Gabapentin attenuates acute hypoxic stress–induced behavioral alterations and oxidative damage in mice: Possible involvement of GABAergic mechanism

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The effect of gabapentin has been investigated on acute hypoxic stress-induced behavioral alterations and oxidative damage in mice. Mice were subjected to hypoxia for 2 hr. Treatment with gabapentin (50 and 100 mg/kg) significantly increased ambulatory movements, exerted anti-anxiety like effect and reduced oxidative damage in mice subjected to acute hypoxic stress. Treatment with picrotoxin (1.0 mg/kg) per se had no significant effect on behavioral and biochemical parameters of stressed mice. Treatment with muscimol (0.05 mg/kg) per se significantly increased the locomotor activity of stressed mice, exerted significant anti anxiety effect and significantly reduced the oxidative damage. Further, pretreatment with picrotoxin (1.0 mg/kg) significantly blocked whereas pretreatment with muscimol (0.05 mg/kg) significantly potentiated the neuroprotective effect of gabapentin. These results suggest that gabapentin produces its neuroprotective effect in mice subjected to acute hypoxic stress through GABA_A receptor mechanism.

Keywords: Anxiety, Gabapentin, Hypoxic stress, Lipid peroxidation, Locomotor activity, Muscimol, Picrotoxin

Hypoxia causes oxidative damage in different body organ systems including brain. Hypoxic stress can cause cellular damage and neuro-degeneration by inducing the reactive oxygen species (ROS) that oxidize vital cellular components such as lipids, proteins and DNA. Gamma–amino–butyric-acid (GABA) is one of the major inhibitory neurotransmitter for fast inhibitory synaptic transmission and regulates many physiological and psychological processes. Role of GABAergic system in stress and related conditions has been well documented. GABAergic system is influenced significantly during stress, particularly the GABA-benzodiazepine receptor binding sites. The antiepileptic drug, gabapentin is a lipophilic analogue of the inhibitory neurotransmitter GABA and being highly lipid soluble, it readily crosses the blood brain barrier. Gabapentin is reported to exhibit neuroprotective activity in posttraumatic stress disorders. However, its exact mechanism of neuroprotective action is still unknown. The present study was designed to investigate the neuroprotective effect of gabapentin on acute hypoxic stress-induced behavioral alterations and oxidative damage in mice and to elucidate the mechanism involved in its neuroprotective action.

Materials and Methods
Albino mice (Laca strain) weighing between 22-30 g bred in Central Animal House (CAH) facility of the Panjab University, Chandigarh, India were used. The animals were housed under standard laboratory conditions and maintained on natural light and dark cycle and had free access to food and water. Animals were acclimatized to laboratory conditions before the experiment. Each group consisted of 6 animals. All experiments were carried out between 0900 and 1500hrs. The experimental protocols were approved by Institutional Animal Ethics Committee (IAEC) and conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals. Hypoxic stress in mice was induced for a period of 2 hr. Slow deprival of oxygen resulted in increased respiratory rate, escape attempts and tremors.

Gabapentin (50 and 100 mg/kg, ip), picrotoxin (1.0 mg/kg) and muscimol (0.05 mg/kg) were dissolved in distilled water. To study their per se effects they were administered (1 ml/100g body weight) intraperitoneally 30 min before animals were subjected to hypoxic stress. For the interaction studies, picrotoxin or muscimol was administered 10
min before gabapentin treatment. Control animals received requisite volume of water (1 ml/100g body weight) intraperitoneally 30 min before being subjected to hypoxic stress. Animals were divided into eight groups, consisting of six animals in each. First and second group were distilled water treated naïve (without any stress) and control (stressed) respectively. Third to sixth group received gabapentin in a dose of 50 mg/kg, and 100 mg/kg, picrotoxin (1mg/kg) and muscimol (0.05 mg/kg) respectively. Seventh group received picrotoxin (1mg/kg) followed 10 min later by gabapentin (50 mg/kg) whereas the eighth group received muscimol (0.05mg/kg) followed 10 min later by gabapentin.

