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Anticonvulsant effect of aqueous extract of *Valeriana officinalis* in amygdala-kindled rats: Possible involvement of adenosine

Mohammad Ebrahim Rezvani*, Ali Roohbakhsh, Mohammad Allahtavakoli, Ali Shamsizadeh

Department of Physiology and Pharmacology, School of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran

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ABSTRACT

Ethnopharmacological relevance: Valeriana officinalis L. (valerian) root extract has been used as an antiepileptic herbal medicine in Iran.
Aim of this study: In the present study the effect of valerian extracts on an experimental model of temporal lobe epilepsy (TLE) was evaluated. Moreover, the involvement of adenosine system in the actions of aqueous extract of valerian was evaluated.
Materials and methods: Bipolar stimulating and monopolar recording electrodes were implanted stereotaxically in the right basolateral amygdala of male Sprague–Dawley rats. After kindling, the effect of aqueous (200, 500 and 800 mg/kg; intraperitoneal) and petroleum ether (PE; 50 and 100 mg/kg; intraperitoneal) extracts of valerian and CPT (selective A_1 receptor antagonist; 10 and 20 μM; intracerebroventricular) on afterdischarge duration (ADD), duration of stage 5 seizure (S5D) and latency to the onset of bilateral forelimb clonuses (S4L) were measured. The effect of CPT (10 μM) on the response of aqueous extract of valerian (500 mg/kg) was also determined.
Results: The results showed that aqueous extract of valerian had anticonvulsant effect. However, PE extract and CPT (20 μM) had proconvulsant effect. Administration of CPT (10 μM) before the administration of aqueous extract decreased the anticonvulsant effect of valerian.
Conclusions: The results showed significant anticonvulsant effect for aqueous but not PE extract of valerian. Moreover, CPT as a selective adenosine A_1 receptor antagonist decreased the anticonvulsant effect of valerian aqueous extract. Therefore, we concluded that part of anticonvulsant effect of valerian probably is mediated through activation of adenosine system.

1. Introduction

Valerian (*Valeriana officinalis* L., Valerianaceae) extracts have been used extensively for the treatment of insomnia worldwide. Considerable evidences exist for anxiolytic and sedative effects of valerian both in humans and animals (Houghton, 1999). Furthermore, valerian root has been used as a rather popular anticonvulsant remedy in Europe and Iran in the past centuries (Gorji and Khaleghi Ghadiri, 2001; Eadie, 2004). The anticonvulsive effects of valerian have been evaluated in limited number of studies. The ethanol extract of valerian has been reported to be effective against picrotoxin but not pentylenetetrazole-induced convulsions (Hiller and Zetler, 1996). However, Petkov and Manolov (1975) reported that valerian could antagonize convulsions induced by pentylenetetrazole and strychnine in mice.

Temporal lobe epilepsy (TLE) is the most common epileptic syndrome in adults, as well as the most difficult to manage (French et al., 1993). Kindling is one of the most commonly used animal models of temporal lobe epilepsy which has been used for preclinical evaluation of antiepileptic drugs (Sato et al., 1990). Kindling is defined as progressive development of electrographic and motor seizures after repeated daily stimulation of particular brain sites (Goddard et al., 1969). The amygdala is an important structure in the brain that is involved in the development of complex partial seizures (Gonçalves Pereira et al., 2005).

Despite intensive research efforts, the pharmacological actions accounting for the CNS suppressant effect of valerian remain unclear. Although this effect is believed to be mediated mainly through GABAergic system (Mennini et al., 1993), the involvement of other mechanisms cannot be excluded. Some studies have shown that serotonin (Dietz et al., 2005), melatonin (Abourashed et al., 2004) or adenosine (Lacher et al., 2007) could be involved in the valerian mediated sedation and anxiolysis. The interaction of valerian extract with adenosine system has been the subject of some recent studies. In these studies, the existence of compounds with partial agonistic or inverse agonistic activity on A_1 adenosine receptors in different valerian extracts has been reported (Lacher et al., 2007; Sichardt et al., 2007).

* Corresponding author. Tel.: +98 391 8220086; fax: +98 391 5225209.
E-mail address: erezvani@rums.ac.ir (M.E. Rezvani).

