Platelet-derived Growth Factor Receptor-induced Feed-forward Inhibition of Excitatory Transmission between Hippocampal Pyramidal Neurons*

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In the central nervous system platelet-derived growth factor receptors (PDGF-R) play a role in the development and survival of neurons, in the regulation of synapses, and in adaptive responses following neuronal injury. These receptors are expressed in the hippocampus, and their activation regulates both excitatory and inhibitory synaptic transmission mediated by NMDA receptors (3) and NMDA receptors (3), respectivley (4). PDGF itself forms dimers (PDGF-AA, -BB, and -AB) that bind to two classes of PDGF-R (α and β) (5), and ligand binding induces receptor dimerization and tyrosine autophosphorylation. Phosphotyrosyl residues of the receptor can then bind a variety of signal transduction molecules via their Src homology 2 domains. These include phospholipase C-γ (PLC-γ), members of the Src family of protein tyrosine kinases including c-Src, the protein tyrosine phosphatase SYP-2, Ras-GTPase-activating protein, and phosphatidylinositol 3-kinase, as well as the adaptor molecules Grb2, Nck, and Shc (5–7). Activation of PDGF-R can therefore signal through various parallel pathways to cell growth and motility. However, recent studies have suggested that these parallel pathways can signal both stimulatory and inhibitory signals (8).

Of the growth factors, there is considerable evidence that PDGF-R receptor activation of Src or PLC-γ leads to stimulation of the mitogen-activated protein kinase (MAPK) cascade and cell mitogenesis (5). Similar activation of the MAPK cascade and mitogenesis has also been reported for G-protein-coupled receptors (9), and we recently reported that several G-protein-coupled receptors act through a sequential activation of PKC and c-Src to up-regulate the activity of N-methyl-D-aspartate (NMDA) receptors (1). This subtype of glutamate receptor is an ion channel that contributes to excitatory synaptic transmission, to plasticity, and to cell growth of central neurons (10). Paradoxically, we also demonstrated that PDGF induces a long term depression of synaptically evoked NMDA receptor-mediated currents at CA1 synapses as well as of NMDA-evoked currents recorded in cultured hippocampal neurons (3).

The PDGF-R-mediated depression of NMDA channels requires activation of the PLC-γ pathway as a tyrosine site (Tyr1092) add-back mutant of the PDGF-β receptor restores this function when receptors are co-expressed in Xenopus oocytes (3). PLC-γ catalyzes the hydrolysis of phosphatidylinositol-4,5-bisphosphate to two potent second messengers: diacylglycerol, which activates various isoforms of protein kinase C (PKC), and inositol triphosphate, which releases intracellular Ca2+ (11). Therefore PDGF-R might be anticipated to activate the PKC/Src signaling cascade in CA1 pyramidal hippocampal neurons responsible for up-regulation of NMDA channel activity. Instead, we show here that the PDGF-R signaling is mediated through a second but functionally opposing pathway. This pathway signals a Ca2+- and PKA-dependent inhibition of the Src regulation of NMDA channels.

**EXPERIMENTAL PROCEDURES**

Preparation of Acutely Isolated Hhippocampal CA1 Neurons—CA1 hippocampal pyramidal neurons were acutely isolated using modified procedures of Wang and MacDonald (12). Briefly, Wistar rats 2–3 weeks
old were decapitated via a guillotine after halothane anesthesia. Hippocampi were removed quickly and placed in a dish containing cold oxygenated external solution consisting of 140 mM NaCl, 1.0 mM CaCl2, 5.4 mM KCl, 25 mM Hapes, 35 mM glucose, 1 mM MgCl2, and 0.0003 mM tetrodotoxin, pH 7.4 (osmolarity, 320–335 mosmol liter−1). The hippocampi were cut into 300–500-μm-thick slices by hand with a razor blade. The hippocampal slices were digested at room temperature (20–22 °C) in the external solution with 5 mg/ml papaya latex (Sigma). The incubation medium was stirred with 95% O2/5% CO2. After 30 min of enzymatic digestion, the slices were rinsed three times with the external solution. The slices were kept in the external solution bubbled with oxygen and used for 8–10 h. The CA1 region was cut out with a scalpel under a phase contrast microscope and triturated with a fire-polished glass pipette.

