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EFFECT OF PRENATAL VALPROIC ACID AND PIRACETAM EXPOSURE ON BEHAVIORAL ALTERATIONS IN SWISS ALBINO MICE

Shamsher Shrestha*[1], Mandavi Singh[1]*, Surendra Pratap Mishra[2]

[1]Department of Anatomy, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005
[2]Department of Biochemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005

ABSTRACT

Valproic acid (VPA) is an antiepileptic drug and when used in pregnancy it acts as a strong teratogen and piracetam (PCT) is a nootropic and neuroprotective agent. In the present study, these two drugs VPA and PCT were administered orally to Swiss albino pregnant mice in the doses of 400mg/kg and 800 mg/kg body weight respectively from 6th to 11th gestation days. The animals from all the groups, treated as well as vehicle control, were allowed to deliver on GD 19. The offspring were subjected to behavioral tests at the age of 8 weeks. The VPA treated mice offspring showed significant decreased locomotor activity, increased anxiety and depressive behavior on the open field, elevated plus-maze and behavior despair test respectively in comparison to control and the other groups, such as treated with piracetam and piracetam with VPA. The offspring of the group treated with VPA alongwith PCT showed significantly increased locomotor activity, decreased anxiety and depression in comparison to VPA treated mice offspring. These findings suggest that prenatal exposure of VPA during critical period of brain development in offspring alters the behavioral function which is reduced by simultaneous piracetam treatment along with valproic acid.

Keywords: Valproic acid, Piracetam, anxiety, depression, behavioral changes

Valproic acid (VPA) is one of the most popular antiepileptic drugs which is used to control all types of seizure disorders and some psychiatric problems. Its mechanism of action includes enhanced neurotransmission of GABA by inhibiting GABA transaminase. It also blocks the voltage-gated sodium and T-type calcium channels. These mechanisms make it a broad spectrum anticonvulsant drug. However, several other mechanisms of action of VPA in neuropsychiatric disorders have been proposed[1].

Valproic acid is also a well established human and animal teratogen[2]. Prenatal exposure of valproic acid induces neural tube defects and congenital malformations in both humans and animals. In rodents, experimental studies have shown that prenatal VPA exposure during critical period of brain development induces neurochemical and structural alterations in various fetal brain areas. It has been reported that exposure of subteratogenic doses of valproic acid may cause microencephaly and behavioral changes such as spatial learning deficiency and locomotor activity in rodents[3,4]. In utero, exposure of rats to valproic acid also causes cerebellar anomalies.[3] In humans, intrauterine exposure to VPA has been associated with mental retardation, cognitive impairment, behavioral deficits and neurodevelopmental delay in children[5,6,7,8].

Piracetam (PCT) is a nootropic or cognitive enhancing agent, used to treat cognitive impairment in aging, brain injuries as well as dementia[9,10]. It is a cyclic derivative of gamma-aminobutyric acid (GABA) but it does not exhibit any GABA- like activity in animals. One of the mechanisms of action of piracetam is to influence the neuronal, vascular and cognitive functions[11]. It also improves the function of the neurotransmitter acetylcholine via muscarinic cholinergic (ACH) receptors, which are implicated in memory process[12]. It has also been reported that

Corresponding Author:
mandavi5@rediffmail.com

piracetam possesses cytoprotective, antioxidant and antihypoxic protective effects[13,14,15,16].

The present study has been undertaken to see the protective effect of piracetam against the valproic acid-induced behavioral alterations in mice offspring.

MATERIALS AND METHODS

1. Animals

Adult Swiss albino mice weighing about 20-30 g were used in the present study. The consent was taken from institutional ethical committee of Banaras Hindu University, Varanasi, India for using these animals. These mice were maintained under standard laboratory conditions (25° ± 2°C, 12 hr L/D cycle, 60 % RH) for 2 weeks for proper acclimatization. All animals were housed in polypropylene cages (39 x 24 x 15 cm) with rice bran bedding. The pelleted food and tap water were provided ad libitum throughout the experiment. The rice bran of each cage was changed regularly to avoid any unhygienic condition.

