The Cognition-Enhancer Nefiracetam Inhibits Both Necrosis and Apoptosis in Retinal Ischemic Models in Vitro and in Vivo

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ABSTRACT
The retinal ischemic-reperfusion stress (130 mm Hg, 45 min) caused neuronal damage throughout all cell layers and reduced the thickness of retinal layer by 30% at 7 days after the stress of mouse retina. The intravitreal injection of 100 pmol of nefiracetam, a cognition-enhancer, completely prevented the damage when it was given 30 min before and 3 h after the stress. Partial prevention was observed when it was given 24 h after the stress, or low dose (10 pmol) nefiracetam was given 30 min before the stress. However, aniracetam had no effect. In the retinal cell line N18-RE-105, the ischemic-reperfusion stress by 2 h culture under the serum-free condition with low oxygen (less of 0.4% O2) and low glucose (1 mM) caused necrosis or apoptosis in the low-density (0.5 x 10^4 cell/cm^2) or high-density (5 x 10^4 cell/cm^2) culture, respectively. The necrosis showed membrane disruption, loss of electron density, and mitochondrial swelling, whereas apoptosis showed nuclear fragmentation and condensation in transmission electron microscopic analyses and in experiments using specific cell death makers. Nefiracetam inhibited both necrosis and apoptosis, whereas brain-derived neurotrophic factor (BDNF) inhibited only apoptosis. The cell-protective actions of nefiracetam were abolished by nifedipine or omega-conotoxin GVIA, L-type and N-type calcium channel blocker, but not by PD98059 or wortmannin, extracellular signal-regulated kinase 1/2 or phosphoinositide 3-kinase inhibitor, respectively, whereas those of BDNF were abolished by PD98059 and wortmannin, but not by nifedipine or omega-conotoxin GVIA. All these findings suggest that nefiracetam inhibit necrosis and apoptosis occurred in the ischemic/hypoxic neuronal injury through an increase in Ca^{2+} influx.

The retina is a part of the central nervous system that is well accessible for quantitative analysis of pathophysiological processes and experimental manipulation (Ismann et al., 2003). Transient ischemia-induced damage of the retina, as well as the brain, results in a prolonged period of neuronal cell death. The mechanisms of ischemia-induced neuronal cell death include an early phase of excitotoxicity through glutamate, leading to necrosis and prolonged phase of apoptosis (Lombardi and Moroni, 1994; Choi, 1996). Necrosis is characterized by the loss of membrane integrity, disruption of intracellular organelles, cellular swelling, and lysis, whereas apoptosis involves reduction of intracellular volume, nuclear condensation, and fragmentation (Kerr et al., 1972; Bredesen, 1995). Although the molecular mechanisms underlying apoptosis are now well characterized (Yuan and Yankner, 2000), and many therapeutic candidates have been proposed, little is known of mechanisms underlying necrosis and its therapeutic candidates. Because cell destructive features of necrosis induce secondary damages of surrounding cells, the inhibition of necrosis would be an important therapeutic target in the sense of minimization of ischemic neuronal injury.

Nefiracetam, N-(2,6-dimethylphenyl)-2-(2-oxo-1-pyrrolidinyl) acetamide, is a member of oxopyrrolidene acetic acid derivatives such as aniracetam, piracetam, and oxiracetam (Sakurai et al., 1989). Several pharmacological studies have shown that the antiamaesic effect of nefiracetam is related to activation of cholinergic, GABAergic, and/or monoaminergic transmitter systems (Luthman et al., 1992; Fukatsu et al., 2002). The electrophysiological studies revealed that nefiracetam activates voltage-dependent N-type and L-type calcium channels, and induces neuronal recovery in ischemic rat hippocampal neurons (Sakurai et al., 1989). The antiamnesic effect of nefiracetam is related to activation of cholinergic, GABAergic, and/or monoaminergic transmitter systems (Luthman et al., 1992; Fukatsu et al., 2002). The electrophysiological studies revealed that nefiracetam activates voltage-dependent N-type and L-type calcium channels, and induces neuronal recovery in ischemic rat hippocampal neurons.
channels, thus promoting neural transmission (Yoshii and Watabe, 1994; Yoshii et al., 1997). Recent studies demonstrate that nefiracetam has protective actions against memory dysfunction, electrophysiological, and metabolic damage in the brain given ischemic stress (Jin et al., 2002; Takeo et al., 2003a,b). Although several neuromodulatory mechanisms underlying antiinflammatory actions of nefiracetam have been proposed in brain ischemia models, little is known of actions on survival activity of neurons. We first reported that nefiracetam protects apoptotic neuronal cell death under serum-free starvation stress condition (Fujita et al., 2001). We have also found that the neurons die by necrosis in the low-density culture under such serum-free condition, whereas they die by apoptosis in the high-density culture (Fujita et al., 2001; Fujita and Ueda, 2003b). However, it remains to be determined whether nefiracetam could inhibit neuronal ischemia in vivo, and how it shows a neuroprotective function in the culture system, particularly about the specificity for necrosis or apoptosis. Here, we examined the neuroprotective actions of nefiracetam on the neuronal damages due to retinal ischemic-reperfusion stress in mice and characterized its neuroprotective actions by use of mouse neuroblastoma × rat neural retina hybrid N18-RE-105 cells.

