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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.

Keywords

kappa opioid antagonist; delta opioid agonist; antidepressant; synergy; forced swim test

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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 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-hr light/dark cycle with food and water available *ad libitum*. Other housing conditions were as follows: cage dimension of 32×18×15 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 × 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, 42cm×20cm×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 identify a specific stereotypic behavior. Activity was measured over 1.5 h post injections with 5-min collection intervals under normal laboratory lighting conditions.

2.6. Data analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni *post hoc* tests (Prism v.5, GraphPad, San Diego, CA) for results of behavioral tests and by paired Student's *t* test for synergy analysis. Each value is expressed as the mean ± S.E.M.

3. Results

3.1. ADL5859 and LY2444296 show dose-dependent antidepressant-like effects

Sixty min following i.p. injection, ADL5859 at 3 and 10 mg/kg, but not at 1 mg/kg, reduced immobility time of mice in the FST in a dose-dependent manner (Fig. 1A). One h after subcutaneous (s.c.) injection of LY2444296, mice displayed significantly decreased

immobility time in the FST at 10 and 30 mg/kg in a dose-dependent fashion, but not 3 mg/kg (Fig. 1B). The data were analyzed with nonlinear regression and produced excellent fits to the dose-effect curves which are $E = 60.7\ D / (D + 2.2)$ for ADL5859 and $E = 81.5\ D / (D + 16.0)$ for LY2444296, where D is dose and E is effect (Fig. 2). The equations so derived provide the potencies.

3.2. Synergistic antidepressant-like effects of combinations of ADL5859 and LY2444296

The potencies of the individual compounds serve as a guide in the selection of the dose ratio to be tested and thus the individual dose – effect curves suggested the dose ratio LY2444296: ADL5859 = 1: 0.28.

Six combinations of the two compounds were tested in mice at this fixed dose ratio (LY2444296: ADL5859=1:0.28) (Fig. 3). Sub-active or active doses of ADL5859 i.p. and LY2444296 s.c. led to significant reduction of mouse immobility for 5 out of 6 drug combinations (Fig.3). The combined active doses ranged from 3.84 mg/kg to 9.0 mg/kg. For each of these dose combinations there is an expected (additive) effect that was calculated from the dose equivalence procedure of (Tallarida and Raffa, 2010) (Fig. 4). Comparison of the observed effects and the expected effects indicates that these two drugs in combination produce significant synergistic antidepressant-like effects at these low doses (Fig. 4). Notably, the combination of 0.84 mg/kg ADL5859 and 3 mg/kg LY2444296 showed as great an effect as higher dose combinations although either one alone barely had any effect (See Fig.1).

3.3. Effects of ADL5859, LY2444296 or combined administration on locomotor activities

We then examined if the reduction in immobility in the FST of ADL5859, LY2444296 or combined administration was due to enhanced locomotor activity. Mice were habituated in the locomotor chambers for 2 h, and then injected with water (i.p.), ADL5859 (i.p., 10 mg/kg), LY2444296 (s.c., 30 mg/kg) or ADL5859 (i.p., 0.84 mg/kg) plus LY2444296 (s.c., 3 mg/kg). After drug administration, mouse activities were recorded continuously for 90 min. Thirty-min cumulative data from 60-90 min post-injections and five-min data 60-65 min post-injections were presented and analyzed, and either drug or the combination did not have significant effects on mouse locomotor activities (Fig. 5A, 5B). Thus, the effect of either drug or combination is not due to enhanced locomotor activity.

4. Discussion

We have shown that LY2444296 and ADL5859 at a fixed dose ratio of 1:0.28 have highly synergistic antidepressant-like effects in the FST at combined doses as low as 3.84 mg/kg. To the best of our knowledge, this is the first to show that a KOP receptor antagonist and a DOP receptor agonist have synergistic antidepressant-like effects. The findings have implications for development of these classes of drugs for clinical applications.

Drug combinations have often been employed clinically; however, rigorous studies on the doses used in combinations that would produce synergy are not performed regularly. Classifying an interaction as we did here is a quantitative pursuit. It does not require a description of mechanism. All that is required are the dose-response curve of each drug

acting alone and the dose-response curve of the combination. In most cases these will be drugs that give overtly similar effects. When a combination of such two drugs or compounds show synergism, both drugs can be used at lower doses, which likely lead to reduced side effects. For example, the combination of tramadol and acetaminophen at a fixed dose ratio have synergistic analgesic effects, and the combination was patented and further developed as the combination drug marketed as ULTRACET®. There are many drug combination studies in animals, in which both drugs are active or only one is active for the pharmacological end point. For example, Fairbanks and Wilcox (1999) demonstrated that either morphine or clonidine administered intrathecally had antinociceptive effect and when given at a dose ratio of 1:20 or 1:2 the combination displayed synergistic antinociceptive effects. Rawls et al. (2004) reported that the NO synthetase inhibitor L-NAME had no effect on body temperature, but it greatly potentiated the hypothermic effect of the cannabinoid agonist WIN 55212-2.