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Based on the above evidences, the present study was conducted to assess the effects of aqueous (polar) and petroleum ether (PE, non-polar) extracts of *Valeriana officinalis* in amygdala-kindled rats and to determine the possible involvement of adenosine system in those effects.

2. Materials and methods

2.1. Plant material

The raw material was collected from Babol, Mazandaran province, Iran. After identification, a voucher specimen (RJ 32-527) was deposited in the Department of Physiology of the Rafsanjan University of Medical Sciences, Rafsanjan, Iran.

2.2. Preparation of extracts

For preparation of petroleum ether extract (PE), 500 g of *Valeriana officinalis* roots were powdered and macerated with petroleum ether (1.5 l) three times (3 × 48 h). After extraction and filtration, the solvent was removed using a rotary evaporator at 40–60 °C (yield 3%). For preparation of aqueous extract, powdered roots (500 g) were added to 90 °C distillated water for 60 min. Then, the aqueous extract was filtered and lyophilized and kept in sealed bottles (yield 14%).

2.3. Animals

Male Sprague–Dawley rats from Razi institute (Iran), weighing 300–350 g at the time of surgery, were used in this study. Animals were housed four per cage, in a room with a 12:12 h light/dark cycle (lights on 07:00 h) and controlled temperature (23 ± 2 °C). Animals had access to food and water ad libitum and they were allowed to adapt to the laboratory conditions for at least 1 week before the surgery. All experiments were performed between 13:00 h and 15:00 h. Seven animals were used in each group of experiments. All experimental procedures were carried out in accordance with the guidelines described by Olfert et al. (1993).

2.4. Surgical and kindling procedure

Rats were anesthetized intraperitoneally (i.p.) with ketamine hydrochloride (100 mg/kg) and xylazine (10 mg/kg) and placed in a Stoelting stereotaxic instrument (Stoelting Inc., Wood Dale, IL). Bipolar stimulating and monopolar recording electrodes were stereotaxically implanted in the basolateral amygdala of the right hemisphere (coordinates: AP, −2.5 mm; ML, 4.8 mm; DV, 7.5 mm) according to Paxinos and Watson (1998). The electrodes (Teflon-coated, 125 μm in diameter; A.M. System Inc., USA) were insulated except at their tips. Two other electrodes were connected to the skull screws and placed above the left cortical surface as earth and differential electrodes. The pins attached to the electrodes were inserted into a socket which was fixed on the skull. A 23-gauge guide cannula was also implanted in the right lateral ventricle (coordinates: AP, −1.0 mm; ML, 1.2 mm; DV, 3.6 mm).

One week after the surgery, afterdischarge (AD) threshold was determined in the amygdala by 2-s, 60 Hz monophasic square wave stimulus of 1 ms per wave. Stimulation was provided by a Nihon Kohden stimulator and Nihon Kohden SS-202J constant-current stimulus isolation unit (Japan). The stimulations were initially delivered at 10 μA and then at 5 min intervals increasing stimulus intensity in increments of 10 μA until at least 5 s of afterdischarges was recorded as previously described (Alasvand Zarasvand et al., 2001). Then, animals were stimulated daily at AD threshold intensity until 5 consecutive stage 5 seizures (fully kindled) according to Racine scales were elicited (Racine, 1972). After repeated kindling stimulations, AD threshold was decreased; thus, supra-threshold stimuli were used to achieve a fully kindled state. In the kindled animals, the recorded parameters were the following: (1) afterdischarge duration (ADD) that was measured from the start of stimulation to the end of afterdischarges; (2) latency to the onset of bilateral forelimb clonuses (S4L) which was measured from the start of the stimulation until the beginning of stage 4 seizure; (3) duration of stage 5 (S5D) that was measured from the start of stage 5 until the end of this stage.

