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AUGMENTATIVE EFFECT OF PROSTAGLANDIN E_1 ON PENTOBARBITAL HYPNOSIS MEDIATED BY 5-HT IN CHICKS

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ABSTRACT : Prostaglandins (PG) are present in different tissues specially in brain tissues endowed with different central nervous system activities. Similarly, 5-hydroxytryptamine (5-HT) a biogenic amine with its presence in different central and peripheral tissues as neurotransmitter plays an important role in the regulation of physiological functions specially hypnosis, convulsions, analgesia in rats, mice, cats and chicks etc. Pentobarbitone (PB) induced sleep appear to be a serotonergic modulator activity in different animals. PGE1 potentiates the pentobarbitone hypnosis also mediated through serotonin. In the present study, PGE_1 induced sleeping time in chicks was evaluated. Drugs affecting 5-HT synthesis, metabolism and receptor activity modulate the potentiating response, while adrenergic receptor antagonists did not showed any response. This study suggest that PGE_1 potentiate PB induced sleep through serotonergic signaling pathway as PGE_1 increased 5-HT synthesis rate in chick brain.

Key words : Prostaglandins, Serotonin, Chick hypnosis.

INTRODUCTION:

Prostaglandins(PG) are found diffusely throughout the central nervous system, which is endowed with complete systems capable of synthesizing and metabolizing distinct PG types. The role of PGs in brain functions is not yet fully explored. PGs have been reported to potentiate hypnosis, analgesis, anticonvulsant effect, gastric secretion inhibitory activity (Sanyal *et al.* 1977 Bhattacharya *et al.* 1975a, 1975b, 1976, 1978; Debnath *et al.* 1978; Sanyal and Debnath 1974). PGE_1 increase brain and stomach 5-HT synthesis rate in rats (Debnath *et al.* 1978). Those activities are mediated through serotonin modulation. Serotonergic signaling appears in general and modulation of various cognitive and behavioral functions such as sleep, mood, pain, addiction,

locomotion, sexual activity, depression, anxiety, alcohol abuse, aggression , inhibition of gastric secretion and diarrhea Learning. Pentobarbitone (PB) hypnosis is reported to be 5-HT mediated response (Chanda *et al.* 1990) in chicks. PB potentiating activity is also modulated by the drugs affecting 5-HT synthesis, metabolism and receptor activity. So far, most of work of the serotonin-sleep-waking problem has been done on the 5-HT_{1A} and 5-HT_2 receptors. Based on the findings of our previous work, the present study was undertaken to investigate whether the PGE_1 potentiate PB induced sleeping time in chicks through neuro-transmitter. (Nan *et al.* 2008).

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MATERIALS AND METHODS :

Broiler chicks (250-300 g) were used in the experiment which were purchased from recognized Government breeding farm. They were housed 7 days in laboratory condition for acclimatization before experiment. They were fed Hind Lever feed and water ad lib. The experiments were conducted between 11 -15 hours. PB (40 mg/kg, i.p). was administered. Loss of righting reflex and regain was recorded as the sleeping time adopting the method reported earlier (Chanda *et al.* 1990). Different drugs affecting 5-HT synthesis and metabolism, PGE synthesis inhibitor (diclofenac) and adrenergic alpha and beta receptor antagonists were administered prior to PB administration.

All the drugs were procured either as gift or purchased, PGE_1 (Upjohn, USA) , methysergide (Sandox, Basel), pCPA (Sigma), Propranol (ICI, UK), Phentolamine (Sigma), 5-HTP (sigma), Pargyline (Abbot, USA), Nembutol (PB, Abbot, India). Statistics: Statistical analysis was done by student "t" test and ANOVA, where applicable.

RESULTS AND DISCUSSION :

PGE_1 in the dose (04 mg/kg s.c) potentiated PB (40 mg/kg) induced sleeping time. PB induced sleeping time and PGE_1 potentiating activity were annulled by methysergide, pCPA and diclofenac while propranolol and phentolamine did not show any appreciable modulatory effect.(Table 1)

Pargyline (75 mg/kg) increased time dependently brain 5-HT. On the other hand, 5-HT synthesis rate was increased from 0.47 to 0.64 µg/g/h brain tissue. of PGE_1 pretreated chicks following Pargyline administration. Similar increase was observed in intestine.(Table 2-4).

