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EFFECT OF ANTIEPILEPTIC DRUGS AND CALCIUM CHANNEL BLOCKER ON HYPERTHERMIC SEIZURES IN RATS : ANIMAL MODEL FOR HOT WATER EPILEPSY

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Abstract: Hyperthermic seizures were elicited in groups of freely ambulant rats with jets of hot water of 55°C on the head for about 10 mins. Bipolar depth EEG from the hippocampus and the behavioural seizures following the stimulation were recorded. The rectal temperature (threshold) for seizure initiation was 41.5°C. The seizures were predominantly clonic jerks accompanied by large spikes and slow waves lasting for 30-60s.

After 3 stimulations (once a day), Phenobarbitone (Pb) 0.02 mg/g daily; Diphenylhydantoin (DPH) 0.001 mg/g, 0.005 mg/g and 0.04 mg/g. daily and Nifedipine (Nif) 0.005 mg/g twice daily were administered intraperitoneally in different rats. During the 10-days injection trials, Pb completely suppressed seizures whereas DPH and Nif did not have any effect. One of the rats with DPH showed increased epileptic activity. After a 10 day 'washout' period' Pb and DPH were interchanged and again the rats were tested for seizures on 10 days.

On changing over to Pb from DPH there was complete suppression of seizures and electrical seizure discharges. Whereas those rats which earlier had no seizure activity with Pb started showing the same on changing over to DPH.

Key words: hyperthermic seizures, depth hippocampal EEG, antiepileptic drugs, calcium channel blocker

INTRODUCTION

Seizures precipitated by hot water bathing is a form of reflex stimulus-sensitive epilepsy, reported in literature as - hot water epilepsy (HWE) (1-4) or water immersion epilepsy (5-10). Though a few isolated cases have been reported from various parts of the world, viz. Japan, U.K., U.S.A. and New Zealand (5-13), reflex epilepsy is found to be highly prevalent in South India (1-4). The pathophysiology of this special type of reflex epilepsy is not clear. The few animal models for hyperthermic seizures such as exposure of rats to hot air (11) infrared lamp (12) or rats allowed to swim in a heated pool (13) do not truly simulate the typical 'hot water bath on head' - in the pattern of stimulation, and initiation of seizure activity as reported in human subjects from South India. Recently, we have developed an animal model (14) where rats exposed to hot water jets over the head developed seizures. The initiation and

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progression of seizures, and the EEG pattern of the epileptic activity in the animal appeared to recapitulate the clinical events in human subjects. We observed that 50°C and 55°C hot water jets, on the head of adult Wistar albino rats resulted in precipitation of seizures, with a threshold rectal temperature of 41.5°C. Cooling of the animals by pouring cold water on the head prevented rapid progression of the seizure activity in the animals (14).

In vitro experimental studies using hippocampal slices, have demonstrated that hippocampus is very sensitive to hyperthermic stimulation (15) resulting in 'seizure like' electrical activity. Based on this observation, we have recorded hippocampal EEG in the rats through various phases of the ictus, using a stereotaxically placed electrode.

Having developed the animal model, in the present study, we evaluated the usefulness of the antiepileptic drugs - Phenobarbitone (Pb) and Diphenylhydantoin (DPH), in abrogating the hyperthermic seizures in the rats. Following the observation that Nifedipine (Nif) - calcium channel blocker - can suppress hippocampal seizures in the animals (16), we also studied the effect of Nif on 'hot water induced' seizures in our rat model as a potential antiepileptic drug.

METHODS

Animals: Fiftyseven male Wistar albino rats, 12-16 weeks old (150-200 g) were housed individually under standard laboratory conditions (25°C, 50% relative humidity; 12h/12h light and dark cycles). The rats had free access to food (standard rat pellets) and water. The experiments were initiated only after a week of taming and handling for acclimatisation.