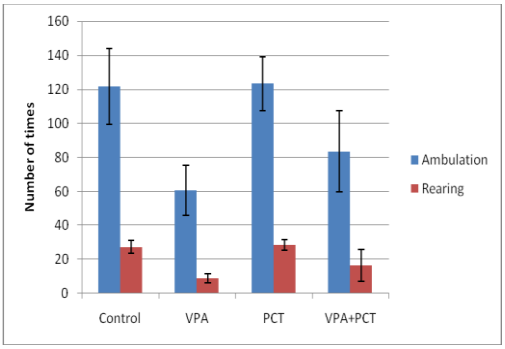

Fig. 1. Effect of VPA and PCT treatment on Ambulation and Rearing.

2. Determination of pregnancy

The male and female mice (1:2) were kept in cages together overnight for mating. On the next morning, presence of the vaginal plug indicated day Zero of gestation.

3. Experimental design and drug exposure

All plug-positive female mice were randomized into four groups. Each group contained eight mice.

Group 1: Control (given equivalent amount of distilled water)

Group 2: Treated with valproic acid (400 mg/kg body weight)

Group 3: Treated with piracetam (800 mg/kg body weight)

Group 4: Treated with a combination of valproic acid and piracetam (dose mentioned above)

All the control and treated mice were given the above mentioned drug in different doses orally from gestation days 6th to 11th.

The pregnant dams from all the groups were allowed to deliver naturally and the pups were reared with their biological mothers. On PND 40 pups were separated from their biological mothers. Male and female pups were separated. This was done in all the groups. At PND 56, 2 male and 2 female pups from each dam were randomly selected from all different groups, i.e., Control (n=16), VPA (n=16), PCT (n=16) and VPA+PCT (n=16) and these pups were subjected to various behavioral tests.

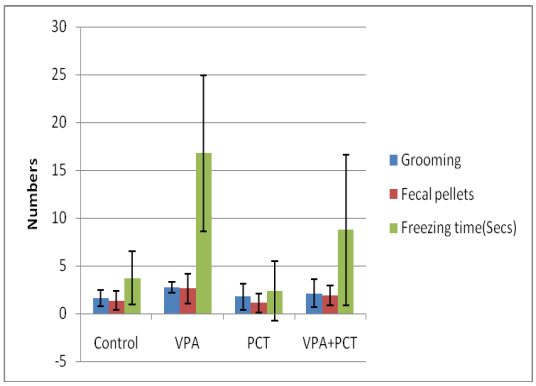

Fig. 2. Effect of VPA and PCT treatment on grooming, fecal pellets and freezing time.

4. Behavioral tests

4.1. Open-field exploratory test

An open field apparatus made of plywood measuring 60.96 X 60.96 X 60.96 cm was used to test the open field exploratory behavior of mice[17]. The floor of the apparatus was divided into 16 evenly spaced squares surrounded by opaque high walls of 60.96 cm. the entire apparatus was painted black except the 6 mm wide white lines that divided the floor into 16 squares. The open field apparatus was illuminated by a 100 W bulb focusing onto field from a height of about 100 cm from the floor. The entire room except the open field was kept dark during the experiment. In the novel test situation, each animal was kept in one corner of apparatus for 5 minutes to observe the following behavioral parameters:

1. Ambulation- The number of squares crossed by the mice.
2. Rearing- The number of times mice stood on its hind limbs.
3. Self- grooming- The number of responses of grooming, scratching, licking and washing made by each individual mice.
4. Freezing time- duration for which mice remains still without any movement.
5. Fecal pellets- The number of fecal pellets exuded by each individual mouse during the trial of 5 minutes.

Before each trial, the floor and wall of apparatus were cleaned with cotton soaked in 70% alcohol. All the trials were performed between 9 am to 11 am. This experiment was done to test the factor of locomotor activity in mice under the effect of drug used in the present study.

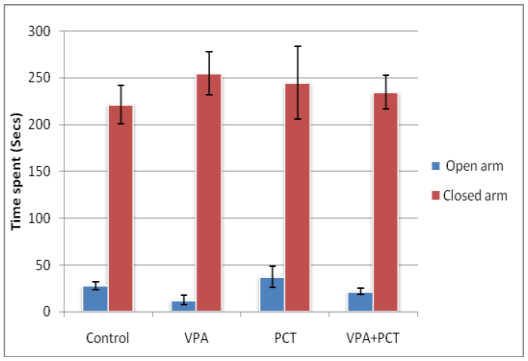

Fig. 3. Exploration scores (time spent in open arm and closed arm) on the elevated plus-maze in different groups.

