Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 4061–4066, March 1999
Neurobiology

Evidence of a role for cyclic ADP-ribose in long-term synaptic depression in hippocampus

(CA1 region/cyclic GMP/ryanodine/synaptic plasticity)

MAGALI REYES-HARDE*, RUTH EMPSON†, BARRY V. L. POTTER‡, ANTONY GALIONE†, AND PATRIC K. STANTON*§

*Departments of Neuroscience and Neurology, Albert Einstein College of Medicine, Bronx, NY 10461; †University Department of Pharmacology, Mansfield Road, University of Oxford, Oxford OX1 3QT, United Kingdom; and ‡Wolfson Laboratory of Medicinal Chemistry, Department of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, United Kingdom

Communicated by Michael V. L. Bennett, Albert Einstein College of Medicine, Bronx, NY, December 31, 1998 (received for review September 23, 1998)

ABSTRACT **Ca^{2+} released from presynaptic and postsynaptic intracellular stores plays important roles in activity-dependent synaptic plasticity, including long-term depression (LTD) of synaptic strength. At Schaffer collateral–CA1 synapses in the hippocampus, presynaptic ryanodine receptor-gated stores appear to mobilize some of the Ca^{2+} necessary to induce LTD. Cyclic ADP-ribose (cADPR) has recently been proposed as an endogenous activator of ryanodine receptors in sea urchin eggs and several mammalian cell types. Here, we provide evidence that cADPR-mediated signaling pathways play a key role in inducing LTD. We show that biochemical production of cGMP increases cADPR concentration in hippocampal slices *in vitro*, and that blockade of cGMP-dependent protein kinase, cADPR receptors, or ryanodine-sensitive Ca^{2+} stores each prevent the induction of LTD at Schaffer collateral–CA1 synapses. A lack of effect of postsynaptic infusion of either cADPR antagonist indicates a probable presynaptic site of action.**

Long-term activity-dependent changes in synaptic efficacy are widely held to be an important method of memory storage in all animals possessing nervous systems. Synaptic transmission and short-term and long-term synaptic plasticity all depend upon the divalent cation Ca^{2+}. To be essential in all these forms of neuronal processing, Ca^{2+} must perform a multitude of distinct functions under differing circumstances. Indeed, both long-term potentiation (LTP) and long-term depression (LTD) of synaptic strength depend on raising intraneuronal Ca^{2+} concentration, making it something of a mystery how raising concentration of the same ion can alter synaptic efficacy in either direction. Differing magnitudes and locations of calcium elevations have been invoked as potential explanations, along with corequirements for other second messengers.

Neurons possess many ways of selectively and locally raising both presynaptic and postsynaptic Ca^{2+} concentration. These include influx through glutamate-gated channels, depolarization-activated channels, and release from both Ca^{2+}- and messenger-gated stores in endoplasmic reticulum. All these sources have been implicated in the induction of LTP and LTD.

Recently, we demonstrated that both presynaptic and postsynaptic Ca^{2+} stores play important roles in the induction of LTD at Schaffer collateral–CA1 synapses in hippocampus (1). Our work suggests necessary roles for the gaseous messenger nitric oxide (NO) (2), cyclic 3′,5′ guanosine monophosphate (cGMP) (2), and release of Ca^{2+} from presynaptic, ryanodine receptor (RyR)-gated Ca^{2+} stores (1). However, it is unclear whether cGMP-stimulated and Ca^{2+}-dependent cascades interact with one another or are independent. A recently discovered messenger molecule, cyclic ADP-ribose (cADPR), offers an intriguing possible connection. In sea urchin eggs (3) and mammalian cells (4, 5), cGMP has been shown to stimulate a cyclase/hydrolase that synthesizes cADPR. There is a cADPR binding site on ryanodine-sensitive channels which, when stimulated, enhances Ca^{2+}-triggered release of Ca^{2+} from this intracellular pool. We tested the hypothesis that cADPR might be a crucial messenger mediating the induction of LTD, by biochemically measuring cADPR synthesis and testing the ability of two selective membrane-permeant cADPR antagonists to impair induction of LTD at Schaffer collateral–CA1 synapses in hippocampal slices *in vitro*.

MATERIALS AND METHODS

Electrophysiological Recordings from Hippocampal Slices. Transverse hippocampal slices were prepared from 14- to 21-day-old Sprague–Dawley rats of either sex sacrificed while they were under deep ether anesthesia. The hippocampus plus entorhinal cortex was dissected out, and 400-μm-thick slices were cut simultaneously with a spring-loaded mechanism (“egg slicer”) that rapidly forces a parallel grid of 20-μm-diameter wires through the tissue. Slices recovered for 1 hr at 33°C in a humidified, oxygenated (95% O_2/5% CO_2) interface recording chamber continuously recirculating 2 ml of artificial cerebrospinal fluid (ACSF). ACSF composition (in mM) was NaCl 126, $NaHCO_3$ 26, NaH_2PO_4 1.25, KCl 5, $CaCl_2$ 2, $MgCl_2$ 2, D-glucose 10, pH 7.4.

Two separate Schaffer collateral-commissural axon populations were isolated by placing stimulating electrodes in the stratum radiatum on opposite sides of the recording site, verified as separate inputs by a lack of paired-pulse facilitation (50-ms interval), and alternately stimulated each 30 s with bipolar stainless steel electrodes (Frederick-Haer; 150-μs dc square pulses). Extracellular recording electrodes (R_E = 2–5 MΩ) were filled with 2 M NaCl. We adjusted stimulus intensity so population excitatory postsynaptic potential (EPSP) amplitude was 50% of maximum (>2 mV), as determined by input/output curves.

