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Prostaglandin $F_{2\alpha}$ Is Required for NMDA Receptor-Mediated Induction of c-*fos* mRNA in Dentate Gyrus Neurons

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Activation of NMDA receptors has been linked to a diversity of lasting physiological and pathological changes in the mammalian nervous system. The cellular and molecular mechanisms underlying permanent modifications of nervous system structure and function after brief episodes of neuronal activity are unknown. Immediate-early genes (IEGs) have been implicated in the conversion of short-term stimuli to long-term changes in cellular phenotype by regulation of gene expression. The intracellular signaling pathways coupling activation of receptors at the cell surface with induction of IEGs in the nucleus are incompletely understood. NMDA produces a striking increase in the IEG c-*fos* in dentate gyrus (DG) neurons *in vitro*; this induction is dependent, in part, on the arachidonic acid cascade. Here we show that NMDA receptor activation triggers the synthesis of the prostaglandins $PGF_{2\alpha}$ and PGE_2, but not PGD_2, in rat cerebral cortical neurons *in vitro*. We further demonstrate that $PGF_{2\alpha}$, but not PGE_2 or PGD_2, is necessary but not sufficient for NMDA induction of c-*fos* mRNA in DG neurons. These findings provide insight into the molecular events coupling activation of the NMDA receptor with regulation of the IEG c-*fos* and identify the diffusable messenger $PGF_{2\alpha}$ as obligatory for NMDA receptor-mediated transcription of a nuclear IEG.

Key words: c-fos; NMDA; prostanoids; immediate-early genes; dentate granule neurons

Many activity-dependent modifications of structure and function in the CNS require synaptic activation of glutamate receptors, in particular those of the NMDA subtype. The cellular and molecular mechanisms by which brief episodes of activity at these synapses produce permanent modifications of neuronal phenotype are not known. Immediate-early genes (IEGs) provide an attractive mechanism by which brief activation of NMDA receptors may produce lifelong changes in neuronal structure and function through regulation of the expression of late response genes. High-frequency stimulation of afferents in the CNS induces both lasting changes in synaptic efficacy and striking increases in mRNA and protein content of the IEG c-*fos* in the hippocampal formation (Dragunow et al., 1987; Simonato et al., 1991; Labiner et al., 1993; Worley et al., 1993). The precise sequence of events leading to the transcriptional activation of IEGs after extracellular stimuli is not fully understood. Defining the intracellular signaling pathways underlying receptor-mediated regulation of IEGs is crucial to understanding the development of activity-dependent modifications in neuronal structure and function.

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The intracellular signaling pathways involved in NMDA receptor-mediated induction of the IEG c-*fos* have been partially defined (Morgan et al., 1986; Szekely et al., 1989; Lerea et al., 1992, 1995; Bading et al., 1993). Our previous studies have focused on the regulation of c-*fos* mRNA after activation of NMDA receptors on dentate gyrus (DG) neurons *in vitro*. We have demonstrated that induction of c-*fos* mRNA by NMDA in DG neurons requires increases of intracellular calcium ($[Ca^{2+}]_i$) and the activation of the arachidonic acid (AA) signaling cascade (Lerea et al., 1992, 1993). Multiple inhibitors of phospholipase A_2 (PLA_2), an enzyme involved in the generation of AA, selectively inhibit NMDA induction of c-*fos* mRNA in DG neurons *in vitro* (Lerea et al., 1993). AA is rapidly metabolized to prostaglandins (PGs) or thromboxanes by cyclooxygenases (COXs), or leukotrienes and HETES by lipoxygenases; many of these metabolites may play a role in receptor-mediated alterations in nervous system structure and function (Piomelli et al., 1987; Williams et al., 1989; Schacher et al., 1993). We demonstrated that inhibitors of COX, but not lipoxygenase, selectively diminished NMDA induction of c-*fos* mRNA in DG neurons *in vitro* (Lerea et al., 1993, 1995). These pharmacological findings formed the basis for two hypotheses: (1) NMDA receptor activation evokes the synthesis of a prostanoid metabolite(s), and (2) the metabolite(s) is required for the transcription of c-*fos* initiated by NMDA receptor stimulation. Our goals were to identify biochemically which PGs are produced in a NMDA-dependent manner from CNS neurons and to test the effects of these PGs on NMDA receptor regulation of c-*fos* transcription using single-cell *in situ* hybridization as described previously (Lerea et al., 1993).

The pioneering work of Dumuis et al. (1988) demonstrated

NMDA receptor-mediated release of AA and some lipoxygenase metabolites from striatal neurons *in vitro* via activation of PLA_2. To date, however, efforts to identify the synthesis of PGs after NMDA receptor activation have been difficult and unsuccessful. The limited amount of tissue available when using neurons derived from discrete regions such as striatum or dentate gyrus has hampered the detection of PG metabolites. To circumvent this problem, we have used neuronal cells obtained from the cerebral cortex together with a highly sensitive and direct detection method, gas chromatography/mass spectrometry (GC/MS), to identify PGs synthesized after NMDA receptor activation. The cerebral cortex provides abundant cellular material to successfully detect released substances such as AA and its metabolites. We report here the induction of two PGs from cerebral cortical cells after addition of NMDA. Only one specific PG, however, $PGF_{2\alpha}$, is able to rescue the induction of c-*fos* mRNA in DG neurons when added in the presence of NMDA *and* a COX inhibitor such as indomethacin or aspirin.

