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Neuronal Sparing and Behavioral Effects of the Antiapoptotic Drug, (−)Deprenyl, Following Kainic Acid Administration

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GELOWITZ, D. L. AND I. A. PATERSON. *Neuronal sparing and behavioral effects of the antiapoptotic drug, (−)deprenyl, following kainic acid administration*. PHARMACOL BIOCHEM BEHAV **62**(2) 255–262, 1999.—(−)Deprenyl is an irreversible inhibitor of monoamine oxidase B (MAO-B) frequently used as an adjunct therapy in the treatment of Parkinson's Disease. Recent evidence, however, has found that deprenyl's metabolites are associated with an antiapoptotic action within certain neuronal populations. Interestingly, deprenyl's antiapoptotic actions appear not to depend upon the inhibition of MAO-B. Due to a paucity of information surrounding (−)deprenyl's ability to spare neurons in vivo, a series of studies was conducted to further investigate this phenomenon within an apoptotic neuronal death model: kainic acid induced excitotoxicity. Results indicated that (−)deprenyl increased hippocampal neuronal survival compared to saline-matched controls following kainic acid insult. Furthermore, it was discovered that (−)deprenyl treatment could be stopped 14 days following CNS insult by kainate, with evidence of neuronal sparing still present by day 28. In open-field locomotor activity testing of kainate-treated animals, those given subsequent (−)deprenyl treatment showed habituation curves similar to control subjects, while saline-treated animals did not. Given deprenyl's antiapoptotic actions, it is proposed that (−)deprenyl may be beneficial in the treatment of a variety of neurodegenerative diseases where evidence of apoptosis exists, such as Parkinson's and Alzheimer's Disease, by slowing the disease process itself. © 1999 Elsevier Science Inc.

Apoptosis Antiapoptotic (−)Deprenyl Deprenyl Kainic acid Kainate Neuron Sparing Locomotor Locomotion Exploratory Neurorescue Hippocampus Excitotoxicity MAO-B Survival Rat Parkinson's Alzheimer's Habituation

(−)DEPRENYL is an irreversible inhibitor of monoamine oxidase B (MAO-B) frequently used as an adjunct therapy in the treatment of Parkinson's Disease (PD) (3,7). Within PD, it is commonly believed that (−)deprenyl may provide symptomatic relief by improving dopaminergic neurotransmission and by reducing neuronal death caused by oxidative free radical damage (3,7). Determining the mechanisms underlying (−)deprenyl's clinical efficacy, however, has proved to be difficult because (−)deprenyl is primarily metabolized in the liver by cytochrome P450 to the active agents of (−)methamphetamine, (−)desmethyldeprenyl, (−)amphetamine, and several other hydroxylated forms [(27) for review]. Though it has been known for some time that cognitive benefits arising from (−)deprenyl administration may be correlated with its amphetamine byproducts (6,11), recent evidence has found that desmethyldeprenyl metabolites are associated with an antiapoptotic action in tissue culture and animal models (17,27), where the action is stereospecific to the (−) or L form (1). Indeed, it has been found that (−)deprenyl reduces the death of motoneurons caused by axotomy in rats (24), and in adult murine facial motoneurons in mice (15), it reduces PC12 cell apoptosis by inducing new protein synthesis (28), it protects rats from DSP-4 toxicity (32,34,35), and it reduced neuronal loss resulting from transient hypoxia–ischemia (16). In tissue culture models, it has been shown that the antiapoptotic action of (−)deprenyl requires new gene expression and pro-

[1]On September 16, 1998 Dr. I. Alick Paterson, age 38, died after a brief, but hard fought battle against metastatic melanoma. He will be greatly missed.

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tein synthesis (28) to prevent mitochondrial dysfunction (18,29), and thus prevent commitment to apoptotic cell death. Further, it has been shown that (−)deprenyl selectively prevents p53-dependent apoptosis (18). It is possible, therefore, that (−)deprenyl, in addition to symptomatic effects, is slowing the disease process itself within neurodegenerative diseases by an antiapoptotic action.

In light of recent antiapoptotic findings with (−)deprenyl, a series of studies were conducted to further investigate this phenomenon within an animal model of apoptotic neuronal death: kainic acid induced excitotoxicity. It has been shown that kainic acid induced seizures produce neuronal death in the hippocampus (2,10,25), including apoptotic cellular death (21), and that this is p53 dependent (12,23). The extent of cell death in the hippocampus is related to the severity of the induced seizure (33).

A number of important questions were to be addressed by the studies presented herein: (a) does (−)deprenyl spare neurons within the kainic acid model of apoptosis; (b) does (−)deprenyl alter behavioral ictal activity in a manner representative of classical anticonvulsants; (c) if neurons are spared are spared by (−)deprenyl following kainic acid administration, are the neurons viable or of functional value to the organism; (d) does (−)deprenyl treatment need to be maintained indefinitely for neuronal sparing to persist following kainic acid insult.