This is the first demonstration that ADL5859 has antidepressant-like effects. This finding is consistent with many previous studies showing that selective DOP receptor agonists reduced depressive-like behaviors in the FST, tail suspension test and learned helplessness. The nonpeptide DOP receptor agonists examined include BW373U86 (Broom et al., 2002b), SNC80 (Broom et al., 2002b; Saitoh et al., 2004), KNT-127 (Nozaki et al., 2014; Saitoh et al., 2011) and AZD2327 (Hudzik et al., 2011). The peptide agonists DPDPE, JOM-13, deltorphin II, and H-Dmt-Tic-NH-CH2-Bid (Torregrossa et al., 2006), NIH11082 (Naidu et al., 2007) and UFP-512 (Vergura et al., 2008) also displayed antidepressant-like effects. Like KNT-127, but unlike BW363U86 and SNC-80, ADL5859 does not cause convulsions (Broom et al., 2002a; Chung et al., 2015; Comer et al., 1993; Saitoh et al., 2011).

Our finding is the first one to show that LY2444496 exhibits antidepressant-like activity. This observation is consistent with several previous reports that KOP receptor antagonists have antidepressant-like activity in animal tests, including norBNI, JDTic, DIPPA, PF-04455242 and LY2456302 (Beardsley et al., 2005; Carr et al., 2010; Grimwood et al., 2011; Mague et al., 2003; Reindl et al., 2008; Rorick-Kehn et al., 2014; Shirayama et al., 2004; Zhang et al., 2007). LY2444496 has a short duration of action, similar to PF-04455242 (Grimwood et al., 2011) and LY2456302 (Rorick-Kehn et al., 2014), but different from long acting KOP receptor antagonists, such as norBNI and JDTic (Carroll et al., 2004; Endoh et al., 1992; Horan et al., 1992).

For the FST, we used C57BL/6J mice from Jackson Labs. This strain has been used previously to demonstrate the anti-depressant-like effects of KOP receptor antagonists in the mouse FST (Falcon et al., 2015; McLaughlin et al., 2003).

The mechanisms underlying this synergistic interaction remain to be determined. The KOP receptor in several mesolimbic areas are involved in KOP receptor-mediated depressive-like behaviors, including the ventral tegmental area, dorsal raphe nucleus, nucleus accumbens, hippocampus and prefrontal cortex [reviewed in (Bruchas et al., 2010; Van't Veer and Carlezon, 2013)]. Carr et al. (2010) reported that the piriform cortex and nucleus accumbens shell in WKY rats play important roles in antidepressant-like effect of the KOP receptor antagonist norBNI. DOP receptor agonists act on the frontal, cingulate and insular cortices,

hippocampus, amygdala and nucleus accumbens to regulate emotional responses [reviewed in (Chung and Kieffer, 2013)]. It is likely that the two drugs may act on different neuronal circuitries to produce synergistic antidepressant-like effects. In addition, there may be interactions of the effects of the two drugs at the cellular levels in some of the regions. The synergistic antidepressant-like effect of ADL5859 and LY2444296 may also be due to pharmacokinetic interactions. Whether this synergistic effect is generally applicable to combinations of other DOP receptor agonists and KOP receptor antagonists remains to be investigated.

In conclusion, ADL5859, a DOP receptor agonist, and LY2444296, a KOP receptor antagonist, produced synergistic antidepressant-like effects in the mouse FST at a fixed dose ratio of 0.28 to 1. Such a synergism will allow use of lower doses of both drugs to achieve desired effects. Studies to examine if these two drugs display synergistic anxiolytic-like effects will be conducted.

