BEHAVIORAL ALTERATION BY AMITRIPTYLINE HYDROCHOLRIDE IN SOCIALLY ISOLATED AND COLONIZED MALE MICE

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ABSTRACT

The present experimentation was designed to find the effect of social isolation on male mice and alteration in behavior by amitriptyline hydrochloride in both socially isolated male mice and colonized male mice. The immobility of mouse in Forced swim test (FST) was considered as the parameter for assessing depression for the mice kept in groups and individually caged for 28 days. Amitriptyline hydrochloride was administered at a dose of 4mg/kg i.p. and 10 mg/kg i.p. before exposure to FST to find its effect on depression. Isolation caused depression which was altered by the tricyclic antidepressant, amitriptyline hydrochloride in a dose dependent manner (4mg/kg i.p. and 10 mg/kg i.p.). Mice in groups did not show any depression which was not changed by amitriptyline hydrochloride. Social isolation cause depression in mice but when mice were in groups either with female counterparts or only with male, the depression did not occur may be due to interaction that reduced the chances of depression. Moreover, as amitriptyline hydrochloride did not reduce the immobility for the control mice so it might be inferred that FST did not develop depressed state and amitriptyline hydrochloride was ineffective in non depressed condition. Amitriptyline hydrochloride was more effective at higher concentration to reduce depression than the lower concentration.

Keywords : Isolated male mice, Forced Swim Test, Amitriptyline, and Depression.

INTRODUCTION

In the recent times depression has been a potential life-threatening disorder affecting major population of the world. The disorder is prevalent to all ages of life. There are multiple symptoms such as sleep and psychomotor disturbances, feeling of guilt, low self-esteem, suicidal motives, autonomic and gastrointestinal disturbances. The excessive search about the pathophysiological cause for the disorder has established it as a complex disorder affecting multiple systems of the brain like altered monoaminergic function, imbalance in hypothalamic-pituitary-adrenal (HPA) axis; brain-derived neurotrophic factor (BDNF), and glutamatergic

neurotransmission [1, 2, 3]. Thorough animal studies have been conducted to elaborate the knowledge about depression. A change in the environment alters the behavioral pattern of animals and the activity changes accordingly [4]. Environmental condition plays a major role in neurobehavioral studies. The housing condition or the acclimatization of the animals is a mandatory requirement for the animals to minimize the abnormal behaviors. Any alteration in the environment provides stress on the animals on experimentation [5]. Emotional change in the animal is a major responsible factor for the loss of memory, impaired cognition, anxiety and depression.

Long-term social isolation is a model for studying the behavioral and neurochemical consequences of rodents deprived of social interaction [6]. Long-term isolation has shown symptoms those observed in depression and anxiety disorders [7]. Offensive and aggressive behavior develops after long-term isolation of male mice [8, 9]. The present study has aimed in finding the depressive condition in the socially isolated male mice and the effect of a tricyclic antidepressant on the depression.

MATERIALS AND METHODS

ANIMALS

Male Swiss Albino mice (obtained from CPCSEA approved animal house Dr B.C. Roy College of Pharmacy and Allied health Sciences, Durgapur, India,) weighing 20-25g were housed in cage (both in groups and individually) under a 12- hr/12-hr light/dark cycle (lights on at 07:00, lights off at 19:00) at a constant temperature of 22 ± 1°C were used for the study. Food and water were available ad libitum for 1 week before the experiments. Mice were handled 1 day before the test. This study was performed according to the guidance of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines and Institutional Animal Ethics Committee (IAEC).

DRUGS AND ADMINISTRATION

Amitriptyline Hydrochloride (Vaikunth Chemicals P. Ltd) was freshly prepared in 0.9% saline at a concentration of 1.6mg/ml. Mice were divided randomly into 7 groups. Group I was colony of 5 male and 3 female mice. Group II was colony of only 5 male mice. Group III was colony of 5 male mice that received Amitriptyline Hydrochloride (4 mg/kg ip). Group IV was colony of 5 male mice that received Amitriptyline Hydrochloride (10 mg/kg ip). In group V, 5 male mice were caged individually. Group VI and VII, separately had 5 male mice in each which were caged individually (one mouse in single cage) and received amitriptyline hydrochloride at a dose of 4mg/kg i.p. and 10 mg/kg i.p. [10], respectively. All groups were housed for 28 days before administration of drug and exposure to Forced swim test. The drugs were administered 30 mins before exposure to Forced swim test to the groups of mice receiving amitriptyline hydrochloride.

