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Biphenyl-indanone A, a Positive Allosteric Modulator of the Metabotropic Glutamate Receptor Subtype 2, Has Antipsychotic- and Anxiolytic-Like Effects in Mice

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ABSTRACT

Previous studies indicate that agonists of the group II metabotropic glutamate receptors (mGluRs), mGluR2 and mGluR3, may provide a novel approach for the treatment of anxiety disorders and schizophrenia. However, the relative contributions of the mGluR2 and mGluR3 subtypes to the effects of the group II mGluR agonists remain unclear. In the present study, we describe an alternate synthesis and further pharmacological characterization of a recently reported positive allosteric modulator of mGluR2 termed biphenyl-indanone A (BINA). In recombinant systems, BINA produced a robust and selective potentiation of the response of mGluR2 to glutamate with no effect on the glutamate response of other mGluR subtypes. In hippocampal brain slices, BINA (1 μM) significantly potentiated the mGluR2/3 agonist-induced inhibition of excitatory synaptic transmission at the medial perforant path-dentate gyrus synapse. BINA was also efficacious in several models predictive of antipsychotic- and anxiolytic-like activity in mice. The behavioral effects of BINA were blocked by the mGluR2/3 antagonist (2*S*)-2-amino-2-[(1*S*,2*S*)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid (LY341495), suggesting that the in vivo effects of BINA are mediated by increased activation of mGluR2. Collectively, these results indicate that BINA is a selective mGluR2 positive allosteric modulator and provide further support for the growing evidence that selective allosteric potentiators of mGluR2 mimic many of the in vivo actions of mGluR2/3 agonists that may predict therapeutic utility of these compounds.

Metabotropic glutamate receptors (mGluRs) are classified into three major groups based on sequence homologies, coupling to second messenger systems, and selectivities for various agonists (Conn and Pin, 1997). Group II mGluR subtypes, mGluR2 and mGluR3, couple to $G_{i/o}$ and associated effector pathways, such as the inhibition of adenylyl cyclase and the regulation of ion channels. Group II mGluRs reduce transmission at glutamatergic synapses in multiple brain regions, where excessive glutamatergic neurotransmission has been implicated in the underlying pathophysiology of anxiety disorders and schizophrenia (Walker and Davis, 2002; Moghaddam and Jackson, 2003). Based on these findings, it has been postulated that selective agonists of group II mGluRs may provide anxiolytic and/or antipsychotic effects through a reduction in glutamatergic neurotransmission within these brain regions.

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ABBREVIATIONS: mGluR, metabotropic glutamate receptor; LY379268, (−)-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylic acid; LY354740, (1*S*,2*S*,5*R*,6*S*)-2-oxabicyclo[3.1.0]hexane-2,6-dicarboxylate monohydrate; LY487379, *N*-(4-(2-methoxyphenoxy)phenyl)-*N*-(2,2,2-trifluoroethylsulfonyl)pyrid-3-ylmethylamine; BINA, biphenyl-indanone A, 3′-[[(2-cyclopentyl-6,7-dimethyl-1-oxo-2,3-dihydro-1*H*-inden-5-yl)oxy]methyl]biphenyl-4-carboxylic acid; **2**, 2′-cyclopentyl-2,3-dimethyl-4-methoxyacetophenone, cyclopentylacetophenone; **3**, 1-(4-methoxy-2,3-dimethylphenyl)-2-cyclopentyl-2-propenone; **4**, 2-cyclopentyl-5-methoxy-6,7-dimethylindan-1-one, methoxyinandone; **5**, 2-cyclopentyl-5-hydroxy-6,7-dimethylindan-1-one, indanol; **8**, ethyl 3′-methylbiphenyl-4-carboxylate, biphenyl; THF, tetrahydrofuran; **9**, ethyl 3′-bromomethylbiphenyl-4-carboxylate; **10**, ethyl 3′-(((2-cyclopentyl-6,7-dimethyl-1-oxo-2,3-dihydro-1*H*-inden-5-yl)oxy)methyl)biphenyl-4-carboxylate; r, rat; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; HEK, human embryonic kidney; CHO, Chinese hamster ovary; h, human; GTPγS, guanosine 5′-[γ-thio]triphosphate; L-AP4, L-(+)-2-amino-4-phosphonobutyric acid; DMSO, dimethyl sulfoxide; ACSF, artificial cerebrospinal fluid; MPP, medial perforant path; fEPSP, field excitatory postsynaptic potentials; DCG-IV, (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine; PCP, phencyclidine; PPI, prepulse inhibition; LY341495, (2*S*)-2-amino-2-[(1*S*,2*S*)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid; ANOVA, analysis of variance; DG, dentate gyrus.

There have been considerable advances in the development of selective agonists of group II mGluRs. Systemic administration of the mGluR2/3 agonists LY379268, LY354740, and their analogs have behavioral effects in several rodent models predictive of anxiolytic and antipsychotic activity (for reviews, see Schoepp and Marek, 2002; Schoepp et al., 2003; Marek, 2004). Moreover, the selective group II mGluR agonist LY354740 has been shown to have anxiolytic activity in several clinical studies (Grillon et al., 2003; Schoepp et al., 2003; Swanson et al., 2005). However, only one structural class of selective group II mGluR agonists has been developed, and these compounds activate both mGluR2 and mGluR3. To date, the relative contributions of mGluR2 and mGluR3 to the actions of the group II mGluR agonists remain unknown. Moreover, the development of tolerance to the effects of the direct-acting group II mGluR agonists has been observed in several rodent behavioral models (Cartmell et al., 2000; Galici et al., 2005; Jones et al., 2005). Thus, it is important to develop novel approaches for the subtype-selective activation of the group II mGluRs.

One alternative approach to direct-acting group II mGluR agonists is the use of subtype-selective positive allosteric modulators of mGluR2 or mGluR3. Johnson et al. (2003) reported the initial discovery of LY487379 as a selective positive allosteric modulator of mGluR2. The pharmacological characterizations of several compounds within the LY487379 structural class have now been reported (Johnson et al., 2003, 2005; Schaffhauser et al., 2003). These compounds are highly selective for mGluR2 relative to other mGluR subtypes. They do not activate mGluR2 directly but act at an allosteric site on the receptor, which induces a leftward shift of the glutamate concentration-response curve (Johnson et al., 2003, 2005; Schaffhauser et al., 2003). Interestingly, these mGluR2 potentiators mimic some of the behavioral effects of mGluR2/3 agonists in animal models used to predict anxiolytic and antipsychotic activity (Johnson et al., 2003, 2005; Galici et al., 2005). However, members of this first series of mGluR2 allosteric potentiators have modest potencies in several behavioral models (Johnson et al., 2003; Galici et al., 2005) and a short duration of action (Galici et al., 2005), which prevents a more systematic characterization of their pharmacologic effects in vivo.

More recently, a novel series of selective allosteric potentiators of mGluR2 have been discovered (Bonnefous et al., 2005; Pinkerton et al., 2005). These compounds are chemically and structurally unrelated to LY487379 or to the group II agonist LY354740. Preliminary studies suggest that some of these newer compounds are centrally penetrant and block ketamine-induced hyperlocomotor activity in rats, an animal model predictive of antipsychotic activity (Rodriguez et al., 2004; Bonnefous et al., 2005; Govek et al., 2005). However, the pharmacological properties of these compounds have not been rigorously evaluated, and no individual compounds have emerged as optimal tools for in vivo and in vitro studies. Interestingly, Rodriguez et al. (2004) presented a preliminary report suggesting that a member of this series, 3′-[[(2-cyclopentyl-6,7-dimethyl-1-oxo-2,3-dihydro-1*H*-inden-5-yl)oxy]methyl]biphenyl-4-carboxylic acid, termed biphenylindanone A (BINA) may provide a useful tool for in vivo studies.

We now report that BINA is a highly selective allosteric potentiator of mGluR2 with robust, long-lasting effects in animal models that have been used to predict antipsychotic- and anxiolytic-like activity of group II mGluR agonists. These studies provide strong support for the growing evidence that mGluR2 is responsible for many of the known in vivo effects of mGluR2/3 agonists and that allosteric potentiators of mGluR2 may provide an exciting alternative to group II mGluR agonists for development of therapeutic agents.

Materials and Methods

Synthesis of BINA

The improved synthesis of BINA was completed via an alternate route (Fig. 1) from the method used by Bonnefous et al. (2005). Cyclopentylacetyl chloride (**1**, 11.3 g) and 2,3-dimethylanisole (8.6 g) in methylene chloride were treated with aluminum trichloride (10.5 g) for 16 h at 20°C to give 16.1 g (98%) of cyclopentylacetophenone (**2**). Treatment of **2** with paraformaldehyde (6.8 g) and dimethylamine hydrochloride (20 g) in acetic acid (1.5 ml) for 18 h at 95°C according to Woltersdorf et al. (1977) gave 6.0 g (84%) of intermediate **3**. Cyclization of **3** in 18 M sulfuric acid (35 ml) for 3 h at 20°C gave 4.9 g (82%) of methoxyindanone (**4**). Demethylation of **4** with 0.5 M boron tribromide in methylene chloride for 4 h at 20°C gave 2.3 g (51%) of the indanol (**5**) after crystallization from methanol. 3-Toluoylzinc bromide (**6**), prepared from 3-bromotoluene (3.2 g), 1.6 M butyl lithium (12 ml), and 0.5 M zinc chloride (38 ml) in anhydrous tetrahydrofuran (THF) and 4-ethoxycarbonylphenyl-bis(triphenylphosphine)palladium (**7**), prepared from ethyl 4-iodobenzoate (4.4 g) and bis(triphenylphosphine)palladium dichloride (0.7 g) in THF, were combined and stirred for 16 h at 20°C according to Klein et al. (1998), yielding 2.8 g (73%) of biphenyl (**8**) after gravity column chromatography on silica in hexane-ethyl acetate (10:1). Free radical-induced bromination of **8** with *N*-bromosuccinimide (2.1 g) and 2,2′-azobisisobutyronitrile (0.2 g) as a free radical initiator in carbon tetrachloride for 16 h at 80°C according to Gillig et al. (2004) yielded 3.2 g (86%) of biphenylmethyl bromide (**9**). Coupling of intermediates **5** (2.4 g) and **9** (3.2 g) with potassium carbonate in dimethylformamide for 14 h at 75°C gave 3.6 g (75%) of the ester **10** after flash column chromatography on silica in hexane-ethyl acetate (10:1). Hydrolysis of **10** with 2 N sodium hydroxide (10 ml) in THF-methanol (1:1, 100 ml) for 2 h at 60°C, followed by extraction with 1 N sodium hydroxide from diethyl ether and neutralization of the aqueous layer with 12 N hydrochloric acid yielded 2.0 g (63%) of BINA. Melting point: 238–241°C. ^{1}H nuclear magnetic resonance (300 MHz in $CDCl_3$): 8.21 (d, 2H, 3,5-CH), 7.73 (d, 2H, 2,6-CH), 7.71 (m, 1H, 2′-CH), 7.64 (dd, 1H, 6′-CH), 7.53 (m, 2H, 4′,5′-CH), 6.81 (s, 1H, 4″-CH), 5.22 (s, 2H, OCH_2), 3.07 (dd, 1H, 2‴-CH), 2.73 (m, 2H, 3‴-CH_2), 2.64 (s, 3H, 7′“-CH_3), 2.33 (m, 1H, 2⁗-CH), 2.23 (s, 3H, 6‴-CH_3), 1.94 (m, 1H, 5⁗-CH), 1.59 (m, 5H, 3⁗,4⁗-CH_2 and 1⁗-CH), 1.42 (m, 1H, 2⁗-CH), 1.09 (m, 1H, 5⁗-CH). Elemental analysis ($C_{30}H_{30}O_4$) C: calculated 79.27%, found 78.52%; H: calculated 6.65%, found 6.73%.

