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ORIGINAL ARTICLE

Involvement of central TRPV1 receptors in pentylenetetrazole and amygdala-induced kindling in male rats

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Abstract Transient receptor potential vanilloid 1 (TRPV1) is a non-selective cation channel that is involved in modulation of diverse physiological processes. The role of this receptor in epilepsy has not been studied well. Therefore, we investigated the role of central TRPV1 receptors on the development of pentylenetetrazole (PTZ) and amygdala-induced kindling in rats. Male Wistar rats received subconvulsive dose of PTZ intraperitoneally, every other day. TRPV1 receptor agonist, OLDA and its antagonist, AMG-9810 were injected intracerebroventricularly 30 min prior to PTZ administration. In electrical kindling, stimulating and recording electrodes were implanted in the right amygdala of male rats. After kindling, the effect of TRPV1 receptor agonist or antagonist on afterdischarge duration (ADD), latency to the onset of bilateral forelimb clonuses (S4L) and duration of loss of equilibrium (stage 5 seizures, S5D) were measured. The results demonstrated that, OLDA at the doses of 0.01, 0.1 and 1 μg/rat, significantly accelerated the incidence of all seizure stages, increased S5D and decreased S4L in the PTZ model of kindling. Also, in amygdala kindling, S5D and ADD were significantly reduced following the administration of AMG-9810. In contrast, OLDA significantly aggravated the indices of seizure in both models of epileptic seizure. This study demonstrated that central TRPV1 receptors may be involved in the development of electrical and PTZ-induced kindling.

Keywords Kindling · Pentylenetetrazole · Amygdala · OLDA · AMG-9810 · TRPV1

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Introduction

Epilepsy is one of the most frequent and heterogeneous neurological disorders with several disabilities which influence behavioral, social and occupational activities [1, 2]. TRPV1 receptors are non-selective cation channels which are believed to be molecular integrators of various stimuli such as pH, high temperature and capsaicin, the pungent compound in chili peppers, causing pain, inflammation and hyperalgesia in peripheral nervous system [3–5]. However, little is known about physiological functions of the central TRPV1 receptors. These channels are expressed in many regions in the brain including hippocampus, cortex, cerebellum, mesencephalon, basal ganglia, thalamus, hypothalamus, periaqueductal gray matter and dentate cerebellar nucleus [6, 7]. Recent data show that these receptors are involved in the brain plasticity [8]. Based on the effects of TRPV1 receptor on cell death, synaptic plasticity and its modulators, Fu et al. [9] hypothesized that this receptor is a potential target for antiepileptogenesis. The role of TRPV1 receptors in seizure has been recently explored by some researchers. For

example, Manna and Umathe showed that capsaicin could act as a proconvulsant chemical and Lee and colleagues reported its anticonvulsant properties in two different animal seizure models [10, 11]. However, their role in the development of kindling has not been investigated yet. Kindling is a chronic model for seizure in which repeated electrical stimulations in the limbic system of rats induce epileptic seizures [12]. Electrical and chemical kindling are two models of epileptic seizure induced by repeated administration of initially subconvulsive chemical or electrical stimuli, respectively. This induction results in an increase in seizure activity and culminating in a generalized seizure [13].

Based on the above evidences, we investigated the probable role of central TRPV1 receptors in the development of PTZ and amygdala kindling.

Materials and methods

Animals

Adult male Wistar rats, weighing 270–310 g, obtained from animal house of Rafsanjan University of Medical Sciences, Rafsanjan, Iran, were used in this study. Animals were housed in standard Plexiglas cages with free access to food and water. The animal house temperature was maintained at 23 ± 2.0 °C with a 12-h light/dark cycle. Animal handling was performed in accordance with EU directive 86/609/EEC for the use of laboratory animals in research and approved by the local ethics committee (The ethics committee of Rafsanjan University of Medical Sciences). We eliminated all distorting factors to minimize the number of animals used and their suffering.

