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Regulation of Growth Hormone (GH) secretion by different glutamate receptor subtypes in the rat*

Review Article

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Summary. It has been firmly established that excitatory amino acids (EAAs), such as glutamate, are pivotal elements in the hypothalamic circuitry involved in the control of pituitary function. The actions of EAAs are mediated by different postsynaptic receptor subtypes, which include N-methyl D-aspartate (NMDA), kainate (KA), 2-amino-3-hydroxy-5 methyl-4-isoxazol propionic acid (AMPA) and metabotropic receptors. In this review, we summarize our 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. In addition, evaluation of the role of hypothalamic GH-releasing hormone (GHRH) in the stimulatory action of NMDA by means of immunoneutralization of endogenous GHRH or destruction of GHRH producing neurons suggested the involvement of signals other than GHRH in this response. Further, evidence was obtained on the modulation of the EAA system by gonadal factors, and on the physiological relevance of EAA pathways in the regulation of pulsatile GH release. 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 life-span.

Keywords: Amino acids – Growth Hormone (GH) – Glutamate – Excitatory amino acids – Inhibitory amino acids – NMDA – KA – AMPA – Receptor – Rat

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1. Introduction

Growth hormone (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 (for a review see Bertherat et al., 1995). Excitatory aminoacids (EAAs), such as glutamate, are the major activating transmitters in the brain (van den Pol et al., 1990). The actions of EAAs are mediated through interaction with different postsynaptic receptors which include ionotropic receptors, as N-methyl D-aspartate (NMDA) receptors, kainate (KA) receptors, and 2-amino-3-hydroxy-5 methyl-4-isoxazol propionic acid (AMPA) receptors, and metabotropic receptors (Collinbridge and Watkins, 1994; Brann and Mahesh, 1997). In recent years, the pivotal role of EAA pathways in the control of neuroendocrine function has been firmly established (for a review see Brann and Mahesh, 1997). The aim of this review is to describe our current knowledge on the physiological role of different subtypes of glutamate receptors in the control of GH secretion, as well as to evaluate future perspectives in this field.

2. Activation of different glutamate receptor subtypes and GH secretion through-out life-span

Compelling evidence indicated that the secretion of several anterior pituitary hormones is under the control of EAA pathways. In this sense, a crucial role for the EAA system in the regulation of gonadotropin secretion and reproductive function had been firmly established (for a review see Brann and Mahesh, 1997). However, analysis of the involvement of this system in the control of pituitary hormones other than gonadotropins had received less attention. Nemeroff et al. (1978) observed diminished serum GH levels after acute administration of monosodium glutamate (MSG), and Mason et al. (1983) 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 (Tena-Sempere et al., 1996; Pinilla et al., 1996, 1999). Our experimental data evidenced that activation of NMDA, KA and AMPA receptors increased GH secretion in neonatal, prepubertal and adult male and female rats (For an example see Fig. 1). Interestingly, age- and sex-dependent differences were detected in the pattern of response to the different agonists. In this sense, the ability of KA, but not of NMDA and AMPA, to stimulate GH release disappeared in the adulthood (Fig. 1), 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 (Fig. 2). 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)

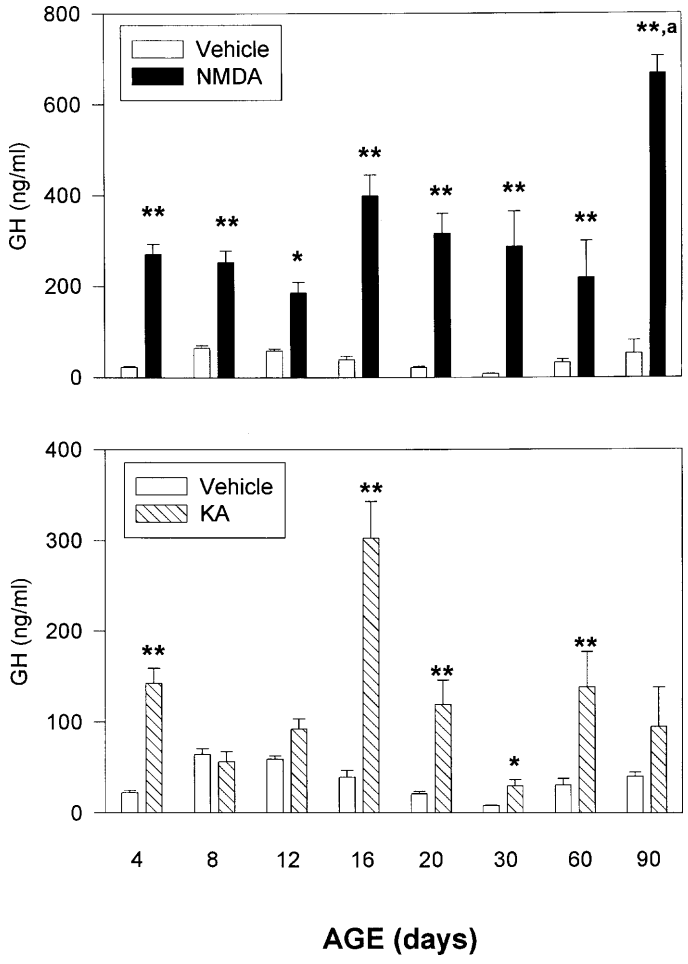

Fig. 1. Serum GH concentrations (ng/ml) in male rats at different ages, 15 min after ip administration of vehicle, NMDA (15 mg/kg) or KA (2.5 mg/kg). Values are given as means ± SEM (10 animals per group). *P ≤ 0.05, **P ≤ 0.01 vs vehicle-injected group; *a* P ≤ 0.01 vs other groups injected with NMDA

