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Protective Effect of Quinine on Chemical Kindling and Passive Avoidance Test in Rats

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Abstract

Background: In humans, convulsive diseases such as temporal lobe epilepsy are usually accompanied by learning and memory impairments. In recent years, the role of gap junction channels as an important target of antiepileptic drugs has been studied and discussed. Quinine, as a gap junction blocker of connexin 36, can abolish ictal epileptiform activity in brain slices.
Objectives: The role of quinine in memory retrieval in pentylenetetrazole (PTZ)-kindled rats was examined using a step-through passive avoidance task.
Methods: Forty rats were used in this experimental study in groups of 10 animals. Quinine (15, 30, and 60 mg/kg, i.p.) and PTZ (35 mg/kg, i.p.) were injected into the rats before the start of the learning test. Then, retention tests were conducted after the treatments ended.
Results: Quinine could attenuate seizure severity at doses of 15, 30 and 60 mg/kg compared with the control at the beginning of the kindling experiment by lowering the mean seizure stages ($P < 0.01$, $P < 0.001$, $P < 0.001$). Quinine at doses of 15 and 30 mg/kg could significantly increase memory retrieval compared with the control in the retention test 24 and 48 hours after training ($P < 0.05$). Quinine at a dose of 60 mg/kg increased latency to enter the dark chamber 24 and 48 hours after training ($P < 0.001$). The results of the retention test one and two weeks after training of quinine were not significant ($P > 0.05$).
Conclusions: Quinine may decrease the severity of seizure and improve the memory retrieval of animals by inhibiting the gap junction channel. However, further studies are needed to evaluate the molecular mechanism underlying the effects of quinine.

Keywords: Seizure, Quinine, Pentylenetetrazole, Passive Avoidance Test

1. Background

Epilepsy is a major focus in the study of neurological disorders. The incidence of epilepsy in the general population is 45/100,000 (1). The pentylenetetrazole (PTZ)- kindling model is a suitable model to show post abnormality of seizure and can be used to find useful treatments for cognitive impairment in human epilepsy (2). Memory impairment has been reported in patients with temporal lobe epilepsy (TLE) (3).

Several studies showed that chemical kindling induced by PTZ could impair memory retrieval (4-8). Communication of neuron via gap junction channels has been considered as an important mechanism in synchronizing neurons in different conditions (9-12). In recent years, the role of glial connexins (Cxs) and pannexins (Panxs), proteins that form gap junctions and membrane hemichannels (connexins) and hemichannels (pannexins), in epilepsy has been discussed. The gap junction seems to be involved in seizure activity (13). Abnormal synchronization of neurons has been determined to occur during epilepsy. Thus, gap junction blockers could inhibit seizure, and openers could enhance it (14, 15). Gap junction channels play a role in synchronizing human neocortical networks and may implement epileptiform activity in focal cortical dysplasia (16). Quinine, an anti-malarial drug, specifically blocks Cx36 and with lesser potency than Cx50 in mammalian cells (17). Quinine could increase the behavioral stimulant effect of cocaine in mice and seems to play a role in increasing the clearance of dopamine (18). It inhibited both electrophysiological parameters, such as amplitude and frequency of spike, and behavioral symptoms of epilepsy (19). Gap junctions responsible for electrotonic coupling between neurons and astrocytes and synchronization of epileptoidal discharges are also considered as a potential target for future antiepileptic drugs (20). The results on the quaternary derivative N-benzylquininium

of quinine indicated that modulating the gating of loop caused the blocking of the gap junction channel rather than its inhibition (21).

2. Objectives

This study aimed to find the possible effects of quinine on memory retrieval in chemical kindling induced by PTZ in rats.

3. Methods

3.1. Animals

Male Wistar rats (250 - 300 g) were obtained from the Razi institute (Karaj, Iran) and housed under standard laboratory conditions. They were kept at a constant room temperature (21 ± 2 °C) under a normal 12 light: 12 dark cycle with free access to food and water. All animal experiments were conducted in accordance with the european communities council directive of November 24, 1986 (86/609/EEC) to minimize the number of animals and their suffering.

