Platelet-derived Growth Factor Induces a Long-Term Inhibition of N-Methyl-D-aspartate Receptor Function*

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Platelet-derived growth factor (PDGF) is a multifunctional protein that plays important roles in many tissues, including the mammalian central nervous system. PDGF and PDGF receptors (PDGFRs) are expressed in virtually every region of the central nervous system where they are involved in the development, survival, growth, and differentiation of both neuronal and glial cells. We now report that a brief activation of PDGFRs produced a long-lasting inhibition of N-methyl-D-aspartate (NMDA)-dependent excitatory postsynaptic currents in CA1 pyramidal neurons in rat hippocampal slices. PDGF also inhibited NMDA receptors (NMDA-Rs) in cultured hippocampal neurons by a mechanism that involves a decrease in single channel open probability. Non-NMDA receptor function was not affected by PDGF in hippocampal neurons. Experiments with mutant PDGFRs and chelation of intracellular Ca2+ in Xenopus oocytes indicate that this inhibition depends on a phospholipase C-γ-induced elevation of intracellular Ca2+ levels. The PDGF-induced inhibition of NMDA-Rs is produced by a mechanism different than the well-characterized phenomenon of Ca2+-dependent NMDA-R rundown because the effect of PDGF was blocked by the phosphatase inhibitor, calyculin A, and was not affected by the microtubule polymerizing agent, phallolidin. Because elevations of PDGF levels are associated with neurotrophic trauma or disease, we propose that PDGF can exert neuroprotective effects by inhibiting NMDA-R-dependent excitotoxicity.

Platelet-derived growth factor (PDGF) is a polypeptide of ~30 kDa that was originally purified from human platelets as a potent mitogen for fibroblasts, osteoblasts, smooth muscle cells, and glial cells (1). Three homo- or heterodimeric disulfide-linked isoforms of PDGF (PDGF-AA, PDGF-BB, and PDGF-AB) and two classes of PDGF receptors (PDGFR-α and PDGFR-β) have been identified (2). PDGFRs are tyrosine kinase-coupled receptors that dimerize upon ligand activation and become autophosphorylated on tyrosine residues. These residues act as binding sites for a group of proteins that contain Src homology 2 (SH2) domains. Phospholipase C-γ (PLC-γ), the protein-tyrosine phosphatase Syp (PTP-Syp), Ras GTPase-activating protein (Ras-GAP), the Src family of protein-tyrosine kinases, phosphatidylinositol 3-kinase (PI3K), and several adaptor-type signal transduction proteins (Shc, Grb2, Shb, and Nck) all bind to activated PDGFR-β via SH2 domains (2-4).

PDGFs are multifunctional proteins that regulate a number of physiological and pathophysiological processes, including embryonic and placental development, wound healing, atherosclerosis, cancer, renal diseases, and arthritis (1). In addition to its role in these processes, PDGF is particularly important for the regulation of both the developing and mature central nervous system. In contrast to the restricted localization of most neurotrophic factors, PDGFs and PDGFRs are expressed in virtually every region of the mammalian central nervous system (5-9). In the developing central nervous system, PDGF is important for the normal formation of neural plates and neural tubes (10), for the differentiation of progenitor oligodendrocyte cells (11), and for the chemotaxis and proliferation of glial cells (8, 12). In the mature central nervous system, PDGF is important in the pathophysiology of several disease states. Cell lines from malignant glioma and other central nervous system tumors express PDGFs and PDGFRs, and it has been suggested that growth of some of these tumors could be mediated by an autocrine PDGF/PDGFR loop (13, 14). Moreover, PDGF levels are elevated in non-neoplastic diseases of the central nervous system such as trauma, stroke, meningitis, cerebral abscesses, and glial and meningeal cysts (13, 15, 16). It is likely that this elevation in PDGF levels is involved not only in the pathogenesis of these conditions but also in the tissue repair processes associated with these diseases. In this regard, it has been shown that PDGF exerts neurotrophic effects on GABAergic and dopaminergic neurons (17, 18) and that it protects hip-...
pocampal neurons against energy deprivation and oxidative injury in vitro (19).