Cell Culture and Transfections

Baby hamster kidney cells stably expressing the rmGluR1a were generously provided by Dr. Betty Haldeman at Zymogenetics Inc. (Seattle, WA). Cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 5% heat-inactivated fetal bovine serum (FBS), 2 mM GlutaMax I, 100 units of penicillin, 100 μg of streptomycin, and 0.25 μg of amphotericin B (antibiotic-antimycotic), 1 mM sodium pyruvate, 20 mM HEPES, and 250 nM methotrexate. Human embryonic kidney (HEK) 293A cells stably expressing the rmGluR5a were grown in DMEM containing 10% heat-inactivated FBS, 2 mM GlutaMax I, antibiotic-antimycotic, 0.1 mM nonessential amino acids, 20 mM HEPES, and 500 μg/ml G418 sulfate. rmGluR2 and the promiscuous G proteins (G_{qi5}) were cotransfected into HEK293A cells using Lipofectamine 2000 and were grown in the same manner as rmGluR5a except that the G418 sulfate was omitted.

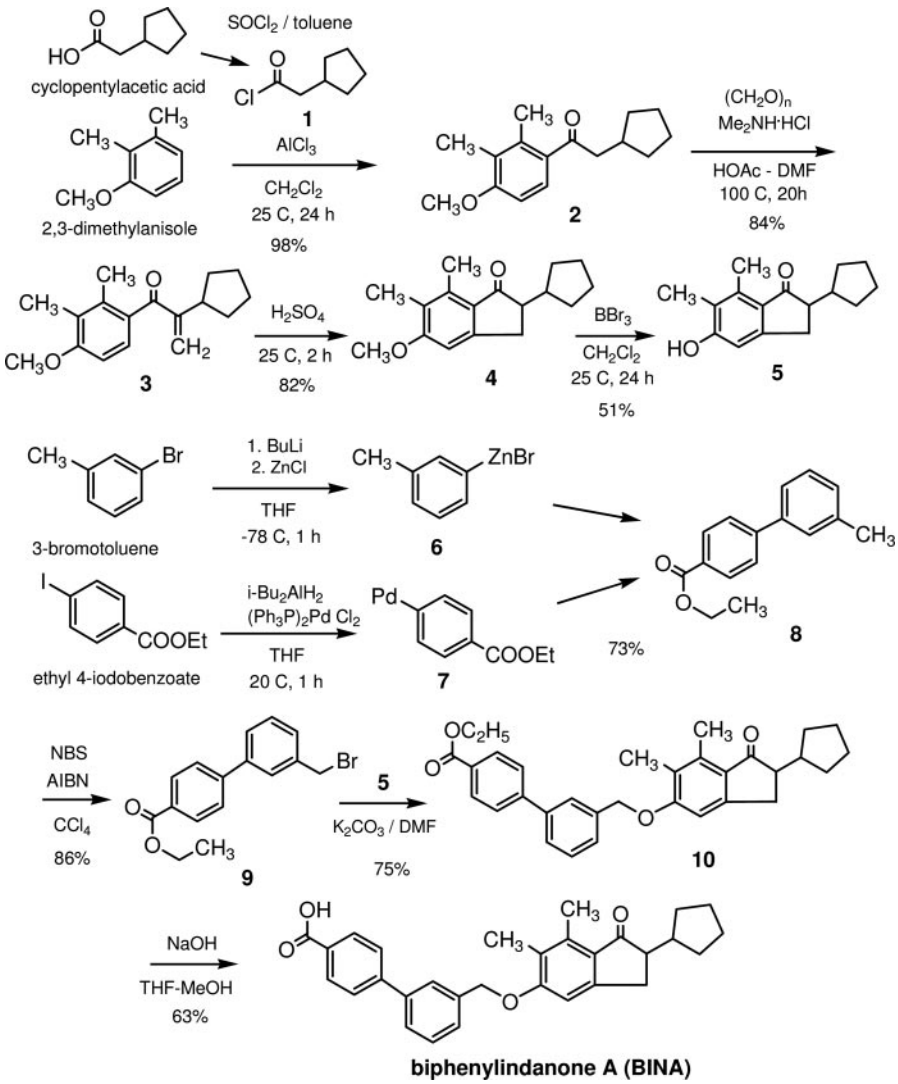

Fig. 1. Synthesis of BINA. BINA was prepared in nine steps from 3-bromotoluene, 2,3-dimethylanisole, ethyl 4-iodobenzoate, and cyclopentylacetic acid in 16% overall yield.

Chinese hamster ovary (CHO) cells stably expressing the hmGluR2 were transiently transfected with G_{qi5}, and CHO cells stably expressing the hmGluR4/G_{qi5} were grown in DMEM containing 10% heat-inactivated, dialyzed FBS, 2 mM GlutaMax I, antibiotic-antimycotic, 1 mM sodium pyruvate, 20 mM HEPES, 5 nM methotrexate, and 20 μg/ml L-proline. CHO cells stably expressing the rmGluR3 were grown in DMEM containing 10% heat-inactivated FBS, 2 mM GlutaMax I, 100 units of penicillin, 100 μg of streptomycin, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, and 20 mM HEPES.

All recombinant cell lines were plated at a seeding density of 7 to 8×10^5 cells/well, in clear-bottomed, poly-D-lysine-coated 96-well plates (Becton Dickinson Labware, Bedford, MA). Cells were then incubated in glutamate-glutamine-free medium overnight at 37°C in 5% CO_2, with the exception of baby hamster kidney cells stably expressing mGluR1a, which were maintained in regular medium.

[^{35}S]GTPγS Binding Assay

Membranes were prepared from CHO cells stably expressing either hmGluR2 or rmGluR3. In brief, cells were washed once with ice-cold 1× phosphate-buffered saline, pH 7.4. Cells were then harvested with a cell scraper and resuspended in ice-cold buffer (20 mM HEPES and 10 mM EDTA, pH 7.4) and were homogenized using a Polytron homogenizer for 2 s. The homogenate was centrifuged at 4°C for 20 min at 40,000*g*. The pellet was resuspended with the same buffer and was homogenized for 10 s. After centrifugation, the pellet was resuspended and homogenized for 5 s in ice-cold buffer containing 20 mM HEPES and 0.1 mM EDTA, pH 7.4. The final pellet was resuspended and homogenized using a glass homogenizer. Aliquots were stored at −80°C until use. Protein concentrations were measured using the Bio-Rad protein assay kit (Bio-Rad, Hercules, CA) using serum albumin (Pierce Chemical, Rockford, IL) as the standard.

After thawing, the membranes were diluted and homogenized using a glass homogenizer in ice-cold assay buffer containing 50 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$, 150 mM NaCl, 1 mM EDTA, 10 μg/ml saponin, and 1 μM GDP. Assay mixtures contained 10 μg of membrane protein, test compound, glutamate, 0.1 nM [^{35}S]GTPγS, and assay buffer to yield a total volume of 100 μl. Nonspecific binding was determined in the presence of 10 μM unlabeled GTPγS. The assay mixtures were incubated at 30°C for 60 min. The binding equilibrium was terminated by rapid filtration through Unifilter-96 GF/B filter plates (presoaked with ice-cold assay buffer), and the filters plates were washed three times with ice-cold assay buffer using a 96-well Brandel harvester (Brandel, Gaithersburg, MD). Filter plates were dried and filled with 30 μl of MicroScint-20, the radioactivity was determined by TopCount NXT Microplate Scintillation and Luminescence Counter (PerkinElmer Life and Analytical Sciences, Downers Grove, IL).

Calcium Mobilization Assay

Cells were loaded with calcium indicator dye (Calcium 3 Assay Kit) from Molecular Devices (Sunnyvale, CA) for 1 h at 37°C. Dye

was removed and replaced with the appropriate volume of assay buffer containing 1× Hanks' balanced salt solution (Invitrogen, Carlsbad, CA), 20 mM HEPES, and 2.5 mM probenecid, pH 7.4. Cells were then preincubated with either vehicle or various concentrations of BINA (5× stock) for 5 min followed by the 1-min stimulation of an EC_{20} of agonists (i.e., glutamate or L-AP4). BINA was dissolved in 100% DMSO and then serially diluted into assay buffer containing 0.1% bovine serum albumin for a 5× stock in 0.5% DMSO. The stock was then added to the assay for a final DMSO concentration of 0.1%. Glutamate and L-AP4 were prepared to a 10× stock solution in assay buffer before addition to the assay plate. Calcium mobilization was measured using the FLEXstation-II (Molecular Devices).

