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Biochemical and Biophysical Research Communications 321 (2004) 510–513
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Acute effect of corticosterone on *N*-methyl-D-aspartate receptor-mediated Ca^{2+} elevation in mouse hippocampal slices

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Received 10 June 2004

Abstract

We examined the rapid effects of corticosterone (CORT) on *N*-methyl-D-aspartate (NMDA) receptor-mediated Ca^{2+} signals in adult mouse hippocampal slices by using Ca^{2+} imaging technique. Application of NMDA caused a transient elevation of intracellular Ca^{2+} concentration followed by a decay to a plateau within 150 s. The 30 min preincubation of CORT induced a significant decrease of the peak amplitude of NMDA-induced Ca^{2+} elevation in the CA1 region. The rapid effect of CORT was induced at a stress-induced level (0.4–10 μM). Because the membrane non-permeable bovine serum albumin-conjugated CORT also induced a similar rapid effect, the rapid effect of CORT might be induced via putative surface CORT receptors. In contrast, CORT induced no significant effects on NMDA-induced Ca^{2+} elevation in the dentate gyrus. In the CA3 region, CORT effects were not evaluated, because the marked elevation of NMDA-induced Ca^{2+} signals was not observed there.

Keywords: Acute effect; Ca^{2+} signals; Corticosterone; Glucocorticoids; NMDA receptor; Hippocampus

Corticosterone (CORT) is a principal glucocorticoid synthesized in the rodent adrenal cortex and secreted in response to stress. There are a series of studies about the chronic and genomic effects of corticosteroids in the hippocampus [1,2]. The stress-induced increase in CORT secretion is known to produce neuronal cell damage. Exogenous application of a high dose of CORT has been shown to elicit the neuronal atrophy in the hippocampus [3]. Rats exposed to restraint stress for three weeks exhibited neuronal atrophy identical to that seen in rats treated with a high dose of CORT for three weeks [4]. In addition to these classical genomic effects, which are actuated via intracellular steroid receptors, glucocorticoids act acutely on neuronal excitability [5,6]. The long-term potentiation (LTP) of the population spike amplitude was also acutely (within 1 h) suppressed by a high concentration of glucocorticoids [7]. It has also been demonstrated that CORT dosage for 20 min significantly suppresses the development of LTP in the CA1 region of 4-week-old rat hippocampal slices [8,9].

It is well known that Ca^{2+} influx via *N*-methyl-D-aspartate (NMDA) receptors plays a crucial role in the induction of LTP. The rapid effects of CORT (appearing within 30 min) on NMDA-induced Ca^{2+} signal transduction, however, have not been well elucidated in the hippocampus. In the present study, we examined the rapid effects of CORT on NMDA receptor-mediated Ca^{2+} signals in mouse hippocampal slices by using Ca^{2+} imaging technique. An advantage of mouse hippocampal slices was that we could measure the CA1, CA3, and dentate gyrus regions simultaneously because the whole hippocampus fell within the microscope field. As a

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0006-291X/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.06.168

result, the 30 min preincubation of CORT induced a significant decrease of the peak amplitude of NMDA-induced Ca^{2+} elevation in the CA1 region. The rapid effect of CORT region was induced at a stress-induced level (0.4–10 μM). In contrast, CORT induced no significant effects on NMDA-induced Ca^{2+} elevation in the dentate gyrus. In the CA3 region, CORT effects were not evaluated, because the marked elevation of NMDA-induced Ca^{2+} signals was not observed there.

Materials and methods

Brain slices (coronal, 300 μm thick) were prepared from 7-week-old male ddY mice after exposure to an overdose of diethyl ether anesthesia. In order to stabilize the plasma glucocorticoid level, mice used in all experiments were decapitated at the same moment (10 a.m.) in the circadian cycle. Following decapitation, the brain was quickly removed and placed in ice-cold oxygenated artificial cerebrospinal fluid (ACSF) (composition in mM: NaCl 124, KCl 5, $CaCl_2$ 2, $NaHCO_3$ 22, $MgSO_4$ 2, NaH_2PO_4 1.24, glucose 10, pH 7.4, and bubbled with 95% O_2/5% CO_2). Slices were then prepared using a microslicer (DTK-1000; Dosaka-EM, Kyoto, Japan). The slices were recovered in ACSF at 30 °C for 60 min and held at room temperature until use. All experiments using animals were conducted in accordance with the institutional guidelines.

