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$mGlu_2$ receptor-mediated modulation of conditioned avoidance behavior in rats

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ABSTRACT

Inhibition of conditioned avoidance behavior in rats is generally considered predictive for antipsychotic activity in man. The present study investigated the $mGlu_2$-mediated modulation of conditioned avoidance and compared $mGlu_2$ agonists with available antipsychotics for their relative effects on conditioned avoidance behavior and locomotion. The $mGlu_{2/3}$ orthosteric agonist 4-amino-2-thiabicyclo [3.1.0]hexane-4,6-dicarboxylic acid 2,2-dioxide (LY-404039) and $mGlu_2$ positive allosteric modulator (PAM) 3-(cyclopropylmethyl)-7-(4-phenylpiperidin-1-yl)-8-(trifluoromethyl)[1,2,4]triazolo[4,3-a]pyridine (JNJ-42153605) inhibited avoidance and blocked escape behavior. The $mGlu_{2/3}$ negative allosteric modulators (NAMs) 7-(dimethylamino)-4-(3-pyridin-3-ylphenyl)-8-(trifluoromethyl)-1,3-dihydro-2H-1,5-benzodiazepin-2-one (JNJ-42112265) and 4-[3-(2,6-dimethylpyridin-4-yl)phenyl]-7-methyl-8-(trifluoromethyl)-1,3-dihydro-2H-1,5-benzodiazepin-2-one (RO-4491533) reversed the LY-404039-induced impairment of avoidance and escape. JNJ-42112265 also reversed the impairment of avoidance and escape induced by the $mGlu_2$-specific PAM JNJ-42153605, suggesting that the effects on conditioned avoidance are specifically $mGlu_2$-mediated. The $mGlu_{2/3}$ antagonist (2-(2-carboxycyclopropyl)-3-(9H-xanthen-9-yl)-D-alanine (LY-341495; s.c.) reversed the LY-404039-induced escape impairment but failed to restore avoidance, suggesting interfering side effects. Like the tested antipsychotics, $mGlu_{2/3}$ orthosteric and allosteric agonists inhibited avoidance behavior and locomotion at similar doses. Hence no clear-cut differences between $mGlu_2$ modulators and currently available antipsychotics in the way they interfere with avoidance behavior in relation to inhibition of locomotion could be established.

1. Introduction

The $mGlu_{2/3}$ orthosteric agonist LY-404039 (4-amino-2-thiabicyclo[3.1.0] hexane-4,6-dicarboxylic acid 2,2-dioxide) has been reported (tested as prodrug LY-2140023) to have antipsychotic activity without weight gain or other side effects associated with currently available antipsychotics (Patil et al., 2007). The antipsychotic potential of LY-404039 was primarily based on the theoretical role of glutamate in schizophrenia but was also supported by animal studies, among others by inhibition of conditioned avoidance behavior (Rorick-Kehn et al., 2007). Although the initial promising results could not be confirmed in a later trial (with an exceptionally high placebo response) (Kinon et al., 2011; Takamori et al., 2003), the use of orthosteric $mGlu_{2/3}$ agonists as novel antipsychotic therapy is still being investigated. Also positive allosteric modulators (PAM) are being studied as an alternative option to stimulate $mGlu_2$ receptors by enhancing the activity of endogenous glutamate, thereby reducing the risk of side effects related to continuous receptor stimulation with orthosteric agonists. JNJ-42153605 (3-(cyclopropylmethyl)-7-(4-phenylpiperidin-1-yl)-8-(trifluoromethyl)[1,2,4]triazolo[4,3-a] pyridine) is a recently discovered $mGlu_2$-specific PAM (Cid et al., 2012).

