0022-3565/01/2981-86–102$3.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 298, No. 1
3792/912924
JPET 298:86–102, 2001
Printed in U.S.A.

Positive Modulation of α-Amino-3-hydroxy-5-methyl-4-isoxazole Propionic Acid (AMPA) Receptors in Prefrontal Cortical Pyramidal Neurons by a Novel Allosteric Potentiator

POLLY J. BAUMBARGER, MARK MUHLHAUSER, JIN ZHAI, CHARLES R. YANG, and ERIC S. NISENBAUM

Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana

Received January 23, 2001; accepted April 2, 2001 This paper is available online at http://jpet.aspetjournals.org

ABSTRACT

Positive modulators of glutamate α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors can enhance cognitive function in several species. The present experiments compared the actions of a novel biarylpropylsulfonamide compound, LY404187, with the prototypical benzoylpiperidine, 1-(quinoxalin-6-ylcarbonyl)-piperidine (CX516), on AMPA receptors of prefrontal cortex (PFC) pyramidal neurons. LY404187 (0.03–10 μM) selectively enhanced glutamate-evoked currents through AMPA receptor/channels of acutely isolated pyramidal neurons with considerably greater potency (EC_{50} = 1.3 ± 0.3 μM) and efficacy (E_{max} = 45.3 ± 8.0-fold increase) than did CX516 (EC_{50} = 2.8 ± 0.9 mM; E_{max} = 4.8 ± 1.4-fold increase). Both LY404187 and CX516 increased the potency of the glutamate concentration-response profile by 6- and 3-fold, respectively. Rapid perfusion experiments demonstrated that LY404187 produced a marked suppression in the magnitude but no change in the kinetics of receptor desensitization; whereas CX516 produced little change in the degree and a modest deceleration of the desensitization process. In PFC slices, both spontaneous and stimulus-evoked AMPA receptor-mediated excitatory postsynaptic potentials were enhanced by nanomolar concentrations of LY404187. Voltage-sensitive *N*-methyl-D-aspartate (NMDA) receptor-dependent synaptic responses also were indirectly augmented as a consequence of greater postsynaptic depolarization. Consistent with the in vitro data, LY404187 was 1000-fold more potent than CX516 in enhancing the probability of discharge of PFC neurons in response to stimulation of glutamatergic afferents from hippocampus in vivo. This potentiation by LY404187 was reduced by both selective AMPA (LY300168, 1 mg/kg, i.v.) and NMDA (LY235959, 5 mg/kg, i.v.) receptor antagonists. Collectively, these results demonstrate that LY404187 is an extremely potent and centrally active potentiator of native AMPA receptors and has a unique mechanism of action. The therapeutic implications of AMPA receptor potentiators are discussed.

An accumulating body of evidence has indicated that recruitment of activity in the prefrontal cortex (PFC) is necessary for higher cognitive functions, particularly those that require short-term working memory (Baddeley, 1992; Frith and Dolan, 1996). In addition, deficits in working memory have been identified in several neurological and psychiatric disorders and are often associated with decreased functioning of the PFC (Baddeley et al., 1991; Weinberger and Berman, 1996). Several lines of evidence suggest that deficits in glutamatergic excitatory transmission in PFC may be involved in working memory impairment. For example, working memory performance in rodents is diminished by reducing glutamate release or its postsynaptic action on α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors of PFC neurons (Romanides et al., 1999). Other experiments have shown that administration of *N*-methyl-D-aspartate (NMDA) glutamate receptor antagonists or agonists can, respectively, reduce or enhance retention times in working memory tasks in monkeys (Dudkin et al., 1997). Analogous brain imaging experiments in humans have shown that administration of NMDA receptor blockers (e.g., phencyclidine, ketamine) disrupt working memory in conjunction with a selective decrease in PFC activity (Krystal et al., 1994). In light of these findings, enhancement of glutamatergic transmission in PFC has been proposed as a strategy for the treatment of certain types of cognitive dysfunction (Yamada, 1998).

Historically, efforts to enhance glutamatergic transmission

ABBREVIATIONS: PFC, prefrontal cortex; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; PSTH, peristimulus time histogram; EPSP, excitatory postsynaptic potential; NMDA, *N*-methyl-D-aspartate; CX516, 1-(quinoxalin-6-ylcarbonyl)-piperidine; LTP, long-term potentiation; BAPTA, 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid; GABA, γ-aminobutyric acid; HβC, 2-hydroxypropyl-β-cyclodextran; ISI, interstimulus interval; APV, DL-2-amino-5-phosphonovaleric acid; AUC, area under the curve, LY235959, (3*R*,4a*R*,6*S*,8a*S*)-(−)-decahydro-6-(phosphonomethyl)-3-isoquinolinecarboxylic acid; LY300168, 5-(4-aminophenyl)-8,9-dihydro-*N*,8-dimethyl-7*H*-1,3-dioxolo[4,5-*H*][2,3]benzodiazepine-7-carboxamide; LY392098, *N*-2-(4-(3-thienyl)phenyl)propyl 2-propanesulfonamide; LY404187, *N*-2-(4-(4-cyanophenyl)phenyl)propyl 2-propanesulfonamide.

have focused on compounds from two chemical classes: pyrrolidinones (e.g., aniracetam, piracetam) and benzothiadiazines (e.g., cyclothiazide), which positively modulate AMPA receptor-mediated currents by suppressing the desensitization process of these receptor/channels (Ito et al., 1990; Copani et al., 1992; Yamada and Tang, 1993). The functional consequence of these compounds is to augment glutamatergic excitatory postsynaptic potentials (EPSPs) by increasing ion flux through AMPA receptors (Ito et al., 1990; Yamada and Tang, 1993). Similar potentiation of AMPA receptor activity and glutamatergic EPSPs has been reported for a newer class of benzoylpiperidine compounds typified by 1-(quinoxalin-6-ylcarbonyl)-piperidine (CX516), which can penetrate the blood-brain barrier (Stäubli et al., 1994a,b; Arai and Lynch, 1998a,b). Moreover, CX516 has been shown to facilitate the induction of NMDA-dependent, long-term potentiation (LTP) in the hippocampus in vivo (Stäubli et al., 1994a). Although not established, the facilitation of LTP has been postulated to arise from an increase in the activity of voltage-sensitive NMDA receptors as a consequence of the greater depolarization produced by AMPA receptor potentiation (Stäubli et al., 1994a,b). Such changes in synaptic plasticity may provide a cellular substrate through which positive modulation of AMPA receptors could promote memory encoding. Consistent with this hypothesis, behavioral studies have demonstrated that CX516 and other potentiators can improve performance in rodents on a variety of memory tasks including those that require working memory (Pontecorvo and Evans, 1985; Stäubli et al., 1994b; Hampson et al., 1998). More importantly, several clinical studies have shown the utility of AMPA receptor potentiators as nootropic agents (Dimond et al., 1979; Oepen et al., 1985; Ingvar et al., 1997). Thus, it is possible that some of the actions of these AMPA receptor potentiators may be mediated in the PFC.

Recently, a novel class of biarylpropylsulfonamide compounds (e.g., LY392098, LY404187) have been developed that positively modulate AMPA receptors (Bleakman et al., 2000; Baumbarger et al., 2001) (Fig. 1). These compounds have been shown to augment the amplitude of the desensitized current recorded from human recombinant homomeric AMPA receptors with nanomolar potency compared with millimolar potency for CX516 (Bleakman et al., 2000). In addition, the potentiation by biarylpropylsulfonamides of both recombinant and native receptors is markedly different from that by CX516 and other potentiators in that the magnitude of the enhancement is time-dependent, increasing with continued agonist exposure (Bleakman et al., 2000; Baumbarger et al., 2001). These data suggest that biarylpropylsulfonamides may affect the desensitization process of AMPA receptors via a distinct mechanism of action. In the present experiments, the actions of the biarylpropylsulfonamide LY404187 were compared with CX516 on the activity of native AMPA receptors of PFC neurons with respect to potency, biophysical mechanisms of action, and ability to potentiate AMPA and NMDA receptor-mediated synaptic responses in vitro and in vivo.

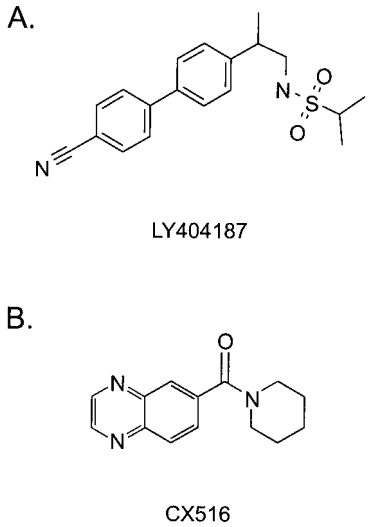

Fig. 1. Chemical structures of LY404187 (A) and CX516 (B).

Materials and Methods

Acutely Isolated PFC Pyramidal Neurons: Preparation and Recording Procedures

Neurons from young (14- to 22-day-old) rats were acutely isolated from the PFC using standard procedures. Male Sprague-Dawley rats were deeply anesthetized with methoxyflurane and decapitated. Their brains were removed rapidly from the skull and immersed in a cold (~2°C) $NaHCO_3$-buffered saline solution (concentrations in mM): NaCl 126.0, KCl 3.0, $MgCl_2$ 1.5, Na_2PO_4 1.25, $CaCl_2$ 2.0, $NaHCO_3$ 26.0, glucose 10.0; pH = 7.4, osmolarity = 300 ± 5 mOsm/l. The brains were blocked, and 400-μm-thick coronal sections were cut through the rostrocaudal extent of the PFC using a Vibroslice (Campden Instruments, London, England). Slices then were incubated at room temperature (20–22°C) for 0.5 to 6 h in a holding chamber containing the continuously oxygenated (95% O_2:5% CO_2) $NaHCO_3$-buffered saline solution. Following the incubation period, slices were transferred to a glass Petri dish containing a low Ca^{2+} HEPES-buffered saline solution (concentrations in mM): $NaHOCH_2CH_2SO_3$ (sodium isethionate) 140.0, KCl 2.0, $MgCl_2$ 4.0, $CaCl_2$ 0.1, glucose 23.0, HEPES 15.0; pH = 7.4, osmolarity = 300 ± 5 mOsm/l and placed under a dissecting microscope. The PFC from each hemisphere was dissected from the surrounding cortex. The tissue was placed into a holding chamber containing protease type XIV (1 mg/ml; Sigma, St. Louis, MO) dissolved in a HEPES-buffered Hanks' balanced salt solution (no. 6136; Sigma) maintained at 37°C and oxygenated (100% O_2), pH = 7.4, osmolarity = 300 ± 5 mOsm/l. Following 30 to 40 min of incubation in the enzyme solution, the cortex was rinsed three times with the low Ca^{2+} HEPES-buffered saline solution and triturated using two fire-polished Pasteur pipettes having tips of decreasing diameter. For experiments examining NMDA responses, tissue was incubated in trypsin (10 mg/ml) and the NMDA receptor antagonist 3-[(*RS*)-2-carboxypiperazin-4-yl]-propyl-1-phosphonic acid (3 μM) to preserve the response. Prior to whole-cell recording, the cell suspension was placed into a 50-mm transparent plastic Petri dish that was mounted onto the stage of an inverted microscope. Pyramidal neurons were selected on the basis of their triangular somatic shape, soma size (~20–30 μm in diameter), and presence of some apical and basal dendrites.

The whole-cell variant of the patch-clamp technique was used for recording current from acutely isolated PFC pyramidal neurons. Electrodes were pulled from borosilicate capillary tubing (Corning 7052, WPI Inc., Sarasota, FL) using a multistage puller (Sutter Instruments Inc., Novato, CA). The electrodes were fire-polished using a microforge (Narishige Inc., Tokyo, Japan) prior to use. The internal electrode filling solution contained (concentrations in mM): *N*-methyl-D-glucamine 160.0, $MgCl_2$ 4.0, HEPES 40.0, BAPTA 3.0, phosphocreatine 12.0, Na_2ATP 2.0, GTP 0.2; pH was adjusted to 7.2 with KOH and osmolarity adjusted to 270 to 280 mOsm/l. The extracellular solution contained (concentrations in mM): sodium is-

ethionate 140.0, KCl 1.0, $BaCl_2$ 5.0, $MgCl_2$ 1.0, HEPES 10.0, tetrodotoxin 0.001; pH adjusted to 7.4 with 1.0 M NaOH; osmolarity adjusted to 300 ± 5 mOsm/l with glucose.