resulted in decreased serum GH levels (Pinilla et al., 1999; González et al., 1999a), as well as delayed growth rate after repetitive administration in prepubertal animals (Veneroni et al., 1990; Cocilovo et al., 1992).

3. Site(s) of action for agonists of different glutamate receptor subtypes

The presence of NMDA, KA and AMPA receptors in the pituitary gland (Herb et al., 1992; Kiyama et al., 1993; Bhat et al., 1995; Villalobos et al., 1996) 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* (Fig. 3), despite previous reports on the ability of NMDA and KA to acutely stimulate GH release by superfused pituitary cells (Lindström and Ohlsson, 1992).

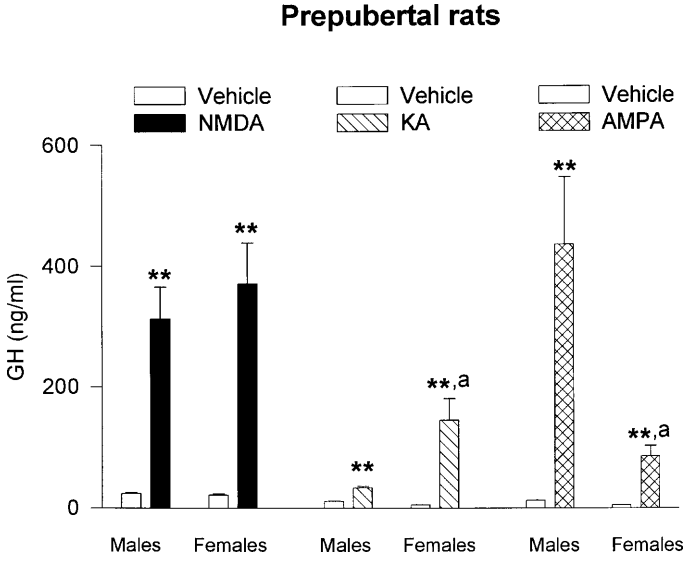

Fig. 2. Serum GH concentrations (ng/ml) in 23 day-old male and female rats 15 min after ip administration of vehicle, NMDA (15 mg/kg), KA (2.5 mg/kg) or AMPA (2.5 mg/kg). Values are given as means ± SEM (10 animals per group). **P ≤ 0.01 vs vehicle-injected group; *a* P ≤ 0.01 vs corresponding groups of males

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 (Cocilovo et al., 1992; Acs et al., 1990). We addressed this question in prepubertal rats. However, our data indicated that a potential increase in GHRH release after NMDA or AMPA administration does not fully explains their effects on GH secretion, as: (1) GHRH induced a very weak response in prepubertal males, while NMDA and AMPA elicited a stronger response (Fig. 4); and (2) the effects of NMDA were not blocked by neonatal destruction of GHRH neurons with MSG (Table 1), neither by pretreatment with GHRH antiserum (Fig. 5). 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 (Acs et al., 1990).