Chemicals

PTZ was purchased from Sigma-Aldrich (UK) and dissolved in 0.9 % sterile saline on a weight/volume basis on the day of use. The specific TRPV1 agonist, OLDA (*N*-oleoyldopamine) and the specific TRPV1 antagonist, AMG-9810 [(E)-3-(4-*t*-butylphenyl)-*N*-(2,3-dihydro-benzo [b][1,4] dioxin-6-yl) acrylamide] were obtained from Tocris Bioscience (Bristol, UK) and dissolved in 0.1 % dimethyl sulfoxide (DMSO) at various concentrations that are described in the next section.

Experiment one: PTZ kindling

The rats were randomly divided into nine experimental groups with ten animals in each group as follows:

Group 1 as control group received 2 µl of 0.1 % DMSO as vehicle (intracerebro-ventricular, i.c.v.) before each 35 mg/kg PTZ (i.p.). Groups 2–6 received OLDA at the doses of 0.0005, 0.001, 0.01, 0.1 or 1 µg/rat (i.c.v.), respectively, plus 35 mg/kg PTZ (i.p.). Groups 7–9 received AMG-9810 at the doses of 0.3, 3 and 30 µg/rat (i.c.v.), respectively, plus 35 mg/kg PTZ (i.p.) [14]. Five minutes after each i.c.v. injection, rats received a subconvulsive dose of PTZ (35 mg/kg, i.p.). Subsequent to PTZ injections, animals were monitored for 30 min and epileptic behaviors were scored as follows: 0, no behavioral changes; 1, facial movements, ear and whisker twitching; 2, myoclonic convulsions without rearing; 3, myoclonic convulsions with unilateral forelimb clonus; 4, myoclonic convulsions with rearing; 5, loss of posture and generalized clonic–tonic seizures.

Surgical procedures

Rats were anesthetized with ketamine hydrochloride (80 mg/kg, i.p.) and xylazine (4 mg/kg, i.p.). One stainless steel guide cannula 23 g was implanted stereotaxically into the right lateral ventricle (coordinates: from bregma—*A*, 0.9 mm; *L*, 1.4 mm; and 3.3 mm below dura) [15]. The guide cannula was fixed in the skull with two eyeglasses screws and dental acrylic cement.

Microinjections and seizure evaluation

Microinjections of DMSO, AMG-9810 and OLDA were administered using a 10 µl Hamilton microsyringe. A volume of 2 µl was injected in the right ventricle over a 60-s period. After 7 days of recovery, the rats received 0.1 % DMSO, AMG-9810 or OLDA as explained above every other day for 30 days.

Experiment two: amygdala kindling

Surgical procedures

This method has been previously described [16]. Briefly, under ketamine (100 mg/kg; i.p.) and xylazine (10 mg/kg; i.p.) anesthesia, animals were stereotaxically implanted with bipolar stimulating and monopolar recording electrodes (stainless steel, Teflon coated, 127 µm in diameter, A.M. system Inc, USA) terminating in the basolateral amygdala of the right hemisphere (coordinates from bregma: *A*, −2.5 mm; *L*, 4.8 mm; and 7.5 mm below dura). Earth and differential electrodes were connected to the skull and fixed on the cortical surface. Also, a stainless steel 23-gage guide cannula was implanted into the right cerebral ventricle according to the aforementioned coordinates. After 10 days, rats were stimulated (60 Hz monophasic square wave, 1 ms duration per wave, for 2 s) in an ascending stepwise fashion to determine the afterdischarge threshold. The stimulus

intensity that produced at least 5 s of afterdischarges was considered as the threshold stimulus. Next, each rat was daily stimulated with the threshold intensity. Stimulation was ceased when rats showed three consecutive stage 5 seizures. Stimulation, isolation and amplification were provided by a WSI apparatus (WSI Co, Iran).

