ANXIOLYTIC ACTIVITY OF GABAPENTIN, PREGABALIN, SODIUM VALPROATE AND ALPRAZOLAM IN WISTAR ALBINO RATS - A COMPARATIVE STUDY

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ABSTRACT

Anxiety is a psychological and physiological disorder. It consists of cognitive, somatic and behavioral components. It is characterized by an unpleasant feeling that is typically associated with uneasiness, fear or worry. The primary objective of the study was to compare anxiolytic activities of sodium valproate, gabapentin, pregabalin and alprazolam in rats. Standard experimental models like elevated plus maze and Open field test were used to assess anxiolytic activity. Total 30 rats divided into five groups and six in each (n=6). Group1 rats received gum acacia 10 ml/kg as control, and Gr. 2, 3, 4, 5 received test drugs, gabapentin 50 mg/kg , pregabalin 1.8 mg/kg , sodium valproate 150 mg/kg, alprazolam 0.08 mg/kg, daily, orally, for 10days respectively. The time spent, number of entries, rears in arms of elevated plus maze and number of entries, time spent in central and peripheral squares in open field test were observed. All test drug treated rats showed significant anxiolytic activity by decreasing the time spent in closed arm and increasing in open arm as compared to control. However, increase or decrease in time spent was not comparable among test drug groups. In open field, time spent by rats treated with alprazolam, sodium valproate, gabapentin, pregabalin was increased in the center but decreased in the periphery as compared to control. Thus

alprazolam, sodium valproate, gabapentin, pregabalin were found to produce anti-anxiety in rats compared to control. However, anxiolytic actions were not comparable between test drug treated rats.

KEYWORDS: elevated plus maze, open field test, behavior.

INTRODUCTION
Major affective and anxiety disorders represent the most common psychiatric illnesses and are observed most often by primary-care clinicians. Anxiety is a symptom of many psychiatric disorders. It is also a component of many medical and surgical conditions. The commonly used classes of drugs for the treatment of anxiety are benzodiazepines, azapirones, selective serotonin reuptake inhibitors (SSRIs) and beta blockers.[1]

The benzodiazepines provide rapid relief of generalized anxiety but have a drawback of producing either sedation or dependence, which limits the usefulness of these drugs.[2] Selective Serotonin Reuptake Inhibitors (SSRIs) appear to be at least as effective in acute and perhaps more effective than benzodiazepines in chronic treatment of anxiety disorders. The adverse effects reported by patients treated with SSRIs are loss of libido, delayed sexual orgasm, diminished arousal, an increase in headache, insomnia, weight gain.[3] Azapirones relieve mild to moderate generalized anxiety, but is ineffective in acute or severe cases, in those showing panic reaction and in obsessive compulsive disorders. Beta blockers provide symptomatic relief not affecting psychological symptoms such as worry, tension, and fear etc.[3]

The exact mechanism by which antiepileptic drugs (AEDs) affect behavior is not clear. It is postulated they may affect behavior by various mechanisms which suppress seizures like increased GABA (the main inhibitory neurotransmitter in the brain) activity and also by inhibiting the fast-conducting sodium channels. The sedative AEDs also possess anxiolytic effects.[4] Preliminary reports have shown clinical utility of gabapentin (enhances release of GABA from glia) and tiagabine in the treatment of anxiety disorders.[5] A study has also shown gabapentin as an effective treatment for social phobia.[6] Hence, this study was undertaken to compare anxiolytic effects of sodium valproate, gabapentin, pregabalin with the standard anxiolytics like alprazolam.

MATERIALS AND METHODS

Animals

Male, Wistar albino rats weighing 150-200 g were used in this study. The study was conducted in the Central Animal House, Manipal, Karnataka approved by the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. The rats were kept in polypropylene cages (U.N. Shah Manufacturers, Mumbai, India) and were maintained under standard environmental conditions on a 12:12 hour light-dark cycle. Experiments were conducted during the dark cycle. Standard food pellets diet (Amrut Lab Animal Feed, Pranav Agro Industries Ltd, Sangli, and Maharashtra, India) and water were provided unlimited.

