**Endocrine,** *vol. 17, no. 3, 193–197, April 2002* 0969–711X/02/17:193–197/$11.25 

# Endogenous Excitatory Amino Acid Neurotransmission Regulates Thyroid-Stimulating Hormone and Thyroid Hormone Secretion in Conscious Freely Moving Male Rats

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**The role of neurotransmission of endogenous excitatory amino acid (EAA) on serum thyroid hormones and thyroid-stimulating hormone (TSH) levels was examined in conscious and freely moving adult male Sprague-Dawley rats. The rats were cannulated at the third ventricle 2 d before the experiments. Several glutamate receptor agonists, such as kainic acid and domoic acid, and antagonists, such as 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and dizocilpine (MK-801) were administered into the third ventricle. Serum TSH levels were assesed by radioimmunoassay, and serum thyroid hormone levels were assessed by enzyme immunoassay. The results showed that the administration of CNQX and MK-801 produced a decrease in serum levels of TSH and thyroid hormones. The administration of kainic acid and domoic acid increased TSH concentrations, whereas CNQX completely blocked the release of TSH induced by kainic acid and domoic acid. These results suggest the importance of endogenous EAA in the regulation of hormone secretion from the pituitary-thyroid axis, as well as the role of the *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors in the stimulatory effect of EAAs on the pituitary-thyroid axis.**

**Key Words:** Excitatory amino acids; thyroid-stimulating hormone; glutamate antagonist.

## Introduction

L-Glutamate (L-Glu) is known to be the major excitatory neurotransmitter in the central nervous system. Evidence suggests that the glutamatergic system is involved not only in fast synaptic transmission, but also in plasticity and higher cognitive functions *(1)*. Recently, attention has been focused on the possible role of glutamate and other excitatory amino acids (EAAs) in the neuroendocrine regulation of the hypothalamic-pituitary axis *(2)*. Several studies have shown that exogenous EAAs such as L-Glu, L-aspartate, *N*-methyl-D-aspartate (NMDA), kainate, and aminohydroxy-5-methyl-4-isoxazole-propionate (AMPA) play an important role in the regulation of the secretion of anterior pituitary hormones such as luteinizing hormone (LH), prolactin, growth hormone (GH) and adrenocorticotropic hormone (ACTH) *(3–5)*. However, there are few available studies about the effects of EAAs on pituitary-thyroid function. Männistö et al. *(6)* studied the effects of several amino acids on the regulation of the stimulated secretion of thyroid-stimulating hormone (TSH), and concluded that glycine, glutamate, and serine inhibit the cold-induced TSH secretion in the male rat.

In male rats and monkeys, both NMDA and AMPA/kainic acid receptor agonists have been shown to be potent stimulators of LH *(5,7)*, suggesting that both types of receptors may have an important effect on the action of EAAs on different endocrine functions.

The studies performed in our laboratory in recent years have dealt with the possible role of EAAs in the regulation of TSH and thyroid hormone (thyroxine [$T_4$] and triiodothyronine [$T_3$]) secretion. These studies have shown that intraperitoneal administration of L-Glu, NMDA, and kainic acid produced an increase in serum levels of both thyroid hormones and TSH *(8)*. Although these studies suggested a possible involvement of EAAs in the regulation of TSH release, they did not conclusively prove this, since only the role of peripheral administration of exogenous EAAs was examined. The use of specific antagonists for glutamate receptors is necessary to assess the possible involvement of endogenous EAAs in neuroendocrine function.

The purpose of the present study was to determine the role of endogenous EAAs on serum TSH, $T_4$, and $T_3$ levels, after the intracerebroventricular administration of both dizocilpine (MK-801) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), antagonists of NMDA and AMPA/kainic acid glutamate receptors, respectively, as well as to examine whether the stimulatory effects induced by exogenous EAA administration may be blocked by antagonist treatment.

Received January 25, 2002; Revised March 15, 2002; Accepted March 15, 2002.

