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European Journal of Pharmacology 458 (2003) 81–89
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Protective effects of selegiline and desmethylselegiline against *N*-methyl-D-aspartate-induced rat retinal damage

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Received 20 June 2002; received in revised form 5 November 2002; accepted 6 November 2002

Abstract

Selegiline, a therapeutic agent of Parkinson's disease, and its metabolite, desmethylselegiline, were explored for their neuroprotective effects against *N*-methyl-D-aspartate (NMDA)-induced cell death in rat retina. Morphometric analysis of the retina revealed that an intravitreal injection of NMDA induced a significant decrease in cell density in the ganglion cell layer and in thickness of the inner plexiform layer, but not of other retinal layers such as the outer nuclear layer. Concurrent intravitreal injection of selegiline with NMDA did not show a significant protective effect, whereas co-injection of desmethylselegiline provided protection from NMDA-induced retinal damage. Parenteral administration (both single and consecutive dosing) of selegiline significantly prevented loss of ganglion cell layer cells. Counting of retinal ganglion cells by fluorescent tracer labeling confirmed that selegiline protected retinal ganglion cells from NMDA toxicity. The selegiline treatment did not produce a significant increase, though it tended to such as effect, in a brain-derived neurotrophic factor (BDNF) level in the retina, when compared with the NMDA-treated control group. These results indicate that parenteral treatment with selegiline rescues inner retinal cells from NMDA-induced neural damage, and that desmethylselegiline may contribute, in part, to the protective activities of selegiline. The neuroprotective effects exerted by selegiline may be attributed partially to a change in the retinal BDNF expression.

Keywords: Selegiline; Desmethylselegiline; NMDA (*N*-methyl-D-aspartate); Retinal degeneration; Cell death; BDNF (brain-derived neurotrophic factor)

1. Introduction

Selegiline, a selective monoamine oxidase-B inhibitor, has been used worldwide for the therapy of Parkinson's disease (Birkmayer et al., 1985; The Parkinson Study Group, 1989). In addition, selegiline has been shown to reduce cell death of different types of neurons, such as neurons of the frontal cortex (Amenta et al., 1994), hippocampus (Barber et al., 1993) and facial motoneurons (Ansari et al., 1993) after various sorts of insults. It has been suggested that selegiline exerts neuroprotective activities through several mechanisms independent of its monoamine oxidase-B inhibitory action (Ansari et al., 1993; Tatton and Chalmers-Redman, 1996a). For example, an anti-oxidant effect per se (Mytilineou et al., 1998), upregulation of anti-oxidant enzymes (Carrillo et al., 1992), preservation of mitochondrial membrane potential (Tatton, 1999) and increases in transcription of trophic factors including brain-derived neurotrophic factor (BDNF) and ciliary neurotrophic factor in cultured astrocytes (Mizuta et al., 2000; Seniuk et al., 1994) have been reported as mechanisms by which neuroprotection may be conferred.

In a number of ophthalmic disorders including glaucoma, the cause and the mechanism underlying retinal neuronal cell death are not well understood. In glaucoma, an elevation in intraocular glutamate levels that leads to glutamate receptor-mediated neurotoxicity is regarded as a key trigger (Yoles and Schwartz, 1998), in conjunction with the subsequent events including influx of Ca^{2+}, activation of various Ca^{2+}-dependent enzymes, nitric oxide and free

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PII: S0014-2999(02)02729-2

radical formation and depletion of ATP (Kristián and Siesjö, 1998). As self-defense mechanisms against such neurotoxicity or neuronal damage, several neurotrophic factors like nerve growth factor (Carmignoto et al., 1989) and BDNF (Mansour-Robaey et al., 1994; Mey and Thanos, 1993) have been reported to promote survival of retinal ganglion cells after axonal damage.

Several research groups have reported that selegiline increases the survival of retinal ganglion cells after optic nerve crush in rats (Buys et al., 1995), and exerts therapeutic effects in some glaucoma patients (Trope et al., 1994). In axotomized animal models, an inadequate supply of neurotrophic factors such as BDNF from the superior colliculus was proposed to result in neurodegenerative processes (Ma et al., 1998). To our knowledge, there are yet no reports about the effects of selegiline on retinal neurons that underwent glutamate receptor-mediated damage. We report here that selegiline and its desmethyl metabolite show some protective effects on glutamate receptor-mediated neurodegenerative processes in adult rat retina.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (7 weeks old, Nihon SLC, Shizuoka, Japan) were maintained in a humidity (55 ± 10%)- and temperature (23 ± 2 °C)-controlled facility under a 12-h light/12-h dark cycle (light on at 8:00 a.m.) with free access to food (MF chow pellets, Oriental Yeast, Tokyo, Japan) and water. The rats were acclimated for 1 week before use in experiments.

All animal procedures were in accordance with the Institutional Guidelines for Care and Use of Laboratory Animals.

