EXPERIMENTAL STUDY

5-HT₁ and 5-HT₂ receptor agonists blunt ($\pm$)- α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)-stimulated GH secretion in prepubertal male rats

Leonor Pinilla, Lucas C Gonzalez, Manuel Tena-Sempere and Enrique Aguilar

Department of Physiology, Faculty of Medicine, Córdoba University, Avenida Menendez Pidal s/n., Córdoba 14004, Spain

(Correspondence should be addressed to Enrique Aguilar; Email: fi1agbee@lucano.uco.es)

Abstract

Objective: Excitatory amino acids, γ -amino butyric acid (GABA), serotonin and catecholamines are involved in the control of GH secretion. The actions of these neurotransmitters are interconnected, and recently we showed that the stimulatory effect of GABA was blocked by MK-801, an antagonist of N-methyl-D-aspartate receptors. The present experiments were carried out to analyze the interrelationships between ($\pm$)- α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors and serotonergic and catecholaminergic pathways in the control of GH secretion in prepubertal (16–23-day-old) male rats.

Design and Methods: The GH response to AMPA was analyzed in animals pretreated with 5-hydroxytryptophan methyl ester (5-HTP) plus fluoxetine (a precursor of 5-hydroxytryptamine (5-HT) synthesis and a blocker of 5-HT re-uptake), R (+)-8-hydroxydipropylaminotetralin hydrobromide (8-OH-DPAT, an agonist of the 5-HT₁ receptors), ($\pm$)-2,5-dimethoxy-4-iodoamphetamine hydrochloride (DOI) and α -methyl-5-hydroxytryptamine (agonists of 5-HT₂ receptors), I-phenylbiguanide (an agonist of 5-HT₃ receptors), or α -methyl-*p*-tyrosine (α -MPT) and diethyldithiocarbamate (DDC) (blockers of catecholamine synthesis).

Results: Basal GH secretion remained unchanged in prepubertal rats after activation of the serotonergic system or blockade of catecholamine synthesis. The stimulatory effect of AMPA on GH secretion was blocked after activation of the serotonergic system, through specific 5-HT₁ and 5-HT₂ receptor agonists. In contrast, activation of 5-HT₃ receptors potentiated AMPA-stimulated GH secretion.

Conclusions: Serotonergic receptors modulate the stimulatory effect of AMPA on GH secretion in prepubertal male rats.

European Journal of Endocrinology 144 535–541

Introduction

In neonatal rats, serum growth hormone (GH) concentrations are high after birth, and then there is a decline by 15–20 days of age (1, 2). In the first days of life, GH secretion is stimulated by γ -amino butyric acid (GABA) (3), thyroliberin (TRH) (4, 5), growth hormone-releasing hormone (GHRH) (6) and excitatory amino acids (EAAs) (7). The excitatory effect of EAAs is mediated by the activation of N-methyl-D-aspartate (NMDA), kainate (KA) and ($\pm$)- α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors (8–10). Serotonin also seems to be stimulatory at this age: the administration of quipazine (a serotonergic agonist) or fluoxetine (an inhibitor of serotonin re-uptake) plus 5-hydroxytryptophan methyl ester (5-HTP) raised the serum GH levels (11), whereas the opposite effect was observed after injection of the serotonergic blocker cyproheptadine (12).

In recent years, different subtypes of 5-hydroxytryptamine (5-HT) receptors have been identified in the brain, and subtype-specific agonist and antagonist drugs have been developed. At least 7 families of 5-HT receptors have been reported so far (13–15). The role of serotonergic neurons in the control of GH secretion remains a matter of debate, basically because of their different roles in different animal species, the multiple receptor subtypes, and the absence (until recently) of specific agonists and antagonists for receptor subtypes.

In adult male rats, the administration of serotonin (16) or 5-HTP (17) stimulated GH secretion, and the administration of the serotonergic antagonists methysergide and metergoline resulted in GH inhibition (18, 19). Serotonin may stimulate GH secretion by increasing hypothalamic GHRH discharge, since somatostatin release into the portal blood was unaffected by the indoleamine (20), or by acting directly

at pituitary level, since serotonin stimulates GH secretion in hypophysectomized autografted animals and in perfused pituitaries (21).

