Abstract

The present study was performed to investigate whether or not carvedilol (a β-adrenoreceptor antagonist) potentiates the anticonvulsive activity of gabapentin against ICES (Increasing current electroshock) and PTZ (Pentylenetetrazole) induced seizures in mice. Further the effect of combination of both the drugs on spatial working memory and locomotor activity on rotarod was also evaluated. The biochemical estimation was done by measuring the lipid peroxidation and reduced glutathione in brain tissue. The results indicate that carvedilol significantly potentiates the anticonvulsive activity of gabapentin in both the models of epilepsy. The combination of both the drugs has no effect on spatial working memory and locomotor activity. In addition carvedilol in combination with gabapentin significantly decreased the level of the lipid peroxidation and increased the level of reduced glutathione (GSH) in brain. So, the present study showed that carvedilol potentiates the anticonvulsive activity of gabapentin, which can be useful for the treatment of epilepsy in patients with hypertension.

Key words: Carvedilol; Gabapentin; Pentylenetetrazole; Increasing current electroshock.

Introduction

Hypertension is the most prevalent modifiable risk factor for both ischemic and hemorrhagic stroke, which is often associated with epilepsy. Importantly, stroke and epilepsy are significant predictors of reduced total and disability-free life expectancy (Hesdorffer et al., 1996). However, classic anti-epileptic drugs (AED) therapy is neither universally effective nor invariably safe (Ali et al., 2004). So far research in epilepsy has focused largely on GABAergic and glutamergic neurotransmission as paradigms of inhibitory and excitatory elements of central nervous system activity (Sarro et al., 1998).

Apart from the well-documented clinical cardiovascular and non-vascular applications of β-adrenoceptor antagonist, there is growing body of evidence showing that the activation of central β-adrenoceptors may also be involved in the development/progress of epileptic phenomena. The high density of β-adrenoceptors occurs in all the subfields of the hippocampus (Reznikoff et al., 1986) known for its low seizure threshold and dominant role in the propagation of seizures (McNamara, 1994). The recent study showed that elevated, endogenous noradrenergic transmission is an etiological factor in some cases of epilepsy (Fitzgerald., 2010). The noradrenergic system was demonstrated to participate in the occurrence of seizures in epileptic EL mice and to increase epileptiform discharges in rat limbic system via. β-adrenergic receptor stimulation (Stoop et al., 2000). The studies show that central β-adrenoceptors activation may lead to the facilitation of excitatory amino acids release and hence, modulate synaptic transmission (Herrero et al., 1996). One study showed that propranolol increased the threshold for lidocaine – induced convulsion by directly acting on brain (Nakamura et al., 2008). Another study showed that propranolol and metoprolol enhance the anticonvulsant action of valproate and diazepam against maximal electroshock (Luchowski et al., 2002). Some β-adrenoceptor antagonists were demonstrated to enhance the antiepileptic activity of swim stress in the model of convulsions generated by picrotoxin administration (Pericic et al., 2000). Moreover, β-adrenoceptor antagonists, especially propranolol display anticonvulsant effects, raising the threshold for electroconvulsions and protecting against maximal electroshock- and pentylenetetrazol-induced seizures (Fischer et al., 1985). The study showed that increased hippocampal noradrenergic neurotransmission increased limbic seizures via α(1A)- and α(1D)-adrenoreceptors (Clinckers et al., 2010).
Carvedilol is an antihypertensive drug with non-selective β-adrenergic and associated pleiotropic effects [antioxidant activity, α-adrenoceptor blocking activity, vasodilatation (Abreu et al., 2000; Savitz et al., 2000), anti-inflammatory (Yaoita et al., 2000), non-competitive inhibitor of N-methyl D-aspartate receptor and calcium channel blockers (Lysko et al., 1992)]. Carvedilol has been shown to exert neuroprotective effects in several models of transient focal stroke (Savitz et al., 2000). One study showed that selective overexpression of the alpha 1β-adrenoceptor is associated with increased in vivo spontaneous interictal epileptogenicity and EEG/behavioral seizures (Kunieda et al., 2002). Gabapentin, an analog of γ-aminobutyric acid, exhibits anticonvulsant properties in both animal models and humans. It is a lipophilic derivative and crosses to the brain. It modifies MES (Maximal electroshock) and inhibits PTZ (Pentylenetetrazole) induced clonic seizures (Loscher et al., 1991). Gabapentin was shown to exert pronounced anticonvulsant effect at non-toxic doses against various types of seizures. Indeed, gabapentin appears to be one of the antiepileptics which might offer advantages to drugs currently used in antiepileptic therapy (Loscher et al., 1988).

