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Full-length article

S-Nitrosoglutathione and glutathione act as NMDA receptor agonists in cultured hippocampal neurons[1]

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Key words

cytosolic Ca^{2+} concentration; *S*-Nitrosoglutathione; glutathione; cultured hippocampal neuron; morphine; glutamate; *N*-methyl-*D*-aspartate receptor

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Abstract

Aim: To characterize the effect of combined pre- and postnatal morphine exposure on *N*-methyl-*D*-aspartate receptor (NMDA) receptor signaling in hippocampal neurons of the offspring of morphine-addicted female rats. **Methods:** Cultured hippocampal neurons and synaptosomes were prepared from neonatal and 2-week-old offspring, respectively, of control or morphine-addicted female rats. The increase in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) of cultured cells was measured using Fura-2, and glutamate release from synaptosomes was measured enzymatically. **Results:** Both glutamate and NMDA caused a dose-dependent increase in the $[Ca^{2+}]_i$. The nitric oxide (NO) donor, S-nitrosoglutathione (GSNO), but not 3-morpholinosydnonimine, sodium nitroprusside, and *S*-nitroso-*N*-acetylpenicillamine, also induced a $[Ca^{2+}]_i$ increase. GSNO and glutathione caused a dose-dependent increase in the $[Ca^{2+}]_i$ with respective EC_{50} values of 56 and 414 μmol/L. Both effects were inhibited by Mg^{2+} or an NMDA receptor antagonist and were unaffected by the presence of a glutamate scavenger. The other glutathione derivatives, oxidized glutathione, *S*-methylglutathione, *S*-ethylglutathione, *S*-propylglutathione, and *S*-butylglutathione, the dipeptides, Glu-Cys and Cys-Gly, and the antioxidants, dithiothreitol and mercaptoethanol, failed to induce a $[Ca^{2+}]_i$ increase. In addition, glutathione caused a dose-dependent increase in glutamate release from synaptosomes. The maximal responses and the EC_{50} values for the glutamate-, NMDA-, GSNO-, and glutathione-induced $[Ca^{2+}]_i$ increases and the glutathione-induced glutamate release were indistinguishable in the neurons of the offspring from control and morphine-addicted female rats. **Conclusion:** GSNO and glutathione act as NMDA receptor agonists and, in contrast to hippocampal brain slice, combined pre- and postnatal morphine exposure does not modulate NMDA receptor signaling in the cultured hippocampal neurons.

Introduction

Clinically, the chronic use of opioid drugs leads to the development of analgesia tolerance and dependence, but the cellular mechanisms underlying these processes are unknown[1,2]. Chronic co-administration of *N*-methyl-*D*-aspartate (NMDA) receptor antagonists and morphine attenuates the development of analgesia tolerance[3,4], showing that glutamatergic transmission is involved in opioid tolerance and dependence. Following NMDA receptor activation, nitric oxide (NO) synthase is stimulated to generate NO[5]. NO synthase inhibitors have similar attenuating effects on analgesia tolerance, supporting a role for glutamatergic transmission in opioid tolerance and dependence[6].

Previously, we have shown that, after combined pre- and postnatal morphine treatment of female rats, the NMDA receptor density in the hippocampal and cortical regions is significantly reduced in the 2-week-old rat brain compared to that in pups from control rats[7]. In electrophysiological studies of hippocampal slices, we also found that the mean

opening time of the NMDA receptor is longer in 2-week-old rats after the same morphine treatment protocol[8]. These results suggest that the functional properties of NMDA receptors during the early life of the offspring are altered by morphine or that the increased opening time of the postsynaptic NMDA receptors may be a result of increased glutamate secretion.

An increase in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) controls a diverse range of cell functions, including neurotransmission, secretion, and contraction. Activation of NMDA receptors results in Ca^{2+} influx and a $[Ca^{2+}]_i$ increase. In the present study, using the same pre- and postnatal morphine exposure protocol as before, we compared Ca^{2+} homeostasis following NMDA receptor activation in cultured hippocampal neurons of neonatal rats from control and morphine-addicted female rats.

Materials and methods

Animal treatment Adult (12-week-old) female Sprague-Dawley rats were used. All procedures employing experimental rats were performed in compliance with the guidelines of the Institutional Animal Care and Use Committee of the National Defense Medical Center and Triservice General Hospital, Taiwan, China. Saline or morphine was injected subcutaneously twice a day (09:00 and 17:00). The starting dose of morphine was 2 mg per kg bodyweight and the dose was progressively increased by 1 mg per kg at 7 d intervals. The rats were mated 1 week after the start of morphine administra-tion, which was continued throughout pregnancy and for the first 2 weeks after birth; the dose of morphine was 5 mg per kg bodyweight at birth and 6 mg per kg bodyweight for the next 14 d as described in previous studies[8,9]. For the preparation of hippocampal neuron cultures, pups were decapitated on the day of birth, while synaptosomes were prepared from 2-week-old pups.

