

RESEARCH ARTICLE

Anticonvulsive effects of nifedipine and flunarizine on maximal electroshock seizures induced seizures

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ABSTRACT

Background: Epilepsy is a common neurological disorder but the mechanism of seizure generation has been only partially unraveled. Currently available antiepileptics have a low therapeutic index and furthermore, provide satisfactory seizures control in only 60–70% of patients. Therefore, the present study was done to review the basic mechanism of seizures with special attention on voltage gated calcium channels and search for new antiepileptic in order to improve the efficacy of current therapies. **Aims and Objective:** The current study aimed to evaluate the anticonvulsant activity of calcium channel blockers. **Materials and Methods:** The animals were treated with Nifedipine (100 µg/100 g ip and 200 µg/100 g ip) and Flunarizine (5mg/kg ip and 10 mg/kg ip) and maximal electroshock seizures (MES) was induced by technoelectro convulsometer, 2 hrs after the administration of drugs, and duration of various phases were noted. Duration of tonic hind limb extension (THLE) was taken as index for antiepileptic activity. **Result:** Nifedipine and Flunarizine when administered in dose of 100 µg/100 g ip and 5 mg/kg respectively did not produce any changes in any phases of the MES induced seizure. But in dose of 200 µg/100 g ip and 10 mg/kg ip respectively, it significantly reduced the duration of THLE. **Conclusion:** Nifedipine and Flunarizine have a significant action against MES induced seizures suggesting an important role of CCBs as future, promising antiepileptic drug.

KEY WORDS: Calcium Channels; Maximal Electroshock Seizures; Nifedipine; Flunarizine; Tonic Hind Limb Extension; Anticonvulsants


INTRODUCTION

Epilepsy is a group of neurological disorders characterized by epileptic seizures.^[1,2] Epileptic seizures are episodes that can vary from brief and nearly undetectable periods to long periods of vigorous shaking.^[3] Epileptic seizures can be

thought of paroxysmal hypersynchronous transient electric discharges in the brain that result from too much excitation or too little inhibition in the area in which abnormal discharges starts. Excitation and inhibition of neurons may be mediated by many different neurotransmitters and γ aminobutyric acid is the principal inhibitory neurotransmitter in the cortex.^[4]

Neurophysiology and Pathophysiology of Epilepsy

Seizures are an extreme form of synchronous brain activity, characterized by enhanced excitation, leading to a transient condition of intense, hypersynchronous neuronal activity. EEG recording such episodes reveals high amplitude ictal discharges in the Berger frequency bands.^[5] Calcium has

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been implicated in the pathophysiology of seizure.^[6-8] The bursting activity is an important stage in the seizure activity and is caused by influx of extracellular calcium, which leads to a relatively long lasting depolarization of the neuronal membrane, opening of the voltage dependent sodium channels and generation of repetitive action potentials. Calcium channels also mediate multiple cellular effects including fusion of neurotransmitter containing vesicles with the presynaptic terminal membrane and allowing release of neurotransmitter.^[9] Voltage gated calcium channels mediate calcium influx that controls both neuronal excitability and regulates calcium sensitive intracellular signaling pathways.

Previous studies have already shown some alterations in Ca_v 3.2 (gene encoding T type Ca⁺⁺ channels), which may induce altered biophysical properties or increase channel expression.^[10-13] The Ca_v 2.1 encodes for both P and Q type calcium channels and these channels are highly expressed presynaptically where they are critically involved in neurotransmission and synaptic efficacy and therefore have a great influence on neuronal excitability.^[14,15]

Studies have also been carried out on genetic epilepsy prone rats, and in them induction of secondary tonic clonic seizure have shown increase in Ca_v 1.3 (L type) and Ca_v 2.3 (R type) protein level, in neurons,^[16] further supporting the role of calcium ions in epileptogenesis.

Thus it may be concluded that calcium current may contribute to epileptogenesis by undergoing burst in pacemaker cells, enhancing postsynaptic excitatory responses in dendrites and somatic nerve cells and providing post burst re-excitation.^[17] Through intensive research, it has also been highlighted that calcium is also involved in neuronal injury which is caused as a result of repeated seizures.^[18] Accumulated evidence shows clear correlation of calcium channel expression with the development and maintenance of seizures. It is found that 30% of patients are resistant to conventional pharmacotherapy and they have lots of side effects so the present study have done to know the alternate therapy with lesser side effect. Thus the present study was undertaken to evaluate the anticonvulsant activity of nifedipine, which is a dihydropyridine calcium channel blocker (CCB), and of flunarizine, which is a difluorinated piperazine derivative, and act as selective calcium entry blocker, on maximal electroshock seizures (MES) induced seizures.