Adrenergic pathways are important elements of the control of GH release. GH secretion is enhanced by α_2 agonists and is inhibited by α_1 - and β -receptor activation (for a review, see 22). Dopamine also inhibited GH secretion in neonatal rats (12). Inhibition of catecholamine synthesis after treatment with α -methyl-*p*-tyrosine (α -MPT) or diethyldithiocarbamate (DDC) suppressed GH secretion in adult rats (23, 24).

Recently, we have described the interplay between GABA and EAAs in the control of GH secretion in rats; the stimulatory effect of GABA was blocked by MK-801, an antagonist of NMDA receptors (7). The present experiments were designed to analyze the interactions between AMPA and the 5-HT₁, 5-HT₂ and 5-HT₃ receptors in the control of GH secretion, using specific agonists of these receptors. An additional aim was the analysis of the interactions between the catecholaminergic system and AMPA receptors. In view of the different roles of dopaminergic, α - and β -adrenergic receptors in the control of GH secretion (22), the first step in the study was the assessment of the net effects of blockade of catecholamine synthesis on AMPA-stimulated GH secretion. Experiments were carried out in animals that were 16–23 days old, this being the age at which GH pulsatility is not established (25), thus facilitating statistical analysis.

Materials and methods

Animals

Male Wistar rats (born in our laboratory) were kept under controlled conditions of light (12 h light:12 h darkness; lights on at 0700 h) and temperature (22 °C), and had free access to pelleted food (Pacsa Sanders, Seville, Spain) and tap water. On day 1 of life, each dam was left with eight pups. The pups were separated from their mothers immediately before the start of any treatment, and they were kept warm by a heating source next to their cage.

All of the experiments were approved by the Cordoba University ethical committee for animal experimentation, and were conducted in accordance with the European Union guidelines for the care and use of experimental animals.

Drugs

AMPA (Research Biochemical Inc., Natick, MA, USA), an agonist of AMPA receptors, was dissolved initially in a few drops of dimethylsulfoxide and thereafter in saline up to the working concentration. A dose of 2.5 mg/kg was injected intraperitoneally 15 min before the animal was killed.

Fluoxetine hydrochloride (Research Biochemical Inc.), a selective serotonin re-uptake inhibitor, was dissolved in saline. A dose of 10 mg/kg was injected intraperitoneally 45 min before the administration of AMPA.

5-HTP (Sigma, Barcelona, Spain), a precursor of serotonin synthesis, was dissolved in saline. A dose of 100 mg/kg was injected intraperitoneally 45 min before the administration of AMPA.

8-OH-DPAT (R (+)-8-hydroxydipropylaminotetralin hydrobromide; Research Biochemical Inc.), an agonist of 5-HT_{1A} receptors, was dissolved in saline. Doses of 5 or 10 mg/kg were injected intraperitoneally 45 min before the administration of AMPA.

DOI ((±)-2,5-dimethoxy-4-iodoamphetamine hydrochloride; Research Biochemical Inc.), an agonist of 5-HT₂ receptors, was dissolved in saline. Doses of 20 or 30 mg/kg were injected intraperitoneally 45 min before the administration of AMPA.

α -Me-HT maleate (α -methyl-5-hydroxytryptamine maleate; Research Biochemical Inc.), an agonist of 5-HT₂ receptors, was dissolved in saline. A dose of 10 mg/kg was injected intraperitoneally 45 min before the administration of AMPA.

1-Phenylbiguanide (Research Biochemical Inc.), an agonist of 5-HT₃ receptors, was dissolved in saline. A dose of 10 mg/kg was injected intraperitoneally 45 min before the administration of AMPA.

α -MPT, an inhibitor of tyrosine hydroxylase (Sigma), was dissolved in saline. A dose of 250 mg/kg was injected intraperitoneally 3 h before the administration of AMPA.

DDC, an inhibitor of dopamine- β -hydroxylase (Sigma), was dissolved in saline. A dose of 500 mg/kg was injected intraperitoneally 3 h before the administration of AMPA.

The doses and timing for drug administration were selected on the basis of previous studies (26–29).

Experimental design

Experiment 1 In order to determine the cross-talk between serotonergic and EAA pathways in the control of GH secretion, 23-day-old males (8–10 per group) were injected intraperitoneally with vehicle or fluoxetine (10 mg/kg) + 5-HTP (100 mg/kg) 45 min before the administration of vehicle or AMPA (2.5 mg/kg). Animals were killed by decapitation 15 min later.

