The Anxiolytic Activity of Gabapentin in Mice

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ABSTRACT
Gabapentin is a new gamma aminobutyric acid (GABA) analogue that has anticonvulsant activity. Preliminary results are promising with gabapentin for the treatment of refractory anxiety disorders. In a supplementary role in several behavioral disorders, especially in acute mania, it has produced significant beneficial results. In this study, the anxiolytic activity of gabapentin was examined in mice. There was a significant decrease in time spent in the central square, an increase in the crossings, and an increase in rearing in the open field with the low-dose gabapentin (10 and 30 mg/kg), implying anxiolysis. Furthermore, potentiation of diazepam with gabapentin was also reported. On screening the gabapentin for learning and memory with passive avoidance response, there was significant prolongation of step-down latency, decrease in step-down error, and decrease in the total time spent by the mice in the shock zone at 10 and 100 mg/kg of gabapentin. Gabapentin was not associated with any adverse effect on the memory of mice.

The lack of effect of gabapentin on memory, exhibiting anxiolytic activity at a low dose, the lack of cognition deficits, lack of drug interaction, alleviating the requirement for plasma monitoring, and its freedom from dependence or abuse liability suggest the potential advantage of gabapentin over the exiting anxiolytics.

INTRODUCTION
Anxiety disorders are the highly prevalent psychiatric disorders, affecting an estimated 25% of the adult population at some point during their lifetime. These psychiatric disorders can be difficult to diagnose in a primary care practice. A large survey found that less than 14% of people with psychiatric disorders receive treatment. Anxiety disorders are highly comorbid, occurring in about 58% of patients with major depressive disorder and 93% of patients with bipolar disorder. Remission is the minimum goal in the treatment of anxiety disorder, which is often chronic. It may take several years before the anxiety disorder is diagnosed. The burden of anxiety disorders is substantial, including not only direct costs of treatment but also the indirect costs of impaired functioning in all aspects of the patient’s life.
In the management of anxiety disorders, medication is used to prevent or reduce the frequency and severity of anxiety attacks and to decrease the anticipatory anxiety precipitated on the withdrawal of the antianxiety drugs. Many anxiety disorders may respond to the treatment but are not treated to remission. The groups of medications commonly used are tricyclic antidepressants (TCAs), benzodiazepines, and monoamine oxidase inhibitors (MAOIs).

Antidepression therapies have produced beneficial affects in the management of anxiety, especially in social anxiety disorder. A large proportion of patients do not tolerate TCAs and MAOIs, whereas benzodiazepines are well tolerated.

These drugs provide only symptomatic relief in certain types of anxiety disorders, and they are usually not effective in preventing relapse and other existing conditions or symptoms with anxiety. Thus, the ultimate goal of therapy full symptom resolution and the patient’s return to normal functioning is unmet with the existing drugs.

Gabapentin is a gamma aminobutyric acid GABA analogue possessing anticonvulsant activity. A recent study shows its secondary role in the treatment of several behavioral disorders. Gabapentin as an adjuvant therapy in treatment of bipolar disorders yielded significant beneficial results.

In posttraumatic stress disorder (PTSD), there are reports in which the addition of gabapentin reduced irritability and increased sleep with reduction in nightmares and flashbacks. The accompanying depression of the patient improved, and there was a reduction in panic attack frequency. One patient with uncontrolled generalized anxiety disorder who was being treated with high doses of diazepam experienced marked improvement in generalized symptoms of worry when the dosage was tapered from diazepam to gabapentin. Moreover, the improvement was sustained at 3 months and the dosage of diazepam was reduced to 10 mg/day.

Gabapentin has also decreased anxiety associated with withdrawal of alcohol. A double-blind, randomized, placebo-controlled trial established the efficacy and safety of gabapentin in relieving the symptoms of social phobia. A significant reduction in the symptoms was observed with gabapentin.

Gabapentin was used to treat 15 alcoholic outpatients who had persistent insomnia after 4 weeks of alcohol abstinence. The dose of gabapentin was titrated to the sleep response of the patients. All patients showed improvement in the quality of sleep and reduction in the daytime anxiety at dosages of 600 to 1500 mg/day of gabapentin. All studies showed that gabapentin was well tolerated.

Several experimental studies have examined the anxiolytic activity and confirmed that it does not impair memory but this needs to be investigated further.

