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Research report

Ascorbic acid attenuates scopolamine-induced spatial learning deficits in the water maze

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ABSTRACT

Vitamin C (ascorbate) has important antioxidant functions that can help protect against oxidative stress in the brain and damage associated with neurodegenerative disorders such as Alzheimer's disease. When administered parenterally ascorbate can bypass saturable uptake mechanisms in the gut and thus higher tissue concentrations can be achieved than by oral administration. In the present study we show that ascorbate (125 mg/kg) administered intraperitoneally (i.p.) 1-h before testing, partially attenuated scopolamine-induced (1 mg/kg i.p.) cognitive deficits in Morris water maze performance in young mice. Cumulative search error, but not escape latency nor path length, was significantly improved during acquisition in ascorbate plus scopolamine-treated mice although performance did not equal that of control mice. During the probe trial, scopolamine led to increased search error and chance level of time spent in the platform quadrant, whereas mice pre-treated with ascorbate prior to scopolamine did not differ from control mice on these measures. Ascorbate had no effect on unimpaired, control mice and neither did it reduce the peripheral, activity-increasing effects of scopolamine. Ascorbate alone increased acetylcholinesterase activity in the medial forebrain area but had no effect in cortex or striatum. This change, and its action against the amnestic effects of the muscarinic antagonist scopolamine, suggest that ascorbate may be acting in part via altered cholinergic signaling. However, further investigation is necessary to isolate the cognition-enhancing effects of ascorbate.

1. Introduction

Vitamin C (ascorbic acid) is of vital importance in the brain, which will sequester it at the expense of other organs in conditions of low ascorbate availability [28]. In fact, in guinea pigs dying of scurvy, when other organs are almost devoid of ascorbic acid, brain levels did not decrease below about 25% of normal [19]. In the first written study of scurvy, British naval surgeon Dr. James Lind reported his surprise in finding that "the brains of these poor people were always sound and entire (p. 245)" [30]. Ascorbate is a powerful antioxidant and thus may help prevent oxidative stress and related damage in a number of neurodegenerative diseases including Alzheimer's disease [15,17]. Furthermore, ascorbate is critical for the synthesis of norepinephrine from dopamine, is involved in regulation of dopamine and glutamate transmission [43] and is also involved in release of acetylecholine from vesicles [27] and thus has also been proposed to have important neuromodulatory functions [44].

We have recently shown that ascorbate (125 mg/kg) administered via intraperitoneal (i.p.) injection reversed some of the learning and memory deficits in aged (12 month) and very old (24 month) APP/PSEN1 transgenic mice, a mouse model of Alzheimer's disease [16]. Twelve consecutive days of daily treatments did not cause any changes in oxidative stress, amyloid plaque level or stored ascorbate levels in brain or liver, measured 24 h following the final treatment. We hypothesized that the effect of treatments was mediated via a short-term pharmacological-like action of ascorbate on neurotransmission. The muscarinic antagonist scopolamine causes short-term learning and memory deficits and has been used to provide a model of pharmacologically induced dementia [47,53]. When administered parenterally, ascorbate improved cognition against age- and scopolamine-induced amnesia in a passive avoidance task, a memory test in the elevated plus maze, and a habituation test in light–dark activity chambers [9,39,49]. The most common form of treatment for Alzheimer's disease involves acetylcholinesterase inhibitors (AChEIs) which work in degenerating cholinergic systems by keeping acetylcholine active for longer periods at the synapse. It has been proposed that ascorbate may have AChEI properties [10] although this has not been confirmed.

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Blueberry extract, another antioxidant compound, administered i.p. for 7 days has also been found to inhibit acetylcholinesterase (AChE) activity and enhance cognitive function in mice [38]. Longer (7 weeks) dietary treatment with antioxidant polyphenols found in tea also reduced AChE activity and attenuated scopolamine-induced amnesia in mice [23]. The present investigation was, therefore, conducted to seek confirmation as to whether ascorbate may act as a modulator of the cholinergic system or AChEI in young wild-type mice.

In the present study young wild-type mice were treated with scopolamine to induce memory deficits in the Morris water maze. Ascorbate treatment (i.p.) was investigated for its ability to reverse these deficits. Mice were also assessed for effects of drug on anxiety and activity to ascertain whether ascorbate mediated the cognitive effects of scopolamine, and to confirm that these could be dissociated from any non-cognitive peripheral effects such as increased locomotor activity. Following behavioral testing, mice were given one final treatment of ascorbate and/or scopolamine and brain tissue, liver, and serum were assessed for any short-term changes in ascorbate level, oxidative stress or AChE activity resulting from the treatments.