Microinjections and seizure evaluation

Forty-two animals were first kindled by daily electrical stimulation of the amygdala until three consecutive stage 5 seizures were elicited. These animals were considered fully kindled. Animal were divided into seven groups of six rats. Seizure parameters of all fully kindled rats in the last stimulation session were recorded as control data. One day later, groups 1–4 received OLDA at the doses of 0.001, 0.01, 0.1 or 1 μg/rat (i.c.v.) and groups 5–7 received AMG-9810 at the doses of 0.3, 3 or 30 μg/rat (i.c.v.) before the stimulation of amygdala. In this experiment, afterdischarge duration (ADD), stage 4 latency (S4L), stage 5 duration (S5D) and convulsive behaviors were recorded.

Experiment three: influence of AMG-9810 pre-treatment on the proconvulsant effect of OLDA on fully kindled seizures

The first aim of this experiment was to determine the possible convulsing properties of these drugs per se. Twenty-four rats were used in this experiment. Groups 1 and 2 received OLDA (1 μg/rat, i.c.v.) and AMG-9810 (30 μg/rat, i.c.v.), respectively. Group 3 that was treated with both the TRPV1 agonist and the TRPV1 antagonist was planned to demonstrate the pharmacological specificity of the possible effects. The rats of group 3 received pharmacologically ineffective dose of AMG-9810 and the rats of group 4 were injected with the inactive dose of AMG-9810 prior to OLDA at its effective dose. The rats in groups 3 and 4 received electrical stimulation 5 min after the i.c.v. injections.

Histology

At the end of all experiments, rats were killed using CO_2. Immediately afterwards, the brains were fixed and cut in coronal sections to confirm the cannula locations. In the case of the presence of any tissue damage or wrong location at the tips of electrodes and cannula, the data from that particular animal were eliminated.

Statistical analysis

Scores of seizure stages were compared using Kruskal–Wallis one-way ANOVA on ranks followed by multiple comparison tests. Results of S4L and S5D were compared using one-way and repeated measure analysis of variance (ANOVA). Tukey's test was employed to compare the differences among treated groups. The differences between each two dependent groups were analyzed using paired Student's t test. The p value <0.05 was considered to be statistically significant.

Results

The effect of AMG-9810 or OLDA on PTZ-induced kindling

The average of seizure scores in vehicle-treated group increased with the number of PTZ injections (Fig. 1). This observation suggests that repeated PTZ injections elicited a progressive increase in seizure severity. Statistical analysis revealed significant difference in seizure stages among 1, 5, 10 and 15 injection sessions ($p < 0.05$). Treatment of rats with AMG-9810 (0.3, 3 and 30 μg/rat) delayed the development of seizure following repeated administration of PTZ ($p < 0.05$). Comparing seizure scores among injection sessions 1, 5, 10 and 15 between vehicle- and AMG-9810-treated groups revealed a significant decrease in seizure score in AMG-9810-treated animals in sessions 10 and 15 ($p < 0.01$) (Fig. 1a). Treatment with either doses of 0.3, 3 or 30 μg/rat of AMG-9810 also prolonged latency to the onset of stage 4 or 5 seizure (Fig. 1c; $p < 0.01$).

Treatment with OLDA (0.01, 0.1 and 1 μg/rat) accelerated the development of seizure activity following repeated administration of PTZ when compared to the vehicle-treated group ($p < 0.05$). Seizure scores of OLDA-treated rats in all injection sessions were significantly increased ($p < 0.05$) (Fig. 1b). However, treatment with 0.001 μg/rat of OLDA prevented the development of seizure activity following repeated administration of PTZ in the last session ($p < 0.05$) (Fig. 1b). Moreover, OLDA at the doses of 0.1 and 1 μg/rat significantly shortened the onset of stage 4 or 5 seizure (Fig. 1c; $p < 0.01$).

