Effects of Vitexin on Scopolamine-Induced Memory Impairment in Rats

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Abstract

Various synthetic derivatives of natural flavonoids are known to have neuroactive properties. The present study aimed to investigate the effects of vitexin (5, 7, 4-trihydroxyflavone-8-glucoside), a flavonoid found in such plants as tartary buckwheat sprouts, wheat leaves phenolome, Mimosa pudica Linn and Passiflora spp, on scopolamine-induced memory impairment in rats. To achieve this goal, we assessed the effects of vitexin on memory retrieval in the presence or absence of scopolamine using a step-through passive avoidance trial. In the first part of the study, vitexin (25, 50, and 100 $\mu$M) was administered intracerebroventricularly (i.c.v.) before acquisition trials. In the second part, vitexin, at the same doses, was administered before scopolamine (10 $\mu$g, i.c.v.) and before the acquisition trials. During retention tests, vitexin (100 $\mu$M) in the absence of scopolamine significantly increased the step-through latencies compared to scopolamine. In addition, vitexin (100 $\mu$M) significantly reversed the shorter step-through latencies induced by scopolamine ($P<0.05$). These results indicate that vitexin has a potential role in enhancing memory retrieval. A possible mechanism is modulation of cholinergic receptors; however, other mechanisms may be involved in its effects in acute exposure.

Key Words: flavonoid, memory retrieval, passive avoidance, scopolamine, vitexin

Introduction

Flavonoids are a group of structurally related phenyl benzopyrones. They are subdivided into the following main classes: flavones, flavanones, flavonols, isoflavones, flavans and flavan-3-ols. Because they are found in all plants, they are constituents of the human diet (13). Flavonoid derivatives have been considered useful in treatment for neurodegenerative disorders such as Alzheimer disease (AD) (45). In recent years, several mechanisms have been examined for signalling pathways that underlie improved cognitive function; flavonoids activate the extracellular signal-regulated protein kinase cAMP-response element-binding protein (ERK-CREB) pathway and the phosphoinositide 3-kinase-mTOR (PI3-kinase-mTOR) cascade, leading to changes in synaptic plasticity. They are also capable of influencing neurogenesis through the activation of PI3-kinase-AkteNOS (44). Moreover, flavonoids have been reported to block oxidative-induced neuronal damage by preventing the activation of caspase-3, thus, providing evidence in support of their potent anti-apoptotic action (37).

In a brain-imaging study of humans, the consumption of flavanol-rich cocoa was shown to enhance cortical blood flow (8). This result is important for increasing cerebrovascular function, especially in the hippocampus, a brain region that is important for memory and that may facilitate adult neurogenesis.
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New hippocampal cells are clustered near blood vessels that proliferate in response to vascular growth factors and may influence memory (29). Several lines of evidence suggest that flavonoids have beneficial effects on memory and learning (39). The effects of flavonoid-rich foods and pure flavonoids on neurocognitive ability have been shown in rodents (14, 16, 18, 47). Rodent models have been used as models of human declarative memory to predict the potential effects of flavonoids on human cognitive performance (34). Previous reports have also established a role for flavonoids in preventing human dementia (5). It has shown that flavonoids have acetylcholinesterase inhibitory activities (20, 42).

Scopolamine is used as a standard drug for inducing cognitive deficits in healthy humans and animals (21). Scopolamine is a muscarinic receptor antagonist with amnestic properties and is a widely used model for characterizing potential cognition-enhancing drugs (8). Blockade of central muscarinic receptors may induce a pattern of cognitive impairment in AD patients (4).

Vitexin (5, 7, 4-trihydroxyflavone-8-glucoside) is a c-glycosilated flavone often found in plants such as Tartary buckwheat sprouts, wheat leaves phenolome, Mimosa pudica Linn and Passiflora spp (25, 31, 46, 48). It has been reported that vitexin has several pharmacological properties, including antinociceptive, antispasmodic, antimyeloperoxidase and α-glucosidase inhibitory activities (11, 12, 23, 32, 33, 38). In addition, vitexin has been shown to have free radical scavenging activity in ultraviolet B (UVB)-irradiated cultured human dermal fibroblasts (19). Recently, we have shown that vitexin has anticonvulsant effects in rats treated with pentylenetetrazole (PTZ) (1).

In the present study, two sets of experiments were conducted. We first investigate the dose-response of vitexin alone, followed by the effects of vitexin with scopolamine interactions.

Materials and Methods

Animals

Male Wistar rats (200-250 g) were obtained from the Razi Institute (Karaj, Iran) and housed in groups of four per cage under standard laboratory conditions. They were kept at constant room temperature (21 ± 2°C) under a normal 12 h light/12 h dark (12L:12D) regime with free access to food and water. All the animal experiments were performed in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC) to minimize the number of animals and their suffering.

