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Synergistic antidepressant-like effects between a kappa opioid antagonist (LY2444296) and a delta opioid agonist (ADL5859) in the mouse forced swim test

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ABSTRACT

Kappa opioid (KOP) receptor antagonists and delta opioid (DOP) receptor agonists have antidepressant-like effects in animal tests and may be useful for treatment-resistant depression in humans. In this study, we examined whether the combination of a KOP receptor antagonist and a DOP receptor agonist would produce a better than additive effect (i.e. synergy). LY2444296 is a short-acting selective nonpeptide KOP receptor antagonist. ADL5859 is a selective nonpeptide DOP receptor agonist which does not produce seizures and EEG disturbances. Each compound and combinations of the two were examined in the forced swim test (FST) one h post injection, a screening test for antidepressant-like effect, in male adult C57BL/6J mice (Jackson Lab). LY2444296 [subcutaneous (s.c.) injection] at 10 and 30 mg/kg, but not 3 mg/kg, significantly decreased immobility time in a dose-dependent manner. Intraperitoneal (i.p.) injections of ADL5859 also reduced immobility time dose-dependently at doses of 3 and 10 mg/kg, but not at 1 mg/kg. An analysis was conducted using the method of Tallarida and Raffa (2010), which employed dose equivalence. The relative potency of the drugs was determined to be LY2444296: ADL5859 = 1:0.28, which was the dose ratio for combination studies. Six combinations of the two compounds were tested in mice at a fixed dose ratio. We found that LY2444296 and ADL5859 yielded significant synergistic effects for the antidepressant-like effect at the combined dose ranging from 3.84 mg/kg to 9.0 mg/kg. ADL5859 (10 mg/kg), LY2444296 (30 mg/kg) and their combined dose (3.84 mg/kg) had no effects on locomotor activities. Since the two drugs have distinct pharmacological profiles, such a synergism will allow use of lower doses of both drugs to achieve desired antidepressant effects with fewer side effects.

1. Introduction

The dynorphin/κ opioid (KOP) receptor system has been demonstrated to mediate negative emotional states. Activation of the KOP receptor by selective agonists produces depression and dysphoria in humans (Barber and Gottschlich, 1997; Pfeiffer et al., 1986) and conditioned place aversion in animals (Shippenberg et al., 2007). In laboratory animals, many stress-induced behavioral responses are mediated by the KOP receptor (McLaughlin et al., 2006; McLaughlin et al., 2003) and prolonged stress produces depression-like behaviors [see Bruchas et al. (2010) for a review]. Several groups have reported that in animal tests KOP receptor antagonists, including norbinaltorphimine (norBNI), JDTic and DIPPA, have antidepressant-like effects (Beardsley et al., 2005; Carr et al., 2010; Mague et al., 2003; Reindl et al., 2008; Shirayama et al., 2004; Zhang et al., 2007). Both nor-BNI and JDTic have slow onsets of maximal KOP receptor antagonist actions (24–48 h) and show very long durations of action, lasting more than two weeks after a single injection (Carroll et al., 2004; Endoh et al., 1992; Horan et al., 1992). In addition, the short-acting KOP receptor antagonists PF-04455242 (Grimwood et al., 2011) and LY2456302 (Rorick-Kehn et al., 2014) were shown to have antidepressant-like effects. The selective KOP receptor antagonist CERC-501 (also known as LY2456302) has passed phase I clinical trial (Lowe et al., 2014) and is currently in phase II trial for adjunctive treatment of major depressive disorder and for substance use disorders (e.g., nicotine, alcohol, and/or cocaine) (http://www.cerecor.com/pipeline/cerc-501.php).

The enkephalin/delta opioid (DOP) receptor system has been shown to be involved in emotional responses. Mutant mice devoid of the DOP receptor or preproenkephalin gene displayed enhanced

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depression- and anxiety-like behaviors (Filliol et al., 2000; Konig et al., 1996; Ragnauth et al., 2001). Several selective DOP receptor agonists reduced depressive-like behaviors in animal tests, including SNC80, NIH11082, UFP512, KNT-127, and AZD2327 [for a review, see Chung and Kieffer (2013)]. The antidepressant-like effects were comparable to those produced by selective serotonin reuptake inhibitors and tricyclic antidepressants (Naidu et al., 2007; Saitoh et al., 2004). DOP receptor agonists decreased anxiety- and depression-like behaviors in mice withdrawn from alcohol and cocaine (Ambrose-Lanci et al., 2010; Perrine et al., 2008; van Rijn et al., 2010).