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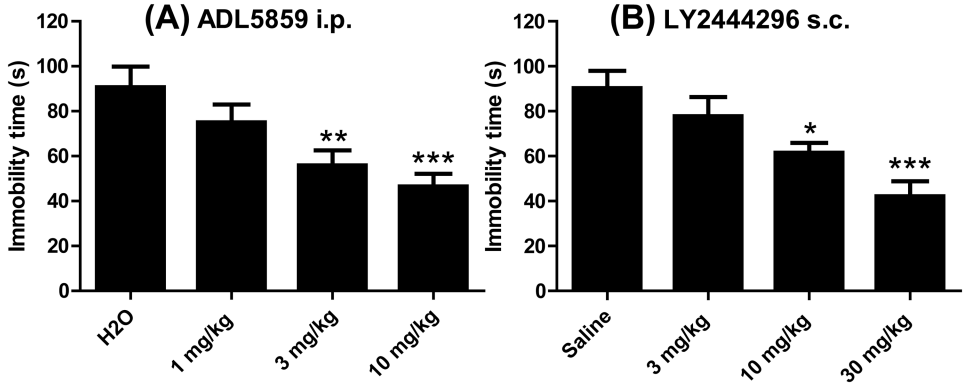


Figure 1. The DOR agonist ADL5859 and the KOR antagonist LY2444296 showed antidepressant-like effects in the FST in C57BL/6J mice

One h after drug administration, (A) ADL5859 (i.p.) and (B) LY2444296 (s.c.) dose-dependently reduced immobility time (in sec), indicating antidepressant-like effects. ***P<0.001, **P<0.01 and *P<0.05, compared with the respective vehicle groups (n=10-15 per group), by one-way ANOVA followed by Bonferroni's multiple comparison tests (Prism 5). The animal numbers for each treatment were as follows: in (A), H_2O (n=14), 1 mg/kg (n=13), 3 mg/kg (n=12) and 10 mg/kg (n=12); in (B), H_2O (n=15), 3 mg/kg (n=12), 10 mg/kg (n=14) and 30 mg/kg (n=10).

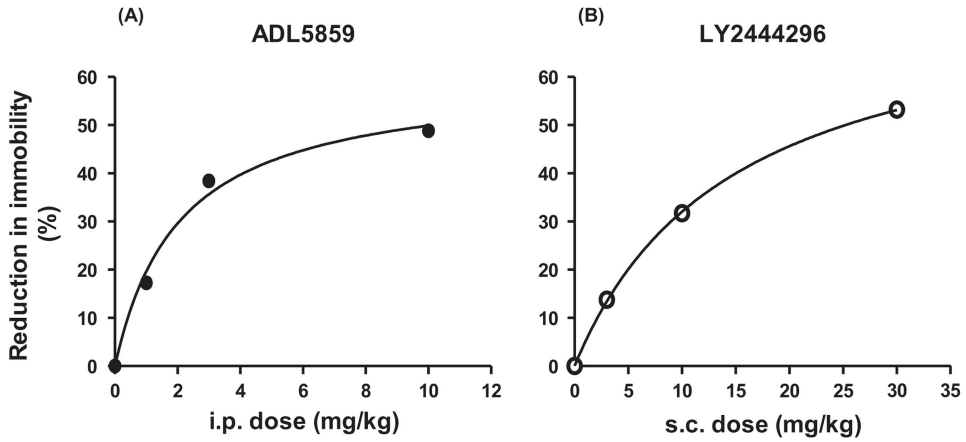


Figure 2. Dose-effect curves of ADL5859 (left) and LY2444296 (right) in the FST where the effect is the magnitude (%) of the decrease in immobility time

The data in Fig. 1 were transformed into % reduction in immobility and were fitted to the dose-effect curves by nonlinear regression.

