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The effect of GABA transporter 1 (GAT1) inhibitor, tiagabine, on scopolamine-induced memory impairments in mice

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ABSTRACT

Background: GABAergic neurotransmission is involved in long-term potentiation, a neurophysiological basis for learning and memory. On the other hand, GABA-enhancing drugs may impair memory and learning in humans and animals. The present study aims at investigating the effect of GAT1 inhibitor tiagabine on memory and learning.
Methods: Albino Swiss (CD-1) and C57BL/6J mice were used in the passive avoidance (PA), Morris water maze (MWM) and radial arm water maze (RAWM) tasks. Scopolamine (1 mg/kg *ip*) was applied to induce cognitive deficits.
Results: In the retention trial of PA scopolamine reduced step-through latency as compared to vehicle-treated mice, and pretreatment with tiagabine did not have any influence on this effect. In MWM the results obtained for vehicle-treated mice, scopolamine-treated group and combined scopolamine + -tiagabine-treated mice revealed variable learning abilities in these groups. Tiagabine did not impair learning in the acquisition trial. In RAWM on day 1 scopolamine-treated group made nearly two-fold more errors than vehicle-treated mice and mice that received combined scopolamine and tiagabine. Learning abilities in the latter group were similar to those of vehicle-treated mice in the corresponding trial block on day 1, except for the last trial block, during which tiagabine + scopolamine-injected mice made more errors than control mice and the scopolamine-treated group. In all groups a complete reversal of memory deficits was observed in the last trial block of day 2.
Conclusions: The lack of negative influence of tiagabine on cognitive functions in animals with scopolamine-induced memory impairments may be relevant for patients treated with this drug.

Introduction

The termination of GABA action is mediated by its neuronal or astrocytic re-uptake. The majority of released GABA is transported into presynaptic nerve endings, whereas a smaller fraction is moved into astrocytes associated with these synapses. GABA taken up into presynaptic nerve endings is re-utilized as a neurotransmitter, but it can also be metabolized, both in neurons and astrocytes [1].

Until now, four plasma membrane GABA transporters (GAT) implicated in GABA re-uptake have been identified, cloned and thoroughly investigated as a potential drug target for the treatment of numerous neurological and psychiatric disorders [2]. In mice these transport proteins are named GAT1-4, whereas in rats and humans they are named GAT-1, BGT-1, GAT-2 and GAT-3, respectively [3].

Among numerous GAT inhibitors that have been synthesized and studied [3–14], there is only one drug that has been introduced into clinic, so far. Tiagabine (Fig. 1), a selective GAT1 inhibitor with IC_{50} of 0.11 μM [10], is used as an add-on therapy of partial seizures in men. Recent animal [15,16] and human [17–21] studies have demonstrated that it can be also effective in the treatment of chronic pain, anxiety or depression. Adverse effects of tiagabine

Abbreviations: CNS, central nervous system; GABA, γ-aminobutyric acid; GAT, GABA transporter; MWM, Morris water maze; PA, passive avoidance; RAWM, radial arm water maze.

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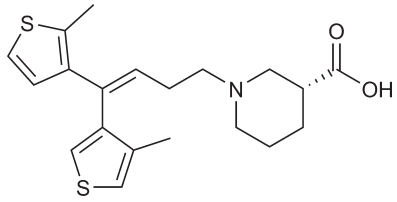

Fig. 1. Chemical structure of tiagabine.

comprise numerous CNS-derived effects, such as sedation, asthenia, dizziness and tremor [22,23].

A number of neurotransmitters differentially involved in the formation and retrieval of memory have been studied [24], and the neurotransmitters, such as glutamate, GABA, dopamine and acetylcholine have been reported to have more powerful impact (81–93%) on cognitive processes than serotonin and norepinephrine (48–55%). In neurodegenerative disorders which affect memory processing, pathological changes have been reported to be related to glutamatergic, cholinergic, noradrenergic and serotonergic neurotransmitter systems [24]. Noteworthy, not only glutamate, but also GABA and GABA-A receptors are involved in long-term potentiation, a phenomenon which is considered a neurophysiological basis for learning and memory processes [24–28]. Moreover, Shi et al. [27] showed that a moderate reduction of GAT1 activity caused cognitive enhancement in GAT1 heterozygous mice. On the other hand, some GABAergic drugs with anticonvulsant properties have been found to seriously impair learning and memory, both in humans [23,29] and experimental animals [30]. In view of these conflicting data, it seems interesting to investigate the effect of tiagabine on cognition. Current literature devoted to the influence of this drug on learning and memory is very limited. Hence, in the present study using three behavioral assays, *i.e.*, the passive avoidance test (PA) which is a fear-motivated task, and two tasks assessing spatial memory in rodents: Morris water maze (MWM) and radial arm water maze (RAWM), we have investigated the potential impact of this GAT1 inhibitor on learning and memory. We have used scopolamine, a nonselective cholinergic M receptor antagonist, a 'gold standard' drug for the induction of cognitive deficits in animals. This drug induces age- and dementia-related cognitive deficits in animals [31]. These cognitive impairments can be recognized by means of several 'land tasks' (*e.g.*, PA task) and 'water maze tasks' (*e.g.*, MWM or RAWM) [32].

