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Research paper

Amelioration of caffeine-induced seizures by modulators of sigma, *N*-methyl-D-Aspartate and ryanodine receptors in mice

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ABSTRACT

Objectives: The aim of this study was to evaluate the antiepileptic effects of opipramol, a sigma receptor agonist, diazepam, ketamine, an *N*-methyl-D-Aspartate (NMDA) receptor antagonist, and dantrolene, a ryanodine receptor antagonist, against caffeine-induced seizures in mice.
Methods: We used caffeine (1000 mg/kg) intraperitoneally for inducing clonic and tonic-clonic seizures in male albino Swiss strain of mice. We used opipramol in three different doses (10, 20 and 50 mg/kg), ketamine (50 mg/kg), dantrolene (40 mg/kg), opipramol (20 mg/kg) plus ketamine (50 mg/kg), opipramol (20 mg/kg) plus dantrolene (40 mg/kg), diazepam (5 mg/kg as a positive control) and the vehicle 30 min before injecting caffeine. We recorded the onset of clonic, tonic-clonic seizures and the time of death of animals after using caffeine.
Results: Animals treated with opipramol at a dose of 50 mg/kg or diazepam had a higher onset of clonic seizure compared with the vehicle-treated group. Dantrolene alone or with opipramol (20 mg/kg) increased the latency of clonic seizure compared with the control group. Opipramol (20 and 50 mg/kg), diazepam, ketamine alone or with opipramol, and dantrolene plus opipramol increased the latency of tonic-clonic seizures in mice. All the treatments except opipramol (10 mg/kg) and dantrolene alone increased the latency of death of animals.
Conclusion: Opipramol attenuated seizures produced by high doses of caffeine. Moreover, the activation of sigma receptors and inhibition of ryanodine receptors may produce synergistic effects against caffeine-induced seizures. Our study may imply that different mechanisms such as inhibition of gamma-aminobutyric acid-A receptors, activation of NMDA and ryanodine receptors may contribute to the caffeine-induced seizures.

1. Introduction

Caffeine, a methylxanthine derivative, is considered as the most widely used central nervous system (CNS) stimulants by man.[1] Caffeine is used in different foods and drinks like tea, coffee, and cola.[2] as well as over-the-counter medications such as headache preparations.[2] Furthermore, methylxanthines in the forms of theophylline and aminophylline, are widely used as medications to treat asthma[3] and apnea, especially in the newborns.[4]

In spite of widespread use of methylxanthines in the medicine and food industry, there are many reports about the serious side-effects of these agents, particularly in the toxic doses. A life-threatening seizure may be an important complication of methylxanthine therapy.[5] Methylxanthines are a trigger of the epileptic seizures in the patients without any history of epilepsy and a risk factor for the patients with underlying epilepsy.[6] More importantly, methylxanthine-induced seizures may be resistant to the conventional and new anti-epileptic drugs.[7,8]

The exact mechanism of methylxanthine-induced seizure is not completely understood. However, the inhibition of adenosine receptors and the activation of ryanodine receptors may be the main mechanisms responsible for the methylxanthine-induced seizure.[9] The ryanodine receptors activation increases the intracellular concentration of calcium in neurons, and this may

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contribute to the convulsant activity of methylxanthines.[10] New generation antiepileptic drugs such as levetiracetam ameliorated peak height of intracellular calcium induced by caffeine.[11] Therefore, drugs that affect intracellular calcium may be useful for managing methylxanthine-induced seizures.

N-methyl-D-Aspartate (NMDA) and ryanodine receptors are actively involved in the intracellular calcium modulation. The ionic channels of NMDA receptors are highly permeable to calcium.[12] and raise intracellular calcium in neurons.[13] Moreover, ryanodine receptors are caffeine-sensitive calcium stores that mobilize calcium from intracellular pools.[10] Thus, the effects of NMDA or ryanodine receptors modulators on the intracellular calcium concentration may be useful for the control of caffeine-induced seizures.

Sigma receptors are chaperone proteins that are located on the sarcoplasmic reticulum.[14] These receptors have important roles in modulating NMDA receptors-mediated glutamate neurotransmission and intracellular calcium.[14],[15] Some reports have implied that sigma receptors may be involved in the pathophysiology of epileptic seizure.[16] Sigma-1 receptor modulators like dextrorphan, carbetapentane, and pentazocine protected animals against kainic acid or maximal electroshock seizures.[17–19] Moreover, a specific sigma receptor agonist produced antiepileptic effects in the rat hippocampal slices.[20] Therefore, sigma receptor modulators may be the potential drugs for managing methylxanthine-induced seizure.

Opipramol is an antidepressant and anti-anxiety drug[21,22] with high affinity for the sigma receptors, particularly the sigma-1 subtype[22] and low affinity for the dopamine and NMDA receptors.[23,24] Opipramol exerted neuroprotective effects in the animal models of ischemia.[25] In our previous work this agent produced an anti-epileptic effect in the pentylenetetrazole (PTZ)-induced seizures.[26] However, there is no other report about the opipramol effects in the caffeine-induced seizure. Therefore, the aim of this study was to evaluate the antiepileptic effects of opipramol, a sigma receptor agonist, against caffeine-induced seizures in mice. We also aimed to show ketamine, a NMDA receptor antagonist, and dantrolene, a ryanodine receptor antagonist, effects against the caffeine-induced seizures in mice and their interaction with opipramol in this animal model.