Materials and Methods

Materials

Nefiracetam and aniracetam were synthesized by Daiichi Pharmaceutical Co. Ltd. (Tokyo, Japan). Dulbecco’s modified Eagle’s medium (DMEM) was purchased from Nissui Pharmaceutical (Tokyo, Japan), and fetal bovine serum (FBS) was from BioSource International (Camarillo, CA). Propidium iodide (PI) and α-conotoxin GVIA were purchased from Sigma (Tokyo, Japan). Nifedipine and wortmannin were purchased from Wako Pure Chemicals (Tokyo, Japan). Dulbecco’s modified Eagle’s medium was from Dainippon Sumitomo Pharmaceutical (Osaka, Japan).

Induction of Ischemic/Reperfusion Injury. We used a modified method of retinal ischemic-reperfusion injury in mice according to previously published methods in rats (Adachi et al., 1998). Briefly, male ICR mice (20–30 g; Tagawa Experimental Animals, Nagasaki, Japan) were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg), and the pupil was fully dilated with 1% atropine sulfate drops. The anterior chamber was cannulated with a 33-gauge needle connected to a container of sterile intraocular irrigating solution (BSS PLUS dilution buffer; Alcon, Fort Worth, TX). Retinal ischemia was induced by elevating the intraocular pressure to generate a hydrostatic pressure of 130 mm Hg for 45 min by lifting a 33-gauge needle connected to a container of sterile intraocular irrigating solution (BSS PLUS dilution buffer; Alcon, Fort Worth, TX).

Results

Neuronal Protective Actions of Nefiracetam in Retinal Ischemic-Reperfusion Model. Retinal ischemic-reperfusion stress was performed by giving hydrostatic-pressure stress of 130 mm Hg for 45 min to the anterior chamber of eye. The retina was then isolated 7 days after this ischemic stress, and used for H&E staining after the fixation with 4% paraformaldehyde (PFA) in PBS at 4°C overnight and observed under a fluorescence microscope (BX50; Olympus; Tokyo, Japan).

Immunocytochemistry of Caspase-3. Cells on 8-well Lab-Tek chamber were stained with PI and Hoechst 33342, fixed with 4% PFA in PBS for 30 min at 25°C, and followed by permeabilization using 50 and 100% methanol for 5 min, respectively. They were incubated in blocking buffer (2% low-fat milk powder, 2% bovine serum albumin, 0.1% Tween 20, in PBS, pH 7.4) for 1 h at 25°C. Anticleaved caspase-3 antibody (1:100; cell signaling) was added to the cells. After 2-h incubation at 25°C and washing, the cells were incubated with FITC-conjugated anti-rabbit IgG (1:200; Cappel, Aurora, OH) for 4 h at 25°C. Immunolabeled cells were observed under a fluorescence microscope.

Transmission Electron Microscopy. Cultured cells were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 1 h at 25°C. The fixed cortical neurons were postfixed with 2% osmium tetroxide for 1 h at 25°C, dehydrated in graded alcohol series, and embedded in Epon 812. Ultrathin sections (80 nm in thickness) were cut with an Ultracut S (Leica, Tokyo, Japan) and then stained with uranyl acetate and lead citrate for 30 and 5 min, respectively. The stained sections were observed under an electron microscope (JEM-1200; JEOL, Tokyo, Japan).

