Glucocorticoid acts on a putative G protein-coupled receptor to rapidly regulate the activity of NMDA receptors in hippocampal neurons

Yanmin Zhang,1,2,∗ Hui Sheng,1,2,∗ Jinshun Qi,3 Bei Ma,1,2 Jihu Sun,1,2 Shaofeng Li,3 and Xin Ni1,2

1Department of Physiology and 2Key Laboratory of Molecular Neurobiology, Ministry of Education, Second Military Medical University, Shanghai; and 3Department of Physiology, Shanxi Medical University, Taiyuan, China

Submitted 20 June 2011; accepted in final form 2 December 2011

Zhang Y, Sheng H, Qi J, Ma B, Sun J, Li S, Ni X. Glucocorticoid acts on a putative G protein-coupled receptor to rapidly regulate the activity of NMDA receptors in hippocampal neurons. Am J Physiol Endocrinol Metab 302: E747–E758, 2012. First published December 6, 2011; doi:10.1152/ajpendo.00302.2011.—Glucocorticoids (GCs), a class of steroid hormones produced in the adrenal gland upon stimulation by the pituitary hormone adrenocorticotropin, are essential for maintenance of homeostasis and enable the organism to prepare for, respond to, and cope with various stresses (25). Classical GC actions, like those of other steroid hormones, are mediated by binding to intracellular receptors. Upon GC binding, the receptors translocate to the nucleus of the cell and regulate gene transcription by either binding to regulatory elements in gene promoters or interacting with other transcription factors (53). In addition to these genomic effects, it has been recognized that GCs can in parallel elicit rapid changes in cellular function incompatible with a slow genomic action, and the nongenomic pathways represent an important facet of GC actions (4).

There is a growing body of literature outlining nongenomic effects of GC in the central nervous system. GCs are known to exert nongenomic feedback effects on the hypothalamo-pituitary-adrenocortical axis (9, 10). The rapid excitatory and inhibitory effects of GC on neuronal electrophysiological activity have been found in the hypothalamus (31, 38), hippocampus (22, 46), and brainstem (6, 38) in rodents. GCs have also been shown to rapidly elicit behavioral changes such as modulation of novelty-related locomotor activity (39), aggression (24, 32), and the acoustic startle response (40). The mechanisms responsible for nongenomic effects of GC in brain remain unknown. The evidence unearthed so far suggests that nongenomic effects of GC are either mediated by a receptor or not. The receptor that is responsible for nongenomic effects of GC could be either the cystolic glucocorticoid receptors (GRs) or membrane-bound receptors (9). The signaling pathways mediating nongenomic GC actions have been demonstrated by a number of studies. Some of the systems implicated in these actions include PKA (44), PKC (7, 16), mitogen-activated protein kinase (MAPK) (18, 29), cAMP (2, 23), and intracellular calcium (2, 43).

The N-methyl-D-aspartate (NMDA) receptors are glutamate-gated ion channels localized to excitatory synapses throughout the brain. NMDA channels are heteromeric assemblies of NMDA receptor 1 and 2 subunits, which are targets of kinases and phosphoprotein phosphatases and downstream signaling proteins (5, 52). There may be conflict regarding GC modulation of NMDA receptors via nongenomic pathway. Our previous study showed that corticosterone rapidly suppresses the NMDA-evoked currents in cultured hippocampal neurons of newborn rats (27). In contrast, Xiao et al. (49) reported recently that corticosterone rapidly enhances NMDA-evoked neurotoxicity in cultured embryonic hippocampal neurons. To further elucidate the nongenomic effect of GC on the activity of NMDA receptors and the possible relevant significance of the GC nongenomic actions, we examined GC modulation of NMDA-evoked current in cultured embryonic hippocampal neurons at first and then defined the signaling pathways involved. Finally, we have investigated the effects of corticosterone on long-term potentiation (LTP) in the CA1 region of the hippocampus in vivo.

MATERIALS AND METHODS

Preparation of hippocampal neuron culture. Primary hippocampal neurons were cultured according to the protocols from the laboratories of Nelson and Gruol (33), with some modifications. All animal procedures were approved by the Ethics Committee of Experimental Animals of the Second Military Medical University, China. Procedures were designed to minimize the number of animals used and their suffering. Pregnant rats were obtained from Sino-British SIPPR/BK Laboratory Animal, Shanghai, China. The hippocampi were dissected from embryonic day 18 (E18) Sprague-Dawley rat fetuses (all of the fetuses were from the same pregnant rat in each culture) in ice-cold

∗Y. Zhang and H. Sheng contributed equally to this work.
Address for reprint requests and other correspondence: X. Ni, Dept. of Physiology, Second Military Medical University, 800 Xiangyin Rd., Shanghai, 200433, China (e-mail: nixin@smmu.edu.cn).
dissection solution containing sucrose-glucose-HEPES (in mM: 136 NaCl, 5.4 KCl, 0.2 Na₂HPO₄, 2 KH₂PO₄, 16.7 glucose, 20.8 sucrose, 0.0012% phenol red, and 10 HEPES, pH 7.4). All of the hippocampi dissected from all embryos were pooled together and then incubated with 0.125% trypsin (Invitrogen, Carlsbad, CA) at 37°C for 15 min. Single-cell suspension was obtained by mechanical dissociation using a Pasteur pipette with a fire-narrowed tip in DMEM (Invitrogen) supplemented with 10% heat-inactivated FBS (Invitrogen) and 10% horse serum (Invitrogen). Cells were then plated at a density of 1 × 10⁴ cells/cm² on poly-l-lysine-coated culture plates (Sigma-Aldrich, St. Louis, MO) or glass coverslips for different experiments. Cultures were maintained in 5% CO₂ at 37°C in DMEM containing 10% heat-inactivated FBS and 10% horse serum overnight, and then the culture media were changed to serum-free B27/neurobasal medium (Invitrogen). One-half of the medium was replaced with fresh medium every 3 days. More than 95% of the cells obtained were neurons that were assessed by immunostaining with neuron-specific markers, microtubule-associated protein-2 (Neomarkers, Fremont, CA), and neuron-specific enolase (Santa Cruz Biotechnology, Santa Cruz, CA). Neurons were cultured 7–10 days before use in experiments.