2.2. Drugs

The following compounds were used: selegiline hydrochloride and desmethylselegiline hydrochloride (Fujimoto Pharmaceutical, Osaka, Japan), dizocilpine maleate ((+)-MK-801; Sigma, St. Louis, MO, USA), *N*-methyl-D-aspartate (NMDA; Nacalai tesque, Kyoto, Japan).

2.3. Animal models with NMDA receptor-mediated retinal neuronal damage

Animal models were made as described by Morizane et al. (1997). Briefly, each Sprague–Dawley rat was anesthetized by an intraperitoneal injection of sodium pentobarbital (50–80 mg/kg), and then given 1% atropine sulfate drops to the right eye to produce full dilation of the pupil. A single intravitreal injection of NMDA (200 nmol in 5 μl of sodium phosphate buffer, containing 0.03 M NaOH) was given in the right eye, using a 33-gauge needle connected to a 25-μl Hamilton syringe. Injection was done slowly over a period of 1 min. Histological sections were prepared from both eyes 7 days after NMDA injection. The control group of rats received 5 μl of vehicle (sodium phosphate buffer, containing 0.03 M NaOH) to the right eyes.

2.4. Schedule for drug administration

Drugs were administered on the following schedule: (1) single intravitreal injection of NMDA with or without (+)-MK-801 (10 nmol), selegiline (0.1, 1, 10 nmol) or desmethylselegiline (0.1, 1, 10 nmol) was administered; (2) selegiline (1 or 10 mg/kg) was subcutaneously administered 30 min before an intravitreal injection of NMDA; and (3) in consecutive administration, the animals were given selegiline (0.1 or 1 mg/kg, as the hydrochloride salt, s.c.) 30 min before an intravitreal injection of NMDA and thereafter daily injections over a period of 7 days (total of eight injections), except for measurement of the BDNF level (10 mg/kg selegiline, total of four injections). The doses of selegiline are expressed as those of the hydrochloride salt.

2.5. Morphometric analysis

Seven days after NMDA injection, the animals were killed and both eyes were enucleated. Eyes were immediately fixed in phosphate-buffered 4% formalin and 1% glutaraldehyde aqueous solution (pH 7.4), followed by phosphate-buffered 10% formalin solution (pH 7.4). After fixation, eyes were embedded in paraffin and subjected to six horizontal meridional sectioning (5 μm thick) through the optic disk of each eye. The sections were then stained with hematoxylin and eosin.

Morphometric analysis was performed to quantify NMDA-induced injury. Three sections were selected randomly for each eye. A microscopic image of each section within 1 mm of the optic disk was scanned digitally with the aid of an image analysis system including a 3CCD camera module (XC-009, Nexus, Tokyo, Japan) and an image analysis processor (nexusQube, Nexus). For assessment of the degree of injury in each eye, the number of cells in the ganglion cell layer was counted for a 1-mm range of the optic disk. The thickness of the inner plexiform layer, inner nuclear layer and outer nuclear layer randomly selected was measured at three points with image analysis software (NIH image, W. Rasband). The number of cells in the ganglion cell layer was calculated as linear cell density (cells/mm, the mean number of cells: 87 ± 2 cells/mm (intact)). Finally, the thickness of the inner plexiform layer (mean thickness: 48.38 ± 0.81 μm (intact)), inner nuclear layer and outer nuclear layer, and the linear cell density in the ganglion cell layer were each expressed as the mean of nine measurements. For each animal, values of the parameters for the right eye were expressed as a ratio (%) to those for the intact left eye. No attempt was made to distinguish the cell types in the ganglion cell layer for counting the cell number.

2.6. Analysis for loss of retinal ganglion cells

Retrograde labeling was performed in a manner similar to that described by Sawada and Neufeld (1999). Three days after an intravitreal injection of NMDA, the animals were anesthetized by an intraperitoneal injection of sodium pentobarbital (50–70 mg/kg), and the head was placed firmly in a stereotaxic apparatus. A solution of 4% aminostilbamidine methanesulfonate (Molecular Probes, Eugene, OR, USA) was microinjected bilaterally into the superior colliculus ($A = -6.3$ mm, $L = \pm 1.5$ mm from bregma and $H = -3.6$ mm from the surface of the skull). Four days later, the animals were killed and both eyes were enucleated. The eyes were fixed for 45 min in 4% paraformaldehyde/phosphate-buffered saline. The retina was divided into six radial cuts and then removed from the sclera and mounted on slides. The regions subjected to counting of retinal ganglion cells were selected from four fields (305×408 μm) of the central area (1–2 mm from the optic disc) and four fields of the peripheral area (4 mm from the optic disc) for each retina. The aminostilbamidine-labeled retinal ganglion cells were counted with NIH image software. For each animal, the survival of retinal ganglion cells in the right retina was expressed as a ratio (%) to those in the intact left retina.