Culture of hippocampal neurons Primary cultures of hippocampal neurons were prepared from neonatal rats as described in a previous study[10]. The hippocampi were dissected from the brain and incubated for 20 min at 37 ºC with trypsin (0.3 g/L), DNAase I (500 U/mL), and 10% horse serum. After mechanical dissociation, the resulting single cell suspension was centrifuged and the cell pellet suspended at an appropriate density and plated on poly-*D*-lysine (30 μg/mL)-coated 24-mm glass coverslips (1×10^5 cells/coverslip). The cells were grown in a humidified incubator with 5% CO_2/95% air at 37 ºC in growth medium consisting of DMEM supplemented with 10% fetal bovine serum, 10% Ham's F12 nutrient mixture, 50 U/mL of penicillin, and 50 μg/mL of streptomycin (Life Technologies, Grand Island, NY, USA). Normally, 20 mmol/L KCl and 10 μmol/L cytosine arabinoside were added to cultures 24 h after plating to minimize the proliferation of non-neuronal cells. In the case of cells prepared from morphine-exposed pups, 10 μmol/L morphine was added to the cultures every day until use. Experiments were performed on cells at 12–14 d after plating.

Preparation of synaptosomes A crude synaptosomal fraction was prepared from the hippocampi of 2-week-old rat pups from control and morphine-treated females as described in a previous study with some modifications[11]. All procedures were at 4 ºC. The hippocampi were homogenized (20 strokes, loose-fitting Dounce type homogenizer) in 9 volumes of 0.32 mol/L sucrose, 5 mmol/L HEPES, pH 7.4, and the homogenate centrifuged at $3000\times g$ for 2 min, then the supernatant was centrifuged at $14\,600\times g$ for 12 min and the resulting pellet saved. The white loosely-packed layer of the pellet, the synaptosome-enriched fraction, was carefully removed with a spatula and resuspended in 0.32 mol/L sucrose, 5 mmol/L HEPES, pH 7.4, at a protein concentration of approximately 10 g/L and stored on ice until use. Glutamate release from the synaptosomal preparation was measured within 6 h after preparation.

Glutamate release Glutamate release from the synaptosomal preparation was measured as described in a previous study[12]. Immediately before measurement of glutamate release, an aliquot of synaptosomes (approximately 1 mg of protein) was washed twice in 3 volumes of HB buffer consisting of NaCl 140 mol/L, KCl 5 mol/L, 2 mol/L $CaCl_2$, 20 mol/L HEPES, 5 mol/L $NaHCO_3$, 1 mol/L $MgCl_2$, 1.2 mol/L Na_2HPO_4, and 10 mol/L glucose, pH 7.4, by centrifugation for 10 s at $10\,000\times g$ in a bench microcentrifuge, and the pellets resuspended in 3 mL of HB buffer containing 1 mmol/L $NADP^+$ and 50 U/mL of glutamate dehydrogenase. The fluorescence of the NADPH generated by enzymatic catalysis in response to stimulation was measured in a stirred cuvette using a spectrofluorimeter (Spex Industries, Edison, NJ, USA) with excitation and emission wavelengths of 340 nm and 460 nm, respectively. The traces were calibrated by addition of 2 nmol of glutamate at the end of each assay and expressed as nmol/mg of synaptosomal protein.

Measurement of the $[Ca^{2+}]_i$ The change in the $[Ca^{2+}]_i$ was measured using the fluorescent Ca^{2+} indicator, Fura-2, as described in a previous study[13]. Cells grown on glass coverslips were loaded with Fura-2 by incubation for 20 min at 37 ºC with 5 μmol/L fura-2 AM (Molecular Probes, Eugene, OR, USA) in loading buffer consisting of 150 mmol/L NaCl, 5 mmol/L KCl, 5 mmol/L glucose, 2.2 mmol/L $CaCl_2$, 1 mmol/L $MgCl_2$, and 10 mmol/L HEPES, pH 7.4, as described in a