Whole cell recording. The experiments of whole cell patch clamp were performed at 22–25°C. A coverslip with cultured neurons was placed in a recording chamber and constantly superfused with the extracellular solution. Whole cell membrane currents were recorded conventionally under voltage clamp conditions at a holding potential of ∼60 mV, using an Axopatch 200B amplifier, Digidata 1322A data acquisition board, and pClamp 9.2 software (Axon Instruments, Foster City, CA). Patch electrodes were pulled from borosilicate glass (Sutter, Novato, CA) with a puller (PC-10, Narishige, Japan), having a resistance of ∼3–5 MΩ when filled with the pipette solution. The series resistance in the recordings was between 6 and 10 MΩ. Recordings in which series resistance varied by 10% were rejected. No electronic compensation for series resistance was used. Current responses were low-pass filtered at 1 kHz and sampled at 5 kHz. The pipette solution was composed of (in mM) 120 K-D-Gluconate, 10 KCl, 5 NaCl, 11 EGTA, 1 CaCl₂, 2 MgCl₂, 10 HEPES, 2 Mg-ATP, and 1 Li-GTP and titrated to pH 7.2 with KOH.

NMDA currents were elicited by the application of 3–300 μM of NMRA plus 10 μM glycine. In all experiments, we monitored rundown and administered drugs after the NMDA had fully stabilized and rundown had subsided. We monitored the holding current of the cell continuously; this varied by <30 pA, indicating that the pharmacological agents used produced little change in resting potential.

Drug application. All drugs used were purchased from Sigma-Aldrich except for H89, G66976, and U73122 (Calbiochem, La Jolla, CA). The water-soluble form of corticosterone and bovine serum albumin-conjugated corticosterone (corticosterone-BSA) was dissolved in sterile water and stored in 1 mM stock at 20°C and achieved their final concentrations during experiments. The drug solutions were freshly prepared by 20 mM absolute ethanol. The mineralocorticoid receptor antagonist spironolactone (10 μM) was prepared in 10 mM dimethylsulfoxide (DMSO). Both drugs were stored at −20°C and diluted to final concentrations by extracellular solution before application. Protein kinase A (PKA) inhibitor H89 (10 μM) and PKCζ inhibitor G66976 (0.1 μM) were stored as 0.1 mM, phospholipase C (PLC) blocker (U73122; 1 μM) was stored as 1 mM, and PKC nonselective blocker chelerythrine (5 μM) and inositol-1,4,5-trisphosphate (IP₃) receptor blocker 2-aminoethoxydiphenyl borate (2APB; 1 μM) were stored as 10 mM in DMSO. All of the above drugs were stored at −20°C and achieved their final concentrations in extracellular solution before application with DMSO <0.01%. The adenylyl cyclase (AC) inhibitor 9-(tetrahydro-2-furanyl)-9H-purin-6-amine (SQ-22536; 10 μM) was dissolved in water and stored in 10 mM. It was freshly diluted by the extracellular solution before use. In most cases, the drugs were tested by delivering pressure to the soma of each recorded neuron via a 100-μm-diameter tip perfusion pipette controlled by a superfusion system and computer interface (DAD-8VSP; ALA Scientific Instruments, Westbury, NY). The nonhydrolyzable guanylyl nucleotide GDP-β-S (500 μM) and, in some cases, corticosterone (0.1 μM) and 1,2-bis(2-aminophenoxy)ethane-N,N,N',N' -tetraacetic acid (BAPTA; 20 mM) were included in the pipette solution for intracellular application. The tips of the patch pipettes were first filled with regular patch solution and then back-filled with the drug solution.

LTP induction. Male adult rats (250–300 g) were anesthetized with urethane (1.5 g/kg ip) and placed in a stereotaxic device. Deep body temperature was maintained at 37 ± 0.5°C with heating pads. Small holes were drilled in the skull at the positions for inserting the electrodes and cannula. A bipolar stimulating electrode was placed in the Schaffer collateral/commisural pathway (4.2 mm posterior to and 3.8 mm lateral to bregma), and the recording electrode was positioned in the stratum radiatum area of CA1 region (3.8 mm posterior to and 2.5 mm lateral to bregma). A cannula was implanted (1.3 mm posterior, 1 mm lateral to bregma, and 4.0 mm below the surface of the dura) into the same hemisphere for intracerebroventricular (icv) injections. Corticosterone was diluted to 0.1 μM with artificial cerebrospinal fluid (CSF) before use. Solutions for icv injection were administered slowly in a 5-μl volume over a 3-min period. Control group was set by injecting same volume of artificial CSF.

The electrodes were slowly lowered through the cortex and the upper layers of the hippocampus and into the CA1 region. The correct placement of electrodes was confirmed via electrophysiological criteria and postmortem identification. The stimulus intensity was then adjusted and fixed to evoke ∼50% of the maximum field excitatory postsynaptic potential (fEPSP) amplitude. Stable baseline fEPSPs were recorded every 30 s (0.033 Hz) for ≥30 min prior to icv injection. Baseline recordings were monitored for an additional 30 min after injection, and then LTP was induced by the high-frequency stimulus (HFS) comprised of three trains of 20 stimuli at 200 Hz, with an intertrain interval of 30 s. fEPSPs were recorded with a computerized stimulating and recording unit. The signals from the recording electrode were filtered at 3 kHz, amplified, and displayed on a poststimulus response. The original evoked responses were plotted by an X-Y recorder (NP-0316) connected to a memory oscilloscope. An electronic stimulus (SEN-3301) and an isolator (ss-102J) were used to give stimulation.