In spite of the importance of PDGF for the development and maintenance of the mammalian central nervous system, little is known about its actions on synaptic transmission. To contribute to this issue, we examined the effects of this growth factor on the function of the N-methyl-D-aspartate subtype of glutamate receptors (NMDA-Rs). NMDA-Rs mediate excitatory synaptic transmission in the central nervous system and play important roles in many physiological and pathophysiological processes such as neuronal development and survival, synaptic plasticity, and neurotoxicity (20). We examined the effects of PDGF on NMDA-Rs in hippocampal slices, cultured hippocampal neurons, and Xenopus oocytes. Electrophysiological experiments with these preparations indicate that PDGF exerts long-lasting modulatory effects on the function of NMDA-Rs and that the mechanism of action of PDGF involves a complex intracellular signal transduction cascade that is triggered by PDGF activation.

**EXPERIMENTAL PROCEDURES**

Electrophysiological Recording from Hippocampal Slices and Cultured Hippocampal Neurons—Unless otherwise indicated, all chemicals were from Sigma. Transverse brain slices (400 μm) were obtained from male Sprague-Dawley rats (120–160 g) as described previously (21). Electrophysiological recording and drug applications were performed exactly as described elsewhere (22), except that the patch pipette solution also contained 5 mM QX-314. Pharmacologically isolated NMDA-excitatory postsynaptic currents (EPSCs) were evoked in the presence of the GABA and glutamate receptor blockers bicuculline methiodide (20 μM) and 6,7-dinitroquinoxaline-2,3(1H,4H)-dione (20 μM), respectively. Pharmacologically isolated non-NMDA-dependent EPSCs were recorded as described above but in the presence of DL-2-amino-5-phosphonovaleric acid (50 μM) instead of 6,7-dinitroquinoxaline-2,3(1H,4H)-dione. The membrane holding potential was -45 mV for NMDA recordings and -70 mV for non-NMDA recordings. Synaptic stimulation was delivered using a bipolar, twisted tungsten wire electrode (0.1-ms pulses of 5–20 V) every 20 s.

Cultured mouse hippocampal neurons were grown according to previously described procedures (23) and were used for patch clamp recordings 12–20 days after plating. Recording electrodes with resistances of 2–5 MΩ were constructed from thin-walled borosilicate glass (1.5 mm diameter, WPI Inc., Sarasota, FL). Patch clamp recordings were made in the perforated patch and cell-attached configurations using Axopatch-1B amplifiers (Axon Instruments, Foster City, CA). For perforated patch recordings, data were digitized, filtered (2 kHz), and acquired on-line using the program pClamp 5.1.5 (Axon Instruments). For cell-attached recordings, single channel currents were filmed on videotape using a digital data recorder (VR-10, Instrument Corp., Mineola, NY) and later played back and acquired using the pClamp 6 program (Axon Instruments). Single channel currents were filtered at 2 kHz and sampled at 5 kHz. Only patches with stable basal activities were used to ensure that the changes in activity were not due to rundown or random fluctuations. To study the effects of PDGF on open probability of NMDA channels, a control period of 5 min to record the basal activity was allowed before the introduction of PDGF. The single channel open probability was determined from the ratio of the time spent in the open state to the duration of recording, Popen = (t0 + t1 + t2 + t3)/ttotal, where t is the amount of time that a channel is open, and N is the maximum number of levels observed in the patch. The extracellular solution contained (in mM) NaCl (140), CaCl2 (1.3), KCl (5.4), HEPES (25), glucose (33), tetraethylene (0.0005–0.001) (pH 7.4, using NaOH, 320–335 mM). For perforated patch recordings, 3 μM glycine and 100 μM NMDA were added to the extracellular solution to evoke NMDA currents. For non-NMDA currents, 200 μM kainate was added to the solution. Perforated patch electrodes were filled with (in mM) KCl (55), MgSO4 (70), HEPES (10), glucose (5), and nystatin 0.3 mg/ml. For cell-attached recording, patch electrodes contained (in mM) NaCl (70), Na2SO4 (70), HEPES (10), CaCl2 (1.3), CsSO4 (5), and glucose (33). Glycine 1–3 and NMDA 10 μM were also added to the electrode solution to induce NMDA channel current. All experiments were performed at room temperature (20–22 °C). A multi-barrel perfusion system was employed to achieve a rapid exchange of solutions.