METHOD

General Methods

Kainic acid model: development and pattern of cellular response. The kainic acid (KA) model developed and employed for all experiments resulted in bilateral damage to rodents within the hippocampus and GFAP activation. The model did, however, prove to be one that was not as easy to advance and implement as first anticipated. For example, only 50–75% of animals typically responded to primary KA infusion, and of the animals responding, 5–10% would often die. The response to primary KA infusion has been referred to as, "all or nothing" (25), and may relate to the drugs' poor ability to cross the blood–brain barrier. In an effort to improve the latter ratio, increase survival rates, and still induce relatively consistent neuronal damage within the hippocampus, a number of preliminary studies were conducted in which room temperature, animal weights, KA dosage, and behavioral seizure stage using Racine's Scale (22) were closely monitored. Optimal conditions were found to be that 8–10 mg/kg IP of KA given to rodents, weighing approx. 200 g and located in a 21.5°C temperature-controlled environment. These conditions resulted in the greatest survival of animals, and consistent loss of neurons within the CA1, CA3, and CA4 fields of the hippocampus. Interestingly, the CA3 field area in the preliminary studies conducted proved to be the most consistently damaged area. Furthermore, it was discovered that animals reaching a stage 4 or 5 seizure rating according to Racine's Scale (22) improved the consistency of neuronal counts within the hippocampal field areas, and lead to the largest increases in GFAP activation (33).

Subjects. Male albino Wistar rats, supplied by Charles River (Montreal, Canada) were used in all experiments conducted. The rats, weighing 150–175 g at the time of delivery, were housed four per cage in wire mesh cages in a controlled environment (19–21°C, 12 L:12 D cycle with lights on at 0700 h). The animals were allowed ad lib access to Purina rat chow and water. All animals were maintained in these controlled conditions prior to use for at least 7 days. All of these procedures employing the animals were in accordance with guidelines established by the Canadian Council on Animal Care and the National Institute of Health for the use and care of experimental animals. The experimental protocols were approved by the University Committee for Animal Care and Supply at the University of Saskatchewan.

Kainic acid administration procedures and behavioral seizure ranking. Rats (200–225 g) were removed from their home cages and brought to a laboratory at a room temperature between 21–22.5°C. On arrival in the laboratory, all animals were weighed and then allowed to wait quietly for a period of 1 h to reduce stress associated with removal from their home cages. After 1 h had passed, all animals were injected with kainic acid [Kainic Acid Hydrate: Sigma Chemical Company K-0250] 8–10 mg/kg IP from syringes and were observed 1 half hour later for the appearance of ictal activity. Thereafter, all animals showing signs of ictal activity were continually monitored and formally ranked for seizure level every half hour for the next 4 h using Racine's Seizure Rating Scale (22). Between formal ranking, it was noted whether an animal reached a stage 4 or 5 seizure level—a seizure level associated with global ictal activity (22). Animals not showing signs of ictal activity were excluded from study.

Histology. The animals used in all experiments were killed by transcardiac perfusion with FAM (Methanol 80%/Formalin 10%/Acetic Acid 10%) fixative under deep anesthesia (chloral hydrate overdose). The brains were then removed, blocked, postfixed overnight in FAM, embedded in paraffin, and stored at room temperature for subsequent sectioning. Coronal sections (10 μm) through the hippocampus were cut on a microtome and mounted onto silane-coated slides. Tissue sections were Nissl stained with either cresyl violet or thionin. For Nissl staining, cell counts reflected the appearance of a clear nucleolus, a fully enclosed nucleus, and an outline of the cells plasma membrane, as indicated by ribosomal staining surrounding the membrane. Cells were counted in separtate coronal tissue sections from both sides of the brain by an observer blind to treatment using an Olympus BH2-RFCA microscope. Cell counts for any hippocampal subfield represents the mean of the individual counts taken for the area in question. On each tissue section, for each hippocampal subfield, neurons were counted in a single field of view, which was 400 μm in diameter. The location for counting CA1 cells started at the CA2/CA1 border, CA3 counts were taken on the "curve" of the CA3 subfield, and CA4 counts were taken in the dentate gyrus area.

Behavioral quantification of locomotor activity. Locomotor testing was conducted in a clear, Plexiglas cage (40 × 40 × 23 cm) positioned inside an Columbus Instruments infrared photobeam recording device, consisting of an array of 12 × 12 photobeams. The photobeam device was interfaced to an Apple II+ Computer, which tabulated horizontal activity scores over time. Horizontal activity was measured by the interruption of any one of the 12 photobeams during a testing period, which was typically 20 min in duration. Test chambers were cleaned with 70% ethanol before and after each test trial.