Experiment 2 To analyze the serotonergic receptor subtype involved in the modulation of AMPA effects on GH secretion, 16- and 23-day-old males (8–10 per group) were injected intraperitoneally with vehicle, 8-OH-DPAT (5 or 10 mg/kg) or DOI (20 or 30 mg/kg) 45 min before the administration of vehicle or AMPA (2.5 mg/kg). The animals were killed by decapitation 15 min later. Other groups of 16- and 23-day-old males (8–10 per group) were injected intraperitoneally

with vehicle, α -Me-HT maleate (10 mg/kg) or I-phenylbiguanide (10 mg/kg) 45 min before the administration of vehicle or AMPA (2.5 mg/kg). The animals were killed by decapitation 15 min later.

Experiment 3 In order to investigate the possible involvement of catecholaminergic pathways in the AMPA effects on GH secretion, 23-day-old males (8–10 per group) were injected with vehicle, α -MPT (250 mg/kg) or DDC (500 mg/kg) 3 h before the administration of AMPA or vehicle. The animals were killed by decapitation 15 min later.

GH determinations

After centrifugation (1600 *g* at 4 °C for 20 min), serum was collected, frozen and stored at –20 °C until use. The concentrations of GH were measured in 10–25 μ l with a double-antibody radioimmunoassay method, using a kit supplied by the National Institutes of Health (NIH, Bethesda, MD, USA). Rat-GH-I-7 was labeled with ¹²⁵I by the Chloramine T method (30), and hormone concentrations were expressed using reference preparation (RP) GH-RP-S2 as the standard. Intra- and interassay variations were 6% and 9% respectively. The sensitivity of the assay was 5 pg/tube. All samples for each experiment were measured in the same assay.

Statistics

Values are expressed as means $\pm$ S.E.M. Differences between groups were analyzed using one- or two-way ANOVA followed by Tukey's test.

Results

Activation of the serotonergic system inhibited AMPA-stimulated GH secretion

The administration of AMPA to 23-day-old male rats induced a significant ($P \leq 0.01$) increase in GH secretion. This response was blunted by the previous activation of the serotonergic system with fluoxetine and 5-HTP. Fluoxetine and 5-HTP *per se* did not affect serum GH concentrations (Table 1).

Activation of the 5-HT_{1A} and 5-HT₂ serotonergic receptors inhibited AMPA-stimulated GH secretion

Male rats (16 days old) injected with 8-OH-DPAT (5 or 10 mg/kg) or DOI (20 or 30 mg/kg) showed serum GH levels similar to those of vehicle-injected animals. AMPA significantly stimulated GH secretion, this effect being blocked by 8-OH-DPAT and DOI (Fig. 1). A similar blockade of the AMPA effect was observed in 23-day-old

Table 1 Effects of AMPA (2.5 mg/kg at –15 min) in 23-day-old male rats with or without pretreatment with fluoxetine (Fx) and 5-HTP (10 and 100 mg/kg respectively, at –60 min). Values are given as means $\pm$ S.E.M. Number of animals are indicated in parentheses.

Treatment	GH (ng/ml)
Vehicle + vehicle	6.59 $\pm$ 3.87 (7) ^a
Vehicle + AMPA	125.00 $\pm$ 20.30 (8) ^b
Fx + 5-HTP + vehicle	5.26 $\pm$ 0.75 (10) ^a
Fx + 5-HTP + AMPA	39.30 $\pm$ 6.30 (7) ^c

Groups with different superscript letters were statistically different ($P \leq 0.01$, using two-way ANOVA followed by Tukey's test).

males injected with 8-OH-DPAT (5 or 10 mg/kg) or DOI (30 mg/kg) (data not shown). The AMPA effect was also blunted in 16- and 23-day-old males pretreated with α -5-HT maleate, another agonist of 5-HT₂ receptors (Figs 2 and 3). In contrast, the administration of I-phenylbiguanide, an agonist of 5-HT₃ receptors, potentiated the stimulatory effect of AMPA on GH secretion in 23-day-old males (Fig. 3).