MATERIALS AND METHODS

Cell preparation. Mixed neuronal and astroglial cell cultures were prepared from embryonic day 18 rat cerebral cortices as described by Patel et al. (1996). Briefly, cerebral cortices were dissected and the meninges and olfactory lobes removed and discarded. The remaining tissue was minced, enzymatically dissociated, rinsed, and dispersed into a single-cell suspension. The cell suspension was centrifuged, and the pellet was resuspended in MEM supplemented to contain 22 mM glucose, 5% bovine serum, 5% fetal horse serum (MEM-G). An estimate of viable cells was obtained using trypan blue dye exclusion; cells were plated in a volume of 4.5 ml/plate at a density of 1.6×10^5 cells/cm^2 on poly-D-lysine-coated 60 mm Falcon plates. Cells were maintained *in vitro* at 37°C in a humidified incubator with 5% CO_2/95% air for 13–14 d before use in experiments.

Dentate gyrus cells were prepared from 4-d-old rats as described by Lerea et al. (1992). Briefly, each hippocampus was dissected and sectioned into 600- to 800-μm-thick transverse slices, and the DG was separated from the hippocampal gyrus by microdissection. All tissue was enzymatically dissociated, rinsed, and dispersed into a single-cell suspension. The cell suspension was centrifuged, and the pellet was resuspended in MEM supplemented to contain 33 mM glucose, 1 mM pyruvic acid, 2 mM $CaCl_2$, 20 mM KCl, and 10% fetal bovine serum. Cells were plated in a small volume at a density of $4–6 \times 10^3$ cells/cm^2 onto poly-D-lysine-coated glass chamberslides. Cells were maintained *in vitro* at 37°C in a humidified incubator with 5% CO_2/95% air for 7–9 d before use.

Analyses of [^{14}C]AA in cortical cells. Growth media was removed and cells were preincubated for 18–24 hr at 37°C with freshly prepared MEM-G containing 1.0 μCi/ml [^{14}C]AA (specific activity 53–57 mCi/mmol). Cells were then rinsed four times with 2 ml of $HBSS^+$ (Ca^{2+}/Mg^{2+}-free HBSS supplemented to contain 2.3 mM $CaCl_2$, 26 mM $NaHCO_3$, 10 mM HEPES, and 5 μM glycine) at 5 min intervals to remove the unincorporated [^{14}C]AA, which represented 5–20% of the total. After a fifth rinse, the cells were equilibrated for 30 min at 37°C before treatment with NMDA or vehicle. MK-801 (final concentration 3 μM) was added to each plate 30 min after addition of NMDA or vehicle to prevent subsequent NMDA receptor activation. Media was removed from each plate at the indicated times post-treatment, and the cells were rinsed with an additional 2 ml of $HBSS^+$ containing 0.05% fatty acid-free BSA. The media and BSA wash were combined from each plate, acidified with formic acid to a final concentration of 0.1%, and extracted twice with ethyl acetate. The organic phases from these extractions were dried under vacuum and resuspended in 45 μl of chloroform–methanol (2:1, v/v). Each sample was spotted onto a preadsorbant zone on Whatman-LK6DF TLC plates and chromatographed with a solvent system of chloroform–methanol–acetic acid–water (90:8:1:0.8, v/v/v/v) until the solvent front migrated 80% of the length of the plate. The relative amounts of [^{14}C]AA released after the designated treatments were quantified with a Molecular Dynamics PhosphorImager by determining the relative intensity of [^{14}C]AA in each sample comigrating with AA standard.

Cerebral cortical cell treatment for PG determination. Cells were rinsed twice in serum-free MEM-G and maintained in this medium in the presence or absence of 3 μM MK-801 for 24 hr. This pretreatment with MK-801 was done to reduce basal NMDA receptor activation in these high-density cultures. Serum-free growth medium was removed, and cells were maintained in $HBSS^+$ at 37°C for 2.5 hr before stimulation. Cells were subsequently incubated with the specified inhibitors and/or receptor agonists for the designated times at 37°C; all drugs were added to the cellular environment as 10× stock solutions. At the designated times, the medium was removed from each plate and transferred to a polypropylene tube. Each plate was rinsed with 1 ml of $HBSS^+$, which was combined with the original media from that plate. The tubes were individually flushed with N_2 gas and frozen at −80°C. Each sample was extracted and analyzed for PG content by stable isotope dilution GC/MS assays as described previously (Parsons III et al., 1988). Absolute PG values varied between experiments because of changes made in the detection assay from experiment to experiment; such changes were aimed at increasing the assay sensitivity so as to detect low levels. All values, therefore, have been normalized to control and are reported as percent increase over control. The remaining cells were frozen at −80°C and subsequently thawed, lysed with 0.1% SDS in PBS, and analyzed for total protein per plate as determined by the Pierce Coomassie Plus Protein Assay. In each experiment, the total protein from each plate differed by no more than 5%. NMDA treatment under the conditions described here were not cytotoxic as assessed by morphology and the lack of LDH release into the media after 90 min of NMDA (our unpublished observations).

*DG cell treatment for c-*fos *mRNA induction.* Dentate gyrus cells were treated as described by Lerea et al. (1992). Briefly, growth medium was removed from each culture well and replaced with $HBSS^+$. Cells were returned to the 37°C incubator for 3–4 hr before stimulation. Cells were incubated with the specified inhibitors and/or receptor agonists for the designated times at 37°C. After treatment, cells were fixed with 4% paraformaldehyde at 4°C for 5 min, rinsed with HBSS containing 10 mM HEPES, and dehydrated through a series of ethanol. Cells were stored at −70°C until used for *in situ* hybridization.