Hot water stimulation: In our earlier study (14), it was found that hot water jets of 55°C on the head, was most consistent in initiating the seizures, while water jets of 4°C, 28°C and 45°C failed to induce seizures and water jets of 50°C precipitated seizures inconsistently. Hence the seizure activity caused by 55°C water jets was employed in the study to investigate the effects of antiepileptic drugs.

The rats were placed in a 40 cm x 30 cm x 15 cm plastic chamber (Fig. 1). The chamber had a fine mesh at the base for continuous drainage of water, thus preventing immersion of the body in water. The rats could move freely in the chamber, above the mesh, during the hot water bath. The hot water jet of 55°C was delivered on the head, with a 10 ml glass syringe. The velocity of the water jet was (determined by Bernoulian Theorem) 13.04 m/s. (mean SD±0.1m) and it was delivered at a rate of 30 jets/min (timed by Metzel metronome) manually. The maximum duration of stimulation was 10 min. The time point was determined in the pilot study, where the animals developed either seizures or appeared exhausted, withdrawing to the corner of the chamber and sitting immobile. Seizure initiation was the end point of stimulation in the rats who developed them. Two min after the seizure, the rectal temperature of the animals was restored to normal resting level by pouring tap water (ambient temperature) over the head and body for 2 min. The animals were gently dried with soft cloth towel, and returned to the home cage to

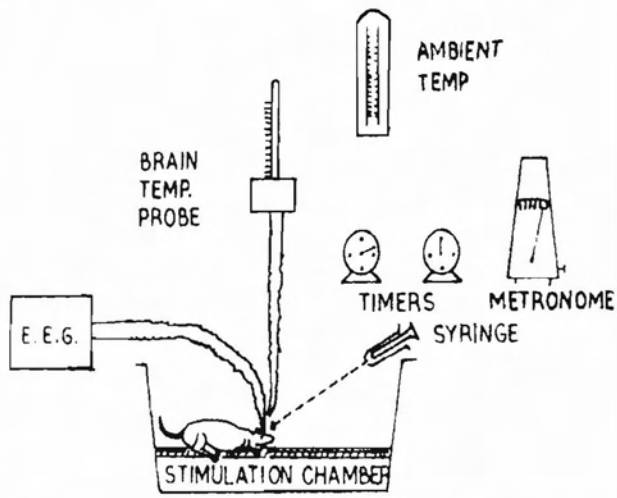

Fig. 1 : Experimental setup showing the animal chamber, the stimulating and recording devices.

mimic hot water bathing in human subjects. The resting temperature, the temperature at seizure initiation (threshold) and after post ictal cooling were recorded. Three such 24 hourly trials were conducted to confirm seizures and establish seizure threshold.

Categorization of subjects: Fifteen of the 57 rats had hippocampal electrodes. Those with electrodes were divided into groups of 3 in each for Pb, DPH, Nif, saline and saline-PEG (PEG = polyethylene glycol). The remaining 42 rats were divided into groups of 6 each - Pb, DPH, (0.001, 0.005, 0.04 mg/g) Nif, saline and saline-PEG.

Electrode implantation: Bipolar electrodes of stainless steel wire (diameter 0.1 mm) with oblique interelectrode distance of 0.5 mm and insulated with epoxy resin, except at the tips, were prepared in the laboratory. They were stereotaxically implanted into the right hippocampus of the rats, under chloral hydrate anaesthesia (400 mg/kg, intraperitoneally), using the standard co-ordinates (17). The electrodes were anchored with eye-glass screws, dental cement and liquid acrylic. On completion of the study, the rats were anaesthetised with chloral hydrate (400 mg/kg. intraperitoneally). The animals were perfused through the heart with normal saline, followed by 10% buffered formalin. The electrode tract was confirmed on histological sections with cresyl violet.

Drugs and dosage: Phenobarbitone was administered intraperitoneally as a single dose of 0.02 mg/g (18), every evening (17.00 h) for 10 days. Diphenylhydantion was administered intraperitoneally in 3 doses of 0.001 mg/g - 0.005 mg/g and 0.04 mg/g every evening for 10 days in a single dose similar to Pb.