AMPA-stimulated GH secretion remained unaffected after blockade of catecholamine synthesis

Basal serum GH levels remained unaffected in 23-day-old males after treatment with α -MPT or DDC, which were also unable to alter the stimulatory effect of AMPA on GH secretion (Fig. 4).

Discussion

The major finding of the present study is that the stimulatory effect of AMPA on GH secretion can be blocked by coactivation of the serotonergic system, through specific 5-HT₁ and 5-HT₂ receptor-mediated pathways. This observation is supported by the reproduction of the results in different experiments, at two ages (16 and 23 days), using different agonists and doses (8-OH-DPAT at 5 and 10 mg/kg; DOI at 20 and 30 mg/kg; α -5-HT maleate at 10 mg/kg).

Activation of the serotonergic system after the administration of 5-HTP plus fluoxetine did not raise GH secretion in prepubertal rats; this contrasts with the stimulatory role observed in neonatal (12) and adult rats (16). We do not have a clear explanation for this unexpected finding, although previous results obtained in 10-day-old male rats by Katz *et al.* (31) appeared conflicting, since fluoxetine plus 5-HTP raised serum GH concentrations, whereas all mixed serotonin agonists suppressed GH secretion (31). It has been previously reported that the role of serotonergic pathways in the neuroendocrine control changes during sexual maturation and that the stimulatory effect of serotonin on gonadotropin secretion in

Figure 1 Effects of AMPA (2.5 mg/kg) on serum GH concentrations in 16-day-old male rats pretreated with 8-OH-DPAT or DOI. ** $P \leq 0.01$ vs vehicle-injected group (two-way ANOVA followed by Tukey's test).

prepubertal rats younger than 20 days of age disappears after this age (32–34). Similar changes have been described for the GABAergic control of gonadotropin secretion (35, 36). It is possible that the stimulatory effect of serotonin on GH secretion is limited to specific periods of development.

The serotonergic 5-HT₁ and 5-HT₂ receptor subtypes have been shown to be involved as stimulatory elements in the control of GH in dogs (37) and in

humans (38), whereas GH secretion seems to be independent of the degree of activation of 5-HT₃ receptors (37). In neonatal rats, it has been proposed that changes in serotonergic activity at 5-HT₂ receptors mediates the inhibition of GH secretion in maternal separation (31). To further the pharmacological characterization of serotonin receptor subtypes involved in the blockade of AMPA-stimulated GH secretion, we used selective agonists of the 5-HT₁,

Figure 2 Effects of AMPA (2.5 mg/kg) on serum GH concentrations in 16-day-old male rats pretreated with α-Me-HT or I-phenylbiguanide. ** $P \leq 0.01$ vs vehicle-injected group (two-way ANOVA followed by Tukey's test).

Figure 3 Effects of AMPA (2.5 mg/kg) on serum GH concentrations in 23-day-old male rats pretreated with α -Me-HT or I-phenylbiguanide. ** $P \leq 0.01$ vs vehicle-injected group; a, $P \leq 0.01$ vs animals injected exclusively with AMPA (two-way ANOVA followed by Tukey's test).

5-HT₂ and 5-HT₃ receptor subtypes. Activation of different 5-HT subtypes alone did not affect GH secretion. This supports data obtained after enhancement of serotonergic tone with 5-HTP plus fluoxetine. Of more relevance is the fact that blockade of AMPA-stimulated GH secretion after the administration of 5-HT₁ and 5-HT₂ agonists was observed.

The mechanisms whereby AMPA stimulates GH secretion have not been explored completely, though

it appears that the hypothalamus is the predominant site of action, since AMPA was unable to stimulate *in vitro* GH secretion by hemipituitaries (10). Our initial hypothesis is that aminoacidergic neurons inhibit somatostatin release via activation of AMPA receptors. The decrease in somatostatin release could raise GH secretion (10). The possibility that AMPA activated the release of other GH secretagogues such as the recently discovered GHrelin (39) is worthy of consideration.

Figure 4 Effects of AMPA (2.5 mg/kg) on serum GH concentrations in 23-day-old male rats pretreated with α -MPT or DDC. ** $P \leq 0.01$ vs vehicle-injected group (two-way ANOVA followed by Tukey's test).

