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An ADP-ribosyltransferase as a potential target for nitric oxide action in hippocampal long-term potentiation

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ABSTRACT **Recent studies of long-term potentiation (LTP) in the CA1 region of the hippocampus have demonstrated that nitric oxide (NO) may be involved in some forms of LTP and have suggested that postsynaptically generated NO is a candidate to act as a retrograde messenger. However, the molecular target(s) of NO in LTP remain to be elucidated. The present study examined whether either of two potential NO targets, a soluble guanylyl cyclase or an ADP-ribosyltransferase (ADPRT; EC 2.4.2.31) plays a role in LTP. The application of membrane-permeant analogs of cGMP did not produce any long-lasting alterations in synaptic strength. In addition, application of a cGMP-dependent protein kinase inhibitor did not prevent LTP. We found that the CA1 tissue from hippocampus possesses an ADPRT activity that is dramatically stimulated by NO and attenuated by two different inhibitors of mono-ADPRT activity, phylloquinone and nicotinamide. The extracellular application of these same inhibitors prevented LTP. Postsynaptic injection of nicotinamide failed to attenuate LTP, suggesting that the critical site of ADPRT activity resides at a nonpostsynaptic locus. These results suggest that ADP-ribosylation plays a role in LTP and are consistent with the idea that an ADPRT may be a target of NO action.**

Long-term potentiation (LTP) in the CA1 region of the hippocampus is an extensively studied form of synaptic plasticity which is initiated by a series of postsynaptic events including Ca^{2+} influx through *N*-methyl-D-aspartate (NMDA) receptor channels (1) but may be maintained, at least in part, by presynaptic mechanisms (1, 2). With standard LTP induction protocols at room temperature (3–5) and with standard (6–8) or weak (9–11) induction protocols at warmer temperatures (but see refs. 5 and 12) the diffusible messenger nitric oxide (NO) is required for LTP production, suggesting that NO may mediate the retrograde communication (2, 13, 14) necessary to bring about a presynaptic component of LTP expression. However, the location and identity of the molecular target(s) of NO are still unknown.

The major target of NO in many areas appears to be a guanylyl cyclase (15–18). In the hippocampus, high-frequency stimulation can produce rises in cGMP that are blocked by NO synthase inhibitors (10). In addition, membrane-permeant analogs of cGMP have been reported to enhance hippocampal synaptic transmission (19) and reduce the inhibition of LTP produced by NO synthase inhibitors (4).

ADP-ribosyltransferases (ADPRTs; EC 2.4.2.31) are enzymes that covalently modify their substrate proteins by attaching ADP-ribose moiety(s) from NAD^+ or $NADP^+$ to specific residues. Poly-ADPRTs, which attach multiple ADP-ribose moieties to their substrates, are primarily nuclear enzymes (20). In contrast, mono-ADPRTs usually attach a single ADP-ribose moiety and possess cytosolic activity (20). Several endogenous mammalian mono-ADPRTs have been described (21–25), but the precise cellular functions of these enzymes remain poorly understood. Early studies suggested that NO-donating compounds can stimulate the ADP-ribosylation of proteins (26, 27), including glyceraldehyde-3′-phosphate dehydrogenase (GAPDH). Although the early studies suggested that the modification of GAPDH was mediated by an endogenous ADPRT, subsequent studies have revealed that the modification is mediated by an autocatalytic mechanism (25, 28–31). NO may also stimulate the auto-ADP-ribosylation of the poly-ADPRT (32, 33). In addition, there are numerous reports that NO can stimulate enzyme-mediated ADP-ribosylation of several neuronal proteins (34–38). In addition, quite recently Duman *et al.* (39) have shown that when *in vitro* assays are performed after LTP induction, less NO-stimulated ADP-ribosylation can be produced in LTP-induced slices than in control slices, suggesting that NO-stimulated ADP-ribosylation may occur during LTP induction. To elucidate the potential mechanisms of action of NO during LTP, we have performed experiments to test the involvement of guanylyl cyclase and/or ADPRT activity.

