sciendo Acta Marisiensis - Seria Medica 2021;67(4):238-243 DOI: 10.2478/amma-2021-0034

RESEARCH ARTICLE

Study of the anxiolytic effect of propranolol and dextromethorphan in mice using a model of psychogenic stress

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Objective: To find the anxiolytic effects in a psychogenic stress model in mice, the present study has investigated the interaction between propranolol and dextromethorphan. **Methods**: 50 Albino Swiss male mice were housed in groups of 10 per cage. The beta-adrenergic receptor blocker propranolol (20 mg/kg bw), the N-methyl-D-aspartate (NMDA) receptor dextromethorphan (30 mg/kg bw), and their combination were administered 10 minutes after exposure to predator odor. The treatments included diazepam as positive control and normal saline as negative control. Anxiety-like behaviors were evaluated using the elevated plus-maze test (EPM) 7 days after stress induction. **Results**: Regarding the length of stay ($F=25.53$; $p<0.0001$)/ number of entries in the open arms ($F=3.533$; $p=0.0416$), time ($F=6.127$; $p=0.0045$)/ number of entries in the closed arms ($F=5.690$; $p=0.0141$), time in center-point ($F=3.577$; $p=0,0295$), and total distance traveled ($F= 4.711$; $p=0.0145$), there was a significant difference among the treated groups. Propranolol and dextromethorphan treated groups expressed lower time in the closed arms vs Placebo ($p=0.0089$, respectively $p=0.0111$). In addition, the time spent in the open arms was higher in propranolol group vs placebo group ($p=0.0215$). **Conclusions**: Considering obtained data, there was a decrease of symptoms in the sympathetic nervous system and the psychological stress disappeared in mice applying a treatment of 20 mg/kg bw propranolol. Our findings indicated that dextromethorphan partially mediated the anxiolytic-like activity. However, the combination of these two drugs did not express anxiolytic effects.

Keywords: elevated plus maze, anxiety, predator odor, psychogenic stress

Received 13 October 2021 / Accepted 23 October 2021

Introduction

This pattern of exposure to the odor of the natural predator triggers a strong innate threat feeling in rodents, corresponding to the types of traumas that commonly cause post-traumatic stress disorder (PTSD) [1,2]. Up to present time, no conclusive diagnostic biomarkers for PTSD have been identified [3]. The use of ethologically relevant stimuli such as cat odor has several advantages, including the fact that "phylogenetically" prepared reactions allow the mapping of the brain regions activated by anxiety [4].

In addition to producing well-defined behavioral effects, a single exposure to cat odor constantly activates a well-defined neural circuit in rats - the hypothalamic medial defensive circuit [5,6]. The smell of cats is processed as "kairomone", a semi-chemical compound released by one species and received by another [7].

Assessments of anxiety states resulting from stress in rodents are mostly assessed by the behavioral test of the elevated plus-maze (EPM). In this test, anxiolytics reduce the animal's natural aversion to open arms and encourage the exploration of those arms. While staying in closed arms indicates increased anxiety [8].

Studies have shown that people with PTSD have high levels of norepinephrine in the cerebrospinal fluid and noradrenergic hyperresponsiveness to various stimuli [9,10]. Some sympatholytic agents such as clonidine and prazosin, are also used to control the symptoms of this disorder [11]. Therefore, in this study we decided to use propranolol for the anxiolytic effect, a non-selective sympatholytic beta-blocker with lipophilic character, which crosses the blood-brain barrier and may inhibit fear or erase memories related to fear. In addition to reducing the psychological stress associated with predatory exposure restores the optimal level of norepinephrine [12,13].

It has been assumed that the long-term NMDA-dependent potentiation of the limbic system circuits that control defensive behavior would be the solution for the sustainable activation of stress-induced anxiety behavior [14]. It is also evident that glutamate NMDA receptor antagonists, such as ketamine, may affect the control of PTSD [15]. Therefore, in the present study, we chose to use dextromethorphan, a synthetic substance derived from thebaine, currently used as a central antitussive drug [16]. Dextromethorphan is also a non-competitive antagonist of NMDA receptors, which can prevent neuronal damage and modulate pain. This substance is used to control somatic and neuropathic pain, mood lability, as well as treatment-resistant mood disorder [16,17].