Whole Cell Recordings—Whole cell recordings were performed with an Axopatch-1B amplifier (Axon Instruments) under voltage-clamp mode. Recording electrodes, with resistance of 3–5 MΩ were constructed from thin walled borosilicate glass (1.5-mm-diameter; World Precision Instruments) using a two-stage puller (PP83, Narishige). Data were digitized, filtered (2 kHz), and acquired on-line using the programs pClamp (Axon Instruments). The standard internal solution for recording electrode consisted of the following 140 mM CsF, 35 mM CsOH, 10 mM Hepes, 2 mM MgCl2, 2 mM tetrathymalummonium, 4 mM Na2ATP, pH 7.3 (osmolarity, 300 mosmol liter−1). A multibarrel fast perfusion system (13) was employed to achieve a rapid exchange of solution. NMDA (50 μM) and glycine (3 μM) in the external solution were applied to the neurons via one barrel for 2 s to evoke NMDA-mediated currents. Isolated hippocampal neurons were first patch clamped and then lifted into the outflow of the control barrel. The distance between the outlet of the barrel and the neuron was within 100 μm. The holding potential was −60 mV. PDGF and other drugs were diluted in the external solution to the required concentrations and applied to the neurons via the control barrel unless otherwise stated. Some hydrophobic drugs were initially dissolved in dimethyl sulfoxide and then diluted to the desired concentration in the external solution. The final concentration of Me2SO applied to the cells was less than 0.1%, and this concentration of Me2SO did not affect NMDA currents.

Miniature Synaptic Currents in Cultured Hippocampal Neurons— Cultures of fetal hippocampal neurons were used for electrophysiological recordings 12–17 days after plating. Spontaneous miniature mEPSCs in cultured hippocampal neurons were recorded after formation of the whole cell patch configuration. The bathing solution was supplemented with 0.5 μM tetrodotoxin, 1 μM strychnine, 10 μM bicuculline methiodide, and 3 μM glycine. Miniature EPSCs were filtered at 2 kHz and averaged on tape prior to their off-line acquisition with an event detection program (SCAN, Strathclyde software; courtesy of Dr. J. Dempster). For detection, the trigger level was set approximately three times higher than the base-line noise. False events were eliminated by subsequent inspection of the raw data.

Single-channel Recordings—Inside-out single channel recordings were carried out on cultured mouse hippocampal neurons (14). After coating the Sylgard the electrodes were washed and filled with the standard external solution containing NMDA (10 μM) and glycine (3 μM). The intracellular solution for bathing the cytoplasmic face of the patch contained 120 mM CsCl, 35 mM CsOH, 10 mM Hapes, 2 mM MgCl2, 11 mM EGTA, 2 mM tetrathymalummonium, 4 mM Na2ATP, pH 7.3 (osmolarity, 300 mosmol liter−1). The intracellular solution was supplemented as required with EPQ(pY)EEIPIA, EPQYEEIPIA, or the catalytic subunit of PKA (cPKA) just before use. During recording, the holding potential of the pipette was +60 mV. Single channel events were recorded 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 sampled at 5 kHz and filtered at 2 kHz.

Calcium Imaging—Cultured hippocampal neurons were incubated in 6 μM fura-2-acetoxymethylester dissolved in Me2SO with pluronic acid in minimal essential for 30–40 min at room temperature. Coverslips with fura-2-loaded cells were transferred to a perfusion chamber on an inverted microscope. Cells were illuminated using a xenon lamp and were observed using a 40× UV filter oil immersion objective on an inverted microscope (Nikon). Video images were obtained using an intensified CCD camera (PTI IC-100). Digitized images were acquired by averaging four frames at video rates using an image processing board (Axon Imagine Lightening) in a computer controlled by Axon Imaging Workbench software (Axon Instruments). The shutter and filter wheels also were controlled by Axon Imaging Workbench software to allow timed illumination of cells at 340 and 380 nm excitation wavelengths. Ratio images were analyzed by averaging pixel ratio values in circumscribed regions of all responding cells in the field of view. The values were exported from Axon Imaging Workbench software to a spreadsheet program (Sigmaplot or Graphpad).