Riboprobe preparation. A full-length rat c-*fos* cDNA insert (generously supplied by J. Morgan and T. Curran) was used to generate antisense and sense c-*fos* riboprobes as described previously (Lerea et al., 1995). Briefly, the full-length c-*fos* cDNA insert was cloned in a pSP65 plasmid containing the SP6 promoter and a single *Xho*I restriction site at base 1353 from the 5′ end. *In vitro* translation of the transcript derived from this plasmid yields the complete FOS protein (Curran et al., 1987). The plasmid was linearized with *Xho*I and riboprobes generated using SP6 polymerase and an *in vitro* transcription assay in the presence of [^{35}S]UTP. Riboprobes were hydrolyzed to ~200 base pairs with sodium carbonate at 60°C. Validation of these riboprobes was assessed in several ways. Hybridization of brain sections prepared from rats killed 30 min after an electrographic seizure results in a discrete pattern of c-*fos* mRNA expression as detected with antisense riboprobe generated from this plasmid (Lerea et al., 1995); this hybridization pattern is identical to the anatomic pattern described previously using radiolabeled oligonucleotide probes prepared to base pairs 270–319 of c-*fos* mRNA (Curran et al., 1987; Simonato et al., 1991). Sense riboprobe generated from this plasmid does not detect any signal. Northern blot analyses of RNA isolated from NMDA-stimulated cerebral cortical neurons maintained *in vitro* or from rat whole cerebral cortex after a pilocarpine (350 mg/kg)-induced seizure when probed with a ^{32}P-labeled c-*fos* antisense riboprobe detect a single band of ~2.2 kb (data not shown), a size consistent with c-*fos* transcripts (Curran et al., 1987).

Riboprobe used for the Northern blot analyses was prepared exactly as described above for the *in situ* experiments except that [^{32}P]UTP was used instead of [^{35}S]UTP. Similar results were obtained with Northern blot analyses using a random-primed DNA probe generated from this plasmid (data not shown).

In situ *hybridization. In situ* hybridization was done as described by Lerea and McNamara (1993). Briefly, cells were incubated for 3–4 hr at 55°C with prehybridization buffer [50% formamide, 10% dextran sulfate, 3× SSC (0.45 M NaCl, 0.045 M citric acid], 5× Denhardt's solution, 500 mg/ml yeast tRNA (Sigma, St. Louis, MO), 500 mg/ml salmon sperm DNA (Sigma), and 10 mM DTT]. Cells were hybridized overnight (~16 hr) at 55°C in the above buffer containing 60 ng/ml ^{35}S-labeled riboprobe. Nonspecific hybridization was determined using ^{35}S-labeled c-*fos* sense riboprobe in adjacent wells. After hybridization, cells were rinsed with 4× SSC (3 times, 15 min each) and treated with RNase A at 37°C for 30 min. Cells were rinsed with 2×, 1×, and 0.5× SSC (15 min each) and 0.1× SSC at 55°C for 30 min. All slides were dipped in NTB-3 liquid emulsion and stored at 4°C for 5–7 d.

Emulsion-coated slides were developed in Kodak D-19, rinsed in water, and fixed in Kodak fixer. Cells were stained for Nissl substance, and silver grains were visualized and counted using bright-field optics on a Zeiss Axiovert microscope interfaced with an Image 1 analysis system (Universal Imaging Corporation, West Chester, PA). Data were collected from morphologically distinct neurons obtained from random fields across each chamberwell. At least three independent chamberslides were used for each experimental condition. Greater than 95% of dentate gyrus neurons respond to glutamate receptor agonists. Data are presented as the mean number of silver grains per single cell ± SEM.

Materials. [^{14}C]AA was purchased from DuPont NEN (Boston, MA) or American Radiolabeled Chemicals (St. Louis, MO). NMDA was purchased from Tocris Neuramin. MK-801 was obtained from Merck (Darmstadt, Germany). Aspirin, indomethacin, and PGs $F_{2\alpha}$, E_2, and D_2 were purchased from Sigma.

RESULTS

NMDA receptor stimulation of AA and eicosanoid synthesis

Previous studies indicated that activation of the NMDA, but not the AMPA/kainate, receptor on DG neurons causes a robust increase in c-*fos* mRNA in a PLA_2- and COX-dependent manner (Lerea et al., 1992, 1993, 1995). We therefore asked whether and which PG(s) is synthesized after AA release from CNS neurons in response to NMDA treatment. AA release from cerebral cortical cells was observed in a concentration-dependent manner with 50 μM NMDA successfully stimulating release; we routinely used saturating concentrations of NMDA (300 μM) to obtain reliable results with the subsequent GC/MS analyses for metabolites. Addition of NMDA (300 μM) to cerebral cortical cells caused a time-dependent release of metabolically labeled AA ([^{14}C]AA). AA release was detected by 10 min after NMDA treatment and continued to increase with maximal levels of release occurring 90 min after addition of NMDA (Fig. 1*a*,*b*). Activation of non-NMDA ionotropic glutamate receptors with kainic acid (30 μM in the presence of APV to block NMDA receptors) did not induce AA release (data not shown). The time course of detectable NMDA-induced AA release provided a framework for measurement of eicosanoid metabolites.

