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Anticonvulsive effects of nimodipine on penicillin-induced epileptiform activity

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Abstract. The common features of all types of epilepsy are synchronized and uncontrolled discharges of nerve cell assemblies. It is believed that calcium ions play an important role in the generation of epileptic activity. Excessive calcium influx into neurons is the first step toward a seizure. The aim of the present study is to investigate whether the calcium channel blocker nimodipine has anticonvulsive effects. The left cerebral cortex was exposed by craniotomy in anaesthetized rats. An epileptic focus was produced by injection of penicillin G potassium (500 units) into the somatomotor cortex. After the epileptiform activity reached maximum frequency and amplitude; nimodipine was injected into the same area. Application of nimodipine caused an inhibition in the electrocorticograms (ECoG). Solvent alone did not affect the epileptiform activity. The results of this study indicate that nimodipine may have anticonvulsant effects.

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Key words: Penicillin, epileptiform activity, calcium, nimodipine, electrocorticogram

INTRODUCTION

Epilepsy is one of the most widespread neurological disorders in the world and current pharmacological therapies remain insufficient to prevent epileptic seizures completely. There are several lines of evidence indicating that Ca^{2+} plays an essential role in epileptogenesis. Excessive calcium influx is thought to be the first step in the generation of epileptic neuronal events. The initiation of epileptogenic activity in the neuron is thought to be connected with the phenomenon known as "intrinsic burst firing", which is activated by an inward Ca^{2+} current (Caspers et al. 1987, Speckmann and Walden 1993, Speckmann et al. 1986). During seizures, the level of extracellular calcium decreases (Heinemann et al. 1977) while the level of intracellular calcium increases (Uematsu et al. 1990).

The effects of several calcium channel blockers have been investigated in different models of epilepsy (Kohling et al. 1993, Kulak et al. 2004, Sullivan and Osorio 1991, Walden et al. 1992). The results obtained from these studies show important discrepancies. A dihydropyridine calcium channel blocker nimodipine potently blocks convulsion induced by pentylenetetrazole, NMDA and Bay K 8644 (Palmer et al. 1993). On the other hand, some studies have revealed that nimodipine administered alone suppressed neither PTZ-induced seizures nor electroconvulsions in mice (Czuczwar et al. 1992, 1994). Nor was it successful in controlling idiopathic epilepsy in dogs (O'Brien et al. 1997).

According to current literature, nimodipine shows differential effects on various epilepsy models. However, there is no experimental study concerning the effects of nimodipine on penicillin-induced focal epilepsy. In this study, we investigated whether nimodipine has anticonvulsant effects on penicillin-induced epileptiform activity in rats.

METHODS

Experiments were performed on anesthetized (urethane 1.25 g/kg i.p.) adult male Wistar rats, weighing 200–250 g (n=30). Rats were divided into three groups: Penicillin+solvent (n=10), penicillin+nimodipine (100 µM, n=10) and penicillin+nimodipine (500 µM, n=10). 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. After the animal was placed in a stereotaxic apparatus (David Kopf, MI), 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). Body temperature was maintained between 36.5 and 37.5°C with a heating pad (Harvard Homeothermic Blanket). Two Ag–AgCl ball electrodes were placed over the left somatomotor cortex (electrode coordinates: first electrode, 2 mm lateral to sagittal suture and 1 mm anterior to Bregma; second electrode, 2 mm lateral to sagittal suture and 5 mm posterior to Bregma). The common reference electrode was fixed on the pinna and ECoG was recorded monopolarly.

