Acta Neurol. Belg., 2011, **111**, 296-305

Influence of carvedilol on anticonvulsant effect of gabapentin

Radha Goel[1]*, Amit Goel[2] and Dr. Yatender Kumar[1]

[1]I.T.S Paramedical College, Ghaziabad, Uttar Pradesh, India; [2]Department of Pharmacology, Faculty of Pharmacy, Jamia Hamdard, Hamdard University, Delhi, India

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 antiepileptic 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 (Luchowsk *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 over expression 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 Alternation 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 × 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:

$$\% \text{ Alternation} = \frac{\text{Actual number of alternations}}{\text{Possible alternations}} \times 100$$

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: -

$$\text{CONCENTRATION} = A \times V/E \times P$$

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

$$Co = A \times D / E$$

Where A is absorbance at 412 nm
D is dilution factor
E is the molar extinction coefficient
($C = 13000 M^{-1} cm^{-1}$)
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.

Table 1

Effect of acute administration of Gabapentin and Carvedilol on seizure threshold in the Increasing Current Electroshock Seizure test in mice

Treatment	Dose (p.o) (mg/kg)	Seizure threshold current (mA) (n = 6)
Control	10 ml/kg	13.16 ± 0.3073
Gabapentin	50	19.66 ± 0.333[a]
Gabapentin	100	20.5 ± 0.2236[a]
Carvedilol	1.25	14.66 ± 0.3333[a]
Carvedilol	2.5	16.66 ± 0.2108[a]
Carvedilol	5.0	18.83 ± 0.3073[a]
Gabapentin + Carvedilol	50 + 1.25	20.83 ± 0.3073[ab]
Gabapentin + Carvedilol	50 + 2.5	21.5 ± 0.2236[ab]
Gabapentin + Carvedilol	50 + 5.0	22.66 ± 0.2108[ac]
Gabapentin + Carvedilol	100 + 1.25	22.33 ± 0.2108[ac]
Gabapentin + Carvedilol	100 + 2.5	23.83 ± 0.3073[ac]
Gabapentin + Carvedilol	100 + 5.0	24.66 ± 0.3333[ac]

Data are presented as the mean ± SEM; n = 6 (Number of animals in each group); p.o: per oral; [a]$p < 0.01$ vs control; [b]$p < 0.01$ vs gabapentin (50 mg/kg); [c]$p < 0.01$ vs gabapentin (100 mg/kg) (ANOVA followed by Dunnett's t-test).

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).

Table 2

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

Treatment	Dose (p.o) (mg/kg)	Latency (min) (n = 6)
Control	10 ml/kg	68.16 ± 0.7491
Gabapentin	50	100.83 ± 5.461[a]
Gabapentin	100	110 ± 3.367[a]
Carvedilol	1.25	85.5 ± 1.708[aa]
Carvedilol	2.5	92.33 ± 2.275[a]
Carvedilol	5.0	97.16 ± 1.327[a]
Gabapentin + Carvedilol	50 + 1.25	105.66 ± 5.251[ab]
Gabapentin + Carvedilol	50 + 2.5	124.33 ± 5.998[ab]
Gabapentin + Carvedilol	50 + 5.0	130.16 ± 7.092[ac]
Gabapentin + Carvedilol	100 + 1.25	111.66 ± 6.427[ac]
Gabapentin + Carvedilol	100 + 2.5	133.83 ± 6.809[ac]
Gabapentin + Carvedilol	100 + 5.0	137.66 ± 6.751[ac]

Data are presented as the mean ± SEM; n = 6 (Number of animals in each group); p.o: per oral; [a]$p < 0.01$ vs control; [b]$p < 0.05$ vs gabapentin (50 mg/kg); [c]$p < 0.05$ 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

