Stimulation of the hippocampal glutamate receptor systems induces stress-like responses

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Abstract

OBJECTIVE: The hypothalamic-pituitary-adrenal (HPA) axis controls glucocorticoid secretion and is in turn controlled by a diverse set of afferents in the brain. However, the precise mechanisms underlying these actions remain to be elucidated. In our previous study, a lesion in the entorhinal cortex, which is the major provider of glutamatergic innervation to the hippocampus, significantly attenuated the elevation of adrenocorticotropic hormone (ACTH) in plasma during immobilization stress. In the present study, we examined the effects of microinjections of glutamatergic agonists into the hippocampus on plasma ACTH and glucose concentrations. We also studied the interactions between glutamate and acetylcholine in this response in the hippocampus.

MATERIALS AND METHODS: NMDA and AMPA subtypes of glutamate agonists were microinjected into the rat hippocampus, and ACTH and glucose levels in plasma were measured. The interaction between cholinergic and glutamatergic systems was investigated pharmacologically.

RESULTS: Both the NMDA and AMPA subtypes of glutamate agonists induced elevations of plasma ACTH and glucose levels in a dose-dependent fashion. These responses were independent of those induced by activation of the hippocampal cholinergic system.

CONCLUSION: Stimulation by the NMDA and AMPA subtypes of glutamate receptors in the hippocampus induced elevations of plasma ACTH and glucose, and these responses were independent of the cholinergic system.

ORIGINAL ARTICLE

Introduction

The hypothalamic-pituitary-adrenal (HPA) axis controls glucocorticoid secretion and is in turn controlled by a diverse set of afferents in the brain. However, precisely how these actions occur remains to be elucidated. We have investigated the involvement of the hippocampus in stress-like responses [14, 15, 16]. Cholinergic stimulation of the hippocampus elicits stress-like responses with HPA axis activation, represented by the release of adrenocorticotropic hormone (ACTH) and the activation of the sympathetic nervous system. The involvement of other neurotransmitter systems, however, has not been investigated.

The entorhinal cortex is the region that provides major glutamatergic innervation to the hippocampus [2]. In our previous study, a lesion in the ento-

rhinal cortex, produced by ibotenic acid, significantly attenuated the elevation of ACTH in plasma during immobilization stress. This suggests that inputs from and/or into the entorhinal cortex, presumably via glutamatergic neurotransmissions, are involved in HPA axis responses during immobilization [1]. The stress-induced elevation of glutamate in the hippocampus has also been reported [6, 9].

In the current study, we examined the effects of microinjections of glutamatergic agonists into the hippocampus on plasma ACTH and glucose concentrations, as well as the interactions between glutamate and acetylcholine in these responses in the hippocampus.

Materials And Methods

Subjects:

We used 9-week-old male Wistar rats (200–300 g) for the experiment. The animals were individually housed under standard laboratory conditions in temperature-controlled rooms (25 °C), and were maintained under a 12 h light/dark cycle (lights on at 08:00) with food pellets and water available *ad libitum*. The rats were cared for in accordance with the ethical guidelines approved by the animal care and use committee of Nagoya University.

Surgery:

The rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and mounted in a stereotaxic frame (Narishige Scientific Instruments Laboratory, Tokyo, Japan). For the insertion of a guide cannula (BAS, Tokyo, Japan), the skull was exposed and a burr hole was drilled overlying the injection coordinates, which were anterior-posterior –2.00 mm, medial-lateral ±1.50 mm, and dorsal-ventral 3.50 mm from the skull surface in accordance with the atlas of Paxinos and Watson [10]. The cannula was then fixed to the skull by dental cement with two anchor screws.

Procedures:

Saline containing pharmacological agents was microinjected in a volume of 1 µl for 1 min using a CMA/100 microinjection pump (BSA, Tokyo, Japan) through the guide cannula into the left dorsal hippocampus of free-moving rats. The pharmacological agents (all purchased from Sigma Chemical, St. Louis, MO) included α-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid hydrate AMPA (8×10^{-8} mol), 6-Cyano-7-nitroquinoxaline-2,3dione disodium salt (CNQX) (8×10^{-8} mol), atropine sulfate salt hydrate (8×10^{-8} mol), neostigmine methylsulfate (5×10^{-8} mol), Dizocilpine hydrogen maleate (MK-801) (8×10^{-8} mol), and N-Methyl-D-aspartic acid (NMDA) (5×10^{-8} mol). To determine the plasma concentrations of ACTH and glucose, blood was intermittently sampled (1.0 ml), starting at time 0, just before injection, and at 15, 30, 60, and 120 min after. To minimize the effect of volume loss, an equal volume of heparinized saline was returned to general circulation at each sampling. The blood samples were kept on ice and centrifuged, after which the plasma was removed and stored at –20 °C in 400 µl aliquots for subsequent determination of ACTH by radioimmunoassay [11]. Plasma glucose concentrations were determined by the immobilized enzyme membrane/H_2O_2 method with a compact glucose Antsense II analyzer (Bayer Medical Co. Ltd, Tokyo, Japan)[14].

