Research article

ANTIDEPRESSANT EFFECTS OF AN INVERSE AGONIST SELECTIVE FOR α5 GABA-A RECEPTORS IN THE RAT FORCED SWIM TEST

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It has been shown in electrophysiological studies that the ligand L-655,708 possesses a binding selectivity and a moderate inverse agonist functional selectivity for α5-containing GABA-A receptors. The present study is aimed to investigate the antidepressant effects of the ligand L-655,708 in the forced swim test (FST) and its impact on locomotor activity in rats. The behavior of the animals was recorded with a digital camera, and the data were analyzed by one-way ANOVA, followed by Dunnett's test. In FST, L-655,708 significantly decreased immobility time at a dose of 3 mg/kg after a single and repeated administration ($p < 0.05$), exerting acute and chronic antidepressant effects. However, it did not induce significant differences in the time of struggling behavior during FST. Furthermore, L-655,708 did not show a significant effect on locomotor activity ($p > 0.05$). These data suggest that negative modulation at GABA-A receptors containing the α5 subunit may produce antidepressant effects in rats. These effects were not confounded by locomotor influences.

Key words: GABA-A receptor, Inverse agonist, Depression, Forced swim test, Locomotor activity.

INTRODUCTION

The majority of fast inhibitory neurotransmissions in the central nervous system (CNS) is governed by the action of γ-aminobutyric acid (GABA) type A receptors (GABA-A). The complex structure and function of GABA-A receptors and benzodiazepine binding sites was recently elucidated [1]. GABA-A receptors are pentameric membrane proteins that operate as GABA-gated Cl⁻ channels. The majority contains a benzodiazepine binding site located at the interface of the γ_2-subunit and the respective α-subunit (α_1, α_2, α_3, α_5) [2].

At these receptors, there are several modulatory sites, which mediate the actions of many drugs, among them benzodiazepines [2]. Three kinds of allosteric modulators

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act through the benzodiazepine binding site: positive (agonist), neutral (antagonist), and negative (inverse agonist) modulators [3]. It is established that agonists at the benzodiazepine site present anxiolytic and amnesic properties, whereas inverse agonists could exert opposite actions [4,5].

Recent genetic studies with mice carrying a point mutation in $\alpha 1$, $\alpha 2$, $\alpha 3$ or $\alpha 5$ subunits have suggested a specific contribution of individual receptor subtypes to the spectrum of behavioral actions of these compounds [6]. These genetic advances have encouraged the synthesis and testing of new, selective BZ site ligands, that possess stronger affinity and/or efficacy profiles [7]. Here, we focus on $\alpha 5$-containing GABA-A receptors, as these receptors are highly expressed in brain regions substantially involved in memory formation and emotion [8-10]. Previous evidence from animal studies has shown that the $\alpha 5$-containing GABA-A receptors were significantly involved in cognition [11-14]. Moreover, it has been shown that a negative modulation of $\alpha 5$ GABA-A receptors may represent an attractive treatment option for cognitive impairments, and potentially positive symptoms, associated with schizophrenia [15].

The goal of the present study was to examine the influence of the inverse agonist 11,12,13,13a-tetrahydro-7-methoxy-9oxo-9H-imidazo [1,5-a]pyrrolo[2,1-c] [1,4] benzodiazepine -1-carboxylic acid, ethyl ester (L-655,708), previously characterized by Atack et al. [11], acting on $\alpha 5$-subunit-containing GABA-A receptor and compare its dose-response effects on locomotor activity and depression-like behavior in the forced swim test.

MATERIALS AND METHODS

Animals

Experiments were carried out on male Wistar rats (Military Farm, Belgrade, Serbia) weighing 180–230 g. The rats were housed in transparent plastic cages, six animals per cage, and had free access to pelleted food and tap water before and after drug administration. The animals were placed in a room kept at a temperature of 22 ± 1 °C, relative humidity 40–70%, and 12/12-h light/dark period (lights on at 0630 h). All handling and testing took place during the light portion of the cycle. The animals (6 rats per treatment group) had been used only once throughout our study, after they were used in other trials. All procedures in the study were confirmed to EEC Directive 86/609 and approved by the Ethical Committee on Animal Experimentation of the Medical Faculty in Belgrade.