Statistical Analysis

All results were subjected to either a *t*-test, one-way, or two-way analysis of variance as appropriate. Any pairwise comparisons that needed to be conducted employed the Newman–Keuls test. Statistical significance was taken at a level of $p < 0.05$ for all statistical analysis performed. All analyses

were conducted using SPSS v6.0 for Microsoft Windows on an IBM 486 with an Intel processor.

Specific Methods

Experiment 1. The purpose of this experiment was to determine whether (−)deprenyl could ameliorate excitotoxin-induced neuronal death following KA administration. The dosage of (−)deprenyl [R(−)-Deprenyl HCl; Research Biochemicals Incorporated M-003] chosen, 0.25 mg/kg SC, was based upon successful neuronal sparing in preliminary studies discussed and other studies (16,19). This dose represents a supramaximal dose in the hypoxia–ischemia model (19). Male rats were administered kainic acid (10 mg/kg IP) dissolved in physiological saline. Following kainic acid treatment, all animals were observed for behavioral ictal activity. Those subjects displaying seizures between stages 4–5 using Racine's Scale (22) were used in the study. After 2.5 h the subjects were injected with either (−)deprenyl (0.25 mg/kg, SC) or saline—a control or no-treatment group was also included. Animals were then observed until all indices of ictal activity subsided. Drug treatment was maintained with daily injections until sacrifice 5 days later. Hippocampal counts were taken from six separate brain regions that were comprised of the left and right CA1, CA3, and CA4 fields. Cell counts from the left and right sides of a given field area, however, were averaged because no significant differences were found between brain sides in neuronal death.

Experiment 2. The effects of (−)deprenyl and pentobarbital [Pentobarbital Sodium; Abbott Laboratories Limited 3/SA-046/5] on behavioral seizure levels following KA administration were assessed. Animals were administered 10 mg/kg (IP) KA, and behavior was monitored continually for the next 5 h, with formal seizure ranking every half hour. Two and half hours following KA administration, however, the animals were split into two groups, with half receiving 0.25 mg/kg (SC) (−)deprenyl, and the other half receiving 12.5 mg/kg of sodium pentobarbital (IP). Once all indices of seizure activity had subsided, all subjects were sacrificed with an overdose of chloral hydrate.

Experiment 3. (A) The purpose of this study was to determine whether (−)deprenyl-treated animals would show differences in habituation scores over time, as indicated by locomotion activity, when compared to saline-treated animals. Preliminary studies revealed that animals that suffer neuronal loss following kainic acid administration are hyperactive when compared to control subjects 1 to 3 days postdrug treatment; therefore, it is important to note that all behavioral testing was carried out 7 days following kainate treatment to eliminate or reduce acute behavioral problems associated with treatment, such as edema and perivenous hemorrhage (25). Consequently, behavioral testing can be considered to have occurred when animals were at a relatively "steady state" behaviorally and in terms of neuronal loss. Animals were initially treated with kainic acid (10 mg/kg IP) and were subsequently treated with (−)deprenyl (0.125 mg/kg SC twice/day) or saline (0.09% twice/day) 4.5 h after kainic acid administration, continuing for the next 12 days. A twice-daily injection schedule (1200 to 2400 h) was adopted, with the expectation that this may further enhance behavioral and neuronal recovery, because more drug would be in the animal's systems for a greater part of any given day. A control group that received similar handling and behavioral testing to that of the (−)deprenyl and saline groups, but no drug administration, was also used. During days 8–12, animal's locomotor activity was observed for 20 min/day in an open field paradigm as previously described. Following the last behavioral test session, all drug-treated subjects were sacrificed. (B) Using the same protocol employed in A, with the expectation of only treating animals for 4 days with drug following kainic acid administration, another experiment was conducted to access locomotor activity scores and neuronal numbers. This procedure was employed to address the question of whether (−)deprenyl metabolites may be influencing behavioral results.

Experiment 4. From experiments conducted, it is evident that (−)deprenyl treatment needs to be maintained in excess of 4 days following kainic acid administration for neuronal sparing to be detected. Therefore, the purpose of this experiment was to provide evidence that (−)deprenyl treatment may be terminated at some point following kainic acid administration, with the sparing of neurons still apparent. The experimental design consisted of kainic acid administration (10 mg/kg) to subjects using the methods previously described, and then dividing the animals into one of three treatment groups to receive various lengths of (−)deprenyl treatment (0.125 mg/kg twice/day): 0 days; 14 days; or 28 days. Sacrifice of all subjects occurred on the same day, which was 28 days following their initial kainic acid injection.