To facilitate detection of PG metabolites from cerebral cortical cells, media samples were taken 90 min after the addition of NMDA (300 μM) and analyzed by GC/MS for PGs $F_{2\alpha}$ ($PGF_{2\alpha}$), E_2 (PGE_2), and D_2 (PGD_2). $PGF_{2\alpha}$ content was increased more than twofold (230% of control) 90 min after addition of NMDA; proportionately lower yet detectable increases were also measured at 30 min (data not shown). Because of the limits of detection of PG metabolites, however, it was not feasible to test earlier time points. Basal levels of $PGF_{2\alpha}$ did not differ between cells pretreated with MK-801 versus no pretreatment. The induction of $PGF_{2\alpha}$ was blocked by MK-801 (3 μM; Fig. 2*a*) as well as by the distinct COX inhibitors indomethacin (10 μM) or aspirin (100 μM) (data not shown). PGE_2 was increased by ~180% after addition of NMDA (Fig. 2*b*); by contrast, PGD_2 was not consistently increased in an NMDA-dependent manner (data not shown). These findings provide direct biochemical evidence for NMDA receptor activation inducing the synthesis and release of distinct PGs.

Role of eicosanoids in NMDA-mediated induction of c-*fos* mRNA

NMDA-induced transcription of c-*fos* in DG neurons is markedly inhibited by COX inhibitors (Lerea et al., 1993, 1995). Because NMDA treatment evoked the synthesis of two PGs in cortical cells, we asked whether $PGF_{2\alpha}$ or PGE_2 was sufficient and/or necessary for the NMDA-evoked transcriptional activation of c-*fos*. $PGF_{2\alpha}$ added directly to DG neurons in the absence of NMDA was not sufficient to induce transcriptional activation of c-*fos* mRNA (Fig. 3*a*). However, addition of $PGF_{2\alpha}$ to DG neurons in the presence of NMDA (50 μM) *and* the COX inhibitor aspirin (used at its IC_{50} concentration of 100 μM) restored the induction of c-*fos* mRNA (Fig. 3*b*). $PGF_{2\alpha}$-mediated rescue of c-*fos* induction occurred in a concentration-dependent manner, saturating between 10 and 30 nM. Similar results were obtained when the structurally distinct COX inhibitor indomethacin was used rather than aspirin (Fig. 3*c*).

Neither PGE_2 nor PGD_2 was able to rescue the inhibition of NMDA-induced c-*fos* mRNA expression by the COX inhibitors aspirin (Fig. 4*a*,*b*) and indomethacin (data not shown). Taken together, these findings demonstrate that $PGF_{2\alpha}$ is necessary, but not sufficient, for NMDA receptor-mediated regulation of the IEG c-*fos* in DG neurons *in vitro*.

DISCUSSION

These findings provide new clues to the chain of molecular events coupling activation of NMDA receptors at the neuronal membrane with regulation of gene expression in the nucleus. We previously demonstrated that NMDA causes a robust increase in $[Ca^{2+}]_i$ in DG neurons and that this increase in $[Ca^{2+}]_i$, along with activation of the AA-signaling cascade, is necessary for the induction of c-*fos* mRNA. The present findings provide direct biochemical evidence for the hypothesis that Ca^{2+} entering through the NMDA receptor evokes activation of PLA_2 and release of AA; AA is metabolized via a COX to a prostanoid that is required for the transcription of the IEG c-*fos*. We identified $PGF_{2\alpha}$ and PGE_2 as specific PGs the synthesis of which is triggered by NMDA, of which only $PGF_{2\alpha}$ is necessary, but not alone sufficient, for NMDA-evoked transcription of c-*fos* in DG neurons.

These findings lead to a number of questions. Which form of COX might be required for the synthesis of $PGF_{2\alpha}$? To date, two forms of COX have been identified by molecular cloning and functional expression, COX1 and COX2 (Yamagata et al., 1993; Breder et al., 1995a,b). The COX inhibitors used in these and other (Lerea et al., 1993, 1995) studies are not sufficiently selective to distinguish the two forms, but expression patterns in rat brain favor COX2. That is, COX2 mRNA and COX2 protein are constitutively expressed in dentate granule cells *in vivo* in both the developing and the mature rat brain; by contrast, COX1 mRNA is not detectable in Northern blot analyses of rat brain (Yamagata et al., 1993).

To which molecule does $PGF_{2\alpha}$ bind in order to promote transcription of c-*fos*? One attractive candidate is the recently cloned $PGF_{2\alpha}$ receptor (Nakao et al., 1993; Kitanaka et al., 1994b; Sugimoto et al., 1994); Northern blot analyses of expression in various organs of rat disclosed the highest levels in brain. Based on sequence homologies, the $PGF_{2\alpha}$ receptor appears to be a G-protein-linked receptor with seven transmembrane domains.

How might activation of the $PGF_{2\alpha}$ receptor participate in the transcription of c-*fos* induced by NMDA? Among the regulatory elements identified in the c-*fos* promoter, the serum response element (SRE) has been suggested to be particularly important for the induction of c-*fos* expression after stimulation of the NMDA receptor in cultured hippocampal neurons (Bading et al., 1993). Calcium flux through the NMDA receptor stimulates ty-