2.5. Drug administration

Lyophilized powder dissolved in sterile saline and evaporated PE extract dissolved in sterile saline using Tween 80. 8-Cyclopenthyl-1,3-dimethylxanthine (CPT; RBI, USA) was used as a selective adenosine A_1 receptor antagonist in this experiment, and was dissolved in artificial cerebrospinal fluid (aCSF). The solutions were sterilized using microfilters (0.2 mm, Minisart, Sartorius, Germany). CPT solution was infused (0.2 μl over 2 min) by means of a microsyringe pump (Stoelting, Wood Dale, IL), via a 30-gauge cannula, terminating 1 mm below the tip of the guide cannula, connected by polyethylene tubing to a 10-μl Hamilton syringe. The injector cannula was left in place for an additional 60 s to allow diffusion of the solution.

2.6. Effect of aqueous extract of valerian in amygdala-kindled rats

Three groups of fully kindled rats received saline (1 ml/kg, i.p.) and aCSF (0.2 μl/rat/2 min; i.c.v.). After 24 h, groups were treated with different doses of aqueous extract of valerian (200, 500 and 800 mg/kg, i.p.) and aCSF (i.c.v.) and were stimulated. The doses of valerian extract were selected according to the study of Hiller and Zetler (1996) and Hattesohl et al. (2008). ADD, S5D and S4L were measured 15, 30 and 90 min after injections as described in the previous section.

2.7. Effect of PE extract of valerian in amygdala-kindled rats

Two groups of fully kindled rats received 1 ml/kg (i.p.) of vehicle and aCSF (0.2 μl/rat/2 min; i.c.v.). After 24 h, groups were treated with PE extract (50 and 100 mg/kg, i.p.) and aCSF (i.c.v.). The doses were selected based on our pilot study. ADD, S5D and S4L were measured 15, 30 and 90 min after injections.

2.8. Effect of CPT, selective A_1 receptor antagonist, in amygdala-kindled rats

Two groups of fully kindled rats received aCSF (0.2 μl/rat/2 min). After 24 h, one group received 10 μM and the other group received 20 μM of CPT (0.2 μl/rat/2 min; i.c.v.). i.c.v. administration was done due to the inability of CPT to cross blood–brain barrier. After administration of either aCSF or CPT, the results were recorded.

2.9. Effect of CPT on anticonvulsive effect of aqueous extract of valerian in amygdala-kindled rats

In this experiment, the anticonvulsive effect of aqueous extract of valerian was evaluated in the presence of ineffective dose of CPT on convulsions. Two groups of fully kindled rats received saline (i.p.). After 24 h, one group received aCSF (i.c.v.) 5 min before i.p. administration of aqueous extract of valerian (500 mg/kg). Other group, received CPT (10 μM, i.c.v.) 5 min before i.p. administration of aqueous extract of valerian (500 mg/kg). The results were recorded as described previously.

2.10. Histology

At the end of the experiments, rats were euthanized and their brains were removed, sectioned and examined under microscope for electrode and cannula positions or the presence of any tissue damage such as lesions. In case of any abnormality, the data from that particular animal was not included in the results.

2.11. Statistical analysis

Data were expressed as mean ± SEM. The comparison between different drug treatments in the same group of animals was made using one-way ANOVA followed by the Tukey's test. Differences with $p < 0.05$ between experimental groups at each point were considered statistically significant.

3. Results

3.1. Effect of aqueous extract of valerian in amygdala-kindled rats

The results showed that i.p. administration of aqueous extract of valerian led to a significant decrease in ADD ($p < 0.01$) and S5D ($p < 0.01$) and a significant increase in S4L at the doses of 500 and 800 mg/kg ($p < 0.01$). These results showed an obvious anticonvulsive effect for aqueous extract of valerian. The results are presented in Fig. 1.

3.2. Effect of PE extract of valerian in amygdala-kindled rats

Fig. 2 shows the effects of PE extract of valerian on ADD, S5D and S4L respectively. The results showed that PE extract increased ADD, S5D ($p < 0.05$), however, S4L parameter did not change significantly. Since 3 rats died after convulsive attacks at the dose of 100 mg/kg; therefore, we did not employ higher doses than 100 mg/kg of PE extract. This implies that the PE extract increased convulsions in the present study.

3.3. Effect of CPT, selective A_1 receptor antagonist, in amygdala-kindled rats

Fig. 3 shows that i.c.v. administration of CPT at the dose of 10 μM did not have any significant effect on ADD, S5D and S4L. However, CPT at the dose of 20 μM increased ADD ($p < 0.05$) and S5D ($p < 0.05$) and did not change S4L significantly. This finding is suggesting a proconvulsant effect for CPT after i.c.v. administration. We selected the non-significant dose of 10 μM for the next experiment.