Statistical analysis:

Data were analyzed by repeated-measures one-factor ANOVA. A level of $P < 0.05$ was accepted as statistically significant.

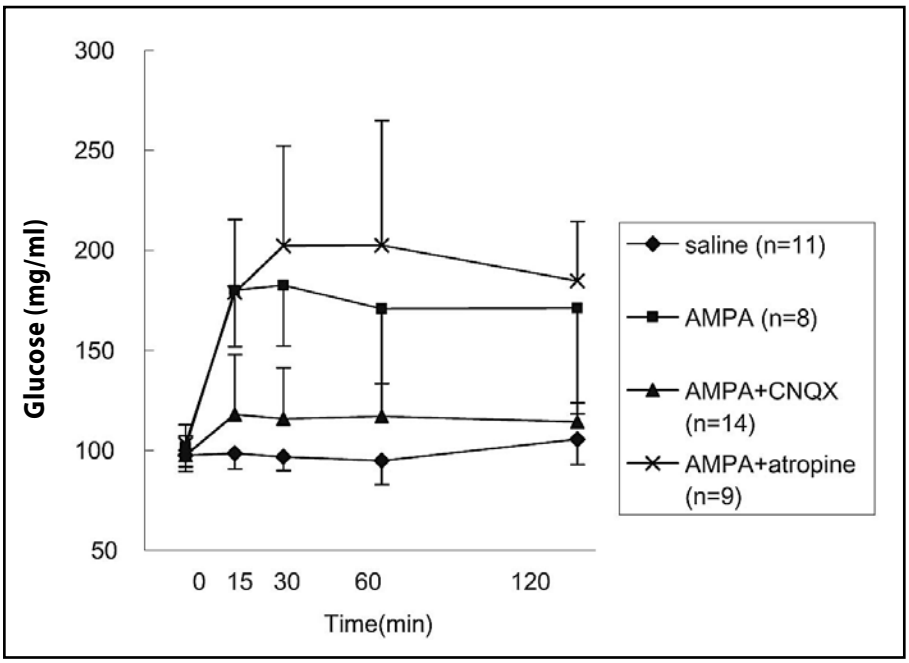

Figure 1A. Effect of hippocampal injection of AMPA on glucose. AMPA 8×10–8 mol/µl (■), AMPA 8×10–8 mol plus CNQX 8×10–8 mol/µl (▲), AMPA 8×10–8 mol plus atropine 8×10–8 mol/µl (×), and saline (1 µl) (◆). Repeated-measures one-factor ANOVA indicated a significant effect with interactions on plasma glucose concentration ($p < 0.0001$). Scheffe's post hoc analysis showed that both the AMPA and AMPA + atropine groups had significantly higher glucose levels than the saline group ($p < 0.0001$ for both groups) and that co-administration of CNQX with AMPA significantly reduced AMPA-induced glucose elevation ($p < 0.0001$).