The DOP receptor and KOPR receptor have distinct distributions in the brain (Mansour et al., 1988). KOPR antagonists and DOPR agonists produce antidepressant-like effects in rodents by acting on some similar and some distinct brain regions. Sites of action of KOPR antagonists include the ventral tegmental area, nucleus accumbens, hippocampus, prefrontal cortex and dorsal raphe nucleus [reviewed in Van't Veer and Carlezon (2013)], whereas those of DOPR agonists are cingulate, frontal and insular cortices, hippocampus, nucleus accumbens, and amygdala [reviewed in Chung and Kieffer (2013)]." We thus hypothesized that a DOP agonist and a KOPR antagonist may have synergistic antidepressant-like effects. In this study, we tested the hypothesis by examining if combinations of a KOP receptor antagonist (LY2444296) and a DOP receptor agonist (ADL5859) exhibited synergistic antidepressant-like effects in the mouse forced swim test. LY2444296, an analogue of LY2456302, is a selective short-acting KOP receptor antagonist with a Ki value of ~1 nM for the KOP receptor and κ/μ and κ/δ selectivity of ~60 and ~350, respectively [compound 25 in Mitch et al. (2011)]. ADL5859 is a highly selective nonpeptide DOP receptor agonist with a Ki value of 0.8 nM for the DOP receptor and δ/κ and δ/μ selectivity > 1000 (Le Bourdonnec et al., 2008). Unlike the prototypic selective nonpeptide DOP receptor agonists BW373U86 and SNC80, ADL5859 does not induce seizures and electroencephalogram (EEG) disturbances in rodents (Chung et al., 2015). In addition, ADL5859 has passed phase I clinical trials demonstrating its safety in humans, but it was shown to be ineffective in reducing osteoarthritis pain in phase II trials (https://clinicaltrials.gov/ct2/show/NCT00979953). Its safety in humans makes it attractive for further investigation as an anti-depressant.

2. Materials and methods

2.1. Drugs

ADL5859 hydrochloride, purchased from MedChem Express (Monmouth Junction, NJ), was prepared in deionized water. LY2444296, a generous gift from Eli Lilly and Co. (Indianapolis, IN), was dissolved in 85% DL- lactic acid (20 μl per mg compound), then diluted with saline by vortex, and lastly added with 1N NaOH (150 μl per mg compound) by vortex for final pH~5. Both drugs were prepared freshly on the test days and mixed thoroughly before syringe aspiration. ADL5859 [intraperitoneal (i.p.) injection], LY2444296 [subcutaneous (s.c.) injection] or their combination was administered in a volume of 10 mL/kg to animals 60 min prior to testing, while control animals received injections of water i.p., saline s.c. or water i.p. plus saline s.c..

2.2. Animals

Male adult C57BL/6J mice (~23 g) were purchased from The Jackson Laboratory (Bar Harbor, ME). The total number of mice used was 230 (see figure legends for details). After arrival, mice were habituated to the animal facility for about 7 days before any experiments. Mice were housed under a 12-h light/dark cycle with food and water available *ad libitum*. Other housing conditions were as follows: cage dimension of $32 \times 18 \times 15$ cm^3, 5 mice per cage without enrichment and housing room temperature of 21 ± 1 °C. Experimental procedures were approved by the Temple University Institutional Animal Care and Use Committee.

2.3. Forced swim test (FST)

FST in mice or rats has been commonly used for screening of antidepressant activities of drugs. It should be noted that it is a screening test, but not a model of depression. FST in mice was performed as described by Lucki et al. (2001). Each mouse was used only once. On the day of experiment, mice were allowed to acclimate to the test room for one h. All experimental sessions were conducted between 1:00 and 6:00 pm. Mice were injected with vehicle or a dose of LY2444296 (s.c.) or ADL5859 (i.p.) or a combination of both drugs. Sixty min later, mice were placed for 6 min in a cylindrical tank (46 cm tall x 20 cm diameter) of 23–25 °C water filled to a depth of 15 cm. The swim sessions were videotaped for later analysis by the experimenter. Some of the FST videos were also scored by other experienced researchers in the lab who were blind to the treatments. The scores obtained were consistent with the data shown. Duration of immobility (minimum movements necessary to stay afloat) in the last 4-min of the swim was measured. A reduction of immobility in the swim session (relative to vehicle controls) is used as a measure of antidepressant-like effects.

2.4. Determination of drug combination effects

From the dose-effect data of the individual compounds, we selected doses for joint application (in a fixed ratio based on the individual potency values). Subsequent analysis of the combination dose-effect data gave combination dose-effect values for comparison with the expected (additive) effect. Getting the additive effect employs the concept of ***dose equivalence*** (the basis of the *isobole* method) (Tallarida and Raffa, 2010). That methodology calculates the expected (additive) effect of each dose combination and thus allows comparison of these effects (statistically) with the observed combination effect. The calculation proceeds from using each dose of ADL5859 in the combination and finding its equivalent dose of LY2444296 (the higher efficacy drug). That equivalent plus the actual quantity of LY2444296 allows calculation of the expected effect by use of LY's dose-effect equation. If the observed effect is greater than the calculated expected effect then that interaction is synergistic. Combinations that give the expected effect are termed *additive*, while those combinations that give effects less than expected are termed *sub-additive. Synergy* means that the observed effect of the combination is greater than the expected.

2.5. Locomotor activities

Motor activities were measured using a Digiscan D Micro System (Accuscan, Columbus, OH, USA) and eight individual activity monitors. A single activity monitor consists of an aluminum frame equipped with 16 horizontal infrared light beams and detectors where the activity chamber (a standard clear plastic animal cage, 42 cm × 20 cm × 20 cm) is placed. As the mouse moves within the chamber, light beams are broken and recorded by a computer connected to the Digiscan system. Activity was recorded as total activity, ambulatory activity and stereotypy. Total activity represents all beam breaks by a single mouse, and is the sum of the ambulatory and stereotypy counts. Ambulatory activity represents successive beam breaks. Stereotypic counts identify repeated breaks of the same beam indicative of a stationary animal engaged in a repetitive behavior as opposed to ambulation, but they do not