Upon placing the recording electrode in the bath, offset potentials were corrected and electrode resistances ranged between 2 and 7 MΩ. Voltage-clamp recordings were made using an Axon Instruments 200B amplifier (Axon Instruments Inc., Foster City, CA). The membrane potential of cells was held at −80 mV unless stated otherwise. Currents were digitized and monitored with pCLAMP software version 8.0 (Axon Instruments Inc.) running on a PC Pentium computer. A small amount of constant positive pressure (2–3 cm of H_2O) was applied to the electrodes as they were advanced through the bath. After achieving the whole-cell configuration, series resistance was compensated (70–85%) and monitored periodically.

Application of drugs was accomplished using one of two methods. A 16-barrel pipette array made from small diameter (~600 μm) glass capillary tubing was used to study the desensitized AMPA responses in PFC neurons. Solutions were contained in 10-ml syringes and positioned approximately 12 inches above the recording chamber. Gravity-induced flow of each solution from the syringe to the corresponding barrel was controlled by electronic valves. The pipette array was positioned 100 to 200 μm from the cell prior to seal formation. The solutions from the drug array were changed (~100 ms) by altering the array position with a d.c. actuator (Newport Inc., Irvine, CA). A fast application system was used to study the desensitization kinetics of AMPA receptor-mediated responses in PFC neurons. The application barrels were constructed from double-barrel borosilicate glass theta tubing. The theta tubing was pulled so that the final diameter of each barrel was approximately 125 μm and the septum between barrels was approximately 80 μm. A piezoelectric actuator (Burleigh Instruments Inc., Fishers, NY) was used to shift the theta tubing. The charging time of the actuator was set to 0.3 ms, and the onset-to-offset time of junction potential measurements (0.1 M and 1.0 M NaCl) was approximately 700 μs. A six-to-one manifold was attached to the input of each barrel of the theta tubing to permit multiple solutions to be applied to an individual neuron. Solutions were delivered to the theta tubing using the gravity-induced flow method described above. All experiments were conducted at room temperature.

PFC Slices: Preparation and Recording Procedures

Coronal PFC slices were prepared from young (14- to 22-day-old) male Sprague-Dawley rats. Animals were sacrificed, and their brains were removed and sectioned (at 300 μm) as described above. Slices were placed into the continuously oxygenated $NaHCO_3$-buffered saline solution warmed to 32°C for 30 min and then maintained at room temperature. After at least 1 h of incubation, individual slices were transferred to a recording chamber mounted on an upright microscope (BX50WI, Olympus Optical Co., Tokyo, Japan) and continuously superfused (2–3 ml/min) with the oxygenated saline solution maintained at 30 ± 0.2°C. Differential interference videomicroscopy was used to visualize pyramidal neurons in the PFC. Whole-cell current-clamp recordings were conducted using procedures similar to those described above. Patch pipettes were fabricated from thin-walled borosilicate glass and had resistances of 1 to 4 MΩ when lowered into the extracellular solution. The pipette solution contained (in mM): K^+-gluconate 130.0, KCl 10.0, $MgCl_2$ 2.0, EGTA 1.0, HEPES 10.0, Na_2ATP 2.0, Na_2GTP 0.3; pH adjusted to 7.3 with 1 M NaOH, osmolarity of 290 to 300 mOsm. For some experiments, voltage-dependent Na^+ currents were blocked by inclusion of QX314 (1 mM) in the internal solution. The extracellular solution contained (in mM): NaCl 125.0, KCl 3.0, $CaCl_2$ 2.4, $MgCl_2$ 1.3, $NaHCO_3$ 26.0, glucose 10.0; pH adjusted to 7.4 with 1 M NaOH, osmolarity of 300 ± 5 mOsm. For some experiments, γ-aminobutyric $acid_{A\ and/or\ B}$ receptors were blocked with bicuculline methiodide (1–2 μM) and SCH59011 (2 μM), respectively. Voltage signals were amplified by an Axoclamp 200B amplifier, low-pass filtered at 5 kHz, and stored on the computer hard-disk for off-line analysis (Clampfit 8.0, Axon Instruments Inc.). Series resistance (10–30 MΩ) compensation was monitored, and recordings displaying >30% change in resistance were not included in subsequent analyses. Voltage errors due to the liquid junction potential were subtracted during analysis. Postsynaptic potentials were evoked by constant current single stimulation pulses (100 μs, 50–500 μA) delivered with a 20-s interstimulus interval using bipolar stimulating electrodes positioned in the cell body layer.

Extracellular Single-Unit Recording from PFC

Male Sprague-Dawley rats (250–320 g) were anesthetized with urethane (1.5 g/kg, i.p.) and mounted on a stereotaxic frame. Core temperature was monitored by a rectal probe and maintained at 37°C by a heating pad. Burr holes were drilled into the skull over the PFC and the ventral subiculum of the hippocampus. The stereotaxic coordinates for the PFC and the ventral subiculum were: PFC: A-P = 2.7 to 3.0 mm anterior to the bregma, L-M = 1 to 1.2 mm, D-V = 2.0 to 3.5 mm; ventral subiculum: A-P = 6.0 mm posterior from the bregma; L-M = 5 mm from the midline, and D-V = 7 mm from the cortical surface. A concentric bipolar stimulation electrode (NE-100, Rhodes Medical Instruments, Inc., Woodland Hills, CA) was positioned stereotaxically in the ventral subiculum. Stimulation currents (20-μs duration, 300–900 μA) were delivered via an optically isolated stimulation unit (Isoflex, A.M.P.I., Jerusalem, Israel). Single stimulation pulses were delivered at 1 Hz.

Conventional extracellular single-unit recordings were made using filament-filled borosilicate glass capillary tubing. The electrodes were pulled from a glass micropipette (o.d. = 1.5 mm, i.d. = 1.17 mm; Hilgenberg GmbH, Malfeld, Germany) using a multistage microelectrode puller (Sutter Instruments Inc.) in two stages. The electrodes were filled with 0.5% sodium acetate in 2% Pontamine Sky blue and 0.5 μM bicuculline methiode to partially block local $GABA_A$ receptor-mediated inhibitory responses. The electrode was advanced by a single-axis Narishige MHW-40 hydraulic micromanipulator (Narishige Inc.) mounted onto the stereotaxic frame.

Extracellular single-unit activity was amplified by an Xcell-3 Plus amplifier (Frederick Haer & Co., Brunswick, ME). Single-unit activity was isolated using a window discriminator (model 74-60-3, Frederick Haer & Co.). The output signals from the window discriminator were digitized and multiplexed by an analog to digital converter (1401 mini, Cambridge Electronics Design Ltd., Cambridge, UK) and were sampled at 10 kHz by a PC-based computer using Spike 3 software (Cambridge Electronics Design Ltd.).

At the end of each recording session, direct current (100 μA for 1 min) was delivered to make an iron deposit at the stimulating electrode tip. Direct current (10 μA for 15 min) also was delivered to iontophorese Pontamine Sky blue through the recording electrode to mark the recording site. The animal then was perfused with saline, followed by buffered formalin. Brain sections (70 μm) containing the PFC and the ventral subiculum were cut using a freezing microtome, and the sections were washed, dehydrated with alcohol, and stained with cresyl violet to permit examination of the recording and stimulation sites.

Drug Preparation and Administration

In Vitro Experiments. Concentrated stock solutions for all compounds were prepared by dissolving in 100% dimethyl sulfoxide. The solution then was aliquoted and stored at −20°C until needed. On the day of recording, solutions were diluted to the desired concentrations in the $NaHCO_3$-buffered saline solution. When low nanomolar concentrations were tested, serial dilutions were performed to assure accuracy.

In Vivo Experiments. Stock solutions of LY404187 (0.1 mg/ml) and CX516 (10.0 mg/ml) were made by dissolving the compound in ethanol (200 μl) and then adding 0.9% NaCl and 15% 2-hydroxypropyl-β-cyclodextran (HβC; Sigma) to bring the final stock solution

volume to 5 ml. The solutions then were aliquoted and stored at −20°C until used.

During each experiment, LY404187 and CX516 were diluted to the desired concentration using 0.9% saline. The drugs were administered intravenously with a syringe via a catheter implanted in the jugular vein. The selective AMPA antagonist LY300168 was dissolved in saline and intravenously infused using an infusion pump at a rate of 0.1 ml/min. The NMDA receptor antagonist LY235959 was dissolved in saline and infused via a catheter inserted into the jugular vein. Only one PFC neuron per animal was studied. No change in spontaneous or synaptically evoked responses was produced by the ethanol/HβC vehicle solution.

Data Analysis

In Vitro Data. Concentration-response profiles for all potentiators were constructed by measuring the peak current amplitude during a 10-s coapplication of compound and glutamate (100 μM), calculating the percent increase relative to the glutamate alone response and plotting the data as a function of potentiator concentration. The plotted points then were fit with a logistic equation of the form: percent potentiation = $E_{max}/(1 + ([LY404187]/EC_{50}))^n$, where the maximal percent potentiation is relative to the current evoked by glutamate alone, EC_{50} is the concentration equal to 50% of the maximally effective concentration, and n is the Hill coefficient. The best fit was chosen using the Marquardt-Levenberg algorithm. Average EC_{50} and E_{max} values were determined and reported as mean ± standard deviation.

In Vivo Data. For in vivo recording experiments, peristimulus time histograms (PSTH, bin width = 1 ms) were constructed for spontaneous and evoked responses of PFC neurons to single-pulse stimulation (at 1 Hz, 300 sweeps) of the ventral subiculum. The mean bin height and the standard deviation 50 ms before the onset of the stimuli was defined as the prestimulus control response for each recording. The beginning of a significant poststimulus response period was defined as the first three consecutive bins whose heights were 1 standard deviation above (for excitatory responses) or below (for inhibitory responses) the mean prestimulus control bin height. Likewise, the end of the significant response period was defined as the first three consecutive bins whose heights were within 1 standard deviation of the control mean.

The average prestimulus spontaneous firing rate and the total number of evoked spikes within the significant poststimulus period were compared between control and drug conditions. Postdrug-evoked or spontaneous firing responses were expressed as a percentage change from the control response. All group data are expressed as mean ± S.E.M. Comparison of group data was made using Student's t test with $p < 0.05$ being significant.

Results

Potency and Time Course of Potentiation by LY404187 and CX516. Initial studies evaluated the potency and efficacy of LY404187 (0.03–10 μM) on the glutamate-evoked responses of PFC neurons by recording the inward current evoked in response to application of glutamate (100 μM, 10-s duration; holding potential = −80 mV) alone and in the presence of the compound. Preliminary experiments demonstrated that this concentration of glutamate was equal to 30% of the maximal response (EC_{30}). Because of the relatively slow solution switching speed (~100 ms) of the actuator used in these experiments, the responses recorded reflect desensitized currents. At all concentrations tested, application of LY404187 alone had no effect on the holding current (Fig. 2A). However, when applied in the presence of glutamate, LY404187 enhanced the evoked current in a concentration-dependent manner (Fig. 2A). As previously reported for biarylsulfonamides on both recombinant and native receptors (Bleakman et al., 2000; Baumbarger et al., 2001), the potentiated response by LY404187 displayed a marked time dependence such that a steady-state level was never achieved during the 10-s glutamate stimulus. Because of this property, the data were expressed as a percent change in peak amplitude from that of the glutamate response alone and plotted as a function of compound concentration. As such, the values for potency and efficacy are estimates. The concentration-response profile shows that LY404187 potentiated glutamate responses with an EC_{50} value of 1.3 ± 0.3 μM and a maximal efficacy (E_{max}) of a 45.3 ± 8.0-fold (n = 6) increase relative to glutamate alone (Fig. 2C). Consistent with a selective effect on AMPA receptors (also see below), the potentiator also enhanced AMPA-evoked currents (5 μM = EC_{30}) with the same time dependence as that of glutamate-evoked currents (Fig. 2A, inset). In addition, the concentration-response profile revealed that LY404187 potentiated AMPA-evoked currents with similar potency (EC_{50} = 1.2 ± 0.4 μM) and efficacy (E_{max} = 45.2 ± 6.8-fold increase, n = 5) to that of glutamate-induced responses.

Consistent with its effects on recombinant receptors (Bleakman et al., 2000), CX516 was less potent and efficacious in potentiating glutamate responses of AMPA receptors in PFC pyramidal neurons. CX516 produced a concentration-dependent enhancement of glutamate responses with an EC_{50} value of 2.8 ± 0.9 mM and an E_{max} value of a 4.8 ± 1.4-fold (n = 4) increase relative to glutamate alone (Fig. 2, B inset and C). The AMPA receptor dependence of the CX516 potentiation also was confirmed by testing the compound with AMPA (5 μM). The potency (EC_{50} = 3.7 ± 1.3 μM) and efficacy (E_{max} = 3.8 ± 1.6-fold increase) (n = 6) of CX516 potentiation of AMPA responses was similar to that for glutamate. Thus, LY404187 is considerably more potent and efficacious than CX516 in potentiating AMPA receptor-mediated responses in PFC neurons.

