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The activation of metabotropic glutamate receptors differentially affects GABA and α-melanocyte stimulating hormone release from the hypothalamus and the posterior pituitary of male rats

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Abstract

The aim of the present study was to investigate the effect of metabotropic glutamate receptor (mGluR) activation on gamma-aminobutyric acid (GABA) and α-melanocyte stimulating hormone (α-MSH) release from hypothalamic fragments and posterior pituitaries. The actions of a number of subtype-selective mGluR agonists were monitored. A group I mGluR agonist, (*S*)-3-hydroxyphenylglycine (3-HPG; 0.5 mM), decreased K^+-induced hypothalamic GABA release. (*RS*)-1-Aminoindan-1,5-dicarboxylic acid (AIDA), a specific group I mGluR antagonist (0.2 mM), blocked the effect of 3-HPG. (2*S*, 1′*S*, 2′*S*)-2-(Carboxycyclopropyl) glycine (L-CCG-I) and L-serine-*O*-phosphate (L-SOP; 0.01–1 mM), agonists of group II and III mGluRs, respectively, did not modify hypothalamic evoked GABA release. Group I mGluR activation decreased, whereas group III increased and group II induced no changes in GABA release from the posterior pituitary. 3-HPG (1 mM) and L-CCG-I (0.1 mM) decreased, whereas L-SOP (0.01–0.1 mM) did not change α-MSH release from hypothalamic fragments. No agonists of the three mGluR groups modified α-MSH release from the posterior pituitary. These results indicate that activation of mGluRs differentially affects GABA and α-MSH release from the hypothalamus and the posterior pituitary.

Keywords: Metabotropic glutamate receptors; Gamma-aminobutyric acid; α-Melanocyte stimulating hormone; Hypothalamus; Posterior pituitary

Glutamate, the major excitatory neurotransmitter in the central nervous system, acts through both ionotropic (iGluRs) and metabotropic (mGluRs) receptors. Glutamate plays an important role in a variety of physiological functions including learning, memory and developmental plasticity, and is also involved in reproductive and neuroendocrine events. The highest glutamate receptor density in the hypothalamus has been localized in regions related to neuroendocrine secretion and autonomic regulation, consistent with reports of glutamate involvement in these functions [11]. mGluRs modulate neurotransmission in synaptic terminals throughout the brain [4] and act presynaptically to reduce transmission at glutamatergic synapses [17].

mGluRs are classified into three groups based on sequence homology, pharmacology and effectors. Group I mGluRs (type 1 and 5 mGluR and splice variants) increase the release of Ca^{2+} from internal stores via inositol triphosphate mobilization and activation of protein kinase C [4]. In contrast, group II mGluRs (mGluR2 and 3) and group III mGluRs (mGluR4, 6, 7 and 8) are linked to the inhibition of adenylyl cyclase and also modulate ion channel activity [4].

Hypothalamic neurons in many regions including the preoptic area, anterior hypothalamus, suprachiasmatic and supraoptic nuclei and periventricular region, express group I mGluRs, though at less density than iGluRs [20]. Group II and III mGluRs are located at supraoptic neurons [7,15]. The presence of group II mGluRs has also been shown in both anterior and posterior lobes of the pituitary gland [15].

Considering that gamma-aminobutyric acid (GABA) and α-melanocyte stimulating hormone (α-MSH) participate in the control of pituitary hormone secretion [2] and given that mGluRs are involved in the regulation of the release of

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neurotransmitters and neuropeptides [14,17], this study has investigated the effect of the activation of subtypes of mGluRs in the release of GABA and α-MSH release from the hypothalamus and the posterior pituitary.

Male Wistar rats (200–250 g body weight) were housed under controlled temperature and light conditions. Food and water were available ad libitum. After decapitation, the posterior pituitary was removed and a hypothalamic explant, including the arcuate and periventricular nuclei, the medial preoptic area and the median eminence, was dissected.

(*S*)-3-Hydroxyphenylglycine (3-HPG), (*RS*)-1-aminoindan-1,5-dicarboxylic acid (AIDA), (2*S*, 1′*S*, 2′*S*)-2-(carboxycyclopropyl) glycine (L-CCG-I), L-serine-*O*-phosphate (L-SOP), (±)-1-aminocyclopentane-*trans*-1,3-dicarboxylic acid (*trans*-ACPD) and (2*S*)-α-ethylglutamic acid (EGLU) were purchased from Tocris Cookson, Inc. (Ballwin, MO) (Table 1). ^{125}I radionucleide and [^{3}H]muscimol were obtained from New England Nuclear™ (Boston, MA).

