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DIHYDROPYRIDINE GROUP OF CALCIUM CHANNEL ANTAGONIST IN EPILEPSY

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Abstract: Effects of intraperitoneally administered dihydropyridine calcium channel blocker-nifedipine (NIF) 5 mg/kg and diphenylhydantoin (DPH) 5 mg/kg were studied on hippocampal kindling and maximum electro shock thresholds (MEST). All the NIF injected rats showed complete supression of behavioral seizure after the 3rd injection. Few of the DPH rats had increased epileptic activity for two days and others - absence of Grade - 5 seizure after the 5th DPH injection. However, all showed partial seizure supression subsequently. Neither NIF nor DPH could suppress the after discharge (AD). MEST were not affected by NIF although DPH showed a complete supression of posterior limb extensor tone in all the rats.

Key words: dihydropyridine calcium channel antagonists epilepsy nifedipine hioppocampus

INTRODUCTION

It is documented that calcium influx is involved in epileptogenesis (1, 2) that is probably evolving through voltage operated calcium channels. Various calcium channel blockers have been tried with this in view using varieties of experimental models of epilepsy (3,4,5). However, there are isolated reports using nifedipine (NIF) and hippocampal kindling as a model (6). This requires an urgent inquiry because kindling is a robust form of epilepsy that cannot be ordinarily controlled with conventional anti epileptics (7). The present study was designed to probe into the effect of NIF as against standard dose of diphenylhydantoin (DPH) on mature kindling and maximum electro shock seizure (MES).

METHODS

Animals: Thirty six male Wistar albino rats weighing between 150-165 g, 10 to 12 weeks of age were housed individually in the animal house with *ad lib* access to food and water. Following a week's handling they were divided randomly into two groups of 12 in each. One group was for kindling trials and the other for maximum electroshock threshold (MEST) testing. Twelve served as control subjects for the kindling and MEST trials (6 each).

Electrode implantation: Bipolar stainless steel electrodes were made in the laboratory using wires of diameter 30 Ga and insulated with apoxy resin excepting at the tips. The oblique inter electrode diameter was 1 mm. They were stereotactically introduced into the right ventral hippocampus of the rats using the standard coordinates (8) 2.6 mm behind bregma, 4·6 mm lateral to and 8.5 mm below the cortical surface, using chloral hydrate anesthesia (400 mg/kg body weight) intraperitoneally. The electrodes were anchored using 3 jewelers screws, dental cement and acrylic liquid. Same electrodes served for stimulation and recording of E.E.G. - after discharges (AD).

Kindling procedure: A train of 100 biphasic square wave pulses of 400 μA; frequency 10 Hz; width 1 msec; interval 100 msec; was administered daily based on our parallel study (9) showing that this pattern of stimulation kindles rapidly, using Nihon Kohden stimulator (SEN 3201) and isolator (SS-302J). After the rats were fully kindled, they were administered the various drugs and tested for seizure and after discharge for a period of 15 days.

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Recording: Seizure AD were recorded using a Polyrite (Medicare System - Ambala) using a custom made switch that would interrupt the recorder from the stimulator when the stimulation was ON. Grading of kindling was done according to Racine and Pinel (10,11), and was considered complete on getting Grade-5 seizures on five successive occasions (Fig.1).

MEST: Twelve subjects were pre-tested for posterior limb extensor tone on administering a MEST. Shocks were administered bitemporally through ear-clip electrodes smeared with 0.6% saline using a Techno Electro Convulsimeter. The MEST was estimated using 60 Hz stimulus of 0.2 sec starting with 36 mA and stepping up by 3 mA every 10 minutes till a Grade-5 seizure (posterior limb extensor tone) was elicited on 3 successive days. Grading was done according to (12). The various drugs were administered for a period of 15 days and the rats tested for seizures with MEST.

Drugs

NIF (5 mg/kg) was injected intraperitoneally twice daily as an infusion made in 40% Polyethylene glycol (400) in 0.9% saline. The solution was made from capsules of Calcigard (Torent Lab.) in a dark wall chamber covered with aluminium foil and the injection syringe was covered with aluminium foil to avoid any form of light influencing the biological activity of NIF.