Thus the present study examines the effect of carvedilol on anticonvulsant effect of gabapentin on two different model of epilepsy for generalized seizures. Spontaneous alternation behavior (SAB) was done to test spatial working memory as it is not representative of other aspects of memory function.

Further, as neurological deficits such as motor impairment are often observed with existing AED’s. We investigated the effect of carvedilol with gabapentin on locomotor activity using the rotarod test. More over the effect of carvedilol with gabapentin was also evaluated on lipid peroxidation and reduced glutathione in brain.

So, the aim of present study is to find out an effective and efficient drug combination for cases where risk factors like hypertension and/or stroke might precipitate seizures, especially for the elderly who are at great risk of cerebrovascular-induced seizures leading to seizures freedom and restoration of confidence and independence.

Materials and Methods

Animals

Total 144 male albino swiss strain mice weighing 18-30 g were used. Animals were housed in groups of 5-10 per cage and maintained at 20-30°C and 50-55% humidity in a natural light and dark cycle, with free access to food and water. The experiments were performed during the light cycle in awake, freely moving animals that were adjusted to laboratory conditions before proceeding with the experiments. Animals were procured from the central animal house, of the institute. The project was undertaken with prior approval from the Institutional Animal Ethical Committee.

Drugs

The drugs used were carvedilol (Carca, Intas Pharmaceuticals, India) gabapentin (Gabapin, Intas Pharmaceuticals, India) and a chemoconvulsant pentylenetetrazole (Sigma, USA). Carvedilol and gabapentin were suspended in 0.25% of carboxy methyl cellulose (CMC) in 0.9% saline solution and was freshly prepared prior to administration. All the drugs were given in volumes of 10 ml/kg. Carvedilol was administered at a dose of 1.25, 2.5 and 5.0 mg/kg p.o while gabapentin was administered at a dose of 50 and 100 mg/kg p.o (Watson et al., 1997). PTZ was administered at a dose of 70 mg/kg i.p (Vohora et al., 2000). The dose of carvedilol was calculated from the equivalent absolute human dose using the surface area ratio of mouse to human (Ghosh, 1984). The drug treatment was given for 4 days and observations were made at the 4th day after drugs treatment. The observations were made at the time of peak effect of the drugs (for carvedilol after 1 hr and for gabapentin after 2 hr). The control animals received 0.25% CMC in 0.9% saline solution. The ICES and PTZ were performed for the evaluation of anticonvulsant effect. The SAB and rotarod test was performed for the neurological disorders. TBARS (Thiobarbituric acid reactive substance) and reduced glutathione were done for the biological estimations. All the parameters were performed to all groups i.e control as well as drugs treated.

Increasing Current Electroshock (ICES) (Kitano et al., 1996)

The Increasing Current Electroshock Seizure test was used to evaluate the anticonvulsant effect of the drugs. Starting with a current of 2 mA, electroshock was delivered to each animal as a single train of pulses (square wave, 5 msec, 20 Hz, 220 V) of linearly increasing intensity from 2 to 30 mA (increment of 0.1 mA/0.1 sec) was applied via ear electrodes. The current at which tonic hind limb extension occurs was recorded as seizure threshold current. If no tonic hind limb extension was observed by a current of 30 mA, electroshock was terminated and this cut off current was used in the analysis.
PENTYLENETETRAZOLE (PTZ) (VOHORA ET AL., 2000)

Seizures were induced chemically with PTZ at a dose of 70 mg/kg i.p, this being the dose that produced seizures in all the animals treated with the drugs i.e. carvedilol and gabapentin. The latency to clonic jerks was observed immediately after PTZ injection for a period of 30 min. In the absence of seizures within 30 min, the latency time was taken as 1800 sec.