Experiment 5. Though many studies have found evidence of apoptosis following kainic acid administration (12,20,21, 23), Experiment 5 was conducted to confirm the appearance of apoptotic neurons within the kainic acid model used in the experiments presented herein. The results of this experiment were expected to be positive. The experimental design consisted of the administration of kainic acid (10 mg/kg) to animals using the procedure previously described. Following this, animals were divided into one or three groups to investigate for evidence of apoptosis 12, 18, or 24 h after kainate administration. All subjects were sacrificed under deep anethesia by transcardiac perfusion using Zamboni's Fixative. The brains were then removed and postfixed overnight in Zamboni's Fixative. The next day, sections of 10 μm were cut from the dorsal hippocampus on a cryostat and stored in a freezer for subsequent staining. Staining was carried out using ApopTag. ApopTag is an In Situ Apoptosis Detection Kit that is commercially available from Oncor Inc., MD (Catalogue #: S7100-Kit). The Kit detects apoptotic cells in research samples by direct immunoperoxidase detection of digoxigenin-labeled genomic DNA in thin sections of fixed tissue.

RESULTS

Experiment 1: Effects of (−)Deprenyl on Neuronal Counts Following Kainic Acid

When animals were treated with (−)deprenyl (0.25 mg/kg SC) 2.5 h after kainic acid injection, and continuing for the next 4 days, neural death was substantially reduced. Daily treatment with (−)deprenyl resulted in significant sparing of CA1, $F(2, 9) = 13.73$, $p < 0.01$, and CA3, $F(2,9) = 25.56$, $p < 0.01$ neurons, but not CA4 neurons (Fig. 1). With saline treatment, the number of surviving neurons per field of view was: CA1, 14 ± 8; CA3, 15 ± 3; CA4, 20 ± 2. With (−)deprenyl treatment the neuronal survival was: CA1, 43 ± 9; CA3, 33 ± 4; CA4, 22 ± 6. With no drug treatment (controls) the neuronal counts were: CA1, 67 ± 2; CA3, 54 ± 4; CA4, 32 ± 3. If neuronal sparing is expressed as percentages relative to controls, then (−)deprenyl-treated animals had 64% of CA1 and 61% of CA3 neurons surviving compared to 21 and 28% in saline-treated subjects.

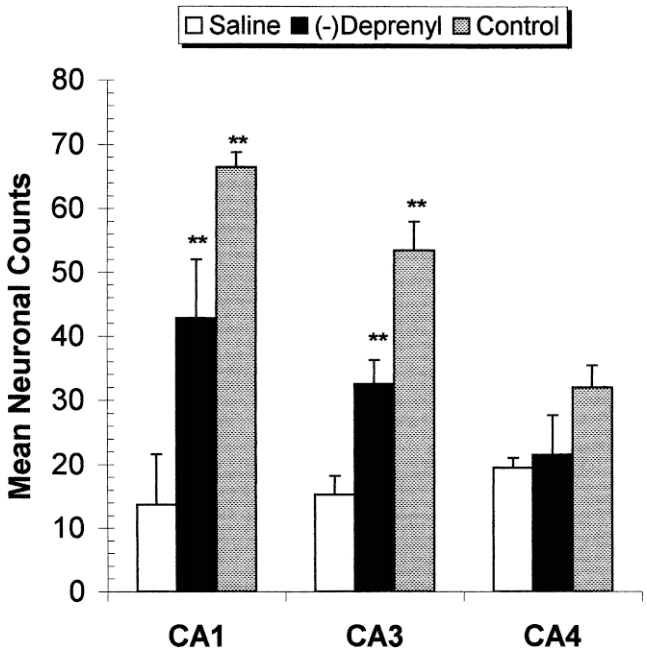

FIG. 1. Neuronal counts in CA1, CA3, and CA4 subfields 5 days following kainic acid for controls and saline and (−)deprenyl-treated animals. Mean neuronal counts in CA1, CA3, and CA4 subfields of the hippocampus for control, and saline and (−)deprenyl-treated animals 5 days following kainic acid administration (10 mg/kg IP) (Nissl staining). (−)Deprenyl (0.25 mg/kg SC) or vehicle was given 2.5 h after kainic acid and then daily until sacrifice on day 5. *Indicates $p < 0.05$ and **$p < 0.01$ significance levels when comparing controls and (−)deprenyl-treated animals to saline-treated animals. Each bar presents the mean and SEM of four animals.

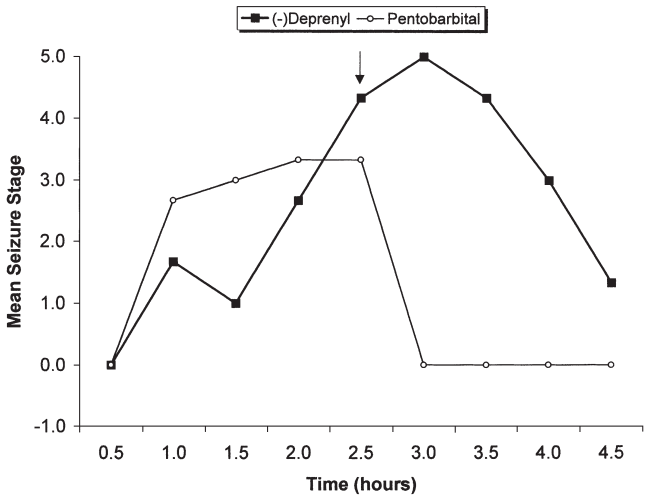

FIG. 2. Seizure rating in pentobarbital and (−)deprenyl-treated animals 2.5 h following kainic acid. Mean seizure rating based upon Racine's (1972) five-stage scale for pentobarbital (12.5 mg/kg IP) and (−)deprenyl (0.25 mg/kg SC)-treated animals. Arrow indicates time of pentobarbital and (−)deprenyl injections at 2.5 h following kainic acid (10 mg/kg IP) administration. Each point represents the mean of three animals.