The effect of AMG-9810 or OLDA on seizure parameters in amygdala kindling

Figure 2 shows behavioral and electrophysiological parameters after application of AMG-9810 and OLDA in different groups of kindled rats. The results showed that i.c.v. administration of AMG-9810 at the doses of 0.3, 3 and 30 μg/rat can elicit a significant shortening in ADD ($p < 0.01$) and a significant prolongation in S4L ($p < 0.05$). S5D in OLDA-treated groups were significantly increased in comparison with vehicle group ($p < 0.01$). Overall, these results show an obvious

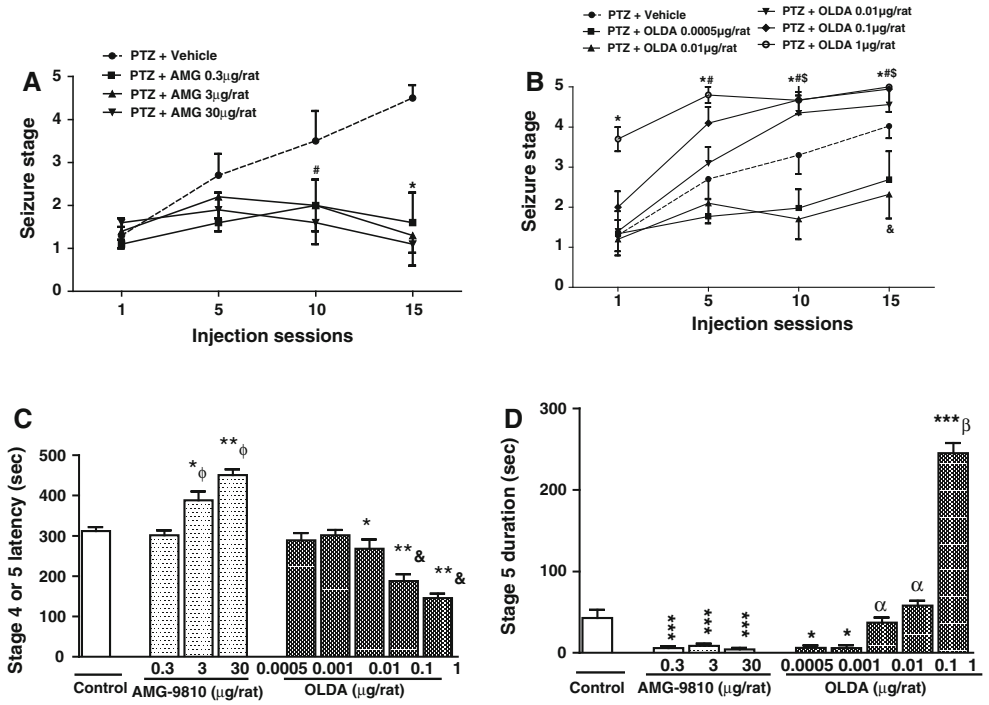

Fig. 1 The effect of pre-treatment with different doses of TRPV1 antagonist (AMG-9810) and agonist (OLDA) on the seizure parameters following PTZ administration. **a** TRPV1 antagonist (AMG-9810), *significant difference between all three doses of AMG-9810 and vehicle groups in 15 injection sessions (all $p < 0.01$). #Significant difference between AMG-9810 at dose of 30 μg/rat and vehicle groups in 10 injection sessions (all $p < 0.01$). **b** TRPV1 agonist (OLDA), *significant difference between OLDA 1 μg/rat and vehicle groups in 1, 5, 10 and 15 injection sessions (all $p < 0.05$), #significant difference between OLDA 0.1 μg/rat and vehicle groups in 5, 10 and 15 injection sessions ($p < 0.001$), $significant difference between OLDA 0.01 μg/rat and vehicle groups in 10 and 15 injection sessions (all $p < 0.05$), &significant difference between OLDA 0.001 μg/rat and vehicle groups in 15 injection sessions ($p < 0.05$). Data are represented as mean ± SEM. In **c** and **d**, $p \leq 0.05$, $^{**}p \leq 0.01$ and $^{***}p \leq 0.001$ mean significant difference between treated groups with control group. ϕSignificant difference when compared to AMG-9810 0.3. αSignificant difference when compared to OLDA 0.0005 or 0.001. βSignificant difference when compared to other doses of OLDA. Values are mean ± SEM

anticonvulsive effect resulted from TRPV1 antagonist, AMG-9810, and the proconvulsant property of TRPV1 agonist, OLDA.