SPONTANEOUS ALTERATION BEHAVIOUR (SAB) (RAGOZZINO ET AL., 1998)

Cognitive function was assessed by measuring percentage alternation on a plus-maze consisted of four arms (height: 50 cm; length: 23.5 cm, breadth: 8 cm; wall height: 10 cm) with a central platform (8 x 8 cm). The arms were labeled as A, B, C and D and percentage alternation was measured. After being placed in the central platform, mice were allowed to move in the maze freely for 6 min. The number and sequence of entries were recorded. A 4/5 alternation was defined as entry into 4 different arms on overlapping quintuple sets. Five consecutive arm choices made up a quintuple set e.g. a quintuple set consisting of arm choices A, B, C, D, B was considered as an alternation, while A, D, C, D, A was not considered as quintuple. Using these procedures percentage alternation was calculated as follows:

Actual number of alternations
% Alternation = ----------------- x 100
Possible alternations

ROTAROD TEST (ALI ET AL., 2004)

Effects on motor function were assessed in a rotarod test using a rod with a diameter of 3 cm rotating at a constant speed of 6 rpm. The mice were placed on the rotating and the time taken to fall was noted.

TISSUE HOMOGENATE PREPARATION

Ten per cent tissue homogenates were prepared by separately homogenizing sufficient amounts of tissue in 0.15 M solution of potassium chloride.

LIPID PEROXIDATION (IN BRAIN) (OHKAWA ET AL., 1979)

One ml of suspension medium was taken from the 10% of tissue homogenate. 1 ml of 30% TCA (Trichloroacetic acid) was added to it, followed by 1 ml of 0.8% TBA reagent. The tubes were covered with the aluminum foil and kept in a shaking water bath for 30 min at 80°C. After 30 min, tubes were taken out and kept in ice-cold water for 30 min. These were then centrifuged at 3000 rpm for 15 min. The absorbance of the supernatant was read at 535 nm at room temperature against the appropriate blank. Blank consists of 1 ml distilled water, 1 ml of 30% TCA and 1 ml of 0.8% TBA (Thiobarbituric acid).

The content of MDA (Malonaldehyde) expressed as n moles formed per mg of protein in the tissue was calculated using the formula:

\[
\text{CONCENTRATION} = \frac{A \times V}{E \times P}
\]

Where A is absorbance.
V is the vol. of solution.
E is extinction coefficient (1.56 \times 10^{-6} m^{-1} cm^{-1}).
P is the protein content of tissue calculated as mg protein/gm.

BRAIN REDUCED GLUTATHIONE ESTIMATION (SEDLAK ET AL., 1968)

To 2 ml of 10% homogenate, which was prepared in KCl (Potassium Chloride) solution, were taken and add 2.5 ml of 0.02 M EDTA (Ethylene diamine tetra acetic acid). Shake it vigorously. Take out 2 ml of the above mixture and add 4 ml of cold distilled water and 1 ml of 50% TCA and shake it for 10 minutes. 10 minutes later the content was transferred to centrifuge tube and centrifuged at 3000 rpm for 15 min. Following centrifugation, 2 ml of the supernatant was mixed with 4 ml of 0.4 M tris buffer (pH 8.9). The whole solution was mixed well and 0.1 ml of 0.01 M ellman’s reagent was added, the absorbance was read with in 5 min of addition of ellman’s reagent at 412 nm against reagent blank with no homogenate. For blank readings, instead of 2 ml of homogenate 2 ml of distilled water was added.