4. Involvement of nitric oxide in the GH-releasing ability of NMDA, KA and AMPA

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

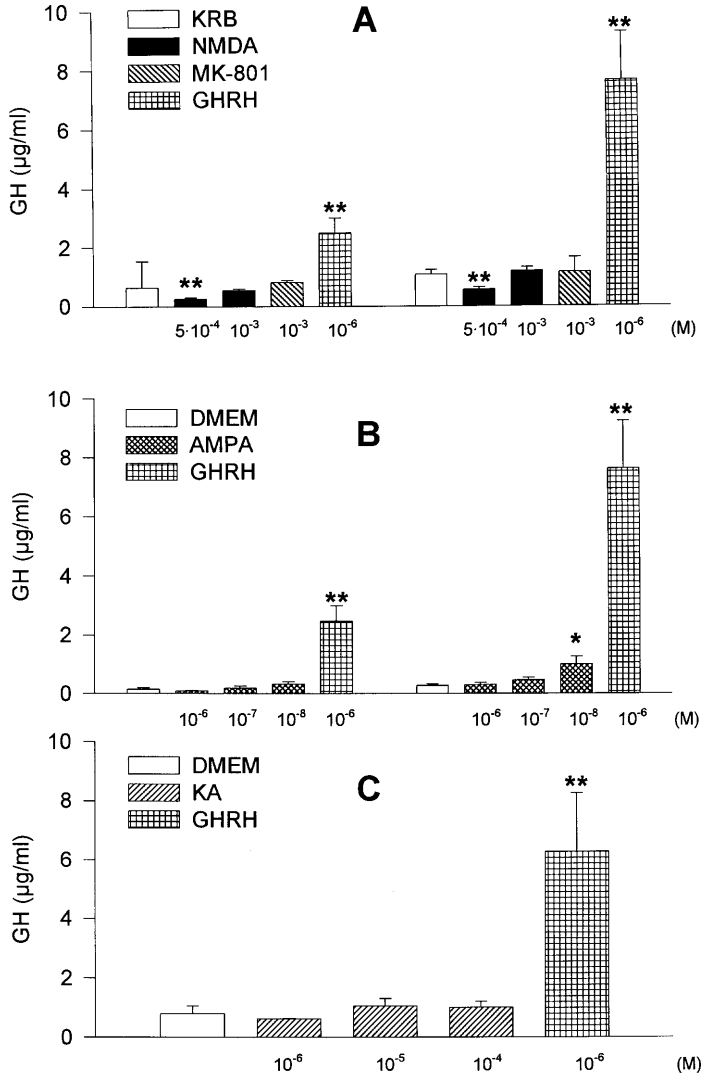

Fig. 3. *In vitro* effects of NMDA, AMPA and KA on GH secretion. In (**A**) and (**B**), GH was measured 60 (left-side) and 120 min (right-side) after incubation of hemipituitaries in presence of NMDA, MK-801, AMPA or GHRH. In (**C**), GH was measured 240 min after incubation of dispersed pituitary cells in presence of KA or GHRH. Values are given as means ± SEM (10 determinations per group). *P ≤ 0.05, **P ≤ 0.01 vs corresponding control group (for more details see Pinilla et al., 1996, 1999; González et al., 1999a)

(Kato, 1992; Ceccatelli et al., 1993; Moretto et al., 1993; Rettori et al., 1993; Mahachoklertwattana et al., 1994; Tena-Sempere et al., 1995). Preliminary data from our laboratory clearly indicated that blockade of nitric oxide synthase 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

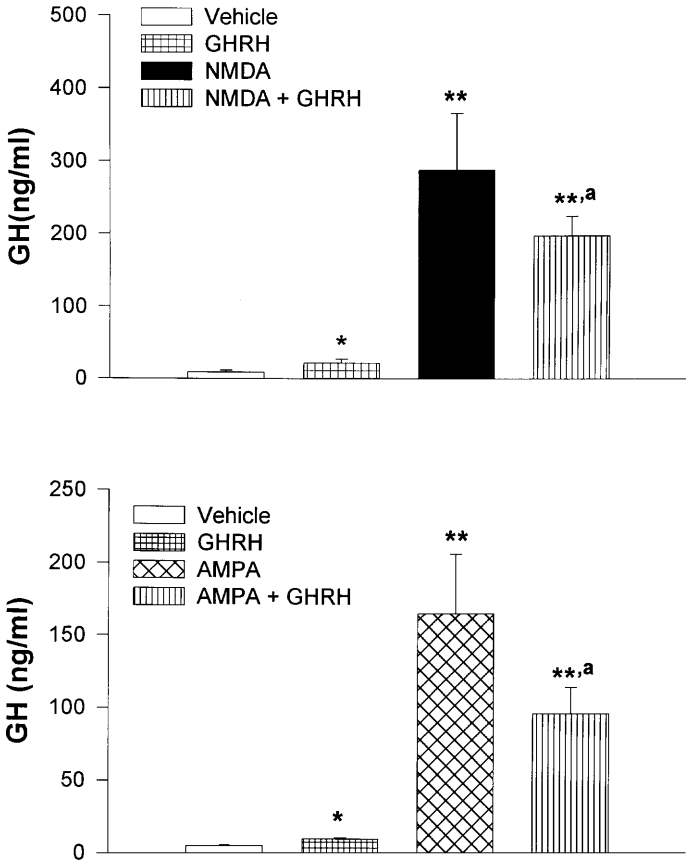

Fig. 4. Serum GH concentrations in prepubertal male rats 15 min after injection of GHRH (500 μg/kg), NMDA (15 mg/kg), NMDA + GHRH, AMPA (2.5 mg/kg) or AMPA + GHRH. Values are given as means ± SEM (n = 8–10 animals per group). *P ≤ 0.05, **P ≤ 0.01 vs corresponding control groups; *a* P ≤ 0.01 vs corresponding groups injected with NMDA or AMPA (for more details see Pinilla et al., 1996, 1999; González et al., 1999a)

Table 1. Serum GH concentrations (ng/ml) in adult male rats decapitated 15 min after i.p. administration of vehicle, NMDA (15 mg/kg) or GHRH (500 μg/kg). Animals were neonatally injected with monosodium glutamate (MSG: 4 mg/g on days 1,3,5,7,9) or vehicle. Values are given as means ± SEM. Each group consisted of 10 animals. **P ≤ 0.01 vs vehicle-injected group (for details see Pinilla et al., 1999)