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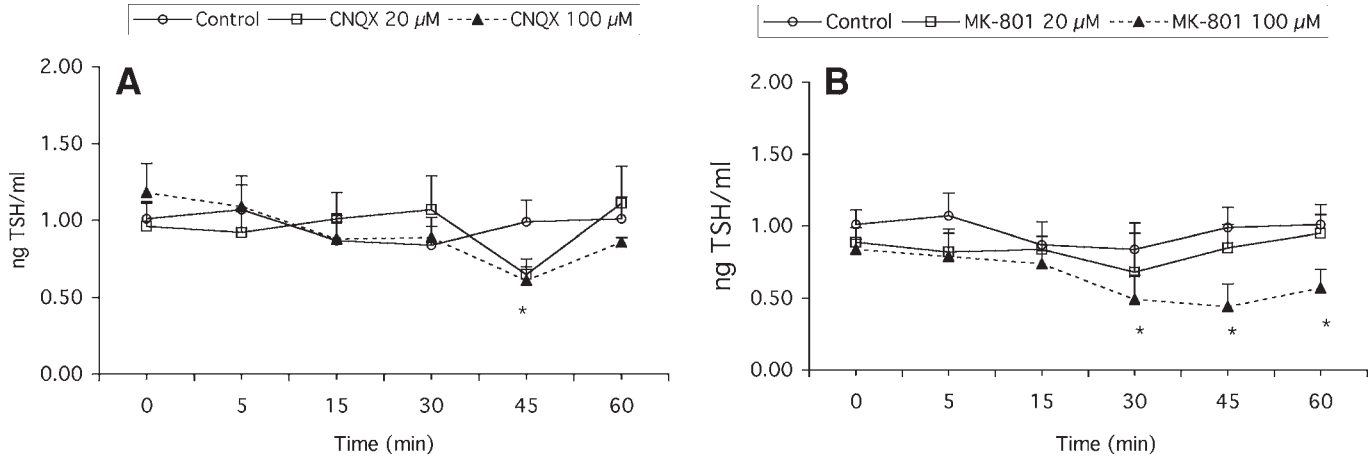


**Fig. 1.** Effects of intracerebroventricular administration of 20 and 100 μ*M* CNQX (**A**) and MK-801 (**B**) on serum TSH levels. Experiments were performed during 1 h in awake freely moving rats. Each point represents the mean ± SEM of data obtained from five to eight rats. **p* < 0.01 vs vehicle-injected rats (control).

## Results

Central administration of CNQX (20 and 100 μ*M*) significantly decreased serum TSH levels (Fig. 1A). The decrease occurred 45 min after CNQX administration (38% compared with control). Serum TSH levels returned to control values 60 min after treatment with both doses of CNQX used. The intraventricular administration of 100 μ*M* MK-801 produced a significant decrease (49% with respect to control group) in serum TSH levels at 30 min (Fig. 1B). Serum TSH levels remained low 45 and 60 min after treatment (55 and 49%, respectively, compared with control). No significant changes in serum TSH were observed with a concentrantion of 20 μ*M* MK-801 (Fig. 1B). The area under the curve (AUC) values (Fig. 2) showed that the inhibitory effect of MK-801 (100 μ*M*) was more relevant than the effect induced by CNQX ($p < 0.01$ with respect to the control and CNQX groups).

A significant decrease (60% with respect to control) in serum $T_3$ levels after 45 min was observed with the dose of 100 μ*M* CNQX (Fig. 3B). These levels remained low after 60 min of treatment. The intraventricular administration of 100 μ*M* MK-801 also significantly decreased serum $T_3$ levels (Fig. 3B). This decrease was 37 and 29% with respect to controls, 45 and 60 min after treatment, respectively. Serum $T_4$ levels decreased (approx 30% with respect to control) 45 min after CNQX administration (20 or 100 μ*M*), although $T_4$ levels returned to control levels with both doses at 60 min (Fig. 3A). However, intraventricular administration of MK-801 (20 or 100 μ*M*) did not change significantly serum $T_4$ levels during the assay (Fig. 3A).

The role of AMPA/kainic acid glutamatergic receptors in TSH and thyroid hormones secretion was also evaluated under the coadministration of AMPA/kainic acid glutamatergic agonists and antagonists. The central administration of kainic acid (10 μ*M*) and domoic acid (1 μ*M*) produced an increase in serum TSH levels at 15 min (76 and 64% compared with control group, respectively), and the values remained elevated after 60 min of treatment (Fig. 4). The AUC values (Fig. 2) showed that similar increases occurred after treatment with both doses of domoic acid and kainic acid used. Both kainic acid– and domoic acid–induced increases in serum TSH levels were significantly suppressed (Fig. 4) by intraventricular administration of CNQX (100 μ*M*). However, the increase in serum TSH levels induced by kainic acid or domoic acid were unaffected by intraventricular injection of MK-801 (Fig. 2).