Thus the content ‘Co’ of GSH was given by

\[
\text{Co} = \frac{A \times D}{E}
\]

Where A is absorbance at 412 nm
D is dilution factor
E is the molar extinction coefficient
\(C = 13000M^{-1}cm^{-1}\)
Co is the concentration of glutathione

STATISTICAL ANALYSIS

The results were presented as Mean ± Standard error of mean (SEM). ANOVA and Dunnett’s t-test where used for the analysis of data p < 0.05 were considered significant.
Results

**EFFECT OF GABAPENTIN AND CARVEDILOL ON INCREASING CURRENT ELECTROSHOCK**

Carvedilol (1.25, 2.5 and 5.0 mg/kg) and gabapentin (50 and 100 mg/kg) alone as well as in combination significantly (p < 0.01) enhanced the seizure threshold as ascertained by ANOVA and Dunnett’s t-test, with the higher dose providing greater enhancement as compared with the control group and with gabapentin groups (Table 1).

**EFFECT OF GABAPENTIN AND CARVEDILOL ON PTZ-INDUCED SEIZURES**

PTZ initially produced fore- and hindlimb clonic jerks 60-70 sec after injection. Carvedilol (1.25, 2.5 and 5.0 mg/kg) (p < 0.01) and gabapentin (50 and 100 mg/kg) (p < 0.01) alone as well as in combination (p < 0.05) significantly prolonged the latency to clonic jerks as ascertained by ANOVA and Dunnett’s t-test, as compared with the control group and with gabapentin groups (Table 2).

**EFFECT OF GABAPENTIN AND CARVEDILOL ON MEMORY**

Carvedilol (1.25, 2.5 and 5.0 mg/kg) significantly enhanced the memory when compared with the control group. Gabapentin (50 and 100 mg/kg) did not affect percentage alternation significantly. The combination of both the drugs also did not affect the percentage alternation significantly (Table 3).

**EFFECT OF GABAPENTIN AND CARVEDILOL ON ROTAROD PERFORMANCE**

No difference was observed in the fall off time with carvedilol (1.25, 2.5 and 5.0 mg/kg), gabapentin (50 and 100 mg/kg) and with the combinations of the drugs (Table 4).

**EFFECT OF GABAPENTIN AND CARVEDILOL ON LIPID PEROXIDATION IN BRAIN**

Gabapentin (50 and 100 mg/kg) and carvedilol (1.25, 2.5 and 5.0 mg/kg) alone showed significant inhibition of lipid peroxidation compared to control (p < 0.01) as well as in combination of gabapentin with the carvedilol showed significant inhibition of lipid peroxidation (p < 0.01) when compared with the control group and with the gabapentin groups (Table 5).

**EFFECT OF GABAPENTIN AND CARVEDILOL ON REDUCED GLUTATHIONE IN BRAIN**

Gabapentin (50 and 100 mg/kg) and carvedilol (1.25, 2.5 and 5.0 mg/kg) alone showed significant increase in the brain GSH level compared to control (p < 0.01) as well as in combination of gabapentin with the carvedilol also showed significant increase in the GSH level in brain (p < 0.01) when compared with the control group and with the gabapentin groups (Table 6).
Discussion

The present study indicates that carvedilol potentiates the anticonvulsant effect of gabapentin in a dose dependent manner. Our published data had revealed that carvedilol has an anticonvulsant effect against ICES and PTZ induced seizures (Goel et al., 2010).

**ANTICONVULSANT EFFECT AND POSSIBLE MECHANISM**

The combination of the both the drugs also significantly increase the seizure threshold and the latency of the seizures in a dose dependent manner. Previous data showed that gabapentin is an agonist of GABA receptor and increase the release of GABA neurotransmission (Locher et al., 1991) but recent study showed that gabapentin directly related to α₂β₂ subunits of calcium channels (Maneuf et al., 2003) and inhibits calcium influx through presynaptic P/Q-type voltage gated calcium channels (Fink et al., 2002). The inhibition of calcium influx reduces potassium-evoked excitatory transmitter release and, thus decreases postsynaptic excitability (Ng et al., 2001). Previous data showed that a synergistic effect between propranolol and gabapentin in dystonia (Palomeràs et al., 2000).