Gabapentin may exert its effects through its structural relationship to GABA, playing an important role in decreasing excitatory input (glutamae)
at the N-methyl-D-aspartate, or NMDA, and [-]α-methyl-3-hydroxy-5-methyl-4-isoxazolepropionic acid, or AMPA, receptors, which are thought to play a role in sensory transmission important in the psychobiology of anxiety in PTSD, arousal, and sleep. On the basis of this limited evidence and the limitations in small sample size and in designing of the reported studies, it is difficult to conclude gabapentin generates anxiolysis. As gabapentin is nonsedating, does not appear to cause cognition deficits or drug interactions, does not require plasma monitoring, and has low dependence or abuse liability, this drug may serve as an alternative in patients where diazepam in contraindicated or is ineffective.30 Gabapentin may be an important adjunct in a complicated psychiatric disorder; however, further studies in a controlled fashion are needed.

There are promising preliminary results with gabapentin in the treatment of refractory anxiety disorders in some patients, but they need to be confirmed. Hence, this study was designed to explore the anxiolytic activity of gabapentin in experimental mice.

METHODS
Research Design and Methodology

A parallel experimental study was performed in the department of pharmacology after approval from the animal ethical committee of the institution.

Subjects
Adult male mice weighing 25 to 30 g bred under the laboratory conditions were used. The animals were housed in plastic cages. Groups of 5 mice per cage were kept under a 12-hour dark and light cycle. They were maintained at a room temperature of 25°C with free access to water and food. The test was conducted between 14 to 17 hours in the daytime (Table 1).

Experimental Models
Habituation in open field
This test utilizes behavioral changes in rodents exposed to novel environments and is used to detect anxiogenic and anxiolytic activity under identical situations. Various types of open-field apparatus have been used to test the mice and rats.

An open-field apparatus, suitable for mice, was composed of a floor space of 40 cm x 40 cm and with 30-cm walls. The floor was colored black and was divided into nine equal squares by white lines. A mouse was placed at the center

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time in Central Square (sec)</th>
<th>Squares Crossed (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.05 ± 1.46</td>
<td>103.25 ± 18.90</td>
</tr>
<tr>
<td>Standard</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(diazepam 1 mg/kg)</td>
<td>4.45 ± 9.83</td>
<td>133.25 ± 39.95</td>
</tr>
<tr>
<td>Test Groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st G-10</td>
<td>0.95 ± 0.39†</td>
<td>134.34 ± 34.11*</td>
</tr>
<tr>
<td>2nd G-30</td>
<td>1.10 ± 1.04*</td>
<td>119.71 ± 26.85*</td>
</tr>
<tr>
<td>3rd G-100</td>
<td>2.9 ± 8.59</td>
<td>104.9 ± 32.12†</td>
</tr>
<tr>
<td>4th D+G (10 mg/kg)</td>
<td>1.05 ± 0.67†</td>
<td>129.8 ± 32.64*</td>
</tr>
</tbody>
</table>

G-10, G-30, and G-100 indicate the test groups at doses of 10 mg/kg, 30 mg/kg, and 100 mg/kg, respectively. D+G (10 mg/kg) indicates the test group receiving diazepam and low-dose gabapentin.

*Significant, P<0.05 as compared with control group.
†Significant, P<0.005 as compared to standard group.
of the field and was left for 2 minutes for acclimatization with the apparatus. Thereafter, for the next 5 minutes, the following parameters were noted: Time spent in the central square, Ambulation (number of squares crossed), Freeze period (periods of immobility) Rearing (number of times the animal stands on the rear paws)

Passive avoidance
This test was performed in a 34 cm x 34 cm x 20 cm chamber with a grid floor through which electric shock of 20 mV was delivered. A shock-free zone (SFZ) was provided in the center of the chamber by the placement of an inverted Petri dish. Mice were placed on the SFZ and when they tried to get down from the SFZ and come in contact with the grid floor, they received an electric shock. Mice gradually learned to avoid shock by staying in the SFZ, curbing their normal exploratory behavior. This is the principle of passive avoidance. The mouse was initially trained until it avoided coming in contact with the shock zone by passively sitting in the SFZ for a minimum of 60 seconds. The mice that did not learn in 5 training sessions were removed from the study.

The parameters noted are:
• Step-down latency (duration for which the animal stays in the SFZ).
• Step-down error (number of attempts the animal makes to come to the shock zone).
• Total time spent in the shock zone.

The animals were administered respective drugs/normal saline intraperitoneally as scheduled, and the test was conducted using experimental models, 30 minutes after administration.

Statistical Analysis
Wilcoxon’s signed rank test was used for comparative analysis of the test groups with the control and standard groups.