Experiment 2: Effects of (−)Deprenyl and Pentobarbital on Behavioral Ictal Activity Following Kainic Acid
Qualitative Analysis

As qualitatively indicated on Fig. 2, (−)deprenyl treatment did not hold anticonvulsant activity, while pentobarbital dramatically halted seizure activity within the first half hour following its administration. To be more specific, seizure activity subsided with a few minutes (10–30) following pentobarbital administration, while the administration of (−)deprenyl had no visible effect on reducing seizure levels throughout the observed period of time. Indices of behavioral seizure activity subsided in the (−)deprenyl-treated animals in a fashion typical of KA administration alone.

Experiment 3A: Effects of Continuous (−)Deprenyl Treatment on Locomotor Activity and Neuronal Numbers Following Kainic Acid

No significant between group differences existed between test days 1 through 5 activity scores; however, significant within-group differences existed for the control and (−)deprenyl groups activity scores. Within-group comparions showed significant reductions in locomotor activity on days 2 to 5 in the control group and on days 4 and 5 in the KA–(−)deprenyl group when compared to day 1 activity scores. Results attested that KA–saline-treated animals maintained a high degree of locomotor activity over all five behavioral test days (no within-group differences), while KA–(−)deprenyl-treated animals displayed the same initial locomotor activity, but significantly reduced activity over the test period, $F(4, 50) = 4.20$, $p < 0.01$, as did control animals, $F(4, 50) = 16.22$, $p < 0.01$ (Fig. 3). The latter within group differences indicate that levels of activity recorded for the (−)deprenyl group were similar to that of control animals, as both groups showed comparable habituation curves over time (Fig. 3). As found previously, neuronal numbers were also significantly affected by drug treatment in some hippocampal field areas: (−)deprenyl significantly enhanced neuronal counts within the CA3, $F(2, 21) = 21.23$, $p < 0.01$, and CA4, $F(2, 27) = 34.78$, $p < 0.01$, fields 5 days following kainic acid administration compared to saline treated animals (Fig. 4). With saline treatment, the number of surviving neurons was: CA1, 39 ± 6; CA3, 15 ± 2; CA4, 19 ± 4. With (−)deprenyl treatment the neuronal survival was: CA1, 52 ± 4; CA3, 34 ± 3; CA4, 31 ± 2.

Experiment 3B: Effects of 4 Days of (−)Deprenyl Treatment on Locomotor Activity and Neuronal Numbers Following Kainic Acid

In contrast to maintaining animals on drug treatment throughout behavioral testing, animals receiving (−)deprenyl or saline for only 4 days following kainic acid administration revealed no significant differences with respect to both locomotor activity and neuronal counts (data not shown).

Experiment 4: Does (−)Deprenyl Need to Be Maintained Indefinitely to Spare Neurons?

The results of this experiment were somewhat surprising (Fig. 5). Though both 14 and 28 days of (−)deprenyl treatment spared more neurons that 0 days of treatment within the CA3, $F(2, 16) = 15.45$, $p < 0.01$, field area, only 14 days of (−)deprenyl treatment spared more neurons that 0 days of treatment within the CA1, $F(2, 16) = 3.57$, $p < 0.05$, and CA4, $F(2, 16) = 4.61$, $p < 0.05$, field areas; however, as previously