Epileptic foci were produced by administration of penicillin G potassium (500 units, in 2.5 µl volume) into the sensory motor cortex. Penicillin was injected 2 mm posterior to Bregma, 3 mm lateral to sagittal suture and 1 mm beneath the brain surface by a Hamilton microsyringe (type 701N) (infusion rate 0.5 µl/min). Nimodipine or solvent was given 30 min after the penicillin injection *via* the same route as the penicillin. The ECoG activity was displayed on a four-channel recorder (Grass 79 F). Control recordings were obtained by injection of solvent (3% DMSO solution) without nimodipine after formation of epileptiform activity in ten animals. Calcium channel blocker nimodipine was administered in 100 or 500 micro molar (in 2-µl volume) concentrations.

Data obtained from recordings during one-minute period prior to drug injections were used as control data for spike frequency and amplitude values. After the injections of nimodipine or solvent, data were recorded in one-minute periods for 30 min. These data were compared to control data. Data are presented as mean ± SEM. Effects of nimodipine on epileptiform activity were analyzed using the Wilcoxon Matched-Pairs Signed-Ranks Test. Whether there was a difference between doses was determined by the Mann Whitney U test.

Urethane, penicillin G, nimodipine and dimetylsulfoxide (DMSO) were obtained from Sigma Aldrich Co. (St. Louis, Mo, USA). Urethane and penicillin G were dissolved in saline. Stock solution of nimodipine was prepared with DMSO and diluted with saline (final concentration of DMSO was 3%). Nimodipine was freshly prepared in colored bottles, and used immediately.

All experiments were carried out according to the guidelines of the European Community Council for experimental animal care.

RESULTS

Intracortical (i.c.) injection of penicillin G (500 units) induced an epileptiform ECoG activity characterized by bilateral spikes. This ECoG activity began within 5.2 ± 1.9 min of application and lasted for 135 ± 17 min (Fig. 1). The mean spike frequency and amplitude were 19.3 ± 2.1 /min and 1246 ± 82 µV at 30 min, respectively (Fig. 1).

100 µM nimodipine (n=10) suppressed the convulsant activity 1.1 ± 0.2 min after application and the suppression lasted for 5.4 ± 1.2 min (P<0.001). Following this inhibition, spikes reappeared but the frequency was 9.6 ± 1.4 /min (P<0.01) and amplitude was 986 ± 159 µV (Fig. 1D). Spike frequency and amplitude reached the pretreatment level within 10.8 ± 1.1 min after nimodipine injection (Figs 2, 3).

500 µM nimodipine (n=10) also suppressed the spike activity 1.0 ± 0.2 min after administration and this effect lasted for 6.8 ± 1.3 min (P<0.001). Following this inhibition, spikes reappeared again but the frequency was 9.1 ± 1.2 /min (P<0.01) and amplitude was 893 ± 171 µV (Fig. 1E). Spike frequency and amplitude reached the pretreatment level within 14.9 ± 1.3 min after nimodipine injection (Figs 2, 3).

