

The effects of rutin on the development of pentylenetetrazole kindling and memory retrieval in rats

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ABSTRACT

Individuals with epilepsy often complain about memory deficits. Various synthetic derivatives of natural flavonoids are known to have neuroactive properties. Rutin is a flavonoid that is an important dietary constituent of foods and plant-based beverages. The aim of the present study was to investigate the effects of rutin on memory retrieval in pentylenetetrazole (PTZ)-kindled rats using a step-through passive avoidance task. We administered rutin and PTZ intraperitoneally every other day prior to the start of training. Two retention tests were subsequently performed to assess memory in these rats. The results suggest that pretreatment with rutin at 50 and 100 mg/kg can attenuate seizure severity during the kindling procedure. Furthermore, rutin administration significantly increased the step-through latency in the passive avoidance paradigm. Taken together, these results indicate that rutin has a potential role in enhancing memory retrieval in kindled rats.

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1. Introduction

Animal models for kindling provide an acceptable approach to quantifying epileptogenesis [1]. Kindling induced with the convulsant pentylenetetrazole (PTZ) represents a model of primary generalized epilepsy [2,3]. The effects of epilepsy on cognition have been reviewed in several articles [4–6], suggesting the presence of at least a mild decline in intellectual performance in children and adults with epilepsy [4]. However, correlating seizures with mental decline requires a careful investigation of the types and frequencies of seizures [5]. Patients with localized epilepsies generally have specific deficits in the cognitive functions controlled by the respective areas, for example, memory impairment associated with temporal lobe epilepsy (TLE) [6]. The PTZ kindling model provides a useful model for postseizure dysfunction, serving as a screen for potential treatments for the cognitive and emotional deficits that are observed in human epilepsy [7]. There are several pieces of evidence for PTZ kindling-induced impairment in shuttle-box performance tasks [8–13].

On the other hand, several studies have shown that flavonoids and other fruit- and vegetable-derived phytochemicals have beneficial effects on learning and memory [14]. Several flavonoid-rich foods including ginkgo biloba, green tea, blueberry, and pomegranate juice have been shown to enhance neurocognitive ability in rodents [15–18]. Rodent models have also been used to study human declarative memory, predicting the potential effects of flavonoids on human cognitive performance [19]. In addition, previous reports have established a role for flavonoids in preventing dementia in humans [20].

Rutin (3, 3', 4', 5, 7-pentahydroxyflavone-3-rhamnoglucoside) is a flavonoid of the flavonol type that is an important dietary constituent of foods and plant-based beverages [21]. Rutin has several noteworthy pharmacological properties, including antioxidant, anticarcinogenic, cytoprotective, antiplatelet, antithrombotic, vasoprotective, cardioprotective, and neuroprotective activities [22–28]. Furthermore, it acts as an anticonvulsant in rats treated with PTZ [29], and has ameliorated ischemia–reperfusion injury in the brain [30]. Recently, we found that rutin may enhance memory retrieval in normal animals [31] and that prolonged supplementation with rutin significantly reverses trimethyltin (TMT)-induced spatial memory impairments and damage to pyramidal neurons in the hippocampal CA3b region. These effects could potentially be related to the antioxidative effects of rutin [32] as well as to suppressed microglial activation and pro-inflammatory cytokines [33]. Thus, in this study, we focused on the possible effects of rutin on memory retrieval in PTZ-kindled rats.

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2. Materials and methods

2.1. 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 a constant room temperature (21 ± 2 °C) under a normal 12 L:12D regimen with free access to food and water. Behavioral observations and evaluations were performed by experimenters who were unaware of the pharmacological treatment. All animal experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) in such a way as to minimize the number of animals and their suffering.

2.2. Drugs

Rutin and PTZ were purchased from Sigma and were dissolved in physiological saline prior to the experiments. All drugs were administered intraperitoneally (ip).

2.3. Experimental procedure

Rats were divided into four groups, each with 10 animals. In the control group, saline was injected 30 minutes before administration of PTZ every other day (35 mg/kg, ip, 13 injections total).

