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Anxiolytic-like effects through a GLU_{K5} kainate receptor mechanism

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Abstract

The hypothesis that kainate receptor blockade would be associated with anxiolytic-like effects was tested with a selective ligand, 3*S*,4a*R*, 6*S*,8a*R*-6-((4-carboxyphenyl)methyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylic acid (LY382884). LY382884 selectively binds the GLU_{K5} kainate receptor subunit ($K_b = 0.6$ μM) and has 30 μM or greater affinity for cloned human AMPA receptor subtypes. The anxiolytic potential of LY382884 was tested in rats responding under a Vogel conflict procedure, a pharmacologically validated model for the prediction of antianxiety efficacy in humans. Both the benzodiazepine anxiolytic chlordiazepoxide and LY382884 increased suppressed licking without affecting rates of non-suppressed licking. In contrast, an AMPA receptor selective antagonist, 7*H*-1,3-dioxolo[4,5-h][2,3]benzodiazepine-7-carboxamide, 5-(4-aminophenyl)-8,9-dihydro-*N*,8-dimethyl-, monohydrochloride (9CI) (GYKI53655), did not increase suppressed responding. The finding that a selective GLU_{K5} receptor antagonist produced anxiolytic-like effects in an animal model predictive of efficacy in humans combined with data in the literature on glutamatergic modulation of anxiety suggests that kainate receptor sensitivity to glutamate might be an important mediating event in the pathophysiological expression of anxiety states. The selective targeting of kainate receptors with an antagonist could therefore be a novel pharmacological mechanism to treat anxiety disorders.

Keywords: Kainate receptors; AMPA receptors; LY382884; GYKI53655; Chlordiazepoxide; Anxiolytic drugs; Rats

Much of our understanding of the neurochemical bases of anxiety disorders, like that in other areas of psychiatric medicine, comes from an analysis of the mechanism of action of drugs used for therapeutic disease modification or management. The majority of drugs currently used to treat anxiety disorders come from only a few pharmacological classes which include selective serotonin uptake inhibitors (SSRIs) like paroxetine, 1,4-benzodiazepine $GABA_A$ receptor agonists of which diazepam is a prototype, and serotonin-1_A receptor partial agonists such as buspirone. Although anxious symptoms can generally be controlled with this set of compounds, there are a host of cases in which the patient community is not fully served, due to either the lack of full efficacy or side-effect liabilities associated with these drugs. Therefore, the search for improvements in anxiolytic medicines is ongoing. To this end, a variety of novel mechanisms have been described including variations on existing themes. Data from a number of areas of inquiry have long pointed to modification of glutamatergic transmission as one such strategy. Recent clinical findings have indeed pointed to the efficacy and safety of an mGlu2/3 agonist in treating generalized anxiety disorder (c.f., Swanson et al., 2005) and mGlu5 antagonists have shown activity in a variety of animal models that also detect the effects of clinically effective anxiolytic drugs (Spooren and Gasparini, 2004). In addition to other data, these findings suggest that direct blockade of ionotropic glutamate receptors might also be a potentially viable mechanism for novel antianxiety treatments.

An AMPA/GLU_{K5} (kainate) receptor antagonist, 3*S*,4a*R*, 6*R*,8a*R*-6-(2-(1(2)*H*-tetrazol-5-l)ethyl)-decahydroisoquinoline-3-carboxylic acid (LY293558), has been shown to produce

