehicle or AMPA (2.5 mg/kg). Values are the mean ± SEM of 10 determinations per group. $**p < 0.01$ vs corresponding vehicle-injected controls (ANOVA followed by Student–Newman–Keuls multiple range test).

and altered its pulsatile profile of secretion: antagonization of NMDA receptors by administration of MK801 or AP-5 abolished pulsatile GH secretion (Fig. 3), whereas administration of NBQX altered the amplitude of GH pulses *(18, 21)*. Similarly, delayed growth rate after repetitive administration of ionotropic antagonists in prepubertal animals strongly suggest a pivotal role of EAA neurotransmission in the control of GH secretion *(22,23)*.

Role of Metabotropic Receptors in the Control of GH Secretion

G protein–coupled metabotropic glutamate (mGlu) receptors function to regulate excitability via pre- and postsynaptic mechanisms. Different mGlu subtypes have been described and are included in three main groups: Group I

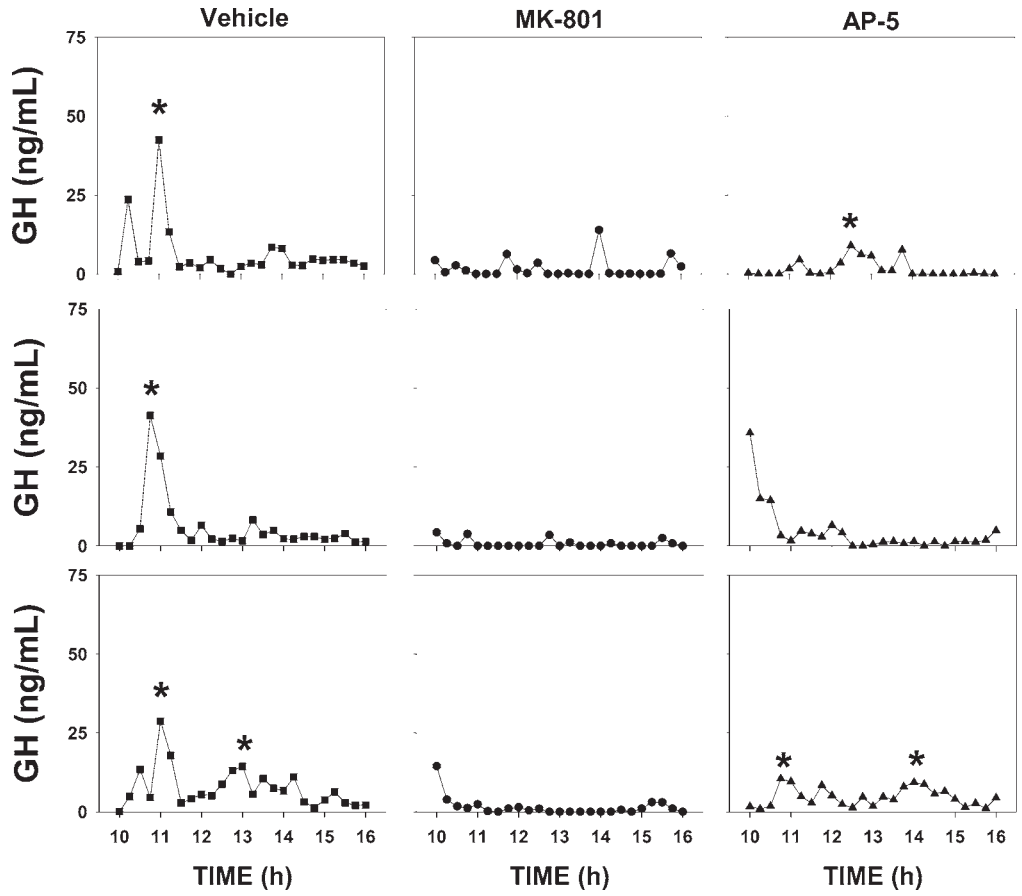

Fig. 3. Representative GH plasma profiles in individual adult male rats after ip injection of vehicle (left panels), or the antagonists of NMDA receptors MK-801 and AP-5 (1 mg/kg divided in three doses). Asterisks denote GH pulses.