In our previous study, PGE_1significantly potentiated the hypnotic activity of pentobarbital in mice and rats. PCPA, and methysergide reduced the sleep latency and increased the sleep time and showed synergic effects with 5-HTP in reversing PCPA's insomnia in pentobarbital-induced sleep in rats and mice ,chicks (Bhattacharya *et al.* 1975, Chanda *et al.* 1990). PGE_1 induced potentiation of hexobarbitone induced sleep in rats , morphine analgesis , anticonvulsant activity was found to be a 5-HT mediated response.(Bhattacharya *et al.* 1975a, 1975b, 1976, 1978).

In albino rats and mice hexobarbitone sleep was inhibited by pCPA, methysegide. Further, 5-HTP and nialamide induced potentiating was also inhibited by pCPA and methysergide but not in mice (Bhattacharya *et al.* 1975). The hypothesis is that central 5-HT has a role in the process of sleep, is strongly supported by evidence that p-chlorophenylalanine, a selective 5-HT synthesis inhibitor, and reserpine regularly precipitate nearly total insomnia in cats, while 5-HTP restores sleep temporarily in pCPA pretreated rats. (Koelle *et al.* 1968, Jouvet 1969). Present investigation on the PGE_1 induced PB sleeping time was inhibited by pCPA, methysergide, diclofenac, while -5-HTP reversed the pCPA induced reduction, may suggest the similar response in chicks being a serotonergic mediated response in brain. It was further substantiated by the facts that PGE_1 increased 5-HT level in brain and intestine of chicks.

The role of 5-HT in pentobarbitone (PB) sleeping time, gross behaviour, electrical activity of the brain and serum 5-HT level was studied in Holtzman strain adult albino rats following treatment with *Moringa oleifera* (MO). MO (350mg/kg) caused inhibition of awareness, touch response, motor activity, righting reflex, and grip strength. It significantly increased the PB sleeping time, serum 5-HT level ($P<0.001$) and alpha-wave activity. These observations indicate that the aqueous extract of MO potentiated PB induced sleeping time and increased the alpha-wave activity through serotonergic pathway (Ray *et al.* 2004).

It has been reported that augmentative effect of tetrandrine on pentobarbital hypnosis in mice may be related to serotonergic system. The present result further showed that augmentative effect of tetrandrine

Table 1 : Prostaglendin E_1 (0.4 mg/kg) potentiation on pentobarbitone Sleeping time in Chicks .

No	Treatment/(Dose) mg/kg	Number	Sleeping Time (Minutes) Mean ± S.E
1	PB (40)	36	81.23 ± 1.19a
2	PGE1 (0.4) + PB	6	108.35 ± 2.79ab***
3	Methy (1.0) + PB	8	61.83 ± 1.68a**
4	Methy + PGE1 + PB	6	78.13 ± 1.54b**
5	PCPA (300x3) +PB	6	48.13 ± 1.16a***
6	PCPA + PGE1 + PB	5	53.89 ± 1.72
7	PCPA + 5-HTP(300) + PGE_1 + PB	5	91.31± 1.65
8	Diclofenac (10) + PB	8	49.51 ± 1.07a***
9	Diclofenac + PGE1 + PB	6	60.87 ± 0.88
10	Propanolol (1) + PB	8	78.31 ± 0.93a
11	Propranolol + PGE1 + PB	6	81.22 ± 2.16b
12	Phentolamine (1) + PB	8	80.13 ± 1.92a
13	Phentolamine + PGE1 + PB	6	79.32± 2.07b

