

**ANTICONVULSANT ACTIVITY WITH NEUROPHARMACOLOGICAL
BENEFITS OF NEBIVOLOL IN MICE**

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Summary

Hypertension is an established risk factor for clinically detected stroke, which is in turn a risk factor for epilepsy. In this study we investigated the anticonvulsant effect of nebivolol (an antihypertensive) on Increasing current electroshock (ICES) and Pentylenetetrazole (PTZ) – induced seizures in mice. Further the effect of nebivolol on memory and rotarod performance was also evaluated. Nebivolol produced a dose-dependent increase in seizures threshold as well as the latency of the seizures in both the models of the epilepsy. Nebivolol has no effect in memory improvement in the SAB model as well as in rotarod performance, suggesting it is devoid of behavioral impairment. So, the present study, showed the anticonvulsant effect of nebivolol, which can be useful for the treatment of hypertension in patients with epilepsy. It also showed the additional neurological benefits.

Key Words- Nebivolol, Rotarod, Pentylenetetrazole, Spontaneous alternation behaviour

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Introduction

Epilepsy and epilepsy syndrome are common neurological diseases which represent an important public health problem that has given rise to marked social and healthcare concerns¹. 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 expectancy². The contribution of nor-adrenergic neurotransmission to the seizure susceptibility and epileptogenesis is gaining more attention recently. The high density of β -adrenoceptors occurs in all the subfields of the hippocampus known for its low seizures and dominant role in the propagation of seizures³. β -adrenoceptors agonists were demonstrated to potentiate the epileptiform abnormalities occurring in slices of pyriform cortex obtained from kindled animals⁴. Moreover, some β -adrenoceptor antagonists display anticonvulsant actions under experimental conditions and could potentiate the protective activity of classic antiepileptic⁵.

Nebivolol (NBV) is a relatively new highly cardioselective, β -adrenergic receptor antagonist that is devoid of intrinsic sympathomimetic activity but possesses vasodilator properties not attributed to blockade of α 1- adrenergic receptors on smooth muscle cells⁶. NBV has antioxidative effect and is a highly lipophilic drug⁷.

Epilepsy is frequently associated with impaired memory and muscle relaxation. Such patients would therefore need additional treatment besides AED therapy, to correct the accompanying neurological deficits⁸.

So, the aim of this study to investigate the protective effects of nebivolol against ICES and PTZ induced seizures, which can be useful for the treatment of hypertension in patients with epilepsy.

Materials and methods

Animals

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. Utmost care was taken to ensure that animals were treated in the most humane and ethically acceptable manner.

Drugs

The drugs used were NBV (Nebicard, Torrent Pharmaceuticals, India) and pentylenetetrazole (Sigma, USA). NBV was 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. NBV was administered at a dose of 0.25, and 0.5 mg/kg p.o⁹. PTZ was administered at a dose of 70 mg/kg i.p¹⁰. The study

was done for 4 days. The observations were made at the time of peak effect of the drug i.e for NBV after 30 min.⁹ The control animals received 0.25% CMC in 0.9% saline solution. ICES and PTZ was performed for the evaluation of anticonvulsant effect. The SAB and rotarod test was performed for the neurological disorders.

Increasing Current Electroshock (ICES)¹¹

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 via a ear electrode as a single train of pulses (for 0.2 sec) with linearly increasing intensity of 2mA/2sec. 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.

Pentylentetrazole (PTZ)¹⁰

Seizures were induced chemically with PTZ at a dose of 70 mg/kg intraperitoneally, this being the dose that produced seizures in all the animals treated with the drug. The latency to clonic jerks was observed immediately after PTZ injection for a period of 30 minutes. In the absence of seizures within 30 min, the latency time was taken as 1800s.

Spontaneous Alternation Behaviour¹²

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:

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

Rotarod Test¹³

Effects on motor function were assessed in a rotarod test (Techno) using a rod with a diameter of 3 cm rotating at a constant speed of 6 rpm. The mice were placed on the rotating rod for 2 min and the time taken to fall was noted.

Statistical analysis

The results were presented as Mean \pm Standard error of mean (SEM). ANOVA and Dunnett's t-test were used for the analysis of data 'p' value < 0.05 were considered significant.

Results**Effect of nebivolol on ICES**

Nebivolol (0.25 and 0.5 mg/kg) 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 (Table-1).

Table-1 Effect of acute administration of nebivolol on seizure threshold in the Increasing Current Electroshock Seizure test in mice.

