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Phenobarbital in the Anticonvulsant Dose Range Does Not Impair Learning and Memory or Alter Brain AChE Activity or Monoamine Levels

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SUDHA, S., M. K. LAKSHMANA AND N. PRADHAN. *Phenobarbital in the anticonvulsant dose range does not impair learning and memory or alter brain AChE activity or monoamine levels.* PHARMACOL BIOCHEM BEHAV **54**(3) 633–638, 1996. – The learning and memory in adult, male Wistar rats were assessed using the T-maze and passive avoidance tests after chronic administration of phenobarbital (PB) at 5, 15, 30, 60, or 75 mg/kg intraperitoneally (IP) for 21 days. The PB levels in plasma, the acetylcholine esterase (AChE) activity in the motor cortex, pyriform cortex, olfactory bulb, striatum, septum, and hippocampus and the levels of serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), dopamine (DA), dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) levels in the hippocampus were measured. There was no significant change in learning and memory, AChE activity, or monoamine levels at plasma PB levels of 1.5, 6.0, 9.0, and 25 μg/ml (corresponding to doses of 5, 15, 30, or 60 mg/kg PB, respectively). However, at a plasma level of 55 μg/ml (75 mg/kg), PB caused impairment in learning and memory. It was associated with an increase in AChE activity and 5-HT levels in the hippocampus. The results indicate that chronic PB administration may not be linked to impaired learning and memory functions at doses used in anticonvulsant therapy.

Phenobarbital Learning and memory AChE activity Monoamines Plasma PB levels

PHENOBARBITAL (PB), the first effective antiepileptic drug introduced in 1912 is widely used against generalized tonic clonic and partial seizures. Its efficacy, low toxicity, and low cost make it an important agent for these types of epilepsy. Many studies have shown the effects of acute administration of antiepileptic drugs on learning and memory (35,40). However, epilepsy is a chronic disorder, and antiepileptic drugs are administered on long-term basis to patients. Optimal therapy would result in complete seizure control without therapy-induced memory disturbances in the patients. Attempts have been made to establish a correlation between cognitive dysfunction and anticonvulsant drugs. It is difficult to attribute mental dysfunctions directly to antiepileptic drugs because other variables such as underlying brain pathology, seizure type, frequency and severity of seizures (44), the antiepileptic drug administered, the blood levels achieved (9), and the number of drugs administered (37) may contribute to cognitive impairments. Normal adult volunteers and persons with epilepsy treated with PB show impaired performance in tests of concentration (21) and in tasks requiring short-term memory (30). But children suffering febrile seizures treated with daily PB do not show lower I.Q. (46). Controlled studies on the effect of chronic administration of antiepileptic drugs on learning and memory need to be carried out (42,43). Clinical investigations on the selective effect of PB are rather difficult to carry out because many patients require treatment by more than one type of anticonvulsant. Experimental research with animals provides opportunities for such investigations that would otherwise not be possible in humans for obvious ethical reasons.

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The central cholinergic system is known to play an important role in learning and memory and is considered crucial for maze learning in rats (3,6,18). Pharmacological modulations of the brain acetylcholinergic systems are also known to alter memory function. Many studies have demonstrated that antimuscarinic agents such as scopolamine and atropine have deleterious effects on learning and memory (23,27). Similarly compounds such as physostigmine that enhance central cholinergic tone by inhibiting the catabolic enzyme acetylcholine esterase can, under certain circumstances enhance performance in learning and memory tasks (1). However, the dissociation between learning and memory capabilities and cortical acetylcholinergic activity has also been presented (14). PB can decrease the release, alter the rate of synthesis, and change the concentration of acetylcholine (Ach) (5,24). However, contributions of cholinergic system to changes in memory function after chronic PB administration are not well defined.

Monoamines may be involved in the mechanism of actions of antiepileptic drugs such as PB. Acute intraperitoneal PB administration increases whole-brain 5-HT (28) and DA levels (38). The central monoamines play a significant role in learning and memory functions. Central DA receptor activation facilitates learning in general. In contrast, the increased serotonergic activity impairs retention of avoidance tasks in rats (11). Drugs like 5-hydroxytryptophan and *p*-Chloramphetamine impair avoidance learning in rats (2,25). Our study assessed the role of central serotonergic and dopaminergic system in learning and memory with chronic PB administration.

METHOD

Animals

Adult male Wistar rats weighing 200–250 g obtained from the Central Animal Research Facility of the National Institute of Mental Health and Neurosciences, Bangalore, India, were used in this experiment. The rats were fed with 18 CW diet (a semisynthetic balanced diet of 18% casein, 78% wheat, and all the vitamins and minerals needed for the normal growth of the rat) and water ad lib. They were housed four per cage in Plexiglas cages and reared in a 12 L : 12 D cycle (lights on at 0700 h), temperature controlled (23 ± 1 degree C) animal house.