previous study[14]. The coverslips were then mounted in a modified Cunningham chamber attached to the stage of a Nikon Diaphot (Shinnagawa-ku, Tokyo, Japan) inverted microscope equipped with a Nikon ×40 Fluor objective, and the fluorescence of the cells monitored using a dual-excitation spectrofluorimeter with a photomultiplier-based detection system (Spex Industries). Using a pinhole diaphragm placed in the image plane in front of the photomultiplier, one cell was selected per coverslip and excited alternately with 340 and 380 nm light, and the emitted fluorescent light collected via the objective through a 510 nm long wave-pass filter. The $[Ca^{2+}]_i$ was expressed as the ratio of the fluorescence using excitation at 340 nm and 380 nm. Changes in the $[Ca^{2+}]_i$ in response to KCl, ATP, leu-enkephalin, or [D-Ala^2, N-Me-Phe^4, Gly^5-ol]-enkephalin (DAMGO) were measured in loading buffer, whereas changes in the $[Ca^{2+}]_i$ in response to known or suspected NMDA receptor agonists were measured in loading buffer lacking Mg^{2+} to avoid Mg^{2+}-dependent block of the NMDA receptor[15] and containing 10 μmol/L glycine, an NMDA receptor coagonist[16]. Experiments were repeated at least six times using different batches of cells; the results of one representative experiment are shown in the Figures 1, 2, and 3. In some experiments, the means±SD values for the 340/380 fluorescence ratio, calculated for *n* experiments, are also shown.

Results

The neuronal character of the cultured hippocampal neurons used was judged by the extent of the NMDA- or KCl-induced $[Ca^{2+}]_i$ increase. In hippocampal neurons prepared from control rats, the addition of 100 μmol/L NMDA or 50 mmol/L KCl resulted in an increase in the $[Ca^{2+}]_i$ from a basal ratio level of 1.2±0.1 (n=37) to 1.9 ± 0.2 (n=6) or 2.5±0.3 (n= 9), respectively, (Figure 1A, traces a and b), showing activation of ionotropic NMDA receptors and voltage-sensitive Ca^{2+} channels. The $[Ca^{2+}]_i$ remained at this level as long as NMDA or KCl was present, and reproducible $[Ca^{2+}]_i$ increases were evoked by repetitive stimulation. In contrast, the amplitude of the $[Ca^{2+}]_i$ increase progressively decreased if the cells were repetitively stimulated by the G protein-coupled receptor agonists, ATP, DAMGO, and leu-enkephalin (Figure 1A, traces c–e), showing desensitization. We next determined whether chronic pre/postnatal morphine exposure modified the effect of glutamate on the $[Ca^{2+}]_i$. As shown in Figure 1B, glutamate caused a dose-dependent $[Ca^{2+}]_i$ increase in cells of pups from both control and morphine-treated rats. The EC_{50} values for glutamate were indistinguishable, being 1.0 μmol/L for control cells and 0.9 μmol/L for the morphine-exposure cells. The same was true for the response to NMDA, the EC_{50} values being 9.0 μmol/L and 8.2 μmol/L, respectively (Figure 1C). We then examined the effect of NO on the NMDA-induced $[Ca^{2+}]_i$ increase in control and morphine-exposed neurons. At NMDA concentrations lower than 30 μmol/L, S-Nitrosoglutathione (GSNO) (300 μmol/L), an NO donor, potentiated the action of NMDA to the same extent in both groups (Figure 1C), while the other NO donors, 3-morpholinosydnonimine (SIN1), sodium nitroprusside (SNP), and S-nitroso-N-acetylpenicillamine (SNAP), had no effect (Figure 1D) suggesting that it was the glutathione, and not the NO, liberated during GSNO degradation that activated the NMDA receptor.

GSNO alone was as effective as NMDA in inducing a $[Ca^{2+}]_i$ increase, the amplitudes of the increase induced by GSNO or MMDA being indistinguishable (Figures 1C, 1D and 2). In addition, the effect of GSNO was inhibited by addition of Mg^{2+} or the NMDA receptor antagonist, AP-5, to the bathing solution (Figure 2A, traces a and b, and 2B, trace b), suggesting that the NMDA receptor was activated by GSNO. Glutathione had a similar effect to GSNO in terms of effectiveness and blockade of the effect by Mg^{2+} and AP-5 (Figure 2B, trace c). As shown in Figure 2C, the effect of both GSNO and glutathione on the increase of $[Ca^{2+}]_i$ was concentration-dependent, the EC_{50} values being 56 and 414 μmol/L, respectively, in both the control and morphine-addicted groups.

These results suggest that glutathione activates the NMDA receptor and NO then modulates its activity. To clarify the role of NO and whether the sulfhydryl groups of the receptor were required for Ca^{2+} flux, we next examined the effect of N-ethylmaleimide on the NMDA-induced $[Ca^{2+}]_i$ increase in the absence or presence of GSNO and found that it had no effect (Figure 3A), showing that sulfhydryl groups were not involved.