[mGlu(1) and mGlu(5)]; group II [mGlu(2) and mGlu(3)], and group III [(mGLU(4),mGlu(7) and mGLU(8)] *(14,24, 25)*. To date, despite the extensive efforts applied to the characterization of the role of ionotropic receptors in the control of neuroendocrine function *(9)*, the role of metabotropic receptors in the control of hypothalamic–pituitary function has remained largely ignored. In the present article we report, for the first time, our initial experiments on the analysis of the role of mGLU receptors in the control of GH secretion. We studied the effect of central and peripheral administration of ibotenic acid (a weak agonist of all metabotropic receptors), t-ACPD (agonist of group I and group II family receptors), L-FCCG-I (agonist of group II), and MCPG (antagonist of groups I and II). The results obtained show minor changes in GH secretion following administration of these compounds to prepubertal animals: a significant decrease in serum GH concentrations after central (icv) administration of t-ACPD, as well as after systemic administration of ibotenic acid (Fig. 4). These inhibitory effects contrast with the potent stimulatory action exerted by stimulation of ionotropic receptors. Thus, it becomes apparent that L-glutamate is able to conduct a dual regulatory action upon GH secretion, which involves a predominant stimulatory effect via iGluRs, as well as a (minor) inhibitory effect via mGluR. Similar findings have been also reported for prolactin *(26)*.

Sites(s) of Action of EAAs in the Control of GH Secretion

The presence of NMDA, KA, AMPA, and metabotropic receptors in the pituitary gland *(26–31)* opened up the possibility that the stimulatory response to different agonists might be carried out directly at pituitary level. However, we were unable to detect stimulatory actions of different doses of NMDA, KA, or AMPA on GH secretion by hemipituitaries incubated in vitro, despite previous reports on the ability of NMDA and KA to acutely stimulate GH release by superfused pituitary cells *(32)*.

Other mechanisms for the effects of different agonists of glutamate receptor subtypes on GH secretion might involve an action at the hypothalamic level, and both an increase in GHRH release and/or a decrease in somatostatin secretion may account for the reported effects on GH release. In this sense, previous experimental evidence suggested that the mechanism whereby NMDA elicits GH release is, at least partially, dependent on an increase in GHRH secretion *(20–22)*. We addressed this question in prepubertal rats. However,

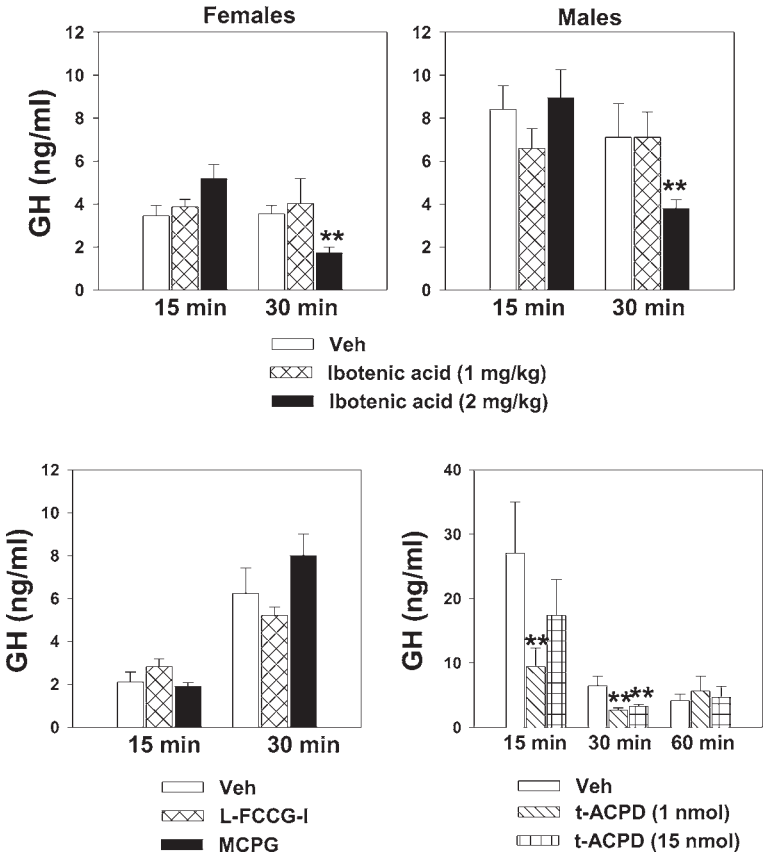

Fig. 4. In the *upper panels*, serum GH concentrations in 23-d-old female and male rats injected ip with 1 or 2 mg/kg of ibotenic acid are presented. In addition, in the *lower panels*, serum GH levels in 23-d-old male rats injected ip with 2 mg/kg of L-FCCG-I or MCPG, or injected icv with 1 or 15 nmol of t-ACPD, are shown. Values are the mean ± SEM of 10 independent determinations. $^{**}p < 0.01$ vs vehicle-injected group (ANOVA followed by Student–Newman–Keuls multiple range test).

