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THE POTENTIATING EFFECT OF MELATONIN ON PHENOBARBITONE IN EXPERIMENTALLY INDUCED SEIZURES IN RATS

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ABSTRACT

TITLE : The potentiating effect of melatonin on phenobarbitone in experimentally induced seizures in rats.

OBJECTIVES : To evaluate the effect of melatonin in potentiating the antiepileptic activity of phenobarbitone(PB) in maximal electroshock (MES)-induced and pentylenetetrazole (PTZ)-induced convulsions in rats.

METHODOLOGY : 72 ,adult, male, Albino rats, were utilised for this study. The effects of melatonin with the antiepileptic phenobarbitone, in different proportions were studied in MES and PTZ induced convulsions in rats. In MES induced rats, abolition of hindlimb tonic extension was taken as the measure of efficacy. In PTZ induced rats, suppression of clonic spasms, was taken as the measure of efficacy. Percentage protection offered was calculated and analysed statistically.

RESULTS : The combination of melatonin and phenobarbitone at a proportion of melatonin 25% + phenobarbitone 75%, offered significant protection in both MES induced and PTZ induced seizures in rats ($p<0.05$).

CONCLUSION : This study suggests that though melatonin itself has got minimal anti-epileptic activity, it significantly potentiates the action of phenobarbitone, which is an anti-epileptic drug with minimal but untolerable adverse effects, that affects the patients day to day life .Thus melatonin could be a potential adjunct to currently available broad spectrum anti-epileptic drug, like phenobarbitone, making them to achieve the therapeutic effect even at lower concentrations, hence limiting their dose related toxicities, which needs further clinical studies for evaluation.

INTRODUCTION :

Epilepsy is the second most common neurological disorder in India[1,2], with an incidence of approximately 0.3 - 0.5% in different populations throughout the world and a prevalence of 5-10 persons per 1000[3]. The term epilepsy refers to the disorder of brain function manifested as periodic and unpredictable occurrences of seizures. The characteristic event in epilepsy, the seizure, is a paradoxical event due to abnormal excessive, hypersynchronous discharges from an aggregate of central nervous system neurons[5].The primary goal of antiepileptic therapy is to achieve complete freedom from seizures without any adverse effect, to reduce morbidity and to improve the quality of life. Though numerous Antiepileptic drugs (AEDs) are currently targeting epilepsy, almost all drugs are with some unwanted effects ranging from minimal CNS impairment till death. And approximately 30% of the people with epilepsy have seizures that do not respond satisfactorily to the conventional AEDs[5].These limitations highlights the need for exploring the drugs that could potentiate the action of these conventional AEDs, making the therapy for epilepsy more effective. It has been postulated that CSF melatonin has been proposed to be as a natural anti convulsant[6]. Moreover, melatonin has been shown to be extremely safe in humans, even at very high doses [7].

Phenobarbitone(PB) was the first effective organic antiseizure agent, that has relatively low toxicity, is inexpensive and is still one of the more effective, broad spectrum antiepileptic drug. But because of its sedative effects and behavioural disturbances in children its use as a primary antiepileptic agent have been reduced[8].

Hence the present study was undertaken to determine the effect of melatonin on phenobarbitone in various proportions (melatonin alone 100%, PB 75% with melatonin 25%, PB50% & melatonin 50%, PB 25% & melatonin & 75%), in maximal electro shock induced (MES) induced and Pentylenetetrazole (PTZ) induced convulsions in rats.

MATERIALS AND METHODS

This randomised, controlled, animal experimental study was conducted in the Institute of Pharmacology, Central animal house, Madurai medical college, Madurai, after obtaining clearance from Instituitional animal Ethical committee.

ANIMALS : 72, Inbred, adult, male albino rats weighing about 200 – 220 gms were used for the study. All the animals were maintained under 12:12 hour light : dark cycles and were fed with standard laboratory chow and water ad libitum. The experiments were carried around the same time each day.