Drugs

Vitexin was purchased from Fluka (Buches, Swetzerland). The other drugs used in this study were ketamine (Rotexmedica, GmbH, Germany) and xylazine (Loughrea Co, Galway, Ireland). Scopolamine was purchased in an injectable form from Daru Pakhsh (Tehran, Iran). Vitexin was dissolved in saline. Vitexin, scopolamine and saline were administered intracerebroventricularly (i.c.v.) in a volume of 5 µl.

Surgery and Experimental Procedures

All the rats were anesthetized with ketamine (60 mg/kg, i.p.) and xylazine (6 mg/kg, i.p.) and placed in a stereotaxic apparatus (Stoelting, Wood Dale, IL, USA). The skull surface was exposed, and the head was oriented such that the bregma and lambda skull sutures were at the same vertical levels. Stereotaxic coordinates for the left lateral ventricle were: anterior-posterior (AP), -0.92 mm from bregma; medial-lateral (ML), 1.6 mm from the midline; and dorsal-ventral (DV), 3.5 mm from the skull surface (30). For chronic i.c.v. cannulation, a stainless steel guide cannula (21-gauge) was implanted to terminate 1 mm above the left lateral ventricle (2.4 mm ventral from the skull surface). This cannula was attached to the skull with a stainless steel screw and dental acrylic cement. Housed individually, the rats were allowed to recover from surgery for at least a week. On the day of the experiment, the rats were transferred to the experimental room 1 h prior to the experiment. For intracerebroventricular drug administration, the rats were gently hand restrained and drug infusions were made using an injection needle (24-gauge) inserted into the guide cannula connected through a polyethylene tube to a 5 µl Hamilton syringe (28).

Experimental Procedure

The rats were divided into eight groups of 10 animals each. The first group was administered saline (5 µl, i.c.v) 30 min before acquisition trials. The second group received scopolamine (10 µg, i.c.v.) 30 min before the acquisition trials. The next three groups received vitexin (25, 50 or 100 µM, i.c.v.) 30 min before the acquisition trials. In the remaining three groups, vitexin (25, 50 or 100 µM, i.c.v.) was administered 15 min before scopolamine (10 µg, i.c.v.) and 45 min before the acquisition trials.

Passive Avoidance Apparatus

The passive avoidance apparatus comprised of
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A learning box consisting of a light (white) and a dark (black) compartment, 20 × 20 × 30 cm each. A guillotine door opening (6 × 6 cm) was made on the floor in the center of the partition between the two compartments. Stainless steel grids (5 mm in diameter) were placed at 1 cm intervals (distance between the centers of grids) on the floor of the dark compartment to produce a foot shock (Borj Sanat, Tehran, Iran).

All the rats were allowed to habituate to the experimental room for at 30 min prior to the experiments. This standard was applied across experiments both in the vitexin dose response and the vitexin with scopolamine treatment. Then, each rat was gently placed in the light compartment of the apparatus. After 5 sec, the guillotine door was opened and the rat was allowed to enter the dark compartment. The latency with which each rat crossed into the dark compartment was recorded. The rats that waited more than 100 sec to cross into the dark compartment were eliminated from the experiments. Once the rat crossed with all four paws into the next compartment, the guillotine door was closed and the rat was returned to its home cage. The acquisition trial was performed 30 min after the habituation trial. The rat was placed in the light compartment, and the guillotine door was opened 5 sec later. As soon as the rat crossed into the dark compartment, the door was closed and a foot shock (0.5 mA intensity, 3 sec) was immediately delivered to the grid floor of the dark room by an insulated stimulator (26). One day after training, retention tests were performed to evaluate memory performance. Each rat was placed in the light compartment for 20 sec, the door was opened, and the step-through latency for entering into the dark compartment was measured. The test session ended when the rat entered the dark compartment or remained in the light compartment for 300 sec. No electric shock was applied during these sessions (27).

Histology

At the end of the experiments, the rats were injected with 2 µl of crystal violet and deeply anesthetized. Their brains were removed and fixed in 10% formaldehyde solution. For histological examination of the cannula and needle placement in the lateral ventricle region, 100 µm-thick sections were taken and the cannula track was examined in each rat. Only those rats whose cannulae were exactly placed in the left ventricle were used for further analysis (28). Thus, only behavioral results for animals with correct cannula placement were considered.

Data Analysis

The data were analyzed three times as follows:

[1] control versus scopolamine treatment analyzed by Student’s t-test, [2] vitexin dose response comparing with the control vs. 25, 50 or 100 µM vitexin analyzed by one-way ANOVA, [3] vitexin with scopolamine treatment analyzed by one-way ANOVA. A probability P < 0.05 was considered statistically significant.

Results

Histological evaluation of the brains of control and drug treated rats confirmed that in all rats in each group, the cannula were in correct position (Fig. 1).