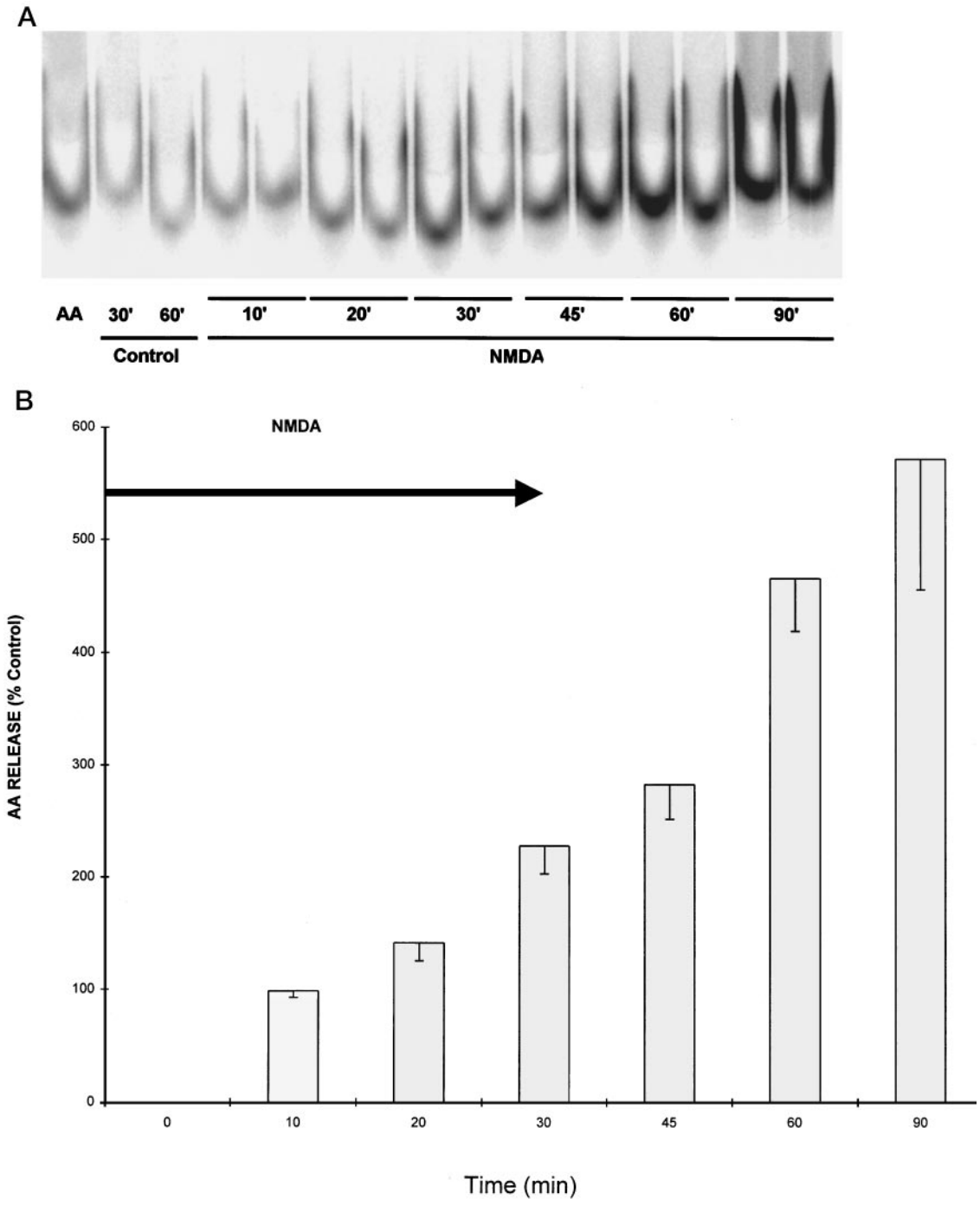

Figure 1. Time course of [^{14}C]AA release from cerebral cortical cells after NMDA treatment. Cerebral cortical cells were pulsed with [^{14}C]AA and treated with vehicle or NMDA (300 μM) for varying lengths of time. Media samples were taken and analyzed for [^{14}C]AA release at the indicated times by TLC analyses as described in Materials and Methods; for the later time points, MK-801 (final concentration 3 μM) was added to the cells 30 min after the initial addition of NMDA to prevent prolonged receptor activation. *A*, Image of a representative TLC plate obtained using a Molecular Dynamics PhosphorImager. Only the region corresponding to AA migration is shown. The *first lane* shows the migration of the [^{14}C]AA standard; each experimental time point was run in duplicate. *B*, Quantification of [^{14}C]AA release after NMDA treatment. The relative intensity of [^{14}C]AA released after NMDA at each time point was compared with the intensity of [^{14}C]AA released under control conditions. Data are expressed as percent of control and were obtained from at least three independent experiments. *Arrow* indicates the duration of NMDA treatment.

rosine and threonine phosphorylation of mitogen-activated protein kinase (MAP kinase) and activates its serine/threonine-specific phosphotransferase activity (Bading et al., 1991). MAP kinase and the MAP kinase-regulated ribosomal protein S6 kinase II can phosphorylate transcription factors that interact with the SRE to promote c-*fos* expression. It has been proposed therefore that Ca^{2+} entering through the NMDA receptor somehow leads to activation of MAP kinase that promotes phosphorylation of