MATERIALS AND METHODS

Animals

Adult, healthy swiss albino mice, aged 6–8 weeks of either sex weighing 20–30 g were used. They were housed under standard laboratory conditions (controlled temperature (around 22 ± 2°C) and humidity (50%) colony room) for 1 week before experiments were started and were kept in

groups of 3–4 in per polypropylene cages. Animals were allowed standardized diet and water ad libitum, except for the period of experimentation.

This study is conducted after approval from institutional animal ethics committee.

Drugs and Chemicals

- I. Nifedipine (JB chemicals, Mumbai) - it was dissolved in propylene glycol, just before use.
- II. Flunarizine (Baroda Pharma Pvt., Ltd., vadodara).
- III. Propylene glycol (Hi Media, Mumbai) - This solvent was used to dissolve the nifedipine and it served as solvent control in nifedipine treated animals.
- IV. Normal saline (0.9% sodium chloride).

Methods

The present study was undertaken to see the antiepileptic activity of nifedipine (DHP CCB) and Flunarizine (difluorinated piperazine derivative, selective calcium entry blocker) against MES induced seizure, in mice.

Maximal electro shock seizure was induced by Techno-electro convulsometer (50 mAmp, 0.1 sec duration) through ear electrodes, via small alligator pinnal clips. Duration of various phases of maximal electroshock seizures (tonic flexion [TF], tonic extension, tonic convulsion, and post tetanic depression) were noted with the help of stop watch.

In the first part of the study, the animals were treated with Nifedipine (100 µg/100 g ip) and (200 µg/100 g ip). MES was induced by technoelectro convulsometer, 2 h after the administration of the drug, and various durations were noted.

Propylene glycol (0.2 ml/100 g ip) treated animals were served as solvent control.

In the second part of the study, the animals were treated with Flunarizine (05 mg/kg ip and 10 mg/kg ip). MES was induced by technoelectro convulsometer, 2 h after the administration of the drug, and various durations were noted.

Saline treated animals were served as solvent control.

Result were statistically analyzed by paired student *t*-test. *P* < 0.05 were considered significant.

RESULTS

The present study was undertaken to explore anticonvulsant effect of nifedipine and flunarizine against MES induced seizure. The experimental study was conducted in mice. Each study was conducted with a control group treated with propylene glycol, for nifedipine and normal saline

for flunarizine. The abolition or reduction of duration of tonic extension was considered as index for antiepileptic activity.

MES Seizure Test

When administered in dose of 100 µg/100 g ip, nifedipine did not produce any significant change in any phase (TF, tonic extension, clonic convulsion (CC), posttetanic depression) of MES induced seizures ($P > 0.05$). When administered in dose of 200 µg/100 g ip, nifedipine significantly reduced the duration of tonic hind limb extension (THLE) ($P < 0.001$), but failed to produce any significant change in any other phase of MES induced seizures ($P > 0.05$) [Table 1]. The tonic hind limb extensor component was found to be reduced significantly in animals pretreated with nifedipine. On the basis of these observations, nifedipine appeared to have a potent antiepileptic effect.

When administered in dose of 05 mg/kg ip, flunarizine did not produce any significant change in any phase (TF, tonic extension, CC, posttetanic depression) of MES induced seizures ($P > 0.05$). When administered in dose of 10 mg/kg ip, Flunarizine significantly reduced the duration of THLE ($P < 0.001$), as well as durations of other phases of MES induced seizures ($P < 0.001$) [Table 2]. The tonic hind limb extensor component was found to be reduced significantly in animals pretreated with flunarizine.

On the basis of these observations, our study demonstrated that both CCBs afford protection against MES induced convulsions, and Flunarizine affords higher degree of protection than Nifedipine.