2.7. Measurement of retinal BDNF levels

The protein level of BDNF in retina and superior colliculus was determined with an enzyme-linked immunosorbent assay (ELISA) (BDNF immunoassay system, Promega, Madison, WI, USA) as described by Nitta et al. (1999). Each retina and superior colliculus was homogenized in the lysis buffer (20 mM Tris–HCl, pH 8.0, containing 137 mM NaCl, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 1 μg/ml leupeptin, 0.5 mM sodium vanadate), and then centrifuged at 15,000 rpm for 10 min. The supernatant was used as tissue extract sample. Multiwell plates to be used for ELISA were incubated for coating with anti-BDNF monoclonal antibody diluted 1000-fold in carbonate buffer (pH 9.7) for 12 h, washed with washing buffer (20 mM Tris–HCl pH 7.6, containing 150 mM NaCl and 0.05% Tween20), and then blocked by incubating with block and sample buffer. A tissue extract sample or BDNF standard solution was added to each antibody-coated well, and incubated for 2 h at room temperature. After five washes with washing buffer, anti-BDNF polyclonal antibody diluted 500 fold was added to each well, and the plates were incubated for 2 h at room temperature. Then, after thorough washing, horseradish peroxidase-conjugated anti-IgY solution diluted 2000-fold was added, and the plates were incubated for 1 h at room temperature. After washing, the remaining horseradish peroxidase activity was assessed by a colorimetric method following incubation with 3,3′, 5,5′-tetramethylbenzidine. Absorbance was measured at 450 nm. For each animal, BDNF levels in the right retina and the left superior colliculus were expressed as a percentage of the level in the corresponding control tissue of the same animal.

2.8. Statistics

Values were expressed as means and S.E.M. for 4–11 animals. Statistical analyses were performed with Dunnett's or Tukey's test, and differences were considered significant at $P < 0.05$.

3. Results

3.1. NMDA-induced retinal neurotoxicity with or without single intravitreal co-injection of (+)-MK-801, selegiline, or desmethylselegiline

In the first set of experiments, we investigated whether selegiline and its metabolite, desmethylselegiline, show an acute neuroprotective effect against NMDA-induced retinal damage when administered by a single intravitreal co-

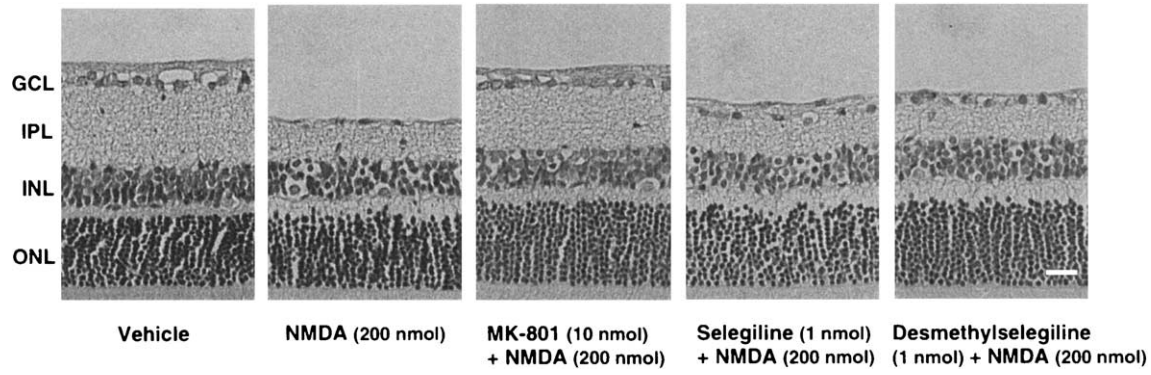

Fig. 1. Light microscopic photographs of hematoxylin/eosin-stained transverse sections from rat retinas 7 days post injection. The retina received an intravitreal injection of vehicle, 200 nmol NMDA, a combination of 200 nmol NMDA and 10 nmol (+)-MK-801, or a combination of 200 nmol NMDA and 1 nmol selegiline and 200 nmol NMDA and 1 nmol desmethylselegiline. Abbreviations: GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; ONL, outer nuclear layer. Scale bar = 20 μm.

Table 1
Effect of MK-801 on NMDA-induced retinal damage 7 days post injection

Treatment	Ganglion cell layer (%)	Inner plexiform layer (%)
Vehicle	102.5 ± 9.0	106.2 ± 3.7
NMDA (200 nmol)	43.3 ± 2.2[a]	43.9 ± 2.9[a]
NMDA (200 nmol) + MK-801 (10 nmol)	67.9 ± 3.5[b]	85.6 ± 7.4[b]

All drugs were intravitreally injected: vehicle, NMDA (200 nmol) and (+)-MK-801 (10 nmol). Each value represents an average for a group of five to eight rats, and the vertical bars represent S.E.M.
[a] $P<0.01$ vs. vehicle-treated group.
[b] $P<0.01$ vs. NMDA-treated group.