Microinjection and Electrophysiological Recording of Xenopus Oocytes—Human NMDA receptor subunits cDNAs (NR1a, NR2A, NR2B) were cloned on the eukaryotic expression vector pCDNA-I-Amp (In-vitrogen Corp., San Diego, CA); the cloning of these subunits is described elsewhere (24). Human wild-type PDGFR-β were cloned on pBS as described by Kazlauskas et al. (25). The construction of the F5 and the Tyr1063 add-back PDGFR-β mutants have been described elsewhere (19). Subcloning of PDGFR-β mutants and in vitro DNA synthesis of PDGF-Rs was performed as described by Valenzuela et al. (22). The methods used for oocyte preparation/culture, cRNA/cDNA microinjection, and drug application/microinjection are the same as those described by Valenzuela et al. (22), except that Mg2+-free modified Barth’s solution was used instead of complete modified Barth’s solution.

**RESULTS**

Effects of PDGF Activation on Glutamate Receptor Function in Hippocampal Slices and Cultured Hippocampal Neurons—We examined the effects of PDGF activation on pharmacologically isolated NMDA-mediated EPSCs in the hippocampus, a brain region where these two types of receptors are known to be coexpressed (7, 19, 26). Application of PDGF-BB (6 nM for 3 min) significantly decreased the amplitude of NMDA-R-mediated EPSCs (by 44 ± 7%, p < 0.001 by two-way ANOVA, n = 7, Fig. 1). The inhibition began soon after the onset of PDGF-BB application and was not reversed even after a 20-min PDGF washout period. No apparent recovery was observed even in cells held for more than 1 h following PDGF treatment. Hippocampal slices treated with vehicle only did not display any inhibition of NMDA-R-mediated EPSCs under the same recording conditions (Fig. 1). Non-NMDA-dependent EPSCs were not affected by PDGF treatment; the value for the non-NMDA-dependent EPSC in the PDGF-treated slices was 110 ± 6% of control (p > 0.05 by t test, n = 5).

Application of PDGF-BB (7 nM for 15 min) to rat cultured hippocampal CA1 pyramidal neurons in the perforated patch configuration significantly decreased the amplitude of NMDA-gated currents (by 23 ± 3%, p < 0.001 by two-way ANOVA, n = 8) (Fig. 2). The inhibitory effects of PDGF-BB appeared gradually over the 15-min application and were not reversible after a 10-min washout period. Control cells treated with vehicle only did not display any rundown of NMDA-R-dependent currents under the same recording conditions (Fig. 2). The ratio of peak/steady state NMDA-gated currents was significantly reduced (p < 0.05 by t test) by PDGF activation; the ratios in control and PDGF-treated neurons were 1.7 ± 0.07 and 1.5 ± 0.1 (n = 9), respectively. Kainate-evoked currents were not affected by PDGF treatment in cultured hippocampal neurons under the same recording conditions (Fig. 2); the values for kainate-gated currents after 5, 10, and 15 min of PDGF-BB application were 99.5 ± 2, 101 ± 3, and 102 ± 4% of control (n = 7).

The effects of PDGF on NMDA-R function were also studied at the single channel level (Table I, Fig. 3). Application of PDGF (7 nM for 6 min) to cultured hippocampal neurons in the cell-attached configuration produced a significant decrease in the open probability of NMDA channels from 0.05 ± 0.01 to 0.03 ± 0.006 (n = 6, p < 0.05 by t test) with no apparent change in short open time (τ1), long open time (τ2), short closed time (τ3), open probability (Po), or mean open time (τo) (Table I).
Inhibition of NMDA Receptors by PDGF

Effects of PDGF on Oocytes Coexpressing NMDA and PDGF Receptors—To study in greater detail the mechanism of the PDGF-induced inhibition of NMDA-Rs, human PDGFR-β subunit cRNA and human NMDA-R subunit cDNAs were coinjected into Xenopus oocytes. Bath application of PDGF-BB (6 nM) to oocytes expressing PDGFR-β produced inward currents that correspond to Ca2+-activated Cl− currents (22). Activation of PDGFR-β with PDGF-BB significantly inhibited (p < 0.001 by two-way ANOVA) NMDA-gated currents in oocytes expressing NR1a/2A subunits; maximum inhibition (50 ± 6%) was reached 10–40 min after PDGF-BB application and was not reversible even after a 230-min washout period (n = 22, Fig. 4A). NMDA receptors composed of NR1a2B subunits were inhibited to the same extent (66 ± 12%, n = 4) as NR1a/2A receptors. PDGF-BB application did not inhibit NMDA-Rs in oocytes expressing only NR1a2A subunits (without PDGFR-β), which indicates that the observed effects required PDGFR activation (n = 17).