Materials and methods

Animals

Eight-week old male Albino Swiss (CD-1) mice weighing between 18 and 22 g were used in the PA test, and C57BL/6J mice were used in the MWM and two-day RAWM tests. For each of these tasks separate groups of mice were used to avoid the possibility that one test may affect the results of another. The animals were housed in groups of 10 mice per cage at room temperature of 22 ± 2 °C, under light/dark (12:12) cycle. The animals had free access to food and water before experiments. The ambient temperature of the room and humidity were kept consistent throughout all the tests. For behavioral experiments the animals were selected in a random way. Each group consisted of 8–10 animals/dose, and each mouse was used only once. The experiments were performed between 8 a.m. and 2 p.m. Immediately after *in vivo* assays the animals were euthanized by cervical dislocation. The maintenance and treatment of laboratory animals were carried out in accordance with guidelines issued by the Local Ethics Committee of the Jagiellonian University in Cracow (ZI/862/2013).

Chemicals used in behavioral assays

Tiagabine (doses: 10 and 30 mg/kg in PA, and 10 mg/kg in MWM and RAWM) was purchased from Tocris Bioscience (Germany). For *in vivo* experiments it was suspended in 1% Tween 80 (Polskie Odczynniki Chemiczne, Poland) and administered intraperitoneally (*ip*) 60 min before the test (for a detailed protocol of drug administration see "Behavioral testing paradigm" section). Control mice were given appropriate amount of vehicle (1% Tween 80). (−)-Scopolamine hydrochloride was purchased from Sigma–Aldrich (Poland). To induce memory impairments it was dissolved in distilled water and administered *ip* at a dose of 1 mg/kg 30 min before the tests.

Behavioral testing paradigm

Passive avoidance task

The effect of tiagabine on acquisition and retention of PA task was conducted according to a previously described method [33]. For this purpose the passive avoidance apparatus (Panlab Harvard Apparatus, Spain) was used. It consists of a large white-painted illuminated compartment (26 cm × 26 cm × 34 cm) and a small black-painted dark compartment (13 cm × 7.5 cm × 7.5 cm) separated from each other by a guillotine gate.

To assess the effect of tiagabine on scopolamine-induced memory impairments the animals underwent two separate trials: an acquisition trial (conditioning phase) and a retention trial (testing phase). The latter was conducted 24 h after the acquisition trial. One hour before the acquisition trial, the mice were pretreated with tiagabine or vehicle. Control animals received 1% Tween 80 solution. Thirty minutes later, the animals were treated with scopolamine hydrochloride. For the acquisition trial, each mouse was initially placed for 30 s in the light compartment (exploration period; guillotine gate is closed). At the end of the exploration period the guillotine door (5 cm × 5 cm) between the light and the dark compartments was opened and the time that elapsed before entering the black chamber was recorded. As soon as the mouse entered the dark compartment, the door automatically closed and an electrical shock (current intensity: 0.2 mA, duration: 2 s) was delivered through the grid floor.

For the retention trial, the mice were placed in the illuminated white compartment again, and the latency time between door opening and entry into the dark compartment was recorded for each mouse. If the mouse did not enter the dark compartment within 180 s (cut off latency), it was concluded that it remembered the foot shock from the acquisition trial. Better memory performance was indicated by longer latency to enter in the black chamber in the test (retention) phase than in the conditioning (acquisition) phase.

Morris water maze test

The Morris water maze (Panlab Harvard Apparatus, Spain) is a circular, plastic and gray-painted pool (120 cm in diameter and 60 cm in height), filled with water (up to about 48 cm below the edge to prevent an animal jumps out) maintained at 23 ± 1 °C. The pool was divided into four equal quadrants (compass locations: NE, NW, SE, SW) by a computerized video tracking system (SMART, ver. 3.0; Panlab, Spain). An escape platform (11 cm in diameter and 47 cm in height) at a fixed location (the center of the NW quadrant, *i.e.*, the target quadrant) was made of transparent Plexiglas, invisible to the swimming animal and was immersed 1 cm under the surface of water. The maze was lighted with the intensity of 45 lx.

During the spatial acquisition trial (6 consecutive days) mice were assigned to training sessions (four training sessions a day; sessions were held at 4 h intervals) in which the mice were trained to escape from water by reaching a hidden platform whose

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