Unfortunately, the localization of different serotonin receptor subtypes in hypothalamic neurons has not been analyzed, making it difficult to explain the present results. One possibility is that serotonergic 5-HT₁ and 5-HT₂ receptors are present in axonic terminals of somatostatin neurons and that their activation stimulates the release of somatostatin, restoring it to levels similar to those present in the absence of activation of AMPA receptors. This possibility, however, seems unlikely since intracerebroventricular administration of 5-HT left unaltered somatostatin in hypophyseal portal blood (20), and 5-HT inhibited somatostatin release from explants of rat medialis hypothalamus (40).

Another possibility is that AMPA receptors involved in the neuroregulation of GH secretion could be regulated by serotonin receptors. In this sense, it is noticeable that 5-HT acting through 5-HT_{1A} and 5-HT_{1B} receptors caused depression of excitatory postsynaptic potentials, mediated mainly by NMDA and AMPA receptors, in entorhinal cortex (41, 42), hippocampal slices (43) and nucleus accumbens (44). If a similar effect were occurring in hypothalamic neurons, the modulation by serotonergic receptors of GH responses to AMPA could be explained.

Activation of α_2 -adrenoreceptors with clonidine stimulates GH secretion in neonatal rats, via release of GHRH, with no effect on the somatotropes (6). However, inhibition of epinephrine and norepinephrine synthesis with DDC or α -MPT did not affect basal GH secretion. This finding is probably explained by elimination of both the stimulatory effect of epinephrine exerted through activation of α_2 -adrenoreceptors and the inhibitory effect mediated by β -adrenergic receptors (45). In addition, AMPA stimulated GH secretion in rats pretreated with α -MPT and DDC, which suggests that the control of GH secretion by EAAs is independent of catecholamine levels.

In conclusion, our results indicate that although basal GH secretion is not under the control of serotonergic and catecholaminergic pathways, AMPA-elicited GH release was blunted by the activation of 5-HT₁ and 5-HT₂ receptors in prepubertal male rats.

Acknowledgements

The authors are very grateful to Rocío Campon and Inmaculada Aguilar for their technical assistance. This work was supported by grants PM98-0163 (DGICYT) and 1-FG-97-0696-02 (FEDER). The NIH supplied materials for luteinizing hormone and prolactin determinations.