In an additional series of experiments, the concentration threshold for potentiation of AMPA (5 μM) responses by LY404187 (3.0 and 10 nM) was tested. The concentration threshold was defined as the lowest concentration of compound that produced a significant increase in AMPA-evoked peak current amplitude. As such, application of 10 nM LY404187 significantly increased the response to AMPA by approximately 350% (AMPA alone = 51.40 ± 23.4 pA, AMPA + LY404187 = 176.0 ± 21.7 pA, n = 5; $F_{2,14}$ = 33.0, $p <$ 0.0001, one-way analysis of variance) (Fig. 2, D and inset).

The responses to AMPA and glutamate in the presence of LY404187 displayed a conspicuous time dependence such that the current never achieved a steady-state level. This effect was in marked contrast to the potentiating actions of CX516 and cyclothiazide, which reached a steady-state value within 2 to 3 s (Fig. 3A). The possibility that the time dependence merely reflected insufficient exposure times was tested by varying the duration (3–40 s) of coapplication of LY404187 (3 μM) and AMPA (5 μM). Application of AMPA alone for 10 s evoked a small inward current that always achieved a steady-state value (Fig. 3B). In contrast, the inward current evoked by combined application of AMPA and LY404187 never reached a steady-state level even when the exposure lasted for 40 s. Longer exposure times (up to 120 s) also were tested on individual neurons with similar results.

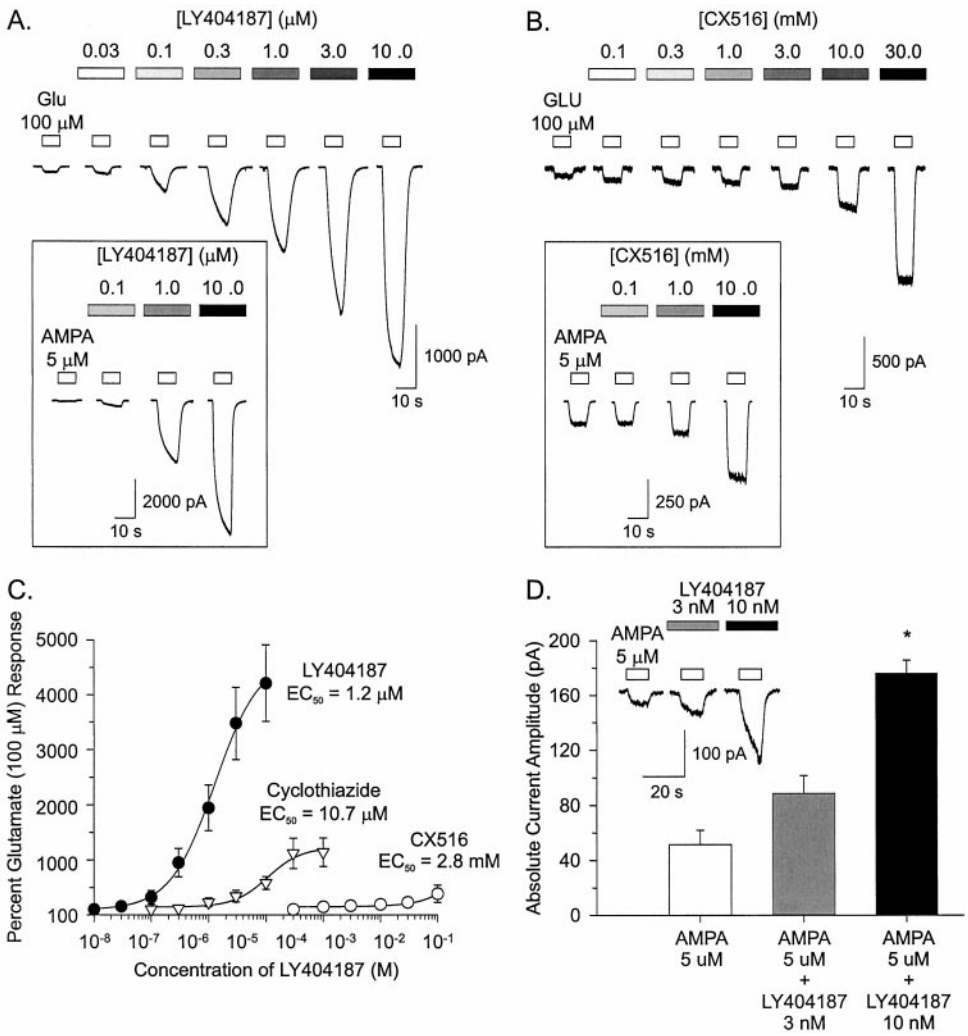

Fig. 2. Concentration-response profile for LY404187 and CX516 potentiation of AMPA receptor-mediated currents. A and B, the concentration-response profiles for LY404187 (0.03–10.0 μM) and CX516 (0.1–30.0 mM) potentiation were constructed by measuring the responses to 100 μM glutamate (GLU) alone and in the presence of potentiator. Both compounds enhanced GLU responses in concentration-dependent manner. Insets, both compounds potentiated responses to AMPA (5 μM) in a manner similar to that of GLU. C, plot of the average degree of potentiation as a percentage of the GLU (100 μM) response for each concentration of compound tested. Analysis revealed that LY404187 was more potent and efficacious than CX516 with the compounds having EC_{50} values of 1.2 μM and 2.8 mM and maximal efficacies of 42- and 5-fold, respectively. The effects of cyclothiazide are presented for comparison. D, the concentration threshold for potentiation of AMPA responses by LY404187 was assessed by comparing the peak responses to application of AMPA (5 μM) alone and in the presence of the potentiator (3.0 and 10.0 nM). Plot of the average peak amplitude response in each condition showed that significant increases in the AMPA-evoked responses were observed with coapplication of 10.0 nM LY404187.

One possible explanation for the time dependence of the potentiation could be that LY404187 acts at an intracellular site that requires the compound to diffuse across the membrane to exert its effect. This possibility was tested by comparing the effects of intracellular and extracellular application of LY404187 (3 μM). For these experiments, the compound was included in the recording pipette solution. After achieving a whole-cell recording, a 5-min period elapsed before the beginning of the experiment to allow time for the compound to diffuse into the cell. When AMPA (5 μM) was applied extracellularly during the next 3 to 5 min, no potentiation was observed. However, subsequent extracellular application of LY404187 always potentiated AMPA responses in these same neurons (Fig. 3C). Thus, the time-dependent nature of LY404187 potentiation does not appear to be the result of a delay in access to its site of action.

Previous studies of positive allosteric modulators of AMPA receptors have shown that cyclothiazide acts rapidly and reversibly (Partin et al., 1994). Similar to cyclothiazide, LY404187 potentiated AMPA receptor activity as soon as agonist was present (Fig. 3, A, B, and C). The reversibility of LY404187 potentiation was examined by recording the responses to AMPA (5 μM, 10-s duration) before, during, and after coapplication of the compound (10 μM) (Fig. 3D). Three applications of AMPA alone delivered every 10 s were used to establish a baseline response. Subsequent exposure with LY404187 alone was without effect but robustly potentiated AMPA-evoked responses upon presentation of the agonist. Following elimination of LY404187, further applications of AMPA every 20 s elicited currents that decreased in amplitude over time. The average peak current amplitudes before, during, and after application of LY404187 were calculated, normalized to the average baseline amplitude before the potentiator, and plotted as a function of time. Results showed that recovery from potentiation occurred within 70 s of elim-

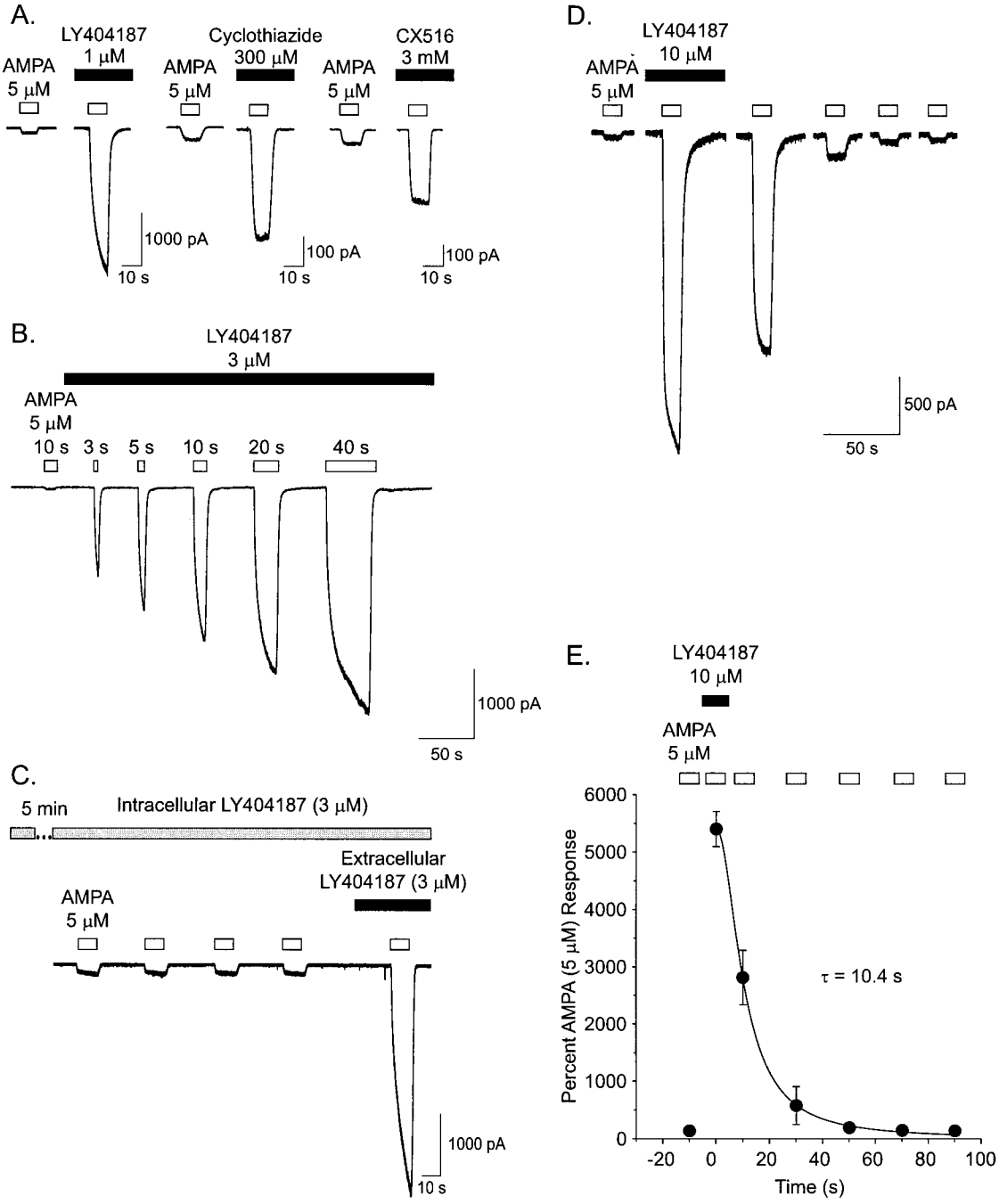

Fig. 3. Activity and time dependence of enhancement by AMPA potentiators. A, the activity and time dependence of the AMPA receptor potentiation produced by LY404187 (1 μM), cyclothiazide (300 μM), and CX516 (3 mM) were evaluated by applying 5 μM AMPA (10-s duration) alone and in the presence of the potentiator. None of the compounds produced an effect alone, but all compounds potentiated the AMPA-evoked currents, suggesting an activity dependence to their action. Unlike cyclothiazide and CX516, the potentiation produced by LY404187 displayed a marked time dependence during the 10-s application. B, this property of the potentiation was explored by applying pulses of AMPA with durations ranging from 3 to 40 s in the presence of 3 μM LY404187. Even in response to a 40-s AMPA pulse, the potentiation by LY404187 never reached a steady-state level. C, inclusion of LY404187 (3 μM) in the recording pipette solution followed by a 5-min dialysis of the neuron did not potentiate extracellular AMPA-evoked responses. However, subsequent application of LY404187 extracellularly did potentiate AMPA responses, indicating an extracellular site of action. D, responses to application of AMPA (5 μM) before, during, and after application of LY404187 (10 μM). Following elimination of LY404187, the responses to AMPA returned to control levels. E, plot of the recovery from potentiation by LY404187. Points reflect the average responses to AMPA before, during, and after application of the compound expressed as a percentage of the response amplitude before potentiator and plotted as a function of time. Points represent mean ± S.D.

ination of the potentiator and that this process could be fit with a single exponential function having a time constant of 10.4 s (Fig. 3D).