Treatments & groups	Vehicle	MSG
Vehicle	21 ± 4	13 ± 2
NMDA	605 ± 104**	35 ± 10**
GHRH	144 ± 37**	18 ± 4

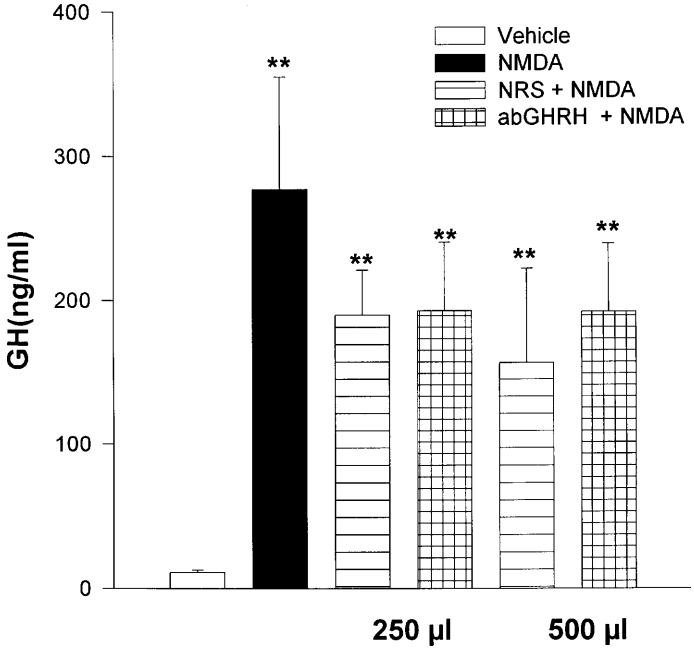

Fig. 5. Serum GH concentrations in prepubertal male rats 15 min after injection of NMDA (15 mg/kg). NRS or GHRH antiserum (250 or 500 μl) were injected 2 h before sacrifice. Values are given as means ± SEM (n = 8–10 animals per group). **P ≤ 0.01 vs vehicle-injected group

shown in Fig. 6, 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 (Tena-Sempere et al., 1995, 1996; González et al., 1999). Assuming that nitric oxide synthase is present in gonadotropes and folliculostellate cells (FS), but not in somatotropes (Ceccateli et al., 1993), our data open two possibilities: (a) the secretagogues act via FS cells to induce GH release, or (b) the secretagogues act on somatotropes in the permissive presence of NO released by FS cells.

5. Role of EAA system in the control of pulsatile GH secretion in adult rats

Previous reports on the actions of EAAs in the control of anterior pituitary secretion have pointed out a clear-cut age-dependency for the effects of different agonists on hormone secretion. For instance, the stimulatory action of NMDA on LH secretion was blunted after puberty (Cicero et al., 1988; Bourguignon et al., 1992; MacDonald et al., 1992; Pinilla et al., 1995). As stated above, our experimental data indicated that the agonists of NMDA and AMPA receptors did induce significant increases in GH secretion throughout life-span (see Fig. 1). Furthermore, evidence was obtained on the

