EFFECT OF DIPHENYLHYDANTOIN AND LITHIUM SEPARATELY AND IN COMBINATION ON ELECTROSHOCK-INDUCED SEIZURES IN MICE

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Summary: Maximal electroshock seizure (MES) was observed in mice at ½, 1, 1½ 2, 3, and 24 hr after the administration of diphenylhydantoin (DPH) alone and in combination with lithium. The duration of the entire seizure was found to be a reliable criterion of the anticonvulsant efficacy of DPH as evidenced by the dose-related reduction in MES duration - the maximum effect being at 1 hr.

Neither acute nor chronic treatment with lithium alone, affected either the duration or the pattern of the MES. Nine day pre-treatment with lithium 0.54 *mEq/kg* significantly enhanced the reduction of MES duration produced by 25 and 75 *mg/kg* DPH. Thus lithium seemed to potentiate the anticonvulsant effect of DPH. The mechanism may be a synergistic action with DPH to further reduce brain intracellular sodium or to raise brain 5-hydroxytryptamine thus protecting animals against electro shock-induced seizures.

Key words: diphenylhydantoin lithium maximal electroshock seizure mice

INTRODUCTION

Salts of lithium are now extensively used to treat manic depressive states. The mode of action of the lithium ion in affective illness remains obscure, most theories relating to interference with electrolyte metabolism. Lithium competes with sodium in sodium transport across membranes (2). In tissues *in vitro*, lithium partly replaces intracellular sodium (11).

Seizure vulnerability is inversely related to the ratio of brain extracellular/intracellular sodium (8). Diphenylhydantoin (DPH) exerts its anticonvulsant effect by increasing active transport of sodium ions out of the cell and by concomitantly preventing the rise in intracellular sodium caused by maximal electroshock seizures (MES) (15). Therefore, combined treatment with lithium and DPH may result in enhanced anticonvulsant activity. In this study, the effects of lithium and DPH separately and in combination on the MES in mice are reported.

MATERIALS AND METHODS

Mice were used for this study because, unlike rats, they invariably exhibit the full pattern of the maximal seizure when shocked, and also exhibit less cumulative postictal refractoriness than rats (13).

Each group consisted of six male mice weighing 25-30 *g* each. MES was induced by delivering an alternating current of 48 mA for 0.1 sec through ear clips. The duration of the

different phases of the seizure was observed before drug treatment and at intervals of ½, 1, 1½, 2, 3, and 24 hr after DPH. The effect of lithium on the alteration in MES produced by two doses of DPH 25 and 75 *mg/kg* (ip) was studied by treating groups with lithium 1.62 *mEq/kg* 1 hr after DPH, lithium 2.5 *mEq/kg* 2 days before DPH, and lithium 0.54 *mEq/kg* 9 days before DPH. Where mice were pre-treated with lithium DPH was given 24 hr. after the last dose of lithium. MES pattern was also studied after lithium treatment and prior to administration of DPH. Doses of DPH and lithium for the study were chosen after preliminary testing of the drugs in varying doses given singly and over a prolonged period of time. Where DPH 75 *mg/kg* was used the mice were subjected to standard neuropharmaco'ogical screening procedures at 1½ and 3 hr after DPH.

Diphenylhydantoin sodium (Parke-Davis) was suspended in 2% tragacanth or dissolved in distilled water to which 1 or 2 drops of 1N sodium hydroxide were added. The drug was injected intraperitoneally in a fixed volume of 0.25 *ml*.

Lithium carbonate (E. Merck) was suspended in distilled water and converted to lithium chloride by adding 1N hydrochloric acid until the pH was neutral. The drug was given orally between 10 and 11 a.m. once daily, in the volume 10 *ml/kg*.