Drugs

Alprazolam 1 mg tab (Torrent Pharmaceuticals Ltd., Gujarat) sodium valproate 200 mg tab (Abbott India Ltd), gabapentin 150 mg cap (Aristo pharmaceuticals pvt ltd) & pregabalin 150 mg tab (Sun Pharmaceuticals Ltd.), were administered in doses based on earlier studies in animals and practical manual of experimental pharmacology.[2, 7, 8] The drugs were procured from the pharmacy store of Kasturba Hospital, Manipal. The control group received 2ml of 2% gum acacia orally. The drugs were dissolved in 2% gum acacia (10 ml/kg) and administered orally. Each drug solution was prepared fresh just before administration.

Experimental design

The study was conducted after obtaining approval by the Institutional Animal Ethics Committee (IAEC/KMC/46/2011-2012). Thirty rats were used and divided into five groups of six in each (n=6). The study was conducted in two phases– acute and chronic. In the acute study, the drugs/vehicle were administered as a single dose 60 minutes prior to the test procedure. In chronic study, drugs/ vehicle were administered orally, once daily, for ten days. The study was carried out 60 minutes after last dose of drugs on the 10th day. To eliminate the possible bias due to odor of previous animal the experimental apparatus was wiped with 10% ethanol after test with each rat.

The drug treatment schedule was as follows

Group 1 - control, received gum acacia10 ml/kg, body weight (b.w.), p.o. (per orally)

Group 2 – received gabapentin 50 mg/kg, (b.w.), p.o.

Group 3 – received pregabalin 1.8 mg/kg (b.w.), p.o.

Group 4 – received sodium valproate 150 mg/kg (b.w.), p.o.

Group 5 – received alprazolam 0.08 mg/kg (b.w.), p.o.

Evaluation of anti- anxiety activity

Two models were used: Elevated plus Maze (EPM) & Open field test (OFT) were used sequentially for each group.

Elevated Plus Maze[9]

The elevated plus maze consists of two open arms (50X10 cm) and two closed arms (50X10X40 cm) elevated 50 cm from the floor with an open roof on central square. The arms of same type are opposite to each other. The drugs were administered to rats as mentioned above. On 10th day, one hour after drug treatment, rat was placed at center of plus maze apparatus facing the open arm. For 5 minutes, following behavior was observed.

A. Number of entries into open and close arms. An entry was defined as the presence of all four paws in the arm. B. Time spent in open and close arms. C. Number of rears in open and close arms.

Open field test[10]

The model consists of a large rectangular box (100 x 80 cm) with 60 cm high walls. The floor had a wired mesh and divided into 25 squares (peripheral 16 and central 9 squares). The box was illuminated with 100 watt bulb placed 60 cm above centre of the field. In the open field test, each animal was placed in one of the peripheral corner square of the box, for 5 minutes, the following behavior was observed.

A. Number of peripheral and central squares crossed. B. Time spent in central squares. C. Number of rears.

STATISTICAL ANALYSIS

All values are expressed as Mean $\pm$ SEM. Data was analyzed using one-way ANOVA followed by Post-hoc Dunnett's test. $P < 0.05$ was considered statistically significant between different groups. The statistical analyses were carried out by using SPSS for Windows (SPSS Software version 17.0).

RESULTS

Table .1: Effect of alprazolam, gabapentin, pregabalin and sodium valproate on the behaviour of rats in elevated plus maze model (acute study)

Group	Dosage and route	No of entries in open arm	No of entries in closed arm	Time spent in open arm (s)	Time spent in closed arm (s)	Rearing
Control	10 ml/kg, (b.w.), p.o.	2.17 ± 0.30	22.33 ± 0.42	40.67 ± 6.0	259.33 ± 6.05	2.17 ± 0.30
Gabapentin	50 mg/kg, (b.w.), p.o.	7.17 ± 0.47*	6.67 ± 0.49*	150.98 ± 7.89*	149.00 ± 7.92*	7.17 ± 0.47*
Pregabalin	1.8 mg/kg (b.w.), p.o.	4.67 ± 0.21*	4.00 ± 0.44	97.00 ± 3.74*	203.00 ± 3.74*	4.17 ± 0.30*
Sodium Valproate	150 mg/kg (b.w.), p.o.	11.17 ± 0.40*	11.00 ± 0.68*	164.33 ± 4.73*	135.67 ± 4.73*	11.00 ± 0.36*
Alprazolam	0.08 mg/kg (b.w.), p.o.	17.00 ± 0.5*ˑ	16.50 ± 0.8*ˑ	228.20 ± 4.66*	71.50 ± 4.83*	2.87 ± 9.32*