Drugs

PB was a gift from Tablets Ltd., India. PB suspended in 0.5% methyl cellulose was administered to groups of rats ($n = 6$) intraperitoneally at doses of 5.0, 15.0, 30.0, 60.0, and 75.0 mg/kg/day for 21 days. Control rats were administered an equal volume of vehicle. Two hours after the drug administration, animals were tested in the T-maze or passive avoidance tasks.

T-maze Procedure

Training. The T-maze had a start arm and a left and right arm (50 × 13 × 35 cm) all painted black. At the extremity of each arm, a food cup (5 cm diameter, 0.5 cm deep) was on the floor. The T-maze was in a dimly illuminated room (25 W light). Starting form the 18th day of chronic PB administration, the animals were given free access to food for only 1 h per day. Rats were familiarized with the maze, food, and food containers on 2 consecutive days before the start of the experiments. On these days, two trials per rat was carried out. At the start of the experimental session, 15 trials per rat were carried out on the first day (i.e., 20 days after the beginning of chronic PB treatment). Only one arm of the T-maze was baited with the food (18 CW diet pellets). The correct choice was the left arm for half of the rats and the right arm for the rest. The rat was placed into the start arm and the door gently raised. When the rat reached one or the other arm it was removed from the maze and placed into a separate waiting box for 10 s and then returned to the maze. A correct trial ended when the rat ate the food. An incorrect trial (error) ended when the rat reached the empty food cup. The time taken for each trial was recorded by a stop watch. The number of errors, the percentage of errors, and average time taken for each trial were recorded.

Testing. The next day trials were conducted for each rat until it attained a criterion of 9 correct choices out of 10 consecutive trials. The number of trials to reach the criterion of nine correct responses, the number of errors, the percentage of errors and the average time taken for each trial were recorded.

Step-Down Passive Avoidance

Training. Naive rats were placed on a plastic platform (10 × 10 × 10 cm) placed in the center of a lighted box (30 × 30 × 30 cm) with an electrifiable grid floor. When the rats stepped off the platform, a constant and continuous electric shock of 0.8 mA was applied through the grid floor for 10 s. The normal reaction of the rat was to jump back on to the platform.

Testing. After 24 h each rat was replaced on the platform and tested again for step-down latency. The test ended when the rat stepped down or refrained from stepping down for at least 60 s. The percentage of rats that refrained from stepping down (i.e., the percentage of rats that learnt) before 60 s was noted.

Plasma PB Levels

Within 1/2 h following the T-maze or passive avoidance tests, the rats were sacrificed by rapid decapitation and the blood was collected. The PB plasma levels were estimated by EMIT assay method using kits from Syva (M/s Syva International, UK).

Brain Dissection

After decapitation, the brains were rapidly removed. Subsequent dissection was performed on ice. Motor cortex, pyriform cortex, olfactory bulb, striatum, hippocampus, and septum were dissected out by a slightly modified method of Glowinski and Iversen (15).

Estimation of Acetylcholine Esterase Activity

Acetylcholine esterase (AChE) activity was measured on the basis of the yellow color produced due to reaction of thiocholine with dithiobis nitro benzoate ion (10).

Estimation of Monoamines

Hippocampal 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), dopamine (DA), dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) levels were assessed by HPLC with electrochemical detection as described previously (39).

Statistical Analysis

The data was analyzed by a statistical package (SYSTAT: SYSTAT Inc., Evanston, IL) on an IBM PC-AT. The mean

TABLE 1

PERFORMANCE OF THE RATS GIVEN DIFFERENT DOSES OF PB AND THE CONTROLS IN THE T-MAZE ON THE FIRST DAY

Parameter	Control	PB Doses				
		5 mg	15 mg	30 mg	60 mg	75 mg
Plasma levels (μg/ml)	–	1.5	6.0	9.0	25.0	55.0
First Day						
Errors	5.167 ± 0.477	6.667 ± 0.843	5.500 ± 0.719	6.833 ± 0.872	6.667 ± 1.453	5.333 ± 0.955
Percentage of errors	34.450 ± 3.181	44.433 ± 5.608	36.667 ± 4.786	45.567 ± 5.818	44.450 ± 9.688	35.550 ± 6.366
Time taken for each trial (s)	43.833 ± 5.620	23.933 ± 4.515	22.767 ± 3.824*	26.733 ± 4.726	22.167 ± 2.750*	28.950 ± 6.020

Means ± SEM.
$n = 6$.
*Significantly different from controls ($p < 0.05$).

and standard error of mean (SEM) was calculated for all the parameters. Comparisons between the various groups were made by the one-way analysis of variance (ANOVA). Further comparisons were made with Tukey's honestly significant difference test at the 5% level of significance.