As glutathione is a tripeptide of glutamate, cysteine, and glycine, it was possible that glutamate, generated by glutathione degradation, activated the NMDA receptor. To exclude this possibility, we measured the glutamate-, glutathione-, and GSNO-induced $[Ca^{2+}]_i$ increases in the presence of 20 U/mL of glutamate pyruvate transaminase and 10 mmol/L pyruvate to scavenge glutamate and found that the effect of glutamate was markedly inhibited, while those of glutathione and GSNO were unaffected (Figure 3B). We also examined the effect of other glutathione derivatives on the $[Ca^{2+}]_i$. When we examined whether the unmodified sulfhydryl group of glutathione was required for the $[Ca^{2+}]_i$ increase, we found that *S*-methylglutathione, *S*-ethylglutathione, *S*-propylglutathione, *S*-butylglutathione, or oxidized

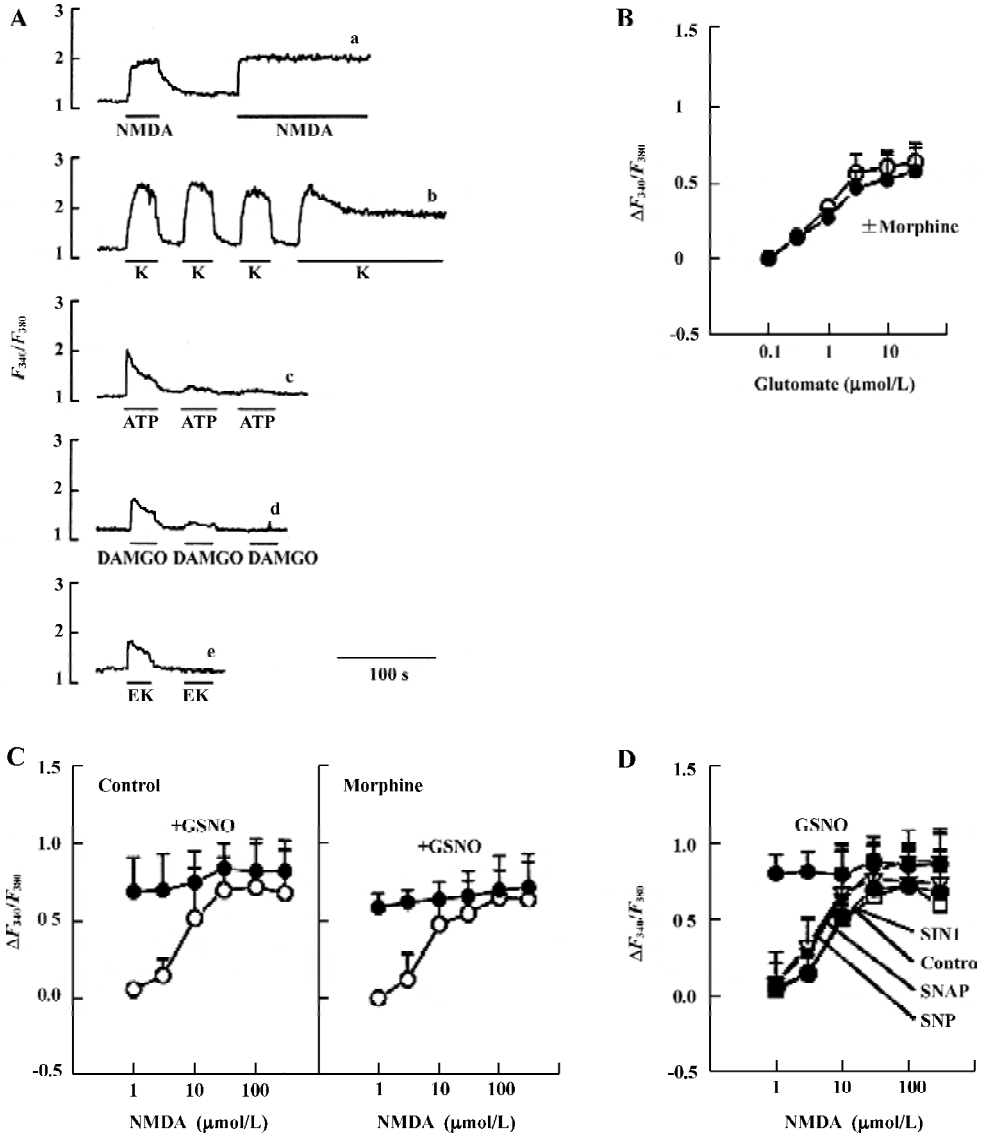

Figure 1. $[Ca^{2+}]_i$ increase induced by various agonists in cultured hippocampal neurons with or without pre/postnatal morphine treatment. (A) $[Ca^{2+}]_i$ changes in Fura-2-loaded cells were measured in response to repetitive stimulation with 100 μmol/L NMDA (trace a), 50 mmol/L KCl (trace b), 1 mmol/L ATP (trace c), 10 μmol/L DAMGO (trace d), or 10 μmol/L leu-enkephalin (EK) (trace e), as indicated by the horizontal bars. The $[Ca^{2+}]_i$ is expressed as the Fura-2 340/380 nm fluorescence ratio. Experiments were repeated six times with similar