The step-through latency times were not significantly different between the experimental and control groups during the acquisition test (Fig. 2A). During the retention test, the step-through latency of the scopolamine-treated group was significantly lower than that of the control group (P < 0.05) (t-test) (Fig. 2B).

The step-through latencies of the groups treated with vitexin alone (25, 50 or 100 µM) were not significant at the studied doses compared to the control (one-way ANOVA, Tukey-Kramer test).

However, vitexin alone at a dosage of 100 µM significantly increased the step-through latency compared to scopolamine treatment (one-way ANOVA, Tukey-Kramer test) (Fig. 2B).

The step-through latencies of the groups treated with vitexin (25, 50 or 100 µM) with scopolamine were not significant at the studied doses compared to the control (one-way ANOVA, Tukey-Kramer test).

Furthermore, in the analysis of the groups treated with vitexin (25, 50 or 100 µM) with scopolamine by
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A one-way ANOVA, vitexin (100 µM) significantly reversed the shorter step-through latencies of scopolamine (*P < 0.05) (one-way ANOVA, Tukey-Kramer test) (Fig. 2B).

Discussion

Data of the present study show that the step-through latency of the scopolamine-treated group was significantly lower than that of the control group in the retention test. Vitexin alone at 100 µM significantly increased the step-through latency compared to scopolamine.

Vitexin administration prior to scopolamine in the acquisition trials is followed by attenuated impairment retention of the avoidance test in rats. Vitexin at a dose of 100 µM significantly reversed scopolamine-induced memory impairment.

Despite some limitations that it fails to replicate the pathological aspects and the progressive degenerative nature of Alzheimer’s disease (43), scopolamine-induced memory impairment, particularly when coupled with a version of the inhibitory avoidance task, provides a relatively rapid phenotypic screening tool for drug discovery in the field of cognition enhancement (7). Similar results have been obtained for other related compounds such as rutin, quercetin, resveratrol and curcuminoind, all of which showed potential protective effects against scopolamine-induced memory impairment (3, 9, 35).

One of the receptors proposed to act as a flavonoid-binding site in neurons is the GABA<sub>A</sub> receptor (2, 15). Muscarinic receptor subtypes are localized in pyramidal and nonpyramidal cells in the hippocampus (22). It has been shown that muscarinic receptors can enhance GABAergic synaptic transmission through a presynaptic and postsynaptic mechanism (49). The rapid release of GABA in response to acetylcholine is mediated by a different subtype of muscarinic receptors (24). Activation of cholinergic receptors seems to cause direct excitation of GABAergic interneurons (17). Moreover, GABAergic inhibition is known to control the flow of information in a cortical circuit that is important for performance of certain forms of memory (6). These findings suggest that vitexin possibly acts via modulation of the GABAergic system to attenuate scopolamine-induced memory impairment. For this reason, the effects of vitexin on memory were more significant in the presence of scopolamine.

As an active component of Urtica circularis, vitexin has shown potent antinociceptive effects in a chemical model of nociception in mice. The possible role of cholinergic activation has been discussed for antinociceptive effects of the extract (13). Furthermore, as the role of acetylcholinesterase inhibitory activity of flavonoids, vitexin could have cholinergic properties (20, 42). As shown by our results, it is pos-
sible that vitexin improves cognition by modulating cholinergic neurotransmission.

The efficacy of flavonoids on memory performance is dependent on the period of their administration. It seems that the mechanisms of acute and chronic flavonoid administration are different (40). Flavonoids have been shown to influence peripheral and central blood flow. Based on both in vitro and in vivo studies, these vascular effects are potentially significant because increased cerebrovascular function is known to facilitate adult neurogenesis in the hippocampus (10, 36). After consumption of flavonol-rich foods, cerebral blood flow rapidly increases in a certain region of the human brain (8). It has been suggested that increased cerebral blood flow exerts impacts on acute cognitive performance (41). Moreover, it has been shown that flavonoids may have the potential to reduce brain amyloid-beta (Aβ) levels by improving cerebral blood flow (45). However, it should be considered that increased cerebral blood flow occurs both on acute and chronic exposure.

In the present study, it is possible that vitexin directly interacts with cholinergic or/and GABAergic neurons and attenuates scopolamine-induced memory impairment in the passive avoidance task. Alternatively, vitexin may act indirectly and activate some signaling pathway by increasing cerebral blood flow. However, further investigation is required to clarify this issue.

Our behavioral study shows that vitexin administration can attenuate scopolamine-induced memory impairment in rats. Vitexin administration prior to training trials in passive avoidance tests resulted in improved memory retrieval, which was observed in the retention tests. It is possible that vitexin acts by several mechanisms that play potential roles in memory retrieval in rats. Further studies are necessary to evaluate the effects of vitexin on memory retention and to determine its molecular mechanisms.

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