RESULTS

In untreated mice, the average durations of the phases of the MES were as follows: (i) tonic flexion lasting 2.3±0.1 sec. (ii) hind limb extension (HLE) lasting 13.0±0.4 sec; (iii) clonic movements lasting 10.0±0.1 sec. The total seizure duration was 56±3.9 sec All the mice developed the full pattern of the MES, and repeated shocking did not affect the seizure pattern. In preliminary studies the duration of the MES was found to vary inversely with the dose of DPH (Fig. 1). Maximum reduction in MES duration was noted 1 hr after all doses of DPH. With 5 and 25 *mg/kg* of DPH, the MES duration began to approach control values from 2 to 24 hr. remaining significantly lower with the latter dose ($P<.01$) even at 24 hr. With higher doses of DPH (75 and 125 *mg/kg*) the MES duration remained significantly low ($P<.01$)

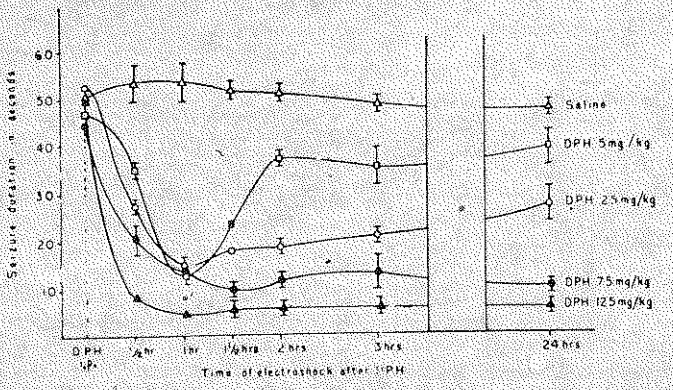

Fig. 1: Effect of DPH on maximal electroshock seizure duration in mice.

TABLE I: Effect of DPH and lithium on electroshock-induced seizure duration in mice.

Treatment	*Before Drugs*	*After Lithium/ saline*	*Time of living electroshock* — *Time after DPH* 1/2 *hr*	1 *hr*	1.1/2 *hr*	2 *hr*	3 *hr*	24 *hr*
1 hr after DPH+								
Saline	57±4.5	—	26±4.6	17±3.4	19±6.0	20±5.0	13±3.0	32±1.2
Lithium 1.62 *mEq/kg*	57±6.0	—	33±5.7	16±5.7	12±3.8	23±5.0	11±6.3	34±6.4
2 days before DPH+								
Saline	56±5.0	54±2.0	23±4.8	20±5.4	20±6.0	19±3.7	18±2.6	34±0.1
Lithium 2.5 *mEq/kg*	54±3.3	54±3.3	22±4.8	20±5.1	16±2.6	19±4.9	13±4.8	36±1.0
9 days before DPH+								
Saline	56±5.0	55±3.0	32±5.0	15±4.0	16±1.7	14±1.7	11±1.0	36±4.0
Lithium 0.54 *mEq/kg*	53±1.5	51±2.0	28±1.1	15±3.2	15±3.2	8±1.1*	5±0.12**	25±3.3
1 hr after DPH++								
Saline	58±3.2	—	15±3.5	13±1.0	13±1.5	9±1.8	10±2.8	10±1.0
Lithium 1.62 *mEq/kg*	56±3.0	—	16±5.0	12±3.8	10±3.2	14±3.0	8±0.1	10±1.0
2 days before DPH++								
saline	62±3.9	53±5.0	21±5.6	14±1.0	10±0.4	8±1.4	15±4.0	10±2.0
Lithium 2.5 *mEq/kg*	52±3.0	48±2.5	21±3.1	13±0.7	12±0.9	13±0.2	15±0.5	10±1.0
9 days before DPH++								
Saline	60±5.0	61±4.0	21±1.5	12±1.5	13±2.5	14±3.2	10±2.5	11±1.2
Lithium 0.54 *mEq/kg*	59±3.0	58±2.5	19±3.0	15±0.1	10±0.7	8±1.0*	7±1.3	4±1.0**

Each value represents the seizure duration in sec ±S.E.

+DPH 25 *mg/kg* ++DPH 75 *mg/kg* *P<0.05 **P<0.01

from 2 to 24 hr. All doses of DPH prevented the appearance of HLE from 1 hr onwards. At 24 hr HLE reappeared in 100% of the mice treated with DPH t5 *mg/kg* and in 50% of the mice treated with DPH 25 *mg/kg* but in none of the mice treated with higher doses of DPH. There was some degree of ataxia in all animals treated with high doses of DPH (75 and 125 *mg/kg*) and the startle response was delayed in 20% of the mice..