RESULTS
Open-field model
The following observations were drawn on the parameters from the open-field habituation test:

Time spent in the central square. The time spent in the central squares in the control and standard (diazepam) groups was $2.05 \pm 1.46$ sec and $4.45 \pm 9.83$ sec, respectively; however, this difference was not significant. There was a signifi-

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**Table 3. Observations in Open-Field Model**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Freezing (sec)</th>
<th>Rearing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.85 ± 3</td>
<td>30.95 ± 9.5</td>
</tr>
<tr>
<td>Standard</td>
<td>2.4 ± 7.06</td>
<td>39.6 ± 12.09</td>
</tr>
<tr>
<td>Test groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st G-10</td>
<td>0.3 ± 1.13</td>
<td>49.9 ± 14.37*</td>
</tr>
<tr>
<td>2nd G-30</td>
<td>1.86 ± 2.03*</td>
<td>33.47 ± 9.26</td>
</tr>
<tr>
<td>3rd G-100</td>
<td>8.05 ± 14.97*</td>
<td>27.2 ± 9.40†</td>
</tr>
<tr>
<td>4th D+G (10 mg/kg)</td>
<td>4.35 ± 11.25</td>
<td>47.25 ± 17.04*</td>
</tr>
</tbody>
</table>

G-10, G-30, and G-100 indicate the test groups at doses of 10 mg /kg, 30 mg/kg, and 100 mg/kg, respectively. D+G (10 mg/kg) indicates the test group receiving diazepam and low-dose gabapentin.

*Significant, $P<0.05$ compared with control group.

†Significant, $P<0.005$ compared with standard group.
cant decrease in time spent in the central square in the test groups that received gabapentin 10 mg/kg, 30 mg/kg as well in the test group that received low-dose gabapentin + diazepam as compared with the control group. Diazepam did not reveal any significant decrease in the times spent in the central square.

The low-dose gabapentin 10 mg/kg alone and in combination with diazepam depicted a significant decrease in time in the central square as compared with the standard diazepam group (Table 2).

**Number of squares crossed.** There was a significant increase in the number of squares crossed with diazepam, and with gabapentin at doses of 10 mg/kg and 30 mg/kg, but not at 100 mg/kg as compared with the control group. A similar finding was observed in the fourth test group with low-dose gabapentin + diazepam.

However, a significant decrease in the locomotor activity was observed with high-dose gabapentin 100 mg/kg compared with diazepam (Table 2).

**Freezing.** There was an increase in the time spent in the freezing behavior of the animals in the test groups receiving 30 mg/kg and 100 mg/kg of gabapentin as compared with the control group (Table 3).

**Rearing.** A significant increase in the rearing behavior of the animals was observed with the 10 mg/kg of gabapentin alone and with the gabapentin + diazepam groups in comparison with the control group. In contrast, a significant decrease in the rearing was observed in the high-dose gabapentin (100 mg/kg) compared with standard group (Table 3).

On the basis of these findings, especially the number of crossings and the incidence of rearing, the anxiolytic activity of gabapentin was observed at 10 and 30 mg/kg as well as in combination with diazepam as compared with control. However, decreases in crossings and rearing were observed at 100 mg/kg of gabapentin compared with standard. This suggests the paradoxical anxiogenic effect of gabapentin at high doses. No dose-dependent potentiation of anxiolytic behavior was observed with gabapentin.

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**Table 4. Observations in Passive Avoidance Test**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Step-Down Latency(s)</th>
<th>Step-Down Error</th>
<th>Time in Shock Zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>107 ± 89.56</td>
<td>11.9 ± 8.5</td>
<td>9.9 ± 11.47</td>
</tr>
<tr>
<td>Standard</td>
<td>188.25 ± 111.83</td>
<td>12.95 ± 6.28</td>
<td>37.85 ± 81.20</td>
</tr>
<tr>
<td>Test groups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; G-10</td>
<td>186.8 ± 123.9&lt;sup&gt;*&lt;/sup&gt;</td>
<td>5.4 ± 4.53&lt;sup&gt;†&lt;/sup&gt;</td>
<td>8 ± 8.93</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; G-30</td>
<td>125.4 ± 120.5</td>
<td>9.1 ± 9.87</td>
<td>13.25 ± 39.08</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt; G-100</td>
<td>228.3 ± 115.77&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>4.7 ± 7.15&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>1.4 ± 3.80&lt;sup&gt;‡&lt;/sup&gt;</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt; D+G</td>
<td>259.25 ± 81.55&lt;sup&gt;‡‡&lt;/sup&gt;</td>
<td>2.3 ± 1.72&lt;sup&gt;‡‡&lt;/sup&gt;</td>
<td>1.1 ± 2.31&lt;sup&gt;‡‡&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

G-10, G-30, and G-100 indicate the test groups at doses of 10 mg /kg, 30 mg/kg, and 100 mg/kg, respectively. D+G (10 mg/kg) indicated the test group receiving diazepam and low-dose gabapentin.