Values are expressed as mean ± SEM, n = 6 in each group, *$p<0.05$ Vs control (One way ANOVA followed by Post-hoc Dunnett's test)

Table. 2 Effect of Alprazolam, Gabapentin, Pregabalin and Sodium Valproate on The Behaviour of Rats In Elevated Plus Maze Model (Chronic Study)

Group/drugs	Dosage and route	No of entries in open arm	No of entries in closed arm	Time spent in open arm (s)	Time spent in closed arm (s)	Rearing
Control	10 ml/kg, (b.w.), p.o.	2.33 ± 0.33	22.83 ± 0.40	28.00 ± 3.01	272.0 ± 3.01	2.33 ± 0.42
Gabapentin	50 mg/kg, (b.w.), p.o.	8.67 ± 0.42*	8.17 ± 0.30*	154.67 ± 7.5*	145.17 ± 7.5*	7.5 ± 0.61*
Pregabalin	1.8 mg/kg (b.w.), p.o.	3.50 ± 0.22	2.83 ± 0.30	79.87 ± 8.14*	220.17 ± 8.14*	3.5 ± 0.42*
Sodium Valproate	150 mg/kg (b.w.), p.o.	11.33 ± 0.33*	11.00 ± 0.44*	193.5 ± 3.67*	106.50 ± 3.67*	11.5 ± 0.42*
Alprazolam	0.08 mg/kg (b.w.), p.o.	16.83 ± 0.60*	16.17 ± 0.54*	251.9 ± 2.70*	48.17 ± 2.7*	17.5 ± 0.42*

Values are expressed as mean ± SEM, n = 6 in each group. *$p<0.05$ as compared to control (One way ANOVA followed by Post-hoc Dunnett's test)

Table.3: Effect of Alprazolam, Gabapentin, Pregabalin And Sodium Valproate on The Behaviour of Rats In Open Field Method (Acute Study)

Group	Dosage and route	No of entries in center	No of entries in periphery	No of lines crossed	Time spent in center (s)	Time spent in periphery (s)	Rearing
Control	10 ml/kg, p.o.	2.50 ± 0.42	22.50 ± 0.42	20.83 ± 2.76	15.5 ± 1.47	284.5 ± 1.47	4.00 ± 0.5
Gabapentin	50 mg/kg, p.o.	4.83 ± 0.47	3.83 ± 0.47	60.67 ± 2.31*	66.00 ± 2.59*	234.0 ± 2.59*	4.17 ± 0.47*
Pregabalin	1.8 mg/kg ,p.o.	6.17 ± 0.54*	5.17 ± 0.54	66.17 ± 1.90*	109.00 ± 3.24*	191.00 ± 3.24*	8.00 ± 0.57*
Sodium Valproate	150 mg/, p.o.	12.67 ± 0.80*	11.67 ± 0.80*	93.17 ± 2.62*	174.17 ± 2.62*	125.83 ± 2.62*	12.33 ± 1.02*
Alprazolam	0.08 mg/kg p.o.	18.83 ± 1.13*	18.50 ± 3.01*	122.67 ± 3.7*	209.00 ± 4.25*	91.00 ± 4.25*	18.67 ± 0.84

Values are expressed as mean ± SEM, n = 6 in each group. *$p<0.05$ as compared to control (One way ANOVA followed by Post-hoc Dunnett's test)

Table.4   Effect of Alprazolam, Gabapentin, Pregabalin And Sodium Valproate on The Behaviour of Rats in Open Field Method (Chronic Study)