Thus, this study aimed to investigate the efficacy of the anxiolytic action of an adrenergic beta-blocker propranolol and antitussive drug dextromethorphan which is an NMDA receptor antagonist, in psychogenic stress model to study PTSD; exposure of rodents to the smell of a natu-

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ral predator (cat), which causes long-term changes in behavior, anxiety, and social withdrawal.

Methods

Ethical approval

The experimental procedures in the study were conducted according to European Directive 2010/63/EU. A total of 50 eight-week-year old albino Swiss mice (male, 20-45 g) from the Animal Facility of our institution were included in the study with the prior approval of the Ethics Committee for Scientific Research of the George Emil Palade University of Medicine, Pharmacy, Science and Technology of Tîrgu Mureș (Protocol number 174/2019) and authorized by National Sanitary Veterinary and Food Safety Authority (Protocol number 39/2019). During the experiment, efforts were made to decrease both the suffering as well as the number of animals used, which was established according to renowned authors [18,19].

Animals and Drugs

The drugs: normal saline solution (placebo), diazepam - vials 10 mg (Diazepam Terapia), propranolol - tablets 40 mg (Propranolol Arena), and dextromethorphan - tablets 20 mg (Tussin Forte).

The animals were housed in standard metal cages (ten animals per cage) and received the same food (standard pellets for rodents) and water ad libitum. The room was kept on a 12-hour light-dark cycle, temperature and humidity were maintained at 21 ± 2°C and 60% ± 10%, respectively. Acclimatization to the housing conditions lasted for one week.

Rodents were randomly divided in 5 groups: I- Ctr (Control group- Diazepam 1,5 mg/kg bw, n = 10); II- Prop (Propranolol 20 mg/kg bw, n = 10); III- Dext (Dextromethorphan 30 mg/kg bw, n = 10); IV- Prop+Dext (Propranolol 20 mg/kg bw and Dextromethorphan 30 mg/kg bw, n = 10); V- Placebo (Normal saline solution 20 mg/kg bw, n = 10). All drugs were injected intraperitoneal (i.p.). All experimental manipulation was carried out between 09:00 and 12:00 a.m.

Stress induction

For the induction of stress like PTSD, mice in groups of 10 were placed in cages and were exposed to a cloth impregnated with cat odor (feces, urine, and cat fluff). Following the acclimation period, the mice were exposed to the cat's odor in their home cages for 10 minutes. Exposure to cat fur odors elicits changes that are qualitatively similar to those elicited by the presence of a cat [20]. After 10 minutes, we weighed animals and administered the treatment for each group with a different substance, as shown in Table I.

After seven days, each group was tested by the EPM test. We weighted each mouse before testing.

Apparatus

The EPM consisted of a plastic four-armed platform, two closed arms with sidewalls, and two open arms. The apparatus was raised 60 cm above the floor. All arms having 5 cm width and 80 cm length were joined in the center to a 5 cm^2 platform. Furthermore, two arms facing each other were covered and surrounded by black plastic walls except at the crossing point, while two remaining arms facing each other were arranged perpendicular to the protected arms which remained open.

At the beginning of each test, animals were transferred with gloved hands to the EPM and placed in the center of the arena with their nose in the direction of one of the open arms where they were freely allowed to explore the apparatus for 5 minutes. The movements were recorded using a top-view camera at 30 fps and stored on a computer. We measured time spent in open and closed arms, the number of entries, and the time in percentage spent in the central area. Additionally, parameters indicative of general activity was analyzed, including the total distance traveled in centimeters and the average running velocity. Therefore, the longer the animals stay in open arms the less nervous they are [21, 22]. After each test, rats were returned to their home cage and the arena was cleaned and wiped dry using 70% alcohol. EPM testing was conducted in the same room as the rest of the experiment. Each animal was tested only once in the plus-maze apparatus.

For data collection, all trials were analyzed with EthoVision XT (Noldus IT, Wageningen, The Netherlands, version 11.5), by monitoring the distance ran by the mice and their active time.

The time spent in the open arm (%), the number of entries in the open arms (%), and the time spent in central area were calculated using the following formulas:

- percentage of time spent in open arm = (time spent in open arm/total time spent in open arm, closed arm, and central point) × 100%;
- percentage of open arm entries = (entry number to open arm/total entry number to open arm and closed arm) × 100%;
- percentage of time spent in central-point= (time spent in central point/ total time spent in open arm, closed arm, and central point) × 100% [23].