<sup>*</sup>Significant, P<0.05 as compared with control group.

<sup>‡‡</sup>Significant, P<0.005 as compared with standard group.
Passive Avoidance Response
The following observations were made from the passive avoidance test (Table 4).

Step-down Latency. A significant prolongation of the step-down latency was observed in the gabapentin 10 mg/kg, gabapentin 100 mg/kg, and low-dose gabapentin + diazepam groups compared with the control group. The combination group receiving low-dose gabapentin + diazepam, only demonstrated a significant prolongation of step down latency as compared with diazepam.

Step-down error. There was a significant decrease in step-down errors in gabapentin 10 mg/kg, gabapentin 100 mg/kg, and low-dose gabapentin + diazepam groups compared with standard and control groups.

Time spent in shock zone. A decrease in the time spent in shock zone was observed in the gabapentin 100 mg/kg and the low-dose gabapentin + diazepam test groups compared with both the standard and control groups. With diazepam, there was an insignificant increase in the time spent by the animals in shock zone as compared to the control group (Table 4).

On the basis of these findings from passive avoidance, anxiolytic activity was observed at 10 mg/kg and 100 mg/kg of gabapentin. There was a potentiation of the response of the low-dose gabapentin + diazepam. No significant effect was demonstrated at 30 mg/kg of gabapentin alone and diazepam in the passive avoidance task.

DISCUSSION
Exposure to a novel environment is associated with emotional disturbance and anxiety. An anxious animal shows reduced locomotion associated with periodic freeze or immobility, a preference for peripheral areas near the boundary, and a reduction in normal behavioral patterns such as rearing and grooming, which are studied in an open-field test. Anxiety is also associated with augmented autonomic activity resulting in increased defecation and urination.

Increase of the time spent in the central area as well as the ratio of central/total locomotion or decreases of latency to enter the central part are indications of anxiolysis in an open field. In the passive avoidance test, the animal avoids punishment by refraining from making a specified response, ie, by staying in the SFZ. A decrease in step-down latency and an increase in step-down errors indicate reduction of normal anxiety associated with exposure to a novel environment. All these effects are accentuated by anxiogenic drugs and attenuated by anxiolytic ones. An open-field test and passive avoidance behavior are some of the standard procedures used to screen the anxiolytic effects of drugs in comparison with a standard such as diazepam. The open-field test and passive avoidance test are simple, sensitive, and reproducible, and they are effective in screening different classes of anxiogenic and anxiolytic agents. However, extreme caution should be exercised in the handling of animals, sound proofing the system, and the doses adopted do not adversely affect motor activity, thereby making the test results unreliable.

This study establishes the anxiolytic effect of gabapentin in animal models of anxiety. However, these effects were pronounced at doses of 10 mg/kg and 30 mg/kg but not at high doses of 100 mg/kg of gabapentin in the open field. This finding is contrary to De-Paris et al reporting maximum anxiolysis at 100 mg/kg of gabapentin. These results are in agreement with Singh and colleagues who reported effective anxiolytic-like
behavior of gabapentin at 30 mg/kg, indicated by an increase in the rearing, number of crossings, and a decrease in the time spent by the mice in central square. In contrast, gabapentin 100 mg/kg did cause a nonsignificant trend to decrease rearing in the open-field training session. This tendency could be attributed to the sedative effect of this drug at this dose.

On screening the drugs for learning and memory with passive avoidance response, it was observed that gabapentin has no effect on memory at all doses used. There was significant prolongation of step-down latency, decrease in step-down error, and time spent by the animal in the shock zone at 10 mg/kg alone and in combination with diazepam and 100 mg/kg of gabapentin. However, with diazepam there was increase in the time spent in shock zone by the mice but this was not significant. An early report exhibited a mixed response of diazepam in PAR, with significant decrease in step-down error, locomotor activity, and an increase in the time in shock zone. The effect of gabapentin at 100 mg/kg and low-dose gabapentin (10 mg/kg) in combination with diazepam was superior to diazepam alone.

In this study we used a simple open-field model that is more sensitive to anxiolytic effect produced by classical benzodiazepines, but it has produced inconsistent results with serotonergic drugs. However, the efficacy of 5-HT\textsubscript{1A} receptor serotonergic agonist in general anxiety disorder is well established and clinical trials suggest anxiolytic action of these agents. A modified open field that can observe drug-induced changes in feeding behavior and locomotor activity would be more appropriate.

The lack of adverse effect of gabapentin on memory suggests its potential advantage over the existing anxiolytics, which show amnesia at doses used for the treatment of anxiety disorders.

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