LY404187 Selectively Potentiates AMPA Receptor-Mediated Currents. The selectivity of LY404187 for potentiating AMPA receptor-mediated currents was tested by attempting to block the potentiation with the selective AMPA receptor antagonist LY300168 (50 μM). Application of 100 μM glutamate elicited a small inward current (101.7 ± 25.6 pA, $n = 7$) that was reduced 90.4% by LY300168 (9.3 ± 6.4

pA). Following wash of the antagonist, subsequent application of LY404187 (3 μM) potentiated the response to AMPA (1689.2 $\pm$ 562.8 pA) in the same neurons. This potentiated response was reduced 98.0% by LY300168 (34.3 $\pm$ 11.3 pA) (Fig. 4, A and C).

The possibility that LY404187 might potentiate NMDA receptor-mediated currents also was explored. For these experiments, the membrane potential was shifted to near 0 mV, and glycine (30 μM) was included in the extracellular solution. Results showed that application of NMDA (200 μM) evoked a small inward current (81.5 $\pm$ 25.0 pA, $n = 4$) that was not altered by LY404187 (3 μM) (81.8 $\pm$ 21.9 pA). However, when the membrane potential of these same neurons was shifted to -80 mV, LY404187 potentiated AMPA responses (Fig. 4, B and D). Additional experiments examined the effects of LY404187 on voltage-gated Na^+ and K^+ currents. Application of LY404187 at concentrations as high as 10 μM did not affect the magnitude or time course of whole-cell K^+ or Na^+ currents in PFC pyramidal neurons (Fig. 4, E and F). Collectively, these data confirm the AMPA receptor specificity of the potentiation by LY404187 on PFC neurons.

LY404187 Increases Agonist Potency. Previous studies have shown that positive modulators of AMPA receptors (e.g., cyclothiazide) can exert their effects in part by produc-

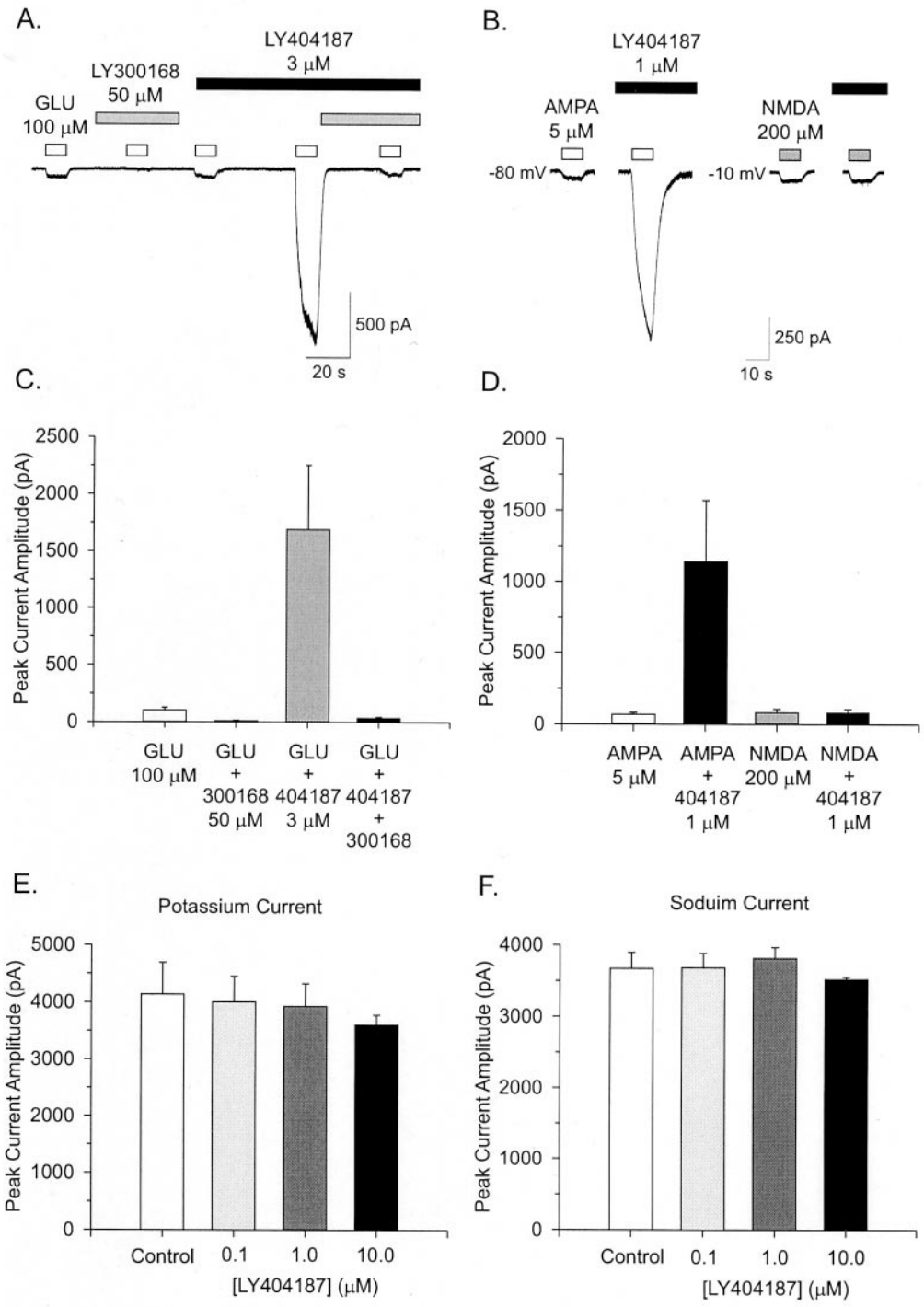

Fig. 4. LY404187 selectively potentiates AMPA receptor-mediated currents. A, the response to application of 100 μM glutamate (GLU) was blocked by 50 μM LY300168. Following elimination of LY300168, subsequent application of 3 μM LY404187 potentiated the GLU response. This potentiated response was blocked by 50 μM LY300168. B, when the membrane potential of neurons was held near 0 mV, application of 200 μM NMDA (in the presence of 30 μM glycine) evoked an inward current that was not potentiated by LY404187. In these same neurons, when the membrane potential was held at -80 mV, the response to 5 μM AMPA was potentiated by 3 μM LY404187. C and D, plots of the average peak amplitudes for each condition in A and B. E and F, plots of the peak whole-cell potassium and sodium currents recorded during control conditions and in the presence of LY404187 (0.1–10.0 μM). No change in these conductances was observed.

ing a leftward shift in the agonist concentration-response relationship (Yamada and Tang, 1993; Partin et al., 1994). The possibility that LY404187 produced similar effects on the responses of pyramidal neurons was investigated. The normalized concentration-response profile for glutamate (0.3–100 μM) alone had an average EC_{50} value of 272.7 ± 178.5 μM (n = 6). The glutamate concentration-response relationship was shifted leftward by LY404187 (1 μM) approximately 6-fold having an EC_{50} value of 44.1 ± 16.1 μM (n = 9) (t = 3.6, $p < 0.01$) (Fig. 5). The magnitude of AMPA receptor responses also was increased dramatically by LY404187; the response to 3 mM glutamate increased by 12-fold from 249.5 ± 68.2 pA (n = 6) during control conditions

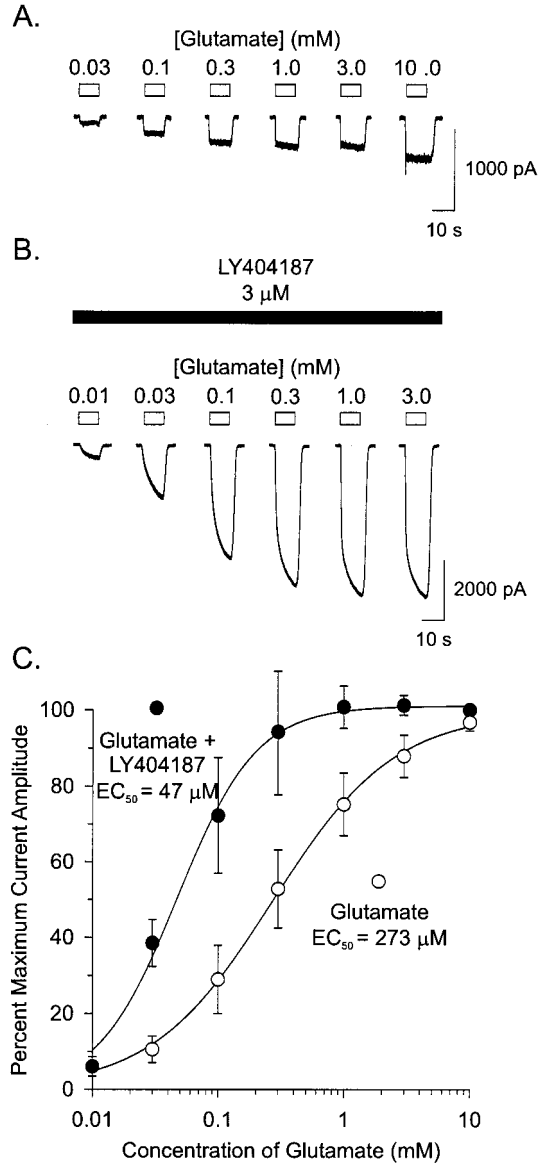

Fig. 5. LY404187 increases the potency of glutamate at PFC AMPA receptors. A, concentration-response of glutamate (0.01–10.0 mM) alone. B, concentration-response of glutamate in the presence of LY404187 (3 μM). Note that the activity and time dependence of LY404187 potentiation is evident regardless of glutamate concentration. C, plot of the normalized peak responses as a function of glutamate concentration in the absence and presence of LY404187 (3 μM). The potentiator produced an approximately 6-fold leftward shift in the concentration-response relationship of glutamate at PFC pyramidal cell AMPA receptors.

to 3057.2 ± 1054.8 pA (n = 9) (t = 6.1, $p < 0.01$) in the presence of the potentiator. Tests for a similar shift in agonist potency by CX516 were conducted. Results showed that the potency of glutamate for PFC AMPA receptors was increased approximately 3-fold by CX516 (3 mM) (EC_{50} = 80.3 ± 20.2 μM; n = 8, t = 8.5, $p < 0.0001$). These data also can be interpreted within the context of results from previous receptor binding experiments with biarylpropylsulfonamides (Lindén et al., 2001). In these studies, specific binding of [^{3}H]LY359153 (a structural analog of LY404187) to cortical membranes was low but increased 5- to 10-fold in the presence of escalating concentrations of AMPA with an EC_{50} value of 5.5 μM. A similar observation was reported for [^{3}H]AMPA binding in the absence and presence of aniracetam (Nicoletti et al., 1992). The present results demonstrate a functional correlate to these binding experiments, such that the potentiating effect of LY404187 on AMPA currents increased as a function of agonist concentration.

LY404187 and CX516 Differentially Alter Desensitization of AMPA Receptors. The pronounced augmentation of glutamate responses by LY404187 suggested that the compound should alter the desensitization process of AMPA receptors in PFC pyramidal neurons, whereas the more modest potentiation by CX516 suggested that it would have much less of an effect on the degree of desensitization. To test these hypotheses, AMPA receptor desensitization was studied in isolated PFC neurons by delivering 500-ms glutamate (1 mM) pulses alone and in combination with the potentiators using a piezoelectric fast perfusion system. The responses to glutamate were due entirely to activation of AMPA receptors as evidenced by their complete blockade in the presence of 50 μM LY300168 (Fig. 6A, inset). The 500-ms glutamate pulse evoked an inward current that reached a peak within milliseconds and then decayed to a steady-state value (measured at the end of the glutamate pulse) that was 8.4 ± 5.5% of the peak response (Fig. 6A). A single exponential function was used to fit the current decay, and the average time constant of desensitization was 15.4 ± 3.7 ms (n = 10) during control conditions. Subsequent application of concentrations of LY404187 (0.1, 0.3, and 1.0 μM) at or lower than the EC_{50} value did not alter the time constant of desensitization (0.1 μM = 15.1 ± 4.8 ms; 0.3 μM = 17.2 ± 6.1 ms; 1.0 μM = 18.2 ± 8.2 ms) (Fig. 6B). However, application of LY404187 enhanced the magnitude of the steady-state current relative to the peak current amplitude in a concentration-dependent manner (0.1 μM = 23.3 ± 9.9%; 0.3 μM = 32.3 ± 13.7%; 1.0 μM = 56.9 ± 19.3%, $F_{3,35}$ = 24.1, $p < 0.0001$) (Fig. 6C). The peak amplitude also was increased by LY404187 relative to control levels (0.1 μM = 17.1 ± 19.2%; 0.3 μM = 39.5 ± 25.0%; 1.0 μM = 72.3 ± 23.2%) ($F_{3,2}$ = 9.5, $p < 0.0001$) (Fig. 6D). The increase in peak amplitude most likely indicates the presence of receptor desensitization in the rising phase of the current during control conditions (Raman and Trussell, 1995; Partin et al., 1996).