Table 1: Effect of nifedipine on duration of various phases of MES induced seizures

Dose of drug	TF	THL	CC	PTD
Control	3.3±0.82	13.9±1.19	9.0±0.81	6.0±0.94
N (100 µg/100g)	3.1±1.01	13.7±1.03	8.9±1.02	5.6±1.05
N (200 µg/100g)	3.2±0.99	4.7±0.76*	8.8±1.02	5.8±1.03

N: Nifedipine, TF: Tonic flexion, THLE: Tonic flexion leg extension, CC: Clonic convulsion, PTD: Post tetanic depression.
* $P < 0.05$. Values are given as mean±SEM ($n=10$). SEM: Standard error mean, MES: Maximal electroshock seizures

Table 2: Effect of flunarizine on duration of various phases of MES induced seizures

Dose of drug	TF	THL	CC	PTD
Control	3.3±0.82	12.48±0.45	24.63±0.56	35.30±0.56
F (5 mg/kg)	2.98±1.01	11.87±1.14	22.73±0.82	36.01±0.15
F (10 mg/kg)	3.1±0.99	5.92±0.14*	17.9±0.13*	14.27±0.26*

F: Flunarizine, TF: Tonic flexion, THLE: Tonic flexion leg extension, CC: Clonic convulsion, PTD: Post tetanic depression.
* $P < 0.001$. Values are given as mean±SEM ($n=10$). SEM: Standard error mean, MES: Maximal electroshock seizures

DISCUSSION

The current experimental study has been planned with an objective to study the antiepileptic effects of Nifedipine and Flunarizine (CCBs) on MES induced seizures. The abolition or reduction of THLE was considered as index of antiepileptic activity. From the results, it was found that Nifedipine, in the dose of 200 µg/100g ip, significantly reduced the duration of THLE ($P < 0.001$) and Flunarizine, in the dose of 10 mg/kg ip, significantly reduced the duration of all the phases (TF, THLE, CC, PTD, $P < 0.001$) of MES induced seizures.

From the experimental results, it was found that Nifedipine significantly reduced the duration of THLE ($P < 0.001$), showing significant anticonvulsant action. The role of calcium in the excitability of muscle and nerve is well established. The influx of calcium into neurons is an important factor in triggering epileptic activity and consequently, the prevention of cellular calcium overload may have anticonvulsant effect.^[6,7] This anticonvulsant action may be based on the facts that, during the episode of epileptic attack there is ischemia and excitation which can cause damage in hippocampus and cerebellar cortex.^[19] Epileptic depolarization in single motor and hippocampus neurons and focal epileptic discharges in neuronal cortical preparations have also been described to be decreased by CCBs and hence CCBs prevents cell damage.^[20] The anticonvulsant effect of nifedipine may also be correlated with the increase in local blood flow due to vasodilatation,^[21] and all these effects of nifedipine may be due to central blockade of calcium entry through dihydropyridine L type calcium channels.^[8] It has been established that even small alterations in the biophysical properties of presynaptic calcium channels could have a significant impact on the firing properties of nerve cells and neuronal networks with the potential to lead to epileptic seizure activity.^[22-24]

In the present study, flunarizine was also found to reduce the various phases (TF, THLE, CC, PTD, $P < 0.001$) of MES induced seizures. The significant anticonvulsant effect of flunarizine can be correlated with previous studies which states that the Flunarizine provides a direct neuroprotective effect against the damaging influx of calcium and prevents neural damage.^[25] It reduces transmission fluxes of calcium in situation where calcium is stimulated to enter the cell in excess, thus preventing the deleterious consequences of calcium overload within the cell.^[26] It readily crosses the blood brain barrier and inhibits entry of calcium into the neurons, primarily under pathophysiological conditions, such as ischemia or seizure activity, without any effect on normal calcium homeostasis.^[27] The cerebrovascular effect of flunarizine could provide a direct neuroprotective effect against the damaging influx of calcium and could also prevent neuronal damage as a result of MES induced seizures.^[28]

The fact that CCBs do not directly inhibit neurotransmitter release, except, in the situation of ischemia and excitation can

encourage the use of these drugs as non sedative anticonvulsants without the risk of catastrophic effect on neurotransmission.^[29] Considerable effect is still going on towards developing new and selective calcium channel blocking compounds aimed at the treatment of epilepsy.^[30] As we already know that many of the currently used antiepileptic drugs have been shown to block the calcium channels and the present study also demonstrated that nifedipine and flunarizine (CCBs) has anticonvulsant action, calcium channels are more commonly viewed as attractive targets for novel epileptic therapies.

CONCLUSION

There is hope that nifedipine and flunarizine could be effective in patients who are refractory to presently available standard antiepileptic medication. In the present study nifedipine and flunarizine are found to have effective antiepileptic activity in animal models, but this is to be confirmed in humans with controlled randomized clinical trials, so further studies need to be carried out to explore the potential of these drugs.

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