Table I. After seven days of acclimatization - the treatment succeeding stress induction

Day	Time	Subjects	Group	Treatment	Testing
1	09:30	10	I (Ctr)	Diazepam 1,5 mg/kg bw, i.p.	day 8
2	09:30	10	II (Prop)	Propranolol 20 mg/kg bw, i.p.	day 9
3	09:30	10	III (Dext)	Dextromethorphan 30 mg/kg bw, i.p.	day 10
4	09:30	10	IV (Prop+Dext)	Propranolol 20 mg/kg bw and Dextromethorphan 30 mg/kg bw, i.p.	day 11
5	09:30	10	V (Placebo)	Saline solution 20 mg/kg bw, i.p.	day 12

Statistical analysis

The Kolmogorov-Smirnov test was used to assess the distribution of collected data. Data were expressed as the mean±SEM or percentage and were analyzed using one-way ANOVA analysis of variance, with the Greisser-Greenhouse correction, followed by Tukey's multiple comparison test. For comparing the weight of mice between testing the non-parametric Wilcoxon test was performed. Probability values below 0.05 were considered statistically significant. Data analysis was performed using GraphPad Prism (GraphPad Software, San Diego, California, USA, version 8).

Results

The mean weight of mice before treatment was 45.72±0.5472 g and the mean weight after the treatment but before testing was 45.23±0.5593 g (p=0.04).

Activity into the closed arm

There was a significant difference between the studied groups regarding the time spent in the closed arms, (F=6.127; p=0.0045). Concerning the entries in the closed arms there was a significant difference between the studied groups (F=5.690; p=0.0141), but no significant difference was seen with Tukey's multiple comparison test (see Figure 1).

Activity into the open arm

Regarding the time spent in open arms, there was a significant difference between the tested groups (F=25.53; p <0.0001). Furthermore, there was a significant difference between the studied groups according to the entries in open arms, (F=3.533; p=0.0416) as illustrated in Figure 2.

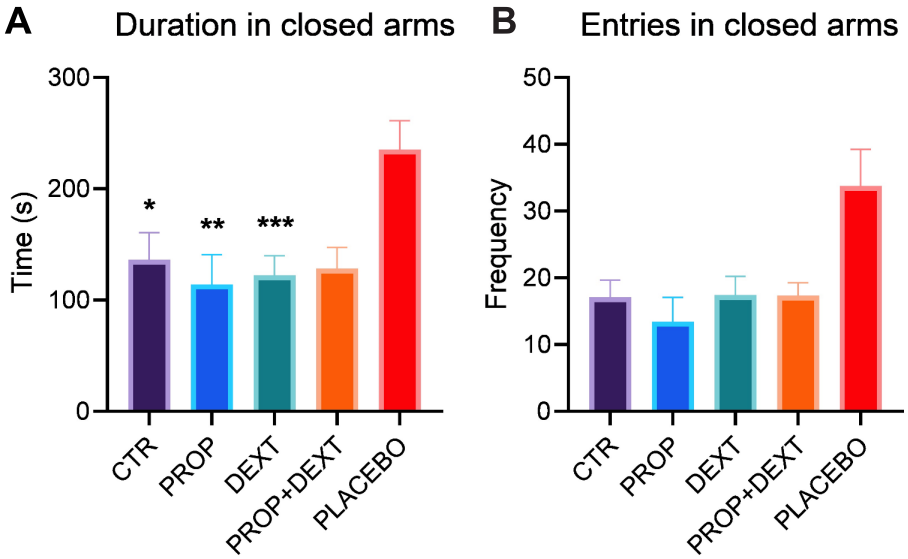

Fig. 1. (A) Time spent in closed arms (s) and (B) number of entries into closed arms in the elevated plus maze test. Data are expressed as the mean ± SEM (one-way analysis of variance and Tukey's post hoc tests). *p=0.0097 vs. Placebo group; **p=0.0089 vs. Placebo group; ***p=0.0111 vs. Placebo group.