Similar analyses were performed on recordings from cells before and after application of CX516. The average glutamate-evoked current recorded in the absence of the compound decayed with a time constant of 16.3 ± 2.2 ms (n = 4) to a steady-state level that was 10.6 ± 5.9% of the peak amplitude (Fig. 6E). In contrast to effects of LY404187, application of CX516 using concentrations at or below the EC_{50} value increased the time constant of desensitization to 22.8 ±

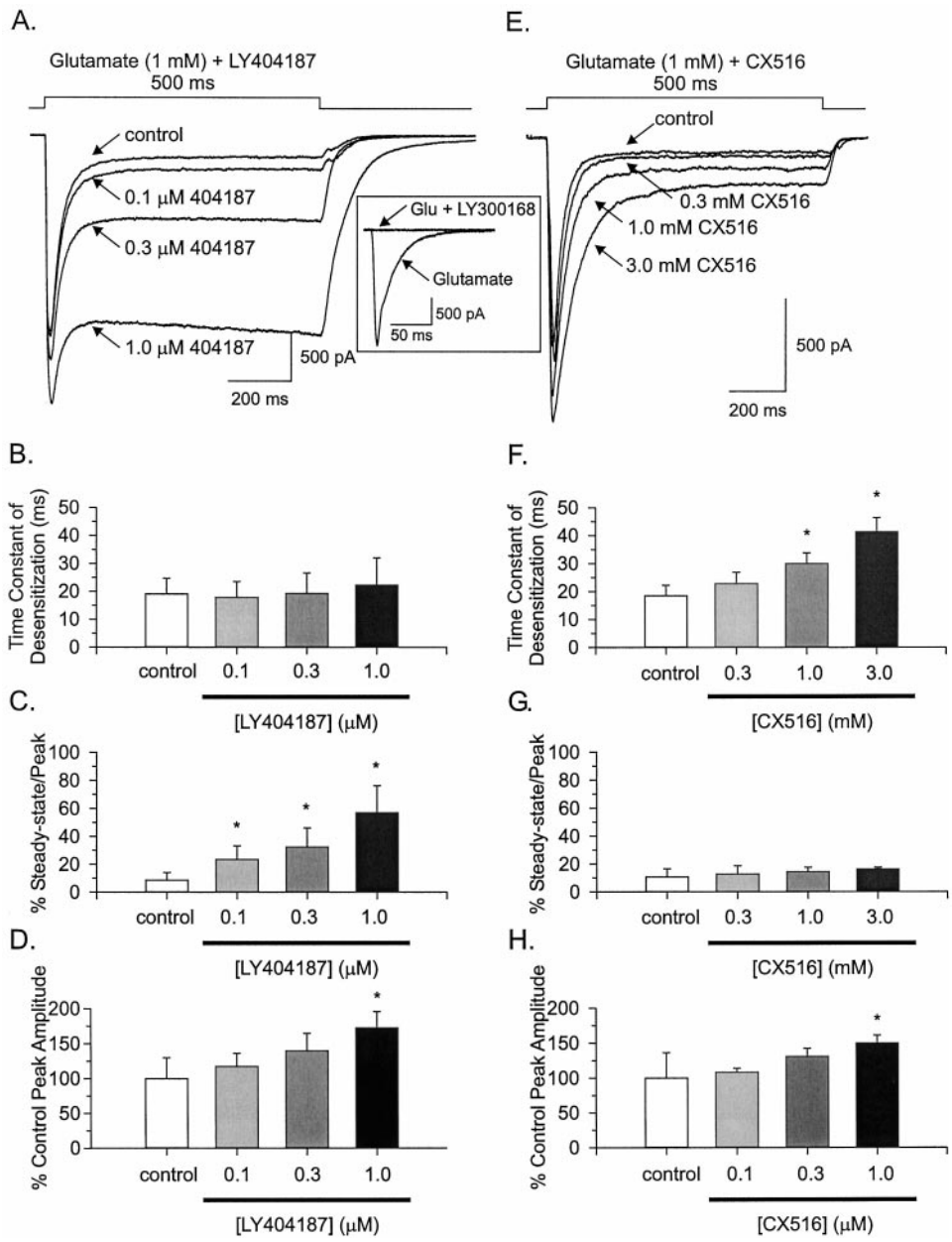

Fig. 6. LY404187 and CX516 differentially affect desensitization of AMPA receptors in PFC pyramidal neurons. A and E, whole-cell responses to 500-ms application of glutamate (1 mM) in the absence and presence of LY404187 (0.1–1.0 μM) and CX516 (0.3–3.0 mM) are presented. Inset, the glutamate-evoked current was completely blocked by LY300168 (50 μM). B and F, the time constant of desensitization of glutamate-evoked responses in the absence and presence of potentiator was determined by fitting the current decay with a single exponential function. Because of the time-dependent nature of LY404187 potentiation, for the 1 μM LY404187 responses, the boundaries of the fit were from the peak of the response to 200 ms after the stimulus. CX516, but not LY404187, increased the time constant of desensitization. C and G, the degree of desensitization was evaluated by expressing the amplitude of the steady-state current as a percentage of the peak current amplitude in the absence and presence of the potentiators. LY404187, but not CX516, significantly decreased the magnitude of desensitization. D and H, a change in the initial peak response to glutamate application was evaluated by expressing the peak amplitude in the presence of each concentration of potentiator as a percentage of the control peak amplitude. LY404187 and CX516 enhanced the peak response, indicating that desensitization occurs during the rising phase of the response.

4.1, 30.0 ± 3.8, and 41.3 ± 5.1 ms ($F_{3,12} = 29.3$, $p < 0.0001$) in the presence of 0.3, 1.0, and 3.0 mM CX516, respectively (Fig. 6F). However, application of CX516 did not decrease the degree of desensitization such that the steady-state current represented 12.7 ± 6.0, 14.4 ± 3.1, and 16.1 ± 1.3% of the peak current in 0.3, 1.0, and 3.0 mM CX516, respectively (Fig. 6G). The peak current amplitude also was enhanced by CX516 relative to control levels (0.3 mM = 8.1 ± 5.8%; 1.0 mM = 30.6 ± 11.8%; 3.0 mM = 49.4 ± 11.3%) ($F_{3,16} = 6.2$, $p < 0.01$) (Fig. 6H). Thus, the two potentiators had opposite effects on the onset kinetics and magnitude of AMPA receptor desensitization.

The contrasting effects of LY404187 and CX516 on the desensitization process suggested that LY404187 would facilitate the recovery from desensitization of AMPA receptors in response to repetitive stimuli to a greater extent than CX516. This hypothesis was tested by applying pairs of brief (10-ms) glutamate pulses [interstimulus intervals (ISIs) = 30–500 ms] and measuring the amplitude of the second response relative to the first response, permitting a recovery ratio to be calculated (Fig. 7, A and C). During control conditions, the amplitude of the second response was correlated with the ISI such that the ratio ranged from 1.0 in response to a 500-ms ISI to approximately 0.2 to a 30-ms ISI, and the time constant of this recovery process was 109.5 ± 57.6 ms ($n = 7$). Application of 1 μM LY404187 ([EC_{50}]) produced a decrease in the time constant of the recovery process (49.8 ± 13.8 ms; $t = 2.9$, $p < 0.05$) coupled with an increase in the recovery ratio at the shortest ISI (30 ms) (control = 0.24 ± 0.14; LY404187 = 0.58 ± 0.13, $t = 2.7$, $p < 0.05$). In contrast to the effects of LY404187, CX516 did not alter the kinetics of recovery from desensitization. During control conditions, the time constant of the recovery process was 105.9 ± 39.0 ms ($n = 6$). Subsequent application of 3.0 mM CX516 ([EC_{50}]) did not significantly affect the kinetics of recovery from desensitization (78.2 ± 23.3 ms) (Fig. 7D).

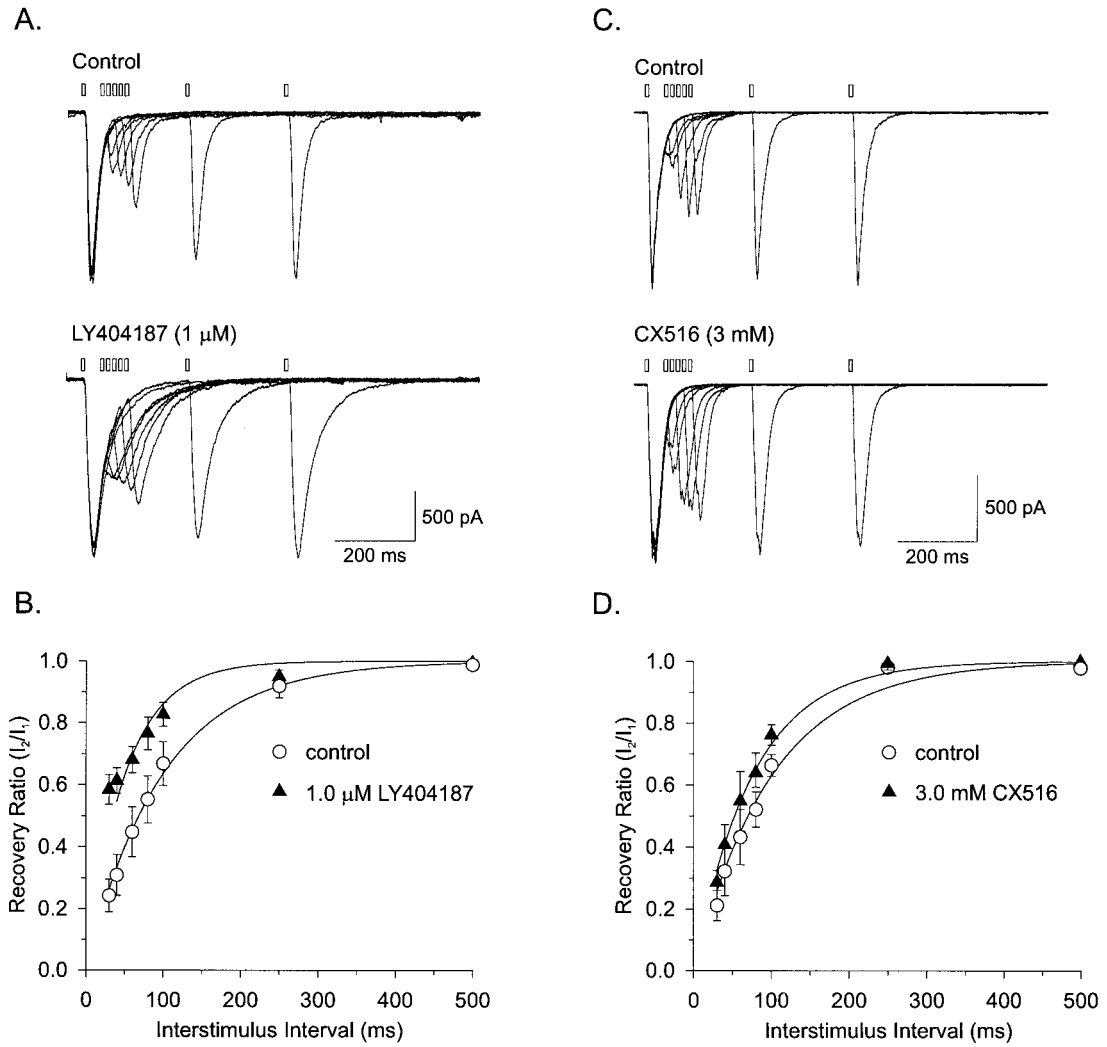

Fig. 7. LY404187 decreases the time course of recovery from desensitization. A and C, pairs of glutamate pulses (10 ms, open boxes) having ISIs of 30 to 500 ms were delivered to PFC pyramidal neurons in the absence and presence of LY404187 (1.0 μM) and CX516 (3.0 mM). B and D, an average recovery ratio was measured for each ISI by dividing the peak of the second response by that of the first response in the absence and presence of potentiator for each cell tested. The average data points for each condition then were fit with a single exponential function to determine the time constant of recovery from desensitization. Results showed that 1.0 μM LY404187 reduced the time course of the recovery process (control = 109.5 $\pm$ 57.6 ms; 1.0 μM LY404187 = 49.8 $\pm$ 13.8 ms).