**Table 2**

Effect of acute administration of Gabapentin and Carvedilol on pentylenetetrazole in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (p.o) (mg/kg)</th>
<th>Latency (min) (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 ml/kg</td>
<td>68.16 ± 0.7491</td>
</tr>
<tr>
<td>Gabapentin</td>
<td>50</td>
<td>100.83 ± 5.461*</td>
</tr>
<tr>
<td>Gabapentin</td>
<td>100</td>
<td>110 ± 3.367*</td>
</tr>
<tr>
<td>Carvedilol</td>
<td>1.25</td>
<td>85.5 ± 1.708*</td>
</tr>
<tr>
<td>Carvedilol</td>
<td>2.5</td>
<td>92.33 ± 2.275*</td>
</tr>
<tr>
<td>Carvedilol</td>
<td>5.0</td>
<td>97.16 ± 1.327*</td>
</tr>
<tr>
<td>Gabapentin + Carvedilol</td>
<td>50 + 1.25</td>
<td>105.66 ± 5.251*</td>
</tr>
<tr>
<td>Gabapentin + Carvedilol</td>
<td>50 + 2.5</td>
<td>124.33 ± 5.998*</td>
</tr>
<tr>
<td>Gabapentin + Carvedilol</td>
<td>50 + 5.0</td>
<td>130.16 ± 7.092*</td>
</tr>
<tr>
<td>Gabapentin + Carvedilol</td>
<td>100 + 1.25</td>
<td>111.66 ± 6.427*</td>
</tr>
<tr>
<td>Gabapentin + Carvedilol</td>
<td>100 + 2.5</td>
<td>133.83 ± 6.809*</td>
</tr>
<tr>
<td>Gabapentin + Carvedilol</td>
<td>100 + 5.0</td>
<td>137.66 ± 6.751*</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± SEM; n = 6 (Number of animals in each group); p.o: per oral; *p < 0.01 vs control; †p < 0.05 vs gabapentin (50 mg/kg); ‡p < 0.05 vs gabapentin (100 mg/kg) (ANOVA followed by Dunnett’s t-test).

**Table 3**

Effect of acute administration of Gabapentin and Carvedilol on spontaneous alternation behaviour in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (p.o) (mg/kg)</th>
<th>Percentage alternation (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 ml/kg</td>
<td>30.16 ± 2.960</td>
</tr>
<tr>
<td>Gabapentin</td>
<td>50</td>
<td>27.66 ± 2.765</td>
</tr>
<tr>
<td>Gabapentin</td>
<td>100</td>
<td>25.5 ± 2.680</td>
</tr>
<tr>
<td>Carvedilol</td>
<td>1.25</td>
<td>45.5 ± 4.537</td>
</tr>
<tr>
<td>Carvedilol</td>
<td>2.5</td>
<td>49.83 ± 4.347</td>
</tr>
<tr>
<td>Carvedilol</td>
<td>5.0</td>
<td>52.16 ± 4.423*</td>
</tr>
<tr>
<td>Gabapentin + Carvedilol</td>
<td>50 + 1.25</td>
<td>31.5 ± 2.232</td>
</tr>
<tr>
<td>Gabapentin + Carvedilol</td>
<td>50 + 2.5</td>
<td>32.33 ± 2.140</td>
</tr>
<tr>
<td>Gabapentin + Carvedilol</td>
<td>50 + 5.0</td>
<td>33.0 ± 2.238</td>
</tr>
<tr>
<td>Gabapentin + Carvedilol</td>
<td>100 + 1.25</td>
<td>29.33 ± 2.552</td>
</tr>
<tr>
<td>Gabapentin + Carvedilol</td>
<td>100 + 2.5</td>
<td>31.66 ± 2.482</td>
</tr>
<tr>
<td>Gabapentin + Carvedilol</td>
<td>100 + 5.0</td>
<td>32.16 ± 2.482</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± SEM; n = 6 (Number of animals in each group); p.o: per oral; *p < 0.01 vs control (ANOVA followed by Dunnett’s t-test).
Whereas carvedilol is a non-selective β-adrenergic, α-adrenergic and NMDA (glutamatergic) receptors blocking agent. It interacts with the NMDA receptor and cause inhibition of the NMDA related responses which are the types of glutamate receptors leads to seizures (Lysko et al., 1992). So, both gabapentin and carvedilol act by their own mechanism of action and complete their own activity and produce synergistic action but there may be pharmacokinetic as well as pharmacodynamic interaction which needs further elucidation. Generally, it is accepted that the drugs with similar mechanism of action produce an additive interaction as a result of summation of the partial effects produced by each component drug in the mixture (Deckers et al., 2000). In contrast, the drugs with diverse mechanism of action may complete their own activities and, thus, produce a synergistically interaction. Considering the possibility of the synergistically application of the combination of both the drugs seems plausible with the different mechanism of action (Deckers et al., 2000).