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anxiolytic-like effects in some animal models (Benvenga et al., 1995; Alt et al., 2006). Based upon the selectivity profile of LY293558, it has been speculated that its antagonist activity at GLU_{K5} receptors might be responsible for its anxiolytic-like behavioral effects (Alt et al., 2006). GLU_{K5} receptors are localized in brain areas regulating anxiety (e.g., basolateral amygdala; see for example H. Li et al., 2001), providing additional support to the hypothesis that specific antagonism of GLU_{K5} receptors might produce anxiolytic-like effects. The present study was conducted with a selective GLU_{K5} receptor antagonist, 3*S*,4a*R*,6*S*,8a*R*-6-((4-carboxyphenyl)methyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylic acid (LY382884), to more clearly define the role of this glutamate receptor subtype in anxiolytic activity. A compound with affinity for AMPA but not kainate receptors, 7*H*-1,3-dioxolo[4,5-h][2,3]benzodiazepine-7-carboxamide, 5-(4-aminophenyl)-8,9-dihydro-*N*,8-dimethyl-, monohydrochloride (9CI) (GYKI53655), was studied for additional insight into the role of GLU_{K5} receptors in engendering anxiolytic-like effects. Both LY382884 and GYKI53655 were also characterized for their affinities to recombinant AMPA/kainate receptor subtypes.

1. Methods

1.1. Cell culture

All cell and tissue culture reagents were from Invitrogen (Grand Island, New York). Recombinant human AMPA receptors (GLU_{A1-4}, both flip (i) and flop (o) variants) were stably expressed in human embryonic kidney (HEK) 293 cell lines, with the exception of GLU_{A3o}, which was stably expressed in AV12 cells. Cells were grown as monolayers under 5% CO_2 at 37 °C in Dulbecco's Modified Eagle Medium with 4.5 g/L D-glucose, L-glutamine and pyridoxine HCl (Invitrogen, Catalog #11965-084), with 10% fetal bovine serum and 250 μg/ml geneticin added. Medium for GLU_{A3o} cells contained 5% fetal bovine serum and 700 μg/ml geneticin and also contained 10 mM HEPES, 1 mM sodium pyruvate, 50 μg/ml streptomycin, 50 units/ml penicillin, and 300 μg/ml hygromycin.

1.2. Measurement of ion influx using Fluo-3

Cells were seeded into poly-D-lysine-coated 96-well plates (Becton Dickinson Labware, Bedford, MA) 1 day prior to experiments at a density of 60,000 cells/well. Cells were washed 3 times with 100 μl assay buffer composed of Hank's Balanced Salt Solution without phenol red (Invitrogen) with 20 mM HEPES and 3.7 mM $CaCl_2$ added (final $[CaCl_2] = 5$ mM). Plates were then incubated for 2 h in the dark at room temperature in 40 μl assay buffer with 8 μM Fluo3-AM dye (Molecular Probes Inc., Eugene, OR). Following dye incubation, cells were rinsed once with 100 μl assay buffer. Finally, 50 μl assay buffer that included the AMPA receptor potentiator LY392098 (10 μM; to prevent desensitization of AMPA receptors) was added to wells and fluorescence measured using a fluorometric imaging plate reader (FLIPR; Molecular Devices, Sunnyvale, CA). A first addition of 50 μl of LY392098-containing assay buffer was followed by a second addition of 100 μl LY392098-containing buffer 3 min later. LY293558 was added in the absence of agonist during the first addition, and in the presence of 100 μM glutamate during the second addition. Because of the high potency of glutamate at GLU_{A4i} receptors ($EC_{50} = 2$ μM, determined from pilot experiments), only 1 μM glutamate was used when testing antagonists at these receptors, in order to provide more accurate estimations of IC_{50} and K_b values.

Affinities of compounds were determined from concentration–response curves for antagonism of glutamate-evoked calcium influx. Curves were analyzed using GraphPad Prism 3.02 Software (San Diego, CA), with slope factor not fixed, and top and bottom fixed at 100% and 0% inhibitions, respectively. The dissociation constant (K_b) was calculated from the IC_{50} value for inhibiting 100 μM glutamate-induced calcium influx (or 1 μM glutamate for GLU_{A4i}) according to Cheng–Prusoff equation (Cheng and Prusoff, 1973): $K_b = IC_{50}/(1+[Glu]/EC_{50Glu})$ where [Glu] is the concentration of glutamate (1 μM or 100 μM). EC_{50Glu} is the EC_{50} value of glutamate for evoking calcium influx in the given cell line, determined from glutamate concentration–response curves run in the same plates as the antagonist concentration–response curves.

