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Central effect of crocin on penicillin-induced epileptiform activity in rats

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Abstract:
In the present study, the effects of separate and combined intracerebroventricular (*icv*) injections of crocin and diazepam were investigated on penicillin-induced epileptiform activity. In urethane-anesthetized rats, epileptiform activity was induced by intracortical (*ic*) administration of penicillin (200 IU, 1 µl) and was analyzed using electrocorticographic (ECoG) recordings. The *icv* injections of crocin at doses of 25, 50 and 100 µg and diazepam at a dose of 10 µg increased the latency time to onset of first spike wave, and decreased the frequency and amplitude of spike waves. Co-administration of an effective dose of crocin (50 µg) with an ineffective dose of diazepam (2.5 µg), increased the latency time to onset of first spike wave and decreased frequency and amplitude of spike waves as compared with crocin (50 µg). These results indicated that crocin and diazepam produced antiepileptic activities at the levels of the brain. Crocin potentiated the antiepileptic effect of diazepam. A $GABA_A$-benzodiazepine receptor-mediated mechanism may be involved in the antiepileptic activity of crocin.

Key words:
crocin, diazepam, penicillin-induced epileptiform activity, rats

Abbreviations: *ic* – intracortical, *icv* – intracerebroventricular, PE – penicillin-induced epilepsy, PTZ – pentylenetetrazole

Introduction

Epilepsy is a complex neurological disorder characterized by recurrent seizures of cerebral origin, presenting with episodes of sensory, motor and autonomic disturbances with or without loss of consciousness [31]. Epileptic seizures result from excessive discharge in a population of hyperexcitable neurons in cortical and hippocampal structures [4, 34]. Conventional treatment of epilepsy consists primarily of anticonvulsant medications [10]. Although these drugs often control or reduce the frequency of seizures, but possess many side effects, and some patients show little or no improvement [15, 20]. Hence, there is a need to address a potent alternative as antiepileptic agent with minimal side effects.

Crocin is one of the active substances of gardenia yellow and saffron, the extracts of *Gardenia jasminoides* fruits and *Crocus sativus* stigmas, respectively [3, 22]. The involvement of crocin in some biological phenomena such as learning and memory, anxiety and pain have been reported [1, 28, 32, 33]. Saffron extracts and safranal (an active constituent of saffron)

exhibited anticonvulsant effects in both pentylenetetrazole (PTZ) and maximal electroshock (MES) models of seizure in mice [18, 19].

Penicillin-induced epileptiform (PE) activity has been established as a model of epilepsy in rats for studying the effects of antiepileptic drugs [6, 11, 13, 16]. The present study was designed to investigate the effects of *icv* injection of crocin on penicillin-induced epilepsy. In addition, to identify the mechanism that possibly mediates the effect of crocin on epilepsy, the contribution of $GABA_A$-benzodiazepine receptor system was assessed using *icv* injection of diazepam (a $GABA_A$-benzodiazepine receptor agonist) with and without crocin.

Materials and Methods

Animals

Healthy adult male Wistar rats, weighing 220–250 g were used in this study. Rats were maintained in polyethylene cages with food and water available *ad libitum* in a laboratory with controlled ambient temperature (22 ± 0.5°C) and under a 12 h light-dark cycle (lights on 7:00 a.m.). Experiments were carried out between 11:00 a.m. and 5:00 p.m. Six rats were used in each experiment. The experimental protocol was approved by the Veterinary Ethics Committee of the Faculty of Veterinary Medicine of Urmia University and was performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

Drugs

Drugs used in the present study included urethane, crocin, diazepam and penicillin G potassium. The drugs were purchased from Sigma-Aldrich Co., St Louis, MO, USA. The drugs were dissolved in normal saline. A drop of Tween 80 was added to diazepam plus normal saline solution.

Treatment groups

The rats were divided into 8 groups with 6 rats in each group. Group A received *icv* normal saline prior to *ic* injection of penicillin. In groups B, C, D and E *icv* injections of crocin at doses of 12.5, 25, 50 and 100 μg were performed before *ic* injection of penicillin, respectively. Groups F and G treated with *icv* injection of 2.5 and 10 μg of diazepam prior to *ic* injection of penicillin. Group H received *icv* co-administartion of an effective dose of crocin (50 μg) with an ineffective dose of diazepam (2.5 μg) before *ic* injection of penicillin. In all groups, the *icv* injection of drug solutions was performed 10 min before *ic* injection of penicillin.

Study protocol

The animals were anesthetized with intraperitoneal (*ip*) injection of urethane (1.2 g/kg), and placed in a stereotaxic apparatus (Stoelting, Wood Lane, IL, USA). Rectal temperature was measured by a digital thermometer and was maintained between 36 and 37°C using a controlled heating pad system. Thereafter, the scalp was incised, and the skull was leveled off around the bregma.

For *icv* injections of normal saline, crocin and diazepam, a hole with 0.8 mm in diameter was drilled in the left parietal bone according to the following coordinates: 0.8 mm posterior to the bergma and 2 mm lateral to the midline [27]. The tip of the needle of a 5 μl Hamilton's syringe was introduced through the hole into the brain and was placed at 4 mm below the surface of the skull in the left lateral ventricle of the brain. The volume of solutions to be injected into lateral ventricle was 1 μl and injection was made over a period of 30 s. The injection needle was left in place for a further 30 s after completion of injection to facilitate diffusion of the drug. After injection, the hole was closed using acrylic cement (Acropars, Tehran, Iran). In the present study, we used *icv* injection route of administration of crocin and diazepam, because it may achieve a greater drug concentration at the epileptogenic area.

The epileptic focus was produced by *ic* injection of penicillin. For this purpose, an additional hole with 0.8 mm in diameter was made in the right parietal bone overlying the right sensory-motor cortex (2 mm posterior to the bregma and 3 mm lateral to the midline) [27]. Penicillin G potassium (200 IU, 1 μl) was injected 1.2 mm beneath the surface of the skull using a 5 μl Hamilton's syringe in a period of 90 s. [5–7, 12].

For ECoG recordings, two 5-mm height pin electrodes (0.5 mm in diameter) were inserted into the right frontal and parietal bones according to the following coordinates: first electrode, 1 mm anterior to the bregma and 2 mm lateral to the midline (frontal