Analysis of DNA Fragmentation. Cells on 9-cm dishes were harvested and centrifuged at 350g for 5 min, and the pellet was used for DNA fragmentation analysis, as described previously (Fujita and Ueda, 2003a). Briefly, the cells were lysed in 250 μl of lysis buffer (50 mM Tris·HCl, 20 mM EDTA, pH 8.0, and 0.5% Triton X-100) with gentle shaking for 15 min at 4°C. The lysates were centrifuged at 6000g for 10 min at 4°C to separate the fragmented DNA (supernatant) and intact chromatin DNA (pellet). The fragmented DNA was extracted with phenol/chloroform/isoamylalcohol, and the DNA in the aqueous phase was precipitated with 2 volume of ethanol after the addition of sodium acetate (final concentration, 0.3 M). The DNA was then collected by centrifugation (14,000g for 10 min at 4°C) and dried. The samples were then dissolved in TE buffer (10 mM Tris·HCl and 1 mM EDTA, pH 8.1), incubated for 1 h at 37°C with RNase A (10 μg/ml), and electrophoresed on a 2% agarose gel. The gel was stained with ethidium bromide and then photographed on an ultraviolet illuminator.

Statistical Analysis. For the statistical analysis of data, Student’s t test following multiple comparisons of the analysis of variance was used. The criterion of significance was set at p < 0.05.
paraformaldehyde and paraffin embedding. There was a reduction by approximately 70% in thickness of retinal cell layers, including ganglion cellular layer (GCL), inner nuclear layer (INL) and outer nuclear layer (ONL) as well as inner plexiform layer after the ischemic-reperfusion stress, compared with sham control (Fig. 1, A and B). The neuronal damages reached maximal 7 days after the ischemic-reperfusion stress. When nefiracetam was intravitreously given 30 min before the ischemic-stress at doses of 10 or 100 pmol in a volume of 1 μl of phosphate buffered solution (pH 7.4), the damage throughout all layers was dose dependently prevented (Fig. 1, C and D). Moreover, when 100 pmol of nefiracetam was given 3 h after the ischemic stress complete prevention was observed, but no or if any weak prevention was observed when it was given 24 h after the stress (Fig. 1, E and F). On the other hand, when aniracetam was intravitreously given 30 min before the ischemic stress at doses of 100 pmol, it did not protect ischemic-induced retinal cell death (Fig. 1G). Moreover, quantification of retinal neurons revealed that 100 pmol of pre- or post-treated (3 h) nefiracetam significantly inhibited loss of neurons under the ischemia stress in GCL, INL, and ONL, respectively (Fig. 1, H–J).

Characterization of Cell Density-Dependent Death Mode Switch of Neuroblastoma × Retinal Neuron Hybrid Cells after Ischemic-Reperfusion Stress. To reproduce the cell density-dependent mode switch from necrosis to apoptosis, which has been originally observed in serum-free culture of cortical neurons (Fujita et al., 2001; Fujita and Ueda, 2003b), we attempted to characterize the cell death under various conditions of culture of neuroblastoma × retinal neuron hybrid cells. When the cell culture in the presence of DMEM containing 10% FBS was given 2 h LOG ischemic stress (serum-free, 0.4% O2 and 1 mM glucose), followed by returning to the starting condition (reperfusion), they successfully showed a cell density-dependent survival activity and cell death mode switch. The cells in the LD culture at 0.5 × 10^4 cells/cm^2 died immediately after the reperfusion, as shown in Fig. 2. The survival activity measured by WST-8 became 55.4% of control 1 h after the reperfusion, whereas it reached to as low as 20% at 3 h. However, the survival activity with WST-8 showed a reversal with the further culture at 18 h and later, possibly because of proliferation of residual cells. On the other hand, the cells in HD culture at 5 × 10^4 cells/cm^2 after the reperfusion died more slowly than the case with LD culture. The recovery of WST-8 activity was also found 18 h after the reperfusion (Fig. 2).

We characterized cell death mode using PI staining and Hoechst 33342 under the LOG ischemic stress. The cells in the LD culture 1 h after the reperfusion showed a marked PI staining (73.3%), but no nuclear fragmentation or condensation stained by Hoechst 33342 (6.2%), as shown in Fig. 3A. On the other hand, the cells in the HD culture showed a marked nuclear fragmentation and condensation (60.7%), but not PI staining (4.4%), 12 h after the reperfusion. The cells in the HD culture showed no significant change in PI (0.8%) or Hoechst 33342 (1.1%) staining 1 h after the reperfusion (data not shown).