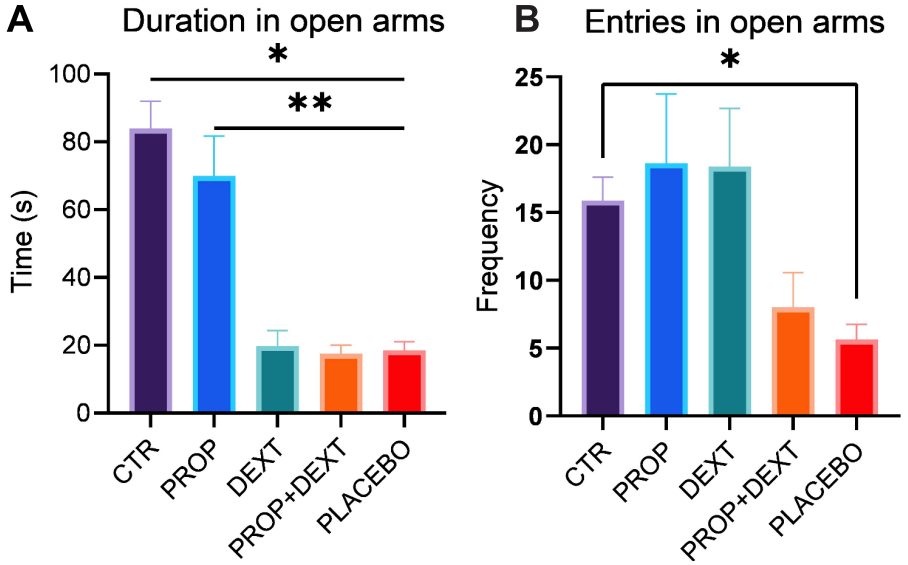

Fig. 2. (A) Time spent in open arms (s) and (B) number of entries into open arms in the elevated plus maze test. Data are expressed as the mean ± SEM (one-way analysis of variance and Tukey's post hoc tests). A: *p<0.05 vs. groups III, IV, and V; **p<0.05 vs. groups III, IV, and V; B: *p=0.0021 vs. Placebo group.

Other mice activities

According to the time spent in center area, there was a significant difference between the studied groups (F=3.577; p=0.0295). Regarding the total distance traveled there was a significant difference between the studied groups (F=4.711; p=0.0145), shown in Figure 3. However, according to the average running velocity, there were no significant differences among the groups (F=1.342; p=0.2854).

The activity of mice is summarized in Table II.

Discussion

The exposure of mice to cat odor induces strong emotional stress caused by significant changes in the expression of endocannabinoid system-related genes in various brain structures [24]. Predator odor stress satisfies many of the diagnostic criteria for PTSD, including enhanced fear, hyperarousal, avoidance, and heightened anxiety. Importantly, these symptoms are persistent, often lasting weeks or months the same as in humans [25].

The validity of EPM in our study was supported by the observation that diazepam which is a classic anxiolytic benzodiazepine, significantly increases the time spent in the open arms and the number of entries in open arms, which was used as a positive control. Additionally, the treated groups showed a significant difference regarding the length of stay in the open arms, number of entries in the open arms, time in the closed arms, number of entries in the closed arms, time in center-point, and total distance traveled.

Our data showed that propranolol had a significant and potent anxiolytic effect with 33.4% of duration spent in open arms and less duration spent in closed arms compared to the placebo group. Beta-adrenoceptor antagonist propranolol, administered systemically or directly into the basolateral structures of the amygdala, blocked the corticosterone-induced memory enhancement [26]. Research suggested that acute propranolol administration in the aftermath of trauma or following the reactivation of a traumatic memory might reduce subsequent physiological responses to trauma-related stimuli [27]. Nielson et al showed that the findings in human subjects were therefore consistent with those of studies using animal models and propranolol can inhibit memory modulation that occurs by endogenous arousal. Consequently, chronic propranolol treatment significantly impairs endogenous memory which normally functions to distinguish important events from trivial ones [28].

