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LY2456302 is a novel, potent, orally-bioavailable small molecule kappa-selective antagonist with activity in animal models predictive of efficacy in mood and addictive disorders

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ABSTRACT

Kappa opioid receptors and their endogenous neuropeptide ligand, dynorphin A, are densely localized in limbic and cortical areas comprising the brain reward system, and appear to play a key role in modulating stress and mood. Growing literature indicates that kappa receptor antagonists may be beneficial in the treatment of mood and addictive disorders. However, existing literature on kappa receptor antagonists has used extensively JDTic and nor-BNI which exhibit long-lasting pharmacokinetic properties that complicate experimental design and interpretation of results. Herein, we report for the first time the *in vitro* and *in vivo* pharmacological profile of a novel, potent kappa opioid receptor antagonist with excellent selectivity over other receptors and markedly improved drug-like properties over existing research tools. LY2456302 exhibits canonical pharmacokinetic properties that are favorable for clinical development, with rapid absorption (t_{max}: 1–2 h) and good oral bioavailability (F = 25%). Oral LY2456302 administration selectively and potently occupied central kappa opioid receptors *in vivo* (ED_{50} = 0.33 mg/kg), without evidence of mu or delta receptor occupancy at doses up to 30 mg/kg. LY2456302 potently blocked kappa-agonist-mediated analgesia and disruption of prepulse inhibition, without affecting mu-agonist-mediated effects at doses >30-fold higher. Importantly, LY2456302 did not block kappa-agonist-induced analgesia one week after administration, indicating lack of long-lasting pharmacodynamic effects. In contrast to the nonselective opioid antagonist naltrexone, LY2456302 produced antidepressant-like effects in the mouse forced swim test and enhanced the effects of imipramine and citalopram. LY2456302 reduced ethanol self-administration in alcohol-preferring (P) rats and, unlike naltrexone, did not exhibit significant tolerance upon 4 days of repeated dosing. LY2456302 is a centrally-penetrant, potent, kappa-selective antagonist with pharmacokinetic properties favorable for clinical development and activity in animal models predictive of efficacy in mood and addictive disorders.

Non-standard abbreviations: CREB, cyclic adenosine monophosphate-response element binding protein; DAMGO, [D-Ala^2, N-MePhe^4, Gly-ol]-enkephalin; DPDPE, [d-Pen^2,d-Pen^5]-enkephalin; ERK, extracellular regulated kinase; GR103545, methyl (3R)-4-[(3,4-dichlorophenyl)acetyl]-3-(pyrrolidin-1-ylmethyl)piperazine-1-carboxylate; JDTic, (3R)-7-hydroxy-N-{(1S)-1-{[(3R,4R)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl}-1,2,3,4-tetrahydro-3-isoquinoline-carboxamide; LY2048978, 4-{4-[(pentylamino)methyl]phenoxy}benzamide; LY2456302, [(S)-3-fluoro-4-(4-((2-(3,5-dimethylphenyl)pyrrolidin-1-yl)methyl)phenoxy)benzamide]; MAP kinase, mitogen-activated protein kinase; MR2034, (2R,11R)-1,2,3,4,5,6-hexahydro-6,11-dimethyl-3-[[(R)-tetrahydrofuran-2-yl]methyl]-2α,6α-methano-3-benzazocin-8-ol; PPI, prepulse inhibition; U-69593, *N*-methyl-2-phenyl-*N*-[(5*R*,7*S*,8*S*)-7-(pyrrolidin-1-yl)-1-oxaspiro[4,5]dec-8-yl]acetamide.

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1. Introduction

Major depression, characterized by negative mood, reduced motivation, and sometimes anhedonia and decreased energy, affects nearly 5% of people worldwide each year. Current antidepressants work well for some patients, but ~60% suffer from unresolved residual symptoms or inadequate treatment response (Thase et al., 2001). As a result, some patients may abuse or misuse alcohol or other drugs in an attempt to reduce their depressive symptoms, often termed "self-medication" (Bolton et al., 2009). A recent study estimated that 16% of depressed patients also have a diagnosable addiction disorder (Sher et al., 2008). Such comorbidity puts patients at greater risk. Comorbid substance use in depressed patients is associated with greater symptom severity, inadequate treatment response, poorer prognosis (including increased risk of suicide), and persistence of depressive symptoms (Thase et al., 2001; Blanco et al., 2012). Therefore, a tremendous need exists for pharmacotherapies effective in treating both depressive symptoms and alcohol dependence.