LTD was evoked by a low-frequency stimulus (LFS) train (150-μs pulses at 2 Hz for 10 min). Maximum initial slopes of field and intracellular EPSPs were calculated by using a six-point interpolation least-squares linear regression method.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “*advertisement*” in accordance with 18 U.S.C. §1734 solely to indicate this fact.

PNAS is available online at www.pnas.org.

Abbreviations: cADPR, cyclic adenosine 5′-diphosphate-ribose; EPSP, excitatory postsynaptic potential; LFS, low-frequency stimuli; LTD, long-term depression; LTP, long-term potentiation; PKA, cAMP-dependent protein kinase; PKG, cGMP-dependent protein kinase; Rp-8pCPT-cGMPS, R_P-8-(4-chlorophenylthio)guanosine 3′,5′-cyclic monophosphorothioate; RyR, ryanodine receptor.

§To whom reprint requests should be addressed at: Department of Neuroscience, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461-1602. e-mail: stanton@aecom.yu.edu.

Each point is from slopes normalized to pre-LFS baselines, averaged over all experiments ± SEM. Changes in synaptic strength were assessed as percent change between baseline averages 10 min preceding LFS and the average of 10 points spanning the 30-min post-LFS period. Statistical significance was determined by a two-tailed Student's t test with significance level $P < 0.05$. Drugs were divided into aliquots and frozen in 1000× stock solutions in distilled H_2O (extracellular), 2 M potassium acetate (intracellular), or DMSO in the case of ryanodine (Research Biochemicals), and they were diluted immediately before use. 7-Deaza-8-Br-cADPR and 8-NH_2-cADPR were synthesized as described (6), purified on a column of Q-Sepharose Fast Flow (Sigma) using a gradient of triethylammonium bicarbonate as buffer, isolated as triethylammonium salts, and fully characterized by UV, NMR, and mass spectroscopy.

Intracellular evoked EPSPs were recorded from CA1 pyramidal neurons (resting membrane potential = −63.6 ± 0.5 mV; R_N = 49 ± 2.5 MΩ; n = 15) impaled with sharp microelectrodes (80–120 MΩ, 2 M potassium acetate). Sharp electrodes were used to prevent rundown of cells caused by washout of intracellular factors. To facilitate infusion of cADPR antagonists into pyramidal neurons, biphasic current pulses of 0.2–0.5 nA magnitude, 200-ms duration were injected at a frequency of 1 Hz for 10–15 min prior to the start of recording baseline EPSPs. In all experiments, simultaneous field recordings were made to monitor slice stability.

Biochemical Measurement of ADP-Ribosyl Cyclase Activity. ADP-ribosyl cyclase activity was measured in hippocampal homogenates by bioassay of activity of product on sea urchin egg homogenate. Hippocampi from five rats were gently homogenized in 0.25 M sucrose/10 mM Hepes buffer, pH 7.2, and centrifuged at 1,000 × g at 4°C. To test for cyclase activity, supernatant was incubated with 2.5 mM β-NAD^+ at 37°C for 10–90 min, and ADP-ribosyl cyclase activity was determined by adding 5 μl of incubation supernatant to sea urchin egg homogenate and measuring evoked increase in Ca^{2+} concentration with Fluo-3 (Molecular Probes). Fluorescence was calibrated daily against signals generated by known concentrations of synthetic cADPR (10–50 nM). All measurements were done in triplicate, on 6 separate days with material from two preparations with protein concentrations between 1.5 and 2 mg/ml. We verified that Ca^{2+} release was due to cADPR, by blockade with prior addition of large amounts of cADPR to desensitize the release apparatus, or of the cADPR antagonist 8-NH_2-cADPR (5 μM; data not shown). In parallel experiments (n = 6), hippocampal homogenates degraded labeled cADPR (75% ± 15% reduction in cADPR concentration after 90 min), consistent with the bifunctional nature of ADP-ribosyl cyclase/hydrolase.

Biochemical Measurement of cADPR Concentration. cADPR concentration was measured in hippocampal slices by a method similar to radioimmunoassay, replacing antibody with purified cADPR receptor from sea urchin egg homogenate. Hippocampal slices (12–14) were prepared by using a Vibroslicer (Camden Instruments) and placed in an interface chamber at room temperature (25°C). After 1-hr preincubation in normal ACSF, slices were treated for 30 min with either zaprinast (20 μM) or zaprinast plus inhibitor H89 (10 μM) to prevent coactivation of cAMP-dependent protein kinase (PKA). After treatment, slices were immersed in ice-cold Ca^{2+}-free ACSF for three volume exchange washes to block synaptic transmission and reduce metabolism, and they were stored at −80°C. Samples were thawed, excess ACSF was removed, and the samples were disrupted vigorously in 200 μl of gluconate-based Ca^{2+}-free buffer. Aliquots were taken from all samples for protein determination by standard methods.

A standard binding curve was generated with 10 nM [^{3}H]cADPR plus various concentrations of unlabeled cADPR (1 nM to 10 μM), in 250 μl of gluconate buffer, plus 10% sea urchin egg homogenate (0.3–0.4 mg/ml). After 25-min incubation on ice, binding was interrupted by filtration over GF/C filters (Sigma) and three washes with 5 ml of ice-cold buffer. Filters were collected and radioactivity was measured in a scintillation counter for 1 min. A standard curve was plotted for cADPR concentration versus radioactivity minus nonspecific binding.

To extract cADPR, 50 μl of slice homogenate was treated with 50 μl of 1 M trichloroacetic acid to precipitate all proteins, and was centrifuged at 1,000 × g for 2 min. Supernatant was neutralized with 25 μl of 1 M Na_2CO_3, and 5- or 10-μl aliquots were added to the radioassay in place of unlabeled cADPR. On the linear portion of the standard curve, cADPR concentration in the deproteinized sample was calculated and corrected to pmol/mg of protein in the original sample.