Hippocampal Slice Electrophysiology

Extracellular field recordings were performed using hippocampal brain slices prepared from male Sprague-Dawley rats, 3 to 4 weeks of age, as previously described with minor modifications (Macek et al., 1996, Marino et al., 1998). After decapitation, brains were rapidly removed and submerged in an ice-cold sucrose replacement solution containing: 200 mM sucrose, 2.5 mM KCl, 1.2 mM NaH_2PO_4, 1.3 mM $MgCl_2$, 8 mM $MgSO_4$, 20 mM glucose, and 46 mM $NaHCO_3$ and equilibrated with 95% O_2-5% CO_2. Coronal hippocampal slices (400 μm) were cut using a microtome (Leica Microsystems, Nussloch GmbH, Nußloch, Germany) and transferred to a holding chamber containing artificial cerebrospinal fluid (ACSF) with 124 mM NaCl, 2.5 mM KCl, 1.3 mM $MgSO_4$, 1.0 mM NaH_2PO_4, 2 mM $CaCl_2$, 20 mM glucose, and 26 mM $NaHCO_3$, equilibrated with 95% O_2-5% CO_2 and maintained at room temperature. For all experiments, both sucrose replacement buffer and holding chamber ACSF buffer were supplemented with 5 μM glutathione, 500 μM pyruvate, and 250 μM kynurenic acid to increase slice viability.

After 1 h of recovery in the holding chamber, hippocampal slices were transferred to the slice recording chamber and maintained fully submerged with continuous perfusion of ACSF (2–3 ml/min). By using a microscope with Hoffman modulation contrast, the middle third of the molecular layer of the dentate gyrus was identified for placement of the recording and stimulation electrodes. Recording electrodes were pulled from borosilicate glass on a Narashige vertical patch pipette puller and filled with 2.5 mM NaCl (0.3–1.0 MΩ resistance). A bipolar tungsten microelectrode (FHC Inc., Bowdoinham, ME) was used for stimulation of the medial perforant path (MPP) fibers; stimuli (100 μs in duration) were delivered at 0.05 Hz using a Grass S48 stimulator and a Grass isolator. The field excitatory postsynaptic potentials (fEPSPs) were recorded using an Axon MultiClamp 700B amplifier (Molecular Devices) in current clamp mode, data were digitized using DigiData 1322A, filtered (2 kHz), and acquired using the pClampex v.9.2 program. An input-output curve was performed for each experiment, and the stimulus intensity was adjusted to produce a fEPSP ~50 to 70% of the maximal amplitude. The fEPSP was analyzed using pClampfit to calculate the slope of the fEPSP. The first six consecutive slope values were averaged to define the baseline fEPSP slope value for a given experiment. The subsequent fEPSP slopes were normalized to the baseline fEPSP slope value and expressed as a percentage. Six averaged consecutive fEPSP slopes after 12 min of application of DCG-IV were averaged and defined as the value for applying DCG-IV. All normalized fEPSP slopes for each concentration of DCG-IV were then expressed as the mean ± S.E.M.

Behavioral Studies

Subjects. All behavioral studies were conducted using C57BL6/J male mice (Charles River Laboratories, Inc., Wilmington, MA) 8 to 10 weeks of age. Subjects were housed in groups of four to five per cage in a large colony room under a 12-h light/dark cycle (lights on at 6:00 a.m.) with food and water provided ad libitum. Test sessions were performed between 6:00 a.m. and 6:00 p.m. All dose groups consisted of 6 to 12 mice. All experiments were conducted in accordance with the National Institutes of Health regulations of animal care covered in *Principles of Laboratory Animal Care* (National Institutes of Health publication 85-23, revised 1985) and were approved by the Institutional Animal Care and Use Committee.

Apparatus. Amphetamine- and phencyclidine (PCP)-induced hyperlocomotor activity studies were conducted in open field chambers (27 × 27 × 20 cm) (Med Associates, Inc., St. Albans, VT) equipped with 16 horizontal (*x*- and *y*-axes) and 16 vertical (*z*-axis) infrared photobeams located 1 and 5.5 cm above the floor of the chamber, respectively. Changes in locomotor activity were measured as the number of photobeam breaks (counts) and were recorded with a Pentium I computer equipped with a mouse activity monitoring system software (Med Associates).

Prepulse inhibition (PPI) studies were conducted in sound-attenuating acoustic startle cubicles (Med Associates) equipped with two speakers, a mouse holder, and a transducer system (a platform with load cells) through which startle responses were recorded. Chambers were connected to an amplifier and to a Pentium IV computer equipped with Startle Reflex software (Med Associates).

For the stress-induced hyperthermia studies, changes in core body temperature were measured rectally using a digital thermometer with an accuracy of 0.1°C using a Physitemp (model BAT-12) digital thermometer (Physitemp Instruments, Inc. Clifton, NJ).

Elevated plus maze studies were conducted using an automated elevated plus maze constructed from black Plexiglas using a design validated by Lister (1987). The maze (Hamilton-Kinder, Poway, CA) consisted of two open arms (37.5 × 5 × 0.3 cm) and two closed arms (37.5 × 5 × 15 cm), which extended from the central platform (5 × 5 cm). The maze was elevated 63 cm above the floor. Each arm was outfitted with four infrared photobeam sensors. Automated software recorded the number of entries and time spent in the open and closed arms of the plus maze by analyzing photobeam breaks using Motor Monitor software (Hamilton-Kinder). A modified zone map was used. The central area was extended to include the first 2.5 cm of each arm; thus the arm area was defined as the most distal (35 cm). An arm entry was recorded when the mouse entered the distal part of the arm based on Lister (1987).

Procedure. *Amphetamine- and PCP-induced hyperlocomotor activity.* Experiments were conducted every 3 to 4 days in the same mice using a within-subject counterbalanced design. Specifically, for each session, half of the mice received an acute injection of vehicle (10 ml/kg i.p.) and the remaining half received a dose of BINA (10 and 32 mg/kg i.p.). Animals were placed in the open field for 60 min (habituation period) and, thereafter, mice received amphetamine (3.2 mg/kg s.c.) or PCP (5.6 mg/kg s.c.), and locomotor activity was measured for an additional 120 min. These pretreatment times for amphetamine and PCP were chosen based on previous open field studies (Galici et al., 2005). The duration of action of BINA (32 mg/kg i.p.) was evaluated in the same mice used for the acute studies by administering BINA at various pretreatment times, specifically 0, 1, 4, 8, or 16 h before PCP administration.

To evaluate the selectivity of the observed behavioral effects of BINA, pretreatment with a dose of BINA (32 mg/kg i.p.) or vehicle for 40 min, was followed by a dose of the orthosteric mGluR2/3 antagonist LY341495 (3 mg/kg, i.p.) before the end of the 60-min habituation period. Thereafter, mice received PCP (5.6 mg/kg s.c.), and locomotor activity was evaluated for an additional 120 min.

In a separate experiment, the effects of BINA (10 and 32 mg/kg i.p.) on locomotor activity when administered alone were assessed using a between-subject design in nonhabituated naive mice. In this experiment, BINA was given 60 min before the habituation period, and locomotor activity was studied for 180 min. For all of the locomotor activity studies, changes in locomotor activity were expressed as the average number of counts or photobeam breaks ± S.E.M.

PCP-induced disruption of PPI of startle reflex. The effects of BINA (32 and 100 mg/kg i.p.) on PCP-induced (5.6 mg/kg s.c.) disruption of PPI was studied using a within-subject design. Eight different treatment conditions were assigned to 16 naive mice in each session. Vehicle and BINA were administered 60 min before

whereas water and PCP were given 15 min before the beginning of the session. These pretreatment times were chosen according to published open field studies in the literature (Geyer and Ellenbroek, 2003). Each session started with a 5-min acclimatization period, during which a 65-dB background noise was continuously present, and included a total of 54 trials. Six different trial types were randomly assigned and delivered (every 15–20 s on average) for 9 times throughout the session: 40-ms broadband 120-dB burst (pulse only), 65-dB background noise (noise only), and 20-ms prepulse of 70, 76, 82, and 88 dB followed by 100 ms of a 120-dB pulse. Sessions were conducted every 3 to 4 days.

Stress-induced hyperthermia. For the stress-induced hyperthermia experiments, a between-subject design was used. Mice were group housed until the day before the experiment and then singly housed overnight. On the test day, mice were pretreated with vehicle or a dose of either BINA (10 or 32 mg/kg i.p.) or the benzodiazepine chlordiazepoxide (2.5, 5, or 10 mg/kg i.p.) for 1 h before the beginning of the experiment. To evaluate the selectivity of BINA, mice were pretreated with a dose of LY341495 (3 mg/kg i.p.) 30 min before a dose of 32 mg/kg BINA i.p. or vehicle and then were tested 60 min later. With the use of a modified method from Van der Heyden et al. (1997) mice were brought into the testing room one at a time, and baseline temperature was taken by inserting a thermistor probe, dipped into mineral oil, 2 cm into the rectum for 20 s to obtain core body temperature (T_1). After a 15-min interval, core body temperature for each mouse was measured a second time (T_2). Stress-induced hyperthermia was calculated as the change in core body temperature between the first and second temperature readings ($\Delta T = T_2 - T_1$).

Elevated plus maze. For the elevated plus maze experiments, a between-subject design was used. On the test day, mice were pretreated with vehicle or a dose of either BINA (10 or 32 mg/kg i.p.) or chlordiazepoxide (2.5, 5, or 10 mg/kg i.p.) and then acclimated in the testing room for 1 h. A 5-min test session began by placing each mouse on the central platform facing the open arm of the elevated plus maze. After each session, the maze was cleaned with 70% ethanol before the next mouse was tested. Data are expressed as either the time spent in the open arms as a percentage of the total testing time, the total number of open arm entries, or the total number of photobeam breaks or ambulations. To evaluate the selectivity of BINA, mice were pretreated with a dose of LY341495 (3 mg/kg i.p.) 30 min before a dose of 32 mg/kg BINA i.p. or vehicle and then tested 60 min later.

Drugs

All reagents and solvents for the synthesis of BINA were obtained from Sigma-Aldrich (St. Louis, MO). All cell culture reagents were obtained from Invitrogen. G418 sulfate was obtained from Mediatech (Herndon, VA). Methotrexate was obtained from Calbiochem (La Jolla, CA). [^{35}S]GTPγS (1250 Ci/mmol), Unifilter-96 GF/B plates, and MicroScint-20 were obtained from PerkinElmer Life Sciences (Boston, MA). Unlabeled GTPγS was obtained from Sigma-Aldrich (St. Louis, MO). Saponin was obtained from Fluka (Buchs, Switzerland). EDTA was obtained from Mallinckrodt Baker, Inc. (Phillipsburg, NJ). L-Glutamic acid, l-AP4, and LY341495 were obtained from Tocris Cookson Inc. (Ellisville, MO). Probenecid, L-proline, PCP hydrochloride, chlordiazepoxide hydrochloride, and amphetamine hydrochloride were obtained from Sigma-Aldrich.