Western blotting analysis. Neurons obtained from embryonic day 18 rats were cultured for the indicated days. The cells were scraped off the plate in the presence of lysis buffer consisting of 60 mM Tris-HCl, 2% sodium dodecyl sulfate (SDS), 10% sucrose, 2 mM phenylmethylsulfonyl fluoride (Merck, Darmstadt, Germany), 1 mM sodium orthovanadate (Sigma-Aldrich), and 10 g/ml aprotinin (Bayer, Leverkusen, Germany). Embryonic neurons of DIV were treated with increasing concentrations of corticosterone or corticosterone-BSA for 4 min and then scraped off plates, as described above.

The lysates were quickly sonicated, boiled for 5 min at 95°C, and centrifuged at 12,000 g for 5 min at 4°C. The supernatant was collected and protein concentration assayed using a modified Bradford assay. The samples were diluted in sample buffer [250 mM Tris-HCl (pH 6.8) containing 4% SDS, 10% glycerol, 2% β-mercaptoethanol, and 0.002% bromophenol blue] and boiled for an additional 5 min. Aliquots of protein were separated by SDS-PAGE (10%) and subsequently transferred to nitrocellulose membranes by electroblotting. The membrane was blocked in 5% skim milk powder in 0.1% Tris-buffered saline-Tween 20 (TBST) at room temperature for 2 h and then incubated with antibodies raised against phosphorylated phospholipase Cβ1 (PLC-β1; Santa Cruz Biotechnology), GR (Santa Cruz Biotechnology), or mineralocorticoid receptor (MR; Santa Cruz Biotechnology) at a dilution of 1:1,000 overnight at 4°C. After three washes with TBST, the membrane was incubated with a secondary
Corticosterone (Cort) rapidly modulates N-methyl-D-aspartate (N)-elicited currents in hippocampal neurons. A, top: representative current traces showing that Cort (0.1 μM) caused a decrease in the amplitude of N-elicited (100 μM) currents from a hippocampal neuron. A, bottom: plot of N currents showing that application of Cort for 4 min reduced peak and steady-state currents of N. The N currents after application of Cort were normalized to the control (Ctl) currents induced by N (100 μM). B: concentration-response curve of Cort-induced decrease in N-methyl-D-aspartate (NMDA) peak currents from 10 hippocampal neurons. The peak currents affected by Cort were normalized to the Ctl. Data are presented as means ± SE. C: the concentration-response relationship of peak current of NMDA in the presence or absence (Ctl) of Cort. All responses were normalized to the peak current of 100 μM NMDA. Each point represents the average response of 7 neurons. Data are presented as means ± SE.

horseradish peroxidase-conjugated antibody (Santa Cruz Biotechnology) for 1 h at room temperature. Immunoreactive proteins were detected using the enhanced chemiluminescence Western blotting detection system (Santa Cruz Biotechnology) and visualized using Sygene Bio Image system (Synoptics). To control sampling errors, the ratio of band intensities to PLC-δ (Santa Cruz Biotechnology) was obtained to quantify the relative protein expression level.

Analysis of Gs protein. The active GTP-bound Gs protein was assayed using commercial kits (NewEast Biosciences). Neurons were treated with increasing concentrations of corticosterone or corticosterone-BSA for 4 min and then scraped off the plate in the presence of lysis buffer that was supplied with the kit. The anti-active Gs monoclonal antibody and the protein A/G agarose bead were added to cell lysates. After incubating at 4°C for 1 h, the beads were pelleted and subsequently resuspended in lysis buffer. Then the samples were centrifuged for 10 s at 12,000 g, and the supernatant containing active GTP-bound Gs protein was collected. The samples were reconstituted in SDS-PAGE sample buffer and boiled for 5 min. Aliquots of samples were separated by SDS-PAGE (10%) and subsequently transferred to nitrocellulose membranes by electroblotting. The membrane was blocked with blocking buffer and then incubated with anti-Gs monoclonal antibody (1:1,000) overnight at 4°C. Then the membrane was washed with TBST three times and incubated with a secondary horseradish peroxidase-conjugated antibody. Immunoreactive proteins were detected using the enhanced chemiluminescence Western blotting detection system (Santa Cruz Biotechnology) and visualized using the Sygene Bio Image system (Synoptics). To control sampling errors, the total Gs protein was determined by Western blotting analysis.

RIA of cAMP. Neurons were treated with increasing concentrations of corticosterone or corticosterone-BSA for 4 min and then terminated by the addition of 0.1 ml of 0.3 M HCl. Cells were frozen overnight, followed by heating of the tubes in boiling water for 5 min. The supernatants were collected by centrifuge and stored at −20°C for later assay for cAMP.

cAMP was assayed using commercially available RIA kits (Shanghai Institute of Biological Product, Shanghai, China). The sensitivity was 0.1 pM. The mean intra- and interassay coefficients of variation were 5.78 and 6.96%, respectively (manufacturer’s data).

IP3 assay. Neurons were treated with increasing concentrations of corticosterone or corticosterone-BSA for 4 min, and then culture media were discarded and replaced by PBS. After the cell solution was frozen and thawed several times, the cell supernatants were collected by centrifuge (3,000 g for 20 min). IP3 content in supernatants was assayed using the IP3 ELISA kits (R & D Systems, Minneapolis, MN).

Statistical analysis. The effect of different reagents on NMDA-induced currents was calculated by dividing the NMDA-evoked current after the application of drugs by the control response to gain the magnitude of changes in the modulation of the NMDA-induced currents by the respective reagents.

eEPSP amplitude was used to measure synaptic efficacy. Baseline was recorded for an average time of 30 min. This value was taken as 100% of the eEPSP slope, and all recorded values were normalized to this baseline value. The statistical comparison of LTP between control and corticosterone groups was carried out at every time point, particularly at 1, 30, and 60 min post-HFS.

All of the results are presented as means ± SE. All of data were tested for homogeneity of variance by Kolmogorov-Smirnov test. The results indicated that the data were distributed normally. Student’s t-test, paired t-test, and one-way ANOVA with Student-Newman-Keuls multiple comparison methods were used. P < 0.05 was considered significant.