Drugs

L-655,708, the inverse agonist selective for $\alpha 5$ GABA$_A$ receptors, was obtained from Sigma-Aldrich (Poole, Dorset, UK). The substance was dissolved in a vehicle containing saline (80%) and dimethylsulphoxide (20% DMSO, from Sigma-Aldrich), and injected intraperitoneally in a volume of 1 ml/kg.

Forced swim test

The forced swimming test, described by Porsolt et al. [16], was performed in a glass cylinder, 45 cm high, 20 cm diameter filled with water up to a height of 30 cm, with a temperature of 21-23 °C. Male Wistar rats were exposed to two swimming sessions (an initial 15-min pretest session, followed 24 h later by a 5-min test session). Time of administration and doses of L-655,708 were chosen based on previous studies by Attack et al. [11]. The animals received i.p. 0.3, 1, and 3 mg/kg of L-655,708 or saline, 30 min before the test session. The duration of immobility (seconds) was scored during the 5-min test session and the rat was considered immobile whenever it floated passively in the water and only made movements necessary to keep its head above the water line. The time of struggling (seconds) during the 5-min test session consists of explosive muscular movements against the apparatus wall, in an attempt to escape from the cylinder. After the test, the rat was removed from the tank, dried with a towel, and placed back in its home cage. The water in the swim tank was changed between rats.

In the experiment with the repeated drug application, on the first day, male Wistar rats were forced to swim for 15 min (pretest session). The rats were removed and randomly divided into two treatment groups. Solvent or L-655,708 (3 mg/kg) were administered, intraperitoneally, once a day for 14 days. Rats were re-exposed to the FST, for a single 5-min session, 24h after the last drug administration.

Spontaneous locomotor activity

The measurement of spontaneous locomotor activity was performed in a clear Plexiglas box (40×25×35 cm), immediately after the application of increasing doses of L-655,708 (0.3, 1, and 3 mg/kg) or solvent. Time of administration and doses of L-655,708 were chosen based on previous studies [11]. To asses the spontaneous locomotor activity, the total distance traveled under dim red light (20 lx), was monitored by a digital camera placed above the box, for a total of 30 min, without any habituation period. Recording ofanimal activity and processing data were conducted by computer software ANY-maze Video Tracking System (Stoelting Co., Wood Dale, IL, USA).

Statistical analysis

All numerical data presented in the figures were given as the mean ± S.E.M. An alpha level of 0.05 was used for all statistical tests. In the forced swim test, the behavior of the animals was recorded by a digital camera, and data were analyzed by one-way ANOVA, followed by Dunnett's test. Spontaneous locomotor activity behavior was recorded by a digital camera, analyzed by the ANY-maze software.

RESULTS

Forced swimming

In the forced swim test, during the test session, ANOVA indicated statistically significant effects of L-655,708 ($F_{(3,20)}=6.42$, $p<0.05$). L-655,708 significantly decreased immobility time at the highest applied dose of 3 mg/kg, exerted acute antidepressant-

like effects (Figure 1). However, L-655,708 did not induce significant differences in the time of struggling behavior during the test (data not shown).