Various behavioral parameters like locomotor activity and mirror chamber test to evaluate anxiety were carried out. The locomotor activity for a period of 5 min was recorded using actophotometer and expressed in terms of total counts per 5 min. Mirror chamber test was used to evaluate anxiety. The apparatus consisted of a wooden box having a mirror chamber enclosed in it. Animals were placed individually at the distal corner of the mirror chamber at the beginning of each 5 min test session in which the following parameters were noted (a) latency to enter the mirror chamber, (b) total time spent in mirror chamber and (c) number of entries in mirror chamber. An anxiogenic response was defined as decreased number of entries and time spent in the mirror chamber.10

At the end of the last behavioral test, all animals were sacrificed by decapitation to carry out the biochemical estimations. The brains were removed, rinsed in isotonic saline and weighed. A 10% (w/v) tissue homogenate was prepared with 0.1 M phosphate buffer (pH 7.4). The post nuclear fraction was obtained by centrifugation of the homogenate at 12000×g for 20 min at 4°C. The biochemical parameters measured were lipid peroxidation, reduced glutathione levels, nitrite levels and catalase activity.

Lipid peroxidation in the whole brain was measured according to the method of Wills.11 The amount of malondialdehyde formed was measured by the reaction with thiobarbituric acid at pH 3.5. The pink color that developed was estimated by measuring the absorbance at 532 nm using Perkin Elmer lambda 20 spectrophotometer. The results were expressed as nanomole of malondialdehyde per milligram protein using the molar extinction coefficient of chromophore (1.56 x 10M⁻¹ cm⁻¹).

Reduced glutathione, a major physiological antioxidant in the brain, was estimated according to the method of Ellman. A 1.0 ml of homogenate was precipitated with 1.0 ml of 4% sulfosalicylic acid by keeping the mixture at 4°C for 1hr and the samples were immediately centrifuged at 1200 × g for 15 min at 4°C. The assay mixture contained 0.1ml of supernatant, 2.7 ml of phosphate buffer of pH 8.0 and 0.2 ml of 0.01M-dithiobisnitrobenzoic acid (DTNB). The yellow colour developed was read immediately at 412nm using Perkin Elmer lambda 20 spectrophotometer. The results were expressed as nanomole GSH/mg protein.

Nitrite is the stable end product of nitric oxide (NO) metabolism in living system. Accumulation of nitrite was measured according to the method of Green et al. Briefly, the supernatant from the brain homogenate was incubated at room temperature for 10 min with Greiss reagent 15 (1% sulphanilamide/ 0.1% naphthylethylediamine dihydrochloride/ 2.5% phosphoric acid) to yield a chromophore. Absorbance was read at 543 nm spectrophotometrically. The nitrite concentration was calculated from a standard curve using sodium nitrite as standard and expressed as micro molar nitrite per milliliter homogenate. Catalase activity was assayed by the method of Luck wherein the breakdown of hydrogen peroxide (H₂O₂) is measured at 240 nm. Briefly, the assay mixture consisted of 3 ml of H₂O₂ phosphate buffer and 0.05 ml of supernatant of tissue homogenate (10%), and the change in absorbance was recorded at 240 nm. The results were expressed as micromole H₂O₂ decomposed per milligram of protein/min. Lastly, the protein content was measured according to the method of Lowry et al using bovine serum albumin as standard. All values are expressed as mean±SE. The data were analyzed by using one way analysis of variance (ANOVA) followed by Tukey’s test. P<0.05 was considered statistically significant.

Results
Animals subjected to 2 hr acute hypoxic stress exhibited significant decrease in locomotor activity (as indicated by decreased ambulatory movements) (Fig 1) and an increase in anxiety like behavior (increased latency to enter in mirror chamber, decreased number of entries and time spent in the mirror chamber) (Table 1) as compared to naïve mice.

Treatment with gabapentin (50 and 100 mg/kg, ip) significantly increased ambulatory movements (Fig. 1) and exerted anti-anxiety like effect (decreased time
latency to enter in mirror chamber, increased number of entries and duration in mirror chamber) in the stressed mice (Table 1). The effects were significant ($P<0.05$) as compared to control mice subjected to hypoxic stress. Treatment with picrotoxin (1 mg/kg) had no significant effect on the locomotor activity of the stressed mice (Fig. 1) and also did not exert significant anti anxiety effect (Table 1). However, pretreatment with picrotoxin (1 mg/kg) blocked the locomotor activity enhancing and anti anxiety effects of gabapentin (50 mg/kg) (Fig. 1, Table 1). Treatment with muscimol (0.05 mg/kg) per se significantly increased the locomotor activity of stressed mice (Fig. 1) and also exerted a significant anti anxiety effect (Table 1). Pretreatment with muscimol (0.05 mg/kg) resulted in further enhancement of locomotor activity and anti anxiety effects of gabapentin (50mg/kg) in the stressed mice and the enhancement of these effects was significant ($P<0.05$) as compared to the per se effects of gabapentin (50 mg/kg) and muscimol (0.05 mg/kg) (Fig. 1, Table 1).

