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Effects of nefiracetam on the levels of brain-derived neurotrophic factor and synapsin I mRNA and protein in the hippocampus of microsphere-embolized rats

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Abstract

Our recent study demonstrated that nefiracetam, *N*-(2,6-dimethylphenyl)-2-(2-oxo-1-pyrrolidinyl) acetamide, prevented impairment of the cyclic AMP (cAMP)/cAMP-responsive element binding (CREB) protein signaling pathway in sustained cerebral ischemia. The purpose of the present study was to determine whether nefiracetam has an effect on the expression of brain-derived neurotrophic factor (BDNF) and synapsin I mRNAs that are believed to be produced via CREB, and the alteration in their protein contents in the hippocampus after cerebral ischemia. Sustained cerebral ischemia was induced by injection of 700 microspheres into the right hemisphere of each rat. The rats were treated once daily with 10 mg/kg nefiracetam, p.o., from 15 h after the operation. Treatment with nefiracetam reduced the prolongation of the escape latency in the water maze test on days 7–9 after microsphere embolism-induced sustained cerebral ischemia, suggesting an improvement in the spatial learning function. Microsphere-embolized rats on day 5 showed decreases in BDNF and synapsin I mRNA levels and their protein contents in the ipsilateral hippocampus. Treatment with nefiracetam partially attenuated the decreases. These results suggest that enhancement of BDNF and synapsin I expression by nefiracetam treatment may be, at least in part, due to the improvement in the CREB binding activity, contributing to the prevention of learning and memory dysfunction after sustained cerebral ischemia.

Keywords: Cerebral ischemia; Microsphere; BDNF; Synapsin I

1. Introduction

Nefiracetam, *N*-(2,6-dimethylphenyl)-2-(2-oxo-1-pyrrolidinyl) acetamide, is a pyrrolidone derivative having a cognition-enhancing effect on several amnesias of animal models (Sakurai et al., 1989; Nabeshima et al., 1990, 1991; Doyle et al., 1993). Therefore, it has been suggested that nefiracetam could be a possible nootropic drug against dementia. Several studies have shown the pharmacological effects of nefiracetam, including increases in the uptake and release of cholinergic and γ-aminobutyric acid (GABA)ergic neurotransmitters (Kawajiri et al., 1994; Yoshii et al., 1997), and enhancement of neuronal transmission through activation of neuronal Ca^{2+} channels (Yoshii and Watabe, 1994) and the nicotinic acetylcholine receptor (Zhao et al., 2001). However, the exact mechanisms for the antiamnesic effects of this agent under pathophysiological conditions, including sustained cerebral ischemia, remain unclear.

Our previous studies demonstrated that treatment with nefiracetam attenuated impairment of the adenylyl cyclase (AC)/cyclic AMP (cAMP)/protein kinase A (PKA)/cAMP-responsive element binding (CREB) protein system and improved learning and memory dysfunction after microsphere embolism-induced sustained cerebral ischemia (Nagakura et al., 2002; Takeo et al., 2003). CREB, which is phosphorylated and activated by PKA, binds to the cAMP response element of target genes (Habener, 1990); these genes are considered to be involved in memory formation (Guzowski and McGaugh, 1997). BDNF, which is regulated by CREB (Tao et al., 1998), is a player in

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neuronal events underlying learning and memory function (Tyler et al., 2002). For example, the brain-derived neurotrophic factor (BDNF) heterozygous mouse shows a deficit in learning and memory function (Linnarsson et al., 1997). A continuous intracerebroventricular infusion of antisense BDNF oligonucleotide in rats results in an impairment of spatial learning (Mizuno et al., 2000). CREB also regulates expression of synapsin I (Lonze and Ginty, 2002), which plays a key role in neurotransmitter release by influencing synaptic vesicle exocytosis (Jovanovic et al., 1996, 2000). Therefore, the improvement in microsphere embolism-induced learning and memory dysfunction by treatment with nefiracetam might be associated with amelioration of BDNF and synapsin I expression via the AC/cAMP/PKA/CREB pathway. In our previous studies, the 2,3,5-triphenyltetrazolium chloride (TTC)-unstained area, which was evaluated as the infarct area, was developed by the third day after the embolism, and light microscopic inspection demonstrated scattered necrotic areas primarily in the parietotemporal cortex, corpus callosum, hippocampus, thalamus, and lenticular nucleus of the ipsilateral hemisphere (Miyake et al., 1993; Kajihara et al., 2001). To further elucidate nefiracetam's mechanisms of action, we investigated the effects of nefiracetam on BDNF and synapsin I expression in the hippocampus, which is known to be vulnerable to ischemia (Kirino et al., 1984) and is involved in spatial memory function (O'Keefe, 1979).