We next determined the effects of PDGFR activation on the NMDA and glycine dose-response curves (Fig. 4, 8 and C). In this batch of oocytes, PDGFR activation produced a 45 ± 8% (n = 9) decrease in the NMDA EC50 (p < 0.001 by two-way ANOVA) with no significant change in the EC80 (p > 0.8 by t test); the NMDA EC50 values before and during PDGFR inhibition were 77 ± 16 and 84 ± 25 μM, respectively. The NMDA Hill coefficients were not affected by PDGFR activation; the values before and after PDGFR activation were 1.7 ± 0.2 and 1.9 ± 1.3, respectively. PDGFR activation produced a significant (p < 0.001 by two-way ANOVA) decrease of 65 ± 7% (n = 10) in the glycine EC50, with no significant change in the EC80 (p > 0.7 by t test); the glycine EC50 values before and during PDGFR inhibition were 4 ± 0.6 and 4 ± 0.7 μM, respectively. The glycine Hill coefficients were not affected by PDGFR activation; the values before and after PDGFR activation were 2 ± 0.3 and 1.9 ± 0.4, respectively.

Finally, we measured the effect of PDGFR activation on the NMDA-R current/voltage relationships. PDGFR activation inhibited NMDA-R currents independently of the membrane holding potential (Fig. 4D). The reversal potentials for the NMDA-R-mediated currents were not significantly (p > 0.5 by t test, n = 9) affected by PDGFR activation; the values before and during PDGFR-induced inhibition were −26 ± 3 and −25 ± 3 mV, respectively.

Effect of PDGFR-β Mutants and Ca2+ Chelation—Two PDGFR-β mutants (3) were expressed in Xenopus oocytes to assess which PDGFR-activated SH2 domain protein mediates the PDGFR inhibitory actions (Fig. 5A). We used the F5 mutant PDGFR-β (3) where tyrosines 740, 751, 771, 1009, and 1021 have been mutated to phenylalanine. This mutant possesses intact intrinsic tyrosine kinase activity but does not bind or activate the following SH2 domain proteins, PI3K, Ras-GAP, Syp, or PLC-γ (Fig. 5A). We also used an “add-back” mutant where Phe1021 was mutated back to tyrosine (Tyr1021 add-back PDGFR-β mutant) (Fig. 5A). This PDGFR-β mutant has restored binding activity for PLC-γ. The F5 PDGFR-β mutant inhibited NMDA-R responses significantly less (p < 0.001 by t test, n = 8–10) than wild-type PDGFR-β (Fig. 5A). Conversely, the Tyr1021 add-back PDGFR-β mutant, with restored activation sites for PLC-γ, inhibited NMDA-R currents to the same extent as wild-type PDGFR-β.

Activation of PLC-γ results in an inositol 1,4,5-triphosphate-dependent elevation of intracellular Ca2+ levels. Consequently, the role of intracellular Ca2+ on the PDGF-induced inhibition of NMDA-Rs was assessed (Fig. 5B). Microinjection of the Ca2+ chelator EGTA (500 μM), before activation of PDGFR-β, significantly reduced the PDGFR-induced maximal inhibition of NMDA-Rs from 66 ± 12 to 25 ± 5% (p < 0.005 by t test, n = 10–11). Conversely, microinjection of the Ca2+ chelator EGTA (500 μM) after activation of PDGFs did not significantly reduce the PDGFR-induced maximum inhibition of NMDA-R responses (Fig. 5B).