our data indicated that a potential increase in GHRH release after NMDA or AMPA administration does not fully explain their effects on GH secretion, because (1) GHRH induced a very weak response in prepubertal males, while NMDA and AMPA elicited a stronger response; and (2) the effects of NMDA were not blocked by neonatal destruction of GHRH neurons with MSG, neither by pretreatment with GHRH antiserum. The latter is in partial agreement with previous results indicating that immunoneutralization of endogenous GHRH did not block the action of NMDA, administered at a dose of 12.5 mg/kg *(20)*. Alternatively, a decrease in hypothalamic release of somatostatin following activation of ionotropic receptors might contribute to their stimulatory action on GH release. Indeed, several experimental approaches have suggested a modulatory role of EAAs on somatostatin release in different biological systems *(33–36)*. This possibility, however, remains to be experimentally tested at the hypothalamus.

Involvement of Nitric Oxide in the GH-Releasing Effect of EAAs

It is well established that the gaseous transmitter, nitric oxide (NO), is an important intracellular and intercellular messenger involved in the control of a wide range of physiological events including neuroendocrine function *(37–41)*. Preliminary data from our laboratory clearly indicated that blockade of NO synthases with L-nitro argininine methyl ester (L-NAME) abolished the releasing effects of GHRH and GH-releasing peptide 6 (GHRP-6) on GH secretion. For this reason, we evaluated whether the stimulatory action of NMDA, KA, and AMPA on GH release is dependent on endogenous NO. As shown in Fig. 5, pretreatment with L-NAME blunted GH responses to NMDA, KA, and AMPA, thus suggesting a pivotal role of NO for the expression of complete GH secretory responses after pleiotropic stimulation, including the recently discovered ghrelin *(21,42–44)*. Assuming that nitric oxide synthase is present in gonadotropes and folliculostellate cells, but not in somatotropes *(37)*, our data open two possibilities: (a) the different secretagogues act via folliculostellate cells to induce GH release, or (b) the secretagogues act on somatotropes in the permissive presence of NO released by folliculostellate cells.

Modulation of EAA System by Gonadal Factors

It has repeatedly been reported that the actions of NMDA and KA on LH release depend on the gonadal environment