The caspase 3 is a prosecutor molecule of apoptosis (Nicholson et al., 1995; Tewari et al., 1995). The immunoreactive caspase 3 was only observed in HD cells 12 h after the reperfusion (57.4%) (Fig. 3B), but not in LD cells 1 h after the reperfusion (3.1%) (Fig. 3B). DNA ladder formation, which is also known as a representative indicator for apoptosis (Walker and Sikorska, 1994), mediated by activated caspase 3, was only observed in the HD cells (Fig. 3C). We used TEM analysis in the present study to confirm in evaluation that PI-Hoechst 33342 or PI-caspase 3 stain showed the necrotic or apoptotic changes in the LD and HD culture. The cell immediately after the 2-h LOG stress did not show any change in morphology in the TEM analysis, compared with the cell without LOG stress (Fig. 4, A and B). However, the cell in the LD culture 1 h after the reperfusion showed typical necrotic features, such as membrane destruct-
Fig. 3. Cell density-dependent death mode switch after the LOG ischemic-reperfusion stress. A, results are representative photographs of double staining of PI and Hoechst 33342. The apoptotic cells were observed as intense signal after staining by Hoechst 33342 (HD, 12 h after reperfusion). B, representative photographs of activated caspase 3 staining. C, ladder formation of DNA derived from cells harvested from control (0 h) or LOG stress-treated culture. Lane M, standard DNA markers; L, LD culture; H, HD culture. In each lane, 10 μg of DNA was applied.

Fig. 4. TEM analysis of cell density-dependent death mode switch in the LOG ischemic-reperfusion stress. Representative photographs of cells cultures in the LD (A–C) or HD (D), without (A) and with ischemic-reperfusion stress (B–D). Insets show high-magnification photographs of mitochondria. Fragmented nuclei are indicated by arrows. Scale bar, 2 μm.

Discussion

A number of studies revealed that nefiracetam has many desirable actions. The cognition-enhancing action has long been discussed in relation to the modulation of neurotransmission (Hiramatsu et al., 1997; Nishizaki et al., 1998; Oyaizu and Narahashi 1999; Nishizaki et al., 2000; Zhao et al., 2001). Nefiracetam also inhibits the development of morphine dependence and tolerance through acute morphine-treatment decrease of intracellular cAMP levels (Itoh et al., 2000). Most recently, Rashid and Ueda reported that nefiracetam shows a potent analgesic action to neuropathic pain, which is resistant to morphine (Rashid and Ueda, 2002). In addition to such neuromodulatory actions, we have also reported that nefiracetam has a neuroprotective action through rapid reduction of WST-8 survival activity of hybrid cells in the LD culture was markedly inhibited by 1 μM nefiracetam, which had been added to the culture before the start of LOG stress, and this inhibition was concentration-dependent between 1 nM and 1 μM of this compound. However, BDNF at 0.1 to 100 ng/ml did not show any survival activity (Fig. 5C). On the other hand, both nefiracetam and BDNF significantly inhibited the slow decrease in the WST-8 activity of HD cells (Fig. 5, D–F). The concentration of nefiracetam required for the survival activity in the ischemia-reperfusion model was 100 times lower than the case with serum-free culture of cortical neurons (Fujita et al., 2001).

Nefiracetam significantly inhibited the PI staining (13.2%) (1 h after the reperfusion of the LD culture, whereas BDNF had no effect (75.4%) (Fig. 5G). Neither nefiracetam nor BDNF has an effect on Hoechst 33342 staining (4.2 nor 6.0%). On the other hand, both nefiracetam and BDNF inhibited the nuclear fragmentation/condensation measured by Hoechst 33342 (5.1 and 3.9%) and caspase 3 activation (7.7 and 4.6%) under the HD culture (Fig. 5H).

In the TEM analyses, on the other hand, it was revealed that the necrotic features observed in the LD culture was inhibited by 1 μM nefiracetam, but not by 100 ng/ml BDNF (Fig. 6, A–C), whereas apoptotic ones in the HD culture was inhibited by both nefiracetam and BDNF (Fig. 6, D–F).