Effect of Calyculin A and Phalloidin—We next tested the effects of the phosphatase inhibitor, calyculin A, and of the microtubule polymerizing agent, phalloidin, to determine...
whether the PDGF-induced inhibition of NMDA-Rs was produced by a similar mechanism to that of the phenomenon of Ca\(^{2+}\)-dependent run down described by Rosenmund and Westbrook (27, 28). These investigators showed that Ca\(^{2+}\)-dependent NMDA-R run down was unaffected by phosphatase inhibitors (27) and blocked by the microtubule polymerizing agent, phalloidin (28). However, microinjection of the potent inhibitor of protein phosphatases 1 (PP1) and 2A (PP2A), calyculin A (20–30 nm), into Xenopus oocytes significantly blocked \(p < 0.05 \text{ by two-way ANOVA, } n = 10\) the PDGF-induced inhibition of NMDA-R function (Fig. 6A). Treatment of cultured hippocampal neurons with calyculin A (40 nm) also significantly blocked the PDGF inhibitory effects on both NMDA-R whole cell currents \(p < 0.001 \text{ by two-way ANOVA, } n = 7\) (Fig. 6B) and single channel open probability (Fig. 6C, Table I). Moreover, phalloidin did not significantly block the effects of PDGF on NMDA-R function in Xenopus oocytes (Fig. 6D, \(p > 0.15 \text{ by two-way ANOVA}\). It should be noted that we also tested the effects of deltamethrin, a potent inhibitor of the Ca\(^{2+}/\text{calmodulin-dependent protein phosphatase 2B, calcineurin.}\) Microinjection of deltamethrin into Xenopus oocytes did not block the inhibitory actions of PDGF; the PDGF-induced maximal inhibition of NMDA-Rs in control and deltamethrin-treated oocytes (200 nm for 6–9 h) was 66 ± 12 and 72 ± 5%, respectively \((n = 7–10)\).

**DISCUSSION**

In spite of being linked by history and name to platelets, PDGF should be considered a “classical” neurotrophic factor from a functional perspective. PDGF, like the neurotrophins, is 1) produced locally and is important for the development, differentiation, proliferation, and survival of neuronal and glial cells (7, 10, 11, 17); 2) coupled to tyrosine kinase receptors that activate complex intracellular signaling pathways (2); and 3) released as part of the compensatory response to central nervous system injury or disease (13, 15, 16). We now report that PDGF exerts another function that is characteristic of the neurotrophic factors, which is the modulation of neurotransmitter receptors in the central nervous system (29).

Regulation of NMDA-R Function by PDGF—Our experiments demonstrate that PDGF is a potent modulator of NMDA-Rs. PDGF activation produced a long-lasting inhibition of NMDA-Rs in cultured hippocampal neurons and in Xenopus oocytes and also inhibited synaptically evoked NMDA-dependent EPSCs in CA1 pyramidal neurons in hippocampal slices. The inhibition gradually appeared within minutes of PDGF application and lasted for at least 20 min in both cultured hippocampal neurons and hippocampal slices and for more than 3 h in the oocytes. At the single channel level, the inhibition was produced by a decrease in the open channel probability and not by a decrease in single channel conductance or open time(s). PDGFR activation decreased the efficacy of both NMDA and glycine but not their potency for NMDA-Rs. PDGFR activation inhibited NMDA-R function independently of the membrane holding potential and did not affect the reversal potential. The inhibitory effects of PDGF were specific for NMDA-Rs since this growth factor did not affect synaptically evoked non-NMDA-mediated EPSCs in CA1 pyramidal neurons in hippocampal slices or kainate-gated currents in cultured hippocampal neurons. Taken together, these findings suggest that PDGF receptor activation specifically affects NMDA-R function and that it does not affect glutamate release.

An effect of PDGF on glutamate release would be expected to affect both NMDA- and non-NMDA-dependent currents.

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**Table I**

| Parameter                      | Control | PDGF-treated |
|--------------------------------|---------|--------------|
| Open time (ms)                 | 1.5 ± 0.4 | 1.6 ± 0.3    |
| Closed time (ms)               | 4.8 ± 1.2 | 4.3 ± 0.7    |
| Current amplitude (pA)         | 3.3 ± 0.2 | 3.1 ± 0.2    |
| Open probability \(a\)         | 0.049 ± 0.01 | 0.028 ± 0.006 |
| + Calyculin A \(b\)           | 0.063 ± 0.015 | 0.054 ± 0.016 |

\(a\) Open probability was calculated as described under “Experimental Procedures.”