Treatment	Dose(p.o)	Seizure Threshold Current (mA) (n=6)
Control	10ml/kg	13.16 \pm 0.3073
Nebivolol	0.25 mg/kg	13.66 \pm 0.3333*
Nebivolol	0.50 mg/kg	15.83 \pm 0.2108*

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

Effect of nebivolol on PTZ-induced seizures

PTZ initially produced fore- and hindlimb clonic jerks 60–70 seconds after injection. Nebivolol (0.25 and 0.5 mg/kg) significantly ($p < 0.01$) prolonged the latency to clonic jerks as compared with the control group (Table-2).

Table-2 Effect of acute administration of nebivolol on pentylenetetrazole in mice

Treatment	Dose (p.o)	Latency (min) (n=6)
Control	10ml/kg	68.16 \pm 0.7491
Nebivolol	0.25 mg/kg	82.16 \pm 1.708*
Nebivolol	0.50 mg/kg	88.33 \pm 6.427*

Data are presented as the mean \pm SEM; n= 6 (Number of animals in each group); p.o: per oral; * p < 0.01 vs grp I (control) (ANOVA followed by Dunnett's t-test).

Effect of nebivolol on memory

The effect of nebivolol treatment on SAB in a plus maze is summarized in Table 3. Nebivolol (0.25 and 0.5 mg/kg) has no effect on memory when compared with the control group.

Table-3 Effect of acute administration of nebivolol on spontaneous alternation behaviour in mice

Treatment	Dose (p.o)	Percentage alternation (n=6)
Control	10ml/kg	30.16 \pm 2.960
Nebivolol	0.25 mg/kg	27.33 \pm 2.552
Nebivolol	0.50 mg/kg	26.16 \pm 2.232

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

Effect of nebivolol on rotarod performance

No difference was observed in the fall-off time with nebivolol (0.25 and 0.5 mg/kg) when compared with the control group (Table 4).

Table-4 Effect of acute administration of nebivolol on rotarod in mice

Treatment	Dose (p.o)	Fall of Time(s) (n=6)
Control	10ml/kg	32.83 \pm 0.3073
Nebivolol	0.25 mg/kg	30.66 \pm 0.2108
Nebivolol	0.50 mg/kg	30.16 \pm 0.2236

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

Discussion

In the present study, nebivolol, a beta blocker drug, was evaluated with respect to its anticonvulsant activity against different models of epilepsy. We provide the first experimental evidence, for nebivolol, of a protective role in electrical and chemical *in vivo* models of epilepsy. However, it provided significant protection at all doses in ICES, a model considered to be more sensitive than MES. We further show that the protective effect of nebivolol against PTZ-induced seizures is dose dependent.

NBV is highly lipophilic agent, easily penetrating the brain, and has antioxidant property. Increasing evidence suggests that central β -adrenergic neurotransmission might also play a modulatory role in epileptic phenomena¹⁴. 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 discharges in hippocampal slices^{14,15}. The involvement of central β -adrenoceptors in genetically programmed seizures has also been demonstrated¹⁶. Moreover, β -adrenoceptor antagonists, especially propranolol display anticonvulsant effects, raising the threshold for electroconvulsions and protecting against maximal electroshock- and pentylenetetrazol-induced seizures¹⁷. There are several reports indicating that β -adrenoceptor antagonists may also potentiate the activity of certain classical antiepileptic drugs. Enhanced norepinephrine-sensitive accumulation of cAMP was observed in cortical slices with iron-induced epileptic activity and the elevation in levels of both cyclic GMP and cyclic AMP was demonstrated to correlate with the development of kindled seizure. Since β -adrenergic blockade leads to the reduced formation of cAMP, it might be hypothesized that β -adrenoceptor antagonists potentiate the activity of antiepileptic drugs that do not diminish the cAMP levels *per se*, such as SVP¹⁸.

Epileptic patients are frequently reported to suffer from neurobehavioral problems such as memory impairment which may have a pathological and/or iatrogenic basis.⁸ 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. Cognitive impairment is frequently associated with epilepsy.¹⁹ 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 retrieval²⁰. The present study demonstrated that there is no effect on memory.

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.²¹ In the present study, nebivolol had no effect on motor parameters, at any of the doses given. Thus, nebivolol appears to be devoid of adverse neurological effects. In conclusion, β -adrenoceptor antagonist nebivolol has an anticonvulsant effect against electroshock and PTZ induced seizures in mice with additional neuropharmacological advantages, it may be advantageous in the treatment of epilepsy in hypertensive patients by improving their seizure control.

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. However, our results are preliminary and further studies are warranted to extrapolate animal data to human situations.

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