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LY404187: A Novel Positive Allosteric Modulator of AMPA Receptors

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ABSTRACT

LY404187 is a selective, potent and centrally active positive allosteric modulator of AMPA receptors. LY404187 preferentially acts at recombinant human homomeric GluR2 and GluR4 versus GluR1 and GluR3 AMPA receptors. In addition, LY404187 potentiates the flip splice variant of these AMPA receptors to a greater degree than the flop splice variant. In both recombinant and native AMPA receptors, potentiation by LY404187 displays a unique time-dependent growth that appears to involve a suppression of the desensitization process of these ion channels. LY404187 has been shown to enhance glutamatergic synaptic transmission both *in vitro* and *in vivo*. This augmentation of synaptic activity is due to the direct potentiation of AMPA receptor function, as well as an indirect recruitment of voltage-dependent NMDA receptor activity. Enhanced calcium influx through NMDA receptors is known to be a critical step in initiating long-term modifications in synaptic function (e.g., long-term potentiation, LTP). These modifications in synaptic function may be substrates for certain forms of memory encoding. Consistent with a recruitment of NMDA receptor activity, LY404187 has been shown to enhance performance in animal models of cognitive function requiring different mnemonic processes. These data suggest that AMPA receptor potentiators may be therapeutically beneficial for treating cognitive deficits in a variety of disorders, particularly those that are associated with reduced glutamatergic signaling such as schizophrenia. In addition, LY404187 has been demonstrated to be efficacious in animal models of behavioral despair that possess considerable predictive validity for antidepressant activity. Although the therapeutic efficacy of AMPA receptor potentiators in these and other diseases will ultimately be determined in the clinic, evidence suggests that the benefit of these compounds will be mediated by multiple mechanisms of action. These mechanisms include direct enhancement of AMPA receptor function, secondary mobilization of intracellular signaling cascades, and prolonged modulation of gene expression.

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INTRODUCTION

Excitatory synaptic transmission in the central nervous system (CNS) is primarily mediated by the amino acid neurotransmitter, glutamate. An accumulating body of evidence has indicated that dysfunction of glutamatergic signaling in the CNS may contribute to the deficits associated with a variety of neurological and psychiatric disorders (27,104,105). As such, a considerable effort is underway to explore therapeutic approaches to these disorders which share the common goal of enhancing glutamatergic synaptic transmission. One of these strategies has focused on compounds that positively allosterically modulate glutamate α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. These receptors are ubiquitous in the CNS and mediate the majority of fast excitatory neurotransmission. Although these compounds do not have any intrinsic activity, in the presence of glutamate (or other agonists), these positive modulators ("potentiators") augment the activity of AMPA receptor/channels by suppressing the biophysical process(es) of desensitization (channel closure in the presence of agonist) and/or deactivation (channel closure upon removal of agonist) (21,78). The functional consequence of these compounds is to enhance glutamatergic excitatory postsynaptic potentials (EPSPs) by increasing ion flux through AMPA receptors/channels (42,103).

Support for the therapeutic potential of AMPA receptor potentiators comes from early clinical studies with the pyrrolidinone compounds, piracetam and aniracetam. These studies showed promising results in treating cognitive deficits associated with several neurological diseases, including Parkinson's disease, Alzheimer's disease, and schizophrenia (20,53,76). The cognitive enhancing effects of these drugs prompted their informal classification as "nootropic agents" (i.e., compounds that facilitate learning and memory). Further exploration of the nootropic action of AMPA receptor potentiators has been pursued using structurally related benzamide compounds (e.g., CX516). These compounds have been demonstrated to improve cognitive function in both healthy and aged subjects (41,62), as well as in patients with schizophrenia when used as an adjunct therapy (32). Consistent with these clinical findings, numerous experimental studies have reported that aniracetam, piracetam, and CX516 can improve performance in animals on a variety of cognitive tasks that require multiple types of mnemonic processes (34,35,81,83,86,87, 93,94,100). Along with their nootropic actions, these positive AMPA receptor modulators have been shown to be active in other models of disease. For example, piracetam is efficacious in several models of anxiety, including the social interaction test, elevated plus maze, and Vogel's conflict test (12,26). CX516 (and structural analogs) has been demonstrated to suppress hyperactivity in animal models of attention-deficit, hyperactivity disorder (ADHD) and positive symptoms of schizophrenia (29,47). In addition, aniracetam has been shown to be effective in the forced swim test, a model of behavioral despair that can be used to detect compounds with antidepressant activity (71). Collectively, these data suggest that positive modulation of AMPA receptors may be efficacious in a wide range of therapeutic targets.

Despite the value of these previous compounds as tools for proof of concept studies, their relatively low potencies (millimolar) and/or poor brain penetration have limited their therapeutic potential. Recently, a novel class of AMPA receptor potentiators, typified by LY404187, has been developed (77). LY404187 is a selective, potent and centrally active positive allosteric modulator of AMPA receptor activity (10,13,30,58,68,99). This article

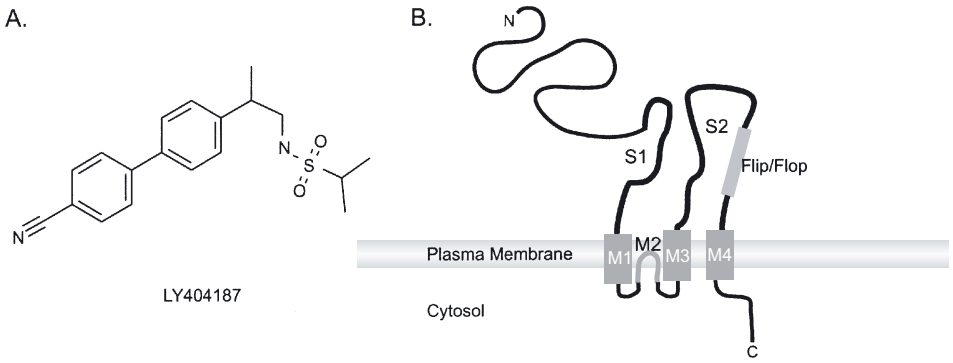

Fig. 1. Chemical structure of LY404187 and structure of AMPA receptor subunit. A, Chemical structure of LY404187; B, structure of AMPA receptor subunit. The transmembrane domains, M1–M4, are labeled in dark gray. M2 is believed to line the pore of the channel and contains the Q/R edited site. The ligand-binding domain consists of S1 and S2, labeled with a thick black line. The location of the flip/flop alternatively spliced exon is labeled in light gray.

will initially provide a brief description of the chemistry of LY404187 and review of the molecular biology of AMPA receptors. Subsequently, a detailed description of the *in vitro* and *in vivo* pharmacological properties of LY404187 and the possible therapeutic targets of positive allosteric modulators of AMPA receptors will be described.

CHEMISTRY

LY404187 is a sulfonamide with the chemical name N-2-[4-(4-cyanophenyl)phenyl]propyl 2-propanesulfonamide and CAS registry number [211311-95-4] (Fig. 1A, Table 1). LY404187 is a racemic mixture of R and S stereoisomers that exists as a chemically stable, white solid with the chemical formula of $C_{19}H_{22}N_2O_2S$ and a molecular weight of 342.45 g/mol. LY404187 has been prepared by the Suzuki coupling of the 4-cyanobenzene boronic acid with N-2-(4-bromophenyl)propyl 2-propanesulfonamide (77). LY404187 possesses a sulfonamide functionality that displays a pK_a of 5.

MOLECULAR BIOLOGY OF AMPA RECEPTORS

Glutamate receptors can be segregated into two distinct classes: ionotropic receptors, which are ligand-gated ion channels and metabotropic receptors, which interact with GTP-binding proteins (G-proteins). The ionotropic glutamate receptors can be further divided into three subtypes on the basis of molecular and pharmacological properties: 1) AMPA, 2) N-methyl-D-aspartic acid (NMDA), and 3) kainic acid (KA) receptors (21, 37). The AMPA receptor family includes four different genes (termed GluR1–4 or GluRA-D) that encode proteins of about 900 amino acids and share approximately 70% amino acid identity. Structurally, these proteins contain a large extracellular N-terminus domain and four hydrophobic domains labeled M1-M4 (Fig. 1B). Evidence suggests that AMPA re-

TABLE 1. List of compounds

Common name	Chemical name	Pharmacological action
Aniracetam	1-anisoyl-2-pyrrolidinone	AMPA receptor potentiator
APV	DL-2-amino-5-phosphonovaleric acid	NMDA receptor antagonist
Bicuculline	[R-(R*,S*)]-6-(5,6,7,8-tetrahydro-6-methyl-1,3-dioxolo[4,5-g] isoquinolin-5-yl)furo[3,4-e]-1,3-benzodioxol- 8(6H)-one	$GABA_A$ receptor antagonist
CNQX	6-cyano-7-nitroquinoxaline-2,3-dione	AMPA receptor antagonist
CX516	1-(quinoxalin-6-ylcarbonyl)piperidine	AMPA receptor potentiator
CX546	1-(1,4-benzodioxan-6-ylcarbonyl)piperidine	AMPA receptor potentiator
Cyclothiazide	6-chloro-3,4-dihydro-3-(2-norbornen-5-yl)-2*H*-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide	AMPA receptor potentiator
LY235959	[3S-(3a,4aa,6b,8aa)]-decahydro-6-(phosphonomethyl)-3-isoquinolinecarboxylic acid	NMDA receptor antagonist
LY300168 (GYKI53655)	5-(4-aminophenyl)-8,9-dihydro-n,8-dimethyl-7h-1,3-dioxolo[4,5-h][2,3]benzodiazepine-7-carboxamide	AMPA receptor antagonist
LY392098	N-2-[4-(3-thionyl)phenyl]-2-propanesulfonamide	AMPA receptor potentiator
LY395153	N-2-(4-benzamidophenylpropyl)-2-propanesulfonamide	AMPA receptor potentiator
LY404187	N-2-[4-(4-cyanophenyl)phenyl]propyl-2-propanesulfonamide	AMPA receptor potentiator
NBQX	2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide	AMPA receptor antagonist
SCH50911	(2S)-(+)-5,5-dimethyl-2-morpholineacetic acid	$GABA_B$ receptor antagonist

ceptor subunits have only three transmembrane domains (M1, M3, and M4 in the original nomenclature) and a reentrant loop (M2) on the cytoplasmic side. This reentrant loop is believed to line the pore of the ion channel, and so is sometimes referred to as the P-domain. This topology indicates that both the large N-terminus and the region between M3 and M4 are extracellular. The region of the N-terminus, immediately adjacent to M1 (designated S1), and the region between M3 and M4 (designated S2) have been crystallized and shown to bind glutamate in a clamshell configuration, similar to metabotropic glutamate receptors (6,7).