Fig. 2. RU-38486 and spironolactone have no effect on the NMDA-evoked currents in hippocampal neurons. A: representative current trace shows that glucocorticoid receptor antagonist RU-38486 (1 μM) had no effect on the NMDA-evoked (100 μM) current from a hippocampal neuron. B: representative current trace shows that mineralocorticoid receptor antagonist spironolactone (10 μM) did not affect the NMDA (100 μM) currents elicited from a hippocampal neuron.
RESULTS

Corticosterone rapid suppression of NMDA-evoked currents is not mediated by the classical GRs. When the holding potential was at −60 mV, application of NMDA (100 μM NMDA + 10 μM glycine in absence of Mg2+) elicited currents that desensitized within ~5 s to a nearly steady-state value on nearly all neurons tested. Application of 0.1 μM of corticosterone significantly suppressed the NMDA-evoked currents over a 4-min treatment period. The peak and steady-state currents were rapidly suppressed (Fig. 1A). At 1 min after administration of corticosterone, the peak and steady-state currents were decreased to 87.9 ± 2.4 and 91 ± 2.6% of control, respectively (n = 15 for 8 independent cultures, P < 0.05 vs. control). The maximal effect was obtained at 3 min after administration to 77.8 ± 1.2% of control for peak current (P < 0.01 vs. control) and 80.1 ± 3.2% of control for steady state (P < 0.01 vs. control). Corticosterone did not change 1steady-state/Ipeak (Istead/Ipeak, from 0.58 ± 0.003 to 0.60 ± 0.005). Full recovery of peak and steady-state currents could be obtained after washout with the extracellular solution. Administration of increasing concentration of corticosterone caused a decrease in NMDA-induced currents in a dose-dependent manner (Fig. 1B). The significant effect of corticosterone was obtained at the concentration 10 nM (n = 10 for 4 cultures, P < 0.01 vs. control). When the holding potential was at −60 mV, application of corticosterone (0.1 nM to 1 μM) did not induce a detectable current (data not shown).

The concentration-response relationships of NMDA currents were determined in the presence and absence of 0.1 μM corticosterone (Fig. 1C). Corticosterone decreased the maximum value of the concentration response of NMDA without affecting threshold concentration. The EC50 value (46 μM) of NMDA in the presence of corticosterone was similar to that of NMDA alone (43 μM), indicating that corticosterone did not change the affinity of NMDA to its receptor.

We then examined whether the inhibitory effect of corticosterone was mediated by the activation of the classical intracellular GRs. At first, the effect of GR antagonist RU-38486 (1 μM) or MR antagonist spironolactone (10 μM) alone on NMDA-evoked currents was examined. It was found that neither RU-38486 nor spironolactone affected the NMDA-evoked current (Fig. 2, A and B). Neurons were administrated with RU-38486 (1 μM) or spironolactone (10 μM) for 4 min, followed by application of corticosterone (0.1 μM). The inhibition of NMDA currents by corticosterone (0.1 μM) remained in the presence of either RU-38486 or spironolactone. In the presence of RU-38486, corticosterone decreased the peak current of NMDA to 74.2 ± 3.2% of the control and steady-state current to 73.8 ± 6.1% of control (n = 8 for 4 cultures, P < 0.01 vs. control; Fig. 3, A and B). In the presence of spirono-

---

**Fig. 3.** The rapid Cort effect is not dependent on intracellular glucocorticoid receptors. **A,** top: representative traces from a neuron showing that glucocorticoid receptor antagonist RU-38486 (RU; 1 μM) did not reverse the Cort-induced depression of NMDA currents. **A,** bottom: plot of NMDA currents showing that application of Cort (0.1 μM) inhibited NMDA-elicited (100 μM) currents and that coapplication of RU and Cort inhibited NMDA currents. **B:** summary histograms showing the effects of RU on Cort-induced inhibition of NMDA currents (n = 8). Data are presented as means ± SE. The NMDA currents affected by respective drugs were normalized to the control currents. **C,** top: representative traces from a neuron showing that mineralocorticoid receptor antagonist spironolactone (Spiro; 10 μM) also failed to block inhibition of NMDA (100 μM) currents by Cort. **C,** bottom: plot of NMDA currents showing that NMDA-elicited current was suppressed by Cort (0.1 μM) and that coapplication of Spiro and Cort reduced NMDA currents. **D:** summary histograms showing the effects of Spiro on Cort-induced inhibition of NMDA currents (n = 8). Data are presented as means ± SE. The NMDA currents affected by respective drugs were normalized to the control currents. **• doi:10.1152/ajpendo.00302.2011 • www.ajpendo.org**
lactone, corticosterone reduced the peak current of NMDA to 78.2 ± 2.8% of control and steady-state current to 81.8 ± 4.8% of control (n = 8 for 4 cultures, P < 0.01 vs. control; Fig. 3, C and D).

**Effect of corticosterone is mediated by a G protein-coupled receptor.** To explore whether the effect of corticosterone on NMDA-evoked currents is mediated by a membrane-bound receptor, the effect of a membrane-impermeable corticosterone-BSA was investigated. As shown in Fig. 4, A–C, corticosterone-BSA (0.1 μM) retained the inhibitory effect of corticosterone on NMDA-mediated currents. The peak value was rapidly reduced to 80 ± 0.4% of control (n = 15 for 8 cultures, P < 0.01 vs. control). Steady-state current was decreased to 78.6 ± 0.7% of control (n = 15 for 8 cultures, P < 0.01 vs. control). Corticosterone-BSA did not significantly change I_{steady-state}/I_{peak} (I_{steady-state}/I_{peak} from 0.61 ± 0.002 to 0.59 ± 0.006). Administration of increasing concentrations of corticosterone-BSA caused a dose-dependent decrease in NMDA-induced currents. The concentration-response relationships of NMDA currents were determined in the presence and absence of 0.1 μM corticosterone-BSA. Corticosterone-BSA decreased the maximum value of the concentration-response of NMDA without affecting threshold concentration. The EC_{50} value (46 μM) of NMDA in the presence of corticosterone-BSA was similar to that of NMDA alone (39 μM).