injection. Fig. 1 shows histopathological images of representative retinas in the control and experimental eyes at 7 days after intravitreal injection of NMDA. No histological alterations following intravitreal injection of vehicle were detectable in the control eyes. Injection of NMDA (200 nmol) resulted in marked cell loss in the ganglion cell layer, and thinning of the inner plexiform layer, but not of the inner nuclear layer and outer nuclear layer, in agreement with previous reports (Morizane et al., 1997; Honjo et al., 2000). Simultaneous intravitreal injection of (+)-MK-801 (10 nmol) with NMDA markedly attenuated the cell loss in the ganglion cell layer and the thickness reduction of the inner plexiform layer compared to those in the NMDA-treated group (Fig. 1, Table 1). When selegiline (0.1–10 nmol) was intravitreally co-injected with NMDA, the density of cells in the ganglion cell layer tended to be preserved, but was not significantly different from that in the NMDA-treated group (Figs. 1 and 2A). In addition, selegiline failed to prevent NMDA-induced inner plexiform layer thinning

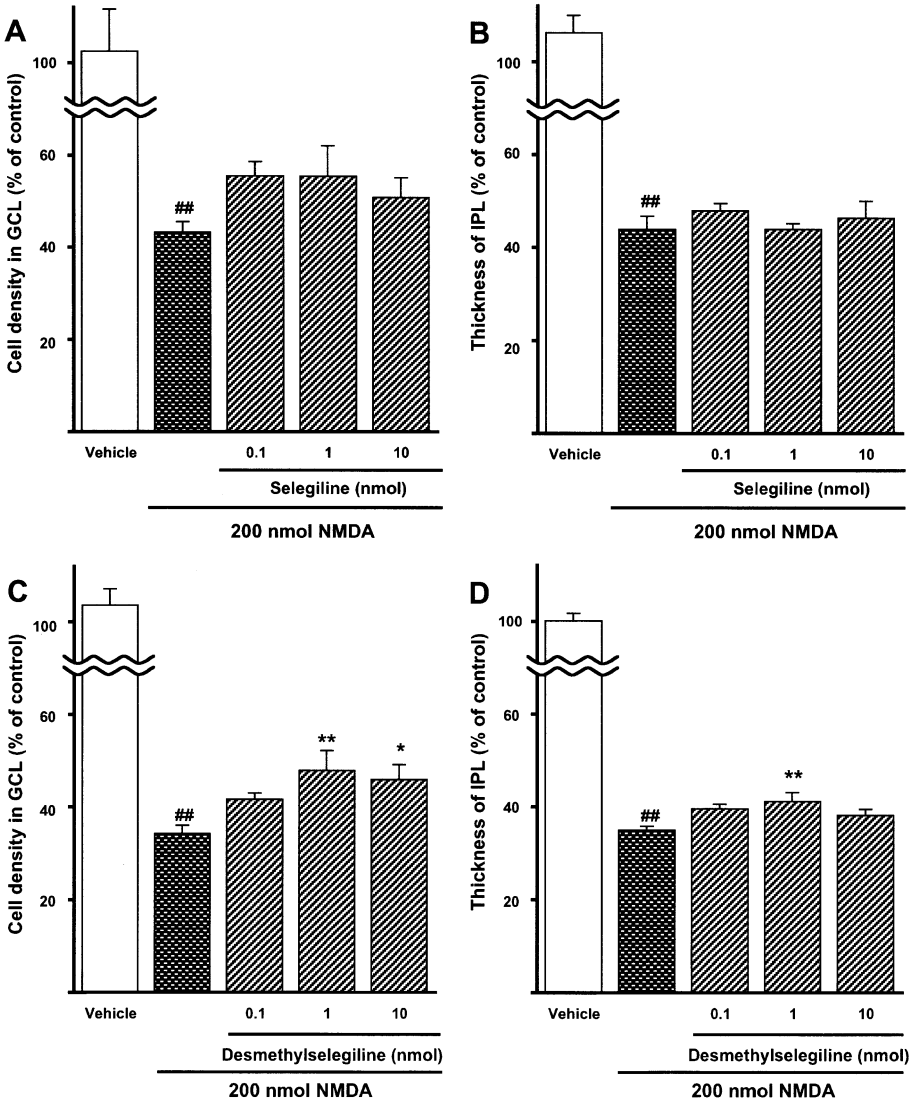

Fig. 2. Effects of selegiline and desmethylselegiline on NMDA-induced retinal damage by a single intravitreal injection. All drugs were intravitreally injected: NMDA (200 nmol), (+)-MK-801 (10 nmol), selegiline (0.1, 1, 10 nmol) and desmethylselegiline (0.1, 1, 10 nmol). Panels A and C and panels B and D show cell density in the ganglion cell layer and thickness of the inner plexiform layer 7 days after the intravitreal injection, respectively. Each value represents an average for a group of 5–11 rats, and the vertical bars represent S.E.M. *$P<0.05$, **$P<0.01$.

(Fig. 2B). In contrast, co-injection of desmethylselegiline significantly reduced NMDA-induced injury in ganglion cell layer and inner plexiform layer, with little effect on the outer nuclear layer (Figs. 1 and 2C,D).