One hypothalamic explant or one posterior pituitary was incubated in a Dubnoff shaker at 37 °C, in 95% O_2–5% CO_2 with 0.5 ml of Krebs–Ringer bicarbonate buffer (KRB), pH 7.4, containing 10 mM glucose, 10 mM HEPES, 1 mM ascorbic acid, 0.1 mM bacitracin and 0.1% bovine serum albumin. Tissues were preincubated for 15 min. Then, the medium was replaced with fresh KRB and the tissues were incubated for 30 min (basal release). For GABA determination, the tissues were incubated for a further 30 min in 40 mM K^+ medium (balanced by reducing Na^+ concentration; evoked release). The agonists and/or antagonists were present during both periods of incubation. At the end of the incubation periods, media and tissues were frozen.

Tissues were homogenized in cold distilled water. The media were heated for 10 min in a 100 °C water bath and centrifuged at 10,000 rev./min for 10 min. GABA concentration was determined by the [^{3}H]muscimol radioreceptor assay described by Bernasconi et al. [1]. This method measures the concentration of endogenous GABA and other $GABA_A$ receptor ligands metabolically related to GABA (sensitivity range: 12.5–200 pmol/ml). α-MSH was determined by radioimmunoassay by the method of Eberle et al. [18], who kindly provided α-MSH antibody, using [^{125}I]α-MSH as the tracer.

Data were expressed as the means ± SEM and were analyzed by the unpaired Student's *t*-test or one-way analysis of variance followed by Dunnett's test for comparisons against the control group or by Student–Newman–Keuls Multiple Comparisons Test for multiple comparisons. Differences were considered statistically significant when $P < 0.05$. All experiments were performed at least twice. Figures represent results of individual experiments.

Table 1
Agonists and antagonists of group I–III mGluRs

	Group I mGluRs	Group II mGluRs	Group III mGluRs
Agonist	3-HPG *trans*-ACPD	L-CCG-I *trans*-ACPD	L-SOP
Antagonist	AIDA	EGLU	

3-HPG (0.5 mM), a specific group I agonist, decreased hypothalamic GABA release. This effect was reduced by AIDA (0.2 mM), a group I antagonist (Fig. 1A). L-CCG-I (0.01–1 mM) and L-SOP (0.01–1 mM), agonists of groups II and III mGluRs, respectively, did not modify GABA release from hypothalamic fragments (Table 2).

In the posterior pituitary, 3-HPG (0.1–1 mM) decreased GABA release. However, this effect was not antagonized by AIDA (0.2 mM; Fig. 1B). *trans*-ACPD, a mixed agonist for groups I and II, decreased GABA release (Control: 1.35 ± 0.08 nmol/mg protein; 1 mM *trans*-ACPD: 1.07 ± 0.09; $n = 5$–6; $P < 0.05$), whereas L-CCG-I (0.1–1 mM) did not modify it (data not shown). On the contrary, L-SOP increased GABA release from the posterior pituitary (Control: 1.11 ± 0.08 nmol/mg protein; 0.01 mM L-SOP: 1.31 ± 0.09; 0.1 mM L-SOP: 1.46 ± 0.05; 1 mM L-SOP: 1.58 ± 0.15*; $n = 7$–8; *$P < 0.01$).

