The Effect of Flunarizine on Penicillin Model Epilepsy in Rats*

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✔ Epilepsy is one of the most important neurological diseases. Calcium ions have an important role on the regulation of cellular functions. It is accepted that calcium flux into the cell is the first step of epileptic neuronal events. In present study, the effect of flunarizine on experimental model epilepsy induced by intracortical (i.c.) penicillin administration was investigated. The left cerebral cortex was exposed by craniotomy in anaesthetised rats. The epileptic focus was produced by injection of penicillin G potassium (500 units) into the somatomotor cortex. Following the epileptiform activity reached maximum frequency and amplitude, flunarizine was injected into the same area by Hamilton mikroinjector. Before flunarizine administration, the average frequency of spikes was 21.1±1.9/min and the average amplitude of spikes was 1080±65 μV. Microinjection of flunarizine (10, 100 μM) into the same area caused an inhibition for 3-4 minutes in electrocorticograms (ECoG) ($p<0.001$). But saline did not affect the epileptiform activity ($p>0.05$). The results of this study suggest that flunarizine may be an anticonvulsant agent in treatment of epilepsy.
Key words: *Calcium antagonist, Flunarizine, epileptiform activity, rat*

✔ **Sıçanlarda Penisilin Modeli Epilepsiye Flunarizinin Etkisi**
Epilepsi en önemli nörolojik hastalıklardan birisidir. Kalsiyum iyonları hücresel fonksiyonların düzenlenmesinde önemli rol oynarlar. Serbest kalsiyum iyonlarının hücre içine girişi epileptik nöronal olayların ilk basamağı olarak kabul edilir. Sunulan çalışmada, intrakortikal (i.c.) penisilin uygulamasıyla oluşturulan deneysel model epilepsiye flunarizinin etkisi araştırıldı. Anestezili sıçanlarda sol serebral korteks kraniotomi ile açıldı. Somatomotor kortekse penisilin G potasyum (500 ünite) verilerek epileptik odak oluşturuldu. Epileptiform aktivite maksimum frekans ve amplitüde eriştikten sonra, aynı bölgeye Hamilton mikroenjektörü ile flunarizin enjekte edildi. Flunarizin uygulanmadan önceki ortalama spike sayısı 21.1±1.9/dk ve ortalama spike yüksekliği 1080±65 μV idi. Aynı bölgeye flunarizin mikroenjeksiyonu (10, 100 μM) elektrokortikogramda (EcoG) 3-4 dakika süreyle inhibisyona neden oldu ($p<0.001$). Oysa serum fizyolojik epileptiform aktiviteyi etkilemedi ($p>0.05$). Çalışmanın sonuçları flunarizinin epilepsi tedavisinde etkili bir antikonvulsan ajan olabileceğini göstermektedir.
Anahtar kelimeler: *Kalsiyum antagonisti, Flunarizin, epileptiform activite, sıçan*

INTRODUCTION

Intracellular free calcium levels have very important roles on the regulation of cellular functions and on ischemia[1,2]. Excessive calcium influx into the cell is the first step of epileptic neuronal events[3-7].

Penicillin model of experimental epilepsy have been used by several researchers. The

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effects of several calcium channel blockers on different epilepsy models have also been investigated[8-12]. Nevertheless, results obtained from these studies show important discrepancies. A dihydropyridine calcium channel blocker nifedipine potently blocks convulsion induced by pentylenetetrazole, NMDA and Bay K 8644[13], but it does not inhibit picrotoxin induced epilepsy[14]. Besides, no studies concerning the effect of intracortically injected flunarizine on electrocorticogram (ECoG) in penicillin model epilepsy have found in the present literature. The aim of this study is to determine the effect of flunarizine on epileptiform activity elicited by administration of penicillin G into the somatomotor cortex.