\(b\) \(p < 0.05 \text{ by } t \text{ test.}\)

\(c\) Neurons were exposed to calyculin A (40 nm) for 10 min.
addition, PDGF not only affected synaptically evoked NMDA currents but also currents produced by both application of NMDA and glycine to cultured hippocampal neurons and Xenopus oocytes.

Studies with PDGFR-β mutants expressed in Xenopus oocytes provided two important pieces of information about the mechanism of the inhibition of NMDA-Rs. First, these experiments indicate that the intrinsic tyrosine kinase activity of
PDGFR-β is not sufficient to produce inhibition of NMDA-Rs because the F5 PDGFR-β mutant, which does not activate a number of SH2 domain proteins but possesses intact intrinsic tyrosine kinase activity, did not inhibit NMDA-R function. Second, these experiments show that the SH2 domain protein that relays the inhibitory signal from PDGFR to NMDA-Rs is PLC-γ because restoration of the activation site for PLC-γ (Tyr1021 PDGFR-β add-back mutant) rescues the inhibitory actions of PDGF. In addition, the effects of PDGFR-β require an elevation of intracellular Ca^{2+} levels because microinjection of EGTA into Xenopus oocytes before PDGF activation blocked its inhibitory actions. Microinjection of EGTA after maximal inhibition was reached did not block the effects of PDGF, suggesting that a transient elevation of intracellular Ca^{2+} levels produces activation of an NMDA-R intracellular modulator. This modulator appears to be PP1 and/or PP2A because the phosphatase inhibitor, calyculin A, blocked the inhibitory effects of PDGF. This signaling cascade is schematically shown in Fig. 7.

Our finding that the PDGFR-induced inhibition of NMDA-R function is blocked by calyculin A is consistent with a number of recent reports showing that protein phosphatases decrease NMDA-R function. Wang et al. (30) reported that NMDA receptor currents are enhanced by calyculin A in cultured hippocampal neurons studied with the perforated patch technique. The authors also found that, like PDGF, PP1 and PP2A decrease the open probability of NMDA-Rs in inside-out patches (30). Inhibition of calcineurin (PP2B) resulted in prolonged single channel openings recorded with the cell-attached patch technique in adult rat dentate gyrus neurons (31). Moreover, calcineurin (PP2B), but not of PP1 and PP2A, appear to be involved in the development of the glycine-insensitive form of NMDA-R desensitization (32, 33). In addition, tyrosine kinase inhibitors decrease NMDA-R function in spinal dorsal horn neurons whereas tyrosine phosphatase inhibitors enhance its function, suggesting that tyrosine phosphatases inhibit NMDA-R function (34). Taken together, these studies indicate that protein phosphatases exert inhibitory actions on NMDA-R function and, consequently, are consistent with our finding that the PDGFR-induced inhibition of NMDA-Rs is mediated by PP1 and/or PP2A. It should be emphasized, however, that whether phosphatases produce NMDA-R inhibition by directly dephosphorylating the receptor or by acting indirectly on a NMDA-R regulatory protein remains to be determined biochemically.