1.3. Plasma and brain exposure

LY382884 was dissolved in 0.06 N NaOH in water at 10 mg/ml and administered i.p. to rats (9 rats per treatment group) at a dose of 30 mg/kg. Fischer male 344 rats weighing between 238 and 257 g were utilized. Blood (~1.0 ml) and brain were collected at 0.167, 0.333, 0.667, 1, 2, 3, 4, 6 and 8 h after dosing. Plasma and brain samples were stored at −70 °C until assayed. Plasma and brain were collected from each rat. There was 1 rat per time point for each treatment group.

To 50 μl of plasma, 75 μl of acetonitrile containing 10 μg/ml internal standard (LY293558) was added. The sample was gently mixed and centrifuged for 10 min at approximately 3200 rpm. Water (125 μl) was added to each sample and subsequently analyzed by LC/MS/MS. To a whole brain, 50 μl 1 N sodium hydroxide, 100 μl internal standard (500 ng LY293558/μl water) and 4 ml acetonitrile were added. The sample was homogenized using a Tissumizer and then centrifuged at ~2000 rpm for 10 min. An aliquot (1 ml) of supernatant was removed and evaporated to dryness. The remaining residue was dissolved in 0.5 ml acetonitrile/water (30:70, v/v), filtered and transferred to a 96-well microtiter plate.

Quantitation was achieved utilizing a Shimadzu model LC-10AD pumps, model Sil-10AXL autosampler and model SCL-10A system controller with an Inertsil column of ODS-3 50 × 2.1 mm, 5 mm. A MicroMass Quattro Electrospray, positive mode, SRM transitions were 317.9 → 272.0 for LY382884 and 279.89 → 138.0 for LY293558 (internal standard). The following mobile phases were used: Mobile phase A: 100 mM formic acid; Mobile phase B: 40 mM formic acid in 10/10/80 water/isopropanol/methanol (1:1, v/v); and Mobile phase C: 40 mM formic acid. Flow rate was 0.2 ml/min and the injection volume was 25 μl. The detection limit for plasma was 5 ng LY382884/ml and for brain it was 5.0 ng/g. A standard curve for plasma was prepared from 5 to 2000 ng/ml while for brain the standard curve was from 5 to 250 mg/ml.

1.4. Vogel conflict test

1.4.1. Subjects

Experimentally naive adult male Sprague–Dawley rats (Harlan Industries, Indianapolis, IN), weighing between 200 and 300 g, were used as subjects. The rats were housed in Plexiglas cages (4 per cage), and given free access to Lab Diet #5001 for rodents (PMI Nutrition International Inc., St. Louis, MO). Water was withheld for 20–24 h prior to the first training session. A 12-h light/dark cycle was maintained, and all experimental sessions were conducted during the light phase of the cycle at about the same time each day. All experiments were conducted in accordance with the NIH regulations of animal care covered in ''Principles of Laboratory Animal Care'', NIH Publication 85-23, and were approved by the Institutional Animal Care and Use Committee.

1.4.2. Apparatus

The experiments were conducted using operant behavior test chambers ENV-007 (Med Associates Inc., Georgia, Vermont, USA), 30.5 × 24.1 × 29.2 cm. The test chambers were contained within light and sound attenuating shells. On the front wall of the chamber, a food trough was mounted 2 cm off the grid floor on the centerline. Two response levers were centered 8 cm off the centerline and 7 cm off the grid floor. Three lights were located above each response lever at 15 cm off the grid floor. Responding on the levers was without consequences for all sessions. On the rear of the chamber, a sipping tube was mounted 3 cm off the grid floor and 3 cm from the door. The sipping tube was wrapped with electrical tape to prevent the circuit from being completed if the animals were holding/touching the tube. All events were controlled and