3.2. Effects of parenteral administration of selegiline on NMDA-induced retinal damage

To examine the effects of pretreatment with selegiline and its consecutive parenteral administration, the animals were given an subcutaneous injection of selegiline 30 min before an intravitreal injection of 200 nmol NMDA (Fig. 3A and B), and thereafter daily injections for over a period of 7 days (Fig. 3C and D). As shown in Fig. 3A and B, retinas from the animals pretreated subcutaneously with 10 mg/kg of selegiline were found to have less cell loss in the ganglion cell layer than the untreated control, but showed no difference in NMDA-induced inner plexiform layer thinning. Pretreatment with a lower single dose of selegiline (1 mg/kg) also ameliorated the cell loss in ganglion cell layer, though recovery in cell density was not statistically significant. However, a significant recovery from the NMDA-induced reduction in ganglion cell layer cell density was observed after pre-and consecutive administration of 1 mg/kg selegiline for 7 days (Fig. 3C and D). With respect to the left eyes (intact), neither the pretreatment nor the addition of consecutive injections of selegiline affected any retinal areas, suggesting no toxic influence of selegiline on retina.

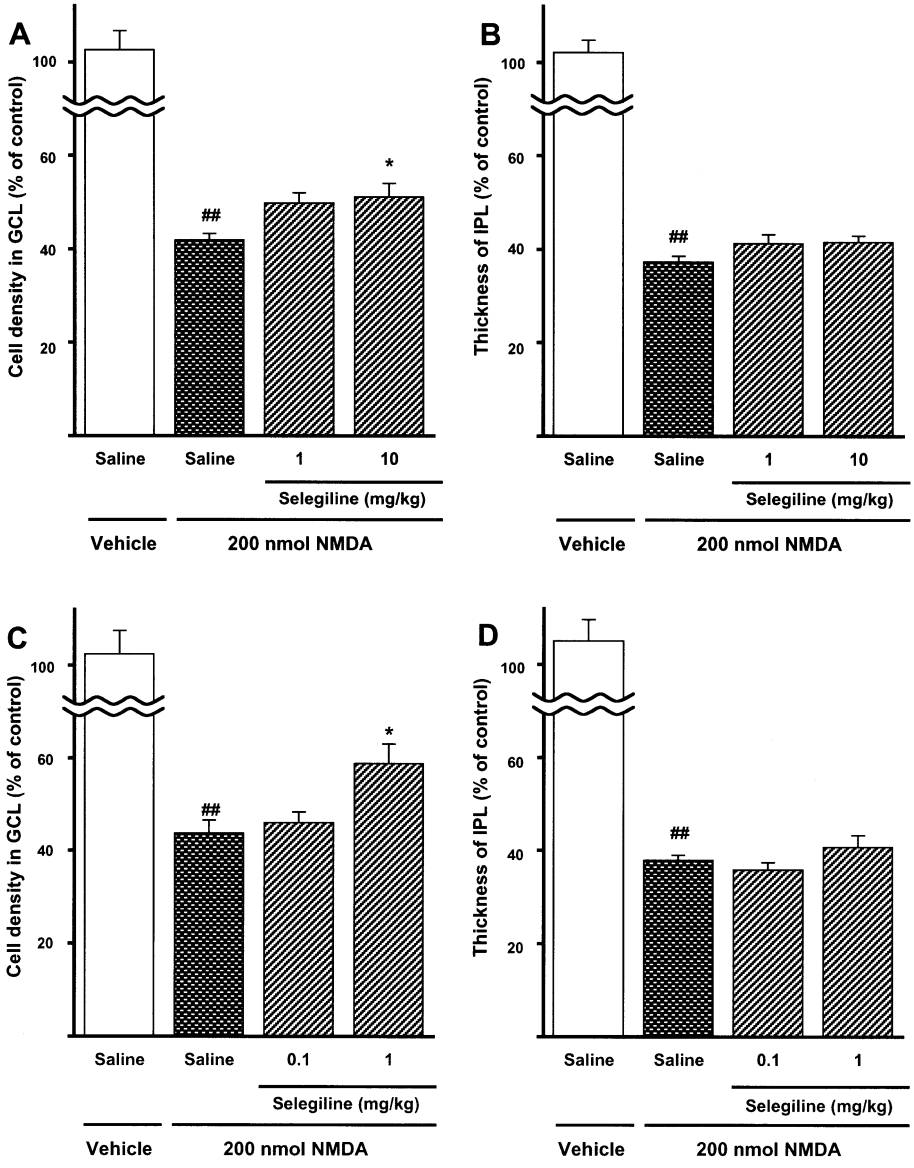

Fig. 3. Effects of parenteral administration of selegiline on NMDA-induced retinal damage. Different doses of selegiline (0.1, 1, 10 mg/kg) were subcutaneously administered 30 min before an intravitreal injection of 200 nmol NMDA (A and B), and thereafter as daily injections for 7 days (C and D). Panels A and C and panels B and D show cell density in the ganglion cell layer and thickness of the inner plexiform layer at 7 days after intravitreal injection, respectively. Each value represents an average for a group of four to nine rats, and the vertical bars represent S.E.M. *$P<0.05$; **$P<0.01$.