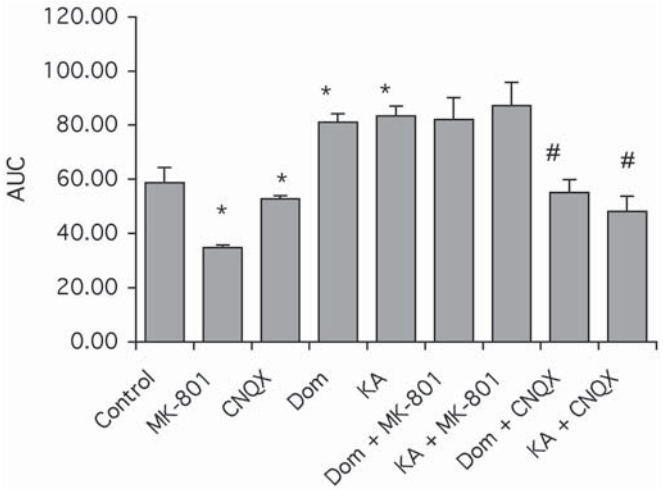


**Fig. 2.** Effects of intracerebroventricular administration of domoic acid or kainic acid with CNQX and/or MK-801 on serum TSH levels expressed as AUC, calculated by the trapezoidal method. Experiments were performed during 1 h in awake freely moving rats. Each point represents the mean ± SEM of data obtained from five to eight rats. **p* < 0.01 vs vehicle-injected rats (control). # *p* < 0.01 vs KA and Dom group.

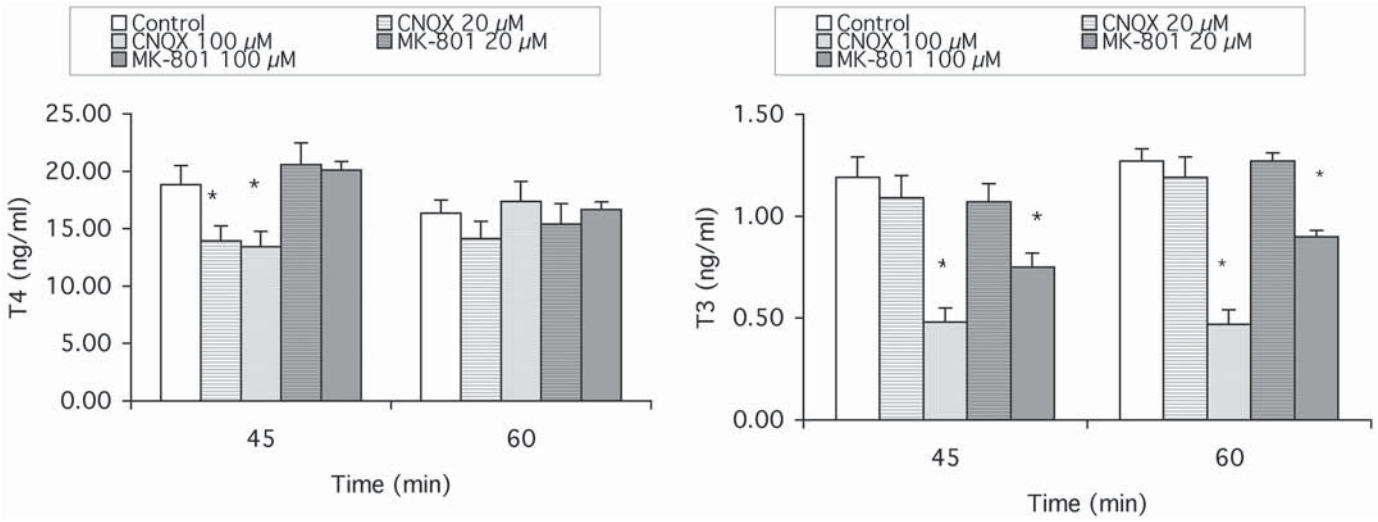


**Fig. 3.** Effects of intracerebroventricular administration of CNQX and MK-801 on serum $T_4$ (**A**) and $T_3$ (**B**) levels. Experiments were performed during 1 h in awake freely moving rats. Each point represents the mean ± SEM of data obtained from five to eight rats. *$p < 0.01$ vs vehicle-injected rats (control).