RESULTS

Fig. 1*A* illustrates normal LTD of synaptic strength at Schaffer collateral-CA1 synapses in hippocampal slices *in vitro*. When a prolonged train of LFS (2 Hz/10 min; solid bar) was applied to Schaffer collateral axons, a reduction in the initial slope of evoked EPSPs was elicited. After LFS, stable LTD was observed which persisted for at least 1 hr (−41% ± 8% decrease from pre-LFS baseline EPSP slope) and was confined to the stimulated set of synapses (control input not shown). Because some of the antagonists used for these studies were in limiting supply, all experiments (including controls) where drugs were bath applied were conducted in a recirculating chamber with a total volume of ≈2 ml.

Release of Ca^{2+} from Ryanodine-Sensitive Stores Is Necessary for Induction of LTD. We have recently shown that ryanodine-sensitive intracellular calcium stores in Schaffer collateral terminals play a necessary role in the induction of LTD (1). Fig. 1*B* confirms this blockade in slices in our recirculating chamber. When 10 μM ryanodine (hatched bar) was bath applied 30 min before LFS (2 Hz/10 min; solid bar), a significant reduction in LTD was observed (−13% ± 12%; $P < 0.05$, Student's t test compared with control LTD). Previously, we showed that postsynaptic infusion of up to 5 mM ryanodine into CA1 pyramidal neurons was unable to block the induction of LTD at Schaffer collateral synapses, consistent with a presynaptic site of action.

Inhibitors of PKG Block Induction of LTD. Evidence suggests that a major component of LTD depends on activation of presynaptic guanylyl cyclase by the retrograde messenger NO (2, 7). In other cells, the second messenger cGMP has been shown to elevate intracellular Ca^{2+} concentration by stimulating production of cADPR by ADP-ribosyl cyclases (8, 9), a process thought to be dependent on activation of PKG. To examine directly the idea that such a cascade plays a role in the induction of LTD, we tested the necessity for PKG activity. Fig. 1*C* illustrates these experiments, in which the cell-permeant, hydrolysis-resistant PKG inhibitor Rp-8pCPT-cGMPS (Bio-Mol, Plymouth Meeting, PA) (10) was bath applied (10 μM; hatched bar) 30 min before LFS (2 Hz/10 min; solid bar). As with ryanodine, inhibition of PKG blocked the induction of LTD at Schaffer collateral–CA1 synapses (−7% ± 5%; $P < 0.05$, Student's t test compared with control LTD). We observed a similar block of induction of LTD with a second PKG inhibitor, KT5823 (11) (10 μM; +2% ± 8%; n = 6; data not shown).

Raising cGMP Concentration in Hippocampal Slices Stimulates Synthesis of cADPR. Given that cGMP, PKG, and RyR-gated calcium stores are all necessary for the induction of LTD, it seemed a reasonable hypothesis that cADPR could be the messenger linking cGMP to release from calcium stores. If this hypothesis is true, the hippocampus should be able to synthesize cADPR, and raising cGMP concentration should