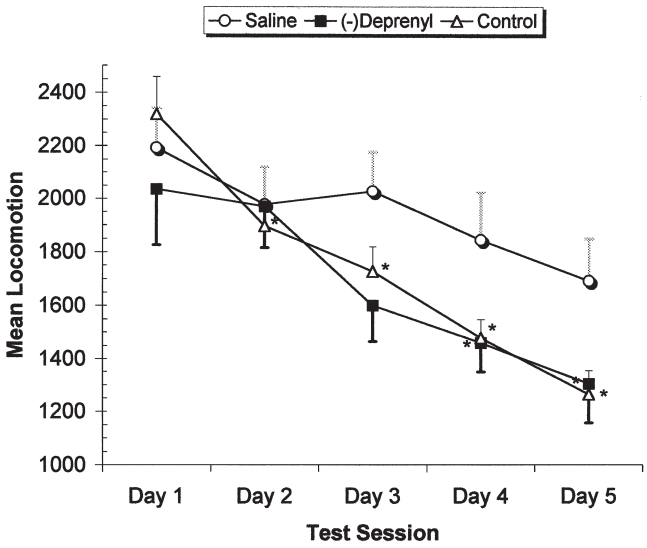

FIG. 3. Locomotor scores 7 days following kainic acid in controls and saline and (−)deprenyl-treated animals. Mean open-field locomotor activity scores during 20-min test sessions in controls, and (−)deprenyl (0.125 mg/kg SC twice/day) and saline-treated animals 7 days following kainic acid (10 mg/kg IP) administration. Drug treatments for saline and (−)deprenyl-treated animals were maintained postkainic acid administration and during behavioral testing until sacrifice 12 days later. No significant between group differences existed; however, within-group comparions showed significant reductions in locomotor activity on days 2 to 5 in the control group and on days 4 and 5 in the KA–(−)deprenyl group when compared to day 1 activity scores. There were no significant differences within the saline group. *Indicates $p < 0.05$ significance level for within group comparisons. Each point represents the mean of 11 animals.

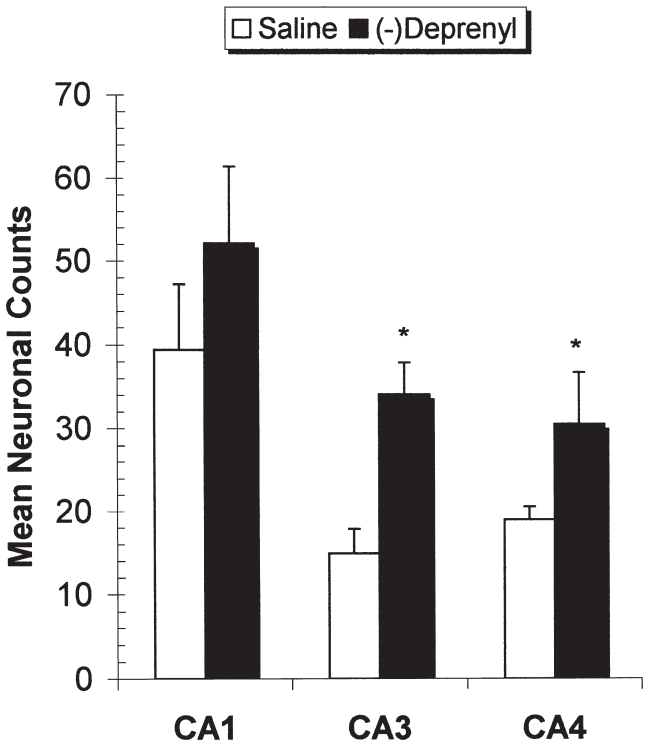

FIG. 4. Neuronal counts in CA1, CA3, and CA4 subfields 12 days following kainic acid in saline and (−)deprenyl-treated animals. Mean neuronal counts in CA1, CA3, and CA4 subfields of the hippocampus for saline and (−)deprenyl (0.125 mg/kg SC twice/day)-treated animals 12 days following kainic acid (10 mg/kg IP) administration (Nissl staining). Drug treatments were administered for 12 days, with animal sacrifice on day 12 as well. *Indicates $p < 0.05$ significance level. Each bar presents the mean and SEM of 9–12 animals.

referred to, the CA3 field area has always shown consistent results, while the CA1 and CA4 field areas are somewhat variable. Neither 14 or 28 days of (−)deprenyl treatment were significantly different from each other in any of the field areas. To be more specific, with 0 days of (−)deprenyl treatment the neuronal survival was: CA1, 15 ± 4; CA3, 27 ± 3; CA4, 20 ± 4; with 14 days of (−)deprenyl treatment the neuronal survival was: CA1, 36 ± 7; CA3, 39 ± 2; CA4, 34 ± 4; and with 28 days of (−)deprenyl treatment the neuronal survival was: CA1, 24 ± 5; CA3, 45 ± 2; CA4, 28 ± 3.

Experiment 5: Detection of Apoptotic Neurons Following Kainic Acid Administration

Results of immunohistochemical staining sections using ApopTag qualitatively revealed evidence of apoptosis within the CA1, CA3, and CA4 field areas of the hippocampus 12, 18, and 24 h following kainic acid administration (photomicrographs not shown). In addition, apoptotic positive cells were also evident within the cortex and dentate gyrus (photomicrographs not shown).

DISCUSSION

Although previous studies have shown apoptotic neuronal death associated with kainic acid induced seizures (2,10,12, 20,21,23,31), the present study is novel because it has shown that a compound with well-characterized antiapoptotic properties, (−)deprenyl (17,18,27–29), can prevent seizure-induced neuronal death. Further, this study has shown a seizure-induced behavioral deficit that was prevented by (−)deprenyl treatment.