In the experiments with repeated administration, a significant effect of treatment $(F(1,18) = 4.779, p < 0.05)$ has been described. The administration of L-655,708, once a day for 14 days, significantly decreased the immobility time in the FST, when compared with the solvent-treated group, confirming antidepressant effects (Figure 2).
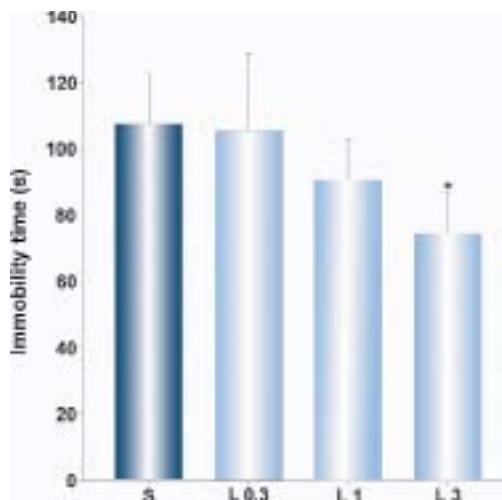

Figure 1. Effects of L-655,708 (L: 0.3, 1, and 3 mg/kg), on the immobility time of the animals in the forced swim test. * p<0.05 compared to solvent group (S).
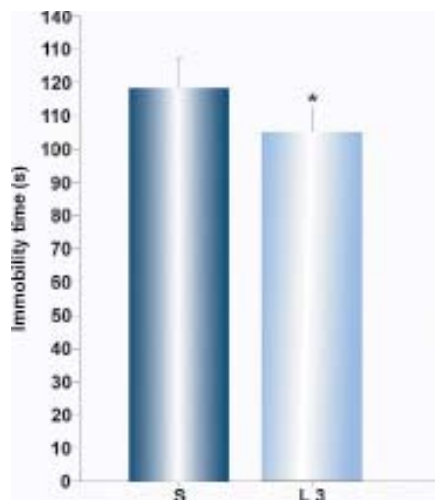Number of animals per treatment was 6.

Figure 2. Effects of repeated application of L-655,708 (L: 3 mg/kg), on the immobility time of rats in the forced swim test. * p<0.05 compared to solvent group (S). Number of animals per treatment was 6.

Spontaneous locomotor activity

Treatment with L-655,708 did not affect the total distance traveled by the animals during 30 min of monitoring spontaneous locomotor activity $(F(3,20)=2.59, p>0.05)$ (Figure 3).
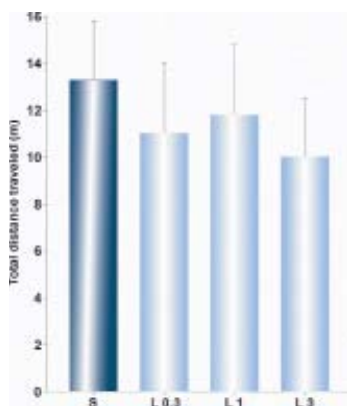

Figure 3. Effects of control (S) and L-655,708 (L: 0.3, 1, and 3 mg/kg) on the total distance traveled in the locomotor activity assay. Number of animals per treatment was 6.

DISCUSSION

It was recently recognized that drugs with antidepressant / mood-stabilizing effects significantly alter GABAergic transmission [17,18]. Namely, modulation in GABA-ergic inhibition has been proposed to play an important role in the pathophysiology of depression [19-21]. Data from experimental models demonstrate that the forced swim test represents a reliable and valuable behavioral research model of depression in rodents and also an important tool to study neurobiological mechanisms involved in antidepressant activity. The substances with antidepressant potential significantly reduce the animals' immobility time, which is considered as a reaction of despair and depressiveness [22].   In our study, decreased immobility was clearly observed with L-655,708 (3 mg/kg), after single and repeated administration, which shows its antidepressant-like properties. Furthermore, there was no effect on struggling time, confirming that these effects were not confounded by change in motor function.

L-655,708 possesses *in vitro* binding selectivity, as well as moderate inverse agonist functional selectivity for the α5-containing GABA-A receptors [11]. Receptors containing the α5 subunit constitute a modest fraction of BZ-sensitive GABA-A receptors (approximately 5%), but are substantially expressed in several brain regions, most notably the hippocampus, the olfactory bulb and layers V and VI of the neocortex [9,10]. Studies with untreated mice with the point mutation at the α5 subunit have indicated the significance of the α5 subunit for learning certain aversively-motivated memory tasks which require temporal integration [23,24]. Our data suggest that negative modulation at GABA-A receptors containing the α5 subunit might have produced the antidepressant effects in rats and that these effects were not confounded by locomotor influences.

