EXPERIMENTAL STUDY

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

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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 (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors and serotoninergic 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), (±)-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 serotoninergic system or blockade of catecholamine synthesis. The stimulatory effect of AMPA on GH secretion was blocked after activation of the serotoninergic system, through specific 5-HT₁ and 5-HT₂ receptor agonists. In contrast, activation of 5-HT₃ receptors potentiated AMPA-stimulated GH secretion.

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

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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 (±)-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 serotoninergic 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 serotoninergic 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 serotoninergic 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 serotoninergic 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 α₂ agonists and is inhibited by α₁- 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 cathecolaminergic 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 07:00 h) and temperature (22 °C), and had free access to pelleted food (Pacsas 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-HT₁A 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.

I-Phenylbiguanide (Research Biochemical Inc.), an agonist of 5-HT₃ receptors, 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 serotoninergic 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 serotoninergic 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 μl 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 ± 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 ≤ 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-HT1A 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 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-HT1 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

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**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 ± S.E.M. Number of animals are indicated in parentheses.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GH (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle + vehicle</td>
<td>Fx + 5-HTP + AMPA</td>
</tr>
<tr>
<td>6.59 ± 3.87 (7)</td>
<td>20.30 (8)</td>
</tr>
<tr>
<td>Vehicle + AMPA</td>
<td>Fx + 5-HTP + vehicle</td>
</tr>
<tr>
<td>125.00 ± 20.30 (8)</td>
<td>7.05 (10)</td>
</tr>
<tr>
<td>Fx + 5-HTP + AMPA</td>
<td>Fx + 5-HTP + vehicle</td>
</tr>
<tr>
<td>39.30 ± 6.30 (7)</td>
<td>3.87 (7)</td>
</tr>
</tbody>
</table>

Groups with different superscript letters were statistically different (P ≤ 0.01; using 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-HT$_1$ and 5-HT$_2$ 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-HT$_3$ receptors (37). In neonatal rats, it has been proposed that changes in serotonergic activity at 5-HT$_2$ 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-HT$_1$, 5-HT$_2$, and 5-HT$_3$ subtypes.
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 serotoninergic tone with 5-HP + 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 GHerlin (39) is worthy of consideration.

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 ≤ 0.01 vs vehicle-injected group; a, P ≤ 0.01 vs animals injected exclusively with AMPA (two-way ANOVA followed by Tukey’s test).

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 ≤ 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 intracebroventricular administration of 5-HT left unaltered somatostatin in hypophyseal portal blood (20), and 5-HT inhibited somatostatin release from explants of rat medibasal 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-HT1A and 5-HT1B 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 α2-adrenoceptors 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 α2-adrenoceptors 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 cathecolaminergic pathways, AMPA-elicted GH release was blunted by the activation of 5-HT1 and 5-HT2 receptors in pubertal male rats.

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