AMPA receptors are tetramers that can be generated by the assembly of one or more of the protein subunits GluR1–4, yielding homomeric or heteromeric receptors. GluR1–4 subunits can be modified post-transcriptionally to further increase the diversity of AMPA receptors. RNA editing occurs at two different positions within the AMPA receptor genes. A glutamine residue (Q; CGA) in the pore region (M2) of GluR2 is edited to give an arginine (R; CGG). In fully developed adult animals, virtually all of the GluR2 subunit is edited at this site. This edited residue significantly reduces calcium permeability, decreases single channel conductance and reduces rectification of the AMPA receptor (38, 101). A second site in the extracellular domain between M2 and M3 is edited from an arginine (R; AGA) to a glycine (G; GGA) in GluR2, GluR3, and GluR4. This edited residue can alter the time course of recovery from desensitization (59).

Additional complexity among AMPA receptors results from alternative splicing in the extracellular S2 region in GluR1–4. This region can contain one of two different exons, referred to as flip (i) and flop (o). The flip and flop exons encode a 38 amino acid sequence that differs between the two isoforms by only 7 amino acids. These different isoforms show distinct cell-specific and developmental expression patterns in the brain (69,92). Furthermore, several functional consequences of this alternative splicing have been identified. In general, receptors composed of flip subunits desensitize more slowly than receptors composed of flop subunits (70). AMPA receptors composed of flip or flop subunits also show differential sensitivity to positive allosteric modulation. For example, cyclothiazide, a benzothiadiazide, preferentially enhances ion flux through flip receptors, whereas the pyrrolidone aniracetam selectively potentiates the activity of flop receptors (46,79). As described above, the sensitivity of AMPA receptors to positive allosteric modulation provides a mechanism by which glutamatergic synaptic transmission can be enhanced in the CNS and may represent a novel approach for the treatment of disease.

AMPA RECEPTOR PHARMACOLOGY

Recombinant Homomeric AMPA Receptors

In initial studies, the relative potency of LY404187 was measured at homomeric GluR1–4i and GluR2o receptors stably transfected in HEK 293 cells. HEK 293 cells do not express endogenous AMPA receptors (68). Although binding studies have not been performed using LY404187, a similar AMPA receptor potentiator, LY395153, has been shown to bind to native AMPA receptors and recombinant GluR4i receptors expressed in HEK cells (57,107). Potentiation of homomeric AMPA receptor activity was assessed by measuring fluorescence changes associated with calcium influx into cells preloaded with Fluo3-AM dye using a FLUOROSKAN II fluorimeter (Labsystems, Needham Heights,

MA, USA). Since this assay depends on calcium influx from the extracellular solution, the unedited (Q) form of the homomeric GluR2 receptor, which is calcium permeable, was used in these experiments. Examination of the different recombinant human homomeric AMPA receptors revealed that the activity of all subtypes of receptors was potentiated by LY404187 in a concentration-dependent manner. LY404187 was active at all receptors in the nanomolar range. However, differences in the relative potency of the compound at each receptor were observed. The rank order of EC_{50} values at flip receptors revealed that LY404187 was more potent at GluR2i (0.15 μM) and GluR4i (0.21 μM) than GluR3i (1.66 μM) and GluR1i (5.65 μM) (68). In addition, potency comparisons of the flip and flop isoforms of the GluR2 receptor showed that LY404187 was approximately 10-fold more potent at GluR2i than the GluR2o (1.44 μM) receptor. Selectivity assays demonstrated that the increase in fluorescence in all AMPA receptor subtypes could be prevented by the addition of the AMPA receptor antagonist, NBQX (50 μM). These results suggest that while LY404187 potentiates all AMPA receptors, it is more potent at GluR2 and GluR4 receptors and has some preference for flip splice variants.

Due to limitations of fluorimetric technology, the kinetics associated with LY404187 potentiation could not be resolved. Therefore, whole-cell voltage-clamp electrophysiology was used to further characterize the effect of LY404187 on recombinant human homomeric receptors. The HEK cell line stably expressing GluR3i was chosen because the relative potency of LY404187 on these receptors was about the average for all the homomeric cell lines tested. In the absence of any agonist, application of LY404187 (0.1–30.0 μM) had no effect on the holding current of HEK cells stably transfected with GluR3i subunits (holding potential –80 mV). However, when applied with the agonist (glutamate at 100 μM), LY404187 potentiated GluR3i receptor responses in a concentration-dependent manner, with a potency similar to that observed using the fluorometric assay (EC_{50} of 3.7 μM; $n = 8$) (Fig. 2). Interestingly, the potentiated responses displayed a marked time-dependent growth, especially at non-saturating concentrations of LY404187. This kinetic property appears to be a unique phenotype of LY404187 potentiation and was also observed in the potentiation of recombinant human GluR4i receptors (30). The time-dependence of LY404187 potentiation will be discussed in more detail below. These whole-cell electrophysiological experiments confirmed the positive allosteric actions of LY404187 described with the fluorimetric screening, and revealed the novel kinetics of this potentiation.

Native AMPA Receptors

Potency and efficacy

Although the precise rules governing assembly of subunits to form AMPA receptor/channels have not been completely elucidated, the different isoforms of GluR1–4 can assemble to form functional heteromeric receptors. Since multiple types of neurons in the CNS have been shown to express mRNA for more than one GluR subunit in a single cell, it has been inferred that native receptors are hetero-oligomers (18,31,52). As such, the following experiments investigated the potency, selectivity and kinetics of potentiation by LY404187 of native (presumably heteromeric) AMPA receptors from acutely isolated pyramidal neurons of the prefrontal cortex (PFC) of rats. These neurons are believed to ex-

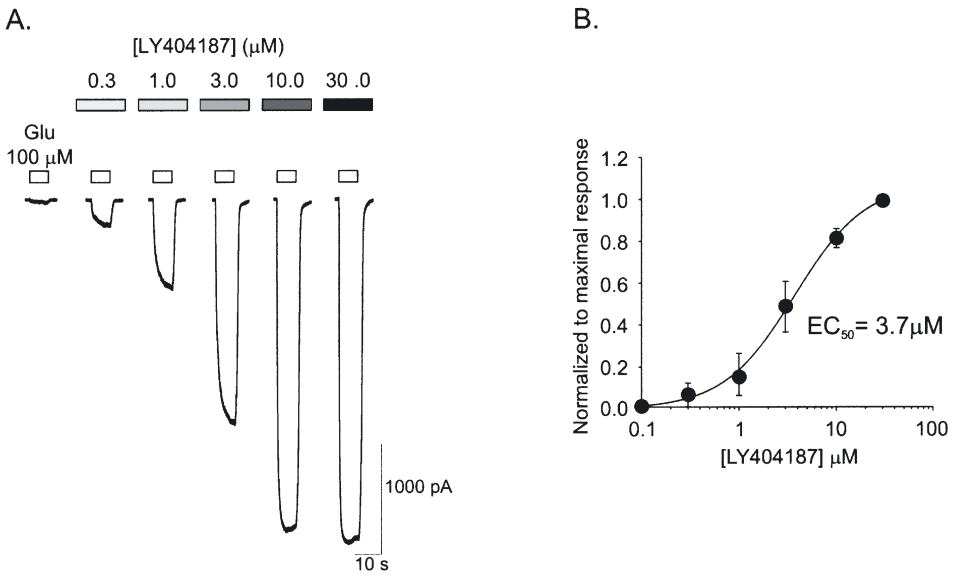

Fig. 2. Concentration-response profile for LY404187 potentiation of glutamate-mediated currents measured from HEK293 cells expressing recombinant human GluR3i. A, The concentration-response profile for LY404187 (0.3–30 μM) was generated by measuring the responses to 100 μM glutamate (GLU) alone and in the presence of LY404187. LY404187 enhanced the response to glutamate in a concentration-dependent manner. B, Concentration-response curve for the degree of potentiation produced by LY404187 normalized to the maximum response (100 μM GLU in the presence of 30 μM LY404187). Points represent mean ± S.D. EC_{50} = 3.7 μM (n = 8).

press high levels of GluR2, modest levels of GluR1 and GluR3 and low levels of GluR4. The pyramidal neurons may express more of the flip isoform of these subunits than the flop isoform (48,52,64,92,96). The potency and efficacy of LY404187 on glutamate-evoked currents was first examined using whole-cell voltage-clamp recording from acutely isolated pyramidal neurons of the PFC. Preliminary experiments demonstrated that 100 μM glutamate was equal to 30% of the maximal response (EC_{30}). Therefore, inward currents were recorded in response to application of glutamate (100 μM, 10 sec duration; holding potential = –80 mV) alone and in the presence of the compound. Application of LY404187 alone had no effect on the holding current at all concentrations tested (0.03–10 μM). However, when applied in the presence of glutamate, LY404187 enhanced the evoked current in a concentration-dependent manner (Fig. 3A). The potentiated response also displayed a marked time-dependence such that a steady-state level was never achieved during the 10 sec glutamate stimulus. Due to this property, 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. 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. 3B). Experiments designed to identify the concentration threshold for potentiation of AMPA (EC_{30} = 5 μM) responses demonstrated that the lowest concentration of