The conditioned avoidance response is considered as an important animal model in the study of antipsychotics (Wadenberg, 2010; Kilts, 2001; Wadenberg and Hicks, 1999; Castagne et al., 2009). In a typical conditioned avoidance experiment, a rat is placed in a two-compartment shuttle box and presented with a neutral conditioned stimulus (e.g. light or tone), followed after a short

Abbreviations: BINA, 3'-{[(2-cyclopentyl-6,7-dimethyl-1-oxo-2,3-dihydro-1H-inden-5-yl)oxy]methyl}biphenyl-4-carboxylic acid; JNJ-42112265, 7-(dimethylamino)- 4-(3-pyridin-3-ylphenyl)-8-(trifluoromethyl)-1,3-dihydro-2H-1,5-benzodiazepin-2-one; JNJ-42153605, 3-(cyclopropylmethyl)-7-(4-phenylpiperidin-1-yl)-8-(trifluoromethyl) [1,2,4]triazolo[4,3-a]pyridine; LY-341495, 2-(2-carboxycyclopropyl)-3-(9H-xanthen-9-yl)-D-alanine; LY-354740, 2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid; LY-404039, 4-amino-2-thiabicyclo[3.1.0] hexane-4,6-dicarboxylic acid 2,2-dioxide; LY-2607540 (THIIC), N-(4-{[3-hydroxy-4-(2-methylpropanoyl)-2-(trifluoromethyl) phenoxy] methyl} benzyl)-1-methyl-1H-imidazole-4-carboxamide); MED, minimum effective dose; NAM, negative allosteric modulator; RO-4491533, 4-[3-(2,6-dimethylpyridin-4-yl)phenyl]-7-methyl-8-(trifluoromethyl)-1,3-dihydro-2H-1,5-benzodiazepin-2-one; PAM, positive allosteric modulator

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delay by an aversive unconditioned stimulus (e.g. foot-shock). The animal can escape from the unconditioned stimulus by jumping from one compartment into the other. After several presentations of the conditioned–unconditioned stimuli pair, the animal typically jumps during the conditioned stimulus, thereby avoiding the unconditioned stimulus. Relatively low doses of antipsychotics inhibit the avoidance without impairing the escape response. This selective disruption of avoidance behavior is characteristic of all antipsychotics and not observed with anxiolytics or antidepressants. Inhibition of avoidance has been shown to be closely correlated with clinical antipsychotic potency.

Though effects were observed with $mGlu_{2/3}$ agonists (Rorick-Kehn et al., 2007; Takamori et al., 2003), effects of $mGlu_2$ PAMs on conditioned avoidance responding have not yet been reported. The present study compares the effects of the $mGlu_{2/3}$ orthosteric agonist LY-404039 and the $mGlu_2$-selective PAM JNJ-42153605 in the assay. Reversibility of the effects was tested by co-administration of the $mGlu_{2/3}$ negative allosteric modulators (NAMs) 7-(dimethylamino)-4-(3-pyridin-3-ylphenyl)-8-(trifluoromethyl)-1,3-dihydro-2H-1,5-benzodiazepin-2-one (JNJ-42112265) and 4-[3-(2,6-dimethylpyridin-4-yl)phenyl]-7-methyl-8-(trifluoromethyl)-1,3-dihydro-2H-1,5-benzodiazepin-2-one (RO-4491533), both originating from Roche; and the orthosteric $mGlu_{2/3}$ antagonist 2-(2-carboxycyclopropyl)-3-(9H-xanthen-9-yl)-D-alanine (LY-341495). Part of these data were previously presented in poster format (Biesmans et al., 2011). LY-404039 has been reported to inhibit avoidance at doses devoid of the motor impairment characteristic of neuroleptics (Rorick-Kehn et al., 2007). To further investigate this statement, we compared two $mGlu_2$ orthosteric agonists (2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (LY-354740; eglumetad) and LY-404039) and three PAMs (3'-{[(2-cyclopentyl-6,7-dimethyl-1-oxo-2,3-dihydro-1H-inden-5-yl)oxy] methyl}biphenyl-4-carboxylic acid (BINA), JNJ-42153605, and N-(4-{[3-hydroxy-4-(2-methylpropanoyl)-2-(trifluoromethyl) phenoxy] methyl} benzyl)-1-methyl-1H-imidazole-4-carboxamide (LY-2607540; THIIC) with eight currently available antipsychotics (chlorpromazine, chlorprothixene, clozapine, haloperidol, olanzapine, perphenazine, risperidone, zotepine) for their relative ability to inhibit conditioned avoidance behavior and spontaneous locomotion. The eight antipsychotics are all neuroleptics acting predominantly via D_2 receptor blockade.

