Studies on anticonvulsant actions of L-deprenyl

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L-Deprenyl (Selegiline), an irreversible inhibitor of MAO type B is being used in the therapy of parkinson’s disease (PD) since 1986 (ref. 1, 2). Besides inhibition of MAO type B, it also inhibits reuptake of dopamine and noradrenaline, increases the turnover of dopamine, enhances striatal enzymes, i.e., superoxide dismutase, catalase activity and is also involved in the detoxification of free radicals. Stimulation of neurotropic factor synthesis and antagonistic modulation of polyamine binding site of N-methyl-D-aspartate (NMDA) -subtype of glutamate receptor by L-deprenyl has also been suggested.

Loscher and Honack were the first to report the anticonvulsant effect of L-deprenyl in kindled rats. It also showed learning and memory improving effects in various cognitive test models in animals as well as in humans. The aim of the present research was to study the protective effect of L-deprenyl in various animal models of epilepsy and memory deficit.

Materials and Methods

Animals—Laca mice of either sex weighing 20-25 g, bred in the Central Animal House facility of Panjab University were used in the present study. The animals were housed under standard laboratory conditions and were provided with food and water ad libitum.

Effect of L-deprenyl in chemical seizures—Acute administration of pentylenetetrazole (PTZ), 80 mg/kg, i.p. produced convulsions and death in control group of mice. Animals were observed for the onset of Straub’s tail, jerks, clonic, tonic phase of convulsions and death/recovery. Diazepam (1, 4 mg/kg, i.p.) was administered in two different groups, 30 min prior to PTZ challenge. In other groups of animals, L-deprenyl (10, 20, 40 mg/kg, i.p.) was given in different doses 30 min prior to PTZ. In combination studies, L-deprenyl (10 mg/kg) and the sub-effective dose of diazepam (1 mg/kg) were given to a group of mice, 30 min before PTZ challenge. All experimental groups consisted of 5-9 animals per treatment group. All experiments were carried out between 0900 and 1300 hrs.

Effect of L-deprenyl in electrical seizures—Application of electric-shock (50 mA for 0.2 sec) through corneal electrodes produced convulsions and death in the control group of mice. Phenytoin (10, 20 mg/kg, i.p.) was administered in two different groups, 30 min prior to the electric-shock and the animals were observed for the onset and duration of tonic
flexion, tonic extensor, clonus, stupor and death/recovery. In other group of animals, L-deprenyl (10, 20 mg/kg, i.p.) was given in different doses 30 min prior to the electric-shock and the animals were observed as for phenytoin groups. In combination studies, L-deprenyl (10 mg/kg) and the lower dose of phenytoin (10 mg/kg) were given to a group of mice, 30 min before the electric shock. All experimental groups consisted of 5-9 animals per treatment group. All administrations were done between 0900 and 1300 hrs, daily.

**Effect of L-deprenyl in status epilepticus**—Status epilepticus (SE) was induced in rats by Li (3 meq/kg, i.p.) following which the animals were observed continuously for the occurrence of behavioral seizures. The animals were kept individually in plexiglas chambers (30 × 90 × 90 cm with partitions). The neuroprotective profile of L-deprenyl (10 mg/kg, i.p.) was studied by administering it 30 min prior to the pilocarpine challenge, subsequently the animals were observed for a period of 90 min to note the onset of forelimb clonus with rearing (FC+R). The mortality (if any) was observed for a period of 48 hrs. Both the experimental groups consisted of 7-9 animals.

**Effect of acute treatment with L-deprenyl on learning and memory**—To study the learning and retention of a learned task (short-term memory), a step-down (passive avoidance) task behavior paradigm was used. The equipment consisted of an electric grid (24 x 30 cm), with a shock-free zone (SFZ) (2 x 3 x 1 cm) in the center and the electric grid having a perflex enclosure. Mice were put individually on the SFZ (a wooden block) and allowed to explore for about 1 min. Then the animal was placed on SFZ and the time it took to step onto the grid was noted. As soon as it stepped on the grid, an electric stimulus (20 V) was given for a fixed period of 80 sec. After 1 hour and 24 hrs of the first trial i.e. learning, each animal was again put individually on the SFZ and the latency in reaching the grid was recorded, in both, the control and the drug treated groups. Drug treated groups consisted that of physostigmine (a cholinergic nootropic), L-deprenyl, scopolamine (an anticholinergic amnesic drug), scopolamine plus physostigmine and scopolamine plus L-deprenyl. All experimental groups consisted of 5 animals per treatment group.