MATERIAL AND METHODS

Experiments were performed on anaesthetised (urethane 1.25 g/kg i.p.) 30 adult male Wistar rats weighing 200-250 g. The right femoral artery was tied off and used to monitor blood pressure in order to assess the general conditions of the animals. The left femoral vein was cannulated. When the blood pressure decreased, rheomacrodex was given by drop infusion. The left cerebral cortex was exposed by craniotomy. Four different corners of the scalp is stitched by surgical threads and stretched in order to form a liquid vaseline pond (37 °C). The head of the animal was immobilised in the stereotaxic head holder (Harvard Instruments). Body temperature was maintained between 36.5 and 37.5 °C with a heating pad (Harvard Homeothermic Blanket). Ag-AgCl ball electrodes were placed over the somatomotor cortex, the common reference electrode being fixed on the pinna and ECoG was recorded monopolarly.

The epileptic focus was produced by injection of penicillin G potassium (500 units, 2.5 µl) into the sensory motor cortex by Hamilton microinjector. The ECoG activity was displayed on a four channel recorder (Grass 79 F). Control recordings were obtained by injection of saline and flunarizine after formation of epileptiform activity in ten animals. Calcium channel blocker flunarizine was administered in 10 and 100 micromolar (µM, 2.5 µl) concentrations. Each dose was applied at least ten animals. The effects of flunarizine on frequency and amplitude of spikes were estimated for each concentrations. Data are presented as mean ± SEM. Effect of flunarizine on epileptiform activity was analysed by Wilcoxon Matched-Pairs Signed-Ranks Test and whether there was a difference between doses was determined by Mann Whitney U test. Urethane and flunarizine dihydrochloride (F-8257) were obtained from Sigma. Both of them were prepared in coloured bottles, soluted with saline and used immediately.

RESULTS

Intracortical (i.c.) injection of penicillin G (500 units) induced an epileptiform ECoG activity characterised by bilateral spikes. This ECoG activity began within 4±2 min of application and lasted 3-4 hours (Fig 1B). The mean of spike frequency and amplitude were 21.1±1.9 /min and 1080±65 µV at 30 min respectively (Fig 1C).

Administration of flunarizine caused an inhibition for 3-4 min ($p<0.001$). Following this inhibition, spikes reappeared again. But frequency was 9.1±0.7 /min for 4 min ($p<0.01$). Differences between two doses of flunarizine were not statistically significant ($p>0.05$). Saline administered via the same way did not affect the epileptiform activity (Fig 1C, $p>0.05$). Figure 1D illustrates the depressant effect of flunarizine on the electrocorticogram, which were regular findings in all experiments.