3.4. Effect of CPT on anticonvulsive effect of aqueous extract of valerian in amygdala-kindled rats

Our results showed that ineffective dose of CPT on convulsive parameters (10 μM) could significantly reverse the decrease in ADD ($p < 0.01$) and S5D ($p < 0.01$) following administration of aqueous extract of valerian. On the other hand, CPT could decrease the raise of S4L parameter induced by administration of aqueous extract of valerian ($p < 0.01$). This means that the anticonvulsant effect of aqueous extract of valerian decreased after administration of an A_1 receptor antagonist. These results are presented in Fig. 4.

4. Discussion and conclusions

Temporal lobe epilepsy or partial seizures have a high frequency of 50% among patients with epilepsy. Current treatments of temporal lobe epilepsy are drug treatment and surgical care. Evidences based on the treatment of this disease using herbal drugs do not exist. Therefore, in the present study, the antiepileptic effect of *Valeriana officinalis* in an animal model of temporal lobe epilepsy have evaluated. The results showed that aqueous but not petroleum ether extract of *Valeriana officinalis* had an obvious anticonvulsant effect in amygdala-kindled rats. In this study, administration of aqueous extract of valerian significantly reduced seizure activity. However, PE extract of valerian at lower doses compared to the aqueous extract, not only did not decrease seizure activity but also intensify it and 3 rats died after convulsive attacks at the dose of 100 mg/kg. The doses of polar extract which were used in this study are similar to the study of Hiller and Zetler (1996) in which they evaluated the anticonvulsant effect of valerian. However, these doses are much higher than the doses that reduce anxiety in humans (600 mg/70 kg; Kennedy et al., 2006). This implies that aqueous extract of valerian has a low potency to control convulsions.

A few corresponding observations exist in the literature concerning the effect of valerian on convulsive behaviors in both animals and humans. In folkloric medicine, valerian has been recognized as an effective anticonvulsant medicinal herb (Gorji and Khaleghi Ghadiri, 2001; Eadie, 2004). Furthermore, the study of Hiller and Zetler (1996) showed that ethanol extract of valerian could decrease convulsions caused by picrotoxin but not pentylenetetrazole. However, another study showed that valerian

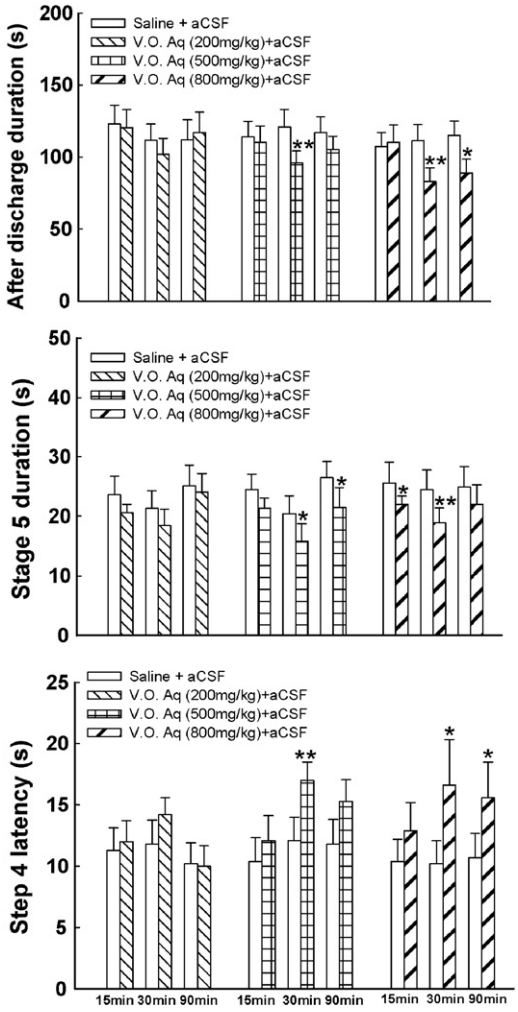

Fig. 1. Effect of aqueous extract of valerian (V.O. Aq) at the doses of 200, 500 and 800 mg/kg on afterdischarge duration, stage 5 duration and step 4 latency after 15, 30 and 90 min of intraperitoneal injections in amygdala-kindled rats ($n = 7$). Values are means ± SEM; $^{*}p < 0.05$ and $^{**}p < 0.01$ in compare with saline treated rats.