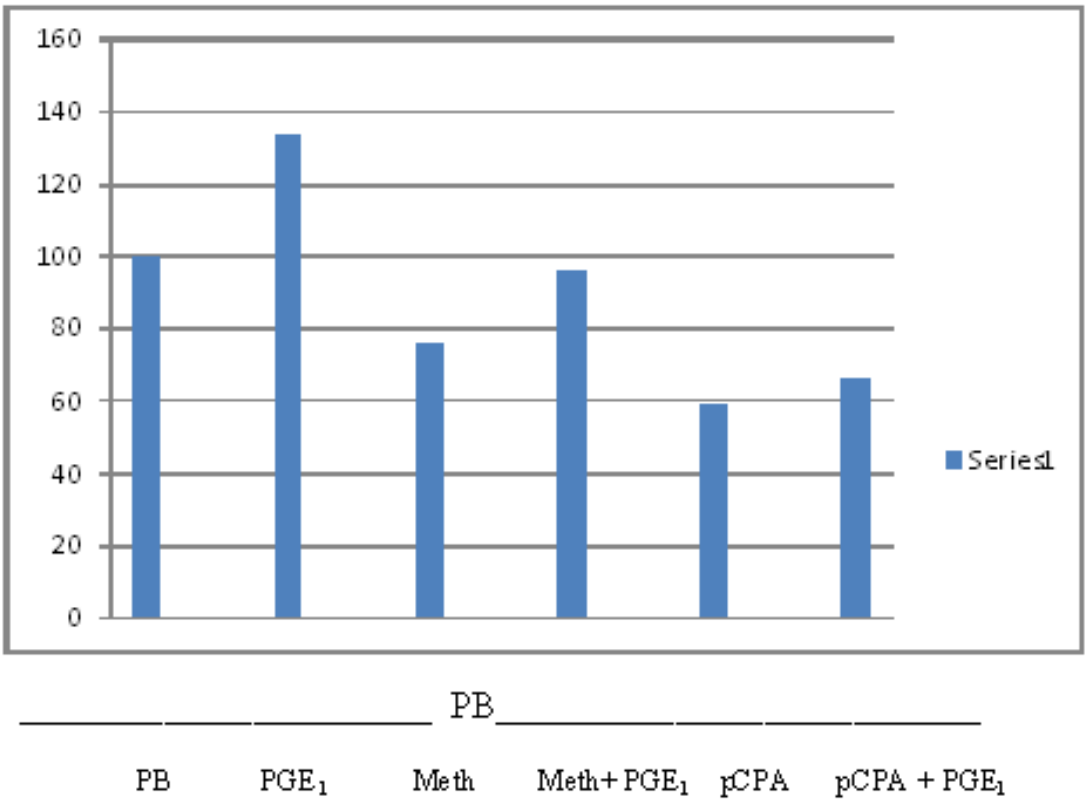
Results are Mean± S.E , Statistical significant **, *** P <0.01 and <0.001 respectively

a –incomparison with PB ; b – incomparison with PGE_1+ PB

PB – Pentobarbitone ; Methy – Methysergide ; pCPA – Para chlorophynylalanine;
Diclofena – Diclofenac Sodium; Prop – Propranolol; Phento – Phentolamine ;
5-HTP -5-hydroxy tryptaphan.

on pentobarbital hypnosis in mice were potentiated by the p-MPPI (5-HT1A receptor antagonist (1 mg/kg, i.p.) and ketanserin (5-HT2A/2C receptor antagonist,1.5 mg/kg, i.p.), respectively. Pretreatment with either 8-OH-DPAT (5-HT1A receptor agonist) (0.1 mg/kg, s.c.) or DOI (5-HT2A/2C receptor agonist,0.2

Fig. 1 : Percent Change in Sleeping time



mg/kg, i.p.) significantly decreased pentobarbital-induced sleeping time, and tetrandrine (60 mg/kg, i.g.) significantly reversed this effect. These results suggest that both the 5-HT1A and 5-HT2A/2C subfamily may be involved in the potentiating mechanism of tetrandrine's effects on pentobarbital hypnosis Pharmacological evidences of serotonergic receptors suggest a role for serotonin (5-HT) in sleep regulation. (Nan *et al.* 2008). In earlier study, it had been shown that PGE1 increases 5-HT synthesis rate and decrease declination rate with the increase of 5-HT turn over in brain not in intestine (Debnath *et al.* 1978). Pharmacological manipulations showed that PCPA prevents the synthesis of serotonin by blocking the enzyme tryptophan hydroxylase, which could produce severe insomnia. However, this could be reversed through administration of small amounts of serotonin precursor 5-HTP in rats.