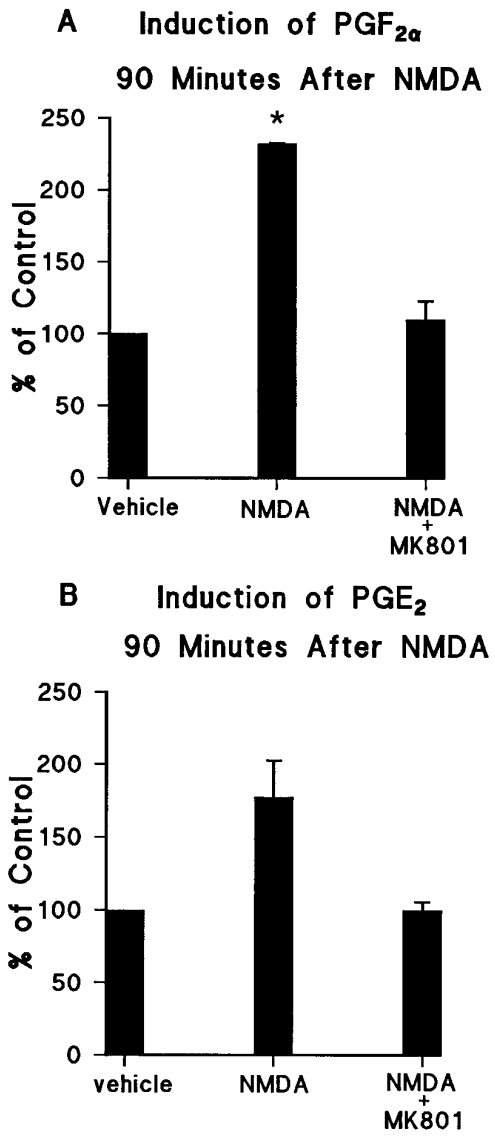

Figure 2. Effects of NMDA treatment on PGs. *A*, Cerebral cortical cells were treated with vehicle or NMDA (300 μM) in the absence or presence of MK-801 (3 μM) for 30 min. After 30 min, cells that had received vehicle or NMDA were treated with MK-801 (3 μM) and all samples were allowed to incubate for an additional 60 min. Media samples were collected 90 min after initial NMDA addition and analyzed for $PGF_{2\alpha}$ as described in Materials and Methods. NMDA treatment resulted in an increase in $PGF_{2\alpha}$ (230% of control) as compared with vehicle-treated cells. This increase in $PGF_{2\alpha}$ was blocked by the NMDA receptor antagonist MK-801. Data are obtained from three independent experiments and are expressed as percent of control; data were analyzed by one-way ANOVA with the Tukey–Kramer *post hoc* test where appropriate ($^*p < 0.001$). *B*, Cerebral cortical cells were treated with vehicle or NMDA as described above. Media samples were collected 90 min after initial NMDA addition and analyzed for PGE_2 as described in Materials and Methods. NMDA treatment resulted in an increase in PGE_2 (180% of control) as compared with vehicle-treated cells. This increase in PGE_2 was blocked by the NMDA receptor antagonist MK-801. Data are expressed as the average values obtained from two independent experiments.

SRE-binding proteins and transcription of c-*fos* (Bading et al., 1991). Interestingly, activation of the $PGF_{2\alpha}$ receptor enhances tyrosine phosphorylation of several proteins including a protein tentatively identified as MAP kinase (Quarles et al., 1993; Watanabe et al., 1994). One possibility is that the cascade of molecular events triggered by NMDA receptor activation leads to synthesis and release of $PGF_{2\alpha}$ and the subsequent activation of the $PGF_{2\alpha}$ receptor which, in turn, leads to the phosphorylation of MAP kinase and promotion of c-*fos* transcription. Interestingly, analyses of c-*fos* expression in transgenic mice disclosed that a point mutation of any one of four regulatory elements in the c-*fos* promoter was sufficient to eliminate stimulus-induced expression of c-*fos*; this underscores the interdependence of different regulatory elements and the transcription factors to which they bind in the regulation of c-*fos* expression (Robertson et al., 1995). Thus, multiple signaling pathways likely act in concert to regulate gene expression. The suggested scenario presented here, if correct, likely represents only part of the molecular events coupling activation of NMDA receptors with c-*fos* transcription; the fact that $PGF_{2\alpha}$ is necessary but not sufficient to activate c-*fos* transcription underscores this idea.

The complex geometry of a neuron imposes spatial constraints on the sequence of molecular events transducing a signal from a receptor on the cell surface to the nucleus. NMDA receptors are enriched in dendrites and preferentially localized to dendritic spines of neurons both *in vivo* and *in vitro* (Pongracz et al., 1992; Petralia et al., 1994; Lau et al., 1995). In the dentate granule cells both *in vivo* and *in vitro*, the most remote extent of the dendritic tree lies ~200 μm from the nucleus in the soma. Activation of NMDA receptors during high-frequency stimulation of CNS afferents is necessary for the full induction of c-*fos* mRNA in dentate gyrus neurons *in vivo* (Labiner et al., 1993). Although the exact site of the NMDA receptors on DG neurons *in vitro* is not known, the interesting question of how a signal triggered by NMDA receptor activation on the membrane surface might be conveyed to the nucleus in the soma to influence gene transcription needs to be asked. It seems plausible that $PGF_{2\alpha}$ might be synthesized locally at or near the site of NMDA receptors (in the dendrites of neurons); COX2, a rate-limiting enzyme likely mediating synthesis of the immediate precursor of $PGF_{2\alpha}$, has been localized exclusively to neurons and, in particular, to dendritic spines (Kaufmann et al., 1996). The cellular and subcellular site at which $PGF_{2\alpha}$ acts to promote c-*fos* transcription is unknown. The diffusible nature of $PGF_{2\alpha}$ might serve a pivotal role in the spatial translocation of the signal from membrane to the nucleus. Although we detected released $PGF_{2\alpha}$ in the media of cultured cortical cells after addition of NMDA, we do not know what concentrations of $PGF_{2\alpha}$ and other AA metabolites remain intracellularly. One possibility, therefore, is that $PGF_{2\alpha}$ acts as an intracellular diffusible signal (intracrine) traveling from the site of synthesis at the membrane to the nucleus; $PGF_{2\alpha}$ may act at a receptor site not yet determined located on the nuclear membrane (Kliewer et al., 1995). Alternatively, $PGF_{2\alpha}$ may be synthesized locally at the site of the NMDA receptor and then diffuse to act extracellularly. In contrast to classical neurotransmitters, PGs are not stored in and released from synaptic vesicles but, instead, are released to the cell exterior where they can diffuse freely. Thus, $PGF_{2\alpha}$ might subsequently activate receptors on the same (autocrine) or neighboring (paracrine) neurons and/or glia. These diffusable messengers may act at both intracellular and extracellular sites. Third, AA released after NMDA receptor activation may diffuse from a neuron to adjacent astroglial cells where it is converted to PGs; $PGF_{2\alpha}$ would then have to diffuse back to the neurons to influence NMDA receptor-mediated