Distinct Mechanisms for Neuroprotective Actions by Nefiracetam and BDNF. The nefiracetam-induced increase in the survival activity of LD or HD culture with LOG stress was significantly inhibited by nifedipine and ω-conotoxin GVIA, L-type, and N-type Ca²⁺ channel inhibitor, respectively (Fig. 7, A and B). However, wortmannin, PI 3-kinase inhibitor, and PD98059, ERK1/2 inhibitor, did not affect the nefiracetam-induced increase in the survival activity in LD or HD culture with the LOG stress. On the other hand, BDNF-induced increase in the survival activity of HD culture was significantly inhibited by wortmannin or PD98059, but not by nifedipine or ω-conotoxin GVIA (Fig. 7B).

The nefiracetam-induced necrosis inhibition (PI staining) in the LD culture and apoptosis inhibition (Hoechst or activated caspase 3 staining) in the HD culture were both significantly inhibited by nifedipine and ω-conotoxin GVIA, respectively (Fig. 7, C and D). However, wortmannin and PD98059 did not affect the nefiracetam-induced necrosis inhibition or apoptosis inhibition. On the other hand, the apoptosis inhibition by BDNF was significantly inhibited by PD98059, but not by ω-conotoxin GVIA (Fig. 7D).
an activation of L- and N-type Ca\(^{2+}\)/H11001 channels (Fujita et al., 2002).

In the clinical, retinal ischemic damages are observed in glaucoma and diabetic retinopathy, and in case of clinical detachment of a retina. In the present study, we have examined the possibility of clinical advantage of nefiracetam against neuronal damages in the retinal ischemic-reperfusion stress model. The model has many advantages in evaluation of neuronal damage and its protection. The pressure stress causes reproducible, quantitative, and time-dependent damages in GCL, inner plexiform layer, INL, and ONL. In addition, topical administration of drugs is available in clinic as well as experiments. Under the present experimental condition, the degree of damage was maximal at 7 days after the stress, and it lasted at least for another week (data not shown). Recently, neuronal stem cells were found in retinal ciliary corpus and ganglion cell layer (Tropepe et al., 2000; Isenmann et al., 2003); however, their regeneration did not recover enough damage from the ischemic-reperfusion stress. The intravitreous injection of nefiracetam 30 min before and 3 h after the stress completely prevented retinal neuronal death (Fig. 1, D and E). Partial prevention was observed when it was given 24 h after the stress, or low-dose (10 pmol) nefiracetam was given 30 min before the stress. Moreover, although it remains to determine whether the parenteral use of nefiracetam is useful for such damage, the topical use of this compound has potential.

The next important issue in this article is the characterization of the mode of neuroprotective action of nefiracetam. For this purpose, we used the retinal cell line neuroblasto\(^{-}/H11003\) retinal neuron hybrid N18-RE-105 cell. Our approaches are aimed to see effects of nefiracetam on either necrosis or apoptosis. In our previous experiments, we first demonstrated that cortical neurons in the LD culture die by necrosis under the serum-free condition without any supplements, whereas they die by apoptosis in the HD culture (Fujita et al., 2001; Fujita and Ueda, 2003b). We have also found that nefiracetam had protective action on the apoptotic cell death in the latter case (Fujita et al., 2002), but it remains to be determined whether this compound has antinecrotic action. In the present study, we first attempted to create both necrotic and apoptotic cell death models, in the

**Fig. 5.** Neuroprotective actions of nefiracetam on necrotic and apoptotic cell death. A and D, time course of nefiracetam (1 \(\mu\)M) or BDNF (100 ng/ml) induced survival activity after the LOG ischemic-reperfusion stress in LD (A) or HD (D) culture. Both compounds were added before the LOG stress until the end of experiments. B, C, E, and F, concentration-dependent survival activities of nefiracetam (B and E) and BDNF (C and F) 3 h or 12 h after reperfusion in LD (B and C) or HD (E and F), respectively. Survival activity was measured by WST-8 reduction activity. Data are the percentage of control activity (0 time after the reperfusion) and expressed as the mean ± S.E.M. from three independent experiments. *, \(p < 0.05\), compared with vehicle treatment.