In rat retina, the ganglion cell layer contains two different neuronal populations: ganglion cells and displaced amacrine cells (Perry, 1981). NMDA receptors are expressed on both cell types in the ganglion cell layer. To specifically evaluate the effect of selegiline on NMDA-induced loss of retinal ganglion cells, we analyzed the retinas isolated from animals that had received an aminostilbamidine injection to the superior colliculus for retrograde labeling of retinal ganglion cells. As shown in Figs. 4 and 5, the number of aminostilbamidine-labeled retinal ganglion cells in the retina treated with NMDA decreased markedly, as compared to the number in the vehicle-treated retina. Parenteral administrations of 1 mg/kg selegiline for 7 days resulted in an increase in retinal ganglion cell survival compared to that in the selegiline-untreated group. These results demonstrated that selegiline protect retinal ganglion cells in the ganglion cell layer from NMDA-mediated neurotoxicity.

3.3. Influence of selegiline on BDNF levels in retina and superior colliculus of the rat with NMDA-induced retinal damage

The above-mentioned results suggest that repeated parenteral administration of selegiline confer neuroprotection to retinal neuronal cells receiving an NMDA insult. To elucidate the mechanisms of these neuroprotective properties of selegiline, we aimed at measuring BDNF levels because several research groups have reported neuroprotective effects of BDNF in the retina (Kido et al., 2000; Vecino et al., 1999) and a selegiline-induced increase in transcription of trophic factors including BDNF in cultured astrocytes (Mizuta et al., 2000; Seniuk et al., 1994).

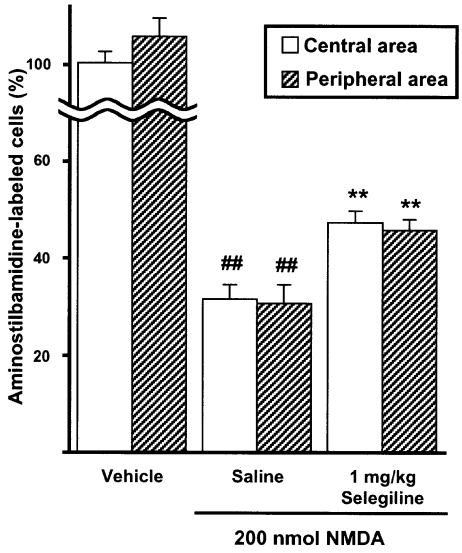

Fig. 5. Effects of parenteral administration of selegiline on loss of aminostilbamidine-labeled retinal ganglion cells at 7 days after intravitreal injection of NMDA. Selegiline (1 mg/kg) was subcutaneously administered 30 min before an intravitreal injection of 200 nmol NMDA, and thereafter as daily injections for 7 days. Each value represents an average for a group of six to eight rats, and the vertical bars represent S.E.M. $^{**}P<0.01$ vs. NMDA and saline-treated control group.

Marked degeneration in the retina was observed 7 days after NMDA injection, suggesting that significant changes of the expression of functional molecules including BDNF are induced by the retinal damage. Moreover, we measured BDNF levels on the third day following NMDA injection, because intravitreal administration of NMDA was expected to upregulate BDNF within a few days. Protein levels of BDNF in experimental (NMDA-injected; right) and intact (left) retinas, and also in the superior colliculus of both