In the experiments presented, parameters were obtained that resulted in behavioral seizures, neuronal death in the pyramidal cells of the CA1, CA3, and CA4 subfields of the hippocampus, and evidence of apoptosis. Following (−)deprenyl treatment, however, animals previously exposed to kainic acid insult revealed significantly increased neuronal numbers within the subfields of the hippocampus when compared to saline-treated animals. If neuronal sparing is expressed as percentages relative to controls (Fig. 1), then (−)deprenyl-treated animals had 64% of CA1 and 61% of CA3 neurons surviving compared to 21 and 28% in saline-treated subjects; that is, (−)deprenyl treatment produced a threefold increase in the number of surviving neurons compared to saline treatment following kainic acid administration.

(−)Deprenyl is a drug with many actions that can influence neurotransmission and neuronal death. As a MAO-B inhibitor and a derivative of amphetamine, (−)deprenyl can alter catecholaminergic neurotransmission resulting in a neuroprotective effect (3,6), yet it also prevents neuronal apoptosis by a mechanism that is independent from MAO-B inhibition. In recent years it has been shown that (−)deprenyl (in particular, the desmethyl metabolite, desmethyldeprenyl) can prevent neuronal apoptosis in a variety of tissue culture models (13,14,18,27–29) and can prevent neuronal death involving

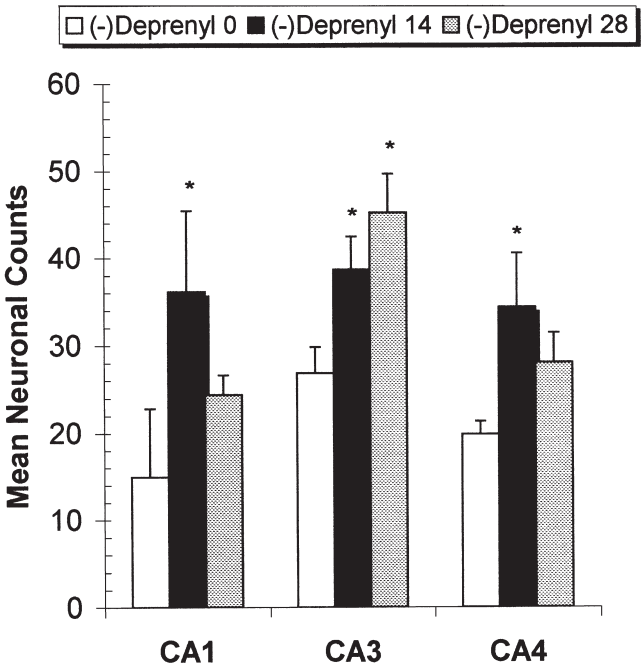

FIG. 5. Neuronal counts in CA1, CA3, and CA4 subfields following kainic acid and (−)deprenyl treatment for 0, 14, and 28 days. Mean neuronal counts in CA1, CA3, and CA4 subfields of the hippocampus for (−)deprenyl (0.125 mg/kg SC twice/day) 0, 14, and 28 days of treatment following kainic acid (10 mg/kg IP) administration (Nissl staining). Days 14 and/or 28 are significantly different than day 0, but not from each other. *Indicates $p < 0.05$ significance level. Each bar presents the mean and SEM of six to seven animals. All subjects were sacrificed on day 28.

apoptosis in the rodent brain (1,15,16,24). This antiapoptotic action involves binding to an intracellular site, possibly the enzyme Glyceraldehyde-3-Phosphate Dehydrogenase or GAPDH (8), to alter gene expression (27,28). One result of this altered gene expression is that mitochondrial function is maintained in the cell, and the cell fails to commit to the apoptotic process (18,29). Interestingly, (−)deprenyl acts selectively on only some forms of neuronal apoptosis. In tissue culture, (−)deprenyl prevents p53-dependent apoptosis (18), and it has been shown that injury-induced neuronal apoptosis in the CNS, and in particular kainic acid-induced neuronal death, is p53 dependent (12,23).

A question arising from administering (−)deprenyl to seizing animals was whether the drug alters behavioral ictal activity in a manner consistent to that of classical anticonvulsants. This questions importance as it relates to the conceptual nature of (−)deprenyl's ability to spare neurons following kainic acid insult. If (−)deprenyl alters behavioral seizure rankings similar to that of pentobarbital, then the (−)deprenyl's neuronal sparing abilities would theoretically be anticonvulsant or ion channels and neurotransmitter altering, and not antiapoptotic or genetic altering. As Fig. 2 indicates, (−)deprenyl had no effect on behavioral seizure stage, while pentobarbital dramatically halted the progression of ictal activity. Though seemingly obvious to many, this finding's theoretical importance should not be understated, and further supports the position that (−)deprenyl's neuronal sparing ability is antiapoptotic in nature. This position may be refuted by some, as it has been reported that (−)deprenyl holds anticonvulsant and antiepileptogenic effects within the kindling model of epilepsy (9); however, because the dosage of (−)deprenyl (10 mg/kg) used in that study was not specific for MAO-B the results may be explained by increased catecholamine release. Indeed, the authors state that at such a high dose (−)deprenyl can increase endogenous noradrenaline; noradrenaline profoundly delays kindling and other forms of epileptiform bursting (26,30). These findings bear little relevance to the experiments presented herein, because the (−)deprenyl dosage used was low in comparison. We conclude that deprenyl is preventing neuronal death induced by kainic acid seizures through an antiapoptotic mechanism. This conclusion is supported by the finding that similar neuronal sparing effects have been seen in this model with another antiapoptotic drug, CGP-3466B, which is devoid of MAO-B inhibitory properties (19).