**Fig. 6.** TEM analyses of nefiracetam-induced inhibition of necrosis and apoptosis. Results are representative TEM photographs of the cell 1 h after the reperfusion under the vehicle (A), nefiracetam (1 \(\mu\)M; B), and BDNF (100 ng/ml; C) in LD culture and 12 h after reperfusion under the vehicle (D), nefiracetam (1 \(\mu\)M; E), and BDNF (100 ng/ml; F) in HD culture. Insets (A–C) show high-magnification photographs of mitochondria. Fragmented nuclei are indicated by arrows. Scale bar, 2 \(\mu\)m.
analogy of cell density-dependent cell death mode switch, observed in the primary culture of cortical neurons. As a result of various trials of stress model, we found that the serum-free culture with LOG stress condition, or low oxygen (0.4%) and low glucose (1 mM) for 2 h (ischemia), followed by replacement with original 10% FBS-containing medium (reperfusion) shows both necrosis and apoptosis when it is performed in the LD and HD culture (Figs. 3 and 4), respectively. Although glucose-deficient condition also shows necrosis in the LD culture, the cell death was so rapid that neuroprotective actions of nefiracetam was hard to be evaluated (data not shown). It was reported that cerebral blood flow decreased to less than 20% of normal cerebral blood flow in ischemic core region in brain (Iadecola, 1999). Moreover, rate of local glucose metabolism in the core region decreased 20% of normal rate of local glucose metabolism (Yao et al., 1995). Therefore, the LOG stress containing 1 mM glucose (cf. 5.5 mM glucose in DMEM) might have some validity for the model of ischemia.

The necrosis observed in the LD culture after the LOG stress was clearly demonstrated in the TEM analysis, showing membrane destruction, loss of electron density, and mitochondrial swelling, and in experiments using PI staining (Figs. 3A and 4C). On the other hand, apoptosis observed in the HD culture after the LOG stress was also clearly demonstrated in the TEM analysis and in experiments using nuclear fragmentation and chromatin condensation by Hoechst 33342, immunocytochemistry of activated caspase 3 and DNA ladder formation (Figs. 3, B–D, and 4D). Nefiracetam prevented all these necrotic and apoptotic features, whereas BDNF prevented only apoptosis (Figs. 5 and 6).

Previously, we have observed that nefiracetam significantly inhibited the cell death due to apoptosis in cortical neurons at concentrations of 1 to 10 μM (Fujita et al., 2002). However, the present study shows that this compound significantly inhibited the cell death due to either necrosis or apoptosis at a concentration of as low as 10 or 100 nM, respectively (Fig. 5, B and E), whereas BDNF did not inhibit the necrotic cell death, but showed a significant inhibition of apoptosis in the present study (Fig. 5, C and F), as reported previously (Han and Holtzman, 2000; Klocker et al., 2000; Fujita and Ueda, 2003a). This strongly indicates that the present experiment is very useful to evaluate selective neuroprotection against necrosis and apoptosis.

Although the details of mechanisms underlying nefiracetam-induced neuroprotection remain to be fully determined, it is evident that the antiapoptotic action of nefiracetam is different from that of BDNF, as shown in Fig. 7 and Table 1. PD98059, a potent inhibitor of ERK1/2, and wortmannin, a potent inhibitor of PI-3 kinase, blocked the BDNF-induced
antiapoptosis action under the HD condition. These results are consistent with the previous findings that BDNF or other neurotrophic factors prevented apoptosis through either PI-3 kinase or ERK1/2 activation in downstream of Trk receptor (for review, see Kaplan and Miller 2000). However, these inhibitors did not affect the nefiracetam’s antiapoptotic actions. On the other hand, nefiracetam-induced antiapoptotic actions were inhibited by nifedipine, a potent inhibitor of L-type Ca$^{2+}$ channel, and ω-conotoxin GVIA, a potent inhibitor of N-type Ca$^{2+}$, respectively, whereas BDNF-induced one was not (Fig. 7; Table 1). Moreover, nefiracetam also showed antinecrotic action through an increase in Ca$^{2+}$ influx under the LD condition (Fig. 7C; Table 1). Thus, BDNF and nefiracetam have different mechanisms for neuroprotection. Recent research has reported that the ischemic preconditioning protects neurons from subsequently induced ischemic damages through an increase in Ca$^{2+}$ influx (Deng et al., 2003). Thus, it is interesting to consider the possibility that nefiracetam-induced neuroprotection is related to such preconditioning mechanisms.

The present study demonstrates that nefiracetam shows a potent neuroprotective action in the retinal ischemia-reperfusion model in vivo and in LOG ischemia-reperfusion stress model of neuroblastoma × retinal neuron hybrid cell in vitro. Nefiracetam inhibited both necrosis and apoptosis in a calcium channel inhibitor-reversible manner.

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References


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