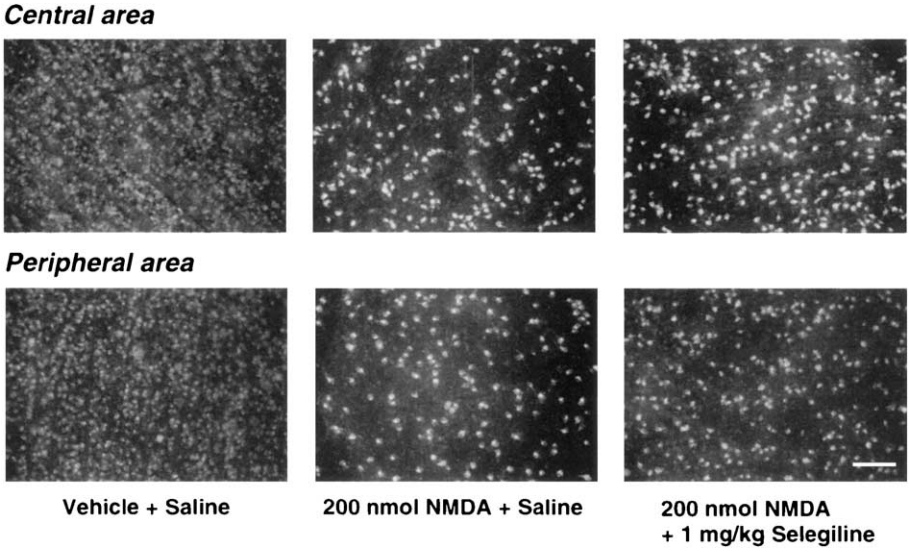

Fig. 4. Fluorescence microscopic photographs of retinal sections from experimental eyes at 7 days after intravitreal injection of NMDA. Selegiline (1 mg/kg) or saline was subcutaneously administered 30 min before an intravitreal injection of 200 nmol NMDA, and thereafter as daily injections for 7 days. Retinal ganglion cells were labeled retrogradely with aminostilbamidine. Scale bar = 100 μm.

sides were measured by ELISA. As shown in Fig. 6A, retinal BDNF levels on the third day following NMDA injection significantly ($P=0.042$) increased in comparison with the levels in the vehicle-injected retina. When selegiline (10 mg/kg) was subcutaneously administered 30 min before NMDA injection and thereafter daily injections for over a period of 3 days, BDNF levels in the retina were markedly elevated ($P=0.0005$) compared to those in the vehicle-treated group. The levels were higher, though not significantly so, than those of the NMDA-injected, selegiline-untreated group. On the other hand, BDNF levels in the superior colliculus remained unchanged regardless of treatments with NMDA or NMDA plus selegiline (Fig. 6B).

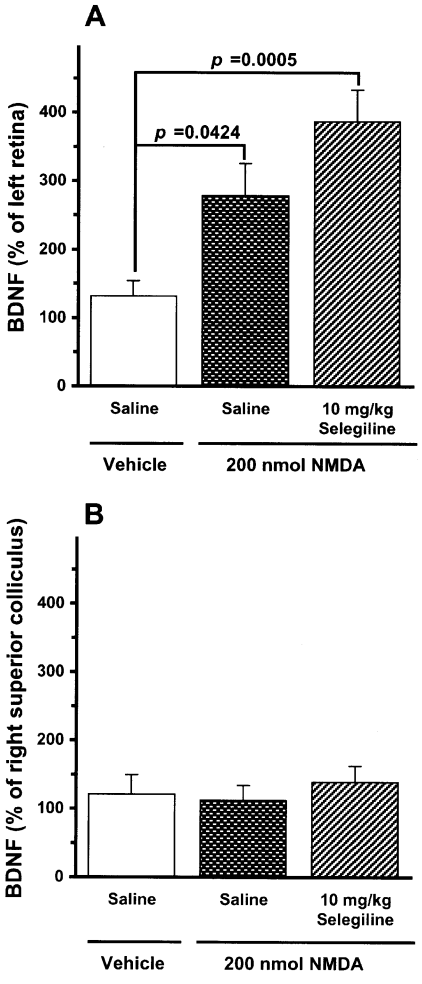

Fig. 6. Effects of selegiline on BDNF content in the retina and superior colliculus at 3 days after an intravitreal injection of NMDA. Selegiline (10 mg/kg) was subcutaneously administered 30 min before an intravitreal injection of 200 nmol NMDA, and thereafter as daily injections for 3 days. (A) The value for the right retina from each animal is expressed as a ratio to the value for the left retina (intact). (B) A value for the left superior colliculus of each animal is expressed as a ratio of the value for the right superior colliculus. Each value represents an average for a group of eight rats, and the vertical bars represent S.E.M. $^{*}P<0.05$, $^{**}P<0.01$.

4. Discussion

In the central nervous system, abnormal release of glutamate occurs upon neurodegenerative insults such as ischemia, which leads to degeneration of a number of neural populations (Choi, 1988; Delbarre et al., 1991). Similarly, glutamate acts as a neurotransmitter in the retina and, under certain conditions, it exerts a neurotoxic effect in the inner retina (Lucas and Newhouse, 1957). Because NMDA receptors are hypothesized to be a common pathway and a predominant route of glutamate-induced neurotoxicity in many neurodegenerative diseases, the rats receiving an intravitreal injection of NMDA were used as an animal model of retinal neurodegeneration in the present study.