For the electrophysiology experiments, BINA was dissolved in DMSO and then diluted to the appropriate concentration using ACSF. LY341495 and DCG-IV were dissolved in double-deionized water with 1 N sodium hydroxide. For behavioral experiments, BINA was dissolved in 10% Tween 80 and 1% lactic acid (Sigma-Aldrich), and the pH was adjusted with 1 N sodium hydroxide (Mallinckrodt, St. Louis, MO). PCP, amphetamine, chlordiazepoxide, and LY341495 were dissolved in double-deionized water. Doses refer to the form of the drug listed. All drugs were administered i.p. in a volume of 10 ml/kg.

Statistical Analysis

For the calcium mobilization assay, all data were normalized to percentage glutamate or L-AP4 maximum defined by 10 μM glutamate or L-AP4 for each cell line, respectively. For the [^{35}S]GTPγS binding assay, all data were normalized to percent glutamate maximum defined as 10 μM glutamate. Data analysis was performed using GraphPad Prism 3.0 software (GraphPad Software, Inc., San Diego, CA). Concentration-response curves were generated from the mean data of at least three independent experiments. Error bars represent ± S.E.M. For the electrophysiology experiments, data were analyzed using Clampfit version 9.2 (Molecular Devices). All results are expressed as means ± S.E.M., and statistical significance was determined using Student's t test. For the locomotor activity time course and antagonist studies, data were analyzed using a repeated measures ANOVA with treatment and time (i.e., 120 min postinjection) as within-group factors followed by analyses of simple main effects and, when appropriate, post hoc analysis with least significant difference test. To quantify the potency and duration of action of BINA, locomotor activity data were calculated as the average counts/5 min that occurred in the first 60 min postinjection (i.e., after habituation time) and were expressed as a percentage of control (i.e., average of two determinations of PCP effects by itself). ED_{50} values were calculated by interpolation (only two data points were available) for each mouse and were expressed as mean ± S.E.M. Startle amplitude data were expressed as the mean value ± S.E.M. during presentation of the background noise only (i.e., 65 dB) and the pulse alone (i.e., 120 dB) and were analyzed with one-way repeated measures ANOVA. Levels of PPI were determined by the formula (100 − [(prepulse pulse/pulse alone) ∗ 100]) and were expressed as percentage PPI ± S.E.M. Data were analyzed using repeated measures ANOVA with the prepulse intensity and treatment as within-group factors, followed by analyses of simple main effects and, when appropriate, post hoc analysis with the least significant difference test. In the stress-induced hyperthermia and elevated plus maze tests, data were expressed as means ± S.E.M. Treatment groups were compared with the appropriate control groups using a one-way ANOVA and Dunnett's t test. Statistical analyses were performed using JMP version 4.04 statistical software (SAS Institute, Cary, NC). For all behavioral data, a probability of $p \leq 0.05$ was taken as the level of statistical significance.

Results

Synthesis of BINA. BINA was synthesized through a nine-step reaction pathway as outlined in Fig. 1 using an alternate synthetic route from that by Bonnefous et al. (2005). The spectral data of BINA and its precursors were consistent with the structure of BINA.

BINA Induces a Selective Allosteric Potentiation of mGluR2 in Recombinant Systems. Activation of mGluR2 was assessed by measuring glutamate-induced increases in intracellular calcium in CHO cells expressing hmGluR2 coexpressed with the G protein (G_{qi5}). Consistent with the effects of other allosteric potentiators of mGluR2, increasing concentrations of BINA induced parallel leftward shifts of the glutamate concentration-response curves in the hmGluR2 CHO cells with no increase in the maximal response to glutamate (Fig. 2A). The EC_{50} value of glutamate in the presence of a maximally effective concentration of BINA was 108.0 ± 15.2 nM. Thus, BINA induced a maximal change in the glutamate EC_{50} of approximately 11-fold (Fig. 2A).

To assess the potency of BINA, we determined the effects of increasing concentrations of BINA on the response to an EC_{20} concentration (200–300 nM) of glutamate in CHO cells expressing hmGluR2/G_{qi5} (Fig. 2B). BINA produced a maxi-

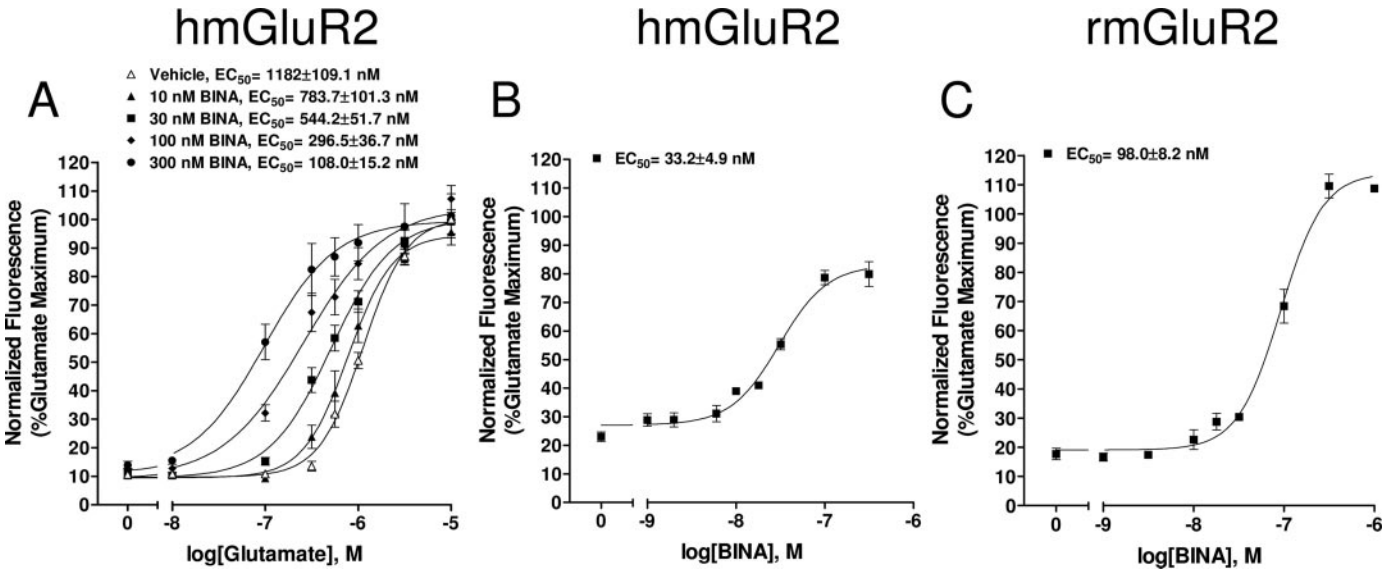

Fig. 2. A, concentration-response curves for glutamate in the absence or presence of increasing concentrations of BINA. B and C, concentration-response curves of BINA in the presence of a submaximal concentration (EC_{20}) of glutamate in CHO cells stably expressing hmGluR2 (B) and rmGluR2 (C). Cells were preincubated with vehicle or BINA for 5 min before the addition of a submaximal concentration (EC_{20}) of glutamate or increasing concentrations of glutamate. Calcium mobilization was measured using FLEXstation-II. The fluorescence responses were normalized as a percentage of the maximal response to glutamate (10 μM) and are the means ± S.E.M. of three independent experiments performed in triplicate.

mal potentiation of approximately 3.5-fold, with an EC_{50} value of 33.2 ± 4.9 nM (Fig. 2B). Similar to the potentiation observed in hmGluR2, BINA also produced a concentration-dependent potentiation of the glutamate response of HEK293A cells expressing rmGluR2/G_{qi5} with a maximal potentiation of approximately 6-fold and an EC_{50} value of 98.0 ± 8.2 nM (Fig. 2C).

To determine the selectivity of BINA for mGluR2 relative to that of other mGluR receptor subtypes, we assessed the effects of this compound on glutamate-induced activation of mGluR3 as well as members of the other mGluR subgroups. In particular, the ability of BINA to shift glutamate-induced [^{35}S]GTPγS binding in membranes expressing either hmGluR2 or rmGluR3 was measured (Fig. 3, A and B). Increasing concentrations of BINA produced a leftward shift in the glutamate-induced [^{35}S]GTPγS binding in the hmGluR2 with a maximal potentiation of approximately 10-fold, resulting in an EC_{50} value for glutamate of 0.50 ± 0.0 μM (Fig. 3A) in the presence of 300 nM BINA. Interestingly, in the absence of glutamate, BINA also increased [^{35}S]GTPγS binding in membranes expressing hmGluR2. In contrast, BINA had no effect on glutamate-induced [^{35}S]GTPγS binding in rmGluR3 (Fig. 3B). Moreover, BINA had no effect on glutamate-induced activation of the other mGluR subtypes tested, including rmGluR1a (Fig. 4A), rmGluR5a (Fig. 4B), or hmGluR4 (Fig. 4C) using a calcium mobilization assay. Furthermore, application of BINA alone did not elicit a calcium response in any of the other cell lines tested. These present data suggest that BINA is a potent and highly selective allosteric potentiator of mGluR2.