In the present study, administration of dextromethorphan significantly reduced the time spent in closed arms, without other observations in the reduction of anxiety. Dextromethorphan might interact dose-dependently with cholinergic, dopaminergic, and serotoninergic neurotransmitter systems to produce the opposite effects on anxiety [29]. On the other hand, another study published by Po KT et al on high dose dextromethorphan (40 mg/kg/day) revealed that it suppressed neurogenesis when administered for 14 days and induced depression-like and anxiety-like

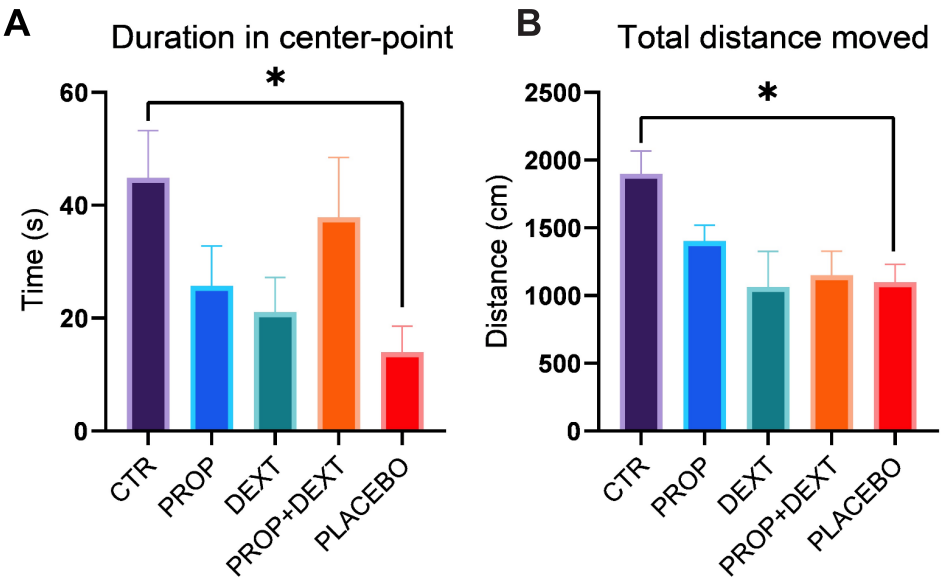

Fig. 3. (A) Cumulative duration in center-point (s) and (B) total distance traveled (cm) in the elevated plus maze test. Data are expressed as the mean ± SEM (one-way analysis of variance and Tukey's post hoc tests). A: *p=0.0142 vs. Placebo group; B: *p=0,0291 vs. Placebo group.

Table II. Mice activity represented in percentage

Groups	Cumulative duration in closed arms (%)	Cumulative duration in open arms (%)	Entry in closed arms (%)	Entry in open arms (%)	Center-point cumulative duration (%)
I- Ctr	51.4	31.7	50.4	49.6	16.9
II- Prop	54.4	33.4	41.9	58.1	12.3
III- Dext	75.0	12.1	48.7	51.3	12.9
IV-Prop+Dext	69.8	9.6	51.3	31.6	20.6
V- Placebo	87.9	6.9	12.9	14.3	5.2

behavior in rats [30]. Salunke et al showed that NMDA receptor agonists aggravated anxiety-like behaviors and NMDA receptor antagonists stimulated anxiolytic-like behaviors in mice [31]. In addition, Engin et al revealed that ketamine (non-competitive receptor antagonist), had anxiolytic-and antidepressant-like properties in animal models [32]. Sub-anesthetic and anesthetic doses of ketamine did not affect anxiety or panic-related behaviors in the Elevated T-maze [33].

Present lab findings suggest an absence of anxiolytic effects after the combination of studied drugs. Concerning the consolidation of anxiogenic effects of predator stress, recent studies indicated a possible convergence of actions of glucocorticoid, mineral corticoid, and β-noradrenergic receptors on long-term potentiation-like neuroplasticity in limbic (amygdala and hippocampus) circuitry [34].

Conclusions

All studied groups exposed to predator odor manifested anxious behavior. Considering our experimental data, there was a decrease of symptoms in the sympathetic nervous system and the psychological stress disappeared in mice applying a treatment of 20 mg/kg bw propranolol. Our findings indicated that dextromethorphan partially mediated the anxiolytic-like activity. However, the combination of these two drugs did not express anxiolytic effects. Further clinical studies are necessary to check the behavioral response and confirm the effectiveness of this drug interaction.

Authors' contribution

RG – Data curation, Investigation, Writing – original draft
SC – Conceptualization, Methodology, Supervision, Writing – review & editing
GGP – Writing – review & editing
RIB – Writing – review & editing

Conflict of interest

None to declare.

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