MATERIALS AND METHODS

Electrophysiology. Hippocampal slices were prepared from male Sprague–Dawley rats, 150–250 g, as described (3). Slices were submerged in a stream of ACSF (119 mM NaCl/2.5 mM KCl/1.3 mM $MgSO_4$/2.5 mM $CaCl_2$/1.0 mM NaH_2PO_4/26.2 mM $NaHCO_3$/11.0 mM glucose) maintained at 25°C and gased with 95% O_2/5% CO_2. Field or intracellular excitatory postsynaptic potentials or currents (EPSPs or EPSCs) measured in stratum radiatum or in CA1 pyramidal cells, respectively, were evoked by stimulation of the Schaffer collateral-commissural afferents (2 or 4 per min). 8-Bromo- or dibutyryl-cGMP (100 μM or 1 mM) and AP5 (50 μM) were applied to slices for at least 20–30 min prior to LTP induction. All other inhibitors were applied to slices for 45–90 min. 8-Bromo- and dibutyryl-cGMP, H-8, nicotinamide (Nic) (Sigma), and AP5 (Research Biochemicals, Natick, MA) were initially dissolved in water; phylloquinone (Phyl) and benzamide (Sigma) were dissolved in dimethyl sulfoxide. Phyl, a fat-soluble compound, was likely applied at ≈50% of the stated concentration, due to difficulties dissolving it at high concentration in dimethyl sulfoxide. Extracellular recording electrodes were filled with 3 M NaCl; intracellular recording electrodes were filled with 2 M $KMeSO_4$ or 2 M cesium acetate including Nic (500 mM). Whole-cell electrode internal solutions consisted of 100 mM cesium gluconate, 0.6 mM EGTA, 5 mM MgCl, 2 mM ATP, 0.3 mM GTP, 40 mM Hepes, and 1 mM QX-314. NMDA-mediated whole-cell EPSCs or extracellular field potentials were recorded from

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Abbreviations: LTP, long-term potentiation; NMDA, *N*-methyl-D-aspartate; ADPRT, ADP-ribosyltransferase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; EPSP, excitatory postsynaptic potential; IPSP, inhibitory postsynaptic potential; EPSC, excitatory postsynaptic current; Nic, nicotinamide; Phyl, phylloquinone; SIN-1, 3-morpholinosydnonimine.

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CA1 pyramidal neurons in the presence of 10 μM 6-cyano-7-nitroquinoxaline (CNQX), 10 μM glycine, and 50 μM picrotoxin. Tetanic stimulation was delivered at the test intensity in 1-s trains at 100 Hz, with one to four trains 15 or 30 s apart. Pairing was accomplished by sustained depolarization of the neuron by dc injection in conjunction with low-frequency (1-Hz) stimulation of the test pathway for 30–45 s. We analyzed the initial slope of the field EPSP or the amplitude of the intracellular EPSP or whole-cell EPSC. Ensemble average plots represent group means of each EPSP, across experiments, aligned with respect to the time of acquisition relative to the tetanic stimulation. Each individual experiment was normalized with respect to the mean value of its EPSP in the 50 responses that preceded the tetanus. Statistical comparisons were made with the Student *t* test, performed on raw data.

Inhibitors. In hen heterophils, Phyl is reported to be a more potent inhibitor of the mono-ADPRT (IC_{50} = 1.9 μM) than the poly-ADPRT (IC_{50} = 520 μM) (40, 41). Nic inhibits both the mono- (IC_{50} = 3400 μM) and poly-ADPRT (IC_{50} = 31 μM) (41). Benzamide is a more potent inhibitor of the poly-ADPRT (IC_{50} = 3.3 μM) than the mono-ADPRT (IC_{50} = 4100 μM) (41). The K_i values of H-8 for cGMP-dependent protein kinases, cAMP-dependent protein kinases, and protein kinase C (PKC) are 0.48 μM, 1.5 μM, and 15 μM, respectively (42). We also attempted to use a more potent and selective inhibitor of cGMP-dependent protein kinase, R_P cGMP (Biolog, La Jolla, CA), but we found that this compound induces a persistent depression of synaptic transmission.