Effect of AMG-9810 pre-treatment on the proconvulsant property of OLDA using amygdala kindling

In amygdala-kindled rats, AMG-9810 at the dose of 0.1 μg/rat did not lead to any change in seizure behaviors. In other groups, vehicle or the ineffective dose of AMG-9810 was i.c.v. administered 2 min prior to OLDA at the dose of 0.1 μg/rat. Ineffective dose of AMG-9810 (0.1 μg/rat) significantly blocked the proconvulsant effect of OLDA (0.1 μg/rat). S4L was significantly longer in AMG-9810 + OLDA-treated rats than vehicle + OLDA ones. Prolongation of S5D induced by OLDA was reversed when rats treated with AMG-9810 + OLDA. Also, ADD elongation elicited by OLDA returned to basal level in rats which were pre-treated with AMG-9810 prior to OLDA (Fig. 3). In addition, animals which were treated with either high doses of OLDA (1 μg/rat) or AMG-9810 (30 μg/rat) did not show any seizure-like behavior, suggesting that neither AMG-9810 nor OLDA has no convulsing effect per se.

Discussion

Results of the present study demonstrated that, activation of TRPV1 receptors by OLDA, accelerated the development of PTZ kindling while inhibition of these receptors by AMG-9810 retarded the development of PTZ kindling.

In agreement with the present findings, a recent work showed that capsaicin, a TRPV1 receptor agonist, decreased the latencies of the onset of the first convulsion induced by PTZ, dose dependently. Another recent study indicated that activation of TRPV1 receptors by capsaicin significantly enhanced dentate gyrus neurons spontaneous and miniature excitatory post-synaptic current frequency in mice with temporal lobe epilepsy; also, it was revealed that activation of TRPV1 receptors increased glutamate release [17]. Another mechanism by which TRPV1 receptors increased the development of PTZ kindling may be