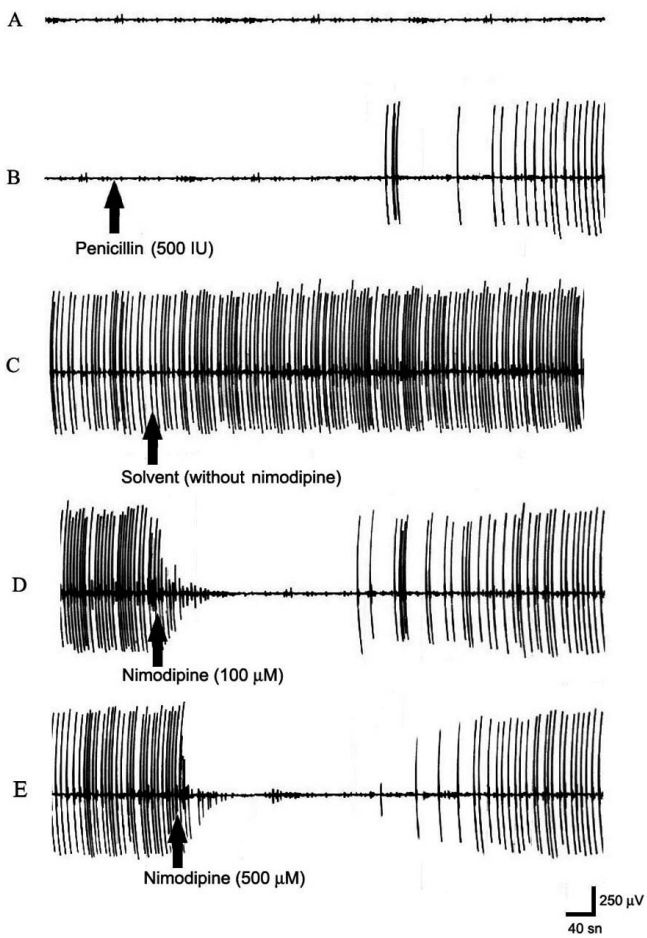

Fig. 1. Effects of nimodipine on penicillin-induced epileptiform activity. ECoG traces represent the recording samples that show the average value for an animal. (A) Control ECoG; (B) Convulsive effect of penicillin G; (C) Solvent injection, 30 min after penicillin injection; (D) Administration of nimodipine (100 µM, i.c.); (E) Administration of nimodipine (500 µM, i.c.).

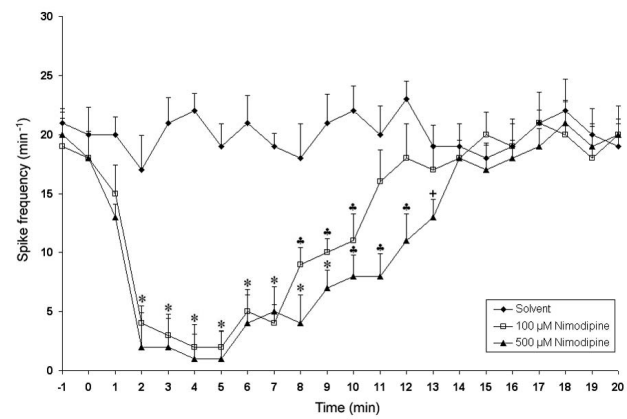

Fig. 2. The effects of the solvent (n=10) and nimodipine (100 µM, n=10 and 500 µM, n=10) on spike frequencies. Zero point shows solvent or nimodipine injection time; (*) P<0.001, (♣) P<0.01, (+) P<0.05.

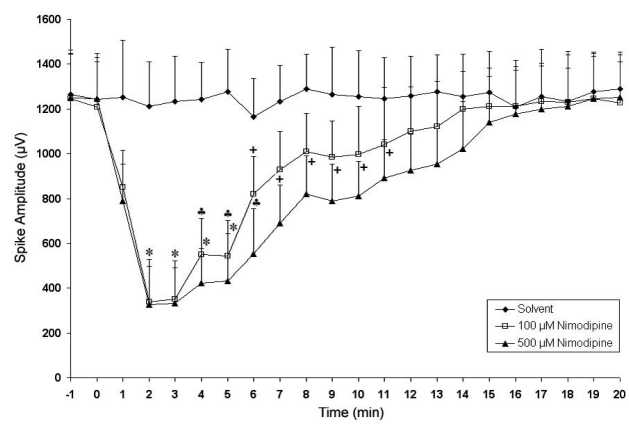

Fig. 3. The effects of the solvent (n=10) and nimodipine (100 µM, n=10 and 500 µM, n=10) on spike amplitudes. Zero point shows solvent or nimodipine injection time; (*) P<0.001, (♣) P<0.01, (+) P<0.05.

The 500 μM dose of nimodipine seemed to be more effective but the difference between two doses was not statistically significant (P>0.05). Solvent (n=10) did not affect the epileptiform activity (Fig. 1C, P>0.05).