2. Materials and methods

2.1. Microsphere embolism

Male Wistar rats (Charles River Japan, Atsugi, Japan), weighing 210–230 g, were maintained in an acclimation room with a 12-h light/12-h darkness cycle, at a temperature of 23±1 °C and a humidity of 55±5% throughout the experiment. The animals had free access to food and water according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Guidelines of Experimental Animal Care issued by the Prime Minister's Office of Japan. All efforts were made to minimize the suffering of the animals, to reduce the number of animals used, and to utilize alternatives to in vivo techniques, if available. The study protocol was approved by the Committee of Animal Care and Welfare of Tokyo University of Pharmacy and Life Science.

Microsphere-induced cerebral embolism was performed by the method described previously (Miyake et al., 1993). In brief, rats were anesthesized intraperitoneally with 35 mg/kg sodium pentobarbital. The right external carotid and pterygopalatine arteries were temporarily occluded with strings. A needle connected to a polyethylene catheter (3 Fr; Atom, Tokyo, Japan) was inserted into the right common carotid artery. Seven hundred microspheres (47.5±0.5 μm diameter, NEN-005; New England Nuclear, Boston, MA, USA), suspended in 20% dextran solution (150 μl), were injected into the right internal carotid artery through a cannula. The needle was removed, and the puncture wound was then repaired with surgical glue (Aron α A; Sankyo, Tokyo, Japan). Thereafter, the strings occluding the right external carotid and pterygopalatine arteries were released. Following the temporal occlusion of the arteries, the blood flow was reestablished within 2–3 min to the areas supplied by the right external carotid and pterygopalatine arteries. Sham-operated rats were prepared in a similar manner by injecting a vehicle without microspheres. Age-matched, nonoperated animals were used as controls.

2.2. Neurological deficits

Fifteen hours after the operation, animal behavior was determined on the basis of paucity of movement, truncal curvature, and forced circling during locomotion as described previously (Miyake et al., 1993). The score of each item (neurological deficit) was rated from 3 to 0 (3, very severe; 2, severe; 1, moderate; 0, little or none). Rats with a total score of seven to nine points were used in the present study. The neurological deficits were determined at 10:00 h up to either day 5 for the examination of biochemical variables including BDNF and synapsin I mRNA levels and their protein contents, or day 9 for the water maze test.

2.3. Treatment with nefiracetam

After stroke-like symptoms of microsphere-injected rats had been detected, the animals were randomly divided into two groups: nefiracetam-treated and untreated groups. Randomization of the operated animals was performed according to the result of the toss of a coin. Nefiracetam at a dose of 10 mg/kg was administered by gavage from 15 h after the operation, once daily to the agent-treated group, whereas a vehicle (0.5% carboxymethylcellulose) was administered by gavage to the untreated group. The dose employed in the present study was based on the data of others (Nabeshima et al., 1990, 1991; Yamada et al., 1999) and those obtained from our preliminary study, which demonstrated that the dose at 10 mg/kg among doses ranging from 3 to 30 mg/kg was most the effective in shortening the escape latency of the microsphere-embolized rats in the water maze test.

2.4. Morris water maze

The water maze test was performed according to the method described by other investigators (Morris, 1981; Miyamoto et al., 1989) and our previous studies (Takagi et al., 1997; Takeo et al., 2003). To eliminate rats that could not swim due to injury after microsphere embolism, we performed a habituation study by placing the rats in a pool with a diameter of 100 cm on day 6 after the

operation. There were no microsphere-embolized rats that could not swim in the habituation test. The water maze test was performed on days 7–9 after the operation. Microsphere-embolized and sham-operated animals were tested using a three-trials-per-day regimen. The water maze apparatus (model TARGET/2; Neuroscience, Tokyo, Japan) consisted of a circular pool with a diameter of 170 cm. A clear acrylic platform circle with a diameter of 12 cm was placed 1.5 cm below the surface of the water (23±1 °C) and kept in a constant position in the center of one of the four quadrants of the pool. The animals were released from three randomly assigned start positions. When an animal mounted the platform, it was kept there for 30 s. If the animal did not reach the platform, it was transferred onto the platform by hand. Data collection was automated by an on-line videotracking device designed to track the object in the field. Escape latency was determined for each trial with a behavioral tracing analyzer (BAT-2; Neuroscience). The cutoff time for each trial was set at 180 s.