Measurement of intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ was performed using the Ca^{2+}-sensitive indicator fura-2. Prior to Ca^{2+} signal measurements, the slices were loaded for 30 min at room temperature with 10 μM fura-2/AM [from 1 mM stock solution in dimethyl sulfoxide (DMSO)] in the presence of 0.01% cremophore EL in 7.2 mL ACSF. After loading with fura-2, the slices were washed in ACSF for 30 min and then preincubated with CORT or bovine serum albumin-conjugated CORT (BSA–CORT) solution for 30 min. CORT and BSA–CORT solutions were prepared at the appropriate dilution with low Mg^{2+} ACSF (control solution) (composition in mM: NaCl 124, KCl 5, $CaCl_2$ 2, $NaHCO_3$ 22, $MgSO_4$ 0.1, NaH_2PO_4 1.24, glucose 10, pH 7.4, and gassed with 5% CO_2/95% O_2) from the stock solution in DMSO. The final concentration of DMSO was less than 0.05% in each case.

For fluorescence measurements of $[Ca^{2+}]_i$, a digital fluorescence microscope system, consisting of an inverted microscope (TE 300; Nikon, Tokyo, Japan) equipped with a xenon lamp for excitation and a CCD camera (C4742-95; Hamamatsu Photonics, Hamamatsu, Japan), was used. Preincubated brain slices were placed in a chamber on the microscope stage. The whole hippocampus fell within the microscope field by using a 4× fluorescence objective (Nikon, Tokyo, Japan). The slices were then perfused with the CORT or BSA–CORT solution kept at 30 °C. At 150 s after the onset of recording the perfusing solution was replaced by the solution containing 1 mM NMDA.

For fura-2 measurements, the excitation wavelength varied discretely between 340 and 380 nm, and $[Ca^{2+}]_i$ was expressed as F340/F380, which is the ratio of the 510 nm fluorescence intensity at 340 nm excitation (F340) to that at 380 nm excitation (F380). In each acquisition trial, consecutive fluorescence images were acquired at 5 s intervals for 350 s. Fig. 1 shows examples of a series of obtained images. The fluorescence images were then analyzed with AQUACOSMOS system (Ver.1.3; Hamamatsu Photonics, Hamamatsu, Japan). Twelve ROIs (regions of interest: 5 × 5 pixels) were put on the cell body layer of each hippocampal subfield, and the fluorescence data of those ROIs were averaged.

The data were expressed as means ± SEM. Student's t test was utilized to test the significance of observed differences between groups. Significance was set at $p < 0.005$.