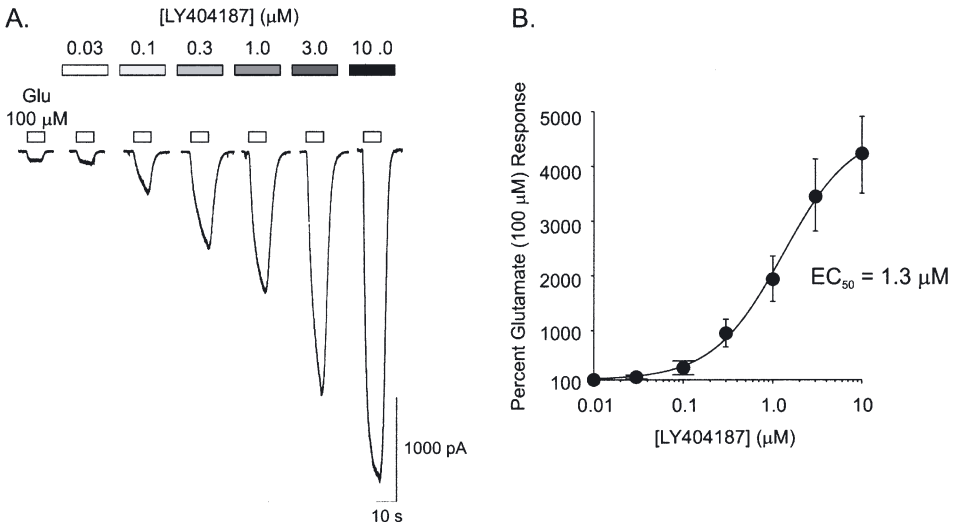

Fig. 3. Concentration-response profile for LY404187 potentiation of glutamate-mediated currents measured from acutely isolated pyramidal PFC neurons. A, The concentration-response profile for LY404187 (0.03–10 μM) was generated by measuring the responses to 100 μM glutamate (GLU) alone and in the presence of LY404187. LY404187 enhanced the response to glutamate in a concentration-dependent manner; B, Concentration-response curve for the degree of potentiation by LY404187 normalized to the GLU (100 μM) alone response. Points represent mean ± S.D. EC_{50} = 1.3 ± 0.3 μM, E_{max} = 45.3 ± 8.0-fold increase (n = 6).

LY404187 required to produce a significant increase in AMPA-evoked current was 10 nM (10). Collectively, these data demonstrate that LY40487 is a highly potent, agonist-dependent potentiator of native AMPA receptor activity in acutely isolated PFC neurons.

Potentiation by LY404187 has also been examined in acutely isolated cerebellar Purkinje neurons. *In situ* hybridization and PCR experiments suggest that Purkinje neurons express different heteromeric AMPA receptors than PFC pyramidal neurons (69,96). Similar to the PFC neurons, application of LY404187 alone had no effect on Purkinje cells, but potentiated the glutamate-evoked responses in a concentration-dependent manner. Also in agreement with the results in PFC neurons, the concentration threshold for potentiation was 10.0 nM in Purkinje cells. As before, LY404187 enhanced glutamate-evoked currents in a time-dependent manner. However, an interesting difference between the effects of LY404187 on the responses of PFC neurons and Purkinje cells was the significant difference in maximal efficacy observed. While LY404187 enhanced glutamate-evoked (100 μM glutamate) currents approximately 45-fold in PFC neurons, the maximal potentiation was approximately 175-fold in Purkinje cells. Although this difference may be in part due to the degree of desensitization produced by glutamate alone on these two neuronal subtypes, LY404187 may potentiate different heteromeric receptor combinations to a greater or lesser extent. If so, one consequence of the receptor heterogeneity among different neuronal subpopulations would be that LY404187 (and positive modulators in general) could selectively augment AMPA receptor function in discrete regions of the brain.

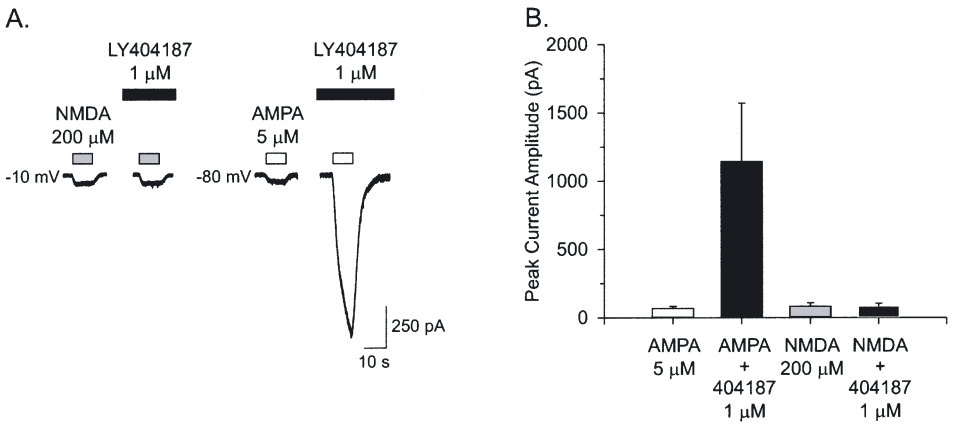

Fig. 4. LY404187 fails to potentiate NMDA receptor-mediated currents measured from acutely isolated pyramidal PFC neurons. A, When the membrane potential of neurons was held at –10 mV and 30 μM glycine was added to the external solution, 200 μM NMDA evoked an inward current that was not potentiated by LY404187 (3 μM). In these same neurons, when the membrane potential was held at –80 mV, application of 5 μM AMPA evoked an inward current that was potentiated by LY404187 (3 μM). B, Plots of the average peak amplitudes (±S.D.) for each condition in A.

Receptor selectivity

The selectivity of LY404187 for potentiating AMPA receptors has been demonstrated in several studies. First, potentiation of glutamate-evoked currents by LY404187 in native receptors can be mimicked with AMPA application (10,30). LY404187 enhanced AMPA-evoked currents (5 μM = EC_{30}) 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 in acutely isolated PFC neurons. Second, the effects of LY404187 on both recombinant receptors and several types of native receptors can be completely blocked by selective AMPA receptor antagonists, including NBQX and LY300168 (10,30,68). Third, LY404187 does not enhance other ionotropic glutamate receptors, including GluR5 recombinant or native KA and NMDA receptors (10,30) (Fig. 4). Finally, LY404187 is without effect on voltage-dependent sodium, calcium or potassium channels (10). Taken together, these data demonstrate that LY404187 is a selective allosteric modulator of ionotropic AMPA receptors.

Kinetics of potentiation

Previous studies of positive allosteric modulators of ionotropic glutamate receptors have shown that the kinetics of onset and recovery from potentiation can vary considerably. For example, the AMPA receptor potentiator, cyclothiazide, enhances current flux rapidly within milliseconds of application in a reversible manner (79). In contrast, the KA receptor modulator, concanavalin A, potentiates more slowly and irreversibly over minutes of exposure (65). The onset and recovery kinetics of LY404187 potentiation was investigated using whole-cell voltage-clamp recording from acutely isolated PFC neurons. Responses to 10-sec 5 μM AMPA pulses before, during and after application of 10 μM LY404187 were recorded. A baseline response to AMPA was established by measuring three applications of AMPA that were separated by 10 sec each (10). Subsequent appli-

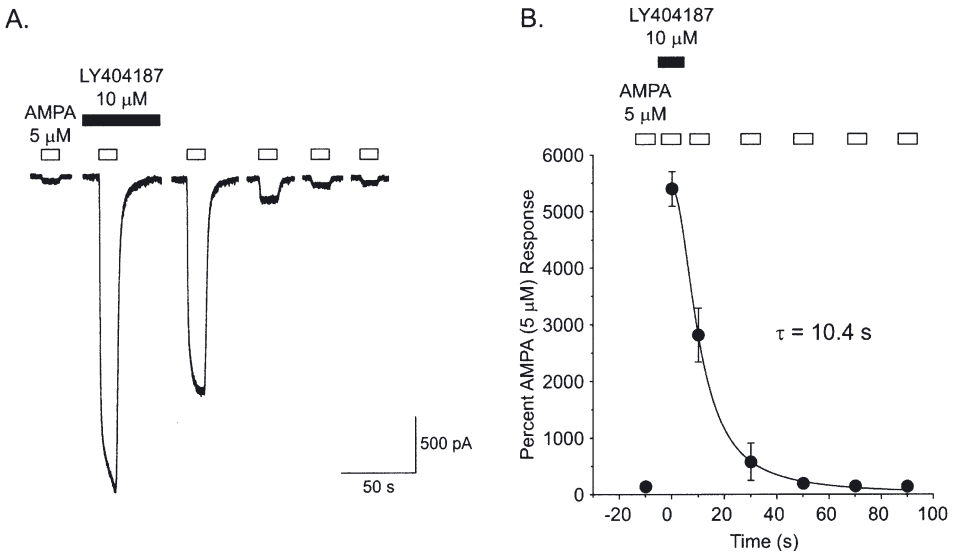

Fig. 5. Kinetics of recovery from LY404187 potentiation. A, Responses to AMPA (5 μM) before, during and after application of 10 μM LY404187 measured from acutely isolated pyramidal PFC neurons. Following removal of LY404187, the responses to AMPA returned to baseline levels. B, Plot of the recovery from potentiation by LY404187. Points reflect the average responses (±S.D.) 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.

cation of LY404187 had no effect, but rapidly potentiated the responses upon co-application of AMPA. After LY404187 was removed, a 10-sec application of AMPA was applied every 20 sec. These AMPA-evoked currents progressively decreased in amplitude back to baseline levels (Fig. 5A). The average peak current amplitudes before, during, and after application of LY404187 were calculated and normalized to the average control response to AMPA before application of LY404187. Recovery from potentiation occurred within 70 sec of removal of LY404187. This recovery process was plotted and fit with a single exponential function having a time constant of 10.4 sec (Fig. 5B). These data indicate that, similar to cyclothiazide, potentiation by LY404187 is rapid and reversible.