2.5. Tissue preparation

Animals were sacrificed by decapitation on day 5 after the operation, and their heads were quickly near-frozen in liquid nitrogen. The hippocampi of the right hemisphere were rapidly dissected and divided into one-half. The divided hippocampi were quickly frozen in liquid nitrogen. Each tissue was used for determination of mRNA levels by reverse transcription polymerase chain reaction (RT-PCR) and of protein contents by Western immunoblotting.

2.6. RT-PCR

Total RNA was purified from the hippocampal tissue using the ISOGEN® reagent (Nippongene, Tokyo, Japan) following the manufacturer's protocol. The level of mRNAs in each tissue was determined by RT-PCR. Total RNA (1 μg) was converted into cDNA using a Reverse Transcription System (Promega, Madison, WI, USA). The following primers were used: BDNF-sense: 5′-AGCCTCCTCTGCTC-TTTCTGCTGGA-3′; BDNF-antisense: 5′-CCACTGTCAT-CAGATGAAATGTTCGTTATCCT-3′ (Promega); Synapsin Ia-sense: 5′-CCCACCCCCACAAGGCCAGCAACA-3′; Synapsin Ia-antisense: 5′-GGTCCCCCGGCAGCAG-CAATGATG-3′ (Safieddine and Wenthold, 1999). The mRNA for β-actin was used as an internal control.

2.7. Western immunoblotting

Frozen tissues were homogenized in ice-cold 4 mM HEPES–NaOH, pH 7.3, 320 mM sucrose, 0.3 mM phenylmethylsulfonylfluoride, 1 mM $MgCl_2$, 1 mM Na_3VO_4, 1 mM NaF, and 5 μg/ml each of aprotinin, antipain, and leupeptin. For immunoblotting analysis, homogenates that had been solubilized by boiling for 5 min in 62.5 mM Tris–HCl, pH 6.8, 2% sodium dodecyl sulfate, 5% β-mercaptoethanol, and 5% glycerol were separated on 10% or 15% polyacrylamide gels and transferred to polyvinylidene difluoride membranes (Immobilon PVDF; Millipore, Bedford, MA, USA). Protein blots were incubated with the indicated antibodies, and bound antibody was detected by the enhanced chemiluminescence detection method (Amersham Pharmacia Biotech, Buckinghamshire, UK) as described by the manufacturer. Quantification was performed using computerized densitometry and an image analyzer. Care was taken to ensure that bands to be semiquantified were linear (Scion Image; Scion, Frederick, MD, USA). Antibodies used were anti-BDNF (1:1000, SC-546; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and antisynapsin I (1:2000; ABR, USA).

2.8. TTC staining

To examine the effect of nefiracetam on the infarct size in the microsphere-embolized rats on day 5 after the operation, TTC staining of brain slices from the nefiracetam-treated and untreated microsphere-embolized rats was performed according to the method described previously (Miyake et al., 1993). In brief, coronal sections with a 2-mm width were incubated for 30 min with 2% TTC in physiological saline. TTC-unstained areas in the ipsilateral hemisphere and hippocampus were measured by the Image analyzer (Scion Image). Infarct areas were expressed as a percentage of the whole hemisphere and hippocampus.

2.9. Statistical analysis

The results are presented as mean±S.E.M. The escape latency of the water maze test and differences in values for biochemical variables among the nefiracetam-untreated sham-operated, nefiracetam-treated sham-operated, nefiracetam-untreated microsphere-embolized, and nefiracetam-treated microsphere-embolized groups were evaluated by using two-way analysis of variance followed by *post-hoc* Fisher's protected least significant difference. Differences with a probability of 5% or less were considered significant ($P<0.05$).