Treatment	Dose (p.o) (mg/kg)	Percentage alternation (n = 6)
Control	10 ml/kg	30.16 ± 2.960
Gabapentin	50	27.66 ± 2.765
Gabapentin	100	25.5 ± 2.680
Carvedilol	1.25	45.5 ± 4.537[a]
Carvedilol	2.5	49.83 ± 4.347[a]
Carvedilol	5.0	52.16 ± 4.4238[a]
Gabapentin + Carvedilol	50 + 1.25	31.5 ± 2.232
Gabapentin + Carvedilol	50 + 2.5	32.33 ± 2.140
Gabapentin + Carvedilol	50 + 5.0	33.0 ± 2.338
Gabapentin + Carvedilol	100 + 1.25	29.33 ± 2.552
Gabapentin + Carvedilol	100 + 2.5	31.66 ± 2.482
Gabapentin + Carvedilol	100 + 5.0	32.16 ± 2.482[c]

Data are presented as the mean ± SEM; n = 6 (Number of animals in each group); p.o: per oral; [a]$p < 0.01$ vs control (ANOVA followed by Dunnett's t-test).

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 $\alpha_2\partial$ 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 (Palomeras *et al.*, 2000).

Table 4

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

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

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

Treatment	Dose (p.o) (mg/kg)	N moles of MDA/mg of protein (n = 6)
Control	10 ml/kg	0.494 ± 0.0023
Gabapentin	50	0.367 ± 0.0016[a]
Gabapentin	100	0.342 ± 0.0004[a]
Carvedilol	1.25	0.308 ± 0.0007[a]
Carvedilol	2.5	0.294 ± 0.0013[a]
Carvedilol	5.0	0.279 ± 0.0015[a]
Gabapentin + Carvedilol	50 + 1.25	0.291 ± 0.0004[ab]
Gabapentin + Carvedilol	50 + 2.5	0.271 ± 0.0008[ab]
Gabapentin + Carvedilol	50 + 5.0	0.257 ± 0.0007[ab]
Gabapentin + Carvedilol	100 + 1.25	0.275 ± 0.0005[ac]
Gabapentin + Carvedilol	100 + 2.5	0.255 ± 0.0005[ac]
Gabapentin + Carvedilol	100 + 5.0	0.240 ± 0.0005[ac]

Data are presented as the mean ± SEM; n = 6 (Number of animals in each group); p.o: per oral; [a]$p < 0.01$ vs control; [b]$p < 0.01$ vs gabapentin (50 mg/kg); [c]$p < 0.01$ vs gabapentin (100 mg/kg) (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

Table 6

Effect of acute administration of Gabapentin and Carvedilol on Brain Glutathione Level in mice brain tissue

Treatment	Dose (p.o) (mg/kg)	N moles of MDA/mg of protein (n = 6)
Control	10 ml/kg	19.83 ± 0.4773
Gabapentin	50	24.66 ± 0.3333[a]
Gabapentin	100	28.16 ± 0.1667[a]
Carvedilol	1.25	32.16 ± 0.5426[a]
Carvedilol	2.5	41.83 ± 0.4014[a]
Carvedilol	5.0	49.16 ± 0.4014[a]
Gabapentin + Carvedilol	50 + 1.25	45.83 ± 0.1667[ab]
Gabapentin + Carvedilol	50 + 2.5	54.5 ± 0.7188[ab]
Gabapentin + Carvedilol	50 + 5.0	62.5 ± 0.4282[ab]
Gabapentin + Carvedilol	100 + 1.25	50.83 ± 0.3073[ac]
Gabapentin + Carvedilol	100 + 2.5	60.83 ± 0.3073[ac]
Gabapentin + Carvedilol	100 + 5.0	70.33 ± 0.5578[ac]

Data are presented as the mean ± SEM; n = 6 (Number of animals in each group); p.o: per oral; [a]$p < 0.01$ vs control; [b]$p < 0.01$ vs gabapentin (50 mg/kg); [c]$p < 0.01$ 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 valpoate 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 adenylyl 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 Ca^{2+} 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/or iatrogenic 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.