Physostigmine and L-deprenyl were administered just after training while scopolamine was administered 30 min after the training, so that the effect of physostigmine and L-deprenyl could be observed 1 hr after training, while that of scopolamine after 30 min of its injection.

**Effect of chronic treatment with L-deprenyl on learning and memory**—The equipment used was same as for acute effect on learning and memory. The animals were trained as for acute study. The experiments were done on three groups and one served as a control. Treatment groups consisted of L-deprenyl (10 mg/kg), scopolamine (0.3 mg/kg) and scopolamine plus L-deprenyl. L-deprenyl was given to group I just after training, scopolamine to group II 30 min after training while group III received L-deprenyl just after training followed 30 min by scopolamine. Same drugs were administered to respective animal groups once a day for 8 days at a gap of 24 hrs. Latency was noted on day one, 1 hr after the training and on days 2 and 8, 1 hr after L-deprenyl injection or 30 min after scopolamine injection.

**Effect of L-deprenyl on anxiety**—Anxiety was measured on elevated plus-maze, a model described and validated by Lister and Sharma and Kulkarni for mice.

L-deprenyl (10, 20 mg/kg, i.p.) and diazepam (1, 2 mg/kg, i.p.) were administered to different groups of animals, and 30 min later, the animals were tested on elevated plus-maze for acute effect. In combination studies, L-deprenyl (10 mg/kg, i.p.) and diazepam (1 mg/kg, i.p.) were administered to a group of animals and 30 min later, the animals were tested on elevated plus-maze for acute effect. Control animals were treated with normal saline. All experiments were done between 0900 and 1300 hrs, daily.

**Drugs**—The drugs used for pharmacological interventions were diazepam injection (Ranbaxy Laboratories Ltd., New Delhi), PTZ (Sigma Chemicals, St. Louis, USA), L-deprenyl (Chinoin, Budapest), phenytoin sodium injection (Caldia healthcare Pvt. Ltd., Ahmedabad, India), Lithium chloride (Merek, Germany), pilocarpine nitrate (Boehringer Ingelheim, Germany), scopolamine (Merck & Co. Inc., Rahway, USA), physostigmine hemisulfate (Sigma Chemical Co., USA). All the drugs were directly dissolved in freshly distilled water except for phenytoin injection which was first wetted in a drop or so of tween-80 and then suspended in distilled water. All the drugs were given intraperitoneally (i.p.) except for pilocarpine hemisulfate which was given subcutaneously (s.c.), in
a constant volume of 1 ml/100 g of body weight for mice and 0.5 ml/100 g of body weight for rats.

Statistical analysis—The non-parametric Mann-Whitney test was used to evaluate the statistical significance of the difference between the values of each group and that of the corresponding control group in chemical and electrical seizure test and in test for status epilepticus and \( P < 0.05 \) was considered statistically significant. For memory and anxiety tests, the data was presented as mean ± SE and analyzed using one way analysis of variance (ANOVA) followed by Dunnet’s test and \( P < 0.05 \) was considered statistically significant.

**Results**

Evaluation of anticonvulsant activity in chemical seizures—L-Deprenyl (10, 20 and 40 mg/kg) showed a significant anticonvulsant activity against PTZ-induced convulsions as it delayed the onset of Straub’s tail and jerks. Diazepam (4 mg/kg) offered complete protection against convulsions while a lower dose of diazepam (1 mg/kg) significantly delayed the onset of Straub’s tail, jerks, clonic convulsions and complete abolition of the tonic extensor phase. The combination of subprotective doses of L-deprenyl (10 mg/kg) and diazepam (1 mg/kg) showed a significant potentiation of the anticonvulsant effect as the combination delayed the onset of jerks, clonic and tonic convulsions.