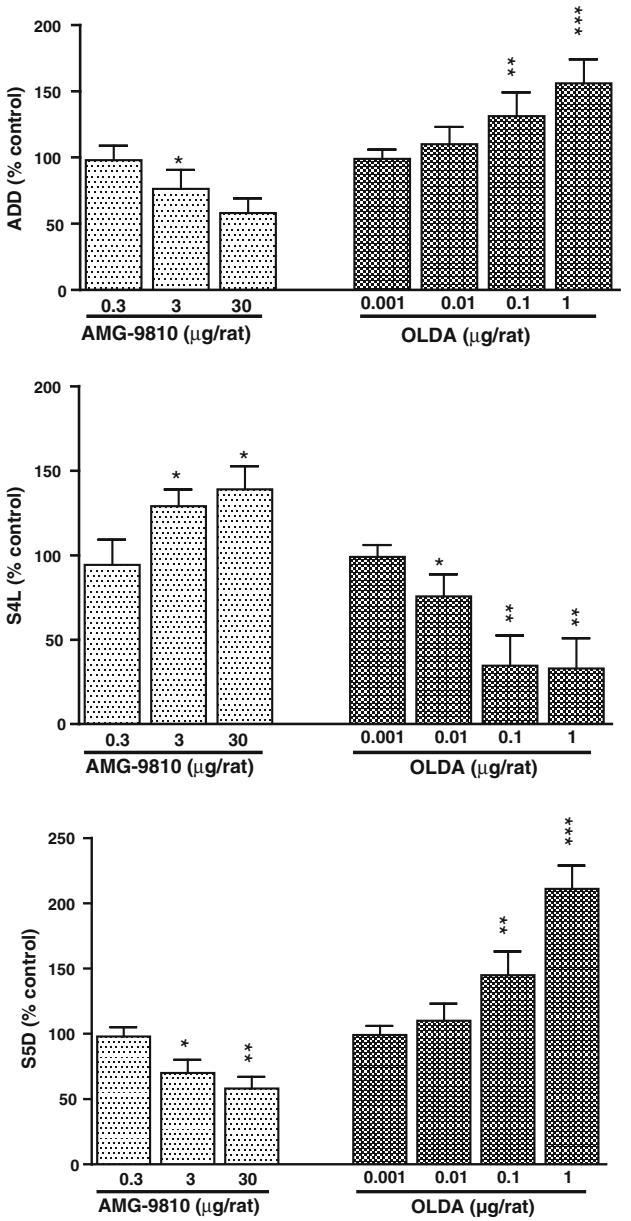

Fig. 2 The effect of different doses of AMG-9810 and OLDA (i.c.v injection) on amygdala afterdischarge duration (ADD), latency to stage 4 (S4L) and duration of stage 5 (S5D) in amygdala-kindled rats. Values are mean ± SEM. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ when compared to vehicle-injected control group by two-tailed paired *t* test

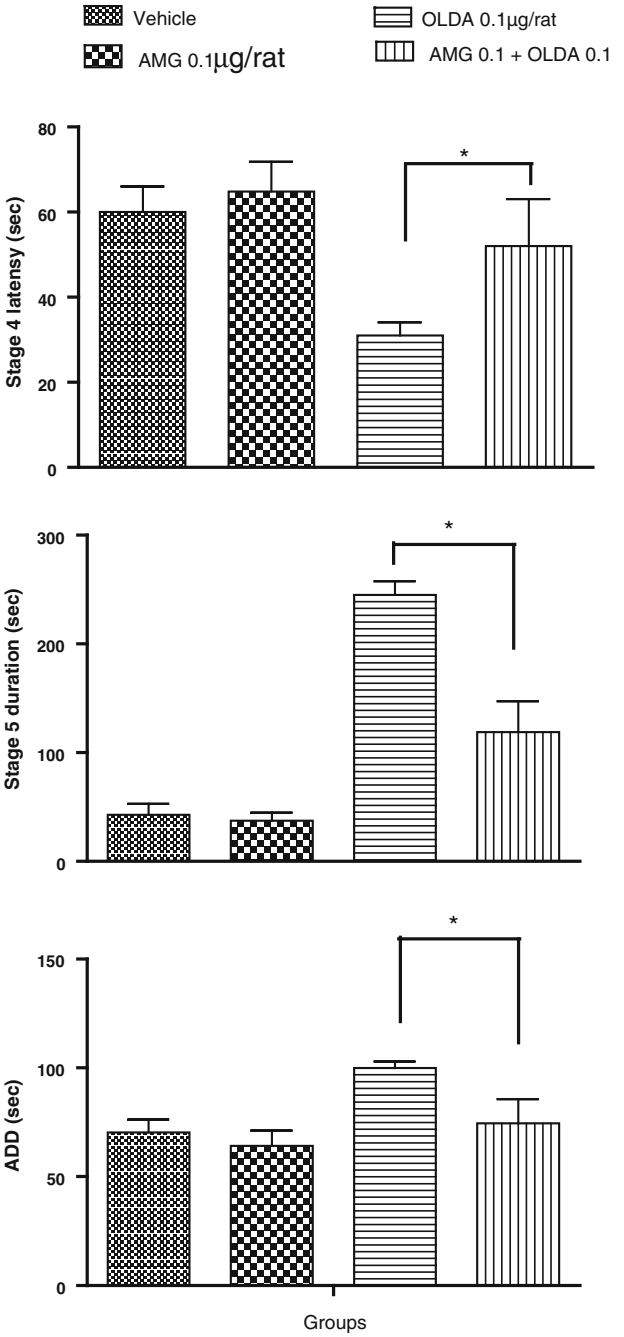

Fig. 3 The effects of AMG-9810 pre-treatment on the proconvulsant effect of OLDA in amygdala-kindled rats. Animals were i.c.v. administered with AMG-9810 (0.1 μg/rat) and 5 min later an injection of OLDA administered by the same route. Rats were stimulated 5 min later. $*p < 0.05$ when compared to OLDA group