Increasing evidence suggests that central β-adrenergic neurotransmission might also play a modulatory role in epileptic phenomena (Rutecki, 1995). Moreover the potentiation of epileptiform abnormalities in slices of pyriform cortex obtained from kindled animals following β-adrenoceptor agonists application. Similarly, β-adrenoceptor activation increased the rate of spontaneous epileptiform

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### Table 4
Effect of acute administration of Gabapentin and Carvedilol on rotarod in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (p.o) (mg/kg)</th>
<th>Fall of time (s) (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 ml/kg</td>
<td>32.83 ± 0.3073</td>
</tr>
<tr>
<td>Gabapentin</td>
<td>50</td>
<td>32.66 ± 0.4216</td>
</tr>
<tr>
<td>Gabapentin</td>
<td>100</td>
<td>32.83 ± 0.4773</td>
</tr>
<tr>
<td>Carvedilol</td>
<td>1.25</td>
<td>34.5 ± 0.8466</td>
</tr>
<tr>
<td>Carvedilol</td>
<td>2.5</td>
<td>35.16 ± 0.833</td>
</tr>
<tr>
<td>Carvedilol</td>
<td>5.0</td>
<td>35.5 ± 0.9574</td>
</tr>
<tr>
<td>Gabapentin + Carvedilol</td>
<td>50 + 1.25</td>
<td>33.33 ± 0.2108</td>
</tr>
<tr>
<td>Gabapentin + Carvedilol</td>
<td>50 + 2.5</td>
<td>33.5 ± 0.2236</td>
</tr>
<tr>
<td>Gabapentin + Carvedilol</td>
<td>50 + 5.0</td>
<td>33.66 ± 0.2108</td>
</tr>
<tr>
<td>Gabapentin + Carvedilol</td>
<td>100 + 1.25</td>
<td>34.33 ± 0.2236</td>
</tr>
<tr>
<td>Gabapentin + Carvedilol</td>
<td>100 + 2.5</td>
<td>34.66 ± 0.2108</td>
</tr>
<tr>
<td>Gabapentin + Carvedilol</td>
<td>100 + 5.0</td>
<td>34.83 ± 0.4773</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± SEM; n = 6 (Number of animals in each group); p.o: per oral (ANOVA followed by Dunnett’s t-test).