Evaluation of anticonvulsant activity in electrical seizures—L-Deprenyl (10 and 20 mg/kg) delayed the onset of extensor phase (the effect being non-significant) while L-deprenyl (10 mg/kg) significantly decreased the duration of extensor phase when compared to the control group. Phenytoin (20 mg/kg and not 10 mg/kg) showed a significant protection against the onset of extensor phase and its duration when compared to control animals. Combined administration of L-deprenyl (10 mg/kg) and phenytoin (10 mg/kg) showed a significant delay in the onset of extensor phase as well as decrease in its duration as compared to the per se effect of both the drugs (Fig 1).

Evaluation of anticonvulsant activity in status epilepticus—Consecutive treatment with L-deprenyl did not affect the time of onset of forelimb clonus and rearing (FC + R) and its duration due to the administration of lithium and pilocarpine, statistically. Also, it did not affect the mortality rate by lithium and pilocarpine.

Acute effect of L-deprenyl on learning and memory—Scopolamine, per se, significantly decreased the latency while physostigmine significantly increased the latency to reach the grid compared to the control animals. L-Deprenyl did not significantly affect the latency. In scopolamine treated animals, L-deprenyl did not significantly affect the latency but hr after its administration as compared to scopolamine treated animals. However, physostigmine significantly increased the latency after

**Fig 1—Effect of various drugs on the duration of tonic extensor phase in MES-induced convulsions in mice. Values are mean ± SE. \( * P < 0.05 \) compared to control, \( ** P < 0.05 \) compared to L-deprenyl (10 mg/kg) and phenytoin (10 mg/kg).**
1 hour of training in scopolamine treated mice (Fig 2). After 24 hr of training, none of the five drug treated animal groups showed any significant difference in latency (Fig 3).

Effect of chronic treatment of L-deprenyl on learning and memory—Scopolamine (0.3 mg/kg) produced a significant memory deficit in mice on days 1, 2 and 8 as compared to control animals. Compared to control animals, L-deprenyl (10 mg/kg) per se did not show a significant effect on latency on day 1, 2 and 8. When given concurrently with scopolamine, L-deprenyl did not show any significant increase in latency compared to scopolamine treated animals on day 1 and 2. However, on day 8, there was a significant increase in latency of mice to step onto the grid (Fig 4).

Effect of L-deprenyl treatment on anxiety response—In the control group, the percentage

![Fig 2](image)

**Fig 2**—Effect of acute treatment with various drugs on learning (tested 1 hr after training) in mice. Values are mean ± SE. * P < 0.05 compared to control

![Fig 3](image)

**Fig 3**—Effect of acute treatment with various drugs on memory paradigm (tested 24 hr after training) in mice. Values are mean ± SE. No significant difference was observed between different groups.
preference of mice for the open arm was 40%. In L-deprenyl (10, 20 mg/kg) treated group, it was 0%. In diazepam (2 mg/kg) group, it was 60% while in diazepam (1 mg/kg) group, it was 0%. Combination of L-deprenyl (10 mg/kg) and diazepam (1 mg/kg) increased the percentage preference for the open arm to 40%.

Diazepam (2 mg/kg) significantly increased the mean total number of entries and the mean total time spent by mice in the open arm of the maze while L-deprenyl (10, 20 mg/kg) and diazepam (1 mg/kg) did not affect both the parameters, significantly. Combination of L-deprenyl (10 mg/kg) and diazepam (1 mg/kg) did not significantly affect the number of entries in the open arm as compared to control or the per se effect of either L-deprenyl (10 mg/kg) or diazepam (1 mg/kg) but significantly increased the mean total time spent in the open arm compared to the per se effects of both the drugs.