ADP-Ribosylation. The CA1 regions of hippocampi were isolated from 150- to 250-g male Sprague–Dawley rats and homogenized in 100 mM Hepes containing 0.5 mM EDTA and a cocktail of protease inhibitors including aprotinin, leupeptin, chymostatin, and pepstatin A, each at 10 ng/ml and 100 μM phenylmethanesulfonyl fluoride. The resulting homogenate was aliquoted, frozen on dry ice, and stored over liquid nitrogen. Protein was measured by the Bradford assay. ADP-ribosylation was carried out with minor modifications of the procedures of Brüne and Lapetina (26, 27). The reaction was performed in a final volume of 65 μl containing 100 mM Hepes buffer (pH 7.5), 1 μM unlabeled NAD^+, 5 μCi (1 Ci = 37 GBq) of [^{32}P]NAD^+, 7.5 mM dithiothreitol, and 100 μg of CA1 homogenate protein. CNBr-activated Sepharose beads were linked to arginine or cysteine by incubation for 3 hr at 4°C with 0.2 M L-arginine or L-cysteine and then washed and resuspended in a Hepes buffer. Twenty microliters of arginine- or cysteine-linked beads was added to a reaction mixture identical to that described above. The mixture was incubated at 37°C for 30 min on a rotator, washed with 500 mM NaCl/10% (vol/vol) glycerol, and then spun 10 s at 10,000 × *g*. Individual reactions were conducted in the presence or absence of hippocampal homogenate to examine the dependence of ^{32}P incorporation on exogenous enzyme. The NO donor 3-morpholinosydnonimine (SIN-1; 500 μM) was also added alone or in the presence of the ADPRT inhibitors Phyl or Nic. ^{32}P incorporation (cpm) was measured with a scintillation counter.

RESULTS

Tests of the Role of cGMP. Previous work indicates that an NO-sensitive guanylyl cyclase activity is present in the hippocampus. To examine the possibility that cGMP may participate in LTP production, phosphodiesterase-resistant membrane-permeant analogs of cGMP (dibutyryl- or 8-bromo-cGMP; 100 μM or 1 mM) were applied extracellularly while the magnitude of the field EPSP was monitored. As has been reported by others (4, 19), cGMP analogs alone failed to induce any enhancement of synaptic transmission [101.6% ± 3.8% (mean percent of baseline ± SEM), 30 min after application] (Fig. 1). Tetanic stimulation delivered to presynaptic afferents in the presence of the cGMP analog and the NMDA receptor antagonist AP5 (50 μM) also failed to produce any synaptic enhancement, although the same tetanic stimulus delivered after the washout of AP5 and the cGMP analog resulted in LTP (Fig. 1 *A* and *B*). To assess the involvement of a cGMP-dependent protein kinase (cGMP-PK) we applied the general protein kinase inhibitor H-8, which, at low concentrations, is most efficacious in inhibiting cyclic nucleotide-dependent protein kinases (42). H-8 (10 μM) did not affect the magnitude or the time course of the potentiation elicited by tetanic stimulation, suggesting that cGMP-PK activity is not required for LTP production (control, 139.7% ± 13.5%; H-8, 152.9% ± 12.5%; n = 9) (Fig. 1 *C* and *D*).

NO-Stimulated ADP-Ribosylation. To test if cells in region CA1 of the hippocampus possess NO-stimulated ADPRT activity, we conducted an *in vitro* assay which examined the ability of CA1 hippocampal tissue to promote the ADP-ribosylation of amino acid residues. Previous studies (43, 44) have shown that isolated amino acids can serve as exogenous ADP-ribose acceptors for purified ADPRTs. In a similar manner, we linked either arginine or cysteine [two common acceptors for ADP-ribose in mono-ADP-ribosylation reactions (20)] to CNBr-activated Sepharose beads and then added the beads to a standard ADP-ribosylation mixture containing [^{32}P]NAD^+. Bead-linked amino acids were then