Recent animal studies with the inverse agonist selective for α5 GABA-A receptors found improved learning and memory function in models of cognitive defect, suggesting distinctive roles of different subunits [25]. The antidepressant and memory-enhancing effects are in agreement with observations that low mood is frequently combined with anxiety and cognitive impairment [26]. A recently found correlation between helpless behavior and cognition [26,27] was fully supported by our results demonstrating that the antidepresant-like effect was obtained by the same selective agent (L-655,708) that previously induced memory-enhancing effects. The overall effect of L-655,708 may thus reflect a better performance based on both cognition and motivational enhancements.

The results of our study are consistent with previously published data, which demonstrate a role for GABA-A receptors in depressive-like behavior [20, 21, 28, 29]. However, while α2-containing GABA-A receptors are known to be involved in regulating anxiety-like behavior [30], our results suggest that α5-containing GABA-A receptors may be involved in depressive-like behavior. To the best of our knowledge, our study is one of the first reports on the influence of a benzodiazepine inverse agonist selective for α5-subunit-containing GABA-A receptors on rat behavior in forced swim test. The observed action was antidepressant and, at first glance, this seems to contradict the view that decreased GABA-A receptor activity enhances depression [17]. But, it should be taken into account, however, that such effects may occur at higher doses of the

drug. The acute antidepressant effect of GABA-A activation observed here, shows striking similarity to the effects of glutamatergic NMDAR antagonists reported by other authors [31-33]. In these papers, the NMDAR antagonists, after only 30 min, significantly reduced immobility in FST compared to vehicle-treated animals, suggesting that NMDAR blockade produces fast-acting antidepressant responses. Here we report a complementary acute antidepressant action by interfering with the counterbalancing, GABA-ergic system. Similar actions were also found in humans [34].

In conclusion, our results experimentally support the finding that inverse agonists selective for α5 GABA-A receptors produce antidepressant effects, and these effects are not confounded by locomotor influences. The molecular substrates linking the actions of specific GABA-benzodiazepine receptor subunits awaits to be further elucidated and linked to human neuropsychiatric disorders.

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ANTIDEPRESIVNI EFEKTI INVERZNOG AGONISTE SELEKTIVNOG ZA α5 GABA-A RECEPTORE U TESTU FORSIRANOG PLIVANJA PACOVA

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U elektrofiziološkim ispitivanjima pokazano je da ligand L-655,708 poseduje selektivnost u vezivanju i umerenu inverznu agonističku aktivnost za GABA-A receptore koji sadrže α5 podjedinicu. Ova studija ima za cilj ispitivanje antidepresivnih efekata liganda

L-655,708 u testu forsiranog plivanja (FST) i njegov uticaj na lokomotornu aktivnost kod pacova. Ponašanje životinja je praćeno pomoću digitalne kamere, a podaci su analizirani primenom jednostepene analize varijanse (ANOVA) i Dunett-ovim testom. U testu forsiranog plivanja L-655,708 statistički značajno smanjuje vreme imobilnosti pri dozi od 3 mg/kg, nakon jednokratne i ponavljane primene ($p < 0,05$), ispoljavajući akutne i hronične antidepresivne efekte. Međutim, nije uticao značajno na vreme provedeno u borbi za preživljavanje tokom FST. Osim toga, L-655,708 nije pokazao značajan uticaj na lokomotornu aktivnost ($p > 0,05$). Ovi podaci ukazuju da negativna modulacija GABA-A receptora koji sadrže α5 podjedinicu može ispoljiti antidepresivne efekte kod pacova. Ovi efekti su bez izraženih lokomotornih uticaja.