3. Results

3.1. Operation

In the present study, microspheres were injected into 48 rats. Thirty-four of the surviving rats (71%) showed stroke-like symptoms with a total score of seven to nine points, and six animals (13%) showed the symptoms of stroke with a score of less than seven points. Eight rats (16%) died before all examinations were completed. Thirty-four microsphere-embolized rats (17 for the nefiracetam-untreated micro-

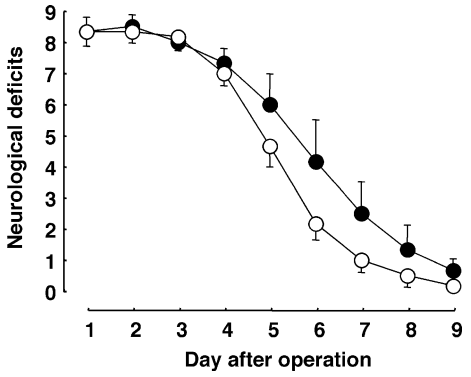

Fig. 1. The time course of changes in neurological deficits of nefiracetam-treated (open circles) and untreated (closed circles) microsphere-embolized rats. Each value represents the mean±S.E.M. of six animals. There were no significant differences in the neurological deficits between the nefiracetam-treated and untreated animals.

sphere-embolized group and 17 for the nefiracetam-treated microsphere-embolized group) and 24 sham-operated rats (12 for the nefiracetam-untreated sham-operated group and 12 for the nefiracetam-treated sham-operated group) were used in the present study.

3.2. Effect of nefiracetam on neurological deficits

The time course of changes in neurological deficits of the nefiracetam-treated and untreated microsphere-embolized rats in the experiment for the water maze test is shown in Fig. 1. The values gradually decreased with time after the operation. There were no significant differences in the neurological deficits between nefiracetam-treated and untreated microsphere-embolized rats [$F(1,10)=1.542$, $P=0.243$]. No neurological deficits were seen in the sham-operated rats, irrespective of treatment with or without nefiracetam in the water maze test series (not shown). Changes in the neurological deficits of the operated animals in the experiments for measurements of mRNA and proteins and in the determination of the infarct size up to 5 days after the operation were similar to those in the water maze test series.

3.3. Effect of nefiracetam on the water maze test

The water maze test was performed on days 7–9 after the operation on the basis of the finding that neurological deficits that might interfere with swimming ability in the water maze task had almost disappeared by this time (Takeo et al., 2003). A significant difference in the escape latency among groups was observed (Table 1). There were significant differences in the escape latency by group [$F(3,20)=25.3$, $P<0.0001$] and by day [$F(8, 160)=15.6$, $P<0.0001$]. The group×day interaction was significant ($P<0.05$). No significant difference in the escape latency between the nefiracetam-treated and untreated sham-operated animals was seen throughout the experiment (Table 1). The escape latency of the microsphere-embolized rats was markedly prolonged compared with that of the sham-operated rats. The escape latency of the microsphere-embolized rats was significantly lengthened compared with that of the sham-operated rats from the second trial on day 7 to the third trial on day 9 ($P<0.05$). Treatment with nefiracetam attenuated the prolonged escape latency of the nefiracetam-untreated microsphere-embolized rats (Table 1). Treatment with nefiracetam reduced the prolongation at the third trial on day 8 and the second and third trials on day 9 ($P<0.05$). Fig. 2 shows the typical swimming loci of the sham-operated, nefiracetam-untreated, and treated microsphere-embolized rats on day 9. The locus to the platform for the sham-operated rats was almost direct. Microsphere-embolized rats took a longer time to climb onto the platform, while nefiracetam-treated microsphere-embolized rats seemed to search for the platform and took a shorter time to climb onto it.