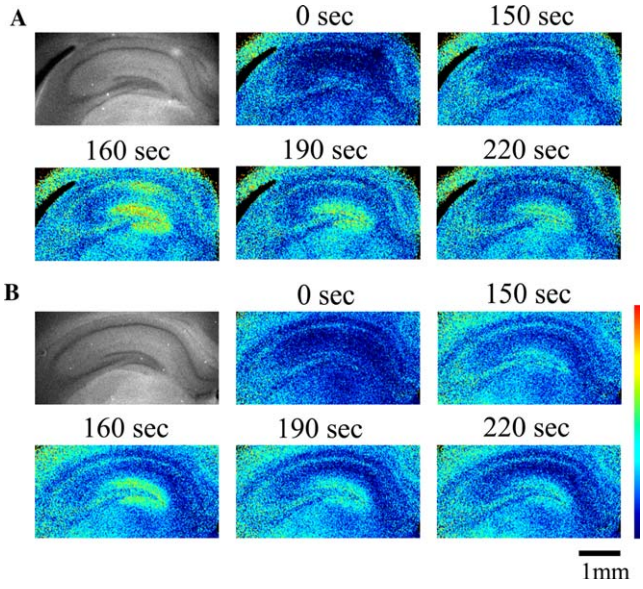

Fig. 1. Fluorescence images of mouse hippocampal slices (A) without the CORT dosage and (B) with the 1 μM CORT dosage. Upper left one of each column is a fluorescence image of a fura-2-loaded slice excited at 380 nm. The other pseudo-colored images demonstrate $[Ca^{2+}]_i$ in the slice. The color bar shows the ratio of the fluorescence intensity of fura-2 excited at 340 and 380 nm, which expresses $[Ca^{2+}]_i$, from blue (low $[Ca^{2+}]_i$) to red (high $[Ca^{2+}]_i$). The time above each pseudo-colored image shows the time from the onset of experiment. At 150 s after the onset of experiment, 1 mM NMDA was applied.

Results

Fig. 2 shows the typical time courses of NMDA-induced Ca^{2+} signal obtained from (A) the CA1 region and (B) the dentate gyrus. The response to continuous NMDA exposure was characterized by a transient elevation in $[Ca^{2+}]_i$ followed by a decay to a plateau within 150 s (see also Fig. 1). The transient elevation in $[Ca^{2+}]_i$ was due to NMDA receptor-mediated Ca^{2+} influx, because no response to NMDA application was observed in the slices preincubated with 100 μM of NMDA antagonist MK-801 (Fig. 2). In the control condition without CORT or BSA–CORT preincubation, the peak amplitudes of $[Ca^{2+}]_i$ elevation, calculated as an increase (Δ(F340/F380)) in F340/F380 from baseline level, were 0.40 ± 0.03 ($n = 7$) in the CA1 region, 0.05 ± 0.01 ($n = 7$) in the CA3 region, and 0.22 ± 0.01 ($n = 7$) in the dentate gyrus. These average values were set to 100%. In the CA1 region, the peak amplitude of $[Ca^{2+}]_i$ elevation was decreased by preincubation with CORT in a dose-dependent manner. The decreased peak amplitudes were $49.4 \pm 9.9\%$ ($n = 5$, $p < 0.005$), $43.0 \pm 7.7\%$ ($n = 9$), and $30.4 \pm 2.0\%$ ($n = 5$) at 0.4, 1, and 10 μM of CORT compared with control (Fig. 3A). In addition, preincubation with 1 μM BSA–CORT (approximately 20 CORT molecules per BSA molecule) also induced a decrease in the peak amplitude ($30.2 \pm 5.0\%$, $n = 5$, $p <$ 0.005). In contrast,