After each injection, the animals were placed individually in plastic boxes. Convulsive behavior was recorded for 20 minutes, and behavioral changes were scored according to Racine's criteria [34]: 0 = no behavioral changes; 1 = facial movements, ear and facial twitching; 2 = myoclonic convulsions without rearing; 3 = myoclonic convulsions with rearing; 4 = clonic convulsions with loss of posture; 5 = generalized tonic-clonic seizures. In the remaining three groups 10, 50, or 100 mg/kg of rutin was administered 30 minutes before PTZ every other day (35 mg/kg, ip, 13 injections total).

Furthermore, for memory testing, a group of animals with unimpaired memory (because they had been injected with saline instead of PTZ) were used. This group was used to elucidate the effects of rutin on memory under baseline conditions, compared with levels that were lower or higher than baseline.

2.4. Passive avoidance apparatus

Forty-eight hours after the last PTZ injection to induce kindling, the animals were tested for learning behavior using a passive avoidance shuttle-box task [35]. The learning box consisted of two compartments, one light (white compartment, $20 \times 20 \times 30$ cm) and the other dark (black compartment, $20 \times 20 \times 30$ cm). 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 the foot shock.

2.5. Training

All of the animals were allowed to habituate in the experimental room for at least 30 minutes prior to the experiments. After habituation, each animal was gently placed in the light compartment of the apparatus; after 5 seconds, the guillotine door was opened and the animal was allowed to enter the dark compartment. The latency to the animal's entry into the dark compartment was recorded. Animals that waited more than 100 seconds to cross into the dark compartment were eliminated from the experiments. Once the animal 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 carried out 30 minutes after the habituation trial. The animal was placed in the light compartment, the guillotine door was opened 5 seconds later, and as soon as the animal crossed into the dark compartment, the door was closed and a footshock (50 Hz, 5 seconds, 0.2-mA intensity) was immediately delivered to the grid floor of the dark room with an insulated stimulator. After 20 seconds, the rat was removed from the apparatus and placed in its home cage. Training was terminated when the rat remained in the light compartment for 120 consecutive seconds [31].

2.6. Retention test

One and two days following training, the retention tests were performed to evaluate memory. Each animal was placed in the light compartment for 20 seconds, the door was opened, and the step-through latency to entry into the dark compartment was measured. The session ended either when the animal entered the dark compartment or when it remained in the light compartment for 300 seconds. During these sessions, no electric shock was applied [31].

2.7. Data analysis

The data were analyzed using a one-way ANOVA and the post hoc Tukey test for multiple comparisons. A level of $P < 0.05$ was considered significant.

3. Results

3.1. Effects of rutin pretreatment on kindling development

As shown in Fig. 1, the repeated application of 35 mg/kg PTZ induced behavioral seizures of increasing severity, culminating in clonic convulsions with loss of posture during stage 4 at the end of the kindling procedure. Pretreatment with rutin (10, 50, and 100 mg/kg) before each kindling injection modified the development of kindling. Pretreatment with rutin at 10, 50, and 100 mg/kg significantly reduced the mean seizure stage during the 13 kindling injections as compared with the saline control ($P < 0.01$, $P < 0.001$, $P < 0.001$) (Fig. 1).

3.2. Effects of rutin pretreatment on passive avoidance test

Administration of rutin induced a significant increase in memory retrieval on the first retention test (1 day after training) as compared