Intravitreal injection of NMDA resulted in a significant cell loss in the ganglion cell layer and thinning of the inner plexiform layer, in agreement with previous reports (Morizane et al., 1997; Honjo et al., 2000). Parenteral administration of selegiline resulted in a lesser degree of NMDA-induced cell loss in the ganglion cell layer. In previous in vitro experiments using an immortalized retinal cell line (E1A-NR3), selegiline significantly increased the survival rate under serum-free and hypoxic conditions at concentrations ranging from 10^{-9} to 10^{-4} M (Ragaiey et al., 1997; Xu et al., 1999). Based on the assumption of a vitreal volume of 60 μl, the intravitreal concentrations of selegiline in the present study can be estimated as $1.7-167$ μM. However, intravitreal co-injection of selegiline and NMDA only tended to increase the density of ganglion cell layer cells compared to that of the NMDA-injected group. It has been reported that plasma concentrations of selegiline in the same range as that mentioned above could be reached within 10 min after subcutaneous administration of 10 mg/kg selegiline to the rat (Melega et al., 1999). In the present study, pretreatment (30 min before NMDA injection) with selegiline (10 mg/kg) ameliorated the cell loss in the ganglion cell layer. Moreover, significant amelioration of the NMDA-elicited cell loss in the ganglion cell layer was observed on pre-and consecutive administration of 1 mg/kg selegiline for 7 days, whereas a single pretreatment (30 min before NMDA injection) with selegiline, 1 mg/kg, did not give rise to a significant recovery. These results suggest that the presence of selegiline in the retina during and after NMDA exposure is necessary for the protective effect. There are many reports indicating that selegiline may exert anti-apoptotic actions that are independent of monoamine oxidase-B inhibition (Tatton et al., 1996b; Mytilineou et al., 1997a). Mytilineou et al. (1997a) reported that selegiline had no effect in ligand binding experiments on NMDA receptors, and speculated that it would act downstream of NMDA receptor activation. The fact that the protective effect of selegiline was significant on the ganglion cell layer, layer of cell bodies, rather than on the inner plexiform layer, layer of dendritic processes, may be relevant to the involvement of an anti-apoptotic action of this compound. Procedures to block apoptosis can preserve neuronal cell

bodies but at the same time fail to prevent neurite degeneration (Sagot et al., 1995). On the other hand, selegiline has been shown to augment transcription of neurotrophic factors, including BDNF and ciliary neurotrophic factor in cultured astrocytes (Seniuk et al., 1994; Mizuta et al., 2000). Thus, we investigated the ability of selegiline to induce the expression of BDNF at a sufficient level to protect retinal neurons from NMDA toxicity. Although Vecino et al. (1999) reported that 40 nmol NMDA transiently induced BDNF expression in rat retina 2 h but not 4 h after injection, there are no reports of BDNF up-regulation by intravitreal injection of NMDA at concentrations that cause histological changes. In the present study, an increase in BDNF levels was induced by 200 nmol NMDA. Although the protection by selegiline could not be explained solely by the change in intraocular BDNF levels, this might have contributed partially to a change in retinal BDNF because there was an obvious tendency for BDNF levels to increase compared to those in the NMDA plus saline-treated group. Intravitreal injection of BDNF (1 μg) has been reported to protect inner retinal cells from NMDA- or elevated intraocular pressure-induced neuronal death (Kido et al., 2000; Ko et al., 2000). As these latter experiments BDNF was exogenously supplied to the retina, large doses of BDNF might be required. It is noteworthy that a combined treatment with BDNF and antioxidants decreased the dosage requirement for BDNF compared to the dosage requirement for treatment with BDNF alone (Ko et al., 2000). As selegiline possesses unique, anti-apoptotic effects as described above, mechanisms for rescuing retinal ganglion cells by selegiline treatment may involve not only up-regulation of BDNF, but also anti-apoptotic responses.

In vivo, selegiline is metabolized mainly by the cytochrome P-450 system and the metabolites identified are desmethylselegiline, *l*-methamphetamine and *l*-amphetamine (Heinonen et al., 1994). Of these metabolites, desmethylselegiline has been reported to enhance neuronal survival under excitotoxic insults (Mytilineou et al., 1997b), the survival of trophically-withdrawn PC12 cells, and the recovery of axotomized immature facial motor neurons (Tatton and Chalmers-Redman, 1996a). In humans, desmethylselegiline is present in plasma within 24 h after a single oral administration of selegiline (Heinonen et al., 1994). In the present study, desmethylselegiline was more effective when co-administered intravitreally with NMDA, while a single intravitreal co-injection of selegiline failed to generate a significant protective effect. Moreover, several investigators have reported that the neuroprotective efficacy of desmethylselegiline is equivalent to, or greater than that, of selegiline (Tatton and Chalmers-Redman, 1996a; Mytilineou et al., 1997b). Together, these observations indicate that part of the neuroprotective effect of selegiline might be attributable to that of desmethylselegiline.

Finally, monoamine oxidase-B inhibition may contribute to the protective effect of selegiline observed above. In this context, Kashii et al. (1994) reported that exogenous dopamine protected cultured retinal neuronal cells against NMDA neurotoxicity. As monoamine oxidase-B activity is detectable in the bovine retina (Arriba et al., 1991) and selegiline and desmethylselegiline inhibit monoamine oxidase-B, further investigations are required to clarify whether or not the neuroprotective effect by selegiline is independent of its monoamine oxidase-B inhibitory action.

In conclusion, treatment with selegiline or its desmethyl metabolite leads to protection of retinal neuronal cells in the inner layers from NMDA receptor-mediated excitotoxicity, and these agents could be clinically useful for retinal diseases associated with neurodegeneration.

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