LY404187 Enhances AMPA Receptor-Mediated Synaptic Responses in Vitro. The robust enhancement of glutamate-evoked currents by LY404187 in the fast perfusion experiments suggested that the compound should similarly potentiate AMPA-mediated synaptic responses in PFC pyramidal neurons in a slice preparation. To test this hypothesis, synaptic responses of PFC pyramidal neurons were evoked by stimulation of fibers in the proximal apical dendritic region of these neurons. Because of the dense recurrent excitatory collateral network among PFC neurons, the synaptic response is comprised of both mono- and polysynaptic components, yielding a complex EPSP (Fig. 8A). In initial experiments, application of 0.1 to 3.0 μM LY404187 enhanced the subthreshold synaptic response in PFC pyramidal neurons to the point of action potential discharge. The effects of LY404187 were reversible within 15 to 30 min of eliminating the potentiator from the extracellular solution (Fig. 8A). Subsequent experiments focused on the selective potentiation of AMPA receptor-mediated EPSPs by recording from slices in which $GABA_{A\ and\ B}$ receptors and NMDA receptors were blocked with bicuculline (1 μM), SCH50911 (2 μM), and APV (50 μM), respectively. In addition, action potentials were blocked by including QX314 (1 mM) in the internal recording solution. Stimulation of glutamatergic inputs elicited a complex EPSP having an average peak amplitude of 9.6 $\pm$ 3.6 mV in control solution. This EPSP was enhanced in a concentration-dependent manner to 11.5 $\pm$ 10.5 mV (19.8%), 15.2 $\pm$ 6.3 mV (58.3%), 18.1 $\pm$ 7.4 mV (88.5%), and 23.2 $\pm$ 6.0 mV (141.7%) in the presence of 0.1, 0.3, 1.0, and 3.0 μM LY404187, respectively ($F_{4,33} = 18.2$, $p < 0.0001$) (Fig. 8, B and D). The selectivity of the effect of LY404187 for AMPA receptors was confirmed by blocking the potentiation with LY300168 (50 μM; $n = 4$) (Fig. 8C).

Because of the complex nature of the recurrent excitatory network in PFC, it was difficult to demonstrate an effect of the potentiator on the monosynaptic component of the AMPA receptor-mediated EPSP evoked by electrical stimulation in normal extracellular solutions. As such, the enhanced responsiveness described above could have been due in part to a recruitment of more excitatory inputs to the recorded neu-

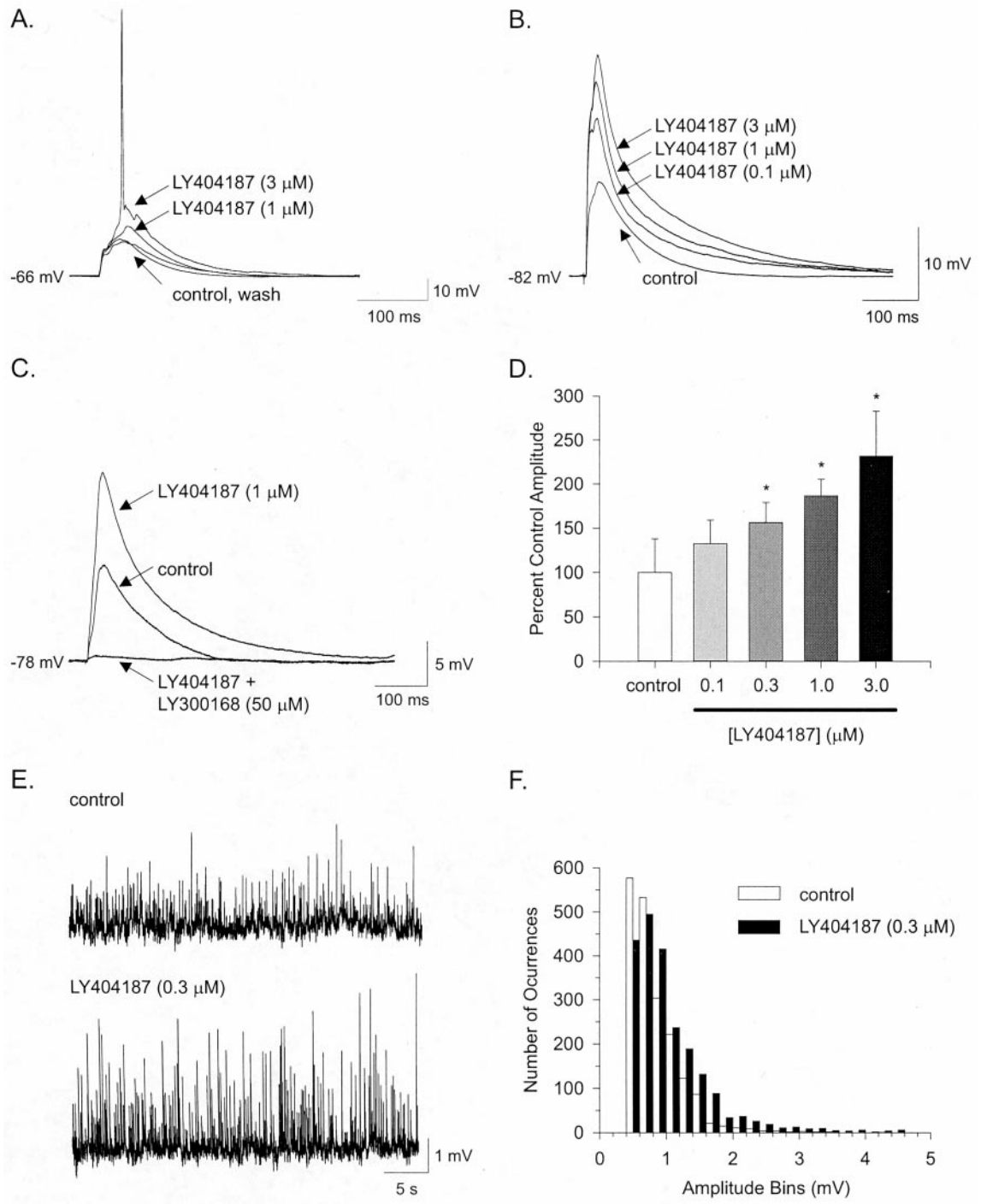

Fig. 8. LY404187 enhances glutamatergic synaptic and spontaneous AMPA receptor-dependent EPSPs in vitro. A, single stimulation of glutamatergic afferents to PFC pyramidal neurons evokes a complex EPSP composed of both mono- and polysynaptic components. Addition of 1 μM and 3 μM LY404187 enhanced the excitatory response to induce spike discharge. B, application of LY404187 (0.1–3.0 μM) increased EPSP amplitude and duration in a concentration-dependent manner. Recordings were made following blockade of $GABA_{A \text{ and } B}$ and NMDA receptors and voltage-dependent sodium channels. C, LY404187 potentiation of the complex EPSP was abolished by the AMPA receptor antagonist LY300168 (50 μM). D, plot of the average AMPA receptor-mediated EPSP amplitude from recordings as in B as a percentage of control amplitude. E, spontaneous EPSPs recorded following blockade of $GABA_{A \text{ and } B}$ and NMDA receptors in an extracellular solution containing elevated potassium concentration (6 mM) in the absence and presence of 0.3 μM LY404187. F, frequency histogram plotting the total number of occurrences of EPSPs recorded as a function of EPSP amplitude for four cells during control and drug conditions. The amplitude threshold for detection was 0.4 mV, and the bin width was 0.2 mV. LY404187 significantly shifted the distribution toward larger amplitudes.

ron in the absence of any potentiation of EPSP amplitude. In an effort to address this issue, the effects of LY404187 were examined on the amplitude of spontaneous monosynaptic EPSPs recorded in the presence of elevated extracellular potassium concentration (6 mM). Spontaneous EPSPs were recorded for 3 min before and during application of 0.3 μM LY404187, and a frequency histogram of EPSP amplitudes (bin width = 0.2 mV, threshold 0.4 mV) was constructed for all cells (n = 4) in each condition (Fig. 8E). For both control and drug conditions, the majority of EPSPs had amplitudes

<1 mV (control = 84.5%; 0.3 μM LY404187 = 72.1%), giving rise to a skewed distribution (Fig. 8F). However, application of LY404187 significantly shifted the distribution of amplitudes toward larger values ($Z = 3.99$, $p < 0.001$; Kolmogorov-Smirnov nonparametric test). No change in the frequency of spontaneous EPSPs was observed between the two conditions. In addition, neither the resting membrane potential (control = -65.6 ± 1.9 mV, 0.3 μM LY404187 = -65.4 ± 2.8 mV; 1.0 μM LY404187 = -65.4 ± 4.2 mV) nor the input resistance (control = 388.6 ± 118.1 MΩ; 0.3 μM LY404187 = 366.4 ± 58.9 MΩ; 1.0 μM LY404187 = 367.9 ± 84.5 MΩ) of PFC pyramidal neurons was altered by LY404187. These data support the hypothesis that the enhancement of synaptic AMPA receptor-mediated EPSPs is due to a potentiation of ion flux through AMPA receptors on PFC pyramidal neurons.

LY404187 Enhances NMDA Receptor-Mediated Synaptic Responses in Vitro. The pronounced enhancement of AMPA receptor-mediated synaptic responses by LY404187 suggested that a secondary effect of the greater depolarization would be to recruit NMDA receptor-dependent input by reducing the Mg^{2+} block of these channels. To test this hypothesis, the NMDA component of the EPSP, defined as the portion of the response sensitive to 40 μM APV, was measured before and during application of LY404187. For these experiments, the monosynaptic component of the EPSP was isolated by raising the extracellular concentrations of Ca^{2+} and Mg^{2+} to 4 mM; $GABA_A$ receptors were blocked with bicuculline (1 μM), and the recording electrode solution contained QX314 (1 mM) to eliminate action potentials. Recordings were performed while the somatic membrane was held at approximately -65 mV with constant current. To better evaluate the contribution of the NMDA conductance, the integrated area under the curve (AUC) delineated by the depolarizing response was measured for each cell in each condition and normalized relative to the control value. During control conditions, single stimulation evoked an EPSP having an average AUC of 1085.1 ± 295.2 mV/ms ($n = 5$). Application of APV (40 μM) reduced the AUC of the response to $78.3 \pm 5.0\%$ (AUC = 853.0 ± 244.7 mV/ms) of the control value (Fig. 9, A and C). APV then was eliminated from the extracellular solution, and the evoked EPSP returned to control levels after 10 to 15 min (Fig. 9A, gray trace). Following the washout period, LY404187 (1 μM) was administered and increased the average AUC of the EPSP to $153.0 \pm 7.2\%$ (AUC = 1664.8 ± 494.1) of the control area ($t = -4.2$, $p < 0.01$) (Fig. 9B). Subsequent application of APV reduced the EPSP to control levels ($95.7 \pm 7.9\%$ of control) (AUC = 1053.1 ± 346.1). Isolation of the APV-sensitive response during control and LY404187 conditions using subtraction procedures revealed that the APV-sensitive response was enhanced in the presence of the potentiator (Fig. 9B, inset). In addition, the EPSP evoked in the presence of LY404187 and APV ($95.7 \pm 7.9\%$ of control) was significantly larger than the response recorded during application of APV alone (gray trace) ($78.3 \pm 5.0\%$) ($t = -4.2$, $p < 0.01$), demonstrating the enhancement of the AMPA receptor-dependent component of the synaptic response (Fig. 9, B and C).

It is important to note that the magnitude of the enhancement of NMDA receptor activity may have been underestimated in these experiments due to the high concentrations of extracellular Mg^{2+} (4 mM) required to eliminate polysynaptic responses and relatively hyperpolarized somatic holding potentials (~ -65 mV) used. Despite these suboptimal conditions, the pronounced augmentation of NMDA receptor activity most likely indicates that neuronal membrane is not isopotential and may be greatly depolarized at the site of synaptic input, particularly in the presence of the potentiator.