explained by its relation with nerve growth factor (NGF). It has been proved that NGF is a regulator of TRPV1 receptors and the important role of NGF in epileptogenesis has also been reported [18–20]. Taking together, one may suggest that activation of TRPV1 receptors is involved in the PTZ-induced kindling and this activation process possibly aggravates epilepsy. In contrast to the present results, Lee et al. reported that administration of capsaicin prevented kainic acid-induced acute epileptogenic seizures in mice [11]. These paradoxical results may be due to different experimental models of seizures, different doses, route of drug administration (i.c.v. vs. subcutaneous) or injection time of TRPV1 agonist. In the present study and the study of Manna and Umathe (2012), TRPV1 agonists, OLDA and capsaicin were injected i.c.v. 30 and 5 min before the injection of PTZ, respectively; whereas, in the study of Lee et al. capsaicin was injected subcutaneously

15 min after kainic acid injection. This controversy possibly shows a modulatory role for TRPV1 receptors in experimental models of seizure. This hypothesis may be further supported by the anticonvulsant effect of OLDA (0.0005 and 0.001 μg/rat) on PTZ-induced kindling as it was shown in the present study. In addition, it has been reported that the electrophysiological characteristics of central and peripheral TRPV1 receptors are dependent on time, type and concentration of the agonist used [21]. However, it looks like that distinct mechanisms mediate the paradoxical effects of low and high doses of the TRPV1 agonist on seizure-like behaviors that warrant further studies.

Amygdala kindling model was used as additional model for further potentiation of the anticonvulsant effect and the spectrum of activity of AMG-9810. Amygdala kindling is a chronic model that induces permanent brain alterations similar to those which are observed in human temporal lobe epilepsy [22].

In this experiment, i.c.v. injection of AMG-9810 significantly reduced the duration of afterdischarge (ADD) recorded from amygdala. In the other words, AMG-9810 reduced the neuronal activity in amygdala and possibly other regions of the brain. Since the seizure protective effect is accompanied with a decrease in S5D and an increase in S4L, it may be concluded that both partial (1–3) and generalized (4 and 5) stages of seizure may be affected by TRPV1 antagonist. The S4L is an index of seizure generalization and its increase represents delays in seizure generalization in rats.

Recent studies reported that hyperthermic seizure threshold in TRPV1$^{-/-}$ mice was significantly higher (41.1 °C) compared with wild-type mice (39.5 °C). They showed that heat-triggered neuronal bursting was influenced mainly in the CA1 and CA3 regions of the hippocampus. They also suggested that the anticonvulsant effect of cannabinoid receptor antagonists on febrile seizures is TRPV1 mediated [23]. On the other hand, in this part of experiment, no dose–response relationship was observed for AMG-9810. This observation may be due to the doses of the drug that we used. The IC50 value of AMG-9810 for rat TRPV1 receptors activation by capsaicin is 85.6 nM and all concentrations of AMG-9810 in the present study (890, 8,900 and 89,000 μM) were much higher than its IC50 value (IC_{50} = 17 nM) [24]. In addition, we showed that proconvulsant effects of OLDA were reversed by the pre-treatment of rat with AMG-9810. This evidence shows that the proconvulsant effects of OLDA were mediated through the TRPV1 receptors.

In conclusion, the results of the present study demonstrated that TRPV1 receptors may have important role in seizure and should be considered in future studies of epilepsy.

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