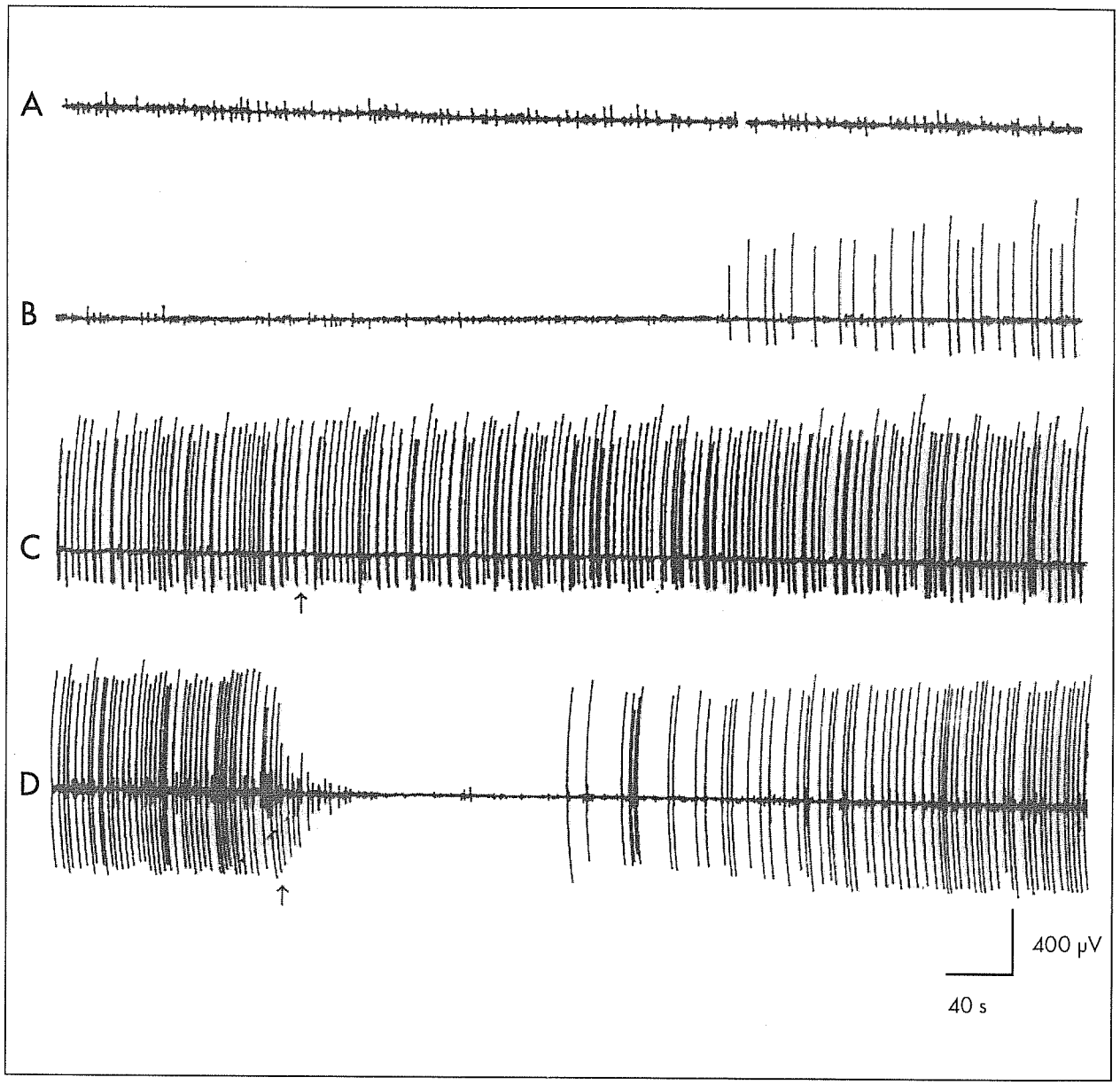

Figure 1. Effect of flunarizine (10, 100 μM) on penicillin-induced epileptiform activity.
A. Control ECoG.
B. Convulsant effect of penicillin, 5 min after penicillin injection.
C. 0 min after penicillin injection (Arrow shows i.c. saline injection time).
D. Effect of flunarizine (10, 100 μM) on penicillin-induced epileptiform activity (Arrow shows i.c. flunarizine injection time).

DISCUSSION

In present study, the effect of calcium channel blocker, piperazine derivative flunarizine on penicillin-induced epileptiform activity was investigated by electro-corticographic recording technique.

Systemic administration of chemical agents may lead to an inhibition of subcortical neurons which may affect on cortical activity. That's why, one can not say that the obtained recordings after systemic administration of substances represent solely the response of cortical neurons. A method which utilizes direct cortical microinjection of drugs eliminates or minimizes the effects of metabolism, binding to plasma proteins, penetrations of the substance and any possible influence of subcortical structures [15].

The penicillin model of experimental epilepsy has been used by several investigators. Sullivan and Osorio[9] induced epileptic activity by injecting penicillin G intraperitoneally. Marangoz et al.[16] reported that intracortical injection of penicillin G (500 units) caused an epileptiform ECoG activity characterised by bilateral spikes and spike-wave complexes. Walden et al.[10] reported that epileptiform potentials can be seen in ECoG 4-5 min after local penicillin administration on cortical surface. In present study, spikes appeared 4±2 min after intracortical penicillin injection.

It is thought that penicillin affects on dendrites prior to interact with GABA transmitter system[17]. Direct penicillin administration into cortex blocks GABA inhibitory system[18]. The inhibition mediated by $GABA_A$ receptor assumed to be the main neuronal inhibitory mechanism in the brain. Weakening of this inhibitory mechanism forms the basis of the convulsing activity [19].