One unique characteristic of potentiation by LY404187 is that, in the presence of agonist, responses display a distinctive time-dependence such that a steady-state level is never achieved at non-saturating concentrations of LY404187. This is in contrast to other classes of AMPA receptor potentiators such as cyclothiazide and CX516 that produce increases in AMPA receptor-mediated responses that reach steady-state values within 2 or 3 sec (Fig. 6A). This time-dependent growth of current is observed in response to co-application of subsaturating concentrations of LY404187 and agonist (e.g., glutamate or AMPA) even with exposure times of up to 120 sec (Fig. 6B). One explanation of this phenotype could be that LY404187 binds to an internal site, and so must cross the plasma membrane to have an effect. In order to test this hypothesis, LY404187 at 3 μM was added to the internal recording pipette solution. After achieving a whole-cell recording, 5 min elapsed to allow time for LY404187 to diffuse into the cell. When AMPA (5 μM) was applied extracellularly during the next 3 to 5 min, no potentiation was observed. In these

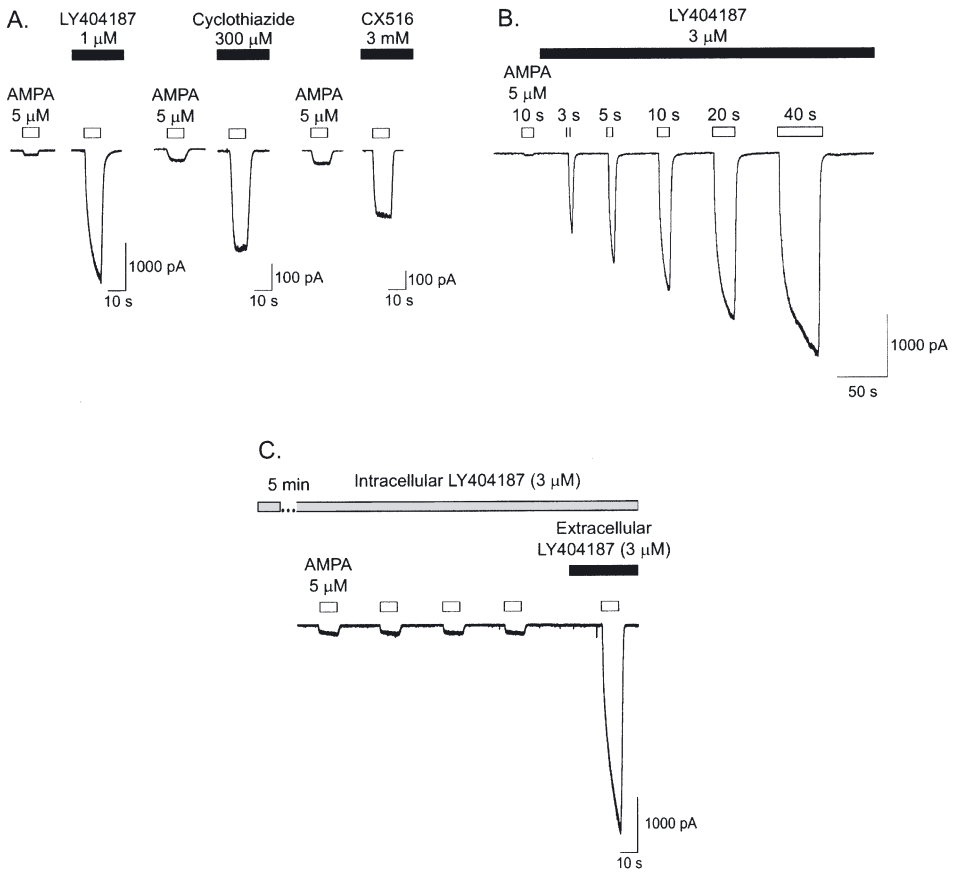

Fig. 6. Activity and time-dependence of enhancement by AMPA potentiators. A, The activity and time-dependence of the AMPA receptor potentiation produced by 1 μM LY404187, 300 μM cyclothiazide, and 3 μM CX516 were evaluated by applying 5 μM AMPA (10 sec duration) alone and in the presence of the potentiator onto acutely isolated pyramidal PFC neurons. None of the compounds produced an effect alone, but all compounds potentiated the AMPA-mediated currents, suggesting a similar activity-dependence to their action. Unlike cyclothiazide and CX516, the potentiation produced by LY404187 displayed a marked time dependence during the 10-sec application. B, Responses to pulses of AMPA (5 μM) of various durations (3–40 sec) in the presence of 3 μM LY404187. Even in response to a 40-sec 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 failed to potentiate the AMPA (5 μM) evoked response. Subsequent extracellular application of LY404187 (3 μM) did potentiate the AMPA evoked response.

same neurons, when LY404187 was applied extracellularly, a robust potentiation to AMPA (5 μM) was always observed (Fig. 6C). Therefore, these results indicate that the unique time-dependence of potentiation is not due to binding to an intracellular site and further suggest that the binding site for LY404187 is extracellular. Although the mechanism of this time-dependent characteristic remains to be determined, it is possible that LY404187 permits AMPA receptor/channels to "resensitize" by shifting the channel from the desensitized state to an agonist-bound open state.

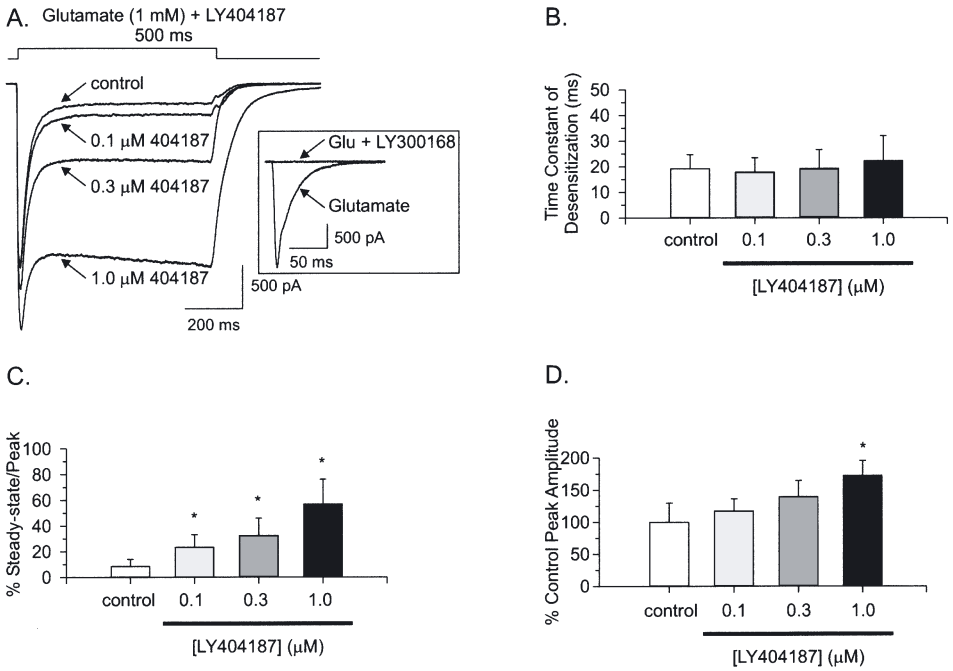

Fig. 7. Desensitization of AMPA receptor-mediated currents is altered by LY404187. A, Whole-cell responses measured from acutely isolated pyramidal PFC neurons to 500 ms application of 1 μM glutamate in the absence and presence of LY404187 (0.1–1.0 μM). Inset, the glutamate-evoked current was completely blocked by 50 μM LY300168. B, 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 responses in the presence of LY404187, the boundaries of the fit were from the peak of the response to 200 ms after the stimulus. C, 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 LY404187. This steady-state value was taken at the end of the 500-ms pulse. Because of the time-dependent nature of LY404187 potentiation, there is not a true steady-state value. D, Change in the initial peak response to glutamate application was evaluated by expressing the peak amplitude in the presence of potentiator as a percentage of the control peak amplitude. The control peaks were then expressed as a percentage of the average control peak. LY404187 enhanced the peak response, possibly by increasing recovery from desensitization during the rising phase of the response. All bars represent mean ± S.D.

The concentration-response profiles described above measured the potentiation of LY404187 on desensitized AMPA receptors. The pronounced enhancement of these currents by LY404187 suggests that this potentiator acts by altering the desensitization kinetics of the AMPA receptor. In order to examine this in greater detail, a piezoelectric fast perfusion system was used to deliver 500-ms pulses of agonist in a whole-cell voltage clamp configuration using pyramidal PFC neurons. Under these conditions, the response to glutamate (1.0 μM) could be completely blocked by LY300168 (50 μM), indicating that the current was entirely due to AMPA receptor activation (Fig. 7A, inset). During control conditions, the response to glutamate 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. 7A). A single exponential function was used to fit the current decay with the average time constant of desensitization being 15.4 ± 3.7 ms

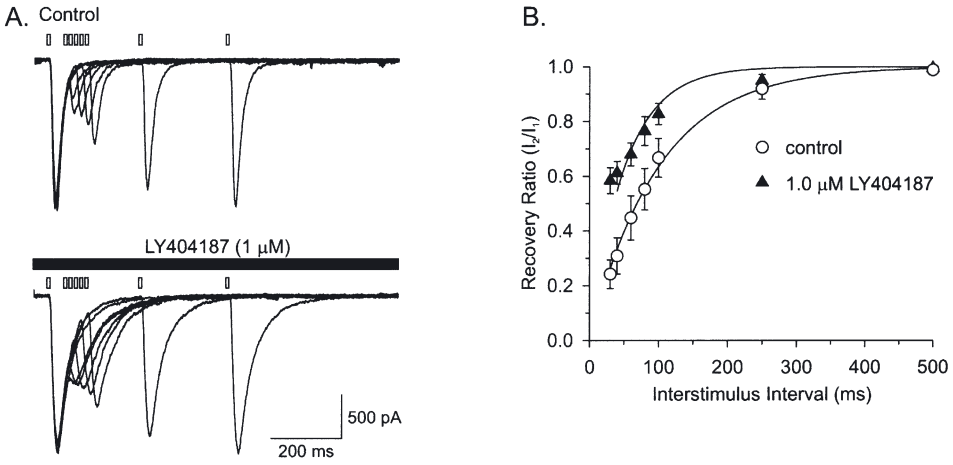

Fig. 8. LY404187 decreases the time course of recovery from desensitization. A, Pairs of glutamate pulses (10 ms, open boxes) having interstimulus intervals (ISIs) of 30 to 500 ms were delivered to acutely isolated PFC pyramidal neurons in the absence and presence of 1.0 μM LY404187. B, An average recovery ratio was measured for each ISI by dividing the peak of the second response by that of the first response in the absence or presence of LY404187. 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 ± 57.6 ms; 1.0 μM LY404187 = 49.8 ± 13.8 ms). Points represent mean ± S.D.