2. Materials and methods

2.1. Chemicals

We bought opipramol and ketamine from Sigma (USA) and caffeine powder from Merck (USA). Diazepam and normal saline were procured from Daru Pakhsh Pharmaceutical Co., Iran. Opipramol, ketamine, dantrolene, diazepam and caffeine were dissolved in saline. We used all compounds by intraperitoneal (*i.p.*) injection 30 min before caffeine administration. All the compounds were used in a volume of 0.1 ml per 10 g of animal's body weight.

2.2. Animals and treatments

Male albino Swiss strain of mice was obtained from Razi Institute (Tehran, Iran). We kept animals in the Plexiglas cages (5 animals per cage) on a regular dark/light cycles (12 h/12 h), controlled temperature (22 ± 2 °C) and free access to food and water. Seventy-two mice were randomly allocated to the nine separate groups (n = 8). We used opipramol in three different doses (10, 20 and 50 mg/kg), ketamine (50 mg/kg), dantrolene (40 mg/kg), opipramol (20 mg/kg) plus ketamine (50 mg/kg), opipramol (20 mg/kg) plus dantrolene (40 mg/kg), diazepam (5 mg/kg as a positive control) and the vehicle 30 min before caffeine injection. The dose selection was mainly according to our previous studies on the dantrolene, opipramol, and ketamine effects against PTZ seizure.[26,27] The diagram in Fig. 1 shows drug using schedule. The experiment was approved by the local Animal Ethics Committee, which follows the European Communities Council to minimize the number and suffering of animals.

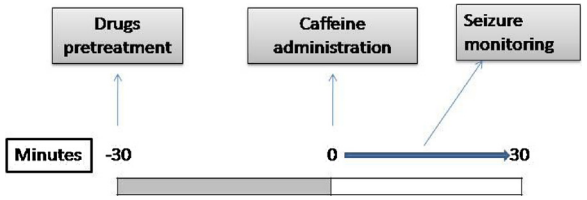

Fig. 1. The schedule of drug administration and seizure monitoring in mice. Mice were treated with drugs (opipramol (10, 20 and 50 mg/kg)), ketamine (50 mg/kg), dantrolene (40 mg/kg), opipramol (20 mg/kg) + ketamine (50 mg/kg), opipramol (20 mg/kg) + dantrolene (40 mg/kg), diazepam (5 mg/kg), and vehicle. All the treatments were used 30 min before the administration of caffeine (1000 mg/kg) and monitored for 30 min for the onset and occurrence of clonic and tonic-clonic seizures, and mortality.

2.3. Caffeine-induced seizure

We used caffeine (1000 mg/kg) to induce the clonic and tonic-clonic seizure in mice. After caffeine injection, mice were placed in the separate cages and watched for 30-min. According to the Łukawski et al. experiment,[28] we considered three seconds clonus of the whole animal body with loss of righting reflex as the clonic seizure. Generalized clonus of animal body with the extension of both forelimb and hindlimb was defined as the generalized tonic-clonic seizure. We recorded the latency of caffeine-induced seizures and death as the onset of clonic and generalized tonic-clonic seizures and the time of death of animals after using caffeine.

2.4. Data analysis

We reported data as the mean ± standard error of the mean (SEM) for the recorded variables. We analyzed the variables with the Kruskal-Wallis test followed by the Mann-Whitney *U* test. The significant level was considered the *p*-value of <0.05. Statistical analysis was performed by the SPSS software version 18.

3. Results

3.1. Effects of different treatments on the onset of caffeine-induced clonic seizure

Animals treated with opipramol at a dose of 50 mg/kg ($\chi^2 = 0.00$, $p = 0.001$) or diazepam ($\chi^2 = 0.00$, $p = 0.001$) had a higher onset of clonic seizure compared with the vehicle-treated group. However, the onset of clonic seizure in the animals treated with opipramol at the doses of 10 and 20 mg/kg was not significantly different from the vehicle-treated group ($\chi^2 = 25.00$, $p = 0.46$; and, $46\chi^2 = 25.50$, $p = 0.49$, respectively). Dantrolene alone or with opipramol (20 mg/kg) increased the latency of clonic seizure compared with the control group ($\chi^2 = 13.50$, $p = 0.05$; and $\chi^2 = 6.00$, $p = 0.006$, respectively). Moreover, the latency of clonic seizure in the animals treated with opipramol + dantrolene was significantly higher than the opipramol- (20 mg/kg) or dantrolene-treated groups ($\chi^2 = 9.00$, $p = 0.02$; and $\chi^2 = 11.00$, $p = 0.03$, respectively). The onset of clonic seizure in the animals treated with ketamine alone or with opipramol (20 mg/kg) was not significantly different from the vehicle-treated group ($\chi^2 = 24.00$, $p = 0.40$; and

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