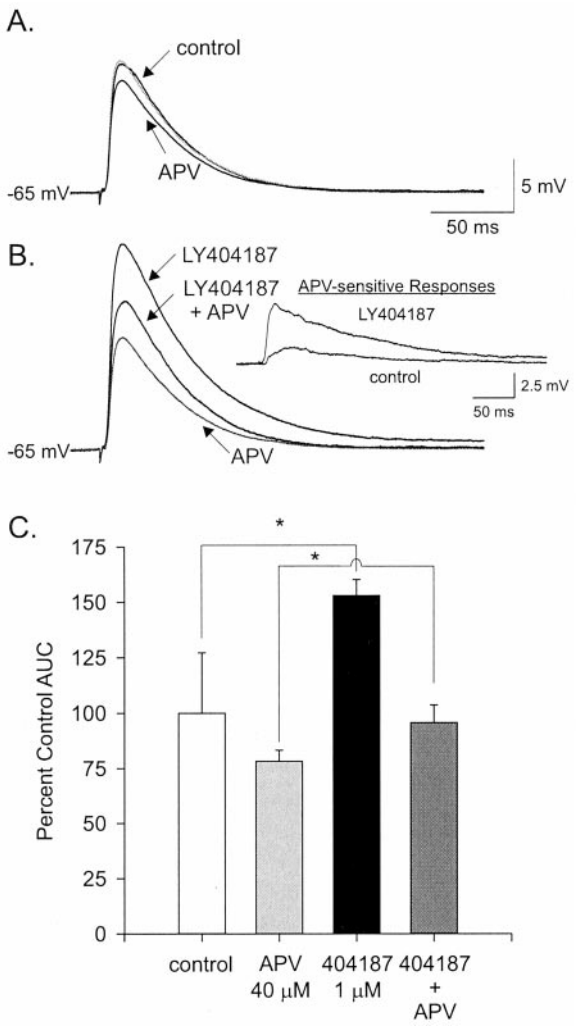

Fig. 9. LY404187 enhances NMDA receptor-dependent synaptic responses in PFC pyramidal neurons. A, single stimulation of glutamatergic afferents to PFC pyramidal neurons in the presence of elevated calcium (4 mM) and magnesium (4 mM) concentrations evoked a monosynaptic EPSP. Application of APV (40 μM) reduced the AUC of the response by approximately 20%. The effects of APV were eliminated within 15 min of washout (gray trace). B, addition of LY404187 (1 μM) enhanced the EPSP by approximately 50%. In the presence of LY404187, APV reduced the EPSP by approximately 50%, indicating an augmentation of the NMDA component of the response. This residual response reflected the potentiated AMPA response and was significantly larger than the control AMPA response (APV trace). Inset, APV-sensitive responses generated during control and LY404187 conditions by subtracting the responses in the presence from those in the absence of APV. C, plot of the average area under the curve for EPSPs recorded during the conditions indicated.

LY404187 Potentiates Excitatory Synaptic Responses of PFC Pyramidal Neurons Evoked by Stimulation of Hippocampal Afferents in Vivo. The results from the in vitro slice experiments demonstrated that LY404187 markedly enhanced excitatory synaptic inputs to PFC pyramidal neurons at nanomolar concentrations, sug-

gesting that this compound should have similar actions on specific inputs to these neurons in vivo provided that LY404187 crosses the blood-brain barrier. The central nervous system permeability and potentiation of synaptic responses by LY404187 were tested in vivo using extracellular recording of spontaneous and evoked action potential discharge of PFC neurons before and during i.v. administration of the compound. Previous neuroanatomical and electrophysiological studies have demonstrated that the ventral subiculum of the hippocampal formations provides monosynaptic, glutamatergic afferents to PFC pyramidal neurons (Jay et al., 1992). In addition, pharmacological experiments have shown that these excitatory synaptic responses are primarily mediated by AMPA receptor activation (Jay et al., 1992). Therefore, electrical stimulation of the ventral subicular inputs to PFC was used to assess the effects of LY404187 and compare with that of CX516, a compound that has previously been shown to be centrally active (Stäubli et al., 1994a).

PSTHs were constructed by measuring the number of action potential discharges per millisecond for 50 ms prior to (spontaneous firing) and 80 ms after single stimuli (evoked firing) delivered at 1 Hz (300 sweeps). Stimulation intensity was adjusted to evoke a response approximately 40% of the time establishing a predrug control baseline (Fig. 10A). Following administration of LY404187 (0.1–100 μg/kg, i.v.), the same stimulation intensity enhanced the probability of discharge without a change in spike latency. The percent change in the maximal probability of evoked discharge increased in a dose-dependent manner by 16.0 $\pm$ 2.3% (0.1 μg/kg), 44.5 $\pm$ 3.8% (1.0 μg/kg), and 113.0 $\pm$ 69.0% (10.0 μg/kg) relative to the average control probability of response ($F_{3,29} = 2.7$, $p < 0.05$) (Fig. 11A). Interestingly, the percent change in probability of response during the highest dose (100.0 μg/kg) of LY404187 tested was not significantly different from control values (92 $\pm$ 48.7%). In addition, because of the high degree of variability in the spontaneous firing rates between individual PFC neurons, no significant change in spontaneous firing was produced by any dose of LY404187 (Fig. 11B).

In a separate set of experiments, the effects of CX516 (0.1–10 mg/kg, i.v.) on evoked and spontaneous discharge of PFC neurons were evaluated. Similar to LY404187, CX516 potentiated the evoked discharge of PFC neurons; however, the dose (1 mg/kg) required to significantly enhance the probability of response was 1000-fold higher than that found for LY404187 (1 μg/kg) (Fig. 11C). A higher dose of CX516 (10 mg/kg) did not further increase the potentiation of evoked spike discharge. The spontaneous firing of PFC neurons also was not enhanced by CX516. Indeed, although not significant, a trend toward suppression of spontaneous activity was observed (Fig. 11D). It should be noted that because the stock solution of CX516 was 100 times greater than that for LY404187 (see *Materials and Methods*), the concentration of HβC also was higher for some CX516 doses, raising the possibility that its lower potency may have reflected an interaction with this solvent. However, when doses of LY404187 (1 μg/kg) and CX516 (100 μg/kg) having identical concentrations of HβC were administered, only LY404187 significantly enhanced evoked discharge. These results support the conclusion that LY404187 is at least 100-fold more potent than CX516 at potentiating evoked responses in vivo.

The demonstration that LY404187 enhanced both the AMPA receptor- and NMDA receptor-dependent synaptic inputs to PFC neurons in vitro suggested that the potentiation of evoked spike discharge in vivo may similarly depend on direct potentiation of AMPA receptors, as well as subsequent recruitment of NMDA channel activity. The AMPA receptor

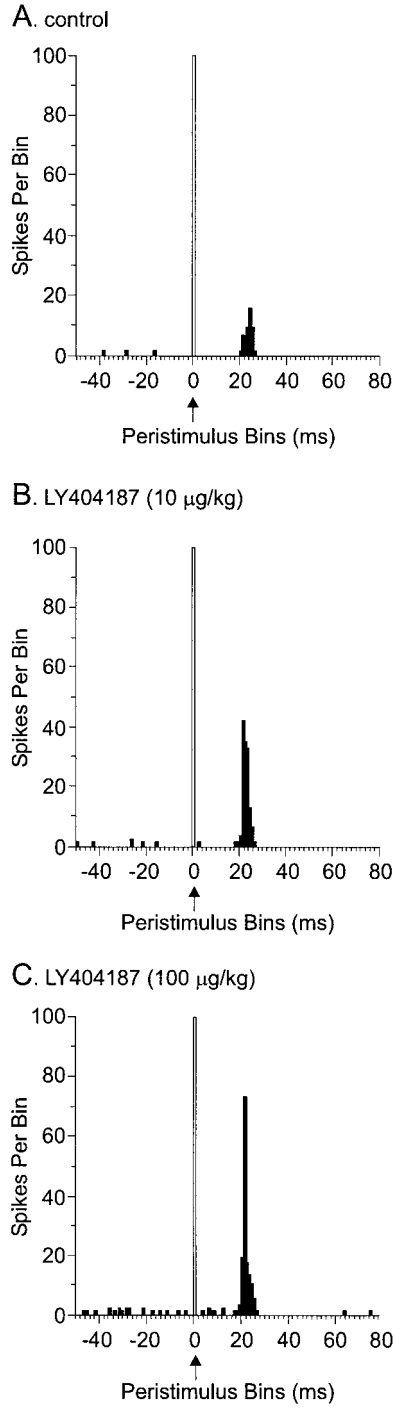

Fig. 10. Dose-dependent potentiation by LY404187 of the excitatory input to PFC neurons from ventral subiculum. A, control PSTH showing the total number of spike discharges of a single PFC neuron in response to stimulation of ventral subiculum (300 trials delivered at 1 Hz). Bin size is 1 ms. Arrow denotes stimulus presentation (time = 0). B and C, LY404187 (10 and 100 μg/kg, i.v.) increased the probability of spike discharge to synaptic stimulation in a dose-dependent manner. Note that spontaneous activity (defined as the number of spikes during the 50-ms prestimulus period) of this neuron also was enhanced by LY404187.

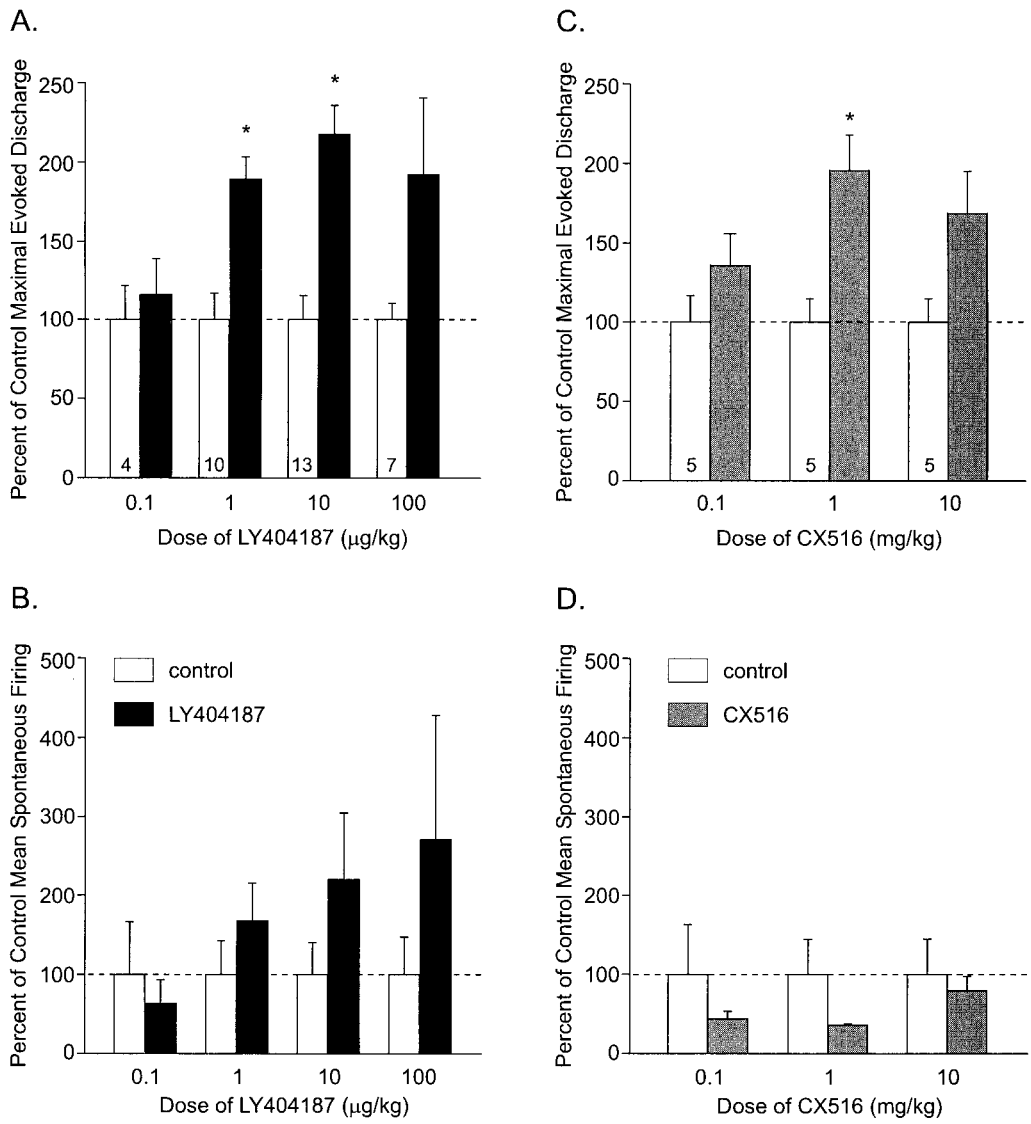

Fig. 11. Group data showing the effects of LY404187 and CX516 on the spontaneous and evoked responses of PFC neurons in vivo. A and C, plots of the average peak probability of evoked spike discharge to stimulation of ventral subiculum as a percentage of the control probability of discharge for LY404187 (0.1–100 μg/kg) and CX516 (0.1–10 mg/kg). The threshold for potentiation of evoked spike discharge by LY404187 (1 μg/kg) was 1000-fold lower than that for CX516 (1 mg/kg). The maximal increase produced by both compounds was approximately 200% of control. B and D, plots of the average peak probability of spontaneous firing as a percentage of the control probability of firing for LY404187 (0.1–100 μg/kg) and CX516 (0.1–10 mg/kg). Neither compound significantly enhanced (or depressed) spontaneous discharge.

specificity of the potentiation of evoked input by LY404187 was tested by attempting to block the increase in probability of discharge with LY300168. As described above, administration of LY404187 (10 μg/kg, i.v.) enhanced the probability of spike discharge to stimulation of the ventral subiculum (Fig. 12A). Subsequent administration of LY300168 (1 mg/kg, i.v.) eliminated the potentiation and reduced the level of evoked firing to control levels (Fig. 12A). Fifteen minutes after termination of LY300168 infusion, the probability of response was similar to that during the control period. No significant effect on spontaneous activity was produced by either compound.