3.2. Chemicals

Quinine anhydrous hydrochloride and PTZ were purchased from Sigma. PTZ was dissolved in a physiological saline solution, and quinine was dissolved in 0.8% (v/v) Tween 80.

3.3. Experiments

Forty rats were used in this experimental study in groups of 10 animals. In group one, saline was administrated 30 minutes prior to the injection of PTZ (35 mg/kg, i.p., 9 injections total). In the other three groups, quinine at doses of 10, 30, 60 mg/kg was administered 30 minutes before PTZ every other day (35 mg/kg, i.p., 9 injections total), respectively. All injections were administrated every other day. After the administration of drugs, the convulsive behavior of animals was recorded as Racine's criteria for 20 minutes (22): Stage 0= no changes, stage 1= facial and ear movements and facial twitching, stage 2= myoclonic convulsions, stage 3= myoclonic convulsions with rearing, stage 4= clonic convulsions and loss of posture, and stage 5= generalized tonic-clonic seizures. Two days after the final injection of PTZ, learning behavior of animals was evaluated using passive avoidance performance (23). The shuttle box consisted of two parts, a light compartment (20 × 20 × 30 cm) and a dark compartment (20 × 20 × 30 cm). A guillotine door opening (6 × 6 cm) was formed on the floor of the partition between the two compartments.

Food shock was produced by stainless steel grids (5 mm in diameter) placed at 1 cm intervals on the floor of the dark compartment. Animals were habituated in the experimental room for at least 30 minutes. The acquisition trial was conducted 30 minutes after the habituation trial. Each animal was placed in the light compartment, and the guillotine door was opened after 5 seconds. Immediately, the animal entered the dark compartment, the door was closed, and a foot shock (50 HZ, 5 seconds, 0.2 mA intensity) was delivered to the grid floor of the dark part. After 20 seconds, the rat was removed from the apparatus and placed in its home cage. The training was terminated when the rat remained in the light compartment for 120 seconds consecutively (24). The retention tests were performed 1, 2, 7, and 14 days after training to evaluate memory function. Each animal was placed in the light compartment, and the session ended when the animal entered the dark compartment or when it remained in the light compartment for 300 seconds. In these sessions, no electric shock was given to the animals (24).

3.4. Data Analysis

One-way ANOVA and the post-hoc Tukey test were used for analyzing data and comparing all groups. P value of less than 0.05 was considered significant.

4. Results

4.1. Pretreatment of Quinine in the Development of Kindling

Chronic administration of PTZ increased the severity of clonic convulsion and caused animals to reach stage 4 during the last days of injection. Administration of quinine (15, 30, and 60 mg/kg) before the injection of PTZ changed the development of the kindling. Quinine at doses of 15, 30, and 60 mg/kg could significantly reduce the mean seizure stage during the 9 kindling injections compared with the control ($P < 0.01$, $P < 0.001$, $P < 0.001$) (Figure 1).

Administration of quinine induced a significant increase in memory retrieval compared with the controls. Quinine at doses of 15 and 30 mg/kg significantly increased latency to enter the dark chamber compared with the control 24 and 48 hours after training ($P < 0.05$) (Table 1). Quinine at a dose of 60 mg/kg increased latency to enter the dark chamber 24 and 48 hours after training ($P < 0.001$) (Table 1). Quinine also increased memory retrieval one and two weeks after training, but this effect was not significant.

5. Discussion

Our results indicate that quinine has anticonvulsant activities in the PTZ kindling model. Pretreatment with quinine at doses of 15, 30, and 60 mg/kg attenuated seizure severity from the beginning of the kindling procedure by

Table 1. Effects of Quinine (15, 30, and 60 mg/kg) on Step-Through Latency in Rats[a,b]

Day	Control	Q15	Q30	Q60
1	38.9 ± 8.20	203.30 ± 61[c]	213 ± 56[c]	251 ± 48.20[d]
2	52.7 ± 28	209.20 ± 43[c]	210 ± 56[c]	300 ± 0.2[d]
7	125.6 ± 31	272.30 ± 27	89.30 ± 36	208.3 ± 58
14	68.6 ± 34	126.60 ± 58	99.30 ± 45	203.2 ± 61

[a]Values are expressed as mean ± SEM, n = 10, Tukey-Kramer test.
[b]Passive avoidance learning tests were performed 24 hours, 48 hours, 1 week, and 2 week after training.
[c]P < 0.05.
[d]P < 0.001 compared with the same-day saline group.