BINA Potentiates Agonist-Induced Inhibition of Excitatory Synaptic Transmission at the Medial Perforant Path-Dentate Gyrus Synapse in Rat Hippocampal Slices. Previous studies have demonstrated that group II mGluRs, located presynaptically within the MPP, inhibit transmission at the MPP-dentate gyrus (DG) synapse by

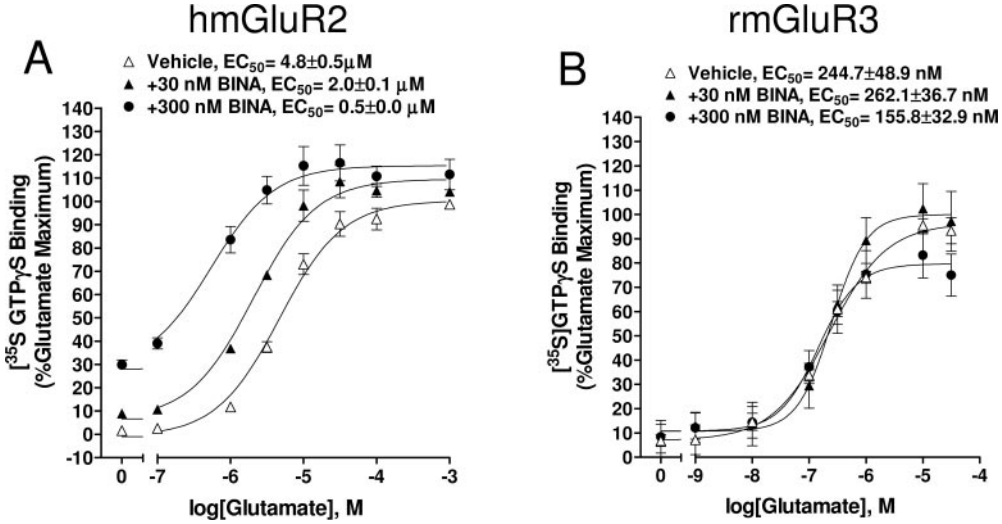

Fig. 3. A, and B, concentration-response curves for glutamate-induced [^{35}S]GTPγS binding to membranes prepared from recombinant cell lines expressing either hmGluR2 (A) and rmGluR3 (B) in the absence or presence of 30 or 300 nM BINA. A [^{35}S]GTPγS binding assay was incubated at 30°C for 60 min, in which the assay mixtures contained 10 μg of membrane protein, BINA, glutamate, and 0.1 nM [^{35}S]GTPγS. Data were normalized as a percentage of the maximal response to 1 mM or 31.6 μM glutamate for hmGluR2 and rmGluR3, respectively. The means of three individual experiments performed in triplicate are shown, and error bars represent S.E.M.

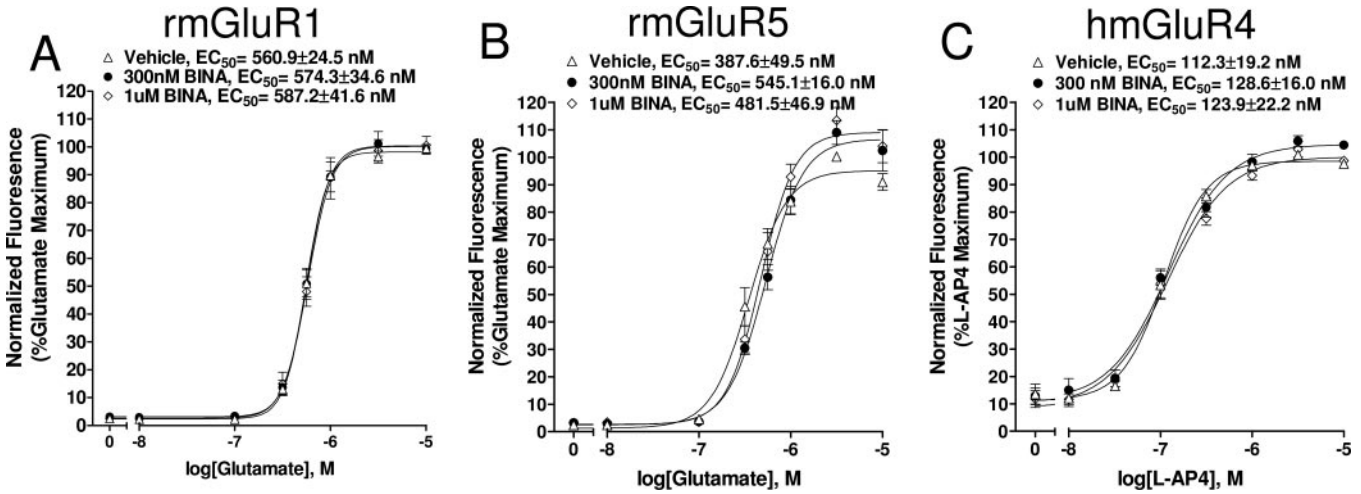

Fig. 4. A–C, concentration-response curves for glutamate in the absence or presence of 300 nM or 1 μM BINA in cell lines expressing rmGluR1a (A), rmGluR5a (B), or hmGluR4 (C). Calcium mobilization was measured using FLEXstation-II, and the signal amplitude was normalized as a percentage of the maximal response to glutamate or L-AP4 (10 μM). The means of three individual experiments performed in triplicate are shown, and error bars represent S.E.M.

reducing glutamate release from presynaptic terminals (Macek et al., 1996, 1998). Furthermore, Schaffhauser et al. (2003) reported that the mGluR2 positive allosteric modulator LY487379 potentiates the effects of group II mGluR agonists at this synapse. These previous findings at the MPP-DG synapses provide an excellent response for determining whether BINA potentiates activation of native mGluR2 at an identified synapse. Thus, extracellular field recordings were performed in the middle third of the molecular layer of the DG to determine whether BINA had effects on synaptic transmission at the MPP-DG synapse. As reported previously (Macek et al., 1996), bath application of the group II mGluR agonist DCG-IV reduced MPP excitatory synaptic responses in a concentration-dependent manner (Fig. 5A), with an inhibition of 70.3% when the concentration of DCG-IV was 3 μM ($n = 18$). The observed effects of DCG-IV were blocked using the group II mGluR antagonist LY341495 (1 μM) (Macek et al., 1998) (data not shown). The effect of BINA was evaluated using a concentration of DCG-IV (100 nM) that induced a submaximal inhibition of the fEPSP slope (18.9 ± 2.6% inhibition; $n = 8$) (Fig. 5, B and C). Consistent with the effects of BINA observed in the recombinant systems, BINA (1 μM) significantly potentiated the effect of DCG-IV on inhibition of the fEPSP slope (33.1 ± 3.9% inhibition; $n = 8$) (Fig. 5, B and C).

BINA Blocks PCP-Induced Hyperlocomotor Activity. The finding that BINA is a highly selective allosteric potentiator of mGluR2 with activity in native systems is encouraging in light of previous studies suggesting that this compound crosses the blood-brain barrier (Rodriguez et al., 2004; Bonnefous et al., 2005). We next performed a series of studies to determine whether BINA could mimic the effects of group II mGluR agonists in animal models used to predict potential antipsychotic-like and anxiolytic activity. The effects of BINA on PCP-induced hyperlocomotor activity were determined using C57BL6 mice (Fig. 6, A–D). A two-way repeated measures ANOVA indicated that there was a significant effect of dose [$F(4,20) = 4.9$, $p < 0.05$]. Post hoc analysis indicated that PCP (5.6 mg/kg s.c.) significantly increased locomotor activity compared with the vehicle control group (Fig. 6A). BINA produced a robust blockade of the PCP-induced hyperlocomotor activity, significant at a dose of 32 mg/kg i.p. (Fig. 6A). The ED_{50} value of BINA for blocking the effects of PCP was 16.8 ± 2.3 mg/kg (Fig. 6B).

The effects of BINA on the PCP-induced hyperlocomotor activity were reversed with pretreatment of the mGluR2/3 antagonist LY341495 (3 mg/kg i.p.), indicating that the effects of BINA were mediated through activation of mGluR2 (Fig. 6A). LY341495 (3 mg/kg i.p.) administered alone had no effects on the PCP-induced hyperlocomotor activity. Two-way repeated measures ANOVA also indicated that there was a time effect (Fig. 4A) [$F(35,175) = 17.6$, $p < 0.05$] and a treatment × time interaction (Fig. 6A) [$F(140,700) = 2.01$, $p < 0.05$]. BINA produced a modest decrease in locomotor activity when administered alone, significant at 32 mg/kg i.p. [$F(2,26) = 4.8$, $p < 0.05$] (Fig. 6C). There was also a significant time effect (Fig. 6C) [$F(35,910) = 34.6$, $p < 0.05$] and a treatment × time interaction (Fig. 6C) [$F(70,910) = 2.7$].

We next determined the time course for the effects of a maximally effective dose of BINA (32 mg/kg i.p.) on PCP-induced hyperlocomotor activity to determine the duration of action of this compound. BINA had no significant effect when it was coadministered with PCP but had a robust effect at a 1-h pretreatment time. This finding suggests that this compound may not fully reach the site of action within the 1st min of administration, but is fully active by 1 h (Fig. 6D). Importantly, BINA had a relatively long duration of action and produced a significant blockade of PCP-induced hyperlocomotor activity that persisted for at least 8 h after administration (Fig. 6D) [$F(6,36) = 8.7$, $p < 0.05$]. However, BINA had no significant effect when pretreated 16 h before PCP.

BINA Has No Effect on Amphetamine-Induced Hyperlocomotor Activity. We next determined the effects of BINA on amphetamine-induced hyperlocomotor activity, another response that has been shown to be sensitive to the effects of group II mGluR agonists (Cartmell et al., 2000) and the mGluR2 allosteric potentiator LY487379 (Galici et al., 2005). Interestingly, BINA had no effect on amphetamine-induced hyperlocomotor activity after coadministration (data not shown) or a 60-min pretreatment time (Fig. 7). Thus, BINA does not share all of the in vivo activities of group II