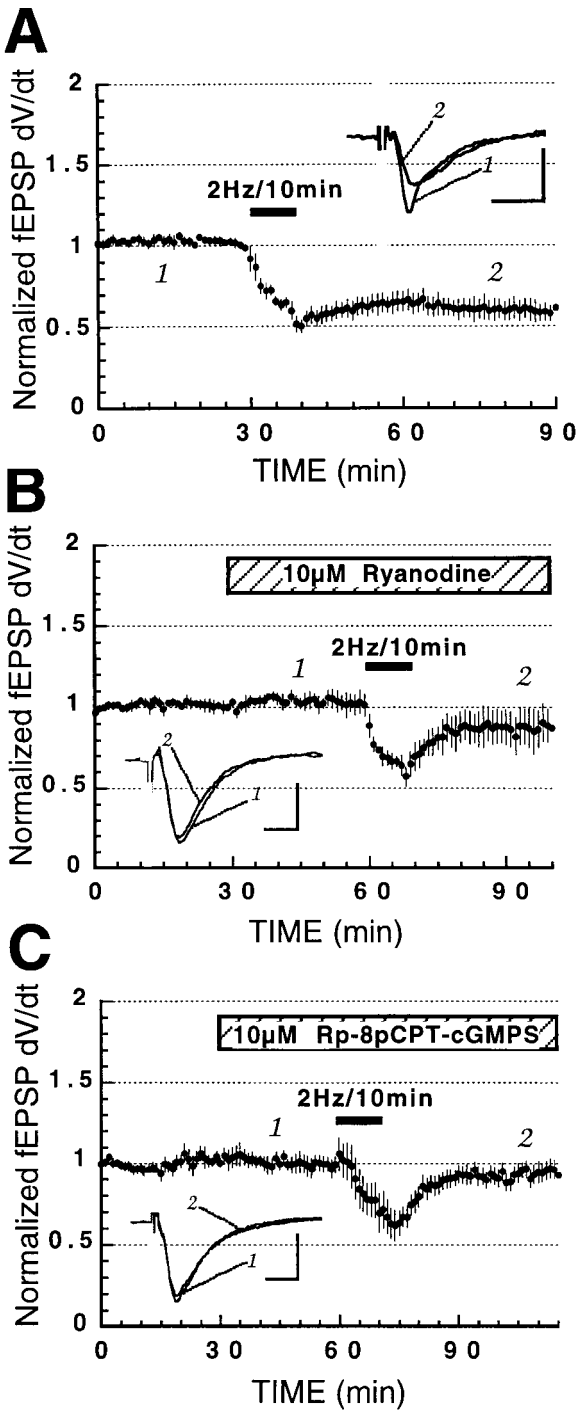

FIG. 1. Blockade of induction of LTD by ryanodine and the cGMP-dependent protein kinase (PKG) inhibitor R_P-8-(4-chlorophenylthio)-guanosine 3′,5′-cyclic monophosphorothioate (Rp-8pCPT-cGMPS). The first derivative of the EPSP (fEPSP, EPSP slope) is plotted as a function of time. (*Insets*) Single extracellular fEPSPs recorded at times indicated. Scales indicate 2 mV and 4 ms. (*A*) Control LTD of synaptic strength at Schaffer collateral–CA1 synapses in hippocampal slices ($n = 5$). Slices were placed in an interface recording chamber continuously recirculating 2 ml of ACSF. After stable baseline EPSPs had been recorded for at least 30 min, LFS (2 Hz/10 min; solid bar) evoked LTD that remained stable throughout the 1-hr recording period after LFS. (*B*) When 10 μM ryanodine (hatched bar) was bath applied ($n = 4$) 30 min before LFS (solid bar), the magnitude of LTD evoked was markedly and significantly reduced ($P < 0.05$, Student's t test compared with control LTD 30 min after LFS). (*C*) When 10 μM Rp-8pCPT-cGMPS (hatched bar) was bath applied ($n = 8$) 30 min before LFS (solid bar), LTD was also markedly reduced in amplitude ($P < 0.05$, Student's t test compared with control LTD).

stimulate cADPR production. Fig. 2*A* illustrates synthesis of cADPR in hippocampal homogenates. To test for ADP-ribosyl cyclase activity, rat hippocampi were homogenized and the $1{,}000 \times g$ supernatant was incubated with β-NAD^+ for periods ranging from 10 to 90 min. cADPR concentration was then bioassayed by application to sea urchin egg homogenate, and Ca^{2+} released from intracellular stores was measured by

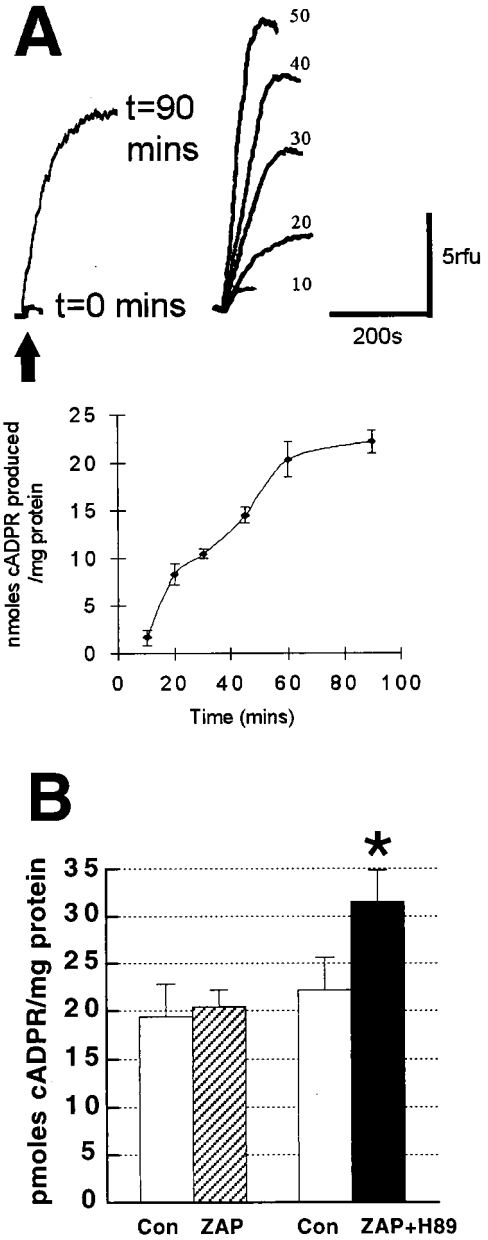

FIG. 2. Hippocampal slices possess cGMP-stimulated ADP-ribosyl cyclase activity. (*A*) (*Upper*) Fluo-3 fluorescence (relative fluorescence units; rfu) increases in sea urchin egg homogenate produced by known concentrations of cADPR (arrow). Numbers next to each trace are cADPR in nmol, against which hippocampal cADPR activity was calibrated. (*Lower*) Time course of cADPR synthesis in hippocampal homogenates incubated with 2.5 mM β-NAD^+ at 37°C. After the indicated times, reaction was stopped and 5 μl of incubation medium was added to sea urchin homogenate ($n = 6$) for fluorescence measurement of amount of cADPR. (*B*) Mean ± SEM amount of cADPR in control hippocampal slices (open bars), versus slices treated with 20 μM zaprinast (ZAP; hatched bar), a type V phosphodiesterase inhibitor that raises cGMP concentration by preventing its degradation, and 20 μM zaprinast plus the PKA inhibitor H89 (10 μM; solid bar). $n = 12$–14 slices per group in three experiments. *, $P < 0.05$, Student's t test compared with control slices.