2. Materials and methods

2.1. Subjects

The current study was performed to further elucidate data and hypotheses obtained in APP/PSEN1 transgenic mice, a model of Alzheimer's disease [16]. The experiments were, therefore, conducted in B6C3F1/J mice, which form the background strain for the APP/PSEN1 mouse line. Twelve male and 12 female B6C3F1/J mice were obtained at 6 weeks of age from The Jackson Laboratory (Bar Harbor, ME). Animals were housed in single-sex cages in groups of four in a light- and temperature-controlled environment on a 12-h light/12-h dark schedule with free access to food and water for the duration of the experiment. Behavioral testing began when mice were aged 12 weeks. A further 12-week-old mice were used for visible platform testing in the water-maze and a final 20 mice were used for additional neurochemistry assays as described below but were not used for behavioral testing. All procedures were approved by the Vanderbilt University Institutional Animal Care and Use Committee and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Groups

For behavioral testing, four test groups each comprised six animals. Equal numbers of male and female mice were used in each group. Groups received the vehicle alone (CON), ascorbate alone (AA), scopolamine alone (SCOP), or ascorbate and scopolamine (AA-SCOP). For additional biochemical assays, a further 20 mice were divided equally among the four groups with approximately equal numbers and male and female mice in each group. Visible platform testing was conducted with only six CON and six SCOP mice.

2.3. Drugs

Ascorbate solutions (125 mg/kg) were prepared immediately before administration in deionized water and adjusted to pH 7. Solutions were kept in the dark in aluminum-foil covered containers and administered within 30 min of preparation in order to minimize the effects of oxidation. Scopolamine hydrochloride (S-1013) was obtained from Sigma–Aldrich (St. Louis, MO, USA) and dissolved in 0.9% physiological saline to a final dose of 1 mg/kg. Ascorbate or the vehicle was administered i.p. 60 min. before each daily behavioral testing session, including the water maze probe trial. Scopolamine was administered i.p. 30 min prior to behavioral testing. Administration volume for both drugs was 10 ml/kg.

3. Behavioral procedures

Differences in anxiety and locomotor activity can negatively impact the ability to perform complex learning tasks such as the water maze. We therefore first tested mice in the zero maze and locomotor activity chambers to provide a more complete behavioral phenotype in order to interpret correctly data from Y-maze and water maze tests.

3.1. Locomotor activity

Locomotor activity was assessed in commercially available activity monitors (ENV-510; MED Associates, Georgia, VT), as previously described [52]. Activity was automatically recorded by the breaking of infrared beams as the mouse explored the chamber, and analyzed using a Windows-based computer. Each session lasted 30 min, and the chambers were cleaned with a 10% alcohol solution between each mouse.

3.2. Zero maze

Anxiety was assessed using a standard Zero maze (San Diego Instruments, CA). A flat, plastic ring (inner edge diameter 51 cm, outer edge diameter 61 cm) was divided into four alternating sections, two of which had 15 cm high walls (closed sections) and 2 of which had just a 1 cm lip to prevent mice falling from the maze (open sections). The apparatus was raised 61 cm from the ground. Mice were released in the center of one of the open sections and permitted to explore the maze for 5 min. Sessions were filmed from above and scored by a trained experimenter for time spent in open and closed sections, and number of transitions between sections.

3.3. Y-maze

Spontaneous alternation behavior was tested in a standard Y-maze made of clear acrylic tubing, as previously described [18]. The number and sequence of arm entries made during a 5-min. session were recorded. Alternations were defined as an entry into each arm within three consecutive arm choices (e.g. ABC or BAC). Percent alternation was calculated as the number of alternations divided by the number of total arm entries minus two.

3.4. Water maze

Hidden-platform testing was conducted in a 107-cm diameter pool with a circular acrylic platform (10 cm diameter) submerged 1 cm below the surface of the water, as previously described [16,18]. Mice were given four acquisition trials per day for 9 days in a massed fashion, i.e. each mouse completed all four trials before the next mouse began its trials. The water maze was located in the centre of a room with distinct, visual cues fixed to the walls that were clearly visible from the pool. These extra-maze cues remained stationary throughout acquisition and probe test sessions. Sessions were captured by an overhead camera and analyzed in real time using an NIH Image macro on a Macintosh computer written specifically for the water maze task [31,32]. Escape latency and path length to reach the hidden platform were recorded during acquisition. Time spent swimming in the periphery (within 8 cm from edge of pool) can indicate increased anxiety and is a non-cognitive behavior that can adversely affect learning and the measurement of learning in the water maze [56]. Swim speed can be a confounding factor for assessing escape latency and path length does not provide any information about where the mouse is swimming in relation to the platform. Therefore, water maze acquisition can also be assessed using cumulative search error (distance from the platform during daily acquisition sessions) calculated as a daily average from each of the four training trials on that day. This can be a more informative measure because it takes into account trial time *and* proximity to the platform.

Twenty-four hours following target acquisition a 60-s probe trial was conducted. The time spent in the target versus non-target quadrants and the average distance from the platform location (search error [6,14]) were the primary dependent measures derived from the probe trial. Swim speed and peripheral swimming were also assessed during the probe trial.