Nifedipine (0.005 mg/g) was administered intraperitoneally twice daily (10.00 h and 17.00 h) in 40% polyethylene glycol (PEG - 400) in 0.9% saline (16, 19). The solution was made from capsules in an amber coloured bottle covered with aluminium foil. Similarly, the injecting syringe was also light protected, to avoid the influence of light on the biological activity of Nif.

Experimental protocol: The study was conducted in four phases -

1. 'pre drug' period of 3 days, followed by
2. 'drug period' of 10 days,
3. 'wash out' period of 10 days and
4. '2nd drug' period of 10 days.

During pre drug and drug periods, hot water stimulation was given every day. There was no stimulation during the wash out period.

In the case of Pb and DPH group, following the wash out period the drugs were interchanged and trials conducted once again for a further period of 10 days.

Recording of seizure activity

Behavioural seizure: The nature of seizure behaviour and its progression with repeated stimulation was observed. The latency and duration of seizure was recorded visually by using two timing devices.

EEG after discharges: EEG was recorded in the resting, ictal and recovery phases using a Polyrite physiograph – EEG recorder. Random interstimulus recordings were done to look for interictal seizure discharges in the initial experiments. Similarly, in the rats under drug trial, the EEG was recorded, at different phases, to evaluate the influence of the drug on electrical activity. Freely moving rats were connected to the EEG machine only during the time of recording.

International Guiding Principles for Biomedical Research involving animals - (1975) were followed throughout the study.

RESULTS

The normal resting rectal temperature of the rat was 37°C (± 1°C SD). All rats developed seizure from the very first stimulation with hot water. Prior to drug administration, the threshold rectal temperature for seizure initiation was 41.5°C (±0.1 SD) and the mean

time taken for seizure initiation (latency) was 3.5 min (±0.1 SD). The seizures were predominantly of continuous clonic jerks lasting for 30-60s, involving all the limbs, trunk and neck. There was no tonic component. Depth EEG recorded from the hippocampus during the seizure demonstrated runs of high voltage spikes (120 μV) interspersed with slow waves lasting about 60s followed by quiescent phase lasting for 45s (Fig. 2).

Following the intraperitoneal administration of Pb, total cessation of the seizure and after discharge were noted in all the rats (Fig. 3) from the first day after injection. This feature lasted during the whole 'drug phase'. On the other hand, control (saline and saline-PEG) rats, and rats with DPH injections continued to demonstrate seizures in the form of clonic movements of all limbs similar to the ones observed prior to injections. One of the rats receiving DPH of 0.005 mg/g consistently manifested seizure activity with 40°C (± 0.3 SD) as against its pre-drug threshold of 41.5°C (± 0.1 SD). Further, its latency had reduced from 3.5 min (± 0.1 SD) to 2.5 min (± 0.2 SD) and seizure duration increased from pre drug value of 45s to 100s after DPH. Out of 6 rats, three that received higher dose of DPH (0.04 mg/g) developed ataxia within 3 days following the administration of the drug.