### Table 5
Effect of acute administration of Gabapentin and Carvedilol on lipid peroxidation in mice brain tissue

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (p.o) (mg/kg)</th>
<th>N moles of MDA/mg of protein (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 ml/kg</td>
<td>0.494 ± 0.0023</td>
</tr>
<tr>
<td>Gabapentin</td>
<td>50</td>
<td>0.367 ± 0.0016</td>
</tr>
<tr>
<td>Gabapentin</td>
<td>100</td>
<td>0.342 ± 0.0004</td>
</tr>
<tr>
<td>Carvedilol</td>
<td>1.25</td>
<td>0.308 ± 0.0007</td>
</tr>
<tr>
<td>Carvedilol</td>
<td>2.5</td>
<td>0.294 ± 0.0013</td>
</tr>
<tr>
<td>Carvedilol</td>
<td>5.0</td>
<td>0.279 ± 0.0015</td>
</tr>
<tr>
<td>Gabapentin + Carvedilol</td>
<td>50 + 1.25</td>
<td>0.291 ± 0.0004</td>
</tr>
<tr>
<td>Gabapentin + Carvedilol</td>
<td>50 + 2.5</td>
<td>0.271 ± 0.0004</td>
</tr>
<tr>
<td>Gabapentin + Carvedilol</td>
<td>50 + 5.0</td>
<td>0.257 ± 0.0007</td>
</tr>
<tr>
<td>Gabapentin + Carvedilol</td>
<td>100 + 1.25</td>
<td>0.275 ± 0.0005</td>
</tr>
<tr>
<td>Gabapentin + Carvedilol</td>
<td>100 + 2.5</td>
<td>0.255 ± 0.0005</td>
</tr>
<tr>
<td>Gabapentin + Carvedilol</td>
<td>100 + 5.0</td>
<td>0.240 ± 0.0005</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± SEM; n = 6 (Number of animals in each group); p.o: per oral; *p < 0.01 vs control; †p < 0.01 vs gabapentin (50 mg/kg); ‡p < 0.01 vs gabapentin (100 mg/kg) (ANOVA followed by Dunnett’s t-test).
discharges in hippocampal slices (Rutecki, 1995). The involvement of central β-adrenoceptors in genetically programmed seizures has also been demonstrated (Khanna et al., 1989). Nebivolol is another beta blocker also displays anticonvulsant effects, raising the threshold for increasing current electroshock induced seizures (Goel et al., 2009).

There are several reports indicating that β-adrenoceptor antagonists may also potentiate the activity of certain classical antiepileptic drugs. Propranolol, pindolol and alprenolol were shown to enhance the anticonvulsant activity of phenobarbital in the maximal electroshock test (Fischer et al., 1988). One study showed that the protective action of diazepam, felbamate, lamotrigine, phenobarbital and valproate against audiogenic seizures is enhanced by co-administration of the mixed β1/β2-adrenoceptor antagonist, propranolol, and the selective β1-adrenoceptor antagonist, metoprolol (Sarro et al., 1998).

The study showed that over expressing α1b-adrenergic receptors leads to spontaneous seizures and widespread degeneration (Zuscik et al., 2000). So, carvedilol blocks α-adrenoceptor and helps to prevent the seizures. Also one of the study showed that lack of α1b-adrenergic receptor protects against epileptic seizures (Pizzanelli et al., 2009).

The study showed that metoprolol alters the pharmacokinetic of diazepam (Klotz et al., 1984). Another study showed that the metabolism of valproate is not affected by coadministration of propranolol (Nemire et al., 1996).

Either pre-or and postsynaptic β-adrenoceptor effects may be responsible for the potentiation of the anticonvulsant activity of excitatory amino acid receptor antagonists. The stimulation of β-adrenoceptors leads to the activation of adenyl cyclase and subsequent phosphorylation of key target proteins by cAMP-dependent protein kinase (Herrero et al., 1996) enhances the phosphorylation of synaptic vesicle associated protein synapsin. Indeed, increased cAMP formation with in the nerve terminals of the cerebral cortex induces spontaneous action potentials and leads to Ca2+ dependent glutamate release (Khanna et al., 1989). It has been demonstrated that the augmented cAMP-dependent protein kinase activity enhances both NMDA-mediated (Raman et al., 1996) and AMPA/kainate mediated post synaptic responses (Herrero et al., 1996) which are the types of glutamate receptor leads to seizures. Thus diminished synthesis of cAMP and decreased cAMP dependent protein kinase-mediated processes, due to β-adrenoceptor antagonist used, may impair glutamate release by interacting with NMDA receptor via the reduction of nerve terminal excitability or interference with vesicular release mechanism, or may reduce postsynaptic responses. In such case the glutamergic blockade would be augmented by the application of β-adrenoceptor antagonists (Lysko et al., 1992).