Chemicals—PDGF-BB provided by Synergen (Boulder, CO) was stored in 10 mM acetic acid plus 0.1% bovine serum albumin at −20 °C as a stock solution and diluted 1:1250 in the external solution immediately before use. Wins41662 was provided by Sanofi Recherche (Tourouvre, France). H7, U73122, U73343, phorbol 12-myristate 13-acetate (PMA), chelerythrine, PKI(5–24), Rp-cAMPs and 3-isobutyl-1-methylxanthine (IBMX) were from Biomol. CPK-A was purchased from Promega. EPQ(pY)EEIPIA, EPQYEEIPIA, Src (40–58), scrambled sSrc (40–58), and anti-cst1 were gifts from Dr. Michael W. Salt (Hospital for Sick Children, University of Toronto, Toronto, Canada). The other chemicals were products of Sigma.

Statistics Analysis—Currents were expressed as the means ± S.E. normalized to the control values before the application of drugs. Values in parentheses refer to the number of different cells used in the statistical analysis. Statistics analyses were performed using either Student’s t test or two-way analysis of variance.

RESULTS

PDGF Receptor Tyrosine Kinase Activity Is Required for PDGF-mediated Depression of NMDA Receptor Function—In acutely isolated hippocampal CA1 pyramidal neurons applications of PDGF (6 nm) substantially depressed peak NMDA-evoked currents (Ip), whereas steady-state currents were much less effected. The maximum effect was reached within 10–15 min of applying PDGF (Fig. 1). I, was inhibited by 29.2 ± 3.7% (n = 9, p < 0.01 by Student’s t test) 20 min following the application of PDGF. Recordings from matched cells that did not receive PDGF demonstrated stable peak responses to NMDA over the same period of recording (Fig. 1). In all subse-
FIG. 2. Tyrosine kinase activity of PDGF receptors and activation of phospholipase C-γ are required for the PDGF-induced depression of NMDA-activated currents. A, Win41662, a specific PDGF receptor tyrosine kinase inhibitor, completely eliminated the depression of NMDA-evoked currents induced by PDGF. Cells were pretreated with Win41662 (3 μM) for 15 min. Then during the recording each neuron was continuously perfused with the same concentration of Win41662. B, a specific PLC-γ inhibitor, U73122 totally blocked the depression of NMDA-evoked currents induced by PDGF. Neurons were pretreated with 10 μM U73122 or U73343 (inactive analogue) for 15 min and recorded in the presence of one of these compounds (inside the patch pipette). Effect of PDGF was blocked by U73122 but not by U73343.

FIG. 3. PDGF-induced increase in intracellular Ca2⁺ and the conversion of the PDGF-induced depression into an enhancement in the presence of heparin. A, time course of PDGF-induced increase in intracellular Ca2⁺. B: mean data for intracellular Ca2⁺ levels in response to applications of PDGF (n = 24, measurement of the peak response within 40 s of PDGF application). C, PDGF-enhanced NMDA evoked currents when heparin (400 μM) was included in the patch pipette.

PDGF Releases Intracellular Ca2⁺—Activation of PLC-γ increases the production of IP3, which induces intracellular Ca2⁺ release in many cells. Therefore, we measured relative changes in intracellular Ca2⁺ in cultured hippocampal neurons using fura-2 ratiometric imaging during applications of PDGF. Absolute values of intracellular Ca2⁺ were not measured. Bath applications of PDGF significantly increased the intracellular Ca2⁺ concentrations within 30 s of applying this growth factor (Fig. 3).

The potential source of this Ca2⁺ signal was then examined. Heparin selectively inhibits the IP3-induced release of Ca2⁺ from intracellular stores. For example it completely blocked the Ca2⁺ release induced following flash photolysis of caged IP3, and it blocked the intracellular Ca2⁺ signal recorded after exposure to PDGF or fibroblast growth factor (16). We anticipated that the PDGF-induced depression of NMDA-evoked currents would be blocked by heparin if an IP3-dependent release of Ca2⁺ were responsible for this effect. Inclusion of heparin (400 μM) in the patch pipette did not alter NMDA-evoked currents on its own, but the inhibitory effect of PDGF was eliminated (Fig. 3C). Unexpectedly, in the presence of heparin applications of PDGF increased Ip by 14.6 ± 1.9% (n = 17, p < 0.05, Student’s t test). This suggests that PDGF-induced potentiation of peak NMDA-evoked currents is masked by the prominent Ca2⁺-dependent depression.