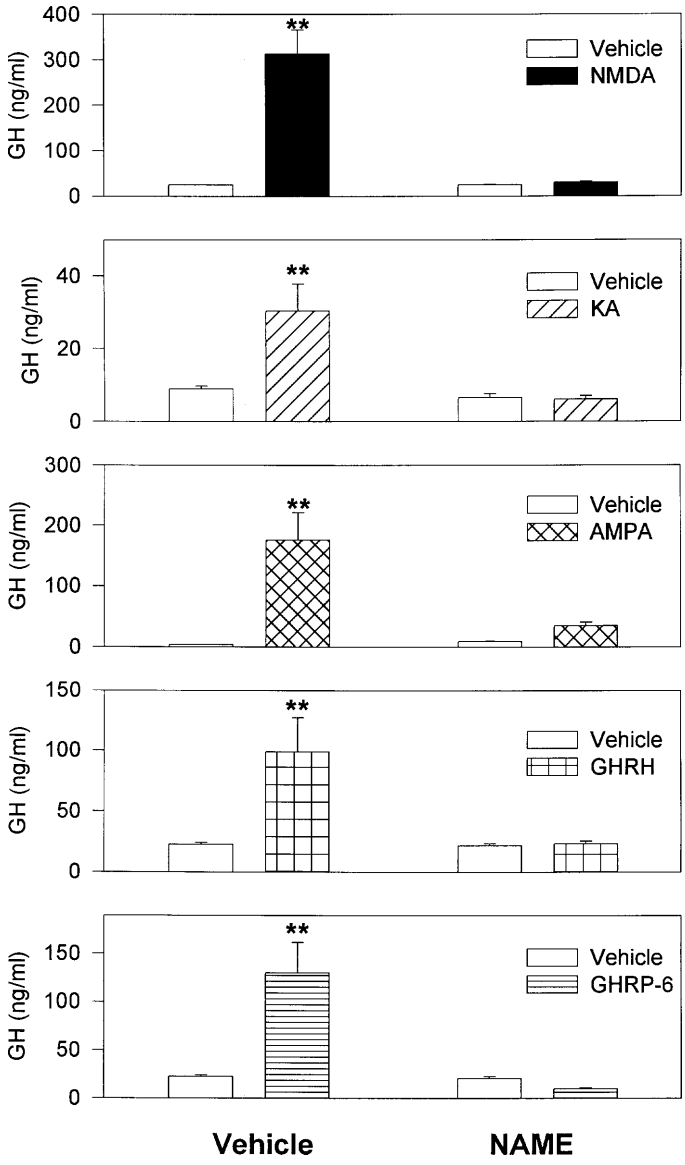

Fig. 6. Serum GH concentrations in prepubertal male rats 15 min after ip injection of vehicle, NMDA (15 mg/kg), KA (2.5 mg/kg), AMPA (2.5 mg/kg), GHRH (500 μg/kg) or GHRP-6 (30 μg/kg). Animals were injected 60 min before sacrifice with vehicle or L-NAME (40 mg/kg). Values are given as means ± SEM (10 animals per group). **P ≤ 0.01 vs corresponding vehicle-injected group (for more details see Tena-Sempere et al., 1996; González et al., 1999a)

physiological relevance of the EAA system in the control of pulsatile GH secretion in adult rats. Antagonization of NMDA receptors by administration of MK-801 or AP-5 abolished pulsatile GH secretion, whereas administration of the antagonist of AMPA receptors, NBQX, altered the amplitude of GH pulses (Figs. 7–10).

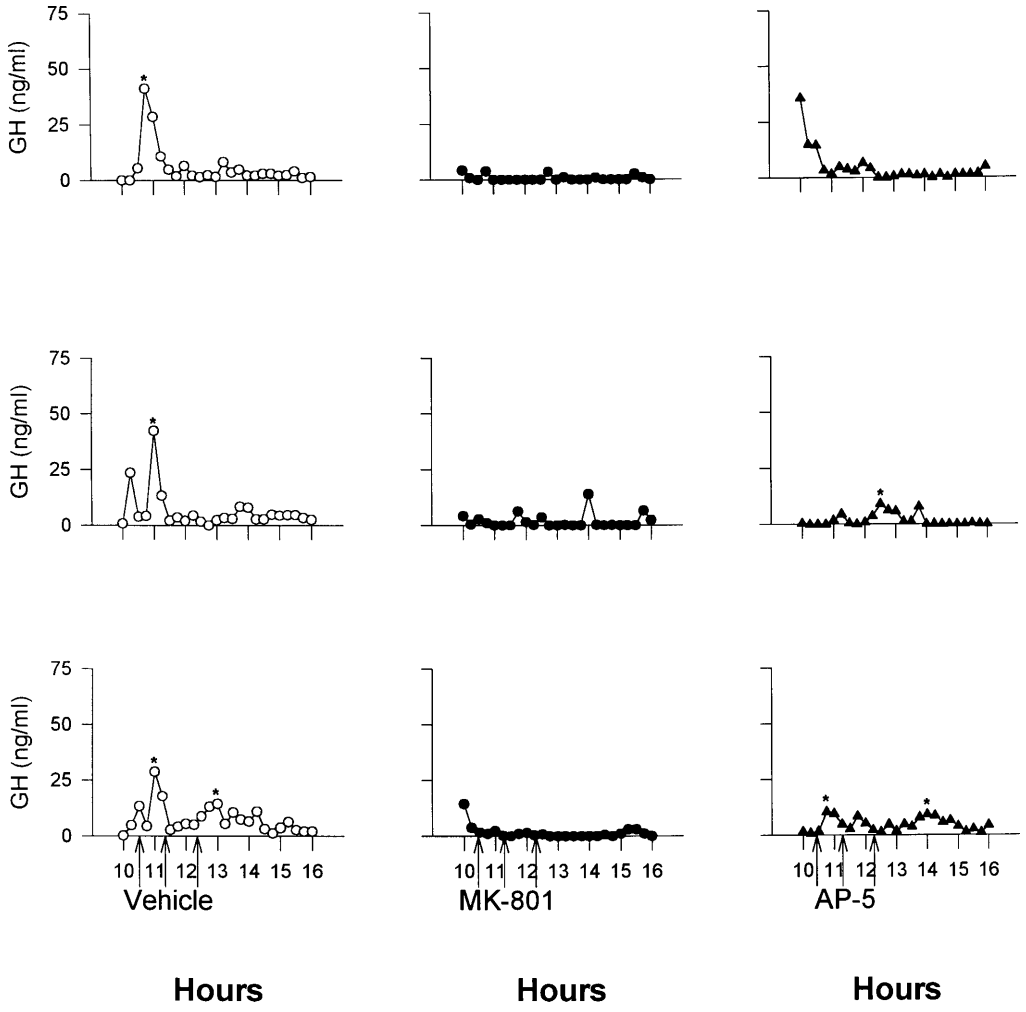

Fig. 7. Representative GH plasma profiles in individual adult male rats after i.p. injection of vehicle (left panels) MK-801 and AP-5 (1 mg/kg divided in three doses). Asterisks denote GH pulses