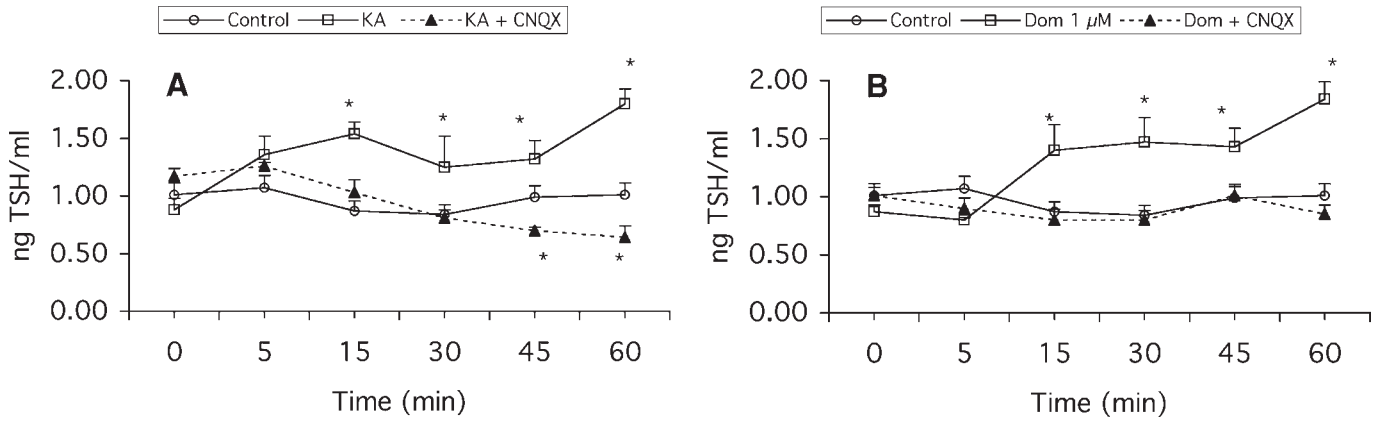


**Fig. 4.** Effects of intracerebroventricular administration of CNQX and MK-801 on increase in serum TSH levels induced by kainic acid (10 μ*M*) and domoic acid (1 μ*M*). Experiments were performed during 1 h in awake freely moving rats. Each point represents the mean ± SEM of data obtained from five to eight rats. *$p < 0.01$ vs vehicle-injected rats (control).

## Discussion

Earlier reports claiming a role for EAA neurotransmission on pituitary hormone secretion (LH, GH, ACTH) were based on pharmacologic studies in which EAA agonists were used to elicit hormone release *(2,9–11)*. Recently, we have reported that ip administration of glutamate receptor agonists increased serum $T_3$, $T_4$, and TSH levels *(8)*. However, the use of specific antagonists for NMDA and AMPA/kainic acid receptors provide tools for a more physiologic evaluation of the relative contribution of EAA neurotransmission in the regulation of neuroendocrine events. The present study confirms our previous results and indicates, for the first time, that endogenous EAAs enhance TSH and thyroid hormone release in conscious and freely moving rats by interacting with NMDA and AMPA/kainic acid receptors.

The global effect of CNQX on serum TSH levels was less relevant than that produced by MK-801. The intraventricular administration of kainic acid and domoic acid clearly confirms the stimulatory effects of these exogenous glutamatergic agonists on TSH secretion, which was reduced by the coadministration of the AMPA/kainic acid receptor antagonist but not by NMDA antagonist. These findings suggest an involvement of AMPA/kainic acid receptors in the control of TSH secretion, although this action seems to be relatively less important than the action mediated by NMDA receptors. Several investigators have observed similar results in other endocrine systems after administration of glutamatergic antagonists, indicating the stimulatory effect of endogenous EAAs on pituitary and peripheral hormone release *(5,12,13)*. Those studies showed that the treatment with agonists produced contrary effects to those observed

after treatment with antagonists and that, in most cases, the latter reverted the effects elicited by the former.

The most likely regulatory site for EAAs appears to be the hypothalamus via modulation of the release of hypothalamic-releasing hormones *(7,14)*, although effects on peripheral sites cannot be discarded. There is no direct evidence on the effects of EAAs on thyrotropin-releasing hormone (TRH). However, the present data obtained by central administration of glutamatergic agonists and antagonists and the presence of EAA receptors in those hypothalamic areas related to TRH/TSH *(15)* suggest that the action of EAAs on TSH secretion may occur at the brain level, interfering with TRH release. A recent study from our laboratory (unpublished data) has shown that glutamate administration partially enhances the TRH-induced TSH release in rats treated with disulfiran (inhibitor of TRH synthesis). This finding may indicate that the stimulatory action of EAAs on TSH and thyroid hormone secretion could only be partially mediated by TRH, and a direct effect at the pituitary level cannot be discarded (experiments in progress). Many different studies have shown that the secretion of TSH and other pituitary hormones is modulated by neurotransmitters, and that EAAs change the synthesis and release of central neurotransmitters *(5,16,17)*. Therefore, several central neurotransmitter systems may be involved in mediating EAA-induced TSH release. In this way, we have previously reported that the effect of domoic acid on TSH and thyroid hormone levels was partially mediated by the hypothalamic serotoninergic system *(18)*.