RESULTS

PB Levels

The average PB levels in the plasma were 1.5, 6.0, 9.0, 25.0, and 55.0 μg/ml for the 5, 15, 30, 60, and 75 mg/kg doses respectively (Table 1).

T-Maze and Passive Avoidance Tests

Performance on the first 2 days on the T-maze and passive avoidance tests is summarized in Tables 1 and 2. Rats administered 5, 15, and 30 mg/kg PB (corresponding to plasma levels of 1.5, 6.0, and 9.0 μg/ml), did not differ from the controls. Although these rats took fewer number of trials to reach criterion when compared to the controls, this effect was not statistically significant. The 60 mg/kg group (plasma level of 25 μg/ml) also did not affect learning and memory. Although this group of rats took more number of trials to reach criterion than the controls, the effect was not statistically significant. Rats administered 75 mg/kg PB (i.e., at 55 μg/ml), showed significant impairments in learning and memory, $F(5, 30) = 7.382$, $p < 0.01$. The 75 mg/kg group also made significantly more errors on the second day when compared to the controls, $F(5, 30) = 12.393$, $p < 0.01$. The PB-treated groups of rats were generally more active and took less time to complete each trial than the controls. But the 75 mg/kg group had an awkward gait as well as general clumsiness. The 15 and 60 mg/kg groups took significantly less time to complete each trial on the first day, $F(5, 30) = 2.994$, $p < 0.05$, and the 5 and 30 mg/kg group took significantly less time to complete each trial on the second day, $F(5, 30) = 4.501$, $p < 0.01$. The rats treated with 75 mg/kg of PB also performed poorly in the passive avoidance test when compared with the controls.

Acetylcholine Esterase Activity

The AChE activity in various brain regions is shown in Table 3. The AChE activity did not differ significantly from the controls in any of the doses of PB administered, except in the hippocampus where the 75 mg/kg group had significantly higher AChE activity when compared to the controls, $F(5, 30) = 6.872$, $p < 0.01$.

TABLE 2

PERFORMANCE OF THE RATS GIVEN DIFFERENT DOSES OF PB AND THE CONTROLS IN THE T-MAZE ON THE SECOND DAY AND THE PASSIVE AVOIDANCE TESTS

Parameter	Control	PB Doses				
		5 mg	15 mg	30 mg	60 mg	75 mg
Second day						
Errors	5.333 ± 0.494	3.333 ± 0.615	4.333 ± 0.715	2.167 ± 0.477	7.667 ± 0.615	8.833 ± 1.195*
Percentage of errors	28.362 ± 1.946	24.333 ± 3.188	25.200 ± 2.766	15.700 ± 2.382*	33.200 ± 2.587	32.550 ± 2.200
Time taken for each trial (s)	53.333 ± 4.620	18.617 ± 1.799*	42.667 ± 9.510	29.650 ± 4.159*	31.333 ± 7.373	31.050 ± 4.333
Trials to criterion	18.667 ± 0.615	16.667 ± 2.404	16.833 ± 1.302	13.333 ± 2.076	22.167 ± 1.424	26.833 ± 2.072*
Passive avoidance % of rats which did not step-down	33.300	33.300	33.300	16.700	0.000	0.000

Means ± SEM.
$n = 6$.
*Significantly different from controls ($p < 0.05$).

TABLE 3

ACTIVITY OF ACETYLCHOLINE ESTERASE IN DIFFERENT REGIONS OF BRAIN OF CONTROL RATS AND RATS GIVEN DIFFERENT DOSES OF PB EXPRESSED AS MICROMOLES HYDROLYZED/G WET WEIGHT/MINUTE

		PB Doses				
Region	Control	5 mg	15 mg	30 mg	60 mg	75 mg
Motor cortex	4.531 ± 0.167	4.961 ± 0.178	4.051 ± 0.316	4.224 ± 0.114	5.024 ± 0.365	4.737 ± 0.240
Pyriform cortex	38.246 ± 3.891	40.171 ± 3.514	49.124 ± 1.890	43.557 ± 3.362	41.706 ± 3.875	41.408 ± 2.954
Olfactory bulb	4.103 ± 0.373	4.370 ± 0.284	4.602 ± 0.196	4.523 ± 0.346	4.927 ± 0.470	5.091 ± 0.150
Striatum	34.047 ± 2.272	42.954 ± 2.981	44.188 ± 1.030	38.147 ± 2.375	44.997 ± 4.637	37.764 ± 5.230
Hippocampus	5.943 ± 0.250	5.777 ± 0.119	5.378 ± 0.132	5.989 ± 0.456	6.645 ± 0.557	7.797 ± 0.183*
Septum	9.004 ± 0.336	13.697 ± 1.197	9.764 ± 0.609	10.080 ± 1.842	8.190 ± 0.184	9.837 ± 1.373

Means ± SEM.
$n = 6$.
*Significantly different from controls ($p < 0.05$).