results; one representative result is shown. (B) $[Ca^{2+}]_i$ increases induced by various concentrations of glutamate in cells prepared from pups from control (white circles) or morphine-addicted (black circles) rats. (C) The experiments were identical to those in (B) except that the $[Ca^{2+}]_i$ increases were induced by various concentrations of NMDA and in the absence (white circles) or presence (black circles) of 300 μmol/L GSNO. (D) $[Ca^{2+}]_i$ increases induced by various concentrations of NMDA in the absence (Control) (white circles) or presence of 300 μmol/L GSNO (black circles), 300 μmol/L 3-morpholinosydnonimine (SIN1) (white squares), 300 μmol/L sodium nitroprusside (SNP) (white inverted triangles), or 300 μmol/L *S*-nitroso-*N*-acetylpenicillamine (SNAP) (black inverted triangles) measured in cells from the control rats. (B–D) The data are the means±SD for at least 26 cells for each concentration of agonist measured in 6 experiments.

glutathione failed to induce a $[Ca^{2+}]_i$ increase (Figure 3B, traces a and b), showing that this group was required. The glutathione-derived dipeptides, Glu-Cys and Cys-Gly, were ineffective (Figure 3B trace d), as were the antioxidants, dithiothreitol and mercaptoethanol (Figure 3B, trace c).

We finally examined whether glutathione acted as an NMDA receptor agonist in inducing glutamate secretion from synaptosomes. As shown in Figure 4A, the fluorescence of