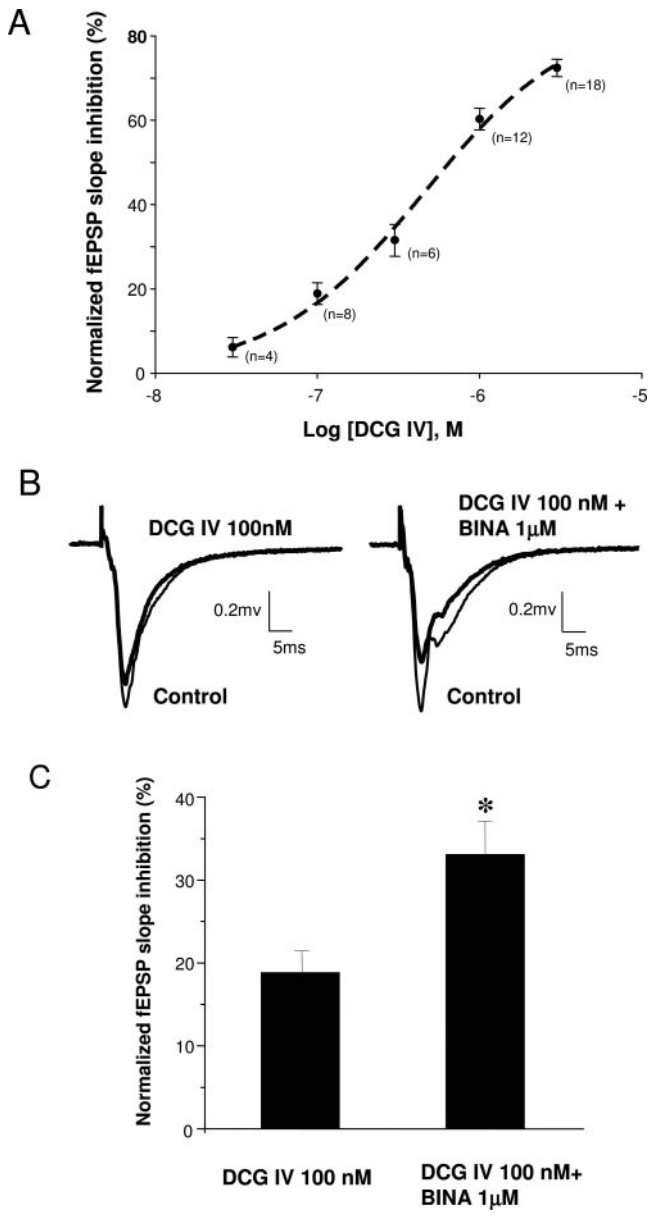

Fig. 5. BINA potentiates the effect of DCG-IV on fEPSPs at MPP-DG synapses in rat hippocampal slices. A, concentration-response curves for the inhibitory effect of DCG-IV on fEPSP slopes by activation of group II mGluR at MPP-DG synapses (n = 50 slices). B, representative traces show the recorded fEPSPs before and after 12 min of application of DCG-IV alone (100 nM, left); or DCG-IV (100 nM) in the presence of BINA (1 μM, right). Each trace is an average of six consecutive evoked responses at MPP-DG synapses. C, bar graph illustrates normalized fEPSP slopes at MPP-DG synapses after a 12-min application of DCG-IV alone (100 nM, n = 8 slices) or DCG-IV (100 nM) in the presence of BINA (1 μM, n = 8 slices). All values are expressed as means ± S.E.M., Calibration bar, 0.2 mV/5 ms. *, $p < 0.01$; Student's t test.

mGluR agonists or LY487379 in models used to assess the activity of potential antipsychotic agents.

BINA Blocks PCP-Induced Disruption of PPI. Another animal model that is often used to assess the potential antipsychotic activity of novel compounds is the reversal of PCP-induced disruption of PPI of the acoustic startle response (Geyer and Ellenbroek, 2003). Consistent with previous studies, PCP (5.6 mg/kg s.c.) produced a robust decrease in PPI across all of the prepulse intensities tested (Fig. 8). Pretreatment with BINA produced a robust blockade of PCP-induced disruption of PPI, which was significant across all prepulse intensities tested after 100 mg/kg s.c. The effect of BINA was also significant with the 82- and 88-dB prepulse intensities after 32 mg/kg s.c. (Fig. 8). Two-way repeated measures ANOVA indicated that there was a treatment effect (Fig. 8) [$F(7,77) = 11.98, p < 0.05$]. In addition, there was a prepulse intensity effect (Fig. 8) [$F(3,33) = 45.8, p < 0.05$], but no treatment × prepulse intensity interaction. One-way ANOVA also indicated that PCP significantly increased the startle amplitude when only the background noise (i.e., 65 dB) was presented (Table 1) [$F(7,77) = 7.74, p < 0.05$). However, there was no effect on startle amplitude when the startle pulse alone (120 dB) was presented under any of the treatment conditions (Table 1).

BINA Decreases Stress-Induced Hyperthermia in Mice. In addition to effects in animal models used to predict antipsychotic efficacy, group II mGluR agonists are also effective in multiple animal models of anxiolytic drug activity (for review, see Swanson et al., 2005). More importantly, mGluR2/3 agonists have been shown to have anxiolytic activity in multiple clinical studies (Grillon et al., 2003; Schoepp et al., 2003; Swanson et al., 2005). Thus, it is critical to determine whether allosteric potentiators of mGluR2 have activity similar to that of mGluR2/3 agonists in these animal models. We determined the effect of BINA on stress-induced hyperthermia, a response that is highly sensitive to mGluR2/3 agonists (Spooren et al., 2002; Swanson et al., 2005). BINA produced an inhibition of stress-induced hyperthermia, as evidenced by a main effect for dose [$F(3,56) = 34.1; p < 0.05$], significant after doses of 32 mg/kg i.p. by a Dunnett's comparison with the vehicle group (Fig. 9A). The effects of BINA (32 mg/kg i.p.) on stress-induced hyperthermia were blocked by pretreatment with a dose of LY341495 (3 mg/kg i.p.), indicating that the effects were mediated by activation of mGluR2 (Fig. 9A). Larger doses of BINA produced substantial decreases in basal body temperature, which precluded further evaluation in the stress-induced hyperthermia model. For comparison, the effects of the benzodiazepine chlordiazepoxide were also evaluated in the stress-induced hyperthermia model. Chlordiazepoxide produced a dose-dependent inhibition of the stress-induced hyperthermia as evidenced by a main effect for dose [$F(3,82) = 25.6; p < 0.05$], significant after 5 and 10 mg/kg i.p. by a Dunnett's comparison with the vehicle group (Fig. 9B).

BINA Produces Anxiolytic-Like Effects in the Elevated Plus Maze Test. Anxiolytic effects of BINA were also evaluated in the elevated plus maze, another animal model that is sensitive to anxiolytic agents. In the elevated plus maze test, BINA increased the percentage of time spent in the open arms of the maze as evidenced by a main effect for dose [$F(3,39) = 3.4; p < 0.05$], significant after a dose of 32 mg/kg i.p. by a Dunnett's comparison with the vehicle group (Fig. 10A). In addition, BINA also increased the number of entries into the open arms of the maze after doses of 10 or 32 mg/kg i.p. by a Dunnett's comparison with the vehicle group (Fig. 10C). When administered alone, BINA had no effect on total ambulations (Fig. 10E). The increased entries and time spent in the open arms of the elevated plus maze after administration of BINA (32 mg/kg i.p.) were blocked with pretreatment of LY341495 (3 mg/kg i.p.), indicating that these effects were mediated through the activation of mGluR2 (Fig. 10, A and C). For comparison, the effects of the chlordiazep-

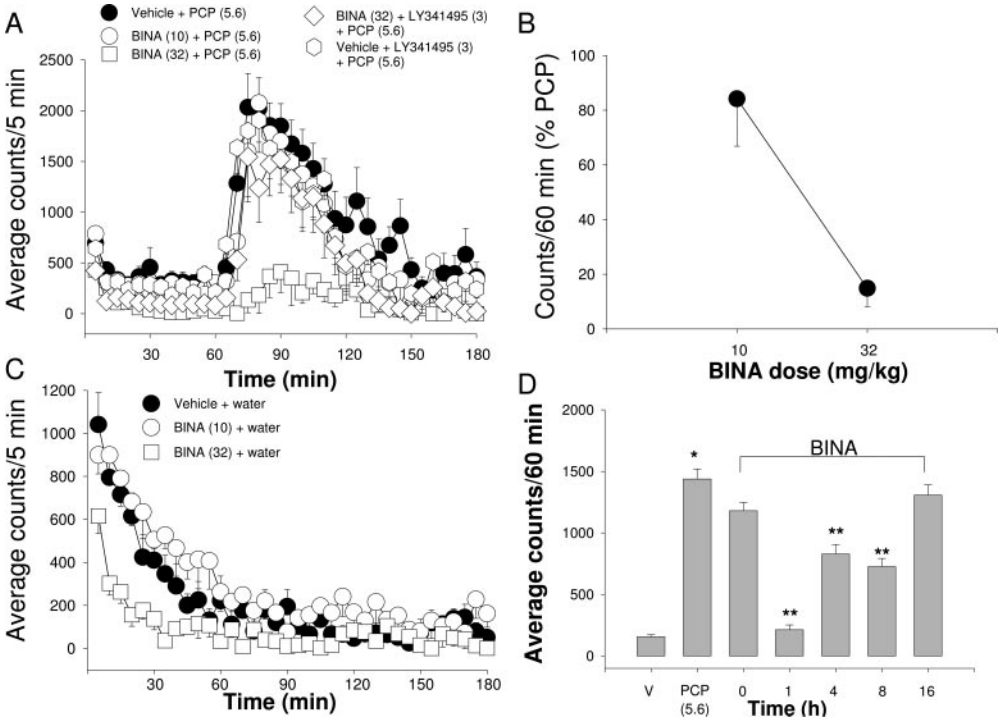

Fig. 6. Effects of BINA on PCP-induced hyperlocomotor activity and spontaneous locomotor activity in mice. A, BINA blocked the effects of PCP-induced hyperlocomotor activity. B, potency of BINA in blocking PCP-induced hyperlocomotor activity. Average counts/60 min, expressed as the mean percentage ± S.E.M of the average of two determinations of PCP effects (i.e., percentage control), are plotted as a function of BINA dose expressed in milligrams per kilogram. C, BINA administered alone (32 mg/kg i.p.) decreased spontaneous locomotor activity. Data are expressed as means ± S.E.M. D, time course effects of BINA on PCP-induced hyperlocomotor activity in mice. BINA was administered with (i.e., at time 0 h) and 1, 4, 8, and 16 h before PCP treatment. Vertical lines represent ± S.E.M. and are absent when less than the size of the point. (n = 7–8 per dose) Ordinate, average counts/5 min. Abscissa, time in minutes. *, $p <$ 0.05 indicates statistical difference from vehicle (V). **, $p < 0.05$ indicates statistical difference from PCP (5.6 mg/kg).