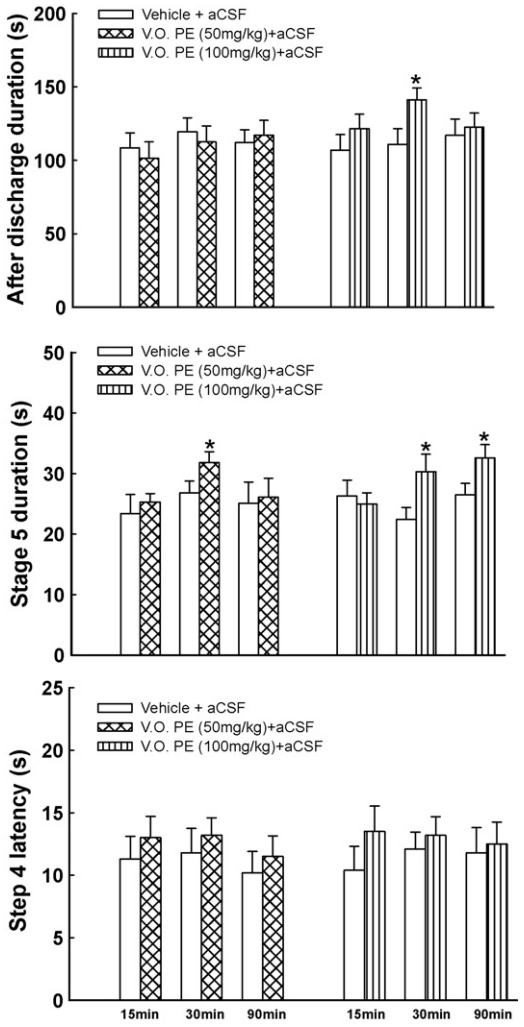

Fig. 2. Effect of petroleum ether extract of valerian (V.O. PE) at the doses of 50 and 100 mg/kg on afterdischarge duration, stage 5 duration and step 4 latency after 15, 30 and 90 min of intraperitoneal injections in amygdala-kindled rats ($n = 7$ for PE 50 mg/kg and $n = 4$ for PE 100 mg/kg). Values are means ± SEM; $^{*}p < 0.05$ in compare with vehicle treated rats.