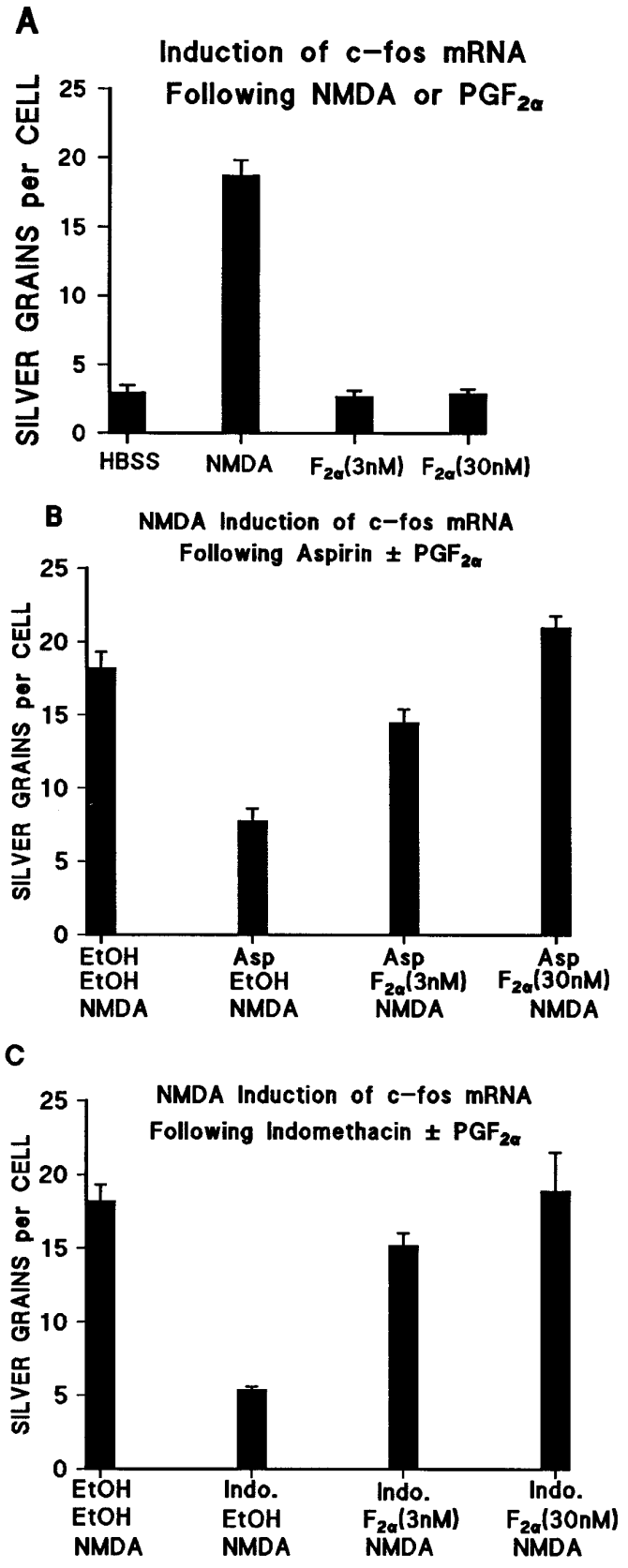

Figure 3. Effects of $PGF_{2\alpha}$ on c-*fos* mRNA induction. *A*, Dentate gyrus cells were treated with vehicle (*HBSS*), NMDA (50 μM), or $PGF_{2\alpha}$ (3 or 30 nM) for 30 min and then processed for c-*fos in situ* hybridization as described in Materials and Methods. Stocks of $PGF_{2\alpha}$ were prepared in 100% EtOH and diluted into HBSS. NMDA was added in the presence of