In a separate series of experiments, the NMDA receptor contribution to the potentiation by LY404187 was explored using the centrally active, selective antagonist LY235959 (5 mg/kg, i.v.). Similar to LY300168, LY235959 reduced the potentiated responses to control levels (Fig. 12A). No effect on spontaneous discharge was observed in the presence of the NMDA receptor antagonist (Fig. 12B) These data suggest that a significant portion of the potentiated evoked response is mediated by recruitment of NMDA receptor activity.

Discussion

Potency Differences between LY404187 and CX516. The present results demonstrate that the biarylpropylsulfonamide LY404187 is a highly potent, selective, and centrally active potentiator of AMPA receptors in PFC pyramidal neurons. In comparison to the prototypic benzoylpiperidine CX516, LY404187 was approximately 3000 times more potent and 10-fold more efficacious at potentiating AMPA receptor responses of isolated PFC neurons. However, previous studies have shown that AMPA receptor modulators can preferentially affect specific receptor subunits and/or splice variants, giving rise to considerably different potency and efficacy values depending on the receptor tested (Partin et al., 1996; Sekiguchi et al., 1997). Molecular cloning techniques have identified four distinct AMPA receptor subunits (GluR1–4 or GluRA–D) with each subunit having two alternative splice variants termed "flip and flop" (Keinänen et al., 1990; Sommer et al., 1990; Hollmann and Heinemann, 1994). Evidence suggests that cortical pyramidal cells predominantly express heteromeric receptors composed of GluR2 and GluR3 subunits and both flip and flop splice variants (Geiger

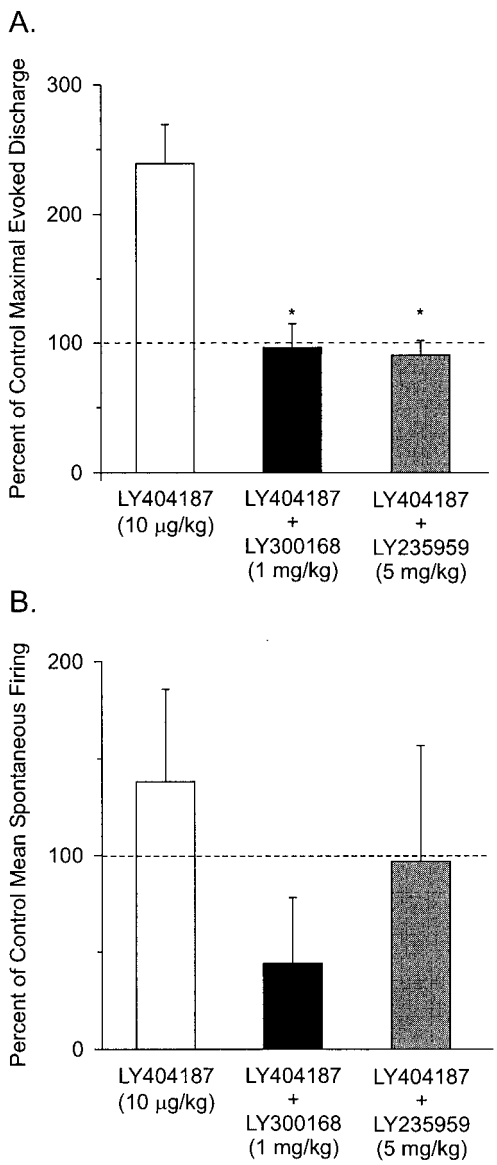

Fig. 12. AMPA and NMDA receptor antagonists suppress the potentiation of evoked spike discharge produced by LY404187. A, plot of the average peak probability of evoked spike discharge to stimulation of ventral subiculum as a percentage of the control probability of discharge for LY404187 (10.0 μg/kg) administered alone or followed by either LY235959 (5 mg/kg, i.v.) or LY300168 (1 mg/kg, i.v.). B, plot of the average peak probability of spontaneous firing as a percentage of the control probability of firing for LY404187 (10.0 μg/kg) administered alone or followed by either LY235959 (5 mg/kg, i.v.) or LY300168 (1 mg/kg, i.v.).

et al., 1995; Kondo et al., 1997). Therefore, some of the disparity between the potencies of LY404187 and CX516 may be attributed to differences in their relative activity at the particular receptors expressed in PFC neurons. Conversely, the low potency of CX516 also has been reported for receptors in hippocampal CA_1 pyramidal neurons (Arai and Lynch, 1998b), which express different subunits and splice variants compared with PFC pyramidal neurons (Sommer et al., 1990). In addition, recent studies showed that LY404187 was similarly more potent and efficacious than CX516 on recombinant homomeric GluR4 flip receptors (Bleakman et al., 2000). Collectively, these data suggest that LY404187 is intrinsically more potent than CX516.

Possible Mechanisms of LY404187 Potentiation. A common attribute of positive AMPA receptor modulators is that they exert their effects by altering the desensitization process. However, evidence from kinetic modeling experiments of GluR1 receptors indicates that AMPA potentiators can suppress the desensitization process through different mechanisms. For example, cyclothiazide potentiates AMPA receptors by directly slowing the onset rate of desensitization and by increasing agonist affinity, which indirectly slows the rate of channel closure (deactivation) (Yamada and Tang, 1993; Partin et al., 1994, 1996). In contrast, aniracetam appears to directly slow the rate of deactivation (without a change in agonist affinity), which indirectly slows the onset of desensitization (Partin et al., 1996).

The extent to which these models apply to heteromeric receptors as presumably expressed in PFC neurons is not presently known. Moreover, a similar kinetic model for LY404187 modulation could not be generated because deactivation rates could not be accurately determined due to the insufficient temporal and spatial resolution of agonist application conferred by recording from pyramidal cell somata and the inability to excise patches of membrane from freshly dissociated tissue. Despite these issues, the present data qualitatively suggest that the mechanism(s) of LY404187 potentiation differ from previously described compounds. Although LY404187 dramatically suppressed the magnitude of desensitization and increased the potency of glutamate at AMPA receptors by approximately 6-fold, similar to cyclothiazide, the compound did not slow the onset rate of the desensitization process. In addition, following entry into the desensitized state, a delayed *increase* in the magnitude of the current was observed (see Fig. 6A) that accounts for the time-dependent nature of the potentiation seen in the slow perfusion experiments (see Fig. 2A). Collectively, these results suggest that LY404187 may act by stabilizing the open state of AMPA receptors in the presence of agonist without altering the rate at which channels desensitize. In addition, the compound may permit desensitized AMPA receptors to make a transition to an open state either directly or through intermediate desensitized and/or closed states. A model consistent with this latter hypothesis has been proposed for AMPA receptors in neurons of the chick nucleus magnocellularis (Raman and Trussell, 1995). Further experiments will be required to clarify these issues.

LY404187 Potentiation of AMPA and NMDA Receptor-Mediated Synaptic Responses. The potentiation of somatic AMPA receptor responses by LY404187 in isolated cell recordings suggested that the compound should similarly enhance synaptic AMPA receptors activated by glutamatergic inputs to PFC neurons. Indeed, results showed that LY404187 potentiated synaptic AMPA receptor-mediated EPSPs in PFC slices in a concentration-dependent manner such that concentrations as low as 300 nM enhanced EPSPs by approximately 60%. In addition, the amplitude, but not the frequency, of spontaneous EPSPs was enhanced by the potentiator, indicating a postsynaptic site of action. Previous studies have demonstrated that the duration and amplitude of AMPA receptor-mediated EPSPs are influenced to a greater extent by modulators that preferentially affect deactivation (e.g., CX516) as opposed to desensitization (e.g., cyclothiazide) (Arai and Lynch, 1998b). Given the robust po-

tentiation by LY404187, these results suggest that LY404187 also modulates the deactivation process.

A predicted consequence of the greater depolarization produced by potentiators is that responses mediated by voltage-sensitive NMDA receptors also should be enhanced. Previous studies have indirectly addressed this hypothesis by demonstrating that positive AMPA receptor modulators can facilitate NMDA receptor-dependent LTP in the CA_1 region of hippocampus (Stäubli et al., 1994a). However, a direct enhancement of NMDA receptor responses has not been demonstrated. Moreover, AMPA receptor potentiators have been shown to increase the expression of neurotrophins (e.g., brain-derived neurotrophic factor), which can influence synaptic plasticity (Lauterborn et al., 2000). In particular, the induction of LTP in CA_1 neurons has been demonstrated to depend on elevation in intracellular brain-derived neurotrophic factor (Patterson et al., 1996), suggesting that the effect of AMPA receptor modulators is not necessarily associated with enhanced NMDA receptor activity. The present experiments directly tested this hypothesis and showed that LY404187 augmented the NMDA receptor component of the EPSP in conjunction with potentiation of the AMPA receptor portion of the response. Thus, these data conclusively demonstrate that a secondary consequence of positive modulation of AMPA receptors is the recruitment of voltage-dependent NMDA receptor activity, providing evidence for an additional mechanism for increasing synaptic strength.

The pronounced potentiation of synaptic AMPA and NMDA receptor responses in vitro suggested that LY404187 should similarly potentiate these responses in vivo provided that the compound is centrally active. Consistent with this hypothesis, administration of LY404187 at doses as low as 1 μg/kg significantly enhanced the probability of spike discharge of PFC neurons by approximately 80% in response to stimulation of the ventral subiculum. As with responses in vitro, this potentiation could be blocked by subsequent administration of LY300168. In addition, delivery of LY235959 reduced potentiation by LY404187, indicating that synaptic NMDA receptor activity also was recruited. Collectively, the results from in vitro and in vivo experiments confirm the central penetration of LY404187 and demonstrate that the compound can directly potentiate the effects of synaptically released glutamate on AMPA receptors, as well as indirectly augment NMDA receptor-dependent responses.

Therapeutic Implications: AMPA Receptor Potentiators Facilitate Working Memory. Considerable evidence has implicated the PFC in a variety of higher cognitive functions, many of which require the mnemonic process of working memory or the ability to "hold information in mind" for later retrieval (Baddeley, 1992). Typically, working memory performance is assessed using delayed-response tasks in which a delay period is introduced between presentation of cue and a required appropriate response. Neurophysiological experiments in primates have shown that subpopulations of PFC neurons exhibit enhanced discharge during the delay period of working memory tasks, suggesting a possible cellular correlate for this form of memory (Funahashi et al., 1989). In addition, lesions of PFC selectively disrupt performance on working memory tasks (Kolb et al., 1982). Clinical studies also have linked activity of the PFC with working memory performance. Brain imaging experiments repeatedly show activation of PFC in association with the successful execution of delayed-response tasks in normal subjects (Frith and Dolan, 1996). Conversely, working memory deficits have been recognized in several disease states and are consistently associated with decreased functioning of the PFC (Baddeley et al., 1991; Weinberger and Berman, 1996).

In light of the relationship between PFC neuronal activity and working memory, positive modulation of AMPA receptors on PFC neurons may be a pharmacological approach through which encoding of interim memories could be enhanced. In support of this hypothesis, previous studies have reported that AMPA receptor potentiators (e.g., CX516) improve performance in variety of delayed-response tasks, implicating an action of these modulators in PFC (Stäubli et al., 1994a,b; Hampson et al., 1998). Similarly, recent behavioral studies have shown that LY392098, a structural analog of LY404187, enhances performance of rodents in a delayed-response water maze task at doses (e.g., 10 μg/kg) that enhance the probability of evoked discharge of PFC neurons in vivo (Kimball et al., 2000; Baumbarger et al., 2001). Consistent with these experimental data, clinical studies have shown that administration of potentiators can improve cognitive function in normal subjects, as well as in some disease states (Dimond et al., 1979; Oepen et al., 1985; Ingvar et al., 1997). The high potency of LY404187, in conjunction with its central activity and efficacy in mnemonic models, suggests that biarylpropylsulfonamides may be therapeutically effective as nootropic agents. At the very least, a growing body of experimental and clinical evidence is providing proof of concept for the use of AMPA receptor potentiators in the treatment of certain forms of cognitive dysfunction.

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Address correspondence to: Eric S. Nisenbaum, Ph.D., Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Drop 0510, Indianapolis, IN 46285. E-mail: ESN@lilly.com