Discussion

In the present study, L-deprenyl exhibited anticonvulsant effect at all the doses studied as it blocked the appearance or delayed the onset of Straub's tail and jerks. However, the onset of clonic convulsions or tonic extensor phase was not significantly delayed due to wide variation in responses of animals within the groups. When L-Deprenyl was administered with a sub-effective dose of diazepam, it potentiated the anticonvulsant effect. The previous studies have reported controversial results. While Loscher and Lehmann found L-deprenyl (1, 2.5 and 5 mg/kg) to significantly increase the myoclonic and clonic convulsive thresholds due to PTZ 30 min after its administration, Hoffman et al. found repeated treatment and not the single dose treatment with L-deprenyl to affect the PTZ-induced convulsions. Similarly, in MES-convulsions, L-deprenyl (10 mg/kg and not 20 mg/kg) pretreatment showed a significant anticonvulsant profile. Co-administration of lower dose of L-deprenyl with phenytoin (10 mg/kg) showed advantage over the per se effect of both the drugs. Thus, L-deprenyl per se or its combination with phenytoin exhibited a potent anticonvulsant activity.

Although, till now, there are no reports of any pathophysiological significance of MAO-B in epilepsy, the finding of anticonvulsant activity of L-deprenyl (MAO-B inhibitor) suggests that the role of MAO-B in epilepsy should be investigated in more detail. Recently, Loscher et al. have proposed that L-deprenyl also inhibits MAO-A at a higher dose (> 10 mg/kg) and it is this inhibition of MAO-A which is responsible for the anticonvulsant effect and not the inhibition of MAO-B mechanism. Hoffman et al. proposed the mechanism for this effect of L-deprenyl not to be associated with dopaminergic or cholinergic mechanism.

Effect of L-deprenyl on lithium-pilocarpine induced convulsions was studied to determine its protective effect in SE and its mechanism of action. Since L-deprenyl did not affect the onset of FC + R or its duration, it suggests that when given as a single dose, it does not protect the animals against SE. This
is supported by a report from Hoffman et al.\textsuperscript{12} who showed that the mechanism for the anticonvulsant effect of L-deprenyl is not related to the changes in acetylcholine levels since prolonged treatment with L-deprenyl did not attenuate the brain sensitivity to pilocarpine-induced seizures. However, in another experiment, chronic treatment with L-deprenyl could improve the concentration of acetylcholine in the CNS that might show protective effect.

Inhibition of cholinergic neurotransmission plays a predominant role in dementia. Scopolamine, a cholinergic muscarinic receptor antagonist is known to produce short term amnesia in humans and animals. In the present study, acute administration of deprenyl per se did not affect acquisition or memory nor did it modify scopolamine-induced memory deficit. In the chronic 8-day treatment schedule, scopolamine produced a significant decrease in acquisition and retention on day 1, 2 and 8. L-deprenyl did not affect learning and memory on day 1 and 2 but significantly improved memory on day 8 in scopolamine treated animals. This is in agreement with the previous reports\textsuperscript{14,15}. Thus, chronic treatment and not the acute treatment with L-deprenyl improved the memory in scopolamine-induced memory deficit. The data suggest that L-deprenyl may have beneficial effect in epilepsy as an adjuvant therapy with major anticonvulsants and it possessed cognitive enhancement abilities. Though the exact mechanism is far from clear, L-deprenyl might be modulating cholinergic activity in the brain.

L-deprenyl, tested at both the doses showed an anxiogenic effect as it decreased the percent preference of animals for the open arm from 40% (in controls) to 0%. However, the combination of L-deprenyl and diazepam (both at lower doses) was anxiolytic as compared to the per se effects of both the drugs. It may be concluded that L-deprenyl (10 mg/kg and 20 mg/kg) per se, increased the anxiety in animals. This effect could be because of the metabolites of L-deprenyl i.e. amphetamine and methamphetamine, which may cause anxiety, insomnia, and other adverse symptoms.\textsuperscript{16} When in combination with diazepam (1 mg/kg), it enhanced its antianxiety effect. A direct mechanism could not be proposed from these studies, however, it seems that deprenyl facilitated the antianxiety effect of diazepam through its central action.

References