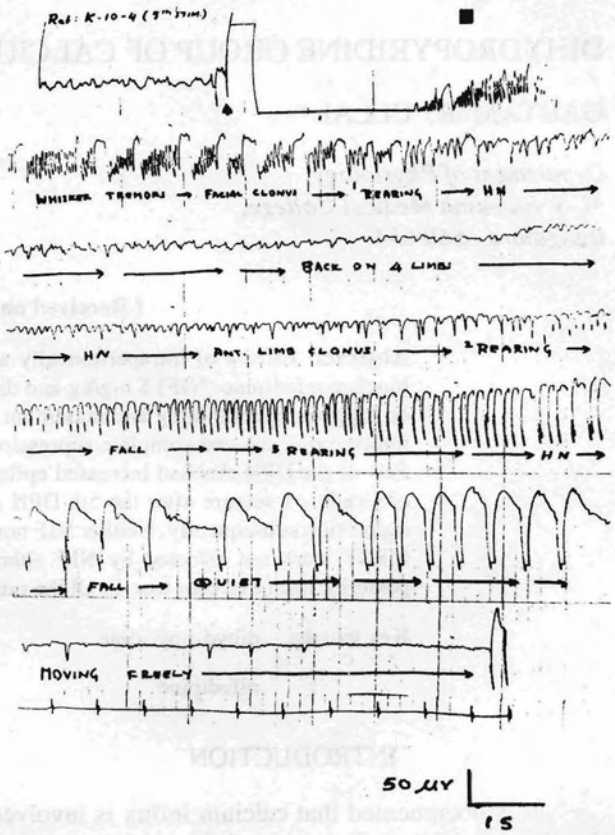

Fig. 1: Representative EEG of a fully kindled rat, with the corresponding behavioural component of seizure indicated below the tracing..

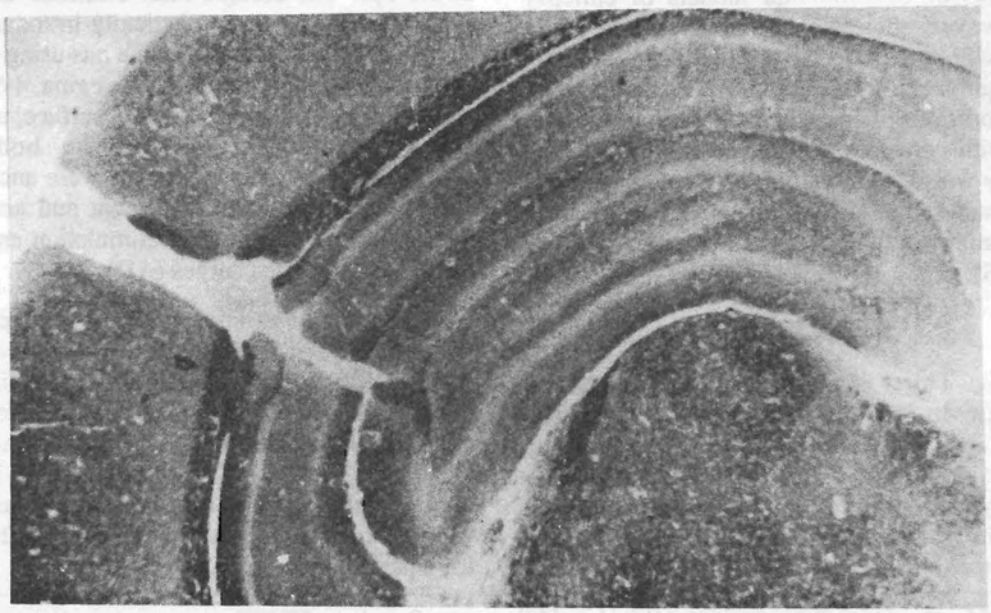

Fig. 2: Section of a hippocampal region in rat showing electrode track in CA1 region.

DPH (5 mg/kg) was injected intraperitoneally directly from a 50 mg ampoule (Eptoin - Cadila Lab.) twice daily.

Control rats were injected 0.5 ml of 0.9% saline.

Histopathology: After the study, kindled rats were anesthetized with chloral hydrate and perfused through the heart with 10% formalin in physiological saline. The brain was sliced and stained with cresyl violet to locate the electrode track (Fig. 2).

RESULTS

Kindling: By 10 days, all the rats had reached full kindling with the stimulus pattern used.

Behavioral aspects: On injecting the 3rd dose of NIF there was a complete absence of behavioral seizures (Table I) except occasional whisker tremors. First and second injections had no effect. DPH injected rats did not show any action for 3 days (5 doses). Following that, 4 rats became more epileptic and showed Grade-7 seizure (11) in that they had repeated Grade-5 seizures and became violent for 10-15 minutes post ictally. This state of increased activity lasted for 2 days. After that they became less epileptic by 3rd and 4th day. By the 5th day they had no behavioral seizure except whisker tremors and facial clonus (Grade-2) following injection. The other two DPH injected rats showed absence of Grade-5 seizures 3 days following the treatment. However, even these rats (like the other 4) had Grade-2 seizure during the treatment period of 15 days.