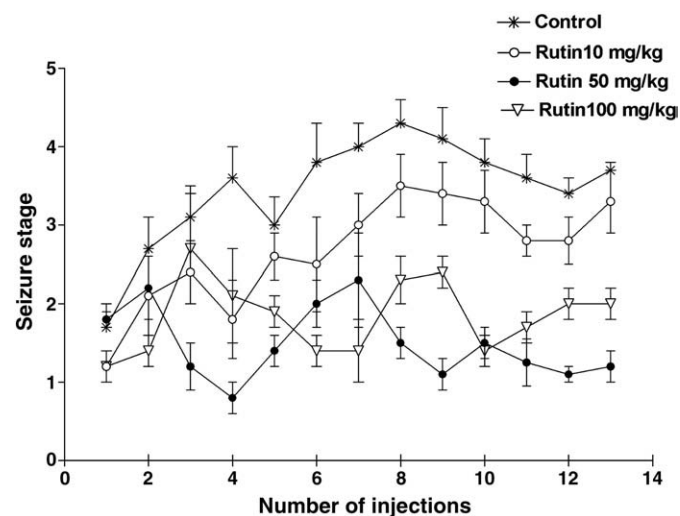


Fig. 1. Effects of repeated administration of rutin (10, 50, and 100 mg/kg) on the development of PTZ-induced kindling (35 mg/kg, ip, 13 injections total), compared with control. Data represent mean seizure stages \pm SEM, $n = 10$.

with controls. Rutin was administered at doses of 50 and 100 mg/kg 30 minutes before the administration of PTZ every other day (35 mg/kg, ip, 13 injections total) prior to training ($P < 0.001$) (Fig. 2). Rutin also increased memory retrieval at a dose of 10 mg/kg; however, this effect was not significant. Moreover, at 50 and 100 mg/kg, rutin significantly increased memory retrieval in the second retention test of the passive avoidance paradigm, compared with the control. This effect was dose dependent ($P < 0.01$, $P < 0.001$) (Fig. 2).

4. Discussion

A survey of more than 1000 patients with epilepsy in the United States revealed that cognitive difficulties rank highest on a list of potential concerns [36]. In humans, convulsive diseases such as temporal lobe epilepsy (TLE) are often accompanied by impairments in learning and memory [37]. Three forms of memory impairment may occur among patients with epilepsy: (1) transient epileptic amnesia, (2) accelerating long-term forgetting, and (3) remote memory impairment [38].

In the present study, we investigated the effect of rutin on the development of PTZ-induced kindling in rats. Pretreatment with rutin at 50 and 100 mg/kg attenuated seizure severity from the beginning of the kindling procedure by lowering the mean seizure stage. Moreover, it appears that the effects of rutin at higher doses are more significant.

Furthermore, pretreatment with rutin before administration of PTZ every other day, and prior to training, is associated with enhanced memory retrieval in rats. Rutin (50 and 100 mg/kg) significantly increased retrieval of memory in the first and second retention tests of a passive avoidance task, compared with controls. The cognitive-enhancing effect of rutin was also observed at 10 mg/kg. The results for the saline group suggest that rutin restored memory to a level higher than baseline.

Several studies have demonstrated that flavonoids have neuroactive properties [13,39,40] and that many of these compounds are ligands for GABA_A receptors in the central nervous system (CNS) [41,42]. Furthermore, they have been found to act like benzodiazepine [43–45]. These findings are supported by behavioral data measuring anxiety, sedation, and convulsive activity in animal models [39,41,46]. In our previous study, intracerebroventricular (icv.) injection of rutin affected minimal clonic seizures (MCSs) in a dose-dependent manner, as well as generalized tonic-clonic seizures (GTCSs), induced with PTZ in rats [29]. The GABAergic system contributed to the anticonvulsant activity of rutin in this study [29]. Moreover, administration of rutin (10 mg/kg) 1 week before the start of training significantly increased

retrieval of memory in the first, second, and third retention tests of a passive avoidance task, compared with controls [31]. Thus, it is possible that by enhancing the activity of the GABAergic and opioid systems, rutin can reduce the severity of seizures resulting from kindling and enhance memory retrieval in kindled rats. However, in this study, it appears that a higher dose of rutin is needed to enhance memory retrieval in kindled rats than in normal rats.