Acknowledgement

The authors thank to institute for providing the adequate facility for carrying out this work.

REFERENCES

Abreu RMV, Santos JSLD, Moreno JMA. Effects of carvedilol and its analog BM-910228 on mitochondrial function and oxidative stress. J Pharmacol Exp Ther. 2000;295:1022-1030.

Ali A, Ahmad FA, Pillai KK, Vohora D. Evidence of the antiepileptic potential of amiloride with neuropharmacological benefits in rodents models of epilepsy and behavior. Epilepsy and behavior. 2004;5:322-328.

Ali A, Pillai KK, Pal SN. Effects of folic acid and lamotrigine therapy in some rodent models of epilepsy and behavior. J Pharm Pharmacol. 2003;55:387-391.

Ali A, Pillai KK, Pal SN. Some neuropsychopharmacological, biochemical and drug interaction effects of lamotrigine. New Delhi. Jamia Hamdard University. 2001;32-39.

Baydas G, Sonkaya E, Tuzcu M, Yasar A, Donder E. Novel role for gabapentin in neuroprotection of central nervous system in streptozotocin-induced diabetic rats. Acta Pharmacologica Sinica. 2005;26: 417-422.

Charles FM, Xiao YX, Zhan WP, Ouyang M, Lee A. *et al.* A distinct role for norepinephrine in memory retrieval. Cell. 2004;117:131-143.

Clinckers R, Zgavc T, Vermoesen K, Meurs A, Michotte Y, Smolders I. Pharmacological and neurochemical characterization of the involvement of hippocampal adrenoreceptor subtypes in the modulation of acute limbic seizures. J Neurochem. 2010;115(6):1595-1607.

Deckers CL, Czuczwar SJ, Hekster YA, Keyser A, Kubova H. *et al.* Selection of antiepileptic drug polytherapy based on mechanism of action: the evidence reviewed. Epilpesia. 2000;41:1364-1374.

Feuerstein GZ, Ruffolo RR. Carvedilol update IV:prevention of oxidative stress, cardiac remodeling and progression of congestive heart failure. Drugs Today. 1997;33:453-457.

Fink K, Dooley DJ, Meder WP, Suman N, Duffy S. *et al.* Inhibition of neuronal ca^{2+} influx by gabapentin and pregabalin in the human neocortex. Neuropharmacology. 2002;42:229-236.

Fischer W, Lasek R, Muller M. Anticonvulsant effects of propranolol and their pharmacological modulation. Pol J Pharmacol Pharm. 1985;37:883-896.

Fischer W, Muller M. Pharmacological modulation of central monoaminergic systems and influence on the anticonvulsant effectiveness of standard antiepileptics in maximal electroshock seizures. Biomed Biochim Acta. 1988;47:631-645.

Fitzgerald PJ. Is elevated norepinephrine an etiological factor in some cases of epilepsy? Seizure. 2010;19(6):311-8.

Ghosh MN. Toxicity studies. In: Ghosh MN, editor. Fundamentals of Experimental Toxicology. Calcutta: Scientific Book Agency. 1984;153-158.

Goel R, Goel A, Sharma M, Kumar Y. Evidence of the antiepileptic potential of carvedilol with neuropharmacological benefits in rodents models of epilepsy and behaviour. Journal of Pharmacy Res. 2010;3:1827-1830.

Goel R, Goel A, Maoncha A, Pllai KK. Influence of nebivolol on anticonvulsant effects of lamotrigine. Indian J Pharmacol. 2009;41:41-46.

Herrero I, Sanchez-Prieto J. c-AMP-dependent facilitation of glutamate release by β-adrenergic receptors in cerbrocortical nerve terminals. J Biol Chem. 1996; 271:30554-30560.

Hesdorffer DC, Hause WA, Annegers JF, Rocca WA. Severe, uncontrolled hypertension and adult-onset Seizures:A case-control study in Rochester. Minnesota, Epilepsia. 1996;37:736-741.