Activation of PKA Reverses PDGF-induced Depression of NMDA Currents—Next we considered the potential target of the elevated intracellular Ca2⁺. This lead us to consider the possibility of a Ca2⁺-dependent activation of adenyl cyclase together with a subsequent activation of PKA as a basis of the depressant response to PDGF. We considered this pathway for a number of reasons. For example, several Ca2⁺-dependent adenyl cyclases (I and VIII) are present in hippocampal neurons (17), and levels of cAMP can be increased in hippocampal neurons through a Ca2⁺-dependent activation of adenyl cyclase (18, 19). In non-neuronal cells the activity of PKA is
PDGF Receptor-induced Feed-forward NMDA Receptor Inhibition

Role of PKC in PDGF-mediated Regulation of NMDA Currents—We next examined the possibility that the PDGF-induced depression masked a PKC-dependent potentiation of $I_p$. If this were the case we would anticipate that blockers of PKC would reduce the depression. We tested the first possibility by including a selective PKC inhibitor chelerythr ine in the patch pipette. Applications of chelerythrine significantly increased the relative PDGF-mediated depression of NMDA-evoked currents (Fig. 5A). The second possibility was examined by pretreating the cells with 4α-PMA, a potent PKC activator. This phorbol ester significantly decreased the relative depression of NMDA-activated currents induced by PDGF (Fig. 5B), whereas the inactive phorbol 4α-PMA was without effect.

The PKC-induced potentiation of peak NMDA-evoked currents in these neurons is mediated via activation of the nonreceptor tyrosine kinase Src (1). We predicted that inhibition of Src kinases should block any potential PKC-dependent PDGF-induced potentiation of $I_p$, leaving only the PDGF-induced depression. Therefore, we included in the pipette anti-cst1 (10 μg/ml), an antibody that selectively inhibits the Src family of kinases (29, 30). An IgG fraction (10 μg/ml) was included in another series of recordings as a control (Fig. 5C). We also examined the effects of a Src$_{40-38}$ peptide fragment that selectively inhibits Src function (29, 30). A scrambled sequence of this peptide was used as the control for these experiments (Fig. 5D). Unexpectedly, both inhibitors of tyrosine kinase Src completely blocked the PDGF-induced depression (Fig. 5, C and D). These results suggested that contrary to our initial hypothesis, the activity of Src is required for the PDGF-induced depression of $I_p$.

PKA Disrupts Src-mediated Up-regulation of NMDA Currents—Tyrosine kinase Src and NMDA receptors co-immunoprecipitate suggesting a close physical proximity of these two proteins at the synapse. Furthermore, the activation of Src enhances NMDA channel activity (29) in inside-out patches and enhances NMDA-mediated synaptic currents in cultured hippocampal neurons (1). Because both PKA and Src activity appeared to be required for the PDGF-induced depression of NMDA-activated currents, we considered the possibility that PKA modulates the Src-induced enhancement of NMDA receptor function. To test this hypothesis we recorded NMDA receptor single-channel currents using inside-out patches. We employed a Src-activating peptide EPQ(pY)EEIPIA (31), which stimulates endogenous Src and enhances NMDA receptor function in such patches (1, 29). The nonphosphorylated form of the peptide EPQYEIIPIA was applied as a control for the activator peptide. In addition, we used the cPKA to simulate activation of endogenous PKA and heat-denatured cPKA was used as a control for the catalytic subunit (1, 29, 31). The results are summarized in Table I. Applications of EPQ(pY)EEIPIA (1 μM) to the cytoplasmic side of the membrane significantly increased the open probability ($P_o$) of NMDA channels (Fig. 6, A, C, and E). The mean open time ($t_o$) was also significantly increased by EPQ(pY)EEIPIA (Table I). The control peptide EPQYEIIPIA (1 μM) did not alter either of these parameters (Table I). In contrast to the active peptide, application of cPKA (100 units/ml) had no significant effect on NMDA channel activity. However, cPKA blocked the effects of EPQ(pY)EEIPIA on NMDA single channel activity (Fig. 6, B, D, and E). Applications of heat-denatured cPKA failed to block the increase in $P_o$ and $t_o$ induced by the active peptide EPQ(pY)EEIPIA (Table I). These results demonstrate that activation of PKA can inhibit the stimulatory actions of endogenous Src upon NMDA channels in these patches.