Group	Dosage and route	No of entries in center	No of entries in periphery	No of lines crossed	Time spent in center (s)	Time spent in periphery (s)	Rearing
Control	10 ml/kg, (b.w.), p.o.	3.67 ± 0.42	23.83 ± 0.40	45.50 ± 3.05	18.3 ± 2.1	281.6 ± 2.1	4.83 ± 0.60
Gabapentin	50 mg/kg, (b.w.), p.o.	7.35 ± 0.22*	6.88 ± 0.25	64.72 ± 0.62*	120.13 ± 1.03*	179.87 ± 1.0*	7.18 ± 0.26
Pregabalin	1.8 mg/kg (b.w.), p.o.	6.38 ± 0.21	5.68 ± 0.21	58.17 ± 0.96*	89.95 ± 1.99*	210.08 ± 1.9*	6.12 ± 0.21
Sodium Valproate	150 mg/kg (b.w.), p.o.	10.07 ± 0.35*	9.93 ± 0.39*	84.40 ± 1.02*	152.77 ± 1.10*	147.23 ± 1.10*	11.93 ± 0.26*
Alprazolam	0.08 mg/kg (b.w.), p.o.	17.15 ± 0.42*	16.05 ± 0.42*	124.5 ± 1.18*	213.13 ± 1.12*	86.87 ± 1.12*	17.28 ± 0.40*

Values are expressed as mean ± SEM, n = 6 in each group. *$p<0.05$ as compared to control (One way ANOVA followed by Post-hoc Dunnett's test)

RESULTS

Elevated Plus Maze

In the Acute study of elevated plus maze model, the number of entries & the time spent in the open arm was significantly ($p<0.05$) increased in alprazolam, sodium valproate, gabapentin, pregabalin treated rats Vs control. The number of entries in the closed arm were significantly decreased in the alprazolam, sodium valproate; gabapentin group Vs control ($p<0.05$). The

time spent in closed arm was significantly decreased (p<0.05) with all the four test drugs treated groups Vs control (Table 1). Whereas, in the chronic study the test drug treated rats i.e. alprazolam, sodium valproate, gabapentin, showed significant increase (p<0.05) in the number of entries in open & closed arm Vs control (p<0.05) (Table 2). When all four drug treated groups of rats were tested, the number of entries and time spent by the rats in open arms were increased and contrarily, these were reduced in the closed arms which was statistically significant Vs control (p<0.05) (Table 1 and 2). However, there was no significant change in the pregabalin treated rats with respect to the number of entries in open arm or closed arm Vs control. There was no significant change in the number of rears in any of the drug treated groups Vs control.

Open Field Test

In both acute and chronic models of open field test, rats treated with alprazolam, sodium valproate, gabapentin showed a significant (p<0.05) decrease in the number of entries and total time spent compared to control in the peripheral area. All the four drugs treated rats exhibited significant increase (p<0.05) in the number of entries and total time spent in center arena Vs control rats (Table 3 and 4). In acute study, all test drugs treated rats showed significant increase in the number of rearing Vs control (p<0.05) (Table 3 and 4) however, chronic study revealed rearing was statistically significant when alprazolam and sodium valproate treated rats were compared Vs control.

DISCUSSION

In the elevated plus maze, due to fear of open spaces rats show preference towards closed arms and avoid open arms.[9,10] Avoidance of the closed arms and increase in the time spent and/or entries into the open arms was due to lack of anxiety.[11] In this study, the increase in time spent in the open arms, decrease in time spent in closed arms and increase in rearing is the result of anxiolytic action exhibited by rats treated with alprazolam, sodium valproate, gabapentin, pregabalin.

The open field test examines anxiety related behavior characterized by the normal aversion of the animal to an open, brightly lit area. Animals removed from their cages when placed in an unknown environment express anxiety, apprehension and fear. Anxiolytic treatments reduce such fearful behavior of animals in open field.[12]

In the open field test, rodents show preference to the periphery of the apparatus.[2] "A decrease in the squares crossed, number of entries and time spent in the central area of the open field indicates anxiety; a decrease in rearing indicates anxiety".[13] In our study, rats successfully increased center zonal activity and also increase in number of rearing. These were the result of anxiolytic action by rats treated with alprazolam, sodium valproate, and gabapentin.

CONCLUSIONS

Anxiolytic activities shown by the drugs sodium valproate, and gabapentin were attributed to the GABA modulatory action in the CNS. However, these results were not comparable to the standard anxiolytic alprazolam. Further, similar studies are needed to authenticate these pharmacological actions of sodium valproate and gabapentin.

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