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=0.0111), there was a significant difference among the treated groups. Propranolol and dextromethorphan treated groups expressed lower time 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

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 mice 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-
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-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 the 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 - tablets 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 weighted 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 cm² 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].

| 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  |

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

![Duration in center-point and Total distance moved](image)

**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                                  |

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]. Nielsen 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 which 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...
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 neuropsychiatry 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.

References
1. Blanchard RJ, Blanchard DC, Rodgers J et al. The characterization and modelling of antipredator defensive behavior. Neurosci Biobehav Rev. 1990;14:463-472.
2. Blanchard RJ, Blanchard DC, Weiss SM et al. The effects of ethanol and diazepam on reactions to predatory odors. Pharmacology Biochemistry and Behavior. 1990;35:775-780.
3. Ronzoni G, Arco A, Mora F et al. Enhanced noradrenergic activity in the amygdala contributes to hyper arousal in an animal model of PTSD. Psychoneuorendocrinology. 2016;70:1-9.
4. Blanchard RJ, Yudko EB, Rodgers RJ et al. Defense system psychopharmacology: an ethological approach to the pharmacology of fear and anxiety. Behav Brain Res. 1993;55:155-165.
5. Canteras NS. The medial hypothalamic defensive system: hodological organization and functional implications. Pharmacol Biochem Behav. 2002;71:481-491.
6. Delineberg RA, Hunt GE, McGregor IS. “When a rat smells a cat”: the distribution of Fos immunoactivity in rat brain following exposure to a predatory odor. Neuroscience. 2001;104:1085-1097.
7. Rutherford JM, Stevens JL, Rich in phenomena-lacking in terms. A classification of karmosomes. Chemoecology. 2002;12:161-167.
8. Rico JL, Bonuti R, Morato S. The elevated gradient of aversion: a new apparatus to study the rat behavior dimensions of anxiety, fear, and impulsivity. Braz J Med Biol Res. 2019;52:e8899.
9. Adamec R, Mur C, Grimes M et al. Involvement of noradrenergic and corticoid receptors in the consolidation of the lasting anxiogenic effects of predator stress. Behav Brain Res. 2007;179:192-207.
10. Strawn JR, Geracioli TD Jr. Noradrenergic dysfunction and the psychopharmacology of posttraumatic stress disorder. Depress Anxiety. 2008;25:260-271.
11. Boehnlein JK, Kinzie JD. Pharmacologic reduction of CNS noradrenergic activity in PTSD: the case for clonidine and prazosin. J Psychiatr Pract. 2007;13:72-78.
12. Brunet A, Orr SP, Tremblay J et al. Effect of post-retrieval propranolol on psychophysiological responding during subsequent script-driven traumatic imagery in post-traumatic stress disorder. J Psychiatr Res. 2008;42:503-506.
13. Deprie J, Ledoux JE. Disruption of reconsolidation but not consolidation of auditory fear conditioning by noradrenergic blockade in the amygdala. Neuroscience. 2004;129:267-272.
14. Adamec RE, Burton P, Shallow T et al. NMDA receptors mediate lasting increases in anxiety-like behavior produced by the stress of predator exposure—implications for anxiety associated with posttraumatic stress disorder. Physiol Behav. 1999;65:723-737.
15. Zhang LM, Zhou WW, Ji YJ et al. Anxiolytic effects of ketamine in animal models of posttraumatic stress disorder. Psychopharmacology (Berl). 2015;232:663-672.
16. Su A, Drachtmann R. Dextromethorphan: a review of N-methyl-d-aspartate receptor antagonist in the management of pain. CNS Drug Rev. 2007;13:96-106.
17. Werling LL, Lauterbach EC, Calif U. Dextromethorphan as a potential neuroprotective agent with unique mechanisms of action. Neurologist. 2007;13:272-293.
18. Lapin IP. Only controls: effect of handling, sham injection, and intraperitoneal injection of saline on behavior of mice in an elevated plus-maze. J Pharmacol Toxicol Methods. 1996;34:73-7.
19. Vogel HG (Ed). Drug Discovery and Evaluation: Pharmacological Assays. Springer-Verlag Berlin Heidelberg, New York, 2008, 629.
20. Muñoz-Abellán C, Armario A, Nadal R. Do odors from different cats induce equivalent unconditioned and conditioned responses in rats? Physiol Behav. 2010;99:388-394.
21. Feyissa DD, Aher YD, Engidawork E et al. Individual Differences in Male Rats in a Behavioral Test Battery: A Multivariate Statistical Approach. Front Behav Neurosci. 2017;11:26.
22. Carola V, D’Olimpio F, Brunamonti E et al. Evaluation of the elevated plus-maze and open-field tests for the assessment of anxiety-related behaviour in inbred mice. Behav Brain Res. 2002;134:49-57.
23. Wu YF, Gao HY, Ouyang SH et al. Predator stress-induced depression is associated with inhibition of hippocampal neurogenesis in adult male mice. Neural Regen Res. 2019;14:2383-2385.
24. Sütter S, Raud S, Areda T et al. Cat odour-induced anxiety—a study of the involvement of the endocannabinoid system. Psychopharmacology (Berl). 2008;198:509-520.
25. Whittaker AM, Gilpin NW, Edwards S. Animal Models of Post-Traumatic Stress Disorder and Recent Neurobiological Insights. Behav Pharmacol. 2014;25:398-405.
26. Valzadegan F, Oryan S, Nasrall M et al. Interaction between morphine and noradrenergic system of basolateral amygdala on anxiety and memory in the elevated plus-maze test based on a test-retest paradigm. Arch Iran Med. 2013;16:281-287.
27. Argolo FC, Cavalcanti-Ribeiro P, Netto LR et al. Prevention of posttraumatic stress disorder with propranolol: A meta-analytic review. J Psychosom Res. 2015;79:89-93.
28. Nielson KA, Czech DA, Laubmeier KK. Chronic administration of propranolol impairs inhibitory avoidance retention in mice. Neurobiol Learn Mem. 1999;71:248-257.
29. Argolo FC, Cavalcanti-Ribeiro P, Netto LR et al. Prevention of posttraumatic stress disorder with propranolol: A meta-analytic review. J Psychosom Res. 2015;79:89-93.
30. Po KT, Siu AM, Lau BW et al. Effect of repeated, high-dose dextromethorphan treatment decreases neurogenesis and results in depression-like behavior in rats. Exp Brain Res. 2015;232:663-672.
33. Silote GP, de Oliveira SFS, Ribeiro DE et al. Ketamine effects on anxiety and fear-related behaviors: Current literature evidence and new findings. Prog Neuropsychopharmacol Biol Psychiatry. 2020;100:109878.

34. Adamec R, Muir C, Grimes M, et al. Involvement of noradrenergic and corticoid receptors in the consolidation of the lasting anxiogenic effects of predator stress. Behav Brain Res. 2007;179:192-207.