Kappa opioid receptors and their endogenous neuropeptide ligand, dynorphin A, are densely localized in limbic and cortical areas comprising the brain reward and stress systems, and play a key role in modulating neurotransmission in these areas (Mansour et al., 1987, 1994; Margolis et al., 2006). In preclinical models, stress produces a prodepressive phenotype that is believed to be associated with the activation of kappa opioid receptors and subsequent downstream signaling events (Pliakas et al., 2001; Newton et al., 2002; McLaughlin et al., 2003; Shirayama et al., 2004; Land et al., 2008). Consistent with this hypothesis, kappa receptor agonists produce anxiogenic- and prodepressive-like effects in animals and humans (Pfeiffer et al., 1986; Todtenkopf et al., 2004; but see also Harden et al., 2012), whereas kappa receptor antagonists reliably exhibit antidepressant-like effects in animal models predictive of efficacy in the domains of mood and affect (Mague et al., 2003; Land et al., 2009; Carr et al., 2010). Unfortunately, there have been no reports of selective kappa receptor antagonists administered in clinical populations.

Kappa-selective antagonists also reduce ethanol intake and reinstatement in a number of preclinical paradigms (Deehan et al., 2012; Walker and Koob, 2008). While nonselective opioid antagonists such as naltrexone, an FDA-approved medication for alcohol dependence, are efficacious in animal models of alcoholism, they do not produce reliable antidepressant- or anxiolytic-like effects in animals or humans, likely due to functional opposition between mu and kappa receptors (Margolis et al., 2003; Spanagel et al., 1992). Similarly, antidepressants are weakly and inconsistently effective at reducing alcohol consumption in depressed patients with comorbid addictive disorders (Kranzler et al., 2006; Pettinati et al., 2010). Because kappa antagonists demonstrate efficacy in animal models predictive of efficacy in mood and addictive disorders, they have the potential to treat depressed patients with comorbid alcohol dependence.

Molecules targeting specific biological mechanisms are powerful tools for elucidating biological function. Most current knowledge of the kappa opioid system comes from studies on the prototypical antagonists, norbinaltorphimine (nor-BNI) and (3R)-7-Hydroxy-N-{(1S)-1-{[(3R,4R)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl}-1,2,3,4-tetrahydro-3-isoquinoline-carboxamide (JDTic), which have unusual pharmacokinetic properties, including delayed onset of centrally-mediated effects (24–48 h) and very long duration of pharmacodynamic effects (28+ days; Munro et al., 2012; Patkar et al., 2013), that complicate research design and interpretation of the results. Long-duration activity of an antagonist can result in biological consequences that are different from that of short-term receptor blockade; differential biochemical modifications occur at the receptor level and at down-stream targets as a result of prolonged gating of agonist from the receptor. In order to expand and crystallize our current understanding of the biological basis of kappa opioid receptor function, the purpose of the present experiments was two-fold: first, to pharmacologically characterize (S)-3-fluoro4-(4-((2-(3,5-dimethylphenyl)pyrrolidin-1-yl)methyl)phenoxy)benzamide (LY2456302) as an improved research tool for studying the kappa opioid receptor system; and second, to examine its antidepressant-like effects and its ability to decrease ethanol consumption.