DISCUSSION

In the present study, we investigated the anticonvulsant properties of the dihydropyridine calcium channel blocker nimodipine in penicillin-induced seizures in rats. This is the first study that demonstrates the effects of nimodipine on penicillin-induced focal epilepsy. According to our findings, nimodipine suppressed penicillin induced epileptiform activity 1.1 ± 0.2 min after application and the suppression lasted for 5–7 min.

In the present study, focal epilepsy was produced by crystallized penicillin administration into the sensory motor cortex. Systemic administration of chemical agents may affect cortical as well as subcortical neuronal activity. Thus one can not say that the recordings obtained after systemic administration of substances represent solely the response of cortical neurons. A method which utilizes direct cortical microinjection of drugs (Ayyildiz et al 2006, Bagirici et al. 1999, Marangoz et al. 1994) eliminates or minimizes the effects of metabolism, binding to plasma proteins, penetrations of the substance and any possible influence of subcortical structures (Moron et al. 1990).

The short duration of anticonvulsant effects of nimodipine in this study may be due to the way that the drugs were applied. The drugs were administered to the tissue once only by intracortical injection in micromolar concentrations. This route of administration results in rapid dilution by diffusion through peripheral tissues *via* local blood circulation of the brain, which has a very high circulation rate, and local concentrations of the drug decrease within minutes.

In previous studies, it has been demonstrated that dihydropyridine calcium channel blockers have anticonvulsant properties in experimental seizures induced by ischemia, bicuculline, pentylenetetrazole, electrical cortical shock, kainic acid, aminophylline, nitrous oxide as well as alcohol withdrawal or high pressure exposure (Chakrabarti et al. 1998, Dolin and Little 1986, Dolin et al. 1988, Kaminski et al. 2001, Kriz et al. 2003, Little et al. 1986, Meyer et al. 1987, Popoli et al. 1988, Zapater et al. 1998). Our findings are consistent with these results. However, other studies have revealed that nimodipine administered alone suppressed neither electroconvulsions nor PTZ-induced seizures in mice (Czuczwar et al. 1992, 1994). It was not successful in controlling idiopathic epilepsy in dogs (O'Brien et al. 1997). These different effects may be due to different epilepsy models. In general, it is thought that blockage of L-type Ca^{2+} channels seems to be associated with control of partial seizures without secondary generalization, while T-type Ca^{2+} channels are associated with efficacy in treating absence seizures (Kulak et al. 2004). The dihydropyridine calcium channel blocker nimodipine blocks especially L-type calcium ion channels (Greenberg 1987).

Penicillin is structurally similar to the GABA antagonist bicuculline. Direct penicillin administration into cortex blocks the GABA inhibitory system and causes focal epilepsy. Weakening the GABA mediated inhibition is of critical importance for the formation and spreading of seizures. Focal epilepsy is believed to be formed by shared effects of decreased GABA mediated inhibition and glutamate mediated excitation in cortex (Martin 1991). Glutamate leads to an influx of Na^{+} and Ca^{2+} ions by stimulating the chemically gated ion channels, especially NMDA channels. Na^{+} influx also activates voltage dependent calcium channels. This excessive Ca^{2+} influx *via* both chemically and voltage-gated channels is assumed to trigger neuronal firing during seizures (Uematsu et al. 1990). It has been shown that the level of extracellular calcium decreases (Heinemann et al. 1977) while level of intracellular calcium increases during seizures (Uematsu et al. 1990). We propose that the suppressive effect of L-type Ca^{2+} channel blocker nimodipine may be due to the prevention of excessive Ca^{2+} flux into cell.

CONCLUSION

Our findings indicate that intracortically injected nimodipine suppresses penicillin-induced epileptiform activity recorded by ECoG in rats. The results of this study suggest that nimodipine may be an anticonvulsant agent. This anticonvulsant effect may be due to the blocking of calcium flux in to the cell. Further studies are needed at the cellular and molecular levels in order to clarify the exact mechanism of the anticonvulsant effect of nimodipine.

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