3.4. Effect of nefiracetam on BDNF and synapsin I mRNA levels in the hippocampus

Our previous studies demonstrated that microsphere-embolized rats induced profound changes in biochemical parameters for a period of at least 5 days (Takeo et al., 1992; Taguchi et al., 1993; Takagi et al., 1996). Therefore, the

Table 1
The escape latency in the water maze test of the sham-operated and microsphere-embolized animals with and without nefiracetam treatment

Day	Trial	S	SN	ME	MN
7	1	131.4±18.6	140.0±18.7	153.5±18.5	178.7±1.3
	2	68.7±22.8	65.6±23.4	143.9±26.1[a]	172.7±5.6
	3	52.1±20.4	43.3±26.2	166.4±13.6[a]	157.9±18.6
8	1	36.6±12.8	47.3±20.2	158.3±19.8[a]	107.5±28.3
	2	27.2±11.9	16.3±5.0	129.0±19.5[a]	80.42±24.7
	3	26.0±3.0	22.2±9.4	151.4±22.2[a]	70.3±24.4[b]
9	1	38.2±18.8	22.3±7.2	114.4±23.2[a]	109.7±23.5
	2	10.5±3.7	5.9±1.1	121.5±29.2[a]	60.2±27.0[b]
	3	18.8±9.7	6.2±1.3	134.8±27.6[a]	40.0±11.6[b]

Each value represents the mean±S.E.M. of nefiracetam-untreated (S) and treated (SN) sham-operated animals and of the nefiracetam-untreated (ME) and treated (MN) microsphere-embolized animals ($n=6$ each).

[a] Significantly different from sham-operated group ($P<0.05$).

[b] Significantly different from microsphere-embolized group ($P<0.05$).

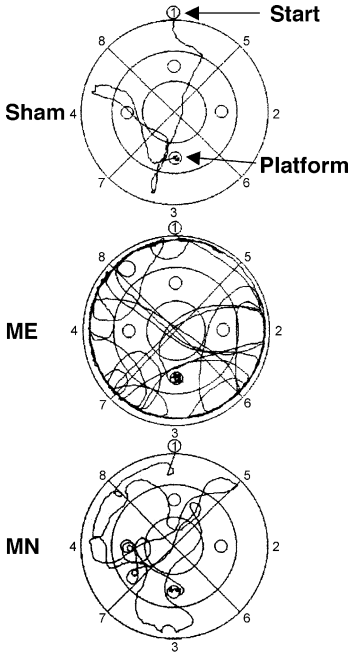

Fig. 2. Typical swimming locus of the nefiracetam-untreated sham-operated (Sham), nefiracetam-untreated (ME), and treated (MN) microsphere-embolized animals on day 9 after the operation.

effects of nefiracetam on BDNF (Fig. 3A), synapsin I (Fig. 3B), and β-actin (Fig. 3C) mRNA expression of the ipsilateral hippocampus were examined on day 5 after the operation. There were no differences in the expression of these mRNAs between the nefiracetam-treated and untreated sham-operated rats. The levels of BDNF and synapsin I mRNA of the microsphere-embolized rats were decreased to approximately 50% and 45%, respectively, of the control rats. Treatment with nefiracetam partially reversed the decreases in BDNF and synapsin I mRNA levels in the ipsilateral hippocampus. No significant alteration in the β-actin mRNA was seen in any of the groups.

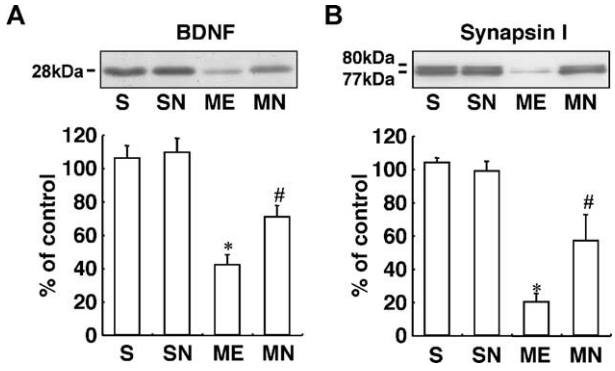

Fig. 4. Representative Western blotting of BDNF (A) and synapsin I (B) in the upper panels indicated the specific bands for BDNF and synapsin I in the ipsilateral hippocampus of the nefiracetam-untreated (S) and treated (SN) sham-operated rats and of the nefiracetam-untreated (ME) and treated (MN) microsphere-embolized rats on day 5 after the operation. Semi-quantified data for the levels of BDNF and synapsin I proteins of the right hippocampus are shown in the lower panels. Each value represents the mean±S.E.M. of six (S and SN) or seven (ME and MN) animals. *Significantly different from the corresponding sham-operated group. #Significantly different from the nefiracetam-untreated microsphere-embolized group when estimated by Fisher's post-hoc PLSD.