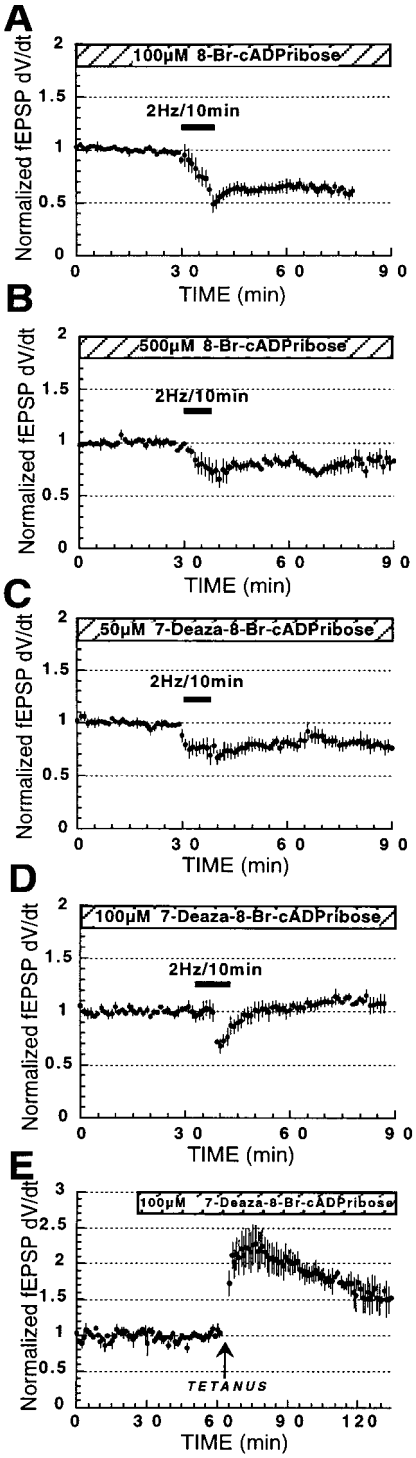

FIG. 3. Blockade of LTD by cell-permeant cADPR receptor antagonists. (*A*) Time course ($n = 5$) of LTD induced by LFS (2 Hz/10 min; solid bar) in the presence of 100 μM 8-Br-cADPR (hatched bar). At this concentration, 8-Br-cADPR had no effect on the magnitude or duration of LTD. (*B*) A 5-fold higher concentration of 8-Br-cADPR (500 μM; hatched bar) produced a partial reduction in the magnitude of LTD ($P < 0.05$, Student's *t* test compared with control LTD; $n = 4$). (*C*) A more potent, nonhydrolyzable cADPR antagonist, 7-deaza-8-Br-cADPR, produced an equivalent partial blockade at a 50 μM concentration (hatched bar; $n = 5$). (*D*) At 100 μM, 7-deaza-8-Br-cADPR (hatched bar) completely blocked the induction of LTD by LFS (solid bar; $P < 0.05$, Student's *t* test compared with Control LTD, 500 μM 8-Br-cADPR, and 50 μM 7-deaza-8-Br-cADPR; $n = 4$). (*E*) In contrast to LTD, 100 μM 7-deaza-8-Br-cADPR (hatched bar) did not impair the induction of LTP by high-frequency stimulation (*TETANUS*; 100 Hz/500 ms in six trains; $n = 4$).

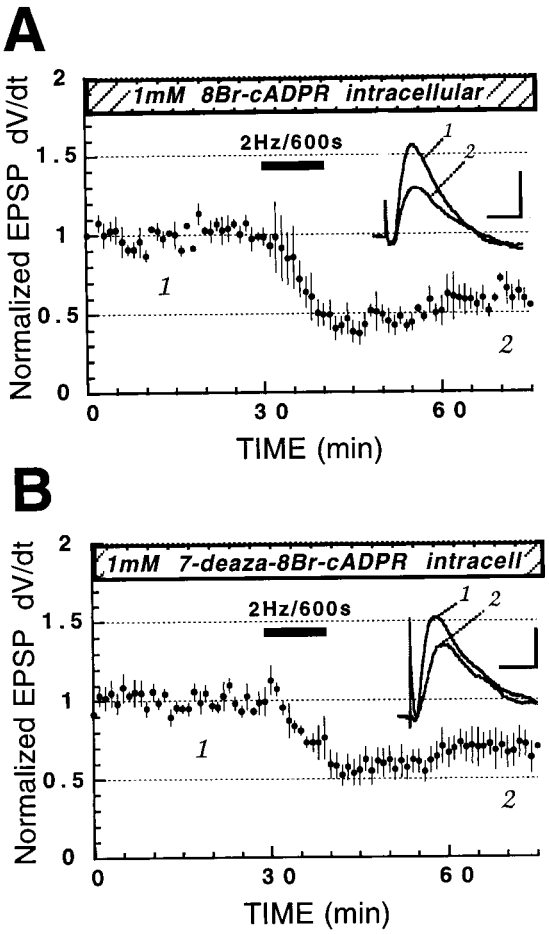

FIG. 4. Postsynaptic intracellular injection of cADPR antagonists into CA1 pyramidal neurons does not affect the induction of LTD. (*A*) Time course of LTD of Schaffer collateral-evoked intracellular EPSPs in CA1 pyramidal neurons ($n = 4$) impaled with microelectrodes containing 1 mM 8-Br-cADPR. Before baseline recording was commenced, 10–15 min of depolarizing current pulse injection (0.2–0.5 nA/200 ms at 1 Hz) was applied to facilitate drug infusion. In contrast to extracellular application, postsynaptic 8-Br-cADPR did not alter the magnitude or duration of LTD. As in controls, LTD was homosynaptic (control input not shown). (*B*) Similarly, postsynaptic intracellular infusion of 1 mM 7-deaza-8-Br-cADPR ($n = 7$) was also unable to block or impair expression of LTD at Schaffer collateral–CA1 synapses. (*Insets*) Single intracellular EPSPs recorded at times indicated. Scale bars represent 5 mV and 10 ms.

Fluo-3 fluorescence and compared with fluorescence induced in the same homogenate by known concentrations of synthetic cADPR. Fig. 2*A Lower* shows the time course of cADPR synthesis, while *Upper* shows fluorescence signals calibrated for concentration. Previous work confirmed that antagonists of cADPR binding sites prevent the increases in intracellular Ca^{2+} concentration evoked in this system (6, 12).