The precise role that Ca^{2+} plays on the PDGFR-induced inhibition of NMDA-Rs is unclear. PP1 and PP2A are not directly regulated by Ca^{2+}, unlike calcineurin (PP2B) which is activated by Ca^{2+} and calmodulin. A signal transduction cascade where calcineurin activates PP1 via dephosphorylation of the endogenous PP inhibitor-1 was recently described (35). However, our results are inconsistent with this mechanism because the calcineurin inhibitor deltamethrin did not block the inhibitory actions of PDGFR-β in Xenopus oocytes. Consequently, the elevation of intracellular Ca^{2+} levels could produce activation of PP1 and/or PP2A by a mechanism different than the calcineurin-dependent dephosphorylation of PP inhibitor-1. The endogenous PP inhibitor-1 and the dopamine/cAMP-regulated phosphoprotein-32 are activated by protein kinase A (36), and elevations in intracellular Ca^{2+} levels mediated by L-type calcium channels have been shown to decrease both adenyl cyclase activity and cAMP levels in cardiac myocytes (37). Therefore, it is possible that the PDGFR-induced elevation of intracellular Ca^{2+} levels results in inhibition of protein kinase A activity which, in turn, could decrease the activities of PP inhibitor-1 and/or dopamine/cAMP-regulated phosphoprotein-32. Whether this is the mechanism by which the elevation in intracellular Ca^{2+} levels results in activation of PP inhibitor-1 and/or PP2A remains to be tested directly; it should be kept in mind that others have shown that elevation of intracellular Ca^{2+} levels stimulate protein kinase A activity (38). Another mechanism by which elevations in intracellular Ca^{2+} levels could result in activation of PP1 and/or PP2 could involve...
tyrosine kinases. Increases in intracellular Ca\textsuperscript{2+} levels are known to activate tyrosine kinases (39, 40), and the activity of PP inhibitor-2 is inhibited by tyrosine phosphorylation in vitro (41). Thus, the elevation in intracellular Ca\textsuperscript{2+} levels could result in activation of tyrosine kinases and inhibition of PP inhibitor-2 activity. It would be interesting to determine whether the PDGF-induced elevation of intracellular Ca\textsuperscript{2+} levels results in activation of PP1 and/or PP2A via these mechanisms or via other, as of yet, unidentified intracellular signaling cascades.

The PDGF-induced inhibition of NMDA-Rs appears to be different from both the Ca\textsuperscript{2+}-dependent inactivation and rundown of NMDA-Rs reported by several laboratories (27, 28, 42-45). Ca\textsuperscript{2+}-dependent inactivation of NMDA-Rs is characterized by transient (10–50 s) inhibition of ~50% that is not modulated by ATP and phosphatase or protease inhibitors and can be triggered by Ca\textsuperscript{2+} entry through NMDA-Rs or voltage-gated Ca\textsuperscript{2+} channels (43, 45). Ca\textsuperscript{2+}-dependent NMDA-R rundown occurs when intracellular Ca\textsuperscript{2+} levels are elevated by repeated (every ~30 s) receptor activation (27, 28). Run down is characterized by inhibition of ~50% that requires minutes to develop and by a reversibility rate that is dependent on the NMDA concentration used. NMDA-R rundown does not occur with infrequent activation of NMDA-Rs, in Ca\textsuperscript{2+}-free media, in the presence of an ATP-regenerating solution or when depolymerization of the actin cytoskeleton is prevented by application of phalloidin (27, 28). Importantly, NMDA-R rundown is not mimicked by intracellular dialysis of protein phosphatases (alkaline phosphatase, PP1, and calcineurin) or blocked by phosphatase inhibitors (okadaic acid and microcystin) (27). Since the PDGF-induced inhibition of NMDA-Rs is blocked by the phosphatase inhibitor, calyculin A, and is not affected by phalloidin, present results suggest that PDGF activation modulates NMDA-R function via a mechanism different from that of the Ca\textsuperscript{2+}-dependent inactivation or the Ca\textsuperscript{2+}-dependent rundown of NMDA-Rs.

Modulation of Glutamate Receptors by Other Growth Factors—Evidence in favor of the importance of growth factors as regulators of glutamate receptor function is beginning to emerge from several laboratories. Basic fibroblast growth factor enhances the elevation of intracellular Ca\textsuperscript{2+} levels produced by activation of AMPA receptors but inhibits Ca\textsuperscript{2+} responses produced by NMDA-R activation (46). Activation of tyrosine kinase-coupled insulin receptors produces a long-lasting potentiation of NMDA-mediated EPSCs in hippocampal slices (47). BDNF and NT-4/5 produce a transient augmentation of AMPA-mediated synaptic currents and a transient increase in the frequency of miniature excitatory postsynaptic currents in cultured embryonic and postnatal rat hippocampal neurons (48). Moreover, Levine et al. (49) showed that BDNF rapidly enhances spontaneous firing rates and excitatory postsynaptic currents in cultured hippocampal neurons, and Kang and Schuman (50) demonstrated that BDNF and NT-3, but not NGF, produced a long-lasting enhancement of excitatory synaptic transmission in the Schaffer collateral-CA1 synapses. Kang and Schuman (50) also found that long term potentiation