ASSESSMENT OF DEPRESSION

The depression in mice was assessed by the Forced Swim Test where the immobility time of mice for the period of 240sec in the water was recorded.

The Forced swim test is the despair method for the study of depression [11]. A cylindrical transparent glass cylinder of height 30cm and diameter 20cm was filled with water upto 15 cm from bottom. The temperature of water was maintained at 23-25°C. The animal was placed slowly and gently in the water by holding the tail. Precautions were being taken such that the animals could not touch the bottom of the cylinder nor it may try to escape from the top. The animal was allowed to remain in water for 6 mins and the immobility time was recorded. A mouse was considered immobile for that fraction of the period when it floated in an upright position without swimming and only small movements were made to keep its head above water for the last 4 mins (240 sec) of the total observation time of 6 mins. After 6 mins the mouse was taken out of the water and the fur was dried with dry cloth and was kept under heat lamp to maintain the temperature at 32°C before transferring to the cage.

STATISTICAL ANALYSIS

The data were analyzed using one-way analysis of variance (ANOVA) followed by Student'st-test. A value of at least 0.05 was considered as the level of significance in all statistical analysis tests.

RESULTS

Table 1: Development of Depression in Different Groups of Mice

Groups	Type of Housing	Immobility Time (sec)
I	Control -Colony of both male and female	87.2 ± 8.1
II	Colony of male mice only	92.6 ± 6.02
III	Colony of male mice receiving amitriptyline 4 mg/kg i.p.	86.8 ± 4.32
IV	Colony of male mice receiving amitriptyline 10 mg/kg i.p.	78.4 ± 5.27
V	Individually caged mice	234.6 ± 9.47 **
VI	Individually caged mice receiving amitriptyline 4 mg/kg i.p.	160.6 ± 11.08**
VII	Individually caged mice receiving amitriptyline 10 mg/kg i.p.	89.8 ± 5.89

*All values are Mean ± SD, n = 5, *P<0.05, **P<0.001 when compared with control*

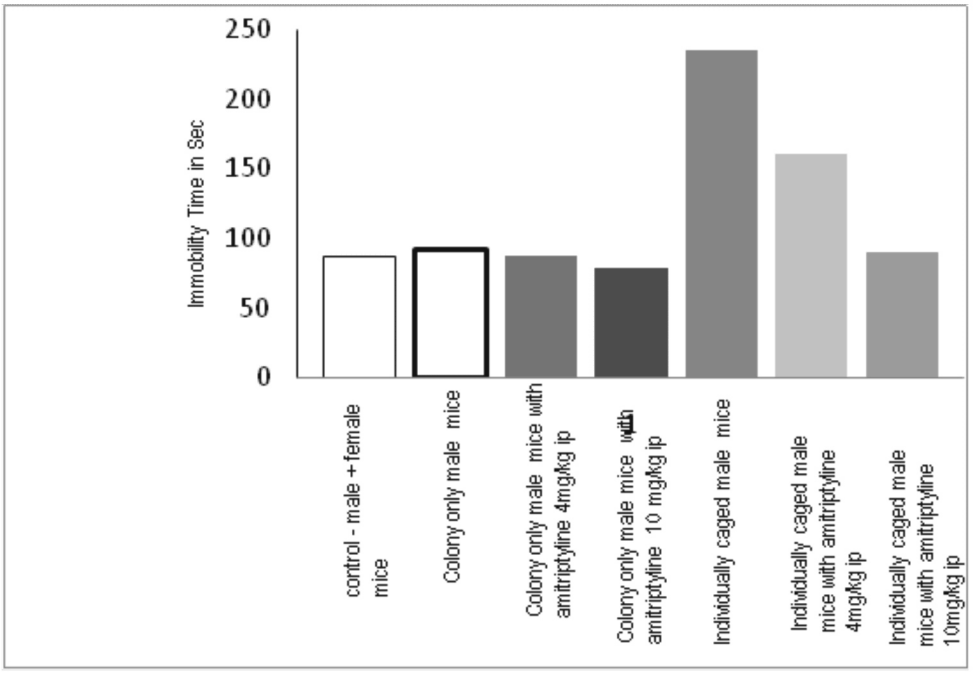

Figure 1: Immobility time in FST for different groups and the effect of amitriptyline