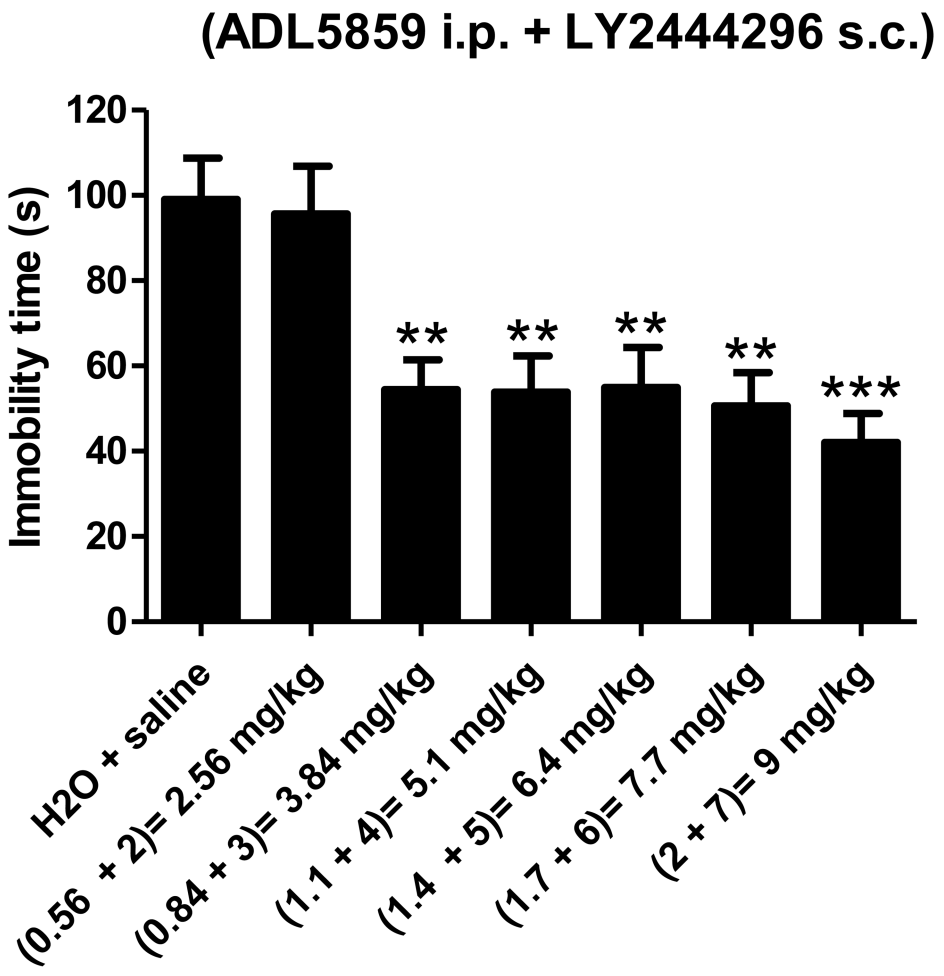


Figure 3. Effects of co-administration of a fixed dose ratio of LY2444296 and ADL5859 (1:0.28) on immobility time in the FST in mice

LY2444296 and ADL5859 were administered at indicated dose combinations and 60 min later, the FST was performed on mice (n=10-15/group). ***P<0.001, **P<0.01, compared with the vehicle group, by one-way ANOVA followed by Bonferroni's multiple comparison tests. The animal numbers for each treatment were as follows: H_2O + saline (n=15), 2.56 mg/kg (n=11), 3.84 mg/kg (n=15), 5.1 mg/kg (n=10), 6.4 mg/kg (n=15), 7.7 mg/kg (n=13) and 9 mg/kg (n=13).

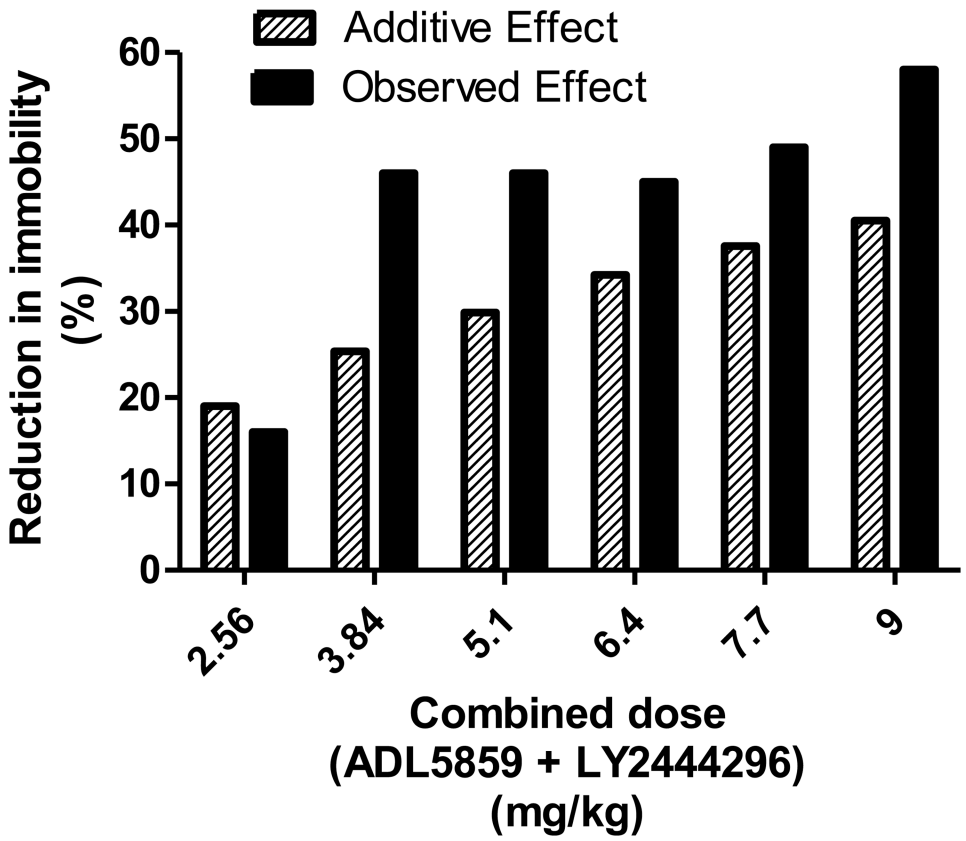
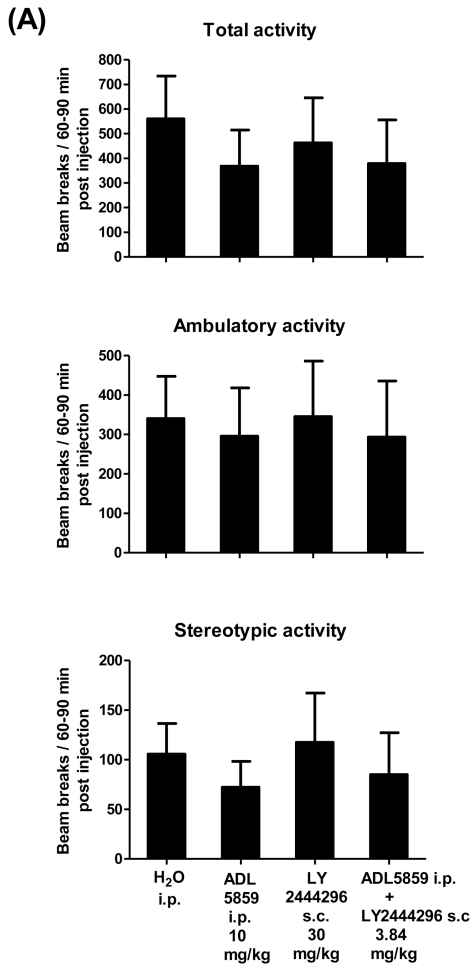