To confirm that the rapid suppression of NMDA-induced currents by corticosterone was not mediated by the intracellular corticosteroid receptors, corticosterone was applied directly into the cytoplasm of hippocampal neurons to bind intracellular corticosteroid receptors. Intracellular administration of corticosterone (0.1 μM) through patch pipette for ~20 min had no effect on the NMDA-mediated (100 μM) currents. The mean peak amplitude of NMDA-induced currents was not different from control neurons (978.4 ± 1.53 vs. 983.5 ± 2.02 pA; n =

---

**Fig. 4.** Cort rapidly suppresses NMDA-elicited currents in hippocampal neurons via a putative membrane receptor. A, top: 1 example of the effect of corticosterone-BSA (Cort-BSA; 0.1 μM) on NMDA-elicited currents from a hippocampal neuron. Application of Cort-BSA reduced the amplitude of both peak and steady-state values of NMDA currents. A, bottom: plot of NMDA currents showing that application of Cort-BSA for 4 min reduced peak and steady-state currents of NMDA. The NMDA currents after application of Cort-BSA were normalized to the Ctrl currents induced by NMDA (100 μM). B: concentration-response curve of Cort-BSA-induced suppression in NMDA peak currents from 10 hippocampal neurons. The peak currents affected by Cort-BSA were normalized to the control. Data are presented as means ± SE. C: the concentration-response relationship of peak current of NMDA in the presence or absence (Ctrl) of Cort-BSA. All responses were normalized to the peak current of 100 μM NMDA. Each point represents the average response of 7 neurons. D, top: representative traces from a neuron showing inhibitory effects of extracellular Cort (0.1 μM) on NMDA current (100 μM) with intracellular infusion with Cort (0.1 μM). D, bottom: plot of peak value of NMDA currents showing inhibitory effects of extracellular corticosterone (0.1 μM) with intracellular infusion with Cort or without intracellular Cort (Ctrl). E: summary histogram of NMDA current before and after application of Cort with intracellular infusion of Cort (Intra Cort) in pipette from 8 neurons. The NMDA currents affected by extracellular Cort were normalized to that without extracellular application of Cort. Data are presented as means ± SE. **P < 0.01 vs. without extracellular application of Cort.
administration of corticosterone, which caused a significant decrease in NMAD currents (to 80.1 ± 4.1% of control for peak current and to 78.6 ± 5.9% of control for steady-state current; n = 7 for 4 cultures, P < 0.01; Fig. 4, D and E).

Previous studies suggested that nongenomic effects of GC were mediated by a G protein-coupled receptor (9). Thus, we also tested whether the rapid actions of corticosterone were G protein dependent. The G protein antagonist GDP-β-S (500 μM) was applied intracellularly via the patch pipette to block G protein activity in the neurons. As shown in Fig. 5, intracellular application of GDP-β-S (10 min) blocked corticosterone-induced (0.1 μM) inhibition of NMAD currents (n = 8 for 4 cultures).

Corticosterone rapidly activates multiple signaling pathways. Our previous study showed that PKA was involved in the GCs’ rapid regulation of NMAD currents (27). Therefore, we first examined whether GCs could activate the Gs-AC signaling pathway. Incubation of neurons with increasing concentrations of corticosterone for 4 min caused an increase in GTP-bound Gs protein (the active Gs protein) in a dose-dependent manner (Fig. 6A). Corticosterone-BSA also dose-dependently activated Gs protein in a concentration range of 10⁻¹⁰ to 10⁻⁶ M (Fig. 6B). Both corticosterone and corticosterone-BSA increased cAMP production significantly in a 4-min incubation time (Fig. 6, C and D).

GC activation of PKC has been demonstrated previously (35, 37). It is known that G protein-coupled receptors can activate PKC via the Gs-PLC signaling pathway. Therefore, we wanted to examine whether corticosterone activates the Gs-PLC signaling pathway. Because there is no commercial kit for Gs detection available, we just determined the activation of PLC-β3 and IP₃, a production of PIP₂ hydrolysis by PLC-β3. As shown in Fig. 6, E–H, incubation of neurons with either corticosterone or corticosterone-BSA for 4 min induced phosphorylated PLC-β3, the active form of PLC-β3, in a dose-dependent manner. Both corticosterone and corticosterone-BSA increased IP₃ production significantly in a 4-min incubation time.

Corticosterone suppression of NMAD-evoked currents is dependent on PLC and its downstream signaling pathways. Because corticosterone activates PLC-β3, we first tested the effect of PLC inhibitor U-73122 on the effect of corticosterone. Application of U-73122 (1 μM) blocked the corticosterone-induced (0.1 μM) decrease of NMAD currents (Fig. 7, A and B). In addition, the application of U-73122 alone enhanced steady-state currents of NMAD (to 109.7 ± 5.2% of control for steady-state current; n = 15 for 5 cultures, P < 0.05 vs. control). Iₛ/Iₚ was increased significantly from 0.59 ± 0.01 to 0.66 ± 0.01 (P < 0.05 vs. control).

It is known that PLC activation catalyzes the hydrolysis of membrane phosphoinositol lipids, which leads to the release of IP₃ and diacylglycerol. IP₃ binding to IP₃ receptors can trigger the release of Ca²⁺ from endoplasmic reticulum. Thus we examined the role of Ca²⁺ in corticosterone regulation of NMAD currents. As shown in Fig. 7, C and D, 2APB (1 μM), a membrane-permeable IP₃ receptor antagonist, blocked the corticosterone-induced decrease of NMAD currents (to 96.9 ± 0.1% of control for peak currents and to 100.3 ± 0.03% of control for steady-state currents; n = 11 for 4 cultures). Iₛ/Iₚ was not changed (from 0.59 ± 0.02 to 0.60 ± 0.02). Dialysis with the Ca²⁺ chelator BAPTA (20 mM) also prevented corticosterone-induced depression of NMAD currents (to 96.7 ± 0.02% of control for peak currents and to 101.3 ± 0.06% of control for steady-state currents; n = 6 for 4 cultures; Fig. 7, E and F). Iₛ/Iₚ was not changed (from 0.57 ± 0.02 to 0.59 ± 0.01).