6. Modulation of the EAA system by testicular factors

It has been repeatedly reported that the actions of NMDA and KA on LH release depend on the gonadal environment and the steroid milieu during neonatal period. Thus, the stimulatory effect of NMDA and AMPA on LH secretion in control female rats drives to an inhibitory effect after ovariectomy (Brann and Mahesh, 1992; Ping et al., 1997), whereas administration of estradiol or testoterone has been shown to change the role of the EAA system in the control of anterior pituitary secretion in male and female rats (Bourguignon et al., 1992; MacDonald et al., 1992; Brann and Mahesh, 1992; Pinilla et al., 1995, 1998; Ping et al., 1997).

In adult rats, *in vivo* pituitary responsiveness to GHRH decreased after orchidectomy (Wehrenberg et al., 1985; Pinilla et al., 1990), and increased

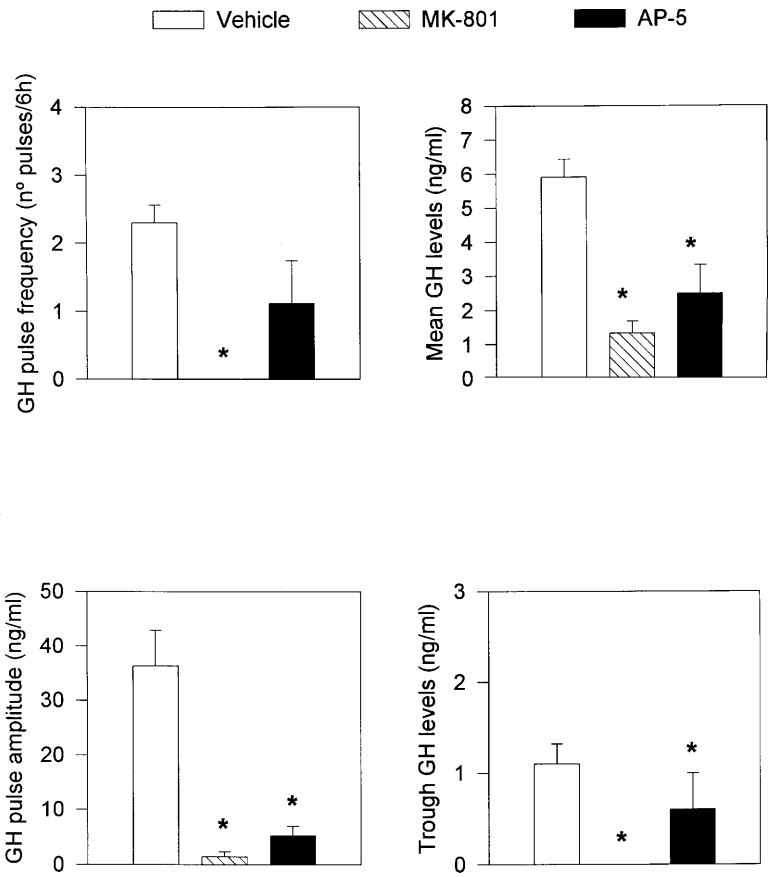

Fig. 8. Effects of i.p. administration of MK-801 and AP-5 on GH pulse frequency, mean GH levels, GH pulse amplitude and trough GH levels over a 6-hour sampling period (n = 12–17 rats per group). Data are expressed as mean ± SEM. *P ≤ 0.05 vs vehicle-injected group

after testosterone administration (Wehrenberg et al., 1985; Aguilar et al., 1992), while orchidectomy enhanced the effectiveness of GHRH in prepubertal males (Pinilla et al., 1992). On the basis of these observations, we found 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 500μg of estradiol benzoate on day 1 of life. Our data indicated that NMDA-induced GH secretion is not dependent of testicular function as it remained after orchidectomy and testosterone replacement (Table 2), as well as in estrogenized males (Pinilla et al., 1999). In contrast, the ability of KA to stimulate GH secretion appeared partially dependent on testicular function, since KA-induced GH release was blunted in orchidectomized males and completely absent in males neonatally estrogenized (Pinilla et al., 1996). Further evidence for the involvement of gonadal factors in the regulation of