METHODOLOGY : The animals were divided into two groups, each group containing 36 animals. One group was utilized for MES method and another group for PTZ method.

MES method :

Here the 36 animals were again divided into 6 equal groups (control, standard, test 1, test 2, test 3, & test 4). One hour prior to the experiment, all the animals were fed orally as shown in Table I.

DRUGS GIVEN TO THE ANIMALS : (TABLE -I)

CATEGORY	TREATMENT
CONTROL	1 ml distilled water orally
STANDARD	Phenobarbitone 30mg/kg (100%) orally
TEST – 1	Melatonin 50 mg/kg (100%) orally
TEST – 2	Phenobarbitone 22.5mg/kg (75%) + Melatonin 12.5 mg/kg (25%) orally
TEST – 3	Phenobarbitone 15 mg/kg (50%) + Melatonin 25 mg/kg (50%) orally
TEST – 4	Phenobarbitone 7.5 mg/kg (25%) + Melatonin 37.5 mg/kg (75%) orally

(Concentration of drugs were so adjusted, that all the groups received the same volume of preparation throughout the study)

Then, Convulsions were induced by electrical stimulation through ear electrodes, previously moistened with saline, with an electroconvulsiometer, which delivered a constant current at a rate of 150 mA at 60 Hz, for a duration of 0.2 seconds. Suppresion of tonic hindlimb extension was taken as a measure of anticonvulsant activity[9].

PTZ method :

Here the remaining 36 animals were again divided into 6 equal groups (control, standard, test 1, test 2, test 3, & test 4). One hour prior to the experiment, all the animals were fed orally as shown in Table I.

The chemical pentylenetetrazole was dissolved in normal saline[10] and was administered at a dose of 70 mg /kg intraperitoneally[11]. All the animals were observed for a period of one hour duration. Suppression of clonic spasms, was taken as the measure of anticonvulsant activity[9].

Statistical analysis : Statistical analysis was carried out using ANOVA method. p value < 0.05 was considered as statistically significant.

RESULTS :

The following results were obtained.

MES method : The animals went through the following phases like latent phase, tonic flexion (Fig : 1), tonic extension (Fig : 2), clonus (Fig : 3) and post ictal depression (Fig : 4). Results are shown in Table II.

Phase of tonic flexion (Fig : 1)

Phase of tonic extension(Fig : 2)

Clonus (Fig : 3)

Post-ictal depression (Fig : 4)

PHASE OF TONIC EXTENSION (in seconds) (TABLE –II)

GROUP	DRUGS	EXTENSION (SECONDS)	No. of rats protected (n= 6) % protection
CONTROL	DISTILLED WATER	15.9	0 (0)
STANDARD	PHENOBARBITONE	0.2	6 (100)
TEST – 1	MELATONIN	2.2	3 (50)
TEST -2	PB + MEL. 75% 25%	1.2	6 (100)
TEST -3	PB + MEL. 50% 50%	2.24	4 (66)
TEST -4	PB + MEL 25% 75%	3.4	4 (66)

All the animals in the test group 2, which received a combination of phenobarbitone 75% & melatonin 25% were protected (100%), whereas further reduction in the dose of phenobarbitone offered only 66% protection.

PTZ method :

All the animals went through a sequence of excitement, myoclonic jerks & clonic seizures. Results are shown in Table III.

PENTYLENETETRAZOLE METHOD RESULTS (TABLE – III)

GROUPS	DRUGS	CLONIC CONVULSIONS (SECONDS)	**No. of rats protected (n= 6) (% protection)**
Control	DISTILLED WATER	46	0 (0)
Standard	PB	0.7	6 (100)
Test -1	MELATONIN	2	3 (50)
Test -2	PB + MEL. 75% 25%	0.8	6 (100)
Test -3	PB + MEL. 50% 50%	1	3 (50)
Test -4	PB + MEL. 25% 75%	1.6	3 (50)

All the animals in test group 2, which received a combination of phenobarbitone 75% & melatonin 25 % showed 100 % protection, whereas further decrease in the dose of PB offered only 50% protection.