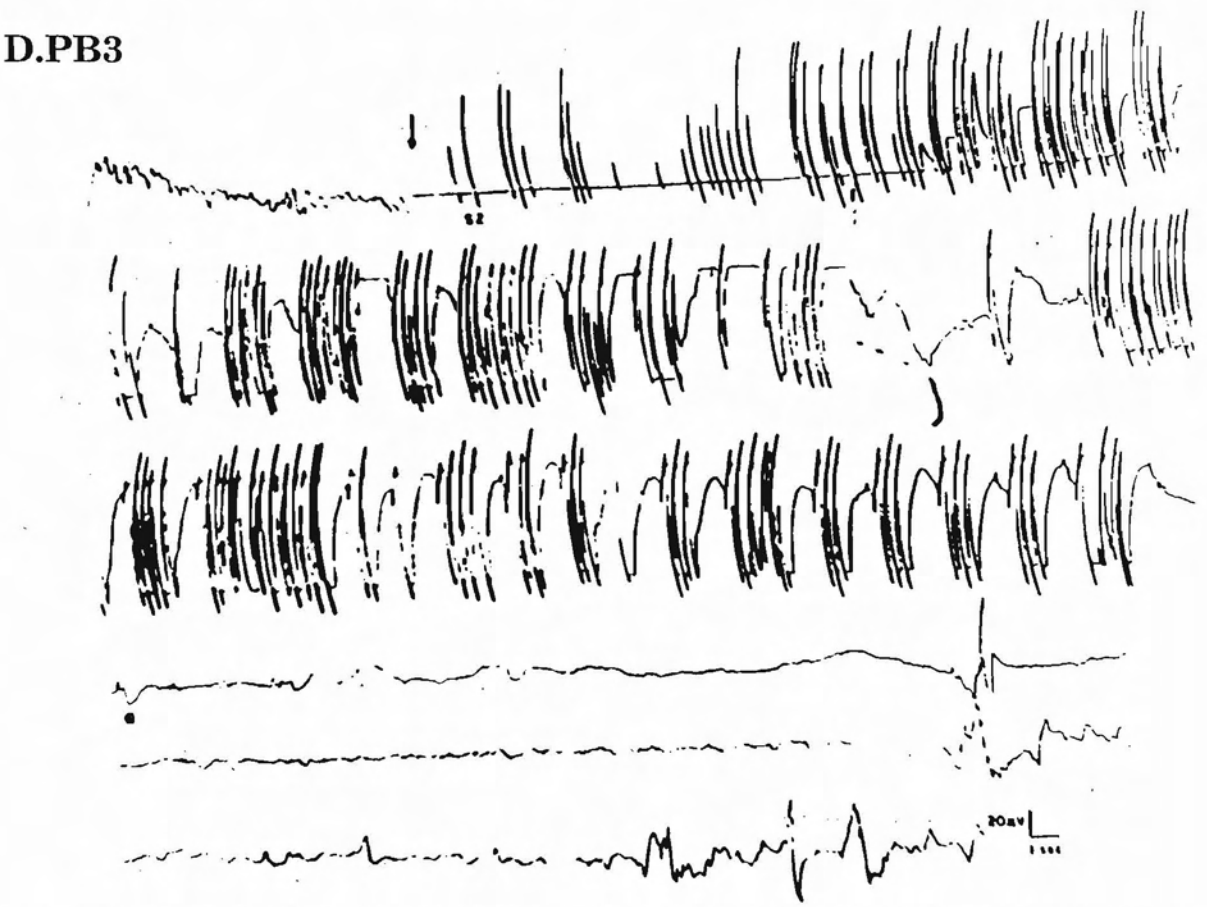

Fig. 2 : Pre-drug phase – EEG recorded through depth electrode from hippocampus of a rat showing the resting, ictal and post–ictal phases of generalised seizure discharge obtained on hot water stimulation on the first day of testing. ('↓' indicates point of commencement of seizure and termination of hot water stimulation).