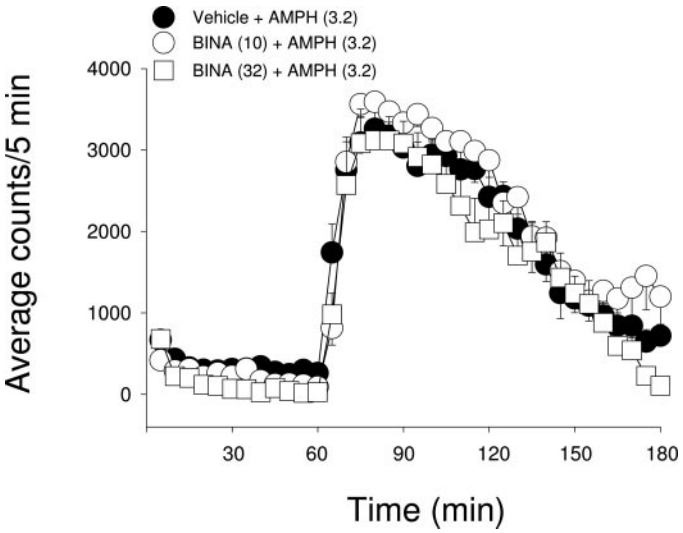

Fig. 7. BINA had no effect on amphetamine-induced hyperlocomotor activity in mice. Data are expressed as means ± S.E.M. Average counts/60 min are plotted as a function of time in hours (n = 7–8 per condition). Vertical lines represent ± S.E.M. and are absent when less than the size of the point. Ordinate, average counts/5 min. Abscissa, time in minutes.

oxide were also evaluated in the elevated plus maze test. Chlordiazepoxide increased the percentage of time spent in the open arms of the maze as evidenced by a main effect for dose [$F(3,39) = 4.8$; $p < 0.05$], significant after doses of 10 mg/kg i.p. by a Dunnett's comparison with the vehicle group (Fig. 10B). In the dose range tested, chlordiazepoxide also produced an increase in the number of entries into the open arms of the maze, significant after doses of 5 and 10 mg/kg i.p. by a Dunnett's comparison with the vehicle group (Fig. 10D). After administration alone, chlordiazepoxide produced a modest increase in total ambulations, significant with a dose of 10 mg/kg (Fig. 10F).

Discussion

We report an alternate synthesis and further pharmacological characterization of the previously reported positive allosteric modulator of mGluR2, termed here biphenyl-indanone A (BINA). Derived from a series of substituted indanones (Bonnefous et al., 2005; Pinkerton et al., 2005), BINA is structurally and chemically distinct from both group II mGluR agonists and the mGluR2 positive allosteric modulator LY487379. In recombinant systems, BINA is a potent and highly selective potentiator of the response of mGluR2 to glutamate with no effect on the glutamate response of the other mGluR receptor subtypes tested. At native mGluR2, BINA functions as a positive allosteric modulator in a manner similar to its actions on recombinant mGluR2. Most encouraging, BINA produces robust and long-lasting effects in mouse behavioral models of antipsychotic- and anxiolytic-like activity that mimic previously observed effects using group II mGluR agonists. Taken together, our findings demonstrate that BINA is a selective tool for dissecting the role of mGuR2 in the observed effects of group II mGluR agonists in vivo.

The development of subtype-selective allosteric modulators for the activation of mGluRs offers many potential advantages over direct-acting agonists, including greater subtype-selectivity maintenance of activity dependent receptor activation. The discovery of LY487379 and related analogs as selective positive allosteric modulators of mGluR2 (Johnson et al., 2003, 2005; Schaffhauser et al., 2003) represented an important breakthrough in the field of mGluR research. However, modest potency and a short duration of action limit the utility of these compounds for in vivo studies (Johnson et al., 2003; Galici et al., 2005). The present findings confirm and extend previously reported studies showing that BINA, a member of a novel series of mGluR2 potentiators (Bonnefous et al., 2005; Pinkerton et al., 2005), is highly selective and

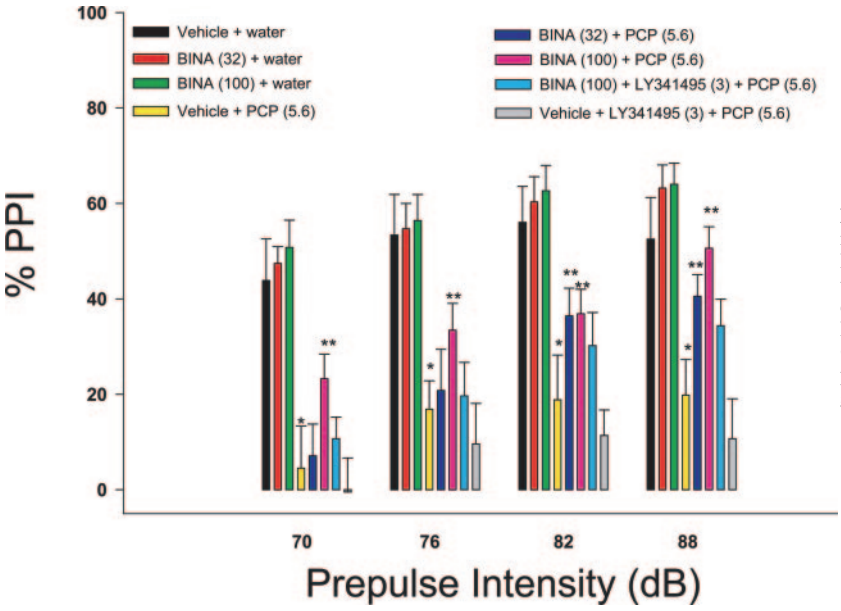

Fig. 8. BINA blocked PCP-induced disruption of PPI in mice. Data are expressed as means ± S.E.M. Percentage PPI is plotted as a function of prepulse intensity (decibels) used. *, $p < 0.05$ indicates statistical difference from vehicle (V). **, $p < 0.05$ indicates statistical difference from PCP (PCP; 5.6 mg/kg). Vertical lines represent ± S.E.M. Ordinate, percentage prepulse inhibition. Abscissa, prepulse intensity amplitude (decibels) (n = 7–8 per dose group).

TABLE 1
Effects of BINA on startle amplitude of the acoustic startle reflex
Data are expressed as means ± S.E.M. when the background noise stimulus (65 dB) or the pulse stimulus (120 dB) was presented alone.

Treatment Dose	Startle Amplitude	
	65	120
	dB	
Vehicle ± water	200.4 ± 28.6	765.3 ± 70.2
BINA (32 mg/kg i.p.) ± water	211.9 ± 31.1	817.3 ± 101.7
BINA(100 mg/kg i.p.) ± water	100.1 ± 10.5	766.9 ± 73.4
Vehicle ± PCP (5.6 mg/kg i.p.)	418.5 ± 80.0*	666.4 ± 59.0
BINA (32 mg/kg i.p.) ± PCP (5.6 mg/kg i.p.)	259.9 ± 37.9	721.2 ± 49.9
BINA (100 mg/kg i.p.) ± PCP (5.6 mg/kg i.p.)	117.3 ± 17.1	654.8 ± 106.5
BINA (100 mg/kg i.p.) ± LY341495 (3 mg/kg i.p.) ± PCP (5.6 mg/kg i.p.)	151.9 ± 13.7	590.0 ± 74.2
Vehicle ± LY341495 (3 mg/kg i.p.) ± PCP (5.6 mg/kg i.p.)	249.4 ± 17.5	548.4 ± 67.5

* $P < 0.05$ compared with control (i.e., vehicle ± water).

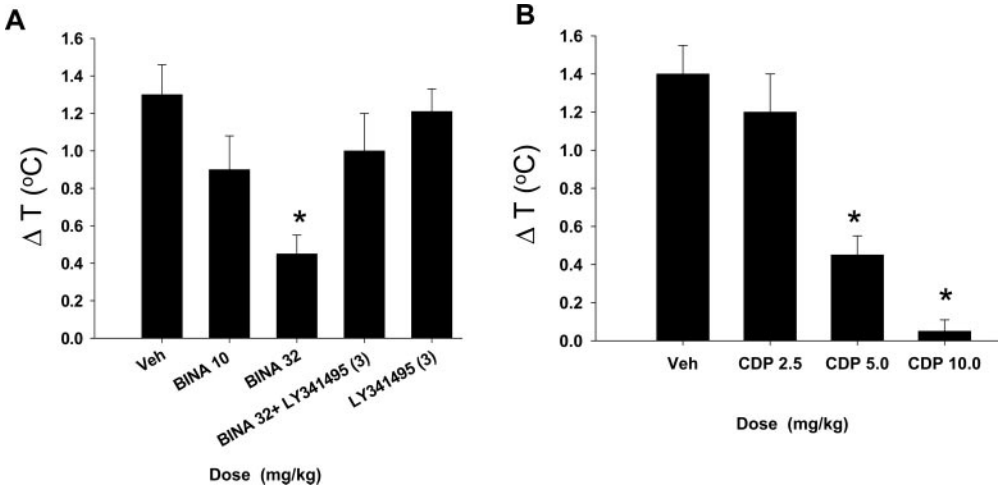

Fig. 9. BINA inhibited stress-induced hyperthermia in mice. The dose-related effects of BINA and the benzodiazepine chlordiazepoxide (CDP) were evaluated on stress-induced hyperthermia. The dose of the mGluR2/3 antagonist LY341495 used was 3 mg/kg. Data are expressed as means ± S.E.M. Bar graphs above Veh represent the effects of vehicle alone. Vertical lines represent ± S.E.M. (n = 10–12 mice per dose group). Abscissa, dose of drug in milligrams per kilogram. Ordinate, ΔT (degrees Centigrade). *, $p < 0.05$ versus vehicle, Dunnett's t test.

has robust in vivo activity, thereby providing an important tool for investigating the positive allosteric modulation of mGluR2.