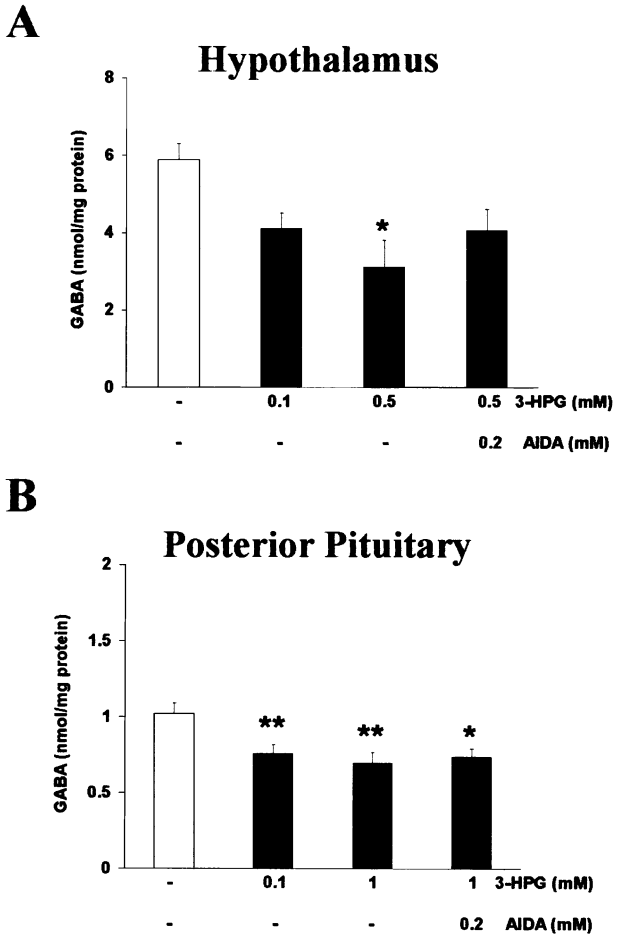

Fig. 1. Effect of 3-HPG (a group I mGluR agonist) in the absence or presence of the group I mGluR antagonist AIDA (0.2 mM) on K^+-induced GABA release from hypothalamus (A) and posterior pituitary (B). *$P < 0.05$; **$P < 0.01$ vs. control.

Table 2
Effect of L-CCG-I[a] and L-SOP[b] on K^+-induced GABA release from hypothalamus

	GABA (nmol/mg protein)	
	L-CCG-I	L-SOP
Control	8.36 ± 0.52 (5)	6.48 ± 0.32 (5)
0.01 mM	10.21 ± 0.89 (4)	6.45 ± 0.66 (6)
0.1 mM	9.33 ± 0.55 (5)	5.93 ± 0.45 (6)
1 mM	8.77 ± 0.52 (6)	7.00 ± 0.34 (6)

[a] A group II mGluR agonist.
[b] A group III mGluR agonist.

Both 3-HPG (1 mM) and L-CCG-I (0.1 mM) decreased α-MSH release from hypothalamic fragments. These inhibitory effects were not observed in the presence of their respective antagonists, AIDA (0.2 mM) and EGLU (0.2 mM; Fig. 2). L-SOP (0.01–0.1 mM) induced no changes in hypothalamic α-MSH release (data not shown).

Neither *trans*-ACPD (0.01–1 mM) nor L-SOP (0.01–1 mM) modified α-MSH release from the posterior pituitary (data not shown).

Our results indicate that mGluR activation affects GABA release from the hypothalamus and the posterior pituitary. mGluR inhibition of GABA release has been described for neurons in several brain areas [4,16]. Chen showed that agonists of the three groups of mGluRs decreased the release of GABA from single axon terminals of suprachiasmatic neurons of the hypothalamus [5]. Our results support the idea that the activation of group I mGluRs decreases inhibitory neurotransmission in the hypothalamus. However, the inhibitory effect of the group I mGluR agonist 3-HPG on GABA release could not be completely reversed by the group I antagonist AIDA. Since AIDA acts preferentially at mGluR1, the inability of AIDA to fully block the inhibitory effects of 3-HPG may possibly be due to a high mGluR5 expression in the hypothalamus, particularly in the astrocytes [12]. Both mGluR1 and mGluR5 localization have been reported in the hypothalamus, but to our knowledge, the only group I mGluR expressed by astrocytes is mGluR5 [3,20]. Astrocytes are in an ideal position to receive excitatory signals from glutamatergic axons. Since astrocytes appear to play a dynamic role in regulating GABA transmission [19], the possibility of glial involvement in the response to group I mGluR activation might be taken into account.

The reduced inhibitory input could contribute to the direct excitatory effects of glutamate through ionotropic receptors, leading to increased excitation of hypothalamic neurons, as has been proposed for other brain areas [9].

The presence of both iGluRs and mGluRs has been detected in the posterior pituitary [2]. We previously demonstrated that glutamate through iGluRs stimulates GABA release from the posterior pituitary [13]. Here, we show that the decrease in GABA release induced by 3-HPG from the posterior pituitary was not blocked by AIDA. The lack of effect of AIDA in this case could be due to a high expression of mGluR5 in the posterior pituitary, although this issue remains to be ascertained. On the contrary, activation of group III mGluRs increased GABA release from the posterior pituitary, suggesting that the stimulatory effect of glutamate on GABA release from the posterior pituitary could also be exerted through this group of mGluRs. Group III mGluRs may not only stimulate GABA release from neurons but also could affect glial cells such as pituicytes and melanotropes, since both types of cells synthesize GABA [10].