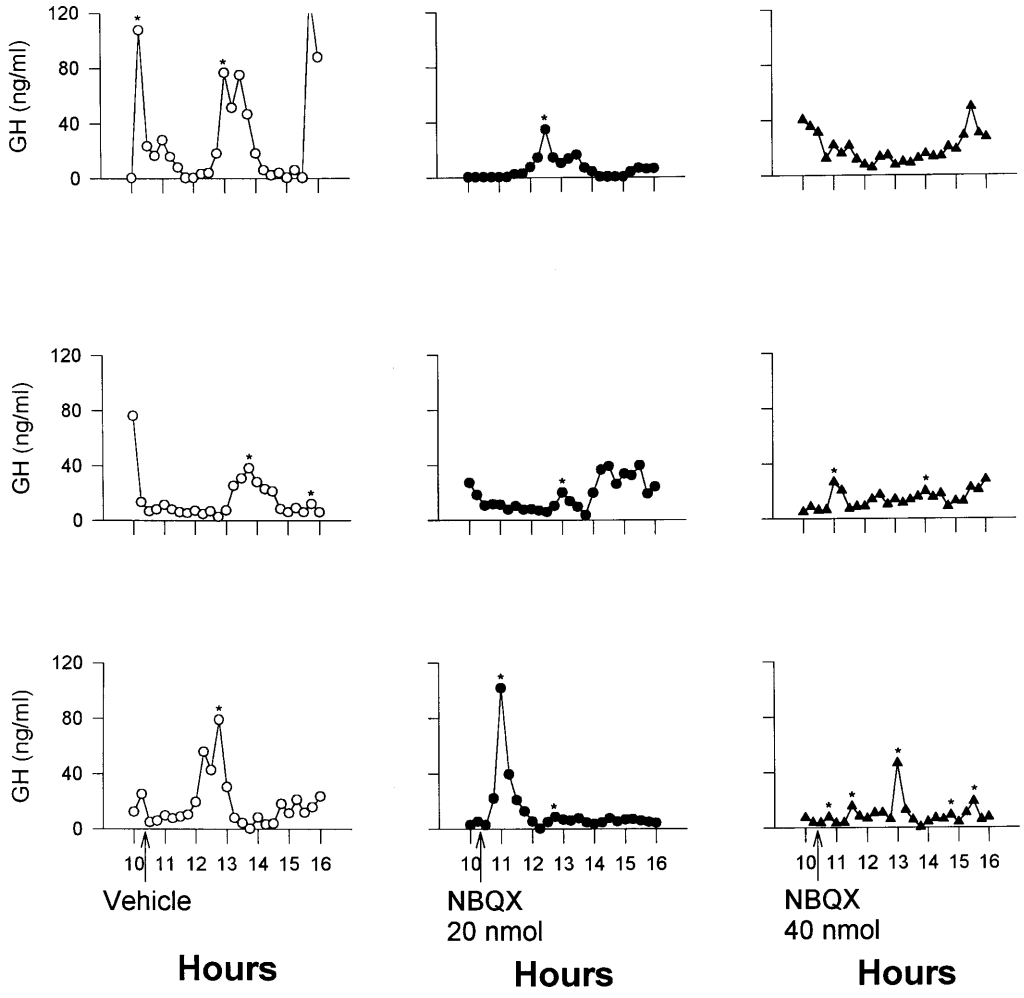

Fig. 9. Representative GH plasma profiles in individual male rats after i.c.v injection of vehicle (left panels) or NBQX (20 nmol: middle panels; 40 nmol: right panels). All the drugs were administered at 10.30 h. Asterisks denote GH pulses

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 (González et al., 1999b).

7. Future perspectives and conclusions

Many neurotransmitters are involved in the control of GH secretion via modulation of GHRH and somatostatin secretion, as well as through their direct actions at pituitary level (for a review see Müller and Nistico, 1989). In this sense, little attention has been paid to the elucidation of potential interactions between EAA pathways and other regulatory neurotransmitter systems in the control of GH secretion. Gamma aminobutyric acid (GABA)