Fig. 4. Observed (red) and expected / additive (black) effects of combinations of ADL5859 and LY2444296 shown on horizontal scale as the dose sum

For each of these dose combinations, expected (additive) effects were calculated from the dose equivalence procedure as described in Methods. Expected and observed effects were compared by the application of *paired Student's t test* and the results shows that the paired differences are significantly different (P = 0.015, comparing all six combined doses; P = 0.0012, comparing five combined doses except for 2.56 mg/kg), thereby indicating synergy.



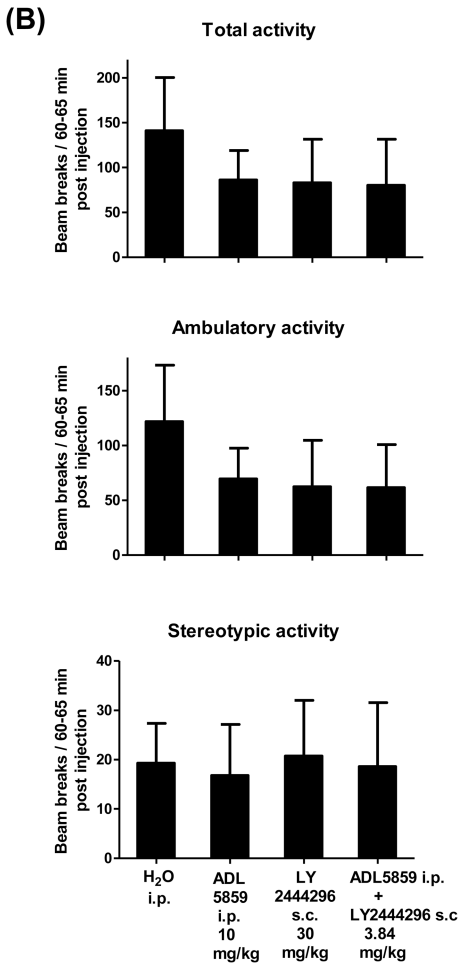


Fig. 5. Effects of ADL5859, LY2444296 or their combined administration vs water on locomotor activity in mice

Mice were habituated in the locomotor chambers for 2 h first and then injected with water or drug(s). Total, ambulatory and stereotypic activities were continuously monitored after injections for 90 min using automated locomotor chambers. Data are expressed as mean ± S.E.M. beam breaks per 30-min period (60-90 min post injections) in (A) or per 5-min period (60-65 min post injections) in (B) and analyzed by one-way ANOVA. n = 8-10/group. The animal numbers for each treatment are as follows: H_2O (n=10), ADL5859 10 mg/kg (n=10), LY2444296 30 mg/kg (n=8) and the combined dose 3.84 mg/kg (n=8).