Monoamine Levels

The levels of monoamines and their metabolites in the hippocampus are shown in Table 4. One-way ANOVA showed a significant difference between the groups in their 5-HT levels, $F(5, 30) = 10.070$, $p < 0.01$. Post hoc tests showed that the 75 mg/kg group had significantly higher 5-HT levels than the controls ($p < 0.05$). All the experimental groups had significantly higher DA and HVA levels than the controls ($p < 0.05$). There was no significant difference in the 5-HIAA and DOPAC levels between the experimental and control rats.

DISCUSSION

Our results indicate that chronic administration of PB at 5, 15, 30, and 60 mg/kg dose with corresponding plasma levels of 1.5, 6.0, 9.0, and 25.0 μg/ml do not impair learning and memory in rats. But PB at 75 mg/kg with a corresponding plasma level of 55 μg/ml causes significant impairment in learning and memory. These results are consistent with earlier reports of acute PB administration, where PB has been shown to cause impairment in retention performance in mice at high doses (100 mg/kg). It has no significant effects on memory at lower doses (35). PB is known to affect learning performance in seizure prone baboons in high doses (45). PB has also been shown to cause deterioration of psychomotor functioning in children (20,21). Therefore, PB is no longer considered as first line of treatment in generalized epilepsy. However, it still remains as the drug of choice in generalized tonic-clonic seizures in many countries because of its low cost.

In the dose range of 5–75 mg/kg, the plasma concentrations of PB in the rats range from 1.5 to 55 μg/ml. The therapeutic range for PB in the plasma lies between 15–40 μg/ml (33). The relationship between doses and plasma levels appears to be almost linear ($r^2 = 0.9873$ for 5–60 mg/kg and $r^2 = 0.9335$ for 5–75 mg/kg dose range). The rise in the plasma level to 55 μg/ml in the 75 mg/kg group may be due to the smaller number of animals used ($n = 6$) in this study. The high plasma PB level of 55 μg/ml produces awkwardness in gait and general clumsiness in motor behavior. Neurological signs of drunkenness including incoordination and ataxia are known to occur at plasma PB concentrations of 40 μg/ml or more (33). Despite observable motor effects, the 75 mg/kg group did not show delay or increased latency in task performance. The poor learning performance of the 75 mg/kg group may be due to a direct action of PB on the CNS and it may not be related to motor impairment.

The AChE activity was measured in different brain regions as a marker enzyme for cholinergic function. Comparative studies with AChE and choline acetyl transferase (ChAT) as a marker enzyme for cholinergic perikarya and their processes (29) show that ChAT immunoreactivity and AChE staining pattern in the hippocampus are virtually identical (32). Quantitative analyses of the two cholinergic marker enzymes,

TABLE 4

LEVELS OF 5-HT, 5-HIAA, DA, DOPAC, AND HVA IN THE HIPPOCAMPUS OF CONTROL RATS AND RATS GIVEN DIFFERENT DOSES OF PB IN ng/mg

		PB Doses				
Parameter	Control	5 mg	15 mg	30 mg	60 mg	75 mg
5-HT	0.222 ± 0.014	0.203 ± 0.015	0.253 ± 0.014	0.240 ± 0.011	0.256 ± 0.017	0.338 ± 0.016*
5-HIAA	0.150 ± 0.021	0.140 ± 0.010	0.137 ± 0.012	0.179 ± 0.010	0.187 ± 0.012	0.185 ± 0.012
DA	0.106 ± 0.010	0.185 ± 0.014	0.192 ± 0.013	0.225 ± 0.011	0.268 ± 0.013	0.307 ± 0.014*
DOPAC	0.401 ± 0.034	0.412 ± 0.025	0.424 ± 0.017	0.425 ± 0.023	0.419 ± 0.015	0.435 ± 0.020
HVA	0.124 ± 0.016	0.285 ± 0.014*	0.316 ± 0.018*	0.303 ± 0.016*	0.311 ± 0.020*	0.313 ± 0.019*

Means ± SEM.
$n = 6$.
*Significantly different from controls ($p < 0.05$).