The control rats continued to get Grade-5 seizures. Five of these rats progressed to Grade-7 seizures within the injection period.

E.E.G. - After discharge (Fig. 1): Neither of the groups (NIF/DPH) showed any suppression of AD.

MEST: The mean (± SD) MEST of all rats was 48 mA before drugging. NIF injected rats did not show any change in the MEST. However, DPH injected rats had complete elimination of posterior limb extensor tone with the threshold stimulation. Three rats had Grade-1, two had Grade-2 and one had Grade-3 seizure 2-3 days following the injection. The controls continued to have posterior limb extensor tone with the same MEST.

TABLE I : Effect of AED on seizure and after discharge.

Injection	*Number of rats*	*Observations*	
		Seizure	*AD*
NIF	6	0†	No change
DPH	6	0**	No change
Saline	6	6	No change

Nifedipine (NIF), diphenylhydantoin (DPH), after discharge (AD).
*Four rats showed initial increase in epileptic activity for 2 days.
†Whisker tremors and facial clonus remained unaffected.

DISCUSSION

In this study, NIF completely suppressed the behavioral seizure produced by hippocampal kindling although the after discharges remained unaffected. DPH on the contrary showed increased epileptic activity, as reported by others (7). However, limbic component of the seizure appeared unaffected by NIF or DPH in that the rats continued to show whisker tremors and facial clonus. After discharge was not influenced by either of the drugs. Suppression of behavioral seizures in absence of AD elimination, indicates that AD being a local phenomenon is robust and cannot be easily suppressed (14).

Studies with MEST, on the contrary, showed that NIF had no effect at all as against DPH, that completely eliminated seizure to a significant level. This indicates that the seizure mechanisms in two epileptic models are different. NIF may have its action only in a seizure that begins from a focus as in kindling and not one that is primarily generalized.

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REFEREMCES

1. Pumain R, Kurcewicz I, Louvel J. Fast extracellular calcium transients: Involvement in epileptic process. *Science* 1983; 222 : 177-179.
2. Heinmann U, Hamon B. Calcium and epiletogenesis. *Exp Brain Res* 1986 ; 65 : 1-10.
3. Meyer FB, Tally PW, Anderson RE, Sundt TM, Yaksh TL, Sharbrough FW. Inhibition of electrically induced seizures by dihydropyridine calcium channel blocker. *Brain Res* 1986; 384: 180-183.
4. Morocutti C, Pierelli F, Sanarelli L, Stefano E, Peppe A, Mattioli GL. Antiepileptic effects of a calcium antagonist (nimodipine) on cefazoline induced epileptogenic foci in rabbits. *Epilepsia* 1986; 27: 498-503.
5. Spedding M, Middlemiss DN. Central effects of Ca2+ anatagonists. *Trends Pharmac Sci* 1985; 309-310.
6. Vezzani A, Wu HQ, Stasi MA, Angelico P, Samanin R. Effect of various calcium channel blockers on three different models of limbic seizures in rats. *Neuropharmacology* 1988; 27: 451-458.
7. Wolfgang L, Rundfeldt C. Kindling as a model of drug resistant partial epileps: Selection of phenytoin resistant rats. *Journal of Pharm and Exper Ther* 1991; 258: 483-489.
8. Pellegrino LJ, Pellegrino AS, Cushman AJ. A stereotactic atlas of the rat brain, 2nd end., Plenum Press, New York, 1979; 32-33.
9. Ullal GR, Mehta MR, Dasgupta C. Frequency dependence of kindling rats- A study revisited. (Manuscript being processed).
10. Racine RJ. Modification of seizue activity by electrical stimulation. I: After discharge threshold. *Electroenceph Clin Neurophysiol* 1972; 32; 269-279.
11. Pinel JPJ. Experimental epileptogenesis: Kindling induced epilepsy in rats: Cortical stimulation. *Exper Neurol* 1981; 72: 559-569.
12. Crossland J. Lewis Pharmacology V Edn. Churchill Livingston 1980; 512-514.
13. Meyer FB, Anderson RE, Sundt TM. Anticonvulsant effect of dihydropyridine Ca2+ antagonists in electrocortical shock seizure. *Epilepsia* 1990; 31(1): 68-74.
14. Racine RJ, MacIntyre W, Burnham WM, Garner JG. First trial motor seizure triggered by amygdaloid stimulation in the rat. *Electroenceph Clin Neurophysiol* 1973; 35: 487-494.