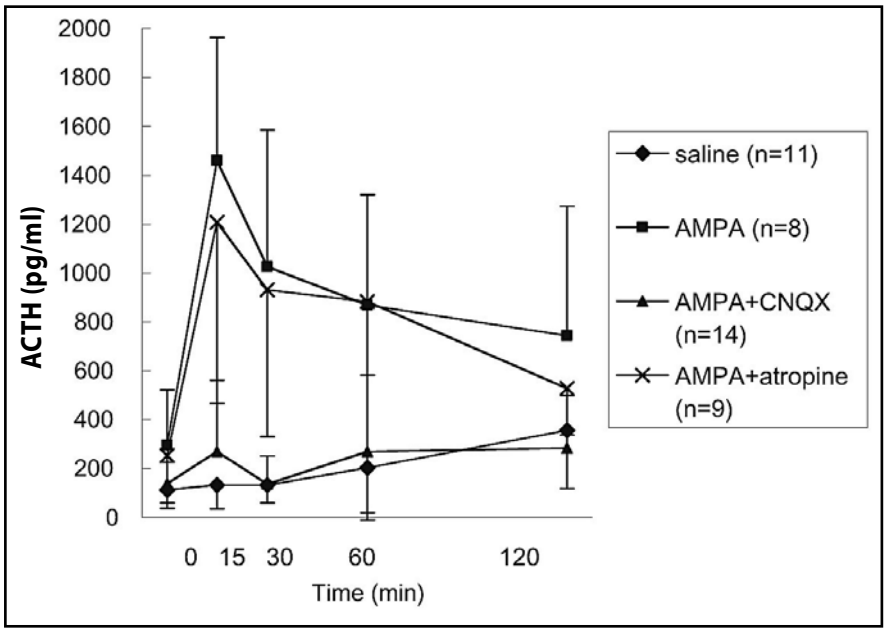

Figure 1B. Effect of hippocampal injection of AMPA on plasma ACTH.
AMPA 8×10–8 mol/µl(■) or AMPA 8×10–8 mol plus CNQX 8×10–8 mol/µl (▲), AMPA8×10–8 mol plus atropine 8×10–8 mol/µl (×), and saline 1 µl (◆).
Repeated-measures one-factor ANOVA indicated a significant effect of interaction on plasma ACTH concentrations (p <0.0001). Scheffe's post hoc analysis showed that both the AMPA and AMPA + atropine groups had significantly higher ACTH concentrations than the saline group (p <0.0001 for both groups) and that co-administration of CNQX with AMPA significantly reduced the AMPA-induced ACTH elevation (p <0.0001).

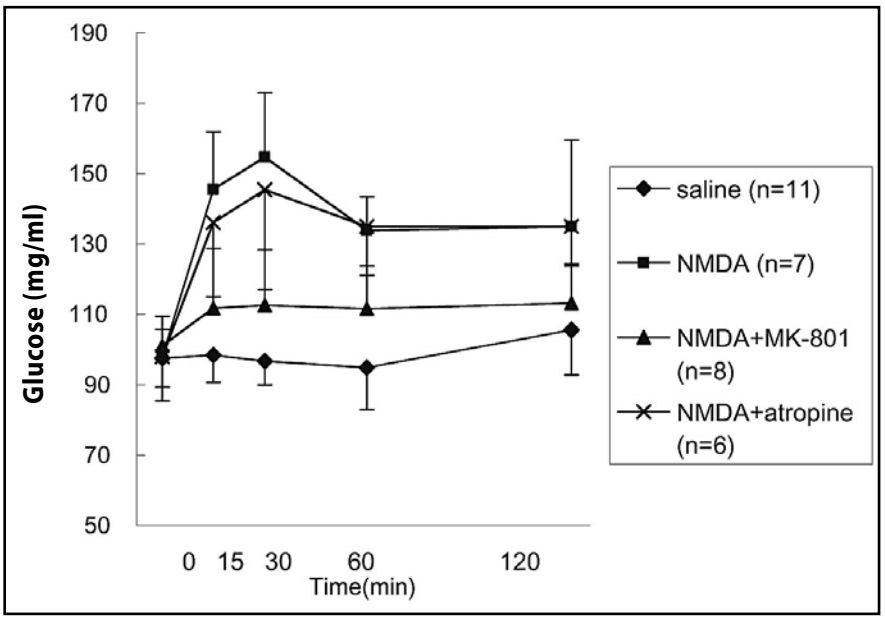

Figure 2A. Effect of hippocampal injection of NMDA on glucose.
NMDA 5×10–8 mol/µl (■), NMDA 5×10–8 mol plus MK-801 5×10–8 mol/µl (▲), NMDA 5×10–8 mol plus atropine 5×10–8 mol/µl (×), and saline 1 µl (◆).
Repeated-measures one-factor ANOVA indicated a significant effect of interaction on plasma glucose concentrations (p <0.0001). Scheffe's post hoc analysis showed that both NMDA and NMDA + atropine groups had significantly higher plasma glucose levels than the saline group (p <0.0001 and 0.0001, respectively) and that co-administration of MK 801 with NMDA significantly reduced the AMPA-induced glucose elevation (p = 0.0029).

