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Research report

Involvement of the serotonergic system in the anxiolytic-like effect of 2-phenylethynyl butyltellurium in mice

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HIGHLIGHTS

- PEBT treatment demonstrated an anxiolytic-like effect in different anxiety models.
- PEBT reduced [^{3}H] 5-HT uptake.
- PEBT selectively inhibited MAO-A activity in cerebral cortex.

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ABSTRACT

Anxiety is a serious disorder with symptoms manifested at the psychological, behavioral, and physiological levels, accompanied by alterations in the serotonergic system and monoaminergic signaling. In this study, the anxiolytic-like effect of 2-phenylethynyl butyltellurium (PEBT), in three well-consolidated anxiety mouse models (light–dark test, novelty suppressed-feeding, elevated plus-maze), was investigated. The involvement of the serotonergic system, synaptosomal [^{3}H] serotonin (5-HT) uptake and monoamine oxidase (MAO A and B) activities on cerebral cortices of mice, was examined. Mice received PEBT (1 mg/kg, by intragastric route, i.g.) or canola oil (10 ml/kg, i.g.) 30 min before behavioral tests. The results showed that PEBT was effective in increasing the time spent by mice in the illuminated side on the light–dark box and in the open arms on the elevated plus-maze. PEBT decreased the latency to begin eating on the novelty suppressed-feeding test, indicating an anxiolytic-like effect of PEBT. Furthermore, PEBT reduced [^{3}H] 5-HT uptake and selectively inhibited MAO-A activity in cerebral cortex, suggesting the involvement of the serotonergic system in the mechanism of action of this tellurium compound.

1. Introduction

Anxiety is a serious disorder in today's society, manifested by disturbances of mood and emotions, as well as of thinking, behavior, and physiological activity. Studies have shown that the monoaminergic neurotransmitter, serotonin [5-hydroxytryptamine (5-HT)], is involved in the pathogenesis of anxiety [1–3]. Furthermore, abnormalities in the serotonergic neurotransmission accompanied by a reduction in monoaminergic signaling [1,4] could increase the breakdown of neurotransmitters, like 5-HT, resulting in a decrease in their availability in the synaptic cleft and this has been implicated in the etiology of several psychiatric disorders [5,6].

In addition, selective 5-HT reuptake inhibitors (SSRIs) have been effective as anxiolytics for long-term therapy, by inhibiting the reuptake into the presynaptic cell, resulting in an increase in the level of 5-HT in the synaptic cleft available to bind to the postsynaptic receptors [1–3].

Monoamine oxidase (MAO) is an enzyme present in mammalian tissues external at mitochondrial membrane, which is responsible for the oxidative deamination of monoamine neurotransmitters. The MAO has two isoforms, MAO-A and MAO-B, which despite catalyze the same reaction, exhibit differences in selectivity for inhibitors, and differences in the amines metabolized [7–9]. MAO-A is selectively inhibited by clorgyline and preferentially desaminates 5-HT and noradrenaline [10] and MAO-B is selectively inhibited by selegiline and metabolizes preferentially phenylethylamine [11]. In this context, the inhibition of MAO-A is associated with the

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metabolism of 5-HT, regulating its intracellular concentration in the brain. Consequently, the abnormal function of this enzyme has been implicated in the etiology and treatment of depression and anxiety disorders [12,13].

Organotellurium compounds have been the subject of research due to their pharmacological properties. Organotellurium compounds have been reported as antioxidants in several animal models of oxidative stress [14–16]. Moreover, these compounds had immunomodulatory and anti-inflammatory actions [17–19]. In addition, the vinyl alkynyl telluride class of compounds demonstrated an antidepressant-like action in mice [20].

Recently, our research group showed the antioxidant effect of different telluroacetylenes *in vitro* [21]. Moreover, specifically 2-phenylethynyl-butyltellurium (PEBT), a telluroacetylene compound, protected against oxidative damage caused by sodium nitroprusside in mouse brain, suggesting an antioxidant *in vivo* effect of this compound [22]. In addition, this compound showed low toxicity *in vitro* [23]. Besides, PEBT significantly ameliorated the scopolamine-induced impairment of long-term memory and Aβ-induced learning deficits in mice, as indicated by a decrease in escape latency and an increase in the number of crossings over the platform location in the Morris Water Maze test. Furthermore, PEBT increased step-down latency in scopolamine-induced memory impairment in mice and Aβ-treated group [24,25].

The need for the development of new therapeutic agents for treating anxiety is of great interest since there are several concerns associated with the use of current anxiolytic therapies, mainly the increased incidence of tolerance, dependence and abuse of benzodiazepines [26]. In this study a possible anxiolytic-like effect of PEBT in different tests predictive of anxiety was investigated in mice. The involvement of the serotonergic system in the anxiolytic-like effect of PEBT, by measuring [^{3}H] 5-HT uptake and the contribution of MAO-A and MAO-B activities in cerebral cortices of mice, were also examined.