References

- Blazquez E, Simon FA, Blazquez M & Foa PP. Changes in serum growth hormone levels from fetal to adult age in the rat. *Proceedings of the Society for Experimental Biology and Medicine* 1974 **147** 780–783.
- Rieutort M. Pituitary content and plasma levels of growth hormone in foetal and wailing rats. *Journal of Endocrinology* 1974 **60** 261–268.
- Acs Z, Lónart G & Makara G. Role of hypothalamic factors (growth-hormone-releasing hormone and gamma-aminobutyric acid) in the regulation of growth hormone secretion in the neonatal and adult rat. *Neuroendocrinology* 1990 **52** 156–160.
- Khorram O, De Palatis LW & McCann SM. Development of hypothalamic control of growth hormone secretion in the rat. *Endocrinology* 1983 **113** 720–728.
- Strbak V, Jurcovicova J & Vigas M. Thyroliberin (TRH) induced growth hormone (GH) release: test of maturation of hypothalamo-pituitary axis in postnatal rat. *Endocrinologia Experimentalis* 1981 **15** 245–249.
- Cella SG, Locatelli V, De Gennaro V, Pellini C, Pintor C & Müller EE. *In vivo* studies with growth hormone (GH)-releasing factor and clonidine in rat pups: ontogenic development of their effect on GH release and synthesis. *Endocrinology* 1986 **119** 1164–1170.
- Tena-Sempere M, Pinilla L, Gonzalez LC & Aguilar E. Regulation of growth hormone (GH) secretion by different glutamate receptor subtypes in the rat. *Amino Acids* 2000 **18** 1–16.
- Pinilla L, Gonzalez L, Tena-Sempere M, Dieguez C & Aguilar E. Gonadal and age-related influences of NMDA-induced growth hormone secretion in male rats. *Neuroendocrinology* 1999 **69** 11–19.
- Pinilla L, Tena-Sempere M, Gonzalez D & Aguilar E. Positive role of non-N-methyl-D-aspartate receptors in the control of growth hormone secretion in male rats. *Journal of Endocrinological Investigation* 1996 **19** 353–358.
- Gonzalez LC, Pinilla L, Tena-Sempere M & Aguilar E. Regulation of growth hormone secretion by amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors in infantile, prepubertal, and adult male rats. *Endocrinology* 1999 **140** 1279–1284.
- Bero LA & Kuhn CM. Differential ontogeny of opioid, dopaminergic and serotonergic regulation of prolactin secretion. *Journal of Pharmacology and Experimental Therapeutics* 1987 **240** 825–830.
- Stuart M, Lazarus L, Smythe GA, Moore S & Sara V. Biogenic amine control of growth hormone secretion in the fetal and neonatal rat. *Neuroendocrinology* 1976 **22** 337–342.
- Hoyer D & Schoeffer P. 5-HT receptors: subtypes and second messengers. *Journal of Receptor Research* 1991 **11** 197–214.
- Hartig PR, Branchek TA & Weinschenk RL. A subfamily of 5-HT_{1D} receptor genes. *Trends in Pharmacological Sciences* 1992 **13** 152–159.
- 1997 Receptor and Ion Channel Nomenclature Supplement (RNS). *Trends in Pharmacological Sciences* 1997 Suppl 1–84.
- Collu R, Fraschini F, Visconti P & Martini L. Adrenergic and serotonergic control of growth hormone secretion in adult male rats. *Endocrinology* 1972 **90** 1231–1237.
- Smythe GA, Brandstater J & Lazarus L. Serotonergic control of rat growth hormone secretion. *Neuroendocrinology* 1975 **17** 245–257.
- Arnold MA & Fernstrom JD. Serotonin receptor antagonists block a natural short term surge in serum growth hormone levels. *Endocrinology* 1978 **103** 1159–1163.
- Martin JB, Durand D, Gurd W, Faille G, Audet J & Brazeau P. Neuropharmacological regulation of episodic growth hormone and prolactin secretion in the rat. *Endocrinology* 1978 **102** 106–113.
- Chihara K, Arimura A & Schally AV. Effect of intraventricular injection of dopamine, norepinephrine, acetylcholine and 5-hydroxytryptamine on immunoreactive somatostatin release into rat hypophyseal portal blood. *Endocrinology* 1979 **104** 1656–1662.
- López F, González D & Aguilar E. Serotonin stimulates GH secretion through a direct pituitary action: studies in hypophysectomized autografted animals and in perfused pituitaries. *Acta Endocrinologica* 1986 **113** 317–322.