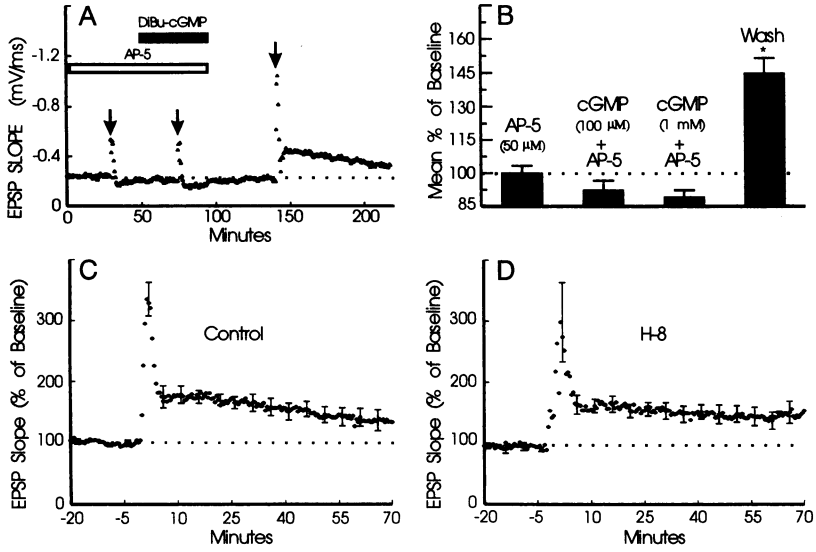

FIG. 1. (*A* and *B*) Extracellular application of cGMP analogs does not induce synaptic enhancement. (*A*) Field EPSP recording of hippocampal slice exposed to tetanic stimulation (arrows) in AP5 alone, in AP5 + dibutyryl-cGMP, and after washing with ACSF. (*B*) Summary data. No significant LTP was produced in AP5 alone, or in AP5 + 100 μM (n = 4) or 1 mM (n = 3) dibutyryl-cGMP; however, LTP was obtained in the same slices after washing with ACSF (n = 7; $P < 0.01$) as measured 20 min (AP5 + cGMP) or 1 hr (wash) after tetanus. (*C* and *D*) H-8, a cGMP-dependent protein kinase inhibitor, does not block LTP. Ensemble averages show a significant amount of potentiation in both control (*C*) ($P < 0.05$) and H-8-treated (*D*) ($P < 0.01$) pathways.

separated from the reaction mixture by a series of centrifugations and also subjected to high-salt washes to remove any ionically bound molecules.

In the absence of hippocampal tissue, a small amount of background incorporation of ^{32}P was observed on the beads; this background activity was not affected by the addition of the NO donor SIN-1 at 500 μM (Fig. 2). However, the addition of CA1 homogenate to the ADP-ribosylation reaction mixture significantly stimulated the ADP-ribosylation of both arginine and cysteine (Fig. 2). This tissue-stimulated ADP-ribosylation was enhanced severalfold by the addition of SIN-1. It is unlikely that the observed ADP-ribosylation is nonenzymatic, since arginine does not undergo this type of reaction (45) and there are no amino groups available for nonenzymatic attachment on cysteine bound to beads. In addition, the NAD glycohydrolase inhibitor isoniazid (20 mM) produced no inhibition of the NO-stimulated ADP-ribosylation (n = 4; data not shown). In contrast, the mono-ADPRT inhibitors Phyl (100 μM) and Nic (10 mM) significantly attenuated the NO-induced enhancement of the ADP-ribosylation of arginine and, to a lesser extent, reduced the ADP-ribosylation of cysteine (Fig. 2). The different sensitivities to the ADPRT inhibitors exhibited by the arginine and cysteine are consistent with the existence of distinct classes of endogenous ADPRTs which preferentially modify arginine or cysteine residues (20, 24).

It has been reported that NO stimulates the automodification of GAPDH, rather than stimulating the activity of endogenous ADPRT (28–30). To test if the NO-stimulated ADP-ribosylation observed in the presence of CA1 tissue could be accounted for by GAPDH present in the hippocampal homogenate, we added purified GAPDH and NO to reaction mixtures in the absence of CA1 tissue. The addition of GAPDH and NO failed to promote the ADP-ribosylation of either arginine or cysteine (Fig. 2). Thus, these data indicate that under these particular experimental conditions NO does not stimulate GAPDH-mediated ADP-ribosylation of exogenous amino acids.