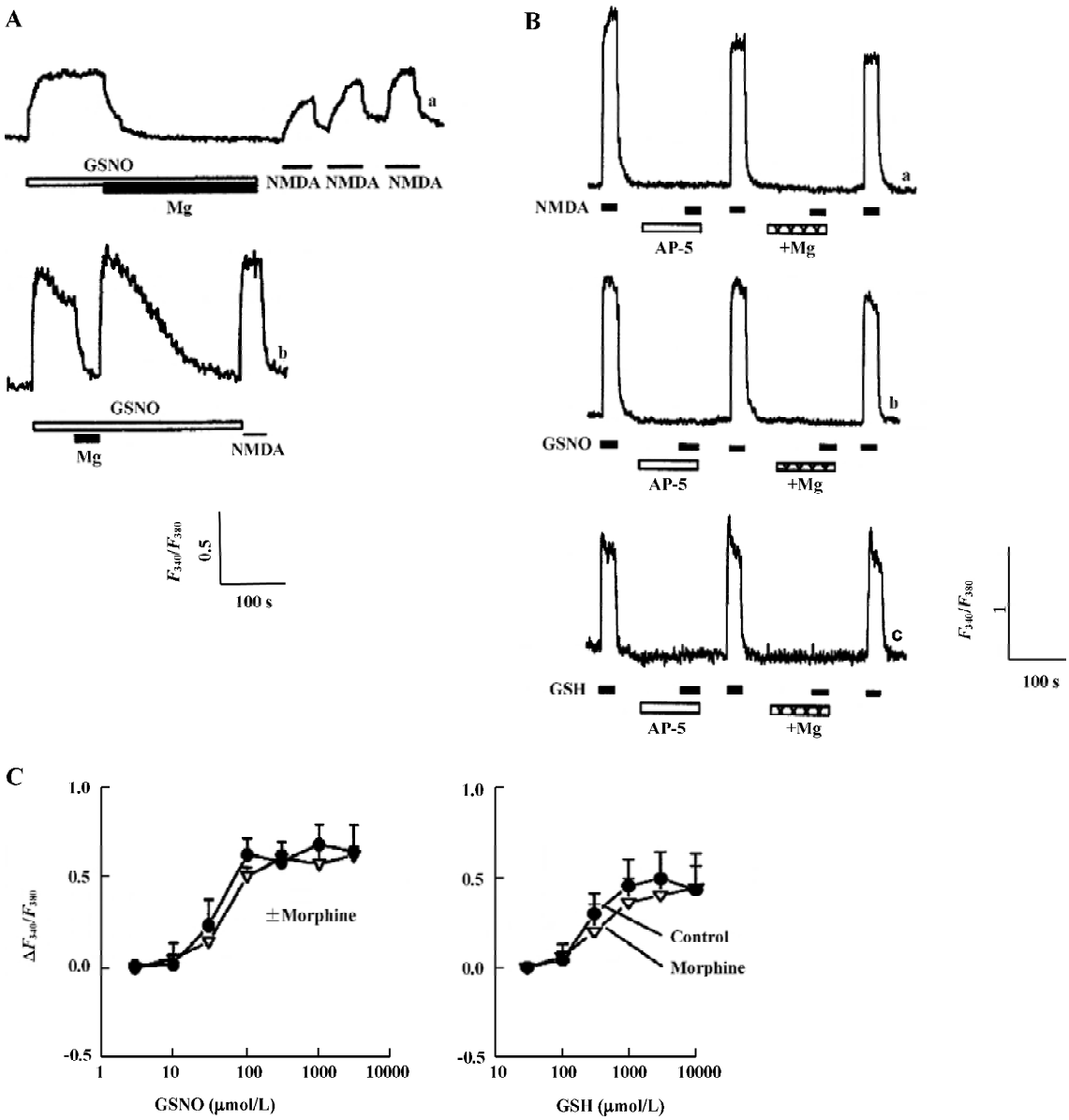

Figure 2. Effect of GSNO or glutathione on the $[Ca^{2+}]_i$ acting via NMDA receptor activation. (A) Representative traces from two cells (a and b) showing $[Ca^{2+}]_i$ increases induced by 300 μmol/L GSNO or 100 μmol/L NMDA, as indicated by the horizontal bars, and the effect of adding back 1 mmol/L $MgCl_2$ to the bathing buffer. Similar results were seen with more than 30 cells. (B) $[Ca^{2+}]_i$ increases on repetitive stimulation by 100 μmol/L NMDA (trace a), 300 μmol/L GSNO (trace b), or 300 μmol/L glutathione (GSH) (trace c), applied as indicated by the black bars. The white and hatched bars show, respectively, inclusion of 10 μmol/L AP-5 or 1 mmol/L $MgCl_2$ in the bathing solution. Experiments were repeated five times with similar results; one representative result is shown. In A and B, the scale is shown in the insert. (C) $[Ca^{2+}]_i$ increases induced by various concentrations of GSNO (left panel) or glutathione (GSH) (right panel) in cells prepared from control (black circles) or morphine-addicted (white inverted triangles) rats. The data are the means±SD for 32 cells for each GSNO concentration and 28 for each glutathione concentration measured in 6 separate experiments.

NADPH, generated by glutamate dehydrogenase in the presence of glutamate, showed a marked increase when the synaptosomes were permeabilized by digitonin, as all the glutamate in the nerve terminals leaked out. A high KCl solution (50 mmol/L), which evoked an increase in the $[Ca^{2+}]_i$ via voltage-sensitive Ca^{2+} channels (Figure 1A, trace b), induced glutamate secretion, as shown by the increased NADPH fluorescence. Glutathione also stimulated glutamate