PKC is a major downstream signaling molecule that can be activated by the PLC signaling pathway. Thus we tested whether the PKC signaling pathway is involved in corticosterone regulation of NMAD currents. Blocking PKC activity with nonselective inhibitor chelerythrine (5 μM) totally blocked the corticosterone-induced depression of NMAD currents (n = 13 for 5 cultures; Fig. 7, G and H). Application of the PKCα/β inhibitor Gö6976 (0.1 μM) partly blocked the corticosterone-induced decrease in NMAD currents (to 89.4 ± 0.04% of control for peak currents and to 92.8 ± 0.02% of control for steady-state currents; n = 9 for 4 cultures, P < 0.05; Fig. 7, I and J). Iₛ/Iₚ was not changed (from 0.60 ± 0.03 to 0.59 ± 0.02).
The role of the AC-PKA signaling pathway in the regulation of NMDA-evoked currents by GCs. We first observed the effects of blocking of AC and PKA on corticosterone-induced suppression of NMDA activity. Application of the PKA inhibitor H89 (10 μM) decreased peak and steady-state currents of NMDA significantly (to 72.3 ± 0.7% of control for peak current and to 84.2 ± 5.9% of control for steady-state current; n = 12 for 5 cultures, P < 0.01 vs. control). NMDA responses nearly recovered to control values after H89 washout (Fig. 8A).

The AC inhibitor SQ-22536 (10 μM) decreased peak and steady-state currents of NMDA significantly (to 84.1 ± 2.6% of control for peak current and to 85.1 ± 2.6% of control for steady-state current; n = 10 for 5 cultures, P < 0.01 vs. control) (Fig. 8B). Iss/Ip of NMDA currents was not changed by either H89 (from 0.59 ± 0.02 to 0.57 ± 0.01) or SQ-22536 (from 0.60 ± 0.01 to 0.60 ± 0.02).

After blockage of PKA with H89, application of corticosterone (0.1 μM) partly reversed the inhibition NMDA currents by H89 (to 83 ± 0.4% of control for peak currents and to 92.9 ± 0.05% of control for steady-state currents, P < 0.05 vs. control; Fig. 8C and D). Iss/Ip was changed from 0.57 ± 0.04 to 0.55 ± 0.02 (P > 0.05 vs. control). After block of AC by SQ-22536, corticosterone (0.1 μM) could partly reverse the inhibition of NMDA currents by SQ-22536 (to 91.7 ± 0.02% of control for peak currents and to 89.2 ± 0.02% of control for steady-state currents, P < 0.05 vs. control; Fig. 8E and F). Iss/Ip was not changed (from 0.56 ± 0.03 to 0.56 ± 0.02).

Corticosterone inhibits LTP in the CA1 region of the hippocampus. To explore the possible physiological significance relevant to the rapid effects of corticosterone on NMDA activity, we tested whether GCs affect LTP in the CA1 region in vivo. The baseline fEPSPs were recorded, and HFS-induced LTP was induced in control and corticosterone-treated animals. The baseline fEPSPs were monitored for 30 min after icv injection, and fEPSPs were further recorded for 1 h after HFS delivery. There was no change in baseline fEPSPs following injection (icv) of artificial CSF. HFS resulted in an increase in fEPSP amplitude to 184.5 ± 5.5, 158 ± 3.9, and 150.3 ± 4.8% of baseline at 1, 30, and 60-min post-HFS, respectively (n = 10). Administration of...
Fig. 7. PLC and its downstream signaling pathways are involved in Cort suppression of NMDA receptors. 

A: plot of normalized NMDA currents showing that Cort (0.1 μM) reduced NMDA-evoked (100 μM) currents and that coapplication of U-73122 and Cort failed to reduced NMDA currents; B: summary histogram showing effects of Cort, U-73122, and U-73122 plus Cort on NMDA currents (n = 15). The NMDA currents affected by the respective drugs were normalized to the control. Data are presented as means ± SE. 

C: plot of normalized NMDA currents showing that Cort (0.1 μM) inhibited NMDA-evoked (100 μM) currents and that coapplication of IP3 receptor antagonist 2-aminoethoxydiphenyl borate (2APB; 1 μM) and Cort failed to reduced NMDA currents. D: summary histogram showing effects of Cort, 2APB, and 2APB + Cort on NMDA currents (n = 11). The NMDA currents affected by the respective drugs were normalized to the control. Data are presented as means ± SE. 

E: effect of BAPTA on Cort-induced inhibition of NMDA currents. Top: representative current traces showing effect of Cort on NMDA currents in the presence of Ca2+ chelator BAPTA (20 mM) in pipette solution; bottom: plot of normalized peak NMDA currents showing that loading with BAPTA blocked the depression on NMDA currents induced by corticosterone. F: summary histogram of NMDA currents before and after application of Cort from 6 neurons. The NMDA currents affected by Cort were normalized to that without Cort application. Data are presented as means ± SE. 

G: plot of normalized NMDA currents showing that Cort (0.1 μM), PKC inhibitor cheleythrine (5 μM) slightly increased NMDA currents, and coapplication of cheleythrine and corticosterone failed to cause a decrease in NMDA currents. H: summary histogram showing effects of Cort, cheleythrine, and cheleythrine + corticosterone on NMDA currents (n = 13). The NMDA currents affected by the respective drugs were normalized to the Ctl. Data are presented as means ± SEM. I: plot of normalized NMDA currents showing the effects of Cort (0.1 μM), PKCo/β inhibitor Gö6976 (0.1 μM) and coapplication of Gö6976 and Cort on NMDA currents; J: summary histogram showing effects of Cort, Gö6976, and Gö6976 + corticosterone on NMDA currents (n = 9). The NMDA currents affected by the respective drugs were normalized to the control. Data are presented as means ± SE. *P < 0.05; **P < 0.01 vs. control; ▲P < 0.05 vs. Cort.
corticosterone had no discernible effect on baseline fEPSPs but significantly suppressed the induction of hippocampal LTP. Application of 0.1 μM corticosterone resulted in HFS-induced fEPSPs to 142.4 ± 1.1, 122.1 ± 2.5, and 114.4 ± 3.6% of baseline at 1, 30, and 60 min post-HFS, respectively (P < 0.05 vs. control group, n = 10; Fig. 9).