In the present study it was observed that the immobility time in the forced swim test for the Group I and Group II were 87.2 ± 8.1 sec and 92.6 ± 6.02 sec, respectively, where Group I was colony of male and female mice and Group II was colony of only male mice. When amitriptyline hydrochloride was administered to the colony of male mice, Group III and Group IV dose dependently showed immobility time as 86.8 ± 4.32 and 78.4 ± 5.27, respectively more or less similar as compared to the control animals. The immobility time of the Group V was 234.6 ± 9.47 sec which increased with a high significance as compared with the control group. Group VI which received amitriptyline hydrochloride (4 mg/kg i.p.) showed immobility time 160.6 ± 11.08 sec. The immobility was lesser than Group V but significantly higher than the control group. The immobility time for Group VII was 89.8 ± 5.89 sec that was similar to the control group of animals (Fig. 1).

DISCUSSION

In the present study, it was observed (Table 1) that social isolation has developed depression in male mice as compared to the control. Tricyclic antidepressant drug, amitriptyline dose dependently reduced the depression. Moreover, it was observed that male mice in colony did not show any depression and administration of amitriptyline hydrochloride did not alter the behavioral pattern. Thus, it seems that isolated state develops depression as found in the other studies.

The depression was analyzed by Forced swimming test (FST) as this is one of the widely used behavioral models for antidepressant drugs [12]. In the FST model the immobility time for Group II is similar to Group I which may be due to no stress development because of interaction of the mice in a colony. But in the FST of Group V the immobility times of mice were significantly longer than in controls (Group I), indicating the development of depression. Studies have been reported regarding the aggressive behavior developed due to anxiety and depression due to isolation [13]. The depression may be due to anxiety developed in the socially isolated mice [14] as during anxiety or stress the Hypothalamus-Pituitary-Adrenal axis (HPA axis) are involved with elevated Corticotropin releasing hormone level in cerebrospinal fluid, increased volume of pituitary and adrenal release of hormones, and it has been studied that high glucocorticoids, are associated in the pathophysiology of depression [15]. HPA axis also has negative influence on brain derived neurotrophic factor (BDNF) release, one of the important biomolecule responsible for reduction of depression [16, 17]. In depression mainly the 3 monoamine neurotransmitters dopamine, norepinephrine and serotonin [18] are involved. The tricyclic anitidepressants (amitriptyline) are the drug of choice for depression which usually inhibit the uptake of the biogenic amines and changes the receptor sensitivity of the amines [19]. In the colonized mice (Group II) the immobility observed in the FST was not further reduced by both the doses of amitriptyline indicating that the bioamine levels may have remained the same as a result the amitriptyline did not have any influence on the uptake mechanism of the bioamines. Moreover, it can also be inferred that FST did not cause depression at a higher extent that could be altered by amitriptyline.

When the amitriptyline was administrered dose dependently there was decrease in the immobility time where 4mg/kg i.p. though reduced immobility time but was significantly higher than the control, whereas the dose of 10 mg/kg i.p. was able to reduce the immobility time similar to the control mice indicating that higher dose was effective in ameliorating the depressant activity. This was because at higher dose the amitriptyline apart from inhibiting the uptake mechanism of bioamines was also involved in the increase of BDNF concentration in the hippocampus of brain and thereby increases the presynaptic protein production [17]. Moreover, amitriptyline down regulates the glucoorticoid receptor [20] as a result the negative influence of corticosteroid on the BDNF was inhibited.

There is a study reporting the contradictory behavioral results [21] as compared to the present study, where

social isolation increased the anxiety level without any change in depression. The result may be so because the strain specificity (interstrain and intrastrain alteration), test specificity, handling, housing of animal has a strong influence behavioral study.

CONCLUSION

From the present study it could be concluded that social isolation plays a major role in the development of depression which is also influenced by the anxiety activity. The tricyclic antidepressants therapy will show altered behavioral change only if depression is developed. Moreover, the tricyclic antidepressant act not only on the bioamines but also influence the BDNF concentration, the antidepressant factor and reduces corticosteroid level. This mechanism is to be elaborated by vast study on the mechanism of action on the central nervous system.

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