The study also demonstrated that two hours of hypoxic stress significantly ($P<0.05$) increased malondialdehyde and nitrite levels and decreased malondialdehyde and nitrite levels and decreased

![Graph showing effect of gabapentin (GBP), picrotoxin (PTX), muscimol (MUS) and combination of GBP with PTX or MUS on locomotor activity of mice subjected to acute hypoxic stress.]

**Table 1**—Effect of gabapentin (GBP), picrotoxin (PTX), muscimol (MUS) and combination of GBP with PTX or MUS as determined by mirror chamber test in mice subjected to acute hypoxic stress.

<table>
<thead>
<tr>
<th>Drug treatment (mg/kg)</th>
<th>Latency to enter mirror chamber (in seconds)</th>
<th>No. of entries in mirror chamber</th>
<th>Time spent in mirror chamber (in seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naïve</td>
<td>39.0±8.81</td>
<td>5.0±0.71</td>
<td>120.0±10.21</td>
</tr>
<tr>
<td>Control (hypoxia)</td>
<td>98.0±4.16</td>
<td>1.56±0.29</td>
<td>1.98±0.86</td>
</tr>
<tr>
<td>GBP (50)</td>
<td>61.0±5.21</td>
<td>3.17±0.29</td>
<td>77.0±5.16</td>
</tr>
<tr>
<td>GBP (100)</td>
<td>45.0±1.6</td>
<td>4.12±0.39</td>
<td>91.0±3.17</td>
</tr>
<tr>
<td>PTX (1.0)</td>
<td>106.0±5.12 NS</td>
<td>0.98±0.047 NS</td>
<td>0.99±0.03 NS</td>
</tr>
<tr>
<td>PTX (1.0) + GBP (50)</td>
<td>88.0±4.78 NS</td>
<td>1.17±0.12 NS</td>
<td>56.0±4.97 NS</td>
</tr>
<tr>
<td>MUS (0.05)</td>
<td>68.0±4.19 b</td>
<td>2.13±0.45 b</td>
<td>68.33±5.12 b</td>
</tr>
<tr>
<td>MUS (0.05) + GBP (50)</td>
<td>47.0±3.79 c</td>
<td>4.99±0.26 c</td>
<td>88.0±6.77 c</td>
</tr>
</tbody>
</table>

*aP<0.05 as compared to naïve, bP<0.05 as compared to control (hypoxia), cP<0.05 as compared to GBP (50 mg/kg), dP<0.05 as compared to GBP (50 mg/kg) and PTX (1 mg/kg), eP<0.05 as compared to GBP (50 mg/kg) and MUS (0.05 mg/kg) (One-Way ANOVA followed by Tukey’s test), NS=non-significant as compared to control
reduced glutathione level and catalase activity as compared to the group of naïve mice. Treatment with gabapentin (50 and 100 mg/kg) significantly ($P<0.05$) reduced the oxidative damage (decreased malondialdehyde and nitrite levels and increased reduced glutathione level and catalase activity) as compared to control group mice subjected to hypoxic stress (Table 2).

Treatment with picrotoxin (1 mg/kg) had no significant effect on the oxidative stress parameters of the stressed mice (Table 2). However, pretreatment with picrotoxin (1 mg/kg) decreased the neuroprotective effect of gabapentin (50 mg/kg) (Table 2). Treatment with muscimol (0.05 mg/kg) exerted significant ($P<0.05$) neuroprotective effects (Table 2). Pretreatment with muscimol (0.05 mg/kg) resulted in further enhancement of neuroprotection afforded by 50 mg/kg gabapentin. The enhancement of these effects was significant ($P<0.05$) as compared to the per se effects of gabapentin (50 mg/kg) and muscimol (0.05 mg/kg) (Table 2).