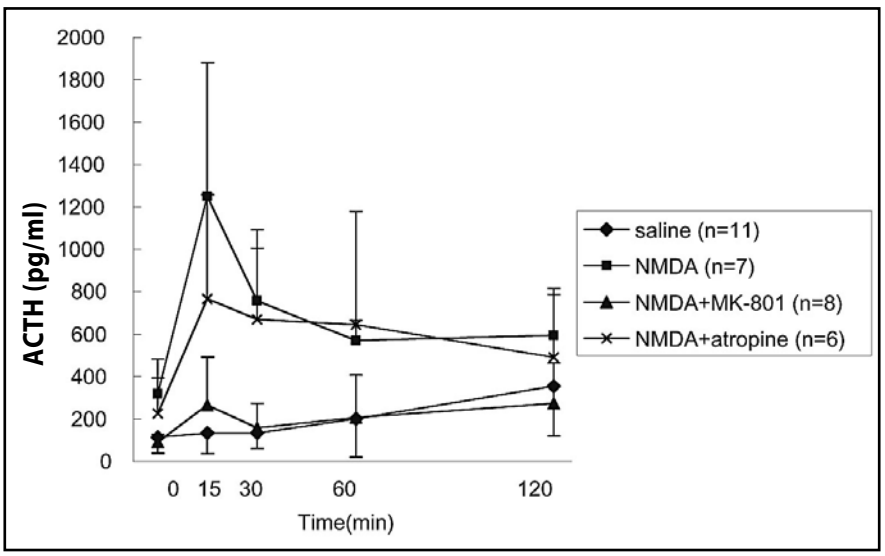

Figure 2B. Effect of hippocampal injection of NMDA on plasma ACTH.
5×10–8 mol/µl (■), NMDA 5×10–8 mol plus MK-801 5×10–8 mol/µl (▲), NMDA 5×10–8 mol plus atropine 5×10–8 mol/µl (×), and saline 1 µl (◆) .
Repeated-measures one-factor ANOVA indicated a significant effect of interaction on plasma ACTH concentrations (p <0.0001). Scheffe's post hoc analysis showed that both NMDA and NMDA + atropine groups had significantly higher plasma ACTH levels than the saline group (p = 0.0004 and 0.0015, respectively) and that co-administration of MK 801 with NMDA significantly reduced the AMPA-induced glucose elevation (p = 0.0013).

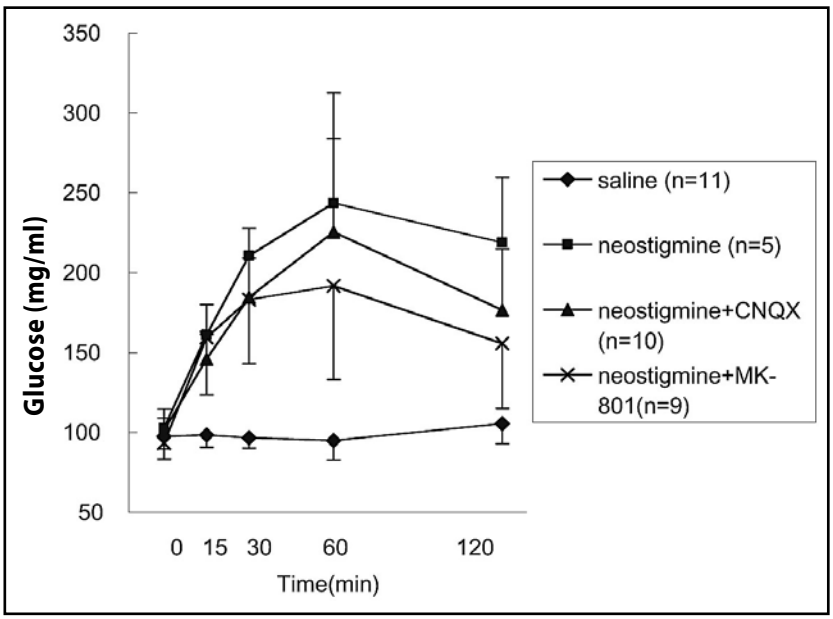

Figure 3A. Effect of hippocampal injection of glutamate antagonists on neostigmine-induced glucose elevation. Neostigmine 8×10–8 mol/µl (■), neostigmine 8×10–8 mol plus CNQX 8×10–8 mol/µl (▲), neostigmine 8×10–8 mol plus MK-801 8×10–8 mol/µl (×), and saline 1 µl (◆). Repeated-measures one-factor ANOVA indicated a significant effect of interaction on plasma glucose concentrations (p <0.0001). Scheffe's post hoc analysis showed that the neostigmine, neostigmine + CNQX, and neostigmine + MK 801 groups all had significantly higher plasma glucose levels than the saline group (p <0.0001 for all groups).