4.2. Elevated plus- maze test

This model was also used to test the anxiety pattern in mice[18]. It consists of two opposite arms 50 X 10 cm, connected with Central Square (10 X 10 cm), giving the apparatus a shape of plus sign. One arm painted white was open arm, whereas the other arm was enclosed with 40 cm high wall and painted black together with walls. The maze was kept in a dimly lit laboratory and was elevated 50 cm above the floor. The experimental animals were placed individually in the centre of the maze facing towards the enclosed arm. The number of entries and the time spent on open and enclosed arms were recorded during the next 5 min for each mouse. The arm entry was recorded when all four paws of the mice entered into the arm. Like the open-field apparatus, the floor and walls of the open and enclosed arms were cleaned with 70% alcohol before each trial. All the trials were made between 9 am to 11 am.

4.3. Behavioral despair test

This maze was used to evaluate the depression status in mice[19,20]. The individual mouse was placed in circular glass chamber, 45 cm in diameter containing 25 cm depth of water, so that mouse could not touch the bottom of the cylinder with its hind limb or climb over the edge of the chamber. Two swim sessions were conducted, initially 15 min pretest, followed by a 5 min test 24 h later. The period of immobility (remain floating in water without struggling) and frequency during 5 min test period was noted and evaluated.

5. Statistical analysis

All data were entered into excel sheet and mean and standard deviation (S.D.) were calculated. One way ANOVA test was done using software SPSS version 16 (statistical package for social sciences). If it was significant, SNK test was used for post hoc analysis. $P < 0.05$ is considered as significant and $p < 0.001$ is highly significant.

RESULTS

1. Open- field behavior

This test was performed to test the locomotor activity in the offspring of all the experimental mice groups at the age of 8 weeks. Prenatal VPA and PCT treatment in mice from GD 6 to 11 induced significant behavioral alterations in open field arena (Figs. 1 and 2). On comparison between the different groups, the ambulation and rearing frequency was observed to be significantly less ($p<0.001$) in group II as compared to groups I and III. The ambulation and rearing frequency were higher in group IV as compared to group II (Fig. 1), though the difference in rearing was significant ($p<0.05$), the ambulation didn't differ significantly ($p>0.05$). The ambulation was significantly ($p<0.05$) decreased in group IV in comparison to groups I and III, the difference was highly significant ($p<0.001$) in comparison to group III and significant ($p<0.05$) in comparison to group I.

The self-grooming, fecal pellets and freezing time were also recorded. There was no significant difference among the different groups in self-grooming and fecal pellets in the open-field arena. But the increase in the parameter of freezing time

was found to be highly significant ($p<0.001$) in group II when compared to groups I and III, though it was not significant in comparison to group IV (Fig. 2).

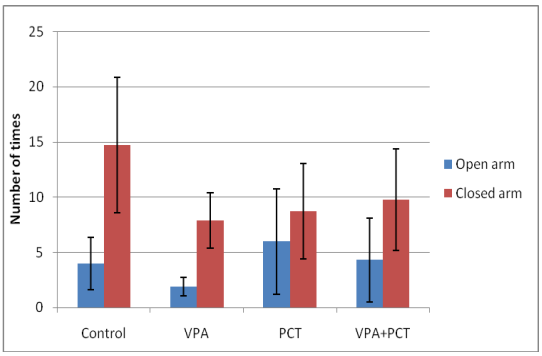

Fig. 4. Exploration scores (Open arm entry and Closed arm entry) on the elevated plus-maze in different groups.

2. Elevated plus maze

This test was performed to test the anxiety and depression in the offspring of all the experimental mice groups at the age of 8 weeks. In the present study, the time spent in open arm by group II animals was less than in groups I and III, it was highly significant ($p<0.001$). In comparison to group IV also, it was significantly reduced ($p<0.05$). The time spent in open arm by group IV was also significantly ($p<0.05$) less in comparison to group I while in comparison to group III, it was highly significant ($p<0.001$). The difference observed in between the different groups in time spent in closed arm was not significant though, group I mice spent least time followed by groups IV, III and II (Fig. 3). On comparing the number of entries in the open and enclosed arms, significant difference was observed in between groups I and II and groups I and III in closed arm entry (Fig. 4). Thus, from the above findings it may be concluded that group II spent least time in open arm displaying anxiogenic behavior which was reduced by simultaneous piracetam treatment along with the valproic acid.