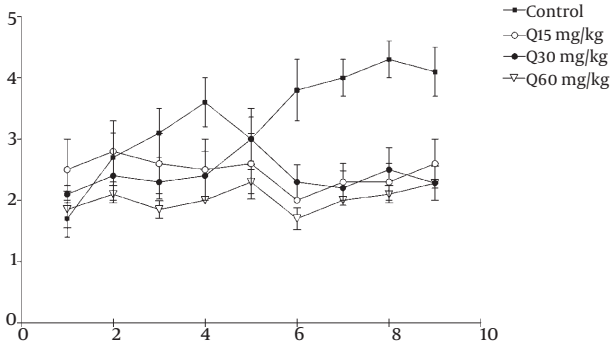

Figure 1. Effects of the repeated administration of quinine (15, 30, and 60 mg/kg) on the development of PTZ-induced kindling (35 mg/kg, i.p., 9 injections total) compared with the control. Data represent mean seizure stages ± SEM, n = 10.

lowering the mean seizure stage. In this study, administration of quinine before the injection of PTZ increased memory retrieval in rats. Quinine at doses of 10, 30, and 60 mg/kg significantly enhanced memory retrieval in the first and second retention tests compared with the control. Our previous studies showed that quinine could inhibit both induction and duration of seizure activity induced by PTZ in animals (25, 26). Similarly, our previous data indicated that quinine such as carbenoxolone has anticonvulsant effects on the PTZ model, and that pretreatment with different doses of trimethylamine (TMA), a gap junction channel opener (i.c.v.), attenuated the anticonvulsant effects of quinine on the latency and duration of generalized tonic-clonic seizure (25, 27). TMA could open the gap junctions as a result of intracellular alkalinization (28, 29). Consistent with these studies, gap junction openers exacerbate seizure activity (30, 31). Quinine was shown to close the gap junction channels in a reversible, concentration-dependent, and connexin-specific manner at an intracellular binding site (17). Administration of quinine into the entorhinal cortex in epileptic animals induced by 4-aminopyridine could reduce the amplitude and frequency of the discharge trains. The proepileptic effect of TMA on epileptiform activity was suggested to depend on the time and route of drug administration (32). As a gap junction blocker, quinine has been recently shown to decrease the mean number of fast ripples (FRs, 250-600 Hz) and the mean number of oscillation cycles per FR event in the hippocampus. FRs are considered potential biomarkers for epilepsy, and FRs can be observed in TLE and in patients with this pathology (33).

Quinine decreased the frequency and amplitude of spikes. It also reduced the score of epileptic behavior in the generalized epileptiform activity of penicillin (19). By preventing the gap junction between neurons, quinine was considered to inhibit convulsion in an in vitro study (19). Thus, by inhibiting the activity of the gap junction channel, quinine may dose-dependently reduce the severity of seizures and enhance memory performance in kindled rats. However, this enhancement did not seem to change by repeating the retention test in this study.

Two other possibilities were discussed for the anticonvulsive effects of quinine on PTZ-induced seizures in mice. First, quinine seems to enhance the threshold of seizure by inhibiting dopamine activity. Second, stability of neural membrane may be increased by increasing the permeability of the potassium channel (34). Nevertheless, quinine could prevent memory impairment associated with epilepsy in animals through its anticonvulsant effects. Quinine may dose-dependently reduce seizure and improve memory retrieval by blocking the gap junction channel. However, quinine seems to be more effective in close memory than in distant memory. Structure activity studies of quinine can be useful to the synthesis of quinine-based derivatives, which are effective in the treatment of seizure disorders and memory dysfunction. Further studies are needed to evaluate the molecular mechanisms of quinine during PTZ kindling.

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Footnotes

Authors' Contribution: Marjan Nassiri-Asl designed the study, conducted and supervised the experiment, and prepared the manuscript. Parichehr Yaghmaei supervised the experiment. Zahra Faridkia conducted the experiment and prepared the manuscript. All authors read and approved the final manuscript.

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