**Effects on Behavioural tests**

Epileptic patients are frequently reported to suffer from neurobehavioral problems such as memory impairment which may have a pathological and/oriatrogenic basis (Ali et al., 2003). Such patients would therefore require additional treatment, besides AED therapy, to correct the accompanying neurological deficits. A better solution would be to use an AED that provides not only seizure protection but also has
a positive effect on memory. Therefore a putative AED should be routinely screened for its neurological effects other than antiepileptic action as part of the drug development process (Ali et al., 2003). In view of these observations we investigated the effect of carvedilol on memory in combination with gabapentin.

Carvedilol alone significantly increases the percentage alternation score in SAB model. While gabapentin did not affect the percentage alternation scores, whereas the combination of both the drugs also did not affect the percentage alternation scores.

Cognitive impairment is frequently associated with epilepsy (Lesser et al., 1986). Moreover the hippocampus has one of the denser inputs of adrenergic terminals (containing NE) in the CNS supporting the hypothesis that the noradrenergic system plays a role in memory retrieval (Charles et al., 2004). The present study demonstrated improvement in memory with carvedilol as shown by the increase in percentage alternation in a plus maze in the SAB test. The SAB model, though not representative of all aspects of cognitive function, is a good measure of spatial memory in rodents (Ali et al., 2001). It is also plausible that increased free radical formation causes macromolecular changes in cholinergic neurons and leads to the increase in acetylcholinesterase activity that contributes to learning and memory deficits. Carvedilol exerts beneficial effects on memory processing that may be attributed to its effect of lowering acetylcholinesterase level and its antioxidative action (Prakash et al., 2009). While the gabapentin has no effect on the percentage alternation score as it increase the acetylcholinesterase level (Thompson et al., 1982). Whereas the combination of both the drugs also had no affect on the percentage alternation score.

The toxicity of AED’s to rodents almost invariably manifests as neurological deficits. Minimal neurological deficits, such as impaired motor function, can be detected and quantitated by standardized tests such as the rotarod test (Loscher et al., 1988). In the present study, carvedilol, gabapentin alone as well as in combinations had no effect on motor parameters, at any of the given doses. Thus, carvedilol as well as gabapentin appears to be devoid of adverse neurological effects.

**EFFECTS ON LIPID PEROXIDATION AND GlUTATHIONE CONTENT**

Studies have reported that oxidative stress exacerbates epilepsy (Baydas et al., 2005). Carvedilol and gabapentin alone as well as in combination shown to inhibit the lipid peroxidation and increase in the level of reduced glutathione in brain which showed that it reduces the oxidative stress.

Carvedilol has been reported to scavenge free radicals and inhibit lipid peroxidation in swine ventricular membranes (Yue et al., 1992) and rat brain homogenates (Yue et al., 1992). It has also been shown to inhibit superoxide ion release from activated neutrophils. Carvedilol has also been shown to preserve the endogenous antioxidant system, that is vitamin E and reduced glutathione, which are normally consumed when tissues or cells are exposed to oxidative stress (Feuerstein et al., 1997). This may explain the fact that carvedilol treatment was able to restore the levels of reduced glutathione in the intracerebroventricular streptozotocin treated rats (Prakash et al., 2009), while gabapentin prevents the oxidative stress by reducing the over production of free radicals (Baydas et al., 2005).

**Conclusion**

β-adrenoceptor antagonist carvedilol potentiates the anticonvulsive activity of gabapentin against electroshock and PTZ induced seizures in mice. However, our results are preliminary and further studies are warranted to extrapolate animal data to human situations.

It is hoped the outcome of this study will lead us to a safe approach to treat epilepsy associated with risk factors, especially for the elderly who are at great risk of epilepsy from hypertension; stroke and other cerebrovascular disease.

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