STATISTICAL ANALYSIS:

The results were analysed statistically using ANOVA method. (Table IV & V). The control group was compared with test group I which received only melatonin and all the other groups were compared with the standard drug phenobarbitone.

MAXIMAL ELECTRO SHOCK METHOD : (Table IV)

GROUPS	CONTROL vs TEST 1	STANDARD vs TEST 1	STANDARD vs TEST 2	STANDARD vs TEST 3	STANDARD vs TEST 4
P – value	< 0.01	> 0.05	< 0.05	> 0.05	> 0.05

P – value < 0.05 (significant)

PENTYLENETETRAZOL METHOD : (Table V)

GROUPS	CONTROL vs TEST 1	STANDARD vs TEST 1	STANDARD vs TEST 2	STANDARD vs TEST 3	STANDARD vs TEST 4
P - value	< 0.01	> 0.05	< 0.05	> 0.05	> 0.05

P – value < 0.05 (significant)

DISCUSSION

Numerous factors are responsible for seizure initiation, but seizures get arrested spontaneously and abruptly and the brain remains seizure free for sometime thereafter, indicating the involvement of some endogenous anticonvulsant substances[12] . It has been postulated that CSF melatonin has been proposed to be as a natural anti convulsant[5]. Melatonin, (N-Acetyl 5 – methoxy tryptamine) is the principal hormone produced and secreted by the pineal gland. It was identified by an American dermatologist, Aaron lerner & colleagues [13] and is a highly conserved molecule present in vertebrates[14]. Pinealectomy, which results in absence of melatonin secretion, has been shown to produce seizures in certain animals within a few hours[15]. Electrophysiological studies have demonstrated that melatonin plays a physiological role in the inhibition of striatal NMDA receptor activity in rat brain[16]. Melatonin is known to depress brain excitability by regulating $Na^{+}K^{+}$ATPase[16] and GABA-BZD receptor complex activities[18]. Besides potentiating brain inhibitory neurotransmission, melatonin blocks glutamatergic dependent brain excitability, thus acting as an anti- excitotoxic compound [19].It has also been suggested that increases in the GABAergic neurotransmission at cerebral level by melatonin may account for some of its anticonvulsant effect[20]. Moreover it has been proposed that melatonin crosses the morphological barriers like blood brain barrier, intracellular and subcellular barriers[21] with ease. Melatonin is rapidly metabolized, chiefly in the liver, by hydroxylation and after

conjugation with sulfuric / glucuronic acid is excreted in the urine. The urinary excretion of 6-sulfatoxy melatonin closely parallels serum melatonin concentrations[22] .

From this study it was observed that melatonin itself has got antiepileptic property ($p<0.01$) in both MES and PTZ induced seizures and when combined with phenobarbitone it potentiated the effects of latter drug. In MES method, the group which received PB 75% + melatonin 25% (test group 2)have shown significant antiepileptic property ($p < 0.05$). In PTZ method also, the group which received PB 75% + melatonin 25%(test group2) have shown significant antiepileptic property ($p < 0.05$).

CONCLUSION

From this study it was observed that melatonin by itself has got anticonvulsant property and when given in different combinations, it significantly potentiated the effects of phenobarbitone. Phenobarbitone, a highly efficacious, inexpensive and one of the broad spectrum antiepileptic drug has comparatively minimal, but unacceptable sideeffects like sedation, ataxia and nystagmus for which it is no longer being used as a primary antiepileptic drug. These side effects are usually absent if the serum levels of phenobarbitone are maintained below 30 µg/ml during long term therapy[7]. If melatonin is combined with phenobarbitone, the dose of the latter drug could be reduced, so that the toxic effects could be prevented and phenobarbitone could be used safely as a first line antiepileptic drug , with least side effects, which needs further clinical studies to be evaluated.

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