2. Materials and methods

2.1. Compounds

All compounds were synthesized in our own laboratories, except clozapine (Smithkline Beecham, UK), LY-341495, LY-354740, LY-404039 (Sequoia Research Products, UK), JNJ-42112265, RO-4491533 and THIIC (WuXi AppTec, China) zotepine (Knoll AG, Ludwigshafen, Germany). The poorly soluble RO-4491533 was prepared as a suspension in distilled water containing 1% polysorbate 80 and therefore dosed orally (p.o.). All other compounds were dissolved, either in distilled water (chlorpromazine, chlorprothixene), in distilled water acidified with tartaric acid (clozapine, olanzapine, haloperidol, risperidone, perphenazine), in distilled water alkalized with NaOH (LY-341495, LY-354740), LY-404039), in 10% hydroxypropyl-β-cyclodextrin acidified with tartaric acid (zotepine) or hydrochloric acid (JNJ-42112265) or alkalized with NaOH (BINA), or in 20% hydroxypropyl-β-cyclodextrin acidified with HCl (JNJ-42153605, THIIC). The formulations were stored in closed containers protected from light at room temperature (except for the less stable JNJ-42153605 which was stored at $<7\,^{\circ}C$ to prevent degradation).

2.2. Animals (species, weight, and gender)

Male Wiga Wistar rats (Charles River Germany; 230 ± 30 g) were housed under standard laboratory conditions ($21 \pm 2\,^{\circ}C$; 50–65% relative humidity; light-dark cycle set at 12 h; lights on at 6.00 h). All rats were fasted overnight prior to the start of the experiments. The food deprivation allows oral dosing on an empty stomach (to reduce variability) and is also used for the subcutaneous route for matter of standardization. Tap water remained available ad libitum. During the test period, the rats were housed in individual cages. The local Ethical Committee in compliance with the Declaration of Helsinki approved all studies.

2.3. Test descriptions

2.3.1. One-way conditioned active avoidance

Whereas conditioned avoidance experiments generally utilize a 2 compartment shuttle box (see Introduction), the present study was carried out using a one-way set up. Rats are initially triggered to jump by an electric shock (1.0 mA) given 10 s after the rat has been placed on the iron grid floor of the jumping box. In subsequent trials, the rats rapidly learn to avoid the electric shock, conditioned by the "fear-eliciting" stimulus, i.e. being placed on the iron grid of the "electric" box. It appeared possible to train the rats during 5 trials at 15-min time intervals over a period of 1 h. This approach allows drug-naïve animals of similar age and body weight to be used, thereby avoiding the risk of drug-induced changes in receptor sensitivity and variation of age and body weight that may affect the study outcome. Data obtained with reference compounds using this set up were in excellent agreement with published data.

2.3.1.1. Apparatus. The apparatus consisted of an inner box surrounded by an outer box. The inner box was composed of four walls of transparent, synthetic material (length × width × height: 30 cm × 30 cm × 30 cm), an open top, and a grid floor made of 15 pairs of iron bars (2 mm diameter; 6 mm inter-bar distance). Odd and even bars were connected with a source of alternative current (1.0 mA; Coulbourn Instruments Solid State Shocker/Distributor), which could be interrupted by a switch. The outer box was composed of the same material (length × width × height: 40 cm × 40 cm × 36 cm), also with an open top, with a distance of 5 cm between inner and outer box at all sides. To decrease the amount of environmental stimuli, three walls of the outer box were made non-transparent. The front wall was left transparent to allow the necessary inspection of the animal during the test. The upper edge of the outer and inner box served as a target for the rats on which to jump with fore- and hind-paws, respectively.

2.3.1.2. Avoidance conditioning and selection of animals. From their arrival in the laboratory on the experimental day, the rats were housed in individual cages provided with bedding material (very important to provide enough contrast with the iron grid of the experimental box). The rats received 5 training sessions at 15-min intervals over a 1-h period. During these training sessions, the rats were conditioned to avoid an electric shock: the rat was placed on the non-electrified grid floor and the grid was electrified 10 s later for not more than 30 s, if the rat did not jump out of the box. Only rats that showed correct avoidance responses in all last 3 training sessions were included for the experiments, and received test compound or solvent immediately after the final training session. Historical data obtained in this test show that 2382 rats out of a total number of 3789 rats (63%) could successfully be trained using this procedure.