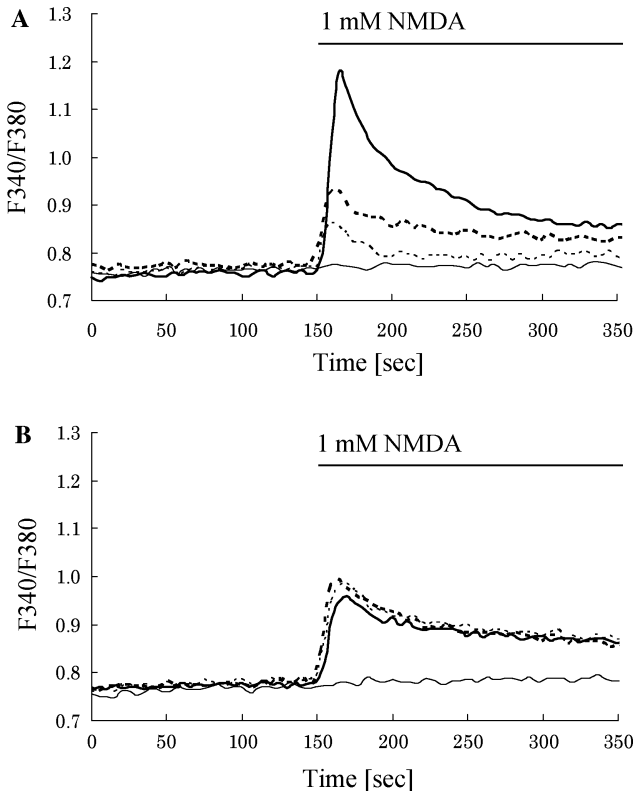

Fig. 2. Typical time courses of NMDA-induced Ca^{2+} signal in (A) the CA1 region and (B) the dentate gyrus. The vertical scale (F340/F380) is the ratio of the fluorescence intensity of fura-2 excited at 340 and 380 nm. Without the CORT dosage, 1 mM NMDA application induced a transient elevation in $[Ca^{2+}]_i$ followed by a decay to a plateau (control: thick line). The Ca^{2+} signal almost disappeared with the 100 μM MK-801 dosage (thin line). With the dosage of CORT, the $[Ca^{2+}]_i$ elevation was decreased compared with control in the CA1 region (1 μM CORT, thick-dotted line; 10 μM CORT, thin-dotted line). In contrast, the CORT dosage induced no significant effect on the Ca^{2+} signal in the dentate gyrus.

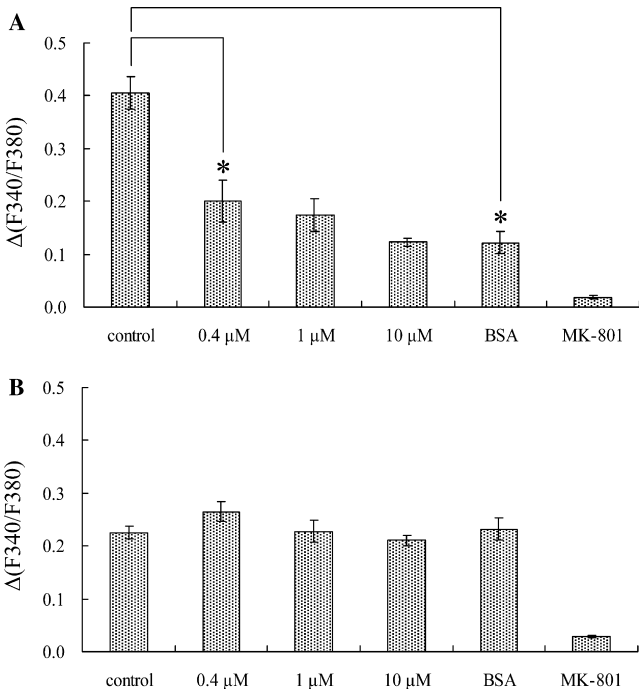

Fig. 3. The effect of CORT on the peak amplitude of NMDA-induced Ca^{2+} signal elevation in (A) the CA1 region and (B) the dentate gyrus. The vertical scale (Δ(F340/F380)) is the peak amplitude of F340/F380 from baseline. The concentrations below the figures show the dosed concentrations of CORT. BSA and MK-801 show the dosage of 1 μM BSA–CORT and 100 μM MK-801, respectively. The data are expressed as means ± SEM. The $[Ca^{2+}]_i$ elevation was significantly suppressed at 0.4 μM CORT and 1 μM BSA–CORT in the CA1 region. *$p < 0.005$ compared from control value.

preincubation with neither CORT nor BSA–CORT significantly changed the peak amplitude of $[Ca^{2+}]_i$ elevation in the dentate gyrus: the peak amplitudes were 117.8 ± 8.5% ($n = 5$), 101.1 ± 9.4% ($n = 9$), and 93.5 ± 4.6% ($n = 5$) at 0.4, 1, and 10 μM of CORT, and 103.2 ± 9.1% ($n = 5$) at 1 μM BSA–CORT compared with control (Fig. 3B). In the CA3 region, CORT and BSA–CORT effects were not evaluated, because the marked elevation of NMDA-induced Ca^{2+} signals was not observed there.