![Image](https://example.com/image.png)

**Fig. 6.** Effect of calyculin A and phalloidin on PDGF-induced inhibition of NMDA-R responses. A, oocytes coexpressing NR1a/2A human NMDA-R subunits and PDGFR-β were microinjected ~30 min before 6 nM PDGF-BB application with ~30 nM calyculin A (■). In some cases, 100 nM calyculin A was bath applied for 30–60 min before PDGF-BB application. Untreated oocytes (□) were from the same batch as the calyculin A-treated oocytes. PDGF (6 nM) was applied for 20 s. Calyculin A significantly blocked the PDGF-induced inhibition of NMDA-Rs (p < 0.05 by two-way ANOVA). Each point represents the mean ± S.E. of 10 (control) and 12 (calyculin A-treated) oocytes. NMDA and glycine concentrations were 100 and 10 μM, respectively. B, treatment of cultured hippocampal neurons with calyculin A (40 nM) blocked the PDGF-induced inhibition of NMDA-activated currents. Calyculin A was perfused 10–20 min before and during the period of recording. Currents were generated by 2-s application of NMDA (100 μM) and glycine (3 μM). Holding potential was −60 mV. Current amplitudes were normalized to that recorded 10 min after the seal formation. Each point represents the mean ± S.E. of 7 (calyculin treated, ■) and 11 (untreated, □) cells (p < 0.001 by two-way ANOVA). C, an example record showing the lack of PDGF effects on single channel activity in calyculin A (40 nM)-treated neurons in cell-attached configuration. The pipette potential (V\textsubscript{p}) was 0 mV, and NMDA (10 μM) and glycine (1 μM) were included in the pipette solution. Calyculin A was perfused 10 min before and during the period of recording. Calyculin A significantly blocked the effects of PDGF on NMDA-R single channel open probability (see Table I). D, oocytes coexpressing NR1a/2A human NMDA-R subunits and PDGFR-β were microinjected 15 min before PDGF-BB (6 nM) application with 100 μM phalloidin (■). Untreated oocytes (□) were from the same batch as the phalloidin-treated oocytes. Each point represents the mean ± S.E. of 7 (control) and 10 (phalloidin-treated) oocytes. Phalloidin did not significantly affect the effect of PDGF (p > 0.15 by two-way ANOVA). NMDA and glycine concentrations were 100 and 10 μM, respectively.
could still be induced in slices where excitatory synaptic transmission had been enhanced by BDNF or NT-3. Interestingly, it was recently reported that hippocampal LTP appears to be impaired in mice deficient for the BDNF gene (51). In the case of PDGF, recent experiments in our laboratory indicate that it does not affect the generation, duration, or magnitude of LTP in CA1 pyramidal hippocampal neurons, mouse brain slices in vitro (54), and application of PDGF to Hydra suppressed a component of the feeding response to glutathione (55). Interestingly, NMDA-Rs appear to be important modulators of the eating response in rats (56). Therefore, it will be important to determine whether the cross-communication between PDGF and NMDA-Rs is part of the system that controls food intake behavior in the brain.

Elevations of PDGF levels in the central nervous system have been detected during neurological diseases associated with excitotoxicity and neuronal death such as infections, trauma, and cerebrovascular ischemic disease (13, 15, 16). Therefore, the inhibitory actions of PDGF on NMDA-R function could also be important in the pathophysiology of these conditions because a decrease in Ca\(^{2+}\) influx through NMDA-Rs may help to restore homeostasis and prevent cell death. The neuroprotective effects of PDGF were recently demonstrated in vitro by Cheng and Matsson (19). The authors showed that PDGF protects cultured hippocampal and cortical neurons against glucose deprivation and oxidative injury-dependent neurotoxicity, and they suggested that this is due to an increase in cellular antioxidant enzymes such as catalase, glutathione peroxidase, and superoxide dismutase. Our results indicate that, in addition to this mechanism, PDGF might exert its neuroprotective actions by inhibiting Ca\(^{2+}\)-influx via NMDA-Rs. A challenging task for future research will be to determine whether PDGF plays these neuroprotective roles in the injured brain in vivo.

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