Fig. 2*B* summarizes direct measurement of cADPR production stimulated by raising cGMP concentration in hippocampal slices. To raise cGMP concentration, we applied the phosphodiesterase inhibitor zaprinast, which selectively inhibits cGMP-selective (type V) phosphodiesterase (13). Zaprinast, when applied alone at 20 μM to hippocampal slices for 30 min (hatched bar), did not significantly change cADPR produced compared with control slices (open bars). However, when zaprinast (20 μM) was applied along with the PKA inhibitor H89 (10 μM) to block PKA activation that might counteract cGMP, it did elicit a sustained increase in cADPR concentration in whole hippocampal slices (+51% ± 16%; $P < 0.05$, Student's *t* test compared with untreated slices). The rationale

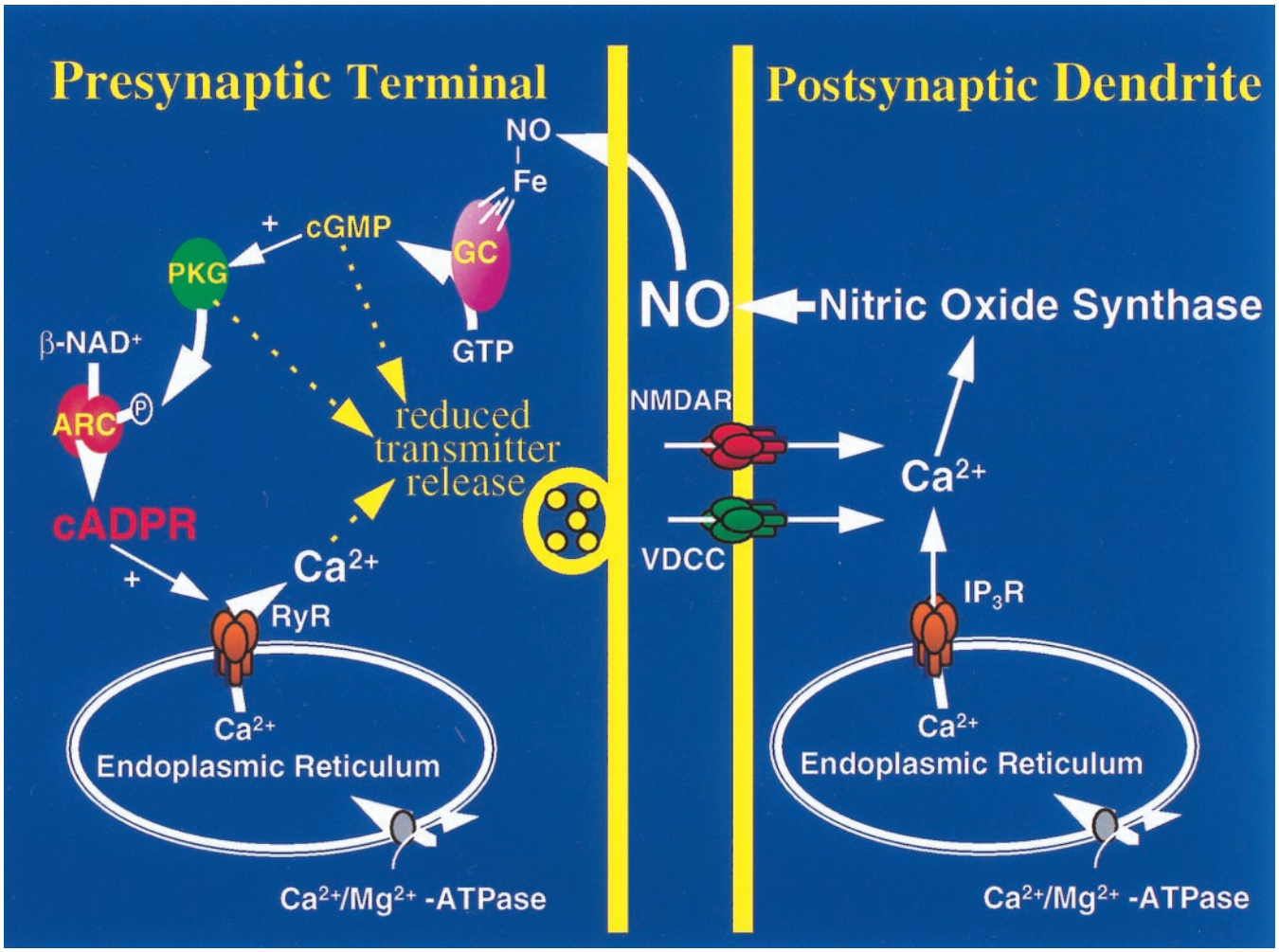

FIG. 5. Proposed presynaptic, NO-triggered cascade for the induction of LTD. The cascade begins with calcium entry via *N*-methyl-D-aspartate receptors (NMDAR), voltage-dependent calcium channels (VDCC), and metabotropic glutamate receptor-triggered, inositol trisphosphate-mediated release of stored calcium, which activates Ca^{2+}/calmodulin-dependent nitric oxide synthase (NOS). NOS produces the retrograde messenger nitric oxide (NO), which diffuses to the presynaptic terminal and activates soluble guanylate cyclase (GC) to generate cGMP. One target of cGMP is PKG, which, when activated, can phosphorylate serine/threonine residues. One potential target of PKG is ADP-ribosyl cyclase/hydrolase (ARC; CD38), which produces cADPR from β-NAD^+. Finally, cADPR facilitates release of calcium from intracellular stores by binding to RyRs on these stores. IP_3R, inositol trisphosphate receptor.

for this co-application protocol arose from our recent discovery that elevating cGMP concentration elicits LTD in field CA1 only when combined with PKA inhibition (14).