Discussion

In the present study, we examined the rapid effects of CORT on NMDA receptor-mediated Ca^{2+} signals in adult mouse hippocampal slices by using Ca^{2+} imaging technique. An advantage of mouse hippocampal slices was that we could measure the CA1, CA3, and dentate gyrus regions simultaneously because the whole hippocampus fell within the microscope field. Concerning rat hippocampal slices, we need to use different slices in order to compare the CA1, CA3, and dentate gyrus regions, resulting in losing accuracy of the relative levels for the $[Ca^{2+}]_i$ elevation. In addition, the heterogeneous effect of CORT between the CA1, CA3, and dentate gyrus regions could not be examined in our previous study using cultured neonatal hippocampal neurons [10], because all the neurons were mixed up there.

The present results showed the rapid effects (within 30 min) on NMDA-induced Ca^{2+} signals particularly in the CA1 region. The transient elevation in $[Ca^{2+}]_i$ induced by NMDA application was significantly suppressed to 49.4% at 0.4 μM CORT in the CA1 region. The increases of CORT concentration to 1.0 and 10 μM gave only a small additional decrease to 43.0 and 30.4%. The CORT effect in the CA1 region is physiologically significant, because rats subjected to immobilization-stress for 1 h were reported to show approximately 2 μM CORT in blood plasma [11]. In our previous study, a similar suppressive effect of CORT was also found in the CA1 region of 4-week-old rat hippocampal slices, in which the peak amplitude of $[Ca^{2+}]_i$ elevation decreased to 79.5% compared with control in the presence of 10 μM CORT (unpublished results).

On the other hand, CORT induced no significant effects on NMDA-induced Ca^{2+} elevation in the dentate gyrus as mentioned above. In the CA3 region, the marked elevation of NMDA-induced Ca^{2+} signals was not observed, although NMDA receptor should also be in the CA3 region. This reason is not clear at this stage.

The rapid effect of CORT on NMDA receptor-mediated Ca^{2+} signals was also found in the cultured neonatal hippocampal neurons in our previous study [10]. It should be noted that in the cultured neonatal hippocampal neurons, CORT induced the extreme prolongation of time duration of NMDA-induced Ca^{2+} elevation. However, the differences in the rapid effect of CORT between the previous study (using dissociated neonatal hippocampal neurons) and the present study (using adult mouse hippocampal slices) may not be controversial, because the age of hippocampal neurons was completely different between these preparations. Different effects of CORT may be due to the differences in molecular types of NMDA receptor subunits which are different between neonatal neurons (containing mainly NR2B) and adult neurons (containing both NR2A and NR2B) [12].

According to the classical view of the steroid action, CORT is thought to penetrate into the cytoplasm of neurons, bind to intracellular receptors, and induce genomic effects through new protein synthesis, resulting in the modulation of Ca^{2+} signals [1–4]. The present results suggested that CORT may bind to putative surface CORT receptors, because the membrane non-permeable BSA–CORT also induced a similar rapid effect. The possibility of genomic mechanisms can be also excluded as an explanation for the present CORT effect, because cycloheximide, an inhibitor of protein synthesis, did not abolish the effect of CORT on NMDA-induced Ca^{2+} signals.

A possible candidate for surface CORT receptors is a classical intracellular glucocorticoid receptor (e.g., GR, a type-2 receptor), which may be bound to plasma membranes. This idea is supported by reports that the immunoreactivity of antibodies against GR was associated with plasma membranes from hippocampal and hypothalamic neurons [13] and that specific CORT binding to neuronal membranes occurred in different brain areas with moderate affinity (K_d = 120 nM) [14,15]. Classical GRs are expressed in the cytoplasm of cultured hippocampal neurons [16]. GRs may drive both classical genomic pathways [17] and non-genomic pathways (the current study) in hippocampal neurons.

It is well known that Ca^{2+} influx via NMDA receptors plays a crucial role in the induction of LTP. The present results may have a good coincidence to the rapid suppressive effect of CORT found by electrophysiological investigations on the LTP in the CA1 region of 4-week-old rat hippocampal slices [8,9].

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