(n = 10). Subsequent application of LY404187 at or below the EC_{50} (0.1–1.0 μM) did not alter the initial time constant of desensitization (15.1 ± 4.8, 0.1 μM; 17.2 ± 6.1 ms, 0.3 μM; 18.2 ± 8.2 ms, 1.0 μM) (Fig. 7A and B). However, application of LY404187 enhanced the magnitude of the steady-state current relative to the peak current amplitude in a concentration-dependent manner (23.3 ± 9.9%, 0.1 μM; 32.3 ± 13.7%, 0.3 μM; 56.9 ± 19.3%, $F_{3,35}$ = 24.1, $p < 0.0001$) (Fig. 7A and C). In addition, the peak amplitude increased in response to LY404187 in a dose-dependent manner (17.1 ± 19.2%, 0.1 μM; 39.5 ± 25.0%, 0.3 μM; 72.3 ± 23.2%, 1.0 μM, $F_{3,2}$ = 9.5, $p < 0.0001$) (Fig. 7A and D), suggesting that desensitization is occurring during the rising phase of the response (80,82).

The marked effect of LY404187 on the desensitization process suggested that recovery from desensitization should proceed more rapidly in the presence of the potentiator. In order to test this hypothesis, brief (10 ms) pairs of glutamate pulses were applied with varying interstimulus intervals (30–500 ms) (Fig. 8A). The amplitude of the second pulse relative to the first pulse was then calculated to give a recovery ratio (Fig. 8B). For glutamate pulses in the absence of LY404187, the time constant for this recovery process was 109.5 ± 57.6 ms (n = 7). For glutamate pulses in the presence of LY404187 at 1 μM, the time constant of recovery significantly decreased (49.8 ± 13.8 ms; t = 2.9, $p < 0.05$). In addition, the recovery ratio at the shortest interstimulus interval (30 ms) significantly increased in the presence of 1.0 μM LY404187 (0.24 ± 0.14 for glutamate alone; 0.58 ± 0.13 in the presence of 1.0 μM LY404187; t = 2.7, $p < 0.05$). Therefore, LY404187 may act to potentiate AMPA receptor activity by allowing faster recovery from desensitization.

POTENTIATION OF EXCITATORY SYNAPTIC RESPONSES *IN VITRO*

Potentiation of AMPA Receptor-Mediated Synaptic Responses

LY404187 has been shown to potently enhance native AMPA receptor activity evoked by exogenously applied agonists. These data suggest that LY404187 should also potentiate AMPA receptor-activity elicited by synaptically released glutamate. To test this hypothesis, the effect of LY404187 on synaptic activity was examined by recording excitatory responses from pyramidal neurons in a rat PFC slice preparation (10). Due to the dense recurrent excitatory collateral network among PFC neurons, the synaptic response recorded is comprised of both mono- and polysynaptic components, yielding a complex EPSP. In initial experiments, application of LY404187 (0.1–3.0 μM) enhanced the subthreshold synaptic response in PFC pyramidal neurons to the point of action potential discharge. These effects of LY404187 could be reversed within 15 to 30 min of elimination of the potentiator. Subsequent experiments focused on the selective potentiation of AMPA receptor-mediated EPSPs by recording from slices in which γ-aminobutyric acid $(GABA)_{A \text{ and } B}$ receptors and NMDA receptors were blocked with bicuculline (1 μM), SCH50911 (2 μM), and APV (50 μM), respectively. In addition, the intracellular sodium channel blocker QX314 (1 μM) was added to the internal recording solution in order to prevent the generation of action potentials. LY404187 significantly enhanced the EPSP amplitude in a dose-dependent manner from a control value of 9.6 ± 3.6 mV to 11.5 ± 10.5, 15.2 ± 6.3, 18.1 ± 7.4, and 23.2 ± 6.0 mV in the presence of 0.1, 0.3, 1.0 and 3.0 μM the potentiator, respectively ($F_{4,33} = 18.2$, $p < 0.0001$; Fig. 9A and B). This potentiated EPSP could then be blocked by the addition of 50 μM LY300168 (Fig. 9A, inset).

The PFC is a complex network of recurrent excitatory connections making it difficult to demonstrate an effect of LY404187 on the monosynaptic excitatory response. As such, the enhanced responsiveness described above could have been due in part to a recruitment of more excitatory inputs to the recorded neuron in the absence of any potentiation of AMPA receptor activity. To address this possibility, the effect of LY404187 on the amplitude of spontaneous monosynaptic EPSPs was recorded in the presence of elevated (6 μM) extracellular potassium for 3 min before and during application of LY404187 (0.3 μM; $n = 4$) (Fig. 9C). EPSP amplitudes were measured, and a frequency histogram of EPSP amplitudes above 0.4 mV (bin width = 0.2 mV) was constructed for each condition (Fig. 9D). In both cases, the majority of EPSPs had an amplitude of <1 mV, leading to a skewed distribution. However, 0.3 μM LY404187 significantly shifted the distribution of amplitudes toward larger values ($Z = 3.99$, $p < 0.001$; Kolmogorov–Smirnov nonparametric test). Consistent with the action of LY404187 on post-synaptic AMPA receptors, no change in the frequency of spontaneous EPSPs was observed. In addition, neither the resting potential nor the input resistance of PFC pyramidal neurons was altered by LY404187. Therefore, these results support the hypothesis that the enhancement of EPSP in the presence of LY404187 is due to a potentiation of the postsynaptic AMPA receptors on PFC pyramidal neurons.

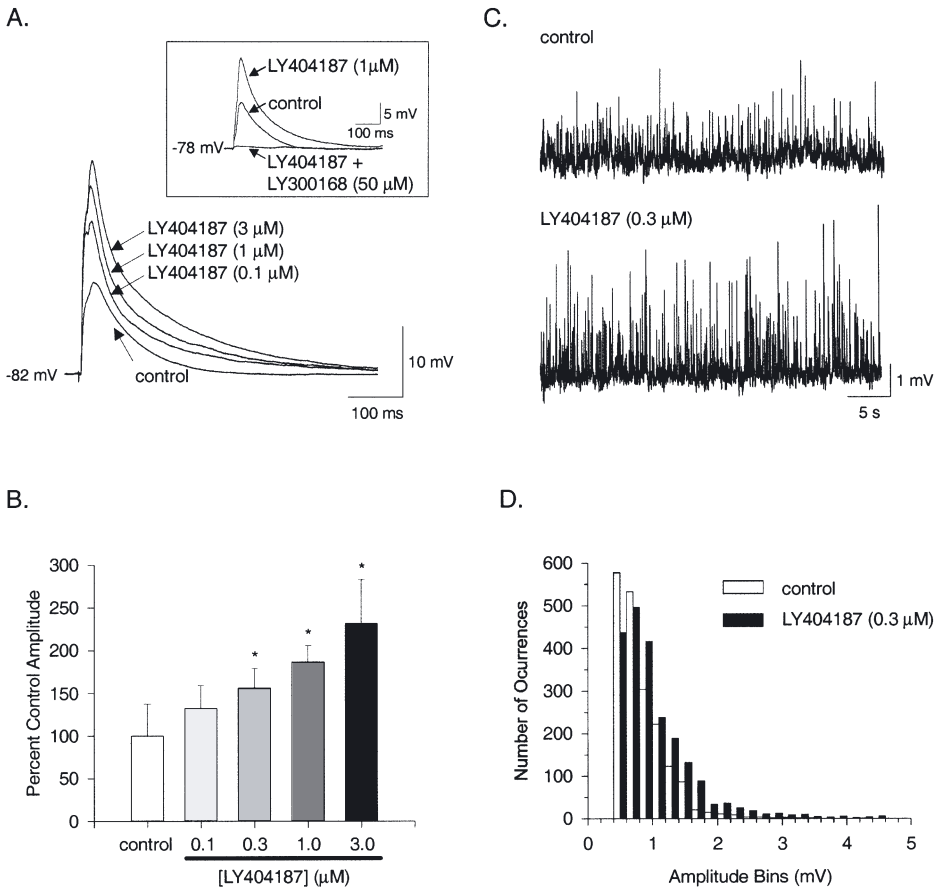

Fig. 9. LY404187 enhances glutamatergic synaptic and spontaneous AMPA receptor-dependent EPSPs in PFC pyramidal neurons from a slice preparation. A, LY404187 (0.1–3.0 μM) increased EPSP amplitude and duration in a concentration-dependent manner. $GABA_A$ and $GABA_B$ receptors, NMDA receptors, and sodium channels were blocked in these experiments. Inset, The potentiated EPSP was completely blocked by LY300168 (50 μM). B, Plot of the average (±S.D.) AMPA receptor-mediated EPSP amplitude as percentage of control amplitude. C, Spontaneous EPSPs in the absence and presence of 0.3 μM LY404187. $GABA_A$ and $GABA_B$ receptors, and NMDA receptors were blocked and 6 μM potassium was added to the external solution. D, Frequency histogram plotting the total number of spontaneous EPSPs recorded as a function of EPSP amplitude for four cells in the presence and absence of 0.3 μM LY404187. 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.