ChAT and AChE, within the hippocampus also indicate that the relative amounts of both enzymes are very similar. Hence, AChE seems to be a reasonably good indicator of cholinergic functions.

Our results show an increase in AChE activity in the hippocampus only in the group of the rats treated with 75 mg/kg of PB. These rats also show impaired performance in the T-maze and passive avoidance tests. Increased AChE activity may decrease the levels of ACh in the brain and may account for the observed impairments in learning and memory. The cholinergic system plays an important role in learning and memory functions (3,6). The septohippocampal cholinergic system in particular plays a crucial role in maze learning. The cholinergic input to the hippocampus modulates hippocampal neuronal excitability (19). Lesions that interfere with cholinergic modulation of the hippocampal neural circuitry result in poor T-maze learning performance (17,26). Anticholinergics like scopolamine and atropine disrupt learning and memory (23,27). PB also has complicated effects on acetylcholine metabolism in the brain. It stimulates the production of free ACh at low concentrations and depresses it at higher concentrations (34,41). At higher doses PB may decrease the release of ACh and alter its rate of synthesis (5,24). The observed impairments in learning and memory may be related to a decreased production or release of ACh.

Chronic PB administration at doses 60 mg/kg or lower has no significant effects on the 5-HT metabolism. However, the 5-HT levels are increased in the hippocampus in the rats treated with 75 mg/kg of PB. The increased activity in central serotonergic systems may impair learning and memory (11, 25). Elevation of 5-HT levels by administration of 5-Hydroxytryptophan 1 h prior to the shock presentation trial and microinjections of 5-HT directly into the hippocampus upto 16 min following shock presentation, result in considerable impairments in a one-trial passive avoidance procedure in the rat (12). Similarly, the electrical stimulation of the dorsal raphe nucleus disrupts memory by a process involving 5-HT (13). PB is known to increase brain 5-HT (28) and tryptophan hydroxylase activity (47). The increased 5-HT levels may contribute to impairment of learning and memory in the 75 mg/kg group.

The 5-HT-related memory impairment caused by PB may be mediated by a dysfunction of ACh transmission. *p*-Chloramphetamine-induced amnesia can be attenuated by AChE inhibitors (31). Therefore, the PB-induced impairment in learning and memory may be partially mediated by a decrease in ACh transmission. PB may increase the levels of 5-HT and thereby inhibit ACh transmission. The learning and memory impairment in the 75 mg/kg group may have been due to cholinergic and serotonergic interactions.

In our study, DA levels are increased in the hippocampus of all the experimental groups. The 75 mg/kg group performed poorly in the T-maze and passive avoidance tests. Dopaminergic mechanisms improve learning and memory, in general. For example, posttraining intrahippocampal injection of the dopaminergic agonists apomorphine and ergometrine improves retention in a brightness discrimination task (16). However, stimulation of postsynaptic dopamine receptors has been reported to retard acquisition of passive avoidance tasks (7,22). Administration of dopamine into the nucleus accumbens disrupts the acquisition of the passive avoidance task (7). It has been shown that DA can regulate AChE release in vivo by a D_2 receptor-mediated inhibitory mechanism and a D_1 receptor-mediated facilitatory one (4). Thus, the impairments of learning and memory seen in our study may also be due to interactions of dopaminergic system with other neurotransmitter systems.

Overall, changes in AChE activity and monoamines are slight, considering the prominent role ascribed to the cholinergic and monoaminergic system in learning and memory (3,36). Although many studies have reported alterations in learning and memory following changes in the cholinergic or monoaminergic system, these alterations must be considered on a relative scale. It would appear that the nature of the deficit would depend on the type of task and the relative amount of a change associated with each neurotransmitter system. Pharmacological studies suggest that interactions of the cholinergic system with monoaminergic or other systems may be critical in memory (8,18). In this view, small changes in monoaminergic or cholinergic function could have synergistic effects with other neural systems in modulating memory function.

In conclusion, chronic administration of PB at doses of 5-60 mg/kg (with plasma levels of 1.5 to 25 μg/ml) does not alter learning and memory, AChE activity or 5-HT levels. The 75 mg/kg PB group (with 55 μg/ml PB in the plasma) shows significant impairments in learning and memory with an increase in AChE activity and an increase in 5-HT levels in the hippocampus. It appears that PB may not cause impairment of learning and memory within the accepted therapeutic range. However, the T-maze and passive avoidance tests may not be predictive of the clinical situation when it comes to cognition and task performance in humans.

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