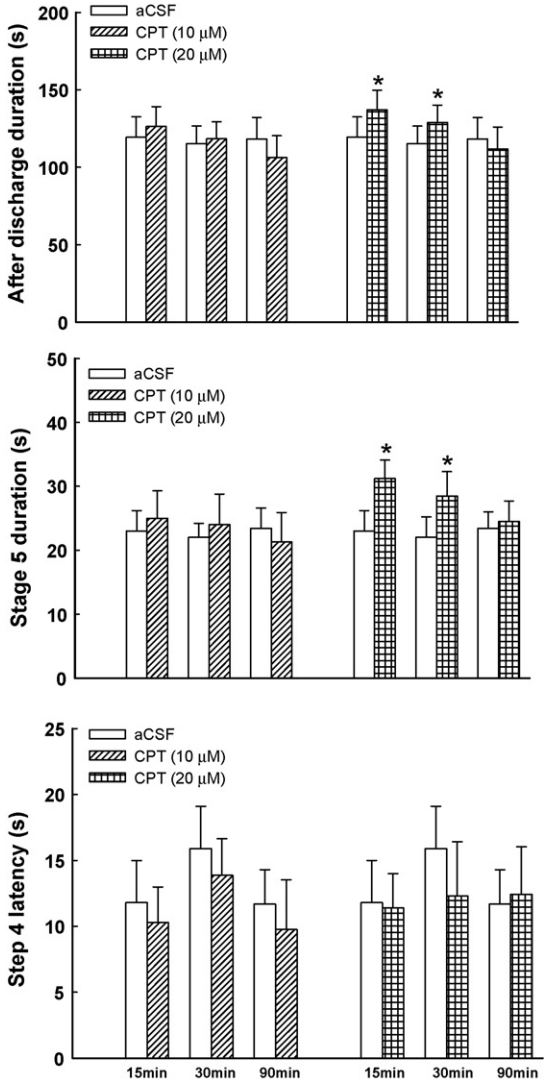

Fig. 3. Effect of CPT (10 and 20 μM) on afterdischarge duration, stage 5 duration and stage 4 latency after 15, 30 and 90 min of intracerebroventricular injections in amygdala-kindled rats ($n = 7$). Values are means ± SEM; $^{*}p < 0.05$ in compare with aCSF treated rats.

extract may have anticonvulsant effects in mice following administration of either pentylenetetrazole or strychnine (Petkov and Manolov, 1975).

The mechanism(s) by which valerian causes CNS suppression has been investigated in some studies. It has been reported that GABA content of aqueous extracts of valerian is involved in the displacement of [^{3}H] muscimol binding, a selective $GABA_A$ receptor agonist (Cavadas et al., 1995). However, GABA does not cross blood–brain barrier (Rosenstein, 1996) and cannot account for the presumed pharmacological effects of valerian extracts. Another study indicated that both valepotriates and valerenic acid that exist in *Valeriana officinalis* are capable of binding to GABA receptors similar to benzodiazepines (Mennini et al., 1993; Benke et al., 2009). Though, valerian does not appear to act similar to benzodiazepines, while some side effects such as impaired mental function, morning hangover and dependency have not been reported with valerian (Pizzorno and Murray, 1999). In addition, valerian compounds which do not bind to GABA receptors have also shown to be sedative. Therefore, other studies proposed the involvement of melatonin (Abourashed et al., 2004), serotonin (Dietz et al., 2005) and adenosine (Lacher et al., 2007) in pharmacological effects of valerian.

Adenosine A_1 receptors are mostly located presynaptically (Rebola et al., 2003) and their activation reduces release of neurotransmitters (Ambrósio et al., 1996). It has been reported that endogenous adenosine through activation of A_1 receptors has anticonvulsant effects (Dragunow, 1988). Therefore, it sounds logical that blockade of A_1 receptors using CPT (20 μM) had proconvulsant effects in this study. Our results also showed that administration of CPT at an ineffective dose on convulsion-related parameters could decrease the anticonvulsant effect of valerian. This finding is suggesting the existence of adenosine ligand(s) in the valerian aqueous extract and activation of A_1 adenosine system is possibly involved in the anticonvulsant effect of aqueous extract of valerian. A variety of natural products have been found to possess affinity to A_1 adenosine receptors, and activation of these receptors has been implicated in the actions of valerian extract. For example, a hydroalcoholic extract of valerian was found to bind to A_1 adenosine receptors in a cortical membrane preparation of the rat brain and competitively displaced the radiolabeled A_1 ligand, [^{3}H]-N6-cyclohexyladenosine, dose dependently (Balduini and Cattabeni, 1989). Furthermore, it has been reported that a lignan isolated from valerian root has partial agonist activity at A_1