Khanna N, Ray A, Alkondon M, Sen P. Effect of β-adrenoceptor antagonists and some related drugs on maximal electroshock seizures in mice. Indian J Exp Biol. 1989;27:128-130.

Kitano Y, Usui C, Takasuna K, Hirohashi M, Nomura M. Increasing current electroshock test:a new method for assessment of anti-and pro-convulsant activities of drugs in mice. J Pharmacl Toxicol Methods. 1996;35:25-29.

Klotz U, Reimann IW. Pharmacokinetic and pharmacodynamic interaction study of diazepam ad metoprolol. Eur J Clin Pharmacol. 1984;26:223-226.

Kunieda T, Zuscik MJ, Boongird A, Perez DM, Lüders HO, Najm IM. Systemic overexpression of the alpha 1B-adrenergic receptor in mice:an animal model of epilepsy. Epilepsia. 2002;43(11):1324-1329.

Lesser RP, Luders H, Wyllie E, Dinner DS, Morris HH. Mental deterioration in epilepsy. Epilepsia. 1986; 27:105-123.

Loscher W, Honack D, Tylor C. Gabapentin increases aminooxyacetic acid- induced GABA accumulation in several regions of rat brain. Neurosci Lett. 1991; 128:150-154.

Loscher W, Schmidt D. Which animal models should be used in the search for new antiepileptic drugs? A proposal based on experimental and clinical considerations. Epilepsy Res. 1988;2:145-181.

Luchowsk E, Luchowski P, Wielosz M, Kleinrok Z, Czuczwer SJ. *et al.* Propranolol and metoprolol enhance the anticonvulsant action of valproate and diazepam against maximal electroshock. Pharmacol Biochem behavior. 2002;71:223-31.

Lysko PG, Lysko KA, Yue TL, Webb CL, Feuerstein GZ. Neuroprotective effects of carvedilol, a new antihypertensive agent, in cultured rat cerebellar neurons and in gerbil global brain ischemia. Stroke. 1992;23:1630-1636.

Lysko PG, Lysko KA, Webb CL, Feuerstein G. Neuroprotective effects of carvedilol, a new antihypertensive, at the N-methyl-D-aspartate receptor. Neuroscience Letters. 1992;148:34-38.

Maneuf YP, Gonzalez MI, Sutton KS, Chung FZ, Pinnock RD. *et al.* Cellular and molecular action of the putative GABA-mimetic, gabapentin. Cell Mol. Life Sci. 2003;60:742-750.

McNamara JO. Cellular and molecular basis of epilepsy. J Neurosci. 1994;14:3413- 3425.

Nakmura T, Oda Y, Takahashi R, Tanaka K, Hase I, Asada A. Propranolol increases the threshold for lidocaine induced convulsions in awake rats:A direct effect on the brain. Anesth Analg. 2008;106(15): 1450-1455.

Nemire RE, Toledo CA, Ramsay RE. A pharmacokinetic study to determine the drug interaction between valproate and propranolol. Pharmacotherapy. 1996;16:1059-1062.

Ng GY, Bertrand S, Sullivan R, ethier N, Wang J. *et al.* γ-aminobutyric acid type B receptors with specific heterodimer composition and post-synaptic actions in the hippocampal neurons are targets of anticonvulsant gabapentin action. Mol Pharmacol. 2001;59:144-152.

Ohkawa H, Ohishi N, Yagi K. Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. Biochem.1979;95:351-358.

Palomeras E, Sanz P, Cano A, Fossas P. Dystonia in a patient treated with propranolol and gabapentin. Arch Neurol. 2000;57:570-571.

Pericic D, Jazvinscak M, Svob D, Mirkovic K. Beta-1 adrenoceptor antagonists potentiate the anticonvulsive effect of swim stress in mice. Pharmacol Biochem Behav. 200;67:507-510.