Extracellular Application of ADPRT Inhibitors. To test whether the activity of an ADPRT participates in LTP production we utilized the same ADPRT inhibitors that attenuated the endogenous hippocampal ADPRT activity (Fig. 2). In each experiment, two afferent pathways in the same slice were utilized to examine both control and inhibitor-treated potentiation. Phyl was also a very effective blocker of LTP, at a concentration (100 μM) well below the IC_{50} for inhibition of the poly-ADPRT reported in other tissue [control potentiation, 161.0% ± 18.4%; Phyl, 111.0% ± 6.4% (mean percent of baseline ± SEM)] (Fig. 3 *A* and *D*). The ADPRT inhibitor Nic also blocked LTP, in a dose-dependent manner: 10 mM Nic was most effective (Fig. 3 *B* and *D*), 1 mM Nic was less effective, and 200 μM was ineffective [control (n = 9), 150.6% ± 9.3%; 10 mM Nic (n = 9), 97.0%

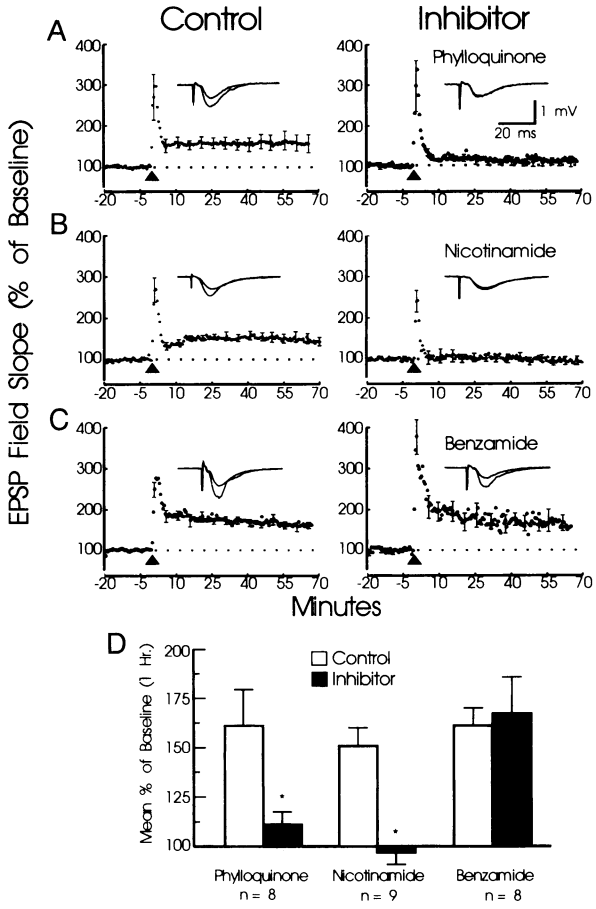

FIG. 3. Extracellular application of mono-ADPRT inhibitors prevents tetanus-induced LTP (arrowheads) of the field EPSP. The *Insets* above each ensemble average show two representative field EPSPs from a slice bathed in control ACSF (*A–C Left*) or in a second afferent pathway after inhibitor application (*A–C Right*), recorded 10 min before and 60 min after tetanic stimulation. (*A–C*) Ensemble averages for control and Phyl (*A*), control and Nic (*B*), and control and benzamide (*C*) experiments. For *A–C* a significant amount of potentiation was observed in all control ($P < 0.01$) and benzamide-treated ($P < 0.05$) pathways, but not in pathways treated with Nic or Phyl. (*D*) Summary. Asterisks indicate that Phyl- and Nic-treated pathways exhibited significantly less LTP than their controls ($P < 0.05$ and $P < 0.01$, respectively).