2. Materials and methods

2.1. Chemicals

PEBT (Fig. 1) was prepared according to the literature methods [27,28]. Analysis of the ^{1}H NMR and ^{13}C NMR spectra showed that the compound synthesized exhibited analytical and spectroscopic data in full agreement with its assigned structure. PEBT was diluted in canola oil. The other chemical reagents utilized for biochemical assays were obtained from Sigma Chemical (St. Louis, MO, USA).

2.2. Animals

The experiments were conducted using male adult Swiss mice (25–30 g) maintained at 22–25 °C with free access to water and food, under a 12:12 h light/dark cycle, with lights on at 7:00 a.m. All manipulations were carried out between 08:00 a.m. and 04:00 p.m and mice were acclimated to the behavioral room at least 2 h before the test. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources of the Federal University of Santa Maria, Brazil (#041/2012). All efforts were made to minimize animals suffering and to reduce the number of animals used in the experiments.

The animals were divided into two groups: (1) control group (40 animals): mice received canola oil (vehicle) by the intragastric (i.g.) route (10 ml/kg); (2) PEBT group (40 animals): mice received PEBT at a dose of 1 mg/kg (i.g.) 30 min before behavioral tests. The animals used in each behavioral test were different and all tests were carried out in different days. The number of animals per group was: 12 animals in the spontaneous locomotor activity test, 9 animals in the light dark and novelty suppressed-feeding tests and 10 animals in the elevated plus maze test.

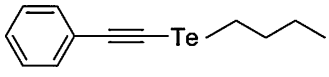

Fig. 1. Chemical structure of 2-phenylethynyl-butyltellurium (PEBT).

2.3. Behavioral tests

2.3.1. Spontaneous locomotor activity

The locomotor activity monitor is a clear acrylic plastic box (50 cm × 48 cm × 50 cm) with a removable plastic lid perforated with holes for ventilation. The monitor contains photocell beams and detectors that are mounted on opposite walls (2 cm above the chamber floor). General locomotor activity and the mouse position in the chamber are detected by breaks of the photocell beams, which are recorded by Software (Insight, Ribeirão Preto, SP, Brazil). Mice were placed in the center of the apparatus and allowed to freely explore the arena. Number of crossings, rearings, fecal pellets, average velocity (mm/s) and total distance traveled (mm) were recorded for a 4 min period.

2.3.2. Rotarod test

The rotarod test was used to investigate motor coordination. Rotarod consisted of a wooden beam covered with masking tape (diameter, 3 cm), used to increase the roughness of the texture and thereby providing a firm grip. The rod was flanked by two cardboard plates to prevent any escape and suspended at a height of 30 cm above the mat-covered table. The mice were placed on top of the already revolving beam (10 rpm) and facing away from the investigator, in the orientation opposite to that of the beam movement in the longitudinal axis, so that forward locomotion was necessary to avoid a fall. Latencies before falling were measured for three trials, with an inter trial interval of 10 min.

2.3.3. Light–dark test (LDT)

The LDT is a sensitive model to detect activity in disorders related to anxiety [29]. The apparatus is an acrylic box (46 cm × 27 cm × 30 cm) divided into light and dark chambers. The light chamber (27 cm × 27 cm) was painted white and was connected *via* an opening (7.5 cm × 7.5 cm) at floor level to the dark chamber (18 cm × 27 cm), which was painted black. A lamp with a 60-W white light was placed 40 cm above the light chamber. Mice were placed in the light chamber facing the opening into the dark chamber, and the following measures were recorded during a 5-min trial: latency to the first transition to the dark chamber, number of zone transitions, and time spent in the light compartment. The measures were recorded manually by a human observer.

2.3.4. Novelty suppressed-feeding (NSF)

The NSF is a conflict test that elicits competing motivations: the drive to eat and the fear of venturing into the center of the brightly lit arena, this is a sensitive model to detect anxiety [30]. The testing apparatus consisted of a square wooden arena (45 cm × 45 cm × 45 cm); the floor was covered with wooden bedding. The test was carried out during a 5 min period according to a previous study [31]. Twenty-four hours before behavioral testing, food was removed from the home cage. At the time of testing, a single pellet of food (regular chow) was placed in the center of the box. The mouse was placed in a corner of the box, and a stopwatch was immediately started. Latency to begin eating (defined as the mouse sitting on its haunches and biting the pellet with the use of forepaws) was used as an index of anxiety-like behavior. Then, the