Discussion
Gabapentin has been reported to exert a neuroprotective effect in posttraumatic stress disorders\(^7\). However, the exact mechanism underlying its neuroprotective action is still unknown. Gabapentin increases the rate of synthesis of GABA in brain and may thus enhance non-vesicular release of GABA\(^16\). Hypoxic-ischemic insult has been reported to influence neurobehavioral performance, including sensorimotor and locomotor function induce anxiety and impair cognitive ability in rat\(^17\). In the present study also, hypoxic stress of two hours caused significant decrease in locomotor activity, induced anxiety like behavior and produced oxidative damage in the animals. Treatment with gabapentin significantly counteracted the locomotor suppressant and anxiety inducing effect of acute hypoxic stress and also reduced the oxidative damage produced by acute hypoxic stress. These results indicate that gabapentin exerts neuroprotective effect in mice subjected to acute hypoxic stress. GABAergic system is involved in stress related defense mechanisms\(^18\). In the present study the GABA\(_A\) receptor antagonist, picrotoxin per se had no significant effect on the locomotor suppressant, anxiety inducing and oxidative damage producing effects of acute hypoxic stress. However, pretreatment with picrotoxin did result in blockade of the neuroprotective effect of gabapentin. Muscimol, the directly acting GABA\(_A\) receptor agonist, per se exerted significant neuroprotective effect in the mice subjected to acute hypoxic stress. Further, pretreatment with muscimol resulted in enhancement of the neuroprotective effect of gabapentin and the enhancement was significant as compared to the per se neuroprotective effects of gabapentin and muscimol. These results indicate the involvement of GABA\(_A\) receptor mechanisms in the neuroprotective action of gabapentin. Several investigations have confirmed that gabapentin has the ability to increase the GABA content of the brain and thereby influence GABA receptor activity\(^19\). An increase in GABAergic activity by gabapentin would promote inhibitory neurotransmission in the central nervous system. This is a potent mechanism by which

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>LPO (moles of MDA/mgpr) (% of control)</th>
<th>Red. GSH (micromoles of GSH/mgpr) (% of control)</th>
<th>Catalase (µ M of H(_2)O(_2)/min/mgpr) (% of control)</th>
<th>Nitrite (µg/ml) (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naïve</td>
<td>100 ± 0.0314</td>
<td>100 ± 0.0018</td>
<td>100 ± 0.033</td>
<td>100 ±2.94</td>
</tr>
<tr>
<td>Control (hypoxia)</td>
<td>386 ± 0.042(^a)</td>
<td>29 ± 0.0023(^a)</td>
<td>20 ± 0.051(^a)</td>
<td>217 ± 3.84(^a)</td>
</tr>
<tr>
<td>GBP (50)</td>
<td>246 ± 0.021(^b)</td>
<td>51 ± 0.0019(^b)</td>
<td>71 ± 0.033(^b)</td>
<td>162 ±3.59(^b)</td>
</tr>
<tr>
<td>GBP (100)</td>
<td>188 ± 0.022(^bc)</td>
<td>81 ± 0.0029(^bc)</td>
<td>76 ± 0.048(^b)</td>
<td>130 ±3.69(^bc)</td>
</tr>
<tr>
<td>PTX (1.0)</td>
<td>422 ± 0.058(^NS)</td>
<td>20 ± 0.0011(^NS)</td>
<td>17 ± 0.011(^NS)</td>
<td>225 ±5.98(^NS)</td>
</tr>
<tr>
<td>PTX (1.0) + GBP (50)</td>
<td>403 ± 0.047(^d)</td>
<td>37 ± 0.0012(^d)</td>
<td>19 ± 0.011(^d)</td>
<td>178 ±4.56(^d)</td>
</tr>
<tr>
<td>MUS (0.05)</td>
<td>288 ± 0.019(^b)</td>
<td>41 ± 0.0016(^b)</td>
<td>54 ± 0.029(^b)</td>
<td>186 ±4.16(^b)</td>
</tr>
<tr>
<td>MUS (0.05) + GBP (50)</td>
<td>207 ± 0.024(^e)</td>
<td>65 ± 0.0033(^e)</td>
<td>78 ± 0.047(^e)</td>
<td>150 ±3.98(^e)</td>
</tr>
</tbody>
</table>

\(^a\)P<0.05 as compared to naïve, \(^b\)P<0.05 as compared to control (hypoxia), \(^c\)P<0.05 as compared to GBP (50 mg/kg),
\(^d\)P<0.05 as compared to GBP (50 mg/kg) and PTX (1 mg/kg), \(^e\)P<0.05 as compared to GBP (50 mg/kg) and MUS (0.05 mg/kg) (One-way ANOVA followed by Tukey’s test), \(^NS\)= non-significant as compared to control

LPO= Lipid peroxidation, MDA= Malondialdehyde, Red GSH= Reduced glutathione
gabapentin acts as a neuroprotector. The other possible explanation for the effect of gabapentin is a drug-induced decrease in glutamatergic neurotransmission\(^2\).  

References