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Research report

Anxiolytic properties of *N*-acetylcysteine in mice

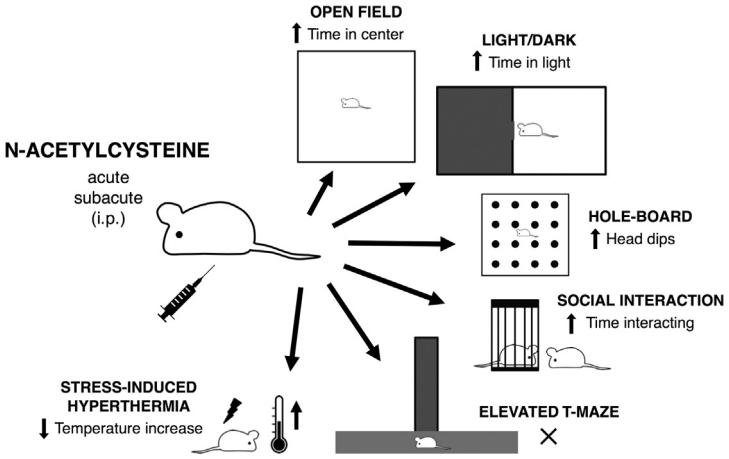
Patrícia Santos[a,b], Ana P. Herrmann[c], Radharani Benvenutti[a,b], Guilherme Noetzold[a], Franciele Giongo[a], Clarissa S. Gama[d,e], Angelo L. Piato[b], Elaine Elisabetsky[a,b,*]

[a] *Laboratório de Etnofarmacologia, Departamento de Farmacologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil*
[b] *Programa de Pós-graduação em Farmacologia e Terapêutica, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil*
[c] *Grupo de Estudos Biológicos e Clínicos em Patologias Humanas, Universidade Federal da Fronteira Sul, Chapecó, SC, Brazil*
[d] *Laboratório de Psiquiatria Molecular, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil*
[e] *Programa de Pós-graduação em Psiquiatria e Ciências do Comportamento, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil*

HIGHLIGHTS

- NAC shows anxiolytic effects on five mice models of anxiety.
- Subacute NAC results in lower effective anxiolytic doses than acute treatment.
- Anxiolytic doses of NAC do not affect locomotion.

GRAPHICAL ABSTRACT



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ABSTRACT

Anxiety disorders are highly prevalent and often result in poor quality of life. Available anxiolytics show significant adverse effects as well as partial efficacy in a sizable part of patients. Innovative treatments with more favorable risk-benefit ratio are sorely needed. A growing body of clinical data indicates the benefits of *N*-acetylcysteine (NAC) in psychiatric conditions. NAC modulates antioxidant, glutamatergic, inflammatory and neurotrophic pathways in the central nervous system, all of which are relevant to anxiety pathology. We evaluated the effects of NAC in mice models commonly used to characterize anxiolytic compounds. Male adult CF1 or BALB/c mice were treated (i.p.) acutely or subacutely (4 consecutive days) with NAC (60–150 mg/kg) 60 min before open field, light/dark, hole-board, social interaction, elevated T-maze or stress-induced hyperthermia tests. Diazepam (2 mg/kg) was used as positive control. We found that NAC presents anxiolytic effects in all models, except for the elevated T-maze. Subacute treatments

* Corresponding author at: Departamento de Farmacologia, Universidade Federal do Rio Grande do Sul, Rua Sarmento Leite 500, sala 305, 90050–170 Porto Alegre, RS, Brazil.
E-mail address: elaine.elisabetsky@gmail.com (E. Elisabetsky).

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resulted in lower effective doses in comparison to acute treatment. The anxiolytic effects of NAC were comparable to diazepam. NAC is a safe and low cost medicine with suggested benefits in psychiatric conditions often presenting co-morbidity with anxiety. This study contributes evidence to support the validity of clinical trials with NAC in the context of anxiety disorders, especially considering the safety profile in comparison to the limitations of diazepam for long term treatment.

1. Introduction

Anxiety can be defined as the emotional anticipation of aversive situations, and is associated with behavioral and endocrine adaptive responses to cope with threatening stimuli [1]. Anxiety can become pathological when unsuccessful in its adaptive function, especially if combined with predisposing factors [2]. Anxiety disorders have an estimated prevalence of 28% [3], resulting in significant impairments in quality of life and high economic costs related to diagnostic problems, inappropriate treatment, and extensive use of health services [4].