3. Behavioral despair test

This test was performed to test the depression status in the offspring of all the experimental mice groups at the age of 8 weeks. In the present study, the immobility time period was more in group II in comparison to groups I, III and IV. It was statistically highly significant ($p<0.001$) in comparison to groups I and III and significant ($p<0.05$) in comparison to group IV. In comparison between group IV and I, the immobility time period was non significantly higher ($p>0.05$) in group IV but in comparison to group III, the increase in immobility time period was highly significant ($p<0.001$) (Fig. 5). From this experiment it does appear that group II mice offspring displayed depressive sign after prenatal valproic acid treatment which was reduced by simultaneous piracetam treatment in group IV.

In the present study, no significant differences were observed in the behavior pattern between male and female mice offspring. Similar findings have been reported earlier also[21].

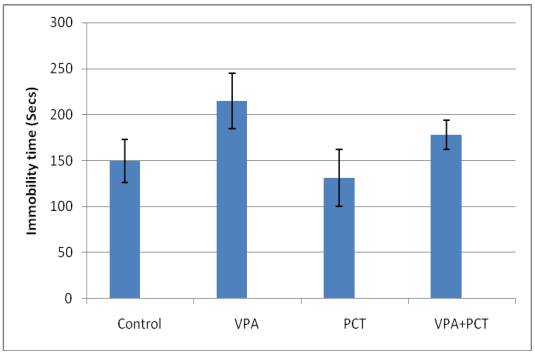

Fig.5. Effect of VPA and PCT treatment on immobility time period.

DISCUSSION

The results from these experiments demonstrate behavioral changes in offspring of mice treated with valproic acid and piracetam. In open field arena, VPA exposed- mice offspring showed significantly decreased ambulation and rearing. In the elevated plus maze test, these offspring made fewer entries and spent less time in open arm and more time in enclosed arm in comparison to groups I and III. In comparison to group IV, the ambulation and rearing frequency also decreased in group II but only in rearing frequency it was significant. A high frequency of ambulation and rearing indicates increased locomotion and lower level of anxiety[17]. In the present study it was found Valproic acid treated offspring showed low frequency of ambulation and rearing indicating decreased locomotor activity and increased anxiety in comparison to control and other groups. In elevated plus maze test the group II offspring spent significantly less time in open arm and more time in closed arm. These behavioral alterations indicate anxiety in offspring[22] and similar findings have been reported by other workers also[23]. Mychasiuk *et al* (2012) reported that PND 105 rat offspring spent more time in open field after

prenatal treatment of valproic acid in comparison to control[24] but in the present study PND 52 mice offspring were used so this observation may not be compared with the present findings.

In the present study depression status in mice offspring was also evaluated by using behavioral despair maze test. The group II mice offspring showed significantly more immobility time period in comparison to control and other groups. These results indicate that the prenatal valproic acid mice offspring have depressive sign.

From these observations it is concluded that in utero exposure to valproic acid induces long lasting effect on neurobevioral alterations in the offspring such as decreased locomotor activity, anxiety and depression. This study is also in agreement with those workers who have suggested that if abnormal offspring were subjected to stressful or new environment, it is difficult for them to cope up with new environment resulting in slower habituation with altered behavioral responses[25].

In the present study, when prenatally valproic acid along with piracetam were administered and the offspring were subjected to behavioral tests they showed better behavioral responses in comparison to those treated with Valproic acid only. Since Piracetam is a nootropic and cognitive enhancing agent, it also improves the function of the neurotransmitter acetycholine via muscarinic cholinergic (ACH) receptors, which are implicated in memory process[26] hence the improvement in behavior responses. It has also been reported that piracetam possesses cytoprotective, antioxidant and antihypoxic protective effects[13,14,15,16]. It also facilitates learning and retrieval of information and protects the brain from physical and chemical noxious agents[27] Piracetam exhibited anxiolytic activity in the open field and elevated maze paradigms[28].

Therefore, decreased anxiety and depression and increased locomotor activity in the group IV mice offspring may be protective effect of piracetam against the valproic acid- induced behavioral alterations in the present study. Since the piracetam is known to possess antioxidant properties, it might neutralise the effects of free oxygen radicals produced by VPA decreasing the number of apoptotic and dead cells. Hence, it may be postulated that piracetam has got protective effect against the induced deleterious effects of VPA in the present study.

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