Recruitment of NMDA Receptor-Mediated Synaptic Responses

Evidence indicates that membrane potential depolarization can remove the voltage-dependent Mg^{2+} block of NMDA receptors, permitting an increase in calcium influx through these channels (66,75). The enhanced intracellular calcium concentration is capable of triggering a variety of intracellular signaling cascades, some of which contribute to synaptic modification such as long-term potentiation (LTP). As described above, potentiation of AMPA receptor activity enhances synaptically evoked responses, suggesting that a consequence of this greater depolarization should be an increase in NMDA receptor-de-

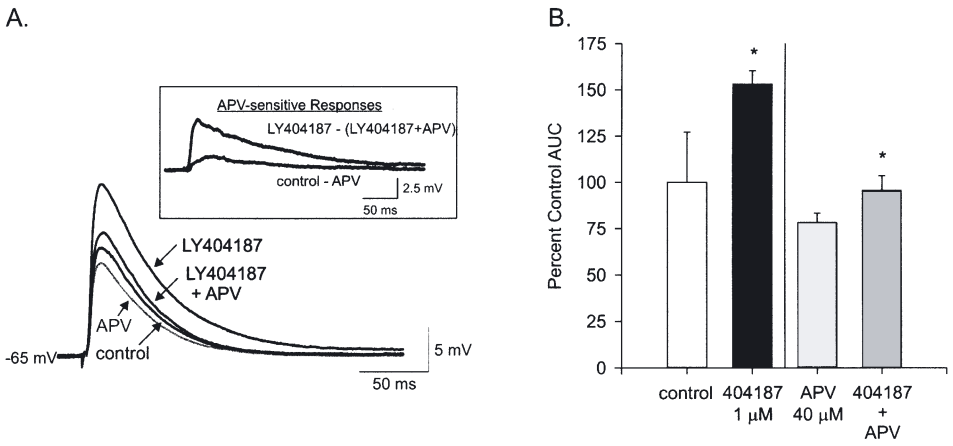

Fig. 10. LY404187 enhances NMDA receptor-dependent synaptic responses in PFC pyramidal neurons from a brain slice preparation. A, Single stimulation of glutamatergic afferents to PFC pyramidal neurons in the presence of elevated calcium (4 μM) and magnesium (4 μM) concentrations evoked a monosynaptic EPSP. Application of APV (40 μM) reduced the EPSP by approximately 20%. The effects of APV were eliminated within 15 min of washout. Application of LY404187 (1 μM) enhanced the EPSP by approximately 50%. Addition of APV with the application of LY404187 reduced this enhancement by approximately 50%. This residual response reflects the potentiated AMPA response, which is significantly larger than the AMPA response (APV alone). Inset, APV-sensitive responses in the presence and absence of LY404187. These responses were calculated by subtracting the APV-sensitive current from the control current. B, Plot of the average area under the curve (±S.D.) for EPSPs recorded during the conditions indicated.

pendent synaptic activity. To examine this possibility, the NMDA receptor antagonist, APV, was used to measure the NMDA receptor-mediated component of the EPSP in PFC pyramidal neurons in the presence and absence of 1.0 μM LY404187 (Fig. 10). For these experiments, $GABA_A$ receptors were blocked with bicuculline (1 μM), the recording electrode solution contained 1 μM QX314 to prevent action potentials in the recorded neuron and extracellular concentrations of Ca^{2+} and Mg^{2+} were raised to 4 μM to isolate the monosynaptic component of the EPSP. Recordings were performed while the somatic membrane was held at approximately –65 mV with constant current. In order to measure the complete NMDA receptor-mediated component of the EPSP, 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. A single stimulation during control conditions evoked an EPSP with an average AUC value of 1085.1 ± 295.2 mV/ms ($n = 5$). APV (40 μM) reduced the EPSP yielding an average AUC value of 853.0 ± 244.7 mV/ms (78.3 ± 5% of control). Following washout of APV (10 to 15 min), application of 1.0 μM LY404187 potentiated the EPSP (AUC = 1664.8 ± 494.1; 153.0 ± 7.2% of the control). Subsequent application of APV (40 μM) reduced the potentiated EPSP to control levels, having an average AUC of 1053.1 ± 346.1 mV/ms (95.7 ± 7.9% of control). Subtracting the APV-insensitive response from the total response in the presence and absence of LY404187 isolated the NMDA receptor-dependent component of the EPSPs. Results showed that LY404187 significantly enhanced both the AMPA and NMDA receptor-mediated components of the synaptic response in PFC neurons. These data support the hypothesis that potentiation of AMPA receptor activity can consequently remove the voltage-dependent block of NMDA receptors and enhance

NMDA receptor-dependent synaptic transmission. It is worth noting that the high extracellular Mg^{2+} concentration (4 μM) and the relatively hyperpolarized somatic holding potentials (–65 mV) used in these experiments would tend to decrease the NMDA receptor-mediated component of the synaptic response. Therefore, the potentiation of NMDA receptor-mediated responses by LY404187 may have been significantly underestimated.

Considerable evidence indicates that recruitment of NMDA receptor activity underlies LTP at many synapses in the CNS (15,73). One implication of the secondary enhancement of NMDA receptor function resulting from positive modulation of AMPA receptor activity is that the threshold for induction of LTP should be reduced and/or the degree of potentiation should be increased. These hypotheses have been explored previously by measuring the effects of the benzamide potentiator, CX516, on the induction of LTP in the CA1 region of the hippocampus *in vitro* and *in vivo*. As predicted, these studies showed that administration of CX516 reduced the stimulation threshold for induction of LTP and enhanced the magnitude of the potentiated response (4,93). Given that LTP has been implicated as a substrate for certain forms of memory, these data suggest that positive modulation of AMPA receptors may facilitate the formation of new memories by enhancing memory encoding (see below).

POTENTIATION OF AMPA AND NMDA RECEPTOR-DEPENDENT RESPONSES *IN VIVO*

Prefrontal Cortex

The results from *in vitro* slice experiments demonstrated that LY404187 markedly enhanced excitatory synaptic inputs to PFC pyramidal neurons at nanomolar concentrations. This suggests that this compound should have similar actions on glutamatergic inputs to these neurons *in vivo* provided that LY404187 crosses the blood-brain barrier. Therefore, *in vivo* electrophysiological studies were initiated to test the ability of LY404187 to cross the blood-brain barrier and potentiate synaptic activity. Extracellular recording of spontaneous and evoked action potential discharge of PFC neurons before and during intravenous (i.v.) administration of the compound was used to test this possibility. Previous neuroanatomical and electrophysiological studies have demonstrated that afferents from the ventral subiculum of the hippocampal formation project glutamatergic, monosynaptic, excitatory inputs onto PFC pyramidal neurons in the prelimbic and medial orbital areas in the prefrontal cortex (44). Electrical stimulation of the ventral subiculum produces an excitatory response in PFC neurons that can be blocked by the AMPA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) but is less sensitive to APV. These results led to the conclusion that this pathway is primarily mediated by AMPA receptor activation (45) and thus was chosen to assess the effects of LY404187 *in vivo*.

For these experiments, single stimulation pulses were delivered at 1 Hz to the ventral subiculum using a stimulation intensity adjusted so that a discharge was evoked approximately 40% of the time during control conditions. The number of action potential discharges per millisecond for 50 ms prior to the stimulus and 80 ms after the stimulus were recorded for 300 trials. LY404187 (0.1–100 mg/kg, i.v.) significantly enhanced the probability of evoked discharge of PFC neurons (Fig. 11A and B). The percent change in the maximal probability of evoked discharge increased in a dose-dependent manner

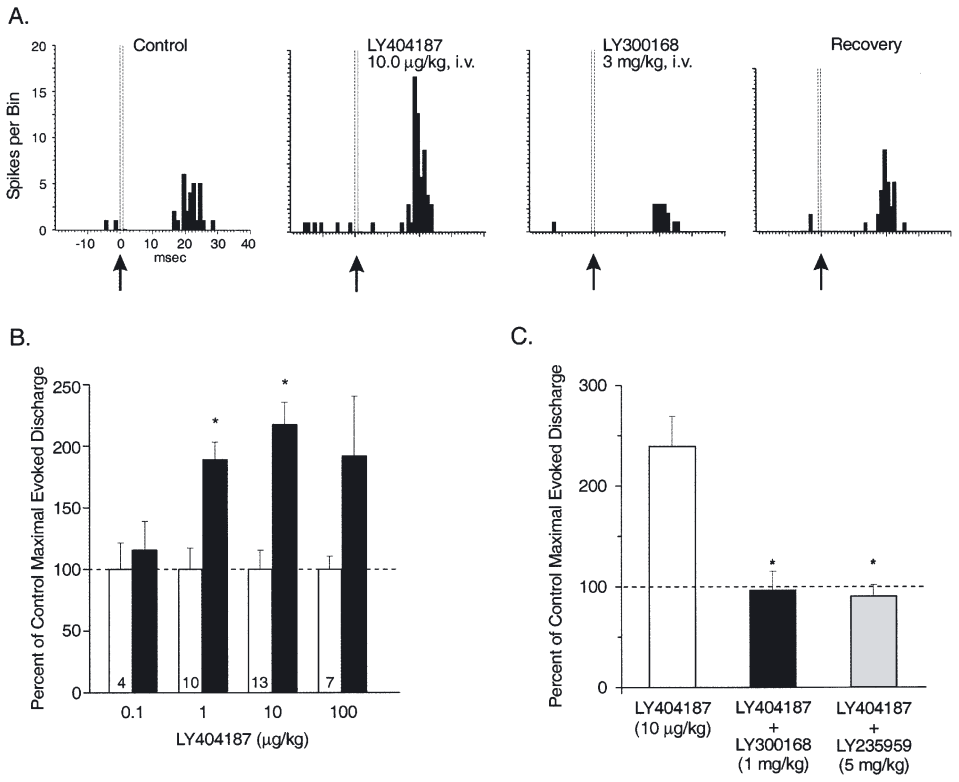

Fig. 11. LY404187 enhances evoke responses of PFC neurons *in vivo*. A, Histograms showing total number of spike discharges of a single PFC neuron in response to stimulation of ventral subiculum (300 trials delivered at 1 Hz). Bin size is 1 ms. Arrow denotes stimulus presentation ($t = 0$). Administration of 10 μg/kg LY404187 i.v., increased the probability of spike discharge. Subsequent administration of LY300168 (3 mg/kg, i.v.) blocked this inhibition. Washout of both compounds allowed the probability of spike discharge to return to predrug control levels. B, Plots of the average (±S.E.M.) 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, i.v.). The maximal increase was approximately 200% of control. C, Plot of the average (±S.E.M.) 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, i.v.) administered alone or followed by either LY300168 (1 mg/kg, i.v.) or LY235959 (5 mg/kg, i.v.).