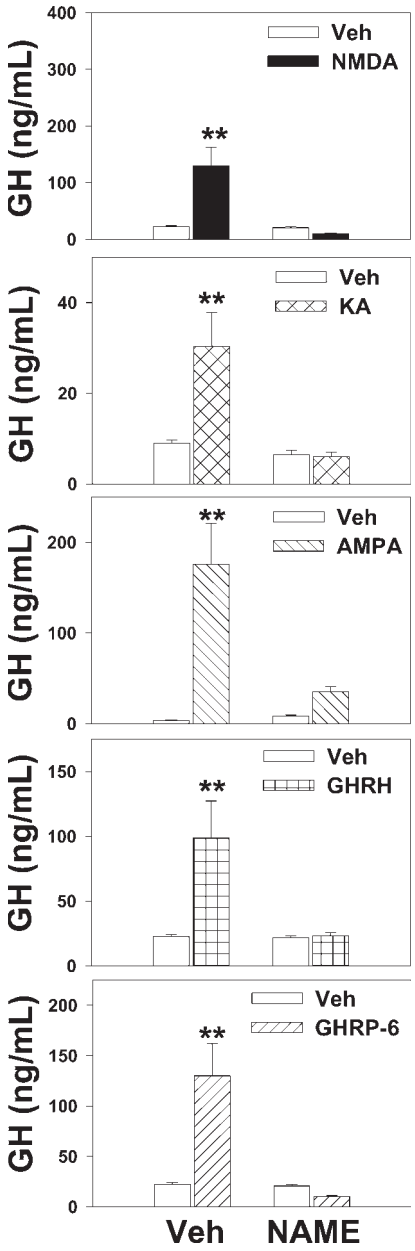

Fig. 5. GH concentrations in prepubertal male rats at 15 min after ip injection of vehicle, NMDA (15 mg/kg), KA (2.5 mg/kg), AMPA (2.5 mg/kg), GHRH (500 μg/kg), and GHRP-6 (30 μg/kg), following pretreatment with the inhibitor of NO synthases NAME (40 mg/kg). Values are the mean ± SEM of 10 independent determinations. $^{**}p < 0.01$ vs the corresponding vehicle-injected groups (ANOVA followed by Student–Newman–Keuls multiple range test).

and the steroid milieu during neonatal period. Thus, the stimulatory effect of NMDA and AMPA on LH secretion in control female rats switch into an inhibitory effect after ovariectomy *(45,46)*, whereas neonatal administration of estradiol or testoterone has been shown to change the role of the EEA system in the control of anterior pituitary secretion in adult male and female rats *(45–49)*.

In adult rats, in vivo pituitary responsiveness to GHRH depended on the estrous phase *(50)*, decreased after orchidectomy, and increased after testosterone administration *(51,52)*, while orchidectomy enhanced the effectiveness of GHRH in prepubertal males *(53)*. On the basis of these observations, we found it interesting to analyze whether the gonadal function can modulate the role of EAAs in the control of GH secretion. For this purpose we evaluated GH responsiveness to NMDA and KA after orchidectomy, testosterone replacement or permanent damage of testicular function after administration of 500 μg of estradiol benzoate on d 1 of life. Our data indicated that NMDA-induced GH secretion is not dependent on testicular function as it remained after orchidectomy and testosterone replacement, as well as in estrogenized males *(18)*. In contrast, the ability of KA to stimulate GH secretion appeared partially dependent on testicular function, because KA-induced GH release was blunted in orchidectomized males and completely absent in males neonatally estrogenized *(16)*. Further evidence for the involvement of gonadal factors in the regulation of the role of the EAA system in the control of GH secretion is provided by the fact that ovariectomy resulted in a significant increase in AMPA-induced GH release in prepubertal females *(54)*.

Interactions Between EAAs and Other Pathways in the Control of GH Secretion

An interesting aspect of the control of GH secretion by EAA pathways is how this system interplays with other well-known neuroendocrine regulators of GH release. In this context, in the last years, we have aimed to analyze at our laboratory the interactions between ionotropic and other neuronal and peripheral inputs controlling GH secretion. These results can be summarized as follows:

Interactions between EAAs and noradrenergic system: The stimulatory role of adrenergic system in the stimulation of GH secretion via α-2 receptors is well known *(55, 56)*. We have tested the effects of AMPA in the control of GH secretion after blockade of noradrenergic and dopaminergic system by administration of α-methyl-*p*-tyrosine (at the dose of 250 mg/kg BW) or diethyldithiocarbamate (at the dose of 500 mg/kg BW). The results obtained clearly showed that the stimulatory effect of AMPA on GH secretion is maintained despite blockade of the catecholaminergic system.

Interactions between EAAs and GABAergic system: Gamma aminobutyric acid (GABA) stimulates GH secretion in adult and neonatal animals *(20,57)*, although inhibitory actions have also been described *(58)*. This stimulatory effect is, at least partially, mediated by a direct action at pituitary level *(59,60)*. Because previous data have demonstrated a cross-talk between excitatory and inhibitory amino acids in the regulation of LHRH *(9)*, we hypothesized that similar interactions might occur in the control of GH secretion. Our experimental data indicate that the stimulatory effect of GABA (25 μg/g) on serum GH concentrations is