PDGF-induced Depression of Miniature Synaptic Currents—We next examined the effects of PDGF receptor activation on the NMDA receptor-mEPSCs in cultured hippocampal neurons (Fig. 7). Applications of this growth factor depressed the NMDA receptor-mEPSCs, and the depression could be enhanced by including chelerythrine in the patch pipette, confirming that synaptically located NMDA receptors are likely simultaneously enhanced by PDGF through a PKC-dependent mechanism (Fig. 7, B and D). In contrast, the depression was eliminated by including the PKA inhibitory peptide in the patch pipette and replaced by a small PDGF-induced enhancement (Fig. 7, C and D).
PDGF Receptor-induced Feed-forward NMDA Receptor Inhibition

DISCUSSION

The primary effect of applications of low concentrations of PDGF on NMDA-evoked currents was to depress $I_p$. This action was mediated via PDGF receptor autophosphorylation as the selective PDGF receptor kinase inhibitor Win41662 blocked it. Furthermore, this inhibition likely takes place through activation of PLC-γ because the depression was also blocked by the PLC-γ inhibitor U73122. Stimulation of PLC-γ through activation of PDGF receptors leads to the hydrolysis of phosphoinositides, the production of IP3 and diacylglycerol (4). The production of IP3 following activation of PDGF receptors is known to release intracellular Ca$^{2+}$ (32), and a similar effect was observed by us in cultured hippocampal neurons. Consistent with this observation, the PDGF-induced depression of $I_p$ in isolated neurons was blocked by including heparin in the patch pipette. The rise in intracellular Ca$^{2+}$ could potentially activate PKA through the stimulation of Ca$^{2+}$-dependent adenylyl cyclases (33, 34). Indeed, PDGF receptor activation is associated with activation of PKA (20, 35). This inhibitory signaling pathway is summarized schematically in Fig. 8.

PDGF Receptor Activation and PKC—In a previous study we demonstrated that G-protein coupled receptors, presumably acting through PLC-γ, enhance $I_p$ through a sequential activation of PKC and c-Src (1). Thus, stimulation of PLC-γ by PDGF-R would also be anticipated to potentiate $I_p$ via a parallel excitatory pathway (Fig. 8). Whether $I_p$ was depressed or enhanced would therefore depend upon the relative strength of activation of inhibitory and excitatory signaling pathways. Our observations that intracellular applications of the selective PKC inhibitor chelerythrine enhanced the PDGF-induced depression, whereas pretreatment of cells with the active phorbol ester β-PMA reduced this depression, are consistent with a block or potentiation, respectively, of the excitatory pathway.

We previously reported that an inhibitor of the phosphatases PP1/PP2A blocked the PDGF-induced inhibition of $I_p$ (3). These inhibitors also dramatically enhance the phorbol-ester induced (PKC- and Src-dependent) potentiation of $I_p$ in these neurons (1). Therefore, inhibition of these phosphatases may have simply favored the PKC-dependent and facilitatory pathway over the PKA-dependent inhibitory pathway (an apparent block of the PDGF-induced inhibition of peak NMDA currents).

Role of PKA in the Regulation of NMDA Receptors—NMDA receptors can be directly phosphorylated by PKA (25, 26). The physiological significance of the PKA-mediated phosphorylation of NMDA channels is nevertheless poorly understood. For example, previous experiments found that intracellular applications of cAMP (36) had little effect on peak NMDA-evoked currents in cultured hippocampal neurons. Applications of cPKA were also reported to have no effect on NMDA single channel activity in outside-out patches taken from cultured neurons (28), and we detected no effect on NMDA channel activity in inside-out patches in the present study. However, the PDGF-induced depression of $I_p$ we observed was blocked by the inhibitory PKA peptide PKI and by the potent and selective PKA inhibitor Rp-cAMPS. Applications of the phosphodiesterase inhibitor IBMX enhanced this depression, also suggesting that activation of PKA is required for this effect even though PKA does not seem to directly modulate the activity of NMDA channels.