Similar results have been observed in rats treated with rutin; both rutin and quercetin improved spatial memory impairment, as assessed with the eight-arm radial maze task, and reduced neuronal death in the hippocampal CA1 area. The same study revealed that, biochemically, the 4-oxo group and the 2,3 double bond in the C ring common to both rutin and quercetin are related to their neuroprotective action. However, it was also demonstrated that rutin has a more significant effect on spatial memory impairment than quercetin [28]. It has already been established that the CA3 subregion of the hippocampus plays an important role in the acquisition of contextual memory, whereas the CA1 subregion plays a role in the consolidation process [47].

Furthermore, the anti-inflammatory effects of flavonoids, including rutin, have been reported in several studies. Their role in the inhibition of nitric oxide production has also been discussed [48–51]. In another study, both rutin and quercetin were found to protect the cell membrane from lipid oxidation, a result that was related to their antioxidant effects [52]. Collectively, all of these mechanisms could contribute to the cognition-enhancing effects of rutin.

The direct role of flavonoids in memory acquisition, consolidation, and storage was described previously in a study that induced activation of neuronal signaling and gene expression in the brain. The results from this study indicate that this may lead to changes in synaptic plasticity and neurogenesis in the brain, ultimately influencing memory, learning, and cognition [53]. Flavonoids have been shown to influence peripheral blood flow in humans [54]; for example, in a brain imaging study, the consumption of flavanol-rich cocoa enhanced cortical blood flow [55]. This result is important when considering mechanisms that increase cerebrovascular function, especially in the hippocampus, a brain region that is important for memory and that may facilitate adult neurogenesis [56]. New hippocampal cells cluster near the blood vessels; they proliferate in response to vascular growth factors and may eventually influence memory [57].

In recent years, several mechanisms for signaling pathways that underlie improved cognitive function have been examined. Some results suggest that 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–Akt–eNOS [58]. It has been shown that quercetin fits into the ATP-binding pocket of the PI3-kinase, inhibiting its activity. This inhibitory action has been proposed as a potential mechanism by which flavonoids modulate neuronal function [59]. Moreover, flavonoids reportedly block oxidation-induced neuronal damage by preventing the activation of caspase-3, thereby providing evidence in support of their potent antiapoptotic action [60].

It has therefore been suggested that rutin not only blocks a memory-destroying effect of kindling, but also has memory-enhancing effects, thereby enhancing memory retrieval in the passive avoidance task.

In our study, pretreatment with rutin before administration of PTZ every other day prior to training decreased the severity of seizures and resulted in increased retrieval of memory, which was observed in kindled rats during the first and second retention tests of the passive avoidance task. This effect was more significant when rutin was administered at doses of 50 and 100 mg/kg. Rutin may mediate memory retrieval in a variety of ways. Further study is necessary to

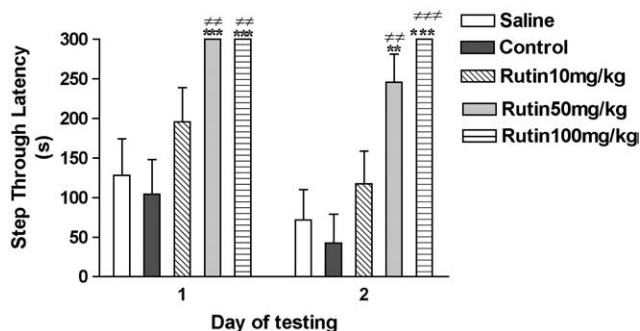


Fig. 2. Effects of rutin (10, 50, and 100 mg/kg) on step-through latency in rats. Values are expressed as means \pm SEM. The control group received PTZ injections every other day (35 mg/kg, ip, 13 injections total) prior to the experiments. The three remaining groups were given 10, 50, and 100 mg/kg rutin 30 minutes before administration of PTZ every other day prior to the experiments. Retention tests were performed 1 and 2 days after training, ** $P < 0.01$, *** $P < 0.001$ compared with the same-day control, ## $P < 0.01$, ### $P < 0.001$ compared with the same-day saline group, $n = 10$, Tukey–Kramer test.

evaluate the effect of rutin on retention of memory and to determine the molecular mechanisms involved in this process.

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