3.5. Effect of nefiracetam on BDNF and synapsin I protein levels in the hippocampus

The effects of nefiracetam on BDNF (Fig. 4A) and synapsin I (Fig. 4B) protein levels of the ipsilateral hippocampus were examined by immunoblotting on day 5 after the operation. The immunoreactivities of BDNF and synapsin I in the ipsilateral hippocampus of the microsphere-embolized rats were decreased to approximately 40% and 20%, respectively, of the control rats. Treatment with nefiracetam partially attenuated the decreases in these protein levels. No significant alterations in the immunoreactivity of these proteins were seen in the nefiracetam-treated or untreated sham-operated rats.

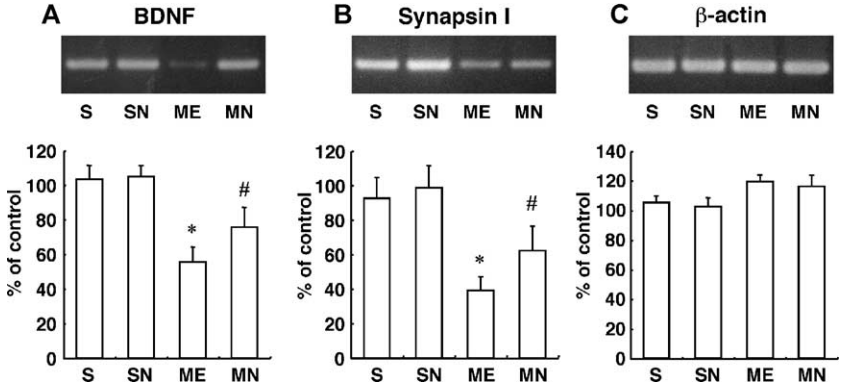

Fig. 3. Representative RT-PCR of BDNF (A), synapsin I (B), and β-actin (C) in the upper panels indicated the specific bands for BDNF, synapsin I, and β-actin in the ipsilateral hippocampus of the nefiracetam-untreated (S) and treated (SN) sham-operated rats and of the nefiracetam-untreated (ME) and treated (MN) microsphere-embolized rats on day 5 after the operation. Semiquantified data for the levels of BDNF, synapsin I, and β-actin mRNAs of the right hippocampus are shown in the lower panels. Each value represents the mean±S.E.M. of six (S and SN) or seven (ME and MN) animals. *Significantly different from the corresponding sham-operated group. #Significantly different from the nefiracetam-untreated microsphere-embolized group when estimated by Fisher's post-hoc PLSD.

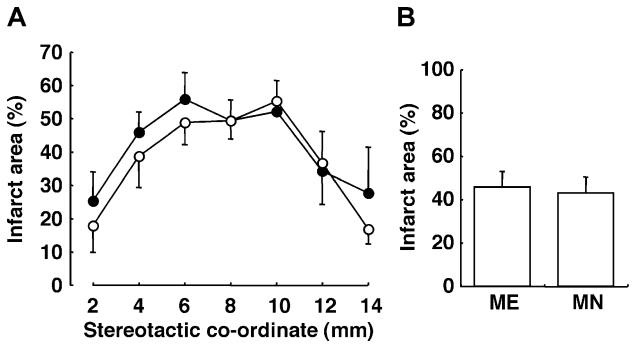

Fig. 5. TTC-unstained area (infarct areas) of the hemispheres at 2, 4, 6, 8, 10, 12, and 14 mm from the forebrain (A) and of the hippocampus (B) of the nefiracetam-untreated (closed circles or ME) and treated (open circles or MN) microsphere-embolized rats on day 5 after the operation. Values are expressed as percentages of the whole hemisphere (A) or hippocampus (B). Each value represents the mean±S.E.M. of four experiments.

3.6. Effect of nefiracetam on infarct size

The effect of nefiracetam on microsphere embolism-induced cerebral infarction was examined on day 5 on the basis of our previous study that a TTC-unstained area had developed by the third day after the embolism, and the extent of the area was comparable thereafter (Miyake et al., 1993). There was no significant difference in the TTC-unstained areas of the brain slices at 2, 4, 6, 8, 10, and 14 mm from the forebrain between the nefiracetam-treated and untreated microsphere-embolized rats (Fig. 5A). In addition, there was no significant difference in the infarct size in the ipsilateral hippocampus between the nefiracetam-treated and untreated microsphere-embolized rats (Fig. 5B). No infarct areas were seen in the brain slices of the nefiracetam-treated and untreated sham-operated rats.