Antagonists of cADPR-Stimulated Ca^{2+} Release Block Induction of LTD, but Not LTP. To test the hypothesis that a cADPR binding site is a necessary factor in the induction of LTD, we utilized two different membrane-permeant cADPR antagonists; 8-Br-cADPR (6, 12), and a more potent analog, 7-deaza-8-Br-cADPR, which is a much poorer substrate for cADPR hydrolase (6). Fig. 3 illustrates the results of these experiments, which were also performed in the recirculating chamber. In Fig. 3*A*, 100 μM 8-Br-cADPR (hatched bar) was present in the bath for at least 60 min before the start of the experiment. After 30 min of baseline recording of extracellular EPSPs, LFS (2 Hz/10 min; solid bar) was applied to Schaffer collateral axons, and normal LTD was elicited (−41% ± 5%). In contrast, when a separate group of slices (Fig. 3*B*) was pretreated with 500 μM 8-Br-cADPR, LTD was markedly reduced, but not completely blocked (−17 ± 7%; $P < 0.05$, Student's *t* test compared with control LTD).

Identical experiments were performed with the more potent, hydrolysis-resistant inhibitor 7-deaza-8-Br-cADPR. At 50 μM, 7-deaza-8-Br-cADPR (Fig. 3*C*) produced a partial attenuation of LTD (−22% ± 6%). However, at a higher concentration (100 μM), the induction of LTD was blocked completely (Fig. 3*D*; +7% ± 8%, $P < 0.05$, Student's *t* test compared with control LTD). In contrast, the same high concentration of 7-deaza-8-Br-cADPR in a separate group of slices ($n = 4$) did not impair LTP induced by high-frequency stimuli (six trains of 100 Hz/500 ms; +57% ± 11% 60 min after tetanus, Fig. 3*E*).

Postsynaptic Infusion of cADPR Antagonists Is Unable to Block LTD. Our previous studies indicate a probable presynaptic locus for NO-stimulated guanylyl cyclase (2), cGMP (2), and ryanodine-sensitive calcium stores (1), necessary for this form of LTD, suggesting that cADPR probably also acts presynaptically. To test this possibility directly, we infused either 8-Br-cADPR (1 mM) or 7-deaza-8-Br-cADPR (1 mM) into single CA1 pyramidal neurons by using intracellular microelectrodes. Fig. 4 illustrates the result of these experiments. After allowing 45–60 min for inhibitor to infuse into each neuron, we applied LFS (2 Hz/10 min; solid bars) to one of two Schaffer collateral inputs. Neither 8-Br-cADPR (Fig. 4*A*) nor 7-deaza-8-Br-cADPR (Fig. 4*B*) affected LTD when injected postsynaptically, supporting the hypothesis that presynaptic cADPR receptors are the likely candidates for involvement in the induction cascade leading to LTD.

DISCUSSION

This study supplies evidence for a physiologic role for the candidate messenger cADPR in mammalian long-term synaptic plasticity. We have shown (*i*) that hippocampal tissue possesses cyclase activity that can synthesize cADPR, (*ii*) that this molecule is, in fact, produced by elevating intracellular cGMP concentration in hippocampal slices, and (*iii*) that blockade of cADPR binding sites prevents the induction of LTD, but not LTP. However, we cannot entirely exclude the possibility that some other, closely related molecule that binds to cADPR-sensitive sites, on RyR or elsewhere, is actually the physiologic mediator of these actions.

The synthetic ADP-ribosyl cyclase and catabolic hydrolase activities are usually colocalized on the same polypeptide, with hydrolase activity exceeding cyclase. A notable exception is *Aplysia californica* ADP-ribosyl cyclase, whose extremely high activity and loose substrate specificity allowed synthesis of the first pharmacologically useful cADPR analogs, including 8-Br-cADPR (12). A single 8-position modification of the adenosine ring abolishes all agonist activity, yielding specific, competitive antagonists of cADPR-sensitive Ca^{2+} release. The synthesis of 7-deaza-cADPR yielded a much poorer substrate for cADPR hydrolase. Combining the two substitutions produced 7-deaza-8-Br-cADPR, a more potent, hydrolysis-resistant competitive antagonist that selectively prevents cADPR-stimulated Ca^{2+} release (6). The potency and extent of blockade of LTD by each compound parallel nicely their efficacies, selectively preventing cADPR-sensitive Ca^{2+} release in many cells, including NO-stimulated Ca^{2+} release in neurons (9).

It should be noted that the phosphodiesterase V inhibitor zaprinast was effective in elevating cADPR concentration only when co-applied with the PKA inhibitor H89. This observation is intriguingly consistent with our recent finding (14) that these two inhibitors together induce an activity-dependent LTD of synaptic transmission at Schaffer collateral–CA1 synapses, whereas zaprinast alone causes only a reversible depression. Both these studies point to a bidirectional kinase regulation of synaptic plasticity, in which cGMP and cADPR are necessary for LTD, whereas cAMP and PKA promote LTP. Only when PKG is stimulated *and* PKA is inhibited is cADPR generated and LTD induced.

In Fig. 5, we propose a specific neuronal cascade for the selective induction of LTD. It is triggered by generation of the gaseous intercellular messenger NO in postsynaptic pyramidal neurons, and its diffusion to presynaptic Schaffer collateral terminals. NO stimulates guanylyl cyclase to produce cGMP and activate PKG. Among many possibilities, PKG causes stimulation of cADPR synthesis, which enhances RyR-mediated release of Ca^{2+} necessary to induce LTD. The fact that inhibition of NO-stimulated guanylyl cyclase, PKG, RyR, and cADPR activity all block induction of LTD supports this model.