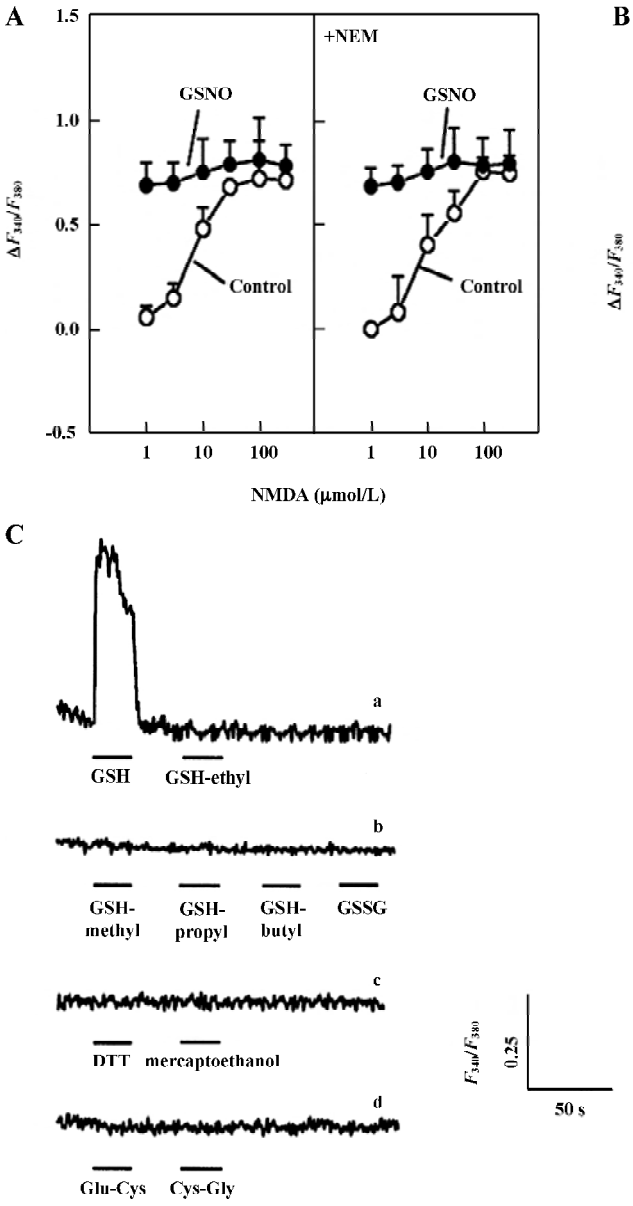

Figure 3. Effect of NMDA, glutathione, or glutathione derivatives on the $[Ca^{2+}]_i$ in the absence or presence of *N*-ethylmaleimide or a glutamate scavenger. (A) $[Ca^{2+}]_i$ increase caused by the indicated concentration of NMDA in the absence (white circles) or presence (black circles) of 300 μmol/L GSNO either in the absence (left panel) or presence (right panel) of 1 mmol/L *N*-ethylmaleimide (NEM). The data are the means±SD for 28 cells for each NMDA concentration measured in 6 separate experiments. (B) $[Ca^{2+}]_i$ increases induced by 100 μmol/L glutamate (Glu), 300 μmol/L glutathione (GSH), or 300 μmol/L GSNO in the absence (white bars) or presence (hatched bars) of 20 U/mL of glutamate pyruvate transaminase (GPT) and 10 mmol/L pyruvate (PA). The data are the means±SD for four experiments using different batches of cells, each performed on at least 14 cells for each group. (C) $[Ca^{2+}]_i$ increases induced by glutathione (GSH) or *S*-ethylglutathione (GSH-ethyl) (trace a), *S*-methylgluta-thione (GSH-methyl), *S*-propylglutathione (GSH-propyl), *S*-butylglutathione (GSH-butyl), or oxidized glutathione (GSSG) (trace b), dithiothreitol (DTT) or mercaptoethanol (trace C), or Glu-Cys or Cys-Gly (trace d), applied as indicated by the horizontal bars. The concentration of all compounds was 300 μmol/L. Experiments were repeated four times with similar results; one representative result is shown.

secretion, although to a slightly lower extent than 50 mmol/L KCl. The effect of glutathione was dose-dependent, with an EC_{50} in the control group of 580 μmol/L (Figure 4B), consistent with the value for inducing the $[Ca^{2+}]_i$ increase (Figure 2C). The potency of glutathione was very similar in the morphine-treated group (EC_{50} 609 μmol/L).

Discussion

To characterize the effect of NO on the interrelationship between chronic morphine exposure and NMDA receptor signaling, we compared its effect on the NMDA-induced $[Ca^{2+}]_i$ increase in the control and chronic morphine-treated groups. As shown in Figure 1, although GSNO potentiated the action of NMDA on the $[Ca^{2+}]_i$ at an NMDA concentration lower than 30 μmol/L, this potentiation was not altered by chronic morphine treatment. Other NO donors tested did not have such effect (Figure 1). These results show that NO does not modulate glutamatergic transmission in hippocampal neurons of neonatal rats from control or morphine-addicted female rats and that the potentiating effect of GSNO is attributable to its glutathione moiety, rather than NO. Our previous study showed that the duration of synaptic NMDA receptor-mediated currents in the hippocampus of the 2-week-