D.PB3

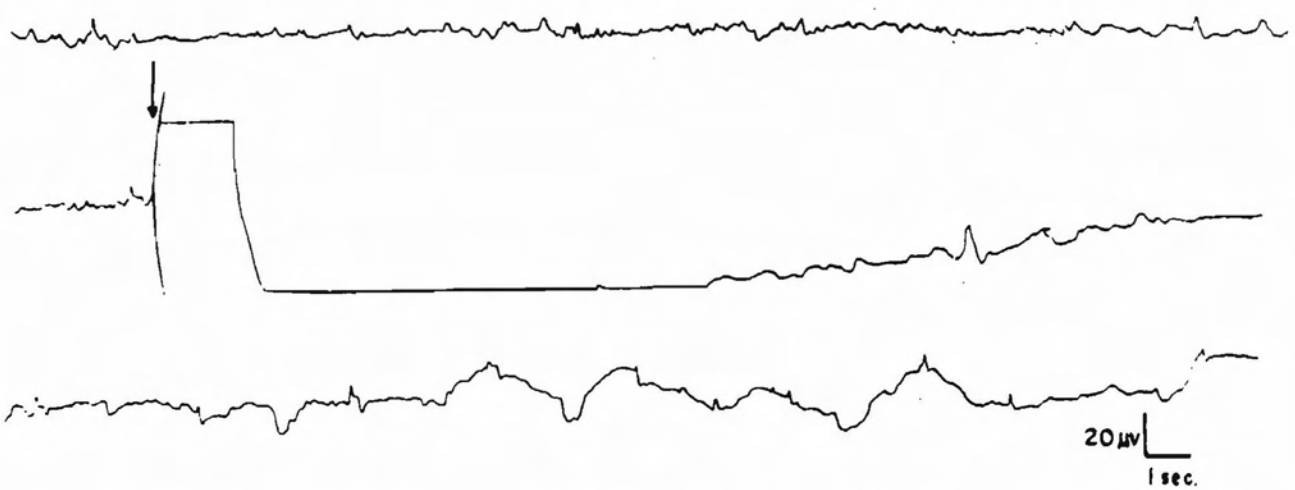

Fig. 3 : Post drug phase – EEG recorded following 10 min continuous hot water stimulation on the third day of phenobarbitone administration, 16 h after the last injection. There was no seizure during the stimulation. ('↓' indicates point of commencement of seizure and termination of hot water stimulation).

Following the wash out period of 10 days, and drug interchange, the animals when administered Pb instead of DPH demonstrated complete cessation of the clinical seizures and disappearance of after discharges in the EEG. On the other hand, in the rats in whom DPH was administered in the place of Pb, the seizures reappeared, at the threshold temperature of 41°C (± 0.1 SD), latency of 3.1 min (± 0.18 SD) for an average duration of 85 sec (± 10 SD).

The group of rats receiving Nif, saline and saline-PEG had seizures and after discharges throughout the period of daily stimulation, with no significant change in threshold temperature, the latency, or the clonic pattern of seizure following hot water stimulation.

None of the rats died during the whole experiment.

DISCUSSION

In this study, we have used our recently developed model of hyperthermic seizures (14) to evaluate the effect of antiepileptic drugs - such as Pb, DPH and calcium channel blocker - Nifedipine.

In our study, we have observed that the seizure induced by hot water stimulation over the head of the rat was generalized clonic seizure involving the extremities and the trunk and there was no tonic phase. When DPH was administered intraperitoneally there was no effect on either behavioural pattern of the seizure or after discharges in any of the rats. This could be explained by the fact that the antiepileptic property of DPH in animals is on tonic phase of a seizure only and further DPH may even prolong and exaggerate clonic phase (20). Olson et al (18), while reporting the effects of anticonvulsants on infrared hyperthermia - induced seizure in the rat have noted that DPH lowers the threshold for this type of seizure. Diphenylhydantoin is also reported to be ineffective in controlling febrile convulsions in young children (21). It is known to produce cerebeller ataxia in toxic doses in the humans (20). Similarly, observation was made in some of our rats. Nifedipine, a member of

dihydropyridine group of calcium channel blockers, was found useful in, lowering hippocampal seizure in animals (16). In view of this, it was tried in the hot water induced seizure in this model. Nifedipine failed to elicit any response in these rats similar to the observation made in maximal electroshock seizure (16, 22). This could suggest that calcium channel antagonist may have antiepileptic activity only on the partial seizure. Phenobarbitone when administered intraperitoneally prior to the hot water stimulation had completely abrogated clinical and electrical seizure discharges thereby suggesting the usefulness of phenobarbitone as antiepileptic drug of choice in this form of reflex epilepsy.

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