DISCUSSION

Our present study demonstrated that GCs rapidly modulated NMDA activity in hippocampal neurons via nongenomic pathways. These effects are mediated by a G protein-coupled membrane receptor and through Gs/AC/PKA and Gq/PLC/IP3 signaling pathways. Intracerebroventricular administration of corticosterone significantly suppressed LTP in the CA1 region of the hippocampus within 30 min in vivo, suggesting the possible physiological significance of the nongenomic effects of GCs on NMDA activity in the hippocampus.

Xiao et al. (49) demonstrated that corticosterone rapidly enhanced NMDA-evoked neurotoxicity and NMDA-induced increment of intracellular free calcium ([Ca2+]i). However, their study did not provide the direct evidence that the boosting effect of GCs on neurotoxicity and [Ca2+]i increment is through enhancement of NMDA receptor activity. In contrast, our previous (27) and present studies suggested that GCs regulate the activity of NMDA receptors because corticosterone suppresses NMDA-evoked currents in hippocampal neurons. It seems that the nongenomic effects of GCs may be dependent on the experimental conditions. Xiao et al. (48) reported that GCs rapidly stimulated ERK1/2 activity in hippocampal neurons. In contrast, another study by their group

Fig. 8. Roles of AC/PKA signaling pathway in the regulation of NMDA currents by Cort. A: effect of H89 on NMDA currents. Top: representative current traces showing that H89 (10 μM) caused a decrease in the amplitude of NMDA currents; bottom: plot of normalized NMDA currents showing the effect of H89 on NMDA currents. B: effect of SQ-22536 (SQ) on NMDA currents. Top: representative traces from a neuron showing that SQ-22536 (AC inhibitor; 10 μM) also suppressed NMDA currents; bottom: plot of normalized NMDA currents showing the inhibitory effect of SQ on NMDA-evoked currents. C: top: representative current traces shown after blockage of PKA with H89; further application of Cort (0.1 μM) restored the inhibition NMDA currents by H89. C: bottom: plot of normalized NMDA currents showing the effects of PKA inhibitor H89 and coapplication of H89 and Cort on NMDA currents. D: summary histogram showing effects of Cort, H89, and H89 + Cort on NMDA currents (n = 12). The NMDA currents affected by the respective drugs were normalized to the Ctl. Data are presented as means ± SE. ▲P < 0.05 vs. H89. E: top: representative current traces shown after block of AC by SQ-22536; Cort (0.1 μM) restored the inhibition of NMDA currents by SQ-22536. E: bottom: plot of normalized NMDA currents showing the effects of AC inhibitor SQ-22536 and coapplication of SQ-22536 and Cort on NMDA currents. F: summary histogram showing effects of Cort, SQ, and SQ + Cort on NMDA currents (n = 10). The NMDA currents affected by the respective drugs were normalized to the control. Data are presented as means ± SE. ▲P < 0.05 vs. SQ; *P < 0.05; **P < 0.01 vs.control.
our findings are in accord with the study by Di et al. (7), where study vs. mice in the study by Karst et al. (22), etc. However, in the study by Karst et al. (22), species differences [rats in our evoked currents in our study vs. glutamate-release probability this discrepancy, for example, target differences [NMDA-differences in experimental conditions might be responsible for effects of corticosterone on NMDA-evoked currents. Some pus depends on the “classical” MRs in mice, which is incon-

enhancement of glutamate release probability in the hippocam-

mechanism in the rapid effect of corticosterone on NMDA-

receptor (7, 8, 30). In agreement with these studies, several lines of evidence in the present study support the involvement of a membrane-associated receptor and G protein-dependent mechanism in the rapid effect of corticosterone on NMDA-evoked currents: 1) application of corticosterone extracellularly induced a rapid suppression of NMDA currents; 2) corticosterone-BSA conjugate maintained the rapid effect; 3) “classic” GR and MR antagonists did not block the effects; and 4) blocking G protein activity reversed the corticosterone effects.

Karst et al. (22) reported recently that corticosterone rapid enhancement of glutamate release probability in the hippocampus depends on the “classical” MRs in mice, which is inconsistent with our finding that MRs are not involved in the rapid effects of corticosterone on NMDA-evoked currents. Some differences in experimental conditions might be responsible for this discrepancy, for example, target differences [NMDA-evoked currents in our study vs. glutamate-release probability in the study by Karst et al. (22)], species differences [rats in our study vs. mice in the study by Karst et al. (22)], etc. However, our findings are in accord with the study by Di et al. (7), where they have demonstrated that “classical” MR antagonist spi-

ronolactone did not block the nongenomic effects of GC on glutamate transmission in hypothalamus.

Various intracellular signaling pathways downstream from the putative membrane GRs have been implicated in the rapid actions of GC. Studies by Han and colleagues (16, 17), Lou and Chen (28), and Qiu and colleagues (36, 37) reported the rapid GC inhibition of voltage-gated and transmitter-evoked calcium transients in different cells via either PKA- or PKC-dependent mechanisms, depending on cell type. More recently, they demonstrated that GCs rapidly activate MAPKs via a G protein/PKC-dependent signaling mechanism in hippocampal neurons (35). Studies by Di et al. (7) and Malcher-Lopes et al. (30) reported that GC rapid stimulation of endocannabinin release from neuroendocrine cells in hypothalamus is through a G protein/cAMP/PLC-dependent mechanism and independent of the Gq protein signaling pathway. Our finding that corticosterone activated the Gq/cAMP and Gq/PLC-β2 signaling pathways suggests that the membrane GR may couple to multiple G proteins, including Gq and Gs1/11. Recent evidence has indicated that a G protein-coupled receptor can couple to multiple G proteins and induce multiple signaling pathways, for instance, corticotropin-releasing hormone receptors (1).