Another mechanism involves in the formation of epileptic activity is the excessive influx of calcium ions[3]. Secretion of excitatory neurotransmitters depends on the amount of calcium ions that enter the cell[20] and administration of calcium agonists exacerbates epileptic activity[21].

Glutamate is the most extensive neurotransmitter in adult central nervous system[22]. Pyramidal neurons utilize glutamate as a neurotransmitter and they are excitatory cells. During epilepsy, routine balance of excitation and inhibition is altered towards the excitation, and a resultant excitatory postsynaptic potential may initiate a burst of dendritic action potentials. Especially Ca^{2+} spikes cause wide and prolonged depolarization[18]. Weakening the GABA mediated inhibition during the seizures has a critical importance for the formation and the spreading of the seizures. Focal epilepsy is believed to be formed by shared effects of decreased GABA mediated inhibition and glutamate mediated excitation in brain cortex[18].

Excessive calcium influx is a very important factor in formation of epilepsy[23]. Depolarisation of a neuron results in a calcium influx through presynaptic voltage-dependent channels and increased concentration of Ca^{2+} then leads to secretion of excitatory neurotransmitters, especially glutamate. This, in turn causes an increased influx of Ca^{2+} via postsynaptic excitatory amino acid channels and postsynaptic voltage-dependent calcium channels[23]. Glutamate leads to an influx of Na^+ and Ca^{2+} ions by stimulating the chemically gated ion channels (NMDA, Kainate and Quisqualate), especially NMDA; and causes an excessive Ca^{2+} influx via voltage dependent calcium channels activated by Na^+ dependent depolarisation[23]. This excessive Ca^{2+} influx is assumed to trigger the neuronal firings

during seizure[23]. It has been shown that the extracellular Ca^{2+} decreases during the seizure[24], while sytosolic Ca^{2+} increases[24]. This important role of calcium in epileptogenesis leads researchers to propose that Ca^{2+} channel blockers might be useful in treatment of epilepsy[25].

In previous studies, following results have been reported: Flunarizine raises the threshold of electroconvulsions in mice[26]; it irreversibly suppresses picrotoxin-induced epileptic activity in hippocampal and neocortical slices in guinea pig[19]; flunarizine and nifedipine have protective effects against pentylenetetrazol (PTZ)-induced seizures in mice[25]; both of them prolong the latent period and reduce the mean duration of PTZ-induced seizures and maximal electroshock seizures (MES). Nifedipine was more potent against PTZ seizures, but flunarizine was against MES[27]; acute administration of flunarizine suppresses the expression of kindled seizure, but there was no effect on the developmental character of kindling[28]; unspecific calcium channel modulator flunarizine depresses epileptiform field potentials in the low Mg^{2+}-model and in the bicuculline model in epileptic and primary non-epileptic neocortical slices in human[29]; piperazine derived flunarizine and papaverine derived verapamil abolish the paroxysmal depolarisation induced by pentylenetetrazol in neurons of organotypic neocortical explants from new-born rats[30]; flunarizine and verapamil depress caffeine-induced epileptic discharges in CA3 neurons of hippocampal slices of the guinea pig[31]; verapamil and flunarizine depress bicuculline-induced epileptic activity in hippocampal and neocortical neurons[32]. In present study, it is shown that intracortically injected flunarizine suppresses penicillin-induced epileptiform activity on ECoG in rats. It has been suggested that flunarizine is effective on E, T, P and N-type voltage dependent ion channels[33,34].

Short duration of anticonvulsant effect of flunarizine in our study is due to the way that the drugs are applied. In our study, drugs administered to the tissue for once by intracortical injection in micromolar concentrations. Administered drug is rapidly diluted by diffusion through peripheral tissues via local blood circulation of the brain which has a very high circulation rate and local concentration of the drugs decreased within minutes. However, all tissue parts are being washed by superfusion of the drugs in hippocampal and cortical preparates in vitro and media contains the slices is filled up with the drug that applied. And in vivo intracerebroventricular studies utilises the continuous pumping of the chemicals into cerebrospinal fluid via infusion method.

The results of this study suggest that flunarizine may be an anticonvulsant agent in treatment of epilepsy.

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