One cannot conclude from the present study that all neurons being spared by (−)deprenyl would have died by apoptosis, nor that (−)deprenyl is sparing all of the apoptotic neurons. Excitotoxins can produce both apoptotic and necrotic cell death (5,20,21), and the interaction between these forms of cell death in a tissue as complicated as the CNS is poorly understood. However, because it has been shown that kainic acid seizures induced apoptosis (12,20,21,23), (−)deprenyl prevents neuronal apoptosis in culture (18,27,28), and (−)deprenyl-like compounds (19) spare neurons following kainic acid elicited seizures, it is reasonable to conclude that (−)deprenyl can prevent neuronal apoptosis in this seizure model.

Other experiments were conducted to access the whether (−)deprenyl treatment could be stopped at some point, and still reveal evidence of neuronal sparing. Although 4 days of (−)deprenyl treatment following kainate administration did not increase neuronal counts when assessed 12 days later, a longer period of (−)deprenyl treatment was able to spare neurons. To elaborate, when animals previously administered kainic acid were placed into 0, 14, or 28 days of (−)deprenyl treatment groups, and then all sacrificed on day 28, statistically significant differences were noted (Fig. 5). Data indicated that animals given 14 days of (−)deprenyl treatment and sacrificed on day 28 spared as many neurons within the CA3 field of hippocampus as animals given 28 days of (−)deprenyl treatment and sacrificed on day 28. Ironically, the 14-day group also revealed statistically significant differences within the CA1 and CA4 fields, while the 28-day group did not. The latter finding is not readily explained, but may be a function of variable response that CA1 and CA4 often manifest in response to kainic acid treatment, while CA3 neurons typically have a more consistent response. It can be concluded, however, that at a point between 4 and 14 days following kainic acid administration, (−)deprenyl treatments may stop, with neuronal sparing still visible 28 days later. Of course, the possibility exists that neurons observed at day 28 may die at some point after this, but considering neurons endured for 14 days without (−)deprenyl treatment would probably preclude this circumstance.

Another meaningful question arising from the sparing of neurons with (−)deprenyl, is whether the sparing of neurons correlates with a functionally significant behavioral effect. Indeed, simply because (−)deprenyl spares neurons when visualized at the light microscope level, it does not necessarily indicate the neurons are of functional value and/or that the (−)deprenyl treatments have a positive effect upon behavior. To address this issue, locomotor activity scores were assessed over a 5-day period in controls and (−)deprenyl and saline-treated animals 7 days following kainic acid insult. The drug

treatments were maintained throughout the period of behavioral testing. To date, such behavioral experimentation has not appeared in the literature. It was anticipated that kainate-insulted animals subsequently treated with (−)deprenyl, would have habituation scores different than that of saline-treated animals. Indeed, results revealed differences in habituation scores within the (−)deprenyl and control groups over a 5-day period when comparing days 1 to days 2–5 scores, while no differences in habituation scores were noted within the saline group (Fig. 3). Not only this, the habituation scores of (−)deprenyl-treated animals were similar to that of control subjects. What these data indicate is that KA–(−)deprenyl treatment produces habituation scores similar to that of control subjects, while KA–saline treatment does not. Furthermore, considering no differences in locomotor activity scores were noted between groups on day 1 testing, it is unlikely that the differences in habituation scores reflect differences in motor system, but rather represent differences in cognitive processing. Although speculative, because (−)deprenyl treatment spared hippocampal neurons following kainate administration, while saline treatment did not, and because the hippocampus is important in spatial memory for rodents (4), it may be that differences in the habituation scores reflect saline-treated animals' inability to remember previously explored spatial locations. However, simply because the sparing of hippocampal neurons is correlated with a significant behavioral event does not necessarily indicate causation. Nevertheless, these results strongly suggest that neurons spared by (−)deprenyl are functional.

To conclude, it is clear from the studies herein that (−)deprenyl treatment spared neurons, that (−)deprenyl treatment does not have to be maintained indefinitely for the neuronal sparing to persist, and that (−)deprenyl treatment has a functionally significant effect on an organisms behavior. These findings suggest that antiapoptotic drugs may prove to be useful tools in animal models that involve neuronal death, and may 1 day prove to be a useful treatment in various neurological disorders, including epilepsy.

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