Our current findings suggest that PGE1 induced potentiation of PB sleeping time was a 5-HT mediated response. by increasing synthesis of 5-HT. Earlior studies , together with these findings suggested that the PGE1 induced potentiation of Pentobarbitone hypnosis and interaction between drugs modulating serotonergic system may participate in the regulation of sleep in chicks .

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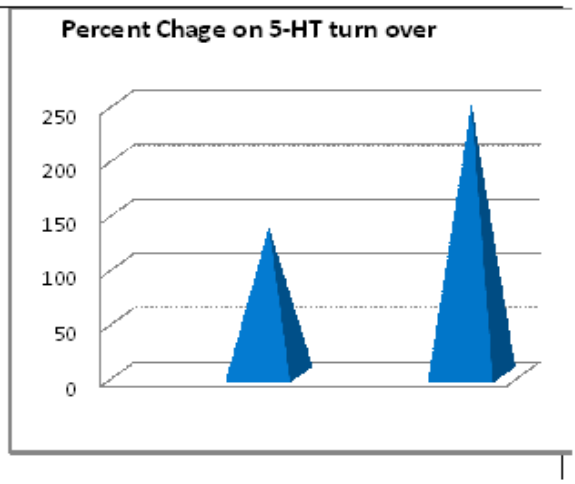
Table 2 : The Effect of PGE_1 (0.4 mg/kg) on brain and intestine 5-HT levels in pargyline (75.0 mg/kg) treated chicks at different time intervals

Time in hours	5-HT µg/g wet tissue			
	Brain		Intestine	
	Pargyline	**Pargyline + PGE_1**	**Pargyline**	**Pargyline + PGE_1**
0	1.06 ± 0.03	1.06 ± 0.03	5.47 ± 0.03	5.47 ± 0.03
½	1.41 ± 0.05	1.54 ± 0.09	6.51 ± 0.10	7.47 ± 0.01
1	1.50 ± 0.10	1.66 ± 0.10	6.66 ± 0.07	7.70 ± 0.10*
2	1.56 ± 0.10	2.19 ± 0.12*	7.19 ± 0.12	8.36 ± 0.15*

Results are Mean ± S.E , n = 4-5 chicks in each group;. *, indicate statistical significance In comparison to control pargyline as P<0.01

Table 3 : The effect of PGE_1 on calculated synthesis rate of 5-HT in brain and intestine

Tissue	Group	Change in 5-HT Synthesis rate* µg /g/h wet tissue	Percent Change (%)
Brain	Control	0.47	136
	PGE_1	0.64	
Intestine	Control	0.79	249
	PGE_1	1.97	



Results expressed as Mean ± SE, P < 0.01

* Rates were expressed as µg serotonin/g/wet tissue/hour. To measure the synthesis rate of accumulation, rats were treated with pargyline (75 mg/kg i.p) and animals were sacrificed at different time intervals thereafter as detailed in method. The rate constant was calculated by plotting the levels of 5-HT at different time on semi logarithmic graph paper. Accumulation rate was calculated simply by observing the rate of accumulation of serotonin at different time intervals (Neff and Tozer, 1968)

Table 4 : Analysis of variance on 5-HT level in chicks brain and intestine after pargyline (75.0 mg/kg i.p) alone and alongwith PGE_1 (0.4 mg/kg sc) for synthesis rate

Tissue*	Source of variance	df	TSS	NSS	F
Brain	Treatment	3	1.10	0.36	36.00**
	Error	12	0.17	0.01	
Intestine	Treatment	3	10.29	3.43	42.87**
	Error	12	1.00	0.08	

**Results expressed as significant $P < 0.01$

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