It has been demonstrated that hypothalamic α-MSH release is stimulated by glutamate through *N*-methyl-D-aspartate (NMDA) receptors [21]. Our results indicate that group I and II mGluR activation decrease hypothalamic α-MSH release. Since endogenous GABA release suppresses α-MSH release from the hypothalamus [6], we would have expected to find an increase in α-MSH release due to the decrease in GABA release induced by the activation of group I mGluRs. However, we found a decrease in both GABA and α-MSH release induced by 3-HPG from the hypothalamus. In this study, the observed decrease in hypothalamic α-MSH release suggests that 3-HPG directly acts on α-MSH neurons and that its effect is not mediated by

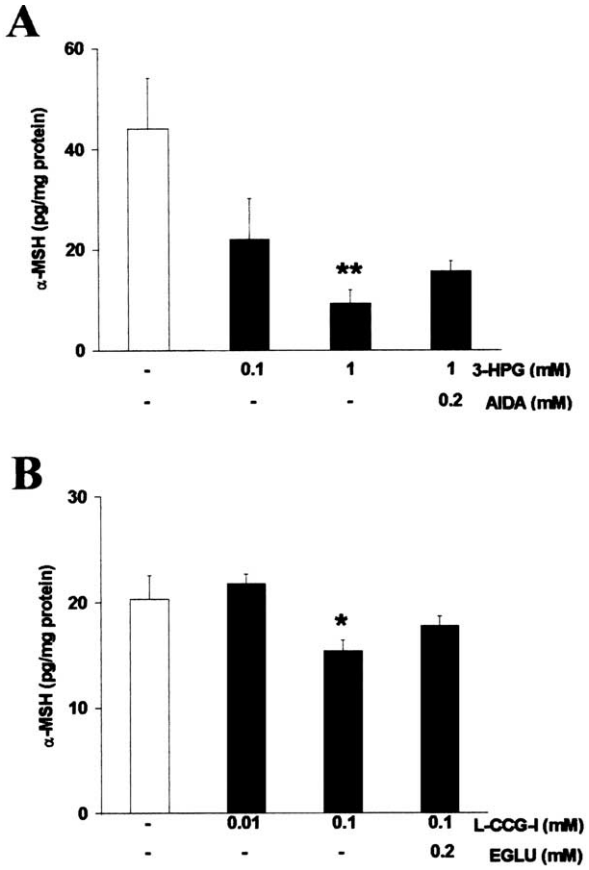

Fig. 2. Effect of: (A), 3-HPG (a group I mGluR agonist); and (B), L-CCG-I (a group II mGluR agonist) in the absence or presence of their respective antagonists (AIDA and EGLU) on hypothalamic α-MSH release. $*P < 0.05$; $**P < 0.01$ vs. control.

GABA. Moreover, L-CCG, a group II mGluR agonist, had no effect on hypothalamic GABA release but decreased α-MSH release, indicating that this agonist also exerts independent effects on GABA and α-MSH neurons. GABA and dopamine inhibit, whereas serotonin stimulates α-MSH release from melanotropes. Although a direct effect of glutamate on α-MSH release from melanotropes has not been described previously, glutamate has been shown to increase the intracellular calcium concentration ($[Ca^{2+}]_i$) in these cells via ionotropic receptors whereas *trans*-ACPD failed to induce a change [6]. In accordance with this, our results show that mGluR agonists do not affect α-MSH release from the posterior pituitary. In the posterior pituitary, ionotropic agonists such as NMDA did not affect α-MSH release (unpublished results).

Glutamate is involved in several neuroendocrine functions, including the regulation of lutenizing hormone and prolactin secretion [2]. The principal site of action of glutamate is the hypothalamus and could involve mediation of prolactin-releasing factors such as oxytocin [14], or of neurotransmitters, such as GABA [8]. Since GABA and α-MSH participate in the control of anterior pituitary hormone secretion, glutamate via both ionotropic and metabotropic receptors could modulate anterior pituitary function.

In conclusion, activation of group I mGluRs decreases GABA release from the hypothalamus and the posterior pituitary while group III mGluRs increase GABA release from the posterior pituitary. On the other hand, both group I and II agonists decrease hypothalamic α-MSH release but do not affect α-MSH release from the posterior pituitary. These modulatory effects may regulate the excitability of the hypothalamic neuron network and may be involved in the neuroendocrine functions of glutamate.

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