- 22 Bertherat J, Bluet-Pajot MT & Epelbaum J. Neuroendocrine regulation of growth hormone. *European Journal of Endocrinology* 1995 **132** 12–24.
- 23 Durand D, Martin JB & Brazeau P. Evidence for a role of α -adrenergic mechanisms in regulation of episodic growth hormone secretion in the rat. *Endocrinology* 1977 **100** 722–728.
- 24 Katakami H, Kato Y, Matsushita N, Hiroto S, Shimatsu A & Imura H. Involvement of α -adrenergic mechanisms in growth hormone release induced by opioid peptides in conscious rats. *Neuroendocrinology* 1981 **33** 129–135.
- 25 Gabriel SM, Rocancio JR & Ruiz NS. Growth hormone pulsatility and the endocrine milieu during sexual maturation in male and female rats. *Neuroendocrinology* 1992 **56** 619–628.
- 26 Pinilla L, Tena-Sempere M & Aguilar E. Effects of 5-HT₂ and 5-HT₃ agonists on PRL secretion in male rats. *Neuroendocrinology Letters* 1995 **17** 209–214.
- 27 Gonzalez LC, Pinilla L, Tena-Sempere M & Aguilar E. Regulation of prolactin secretion by α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors in male rats. *Journal of Endocrinology* 2000 **166** 669–675.
- 28 Negro-Vilar A, Ojeda SR, Advis JP & McCann SM. Evidence for noradrenergic involvement in episodic prolactin and growth hormone release in ovariectomized rats. *Endocrinology* 1979 **105** 86–91.
- 29 Martin AI, Fernandez-Ruiz J & Lopez Calderon A. Effects of catecholamine synthesis inhibitors and adrenergic receptor antagonists on restraint-induced LH release. *Journal of Endocrinology* 1995 **144** 511–515.
- 30 Greenwood FC, Hunter WM & Glover JS. The preparation of ¹³¹I labelled human growth hormone of high specific radioactivity. *Biochemistry Journal* 1963 **89** 114–123.
- 31 Katz LM, Nathan L, Kuhn CM & Schanberg SM. Inhibition of GH in maternal separation may be mediated through altered serotonergic activity at 5-HT_{2A} and 5-HT_{2C} receptors. *Psychoneuroendocrinology* 1996 **21** 219–235.
- 32 Arias P, Szwarcfarb B, Rondina DC, Carbone S, Sverlik R & Moguilevski JA. *In vivo* and *in vitro* studies on the effect of the serotonergic system on luteinizing hormone and luteinizing hormone releasing hormone secretion in prepubertal and peripubertal female rats. *Brain Research* 1991 **523** 57–61.
- 33 Carbone S, Szwarcfarb B, Lasaga M, Duvilansky B, Seilicovich A & Moguilevski JA. Effect of serotonergic system on the hypothalamic GABAergic activity during sexual maturation in female rats. Its relation with the control of LH secretion. *Neuroendocrinology Letters* 1993 **15** 183–190.
- 34 Moguilevski JA, Faigon MR, Scacchi P & Szwarcfarb B. Effect of serotonergic system on luteinizing hormone secretion in prepubertal female rats. *Neuroendocrinology* 1985 **40** 135–138.
- 35 Moguilevski JA, Carbone S, Szwarcfarb B & Rondina D. Sexual maturation modifies the GABAergic control of gonadotropin secretion in female rats. *Brain Research* 1991 **563** 12–16.
- 36 Szwarcfarb B, Carbone S, Stein ML, Medina JA & Moguilevski JA. Sexual differences in the effect of GABAergic system on LH secretion and in the hypothalamus ontogenesis of GABA-A receptors in prepubertal rats. *Brain Research* 1994 **646** 351–355.
- 37 Valverde I, Peñalva A & Dieguez C. Influence of different serotonin receptor subtypes on growth hormone secretion. *Neuroendocrinology* 2000 **71** 145–153.
- 38 Mota A, Bento A, Peñalva A, Pombo M & Dieguez C. Role of the serotonin receptor subtype 5-HT_{1D} on basal and stimulated growth hormone secretion. *Journal of Clinical Endocrinology and Metabolism* 1995 **80** 1973–1977.
- 39 Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H & Kangawa K. Ghrelin is a growth hormone-releasing acylated peptide from stomach. *Nature* 1999 **402** 656–660.
- 40 Richardson SB, Hollander CS, Prasad JA & Hirooka Y. Somatostatin release from rat hypothalamus *in vitro*: effects of melatonin and serotonin. *Endocrinology* 1981 **109** 602–606.
- 41 Schmid D, Gloveli T, Empson RM, Draguhn A & Heinemann U. Serotonin reduces synaptic excitation in the superficial medial entorhinal cortex of the rat via a presynaptic mechanism. *Journal of Physiology* 1998 **508** 119–129.
- 42 Schmitz D, Gloveli T, Empson RM & Heinemann U. Potent depression of stimulus evoked field potential responses in the medial entorhinal cortex by serotonin. *British Journal of Pharmacology* 1999 **128** 248–254.
- 43 Pugliese AM, Passani MB & Corradetti R. Effect of the selective 5-HT_{1A} receptor antagonist WAY 100635 on the inhibition of e.p.s.ps produced by 5-HT in the CA1 region of rat hippocampal slices. *British Journal of Pharmacology* 1998 **124** 93–100.
- 44 Muramatsu M, Lapid MD, Tanaka E & Grenhoff J. Serotonin inhibits glutamate currents in rat nucleus accumbens neurons via presynaptic 5-HT_{1B} receptors. *European Journal of Neuroscience* 1998 **10** 2371–2379.
- 45 Krieg RJ, Perkins SN, Johnson JH, Rogers JP, Arimura A & Cronin MJ. Beta-adrenergic stimulation of growth hormone (GH) release *in vivo*, and subsequent inhibition of growth hormone (GH) releasing factor induced GH secretion. *Endocrinology* 1988 **122** 531–537.

Received 17 July 2000

Accepted 29 November 2000