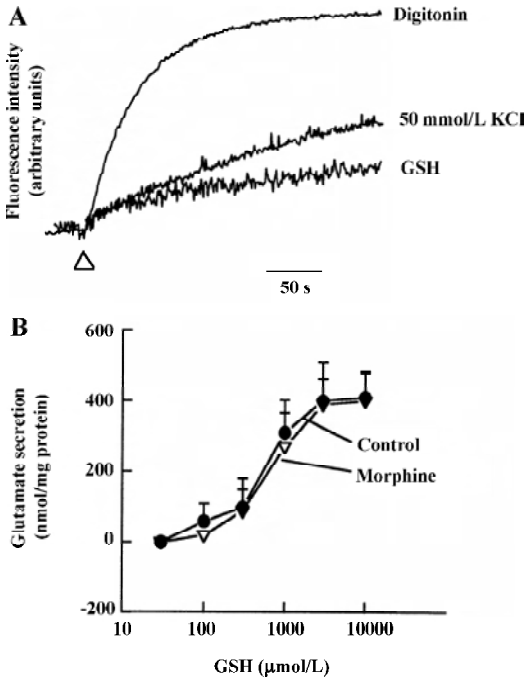

Figure 4. Glutathione-induced glutamate release from synaptosomes with or without pre/postnatal morphine treatment. Glutamate released from synaptosomes was measured enzymatically and is expressed as the NADPH fluorescence intensity (A) or normalized by addition of 2 nmol of glutamate at the end of each assay and divided by the amount of synaptosomal protein (B). (A) Fluorescence increase with synaptosomes from control rats in response to addition of 300 μmol/L glutathione (GSH), 50 mmol/L KCl, or 0.02% digitonin, applied as indicated by the triangle. The experiment was performed four times with similar results; one representative result is shown. (B) Glutamate release induced by various concentrations of GSH from synaptosomes prepared from control (black circles) or morphine-addicted (white inverted triangles) rats. The data are the means±SD for four different experiments.

old offspring of morphine-treated rats is extended, presumably resulting in increased Ca^{2+} entry through NMDA receptor channels[8]. However, the present study, using the same pre/postnatal exposure protocol, showed that the $[Ca^{2+}]_i$ increase evoked by activation of NMDA receptor channels was not significantly different in control and morphine-treated rats (Figure 1) and that pre/postnatal morphine treatment has no effect on glutamate release from the hippocampal nerve terminals of the offspring (Figure 4). This difference may be because of the fact that hippocampal slices prepared from 2-week-old offspring were used in the previous study, while, cultured hippocampal neurons prepared from neonatal rats were used in the present study.

Glutathione, acting as a neuromodulator, displaces ionotropic glutamate receptor ligands from their binding sites and regulates calcium influx through the NMDA receptor[17–19]. Our results showed that glutathione also acted as an NMDA receptor agonist. Not only did it cause a dose-dependent $[Ca^{2+}]_i$ increase and the response was blocked by Mg^{2+} and AP-5 (Figure 2), but it also evoked glutamate release from nerve terminals (Figure 4). Of all the glutathione derivatives and antioxidants tested, glutathione was the only species to activate the NMDA receptor (Figure 3). S-nitrosylation of NMDA receptor thiol groups by NO results in channel inactivation[20]. Furthermore, in a ligand binding study on pig cerebral cortical synaptic membranes, GSNO was shown to act as an NMDA receptor ligand[21]. If the effect of GSNO on the $[Ca^{2+}]_i$ were attributable to its degradation product, glutathione, GSNO would be expected to be less effective than glutathione, because the NO released by GSNO breakdown would inactivate the NMDA channel once activated by glutathione and, in addition, GSNO would not be completely degraded to glutathione. However, this was not the case, as GSNO was actually more potent than glutathione, the EC_{50} values for inducing a $[Ca^{2+}]_i$ increase being 56 μmol/L and 414 μmol/L for GSNO and glutathione, respectively (Figure 2). Pretreatment of the cells with N-ethylmaleimide did not inhibit the potentiation effect of GSNO on the NMDA-induced $[Ca^{2+}]_i$ increase, ruling out the possibility that the effect was because of nitrosylation caused by GSNO. Thus, GSNO itself also acts as an NMDA receptor agonist. The effect of GSNO was inhibited by the addition of Mg^{2+} or an NMDA receptor antagonist (Figure 2). In addition, repetitive stimulation with either GSNO or glutathione induced $[Ca^{2+}]_i$ increases (Figure 2), a typical character of NMDA receptor activation[14], and both retained their ability to increase the $[Ca^{2+}]_i$ in the presence of a glutamate scavenger (Figure 3) suggesting that the molecules themselves, rather than the degradation product, glutamate, are the active species. Taken together, our data show that GSNO and glutathione are endogenous NMDA receptor agonists.

In conclusion, in an attempt to characterize the interrelationship between chronic pre- and post-natal morphine exposure and the glutamatergic neurotransmission, we measured NMDA receptor agonist-induced $[Ca^{2+}]_i$ increase and glutamate secretion in hippocampal neurons of the offspring of morphine-addicted female rats. Our results indicate that combined pre- and postnatal morphine exposure does not modulate NMDA receptor signaling in the cultured hippocampal neurons. However, we also found that GSNO and glutathione act as an endogenous NMDA receptor agonist.

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