EFFECT OF DIPHENYLHYDANTOIN AND LITHIUM ON WHOLE BRAIN SEROTONIN SODIUM AND POTASSIUM IN MICE

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Summary: The effect of lithium and Diphenylhydantoin separately and in combination on whole brain serotonin, sodium and potassium were studied in mice. 9 day treatment with lithium was found to significantly raise brain serotonin. Lithium enhanced the sodium lowering effect of diphenylhydantoin. Though neither drug affected brain potassium, combination of the two drugs significantly lowered brain potassium. The possible mechanisms of these effects are discussed.

Key words: Lithium Diphenylhydantoin Serotonin Sodium Potassium

INTRODUCTION

In a previous study (10) in mice 9 day pretreatment with lithium 0.54 *mEq/kg* was found to enhance the anticonvulsant effect of diphenylhydantoin (DPH) 25 and 75 *mg/kg* at 2 and 3 hr after DPH. DPH action is influenced by the state of the brain Na-K pump, and brain electrolyte distribution (11). The intracellular ionic balance is regulated by a biochemical system in which serotonin is important (2). Lithium increases serotonin turnover rate in the brain (8) and affects ATPase activity (5). Whole brain electrolytes and serotonin were therefore estimated at relevant periods after DPH on mice pretreated with lithium, with a view to elucidate the mechanism of potentiation of DPH activity by lithium.

MATERIAL AND METHODS

Laboratory bred albino mice weighing between 25-30 g were used in groups of twelve. Lithium chloride was prepared and administered orally as described earlier (10) in the dose 0.54 *mEq/kg* for 9 days.

Control groups received saline (1 *ml*/100 *g* B.W.). DPH in doses of 25 and 75 *mg/kg* was given i.p. 24 hrs after the last dose of lithium. The animals were sacrificed by cervical dislocation at 3 and 24 hrs after DPH. The brains were removed rapidly, blotted well and weighed. Estimations of serotonin (5-HT), Sodium (Na) and potassium (K) were done on pooled homogenates of 3 brains.

Estimation of 5-HT: Brain serotonin was extracted and estimated according to the method of Bagdanski *et al* (1). Fluorescence was read in a Beckman Ratio Fluorometer.

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Estimation of Na and K: Tissues were digested in concentrated nitric acid. The extracts were diluted 1: 250 with glass distilled water and the Na and K estimated in a Lange Flame Photometer model 6.A.

RESULTS

Brain 5 HT: 9 day treatment with lithium 0.54 *mEq/kg* significantly raised brain 5-HT ($P<.01$). However, when DPH was given after lithium treatment this effect was reversed, the 25 *mg/kg* dose bringing it back to normal and the 75 *mg/kg* dose markedly lowering brain 5-HT levels three hours after its administration ($P<.01$). These effects did not last for 24 hrs. DPH by itself in either dose employed did not alter brain 5-HT levels.

Brain Na: Lithium by itself did not alter brain Na levels, whereas DPH in either dose used, significantly lowered brain sodium, 3 hr after administration ($P<.01$), the values returned to control levels in 24 hrs. Pre-treatment with lithium enhanced this Na lowering effect of DPH ($P<.05$).

Brain K: Neither lithium nor DPH by itself altered brain K, whereas in combination K levels were significantly reduced, both at 3 and 24 hrs after administration, (Table I).

TABLE I: Effect of 9 day pre-treatment with lithium 0.54 *mEq/kg* on whole brain 5-HT, Na and K at 3 and 24 hr after DPH.

Pretreatment 9 *days*	*Treatment**	*5-HT* $\mu g/gm \pm SE$		*Na* $mEq/kg \pm SE$		*K* $mEq/kg \pm SE$	
		3 *hr*	24 *hr*	3 *hr*	24 *hr*	3 *hr*	24 *hr*
Saline	Saline	0.81±0.04	0.83±0.02	58.72±0.35	63.87±0.32	125.15±4.8	130.95±4.80
Lithium 0.54 *mEq/kg*	Saline	0.93±0.03**	0.96±0.05**	60.31±0.21	62.71±0.59	122.10±4.3	138.08±2.94
Saline	DPH 25 *mg/kg*	0.75±0.03	0.78±0.04	41.96±1.40**	61.05±0.12	119.6±3.7	121.50±1.00
Lithium 0.54 *mEq/kg*	DPH 25 *mg/kg*	0.67±0.05	0.72±0.02	30.35±1.06++	66.67±0.84	66.92±3.60*	72.94±4.47+
Saline	DPH 75 *mg/kg*	0.71±0.02	0.79±0.08	40.87±0.59**	76.20±0.86	114.50±6.50	126.77±1.39
Lithium 0.54 *mEq/kg*	DPH 75 *mg/kg*	0.39±0.03+	0.85±0.04	31.60±0.32++	75.47±0.71	70.79±1.80+	72.90±4.28+

* Treatment given i.p 24 hours after the last dose of pre-treatment drug.
** $P<.01$ as compared to control.
\+ $P<.01$ as compared to effect of DPH alone.
++ $P<.05$ as compared to effect of DPH alone.

DISCUSSION

It has been stated that lithium treatment does not alter brain monoamines (9). In our experiments we have noted that chronic administration of lithium 0.54 *mEq/kg* for 9 days does increase whole brain serotonin levels in mice ($P<.01$) and these are partially consistent with the report of other workers, who have reported stimulation of serotonin synthesis after acute administration of lithium (8). A recent report has indicated that lithium carbonate treatment was accompanied by a significant increase in the uptake of serotonin by platelets (7). The reversal of lithium induced increase in 5-HT to normality produced by the lower dose of DPH, and the marked reduction produced by the higher dose of DPH may imply that DPH and lithium have a similar site of action. Both lithium and DPH are known to affect ionic movements and the Na K ATPase activity (5, 11). However, it is not clear whether the state of the Na-K-ATPase pump or the specific ionic conditions is the more critical determinant for lithium induced serotonin stimulation and its reversal by DPH.

Though lithium by itself did not affect whole brain Na it enhanced the reduction of brain Na produced by DPH. Lithium is known to replace Na from intracellular sites. The anticonvulsant property of DPH is generally considered to be due to stimulation of the brain Na-K pump (4). DPH after crossing the blood brain barrier and building up an effective membrane concentration increases Na turnover while it prevents the rise in intracellular Na caused by maximal electroshock. Under normal conditions efflux of Na is accompanied by an influx of K. It has been stated that intracellularly accumulated lithium is extruded only 1/10-1/25 as fast as sodium. Furthermore extrusion of lithium is not accompanied by an obligatory influx of K as occurs when Na is displaced. The net result is intracellular accumulation of lithium resulting in an equal displacement of Na and K (6). These effects have been observed after acute administration of lithium. In our experiments where lithium was given for a prolonged period of time neither Na nor K concentration of brain was affected.

DPH did not affect K concentration and this is in accordance with earlier reports (3). The combined effect of DPH and lithium was a marked reduction in whole brain K. The acute administration of DPH after lithium probably upsets the state of the Na-K-ATPase pump which in turn affects the ionic movements probably leading to a reduction in whole brain K. Whole brain analysis prevents adequate assessment of the intracellular/extracellular ratio of ions, the determination of which is necessary before any conclusion can be drawn about the effects of these drugs.

ACKNOWLEDGEMENTS

We are grateful to Prof. Ranita Aiman, Professor of Pharmacology for valuable advice, and to Dr. Sampath, National Dairy Research Institute for permission to use the Flamephotometer. Part of this work was carried out on a Junior Research Fellowship granted by the the Council of Scientific and Industrial Research.

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