![FIG. 5. Role of PKC and Src in the PDGF-mediated response of NMDA channels. A, inclusion of chelerythrine (10 μM) in the pipette enhanced the PDGF-induced depression. B, activation of PKC using phorbol ester attenuated the inhibitory effect of PDGF. After treatment of the neurons with 4β-PMA (100 nM, inactive) or 4α-PMA (100 nM, active) for 10 min, PDGF was applied (in the continuous presence of the phorbol ester) for 20 min. In the presence of 4β-PMA but not 4α-PMA, the inhibitory effect of PDGF on $I_p$ currents was diminished. C, inclusion of the Src antibody, anti-cst1 (10 μg/ml) in the pipette completely blocked the depression of NMDA-evoked currents by PDGF (n = 11, squares, p < 0.05, Student’s t test); whereas control Ig-G antibody (10 μg/ml) had no effect on this depression (n = 9, circles, p > 0.05, Student’s t test). D, effects of PDGF on NMDA-activated currents were blocked by the inclusion of the unique domain peptide fragment Src(40–58) (25 μg/ml, n = 8, p < 0.05, Student’s t test) but not by a scrambled Src (sSrc, 25 μg/ml, n = 7, p < 0.01, Student’s t test). In each series of recordings PDGF was applied for 20 min with the onset of the application set at time 0.

TABLE I

| Single channel parameters |
|---------------------------|
| n | Open probability | Mean open time | % of control | % of control |
|---|-----------------|----------------|-------------|-------------|
| EPQ(pY)EEPIA | 6 | 230.4 ± 18.3 | 167.5 ± 12.6 | 3.8 | 98.0 |
| EPQYEPIA | 5 | 111.6 ± 5.1 | 103.4 ± 2.5 | 3.8 | 98.0 |
| cPKA | 6 | 101.3 ± 11.2 | 90.9 ± 3.0 | 3.8 | 98.0 |
| cPKA + EPQ(pY)EEPIA | 6 | 104.3 ± 23.2 | 87.3 ± 12.2 | 3.8 | 98.0 |
| dPKA | 5 | 103.9 ± 23.8 | 98.0 ± 1.3 | 3.8 | 98.0 |
| dPKA + EPQ(pY)EEPIA | 5 | 234.5 ± 10.7 | 106.3 ± 9.6 | 3.8 | 98.0 |

*p < 0.05, **p < 0.01.

dPKA indicates heat-denatured cPKA.
Role of c-Src in the PDGF-induced Depression—Intracellular applications of either the c-Src selective inhibitory peptide (Src40–58) or the Src family inhibitory antibody (anti-cst1) but not appropriate controls blocked the PDGF-induced depression of I_NMDA. These results were surprising because we anticipated that inhibitors of c-Src would block the excitatory pathway and shift the effects of PDGF toward a predominance of the inhibitory one (Fig. 8). One possible explanation is that the inhibitory pathway also requires activation of c-Src. For example, auto-phosphorylation of the PDGF-R at Tyr695 (and Tyr704) is known to stimulate binding of c-Src to the receptor through an interaction of this phosphotyrosine with the Src homology 2 domain of c-Src (7). This interaction leads to activation of c-Src, and in turn Src phosphorylates residue Tyr579 of the PDGF receptor (37). This sequence of events is thought to activate the MAPK kinase cascade by mechanisms that are not well understood. However, we previously demonstrated that only Tyr1023 is required for the PDGF-induced depression of NMDA-evoked currents (3) and that the depression is also blocked by the PLC-γ inhibitor Win41662; therefore, a Src-dependent phosphorylation of the PDGF receptor is unlikely to be required.