Pizzanelli C, Lazzeri G, Fulceri F, Giorgi FS, Pasquali L. *et al.* Lack of α1b-adrenergic receptor protects against epileptic seizures. Epilepsia. 2009;50 (Suppl.1):S59-S64.

Prakash AK, Kumar A. Effect of chronic treatment of carvedilol on oxidative stress in an intracerbroventriclar streptozotocin induced model of dementia in rats. J Pharm Pharmacol. 2009;61:1665-1672.

Ragozzino ME, Pal SN, Unick K, Steigani MR, Gold PE. Modulation of hippocampal acetylcholine release and spontaneous alternation scores by intrahippocampal glucose injection. J Neurosci. 1998; 18:1595-1601.

Raman IM, Tong G, Jahr CE. β-adrenergic regulation of synaptic NMDA receptors by cAMP-dependent protein kinase. Neuron. 1996;16:415-421.

Reznikoff GA, Manaker S, Rhodes CH, Winokur A, rainbow TC. Localization and quantification of β-adrenergic receptors in human brain. Neurology. 1986;36:1067-1073.

Rutecki PA. Noradrenergic modulation of epileptiform activity in the hioppocampus. Epilepsy Res. 1995; 20:125-136.

Sarro GD, Spagnolo C, Gareri P, Gallelli L, Sarro AD. Gabapentin potentiates the antiseizure activity of certain anticonvulsants in DBA/2 mice. Eur J Pharmacol. 1998;349:179-185.

Savitz SI, Rosenbaum DM, Dinsmore JH. The novel β-blocker, carvedilol, provides neuroprotection in transient focal stroke. J Cereb Blood Flow Metab. 2000;20:1197-1204.

Sedlak J, Lindsay RH. Estimation of total protein bound and non- protein bound sulphydryl groups in tissues with ellman's reagent. Anal Biochem. 1968;25: 192-197.

Stoop R, Epiney S, Meier E, Pralong E. Modulation of epileptiform discharge in rat limbic system in vitro by noradrenergic agents. Neurosci Lett 2000;287:5-8.

Thompson PJ, Trimble MR. Anticonvulsant drugs and cognitive functions. Epilepsia. 1982;23:531-534.

Vohora D, Pal SN, Pillai KK. Thioperamide, a selective histamine H_3 receptor antagonist, protects against PTZ-induced seizures in mice. Life Sci. 2000;66: 297-301.

Watson WP, Robinson E, Little HJ. The novel anticonvulsant, gabapentin, protects against both convulsant and anxiogenic aspects of the ethanol withdrawl syndrome. Neuropharmacology. 1997;36:1369-1375.

Yaoita H, Sakabe A, Maehara K, Maruyama Y. Different effects of carvedilol, metoprolol and propranolol on left ventricular remodeling after coronary stenosis or after permanent coronary occlusion in rats. Circulation. 2002;105:975-980.

Yue TL, Cheng HY, Lysko PG, McKenna PJ, Feuerstein R. *et al.* Carvedilol, a new vasodilator and beta adrenoreceptor antagonist, is a antioxidant and free radical scavenger. J Pharmacol Exp Ther. 1992;263:92-98.

Yue TL, Feuerstein GZ. Carvedilol, a new vasodilator and β-adrenoceptor antagonist, inhibits oxygen radical-mediated lipid peroxidation in swine ventricular membranes. Pharmacol Commun. 1992;1:27-35.

Zuscik MJ, Sands S, Ross SA, Waugh DJ, Gaivin RJ. Overexpression of the α1b-adrenergic receptor causes apoptotic neurodegeneration:multiple system atrophy. Nat Med. 2000;6:1388-1394.

Radha Goel,
I.T.S Paramedical College,
Delhi-Merrut Road,
Ghaziabad
Uttar Pradesh-201206 (India).
E-mail: radhamit2006@gmail.com