4. Discussion

We demonstrated that treatment with nefiracetam attenuated the sustained cerebral ischemia-induced prolongation of the escape latency in the water maze test. The result is consistent with those of our previous studies (Fukatsu et al., 2002; Takeo et al., 2003) and the effective doses of nefiracetam in the present and previous studies were comparable to those of others obtained in the passive avoidance test (Nabeshima et al., 1990, 1991; Yamada et al., 1999). These results suggest that nefiracetam has the potential to improve spatial memory dysfunction after cerebral ischemia. Furthermore, as there was no influence on the escape latency between the nefiracetam-treated and untreated sham-operated rats, this agent seems to be effective in the learning function under pathophysiological conditions.

Various molecular and cellular mechanisms regulate the learning and memory functions. CREB plays a key role in gene expression, which is thought to be involved in memory formation following new protein synthesis (Guzowski and McGaugh, 1997). Indeed, long-term memory of fear conditioning and spatial learning were disrupted in CREB knockout mice (Bourtchuladze et al., 1994) and the infusion of CREB antisense oligonucleotides into the hippocampus disturbed long-term spatial memory (Guzowski and McGaugh, 1997). We previously demonstrated that microsphere embolism decreased Ca^{2+}/calmodulin-sensitive AC I activity, cAMP content, levels of nuclear PKA, phosphorylation of CREB, and DNA-binding activity of CREB in the ipsilateral hippocampus, and that nefiracetam attenuated these decreases (Takeo et al., 2003). These results suggest a possible relationship between improvements in the cAMP/CREB pathway and amelioration of learning and memory dysfunction by nefiracetam treatment.

It has been shown that various CREB-mediated gene expressions are involved in learning and memory function (Lonze and Ginty, 2002). Of these, BDNF is expressed abundantly in the central nervous system (Lewin and Barde, 1996) and is believed to play a pivotal role in learning and memory function, including the induction of the hippocampal long-term potentiation (Thoenen, 1995; Black, 1999). Furthermore, a selective induction of BDNF expression in the hippocampus during contextual and spatial learning was recently reported (Kesslak et al., 1998; Hall et al., 2000; Schaaf et al., 2001). These findings suggest that an impairment in BDNF expression would be a cause of learning and memory disturbance. Accordingly, we focused on the effect of nefiracetam on the expression of BDNF in the hippocampus after sustained cerebral ischemia. Treatment with nefiracetam appreciably improved BDNF mRNA and protein levels in the ipsilateral hippocampus. BDNF has been shown not only to reduce infarct size after transient focal cerebral ischemia (Schabitz et al., 1997), but also to improve long-term potentiation and cognitive functions after transient global ischemia (Kiprianova et al., 1999), and to recover functional motor activity after photoembolic stroke (Schabitz et al., 2004). In the present study, improvement in learning and memory function with nefiracetam treatment after sustained cerebral ischemia is unlikely to be attributable to the decrease in the infarct areas, as nefiracetam did not influence the TTC-unstained areas.

In addition to BDNF, synapsin I expression is also regulated by CREB and has been well characterized as a key protein in the release of neurotransmitters from nerve terminals and in synapse formation. We demonstrated marked decreases in synapsin I mRNA and protein levels in the ipsilateral hippocampus of microsphere-embolized rats. Treatment with nefiracetam attenuated these decreases after sustained cerebral ischemia. Therefore, the amelioration of synapsin I mRNA and protein by treatment with nefiracetam may contribute to functional recovery of synaptic transmission. Although other molecular and cellular mechanisms may also play an important role in learning and memory dysfunction after sustained cerebral ischemia (Takagi et al., 1997, 2002), our previous and present results suggest that microsphere embolism impairs

CREB-mediated transcription and translation of proteins, including BDNF and synapsin I. These alterations were partially restored by nefiracetam treatment. This improvement may lead to amelioration of spatial memory function in microsphere-embolized animals.

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