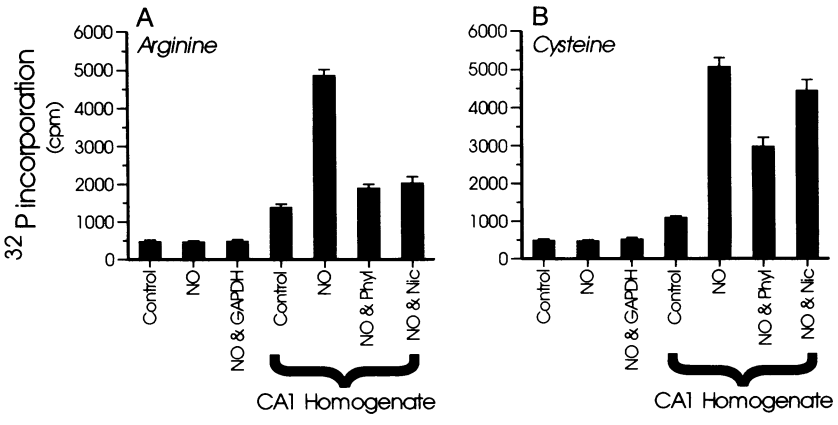

FIG. 2. NO stimulates the enzyme-dependent ADP-ribosylation of arginine and cysteine linked to CNBr-activated Sepharose beads. In the absence of CA1 homogenate a small amount of ^{32}P is bound to arginine- (*A*) or cysteine- (*B*) linked beads. Addition of SIN-1 (or sodium nitroprusside; data not shown) does not significantly enhance this basal ADP-ribosylation, nor did the addition of SIN-1 + purified GAPDH. However, the addition of hippocampal homogenate resulted in a significant ADP-ribosylation of arginine and cysteine (mean increase: arginine, 293.3%, $P < 0.001$, n = 20; cysteine, 229.5%, $P < 0.001$, n = 20). The addition of SIN-1 to these reactions caused a dramatic enhancement of the ADP-ribosylation (arginine, 351.5%, $P < 0.001$, n = 20; cysteine, 458.9%, $P < 0.001$, n = 20). The homogenate-dependent increase in arginine ADP-ribosylation induced by SIN-1 was significantly attenuated by the ADPRT inhibitor Phyl (mean inhibition, 61.2%, $P < 0.001$, n = 12) and Nic (58.4%, $P < 0.001$, n = 12). The homogenate-dependent increase in cysteine ADP-ribosylation induced by SIN-1 was also significantly attenuated by the ADPRT inhibitor Phyl (41.4%, $P < 0.001$, n = 12) but not Nic (12.5%, not significant, n = 12).

± 6.0%; 1 mM Nic (n = 9), 109.1% ± 8.7%; 200 μM Nic (n = 8), 143.1% ± 7.3%]. The observed dose–response profile fits with the interpretation that Nic is working by inhibiting a mono- rather than a poly-ADPRT. Nic (1–5 mM) also blocked LTP when experiments were conducted at 30°C rather than room temperature [91.7% ± 11.7% (n = 6)]. To directly assess the potential involvement of a poly-ADPRT we used the poly-ADPRT inhibitor benzamide at 100 μM (40, 41). In contrast to the block of LTP observed with Phyl and Nic, benzamide did not prevent tetanus-induced enhancement of synaptic transmission (control, 161.0% ± 9.1%; benzamide, 167.1% ± 18.6%) (Fig. 3 *C* and *D*). Taken together, these data suggest that mono- rather than poly-ADPRT activity is necessary for the induction of LTP.

A blockade of LTP was observed with two different ADPRT inhibitors (Phyl and Nic) with distinct chemical structures, arguing against a nonspecific pharmacological effect being responsible for the prevention of LTP. As illustrated in Fig. 4*A*, neither of the ADPRT inhibitors that were effective in blocking LTP had any significant effect on basal excitatory synaptic transmission. In addition, neither inhibitor had any significant effect on either the inhibitory postsynaptic potential (IPSP) recorded in pyramidal neurons (mean of control IPSP ± SEM: Phyl, 95.7% ± 1.3%, n = 3; Nic, 91.8% ± 5.4%, n = 4) (Fig. 4*B*) or on the NMDA-mediated component of the EPSC measured with whole-cell recording (mean of control current ± SEM: Phyl, 101.3% ± 9.4; Nic, 96.8% ± 8.7). Fig. 4 also illustrates that the NO synthase inhibitor L-methylarginine, previously reported to block LTP, has no effect on the amplitude of the NMDA current. In addition, L-nitroarginine (10–100 μM) does not affect the pharmacologically isolated NMDA-receptor-mediated extracellular field potential (data not shown). The inability of Phyl and Nic to alter basal excitatory, inhibitory, and NMDA-mediated synaptic transmission is consistent with the interpretation that these drugs block LTP by interfering with the ADPRT activity.