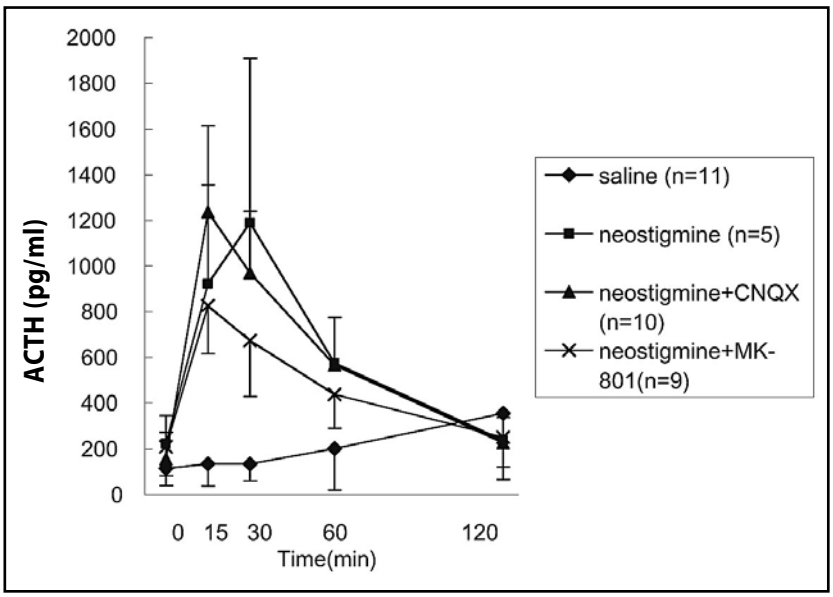

Figure 3B. Effect of hippocampal injection of glutamate antagonists on neostigmine-induced ACTH elevation. Neostigmine 8×10–8 mol/lµl (■), neostigmine 8×10–8 mol plus CNQX 8×10–8 mol/µl (▲), neostigmine 8 ×10–8 mol plus MK-801 8×10–8 mol/µl (×), and saline 1 µl (◆) Repeated-measures one-factor ANOVA indicated a significant effect of interaction on plasma ACTH concentrations (p <0.0001). Scheffe's post hoc analysis showed that the neostigmine, neostigmine + CNQX, and neostigmine + MK 801 groups all had significantly higher plasma ACTH levels than the saline group (p <0.0001, <0.0001, and 0.0002, respectively).

Results

Figures 1 and 2 show the responses of glucose (A) and ACTH (B) in plasma induced by the hippocampal injection of AMPA and NMDA, respectively. Both AMPA and NMDA induced significant elevations in glucose and ACTH, which were suppressed by their respective pharmacological antagonists, CNQX and MK-801. Atropine, the nicotinic cholinergic antagonist, had no effects on glutamate agonist-induced responses.

The injection of neostigmine into the hippocampus induced elevations in plasma glucose (Fig. 3A) and ACTH (Fig. 3B), as we reported previously [14,15,16]. The pharmacological antagonists of both glutamate receptor subtypes did not affect neostigmine-induced responses.

Discussion

The present study demonstrated that both the NMDA and AMPA subtypes of glutamate agonists induced elevations of plasma ACTH and glucose levels in a dose-dependent fashion. These responses were independent of those induced by the activation of the hippocampal cholinergic system, which we have repeatedly reported [14,15,16].

The effects of stress on brain structures mainly mediated by glucocorticoid hormone, especially on the hippocampus have accumulated much interest [7]. Our series of studies has raised the possibility that the hippocampus is involved in stress responses. The hippocampus, which expresses glucocorticoid receptors [3, 8], hypothetically has a suppressive role in stress responses

by a negative feedback-mechanism of glucocorticoid metabolism [13]. The present results showed that activation of the glutamate receptor system, which is the main neurotransmitter in the hippocampus and the input from the entorhinal cortex or other regions [4], induces the activation of stress-like responses. Therefore, the glutamatergic neurons in the hippocampus may be involved in the early activation phase of stress responses. The functional and/or structural changes of the hippocampus may induce changes in the stress responses. Further investigations into the profiles of stress responses, under the conditions that accompany hippocampal alterations, are necessary.

Glutamate receptors include two major subtypes – ionotropic and metabotropic – with the former further divided into NMDA, AMPA, and kinate subtypes according to their pharmacological characteristics [12]. In the present study, pharmacological stimulation of two ionotropic subtypes of glutamate receptors in the hippocampus produced similar reactions. The involvement of other subtypes of glutamate receptors will be investigated in a future study.

The activation of cholinergic systems also induces stress-like responses. The present results suggest that the cholinergic and glutamatergic systems in the hippocampus are mutually independent in terms of the elevations of plasma ACTH and glucose. The involved circuits in the brain may depend on the kinds of stressors involved [5, 14]. The role of each neurotransmitter system in stress responses to a variety of stimuli should be studied.

In conclusion, stimulation of glutamate receptors in the hippocampus by NMDA and AMPA subtypes induced elevations of plasma ACTH and glucose.

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