2. Materials and methods

2.1. Drugs and reagents

(S)-3-fluoro-4-(4-((2-(3,5-dimethylphenyl)pyrrolidin-1-yl)methyl)phenoxy) benzamide (LY2456302; Diaz Buezo et al., 2009; Mitch et al., 2011), LY2048978, JDTic, and GR103545 were synthesized at Lilly Research Laboratories. Naltrexone HCl, DAMGO acetate, DPDPE hydrate, naltriben methanesulfonate hydrate, U-69593, morphine sulfate, imipramine HCl, chlordiazepoxide HCl, phencyclidine HCl, and formalin solution (10%, diluted to 5%) were purchased from Sigma Aldrich (St. Louis, MO). LY2456302, LY2048978, JDTic, and naltrexone were dissolved in water with the addition of 85% lactic acid. U-69593 was dissolved in 12.5% 2-hydroxypropyl-β-cyclodextrin (formalin study) or sterile water (prepulse inhibition studies). Morphine, phencyclidine, chlordiazepoxide, and imipramine were prepared in sterile water. For *in vitro* assays, DAMGO, U-69593, DPDPE, and naltriben were prepared in a buffer consisting of 20 mM HEPES, pH 7.4, 5 mM $MgCl_2$, 100 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol. Drugs were mixed fresh on the day of dosing. Doses were administered to rats in a volume of 1 or 2 ml/kg; doses were administered to mice in a volume of 10 ml/kg. Doses, routes of administration, and pretreatment times are indicated separately for each experiment. Unless otherwise indicated, experiments were conducted in non-fasted animals. All experiments were conducted in compliance with the Guide for the Care and Use of Laboratory Animals under protocols approved by a local animal care and use committee.

2.2. In vitro receptor binding and functional activity

In vitro opioid receptor binding and [^{35}S]-GTPγS binding experiments were conducted as previously described (Mitch et al., 2011). Briefly, radioligand displacement studies with [^{3}H]diprenorphine were carried out using membranes prepared from CHO cells expressing cloned human κ and μ opioid receptors or HEK293 cells expressing the cloned δ opioid receptor. Concentrations causing 50% inhibition (IC50) of [^{3}H]-diprenorphine binding were determined from 11-point concentration response curves in assay buffer containing sodium and guanosine diphosphate (GDP). Naltrexone was included as a control at 10 μM to define nonspecific binding and was also tested as a comparator molecule in concentration response curves.

2.3. Rat and mouse plasma exposure and unbound fraction in plasma and brain

Three male cannulated rats were administered a single 1 mg/kg intravenous (IV) and 10 mg/kg oral (PO) dose of LY2456302 to determine the pharmacokinetic parameters. Plasma samples were collected at 0.08 (IV only), 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h post-dose and analyzed by liquid chromatography coupled to tandem mass spectral detection (LC–MS/MS) to determine the concentrations of LY2456302. Male mice (n = 3 per time point) were administered a single 10 mg/kg PO dose of LY2456302 to determine the pharmacokinetic parameters. Plasma samples were collected at 0.5, 1, 2, 4, 8, and 24 h post-dose and analyzed by LC–MS/MS to determine the concentrations of LY2456302. The plasma and brain binding of LY2456302 was determined by equilibrium dialysis at 1 μM.

2.4. In vivo receptor occupancy

The ability of orally administered LY2456302 to occupy brain mu, delta, and kappa opioid receptors *in vivo* was assessed in male Sprague-Dawley (SD) rats (Harlan, Indianapolis, IN), weighing 250–300 g, n = 4/dose. Kappa opioid occupancy was measured in male NIH-Swiss mice (Harlan, Indianapolis, IN), weighing 25–30 g, n = 4/dose. Receptor occupancy (RO) was determined 90 min after a PO dose of LY2456302 by measuring displacement of unlabeled tracers by LC–MS/MS (Need et al., 2007). Tracers for mu, kappa, and delta receptors, were naltrexone (10 μg/kg), naltriben (10 μg/kg), and GR103545 (1.5 μg/kg), respectively, administered as a single IV injection (Need et al., 2007). In a separate study, RO was determined by LC–MS/MS in male SD rats (Harlan, Indianapolis, IN, 250–300 g, n = 4/dose) at 1, 4, 8, and 48 h after a 10 mg/kg PO dose of LY2456302. Total binding was represented by levels of tracers in the striatum for delta and kappa receptors, and thalamus for the

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