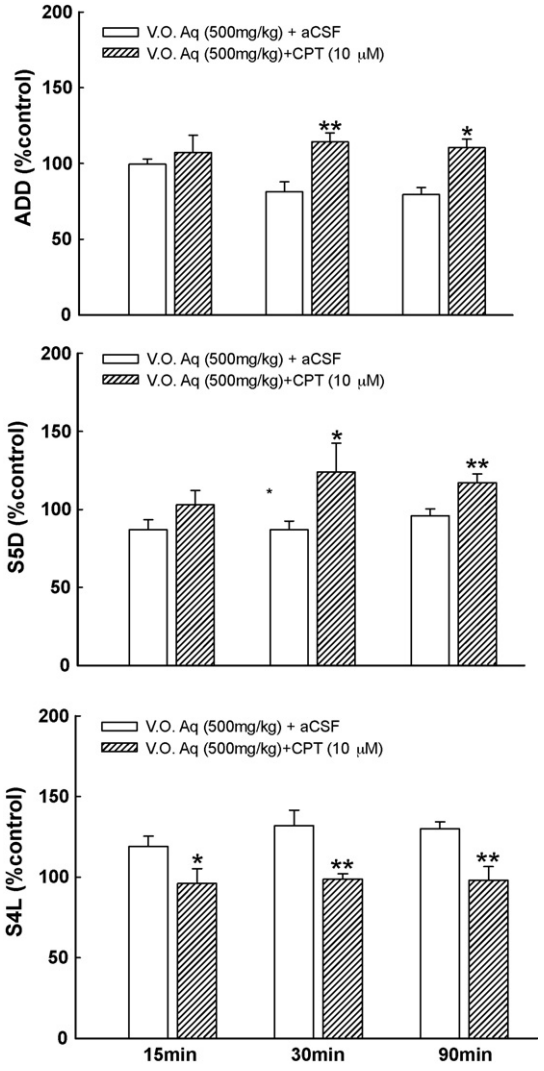

Fig. 4. Effect of aqueous extract of valerian (V.O. Aq, 500 mg/kg) alone or in the presence of CPT (10 μM) on afterdischarge duration, stage 5 duration and stage 4 latency after 15, 30 and 90 min of intracerebroventricular injections in amygdala-kindled rats ($n = 7$). Values are means ± SEM; $^{*}p < 0.05$ and $^{**}p < 0.01$ in compare with valerian treated rats.

adenosine receptors (Schumacher et al., 2002). The lignan 4′-*O*-β-D-glucosyl-9-*O*-(6″-deoxysaccharosyl)olivil was isolated from a polar (methanol) extract of the roots of *Valeriana officinalis* and was found to be a potent partial agonist at rat and human adenosine A_1 receptors exhibiting A_1 affinity in very low concentrations. Moreover, an interaction of caffeine and the fixed combination of valerian and hops (Ze91019) was reported in a clinical study; Schellenberg et al. (2004) showed that fixed combination of valerian and hops decreased or even inhibited the arousal induced by caffeine. Since, caffeine mediates its effects by a blockade of A_1 and A_{2A} adenosine receptors in the brain, these finding may also confirm that valerian possibly activates central adenosine receptors. The obvious difference between anticonvulsant effect of PE and aqueous extracts may be explained by the finding of Lacher et al. (2007); they investigated valerian extracts of different polarities for their affinities to adenosine A_1 and A_{2A} receptors. Polar as well as non-polar extracts were found to interact with adenosine A_1 receptors. While polar extracts activated A_1 receptors, non-polar extracts were antagonists or even inverse agonists at A_1 receptors. This important finding that was further confirmed by Sichardt et al. (2007) may explain the proconvulsant effect of PE extract of valerian. Isovaltrate is the compound that has been found in lipophilic extracts of valerian and is a full inverse agonist of A_1 receptors (Lacher et al., 2007). Thus, it could be suggested that isovaltrate may induce stimulatory effects on the central nervous system by blockade of tonically activated adenosine A_1 receptors in the brain.

The effects of medicinal herbs in the treatment of temporal lobe epilepsy have not been evaluated yet. For the first time, the present findings showed that aqueous extract but not PE extract of valerian had anticonvulsant effects in an animal model of temporal lobe epilepsy. However, this effect was manifested at a relative high dose. Using pharmacological tools, we showed that an A_1 receptor antagonist could decrease the anticonvulsant effect of aqueous extract of valerian. This means that this extract possibly has anticonvulsive effect through activation of adenosine system. This finding is in line with the previous reports regarding the existence of an interaction between different extracts of valerian and adenosine system. More research such as binding studies is needed to confirm these findings. Since, valerian is on the FDA's Generally Recognized as Safe (GRAS) list (Kumar, 2006) and despite most common antiepileptic drugs, valerian has no interaction with CYP 3A4, 1A2, 2D6 and 2E1 liver enzymes (Donovan et al., 2004; Gurley et al., 2005), and it does not have myorelaxant activity (Hattesohl et al., 2008), the aqueous extract of *Valeriana officinalis* may be suggested as a good candidate for the treatment of complex partial seizures without interactions with other drugs and with minimum side effects.

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