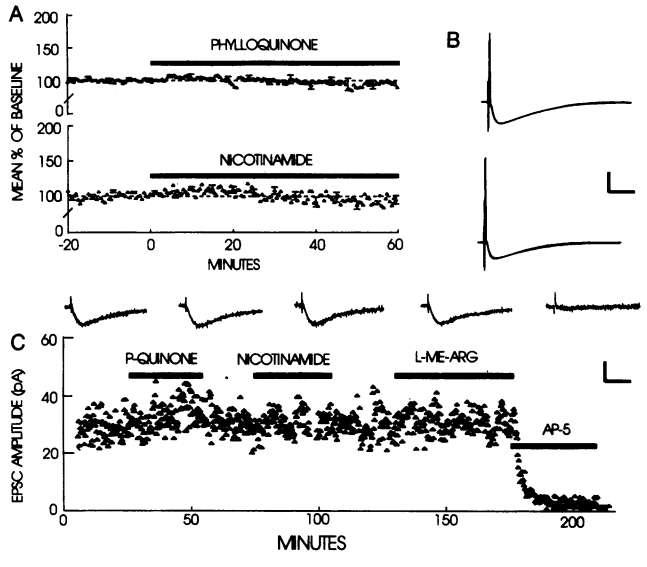

FIG. 4. ADPRT inhibitors do not affect basal excitatory or inhibitory synaptic transmission or the magnitude of NMDA-mediated EPSCs measured with whole-cell recording. (*A*) Ensemble average showing baseline synaptic transmission for all experiments before and after the addition of inhibitors. (*B*) Superimposed averaged IPSPs before and after Phyl (*Upper*) and Nic (*Lower*). Scale: 10 mV/200 ms. (*C*) NMDA-mediated EPSC amplitude before and after the addition of Phyl, Nic, and the NO synthase inhibitor L-methylarginine. Traces are the average of 10 consecutive traces taken during the last 5 min of control and inhibitor-treated periods. The current was abolished by the application of AP5, indicating that the EPSC was mediated by the NMDA receptor channel. Scale: 30 pA/10 ms.

Postsynaptic Injection of ADPRT Inhibitor. To ascertain whether postsynaptic ADPRT activity is important for LTP, Nic was injected directly into an individual postsynaptic CA1 pyramidal neuron through the intracellular recording microelectrode (Fig. 5). In contrast to the blockade of LTP observed when Nic was applied in the bath, intracellular postsynaptic Nic did not prevent pairing- or tetanus-induced synaptic enhancement of the intracellular EPSP [200.8% ± 26.1% or 214.0% ± 18.7%, 1 hr after pairing (n = 4; data not shown) or tetanus, respectively] (Fig. 5*B*). However, tetanus-induced LTP of a second afferent pathway in the same slice was blocked after Nic (5–10 mM) was added to the bath (90.1% ± 4.7%) (Fig. 5 *B* and *C*). The possibility that postsynaptically introduced Nic failed to reach sufficient concentrations in the dendrites cannot be ruled out at this time. However, it is attractive to suggest that Nic's potency in preventing LTP when applied in the bath may be attributed to inhibition of ADPRT activity at a site other than the postsynaptic cell, perhaps the presynaptic nerve terminal, or another compartment such as glial cells.

Induction vs. Maintenance. In several experiments, Nic or Phyl was added to the bath after LTP induction to examine whether continuous ADPRT activity is necessary to maintain potentiation. As illustrated in Fig. 5, bath application of Nic after induction of LTP produced little decline in the field or intracellular EPSP. Potentiated pathways exposed to Nic or Phyl beginning 10–20 min after tetanus exhibited 11.6% ± 3.0% (n = 15) or 3.9% ± 6.7% (n = 8) decline, respectively, measured 40–50 min after the initial application of the inhibitor. Potentiated pathways not exposed to an inhibitor exhibited 12.2% ± 3.4% (n = 10) decline. Thus, inhibition of ADPRT activity after LTP induction does not affect estab-