16.0 ± 2.3% (0.1 μg/kg), 44.5 ± 3.8% (1.0 μg/kg), and 113.0 ± 69.0% (10.0 μg/kg) above the average control probability of response ($F_{3,29} = 2.7$, $p < 0.05$). However, at the highest dose (100.0 μg/kg) of LY404187 tested, the variability of response was high and the percent change (92 ± 48.7%) in the maximal probability of evoked discharge was not significantly different from control values. While not significant, a trend toward an increase of spontaneous activity was also observed.

Although responses evoked by stimulation of the ventral subicular inputs to the PFC have been shown to be mediated primarily by CNQX-sensitive receptors, an APV-sensitive component was also described (45). Furthermore, the slice experiments described above demonstrated that LY404187 could potentiate both AMPA and NMDA receptor-dependent synaptic responses. Therefore, the AMPA and NMDA receptor-mediated contri-

butions to the enhanced probability of discharge were evaluated using selective, centrally active antagonists. As described above, intravenous administration of 10.0 μg/kg LY404187 potentiated spike discharge of PFC neurons. Subsequent administration of LY300168 (1.0 mg/kg, i.v.) blocked the potentiation and reduced the probability of evoked firing to control levels (Fig. 11A and C). These results indicate that LY404187 potentiates evoked discharge by enhancing AMPA receptor activity. The extent to which the greater depolarization produced by potentiation of AMPA receptor function resulted in a recruitment of NMDA receptor activity was also investigated. In a separate set of neurons, the NMDA receptor antagonist, LY235959 (5.0 mg/kg, i.v.), was administered after the enhancement of the evoked response produced by of LY404187 (10.0 μg/kg, i.v.). Similar to the effects of LY300168, LY235959 reduced the potentiated response to control levels (Fig. 11C). Taken together, these data indicate that, although NMDA receptor activity makes little contribution to the synaptic responses of PFC pyramidal neurons following stimulation of subicular afferents during control conditions, recruitment of NMDA receptors may account for a significant portion of the potentiated response produced by LY404187.

Hippocampus

Additional *in vivo* electrophysiological recording experiments have examined the effect of LY404187 on hippocampal CA1 neurons (99). In these studies, the effect of LY404187 on responses of CA1 neurons to iontophoretically applied AMPA was examined. The current and duration of AMPA ejection was adjusted so that similar submaximal increases in firing rates were achieved for all cells. LY404187 (1.0–100.0 μg/kg, i.v.) produced a dose-dependent increase in the firing rate of CA1 neurons in response to AMPA application. The spontaneous firing rate also increased in the presence of LY404187. The firing rate in response to iontophoretically applied NMDA, was also be increased by LY404187, but to a much lower degree. Presumably, potentiation of the response to NMDA is due to the enhanced depolarization produced by potentiation of AMPA receptor activity. This hypothesis was tested using LY392098, another AMPA receptor potentiator. Results showed that LY300168 (1 mg/kg, i.v.) abolished the increase in the firing rate in response to iontophoretically applied NMDA produced by LY392098 (100.0 μg/kg i.v.) (99). In conjunction with the data from PFC recordings, these results indicate that LY404187 can permeate the blood-brain barrier and potentiate the firing rate of central neurons through a direct enhancement of AMPA receptor activity, as well as through the recruitment of NMDA receptors.

THERAPEUTIC POTENTIAL OF POSITIVE MODULATION OF AMPA RECEPTORS

Cognitive Deficits

Schizophrenia

Schizophrenia is one of the most debilitating psychiatric disorders. It is characterized by a variety of symptoms that can be clustered into three categories: positive symptoms

such as hallucinations and delusions, negative symptoms typified by emotional and social withdrawal and disorganized thought, including inattention and deficits in several mnemonic processes, particularly working memory (56,102). Although the pathophysiology of schizophrenia has not been elucidated, several lines of evidence have suggested that dysfunction of glutamatergic neurotransmission within specific circuits in the brain may contribute to the symptoms of this disease. Much of the support for this "glutamate hypothesis" has rested upon the observation that administration of phencyclidine (PCP), a noncompetitive NMDA receptor antagonist (3), can produce a psychotomimetic state in healthy individuals that includes positive and negative symptoms and cognitive deficits (61). In addition, ketamine, a congener of PCP, has been shown to have similar effects in normal subjects and to exacerbate psychosis in schizophrenic patients (50,51). Further support for a role for glutamatergic dysfunction in schizophrenia comes from postmortem studies of schizophrenic brains that have demonstrated a reduction in mRNA and protein for AMPA GluR1 and GluR2 receptor subunits in the medial temporal lobe (23). Other studies have reported a decrease in the expression of mRNA flop and, to a lesser extent, flip splice variants in the hippocampal formation (24). These reductions in mRNA levels have been recently replicated using quantitative immunocytochemical analyses (25). Similar decreases in the mRNA of the obligate NMDA receptor subunit, NR1, have been reported in postmortem tissue from schizophrenic patients not treated with neuroleptic (behavior modifying) drugs (91). However, in addition to the inherent confounds associated with postmortem analyses (e.g., variability in postmortem conditions, unknown perimortem factors, etc.), it is important to note that other studies have found conflicting results (for review see ref. 67). Despite these discrepancies, the possibility that a decrease in glutamatergic neurotransmission contributes to the symptoms of schizophrenia remains a viable working hypothesis (1,16,19,95).

A cardinal feature of the deficit in higher cognitive functions in schizophrenia is an impairment in the mnemonic process of working memory, a form of memory which permits information to be "held in mind" for short periods of time (8,28). 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. Evidence from electrophysiological studies in primates has indicated that changes in the activity of neurons in the PFC are required for execution of working memory tasks (33). In addition, clinical studies 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 (28). Conversely, decreased functioning of the PFC in schizophrenic patients has consistently been demonstrated concomitant with poor performance on working memory tasks (102). Although the source of PFC dysfunction is unknown, evidence suggests that deficits in glutamatergic excitatory transmission may be involved. For example, working memory performance in rodents is impaired by reducing glutamate release or its postsynaptic action on AMPA receptors of PFC neurons (84). Other experiments have shown that administration of NMDA glutamate receptor antagonists or agonists can respectively reduce or enhance retention times in working memory tasks in monkeys (22). Analogous imaging experiments in humans have shown that administration of PCP or ketamine will disrupt working memory in conjunction with a selective decrease in PFC activity (43,49).

In light of the evidence linking glutamatergic hypofunction and cognitive deficits, enhancement of glutamatergic transmission in PFC may be a therapeutic strategy to improve

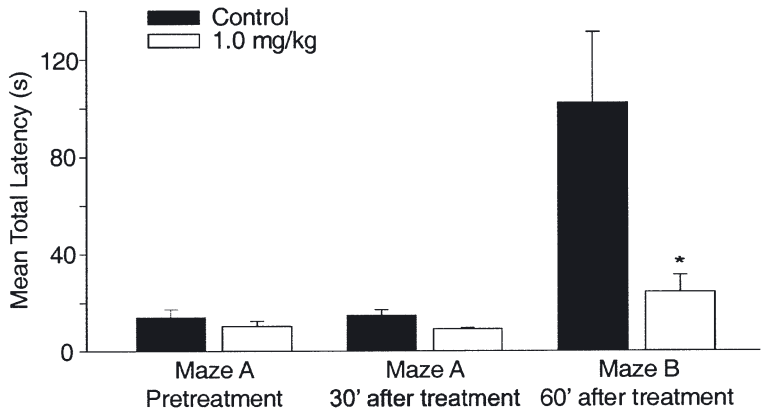

Fig. 12. LY404187 enhances performance of young rats in water maze. Fisher 344 rats were trained in a simple water maze (maze A). On the test day, animals were tested in maze A prior to and 30 min after treatment with LY404187 (1.0 μg/kg, p.o.) or saline. A novel, more complex maze (maze B) was used 60 min after LY404187 was administered to the rats. Plot of the average (±S.E.M.) time to complete each maze for control rats and rats administered LY404187.

cognitive function in schizophrenia. Toward this end, positive modulation of AMPA receptors may be a fruitful approach. Indeed, the data presented above has demonstrated that LY404187 is a potent and efficacious potentiator of AMPA receptor-dependent synaptic transmission in PFC *in vitro* and *in vivo*. The enhanced postsynaptic depolarization produced in the presence of this compound is sufficient to augment the voltage-dependent activity of NMDA receptor-mediated transmission. This would provide a possible mechanism for synaptic plasticity (e.g., LTP) and a cellular substrate through which positive modulation of AMPA receptors could promote memory encoding. Consistent with this hypothesis, LY404187 has recently been shown to enhance cognitive function in a water maze task that requires working memory (J. Tizzano, personal communication). In this assay, rats learn to navigate through alleyways in the maze to an escape platform by the use of spatial cues located throughout the room. Alleyways were created by removable doorways thereby obtaining different maze configurations. Rats initially were trained to a criterion (complete in ≤40 seconds or with ≤3 errors) in maze A, which consists of 4 error zones. When all rats had met the criterion, usually between 2 and 3 days, animals were randomly assigned to a vehicle or drug group (LY404187 1.0 mg/kg, p.o.). Twenty-four hours following the last training session and thirty min after dose administration rats were presented with maze A in order to evaluate disruptions associated with the compound on an already established learned behavior. Thirty min later, rats were tested in maze B, which consisted of 7 error zones, but contained the identical escape path as maze A. The latency to reach the escape platform was measured. LY404187 did not disrupt performance in maze A. However, the compound enhanced performance in maze B by reducing the amount of time required to navigate through the maze to the escape platform (Fig. 12).