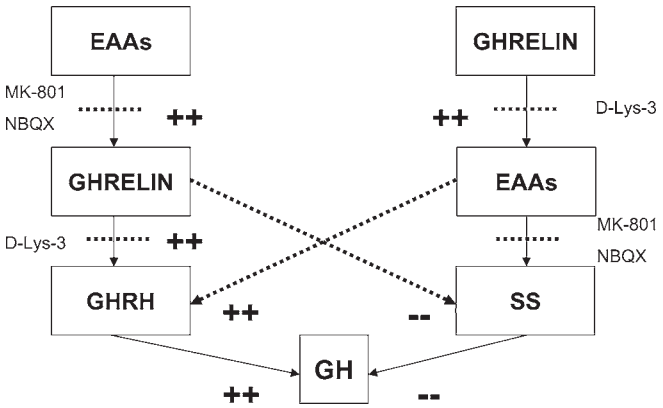

Fig. 6. Proposed model for the reciprocal interaction between ghrelin and EAA systems in the hypothalamic control of GH secretion. The stimulatory effect of ghrelin presumably involves activation of ionotropic EAA receptors, because the blockade of NMDA and AMPA receptors with MK-801 or NBQX, respectively, abolished the GH-releasing effect of ghrelin. Conversely, the EAA system elicits GH release through a mechanism involving ghrelin actions, because pretreatment with an antagonist of the cognate ghrelin receptor (D-lys-3-GHRP-6) also blocked the stimulatory effect of NMDA and AMPA, agonists of EAA receptors. To explain such a reciprocal cross-talk, permissive effects of ghrelin and EAAs on each other actions are hypothesized (dotted lines). SS: somatostatin.

blocked by treatment with MK-801 (0.25 μg/kg at −60 min) but not by NBQX *(61,62)*. Similarly, the stimulatory effect of AMPA on GH secretion was blocked by pretreatment with phaclofen (an antagonist of GABAB receptor) or bicuculline (an antagonist of GABAA receptor), thus suggesting the existence of functional interactions between EAA and inhibitory amino acid systems.

Interactions between EAAs and ghrelin: The stimulatory effects of ghrelin on GH secretion were blocked by pretreatment with the antagonist of ionotropic receptors MK-801 and NBQX. In addition, the potent releasing activity of the EAA agonists NMDA and AMPA was blunted by pretreatment with D-Lys3-GHRP-6, a selective antagonist of the cognate ghrelin receptors *(44)*. These data demonstrated the cross-talk between EAAs and ghrelin, as depicted in the proposed model of Fig. 6. In addition, a proper serotoninergic input is needed for the stimulatory role of ghrelin, as depletion of serotonin stores after treatment with PCPA (*p*-chrolophenylalanine methyl ester, a blocker of serotonin synthesis) blunted the GH-releasing effect of ghrelin.

Conclusion

On the basis of the above data, we propose that the physiological control of GH secretion requires the appropriate interactions between different neurotransmitters and neuropeptides, where EAA pathways do play a prominent role. Such interactions might take place at the level of GHRH and/or somatostatinergic neurons. Alternatively, each signal could modulate the release of other neurotransmitters involved in the control of GH secretion (Fig. 7).

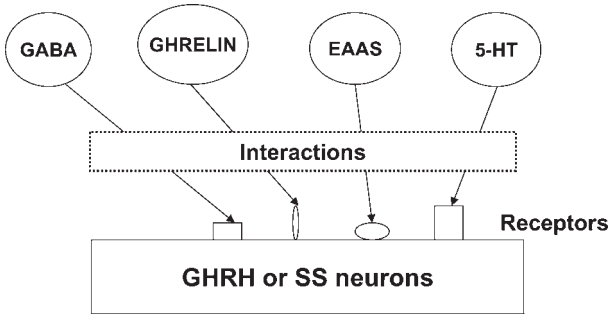

Fig. 7. Proposed model for the integral control of GH secretion and tentative role of EAA neurotransmission. The GHRH and somatostatinergic neurons govern the pulsatile GH secretion. These neurons express multiple receptors for different neurotransmitters. The appropriate secretion of GHRH and somatostatin (SS) is controlled by different nerurotransmitters and neuropeptides, including glutamate and other EAAs, acting on their specific receptors. Changes in the secretion and/or action of one of the factors controlling GHRH and SS neurons may modify the pituitary GH secretion.

Acknowledgments

The authors are indebted to R. Fernández-Fernández for his skillful assistance during preparation of this manuscript. Likewise, the authors are grateful to L.C. Gonzalez and other present and former members of the research group who importantly contributed to the experimental work reviewed here. This work was supported by grants PM98-0163 (CICYT, Spain) and 1FD97-0696-92 (FEDER).

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