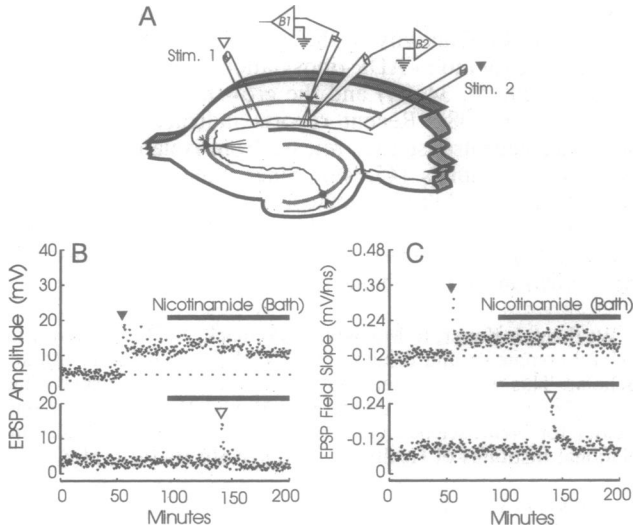

FIG. 5. Injection of Nic into the postsynaptic cell does not block LTP. (*A*) Schematic diagram. Two stimulating electrodes, an extracellular recording electrode (B2), and an intracellular microelectrode containing 500 mM Nic (B1) were placed as shown in a hippocampal slice. (*B* and *C*) Nic was injected into the postsynaptic cell prior to tetanic stimulation of one pathway and subsequently applied in the bath prior to tetanic stimulation of a second pathway. (*B*) Intracellular EPSP amplitudes elicited by stimulation of two independent (*Upper* and *Lower*) afferent pathways. (*C*) Field EPSP slopes for stimulation of two independent (*Upper* and *Lower*) populations of afferent fibers. ▼s, show that tetanic stimulation by electrode 2 resulted in robust LTP in both the intra- (*B Upper*) ($P < 0.01$) and extracellular (*C Upper*) ($P < 0.01$) recording (n = 7). Solid bar indicates the addition of 5 mM Nic into the ACSF. At ▽, tetanic stimulation was delivered from electrode 1. Bath-applied Nic blocked LTP of both the intra- (*B Lower*) and extracellular (*C Lower*) LTP (n = 7).

lished LTP, suggesting that ADPRT activity is important only during a window of time surrounding LTP induction.

DISCUSSION

The inability of membrane-permeant cGMP analogs to produce potentiation and the inability of H-8 to attenuate LTP suggests that cGMP-dependent processes are neither necessary nor sufficient for LTP production. It should be noted that our results differ from the recently published studies of Zhou *et al.* (19). There are several differences in the experimental protocols used that could account for the different results obtained, including the type of chamber used (submerged vs. interface), the recording temperature (22–25°C vs. 30°C), and the stimulation frequency (0.15–0.33 Hz vs. 0.02 Hz). We have not been able to directly address the role of cGMP, since available inhibitors of guanylyl cyclase—e.g., methylene blue and LY83583—depress synaptic transmission (unpublished observations) or inhibit NO production (46), respectively.

The experiments performed with arginine and cysteine provide simple and clear evidence that the CA1 region of the hippocampus possesses an ADPRT activity that can be significantly enhanced by NO and attenuated by inhibitors of mono-ADP-ribosylation. These data support observations from other laboratories that NO can stimulate the activity of endogenous cellular ADPRTs (34, 35). The electrophysiological data provide evidence that ADPRT activity may be an important step in LTP production. Two chemically distinct ADPRT inhibitors prevented LTP, without affecting basal excitatory or inhibitory synaptic transmission or affecting the magnitude of the NMDA-mediated whole-cell currents. Naturally, our interpretation of these data relies on the specificity of the inhibitors we have used; in future experiments it will be necessary to examine directly whether ADP-ribosylation is involved in LTP.

It is interesting to speculate on potential mechanisms that involve ADPRT activity in the presynaptic terminal. Common targets for mono-ADP-ribosylation include GTP-binding proteins (20, 24, 34, 47) and the growth-associated protein GAP-43/B-50 (48). Recent reports indicate that NO can stimulate neurotransmitter release from isolated hippocampal nerve terminals (45, 49). Since both G proteins and GAP-43 may modulate exocytosis (50, 51), the regulation of these protein(s) by NO-stimulated ADP-ribosylation could potentially lead to increases in neurotransmitter release that may function as a mechanism of LTP expression.

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