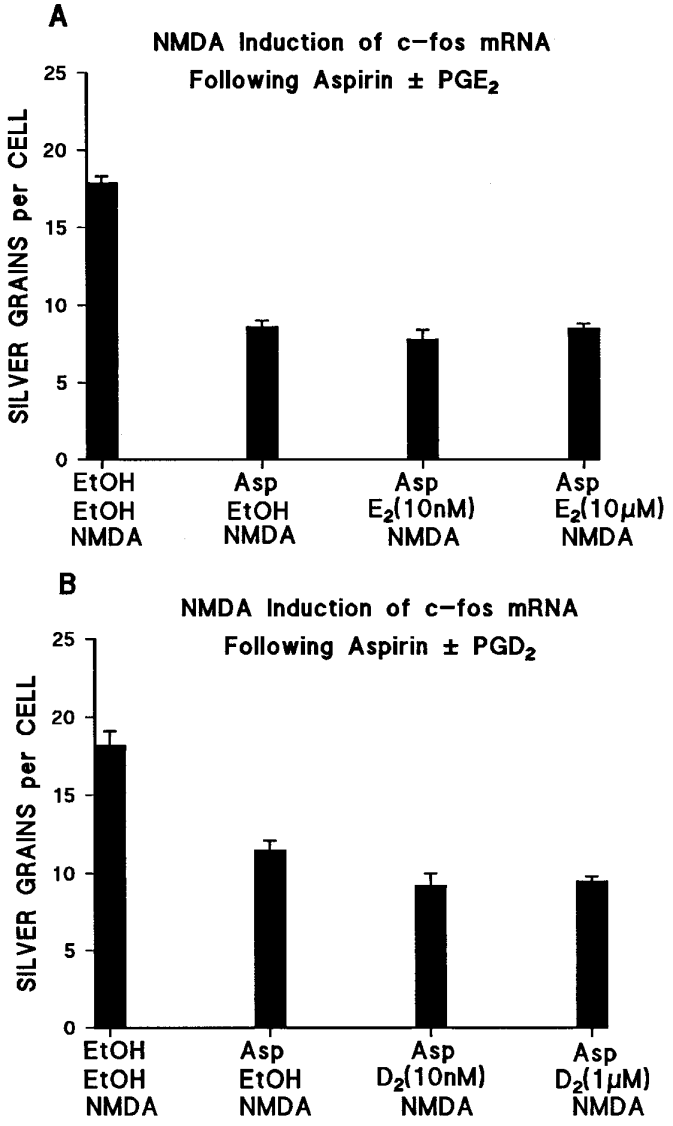

Figure 4. Effects of PGE_2 and PGD_2 on c-*fos* mRNA induction. *A*, *B*, Dentate gyrus cells were pretreated with vehicle or aspirin (100 μM) for 15 min before receiving NMDA (50 μM) or NMDA + PGE_2 (*A*) or PGD_2 (*B*). Cells were processed for c-*fos in situ* hybridization 30 min after NMDA addition as described. NMDA caused a fivefold increase in c-*fos* mRNA; aspirin reduced NMDA induction of c-*fos* mRNA. Neither PGE_2 (*A*) nor PGD_2 (*B*) was able to restore the induction of c-*fos* mRNA by NMDA in the continued presence of aspirin. Each experiment was repeated at least three times.

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10 μM 6-cyano-2,3-dihydroxy-7-nitroquinoxaline (CNQX). Each data point represents silver grain counts over at least 20 individual neurons. NMDA caused a fivefold increase in c-*fos* mRNA; $PGF_{2\alpha}$ alone did not induce c-*fos* mRNA. Each experiment was repeated at least three times. *B*, *C*, Dentate gyrus cells were pretreated with vehicle, the IC_{50} concentration of the COX inhibitor aspirin (100 μM; *B*) or the COX inhibitor indomethacin (1 μM; *C*) for 15 min before receiving NMDA (50 μM) or NMDA + $PGF_{2\alpha}$ (3 or 30 nM). Cells were processed for c-*fos in situ* hybridization 30 min after NMDA addition as described. Each data point represents silver grain counts over at least 25 individual neurons. NMDA caused a fivefold increase in c-*fos* mRNA; both aspirin and indomethacin reduced NMDA induction of c-*fos* mRNA. $PGF_{2\alpha}$ restored the induction of c-*fos* mRNA by NMDA in the presence of aspirin or indomethacin in a concentration-dependent manner. Each experiment was repeated at least three times.

c-*fos* transcription. Although we have never observed NMDA receptor-mediated responses (i.e., intracellular calcium influx, induction of c-*fos* mRNA) in astroglia, astroglial cells have been demonstrated to release AA and metabolites after treatment with a calcium ionophore or glutamate. Glutamate-induced release of AA from glial cells, however, is not blocked by either MK-801 or APV, two NMDA receptor antagonists, and no detectable release is observed after treatment with NMDA (Stella et al., 1994). A potential role of non-neuronal cells, however, must be considered because $PGF_{2\alpha}$ does stimulate phosphoinositide hydrolysis in astroglial cells maintained in primary culture (Kitanaka et al., 1994a).

Pathological activity is a highly effective stimulus for inducing both c-*fos* expression and PG synthesis *in vivo*. Activity-induction of c-*fos* expression exhibits a threshold *in vivo* in that the synchronous activation of NMDA receptors that is sufficient to induce LTP is not always sufficient to consistently induce expression of c-*fos* in the dentate granule cells (Worley et al., 1993). However, pathological activity in the form of seizures produces rapid and dramatic induction of c-*fos* expression in the dentate granule cells *in vivo* (Dragunow et al., 1987; Morgan et al., 1987) and NMDA receptor activation, not simply firing of action potentials, is required for this expression (Labiner et al., 1993). Seizures are also a highly effective stimulus for the release of AA (Bazan, 1989) and synthesis of PGs, producing increases of $PGF_{2\alpha}$ content of >60-fold in the hippocampus with peak levels obtained 6 min after seizure onset (Hertting et al., 1990). Whether LTP-inducing stimuli can evoke PG synthesis and whether NMDA receptor activation is required for the seizure-induction of PG synthesis *in vivo* are unknown. If so, synthesis of PGs may serve as the rate-limiting step in c-*fos* expression *in vivo*.

NMDA receptor activation is intimately associated with activity-determined long-lasting changes in nervous system structure and function, including formation of normal synaptic connections during development and pathological synaptic connections in the adult. The molecular events linking NMDA receptor activation to lasting changes in cell phenotype are not well defined but almost certainly require alterations of gene expression. Insights derived from intensive study of the c-*fos* promoter render c-*fos* an informative model with which to elucidate some of the molecular events linking activation of a surface receptor to the transcriptional activation of nuclear genes. The present findings shed additional light on the molecular events underlying NMDA receptor-mediated regulation of gene expression.

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