In conclusion, our study demonstrates, for the first time, that EAA neurotransmission may play an important role in regulating TSH and thyroid hormone secretion. This stimulatory effect is mediated by both types of ionotrophic glutamate receptors (NMDA and AMPA/kainic acid), although the more important effects were those produced by NMDA receptors. Further studies are in progress to examine the role of the catecholaminergic system in the effects of EAAs on TSH release and to determine the effect of glutamatergic agonists on TSH release from cultured rat pituitary cells.

## Materials and Methods

### *Animals*

Adult male Sprague-Dawley rats, weighing 200–250 g, were used. The animals were housed in a light- and temperature-controlled room, with a lighting schedule of 12 h of light, 12 h of darkness (lights on at 8:00 AM) and a temperature of 20–22ºC. They were fed with Purina pelleted chow and received tap water ad libitum.

### *Administration of Glutamate Receptor Agonists and Antagonists*

Two days prior to the experiments, third-ventricular cannulae (PE-50; Becton Dickinson) were implanted (2 mm posterior to Bregma and 9 mm down from the duramater in the midline) following the Pellegrino stereotaxic atlas *(19)*. Twenty-four hours before bleeding, a PE-50 cannula (covered at the front with a sylastic tube with an id of 0.025 in.) was implanted in the jugular vein of the rats. The NMDA antagonist MK-801 (20 and 100 μ*M*), the AMPA/kainic acid antagonist CNQX (20 and 100 μ*M*), and the glutamatergic agonists kainic acid (10 μ*M*) and domoic acid (1 μ*M*) were dissolved in Ringer solution (145 m*M* NaCl; 5 m*M* KCl; 11 mM $NaHCO_3$; 1.2 m*M* $NaH_2PO_4$; 1.2 m*M* $CaCl_2$; 10 m*M* glucose; pH 7.6) and intraventricularly administered using an injection pump at a flow rate of 3.4 μL/min over a 5-min period, in order to avoid any volume-induced effect. Animals perfused with Ringer solution were used as controls. Animals did not show appreciable behavioral alterations with all doses assayed.

### *Assay Procedures*

Blood samples were sequentially collected through the jugular cannula in conscious and freely moving animals 0, 5, 15, 30, 45, and 60 min after treatment. The serum was obtained after centrifugation of blood at 2000*g* for 15 min and was kept at –20ºC until assay. Serum thyroid hormone concentrations were determined by enzyme immunoassay using commercial kits (Biomérieux) validated in our laboratory *(20)*. The assay is based on solid-phase and competitive principle. Separation of $T_4$ and $T_3$ from carrier proteins was performed with 8-aniline-1-naphtalenesulfonic acid. Hormones were conjugated with horseradish peroxidase, with *o*-phenylenediamine as the cromogen and $H_2O_2$ as the substrate. The intraassay coefficients of variation were 3–9 and 7–10% for $T_4$ and $T_3$, respectively. The interassay CVs were 8–12 and 1.8–5% for $T_4$ and $T_3$, respectively. TSH concentrations were determined in serum using a double-antibody radioimmunoassay (RIA) with r-TSH-I-9, r-TSH-RP-3, and anti-rTSH-RIA-6 purchased by the National Institute of Diabetes and Digestive and Kidney Diseases's (NIDDKs) National Hormone & Pituitary Program (National Institutes of Health [NIH], Bethesda, MD). The rat TSH antigen was labelled with $^{125}I$ by the chloramine-T method. The second antibody (rabbit anti-γ-globulin) was provided by Sigma. The intra- and interassay CVs of the RIA assay were 3.7 and 4.9%, respectively.

### *Statistical Analyses*

Data are shown as the mean ± SEM ($n$ = 5–8 animals) and were analyzed by Kruskal-Wallis and Mann-Whitney tests. *$p < 0.01$ was considered statistically significant. To determine the global effect of the administration of the different treatments, the AUC was calculated using the trapezoidal method, according to the following formula:

$$\mathrm{AUC} = \Delta t \sum_{n=1}^{5} (X_{n-1} + X_n/2)$$

in which $X_o$ denotes basal TSH levels at 0 min; and $X_1$, $X_2$ . . . $X_5$ are TSH values at 5, 15 . . ., and 60 min, respectively.

## Acknowledgments

We wish to express our gratitude to the NIDDK (NIH) for the provision of the TSH RIA kits. This work was supported by research grants from Xunta de Galicia and the University of Vigo (Spain).

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