A more plausible explanation is that the PDGF-R receptor modulates the ability of c-Src to directly enhance NMDA channel activity. We suggest that blockers of Src likely decrease an already tonic potentiation mediated by Src (1) and occlude the effects of PDGF. This is strongly supported by our observation that cPKA inhibits the ability of the Src activator peptide to enhance NMDA channel activity and by our observation that

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**Fig. 6.** The enhancement of NMDA channel activity by Src is inhibited by cPKA in inside-out patches taken from cultured hippocampal neurons. A, continuous record of channels activity recorded prior to the application of cPKA. B, application of cPKA (100 units/ml) to the cytoplasmic face of the same patch did not alter NMDA channel activity. C, in contrast, applications of the Src activator peptide EPQ(pY)EEIPIA (1 mM) increased the frequency of channel activity in this patch. D, NMDA channel activity of the same patch during the co-application of cPKA (100 units/ml) and the peptide activator of Src family kinases, EPQ(pY)EEIPIA (1 mM). In the presence of cPKA, applications of EPQ(pY)EEIPIA failed to enhance NMDA channel activity. E, a continuous recording of NMDA channel open probability (P_o) before and during cytoplasmic application of EPQ(pY)EEIPIA. NMDA channel activity was strongly inhibited by PDGF in the presence of this inhibitor of PKC. F, inclusion of the specific PKA inhibitor PKI5–24 (15 nM) in the patch pipette both before and during the application of PDGF. Note that the NMDA component was slightly enhanced by PDGF under these conditions. G, pooled data for the effects of the intracellular applications of inhibitors on PDGF receptor-mediated modulation of the NMDA receptor component. Applications of PDGF inhibited the NMDA component of mEPSCs to 71 ± 5% of the control (n = 6, p < 0.01). In the presence of chelerythrine, PDGF inhibited NMDA mEPSCs to 42 ± 9% of the control (n = 6, p < 0.01). Co-applications of PKI5–24 and PDGF enhanced this component to 123 ± 5% of the control (n = 6, p < 0.05).

**Fig. 7.** Role of PKA and PKC in the PDGF-mediated modulation of synaptic transmission in cultured hippocampal neurons. A, mEPSCs from a cultured hippocampal neuron before and during the application of PDGF (6 nM). The NMDA receptor-mediated component (trace 3) was isolated by subtracting the mEPSC in the presence of the competitive NMDA receptor antagonist AP5 (trace 2) from the mEPSC in the absence of AP5 (trace 1). Applications of PDGF depressed the NMDA component. B, the specific PKC inhibitor chelerythrine (10 μM) was included in the patch pipette and mEPSCs recorded from a neuron before and during the application of PDGF. Note the NMDA component was strongly inhibited by PDGF in the presence of this inhibitor of PKC. C, inclusion of the specific PKA inhibitor PKI5–24 (15 nM) in the patch pipette both before and during the application of PDGF. Note the NMDA component was slightly enhanced by PDGF under these conditions. D, pooled data for the effects of intracellular applications of inhibitors on PDGF receptor-mediated modulation of the NMDA receptor component. Applications of PDGF inhibited the NMDA component of mEPSCs to 71 ± 5% of the control (n = 6, p < 0.01). In the presence of chelerythrine, PDGF inhibited NMDA mEPSCs to 42 ± 9% of the control (n = 6, p < 0.01). Co-applications of PKI5–24 and PDGF enhanced this component to 123 ± 5% of the control (n = 6, p < 0.05).
inhibitors of c-Src block the inhibitory modulation of \( I_p \) by PDGF. Therefore, the PDGF-induced depression of \( I_p \) is exerted indirectly at least in part through activation of PKA and inhibition of the effects of c-Src on NMDA channels (Fig. 8).

One possible mechanism is that PKA-induced phosphorylation of the NMDA channel alters the configuration of the channel and renders it insensitive to the enhancement by c-Src. Alternatively there is evidence that c-Src is phosphorylated by PKA at serine 17 (38, 39), and this phosphorylation is known to decrease the hydrophobicity of c-Src and enhance the release of c-Src from the cytoplasmic membrane to the cytosol (21, 40, 41). PKA may therefore increase translocation of c-Src from the cell membrane to the cytosol, thus decreasing its ability to phosphorylate a relevant substrate and modulate NMDA channels.

This possibility is consistent with co-immunoprecipitation of c-Src and NMDA receptor proteins from the postsynaptic density of central synapses (29, 42). This latter mechanism is also consistent with serine-threonine phosphorylation and altered affinity of c-Src and NMDA receptor proteins from the postsynaptic density of central synapses (29, 42). This latter mechanism is also consistent with co-immunoprecipitation of c-Src and NMDA receptor proteins from the postsynaptic density of central synapses (29, 42).

PKA at serine 17 (38, 39), and this phosphorylation is known to...