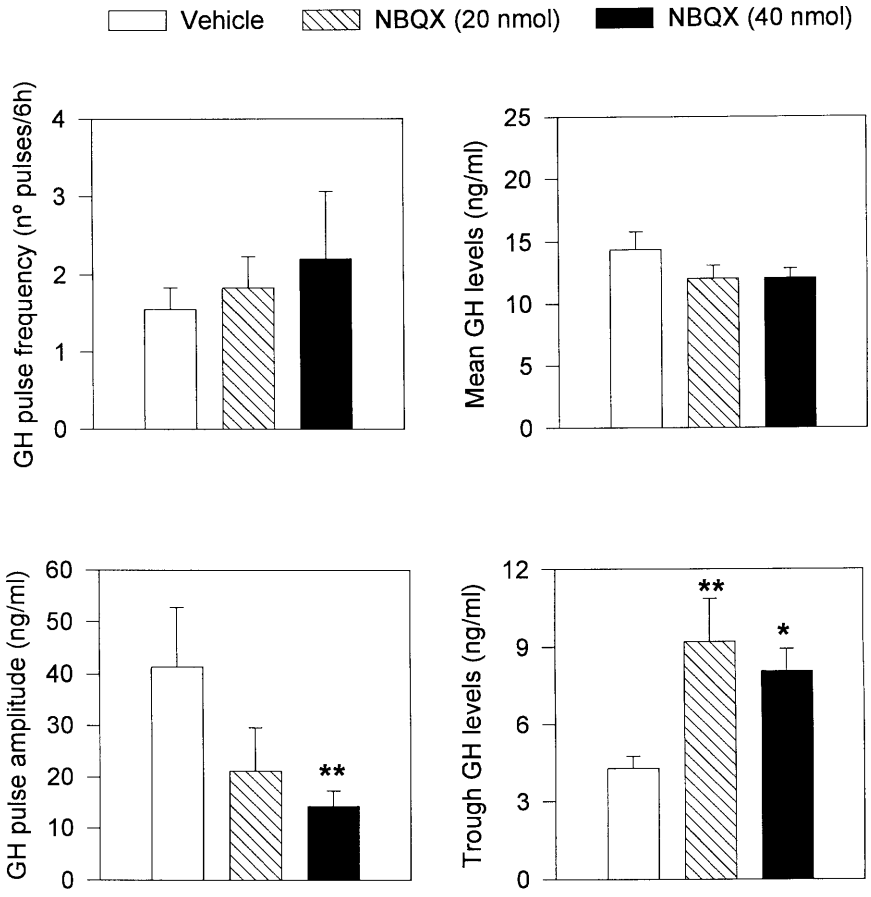

Fig. 10. Effects of intracerebroventricular administration of NBQX (20 and 40 nmol) on pulse GH frequency, mean GH levels, pulse amplitude and trough GH levels over a 6-hour sampling period (n = 12–17 rats per group). Data are expressed as mean ± SEM. *P ≤ 0.05, **P ≤ 0.01 vs vehicle-injected group

Table 2. Serum GH and testosterone concentrations (ng/ml) in prepubertal and adult male rats, 15 min after injection of vehicle or NMDA (15 mg/kg). Groups of animals were orchidectomized and orchidectomized and treated with testosterone. Sham-operated rats served as controls. Animals were orchidectomized or sham-operated a week before use. Orchidectomized males were implanted with silastic capsules (T1,T2 and T3: length 0.5, 1 and 1.5 cm, respectively). Values are given as means ± SEM. Each group consisted of 10 animals. *ND* not detectable. *P ≤ 0.05; **P ≤ 0.01 vs vehicle-injected group. [a]P ≤ 0.01 vs intact group injected with NMDA

Treatment	30 days		90 days	
	Testosterone	GH	Testosterone	GH
Intact + Veh.	0.50 ± 0.06	8.00 ± 2.00	2.75 ± 0.40	53.0 ± 29.0
Intact + NMDA	0.62 ± 0.08	287 ± 78.0**	3.54 ± 0.50	667 ± 38.0**
Orch. + NMDA	ND	285 ± 36.0**	ND	666 ± 90.0**
Orch. + T1 + NMDA	1.17 ± 0.11*	278 ± 30.0**	1.28 ± 0.11**	817 ± 54.0**
Orch. + T2 + NMDA	2.08 ± 0.10**	478 ± 37.0**,a	1.34 ± 0.25**	647 ± 121**
Orch. + T3 + NMDA	3.50 ± 0.24**	444 ± 43.0**,a	2.75 ± 0.40	837 ± 75.0**

stimulates GH secretion in adult and neonatal animals (Takahara et al., 1980; Acs et al., 1987), although inhibitory actions have been also described (Fiok et al., 1984). This stimulatory effect is, at least partially, mediated by a direct action at pituitary level (Anderson and Mitchell, 1986; Acs et al., 1992). Since previous data have demonstrated a cross-talk between excitatory and inhibitory amino acids (IAA) in the regulation of LHRH (Donoso et al, 1992), we hypothesized that similar interactions might occur in the control of GH secretion. Our preliminary experimental data indicate that the stimulatory effect of GABA (25 μg/g) on serum GH concentrations is blocked by treatment with MK-801 (0.25 μg/kg at -60 min) but not by NBQX (Pinilla, Gonzalez, Tena-Sempere, and Aguilar, in preparation). Similarly, the stimulatory effect of AMPA on GH secretion was blocked by pretreatment with Phaclofen (an antagonist of $GABA_B$ receptor) or bicuculline (an antagonist of $GABA_A$ receptor), thus suggesting the existence of functional interactions between EAA and IAA systems in the control of GH secretion. Other neurotransmitter involved in the control of GH secretion is serotonin, and stimulatory effects for this amine have been reported (Collu et al., 1972; Smythe et al., 1975; López et al., 1986). We have undertaken the characterization of potential interactions between EAAs and serotonin in the regulation of GH release. Surprisingly, the stimulatory effect of NMDA on GH secretion was partially blocked after activation of serotoninergic system with fluoxetine and 5-hyroxytryptophan (our personal observations).

The present review aimed to briefly summarize our knowledge on the participation of the EAA system in the physiological control of GH secretion. Although current data strongly support a relevant role of EAA pathways in the neuronal circuitry governing GH secretion, further elucidation of the mechanisms of action and interplay between EAAs and other neurotransmitters in the regulation of GH release will help us to expand our present understanding of physiological and pathophysiological aspects of GH secretion.

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