Lithium 0.54 *mEq/kg* given for 10 days was non-toxic. There was little or no weight loss. A remarkable reduction of fighting behaviour was seen in these mice. Lithium 10.8 *mEq/kg* administered for 10 days was observed to be extremely toxic. Mice showed progressive emaciation loss of appetite, lowering of body temperature, and profuse salivation and urination accompanied by dehydration and extreme thirst. On administering electroshock, clonic movements were extremely weak and some mice died of respiratory arrest. Hence these values were discarded.

A perusal of Table I shows that lithium given alone in doses used in this study did not alter the duration of the MES. Neither did it alter the effect of either doses of DPH when given 1 hr after DPH or for 2 days prior to DPH. But in the dose 0.54 *mEq/kg* for 9 days prior to either doses of DPH it significantly ($P<0.01$) enhanced the effect of DPH in reducing the MES duration at 2 and 3 hr. This potentiation was not seen at 24 hr with the lower dose of DPH though it continued to be so with the higher dose.

DISCUSSION

The duration of the entire seizure was found to be a reliable criterion of the anticonvulsant efficacy of a wide range of doses of DPH (Fig. 1). While the effect of smaller doses of DPH began to decrease after 2 hr the effect of higher doses lasted for 24 hr. This is because the biological half life of DPH becomes longer as the dose of the drug increases and the change in the disappearance of the drug is exponential and closely follows first order kinetics throughout (6). While in our study a dose of 10.8 *mEq/kg* administered for 10 days proved to be highly toxic the same dose given to pregnant female mice resulted in serum lithium levels which correspond to therapeutically effective lithium levels in man (14). The report of Schou *et al.* (12) that pregnancy causes a fall in serum lithium levels may probably explain the altered susceptibility of our mice. The MES duration approaches control values from 2 hr after DPH 25 *mg/kg* when brain levels of the drug are presumably falling, thereby reducing the intensity of its anticonvulsant action. Lithium which had no effect on the maximal action of this dose of DPH seems to potentiate its effect when the effect of DPH is wearing off. With DPH 75 *mg/kg* the anticonvulsant action seems to be prolonged upto 24 hr and lithium has further enhanced its action from 2 to 24 hr though the brain levels of DPH still seems to be effective. Probably with the higher doses the delicate biochemical mechanisms in the brain is more complexly affected.

These potentiating effects of lithium may be due to inhibition of transport of DPH out of the brain, or by prolongation of the effect of the drug on cellular amines and electrolytes. DPH stimulates membrane sodium-potassium adenosine triphosphatase (Na-K-ATP-ase) (5),

and reduction of intracellular sodium is probably the mechanism of its anticonvulsant action (15). Lithium modifies brain ATP-ase activity and consequently lowers whole brain sodium in mice (7). Also it partly replaces intracellular sodium (11). Intracellular ionic balance in turn is regulated by a biochemical system in which 5-hydroxytryptamine (5-HT) is important (3). Raising brain 5-HT levels was observed to abolish the HLE component of the MES (10). While DPH in higher doses of 100 *mg/kg* on chronic administration, raised brain 5-HT levels in rats (1) lower doses of 25 and 75 *mg/kg* did not alter brain 5-HT though they effectively blocked the HLE (10). Lithium increases the rate of 5-HT synthesis and turnover (9). DPH treatment superimposed on a state of altered 5-HT balance may affect the brain 5-HT levels.

The initial anticonvulsant effect of DPH is probably due to reduction of intracellular sodium and the enhancing effect of lithium due to alteration of brain 5-HT levels, since brain 5-HT is apparently involved in electroshock-induced seizures. Lithium action on 5-HT could be secondary to ionic effect since sodium and potassium have been shown to be essential ingredients in the transport of 5-HT (4). Lithium has such a wide range of actions and it is difficult to demarcate electrolyte and amine changes. Estimation of whole brain sodium potassium, and 5-HT at significant times after lithium and DPH which is now in progress may help to explain the complex interactions between these two drugs.

ACKNOWLEDGEMENTS

We are grateful to Dr. Ranita Aiman, Professor of Pharmacology, for valuable advice. This work was carried out on a Junior Research Fellowship granted by the Council of Scientific and Industrial Research.

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