In addition to inducing a leftward shift of the glutamate concentration response curve, BINA induced a modest activation of mGluR2 in the absence of glutamate when [^{35}S]GTPγS binding was measured in membranes from cells expressing mGluR2. Thus, BINA has modest intrinsic mGluR2 agonist activity in this assay. It is unlikely that this is due to potentiation of the response to low concentrations of glutamate present in the medium because the [^{35}S]GTPγS binding assay is performed in a washed membrane preparation in which glutamate should not be present. This direct activation of mGluR2 by BINA is reminiscent of the previously reported effects of 3-cyano-*N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamide, a positive allosteric modulator of mGluR5

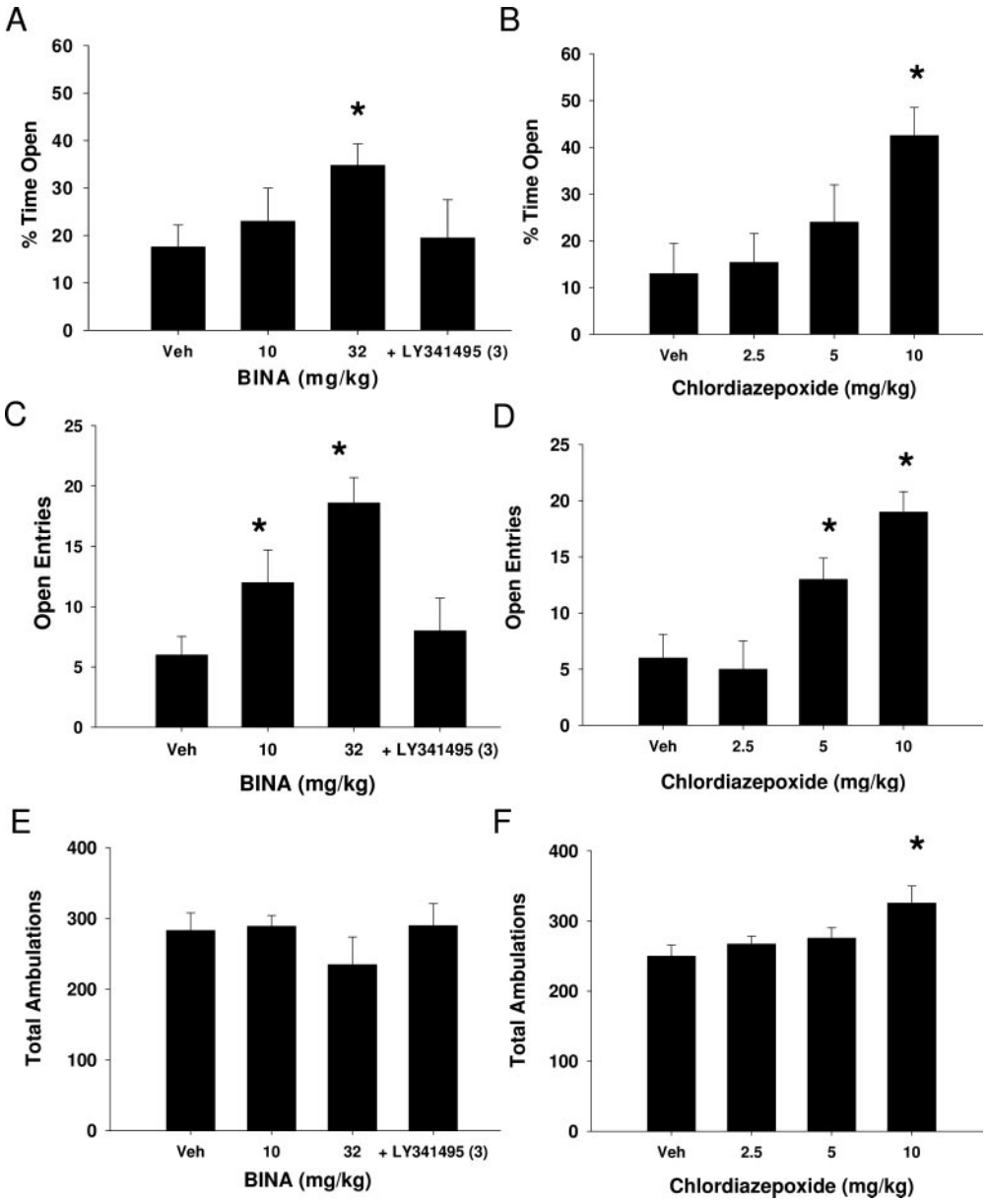

Fig. 10. BINA produced anxiolytic effects in the elevated plus maze test in mice. The dose-related effects of BINA and the benzodiazepine chlordiazepoxide were evaluated on time spent in open arms as a percentage of total testing time (A and B), total number of entries into the open arms (C and D), or total ambulations (E and F). Bar graphs above ±LY341495 (A, C, and E) represent the effects of the mGlu2/3 antagonist LY341495 (3 mg/kg) given in combination with a 32 mg/kg dose of BINA. Data are expressed as means ± S.E.M. Bar graphs above Veh represent the effects of vehicle alone. Vertical lines represent ± S.E.M. Abscissa, dose of drug in milligrams per kilogram (n = 10–12 mice per dose group). Ordinate, percentage of time in the open arms, total number of entries into the open arm, or total ambulations (total number of beam breaks/5 min). *, $p < 0.05$ versus vehicle, Dunnett's t test.

that also exhibits modest intrinsic agonist activity at mGluR5 (Kinney et al., 2005). Furthermore, previous studies have yielded direct allosteric agonists for mGluR7 (Mitsukawa et al., 2005) and the M1 muscarinic receptor (Spalding et al., 2002; Sur et al., 2003). However, it is not yet clear whether this direct activation of mGluR2 by BINA is relevant in vivo or is an artifact of this expression system. Importantly, BINA did not appear to have direct agonist activity in the calcium mobilization assay or at native mGluR2 at the MPP synapse. However, this does not necessarily imply that BINA is incapable of direct activation of this receptor at other synapses or in other neuronal populations.

In addition to providing insights into the pharmacological properties of the positive allosteric modulation of mGluR2 in recombinant systems, BINA also served as a valuable ligand for the study of physiological and behavioral responses mediated by native mGluR2. BINA potentiated mGluR2 agonist effects on excitatory synaptic responses at the MPP-DG synapse. Potential hyperactivty of glutamatergic transmission within the MPP-DG synapse is one region of the CNS thought to be associated with the pathogenesis of anxiety and fear conditioning (Walker and Davis, 2002; Bergink et al., 2004). Thus, our data provide important evidence to further support the role for mGluR2 in the physiological responses mediated by group II mGluR activation in systems thought to be relevant for anxiolytic and antipsychotic action of these compounds.

In the present study, BINA also mimicked the effects of group II mGluR agonists and mGluR2 allosteric potentiators in animal models used to predict potential antipsychotic-like and anxiolytic-like activity. Previous studies have shown that the *N*-methyl-D-aspartate receptor antagonist PCP and the indirect acting dopamine agonist amphetamine produce behavioral changes in animals, such as increases in locomotor activity and disruption of prepulse inhibition, which are reversed by currently available antipsychotic therapies (for review, see Geyer and Ellenbroek, 2003). Systemic administration of BINA produced a dose-related inhibition of PCP-

induced hyperlocomotor activity and disruption of PPI. For the most part, the effects of BINA in these models were similar to those previously shown for group II mGluR agonists or the mGluR2-specific allosteric potentiator LY487379 (Cartmell et al., 1999; Spooren et al., 2000; Galici et al., 2005). Consistent with mediation by mGluR2, each of the effects of BINA were blocked by the mGluR2/3 antagonist LY341495. However, unlike the short-acting LY487379 (Galici et al., 2005), the effects of BINA were long-lasting over a duration of at least 8 h, indicating that this compound is suitable for a further pharmacologic evaluation in vivo (i.e., chronic studies).

Although the effects of BINA and the structurally distinct mGluR2 potentiator LY487379 in animal models of antipsychotic-like activity were comparable, there were some important differences. For instance, BINA did not reverse amphetamine-induced hyperlocomotor activity whereas, group II mGluR agonists (Cartmell et al., 1999, 2000) and LY487379 (Galici et al., 2005) inhibit amphetamine-induced hyperlocomotor activity. Furthermore, in contrast to LY487379 (Galici et al., 2005), BINA blocked the disruptive effects of PCP on PPI. Although the reasons for these important differences between the effects of BINA and LY487379 are unclear, previous studies revealed that different orthosteric agonists can differentially activate different signaling pathways of a single receptor, a phenomenon referred to as agonist receptor trafficking (Brink et al., 2000; Gazi et al., 2003). Based on this finding, it has been suggested that structurally distinct agonists can have different in vivo effects by actions at a single receptor. Interestingly, we recently showed that the same principle applies to allosteric potentiators and that different classes of allosteric potentiators of mGluR5 can have different effects on coupling of this receptor to activation of calcium transients versus extracellular signal-regulated kinase 2 phosphorylation (Zhang et al., 2005). It is conceivable that potentiation of mGluR2 by different classes of allosteric modulators could have distinct effects on coupling of this receptor to different cellular responses. If so, this could lead to different in vivo effects of different classes of allosteric potentiators. It is unlikely that the effects of BINA and LY487379 are due to off-target activity because they are both blocked by the group II mGluR antagonist LY341495. Thus, the present data provide the first evidence that two classes of allosteric potentiators of an mGluR have subtly different effects in vivo. The combined actions of BINA reported here provide further preclinical evidence that mGluR2 may play a key role in the antipsychotic-like effects of mGluR2/3 agonists. Although it is tempting to speculate that selective positive allosteric potentiators of mGluR2 may provide a novel therapeutic approach for the treatment of schizophrenia, particularly as an important alternative to group II mGluR agonists (Johnson et al., 2005), further characterization of BINA in other preclinical models predictive of antipsychotic activity is warranted.

Finally, we report that BINA has activity in two preclinical models of anxiety, specifically the elevated plus maze and stress-induced hyperthermia. These findings are especially exciting in light of recent clinical studies revealing that group II mGluR agonists have anxiolytic effects in humans (Grillon et al., 2003; Schoepp et al., 2003; Swanson et al., 2005). In the present study, the magnitude of the observed increase in time spent and number of entries into the open arm of the elevated plus maze after pretreatment with BINA were similar to effects observed previously with group II mGluR agonists (Helton et al., 1998; Linden et al., 2004). BINA also prevented the development of stress-induced hyperthermia as observed using both group II mGluR agonists and N-[4′-cyanobiphenyl-3-yl)-*N*-(3-pyridinylmethyl)ethanesulfonamide hydrochloride, a structural analog of LY487379 (Johnson et al., 2005; Rorick-Kehn et al., 2006). In both preclinical anxiety models, the effects of BINA were blocked by LY341495, confirming a role for the activation of mGluR2. Thus, our findings extend previous studies suggesting a role for mGluR2 in anxiety disorders and support the fundamentally novel approach to treatment of anxiety disorders using allosteric potentiators of mGluR2.

In summary, BINA constitutes a highly selective positive allosteric modulator of mGluR2 with a long duration of action and robust efficacy in several preclinical models used to predict anxiolytic and antipsychotic-like activity. Our findings confirm and extend a role for the mGluR2 receptor in many of the documented effects of mGluR2/3 agonists in vivo and suggest that positive allosteric modulators of mGluR2 provide an important alternative to group II mGluR agonists for the treatment of anxiety disorders and schizophrenia.

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