LY404187 has also been shown to be efficacious in an amnesic model of passive-avoidance behavior (J. Tizzano, personal communication). Rats were treated with vehicle or LY404187 (0.0001–1.0 mg/kg, p.o.) in combination with either another vehicle solution or the selective AMPA receptor antagonist, GYKI52466 (1 mg/kg, i.p.). They were

then tested in a 1-trial passive avoidance procedure with a 24 to 48-h retention test. On the test day, animals treated with vehicle followed by GYKI52466 displayed significantly shorter latencies to crossing into the chamber that previously was associated with a shock. This indicates impairment in their ability to remember the aversive experience. In contrast, when administered in combination with GYKI-52466, LY404187 reversed the memory deficit such that the latency to cross was equivalent to control levels. The minimal effective dose (MED) for this effect was 0.001 mg/kg.

Collectively, these behavioral results demonstrate that LY404187 is active in models of cognitive function that require several mnemonic processes, including working memory. In conjunction with the electrophysiological data, these results are consistent with the hypothesis that AMPA receptor potentiators may be beneficial in the treatment of cognitive deficits associated with PFC dysfunction such as schizophrenia. Although this hypothesis has not been tested directly, it is important to note that in a recent clinical study, administration of the benzamide potentiator, CX516, in combination with the atypical antipsychotic, clozapine, improved measures of attention and memory in schizophrenic subjects (32). These studies support the hypothesis that AMPA receptor potentiators may be therapeutically beneficial in treating the cognitive symptoms of schizophrenia.

Age-associated memory deficits

Age-related memory deficits can range from mild cognitive impairment to the marked dementia associated with Alzheimer's disease (AD). Although multiple biochemical changes most certainly contribute to this wide range of cognitive dysfunction, recent evidence has suggested that age-related loss of AMPA receptors may be an important factor. Early studies in rats demonstrated that AMPA receptor levels in brain decline with age (63). More recent studies have described a selective reduction of AMPA GluR2/3 subunits in the magnocellular cholinergic neurons of the nucleus basalis of Meynert. This area has long been recognized as a site of degeneration in AD (5,40). A similar loss of GluR2/3 subunits in the entorhinal cortex and CA1/subiculum regions of the hippocampal formation has been reported in postmortem studies of AD brains (39).

Although speculative, AMPA receptor potentiators may provide a novel therapeutic approach to treating cognitive deficits associated with aging and/or AD by increasing glutamatergic signaling through the remaining AMPA receptors. Consistent with this hypothesis, AMPA receptor potentiators have been shown to enhance performance of aged (10 months to 24 months) rats in behavioral tasks requiring different types of memory to levels of young (2 to 3 months) rats (34,86,87). More importantly, administration of CX516 to aged human subjects (65–76 years) has been shown to enhance recollection of nonsense syllables after a 5-min delay (62). In conjunction with the data reviewed above indicating that AMPA potentiators can facilitate forms of synaptic plasticity that may subserve memory encoding, these data support the hypothesis that AMPA receptor potentiators may be beneficial in treating the cognitive decline associated with aging.

Attention Deficit Hyperactivity Disorder (ADHD)

ADHD is one of the most commonly diagnosed childhood psychiatric disorders. Controversy surrounds the prevalence of this disorder with estimates ranging from 3–5% of children to some studies suggesting the prevalence may be as high as 20% (14). Symptoms of ADHD include impulsive behavior, danger-seeking, high levels of inattentiveness,

and locomotor hyperactivity. In about half of the children with the diagnosis of ADHD, these symptoms persist after the age of 20. The primary deficit of ADHD is believed to be failure to control motor activity (98). This dyscontrol of the motor cortex may be caused by deficits in the activity of the PFC (9,11). Consistent with this hypothesis, Rett Syndrome (RS), which is characterized by a frontal lobe that is smaller in size, displays behavior symptoms similar to ADHD (74). Although there are no gross anatomical deficiencies in the frontal lobe in patients diagnosed with ADHD, functional brain imaging studies indicate that activity is reduced in this cortical region. Frontal lobe glutamatergic neurons projecting to the striatum have been shown to be *hypoactive* by SPECT (2,60,88), PET (106) and electrical imaging (89). Thus, although the symptoms of ADHD result from hyperactivity of the motor cortex, the underlying cause of ADHD may be a hypoactivity of the PFC. These observations have led to the proposal that AMPA receptor potentiators may increase the glutamatergic synaptic transmission in the PFC and thus indirectly suppress activity in the motor cortex of ADHD patients (17).

Indirect support of this hypothesis was recently reported using knockout mice lacking the dopamine transporter. These mice display locomotor hyperactivity that is inhibited by psychostimulants and serotonergic drugs, and thus represent an animal model of ADHD. In these mice, AMPA receptor potentiators from two different classes of compounds, pyrrolidinones (aniracetam) and benzamides (CX516, CX546) significantly reduced the locomotor activity in a dose-dependent manner (29). These AMPA receptor potentiators had no effect on the high basal levels of dopamine in these knockout animals. This suggests that the potentiators did not reduce the hyperactivity merely by reducing the high accumulation of dopamine in these animals. Rather, these results illustrate the interactions between the dopaminergic and glutamatergic systems. The proper balance between these two systems may be a crucial key for normal cognitive function.

Depression

The biochemistry underlying depression is complex and poorly understood. Nonetheless, antidepressants have been available for nearly four decades, and improved antidepressants are constantly being introduced. One problem associated with current antidepressant therapies is the considerable lag time (greater than two weeks) between beginning of treatment and alleviation of the symptoms for most patients. In addition, a subset of patients fails to respond to the medication entirely. This observation has led to the belief that these antidepressants do not directly treat the pathophysiology of depression, but rather modulate systems that, with time, alter gene expression. These changes in gene expression then are suspected to be the final targets of these antidepressants. One such target that has received much attention is the neurotrophin, brain derived neurotrophic factor (BDNF) (97). BDNF is a member of the nerve growth factor (NGF) family of growth factors and supports the development, growth, and maintenance of neurons. In rats, mRNAs for both BDNF and the receptor that it binds to, trkB, have been shown to be upregulated with the chronic treatment of antidepressants (72). Furthermore, BDNF mRNA is downregulated in rats tested in animal models of behavioral despair, including restraint stress and the forced swim test and this reduced expression can be reversed by antidepressant treatment (72,85). In addition, central administration of BDNF produces antidepressant-like activity in learned helplessness and forced swim tests (90). These converging lines of evidence suggest that regulation of BDNF expression may be an important target in the treatment of depression.

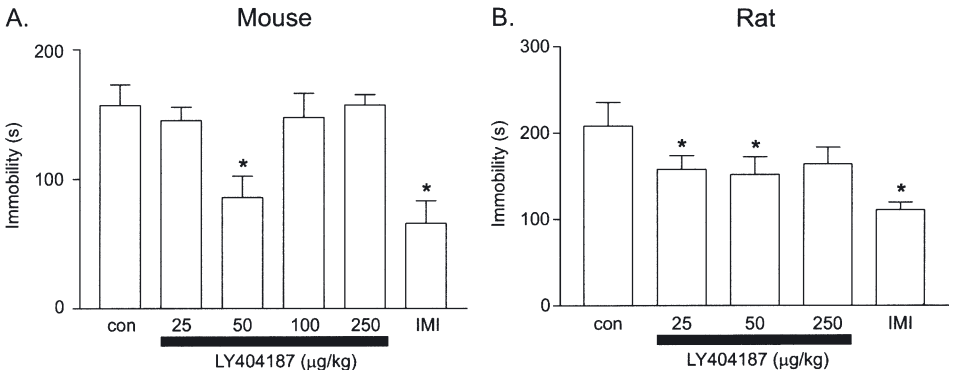

Fig. 13. LY404187 decreases immobility in the forced swim test in both rats and mice. A, Plot of the results for mice. One way ANOVA, followed by Bonferroni post hoc test. Asterisk denotes $p < 0.01$. The values are mean ± S.E.M. for 8 mice in the control group and 5 or 6 mice in each test group. B, Plot of the results for rats. IMI, imipramine. The values are mean ± S.E.M. for 11 rats in the control group and 6 rats in each test group. One way ANOVA followed by Bonferroni post hoc test, asterisk denotes $p < 0.5$.

Multiple intracellular signaling pathways regulate BDNF levels. Antidepressants that increase norepinephrine release may enhance BDNF through the CREB pathway. Recruitment of the MAP kinase pathway can also increase BDNF mRNA levels. Lyn, a member of the Src-family of protein tyrosine kinases, can activate the MAP kinase pathway. Lyn has been shown to be physically associated with AMPA receptor GluR2 and/or GluR3 subunits and can be activated by robust stimulation of AMPA receptors. In addition, studies in primary cerebellar cultures have demonstrated that while cyclothiazide alone had no effect on Lyn activation, when co-applied with AMPA, Lyn activation was enhanced (36). Therefore, by potentiating AMPA receptors, BDNF levels are proposed to increase through the activation of Lyn (36). This hypothesis has been confirmed by studies in neuronal cultures demonstrating that LY392098, added in the presence of AMPA or glutamate, produced an increase of BDNF mRNA and protein levels (54). Collectively, these data suggest that positive modulation of AMPA receptors may be beneficial in the treatment of depression by increasing the levels of BDNF through the MAP kinase pathway.

An initial exploration of the utility of AMPA receptor potentiators in depression has been pursued. The activity of LY404187 was tested in the forced swim test model of behavioral despair that can be used to detect antidepressant compounds (P. Skolnick, personal communication). Results showed that LY404187 reduced immobility in the test in both mice and rats with a MED of 0.05 and 0.025 mg/kg (p.o.), respectively (Fig. 13). Imipramine (15 mg/kg, i.p.) served as a positive control in this test. LY404187 did not affect motor activity, indicating that the efficacy of this compound in the forced swim test is not related to a motor stimulant action. Similar results have been reported for LY392098 (55). In addition, the activity of LY392098, but not imipramine, was blocked by systemic administration of LY300168, indicating that the effect of the potentiator was mediated through AMPA receptors (55). Collectively, these findings demonstrate that positive modulators of AMPA receptors are active in animal models capable of detecting clinically effective antidepressants. This supports the hypothesis that this class of compounds may represent a novel class of antidepressants.

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