Multiple observations support a presynaptic site for this cascade. Both postsynaptic chelation of calcium (15) and extracellular chelation of NO (7, 13) have been reported to prevent the induction of LTD, suggestive of diffusion of postsynaptically synthesized NO to a transsynaptic target. In inhibitor studies, postsynaptic injection of an NO-stimulated guanylyl cyclase inhibitor (2), PKG inhibitor (2), ryanodine (1), or cADPR antagonists all failed to block induction of LTD at Schaffer collateral–CA1 synapses, even though postsynaptic injection of dye (1) and other inhibitors (1, 2, 16) verified dendritic infusion. However, a study in dentate granule cells (17) has reported block of LTD by postsynaptic infusion of the RyR antagonist ruthenium red, suggesting that different neuronal types may utilize differing cascades.

Other investigators have postulated a different cascade involving Ca^{2+}-activated protein phosphatases (18), which they believe act postsynaptically (19). We propose that at least two distinctly, and computationally, different cascades exist on opposite sides of the synapse, separately responsible for altering presynaptic transmitter release and postsynaptic sensitivity, with both necessary for full expression of LTD (refs. 1 and 2; Fig. 5). Indeed, a recent report (20) has demonstrated that both *N*-methyl-D-aspartate and metabotropic glutamate receptor-dependent forms of LTD can be preferentially induced by different stimulus protocols, suggesting that different presynaptic activity patterns might trigger one form in preference to another.

It remains to be determined what targets are downstream of cADPR-triggered Ca^{2+} release. One candidate is presynaptic Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII). We have recently shown (16) that bath application of a membrane-permeant inhibitor of CaMKII prevents the induction of both LTD and LTP, whereas postsynaptic injection blocks only LTP. Clearly, synaptic vesicle proteins are appealing targets for both CaMKII and PKG. However, cADPR-mediated Ca^{2+} release has been shown to be calmodulin-dependent (21), and CaMKII has been shown to phosphorylate RyR directly (22), offering other possible targets of presynaptic regulation by CaMKII. Elucidation of the functional roles for cADPR in long-term synaptic plasticity is just beginning.

This work is dedicated to the memories of Lewis N. Stanton, Sr., and Vilma Maldonado. We thank A. Kyrozis, S. Nawy, T. Opitz, and A. Peinado for helpful discussions, and V. C. Bailey and G. A. Ashamu for ligand synthesis. This work was supported by Whitehall Foundation Grant A98-32 (to P.K.S.), Wellcome Trust Grant 045491 (to B.V.L.P.), and National Institutes of Health Medical Scientist Training Program Grant F31GM16379 (to M.R.H.).

1. Reyes, M. & Stanton, P. K. (1996) *J. Neurosci.* **16,** 5951–5960.
2. Gage, A. T., Reyes, M. & Stanton, P. K. (1997) *Hippocampus* **7,** 286–295.
3. Galione, A., Lee, H. C. & Busa, W. B. (1991) *Science* **253,** 1143–1146.
4. Berridge, M. J. (1993) *Nature (London)* **365,** 388–389.
5. Lee, H. C., Walseth, T. F., Bratt, G. T., Hayes, R. N. & Clapper, D. L. (1989) *J. Biol. Chem.* **264,** 1608–1615.
6. Sethi, J. K., Empson, R. M., Bailey, V. C., Potter, B. V. L. & Galione, A. (1997) *J. Biol. Chem.* **272,** 16358–16363.
7. Izumi, Y. & Zorumski, C. F. (1993) *NeuroReport* **4,** 1131–1134.
8. Galione, A., White, A., Willmott, N., Turner, M. & Potter, B. V. L. (1993) *Nature (London)* **365,** 456–459.
9. Willmott, N., Sethi, J. K., Walseth, T. F., Lee, H. C., White, A. M. & Galione, A. (1996) *J. Biol. Chem.* **271,** 3699–3705.
10. Butt, E., Eigenthaler, M. & Genieser, H. G. (1994) *Eur. J. Pharmacol.* **269,** 265–268.
11. Kase, H., Iwahashi, K., Nakanishi, S., Matsuda, Y., Yamada, K., Takahashi, M., Murakata, C., Sato, A. & Kaneko, M. (1987) *Biochem. Biophys. Res. Commun.* **142,** 436–440.
12. Walseth, T. F. & Lee, H. C. (1993) *Biochim. Biophys. Acta* **1178,** 235–242.
13. Boulton, C. L., Irving, A. J., Southam, E., Potier, B., Garthwaite, J. & Collingridge, G. L. (1994) *Eur. J. Neurosci.* **6,** 1528–1535.
14. Santschi, L. A. & Stanton, P. K. (1997) *Soc. Neurosci. Abstr.* **23,** 1175.
15. Mulkey, R. M. & Malenka, R. C. (1992) *Neuron* **9,** 967–975.
16. Stanton, P. K. & Gage, A. T. (1996) *J. Neurophysiol.* **76,** 2097–2101.
17. Wang, Y., Rowan, M. J. & Anwyl, R. (1997) *J. Neurophysiol.* **77,** 812–825.
18. Mulkey, R. M., Herron, C. E. & Malenka, R. C. (1993) *Science* **261,** 1051–1055.
19. Mulkey, R. M., Endo, S., Shenolikar, S. & Malenka, R. C. (1994) *Nature (London)* **369,** 486–488.
20. Oliet, S. H. R., Malenka, R. C. & Nicoll, R. A. (1997) *Neuron* **18,** 969–982.
21. Lee, H. C., Aarhus, R., Graeff, R., Gurnack, M. E. & Walseth, T. F. (1994) *Nature (London)* **370,** 307–309.
22. Takasawa, S., Ishida, A., Nata, K., Nakagawa, K., Noguchi, N., Tohgo, A., Kato, I., Yonekura, H., Fujisawa, H. & Okamoto, H. (1995) *J. Biol. Chem.* **270,** 30257–30259.