We found that GC-induced suppression of NMDA currents was totally prevented by blockage of either PLC or its down-

stream signaling, including IP3, Ca2+, and PKC. PLC signaling pathway downregulation of NMDA receptors has been found in some regions of the brain. Gu et al. (15) reported that activation of PLC/IP3/Ca2+ signaling pathways inhibits the functions of NMDA receptor in prefrontal cortex. Grishin et al. (12) demonstrated that the M1 acetylcholine receptor that couples to the Gq protein reduces NMDA responses in CA3 hippocampal neurons via Ca2+-dependent signaling pathway. Our study also showed that PLC inhibitor U-73122 and PKC inhibitor chelerythrine increased Iss/IP, suggesting that the PLC/PKC signaling pathway is involved in the desensitization of NMDA receptors, which is consistent with the study by Jackson et al. (20), where it was demonstrated that PKC activation is associated with desensitization of NMDA receptors. Our results demonstrated that GCs did not affect Iss/IP but recovered U-73122-induced NMDA receptor desensitization. Thus it would suggest that, besides PLC signaling, other signaling pathways might be involved in GC regulation of NMDA receptor activity. The effect of GC on NMDA receptor desensitization needs to be investigated further.

NMDA receptors are the molecular targets of the cAMP/ PKA signaling cascade, and PKA phosphorylation increases NMDA receptor activity (26, 42, 52). In the present study, we found that blockage of AC and PKA reduced NMDA-evoked currents, suggesting that NMDA receptors might be phosphory-

lated by basal PKA, and when PKA is blocked, phosphatases rapidly dephosphorylate NMDA receptors, receptor-associated proteins, or both. These data are consistent with the study by Skeberdis et al. (41), where it was shown that H89 and SQ-22536 inhibited NMDA currents in cultured hippocampal neurons. Interestingly, we also found that corticosterone could partially reverse the inhibition of NMDA currents by blockage of AC and PKA. It is hard to understand why corticosterone could potentiate NMDA currents in the presence of AC and PKA inhibitors. Further study is required to explore the mech-

![Figure 9](https://example.com/figure9.png)

**Figure 9.** Cort impaires hippocampal long-term potentiation (LTP) in vivo. *Top:* sample records of field excitatory postsynaptic potentials (EPSP) for Ctl (1, 2) and Cort (0.1 μM) groups (3, 4) at times indicated before and after induction of LTP [60 min after high-frequency stimulus (HFS)]. *Bottom:* scatter plots showing the suppressive effects of Cort on HFS-induced LTP.
anism that corticosterone reversed the inhibition of NMDA activity by PKA and AC blockers.

LTP is evoked by HFS of selected afferent pathways, resulting in a long-lasting enhancement of synaptic efficacy (14). GCs have been shown to suppress hippocampal synaptic potentiation in vivo (50, 51). In the present study, we showed that intracerebroventricular administration of corticosterone significantly suppresses LTP elicited in the CA1 region of the hippocampus in vivo. At the hippocampal Schaffer collateral-CA1 synapses, the LTP in these inputs is mediated mainly by NMDA receptors and the α-aminooxy-3-hydroxy-5-methylisoxazole-4-propanoic acid receptors (3, 11, 13, 47, 54). Given that gene-mediated stress effects by GC in hippocampus develop typically with a delay of 1–2 h (21), our data that LTP was suppressed at 30 min after administration of corticosterone would suggest that the nongenomic effect of GC on NMDA activity might influence LTP formation. The studies of Wiegert and colleagues (45, 46) have demonstrated that, in mouse hippocampus slice preparation, the corticosterone could rapidly enhance LTP through a nongenomic mechanism, whereas it suppressed LTP 1–6 h after the application of corticosterone and was blocked by RU-486, suggesting that suppressive effect of corticosterone on LTP in hippocampus slice is through a genomic mechanism. The discrepancy between our study and their studies might be due to some differences in study conditions, for example, experimental setting differences [in vivo in our study vs. in vitro in the studies by Wiegert and colleagues (45, 46), intact animals used in our study vs. brain slices used in the studies by Wiegert and colleagues (45, 46), and intracerebroventricular administration of corticosterone vs. direct application of corticosterone to hippocampus in studies by Wiegert and colleagues (45, 46)] and species differences [rats in our study vs. mice in the studies by Wiegert and colleagues (45, 46)].

In conclusion, GCs act on a G protein-coupled receptor to activate multiple signaling pathways, and the rapid suppression of NMDA activity by GCs is dependent on PLC and downstream signaling. GCs inhibit LTP in hippocampus in vivo, implicating the possibly physiological significance of rapid effects of GCs on NMDA receptors.

ACKNOWLEDGMENTS

We thank Prof. Yizhang Chen for advice during preparation of this article.

GRANTS

This work was supported by the National Basic Research Program of China (2007CB512303), the Natural Science Foundation of China (nos. 30900434 and 31100840), and the Technology Commission of Shanghai Municipal (09XD1405600, 09ZR1439800, and 11ZR1446700).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Y.Z. performed the experiments; H.S. prepared the figures; J.Q., B.M., J.S., S.L., and X.N. approved the final version of the manuscript; X.N. did the conception and design of the research; X.N. edited and revised the manuscript.

REFERENCES

15. Gu Z, Jiang Q, Fu AK, Ip NY, Yan Z. Regulation of NMDA receptors by neuregulin signaling in prefrontal cortex. J Neurosci 25: 4974–4984, 2005.
E758 RAPID REGULATION OF NMDA RECEPTOR BY GLUCOCORTICOID