Current available anxiolytics act on GABAergic and serotonergic systems, including benzodiazepines (BZDs), partial 5-HT_{1A} receptor agonist (buspirone) and selective serotonin reuptake inhibitors (SSRIs). The adverse effects of BZDs and SSRIs [5], the slow onset of action and limited scope of buspirone [6], as well as the various forms of treatment resistant anxiety [7,8], reinforce the need for innovative treatments. While congruent with the less than desirable efficacy of typical treatments, the recognition that anxiety disorders may result from hyperactivity of the excitatory glutamatergic system [1,9] and is accompanied by neuroinflammation [10,11] and oxidative stress [12–14] has opened new scenarios in the field.

N-acetylcysteine (NAC), a precursor of glutathione, possesses an original mechanism of action composed by the modulation of antioxidant, glutamatergic, inflammatory and neurotrophic pathways [15,16]. Long marketed as treatment for paracetamol poisoning, chronic obstructive pulmonary disease and contrast-induced nephropathy [16], a growing body of clinical data bespeaks on the benefits of NAC in psychiatric conditions. Favorable evidence for NAC has been reported for schizophrenia, autism, Alzheimer's disease, drug-induced neuropathy, progressive myoclonus epilepsy, bipolar disorder, depression, addiction (cocaine, heroin, cigarettes and marijuana), obsessive compulsive disorder, trichotillomania, nail biting, and skin picking [15–21]. Relevant to this study, marked anxiety is frequently presented in several of the conditions where NAC seems to be clinically useful and a core symptom in obsessive-compulsive disorder, trichotillomania, nail biting, and skin picking.

Considering the paucity of data on properties of NAC on anxiety [22,23], the purpose of this study was to verify the effects of NAC in mice. The effects of various doses of acute and subacute (4 days) NAC were evaluated in the open field, light/dark, hole-board, social interaction, elevated T–maze and stress-induced hyperthermia models.

2. Materials and methods

2.1. Animals

Two month-old male mice were used: CF1 (from Universidade Federal do Rio Grande do Sul, UFRGS) or BALB/c mice (from Universidade Federal de Pelotas, UFPel). Mice were housed in groups of 4 animals per cage (30 × 19 × 13 cm), under controlled environmental conditions (22 ± 2 °C, 12-h light/dark cycle, lights on 07:00 h, food and water *ad libitum*). The animals were kept in our animal facility for at least 14 days before experiments. All procedures were carried out according to institutional policies on experimental animals handling and approved by the University Ethics Committee (approval #22308 and #27553). A total of 535 mice were used in this study (447 CF1 and 88 BALB/c).

2.2. Drugs

N-acetylcysteine (NAC) was purchased from Sigma-Aldrich (St Louis, Missouri, USA). Diazepam (DZP) was used from commercial source (injectable ampoules from Teuto Laboratories, GO, Brazil). All drugs were solubilized in saline (NaCl 0.9%), which was used as the negative control. Injection volume was 0.1 ml/10 g of body weight. All drugs were administered intraperitoneally.

2.3. Experimental design

Mice were habituated to the experimental room for at least 30 min before behavioral testing. Except for stress-induced hyperthermia, tests were performed in dimly lit (red light 26 W) experimental room. Animals were randomly assigned to the treatment groups. All parameters were quantified by researchers blind to the treatment groups. Behavioral tests were performed in separate groups of animals; each mouse was used only once.

Acute treatment: animals were treated with saline, DZP 1 or 2 mg/kg (as specified), or NAC 60, 100 or 150 mg/kg. NAC was administered 60 min and DZP 30 min before behavioral tests, except for stress-induced hyperthermia where all treatments were administered 60 min before tests (because temperature returns to basal levels 60 mins after injections [24]).

Subacute treatment: animals were treated for four consecutive days with saline, DZP 1 or 2 mg/kg (as specified), or NAC 10, 30, 60 or 100 mg/kg. The last administration of NAC was realized 60 min, and that of DZP 30 min, before behavioral tests, except for the stress-induced hyperthermia where all treatments were administered 60 min before tests. The time course for drug administration was chosen according to specificities in pharmacokinetics, the literature and upon pilot experiments showing that NAC was effective 1 h after i.p. administration whereas diazepam within 30 min [25–31].

2.4. Behavioral tests

2.4.1. Open field

To discard non-specific reactions to the pharmacological treatments or changes in locomotion, CF1 mice (n = 7–10) were tested in a gray wooden apparatus (40 × 40 × 40 cm). In addition to locomotor activity, the time spent in the center of the open field was assessed to index anxiety levels [32]. Animals were allowed to explore the arena for 15 min; the first 5 min were considered as exploratory behavior and the last 10 min as locomotion. The experiment was recorded by a digital camera installed above the arena and videos were analyzed using ANY-Maze tracking software (Stoelting Co., Wood Dale, IL, USA).